# POTENTIAL OF SEED BIOPRIMING IN MANAGEMENT OF DAMPING-OFF DISEASE IN SOLANACEOUS VEGETABLE CROPS

*Thesis*

**by**

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**(H-2019-30-D)**

**submitted to**

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# CERTIFICATE-I

This is to certify that the thesis titled **"Potential of seed biopriming in management of damping-off disease in solanaceous vegetable crops"** submitted in partial fulfillment of the requirements for the award of the degree of **Doctor of Philosophy Plant Pathology** in the discipline of **Plant Protection** to Dr. Yashwant Singh Parmar University of Horticulture & Forestry, (Nauni) Solan (HP)-173 230 is a bonafide research work carried out by **Ms. Deepika Sharma (H-2019-30-D)** daughter of Shri Sarwan Kumar under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been fully acknowledged.

**Place: Nauni, Solan**
**Dated:**

**Dr. H.R. Gautam**
**Major Advisor**

# CERTIFICATE - II

This is to certify that the thesis titled, "**Potential of seed biopriming in management of damping-off disease in solanaceous vegetable crops**" submitted by **Ms. Deepika Sharma (H-2019-30-D)** daughter of Shri Sarwan Kumar to the Dr. Yashwant Singh Parmar University of Horticulture and Forestry, (Nauni), Solan (HP)-173 230, India in partial fulfilment of the requirements for the award of degree of **Doctor of Philosophy Plant Pathology** in the discipline of **Plant Protection** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

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*To err is human. So all the mistakes are mine*

**Place: Nauni, Solan** **(Deepika Sharma)**
**Dated:**

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# LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| % | : | Per cent |
| @ | : | At the rate of |
| µg | : | Microgram |
| µm | : | Micro meter |
| $^{0}$C | : | Degree Celsius |
| ANOVA | : | Analysis of Variance |
| BCAs | : | Biocontrol agents |
| BOD | : | Biological Oxygen Demand |
| CD | : | Critical Difference |
| cfu | : | Colony forming units |
| CMC | : | Carboxy methyl cellulose |
| CRD | : | Completely randomized design |
| cv. | : | Cultivar |
| EB | : | Endophyte bacterial |
| EF | : | Endophyte fungal |
| *et al*. | : | Etalia (co-workers) |
| etc. | : | Etcetera |
| g | : | Grams |
| hrs | : | Hours |
| *i.e.* | : | Id est (that is) |
| mg | : | Milligrams |
| min. | : | Minutes |
| ml | : | Milliliters |
| mm | : | Millimeter |
| MT | : | Metric tons |
| OD | : | Optical Density |
| PDA | : | Potato Dextrose Agar |
| PGPR | : | Plant growth promoting rhizobacteria |
| psi | : | Per square inch |
| RBD | : | Randomized block design |
| SVI-L | : | Seedling vigour index-length |
| SVI-M | : | Seedling vigour index-mass |
| v/v | : | Volume/volume |
| viz. | : | *Videlicet* Namely |
| w/v | : | Weight/volume |

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# *Chapter-1*

# INTRODUCTION

Vegetables have an important role in human diet as these are the important source of vitamins, minerals, phytochemical compounds and dietary fibre content. India ranks 2nd in vegetable production worldwide and thus accounts for about 15 per cent of the world's vegetable production. In India, vegetables are cultivated over an area of 11 million ha with a production of around 205 million MT (Anonymous 2021). In Himachal Pradesh, total area under vegetable cultivation is 91.99 thousand ha with a production of about 1.88 million MT (Anonymous 2021). While among different vegetables in the platter, tomato, capsicum and chilli are important part of our daily food cuisine and culinary. In India, total production of tomato is around 20.33 million MT, total production of chilli and capsicum is around 4.7 million MT and these crops are grown mainly in states of Madhya Pradesh, Andhra Pradesh, Karnataka and West Bengal (Anonymous 2021).

Tomato is a rich source of nutrition and contains 1.1 g protein, 4.7 g carbohydrates, 900 IU vitamin A, 23 mg vitamin C, 0.7 mg niacin, 13 mg calcium, 27 mg phosphorus and 0.5 mg iron per 100 g of fresh fruit. Chilli is another important solanaceous crop having pungency due to capsaicin content and red colour due to the pigment capsanthin. If we analyze 100 g of green chilli fruits, it contains 85.7 per cent moisture, 2.9 g proteins, 0.6 g fat, 6.8 g fibre, 3.0 g carbohydrates, 30 mg calcium, 80 mg phosphorus, 217 mg potassium, 1.2 mg iron, 0.19 mg thiamine, 0.39 mg riboflavin, 67 mg oxalic acid, 0.9 mg nicotinic acid, 292 IU vitamin A and 111 mg vitamin C. Capsicum also known as bell pepper is an important source of carotenoids such as carotene, lycopene, lutein and zeaxanthin. It provides about 12 per cent of the total zeaxanthin and 7 per cent of the total vitamin C consumption. Further, 100 g of green bell pepper fruits contains 86 per cent moisture, 2.2 g protein, 0.8 g fat, 10.0 g carbohydrates, 2.6 g fibre, 29 mg calcium, 61 mg phosphorus, 2.6 mg iron, 0.12 mg thiamine, 0.15 mg riboflavin, 2.2 mg niacin and 140 mg vitamin C (Dhaliwal 2017).

Raising of healthy seedlings in nursery is important for the successful production of vegetable crops which are transplanted like tomato, chilli and capsicum. Hence, these nursery raised solanaceous vegetable crops are prone to attack by several soil-borne pathogens pre-dominantly damping-off disease which is a devastating disease under nursery conditions.

Damping-off disease in vegetable nurseries is caused by fungal pathogens namely *Pythium*, *Phytophthora*, *Fusarium*, *Rhizoctonia*, *Sclerotium*, *Phomopsis*, *Colletotrichum* etc. In solanaceous vegetable crops, pathogens like *Pythium*, *Fusarium*, *Rhizoctonia* and *Sclerotium* are reported to cause damping-off disease under different conditions. Damping-off pathogens are reported to cause huge economic losses (41.33-85 %) and high mortality of seedlings (Jayaraj et al. 2006; Kumar et al. 2018). Damping-off incited by pathogens like *Pythium*, *Rhizoctonia*, *Sclerotium*, *Fusarium* were reported to cause more than 60 per cent mortality of seedlings in nurseries as well as in main fields. Elshahawy et al. (2018) reported that tomato seedlings infected with *Pythium aphanidermatum* caused severe losses of 35 per cent at vegetative stage. Majeed et al. (2019) reported mortality of chilli seedlings to an extent of 27-38 per cent due to damping-off disease. Damping off caused by *Pythium ultimum*., has been reported to cause seedling mortality of upto 90 per cent in brinjal (Gholve et al. 2014). Punja and Yip (2003) reported that seedling mortality due to *Pythium aphanidermatum* was over 80 per cent after about 15 days of inoculation due to the conducive environmental conditions.

In Himachal Pradesh, damping-off disease is of major concern in solanaceous vegetable crops as pre-emergence and post-emergence damping-off incidence of 40-52.21 per cent and 16.67-65.7 per cent in tomato was reported by various research workers (Raj et al. 1997; Kumar 2017; Bhardwaj 2019). Symptoms of damping-off include poor germination of seeds during pre-emergence phase wherein radicle and plumule of young seedlings undergo decay before emerging out of soil. In post-emergence phase of damping-off, hypocotyls were attacked by the pathogens that forms water-soaked lesions near soil surface which ultimately led to withering and death of plants (Kumar et al. 2018).

Chemicals have been used either as fumigants or as drenching or both for the management of the soil-borne pathogens in the nursery but use of chemicals for the management of soil-borne pathogens has adversely affected human health and environment. There are some alternative methods like application of soil amendments, soil solarization and bio-control agents which are reported effective in managing damping-off disease, Use of soil amendments and different seed biopriming treatments are also gaining importance as effective alternatives to chemical control. Out of 16 per cent, annual crop losses due to plant diseases, at least 10 per cent loss is incurred due to seed-borne pathogens. Seed priming refers to a approach for seed treatment in which amount of water absorption is controlled in order to initiate metabolic and physiological activities for seed germination but emergence of

radicle is prohibited. Callan et al. (1990) described priming of seeds with beneficial microorganisms (biopriming) as a type of seed priming in which hydration of seed (physiological mechanism) is integrated with bio-inoculation (biological mechanism). Rapid and uniform seed emergence are essential for healthy and high-quality seedling production particularly in transplanted vegetable crops. Thus, seed treatment like seed biopriming carried out before sowing is highly useful to achieve rapid and uniform germination along with protection against seed and soil-borne pathogens. Biopriming of seeds with bioagents will help to increase the population load of bioagents to many folds on the seeds once they are primed, thus protecting the spermosphere of seeds from the ingress of soil-borne pathogens. Seed biopriming is an economic, highly effective, simple and green approach for improving plant acclimatization and simultaneously to counter biotic and abiotic stress conditions, which will lead to consistent performance of bio-control agents, ensuring uniform seed germination rapid emergence, better crop stand establishment and improved plant growth.

Seed biopriming with bio-control agents and microbial consortia is an economic and eco-friendly alternative to the chemicals. Thus, keeping in view, the damage caused by the damping-off disease, the study was done with the following objectives:

i) To isolate the pathogens associated with damping-off disease
ii) To study efficacy of seed biopriming with microbial inoculants, plant extracts and endophytes against damping-off disease
iii) Integration of seed biopriming with soil amendments and organic extracts
iv) To study efficacy of seed biopriming in activation of defence related compounds in plants against disease

# *Chapter-2*

# REVIEW OF LITERATURE

Vegetables are important for human life as they provide nutrition to human body being rich source of vitamins, minerals, dietary fibre etc. Among vegetables, solanaceous crops are particularly important due to their daily use and nutritional benefits in the human diet. The productivity of solanaceous crops is adversely affected due to the high incidence of fungal, bacterial and viral diseases. Soil-borne pathogens like *Pythium*, *Phytophthora*, *Rhizoctonia*, *Fusarium*, *Sclerotium*, *Sclerotinia*, *Colletotrichum*, *Verticillium* are important which infect the crops at various stages of growth. However, pathogens like *Pythium*, *Phytophthora*, *Fusarium*, *Sclerotium* and *Rhizoctonia* cause pre-dominantly pre and post emergence damping-off at the nursery stage.

Healthy nursery is essentially required for raising a healthy crop. However, to raise healthy nursery farmers face major problem of damping-off incited by various pathogens like *Pythium* sp., *Phytophthora* sp., *Fusarium oxysporum*, *Sclerotium rolfsii*, *Rhizoctonia solani* etc. Damping-off disease has been reported to cause incidence of 25-75 per cent depending upon host variety and environmental conditions (Gupta and Paul 2001). Damping-off disease caused by *Pythium* sp., *Rhizoctonia* sp., *Sclerotium* sp. and *Fusarium* sp. has been reported to be most devastating at the nursery stage which has been reported to cause seedling mortality of 50-60 per cent (Singh and Srivastava 1953). Damping-off disease caused by *Pythium aphanidermatum* has been reported as serious disease in tomato causing high mortality of seedlings grown in nursery and field conditions (Jayaraj et al. 2006).

Damping-off caused by *Pythium aphanidermatum* in vegetable nurseries is a major problem in solanaceous vegetables with about 60-75 per cent mortality of tomato and chilli seedlings (Manoranjitham et al. 2000; Jadhav and Ambadkar 2007). Ramamoorthy et al. (2002) reported 62 per cent mortality of chilli seedlings due to *Pythium aphanidermatum* during nursery raising. Further, while *Sclerotium rolfsii* resulted in 13 per cent mortality due to rotting of seedlings, *Rhizoctonia solani* caused 20 per cent seedling blight in tomato nurseries (Sri et al. 2017). In other report, *Sclerotium rolfsii* resulted in approximately 30 per cent crop loss in greenhouse and field conditions during warm and humid conditions due to collar rot in tomato (Rajput et al. 2020).

**Geographical distribution**

Damping-off disease caused by various fungal pathogens has worldwide distribution with vast host range. Among oomycetes, the genus *Pythium* is one of the largest genus that consists of 130 species which have been isolated from different crops in different regions of the world. In tomato, damping-off disease is also a serious problem and pathogens like *Pythium*, *Rhizoctonia*, *Phytophthora* have been found to be associated (Anita et al. 2012). *Pythium* sp. was reported to be responsible for incidence to extent of 50 per cent in tomato (Bisht et al. 1997).

Among *Pythium* spp., *P. aphanidermatum* is one of the most pathogenic species with extensive host range and cause severe damage in economically important crops (Nene et al. 1996; Dey 2005; Kubota 2010). *Pythium aphanidermatum* has been reported to cause damping-off in Egypt for the first time (Elshahawy et al. 2018). In India, *Pythium*, *Fusarium*, *Rhizoctonia* and *Macrophomina* have been found to be associated with damping-off disease (Jiskani et al. 2007; Awasthi et al. 2019). Damping-off disease caused by *Rhizoctonia solani* in tomato has been reported to be an important disease in Sri Lanka (Abeysinghe 2009). Jiskani et al (2007) reported the association of *Fusarium oxysporum* and *Rhizoctonia* solani in high frequency from the infected root portions of tomato seedlings causing seedling blight and root rot in tomato. *Rhizoctonia solani* has been found associated with damping-off disease in tomato under moist infected soil with around 20 per cent incidence in field (Marzouk et al. 2021). Damping-off disease caused by *Fusarium oxysporum* has been reported to be the most prevalent disease of tomato (Sudhamony et al. 2009). *Sclerotium rolfsii* has also been reported to be found associated with damping-off disease in tomato along with other soil-borne pathogens like *Fusarium oxysporum* and *Macrophomina phaseolina* (Siddiqui et al. 2016). *Sclerotium rolfsii* has been reported as the causal agent of damping-off disease in tomato from Nigeria and the fungus was found as a white mycelial mat on infected roots producing reddish or dark brown sclerotia (Okereke and Wokocha 2006).

In chilli, damping-off incited by *Pythium* spp. is responsible for upto 90 per cent mortality as pre and post emergence damping-off in nurseries and fields (Sowmini 1961; Zagade et al. 2012). In addition, fungal pathogens like *Alternaria*, *Colletotrichum*, *Phytophthora* and *Fusarium* were also found associated with the disease in chilli. Among different diseases, damping-off disease of chilli incited by *Pythium* sp. reported to cause more

than 90 per cent plant death which limits the cultivation of chilli leading to reduction in yield (Zagade et al. 2012; Jain et al. 2014). Damping-off disease has also been reported from Punjab (Pakistan) in chilli growing areas with disease incidence ranging from 13.8 to 45.4 per cent (Hyder et al. 2018). *Pythium aphanidermatum* was found to be associated with damping-off of chilli and brinjal seedlings in India (Kavitha et al. 2005; Bohra et al. 2006). *Pythium* sp. has also been found to be associated with damping-off from major chilli growing areas of Tamil Nadu (Muthukumar et al. 2010). Association of different *Pythium* sp with damping-off disease in tomato has been reported by various workers (Yadav and Joshi 2012; Gilardi et al. 2013; Lai et al. 2015). Further, *Sclerotium rolfsii* has also been reported to cause severe incidence of root rot in chillies for the first time from Rajasthan causing huge economic losses (Kamlesh and Gurjar 2001). Chilli seedlings were also found to be attacked by *Rhizoctonia solani* causing damping-off disease (Ananthi et al. 2017).

**Symptomatology**

Damping-off disease appear in two phases based upon the time of infection. Pre-emergence damping-off symptoms have been associated with seeds before their emergence from soil. In pre-emergence damping-off, infection of the germinating seeds results in seed rotting, water-soaked lesions are produced on roots and stems of emerging seedlings which ultimately lead to seedling death (Singh 2000). In post-emergence damping-off, stem becomes thin with brown lesions, crown rots, hypocotyl become water soaked, cotyledonary leaves roll downward, roots of the infected plant rot which eventually led to death of the seedlings (Gupta and Thind 2006).

*Pythium* sp. are ubiquitous in soil and considered as most damaging plant pathogens in nursery, greenhouse and field conditions. Among different *Pythium* spp., *Pythium aphanidermatum*, is most destructive causal agent of damping-off in tomato in nurseries. Elshahawy and El-Mohamedy (2019) reported the symptoms of damping-off in tomato caused by *Pythium* sp. as patchy appearance of seedlings in the field as the hypocotyl gets infected before seed emergence and plants wither and died during the later stages. *Rhizoctonia solani* was also reported to cause damping-off disease in tomato plants where symptoms of pre-emergence and post emergence were recorded (Ismael and Mahmood 2016). Symptoms on tomato seedlings due to *Rhizoctonia solani* appears in early stage as typical brown discoloration in roots and lesions also appear at collar region. These lesions later coalesce that leads to rotting of roots and the plant then ultimately died (Jiskani et al. 2007).

*Sclerotium rolfsii* causes damping-off of seedlings in tomato as well as stem rot and blight in adult plants. This fungus attacks the lower portion of the seedlings near the soil surface and causes root rot which is more problematic in warm and moist conditions (De Curtis et al. 2010).

Symptoms of damping-off in brinjal initiates as the tissue of stem portion become soft, water-soaked lesions appear and then rotting of collar portion occur which leads to collapse and death of seedlings (Gholve et al. 2014; Awasthi et al. 2019). Tiwari et al. (2017) reported wilting of lower leaves followed by vascular discoloration of xylem extending upwards throughout the seedlings in damping-off affected seedlings of eggplant. Muthukumar et al. (2010) reported rotting of seeds and seedlings before actual emergence from the soil due to damping-off symptoms in chilli caused by *Pythium* sp. Post-emergence damping-off is severe when seedlings were in cotyledonary stage. In chilli, attack by *Fusarium oxysporum* at nursery stage resulted in stunting, chlorosis and wilting of leaves (Ferniah et al. 2014). Dube (2014) reported that *Rhizoctonia solani* attacks the seedlings at the base of stem near the soil line causing necrosis of tissues of stem which ultimately result in fall of seedlings. Damping-off in chilli plants due to *Rhizoctonia solani* has been reported to cause necrotic lesions at the collar regions (Abeysinghe 2009).

**Pathogenicity**

Hassanisaadi et al (2021) carried out pathogenicity assessment experiment and observed that *Pythium aphanidermatum* was pathogenic to tomato (cv. Moneymaker) with disease incidence of 88.89 per cent. Different *Pythium* species were isolated from tomato plant and their association with damping-off disease was proved with sick pot method and the symptoms of the disease were recorded after 30 days of sowing (Elshahawy et al. 2018; Karmel and Muthukumar 2019). Ismael and Mahmood (2016) proved the pathogenicity of *Fusarium oxysporum* and *Rhizoctonia solani* on tomato cultivar “Super Queen” by soil inoculation with spore suspension method for 30 days and reported that damping-off disease appeared to be most severe in affecting seedling health and quality. Jiskani et al. (2007) reported association of *Rhizoctonia solani* causing damping-off in tomato by soil inoculation method. They reported that *Rhizoctonia solani* infected plants were obtained after 30 days of sowing from the infested soil with disease incidence of 63.63 per cent.

*Pythium aphanidermatum* has been found to be responsible for damping-off of brinjal seedlings (Ramesh 2004). Pathogenicity of five different isolates of *Pythium* from chilli

seedlings was proved for their association with damping-off in chilli by using sick pots and symptoms of the disease appeared after 10 days of sowing (Muthukumar et al. 2010). Hyder et al. (2018) reported that the chilli plants inoculated with *Pythium mriotylum* exhibited damping-off and root rot symptoms on chilli cv. Sanam under greenhouse conditions at 30°C after 15 days of sowing. Ramesh (2004) and Jiskani et al. (2007) proved the pathogenicity of *Pythium aphanidermatum* with damping-off in brinjal and chilli. Pathogenicity of *Fusarium oxysporum* on chilli seedlings were proved by Ferniah et al (2014) by using root dip method in conidial suspension of pathogen and the symptoms of the damping-off appeared in 15 days of inoculation. Abeysinghe (2009) proved pathogenicity of *Rhizoctonia solani* isolated from the necrotic lesions at collar region of chilli plants by soil inoculation method. Pathogenicity of five different isolates of *Sclerotium rolfsii* was proved in pot culture experiment in chilli and seedling mortality was observed at 10-20 days after sowing of seeds in infected soil under pot conditions (Sultana et al. 2012).

**Disease management**

Use of chemical fungicides for the management of soil-borne pathogens result in soil pollution which disturbs the biological balance of soil by destroying non-target and beneficial microorganisms. Increasing awareness of long-term adverse effects of chemicals in soil to non-target soil microflora is creating attention towards biological methods of disease management (Moorman et al. 2002; Lamichhane et al. 2017). Biological methods of disease management are emerging as promising and sustainable approach in managing plant diseases. Researchers are focussing their efforts on developing environmentally safe, long lasting and effective biological methods for management of damping-off disease and other soil-borne pathogens (Rani and Satheesh 2007; Hooda et al. 2011; Chavan et al. 2012; Singh and Yaduman 2016; Nirmalkar et al. 2018; Singh et al. 2020a). Seed priming with bio-inoculants is one of such novel approach which has a potential role in disease suppression by utilizing various biological mechanism to replace the chemical methods of disease management (Pathak et al. 2016; Entesari et al. 2013).

**a) Biocontrol agents**

Biological control is one of the promising environment friendly alternatives for protecting plants against soil borne pathogens (Singh et al. 2011; Singh and Singh 2012). Use of native rhizosphere microorganisms as biological agents is an effective method of disease management. Biological control agents are extensively used for the management of soil-

borne diseases and their efficacy has been reported against number of pathogens (Paulitz and Belanger 2001; Dhanasekaran et al. 2005; Gravel et al. 2005; Hyder et al. 2021). Biological seed treatment is effective method in protecting plants against soil-borne pathogens and thus reduce dependence on chemical fungicides for disease management (Callan et al. 1997; Jain et al. 2014; Singh et al. 2016). Biological control agents (BCAs) and plant growth promoting rhizobacteria (PGPRs) are effective plant conditioners in improving plant health. Biocontrol agents have high effectiveness, are inexpensive with long shelf life and suitable delivery method (Someshwar et al. 2013)

Different fungal and bacterial agents like *Trichoderma*, *Serratia*, *Pseudomonas*, *Streptomyces*, *Bacillus subtilis*, *Bacillus cereus*, *Talaromyces variabilis, Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Burkholderia* and *Serratia* spp. have been reported as effective biocontrol agents against different soil borne pathogens (Liu et al. 2007; Chen et al. 2009; Labuschagne et al. 2010; Mishra 2010; Souza et al. 2015; Halo et al. 2018; Hassanisaadi et al. 2021). PGPRs have biocontrol potential against *Pythium* spp. along with their plant growth promoting activity in crops like tomato, potato and cucumber as reported by various workers (Al-Hussini et al. 2019; Kenawy et al. 2019; El Tarabily et al. 2009). Among different biocontrol agents, *Trichoderma* spp. are considered as potent bioagents against soil-borne diseases (Sharma et al. 2014). *Trichoderma harzianum* and *T. lignorum* isolates were found effective against various soil-borne plant pathogens (Spiegel and Chet 1998). Further, *Trichoderma viride*, *T. harzianum*, *T. hamatum*, *T. reesei*, *T. koningii*, *Pseudomonas* sp. and *Bacillus* sp. have been used effectively for seed and soil treatments against *Pythium* sp. (Hazarika et al. 2000; Pandey and Pandey 2005).

Rani and Satheesh (2007) reported inhibition of *Pythium aphanidermatum* causing damping-off in tomato by *Trichoderma viride* (71.85 %) followed by *T. virens* with inhibition of 70.11 per cent. Dania and Omidiora (2019) reported that the inoculation of *Trichoderma viride* reduced the radial mycelial growth of *Pythium aphanidermatum* in tomato to 64.8-67.2 per cent. Among various antagonists tested against *Pythium ultimum*, *Trichoderma harzianum* was found to be significantly superior in suppressing the growth of *Pythium ultimum* followed by *T. hamatum* and *T. konigii* while *T. lignorum* was least effective (Chavan et al. 2012). *Bacillus subtilis* RB14, has been reported effective against *Rhizoctonia solani* causing damping-off disease in tomato (Asaka and Shoda 1996). *Streptomyces* strains have been

reported effective in reducing the incidence of damping-off in tomato seedlings due to *Rhizoctonia solani* (Dhanasekharan et al. 2005).

Srivastava et al. (2010) observed that among different isolates of *Trichoderma*, isolate 35 inhibited the mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* by 48 per cent as compared to other isolates having inhibition of 30-45 per cent. Among bacterial bio-agents, four *Pseudomonas* isolates inhibited more than 45 per cent growth. Antagonists like *Bacillus* sp., *Pseudomonas fluorescens*, *Trichoderma harzianum* when inoculated in soil, resulted in suppression of damping-off incidence in tomato seedlings along with increment in plant biomass in comparison to untreated pots (Kavitha et al. 2005; Sivakumar and Sharma 2007; Jayaraj and Radhakrishnan 2008).

*Trichoderma* species like *T. harzianum, T, viride* and *T. koningii* were evaluated for their efficacy against *Sclerotium rolfsii* under *in vitro* conditions and *T. harzianum* was found most effective with mycelial inhibition of 61.5 per cent (Kamel et al. 2020). *Pseudomonas* sp. strain PC12 has been reported effective against damping-off in tomato caused by *Sclerotium rolfsii* (Pastor et al. 2010). *Trichoderma* species inhibit *Pythium* spp in seedlings of sweet pepper with the mechanism of hyper parasitism, while *Bacillus subtilis* causes apparent lysis of the pathogen mycelia (Howell 2003; Harman et al. 2004; Idowu et al. 2016). Gholve et al. (2014) reported that among different antagonists evaluated *Trichoderma viride* was found most effective with highest mycelial inhibition of 69.44 per cent followed by *Trichoderma koningii* and *T. hamatum* with 67.32 and 63.99 per cent of mycelial inhibition against *Pythium ultimum* causing damping-off in brinjal.

Mahmoud and Abdalla (2018) reported that application of *Trichoderma harzianum* reduced the disease severity by 53.88 per cent and *Gliocladium catenulatum* by 39 per cent. Application of rapeseeds with *Serratia plymuthica* and *Pseudomonas chlororaphis* applied either alone or in combination against the pathogen *Leptosphaeria maculans* was effective with mean disease reduction of 71.6 per cent for *S. plymuthica* and 54 per cent for *P. chlororaphis* (Abuamsha et al. 2012).

**b) Botanical extracts**

Plants and their products have the potential of being used as cost-effective and environmentally safe alternative for disease management (Hussain et al. 2009). Plants are a valuable source of bioactive compounds like secondary metabolites, phenols, phenolic acids,

quinones, flavones and tannins etc. as these group of compounds have antimicrobial activity against plant pathogens and thus these are important components of plant defense against the pathogenic microorganisms. Plant extracts have been used for management of plant pathogens by various workers (Gachomo and Kotchoni 2008; Thobhunluepop 2009; Duru and Onyedineke 2010, Monaim et al. 2011).

Leaf extract of *Lantana camara* and *Cassia auricularia* inhibited sclerotial production of *Sclerotium rolfsii* (Manian et al. 1988). Further, *Azadirachta indica* and *Piper betle* reduced the sclerotial production of *Rhizoctonia solani*. Leaf extracts of *Lantana camara* reported to have fungitoxic activity against *Fusarium oxysporum* and *Pythium debaryanum* (Kumar et al. 1996). Pattnaik et al (2012) reported that leaf extracts of *Aegle marmelos*, *Tagetes patula*, *Piper nigrum* and *Ageratum conyzoides* inhibited mycelial growth of *Pythium debaryanum*. Leaf extracts of cinnamon, moringa and clove at three concentrations (1-3 %) reported to have inhibitory effect against *Rhizoctonia solani* causing damping-off disease in tomato.

Hooda et al. (2011) reported, maximum control of pre-emergence rot caused by *Pythium aphanidermatum* in tomato with *Lantana camara* (40.4 %) followed by *Sapium* sp. (32.9 %), *Urtica parviflora* (31.4 %) and *Ligustrum nepalensis* (30.4 %). Hossain et al. (2011) reported that six extracts obtained from *Allium cepa*, *Azadirachta indica*, *Cassia alata*, *Curcuma longa*, *Datura innoxia* and *Polygonum orientale* were found to inhibit the growth of *Fusarium oxysporum* and *Rhizoctonia solani*. They reported that the maximum inhibition (100.0 %) of different *R. solani* isolates was found with plant extracts of *A. sativum* and *D. innoxia* followed by *A. racemosus* (59.29%).

Zagade et al. (2012) reported that against chilli damping-off, ginger rhizome extract and turmeric rhizome extract were found effective in suppressing the growth of *Pythium ultimum.* Gholve et al. (2014) reported that out of ten botanicals evaluated at 10-20 per cent concentration against *Pythium ultimum*, garlic was found most effective with highest mycelial growth inhibition of 94.83 per cent followed by datura with 60.65 per cent of mycelial inhibition.

Singh and Yaduman (2016) reported that 40 and 60 per cent concentrations of *Azadirachta indica* were effective against *Pythium aphanidermatum* with lowest incidence. They reported that decrease in mortality rate of seedlings was observed with priming of seeds

with extracts of wood apple, neem, *Vinca rosea* and *Datura stramonium* which inhibited the growth of *Fusarium oxysporum* by 72 per cent. Islam and Faruq (2012) studied the effect of plant extracts against damping-off disease in solanaceous vegetable crops and reported that neem leaf extract followed by garlic extract were effective in suppressing damping-off disease in chilli, brinjal and tomato. They also reported that seed treatment with effective plant extracts was also effective with highest seed germination recorded in tomato (86.67 %) followed by brinjal (86.33 %) and chilli (90.33 %).

**c) Endophytes**

The term "endophyte" is derived from a Greek word "endon" meaning inside and "phyte" meaning plants. Thus, endophytes can be defined as those microorganisms that colonize the internal tissues of plants and cause no harm or negative effect on their host (Schulz and Boyle 2006; Rodriguez et al. 2009). Endophytic microorganisms reside inside the plant cell or vascular system without producing any external symptoms (Hallmann et al. 1997). Endophytes colonize the plant surface, produce enzymes to hydrolyze plant cell walls in order to gain entry inside plants. Potential of endophytes as biocontrol agents in agriculture has been explored by various workers (Lahlali and Hijri 2010; Bae et al. 2011; Boshra et al. 2020).

Different endophytic microorganisms produce secondary metabolites and some have antifungal and antibacterial activity against plant pathogens (Arnold et al. 2003). These endophytic microbes have an ecological importance and ability to influence the tolerance of plants against biotic and abiotic stresses. Endophytes can be of fungal and bacterial origin. Fungal endophytes were reported to benefit the plants by promoting plant growth, improving resistance to multiple stress and protection from diseases (Malinowski et al. 2004; Tanaka et al. 2005). Different endophytic fungi have potential to suppress plant pathogenic fungi. Some important examples of endophytic fungi are *Acremonium* sp., *Trichoderma harzianum*, *Curvularia* spp. and *Tolypocladium* spp. found effective against soil borne pathogens *Pythium myriotylum*, *Pythium aphanidermatum*, *Rhizoctonia solani* and *Phytophthora palmivora*, respectively as reported by various research workers (Anisha and Radhakrishnan 2015; Vinayarani and Prakash 2018; Alsultan et al. 2019). Lahlali and Hijri (2010) reported that *Trichoderma atroviride* and *Epicoccum nigrumn* have significant inhibition against *Rhizoctonia solani*. *Pseudomonas aeruginosa* isolated from the roots of wheat plant was reported to have antagonistic activity to root rotting fungi *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium solani* (Tariq et al. 2014).

Similarly, endophytic bacteria isolated from the surface of the disinfected parts of healthy plants have also been reported to have potential as biocontrol agent. Bacterial endophytes have been reported from genera like *Burkolderia*, *Cronobacter*, *Enterobacter*, *Pantoea* and *Pseudomonas*. Gram negative endophytic bacteria like *Chromobacterium violaceum*, *Pseudomonas fluorescens* were reported to be effective against *Sclerotium rolfsii* and *Rhizoctonia solani* (Nagarajkumar et al. 2004). Nine bacterial endophytes isolated from the stem and root portions of chilli plant were reported to be effective against the chilli damping-off pathogen *Pythium aphanidermatum* under glasshouse conditions (Muthukumar et al. 2010). Among all these bacterial endophytes EBC 5, EBC 7 and EBC 6 recorded maximum inhibition of pathogen over control. Endophytic isolates of *Pseudomonas fluorescens* were reported to enhance the germination of tomato seeds by 88-93 per cent in 10 days period (Sundaramoorthy and Balabaskar 2012). Fungal endophytes like *Trichoderma ovalisporum*, *T. theobromica*, *T. hamatum*, *T. stilbohypoyli* and *T. caribeaum* isolated from plants had a role in protecting the pepper plants against *Phytophthora capsici* (Bae et al. 2011).

Priyanka et al. (2019) reported that among thirty different isolates tested for their efficacy against *Pythium aphanidermatum*, *Pseudomonas aeruginosa* and *Achromobacter denitrificans* showed maximum inhibition of the damping-off pathogen in cucumber seedlings. Huang et al. (2018) reported that the endophytic bacteria *Bacillus mycoides* has potential antagonistic effect against the damping-off pathogen *Pythium aphanidermatum* on cabbage seedlings. Boshra et al. (2020) reported the suppression of *Pythium aphanidermatum* causing damping-off in cucumber by an endophytic fungus *Cladosporium omanense*. This endophytic fungus also increased the surviving ability of cucumber seedlings upto an extent of 58.9 per cent. The culture filtrate of *Cladosporium omanense* consisted of cellulose, β-1,3 glucanase, siderophores that induces cellular leakage and which also inhibited oospore production.

**d) Seed biopriming**

The concept of seed priming was proposed by Heydecker and his co-workers in 1973. Seed priming is known as the controlled hydration process, which is widely used for improving the seed performance by improving the rate and uniformity of germination and thus decreasing sensitivity of seeds to external factors. Among the different pre-sowing techniques for disease management, biopriming has emerged out as a novel, most simple,

economical and eco-friendly delivery system of beneficial microorganisms in the agroecosystem (Sarkar et al. 2017; Singh et al. 2016; Sarkar et al. 2020). Bio-priming is a process that involves treatment of seeds with beneficial microbial inoculants under controlled hydration conditions without emergence of radicle.

Biopriming is a new technique that integrates biological and physiological aspects of disease management strategies for the effective management of various seed and soil-borne pathogens (Callan et al. 1990; Ananthi et al. 2014; Singh et al. 2016; Devika et al. 2021). Seed biopriming is innovative among other methods of disease management as not only it enhances the germination of seed, seedling vigour but also reported to be useful in combating biotic as well as abiotic stress (Someshwar and Sitanshu, 2010; Sukanya et al. 2018; Prabha et al. 2019). The expression of physiological process associated with seed biopriming involves increase in hydrolytic enzyme activity, reactive oxygen species (ROS), detoxifying enzyme activity and changes in hormonal levels along with differential gene expression that induces to resistance against biotic and abiotic stress (Deshmukh et al. 2020).

Seed priming is the technique which is based on the progress of germination in three phases *i.e.,* imbibition phase, transition phase and growth phase. Water uptake by seeds follows triphasic pattern involving rapid imbibition phase (phase I), followed by lag phase (phase II) and final water uptake phase (phase III) associated with radicle growth (Bennette et al. 2013). The basis of seed priming is to allow a controlled water uptake by seeds till the end of phase II, before radicle emergence from the seed coat. Since most of the seeds are desiccation tolerant upto this stage, the germination process can be arrested by drying. Thus, seeds that have passed this critical stage in the priming process can only germinate in a wider range of environmental conditions as compared to non-primed seeds.

Bio-priming is a potentially prominent technique that induces changes in characteristics of plants and thus promotes uniform seed germination and plant growth (Ibrahim 2016; Devika et al. 2021; Sarkar et al. 2020). Among various modes of bio-agents application, seed biopriming has been found to be very effective due to its characteristics like enhancing rate of germination, uptake of inorganic phosphates and induction of defense activity etc (Keswani et al. 2014; Bisen et al. 2015). Role of PGPR like *Bacillus licheniformis*, *Bacillus amyloliquifaciens* as seed priming agents against biotic and abiotic stress and in improving seed quality parameters like seed germination, root length, shoot

length has been reported by various research workers (Nemec et al. 1996; Gowthamy and Manonmani 2018).

Rakshit et al. (2014) reported that seed biopriming can promote rapid and uniform seed germination, plant growth and augment seedling strength to combat biotic and abiotic stresses and improve the crop performance. They reported that biopriming of seeds with *Trichoderma viride*, *Trichoderma harzianum*, *Bacillus subtilis* and *Pseudomonas fluorescens* resulted in the reduction of root rot incidence (*Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium solani*) and thus considered this process as safe, cheap and reliable biocontrol method against the soil-borne pathogens. Seeds bioprimed with different *Trichoderma* spp. were reported to have minimum root rot and damping-off incidence in tomato (Kumar et al. 2012). Reddy (2013) reported that seed biopriming with *Pseudomonas aeruginosa* resulted in reduction of damping-off disease by 48.6-65 per cent.

Biopriming with *Clonostachys rosea* was found effective against pre-emergence and post-emergence damping-off caused by seed-borne pathogens *Alternaria dauci* and *A. radicina* effectively (Jensen et al. 2004). Priming of tomato seeds with plant extracts of *Azadirachta* (rich in terpenoids, steroids), *Chlorophylum* (saponins and alkaloids) and *Vinca rosea* was found to reduce the plant mortality of tomato along with high seedling vigour (Prabha et al. 2016). Seed biopriming of chilli seeds with *Pseudomonas fluorescence* resulted in maximum seed germination (59.4 %) and disease incidence of damping-off was reduced by 21.6 per cent (Chauhan and Patel 2017).

Ananthi et al. (2014) reported that the chilli seeds primed with *Pseudomonas fluorescens* 60 per cent (w/v) for 12 hrs have high germination with an increase of 31 per cent over non-primed seeds. They also reported that seeds bioprimed with *T. viride* for 3 hrs has 93 per cent germination as compared to non-primed seeds. Seed biopriming has been reported to significantly reduce the seed-borne diseases like collar rot (*Sclerotium rolfsii*) and Fusarium wilt in chickpea (Singh et al. 2013). Seed priming with endophytic microorganisms helps due to inherent properties of endophytes like growth promotion, phytohormone production and induction of resistance, tolerance to abiotic and biotic stresses. Irabor and Mmbaga (2017) reported that isolated endophytic bacterial isolates of *Bacillus* have superior inhibitory effects against *Phytophthora capsici*. Seed treatment with these isolates reduced the disease severity and also increased the plant shoot length, fresh weight and fruit yield significantly.

Biopriming of sweet corn seeds with *Pseudomonas fluorescens* at $3.3x10^8$ cfu was found effective against damping-off disease under field experiment (Callan et al. 1990). Seed biopriming in rice with *Trichoderma harzianum*, *T. viride*, *T. virens*, *Pseudomonas fluorescens* and mixed formulations of *T. harzianum* and *Pseudomonas fluorescens* at 5-10 g/kg of seed has been reported to give effective control of various seed and soil-borne diseases, especially against *Rhizoctonia solani*, *Pythium* sp., and *Helminthosporium oryzae* (Mastouri et al. 2010; Swain et al. 2018).

Biopriming of seeds resulted in reduction in root rot incidence caused by *Fusarium solani*, *Macrophomina phaseolina* and *Rhizoctonia solani* in cowpea by 64 and 45.3 per cent at pre-emergence stage and by 68 and 60.1 per cent at post-emergence stage (El-Mohamedy et al. 2006). Control of these root rot pathogens due to seed biopriming could be attributed due to increase in activity of indigenous microflora resulting in suppression of pathogen population, release of compounds like $CO_2$, ammonia, nitrites, saponin or enzymes etc. which are generally toxic to pathogens.

**e) Integration of seed biopriming with microbial inoculants, plant extracts and soil amendments with soil solarization**

Soil-borne pathogens can be more effectively managed by integration of different eco-friendly management strategies like soil solarization, biocontrol agents, botanicals and endophytes (Ramamoorthy et al. 2002; Akhtar et al. 2008; Yadav et al. 2013; Tiwari et al. 2017). Damping-off disease of tomato due to *Pythium*, *Sclerotium* and *Rhizoctonia* were effectively managed by integration of different biological resources (Pandey et al. 2005). Soil solarization has been reported to be effective in reducing the incidence of damping-off disease in solanaceous crops viz. tomato, chilli and brinjal (Pandey and Pandey 2005). Soil solarization for 30 days in nurseries of tomato and chilli resulted in lower incidence of damping-off disease (Rahman et al. 2003; Akhtar et al. 2008; Kadam et al. 2018). Akhtar et al (2012) reported that soil solarization for 8 weeks in integration with FYM @ $0.2kg/m^2$ and *Trichoderma harzianum* @ 5g/kg of seed resulted in reduction of pre and post emergence damping-off incidence in tomato, chilli and brinjal by 15.6 and 2.0 per cent, 11 and 2.3 per cent, 17.3 and 3.3 per cent respectively as compared to control.

Jayaraj and Radhakrishnan (2008) reported that soil solarization in combination with soil treatment with biocontrol agents like *Pseudomonas fluorescens* and *Trichoderma harzianum* was effective in managing damping-off disease of tomato. Application of

*Trichoderma viride* in solarized soil reduce the pre and post emergence damping-off in tomato by 75.2 and 88.3 per cent respectively (Gnanavel and Jayaraj 2003). Integration of soil solarization with organic amendments, bioagents reported to be effective in reducing damping-off disease incidence in tomato (Sofi et al. 2009). Rahman et al (2003) reported that soil solarization resulted in lowest incidence of damping-off in tomato (3.9 %) and chilli (1.3 %) in comparison to control. Akhtar et al. (2008) also observed that soil solarization for 30 days resulted in reduction of damping-off incidence in tomato.

Combination of biocontrol agents (*Trichoderma viride*, *Trichoderma harzianum* and *Bacillus subtilis*) and extracts of garlic (*Allium sativum*) were found to be more effective in reducing the mycelial growth of damping-off pathogen (*Pythium aphanidermatum*) in tomato with mycelial inhibition of 77.6-91.2 per cent as compared to their alone application (Dania and Omidiora 2019). Singh et al. (2003) evaluated PGPR *Pseudomonas aeruginosa* strain Pag and *Pseudomonas fluorescens* strain Pf4 against *Sclerotium rolfsii* in chickpea and found that they gave protection to seedlings (mortality of 16 %) as compared to control (mortality of 24 %). Seed treatment with *Trichoderma harzianum* exhibited maximum seed germination of 91.29 per cent followed by soil treatment with *Trichoderma harzianum* (90.40 %) and seed treatment with *Trichoderma viride* (89.33 %). Seed treatment followed by soil application of *Trichoderma* spp in combination with FYM was found to be more effective as compared to seed treatment with carbendazim and soil drenching with *Trichoderma* spp. (Nazir et al. 2011).

Pandey et al. (2016) reported the efficacy of integration of biocontrol agents (*T. harzianum*, *T. hamatum*, *T. asperellum*, *Bacillus subtilis* and *Pseudomonas fluorescens*) with four botanicals (*Vinca rosea*, *Lantana camara*, *Eucalyptus globulus* and *Lawsonia intermis*) against damping-off disease of chilli caused by *Pythium aphanidermatum* under field conditions. Rajkumar et al. (2008) reported that seed treatment with *T. viride* along with application of FYM in the soil was found effective in controlling the pre-emergence and post-emergence damping-off in chilli seedlings. Improvements in plant growth and crop productivity has been recorded when seeds were bio-primed with *Pseudomonas fluorescens* (60 %) for 12 hrs in combination with application of foliar sprays of *Pseudomonas fluorescens* after 45 days of sowing (Mariselvam 2012).

Muthukumar et al. (2010) reported that combined application of *Trichoderma viride*, *Pseudomonas fluorescens* and Zimmu leaf extract as seed treatment resulted in lower pre and

post-emergence damping-off incidence (8.3 and 17.0 % respectively) in chilli with increased plant growth (shoot length and root length) and yield of chilli. Huang et al. (2020) reported that biopriming with *Trichoderma harzianum* (10 %) was found effective in reducing the incidence of *Fusarium* root rot by 73.9 per cent, *Rhizoctonia* root rot by 78.6 per cent and charcoal root rot by 70.8 per cent. They tested various commercially formulated biocontrol agents like *Streptomyces griseovirdis* strain K61, *Trichoderma harzianum* T-22, *Trichoderma virens* strain GL-21 and *Gliocladium catenulatum* strain J1446 for their efficacy against this disease. Among these formulated products *G. catenulatum* significantly reduced disease incidence of soil-borne pathogens namely *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Fusarium oxysporum* and also have positive correlation with enhanced plant height and fresh weight.

Seed treatment with *Trichoderma* spp. along with application of soil amendments like vermicompost, sawdust, cow dung was found effective against damping-off disease in tomato and egg plant seedlings (Uddin et al. 2009). Nemec et al. (1996) reported that soil amendments with biocontrol agents like *Trichoderma harzianum*, *Bacillus subtilis*, *Gliocladium virens* and *Streptomyces* sp. reduced the root and crown rot diseases (*Pythium*, *Fusarium, Thielaviopsis* and *Phytophthora*) in tomato, bell pepper, celery and citrus. Seed priming with PGPRs has been reported to increase the germination and improve the seedling establishment. Seed biopriming results in initiation of physiological process which helps in establishment and proliferation of PGPR on the spermosphere (Taylor and Harman 1990). Biopriming has been reported to have beneficial effects in several vegetable crops like in tomato, seed priming improved the germination rate, seedling emergence and growth (Cayuela et al. 1996). Hooda et al. (2011) reported 60.1, 45.5 and 44.5 per cent higher seedling emergence when seeds were treated with *Lantana camara*, *Urtica parviflora* and *Ligustrum nepalensis* respectively. Vigour index was also recorded higher when seeds were treated with *Lantana camara* (81.3 %) followed by *Thuja compacta* (75.4 %), *Parthenium hysterophorus* (69.5 %), *Tagetes minuta* (69.5 %), *Eucalyptus* sp. (64.3 %) and *Azadirachta* (64.1 %).

*Trichoderma atroviride* when applied as seedling dip also found to increase the shoot and root dry weight of seedlings by threefold and an increase in fruit quality in healthy tomato plant (Gravel et al. 2007). Biopriming process involves potential advantages over simple seed coating as reported by Mathre et al. (1994). They reported that biopriming of sweet corn with *Pseudomonas aureofaciens* AB254 resulted in rapid and uniform seedling emergence and it was also found useful under adverse soil conditions. El-Mohamedy et al.

(2006) reported that biopriming with *Trichoderma harzianum* (10.0 %) was found effective in cowpea under field conditions as it reduced the *Fusarium* root rot by 73.9 per cent, *Rhizoctonia* root rot by 78.6 per cent and charcoal root rot by 70.8 per cent respectively.

Seed biopriming with *Trichoderma harzianum*, *Bacillus subtilis* and *Pseudomonas fluorescens* enhanced the effectiveness in control of root rot pathogens *i.e., Fusarium solani* and *Rhizoctonia solani* (El-Mohamedy and Abd El-Baky 2008). Seed treatment has also been reported to improve seed germination, seed emergence, vigour index, plant height, yield and seedling weight as well. Biopriming with *Pseudomonas fluorescens* has been reported to improve seedling vigour and growth in sunflower (Moeinzadeh et al. 2010). Abeysinghe (2009) reported that conjoint application of first *Bacillus subtilis* CA32 and then soil application of *Trichoderma harzianum* RU01, provided protection against *Rhizoctonia solani* with lowest disease severity.

Dabire et al. (2016) reported that seed coating and spraying the seedbeds with *Trichoderma harzianum* resulted in significant reduction in seedling damping-off due to *Fusarium* strains causing damping-off in onion along with significant increase in the seedling length, root length and fresh weight of seedlings. Yadav et al. (2013) reported that biopriming with *Pseudomonas fluorescens*, *Trichoderma asperellum* and *Rhizobium* sp., individually as well as in combination in chickpea and *Phaseolus vulgaris* resulted in increased seed germination and accumulation of biomass in crops both under pot and field conditions. Tancic et al. (2020) reported increase of about 24 per cent of root dry weight and 45 per cent of germination after seed biopriming of sweet pepper and cucumber seedlings with *Trichoderma harzianum*.

**f) Role of seed bio-priming in defense**

Beneficial rhizospheric and endophytic microbes trigger plant resistance, which has beneficial effects on plant growth and activates plant defense responses. Biopriming promotes tolerance level of seeds to environmental stress (Sarkar et al. 2018). Bioprimed plants tend to accumulate organic molecules (sugar, polyamines) and secondary metabolites (polyphenols, flavonoids) as a defense mechanism under stress. PGPR suppress plant pathogens by various mechanism involving induction of systemic resistance which activates multiple defense related enzymes to counter broad spectrum of phytopathogens. Seed biopriming with *Trichoderma* sp., *Pseudomonas* sp. have been reported to augment in anti-oxidant activity and also with defense related compounds by Jayapala et al. (2019).

*Trichoderma* isolates stimulated systemic defense responses in roots of tomato plants under field conditions by activating defense enzymes including peroxidase, polyphenol oxidase and chitinase (Ramamoorthy et al. 2001).

Inoculation of *Trichoderma harzianum*, *Trichoderma asperellum* and *Trichoderma virens* in tomato roots has been found to stimulate higher activities of peroxidase, polyphenoloxidase and chitinase to about 49.3, 56.6 and 58.3 per cent, respectively over the control (Elshahawy and El-Mohamedy 2019). Ramamoorthy et al. (2002) reported increased activities of phenyl ammonia lyase (PAL), peroxidase (PO) and polyphenol oxidase (PPO) when tomato and hot pepper plants were pre-treated with *Pseudomonas fluorescens* strain Pf1 pre-treated tomato and hot pepper plants against *Pythium aphanidermatum.* PGPR strains when applied as seed treatment have been reported to induce synthesis of specific phenolic acids (gallic, ferulic and chlorogenic), salicylic acid and total phenol content at various stages of growth of chickpea and thus alleviates physiological response against *Sclerotium rolfsii* (Singh et al. 2003).

Root colonisation by various *Trichoderma* spp. like *T. asperellum, T. hamatum, T. harzianum* and *T. virens* has been found to trigger the Systemic Acquired Resistance (SAR) against different diseases. During root colonisation, biocontrol agents develop itself within the intercellular root vessels and cortex region, thus contact with the pathogen during attack and also able to induce the systemic responses (Harman et al. 2004). Seed treatment with *Trichoderma harzianum* strain T22 has been reported to reduce the damage resulting from accumulation of ROS (Reactive Oxygen Species) in stressed plants infected with *Pythium ultimum* (Mastouri et al. 2010). Srivastava et al. (2010) reported that the bio-priming of seeds with combination of effective bioagents of *Trichoderma* and *Pseudomonas* (T35 + P16) enhanced the seed germination by 48 per cent as compared to non-primed seeds against the pathogen.

Devi et al. (2013) reported that seed biopriming with PGPRs (*Bacillus megaterium*, *Azospirillum brasilences* and *Azotobacter chroococcum*), BCAs (*Trichoderma harzianum* and *Pseudomonas fluorescens*) resulted in increased activity of defense enzymes at all time intervals and thus seed biopriming gave alternate way for organic management of damping-off disease. Bekkar et al. (2018) reported that application of *B. tequilensis* followed by *B. amyloliquefaciens* in roots of chickpea plants resulted in reduction of *Fusarium* wilt of chickpea along with significant increase in seedling growth.

Singh et al. (2013) studied the efficacy of rhizospheric microbial consortium consisting fluorescent *Pseudomonas* (PHU094), *Trichoderma* (THU0816) and *Rhizobium* (RL091) on defense response activation in chickpea against biotic stress caused by *Sclerotium rolfsii*. They observed that PAL activity was significantly higher in triple consortium treatment with (PHU094+THU0816+RL091) after 72 hrs of inoculation and the activity was found 1.50 and 2.66 times higher than the pathogen challenged and non-challenged controls, respectively. PPO activity was also higher in triple consortium treatment at 1.77 and 4.02 times increase over pathogen challenged and non-challenged control plants respectively.

Toribio et al. (2021) reported the efficacy of different cyanobacterial extracts applied as seed biopriming treatment from *Anabaena* spp., *Tolypothrix* spp., *Nostoc* and *Trichormus* against *Pythium ultimum* in cucumber seedlings. Mehmood et al. (2021) observed that *Azospirillum lipoferum* AL-3 reduced disease incidence of *Rhizoctonia solani* in tomato by 52.7 per cent. Seeds bioprimed with *Pseudomonas aeruginosa* MF-30 against *Rhizoctonia solani* resulted in significant increase in activities of anti-oxidative enzymes *i.e.*, PAL (phenylalanine ammonia lyase), ascorbate peroxidase, peroxidase, superoxide dismutase and catalase. *Pseudomonas aeruginosa* is an excellent endo-symbiont and has myco-parasitic ability, protect plants directly from pathogen attack and is well known for its ability to stimulate plant growth and development (Singh et al. 2020b). The endophytes employ several mechanisms to suppress pathogen growth and colonization by direct parasitism, antibiosis and competition for resources on infection sites. The indirect mechanism involves elicitation of systemic resistance responses in host. Plants primed with endophytes elicited Induced Systemic Resistance (ISR) via modulation of defense pathways for resistance against upcoming pathogen attacks.

# *Chapter-3*

# MATERIALS AND METHODS

The present studies entitled **"Potential of seed biopriming in management of damping-off disease in solanaceous vegetable crops"** were carried out in the different laboratories and at the experimental farm of Department of Plant Pathology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (Himachal Pradesh) during the year 2020-2023. The methodologies adopted during course of studies are described as follows:

**Glasswares used**

All the glasswares comprising mainly of Petri plates (90 mm diameter), test tubes (10 and 20 ml), microscopic slides (24x7.5x1 mm), Erlenmeyer flasks (100, 250 and 500 ml), beakers (250 and 500 ml), measuring cylinders (10 ml, 50 and 500 ml) were used of Borosil make.

**Equipments and other materials used**

Different materials used for research work were weighed on an electrical balance (Narang Scientific Works Pvt Ltd.). Culture of the pathogens in different experiments was incubated in BOD incubator (REMI instruments Ltd.). Pots of 6-inch diameter were used for conducting different pot experiments. Autoclave (Narang Scientific Works Pvt. Ltd.) for the sterilization of different media, Hot air oven (Lab Equipment industry) for sterilization of glasswares and drying of materials, Laminar airflow (Narang Scientific Works Pvt. Ltd.), Spectrophotometer (Thermo Spectronic 20D +) and Centrifuge (MPW-260R) were used in various research experiments. Cultures were kept in the refrigerator at 4˚C for use during the research experiments. Thin transparent polythene sheet of 25 µm thickness was used for soil solarization. Seeds of tomato (var. Solan Lalima), chilli (var. DKC-8) and capsicum (var. Solan Bharpur) were obtained from Department of Seed Science and Technology. Different bioformulations of PGPR were obtained from Department of Soil Science and Water Management, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (Himachal Pradesh). Soil amendments used in the experiments were procured from the local market.

**Sterilization**

Sterilization of glasswares was carried out in hot air oven at 180˚C for 2 hrs. Different culture media were sterilized by autoclaving them at 15 p.s.i for 20 min. Soil used in the studies for pot experiments was sterilized with 40 per cent formaldehyde diluted with water in 1:7 ratio. After soil treatment with formaldehyde, it was covered with polythene sheet for five days and later the sheet was removed and soil was exposed to air and raked daily for 10-15 days for eliminating the fumes of formaldehyde. The sterilized soil was used for pot experiments. All *in vitro* experiments were carried out under aseptic conditions under laminar air flow.

## 1. Symptomatology

Symptoms of pre and post-emergence damping-off of tomato, chilli and capsicum seedlings including above and below ground symptoms were studied by taking observation on germination starting after one week of sowing at regular intervals for four weeks. Infected seeds without germination from underneath the soil and infected seedlings after the post-emergence damping-off were collected from the field for examination in the laboratory.

## 2. Isolation and Identification of pathogens

**Isolation**

In order to isolate the pathogens associated with damping-off in tomato, chilli and capsicum, infected seedlings were first collected from the nurseries and brought to the laboratory in paper bags. Seedlings having characteristic symptoms of damping-off disease at the collar regions were selected. The infected seedlings were washed in running tap water in the laboratory to remove the clinged soil particles. Small bits of 2-5mm were cut from the juncture of healthy and diseased collar portion of the infected seeds and seedlings with the help of a sterilized blade. The bits were then surface sterilized by dipping in sodium hypochlorite solution (1%) for 30 sec using sterilized forceps. Afterwards, the sterilized bits were washed thrice in distilled water and kept on sterilized filter paper in order to remove the excessive moisture. Subsequently, these bits were then aseptically transferred to Petri-plates containing Oat Meal Agar (OMA) media specific for *Pythium* sp. and Potato Dextrose Agar (PDA) media for *Fusarium* sp., *Sclerotium* sp., *Rhizoctonia* sp. Antibiotic streptocycline (30 µg/l) was added in the molten media at the time of pouring in Petri-plates after sterilization to avoid bacterial contamination. After inoculation, Petri-plates were incubated at 25±2 ˚C in

BOD incubator. Petri-plates were observed daily for any mycelial growth and pure culture of the pathogens was obtained by repeated transfer of hyphal tips from the margins of growing culture. Pure culture of the pathogen was maintained at 4±1˚C in the refrigerator and all the cultures were regularly sub cultured after 15-20 days interval.

**Identification of pathogens**

In order to study the cultural and morphological characteristics, isolated pathogens were raised on the PDA media under *in vitro* conditions. Fungal cultures were examined under microscope (Nikon 200) for septation of hyphae, shape of spores and other distinguishable morphological features. Cultural and morphological characters of the fungi like pigmentation, appearance of colony, shape, size, colour, septation of conidia and formation of chlamydospores and sclerotia were also observed. The recorded characters were then compared with standard descriptions as detailed by Waterhouse (1967) for *Pythium* sp., Booth (1971) for *Fusarium* sp., Parameter (1970) for *Rhizoctonia* sp., Barnett and Hunter (1972) for *Sclerotium* sp. Based on these keys, pathogens were identified as *Pythium* sp., *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsii*.

**3. Pathogenicity**

To establish the association of isolated pathogens with pre-emergence and post-emergence damping-off disease in tomato, capsicum and chilli, pathogenicity was proved under polyhouse conditions using soil inoculation method as per procedure mentioned below:

**Mass culture preparation of pathogens**

The mass culture of pathogens was prepared on corn: sand meal medium (Dohroo 1988). While preparing the mass culture, the maize grains were boiled for 35-40 min till they get soften and afterwards the grains were slightly crushed. Later the grains were put on the blotting paper to remove the excess water and then dried. Grains were mixed with sand (3:1) along with 2 per cent of sucrose. This mixture was then filled in conical flasks (500 ml) and plugged with non-absorbent cotton. These flasks filled with corn sand meal medium were autoclaved at 15 p.s.i for one hr for 3 consecutive days. Later, these sterilized media flasks were inoculated with 3-4 mycelial bits of 0.25-0.5 cm size of the pathogens and incubated at 25±2˚C in BOD incubator for 15 days. The inoculated flasks were shaken regularly after 3-4 days so that the culture grows uniformly till 15 days and then mass culture of pathogens were used for carrying out various pot experiments.

**Proving pathogenicity**

Soil inoculation method was used for proving pathogenicity of pathogens associated with damping-off disease in tomato, chilli and capsicum. In the beginning, seeds of tomato, chilli and capsicum were surface sterilized with 1per cent sodium hypochlorite ($NaOCl_2$) solution for 5 min followed by their rinsing in sterilized distilled water and air drying. Soil is mixed with FYM in equal proportions and sterilization of soil was carried out with formalin (5%) as described earlier. After treatment, soil was kept open for 3-4 days for removing the fumes of formaldehyde and then the soil was filled in pots (6-inch diameter). Mass culture of the isolated pathogens were mixed in soil @ 10g/350 g of sterilized soil at about 5 cm depth. Mass culture of the isolated pathogens was added individually in some pots having sterilized soil mixture and also combined mass culture of all pathogens was added together in different pots containing sterilized soil mixture. After inoculation, pots were watered slightly and kept covered with polythene sheet for 5-7 days for buildup of the inoculum of different pathogens. Pots without inoculum served as control. Experiment was conducted in two sets. In the first set, about 10 seeds each of tomato, chilli and capsicum were sown in different pots. Pots were then observed for pre-emergence damping-off symptoms, seed rotting and patchy germination under field conditions. In the second set, ten healthy seedlings of tomato, chilli and capsicum were transplanted in pots with sick soil and these pots with transplanted seedlings were kept under polyhouse and observed for post-emergence damping-off symptoms in the seedlings. Incubation period (days) taken for appearance of symptoms (post-emergence damping-off) and data on disease incidence (%) was recorded as per formula given below:

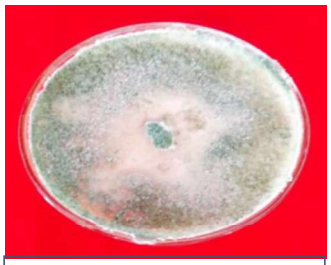
$$\text{Disease incidence (\%)} = \frac{\text{Number of plants infected}}{\text{Number of plants observed}} \text{ X } 100$$

## 4. Disease Management

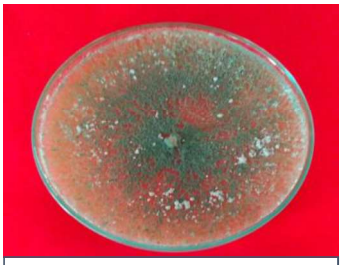
### 1. *In vitro* evaluation of biocontrol agents against test pathogens

Fungal biocontrol agents namely, *Trichoderma harzianum*, *T. atroviride*, *T. virens*, yeast bioagents, *Saccharomyces cerevisiae, Saccharomyces* sp. and bacterial biocontrol agents *Bacillus licheniformis*, *Bacillus* sp, *B. subtilis*, *Pseudomonas* sp, procured from the Department of Plant Pathology, Dr YS Parmar University of Horticulture and Forestry, Nauni Solan HP were evaluated for their efficacy against damping-off pathogens under *in vitro* conditions (Plate 1). While fungal antagonists and yeast bioagents were maintained on PDA,

*Trichoderma harzianum*



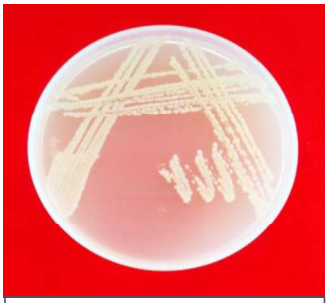
*Trichoderma atroviride*



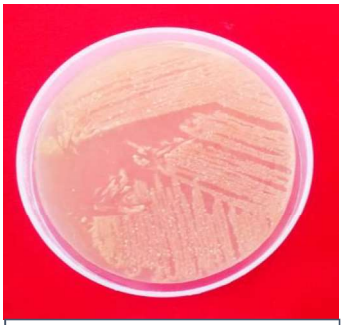
*Trichoderma virens*

*Saccharomyces cerevisiae*

*Saccharomyces* sp.



*Bacillus subtilis*



*Pseudomonas fluorescens*

*Bacillus licheniformis*

*Bacillus* sp.

**Plate 1: Antagonistic fungal and bacterial biocontrol agents**

bacterial antagonists were maintained on Nutrient Agar. These cultures were maintained in refrigerator at 4˚C for further studies. Fungal biocontrol agents and yeast bioagents were evaluated against damping-off pathogens i.*e*., *Pythium* sp., *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsii* for their antagonistic activity by dual culture method (Huang and Hoes 1976) and bacterial biocontrol agents were evaluated by streak plate method (Utkhede and Rahe 1983).

Culture discs (5mm) each from the fungal antagonists and damping-off pathogens were taken from the margins of the actively growing cultures and transferred to PDA medium containing Petri-plates (90mm) on opposite sides approximately 1 cm away from wall of the Petri-plate. Similarly, the yeast bioagents and bacterial biocontrol agents were streaked on one side of Petri-plate opposite to mycelial disc of the pathogen. Control Petri-plate having the inoculation of test pathogens only was also kept for comparison. The Petri-plates were then incubated in BOD incubator at 25±2˚C till the control plates were completely covered with the pathogens. Experiment was carried out in completely randomized design with three replications for each treatment. Colony diameter of the test fungi as well as each antagonist up to zone of inhibition was recorded and the per cent growth inhibition of the test fungi over control was calculated according to Vincent (1947) as under:

$$I = \frac{C - T}{C} X\ 100$$

Where,

I – Per cent inhibition of mycelial growth
C – Linear mycelial growth in control (mm)
T– Linear mycelial growth in treatment (mm)

**2. Efficacy of seed biopriming with biocontrol agents (BCAs)**

Effective biocontrol agents against the damping-off pathogens were selected based on the *in vitro* studies. Effective biocontrol agents were then further tested by seed biopriming technique for their efficacy against the damping-off disease in tomato, chilli and capsicum. Seed biopriming involves a technique in which hydration of seeds was carried out along with treatment with bioinoculants. Thus, to proceed with seed biopriming of tomato, chilli and capsicum seeds, first concentration of biocontrol agents and duration of hydration (soaking)

was standardized for better efficiency by using rolled paper towel method. The procedure of both the processes is described below:

**Preparation of suspensions of biocontrol agents (BCAs)**

Suspensions of fungal biocontrol agents were prepared from fully grown fresh cultures. Firstly, 1 ml of sterilized distilled water was added to each of these fungal cultures followed by scrapping of these cultures to produce slurry. Then, 1 ml of this slurry was transferred to 9 ml of distilled water to make suspension. After that, 1 ml of this suspension was pipette out and dispense in another test tube containing 9 ml of sterile distilled water to obtain a $10^{-2}$ dilution. This process is repeated in the same way until $10^{-8}$ dilution was obtained for each fungal antagonist culture. Then, 500 µl was pipette out from each of $10^{-6}$, $10^{-7}$ and $10^{-8}$ dilutions and added to separate Petri-plates containing 15-20 ml of cool and molten PDA media and spread evenly in all directions. Petri-plates were then incubated at 28±2˚C in BOD for about one week. After about one week fully grown cultures of doses ($10^6$ cfu per ml, $10^7$ cfu per ml and $10^8$ cfu per ml) were further diluted in 250 ml of sterilized distilled water for biopriming as per procedure followed by Singh et al. (2020b). For bacterial biocontrol agents, firstly they were multiplied on Nutrient Broth followed by their further dilutions ($10^6$ cfu per ml, $10^7$ cfu per ml and $10^8$ cfu per ml) using serial dilution method in the same way as for fungal biocontrol agents and then the required doses of suspensions ($10^6$ cfu per ml, $10^7$ cfu per ml and $10^8$ cfu per ml) were further diluted in 250 ml of Nutrient Broth for carrying out biopriming of tomato, chilli and capsicum seeds.

**Standardization of concentration of BCAs suspension for seed biopriming**

Before carrying out the experiments on seed biopriming in field conditions, it was necessary to standardize the concentration of effective BCAs suspension for carrying out biopriming of seeds. Healthy seeds without cracks and any visible deformities of tomato, chilli and capsicum were surface sterilized in 1% sodium hypochlorite solution for 5 min and then rinsed 2-3 times in distilled water followed by air drying. Seeds of tomato, chilli and capsicum were bioprimed by dipping them individually in suspensions of biocontrol agents at different concentrations ($10^6$ cfu per ml, $10^7$ cfu per ml, $10^8$ cfu per ml) for 12 hrs at 25±2˚C. The priming suspensions of biocontrol agents were then drained off after 12 hrs of dipping and bioprimed seeds were dried under laminar air flow chamber on sterilized blotting paper until they obtain their original moisture content. After drying of seeds, “Rolled paper towel

method" was used to carry out the germination and vigour tests of bioprimed seeds of tomato, chili and capsicum germinated under seed germinator at 28±2˚C (Plate 2).

Rolled paper towel method was used to study the effect of different biopriming treatments on seed vigour and seed health as per the standardized procedure suggested by International Seed Testing Association (Anonymous 1999). Germination was tested *in vitro* using layers of water-soaked germination paper and wax paper. In this experiment, 100 seeds were placed in each replication per treatment. Seeds were placed in 10 rows with 10 seeds in a row at equi-distance in between two layers of moist germination paper, covered with layer of wax paper. These were then kept in seed germinator and incubated at 28±2˚C for 14 days and experiment was replicated three times in each treatment. Germination of the seeds was recorded on $7^{th}$ day and final count was taken on $14^{th}$ day. Seeds without any treatment served as control for comparison of the treatments. Experiment was carried out in completely randomized design with three replications each. Data on seed germination (%), seedling length (cm), seedling dry weight (mg) were recorded for each treatment. Following observations were recorded:

**i) Germination (%)**

Germination percentage was calculated using the following formula:

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings germinated}}{\text{Total number of seeds kept for germination}} \text{ X } 100$$

**ii) Seedling length (cm)**

Ten normal seedlings were randomly selected for measurement of seedling length. In the selected seedlings, length of the roots and shoot were measured with the help of a measuring scale. Then, mean value was calculated for the shoot and root length.

**iii) Seedling dry weight (mg)**

Ten seedlings selected for measuring seedling length were also used to find out the seedling dry weight. Seedlings were dried in oven at 60˚C for 24 hrs and then weighed using weighing balance. Then mean value was expressed in mg.

**iv) Seedling vigour index-L**

Seedling vigour index-L was calculated as per the formula given by Abdul-Baki and Anderson (1973) as:

$$\text{Seedling vigour index} - \text{L} = \text{Germination (\%)} \times \text{Seedling length (cm)}$$

**v) Seedling vigour index-M**

Seedling vigour index-M was calculated as per formula given by Abdul-Baki and Anderson (1973) as:

$$\text{Seedling vigour index} - \text{M} = \text{Germination (\%)} \times \text{Seedling dry weight (mg)}$$

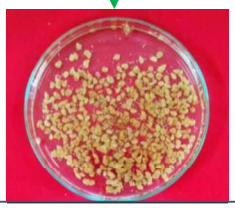
**Standardization of duration of seed biopriming with BCAs**

After standardizing the concentration of priming suspensions of BCAs for biopriming of tomato, chilli and capsicum seeds, duration of seed biopriming with suspensions of biocontrol agents was also standardized. Healthy seeds without cracks and any visible deformities were surface sterilized in 1 per cent sodium hypochlorite solution for 5 min and then rinsed 2-3 times in distilled water followed by air drying. Seeds of tomato, chilli and capsicum were bioprimed by dipping them in suspensions of biocontrol agents at concentration of $10^7$ cfu per ml (tomato and chilli seeds) and $10^8$ cfu per ml (capsicum seeds) in case of fungal biocontrol agents and at concentration of $10^8$ cfu per ml (tomato, chilli and capsicum) in case of bacterial biocontrol agents for 6, 8 and 12 hrs duration of soaking in suspensions. Then priming suspensions were drained off and bioprimed seeds were dried under laminar air flow chamber on sterilized blotting paper until they obtain their original moisture content. "Rolled paper towel method" was used to carry out the germination and vigour tests of treated seeds of tomato, chilli and capsicum as described earlier (Plate 2). Experiment was carried out in completely randomized design with three replications each. Data on seed germination (%), seedling length (cm), seedling dry weight (mg) was recorded for each treatment.

**3. Seed biopriming with effective biocontrol agents and PGPR bio-formulations under field conditions**

Seed biopriming with effective biocontrol agents was carried out in integration with some PGPR bio-formulations of *Azotobacter*, *Azospirillum* and *Pseudomonas aeruginosa* in order to evaluate their efficacy against damping-off disease under field conditions. Healthy seeds without cracks and any visible deformities were surface sterilized in 1 per cent sodium hypochlorite solution for 5 min followed by their rinsing in distilled water and air drying. Then, seeds were bioprimed with suspensions of fungal biocontrol agents prepared in 1 per cent Carboxymethyl cellulose (CMC) at a concentration of $10^7$ cfu per ml (tomato and chilli)

Seeds of chilli, capsicum and tomato



Surface sterilization of seeds in 1 % NaOCl

Shade drying of surface sterilized seeds

Seeds soaking in suspensions at BCAs/Plant extracts/Endophytes

Drying of bioprimed seeds under laminar air flow

Germination test using paper roll towel method

**Plate 2: General procedure of seed biopriming**

and $10^8$ cfu per ml (capsicum) for 8 hrs as per results of *in vitro* experiments. For bacterial biocontrol agents and bioformulations, seeds of tomato, chilli and capsicum were bioprimed with suspensions prepared in 1 per cent CMC at a concentration of $10^8$ cfu per ml for 8 hrs as per results of *in vitro* experiments. After biopriming, suspensions were drained off and seeds were dried under laminar air flow chamber on sterilized blotting paper to bring down the moisture content to original concentration (Singh et al. 2020b). Bioprimed seeds were then sown in nursery beds of size 1.5x1.5 $m^2$ under natural field conditions. The experiment was carried out in randomized block design with four replicates each. Data pertaining to disease incidence (%), seed germination (%), shoot length (cm), root length (cm) was recorded after 15 days of sowing at weekly intervals based upon the disease development and collection of shoots and roots from each treatment was carried out.

**4. *In vitro* evaluation of plant extracts against test pathogens**

Efficacy of various botanical extracts from commonly available flora namely *Aloe barbadensis*, *Cymbopogon citratus*, *Datura stramonium*, *Hibiscus rosa-sinensis*, *Thymus vulgaris*, *Melia azedarach*, *Tagetes* sp., *Lantana camara*, *Parthenium hysterophorus*, *Ocimum* sp. and *Roylea elegans* on mycelial growth of pathogens was studied by poisoned food technique (Nene and Thapliyal 2000) (Plate 3).

**Preparation of botanical extracts**

Fresh mature leaves of the above-mentioned plants were collected and washed under tap water. In case of every plant, 100 g of plant leaves were crushed in 100 ml distilled water (1:1 w/v) in mortar and pestle. The filtrate was then centrifuged at 5000 rpm for 15 min and the supernatant was collected. Supernatant was strained through whatman filter paper and the filtrate obtained was used as stock solution with 100 per cent concentration (Gholve *et al*. 2014). Tyndalization of filtrate was done for three consecutive days at 10 p.s.i for 15 min. Thus, prepared plant extracts were evaluated @ 10 per cent using poisoned food technique (Nene and Thapliyal 2000) in which media containing plant extracts (10 %) was poured in the sterilized Petri-plates under aseptic conditions. The Petri-plates were then inoculated with mycelial discs (5 mm) of fresh culture of pathogens. Control plates without poisoning of media with botanical extracts were kept under *in vitro* conditions for comparison. Each treatment was replicated three times in completely randomized design and inoculated plates were incubated at 25±2˚C in incubator for 7 days. The colony diameter of the pathogens was recorded till the control plates were full with mycelium of the damping-off pathogens. Data

on per cent mycelial growth inhibition of the test fungi over control was calculated according to Vincent (1947).

**5. Efficacy of seed biopriming with plant extracts**

Effective plant extracts against the damping-off pathogens were selected based on *in vitro* studies and were further tested by seed biopriming technique for their efficacy against the damping-off disease in tomato, chilli and capsicum. Thus, to proceed with seed biopriming of tomato, chilli and capsicum seeds with effective plant extracts, first concentration of plant extracts (5 and 10 %) and duration of hydration (6, 8 and 12 hrs) was standardized for better efficiency as per method described in earlier experiment by using rolled paper towel method (Plate 2).

**6. Seed biopriming with effective plant extracts under field conditions**

Seed biopriming with effective plant extracts was carried out in order to evaluate their efficacy against damping-off disease under field conditions. Healthy seeds without cracks and any visible deformities were surface sterilized in 1 per cent sodium hypochlorite solution for 5 min followed by their rinsing in distilled water and air drying. Then, tomato, chilli and capsicum seeds were primed with plant extracts at a concentration of 10 per cent for 12 hrs as per results of *in vitro* experiments. After application of the treatments, bio-primed seeds were dried under laminar air flow chamber on sterilized blotting paper to bring down the moisture content to original concentration (Singh et al. 2020b). Bioprimed seeds were then sown in nursery beds of size 1.5x1.5 $m^2$. The experiment was carried out in randomized block design with four replicates. Data pertaining to disease incidence (%), seed germination (%), shoot length (cm), root length (cm) was recorded for each treatment.

**7. Integration of seed biopriming with biocontrol agents and plant extracts under field conditions**

Seed biopriming was carried out using effective biocontrol agents and plant extracts in integration in two stages. Combination of treatments were selected based upon their compatibility with each other tested by poisoned food technique and also based upon their effectiveness against damping-off pathogens under *in vitro* conditions. Healthy seeds of tomato, chilli and capsicum without cracks and any visible deformities were surface sterilized in 1 per cent sodium hypochlorite solution for 5 min followed by their rinsing in distilled water and air drying. In first stage, seed biopriming with effective plant extracts at 10 per cent

*Datura stramonium*

*Hibiscus rosa-sinensis*

*Lantana camara*

*Roylea elegans*

*Parthenium hysterophorus*

*Aloe barbadensis*

*Ocimum sp.*

*Melia azedarach*

*Cymbopogon citratus*

*Thymus vulgaris*

*Tagetes sp.*

**Plate 3: Parts of native plants used for preparation of botanical extracts**

concentration for 12 hrs was carried out. After application of the treatments, bio-primed seeds were dried under laminar air flow chamber on sterilized blotting paper to bring down the moisture content to original moisture concentration. In the second stage, same set of seeds of tomato, chilli and capsicum seeds were again bioprimed with effective fungal biocontrol agents at a concentration of $10^7$ cfu per ml (tomato and chilli) and $10^8$ cfu per ml (capsicum) for 8 hrs. For effective bacterial biocontrol agents, seeds of tomato, chilli and capsicum were bioprimed with them at a concentration of $10^8$ cfu per ml for 8 hrs. After biopriming, suspensions were drained off and seeds were dried under laminar air flow chamber on sterilized blotting paper to bring down the moisture content to original moisture concentration. Bioprimed seeds were then sown in nursery beds of size 1.5x1.5 $m^2$. Experiment was carried out in randomized block design. Data on disease incidence (%), seed germination (%), shoot length (cm) and root length (cm) was recorded for each treatment.

**8. Efficacy of seed biopriming with endophytes**

Endophytes are the microorganism that lives inside the host plant which help the plants in strengthening their defense against biotic and abiotic stresses and also improve the plant growth. Endophytes were isolated from medicinal plants namely, *Mentha* sp, *Ocimum* sp, *Vinca rosea* and *Hibiscus rosa-sinensis* and evaluated for their efficacy in managing damping-off disease of solanaceous vegetable crops.

**Isolation of endophytes**

Endophytes usually resides in living tissues of the plant. These can be of fungal and bacterial origin. For isolation of fungal endophytes stem samples collected were cut into 1cm long segments and washed with running tap water thrice. For isolation of endophytes, surface sterilization is crucial so as to kill any microbe present on plant surface without affecting internal tissues of host plant and endophytic microorganism. Thus, to improve the effectiveness of surface sterilization procedure, combination of sterilizing agents was used. These segments were then surface sterilized with 70 per cent v/v ethanol for 1 min followed by sterilization with 5% v/v sodium hypochlorite for 5 min and again treated with 70 per cent ethanol for 1 min. The efficiency of surface sterilization procedure was confirmed by plating the final rinse water on PDA medium to check for any growth. Then, all the segments were washed 2-3 times with sterilized distilled water and air dried. The processed segments were placed in the Petri-plates containing PDA media in aseptic conditions and incubated at 27˚C for the growth of fungal endophytes. The fungi grown out from the inoculated plant segments were transferred to Petri-plates containing PDA media and subsequently pure cultures of

endophytes were obtained. These cultures, were maintained on PDA slants at 4˚C in refrigerator for further use (Khiralla et al. 2016).

For isolation of bacterial endophytes, collected stem samples were washed in running water and then the surface of the leaves were disinfected with 70 per cent alcohol for 1 min, sodium hypochlorite 2.5 per cent for 4 min, ethanol for 30 sec and finally 3 rinses in sterilized distilled water. To improve the effectiveness of surface sterilization procedure which is crucial for isolation of endophytes, combination of sterilizing agents was used. Samples were grounded with 6 ml aqueous solution of 0.9 per cent NaCl using a sterile mortar and pestle. The tissue extract was subsequently incubated at 28˚C for 3 hrs to allow the complete release of endophytic microorganisms from the host tissue. The tissue extract was diluted in an aqueous solution (0.9 % NaCl) and plated on 10 per cent NA plates for each dilution ($10^{-1}$ and $10^{-2}$). To confirm the sterilization protocol, aliquots of water used in final rinse was plated on NA media containing Petri-plates and these were then examined for any growth. The plates were incubated for 15 days at 28˚C. Colonies emerging in the Petri-plates were selected on 5-10 days of incubation and purified again in NA medium. For each Petri-plate evaluated, the colonies were selected according to their time of growth and morphology (colour, size and shape). After 10 days of incubation, all of the colonies were counted and colonies were maintained on Petri-plates containing NA media (Anjum and Chandra 2015; Costa et al. 2012).

**Identification of endophytes**

The isolated fungal endophytes were studied and identified as per the standard protocols based on different morphological and cultural features of mycelial growth of the fungi. The mycelium of fungal endophytes was stained with lactophenol cotton blue for 10 min. Thereafter, stained microscopic slides were visualized at 40X magnification using Nikon 200 compound microscope and different morphological characters were recorded.

**Biochemical characterization of bacterial endophytes**

The bacterial endophytes were identified and characterized based upon their biochemical traits analyzed during the present investigation namely Gram's reaction, KOH test, oxidase test, methyl red test and catalase test. The description of the tests is given below:

**i) Methyl red test**

The pure culture of bacteria to be tested was inoculated into glucose phosphate broth, which contained glucose and a phosphate buffer and incubated at 37˚C for 48 hrs. After

incubation, the pH of the medium is tested (change in colour) by the addition of five drops of Methyl Red reagent (pH indicator). Methyl Red positive organism produces red colour (pH below 4.4) while Methyl Red negative organism produces yellow colour (pH above 6) after addition of Methyl Red indicator.

**ii) Catalase test**

To perform the catalase test, loopful of bacterial suspension culture was smeared onto a glass slide by using a sterile inoculating needle. Then, a drop of 3% $H_2O_2$ was put on to the slide and the same was mixed gently. Rapid evolution of oxygen by bubbling after addition of $H_2O_2$ depicts positive reaction.

**iii) KOH test**

To perform the KOH test, one drop of 3 per cent KOH solution was kept on a clean microscope slide. Then, a few colonies of the test bacterial endophyte were spread on the drop of 3% KOH to make a dense suspension. Then, it was stirred continuously for 60 sec and loop was pulled away from the suspension to observe any changes. If the KOH test is positive organism become thick, stringy and form long strands while negative organism leaves the suspension unaltered.

**iv) Oxidase test**

The oxidase test was performed to identify the bacteria that produce cytochrome C oxidase, enzyme of the bacterial electron transport chain. When it is present, the cytochrome C oxidase oxidizes the reagent (tetramethyl-p-phenylenediamine dihydrochloride) to indophenols and consequently, a purple or dark blue colour end product. When the enzyme is not present, the reagent remains reduced and is colourless.

To perform this test, small piece of filter paper was soaked in 1 per cent Kovac's reagent and then it was left for drying, Then, using a sterile loop a well isolated colony was picked from the pure culture of test bacterial endophyte and then rubbed onto the treated and dried filter paper disc and observed for any changes in colour. Oxidase positive organism changes the colour of disc to dark purple in 5-10 sec. However. delayed oxidase positive organism changes colour to purple in 60-90 sec. Oxidase negative organism does not change the colour.

**Molecular characterization of effective endophytes**

On the basis of efficacy of endophytes in managing damping-off disease of tomato, chilli and capsicum under field conditions, the two most effective endophytes (EF1 and EB5) were further selected for their molecular identification based on 16S rRNA and 18S rRNA gene sequence analysis.

**Isolation of fungal endophyte (EF1) genomic DNA**

Extraction of genomic DNA of fungal endophyte was carried out using Cetyl Trimethyl Ammonium Bromide (CTAB) method as per procedure described by Priyanka et al. (2019). For DNA extraction, fungal tissues were harvested from about seven days old culture of fungal endophyte grown in Potato Dextrose Broth. Extracted fungus mycelial mat was filtered by sterile filter paper and ground to a fine powder in liquid nitrogen. Approximately 1g of ground mycelium was taken into a microcentrifuge tube containing 1ml of pre-warm (65˚C) DNA extraction buffer (DNA extraction buffer: 100mM Tris-HCl (pH 8.0), 20Mm EDTA (pH 8.0), 1.4M NaCl, 2 per cent (w/v), CTAB 0.2 per cent (v/v), 2-Mercaptoethanol) and mixed by gentle inversion. The sample was incubated at 65˚C for 1 hr and it was mixed twice during incubation by inverting tube. In this sample, 1ml chloroform: Isoamyl alcohol was added (24:1, v/v) was added and mixed by inversion to emulsify. Centrifugation was carried out at 12,000 rpm for 20 min at room temperature. Aqueous phase was pipetted out gently without disturbing the interphase to another tube. The 2/3$^{rd}$ volume of isopropanol was added and mixed by gentle inversion. DNA pellet was spined down by centrifugation at 12,000 rpm for 10 min at 4˚C. It was then washed twice with 70 % ethanol and again centrifugation was done at 10,000 rpm for 5-10 min at 4˚C. Pellet was dried and dissolved in 50-100 µl TE buffer (pH 8.0) depending upon the yield of DNA. Isolated DNA was checked on 0.8 per cent of agarose.

Genomic DNA after extraction was subjected to the PCR amplification of the Internal Transcribed Spacer (ITS) region using the primer ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). PCR reactions were carried out on a thermal cycler (Eppendorf). PCR consists of 40 cycles with initial denaturing phase at 95 ˚C for 10 min followed by annealing phase at 95˚C for 1 min, 60˚C for 30 s, 72˚C for 1 min and final extension phase by 72˚C for 10 min. The PCR products were analyzed on 1.2 per cent gel electrophoresis at 110 V for 1h. The gel was visualized employing gel documentation

system. The size of the PCR products was determined by the 1 kb molecular marker. The amplified product was subjected to sequencing.

**Isolation of bacterial endophyte genomic DNA**

Extraction of genomic DNA of bacterial endophyte was carried out by following method adopted by Khanna et al. (2022). Bacterial endophyte culture was multiplied in King's B Broth for 24 hrs at 28 ˚C. Bacterial suspension was then centrifuged at 10,000 rpm for 10 min and the pellet was resuspended in 650 µl extraction buffer followed by its incubation at 65˚C for 30 min. Then, 100 µl of Potassium Acetate and 750 µl of isoamyl alcohol: chloroform (1:24) was added to the centrifuge tube containing pellet and centrifuged at 10,000 rpm for 5 min. After centrifugation, supernatant was collected in 2 ml of Eppendorf tube and 600 µl of chloroform was added. The mixture was then subjected to centrifugation at 10,000 rpm for 5 min and the supernatant was transferred to 1.5 ml Eppendorf tube. To this, 600 µl of ice-cold isopropanol was added to the same Eppendorf tube that carried out precipitation of nucleic acid and tube was shaken back and forth unless white DNA was observed. This mixture after shaking was subjected to centrifugation at 10,000 rpm for 10 min at 4˚C. After centrifugation, supernatant was discarded and the pellet was washed twice with 500 µl of ethanol (70 %) and was then air-dried. The air-dried DNA pellet was then resuspended in 100 µl TE buffer and stored at -20°C for further use.

Isolated bacterial genomic DNA was then subjected to quantification by agarose gel electrophoresis. Universal 16S rDNA primers (27F and 1492R) were used for carrying out the amplification of 16S rDNA from selected bacterial isolates. The amplified product obtained with universal primers were then used for sequencing.

**DNA sequencing and sequence analysis**

For DNA sequencing, purified and amplified DNA products of bacterial and fungal endophytes were sent to Biokart India Pvt Ltd- Bangalore (Karnataka) India under refrigerated conditions using gel packs. Sequenced data so obtained was further analyzed using tools like BLAST (Basic Local Alignment Search Tool) of NCBI (National Centre for Biotechnology Information) for homology search and phylogenetic tree was constructed using MEGA 11 software.

**Studies on compatibility between bacterial endophytes and bacterial biocontrol agents**

Experiment was also conducted to check the compatibility between endophytes and biocontrol agents for their possible conjoint use for the management of damping-off disease. Compatibility between effective bacterial endophytes (EB 2, EB3 and EB5) and effective bacterial biocontrol agents (*Pseudomonas* sp., *Bacillus licheniformis*) was determined by disc diffusion method (Irabor and Mmbaga 2017). Bacterial biocontrol agents were multiplied on Nutrient Broth (NB) and incubated for 24 hrs on an incubator shaker at 200 rpm at 30˚C. The concentration of the bacterial biocontrol agents was then adjusted to $10^8$ cfu/ml using serial dilution method. Suspensions of test endophytes were prepared by multiplying them on NB. Then, 8 mm sterilized discs of Whatman filter paper were taken and dipped aseptically in the suspensions of endophytes followed by air drying under laminar air flow chamber on the sterilized Petri-plates for 30 min. The bacterial biocontrol agents were swabbed uniformly over the NA media plates using a sterile cotton applicator. Discs smeared with endophytes were then gently pressed and kept over the NA plates swabbed with bacterial bioagents at four equidistant positions using sterile forceps. The plates were then incubated at 28±2˚C and observed for 48-72 hrs as the incompatible endophytes were identified by formation of zone of inhibition between them.

**9. *In vitro* evaluation of endophytes against test pathogens**

Efficacy of fungal endophytes (EF1, EF2, EF3) against the test pathogens (*Pythium* sp., *Fusarium oxysporum*, *Rhizoctonia solani*, *Sclerotium rolfsii*) was evaluated by dual culture method (Huang and Hoes, 1976) and for bacterial endophytes (EB2, EB3 and EB5) by streak plate method (Utkhede and Rahe, 1983). Culture discs (5 mm) each from the fungal antagonist and test pathogens were taken from the margins of the actively growing cultures and transferred to PDA medium containing Petri-plates (90 mm) on opposite sides approximately 1 cm away from wall of the Petri-plates. Similarly, the yeast biocontrol agents and bacterial biocontrol agents were streaked on one side of Petri-plate opposite to mycelial disc of the pathogen. A control having the test pathogens only was also kept for comparison. The Petri-plates were then incubated in BOD incubator at 25±2˚C till the control plates were completely covered with the test pathogens. The experiment was carried out in completely randomized design with each treatment replicated three times. Colony diameter of the test fungi as well as each antagonist up to zone of inhibition was recorded and the per cent growth inhibition of the test fungi over control was calculated according to the below mentioned formula given by Vincent (1947).

## 10. Seed biopriming with effective endophytes under field conditions

Endophytes which were found effective against the damping-off pathogens, based on *in vitro* studies were selected. These effective endophytes namely, EF1, EF3, EB2, EB3, EB5 were further evaluated by seed biopriming technique for their efficacy against the damping-off disease of tomato, chilli and capsicum in field conditions. Suspensions of effective fungal and bacterial endophytes used for seed biopriming were prepared by same way as for biocontrol agents as described earlier. To initiate the process of seed biopriming of tomato, chilli and capsicum seeds, concentration of suspensions of endophytes ($10^6$, $10^7$, $10^8$ cfu per ml) and duration of hydration (6, 8 and 12 hrs) was first standardized for better efficiency as per method described earlier by using rolled paper towel method (Plate 2).

Healthy seeds without cracks and any visible deformities were surface sterilized in 1 per cent sodium hypochlorite solution for 5 min followed by their rinsing in distilled water and air drying. Then, tomato, chilli and capsicum seeds were primed with suspensions of endophytes prepared in 1per cent CMC at a concentration of $10^8$ cfu per ml for 12 hrs (fungal endophytes) and at a concentration of $10^7$ cfu per ml for 12 hrs (bacterial endophytes) as per results of previous experiments. After application of the treatments, the microbial suspensions smeared on the seeds were drained off and bio-primed seeds were dried under laminar air flow chamber on sterilized blotting paper to bring down the moisture content to original concentration (Singh et al. 2020b). Bioprimed seeds were then sown in nursery beds of size 1.5x1.5 $m^2$. The experiment was carried out in randomized block design with four replicates. Data pertaining to disease incidence (%), seed germination (%), shoot length (cm), root length (cm) was recorded for each treatment.

## 11. Conjoint application of seed biopriming with effective biocontrol agents and soil amendments

Seed biopriming with effective biocontrol agents in combination with soil amendments (vermiwash, NSKE (5%), compost tea) were evaluated to check their efficacy in managing damping-off disease in tomato, chilli and capsicum under pot conditions. Seeds of tomato (Solan Lalima), capsicum (Solan Bharpur) and chilli (DKC-8) were first surface sterilized with 1% sodium hypochlorite solution for about 5 min followed by their subsequent rinsing in sterilized distilled water thrice and air drying.

| S. No. | Treatments |
|---|---|
| 1. | Seed biopriming with BCA-1+ sowing of seeds in soil amended with NSKE (5%) |
| 2. | Seed biopriming with BCA-1+ sowing of seeds in soil amended with compost tea |
| 3. | Seed biopriming with BCA- 1+ sowing of seeds in soil amended with vermiwash |
| 4. | Seed biopriming with BCA-2+ sowing of seeds in soil amended with NSKE (5%) |
| 5. | Seed biopriming with BCA-2+ sowing of seeds in soil amended with compost tea |
| 6. | Seed biopriming with BCA-2+ sowing of seeds in soil amended with vermiwash |
| 7. | Seed biopriming with BCA-3+ sowing of seeds in soil amended with NSKE (5%) |
| 8. | Seed biopriming with BCA-3+ sowing of seeds in soil amended with compost tea |
| 9. | Seed biopriming with BCA-3+ sowing of seeds in soil amended with vermiwash |
| 10. | Seed biopriming with BCA-4+ sowing of seeds in soil amended with NSKE (5%) |
| 11. | Seed biopriming with BCA-4+ sowing of seeds in soil amended with compost tea |
| 12. | Seed biopriming with BCA-4+ sowing of seeds in soil amended with vermiwash |
| 13. | Seed biopriming with BCA-5+ sowing of seeds in soil amended with NSKE (5%) |
| 14. | Seed biopriming with BCA-5+ sowing of seeds in soil amended with compost tea |
| 15. | Seed biopriming with BCA-5+ sowing of seeds in soil amended with vermiwash |
| 16 | Control |

Biopriming of seeds was then carried out with suspensions of effective fungal biocontrol agents prepared in 1 per cent Carboxymethyl cellulose CMC at a concentration of $10^7$ cfu per ml (tomato and chilli) and $10^8$ cfu per ml (capsicum) for 8 hrs. Biopriming of tomato, chilli and capsicum seeds with suspensions of effective bacterial biocontrol agents at concentration of $10^8$ cfu per ml prepared in 1 per cent Carboxymethyl cellulose (CMC) for 8 hrs was carried out. After application of the treatments, the microbial suspensions smeared on the seeds were drained off and bioprimed seeds were dried under laminar air flow chamber on sterilized blotting paper to bring down the moisture content to original concentration (Singh et al. 2020b).

Sick pots were prepared by filling the soil mixture containing sandy soil, vermi-compost and FYM in the ratio of 2:1:1 after sterilization with formalin into the pots (6-inch diameter). Inoculum of the pathogens namely *Pythium* sp., *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsii* prepared on corn sand meal media was added into the soil mixture filled in the pots @ of 10g/350g of soil and kept covered with polythene sheet for 7 days for build-up of inoculum (Dohroo, 1988). Afterwards, the bioprimed seeds of tomato, chilli and capsicum of the treatments mentioned below were sown into separate pots. Soil amendments like NSKE (5%), compost tea and vermiwash were amended in soil by using soil inoculation method at weekly interval from 15 days of sowing of bioprimed seeds. Experiment was carried out in completely randomized design with three replications in each case. Data pertaining to disease incidence (%), seed germination (%), shoot length (cm) and root length (cm) was also recorded.

## 12. Efficacy of integration of seed biopriming with effective bioinoculants (BCAs, plant extracts, endophytes) and soil solarization

Seed biopriming with effective bioinoculants like BCAs, plant extracts and endophytes followed by their plantation in solarized soil was done to evaluate the efficacy of combination in managing damping-off disease in tomato, chilli and capsicum nurseries. The field experiment was carried out in randomized block design with three replications in each case and different treatment combinations were evaluated to record the incidence of damping-off disease. The experiment was conducted in solarized and unsolarized conditions. Solarization was done by covering nursery beds with thin transparent polythene sheet of 25µm (100 gauge) thickness during the months of May-June for a period of 30 days. While, half of the beds were selected for soil solarization and remaining beds were kept unsolarized. Seeds biopriming was done with effective biocontrol agents at a concentration of $10^7$ cfu per ml (tomato and chilli) and $10^8$ cfu per ml (capsicum) for 8 hrs for fungal biocontrol agents and at $10^8$ cfu per ml for 8 hrs for bacterial biocontrol agents. Seed biopriming was done with effective plant extracts @ 10 % for 12 hrs and with fungal endophytes at a concentration of $10^8$ cfu per ml for 12 hrs and for bacterial endophytes at a concentration of $10^7$ cfu per ml for 12 hrs. Bioprimed seeds were then sown separately in solarized and unsolarized nursery beds of size 1.5x1.5 $m^2$. Neem cake and vermicompost was applied at the time of preparation of nursery beds and as well as during sowing of bioprimed seeds at 40 grams per $m^2$ concentration. Data pertaining to disease incidence (%), seed germination (%), shoot length (cm) and root length (cm) was recorded for each treatment.

**Treatments applied separately in solarized and unsolarized beds**

| S. No. | Treatments |
|---|---|
| **1.** | Seed bio priming with BCA 1+ Soil application of Neem cake |
| **2.** | Seed bio priming with BCA 1+ Soil application of Vermicompost |
| **3.** | Seed bio priming with BCA 2+ Soil application of Neem cake |
| **4.** | Seed bio priming with BCA 2+ Soil application of Vermicompost |
| **5.** | Seed bio priming with Plant extract 1 + Soil application of Neem cake |
| **6.** | Seed bio priming with Plant extract 1 + Soil application of Vermicompost |
| **7.** | Seed bio priming with Plant extract 2+ Soil application of Neem cake |
| **8.** | Seed bio priming with Plant extract 2+ Soil application of Vermicompost |
| **9.** | Seed bio priming with Endophyte 1+ Soil application of Neem cake |
| **10.** | Seed bio priming with Endophyte 1+ Soil application of Vermicompost |
| **11.** | Seed bio priming with Endophyte 2 + Soil application of Neem cake |
| **12.** | Seed bio priming with Endophyte 2 + Soil application of Vermicompost |
| **13.** | Control |

## 13. Biochemical estimation of enzyme activity in bioprimed plants

Chilli, capsicum and tomato seedlings obtained from different treatments emerging out of bio-primed seeds were uprooted after 20 days of sowing of bioprimed seeds. These seedlings were examined for the estimation of the enzyme activity. Inducible changes in the enzyme activity were then examined by following methods described by Jain et al. (2012).

### Sample collection for biochemical analysis

Plants were selected randomly from each treatment, and nodal leaves ($3^{rd}$ to $5^{th}$ nodes) were collected as samples after 20 days. Collected leaves were then washed in running tap water, dried with blotting paper and stored at 4˚C temperature for further use.

### Determination of total phenol content (TPC)

For the estimation of Total Phenol Content (TPC) in leaves, 100 mg sample of leaf tissue from different treatments were taken and were then homogenized separately in 5 ml of 95 per cent ethanol. The homogenized samples were then centrifuged at 13,000 rpm for 10 min and the supernatant was collected. Reaction mixture was prepared with 1 ml of the supernatant, 1 ml of 95 per cent ethanol and 5 ml of sterile distilled water and 0.5 ml of 50% Folin–Ciocalteau reagent. Folin–Ciocalteau is a sensitive reagent that contains phosphomolybdate and phosphotungstate that form blue-complex in alkaline solution by the reduction of phenols. After about 5 min. 1 ml of 5 per cent sodium carbonate was added to the reaction mixture and the reaction mixture was allowed to stand for 1 hr. The absorbance value for each treatment was taken at 765 nm using methanol as blank. Gallic acid at different concentrations was used as the standard. Standard curves were prepared for assay using various concentrations of gallic acid (GA) in 95 per cent ethanol. Absorbance values were then converted to mg GA equivalents (GAE) per g fresh weight (FW) for phenolic content estimation.

### Phenylalanine ammonia lyase (PAL) assay

For the estimation of PAL activity, leaf sample of 100 mg from each of the treatments were taken and were homogenized separately in 2 ml of 0.1M sodium borate buffer (pH 7) containing 1.4 mM β-mercaptaethanol and the homogenate was centrifuged at 16,000 rpm at

4˚C for 15 min. The supernatant collected was used as enzyme source. The reaction mixture was prepared with 0.2 ml of enzyme extract, 0.5 ml of 0.2M borate buffer (pH 8.7) and 1.3 ml of distilled water. The reaction was initiated by the addition of 1 ml of 0.1M L-phenylalanine (pH-8.7) and incubated for 30 min at 32˚C. The reaction was later terminated after incubation by addition of 0.5 ml of trichloroacetic acid (TCA, 1M). PAL activity was measured by the formation of *trans*-cinnamic acid at 290 nm and was expressed in terms of μmol $l^{-1}$ TCA per g fresh weight (FW).

**Polyphenol oxidase (PPO) assay**

For the estimation of Polyphenol oxidase activity, 100 mg leaf samples from each treatment were homogenized separately in 2 ml of ice-cold phosphate buffer (0.1M, at pH 6.5). The homogenate was then centrifuged at 16,000 rpm for 30 min at 4˚C, and the supernatant was collected which was used directly in the enzyme assay. The reaction mixture was prepared with 0.4 ml catechol (1mM) in 3 ml of 0.05 M sodium phosphate buffer (pH 6.5) and 0.4 ml enzyme extract. Reaction mixture which contained only the substrate without enzyme extract was used as control. Catechol was used as substrate for PPO and the increment in absorbance was recorded at 405 nm. The PPO activity was expressed as change in Optical Density (OD) per min per g fresh weight (FW).

**Peroxidase assay**

For the estimation of peroxidase assay, 100 mg leaf samples from each treatment were homogenized separately in 2 ml of 0.1 M phosphate buffer (pH 7) and then centrifuged at 16,000 rpm for 15 min at 4˚C and the supernatant collected was used as enzyme source. The reaction mixture was prepared with 1.5 ml pyrogallol (0.05 M), 0.05 ml enzyme extract and 0.5 ml $H_2O_2$ (1% v/v). Reaction mixture without enzyme extract was used as control. The enzyme activity with respect to absorbance at 420 nm was expressed as change in the U per min per g fresh weight (FW).

## 5. Statistical analysis

Data recorded from *in vitro* and field experiments were statistically analyzed. The differences among treatments in various experiments were tested for their significance using procedure described by **(**Panse and Sukhatme, 1984; Gomez and Gomez, 1984). Data was

analyzed by analysis of variance (ANOVA) and treatment means were compared using Duncan Multiple Range Test (DMRT) and by using Least Significance Difference (LSD) at P values $\leq 0.05$ using SPSS version 16, WASP 2.0 and OPSTAT statistical tool.

# *Chapter-4*

# RESULTS AND DISCUSSION

The present studies entitled **"Potential of seed biopriming in management of damping-off disease in solanaceous vegetable crops"** were carried out in the different laboratories and at the experimental farm of Department of Plant Pathology, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (Himachal Pradesh) during the year 2020-2023. The results obtained from various experiments during the study are presented and discussed below:

## 1. SYMPTOMATOLOGY

Typical symptoms of the damping-off disease in solanaceous vegetable crops namely tomato, chilli and capsicum were recorded. Symptoms of the disease appeared in two phases *i.e*., pre-emergence damping-off and post-emergence damping-off. In case of pre-emergence damping-off, rotting of seeds occurred which led to failure in their germination resulting in patchy appearance in the field (Plate 4). In case of post-emergence damping-off, water-soaked brown lesions appeared on hypocotyl portion near soil level which later caused rotting, brown discoloration and constriction at the infected portion which consequently led to collapse and decay of young seedlings (Plate 4). The characteristic symptoms of the damping-off disease mentioned above were recorded in tomato, chilli and capsicum which were found similar as reported by various research workers (Gattani and Kaul 1951; Rangaswami and Mahadevan 2004; Horst 2013; Gholve et al. 2014; Patel et al. 2014; Shinde et al. 2016; Singh et al. 2016; Elshahawy and El-Mohamedy 2019; Hyder et al. 2021; Hassanisaadi et al. 2021; Varma and Kumhar 2022).

## 2. ISOLATION AND IDENTIFICATION OF THE PATHOGENS

The pathogens associated with damping-off disease of tomato, chilli and capsicum were isolated from the infected seedlings by standard tissue isolation method and were further purified by hyphal tip culture method. The purified pathogens were later identified based upon their cultural and morphological characters under the microscope. Their morphological characters were later compared with standard descriptions as described by Waterhouse (1967) for *Pythium*; Booth (1971) for *Fusarium*; Parameter (1970) for

*Rhizoctonia* and Barnett and Hunter (1972) for *Sclerotium*. Based on these identification keys, associated pathogens were identified as *Pythium* sp., *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsii*. Cultural and morphological characters of the isolated pathogens observed during the studies have been described below:

***Pythium* sp.**

The culture of *Pythium* sp. was white in colour with dense aerial mycelial on PDA medium. Pathogen was fast growing which took around 5 days for completely covering the Petri-plate (90 mm) with mycelium (Plate 5). Microscopic observations (400X) indicated that the hyphae of the fungus were hyaline and coenocytic (8-15 µm long with width ranged from 4.25-8.5µm). Sporangia were globular and ovoid without any papilla with size varying from 24.08-32.85 x 22.3-33.56 µm. Zoospores were also observed in the sporangia which were found differentiating inside the vesicle (Plate 5). Oospores were thick walled and smooth formed after 8 days of inoculation in media (Plate 5).

***Fusarium oxysporum***

The culture of *Fusarium oxysporum* was white in colour with aerial growth on the PDA medium. The fungus completely covered the Petri-plate (90 mm) on the media within 7 days. No pigmentation was observed on the medium (Plate 5). Mycelium of the fungus was hyaline septate (9.3-15.7 µm long with width of 2.5-4 µm) with presence of both macro and micro conidia (Plate 5). Macroconidia were hyaline, falcate with 3-5 septation and pointed curved ends (25-35 x 2.5-4.8 µm). Microconidia were ovoid without any septation (5-10 x 2.1-3.2 µm). Chlamydospores were thick walled which were terminal as well as intercalary.

***Sclerotium rolfsii***

The mycelium of pathogen appeared white in colour with viscid appearance in culture (Plate 6). The fungus completely covered the Petri-plate (90 mm) on the media within 5 days. The hyphae of the fungus were hyaline, thin walled with sparse septation in young hyphae (5.29-8.5 µm long with width of 5-6.5µm). Sclerotia were light brown initially turning dark brown with hard and round appearance of size 1-2 mm (Plate 6).

***Rhizoctonia solani***

The fungus had initially sparse white mycelium on the surface of Petri-plate which radially turns brown in colour in old cultures and covered the complete Petri-plate within 6 days (Plate 6). The mycelium was septate (6-25 µm long and width of 2-8 µm) with typical characteristic right-angled branching and the branches were constricted at the point of their

Non-uniform germination

Toppling over of infected seedlings

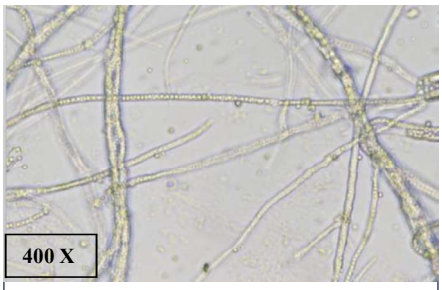
Soft water soaked lesions on stem

Brown decolouration with constriction

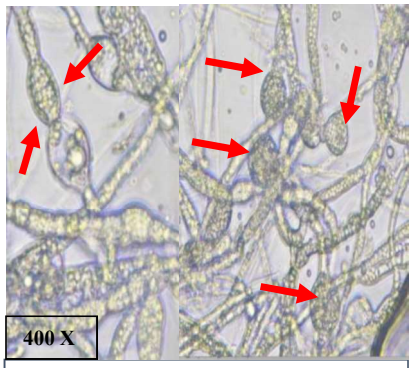
Decay of seedlings

Plate 4: Symptoms of damping-off disease

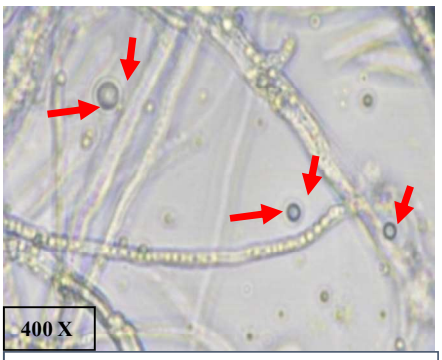
Pure culture




Coenocytic mycelium



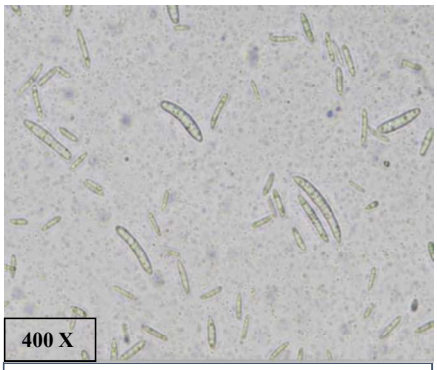

Sporangia with vesicle



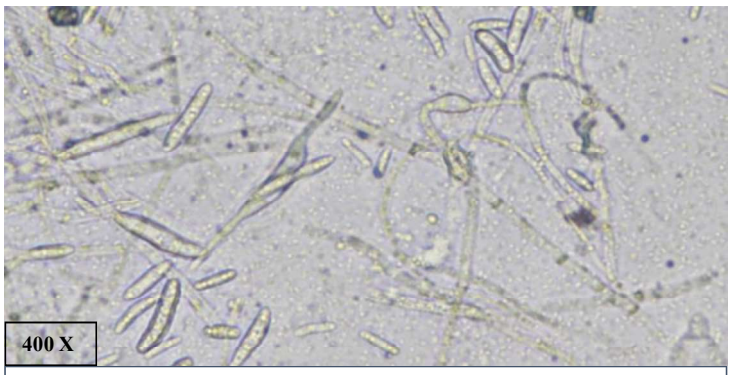

Oospore formation

*Pythium* sp.

Pure culture



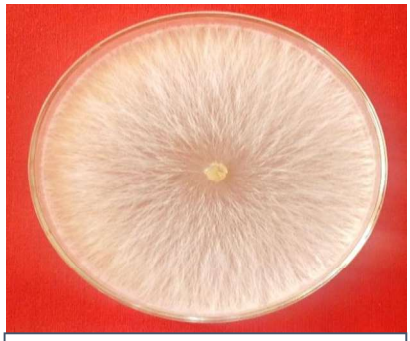

Macro conidia




Septate mycelium with macro and micro conidia

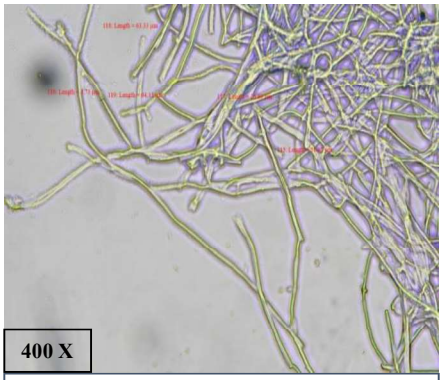
*Fusarium oxysporum*

**Plate 5: Cultural and morphological characters of *Pythium* sp. and *Fusarium oxysporum***



Pure culture

Sclerotia

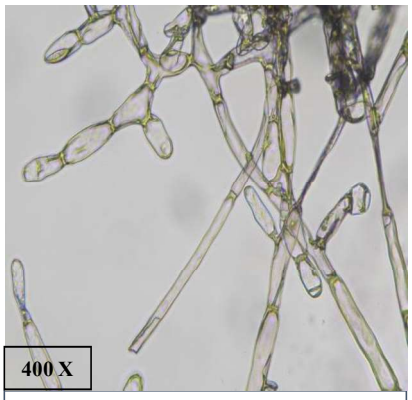



Mycelium

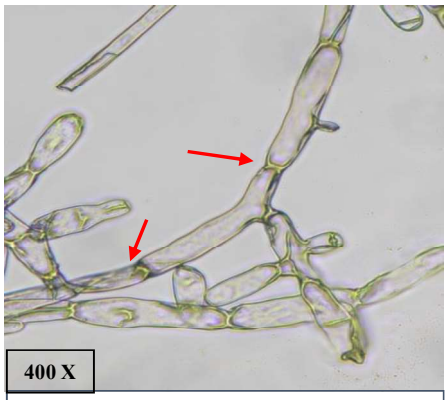
*Sclerotium rolfsii*

Pure culture

Pure culture with sclerotia




Mycelium




Branching at right angle

*Rhizoctonia solani*

**Plate 6: Cultural and morphological characters of *Sclerotium rolfsii* and *Rhizoctonia solani***

origin from the septa as appeared under microscope (Plate 6). Sclerotia were light brown in colour with rough appearance and formed within 10-12 days in the pure culture of size 0.4-2.5 mm (Plate 6).

## 3. PATHOGENICITY

The association of the isolated pathogens with damping-off disease in tomato, chilli and capsicum was tested using sick soil inoculation method. The pathogens produced typical damping-off symptoms in the sick soil pots inoculated with the pathogens individually as well as in combination. Data pertaining to their incubation period *i.e.,* time taken by pathogen for symptom development upto post-emergence stage, pre-emergence (based on number of seeds germinated) and post-emergence (based on infected seedlings after germination) damping-off incidence was recorded which indicate that all the four isolated pathogens were associated with damping-off disease in tomato, chilli and capsicum.

In tomato seedlings, combined association of all the four pathogens resulted in highest pre-emergence disease incidence of 66.67 per cent and post-emergence damping-off incidence of 75.42 per cent with incubation period of 7 days (Plate 7). Among the individual pathogens, *Pythium* sp. was found most severe with pre-emergence incidence of 52.47 per cent, post-emergence incidence of 62.61 per cent with incubation period of 8 days followed by *Rhizoctonia solani* with pre-emergence incidence of 40.25 per cent, post emergence incidence of 54.35 per cent with incubation period of 9 days. Inoculation with *Fusarium oxysporum* resulted in pre-emergence disease incidence of 31.54 per cent and post-emergence incidence of 54.88 per cent with incubation period of 9 days. However, *Sclerotium rolfsii* resulted in least severe infection with pre-emergence disease incidence of 28.51 per cent, post-emergence incidence of 42.87 per cent with incubation period of 14 days (Table 1, Plate 7).

**Table 1: Damping-off incidence in tomato under artificially inoculated conditions**

| Fungal pathogens | Incubation period (days) | Disease incidence (%) | |
|---|---|---|---|
| | | Pre-emergence damping off | Post- emergence damping-off |
| ***Pythium* sp. (P)** | 8 | 52.47 (46.40) | 62.61 (52.28) |
| ***Fusarium oxysporum* (F)** | 9 | 31.54 (34.15) | 54.88 (47.78) |
| ***Sclerotium rolfsii* (S)** | 14 | 28.51 (32.26) | 42.87 (40.88) |
| ***Rhizoctonia solani* (R)** | 9 | 40.25 (40.52) | 54.35 (47.47) |
| **P+F+ S+R** | 7 | 66.67 (54.72) | 75.42 (60.26) |
| **CD $_{(0.05)}$** | **-** | **1.17** | **0.96** |

Figures in the parenthesis are arc sine transformed values

Pathogenicity test carried out for chilli, indicate that combined inoculation of all the four pathogens resulted in highest pre-emergence disease incidence of 72.65 per cent and post-emergence incidence of 84.33 per cent with incubation period of 6 days (Plate 7). Among the individual pathogens, *Pythium* sp. was found most severe with pre-emergence incidence of 63.42 per cent and post-emergence incidence of 69.89 per cent with incubation period of 9 days followed by *Rhizoctonia solani* with pre-emergence incidence of 48.15 per cent and post-emergence incidence of 66.78 per cent with incubation period of 7 days. *Fusarium oxysporum* resulted in pre-emergence damping-off disease incidence of 41.62 per cent and post-emergence incidence of 64.17 per cent with incubation period of 10 days. However, *Sclerotium rolfsii* resulted in least severe infection with pre-emergence disease incidence of 31.51 per cent and post-emergence incidence of 42.71 per cent with incubation period of 12 days (Table 2, Plate 7).

**Table 2: Damping-off incidence in chilli under artificially inoculated conditions**

| Fungal pathogens | Incubation period (days) | Disease incidence (%) | |
|---|---|---|---|
| | | Pre-emergence damping off | Post- emergence damping-off |
| ***Pythium* sp. (P)** | 9 | 63.42 (52.76) | 69.89 (56.70) |
| ***Fusarium oxysporum* (F)** | 10 | 41.62 (40.16) | 64.17 (53.21) |
| ***Sclerotium rolfsii* (S)** | 12 | 31.51 (34.14) | 42.71 (40.78) |
| ***Rhizoctonia solani* (R)** | 7 | 48.15 (43.92) | 66.78 (43.92) |
| **P+F+ S+R** | 6 | 72.65 (58.44) | 84.33 (58.44) |
| **CD $_{(0.05)}$** | **-** | **0.69** | **1.17** |

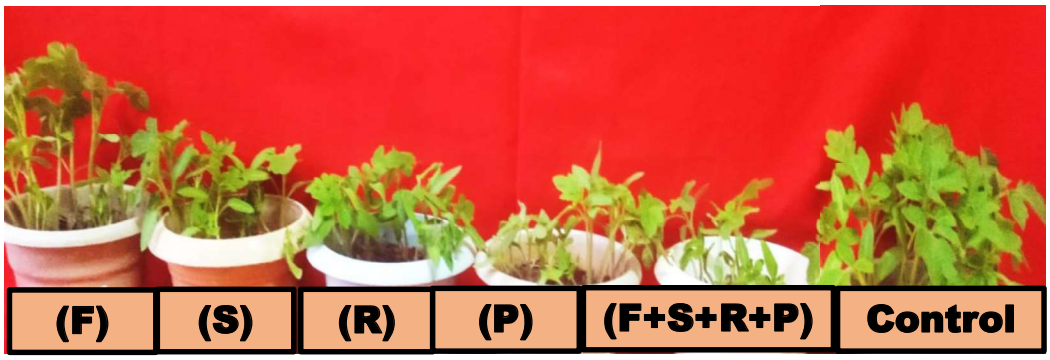
Figures in the parenthesis are arc sine transformed values

Pathogenicity test carried out in capsicum seedlings indicate that combined inoculation of all the four pathogens resulted in highest pre-emergence disease incidence of 72.16 per cent and post-emergence incidence of 81.97 per cent with incubation period of 6 days (Table 3, Plate 7).

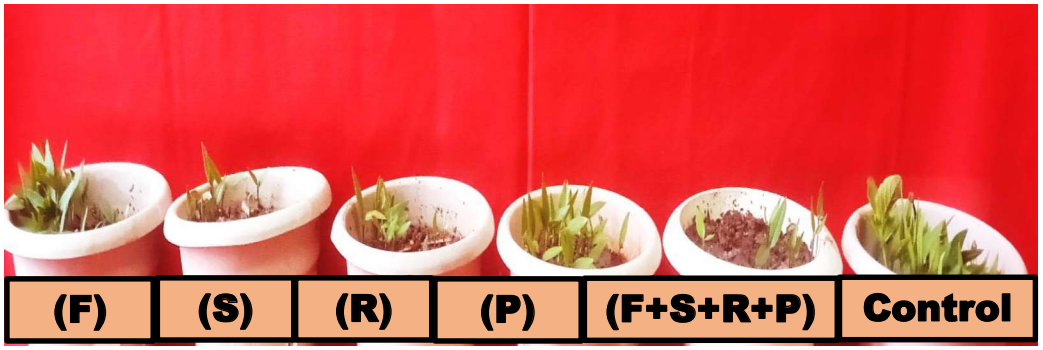
**Table 3: Damping-off incidence in capsicum under artificially inoculated conditions**

| Fungal pathogens | Incubation period (days) | Disease incidence (%) | |
|---|---|---|---|
| | | Pre-emergence damping off | Post- emergence damping-off |
| ***Pythium* sp. (P)** | 8 | 60.56 (51.08) | 67.21 (55.05) |
| ***Fusarium oxysporum* (F)** | 9 | 30.29 (33.37) | 55.77 (48.29) |
| ***Sclerotium rolfsii* (S)** | 11 | 46.77 (43.13) | 65.77 (54.17) |
| ***Rhizoctonia solani* (R)** | 7 | 60.44 (51.01) | 68.54 (55.86) |
| **P+F+ S+R** | 6 | 72.16 (58.13) | 81.97 (64.85) |
| **CD $_{(0.05)}$** | - | **0.58** | **1.14** |

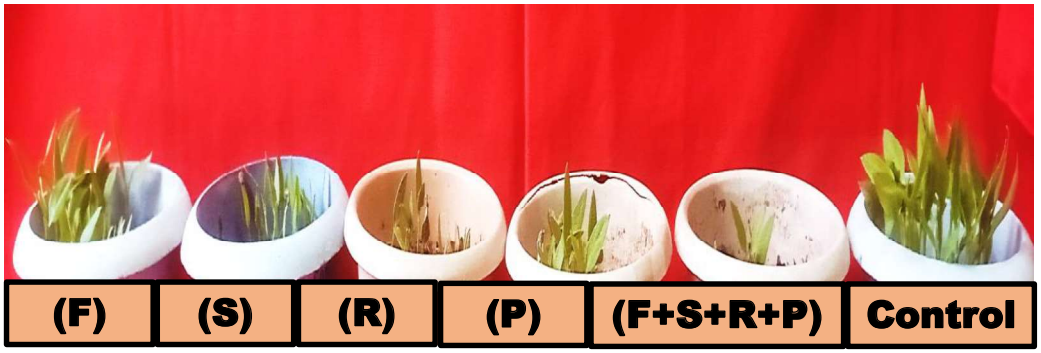
Figures in the parenthesis are arc sine transformed values




**Tomato**




**Chilli**




**Capsicum**

**Plate 7: Proving pathogenicity of pathogens associated with damping-off disease in tomato, chilli and capsicum**

Among the individual pathogens, *Pythium* sp. was most severe with pre-emergence incidence of 60.56 per cent, post-emergence incidence of 67.21 per cent with incubation period of 8 days followed by *Rhizoctonia solani* with pre-emergence incidence of 60.44 per cent, post emergence incidence of 68.54 per cent with incubation period of 7 days. Further, *Fusarium oxysporum* was found least severe with pre-emergence disease incidence of 30.29 per cent, post-emergence incidence of 55.77 per cent with incubation period of 9 days. For *Sclerotium rolfsii*, pre-emergence and post-emergence incidence was found 46.77 and 65.77 per cent, respectively with incubation period of 11 days among all the pathogens (Plate 7).

Similar findings have been reported by different research workers while proving the pathogenicity by sick soil method (Sowamini 1961; Bisht et al. 1997; Ramamoorthy et al. 2002; Jiskani et al. 2007; Abeysinghe 2009; Shinde et al. 2016; Ismael and Mahmood 2016; Bhardwaj 2019; Hassanisaadi et al. 2021). Sultana et al. (2012) reported the post-emergence mortality in chilli seedlings due to damping-off disease after 10 days of sowing of seeds in infected soil under pot conditions. Hyder et al. (2018) reported that symptoms of disease development appeared after 10 days of sowing of chilli cv. Sanam under greenhouse conditions. Reetha and Muthukumar (2019) proved pathogenicity of different *Pythium* isolates on tomato cv. PKM-1 and reported that maximum disease incidence of 43.65 per cent and 67.98 per cent was recorded after 14 days of incubation. Navi et al. (2019) reported 79.80 per cent incidence of damping-off after 6 days of incubation under greenhouse conditions due to *Pythium ultimum* var. *ultimum*. Muthukumar et al. (2010) reported that disease incidence varied from 34.0 to 65.0 per cent and the symptoms of damping-off disease in chilli appeared after 14 days of sowing.

## 4. Disease management

### 1. *In vitro* efficacy of biocontrol agents against damping-off pathogens

Antagonistic activity of the fungal and bacterial biocontrol agents was tested against the isolated damping-off pathogens. Biocontrol agents which were found effective against damping-off pathogens were selected for seed biopriming treatment to further evaluate their potential in the management of damping-off disease in tomato, chilli and capsicum.

All the biocontrol agents inhibited the mycelial growth of damping-off pathogens ranging from 11.35 to 67.89 per cent (Table 4). *Trichoderma harzianum* was found most effective in inhibiting the mycelial growth of the pathogens followed by *Trichoderma virens*

and *Trichoderma atroviride* (Plate 8). Among all the biocontrol agents evaluated against *Pythium* sp., *T. virens* was found most effective and significantly superior with 88.70 per cent mycelial inhibition followed by *T. harzianum* and *T. atroviride* with 83.85 and 52.41 per cent inhibition, respectively. Among, bacterial biocontrol agents, *Pseudomonas fluorescens* was found next in efficacy with 52.11 per cent mycelial inhibition (Plate 9). *Bacillus* sp. and *B. subtilis* were found least effective against *Pythium* sp. in inhibiting radial mycelial growth. Further, *T. harzianum* inhibited the mycelial growth of *Fusarium oxysporum* by 86.55 per cent followed by *T. virens* with inhibition of 84.26 per cent and both were found statistically at par with each other.

**Table 4: *In vitro* efficacy of biocontrol agents against damping-off pathogens**

| | | Mycelial inhibition (%) | | | | |
|---|---|---|---|---|---|---|
| **S. No.** | **Treatments** | ***Pythium* sp.** | ***Fusarium oxysporum*** | ***Sclerotium rolfsii*** | ***Rhizoctonia solani*** | **Mean** |
| **1.** | ***Trichoderma harzianum*** | 83.85 (66.32) | 86.55 (68.52) | 36.74 (37.30) | 64.40 (53.37) | **67.89 (56.38)** |
| **2.** | ***Trichoderma virens*** | 88.70 (70.36) | 84.26 (66.73) | 34.64 (36.04) | 53.81 (47.18) | **65.35 (55.08)** |
| **3.** | ***Trichoderma atroviride*** | 52.41 (46.38) | 62.77 (52.40) | 36.48 (37.14) | 53.35 (46.92) | **51.25 (45.71)** |
| **4.** | ***Bacillus* sp.** | 7.51 (15.83) | 28.66 (32.32) | 16.33 (23.83) | 26.49 (30.96) | **19.75 (25.74)** |
| **5.** | ***Bacillus licheniformis*** | 33.18 (35.16) | 44.44 (41.80) | 35.16 (36.36) | 26.20 (30.78) | **34.75 (36.02)** |
| **6.** | ***Bacillus subtilis*** | 6.96 (15.25) | 23.33 (28.87) | 7.47 (15.79) | 7.62 (15.95) | **11.35 (18.97)** |
| **7.** | ***Pseudomonas fluorescens*** | 52.11 (46.21) | 25.37 (30.23) | 14.03 (21.97) | 33.73 (35.50) | **31.31 (33.43)** |
| **8.** | ***Saccharomyces* sp.** | 23.59 (29.05) | 17.22 (24.49) | 13.37 (21.41) | 13.51 (21.53) | **16.92 (24.12)** |
| **9.** | ***Saccharomyces cerevisiae*** | 25.55 (30.34) | 15.39 (23.09) | 15.77 (23.39) | 16.40 (23.86) | **18.23 (25.17)** |
| | **Mean** | **37.37 (35.49)** | **38.79 (36.85)** | **20.99 (25.32)** | **29.55 (30.61)** | |
| | **CD $_{(0.05)}$** | **2.09** | **2.93** | **2.28** | **2.18** | |

Figures in the parenthesis are arc sine transformed values

However, yeast antagonists *Saccharomyces* sp. and *S. cerevisiae* were found least effecting in inhibiting mycelial growth of *Fusarium oxysporum*. All biocontrol agents were found comparatively less effective in inhibiting the growth of *Sclerotium rolfsii* with mycelial inhibition of only 36.74 per cent by *Trichoderma harzianum*. *Trichoderma atroviride* and *Bacillus licheniformis* resulted in 36.48 per cent and 35.16 per cent inhibition, respectively.

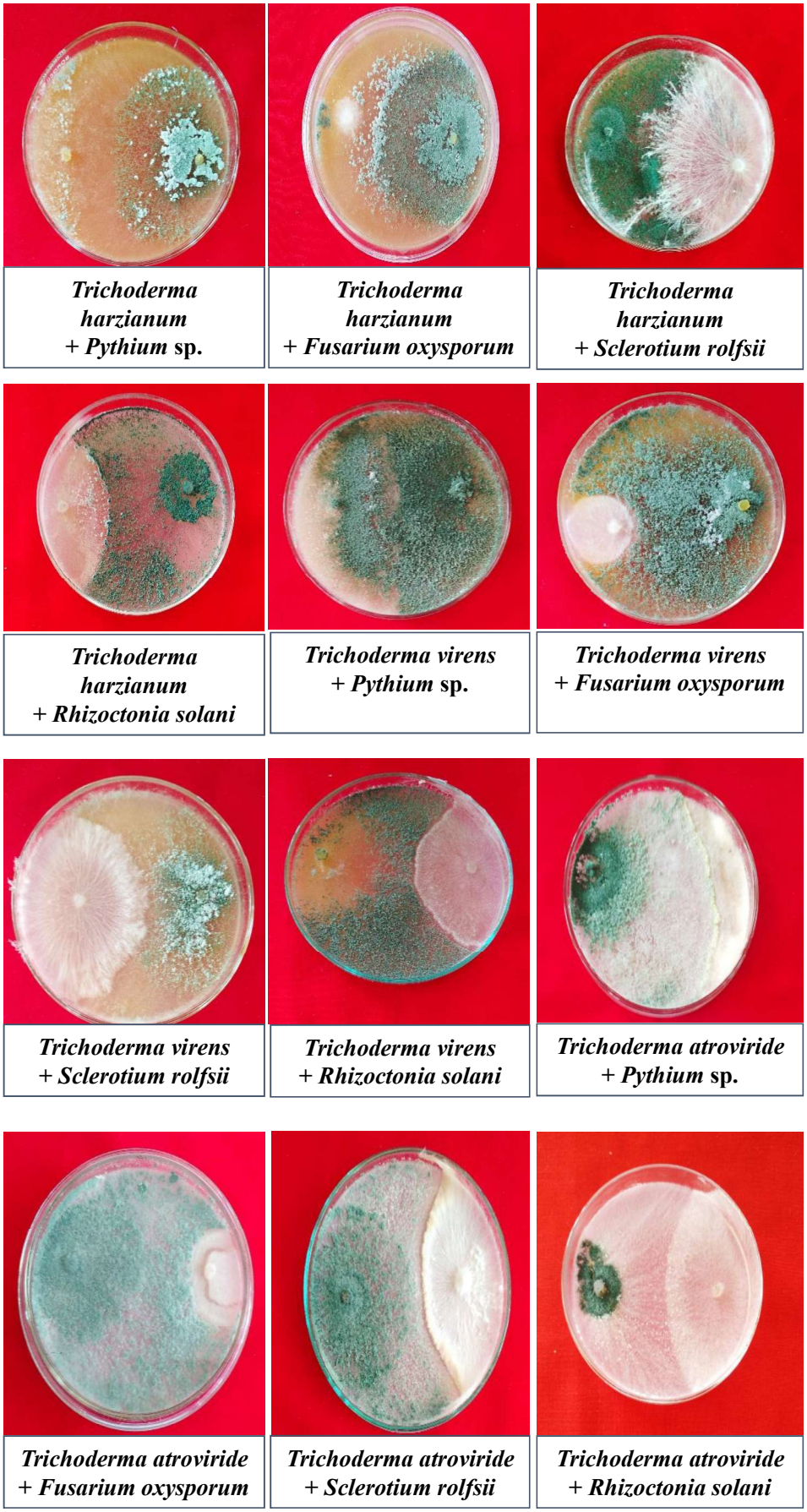



**Plate 8: *In vitro* evaluation of fungal biocontrol agents against damping-off pathogens**

*Bacillus licheniformis* + *Pythium* sp.

*Bacillus licheniformis* + *Fusarium oxysporum*

*Bacillus licheniformis* + *Sclerotium rolfsii*

*Bacillus licheniformis* + *Rhizoctonia solani*

*Pseudomonas fluorescens* + *Pythium* sp.

*Pseudomonas fluorescens* + *Fusarium oxysporum*

*Pseudomonas fluorescens* + *Sclerotium rolfsii*

*Pseudomonas fluorescens* + *Rhizoctonia solani*

**Plate 9: *In vitro* evaluation of bacterial biocontrol agents against damping-off pathogens**

*Trichoderma harzianum* resulted in maximum inhibition of (64.40 %) mycelial growth of *Rhizoctonia solani* followed by *T. virens* and *T. atroviride* with mycelial inhibition per cent of 53.81 and 53.35 per cent, respectively. *Bacillus subtilis* was found least effective against *Rhizoctonia solani* with mycelial inhibition of 7.62 per cent. Similar results have been reported by the researchers from other parts of the country (Manoranjitham et al. 2000; Chakrabarti et al. 2005; Jeyaseelan et al. 2012). Dar et al. (2015) reported that *Trichoderma viride* (isolate TK1) and *Trichoderma harzianum* (isolate TK8) resulted in 61.2-73.2 per cent and 62.3-67.5 per cent inhibition against *Pythium* sp., *Sclerotium rolfsii*, *Fusarium oxysporum* and *Rhizoctonia solani*. Amin et al. (2010) and Naik et al. (2009) also reported that the *Trichoderma* sp. effectively inhibited the growth of *Sclerotium rolfsii*, *Rhizoctonia solani*, *Pythium* sp. and *Fusarium oxysporum* in vegetable crops. Anita et al. (2012) reported that, *Trichoderma viride*, *Trichoderma harzianum* and *Trichoderma flavofuscum* were found most effective with mycelial inhibition of 87.78, 80.11 and 80.0 per cent, respectively against damping-off pathogen *Pythium aphanidermatum* in tomato seedlings. Shinde et al (2016) reported that *Trichoderma harzianum* and *Pseudomonas fluorescens* were effective in inhibiting the mycelial growth of damping-off pathogen (*Pythium aphanidermatum*) in tomato. Jamal et al. (2021) reported that among 76 yeast isolates tested against *Rhizoctonia solani*, 75 isolates inhibited the mycelial growth of *Fusarium oxysporum.* Antagonistic yeast *Saccharomyces* sp. in combination with *Trichoderma viride* and *Trichoderma album* completely suppressed the mycelial growth of *Rhizoctonia solani* and *Fusarium solani*, respectively in bean causing damping-off disease in comparison to other treatments (El-Sayed 2022).

## 2. Efficacy of seed biopriming with biocontrol agents (BCAs)

Effective biocontrol agents (*Trichoderma harzianum*, *T. virens*, *T. atroviride, Pseudomonas fluorescens* and *Bacillus licheniformis*) under *in-vitro* conditions were then evaluated for their efficacy in seed biopriming against damping-off disease in tomato, chilli and capsicum. At first, the optimization of concentration of suspensions of biocontrol agents and duration for seed biopriming was carried out.

### Standardization of effective concentration of BCAs for seed biopriming

The experiment was conducted for optimization of effective concentration of biocontrol agents out of the three concentrations *i.e.*, $10^6$ cfu per ml, $10^7$ cfu per ml and $10^8$ cfu per ml evaluated for seed biopriming. For standardization of effective concentration of

biocontrol agents for seed biopriming of tomato, chilli and capsicum, germination test was carried out using the paper roll towel method of International Seed Testing Association (Anonymous 1999).

Present findings, indicate that seed health attributes like seed germination per centage, seedling length, seedling dry weight, Seedling Vigour Index-Length (SVI-L) and Seedling Vigour Index-Mass (SVI-M) significantly improved under *in vitro* conditions. It is evident from the data presented in Table 5 that out of the three concentrations of suspensions of biocontrol agents tested, $10^7$ cfu per ml was found optimum and effective for seed germination in case of fungal biocontrol agents and concentration of $10^8$ cfu per ml was found effective for bacterial biocontrol agents in tomato. Maximum seed germination of 76.0 per cent was found at $10^7$ cfu per ml concentration in *Trichoderma harzianum* which is 18.42 per cent higher than control. *Trichoderma atroviride* and *Trichoderma virens* were found next in efficacy with germination of 75.67 and 71.67 per cent, respectively at the similar concentration of $10^7$ cfu per ml (Plate 10). In case of bacterial biocontrol agents, maximum seed germination of 88.33 per cent was found at $10^8$ cfu per ml concentration in *Bacillus licheniformis* followed by *Pseudomonas fluorescens* with germination of 82.0 per cent which was 29.80 and 24.39 per cent higher than control.

Seed biopriming with *Bacillus licheniformis* at $10^8$ cfu per ml concentration resulted in highest seedling length (16.56 cm) followed by the treatment of *Pseudomonas fluorescens* at $10^8$ cfu per ml and *Trichoderma atroviride* at $10^8$ cfu per ml with seedling length of 16.50 cm and 15.98 cm, respectively. Seed biopriming with *Trichoderma harzianum* followed by *Trichoderma virens* at lower concentration of $10^6$ cfu per ml was found next in efficacy with seedling length of 15.89 cm and 15.49 cm, respectively. Seedling dry weight was found maximum (43.67 mg) in seed biopriming treatment with *T. harzianum* at $10^8$ cfu per ml followed by *Pseudomonas fluorescens* at $10^6$ cfu per ml and *Trichoderma atroviride* at $10^6$ cfu per ml with dry weight of 41.17 mg and 33.93 mg, respectively. SVI-L was found maximum (1464.57) in *Bacillus licheniformis* at $10^7$ cfu per ml which is 63.13 per cent higher than control followed by *Pseudomonas fluorescens* at $10^8$ cfu per ml and *Trichoderma atroviride* at $10^7$ cfu per ml with values of 1354.33 and 1206.52, respectively. SVI-M was found maximum (2799.22) in biopriming treatment with *Pseudomonas fluorescens* at $10^6$ cfu per ml followed by *Trichoderma harzianum* at $10^8$ cfu per ml and *Trichoderma virens* at $10^7$ cfu per ml with value of 2750.33 and 2224.40, respectively.

**Table 5: Standardization of effective concentration of biocontrol agents for seed biopriming in tomato**

| S. No | Treatments (Seed Biopriming) | Seed germination (%) | Seedling length (cm) | Seedling dry weight (mg) | Seedling Vigour Index-L | Seedling Vigour Index-M |
|---|---|---|---|---|---|---|
| 1. | *Trichoderma harzianum* ($10^6$ cfu per ml) | 66.33 (54.52) | 15.89 | 27.30 | 1053.86 | 1809.8 |
| 2. | *Trichoderma harzianum* ($10^7$ cfu per ml) | 76.00 (60.64) | 15.22 | 34.33 | 1157.36 | 2609.20 |
| 3. | *Trichoderma harzianum* ($10^8$ cfu per ml) | 63.00 (52.51) | 14.60 | 43.67 | 920.35 | 2750.33 |
| 4. | *Trichoderma atroviride* ($10^6$ cfu per ml) | 67.33 (55.17) | 15.30 | 33.93 | 1031.27 | 2282.10 |
| 5. | *Trichoderma atroviride* ($10^7$ cfu per ml) | 75.67 (60.42) | 15.95 | 20.60 | 1206.52 | 1559.20 |
| 6. | *Trichoderma atroviride* ($10^8$ cfu per ml) | 72.67 (58.47) | 15.98 | 26.50 | 1161.63 | 1928.50 |
| 7. | *Trichoderma virens* ($10^6$ cfu per ml) | 55.67 (48.24) | 15.49 | 26.63 | 863.71 | 1485.47 |
| 8. | *Trichoderma virens* ($10^7$cfu per ml) | 71.67 (57.82) | 14.94 | 31.03 | 1072.19 | 2224.40 |
| 9. | *Trichoderma virens* ($10^8$ cfu per ml) | 68.67 (55.96) | 11.83 | 11.90 | 811.33 | 816.00 |
| 10. | *Pseudomonas fluorescens* ($10^6$ cfu per ml) | 68.00 (55.53) | 15.64 | 41.17 | 1062.82 | 2799.20 |
| 11. | *Pseudomonas fluorescens* ($10^7$cfu per ml) | 74.33 (59.58) | 15.21 | 19.00 | 1132.47 | 1413.67 |
| 12. | *Pseudomonas fluorescens* ($10^8$ cfu per ml) | 82.00 (64.89) | 16.50 | 12.03 | 1354.33 | 986.80 |
| 13. | *Bacillus licheniformis* ($10^6$ cfu per ml) | 77.00 (61.32) | 15.54 | 11.50 | 1197.02 | 884.67 |
| 14. | *Bacillus licheniformis* ($10^7$cfu per ml) | 83.33 (70.09) | 13.98 | 12.33 | 1464.57 | 1029.33 |
| 15. | *Bacillus licheniformis* ($10^8$ cfu per ml) | 88.33 (65.94) | 16.56 | 21.00 | 1163.57 | 1856.67 |
| 16. | Hydropriming | 72.67 (58.46) | 12.09 | 12.57 | 879.46 | 912.68 |
| 17. | Control | 62.00 (51.92) | 8.71 | 10.23 | 540.02 | 620.00 |
| | **CD $_{(0.05)}$** | **2.78** | **1.69** | **1.84** | **156.09** | **178.16** |

Figures in the parenthesis are arc sine transformed values

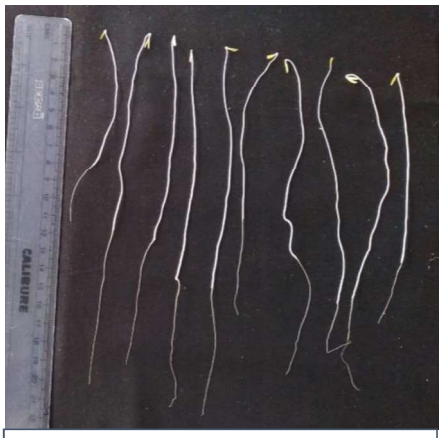
Among the three concentrations of biocontrol agents tested to find the optimum concentration for seed biopriming in chilli, $10^7$ cfu per ml concentration was found more

effective for seed germination in case of fungal biocontrol agents and concentration of $10^8$ cfu per ml was found effective for bacterial biocontrol agents (Table 6, Plate 11). Among the three concentrations of biocontrol agents evaluated, $10^8$ cfu per ml of *Bacillus licheniformis* was found most effective and maximum seed germination of 92.33 per cent with increase of 32.84 per cent in comparison to control. *Pseudomonas fluorescens* and *Trichoderma virens* were found next in efficacy with germination of 90.33 and 85.33 per cent, respectively. However, seed biopriming at $10^6$ cfu per ml concentration with *Bacillus licheniformis* resulted in highest seedling length (15.81 cm) followed by *Trichoderma harzianum* at $10^8$ cfu per ml and *Trichoderma virens* at $10^7$ cfu per ml with seedling length of 15.07 cm and 14.80 cm, respectively.

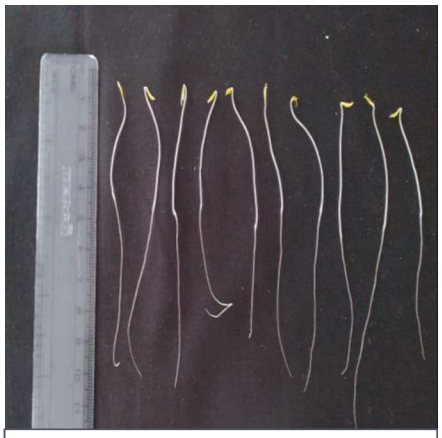
Seed biopriming with *Trichoderma virens* at $10^8$ cfu per ml followed by *Pseudomonas fluorescens* at $10^7$ cfu per ml were found next in efficacy with seedling length of 14.80 cm and 13.83 cm, respectively. Seedling dry weight was found maximum in seed biopriming at $10^7$ cfu per ml with *T. virens* followed by *Pseudomonas fluorescens* at $10^8$ cfu per ml and $10^6$ cfu per ml concentration, respectively. SVI-L was found maximum (1330.47) in *Bacillus licheniformis* at $10^6$ cfu per ml concentration followed by *Trichoderma virens* and *Pseudomonas fluorescens* at $10^8$ cfu per ml concentration with values of 1262.97 and 1229.17, respectively. SVI-M was found maximum (2245.7) in biopriming at $10^8$ cfu per ml concentration with *Trichoderma virens* followed by *Trichoderma atroviride* at $10^6$ cfu per ml and *Pseudomonas fluorescens* at $10^8$ cfu per ml concentration with values of 2224.7 and 2201.7, respectively.

In capsicum, spore count of $10^8$ cfu per ml was found effective for fungal and bacterial biocontrol agents (Table 7, Plate 12). Among the three concentrations of biocontrol agents evaluated, maximum seed germination of 75.0 per cent was found with $10^8$ cfu per ml concentration of *Trichoderma harzianum* with an increase of 29.33 per cent in comparison to control. Seed biopriming with *Trichoderma virens* at $10^8$ cfu per ml and $10^7$ cfu per ml with germination of 73.0 and 67.33 per cent, respectively.

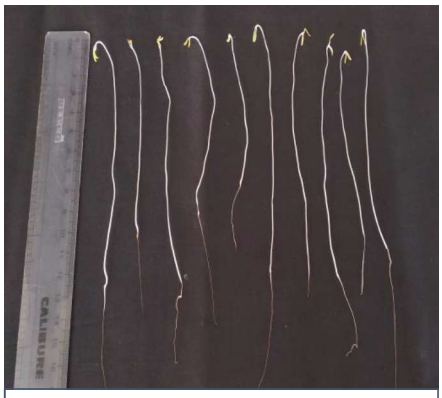
Seed biopriming at concentration of $10^7$ cfu per ml of *Trichoderma atroviride* resulted in highest seedling length (14.11 cm) followed by *Pseudomonas fluorescens* with $10^8$ cfu per ml and hydropriming with seedling length of 13.40 cm and 12.51 cm, respectively. Seed biopriming treatment with $10^7$ cfu per ml of *Pseudomonas fluorescens* was found least effective with seedling length of 9.62 cm.



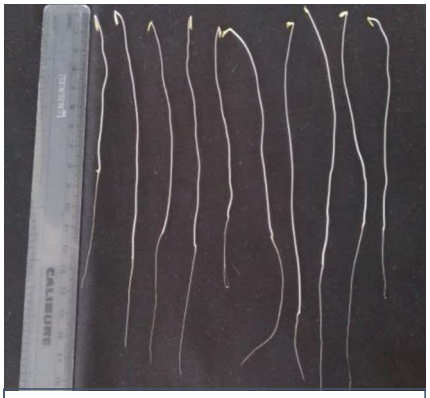
***Trichoderma harzianum***
**($10^7$ cfu per ml ) 8 hrs**



***Trichoderma virens***
**($10^7$ cfu per ml ) 8 hrs**



***Trichoderma atroviride***
**($10^7$ cfu per ml ) 8 hrs**



***Pseudomonas fluorescens***
**($10^8$ cfu per ml ) 8 hrs**

***Bacillus licheniformis***
**($10^8$ cfu per ml ) 8 hrs**

**Plate 10: Standardization of effective concentration of biocontrol agents and duration for seed biopriming in tomato**

**Table 6: Standardization of effective concentration of biocontrol agents for seed biopriming in chilli**

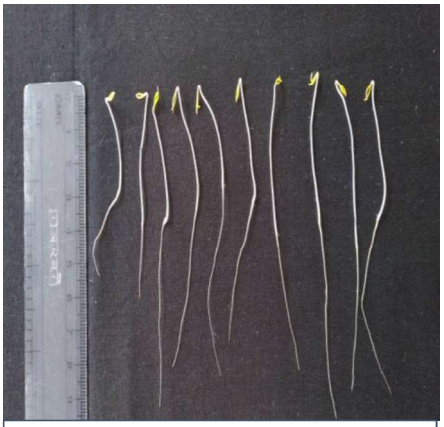
| S. No. | Treatments (Seed Biopriming) | Seed germination (%) | Seedling length (cm) | Seedling dry weight (mg) | Seedling Vigour Index-L | Seedling Vigour Index-M |
|---|---|---|---|---|---|---|
| 1. | *Trichoderma harzianum* ($10^6$ cfu per ml) | 76.67 (61.11) | 14.00 | 26.33 | 1071.30 | 2021.00 |
| 2. | *Trichoderma harzianum* ($10^7$ cfu per ml) | 84.00 (66.48) | 14.60 | 14.50 | 1227.80 | 1221.20 |
| 3. | *Trichoderma harzianum* ($10^8$ cfu per ml) | 72.33 (58.26) | 15.07 | 14.00 | 1090.33 | 1014.00 |
| 4. | *Trichoderma atroviride* ($10^6$ cfu per ml) | 82.33 (65.16) | 11.44 | 27.00 | 940.77 | 2224.70 |
| 5. | *Trichoderma atroviride* ($10^7$ cfu per ml) | 85.00 (67.19) | 13.54 | 15.57 | 1151.39 | 1322.80 |
| 6. | *Trichoderma atroviride* ($10^8$ cfu per ml) | 82.67 (65.41) | 13.41 | 13.33 | 1107.21 | 1103.70 |
| 7. | *Trichoderma virens* ($10^6$ cfu per ml) | 85.00 (67.22) | 12.01 | 16.70 | 1020.85 | 1419.50 |
| 8. | *Trichoderma virens* ($10^7$cfu per ml) | 85.33 (67.47) | 14.80 | 26.33 | 1262.97 | 2245.70 |
| 9. | *Trichoderma virens* ($10^8$ cfu per ml) | 77.67 (61.79) | 14.56 | 11.80 | 1131.92 | 916.67 |
| 10. | *Pseudomonas fluorescens* ($10^6$ cfu per ml) | 67.33 (55.13) | 13.63 | 22.67 | 917.98 | 1527.70 |
| 11. | *Pseudomonas fluorescens* ($10^7$cfu per ml) | 84.67 (66.94) | 13.83 | 14.00 | 1172.92 | 1187.3l |
| 12. | *Pseudomonas fluorescens* ($10^8$ cfu per ml) | 90.33 (71.89) | 13.61 | 24.33 | 1229.17 | 2201.70 |
| 13. | *Bacillus licheniformis* ($10^6$ cfu per ml) | 84.33 (66.68) | 15.81 | 20.40 | 1330.47 | 1718.30 |
| 14. | *Bacillus licheniformis* ($10^7$cfu per ml) | 86.00 (68.01) | 12.89 | 14.00 | 1107.94 | 1204.70 |
| 15. | *Bacillus licheniformis* ($10^8$ cfu per ml) | 92.33 (74.04) | 12.28 | 16.13 | 1134.67 | 1490.50 |
| 16. | Hydropriming | 71.00 (57.39) | 10.60 | 22.33 | 752.59 | 1586.30 |
| 17. | Control | 62.00 (51.92) | 10.30 | 14.33 | 623.72 | 868.00 |
| | **CD $_{(0.05)}$** | **2.69** | **1.80** | **2.85** | **151.69** | **268.43** |

Figures in the parenthesis are arc sine transformed values

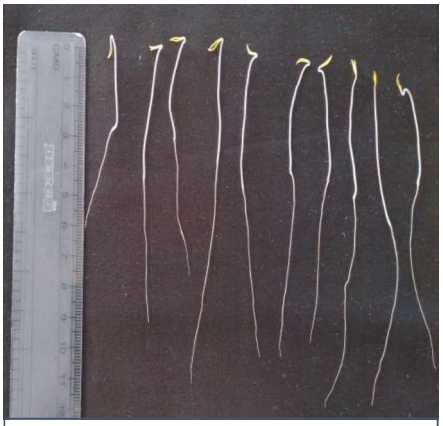
**Table 7: Standardization of effective concentration of biocontrol agents for seed biopriming in capsicum**

| S. No. | Treatments (Seed Biopriming) | Seed germination (%) | Seedling length (cm) | Seedling dry weight (mg) | Seedling Vigour Index-L | Seedling Vigour Index-M |
|---|---|---|---|---|---|---|
| 1. | *Trichoderma harzianum* ($10^6$ cfu per ml) | 65.00 (53.72) | 11.05 | 17.30 | 719.10 | 1123.00 |
| 2. | *Trichoderma harzianum* ($10^7$ cfu per ml) | 66.33 (54.52) | 11.20 | 24.97 | 743.38 | 1656.67 |
| 3. | *Trichoderma harzianum* ($10^8$ cfu per ml) | 75.00 (60.21) | 10.60 | 19.17 | 794.70 | 1437.50 |
| 4. | *Trichoderma atroviride* ($10^6$ cfu per ml) | 63.00 (52.52) | 10.77 | 22.03 | 678.95 | 1388.90 |
| 5. | *Trichoderma atroviride* ($10^7$ cfu per ml) | 63.33 (52.72) | 14.11 | 14.02 | 894.05 | 888.13 |
| 6. | *Trichoderma atroviride* ($10^8$ cfu per ml) | 65.00 (53.71) | 10.61 | 23.70 | 689.92 | 1539.97 |
| 7. | *Trichoderma virens* ($10^6$ cfu per ml) | 63.67 (52.91) | 12.10 | 22.38 | 770.08 | 1423.77 |
| 8. | *Trichoderma virens* ($10^7$cfu per ml) | 67.33 (55.13) | 12.04 | 21.10 | 810.72 | 1419.13 |
| 9. | *Trichoderma virens* ($10^8$ cfu per ml) | 73.00 (58.69) | 11.41 | 23.40 | 833.67 | 1705.90 |
| 10. | *Pseudomonas fluorescens* ($10^6$ cfu per ml) | 63.00 (52.52) | 10.50 | 22.31 | 661.31 | 1406.45 |
| 11. | *Pseudomonas fluorescens* ($10^7$cfu per ml) | 57.33 (49.19) | 9.62 | 27.98 | 551.43 | 1604.03 |
| 12. | *Pseudomonas fluorescens* ($10^8$ cfu per ml) | 66.67 (54.72) | 13.40 | 34.83 | 893.17 | 2321.00 |
| 13. | *Bacillus licheniformis* ($10^6$ cfu per ml) | 57.33 (49.19) | 12.48 | 24.68 | 715.20 | 1416.12 |
| 14. | *Bacillus licheniformis* ($10^7$cfu per ml) | 54.67 (47.66) | 10.73 | 21.67 | 586.41 | 1185.50 |
| 15. | *Bacillus licheniformis* ($10^8$ cfu per ml) | 57.67 (49.39) | 11.06 | 23.50 | 638.20 | 1355.00 |
| 16. | Hydropriming | 56.67 (48.81) | 12.51 | 31.33 | 709.41 | 1775.00 |
| 17. | Control | 53.00 (46.40) | 10.63 | 23.33 | 561.80 | 1325.00 |
| | **CD $_{(0.05)}$** | **1.78** | **0.68** | **2.43** | **52.06** | **152.38** |

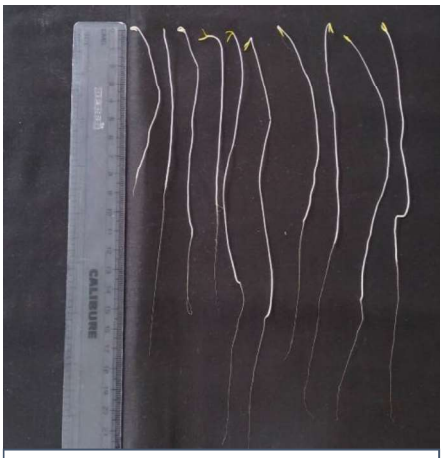
Figures in the parenthesis are arc sine transformed values



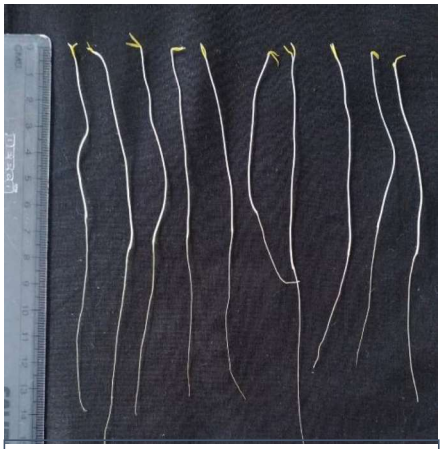
***Trichoderma harzianum***
**($10^7$ cfu per ml ) 8 hrs**



***Trichoderma virens***
**($10^7$ cfu per ml ) 8 hrs**



***Trichoderma atroviride***
**($10^7$ cfu per ml ) 8 hrs**

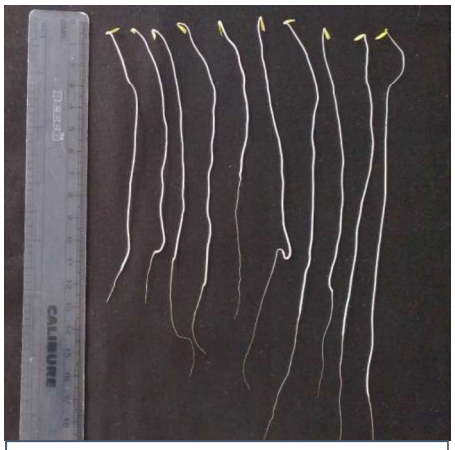


***Pseudomonas fluorescens***
**($10^8$ cfu per ml ) 8 hrs**

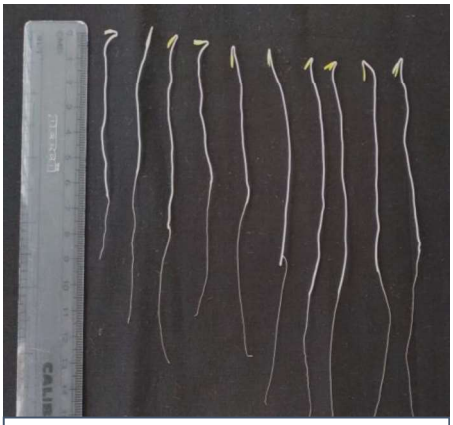
***Bacillus licheniformis***
**($10^8$ cfu per ml ) 8 hrs**

**Plate 11: Standardization of effective concentration of biocontrol agents and duration for seed biopriming in chilli**

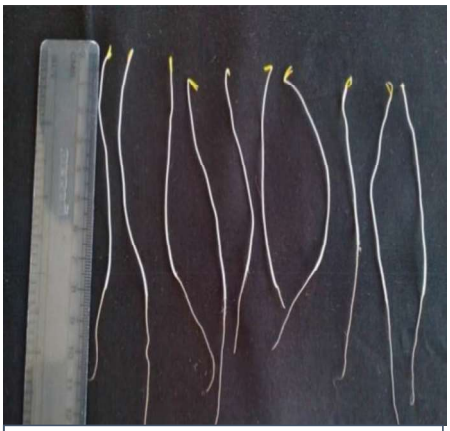
*Trichoderma harzianum*
**($10^7$ cfu per ml ) 8 hrs**



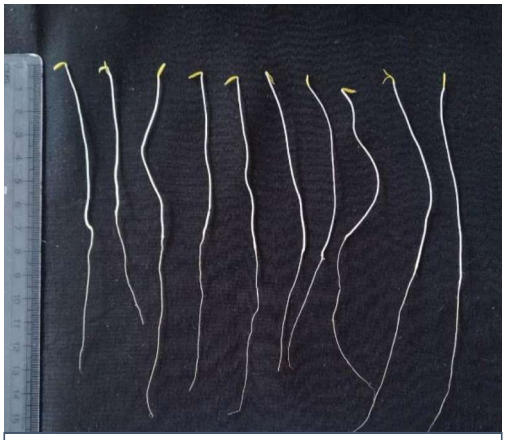
*Trichoderma virens*
**($10^7$ cfu per ml ) 8 hrs**



*Trichoderma atroviride*
**($10^7$ cfu per ml ) 8 hrs**



*Pseudomonas fluorescens*
**($10^8$ cfu per ml ) 8 hrs**



*Bacillus licheniformis*
**($10^8$ cfu per ml ) 8 hrs**

**Plate 12: Standardization of effective concentration of biocontrol agents and duration for seed biopriming in capsicum**

Seedling dry weight was found maximum (34.83 mg) in seed biopriming treatment with $10^8$ cfu per ml of *Pseudomonas fluorescens*. SVI-L was found maximum (894.05) in seeds bio-primed with *Trichoderma atroviride* at $10^7$ cfu per ml followed by *Pseudomonas fluorescens* with $10^8$ cfu per ml concentration and *Trichoderma virens* at $10^8$ cfu per ml with values of 893.17 and 833.67, respectively. SVI-M was found maximum (2321) in biopriming treatment at $10^8$ cfu per ml concentration of *Pseudomonas fluorescens*.

Singh et al. (2020b), reported that among the different concentrations of priming solution of antagonists tested for seed biopriming in tomato, spore dose of $1x10^7$ cfu per ml of *Trichoderma asperellum* was found most effective with maximum seed germination of 91.0 per cent. In case of bacterial antagonist *Ochrobactrum* sp. spore concentration of $1x10^8$ cfu per ml was reported most effective with germination per cent of 98.33. Ananthi et al. (2014) reported that biopriming of chilli seeds with *Trichoderma viride* and *Pseudomonas fluorescens* at 60 per cent w/v concentration resulted in 90-90.2 per cent germination and vigour index of 1273 and 1361, respectively. Raj and Sundareswaran (2016) reported that seed biopriming treatment with *Pseudomonas fluorescens* Pf 1 @ 8 per cent concentration resulted in 14.0, 13.0 and 55.0 per cent increase in dry matter production and vigour index, respectively in tomato cv. PKM1 in comparison to control. Similar results were obtained in studies carried out by other research workers (Amjad et al. 2007; Srivastava et al. 2010; Mona et al. 2017).

**Standardization of duration of seed biopriming with BCAs**

The experiment was conducted for optimization of duration out of 6, 8 and 12 hrs treatment of seed biopriming with the suspensions at concentration of $10^7$ cfu per ml and $10^8$ cfu per ml, respectively for fungal and bacterial biocontrol agents in tomato, chilli and at concentration of $10^8$ cfu per ml for fungal and bacterial biocontrol agents in capsicum. Paper roll towel method was used for optimization of duration for seed biopriming of tomato, chilli and capsicum seeds. Seed biopriming with biocontrol agents for 8 hrs duration was found most effective to other duration of 6 hrs and 12 hrs. Seed biopriming for 8 hrs with *Trichoderma virens* resulted in 93.0 per cent seed germination which is 44.09 per cent higher than control followed by *Trichoderma harzianum* and *Trichoderma atroviride* with germination of 89.33 and 77.67 per cent, respectively (Table 8). However, seed biopriming of tomato seeds with *Bacillus licheniformis* was found least effective for 8 hrs duration with germination of 56.0 per cent (Table 8). Seedling length was found maximum (17.34 cm) in

case of seed biopriming with *Trichoderma atroviride* for 8 hrs duration followed by *Trichoderma virens* and *Trichoderma harzianum* with seedling length of 16.55 and 16.43 cm, respectively (Plate 10).

Seed biopriming with *Pseudomonas fluorescens* for 8 hrs was found least effective with respect to its effect on seedling length (11.33 cm) in comparison to hydropriming treatment and control. Seed biopriming with *Trichoderma atroviride* for 8 hrs was found most effective with respect to seedling weight of 53.6 mg in case of tomato seedlings. Biopriming with *Bacillus licheniformis* for 8 hrs was found next in efficacy with seedling weight of 51.33 mg followed by *Trichoderma virens* at same duration of biopriming with seedling weight of 44.17 mg (Table 9). Seed biopriming with *Trichoderma harzianum* for 8 hrs also resulted in maximum SVI-L (1472.47) and SVI-M (4558.33).

**Table 8: Effect of duration of seed biopriming with biocontrol agents on seed germination and seedling length in tomato**

| S. No. | Treatments (Seed Biopriming) | Seed germination (%) | | | | Seedling length (cm) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 6h | 8h | 12h | Mean | 6h | 8h | 12h | Mean |
| **1.** | *Trichoderma harzianum* | 62.67 (52.32) | 89.33 (71.08) | 82.33 (65.16) | **78.11 (62.85)** | 16.16 | 16.43 | 14.44 | **15.68** |
| **2.** | *Trichoderma atroviride* | 56.00 (48.43) | 77.67 (61.79) | 53.67 (47.08) | **62.44 (52.44)** | 16.15 | 17.34 | 11.31 | **14.93** |
| **3.** | *Trichoderma virens* | 53.33 (46.89) | 93.00 (74.79) | 84.33 (66.72) | **76.89 (62.80)** | 15.07 | 16.55 | 16.07 | **15.89** |
| **4.** | *Pseudomonas fluorescens* | 52.67 (46.51) | 57.33 (49.20) | 55.00 (47.85) | **55.00 (47.86)** | 16.40 | 11.33 | 17.29 | **15.01** |
| **5.** | *Bacillus licheniformis* | 55.00 (47.85) | 56.00 (48.43) | 36.67 (37.25) | **49.22 (44.51)** | 15.99 | 12.96 | 11.37 | **13.44** |
| **6.** | Hydropriming | 63.67 (52.91) | 62.67 (52.36) | 66.00 (54.32) | **64.11 (53.19)** | 11.73 | 12.46 | 12.48 | **12.22** |
| **7.** | Control | 52.00 (46.13) | 52.00 (46.13) | 52.00 (46.13) | **52.00 (46.13)** | 8.71 | 8.71 | 8.71 | **8.71** |
| | **Mean** | **56.48 (48.72)** | **69.71 (57.68)** | **61.43 (52.07)** | | **14.32** | **13.68** | **13.09** | |
| | **$CD_{(0.05)}$**<br>**Treatment (T)**<br>**Duration (D)**<br>**T X D** | **1.74**<br>**1.14**<br>**3.01** | | | | **0.64**<br>**0.42**<br>**1.11** | | | |

Figures in the parenthesis are arc sine transformed values

**Table 9: Effect of duration of seed biopriming with biocontrol agents on seedling dry weight and seedling vigour in tomato**

| S. No. | Treatments (Seed Biopriming) | Seedling dry weight (mg) | | | | Seedling vigour Index-L | | | | Seedling vigour Index-M | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 6h | 8h | 12h | Mean | 6h | 8h | 12h | Mean | 6h | 8h | 12h | Mean |
| **1.** | *Trichoderma harzianum* | 15.23 | 51.00 | 14.70 | **26.98** | 1012.26 | 1472.47 | 1188.97 | **1224.56** | 954.90$^{c}$ | 4558.33$^{a}$ | 1210.57$^{b}$ | **2241.28** |
| **2.** | *Trichoderma atroviride* | 35.37 | 53.60 | 15.20 | **34.72** | 904.50 | 1346.74 | 607.61 | **952.95** | 1978.63$^{b}$ | 4165.20$^{b}$ | 816.30$^{c}$ | **2320.04** |
| **3.** | *Trichoderma virens* | 15.07 | 44.17 | 21.97 | **27.07** | 803.97 | 1539.55 | 1355.09 | **1232.87** | 804.10$^{c}$ | 4106.00$^{b}$ | 1854.37$^{a}$ | **2254.81** |
| **4.** | *Pseudomonas fluorescens* | 41.67 | 34.67 | 24.27 | **33.53** | 863.86 | 649.92 | 952.25 | **822.01** | 2193.17$^{a}$ | 1987.83$^{d}$ | 1334.37$^{b}$ | **1838.46** |
| **5.** | *Bacillus licheniformis* | 32.33 | 51.33 | 16.28 | **33.31** | 880.44 | 726.71 | 417.18 | **674.78** | 1777.33$^{b}$ | 2875.83$^{c}$ | 599.96$^{cd}$ | **1751.04** |
| **6.** | Hydropriming | 12.83 | 12.00 | 11.50 | **12.11** | 746.52 | 785.25 | 823.21 | **784.99** | 815.83$^{c}$ | 750.33$^{e}$ | 758.33$^{cd}$ | **774.83** |
| **7.** | Control | 10.00 | 10.00 | 10.00 | **10.00** | 452.92 | 452.92 | 452.92 | **452.92** | 520.00$^{d}$ | 520.00$^{e}$ | 520.00$^{d}$ | **520.00** |
| | **Mean** | **23.21** | **36.68** | **16.27** | | **809.21** | **996.22** | **828.18** | | **1291.99** | **2709.08** | **1013.41** | |
| | **CD $_{(0.05)}$**<br>Treatment (T)<br>Duration (D)<br>T X D | | **1.31**<br>**0.86**<br>**2.27** | | | | **71.49**<br>**46.80**<br>**123.82** | | | | **372.62**<br>**243.93**<br>**645.39** | | |

Seed biopriming of chilli with biocontrol agents at 8 hrs duration was found most effective in comparison to 6 and 12 hrs. Among the different treatments of seed biopriming evaluated at 8 hrs, maximum seed germination was found in *Trichoderma harzianum* (90.67 %) which was 25.0 per cent higher than control Seed biopriming with *Bacillus licheniformis* and *Trichoderma virens* with germination of 88.33 and 87.33 per cent, respectively were found next in efficacy and both were found statistically at par (Table 10, Plate 11).

**Table 10: Effect of duration of seed biopriming with biocontrol agents on seed germination and seedling length in chilli**

| S. No. | Treatments (Seed Biopriming) | Seed germination (%) | | | | Seedling length (cm) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 6h | 8h | 12h | Mean | 6h | 8h | 12h | Mean |
| **1.** | *Trichoderma harzianum* | 84.33 (66.68) | 90.67 (72.59) | 56.67 (48.81) | **77.22 (62.69)** | 13.19 | 8.23 | 11.64 | **11.03** |
| **2.** | *Trichoderma atroviride* | 51.00 (45.56) | 82.33 (65.16) | 89.67 (71.61) | **74.33 (60.77)** | 9.34 | 10.48 | 8.61 | **9.48** |
| **3.** | *Trichoderma virens* | 74.67 (59.84) | 87.33 (69.18) | 85.00 (67.25) | **82.33 (65.43)** | 9.64 | 11.05 | 9.25 | **9.97** |
| **4.** | *Pseudomonas fluorescens* | 68.67 (56.02) | 89.00 (71.02) | 83.67 (66.15) | **80.44 (64.40)** | 11.96 | 17.56 | 12.84 | **14.12** |
| **5.** | *Bacillus licheniformis* | 64.67 (53.53) | 88.33 (70.66) | 87.00 (68.96) | **80.00 (64.38)** | 11.67 | 11.78 | 12.34 | **11.93** |
| **6.** | Hydropriming | 67.67 (55.33) | 73.00 (58.67) | 67.33 (55.12) | **69.33 (56.38)** | 9.73 | 11.16 | 9.89 | **10.26** |
| **7.** | Control | 68.00 (55.53) | 68.00 (55.53) | 68.00 (55.53) | **68.00 (55.53)** | 10.06 | 10.06 | 10.06 | **10.06** |
| | **Mean** | **68.43 (56.07)** | **82.67 (66.11)** | **76.76 (61.92)** | | **10.80** | **11.48** | **10.66** | |
| | **$CD_{(0.05)}$ Treatment (T)** | **2.99** | | | | **0.99** | | | |
| | **Duration (D)** | **1.96** | | | | **0.65** | | | |
| | **T X D** | **5.19** | | | | **1.73** | | | |

Figures in the parenthesis are arc sine transformed values

Priming of seeds with water (hydropriming) for 8 hrs was found least effective with germination of 73 per cent in comparison to other biopriming treatments for 8 hrs duration. Seedling length was found maximum (17.56 cm) with 8 hrs of seed biopriming with *Pseudomonas fluorescens* followed by *Bacillus licheniformis* and hydropriming treatment for 8 hrs with seedling length of 11.78 and 11.16 cm, respectively. Seed biopriming with *Trichoderma harzianum* for 8 hrs was found least effective with seedling length of 8.23 cm in comparison to control. Seed biopriming for 8 hrs with *Trichoderma virens* was found most effective with seedling weight of 81.0 mg in chilli seedlings (Table 11).

**Table 11: Effect of duration of seed biopriming with biocontrol agents on seedling dry weight and seedling vigour in chilli**

| S. No. | Treatments (Seed Biopriming) | Seedling dry weight (mg) | | | | Seedling vigour Index-L | | | | Seedling vigour Index-M | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 6h | 8h | 12h | Mean | 6h | 8h | 12h | Mean | 6h | 8h | 12h | Mean |
| **1.** | *Trichoderma harzianum* | 64.67 | 72.33 | 55.67 | **64.22** | 1111.57 | 777.22 | 660.42 | **849.74** | 5451.33 | 6560.67 | 3152.00 | **5054.67** |
| **2.** | *Trichoderma atroviride* | 47.67 | 51.67 | 67.33 | **55.56** | 475.69 | 862.08 | 737.41 | **691.73** | 2441.00 | 4263.67 | 6048.00 | **4250.89** |
| **3.** | *Trichoderma virens* | 34.67 | 81.00 | 62.00 | **59.22** | 720.66 | 964.20 | 787.25 | **824.04** | 2601.33 | 7060.00 | 5271.00 | **4977.44** |
| **4.** | *Pseudomonas fluorescens* | 39.00 | 68.00 | 38.00 | **48.33** | 822.01 | 1469.27 | 1146.28 | **1145.85** | 2677.33 | 5683.67 | 3382.67 | **3914.56** |
| **5.** | *Bacillus licheniformis* | 45.67 | 55.33 | 45.33 | **48.78** | 751.73 | 1043.33 | 1076.12 | **957.06** | 2971.00 | 4875.00 | 3939.33 | **3928.44** |
| **6.** | Hydropriming | 32.33 | 44.67 | 41.33 | **39.44** | 657.85 | 814.70 | 665.93 | **712.83** | 2190.33 | 3259.00 | 2780.00 | **2743.11** |
| **7.** | Control | 15.00 | 15.00 | 15.00 | **15.00** | 684.08 | 684.08 | 684.08 | **684.08** | 1020.00 | 1020.00 | 1020.00 | **1020.00** |
| | **Mean** | **39.86** | **55.43** | **46.38** | | **746.23** | **944.58** | **843.75** | | **2764.62** | **4574.57** | **3656.14** | |
| | **CD $_{(0.05)}$**<br>Treatment (T)<br>Duration (D)<br>T X D | | | **5.18**<br>**3.39**<br>**8.97** | | | | **96.28**<br>**63.03**<br>**166.75** | | | | **461.16**<br>**301.90**<br>**798.76** | |

Biopriming treatment with *Trichoderma harzianum* for 8 hrs was found next in efficacy with seedling weight of 72.33 mg followed by biopriming with *Pseudomonas fluorescens* for 8 hrs with seedling weight of 68.0 mg. Seedling vigour index-I was found maximum (1469.27) in seed biopriming with *Pseudomonas fluorescens* for 8 hrs. Seedling vigour index-II was found maximum (6560.67) in seed biopriming treatment with *Trichoderma harzianum* for 8 hrs (Table 11).

Among the different treatments of seed biopriming of capsicum evaluated at 8 hrs, maximum seed germination was found in *Trichoderma harzianum* (83.67 %) which was 36.66 per cent higher than control followed by *Trichoderma virens* and *Trichoderma atroviride* with germination of 69.67 and 66.33 per cent, respectively (Table 12, Plate 12). Hydropriming for 8 hrs was found least effective with germination of 58.33 per cent in comparison to control. Seedling length was found maximum (12.65 cm) in case of seed biopriming for 8 hrs with *Trichoderma harzianum* followed by *Trichoderma virens* and hydropriming with seedling length of 11.34 and 11.31 cm, respectively and both were found statistically at par.

**Table 12: Effect of duration of seed biopriming with biocontrol agents on seed germination and seedling length in capsicum**

| S. No. | Treatments (Seed Biopriming) | Seed germination (%) | | | | Seedling length (cm) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 6h | 8h | 12h | Mean | 6h | 8h | 12h | Mean |
| **1.** | *Trichoderma harzianum* | 72.33 (58.25) | 83.67 (66.15) | 63.33 (52.71) | **73.11 (59.04)** | 11.12 | 11.31 | 10.90 | **11.11** |
| **2.** | *Trichoderma atroviride* | 62.67 (52.32) | 66.33 (54.52) | 64.00 (53.11) | **64.33 (53.32)** | 9.56 | 10.64 | 8.43 | **9.54** |
| **3.** | *Trichoderma virens* | 65.67 (54.12) | 69.67 (56.57) | 61.00 (51.36) | **65.44 (54.01)** | 9.64 | 11.34 | 11.53 | **10.84** |
| **4.** | *Pseudomonas fluorescens* | 57.00 (49.01) | 58.33 (49.78) | 56.00 (48.43) | **57.11 (49.07)** | 9.47 | 8.28 | 10.85 | **9.53** |
| **5.** | *Bacillus licheniformis* | 60.00 (50.75) | 62.67 (52.32) | 59.33 (50.36) | **60.67 (51.14)** | 9.65 | 10.66 | 9.62 | **9.98** |
| **6.** | Hydropriming | 55.67 (48.24) | 56.33 (48.62) | 53.67 (47.08) | **55.22 (47.98)** | 10.44 | 12.65 | 10.54 | **11.21** |
| **7.** | Control | 53.00 (46.70) | 53.00 (46.70) | 53.00 (46.70) | **53.00 (46.70)** | 10.50 | 10.50 | 10.50 | **10.50** |
| | **Mean** | **60.91 (51.34)** | **64.29 (53.52)** | **58.62 (49.96)** | | **10.06** | **10.77** | **10.34** | |
| | **CD $_{(0.05)}$** | | | | | | | | |
| | **Treatment (T)** | **0.85** | | | | **0.61** | | | |
| | **Duration (D)** | **0.59** | | | | **0.39** | | | |
| | **T X D** | **1.48** | | | | **1.05** | | | |

Figures in the parenthesis are arc sine transformed values

Seed biopriming with *Pseudomonas fluorescens* for 8 hrs was found least effective with seedling length of 8.28 cm in comparison to hydropriming treatment and control. Seed biopriming with *Pseudomonas fluorescens* for 8 hrs was found most effective with seedling weight of 45.07 mg in capsicum seedlings. Further, *Trichoderma* was found next in efficacy with seedling weight of 45 mg followed by *Trichoderma virens* with seedling weight of 44.93 mg when capsicum seeds were bio-primed for 8 hrs. SVI-L had maximum value in 8 hrs seed biopriming with *Trichoderma harzianum* with value of 946.93 and SVI-M was found maximum in 12 hrs seed biopriming with *Trichoderma virens* with value of 4674.8 (Table 13).

Ananthi et al. (2014) also reported that among different durations of seed biopriming evaluated, biopriming with *Trichoderma viride* at 60 per cent v/v concentration for 3 hrs and with *Pseudomonas fluorescens* at similar concentration for 12 hrs was most significant with germination of 93.0 and 95.0 per cent, respectively. Similar findings have been reported in studies carried out by other research workers where biopriming seed treatments with *Trichoderma harzianum* and *Bacillus subtilis* improved seed germination of okra, tomato and chilli seeds (El-Mohamedy 2004; Mariselvam et al. 2012; Dhanalaksmi 2013). Jaiman et al. (2020) reported that out of the three biopriming durations of tomato and chilli seeds, *Trichoderma harzianum* for 12 hrs was found most effective with germination per cent of 92.92, 90.77, seedling length of 32.38 cm, 29.35 cm and seedling vigour index of 3008.11, 2664.0 in tomato and chilli, respectively.

### 3. Seed biopriming with effective biocontrol agents and PGPR bio-formulations under field conditions

Seed biopriming treatments of fungal biocontrol agents (*Trichoderma harzianum*, *T. virens*, *T. atroviride*) at effective concentration of $10^7$ cfu per ml (tomato and chilli) and $10^8$ cfu per ml (capsicum) and duration of 8 hrs under *in vitro* experiments were then applied under field conditions. Seeds of tomato, chilli and capsicum were bioprimed with the suspensions of bacterial biocontrol agents (*Pseudomonas fluorescens*, *Bacillus licheniformis)* and PGPR bioformulations (*Azospirillum*, *Azotobacter*, *Pseudomonas aeruginosa*) at a concentration of $10^8$ cfu per ml for 8 hrs and then, these bioprimed seeds sown in nursery beds to evaluate their efficacy in managing the damping-off disease. Among all the treatments, seed biopriming with *Trichoderma harzianum* and *Trichoderma virens* was found most effective against the damping-off disease in tomato, chilli and capsicum (Table 14, Plate 13).

Biopriming of tomato seeds with *Trichoderma virens* was found most effective against damping-off disease with 58.29 per cent reduction in seedling mortality followed by treatment of *Trichoderma harzianum* with reduction of 57.87 per cent in comparison to control. *Trichoderma atroviride* was found next in efficacy with seedling mortality of 21.48 per cent. Seed biopriming with *Trichoderma harzianum* was found most effective with respect to seed germination of 76.79 per cent followed by *Trichoderma virens* and *Trichoderma atroviride* with germination of 67.71 and 57.46 per cent, respectively. Seed biopriming with *Pseudomonas fluorescens* and *Trichoderma harzianum* were found most effective with respect to its effect on average shoot length (18.66 cm) and root length (8.09 cm), respectively. Seed biopriming with *Azotobacter* was found least effective with only 10.39 cm average shoot length and 4.81 cm root length of tomato seedlings.

In chilli, seed biopriming with *Trichoderma virens* was found most effective in the management of damping-off disease with 54.71 per cent reduction in seedling mortality followed by treatment with *Trichoderma harzianum* with 46.16 per cent reduction in seedling mortality in comparison to control (Table 14, Plate 13). Seed biopriming with *Trichoderma harzianum* was found most effective with seed germination of 72.41 per cent followed by treatment of *Trichoderma atroviride* and *Trichoderma virens* with seed germination of 64.30 and 62.12 per cent, respectively.

Among all the biopriming treatments, seed biopriming with *Azotobacter* was found least effective with seed germination of 52.39 per cent in comparison to control. Seed biopriming with *Trichoderma harzianum* was found most effective in chilli seedlings with average shoot length and root length of 13.29 cm and 8.36 cm, respectively. Seed biopriming with *Trichoderma virens* was found next in efficacy with shoot length of 10.57 cm and root length of 9.31 cm. Seed biopriming with *Azotobacter* and *Pseudomonas aeruginosa* was found least effective in chilli seedlings with average shoot and root length of 8.38 cm and 4.46 cm, respectively in comparison to control.

Seed biopriming with *Trichoderma atroviride* was found effective in capsicum in the management of damping-off disease with 55.51 per cent reduction in seedling mortality followed by *Trichoderma virens* and *Trichoderma harzianum* with 49.34, 44.55 per cent reduction in seedling mortality, respectively (Table 14, Plate 13).

**Table 13: Effect of duration of seed biopriming with biocontrol agents on seedling dry weight and seedling vigour in capsicum**

| S. No. | Treatments (Seed Biopriming) | Seedling dry weight (mg) | | | | Seedling vigour Index-L | | | | Seedling vigour Index-M | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 6h | 8h | 12h | Mean | 6h | 8h | 12h | Mean | 6h | 8h | 12h | Mean |
| **1.** | *Trichoderma harzianum* | 35.47 | 45.00 | 55.27 | **45.24** | 803.01 | 946.93 | 691.02 | **813.66** | 2566.00 | 3763.83 | 3499.67 | **3276.50** |
| **2.** | *Trichoderma atroviride* | 65.17 | 44.17 | 74.67 | **61.33** | 628.52 | 742.02 | 514.41 | **628.32** | 4276.83 | 3079.00 | 4553.33 | **3969.72** |
| **3.** | *Trichoderma virens* | 63.17 | 44.93 | 73.07 | **60.39** | 603.83 | 752.64 | 737.71 | **698.06** | 3958.50 | 2981.40 | 4674.83 | **3871.58** |
| **4.** | *Pseudomonas fluorescens* | 59.50 | 45.07 | 26.83 | **43.80** | 539.87 | 482.93 | 607.18 | **543.33** | 3393.00 | 2629.00 | 1502.33 | **2508.11** |
| **5.** | *Bacillus licheniformis* | 44.67 | 42.77 | 25.03 | **37.49** | 579.07 | 669.15 | 571.43 | **606.55** | 2679.83 | 2678.93 | 1486.07 | **2281.61** |
| **6.** | Hydropriming | 30.80 | 33.33 | 31.17 | **31.77** | 581.23 | 713.02 | 566.82 | **620.35** | 1714.93 | 1879.00 | 1667.83 | **1753.92** |
| **7.** | Control | 22.00 | 22.00 | 22.00 | **22.00** | 556.50 | 556.50 | 556.50 | **556.50** | 1166.00 | 1166.00 | 1166.00 | **1166.00** |
| | **Mean** | **45.82** | **39.61** | **44.01** | | **613.15** | **694.74** | **606.44** | | **2822.16** | **2596.74** | **2650.01** | |
| | **CD $_{(0.05)}$**<br>Treatment (T)<br>Duration (D)<br>T X D | | | **2.08**<br>**1.36**<br>**3.61** | | | | **43.99**<br>**28.80**<br>**76.21** | | | | **133.52**<br>**87.41**<br>**231.26** | |

**Table 14: Effect of seed biopriming with effective biocontrol agents and bioformulations on damping-off and plant growth in tomato, chilli and capsicum under field conditions**

| S. No. | Treatments (Seed biopriming) | Tomato | | | | Chilli | | | | Capsicum | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Disease incidence (%) | Seed germination (%) | Shoot length (cm) | Root length (cm) | Disease incidence (%) | Seed germination (%) | Shoot length (cm) | Root length (cm) | Disease incidence (%) | Seed germination (%) | Shoot length (cm) | Root length (cm) |
| 1. | *Trichoderma harzianum* | 16.15 (23.66) | 76.79 (61.19) | 16.37 | 8.09 | 23.99 (29.28) | 72.41 (58.32) | 13.29 | 8.36 | 17.26 (24.55) | 74.73 (59.84) | 14.13 | 8.53 |
| 2. | *Trichoderma virens* | 15.99 (23.55) | 67.71 (55.36) | 18.21 | 6.15 | 20.18 (26.68) | 62.12 (52.01) | 10.57 | 9.31 | 15.77 (23.38) | 60.69 (51.23) | 13.97 | 8.14 |
| 3. | *Trichoderma atroviride* | 21.48 (27.61) | 57.46 (49.27) | 14.63 | 6.39 | 24.76 (29.77) | 64.30 (53.33) | 12.43 | 6.34 | 13.85 (22.04) | 50.42 (45.22) | 12.12 | 7.04 |
| 4. | *Bacillus licheniformis* | 28.73 (32.41) | 55.72 (48.27) | 15.63 | 6.42 | 32.91 (34.99) | 62.01 (51.95) | 8.64 | 6.05 | 22.92 (28.59) | 46.95 (43.23) | 11.95 | 6.55 |
| 5. | *Pseudomonas fluorescens* | 21.88 (27.89) | 42.76 (40.82) | 18.66 | 5.79 | 35.35 (36.48) | 52.79 (46.59) | 9.14 | 4.84 | 22.57 (27.67) | 54.53 (47.58) | 11.59 | 5.39 |
| 6. | *Pseudomonas aeruginosa* | 25.58 (30.38) | 42.89 (45.57) | 17.58 | 5.35 | 39.16 (38.74) | 53.73 (47.14) | 9.50 | 4.46 | 24.34 (29.56) | 54.95 (47.83) | 10.21 | 6.48 |
| 7. | *Azospirillum* | 23.55 (29.02) | 51.02 (45.57) | 14.77 | 5.66 | 39.87 (39.16) | 56.55 (48.77) | 9.98 | 5.97 | 29.88 (33.14) | 61.34 (51.53) | 10.13 | 5.59 |
| 8. | *Azotobacter* | 33.14 (35.17) | 47.14 (43.34) | 13.35 | 4.93 | 41.49 (40.10) | 52.39 (46.37) | 8.38 | 4.83 | 27.29 (31.49) | 61.18 (51.44) | 9.01 | 5.23 |
| 9. | Control | 38.34 (38.26) | 40.39 (39.46) | 10.39 | 4.81 | 44.56 (41.88) | 49.98 (44.99) | 8.03 | 4.16 | 31.13 (33.91) | 41.42 (40.04) | 7.58 | 4.53 |
| | **$CD_{(0.05)}$** | **1.42** | **1.75** | **0.69** | **0.72** | **2.29** | **2.23** | **1.64** | **1.40** | **1.14** | **2.78** | **1.05** | **1.03** |

Figures in the parenthesis are arc sine transformed values

Seed biopriming with *Trichoderma harzianum*

Seed biopriming with *Trichoderma virens*

Seed biopriming with *Trichoderma atroviride*

Control

Seed biopriming with *Trichoderma virens* in chilli

Seed biopriming with *Trichoderma virens* in capsicum

**Plate 13: Seed biopriming with effective biocontrol agents under field conditions**

Seed biopriming with *Azospirillum* was found least effective with seedling mortality of 29.88 per cent in comparison to control. Seed biopriming with *Trichoderma harzianum* was found most effective in enhancing the seed germination to 74.73 per cent followed by treatment with *Trichoderma virens* and *Trichoderma atroviride* with seed germination of 60.69 and 50.42 per cent, respectively. Among all the biopriming treatments, seed biopriming with *Azotobacter* was found least effective with respect to seed germination of 61.18 per cent in comparison to control. Seed biopriming with *Trichoderma harzianum* was also found most effective in capsicum seedlings with shoot and root length of 14.13 cm and 8.53 cm in capsicum seedlings, respectively. Seed biopriming with *Trichoderma virens* was found next in efficacy with shoot length of 13.97 cm followed by *Trichoderma atroviride* with shoot length of 12.12 cm. Seed biopriming with *Azotobacter* was found least effective with root length of 5.23 cm in capsicum in comparison to control.

Nirmalkar et al. (2018) reported that *Trichoderma harzianum* when applied as seed treatment resulted in seed germination of 80.32 per cent and this treatment significantly increased the growth attributes of tomato. Zagade et al. (2012) reported that *Trichoderma* sp. significantly increased seed germination and effectively reduced seedling rotting in chilli. Idowu et al. (2016) reported that seeds of sweet pepper treated with *Trichoderma atroviride* and *Trichoderma harzianum* had higher germination in comparison to untreated seedlings. Similar findings of increased growth rate of seedlings in nurseries due to treatment with *Trichoderma* sp. have been reported by Champawat and Sharma (2003); Srivastava (2004); Shabir and Rubina (2010) and Jena (2012).

Singh et al. (2020b) reported that seed biopriming of tomato seeds with *Trichoderma asperellum* and *Ochrobactrum* sp. at $10^7$ cfu per ml and $10^8$ cfu per ml for 24 hrs resulted in 2.31 times higher seed germination, 0.88 times higher shoot length and 1.41 times higher root length in comparison to control. They reported that seed biopriming with *Trichoderma asperellum* at 40 per cent v/v concentration and *Ochrobactrum* sp. at 66 per cent concentration resulted in higher reduction in disease incidence against *Fusarium oxysporum* in tomato.

### 4. *In vitro* evaluation of plant extracts against damping-off pathogens

Extracts of eleven native plants were evaluated under *in-vitro* conditions against the damping-off pathogens. Different plant extracts at 10 per cent concentration inhibited the

mycelial growth of damping-off pathogens ranging from 2.81 to 55.55 per cent (Table 15). Among the different plant extracts evaluated, extracts of *Roylea elegans* was found most effective with inhibition of 36.78 per cent followed by *Melia azedarach, Datura stramonium* and *Lantana camara* with mycelial inhibition of 36.38, 30.78 and 23.81 per cent, respectively (Plate 14, Plate 15). *Roylea elegans* extract was found most effective with 23.89 per cent inhibition of *Pythium* sp. followed by *Melia azedarach* and *Datura stramonium* with mycelial inhibition of 15.85 and 14.11 per cent, respectively (Plate 14a, Plate 14b). Extract of *Tagetes* sp. was found least effective with mycelial inhibition of 3.29 per cent. *Melia azedarach* extract was found most effective against *Fusarium oxysporum* with inhibition of 55.55 per cent followed by *Datura stramonium* with inhibition of 52.71 per cent. *Thymus vulgaris* extract was found next in efficacy with mycelial inhibition of 48.02 per cent.

**Table 15: *In vitro* efficacy of plant extracts against damping-off pathogens**

| S. No. | Treatments @ 10% concentration | Mycelial inhibition (%) | | | | |
|---|---|---|---|---|---|---|
| | | ***Pythium* sp.** | ***Fusarium oxysporum*** | ***Sclerotium rolfsii*** | ***Rhizoctonia solani*** | **Mean** |
| **1.** | *Aloe barbadensis* (Aloe vera) | 2.81 (9.64) | 15.63 (23.27) | 5.67 (13.77) | 29.29 (32.71) | **13.35 (19.85)** |
| **2.** | *Cymbopogon citratus* (Lemon grass) | 5.16 (13.12) | 25.93 (30.55) | 8.52 (16.96) | 26.15 (30.73) | **16.44 (22.84)** |
| **3.** | *Datura stramonium* (Datura) | 14.11 (21.83) | 52.71 (46.53) | 42.41 (40.61) | 13.89 (21.86) | **30.78 (32.71)** |
| **4.** | *Hibiscus rosa-sinensis* (Hibiscus) | 7.47 (15.79) | 23.92 (29.26) | 13.70 (21.69) | 34.26 (35.79) | **19.84 (25.63)** |
| **5.** | *Thymus vulgaris* (Thyme) | 5.53 (13.59) | 48.02 (43.84) | 17.19 (24.48) | 19.44 (26.13) | **22.55 (27.01)** |
| **6.** | *Melia azedarach* (Darek) | 15.85 (23.45) | 55.55 (48.12) | 33.89 (35.57) | 40.22 (39.34) | **36.38 (36.62)** |
| **7.** | *Tagetes* sp. (Marigold) | 3.29 (10.43) | 15.74 (23.28) | 14.08 (21.96) | 8.22 (16.62) | **10.33 (18.04)** |
| **8.** | *Lantana camara* (Wild sage) | 12.29 (20.49) | 44.63 (41.89) | 13.89 (21.82) | 24.41 (29.56) | **23.81 (28.44)** |
| **9.** | *Parthenium hysterophorus* (Congress grass) | 5.34 (13.35) | 35.00 (36.23) | 15.37 (22.94) | 15.67 (23.02) | **17.85 (23.88)** |
| **10.** | *Ocimum sanctum* (Tulsi) | 5.03 (12.94) | 24.52 (29.64) | 13.62 (21.61) | 30.55 (33.51) | **18.43 (24.43)** |
| **11.** | *Roylea elegans* (Karvya) | 23.89 (29.23) | 44.33 (41.73) | 29.08 (32.53) | 49.81 (44.87) | **36.78 (37.11)** |
| | **Mean** | **9.16 (16.01)** | **35.09 (35.85)** | **18.86 (24.90)** | **26.54 (30.37)** | |
| | **CD $_{(0.05)}$** | **2.56** | **3.03** | **3.33** | **3.89** | |

Figures in the parenthesis are arc sine transformed values

*Pythium sp.*

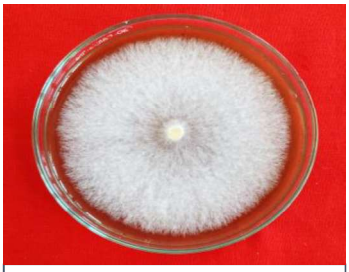
*Fusarium oxysporum*

*Sclerotium rolfsii*

*Rhizoctonia solani*

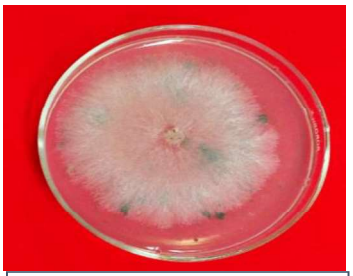
***Datura stramonium* extract**

*Pythium sp.*



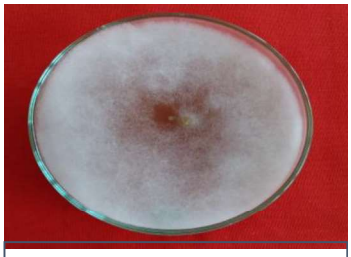
*Fusarium oxysporum*

*Sclerotium rolfsii*



*Rhizoctonia solani.*

***Hibiscus rosa-sinensis* extract**

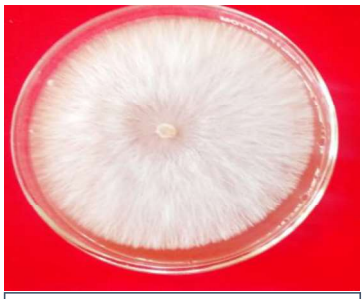


*Pythium sp.*

*Fusarium oxysporum*

***Lantana camara* extract**

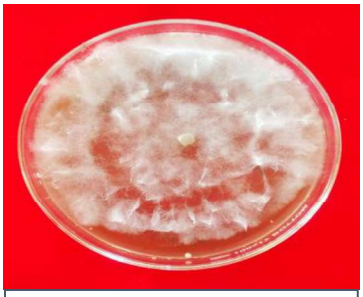
**Plate 14a: *In vitro* evaluation of native plant extracts against damping-off pathogens**



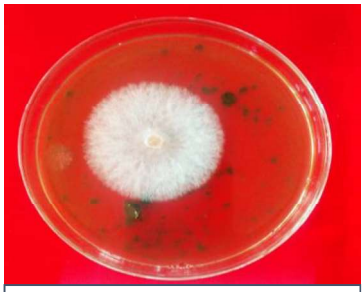
*Sclerotium rolfsii*

*Rhizoctonia solani.*

***Lantana camara* extract**



*Pythium sp.*

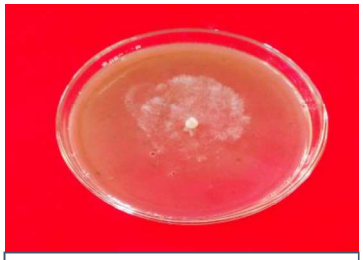


*Fusarium oxysporum*

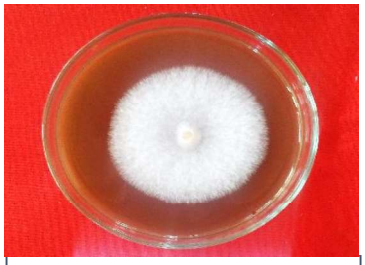
*Sclerotium rolfsii*

*Rhizoctonia solani.*

***Melia azedarach* extract**



*Pythium sp.*



*Fusarium oxysporum*

*Sclerotium rolfsii*

*Rhizoctonia solani.*

***Roylea elegans* extract**

**Plate 14b: *In vitro* evaluation of native plant extracts against damping-off pathogens**

*Datura stramonium* extract was found most effective followed by *Melia azedarach* extract with 42.41 and 33.89 per cent inhibition of mycelial growth of *Sclerotium rolfsii*, respectively. Extracts of *Aloe barbadensis* and *Cymbopogon citratus* were found least effective against *Sclerotium rolfsii* with mycelial inhibition of 5.67 and 8.52 per cent, respectively. *Roylea elegans* extract was found most effective against *Rhizoctonia solani* with per cent mycelial inhibition of 49.81 followed by *Melia azedarach* with mycelial inhibition of 40.22 per cent. *Tagetes* sp. was found least effective against *Rhizoctonia solani* with mycelial inhibition of 8.22 per cent only.

Efficacy of different botanicals used in this study have also been reported against the damping-off pathogens by several workers (Kumar et al. 1996; Rodriguez and Montilla, 2002; Hooda et al. 2011; Pattnaik et al. 2012). However, in contrast to our findings, Gholve et al. (2014) reported the efficacy of extracts of datura and neem in inhibiting mycelial growth of *Pythium ultimum* by 60.65, 31.60, 50.59 per cent, respectively. Siva (2008) reported that extract of tulsi significantly reduced the growth of *Fusarium oxysporum* in *Solanum melongena*. Yadav et al. (2021) reported the efficacy of tulsi extracts at 10 per cent concentration in suppressing the mycelial growth of *Rhizoctonia solani*. Siddiqui et al. (2014) reported that extracts of *Melia azedarach* at 5 per cent concentration showed maximum antifungal activity with 20-70 per cent reduction in fungal biomass of *Rhizoctonia solani*. Hooda et al. (2011) also reported the efficacy of extracts of *Lantana*, *Sapium* sp. and *Urtica parviflora* against damping-off disease in tomato in managing damping-off disease. Sharma and Trivedi (2002) reported 72.0 per cent inhibition of *Fusarium oxysporum* with extracts of *Datura stramonium*. Efficacy of leaf extracts of *Chlorophytum*, *Vinca rosea*, neem leaves against plant pathogens have also been reported (Chakraborty et al. 2014; Tiong et al. 2013, Raphael 2012).

**Standardization of effective concentration of plant extracts for seed biopriming**

Plant extracts which were found most effective in inhibiting the mycelial growth of damping-off pathogens under *in vitro* conditions (*Roylea elegans*, *Melia azedarach*, *Lantana camara*, *Datura stramonium*, *Thymus vulgaris*, *Hibiscus rosa sinensis*) were selected. Comparative efficacy of 5 and 10 per cent concentration of plant extracts was tested for seed biopriming of tomato, chilli and capsicum by using rolled paper towel method.

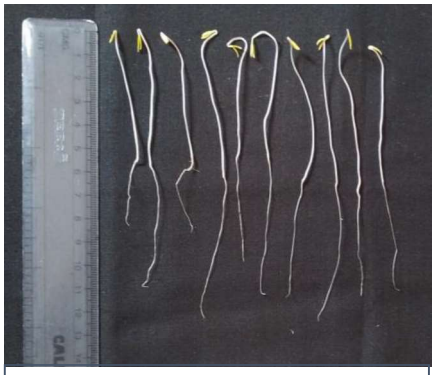
Biopriming with both the concentrations (5 and 10 %) had a significant difference in germination per centage of bioprimed seeds of tomato. However, biopriming at 10 per cent concentration with plant extracts, was found most effective and significant (Table 16, Plate 15). Maximum seed germination of 92.5 per cent was recorded when seeds of tomato were bioprimed at 10 per cent concentration with *Melia azedarach* while 92.0 per cent seed germination was recorded at 5 per cent concentration which were found 43.78 and 43.48 per cent higher than control. Minimum germination of 57.67 per cent was recorded in biopriming treatment with *Thymus vulgaris* at 5 per cent concentration. Biopriming with plant extracts significantly improved the seedling length, Maximum seedling length of 15.25 cm was observed in seed biopriming treatment with *Lantana camara* (5 %) followed by *Thymus vulgaris* (10 %) and *Melia azedarach* (10 %) with seedling length of 14.62 and 13.60 cm, respectively.

**Table 16: Standardization of effective concentration of plant extracts for seed biopriming in tomato**

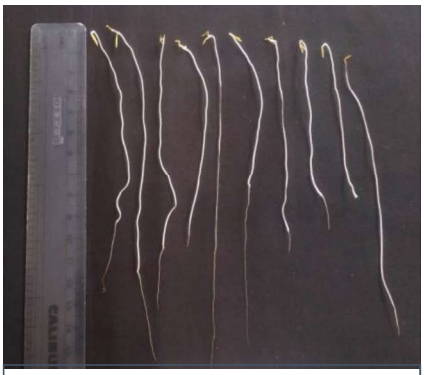
| S. No. | Treatments (Seed biopriming) | Seed germination (%) | Seedling length (cm) | Seedling dry weight (mg) | Seedling Vigour Index-L | Seedling Vigour Index-M |
|---|---|---|---|---|---|---|
| 1. | *Roylea elegans* @ 5% | 85.33 (67.47) | 10.75 | 42.30 | 917.10 | 3610.13 |
| 2. | *Roylea elegans* @ 10 % | 85.67 (67.76) | 11.97 | 21.20 | 1026.57 | 1815.50 |
| 3. | *Melia azedarach* @ 5% | 92.00 (73.56) | 9.89 | 17.90 | 909.83 | 1645.53 |
| 4. | *Melia azedarach* @ 10 % | 92.50 (73.62) | 13.60 | 15.20 | 1251.55 | 1398.67 |
| 5. | *Lantana camara* @ 5% | 73.67 (59.11) | 15.25 | 41.93 | 1123.71 | 3090.07 |
| 6. | *Lantana camara* @ 10 % | 82.67 (65.41) | 13.45 | 13.60 | 1113.17 | 1123.27 |
| 7. | *Datura stramonium* @ 5 % | 75.33 (60.22) | 9.46 | 14.97 | 713.89 | 1122.63 |
| 8. | *Datura stramonium* @ 10 % | 83.33 (65.88) | 12.86 | 20.47 | 1072.21 | 1705.60 |
| 9. | *Hibiscus rosa-sinensis* @ 5% | 82.33 (65.16) | 12.78 | 10.20 | 1052.05 | 840.37 |
| 10. | *Hibiscus rosa-sinensis* @ 10 % | 84.00 (66.50) | 9.89 | 22.03 | 830.92 | 1851.60 |
| 11. | *Thymus vulgaris* @ 5 % | 57.67 (49.39) | 12.07 | 31.80 | 697.67 | 1834.43 |
| 12. | *Thymus vulgaris* @ 10 % | 63.00 (52.52) | 14.62 | 11.63 | 922.45 | 730.33 |
| 13. | Hydropriming | 62.33 (52.12) | 11.73 | 12.83 | 731.60 | 800.50 |
| 14. | Control | 52.00 (46.13) | 8.71 | 10.00 | 452.92 | 520.00 |
| | **CD $_{(0.05)}$** | **2.72** | **1.30** | **2.51** | **124.42** | **201.49** |

Figures in the parenthesis are arc sine transformed values

Maximum seedling dry weight of 42.30 mg was observed in biopriming with *Roylea elegans* (5 %) followed by *Lantana camara* with seedling dry weight of 41.93 mg at similar



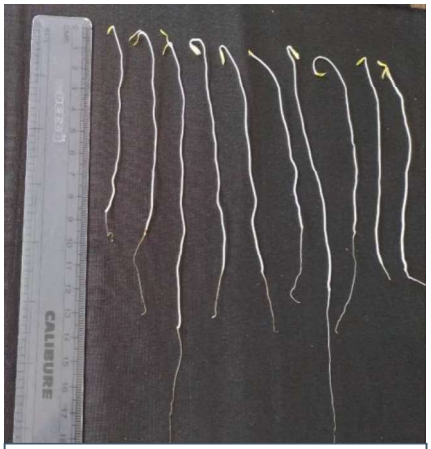
***Roylea elegans* @ 10 % for 12 hrs**



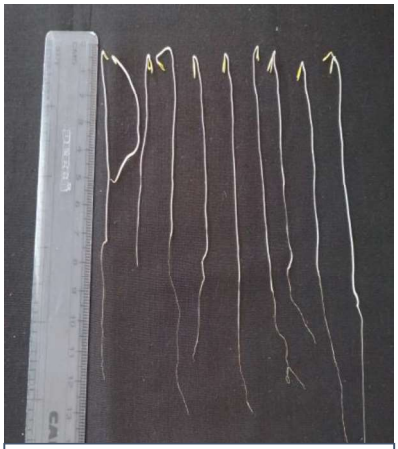
***Melia azedarach* @ 10 % for 12 hrs**

***Lantana camara* @ 10 % for 12 hrs**

***Datura stramonium* @ 10 % for 12 hrs**



***Hibiscus rosa sinensis* @ 10 % for 12 hrs**



***Thymus vulgaris* @ 10 % for 12 hrs**

**Plate 15: Standardization of effective concentration of plant extracts and duration for seed biopriming in tomato**

concentration and both were found statistically at par with each other (Table 16). Seed biopriming with *Thymus vulgaris* (5 %) was found next in efficacy with dry weight of 31.80 mg followed by *Hibiscus rosa-sinensis* (10 %) with dry weight of 22.03 mg.

**Table 17: Standardization of effective concentration of plant extracts for seed biopriming in chilli**

| S. No. | Treatments (Seed biopriming) | Seed germination (%) | Seedling length (cm) | Seedling dry weight (mg) | Seedling Vigour Index-L | Seedling Vigour Index-M |
|---|---|---|---|---|---|---|
| 1. | *Roylea elegans* @ 5% | 92.00 (73.62) | 11.85 | 25.50 | 1090.57 | 2346.67 |
| 2. | *Roylea elegans* @ 10 % | 92.33 (74.04) | 8.31 | 25.63 | 766.67 | 2367.67 |
| 3. | *Melia azedarach* @ 5% | 84.00 (66.50) | 10.82 | 33.97 | 909.08 | 2856.13 |
| 4. | *Melia azedarach* @ 10 % | 85.67 (67.76) | 9.99 | 22.73 | 855.84 | 1946.03 |
| 5. | *Lantana camara* @ 5% | 83.33 (65.89) | 10.89 | 19.13 | 909.65 | 1593.70 |
| 6. | *Lantana camara* @ 10 % | 83.67 (66.19) | 9.59 | 32.65 | 801.11 | 2735.90 |
| 7. | *Datura stramonium* @ 5 % | 76.67 (61.09) | 13.24 | 34.90 | 1015.63 | 2675.00 |
| 8. | *Datura stramonium* @ 10 % | 82.67 (65.41) | 11.11 | 23.29 | 918.36 | 1923.00 |
| 9. | *Hibiscus rosa-sinensis* @ 5% | 73.33 (58.91) | 12.19 | 25.35 | 897.08 | 1858.50 |
| 10. | *Hibiscus rosa-sinensis* @ 10 % | 76.00 (60.65) | 14.19 | 23.15 | 1079.04 | 1758.70 |
| 11. | *Thymus vulgaris* @ 5 % | 76.67 (61.09) | 9.33 | 18.20 | 715.73 | 1395.90 |
| 12. | *Thymus vulgaris* @ 10 % | 77.33 (61.56) | 10.91 | 23.05 | 844.28 | 1779.17 |
| 13. | Hydropriming | 67.67 (55.33) | 9.73 | 19.00 | 657.85 | 1285.00 |
| 14. | Control | 65.00 (53.71) | 10.06 | 15.00 | 653.90 | 975.00 |
| | **CD $_{(0.05)}$** | **3.04** | **2.16** | **2.37** | **176.79** | **229.34** |

Figures in the parenthesis are arc sine transformed values

Maximum SVI-M (3610.13) was observed in biopriming treatment with *Roylea elegans* (5 %). Biopriming treatment with *Melia azedarach* (10 %) resulted in maximum SVI-L of 1251.55 followed by seed biopriming treatment with *Lantana camara* (5%) with vigour index-1 of 1123.71 and both were found statistically at par with biopriming treatment of seed biopriming with *Datura stramonium* (10 %) with seedling vigour index-1 of 1072.21 (Table 16).

Seed biopriming of chilli with plant extracts at the rate of 10 per cent concentration was found most effective and significant. Maximum seed germination of 92.33 per cent was recorded in biopriming with *Roylea elegans* (10 %) which was found 29.60 per cent higher than control followed by 92.0 per cent germination in biopriming with *Roylea elegans* at 5 % concentration (Table 17, Plate 16). However, minimum germination of 67.67 per cent was recorded in hydropriming treatment in comparison to control. Biopriming with plant extracts

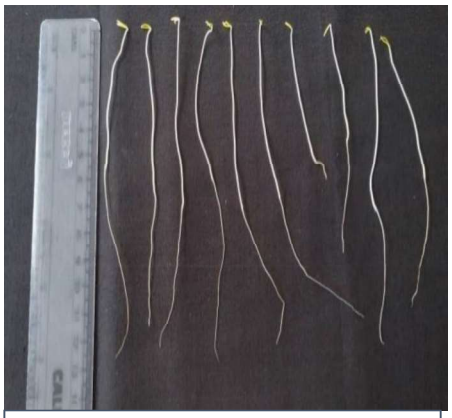
(5 and 10 %) significantly improved the seedling length. Maximum seedling length of 14.19 cm was observed in seed biopriming with *Hibiscus rosa-sinensis* (10 %) followed by biopriming with *Datura stramonium* (5 %) and *Hibiscus rosa-sinensis* (5 %) with seedling length of 13.24 and 12.19 cm, respectively.

In other plant growth traits, maximum seedling dry weight (34.90 mg) was observed in seeds bioprimed with *Datura stramonium* (5 %) followed by *Melia azedarach* (5 %) with seedling dry weight of 33.97 mg and *Lantana camara* (10 %) with dry weight of 32.65 mg and both were found statistically at par with each other. Results indicated that there was a significant difference in seedling vigour index in bioprimed treatments. Maximum SVI-L of 1090.57 was recorded at 5 per cent concentration with *Roylea elegans*. However, *Hibiscus rosa-sinensis* was found next in efficacy with SVI-L of 1079.04 at 10 per cent concentration and both were found statistically at par with each other. Maximum SVI-M of 2856.13 was observed at 5 per cent concentration with *Melia azedarach* followed by *Lantana camara* with SVI-M of 2735.90 at 10 per cent concentration and both were found statistically at par with each other.

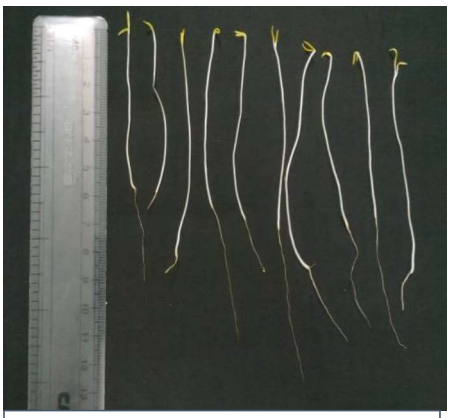
**Table 18: Standardization of effective concentration of plant extracts for seed biopriming in capsicum**

| S. No. | Treatments (Seed biopriming) | Seed germination (%) | Seedling length (cm) | Seedling dry weight (mg) | Seedling Vigour Index-L | Seedling Vigour Index-M |
|---|---|---|---|---|---|---|
| 1. | *Roylea elegans* @ 5% | 85.33 (67.51) | 9.65 | 39.33 | 823.07 | 3355.00 |
| 2. | *Roylea elegans* @ 10 % | 86.33 (68.33) | 8.53 | 24.09 | 736.47 | 2079.48 |
| 3. | *Melia azedarach* @ 5% | 75.00 (59.97) | 10.4 | 34.97 | 780.56 | 2622.97 |
| 4. | *Melia azedarach* @ 10 % | 92.00 (73.62) | 9.84 | 24.42 | 904.60 | 2243.93 |
| 5. | *Lantana camara* @ 5% | 83.00 (65.66) | 10.14 | 23.90 | 842.74 | 1985.57 |
| 6. | *Lantana camara* @ 10 % | 84.33 (66.67) | 13.43 | 27.77 | 1132.77 | 2342.57 |
| 7. | *Datura stramonium* @ 5 % | 82.33 (65.13) | 7.00 | 16.56 | 578.01 | 1362.05 |
| 8. | *Datura stramonium* @ 10 % | 87.33 (69.15) | 9.99 | 40.73 | 872.58 | 3557.40 |
| 9. | *Hibiscus rosa-sinensis* @ 5% | 73.33 (58.91) | 7.71 | 30.57 | 562.39 | 2244.33 |
| 10. | *Hibiscus rosa-sinensis* @ 10 % | 82.00 (64.91) | 7.37 | 19.33 | 604.87 | 1583.83 |
| 11. | *Thymus vulgaris* @ 5 % | 74.33 (59.58) | 11.48 | 31.91 | 853.40 | 2372.07 |
| 12. | *Thymus vulgaris* @ 10 % | 82.67 (65.41) | 11.68 | 25.97 | 966.63 | 2149.73 |
| 13. | Hydropriming | 67.67 (55.33) | 10.77 | 18.85 | 729.54 | 1274.42 |
| 14. | Control | 60.00 (50.75) | 10.50 | 12.02 | 630.00 | 721.20 |
| | **$CD_{(0.05)}$** | **2.93** | **1.44** | **2.90** | **121.71** | **269.55** |

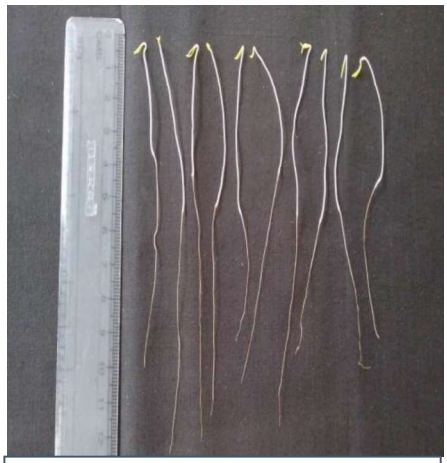
Figures in the parenthesis are arc sine transformed values



***Roylea elegans* @ 10 % for 12 hrs**

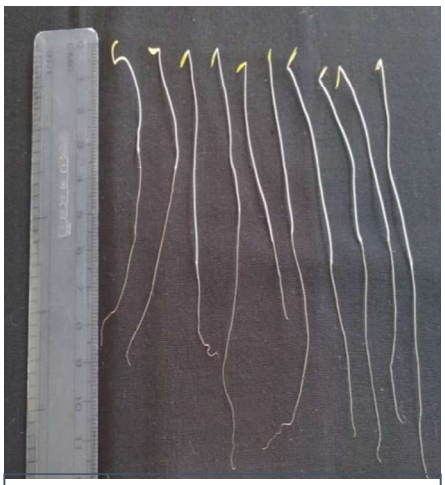


***Melia azedarach* @ 10 % for 12 hrs**

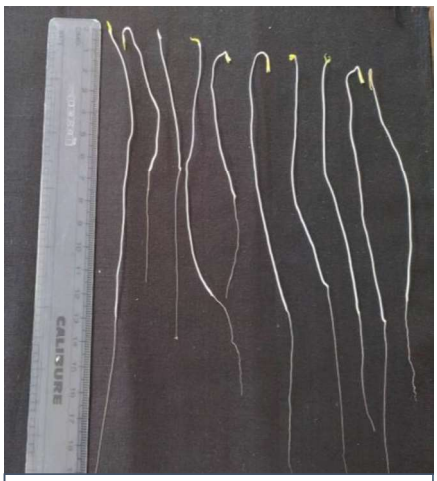


***Lantana camara* @ 10 % for 12 hrs**

***Datura stramonium* @ 10 % for 12 hrs**

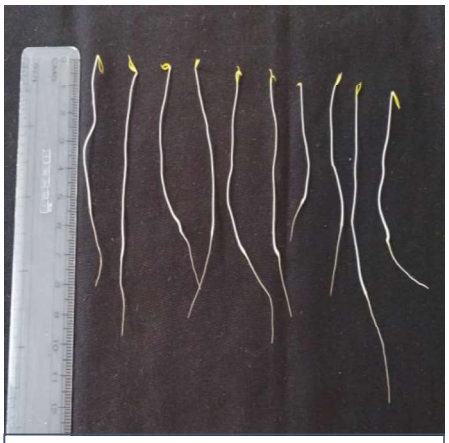


***Hibiscus rosa sinensis* @ 10 % for 12 hrs**

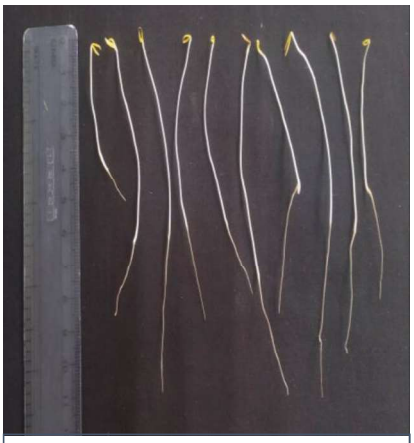


***Thymus vulgaris* @ 10 % for 12 hrs**

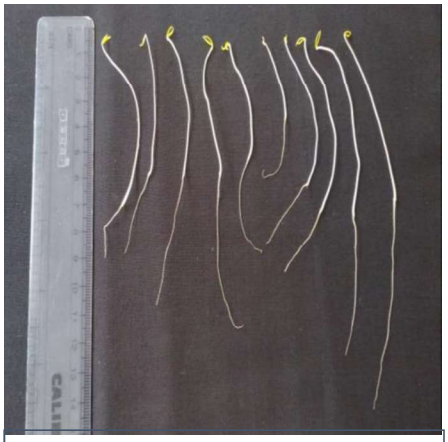
**Plate 16: Standardization of effective concentration of plant extracts and duration for seed biopriming in chilli**



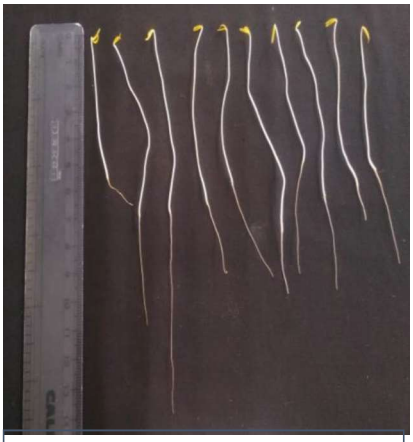
***Roylea elegans* @ 10 % for 12 hrs**



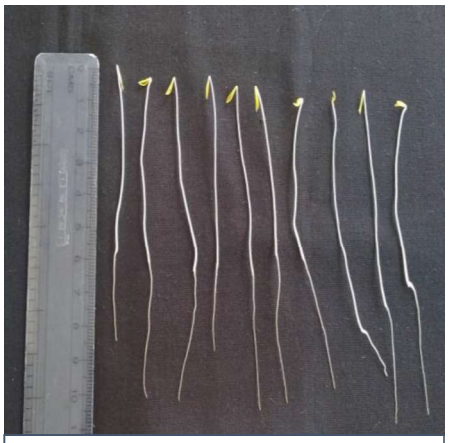
***Melia azedarach* @ 10 % for 12 hrs**



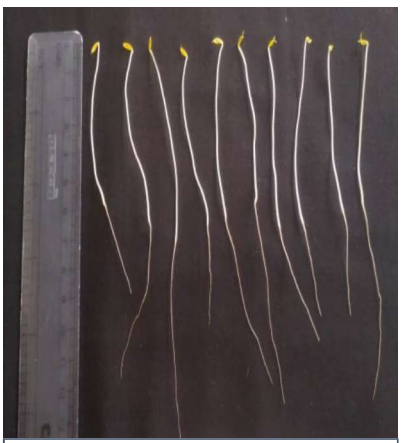
***Lantana camara* @ 10 % for 12 hrs**



***Datura stramonium* @ 10 % for 12 hrs**



***Hibiscus rosa sinensis* @ 10 % for 12 hrs**



***Thymus vulgaris* @ 10 % for 12 hrs**

**Plate 17: Standardization of effective concentration of plant extracts and duration for seed biopriming in capsicum**

In capsicum, biopriming with *Melia azedarach* at 10 % concentration resulted in maximum seed germination of 92.0 per cent which was 34.78 per cent higher than control followed by 87.33 per cent germination in biopriming with *Datura stramonium* at similar concentration (Table 18, Plate 17). *Roylea elegans* (10 %) was found next in efficacy with germination of 86.33 per cent. However, minimum germination of 67.67 per cent was recorded in hydropriming followed by biopriming with *Hibiscus rosa-sinensis* (5 %) with germination of 73.33 per cent.

Further, maximum seedling length of 13.43 cm was observed in seed biopriming treatment with *Lantana camara* (10 %). Seedling length of 11.48 cm and 11.68 cm was observed in biopriming treatment with *Thymus vulgaris* at 5 and 10 per cent concentration, respectively. Maximum seedling dry weight of 40.73 mg was observed in biopriming with *Datura stramonium* (10 %) followed by dry weight of 39.33 and 34.97 mg with biopriming treatment of *Roylea elegans* and *Melia azedarach* at 5 per cent concentration, respectively. Maximum SVI-L of 1132.77 was recorded in biopriming with *Lantana camara* at 10 per cent concentration. Seed biopriming treatment with *Thymus vulgaris* at 10 per cent concentration was found next in efficacy with SVI-L of 966.63. Maximum SVI-M of 3557.40 was observed in biopriming treatment with *Datura stramonium* (10 %) followed by SVI-M of 3355 in biopriming with *Roylea elegans* at 5 per cent concentration.

**Standardization of time duration of seed biopriming with effective plant extracts**

Plant extracts (*Roylea elegans*, *Melia azedarach*, *Lantana camara*, *Datura stramonium*, *Thymus vulgaris*, *Hibiscus rosa sinensis*) at 10 per cent concentration were further evaluated to find the best time duration (6, 8 and 12 hrs) for seed biopriming of tomato, chilli and capsicum.

Duration of seed biopriming with plant extracts at optimum concentration of 10 per cent significantly improved the seed germination and plant growth characteristics like seedling length, seedling dry weight and seedling vigour indexes. Biopriming of tomato seeds for 12 hrs was found more effective with better plant health attributes in comparison to biopriming for 6 and 8 hrs (Table 19). Seeds of tomato bioprimed for 12 hrs with extracts of *Melia azedarach* (10 %) resulted in 37.97 per cent higher seed germination followed by biopriming with *Roylea elegans* (10 %) for 12 hrs with 36.29 per cent higher seed germination in comparison to control.

Seed biopriming with *Thymus vulgaris* (10 %) for 12 hrs was found least effective with seed germination of 61.0 per cent in comparison to control. Seeds bioprimed for 12 hrs with extracts of *Lantana camara* (10 %) had the higher seedling length of 14.54 cm, while the shortest seedling length of 8.71 cm was observed in seedlings from non-primed (control) treatment (Plate 16). Seeds bioprimed with *Roylea elegans* (10 %) for 12 hrs accumulated more biomass (44.17 mg) followed by dry weight of 33.87 mg in those seedlings which were bioprimed with extracts of *Hibiscus rosa-sinensis* for 12 hrs at 10 % concentration (Table 20).

**Table 19: Effect of duration of seed biopriming with plant extracts on seed germination and seedling length in tomato**

| S. No. | Treatments (Seed biopriming) | Seed germination (%) | | | | Seedling length (cm) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 6h | 8h | 12h | Mean | 6h | 8h | 12h | Mean |
| **1.** | *Roylea elegans* | 58.00 (49.62) | 80.00 (63.52) | 86.33 (68.29) | **74.78 (60.47)** | 10.35 | 9.38 | 13.97 | **11.24** |
| **2.** | *Melia azedarach* | 72.67 (58.81) | 81.67 (64.65) | 88.67 (71.09) | **81.00 (64.85)** | 9.93b | 11.11 | 13.47 | **11.50** |
| **3.** | *Lantana camara* | 73.33 (58.89) | 71.33 (57.63) | 81.67 (64.66) | **75.44 (60.39)** | 11.58 | 9.72 | 14.54 | **11.95** |
| **4.** | *Datura stramonium* | 68.67 (55.96) | 76.67 (61.23) | 82.33 (65.35) | **75.89 (60.85)** | 8.39 | 10.44 | 12.62 | **10.48** |
| **5.** | *Hibiscus rosa sinensis* | 69.67 (56.64) | 71.00 (57.58) | 80.33 (63.75) | **73.67 (59.32)** | 9.54 | 9.60 | 13.63 | **10.92** |
| **6.** | *Thymus vulgaris* | 76.00 (60.93) | 78.67 (62.53) | 61.00 (51.41) | **71.89 (58.28)** | 11.21 | 11.34 | 11.82 | **11.46** |
| **7.** | Hydropriming | 61.67 (51.73) | 66.67 (54.74) | 71.33 (57.67) | **66.56 (54.71)** | 11.70 | 12.03 | 12.48 | **12.08** |
| **8.** | Control | 55.00 (47.85) | 55.00 (47.85) | 55.00 (47.85) | **55.00 (47.85)** | 8.71 | 8.71 | 8.71 | **8.71** |
| | **Mean** | **66.88 (55.05)** | **72.63 (58.72)** | **75.83 (61.26)** | | **10.18** | **10.29** | **12.66** | |
| | **CD (0.05)** | | | | | | | | |
| | **Treatment (T)** | **3.72** | | | | **0.44** | | | |
| | **Duration (D)** | **2.28** | | | | **0.27** | | | |
| | **T X D** | **6.44** | | | | **0.76** | | | |

Figures in the parenthesis are arc sine transformed values

Biomass production was lowest (12.0 mg) in seeds which were hydro primed for 8 hrs duration in comparison to non-primed seeds with dry weight of 10.0 mg. Seed biopriming treatment of tomato seeds with *Melia azedarach* (10 %) for 12 hrs resulted in SVI-L of 1206.19 in comparison to control with least SVI-L of 278.72.

**Table 20: Effect of duration of seed biopriming with plant extracts on seedling dry weight and seedling vigour in tomato**

| S. No. | Treatments (Seed biopriming) | Seedling dry weight (mg) | | | | Seedling vigour Index-L | | | | Seedling vigour Index-M | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 6h | 8h | 12h | Mean | 6h | 8h | 12h | Mean | 6h | 8h | 12h | Mean |
| **1.** | *Roylea elegans* | 17.28 | 14.27 | 44.17 | **25.24** | 721.77 | 906.67 | 1193.39 | **940.61** | 1005.77 | 1141.67 | 3812.83 | **1986.76** |
| **2.** | *Melia azedarach* | 14.77 | 17.77 | 14.20 | **15.58** | 432.39 | 751.05 | 1206.19 | **796.54** | 1070.30 | 1451.97 | 1273.53 | **1265.27** |
| **3.** | *Lantana camara* | 24.07 | 15.90 | 15.53 | **18.50** | 849.33 | 693.25 | 1186.80 | **909.79** | 1767.00 | 1133.00 | 1266.83 | **1388.94** |
| **4.** | *Datura stramonium* | 22.53 | 15.47 | 22.70 | **20.23** | 577.49 | 800.97 | 1038.08 | **805.51** | 1544.97 | 1183.57 | 1864.37 | **1530.97** |
| **5.** | *Hibiscus rosa sinensis* | 14.40 | 29.17 | 33.87 | **25.81** | 663.49 | 681.70 | 1095.37 | **813.52** | 1003.87 | 2072.70 | 2719.63 | **1932.07** |
| **6.** | *Thymus vulgaris* | 26.43 | 17.27 | 31.50 | **25.07** | 854.38 | 892.51 | 725.58 | **824.16** | 2001.73 | 1357.30 | 1919.20 | **1759.41** |
| **7.** | Hydropriming | 12.83 | 12.00 | 11.50 | **12.11** | 746.52 | 785.25 | 823.21 | **784.99** | 815.00 | 761.17 | 758.33 | **778.45** |
| **8.** | Control | 10.00 | 10.00 | 10.00 | **10.00** | 278.72 | 278.72 | 278.72 | **278.72** | 1312.00 | 1312.00 | 1312.00 | **1312.00** |
| | **Mean** | **17.79** | **16.48** | **22.93** | | **640.51** | **723.77** | **943.42** | | **1315.18** | **1301.67** | **1865.84** | |
| | **$CD_{(0.05)}$**<br>**Treatment (T)**<br>**Duration (D)**<br>**T X D** | **1.18**<br>**0.73**<br>**2.05** | | | | **98.36**<br>**60.24**<br>**170.37** | | | | **147.03**<br>**90.04**<br>**254.66** | | | |

**Table 21: Effect of duration of seed biopriming with plant extracts on seed germination and seedling length in chilli**

| S. No. | Treatments | Seed germination (%) | | | | Seedling length (cm) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 6h | 8h | 12h | Mean | 6h | 8h | 12h | Mean |
| **1.** | *Roylea elegans* | 81.00 (64.76) | 82.33 (65.17) | 93.33 (75.21) | **85.56 (68.38)** | 11.53 | 12.78 | 13.21 | **12.50** |
| **2.** | *Melia azedarach* | 83.67 (66.19) | 86.00 (68.03) | 89.00 (70.75) | **86.22 (68.33)** | 7.69 | 10.73 | 12.63 | **10.35** |
| **3.** | *Lantana camara* | 77.33 (61.64) | 82.00 (64.91) | 87.67 (69.42) | **82.33 (65.32)** | 8.48 | 12.06 | 13.41 | **11.32** |
| **4.** | *Datura stramonium* | 70.33 (57.03) | 82.67 (65.38) | 85.00 (67.22) | **79.33 (63.21)** | 8.48 | 7.66 | 12.58 | **9.57** |
| **5.** | *Hibiscus rosa sinensis* | 84.00 (66.42) | 82.00 (64.91) | 74.33 (59.58) | **80.11 (63.64)** | 12.39 | 11.83 | 12.55 | **12.26** |
| **6.** | *Thymus vulgaris* | 76.00 (60.89) | 82.67 (64.78) | 87.67 (69.43) | **81.78 (65.04)** | 13.92 | 12.63 | 10.95 | **12.49** |
| **7.** | Hydropriming | 67.67 (55.33) | 73.00 (58.69) | 67.33 (55.12) | **69.33 (56.38)** | 9.73 | 11.56 | 12.60 | **11.29** |
| **8.** | Control | 68.00 (55.53) | 68.00 (55.53) | 68.00 (55.53) | **68.00 (55.53)** | 10.06 | 10.06 | 10.06 | **10.06** |
| | **Mean** | **76.00 (60.98)** | **79.71 (63.43)** | **81.54 (65.28)** | | **10.29** | **11.16** | **12.25** | |
| | **CD $_{(0.05)}$** | | | | | | | | |
| | **Treatment (T)** | **2.77** | | | | **0.45** | | | |
| | **Duration (D)** | **1.69** | | | | **0.29** | | | |
| | **T X D** | **4.80** | | | | **0.83** | | | |

Figures in the parenthesis are arc sine transformed values

SVI-M was observed maximum (3812.83) in seed biopriming for 12 hrs with *Roylea elegans* (10 %) in comparison to non-primed seeds with SVI-M of 1312. Biopriming of chilli seeds for 12 hrs duration at 10 per cent concentration was found more effective with respect to seed health attributes as compared to biopriming for 6 and 8 hrs (Table 21).

Seeds bioprimed with extracts of *Roylea elegans* (10 %) for 12 hrs resulted in 27.14 per cent higher seed germination in comparison to control. Seed biopriming for 12 hrs duration with *Thymus vulgaris* (10 %) resulted in 87.67 per cent germination which were statistically at par with each other. Seed biopriming with *Hibiscus rosa-sinensis* (10 %) for 12 hrs was found least effective with seed germination of 74.33 per cent only in comparison to control treatment with seed germination of 67.0 per cent.

Seeds bioprimed with extracts of *Roylea elegans* (10 %) for 12 hrs had the maximum seedling length of 13.21 cm, while biopriming treatment with *Datura stramonium* (10 %) for 12 hrs duration resulted in shortest seedling length of 8.48 cm (Plate 17). Seeds bioprimed with *Melia azedarach* (10 %) for 12 hrs accumulated more biomass (34.07 mg) followed by those bioprimed with extracts of *Hibiscus rosa-sinensis* (10 %) for 12 hrs which had only

**Table 22: Effect of duration of seed biopriming with plant extracts on seedling dry weight and seedling vigour in chilli**

| S. No. | Treatments | Seedling dry weight (mg) | | | | Seedling vigour Index-L | | | | Seedling vigour Index-M | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 6h | 8h | 12h | Mean | 6h | 8h | 12h | Mean | 6h | 8h | 12h | Mean |
| **1.** | *Roylea elegans* | 27.60 | 32.70 | 32.79 | **31.03** | 933.99 | 1040.96 | 1232.07 | **1069.01** | 1936.80 | 2704.20 | 2708.60 | **2449.87** |
| **2.** | *Melia azedarach* | 26.67 | 32.80 | 34.07 | **31.18** | 594.61 | 880.08 | 1106.99 | **860.56** | 2061.97 | 2695.03 | 2986.47 | **2581.15** |
| **3.** | *Lantana camara* | 22.90 | 26.30 | 25.57 | **24.92** | 709.95 | 1036.77 | 1193.74 | **980.15** | 1861.97 | 2169.03 | 2386.17 | **2139.05** |
| **4.** | *Datura stramonium* | 22.07 | 25.43 | 31.85 | **26.45** | 596.16 | 633.54 | 1069.09 | **766.27** | 1848.50 | 2189.27 | 2917.50 | **2318.42** |
| **5.** | *Hibiscus rosa sinensis* | 28.10 | 27.57 | 32.85 | **29.50** | 1041.15 | 969.25 | 932.54 | **981.02** | 2361.20 | 2257.47 | 2445.30 | **2354.66** |
| **6.** | *Thymus vulgaris* | 32.17 | 29.17 | 13.37 | **24.90** | 1048.20 | 1031.17 | 959.66 | **1013.01** | 2425.33 | 2384.83 | 1171.63 | **1993.93** |
| **7.** | Hydropriming | 22.33 | 24.67 | 24.67 | **23.89** | 657.85 | 843.29 | 848.42 | **783.19** | 1513.67 | 1799.00 | 1660.00 | **1657.56** |
| **8.** | Control | 15.00 | 15.00 | 15.00 | **15.00** | 684.08 | 684.08 | 684.08 | **684.08** | 1020.00 | 1020.00 | 1020.00 | **1020.00** |
| | **Mean** | **24.60** | **26.70** | **26.27** | | **783.25** | **889.89** | **1003.34** | | **1878.68** | **2152.35** | **2161.96** | |
| | **CD $_{(0.05)}$**<br>**Treatment (T)**<br>**Duration (D)**<br>**T X D** | | **2.02**<br>**1.23**<br>**3.51** | | | | **46.47**<br>**28.45**<br>**80.49** | | | | **193.10**<br>**118.25**<br>**334.47** | | |

32.85 mg of biomass. Biomass production was lowest (15.0 mg) in non-primed chilli seeds. Seed biopriming treatment of chilli seeds with *Roylea elegans* (10 %) for 12 hrs resulted in maximum SVI-L (1232.07) in comparison to control (Table 22). SVI-M (2986.47) was observed maximum in seed biopriming treatment of *Melia azedarach* (10 %) for 12 hrs in comparison to non-primed seeds with SVI-M of 1005.

Capsicum seeds bioprimed with extracts of *Roylea elegans* (10 %) for 12 hrs resulted in 41.76 per cent higher seed germination followed by 90.33 per cent germination in seed biopriming with *Lantana camara* (10 %) for 12 hrs and both were found statistically at par with each other (Table 23). Hydropriming for 6 hrs was found least effective with seed germination of 53.67 per cent in comparison to control with seed germination of 53.0 per cent. Seeds bioprimed with extracts of *Roylea elegans* (10 %) for 8 hrs had the maximum seedling length (12.13 cm), while the shortest seedling length (7.42 cm) was observed in seedlings bioprimed with *Lantana camara* (10 %) concentration for 6 hrs (Plate 17).

**Table 23: Effect of duration of seed biopriming with plant extracts on seed germination and seedling length in capsicum**

| S. No. | Treatments | Seed germination (%) | | | | Seedling length (cm) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 6h | 8h | 12h | Mean | 6h | 8h | 12h | Mean |
| **1.** | *Roylea elegans* | 82.67 (65.39) | 82.67 (65.41) | 91.00 (72.53) | **85.40 (67.78)** | 8.08 | 12.13 | 9.48 | **9.89** |
| **2.** | *Melia azedarach* | 77.67 (61.79) | 83.00 (65.64) | 84.67 (66.94) | **81.78 (64.79)** | 8.18 | 11.43 | 8.91 | **9.51** |
| **3.** | *Lantana camara* | 82.67 (65.39) | 67.33 (55.13) | 90.33 (71.89) | **80.11 (64.14)** | 7.42 | 10.25 | 10.07 | **9.25** |
| **4.** | *Datura stramonium* | 74.00 (59.33) | 72.00 (58.05) | 84.00 (66.44) | **76.67 (61.28)** | 11.46 | 8.58 | 11.17 | **10.40** |
| **5.** | *Hibiscus rosa sinensis* | 71.00 (57.42) | 75.67 (60.43) | 85.33 (67.55) | **77.33 (61.81)** | 10.88 | 11.05 | 8.54 | **10.16** |
| **6.** | *Thymus vulgaris* | 77.00 (61.34) | 82.67 (65.39) | 89.67 (71.44) | **83.11 (66.06)** | 11.85 | 11.84 | 12.17 | **11.96** |
| **7.** | Hydropriming | 53.67 (47.08) | 57.00 (49.01) | 60.00 (50.75) | **56.89 (48.95)** | 10.77 | 12.65 | 10.54 | **11.32** |
| **8.** | Control | 53.00 (46.70) | 53.00 (46.70) | 53.00 (46.70) | **53.00 (46.70)** | 10.50 | 10.50 | 10.50 | **10.50** |
| | **Mean** | **71.46 (58.06)** | **71.67 (58.22)** | **79.75 (64.28)** | | **9.89** | **11.06** | **10.18** | |
| | **$CD_{(0.05)}$** | | | | | | | | |
| | **Treatment (T)** | **1.61** | | | | **0.88** | | | |
| | **Duration (D)** | **0.99** | | | | **0.54** | | | |
| | **T X D** | **2.79** | | | | **1.53** | | | |

Figures in the parenthesis are arc sine transformed values

**Table 24: Effect of duration of seed biopriming with plant extracts on seedling dry weight and seedling vigour in capsicum**

| S. No. | Treatments | Seedling dry weight (mg) | | | | Seedling vigour Index-L | | | | Seedling vigour Index-M | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 6h | 8h | 12h | Mean | 6h | 8h | 12h | Mean | 6h | 8h | 12h | Mean |
| **1.** | *Roylea elegans* | 25.91 | 44.30 | 27.67 | **32.63** | 667.62 | 1003.86 | 863.81 | **845.09** | 2139.66 | 3662.57 | 2516.67 | **2772.96** |
| **2.** | *Melia azedarach* | 36.30 | 32.94 | 24.67 | **31.30** | 847.15 | 619.18 | 935.33 | **800.56** | 2682.90 | 2368.73 | 2067.13 | **2372.92** |
| **3.** | *Lantana camara* | 14.12 | 24.20 | 23.35 | **20.56** | 612.25 | 689.43 | 909.52 | **737.07** | 1167.23 | 1630.30 | 2109.77 | **1635.77** |
| **4.** | *Datura stramonium* | 23.47 | 17.40 | 32.70 | **24.52** | 627.37 | 950.22 | 759.88 | **779.16** | 1820.93 | 1442.00 | 2787.47 | **2016.80** |
| **5.** | *Hibiscus rosa sinensis* | 23.60 | 38.13 | 24.87 | **28.87** | 774.01 | 836.06 | 730.64 | **780.23** | 1672.77 | 2881.50 | 2124.70 | **2226.30** |
| **6.** | *Thymus vulgaris* | 22.50 | 22.63 | 14.05 | **19.73** | 912.07 | 979.39 | 1086.63 | **992.69** | 1731.83 | 1871.47 | 1256.11 | **1619.80** |
| **7.** | Hydropriming | 22.50 | 33.33 | 31.17 | **29.00** | 380.58 | 518.92 | 438.98 | **446.16** | 1208.50 | 1900.33 | 1865.33 | **1658.06** |
| **8.** | Control | 19.50 | 19.50 | 19.50 | **19.50** | 241.50 | 241.50 | 241.50 | **241.50** | 1033.50 | 1033.50 | 1033.50 | **1033.50** |
| | **Mean** | **23.49** | **29.05** | **24.76** | | **632.82** | **729.82** | **745.79** | | **1682.17** | **2098.80** | **1967.57** | |
| | **CD $_{(0.05)}$**<br>**Treatment (T)**<br>**Duration (D)**<br>**T X D** | **2.48**<br>**1.52**<br>**4.30** | | | | **71.06**<br>**43.51**<br>**123.07** | | | | **177.08**<br>**108.44**<br>**306.71** | | | |

Seeds bioprimed with *Roylea elegans* (10 %) concentration for 12 hrs accumulated more biomass (44.30 mg) followed by those bioprimed with extracts of *Hibiscus rosa-sinensis* (10 %) for 8 hrs (38.13 mg). Biomass production was lowest (19.5 mg) in non-primed capsicum seeds followed by hydropriming for 6 hrs with biomass of 22.50 mg (Table 24). Seed biopriming treatment of capsicum seeds with *Thymus vulgaris* (10 %) for 12 hrs resulted in maximum SVI-L of 1086.63 in comparison to control.

Singh and Yaduman (2016) reported that application of neem leaf extract at 40 and 60 % concentration was effective against *Pythium aphanidermatum*. Prabha et al. (2016) reported that priming of tomato seeds with extracts of *Azadirachta indica* and *Vinca rosea* at 2 per cent concentration for 4 hrs significantly reduced seedling mortality in tomato. However, very less work was carried out on seed biopriming with plant extracts. Murtaza et al. (2015) studied the antifungal potential of medicinal plants like *Artemisia*, *Thyme, Mentha*, *Tectona*, *Cymbopogon*, *Ocimum* against fungal pathogens.

**5. Seed biopriming with effective plant extracts against damping-off disease under field conditions**

Effective seed biopriming treatments of plant extracts under *in-vitro* studies were applied under field conditions. Seeds of tomato, chilli and capsicum were bioprimed with the leaf extracts of *Roylea elegans*, *Melia azedarach*, *Lantana camara, Datura stramonium* and *Hibiscus rosa-sinensis* at a concentration of 10 per cent for 12 hrs and then these bioprimed seeds were sown in nursery beds and data pertaining to disease incidence (%) and plant growth characteristics were recorded.

Seed biopriming with *Roylea elegans* was found most effective in the management of damping-off disease in tomato with 54.73 per cent reduction in seedling mortality in comparison to control (Table 25, Plate 18). Seed biopriming with *Melia azedarach* was found next in efficacy with seedling mortality of 23.27 per cent. Seed biopriming with *Datura stramonium* was found next in efficacy in managing damping-off disease in tomato with seedling mortality of 31.19 per cent. Seed biopriming with *Roylea elegans* was also found most effective in improving seed germination of tomato to 75.75 per cent followed by seed biopriming with *Melia azedarach* and *Lantana camara* with seed germination of 65.50 and 60.25 per cent, respectively. Among all the treatments, seed biopriming with *Roylea elegans* was found most effective in improving seedling growth with average shoot and root length of 24.84 and 8.21 cm, respectively. Seed biopriming with *Melia azedarach* was next in efficacy

**Table 25: Effect of seed biopriming with effective plant extracts on damping-off and plant growth in tomato, chilli and capsicum under field conditions**

| S. No. | Treatments (Seed biopriming) | Tomato | | | | Chilli | | | | Capsicum | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Disease incidence (%) | Seed germination (%) | Shoot length (cm) | Root length (cm) | Disease incidence (%) | Seed germination (%) | Shoot length (cm) | Root length (cm) | Disease incidence (%) | Seed germination (%) | Shoot length (cm) | Root length (cm) |
| 1. | *Roylea elegans* | 18.27 (25.28) | 75.75 (60.49) | 24.84 | 8.21 | 21.57 (27.66) | 83.75 (66.24) | 21.84 | 5.73 | 14.26 (22.18) | 64.50 (53.42) | 23.21 | 8.32 |
| 2. | *Melia azedarach* | 23.27 (28.83) | 65.50 (54.02) | 24.11 | 7.82 | 17.26 (24.53) | 80.00 (63.45) | 18.82 | 5.43 | 19.19 (25.98) | 71.50 (57.73) | 16.65 | 7.48 |
| 3. | *Lantana camara* | 31.54 (34.15) | 63.00 (52.52) | 18.49 | 5.99 | 26.94 (31.25) | 76.75 (61.16) | 17.78 | 4.52 | 25.19 (30.11) | 61.60 (51.69) | 18.01 | 6.35 |
| 4. | *Datura stramonium* | 31.19 (33.93) | 60.25 (50.89) | 17.21 | 5.36 | 31.78 (34.29) | 67.00 (54.92) | 16.65 | 4.73 | 32.07 (34.48) | 59.63 (50.53) | 21.06 | 7.49 |
| 5. | *Hibiscus rosa sinensis* | 36.55 (37.18) | 59.00 (50.17) | 16.80 | 4.56 | 33.52 (33.36) | 75.50 (60.32) | 12.58 | 3.52 | 31.75 (34.28) | 52.50 (46.42) | 18.33 | 6.53 |
| 6. | Control | 40.21 (39.34) | 56.00 (48.43) | 14.77 | 4.51 | 39.54 (38.95) | 61.00 (51.34) | 7.46 | 3.28 | 37.92 (37.97) | 57.25 (49.15) | 12.79 | 5.35 |
| | **$CD_{(0.05)}$** | **1.12** | **1.93** | **1.34** | **0.62** | **1.20** | **1.78** | **1.25** | **1.32** | **1.54** | **1.74** | **2.07** | **0.33** |

Figures in the parenthesis are arc sine transformed values

with shoot length of 24.11 cm and root length of 7.82 cm followed by *Hibiscus rosa-sinensis* with average shoot length and root length of 16.80 cm and 4.56 cm, respectively.

In chilli, seed biopriming with *Melia azedarach* was found most effective in the management of damping-off disease with 56.34 per cent reduction in seedling mortality followed by *Roylea elegans* with 45.44 per cent reduction in comparison to control (Table 25, Plate 18). Seed biopriming with *Lantana camara* was found next in efficacy in the management of damping-off disease with seedling mortality of 26.94 per cent.

Further, seed biopriming with *Roylea elegans* was also found most effective in improving seed germination to 83.75 per cent. Seed biopriming with *Melia azedarach* followed by *Lantana camara* were next in efficacy with seed germination of 80 and 76.75 per cent respectively. Seed biopriming with *Datura stramonium* was found least effective with seed germination of 67 per cent in comparison to control.

Seed biopriming with *Roylea elegans* was found most effective in improving different plant growth characteristics with average shoot and root length of 21.84 and 5.73 cm in chilli seedlings, respectively. Seed biopriming with *Melia azedarach* was found next in efficacy with shoot length of 18.82 cm and root length of 5.43 cm. Seed biopriming with *Hibiscus rosa-sinensis* was found least effective with shoot and root length of 12.58 cm and 3.52 cm in chilli seedlings, respectively in comparison to control.

Seed biopriming was also found effective in capsicum. Seed biopriming treatment with *Roylea elegans* was found most effective in the management of damping-off disease with 62.39 per cent reduction in seedling mortality followed by seed biopriming treatment with *Melia azedarach* with reduction of 49.39 per cent in comparison to control (Table 25, Plate 18). Seed biopriming with *Lantana camara* was found next in efficacy in the management of damping-off disease with seedling mortality of 25.19 per cent in comparison to control. Seed biopriming with *Melia azedarach* was also found most effective in enhancing the seed germination to 71.5 per cent followed by seed biopriming treatments with *Roylea elegans* and *Lantana camara* with seed germination of 64.50 and 61.6 per cent, respectively. Among all the biopriming treatments, seed biopriming with *Hibiscus rosa-sinensis* was found least effective with seed germination of 52.50 per cent in comparison to control. Seed biopriming with *Roylea elegans* was also found most effective with respect to seedling growth in capsicum with average shoot and root length of 23.21 and 8.32 cm, respectively. Seed biopriming with *Lantana camara* was found next in efficacy with shoot length of 18.01

**Seed biopriming with *Roylea elegans* in tomato**

**Control**

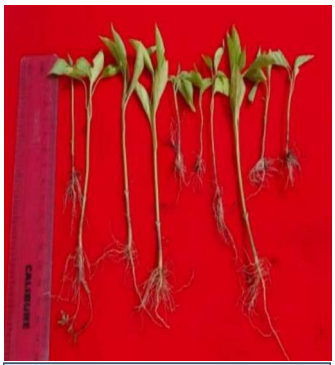
**Seed biopriming with *Melia azedarach* in capsicum**

**Control**

**Seed biopriming with *Roylea elegans* in chilli**

**Control**

**A**



**B**

**C**

***Roylea elegans* bioprimed seedlings of A) Tomato B) Chilli C) Capsicum**

**Plate 18: Seed biopriming with effective plant extracts under field conditions**

cm followed by *Melia azedarach* with shoot length of 16.65 cm. Seed biopriming with *Hibiscus rosa-sinensis* was found least effective with root length of 6.53 cm in capsicum in comparison to control.

Potential of plant extracts in the management of damping-off disease had been reported by various workers (Hooda et al. 2011; Hossain and Bashar 2011; Zagade et al. 2012; Gholve et al. 2014; Singh and Yaduman 2016). However, there are scanty reports about the use of plant extracts for seed biopriming in the management of damping-off disease. Islam and Faruq (2012) studied the efficacy of neem leaf, garlic clove, bel leaf, allamanda leaf extracts against damping-off disease and reported the efficacy of allamanda leaf extract and neem leaf extract in reducing damping-off incidence in tomato, eggplant and chilli seedlings with increased germination of seeds. However, efficacy of direct application of plant extracts in managing soil borne pathogens under field conditions has been reported by various workers (Bouzidi and Mederbal 2016; Sanaullah et al. 2018; Carvalho et al. 2022).

## 6. Integration of seed biopriming with biocontrol agents and plant extracts under field conditions

Effective seed biopriming treatments of biocontrol agents and plant extracts were conjointly applied as seed biopriming to check their efficacy in managing damping-off disease. To do the same, first seeds of tomato, chilli and capsicum were bioprimed with effective biocontrol agents (*Trichoderma harzianum*, *Trichoderma virens*, *Trichoderma atroviride*, *Pseudomonas fluorescens*, *Bacillus licheniformis*) followed by their priming with effective plant extracts (*Roylea elegans*, *Melia azedarach*, *Datura stramonium*, *Lantana camara*, *Hibiscus rosa sinensis*). In these treatments of conjoint application, seed biopriming treatment with integration of *Roylea elegans* with *Trichoderma harzianum* and *Melia azedarach* with *Trichoderma virens* were found most effective in the management of damping-off disease with significant effect on seed health attributes.

Seed biopriming of tomato seeds with conjoint application of *Roylea elegans* and *Trichoderma harzianum* was found most effective against damping-off disease with 48.05 per cent reduction in seedling mortality followed by conjoint application of *Melia azedarach* and *Trichoderma virens* with reduction of 42.79 per cent in comparison to control (Table 26). Conjoint application of seed biopriming with *Lantana camara* and *Trichoderma atroviride* was found next in efficacy with 37.70 per cent reduction in seedling mortality. Seed biopriming with conjoint application of *Roylea elegans* and *Trichoderma harzianum* was

**Table 26: Effect of conjoint application of seed biopriming with effective biocontrol agents and plant extracts on damping-off and plant growth in tomato, chilli and capsicum under field conditions**

| S. No. | Treatments (Seed biopriming) | Tomato | | | | Chilli | | | | Capsicum | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Disease incidence (%) | Seed germination (%) | Shoot length (cm) | Root length (cm) | Disease incidence (%) | Seed germination (%) | Shoot length (cm) | Root length (cm) | Disease incidence (%) | Seed germination (%) | Shoot length (cm) | Root length (cm) |
| 1. | *R. elegans* + *T. harzianum* | 18.20 (25.23) | 82.91 (65.59) | 22.64 | 8.83 | 16.86 (32.24) | 78.82 (62.58) | 20.14 | 9.41 | 14.48 (22.36) | 73.50 (58.99) | 18.74 | 10.03 |
| 2. | *M. azedarach* + *T. virens* | 20.12 (26.65) | 78.38 (62.29) | 20.84 | 9.25 | 20.70 (26.98) | 74.34 (59.54) | 18.9 | 7.19 | 21.68 (27.74) | 65.30 (53.91) | 19.79 | 7.62 |
| 3. | *L. camara* + *T. atroviride* | 21.91 (27.91) | 70.80 (57.30) | 17.44 | 7.01 | 23.59 (29.05) | 70.24 (56.91) | 16.21 | 6.02 | 28.89 (32.47) | 48.49 (44.12) | 16.17 | 6.45 |
| 4. | *D. stramonium* + *B. licheniformis* | 25.01 (30.01) | 58.38 (49.82) | 22.83 | 6.61 | 27.69 (31.74) | 64.07 (53.16) | 13.73 | 4.79 | 19.88 (26.47) | 57.91 (49.53) | 16.93 | 5.94 |
| 5. | *H. rosa sinensis* + *P. fluorescens* | 28.15 (32.03) | 50.13 (45.05) | 21.14 | 7.09 | 24.60 (29.73) | 64.19 (53.23) | 14.79 | 4.94 | 25.29 (30.19) | 51.52 (45.86) | 17.74 | 5.63 |
| 6. | Control | 35.17 (36.37) | 52.74 (46.55) | 15.31 | 5.29 | 34.55 (35.99) | 46.50 (42.98) | 11.13 | 4.61 | 32.32 (34.64) | 35.08 (36.30) | 12.66 | 4.92 |
| | **CD $_{(0.05)}$** | **1.36** | **3.07** | **1.32** | **1.37** | **2.23** | **1.41** | **1.33** | **1.18** | **2.04** | **1.66** | **1.82** | **1.07** |

Figures in the parenthesis are arc sine transformed values

found most effective with an increase of 36.38 per cent in seed germination followed by conjoint application of seed biopriming with *Melia azedarach* and *Trichoderma virens* with increase of 32.71 per cent in comparison to control. Conjoint application of seed biopriming with *Hibiscus-rosa sinensis* and *Pseudomonas fluorescens* was found least effective with seed germination of 50.13 per cent in bioprimed tomato seeds. Conjoint application of seed biopriming with *Roylea elegans* and *Trichoderma harzianum* was found most significant with average shoot length of 22.64 cm followed by *Melia azedarach* and *Trichoderma virens* with average shoot length of 20.84 cm. Seed biopriming with conjoint application of *Melia azedarach* and *Trichoderma virens* was found most effective with root length of 9.25 cm followed by treatment combination of *Roylea elegans* and *Trichoderma harzianum* with root length of 8.83 cm in comparison to control.

Seed biopriming of chilli seeds with conjoint application of *Roylea elegans* and *Trichoderma harzianum* was most effective against damping-off disease with 51.20 per cent reduction in seedling mortality in comparison to control (Table 26). Seed biopriming with treatment combination of *Melia azedarach* and *Trichoderma virens* was found next in efficacy with 40.08 per cent reduction in seedling mortality followed by treatment of conjoint application of *Lantana camara* and *Trichoderma atroviride* with seedling mortality of 23.59 per cent. Seed biopriming with conjoint application of *Roylea elegans* and *Trichoderma harzianum* was found most effective with 78.82 per cent increase in seed germination followed by *Melia azedarach* and *Trichoderma virens* with seed germination of 74.34 per cent. Conjoint application of *Datura stramonium* and *Bacillus licheniformis* was found least effective with seed germination of 64.07 per cent in bioprimed chilli seeds. Seed biopriming with treatment combination of *Roylea elegans* and *Trichoderma harzianum* was found most effective with average shoot length of 20.14 cm while average root length of 7.19 cm was found in conjoint application of *Melia azedarach* and *Trichoderma virens*. Seed biopriming with conjoint application of *Lantana camara* and *Trichoderma atroviride* was found next in efficacy with average root length of 6.02 cm in comparison to control.

Seed biopriming with combination of *Roylea elegans* and *Trichoderma harzianum* was found most effective against damping-off disease in capsicum with 55.19 per cent reduction of seedling mortality followed by 32.92 per cent reduction in treatment combination of *Melia azedarach* and *Trichoderma virens*. Treatment combination of *Datura stramonium* and *Bacillus licheniformis* was found next in efficacy with 38.49 per cent

reduction in seedling mortality. Seed biopriming with treatment combination of *Roylea elegans* and *Trichoderma harzianum* was found most effective with 52.27 per cent higher seed germination followed by conjoint application of *Melia azedarach* and *Trichoderma virens* with 46.28 per cent higher seed germination.

Seed biopriming with treatment combination of *Lantana camara* and *Trichoderma atroviride* was found least effective with only 51.52 per cent seed germination in capsicum seeds. Seed biopriming with conjoint application of *Melia azedarach* and *Trichoderma virens* was found most significant with 36.02 per cent increase in average shoot length followed by treatment combination of *Roylea elegans* and *Trichoderma harzianum* with 32.44 per cent increase. Further, seed biopriming with treatment combination of *Roylea elegans* and *Trichoderma harzianum* was found most effective with 50.94 per cent increase in average root length.

Dania and Omidora (2019) reported that conjoint application of biocontrol agents (*Trichoderma viride*, *Trichoderma harzianum*, *Bacillus subtilis*) and leaf extract of *Allium sativum* (25 %) were most effective in reducing damping-off incidence in comparison to untreated control. Gholve et al. (2014) also reported the efficacy of *Trichoderma viride* and garlic extract against damping-off disease in brinjal. Conjoint application of *Trichoderma harzianum* and garlic extract has also been reported effective against damping-off disease in bean (Khalifa et al. 2021). Efficacy of *Trichoderma harzianum* and neem extracts has been reported for the control of soil-borne pathogens by many researchers (Mnajula et al. 2004; Muthukumar et al. 2010; Khan et al. 2020).

## 7. Isolation and identification of endophytes

Endophytic microorganisms were isolated and further evaluated for their biocontrol potential against damping-off disease. Medicinal plants namely *Mentha* sp., *Ocimum* sp, *Datura stramonium*, *Vinca rosea, Hibiscus-rosa sinensis* were selected for isolation of endophytes from their leaves and stem. Isolated fungal endophytes (EF1 from leaves of *Mentha* sp., EF2 from leaves of *Datura stramonium* and EF3 from stem of *Vinca rosea*) and bacterial endophytes (EB1 and EB2 from leaves of *Hibiscus rosa sinensis*, EB3 from steam of *Datura stramonium,* EB4 from stem of *Vinca rosea* and EB5 from leaves of *Ocimum* sp.) were identified based upon their morphological, cultural and biochemical characters.

**Morphological and cultural characterization of fungal endophytes**

**EF1 (Fungal Endophyte 1)**

The culture of fungal endophyte EF1 was initially white in colour with presence of yellow pigment in center. The colour of mycelium in later stage turned greenish with aerial mycelial growth on PDA medium. Endophyte was fast growing and covered the entire Petri-plate (90 mm) in 6 days. Brown pigment was present on the reverse side of culture in later stage. Phialides measuring 5-7 µm in length were visible under microscope (400X) along with globular greenish conidia varying from 3.68-4.68 µm (Plate 19). Based upon the morphological and microscopic characters, the endophyte was identified as *Penicillium* sp. (Visagie et al. 2014).

**EF2 (Fungal Endophyte 2)**

The culture of fungal endophyte EF2 was pure white in colour with no pigmentation on the PDA medium. Endophyte was slow growing and it took around 9 days for covering the entire Petri-plate (90 mm). Microscopic observations showed the presence of hyaline septate mycelium with macro and micro conidia. Chlamydospores were also present which were terminal as well as intercalary of 8.15-11.26 µm size (Plate 19). Based upon the morphological and cultural characters, the endophyte was identified as *Fusarium oxysporum* (Kumar et al. 2013).

**EF3 (Fungal Endophyte 3)**

The culture of fungal endophyte EF3 was initially green which later turned black in colour with no pigmentation on the PDA medium. Endophyte was slow growing and it took 8 days for covering the entire Petri-plate (90 mm). Microscopic observations showed the presence of dark septate mycelium with dimensions of 14.78-20.45 µm long and 2.25-4.45 µm wide with presence of dark conidia of 2-3 septations of 15.23-23.43 µm size (Plate 19). Based upon the morphological and cultural characters, the endophyte was identified as *Curvularia* sp. (Khiralla et al. 2016).

**Biochemical characterization of bacterial endophytes**

Purified bacterial endophytes were later subjected to biochemical characterization. Out of five bacterial endophytes, only one was found gram positive (EB 4) while others were gram negative (Plate 20b). Bacterial endophyte EB4 showed negative results while others were positive for KOH test. With the exception of EB3 all other bacterial endophytes were oxidase negative. All the tested bacterial endophytes were catalase positive and were Methyl Red negative (Plate 20a, Plate 20b).

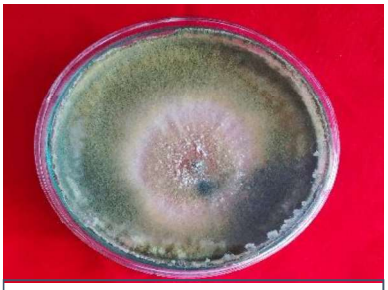
**8. *In vitro* evaluation of native endophytes against damping-off pathogens**

Antagonistic activity of the isolated and purified endophytes was tested against damping-off pathogens. Fungal and bacterial endophytes inhibited the mycelial growth of damping-off pathogens under *in-vitro* conditions ranging from 20.48 to 72.59 per cent (Table 27). Among all the endophytes, fungal endophyte EF1 was found most effective against damping-off pathogens followed by bacterial endophyte EB5 and EB3 (Plate 21).

Among all the endophytes evaluated against *Pythium* sp., EF1 (Endophyte Fungal 1) was found most effective with 94.44 per cent mycelial inhibition followed by EB 5 (Endophyte Bacterial 5) and EF3 with 48.40 and 36.35 per cent inhibition, respectively. Bacterial endophyte EB1 was found least effective with mycelial inhibition of 5.33 per cent. Further, EF1 also resulted in maximum (77.7 %) mycelial inhibition followed by 56.96 and 38.13 per cent inhibition with EB2 and EF2, respectively.

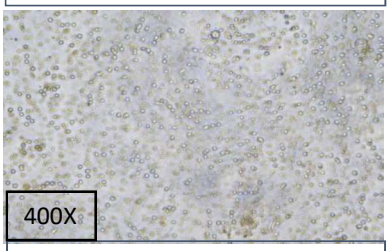
Endophyte EF1 resulted in maximum inhibition of 46.37 per cent against *Sclerotium rolfsii* followed by 34.66 per cent with EB3 and 33.21 per cent with EB5. However, EB 4 was found least effective against *Fusarium oxysporum* with mycelial inhibition of 13.87 and 4.59 per cent against *Sclerotium rolfsii*. Fungal endophyte EF1 resulted in maximum mycelial inhibition of 71.85 per cent against *Rhizoctonia solani* followed EB3 and EB4 with mycelial inhibition of 65.25 and 58.08 per cent, respectively. Fungal endophyte EF2 was found least effective against *Rhizoctonia solani* with mycelial inhibition of 34.33 per cent.

There are numerous reports of isolation of fungal and bacterial endophytic strains from medicinal plants (Khan et al. 2010; Huang et al. 2008; Nascimento et al. 2015). Khiralla et al. (2016) isolated different endophytic fungi viz. *Curvularia aeria*, *Cladosporium,*

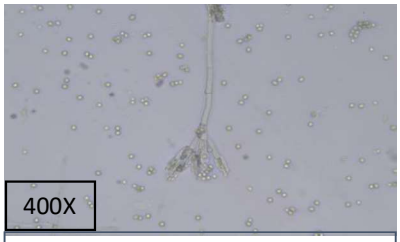


Pure culture

Brown pigment at dorsal side



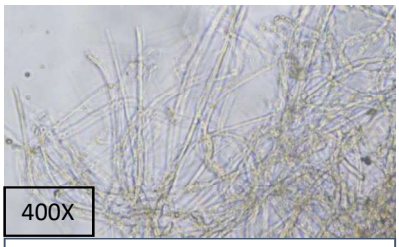

conidia



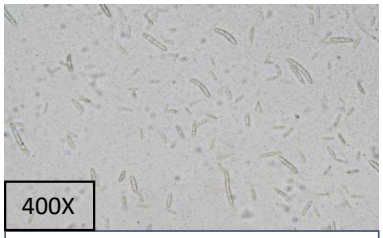

phialide

**EF1 (*Penicillium sp.*)**

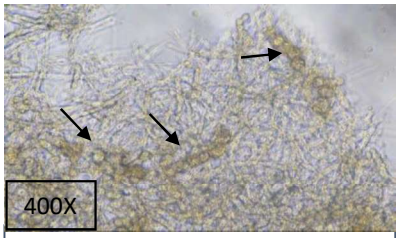
Pure culture




Septate mycelium



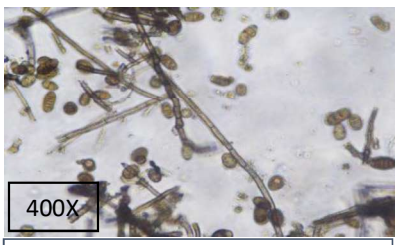

Macro and micro conidia



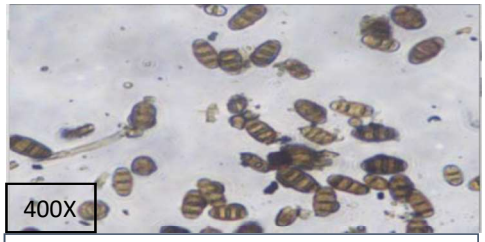

Chlamydospores

**EF2 (*Fusarium oxysporum*)**

Pure culture



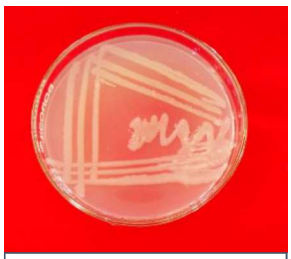

Septate mycelium



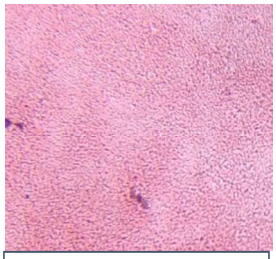

Pigmented septate conidia

**EF3 (*Curvularia* sp.)**

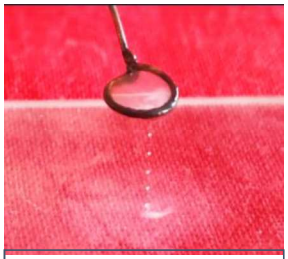
**Plate 19: Cultural and morphological characters of fungal endophytes**



Pure culture



Gram negative



KOH positive

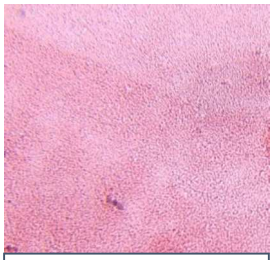
Oxidase negative

Catalase positive

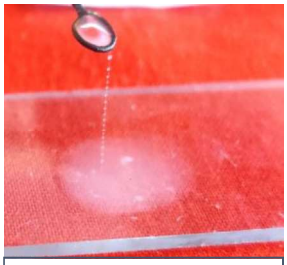
Methyl Red negative

**Bacterial endophyte 1 (EB 1)**

Pure culture

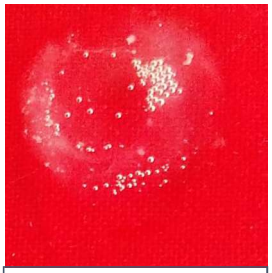


Gram negative



KOH positive

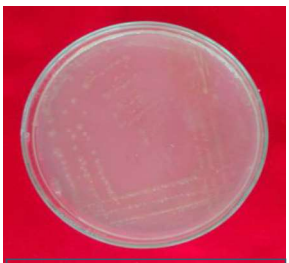
Oxidase negative



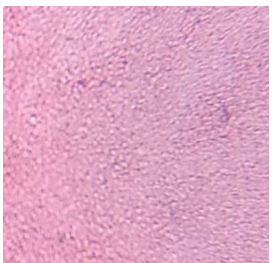
Catalase positive

Methyl Red negative

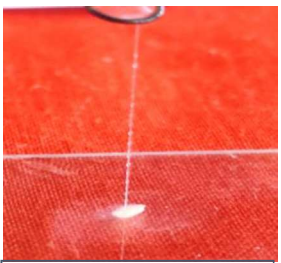
**Bacterial endophyte 2 (EB 2)**



Pure culture



Gram negative

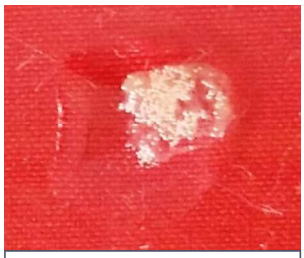


KOH positive

**Bacterial endophyte 3 (EB 3)**

**Plate 20a: Biochemical characterization of isolated bacterial endophytes**

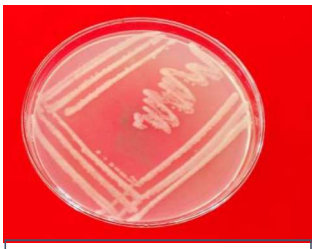
Oxidase positive



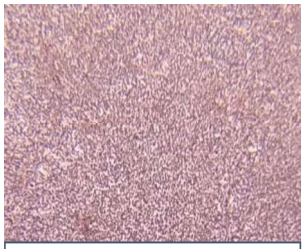
Catalase positive

Methyl Red negative

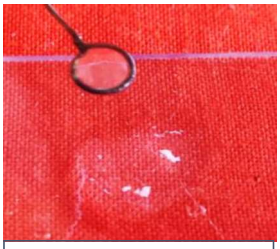
**Bacterial endophyte 3 (EB 3)**



Pure culture



Gram positive



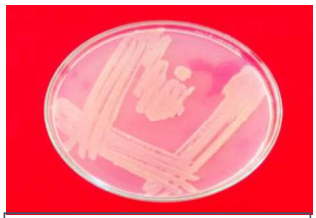
KOH negative

Oxidase negative

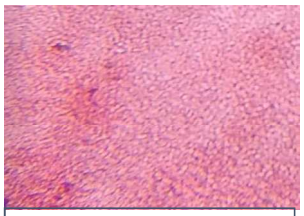
Catalase positive

Methyl Red negative

**Bacterial endophyte 4 (EB 4)**



Pure culture



Gram negative

KOH positive

Oxidase negative

Catalase positive

Methyl Red negative

**Bacterial endophyte 5 (EB 5)**

**Plate 20b: Biochemical characterization of isolated bacterial endophytes**

*Chaetomium, Aspergillus*, *Phoma*, *Penicillium*, *Alternaria* from medicinal plants and reported their antibiotic potential against different soil borne pathogens.

**Table 27: *In vitro* efficacy of native endophytes against damping-off pathogens**

| S. No | Treatments | Mycelial inhibition (%) | | | | |
|---|---|---|---|---|---|---|
| | | ***Pythium* sp.** | ***Fusarium oxysporum*** | ***Sclerotium rolfsii*** | ***Rhizoctonia solani*** | **Mean** |
| 1. | EF 1 | 94.44 (76.34) | 77.70 (61.80) | 46.37 (42.90) | 71.85 (57.93) | **72.59 (59.74)** |
| 2. | EF 2 | 20.56 (26.91) | 38.13 (38.11) | 15.56 (23.18) | 34.33 (35.84) | **27.15 (31.01)** |
| 3. | EF 3 | 36.35 (37.06) | 26.52 (30.98) | 23.89 (29.23) | 57.22 (49.15) | **35.99 (36.61)** |
| 4. | EB 1 | 5.33 (13.35) | 24.85 (29.88) | 12.89 (20.97) | 41.47 (40.07) | **21.13 (26.07)** |
| 5. | EB 2 | 25.74 (30.46) | 56.96 (48.98) | 17.17 (24.45) | 25.81 (30.52) | **31.42 (36.61)** |
| 6. | EB 3 | 26.35 (30.86) | 26.67 (31.05) | 34.66 (36.05) | 65.25 (53.86) | **38.23 (37.96)** |
| 7. | EB 4 | 5.36 (13.37) | 13.87 (21.86) | 4.59 (12.32) | 58.08 (49.63) | **20.48 (24.29)** |
| 8. | EB 5 | 48.40 (44.07) | 29.08 (32.61) | 33.21 (35.16) | 56.07 (48.46) | **41.69 (40.08)** |
| | **Mean** | **29.17 (30.27)** | **32.64 (32.81)** | **20.92 (24.92)** | **45.56 (40.60)** | |
| | **CD $_{(0.05)}$** | **1.98** | **1.90** | **2.61** | **2.25** | |

Figures in the parenthesis are arc sine transformed values

Kharwar et al. (2008) recovered different endophytic fungi namely *Curvularia*, *Chaetomium* and *Aspergillus* from *Catharanthus roseus*. Karunasinghe et al. (2020) isolated five isolates of endophytic fungi from medicinal plant *Sesuvium portulacastrum* which resulted in around 50 % suppression of *Pythium aphanidermatum* under dual culture test. Dunne et al. (2000) reported the biocontrol activity of *Stenotrophomonas maltophilia* W81 isolate against *Pythium ultimum.* Zouari et al. (2016) reported the biocontrol activity of endophytic bacteria *Bacillus amyloliquefaciens* against *Pythium aphanidermatum* causing damping-off in tomato.

. **Studies on compatibility between bacterial endophytes and bacterial biocontrol agents**

Compatibility studies were carried out between effective bacterial biocontrol agents and effective bacterial endophytes using disk diffusion method. Combinations of effective bacterial endophytes (EB2 and EB3) were found compatible with bacterial biocontrol agents

(*Pseudomonas fluorescence*, *Bacillus licheniformis*) with no zone of inhibition as both grew well together without any antagonism. However, combination of bacterial endophyte EB5 with *Pseudomonas fluoresecens* and EB5 with *Bacillus* sp. were found incompatible with each other as they had a clear zone of inhibition between them (Plate 22).

**Standardization of effective concentration of endophytes for seed biopriming**

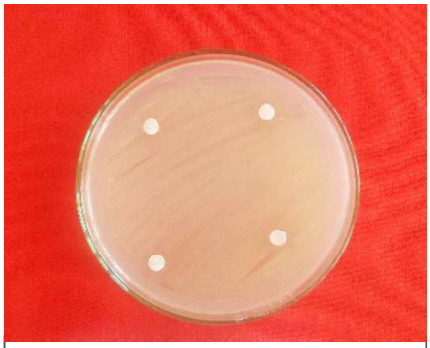
Effective endophytes were further evaluated in tomato, chilli and capsicum for the estimation of most effective concentration for seed biopriming out of the three concentrations of $10^6$ cfu per ml, $10^7$ cfu per ml and $10^8$ cfu per ml. Seed biopriming of tomato at $10^7$ cfu per ml with bacterial endophyte EB3 resulted in maximum seed germination of 93.33 per cent (Table 28, Plate 23).

Biopriming of tomato seeds with bacterial endophyte EB5 at $10^7$ cfu per ml was found next in efficacy with seed germination of 88.67 per cent followed by seed biopriming treatment with fungal endophyte EF3 at $10^8$ cfu per ml with germination of 86 per cent. Seed biopriming at $10^6$ cfu per ml concentration with bacterial endophyte EB3 was found least effective in tomato with germination of 71.0 per cent. Seed biopriming with endophytes, significantly increased the growth parameter *i.e.,* seedling length and seedling dry weight of the seedlings. Maximum seedling length was observed in treatment with bacterial endophyte EB3 at $10^6$ cfu per ml (13.59 cm) which was 19.79 per cent higher than control. Biopriming with EF3 at $10^8$ cfu per ml resulted in seedling length of 13.38 cm and both were found statistically at par with each other. Seed biopriming with fungal endophyte EF3 at $10^6$ cfu per ml was found least effective with seedling length of 10.19 cm.

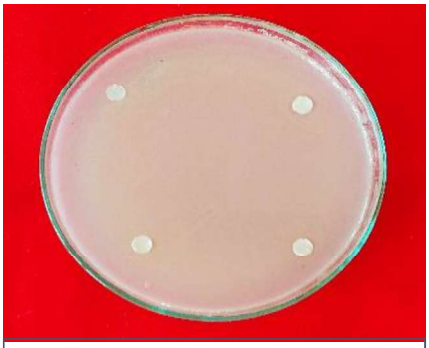
Seedling dry weight was found maximum (23.70 mg) at a concentration of $10^7$ cfu per ml with bacterial endophyte EB5 which was 67.93 per cent higher than control followed by biopriming treatment with fungal endophyte EF1 at similar concentration with seedling dry weight of 22.60 mg. SVI-L was found maximum (1161.11) in seed biopriming with fungal endophyte EF1 at $10^8$ cfu per ml which was 45.55 per cent higher than control followed by SVI-M of 1152.10 in seed biopriming with fungal endophyte EF3 at similar concentration. Seedling vigour index-M was found maximum (2104.70) at $10^7$ cfu per ml with bacterial endophyte EB3 followed SVI-M of 2099.27 in bacterial endophyte EB5 at similar concentration.

| | | |
|---|---|---|
| **EF 1 + *Pythium* sp.** | **EF 1 + *Fusarium oxysporum*** | **EF 1 + *Sclerotium rolfsii*** |
| **EF 1 + *Rhizoctonia solani*** | **EB 3 + *Pythium* sp.** | **EB 3 + *Fusarium oxysporum*** |
| **EB 3 + *Sclerotium rolfsii*** | **EB 3 + *Rhizoctonia solani*** | **EB 5 + *Pythium* sp.** |
| **EB 5 + *Fusarium oxysporum*** | **EB 5 + *Sclerotium rolfsii*** | **EB 5 + *Rhizoctonia solani*** |

**Plate 21: *In vitro* efficacy of native endophytes against damping-off pathogens**

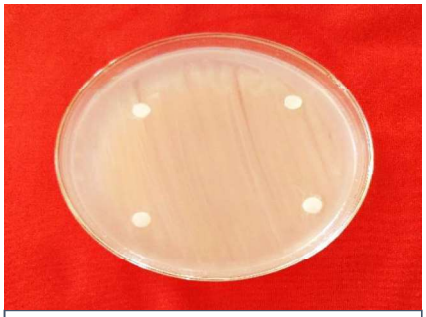


*Bacillus licheniformis*

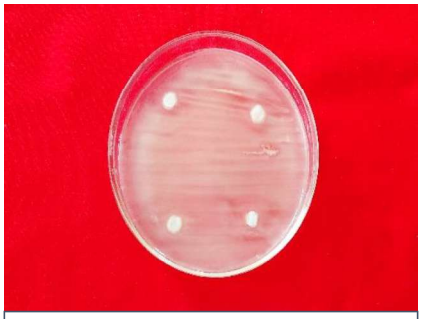


*Pseudomonas fluorescens*

**Bacterial endophyte 2 (EB 2)**

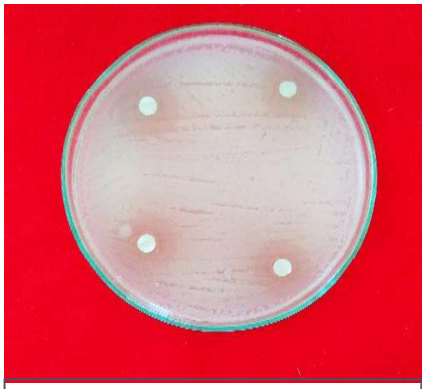


*Bacillus licheniformis*

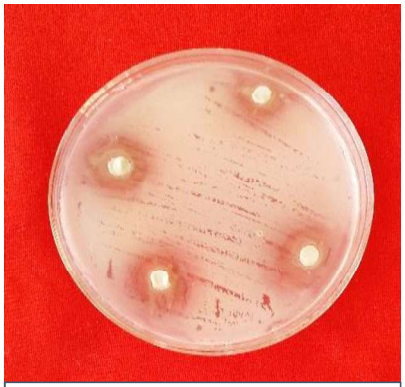


*Pseudomonas fluorescens*

**Bacterial endophyte 3 (EB 3)**



*Bacillus* sp.



*Pseudomonas fluorescens*

**Bacterial endophyte 5 (EB 5)**

**Plate 22: Compatibility between bacterial biocontrol agents and bacterial endophytes**

**Table 28: Standardization of effective concentration of endophytes for seed biopriming in tomato**

| S. No. | Biopriming treatments | Seed germination (%) | Seedling length (cm) | Seedling dry weight (mg) | Seedling Vigour Index-L | Seedling Vigour Index-M |
|---|---|---|---|---|---|---|
| 1. | EF 1 $10^6$ cfu per ml | 78.00 (62.01) | 14.05 | 22.60 | 1095.75 | 1761.53 |
| 2. | EF 1 $10^7$ cfu per ml | 65.00 (53.72) | 13.34 | 21.17 | 868.14 | 1376.13 |
| 3. | EF 1 $10^8$ cfu per ml | 85.00 (67.19) | 13.67 | 10.73 | 1161.11 | 912.47 |
| 4. | EF 3 $10^6$ cfu per ml | 81.33 (64.39) | 10.19 | 10.73 | 828.20 | 872.73 |
| 5. | EF 3 $10^7$ cfu per ml | 74.67 (59.76) | 12.79 | 15.53 | 955.16 | 1158.97 |
| 6. | EF 3 $10^8$ cfu per ml | 86.00 (68.02) | 13.38 | 15.40 | 1152.10 | 1327.30 |
| 7. | EB 2 $10^6$ cfu per ml | 74.33 (59.55) | 11.11 | 12.87 | 826.10 | 956.83 |
| 8. | EB 2 $10^7$ cfu per ml | 85.67 (67.76) | 11.07 | 13.60 | 949.98 | 1164.10 |
| 9. | EB 2 $10^8$ cfu per ml | 85.00 (67.19) | 11.51 | 10.80 | 977.29 | 918.07 |
| 10. | EB 3 $10^6$ cfu per ml | 71.00 (57.39) | 13.59 | 11.53 | 965.70 | 818.93 |
| 11. | EB 3 $10^7$ cfu per ml | 93.33 (75.04) | 11.74 | 22.55 | 1095.27 | 2104.70 |
| 12. | EB 3 $10^8$ cfu per ml | 85.67 (67.76) | 11.80 | 14.10 | 1010.95 | 1208.97 |
| 13. | EB 5 $10^6$ cfu per ml | 83.67 (66.17) | 13.45 | 14.33 | 1125.81 | 1198.00 |
| 14. | EB 5 $10^7$ cfu per ml | 88.67 (70.36) | 11.76 | 23.70 | 1042.64 | 2099.27 |
| 15. | EB 5 $10^8$ cfu per ml | 76.00 (75.04) | 11.10 | 22.03 | 843.92 | 1673.57 |
| 16. | Hydropriming | 75.67 (60.43) | 11.62 | 9.03 | 879.77 | 684.83 |
| 17. | Control | 58.00 (49.58) | 10.90 | 7.60 | 632.20 | 440.80 |
| | **CD $_{(0.05)}$** | **2.20** | **1.36** | **2.37** | **122.11** | **195.34** |

Figures in the parenthesis are arc sine transformed values

In chilli, highest seed germination of 95.67 per cent was recorded $10^8$ cfu per ml with seed biopriming with fungal endophyte EF1 (Table 29. Plate 24). Biopriming with bacterial endophyte EB5 at $10^7$ cfu per ml was found next in efficacy with germination of 90.67 per cent followed by biopriming with bacterial endophyte EB3 at similar concentration with germination of 88.0 per cent, respectively. Hydropriming treatment was found least effective with seed germination of 64 per cent followed by biopriming at $10^6$ cfu per ml with bacterial endophyte EB2 with germination of 71.33 per cent. Maximum seedling length of 12.59 cm was observed in seed biopriming with bacterial endophyte EB5 at $10^7$ cfu per ml followed by biopriming with EF1 with at $10^8$ cfu per ml with seedling length of 11.86 cm.

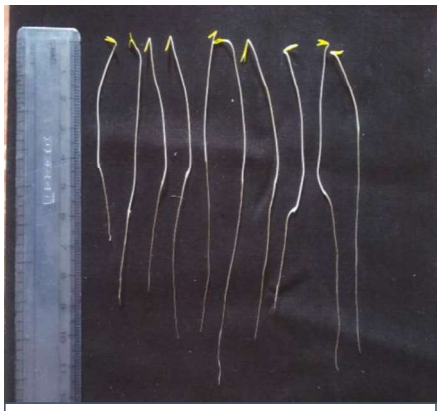
**Table 29: Standardization of effective concentration of endophytes for seed biopriming in chilli**

| S. No. | Biopriming treatments | Seed germination (%) | Seedling length (cm) | Seedling dry weight (mg) | Seedling Vigour Index-L | Seedling Vigour Index-M |
|---|---|---|---|---|---|---|
| 1. | EF 1 $10^6$ cfu per ml | 73.33 (58.89) | 9.45 | 34.40 | 693.18 | 2523.20 |
| 2. | EF 1 $10^7$ cfu per ml | 82.33 (65.13) | 10.55 | 24.83 | 866.89 | 2046.07 |
| 3. | EF 1 $10^8$ cfu per ml | 95.67 (77.97) | 11.86 | 15.47 | 1134.93 | 1480.07 |
| 4. | EF 3 $10^6$ cfu per ml | 67.33 (55.13) | 9.31 | 23.47 | 625.74 | 1578.53 |
| 5. | EF 3 $10^7$ cfu per ml | 75.67 (60.47) | 10.55 | 9.57 | 797.94 | 722.37 |
| 6. | EF 3 $10^8$ cfu per ml | 83.00 (65.64) | 10.75 | 25.50 | 892.69 | 2114.10 |
| 7. | EB 2 $10^6$ cfu per ml | 71.33 (57.61) | 10.00 | 32.03 | 713.93 | 2285.07 |
| 8. | EB 2 $10^7$ cfu per ml | 82.33 (65.15) | 9.84 | 16.60 | 809.25 | 1362.47 |
| 9. | EB 2 $10^8$ cfu per ml | 76.33 (60.87) | 11.50 | 34.10 | 877.94 | 2604.23 |
| 10. | EB 3 $10^6$ cfu per ml | 77.00 (61.34) | 9.93 | 16.20 | 764.47 | 1246.53 |
| 11. | EB 3 $10^7$ cfu per ml | 88.00 (66.44) | 9.37 | 18.33 | 787.43 | 1540.63 |
| 12. | EB 3 $10^8$ cfu per ml | 84.00 (69.74) | 10.94 | 17.77 | 963.12 | 1562.73 |
| 13. | EB 5 $10^6$ cfu per ml | 84.67 (66.92) | 11.14 | 21.43 | 943.22 | 1813.76 |
| 14. | EB 5 $10^7$ cfu per ml | 90.67 (72.26) | 12.59 | 14.77 | 1140.80 | 1350.37 |
| 15. | EB 5 $10^8$ cfu per ml | 86.67 (68.59) | 10.46 | 17.67 | 907.07 | 1532.83 |
| 16. | Hydropriming | 64.00 (53.11) | 10.85 | 9.03 | 694.41 | 578.87 |
| 17. | Control | 58.00 (49.58) | 10.90 | 5.60 | 632.20 | 324.80 |
| | **CD $_{(0.05)}$** | **2.49** | **0.93** | **3.44** | **76.32** | **284.40** |

Figures in the parenthesis are arc sine transformed values

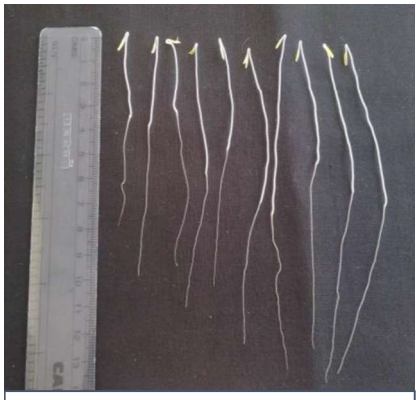
Seed biopriming with fungal endophyte EF3 at $10^6$ cfu per ml was found least effective with seedling length of 9.31 cm. Seedling dry weight was found maximum (34.40 mg) in biopriming treatment at $10^6$ cfu per ml with fungal endophyte EF1 followed by seedling dry weight of 34.16 mg in biopriming treatment with bacterial endophyte EB2 at $10^8$ cfu per ml. SVI-L was found maximum (1140.80) in seed biopriming at $10^7$ cfu per ml with bacterial endophyte EB5 followed by SVI-L of 1134.93 in seed biopriming at $10^8$ cfu per ml concentration with fungal endophyte EF1. SVI-M was found maximum (2604.23) at $10^8$ cfu per ml in biopriming treatment with bacterial endophyte EB2 followed by SVI-M of 2523.20 in treatment with fungal endophyte EF1 at $10^6$ cfu per ml.

In capsicum, biopriming treatment with bacterial endophyte EB5 resulted in maximum seed germination of 89.0 per cent (Table 30, Plate 25). Biopriming with bacterial

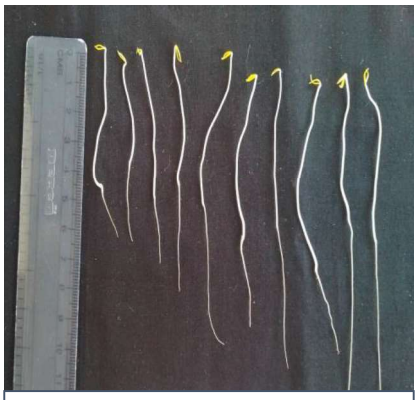


EF 1 ($10^8$ cfu per ml ) 12 hrs

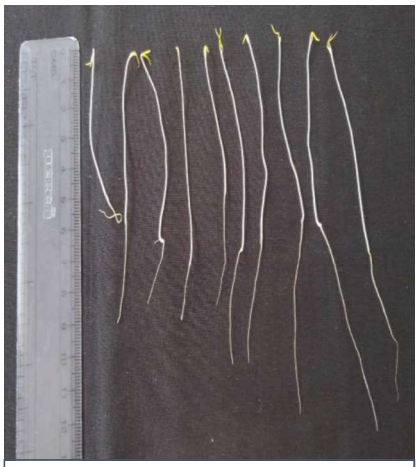
EF 3 ($10^8$ cfu per ml ) 12 hrs



EB 2 ($10^7$ cfu per ml ) 12 hrs

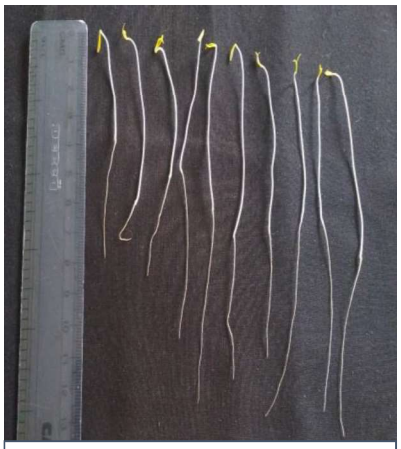


EB 3 ($10^7$ cfu per ml ) 12 hrs

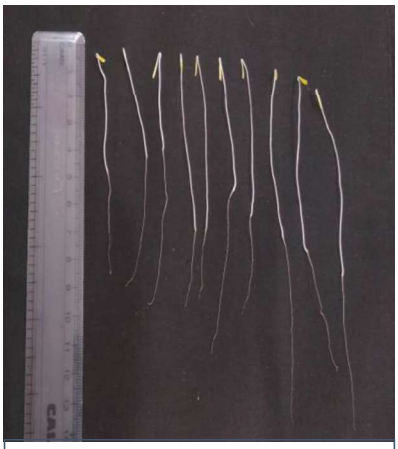


EB 5 ($10^7$ cfu per ml ) 12 hrs

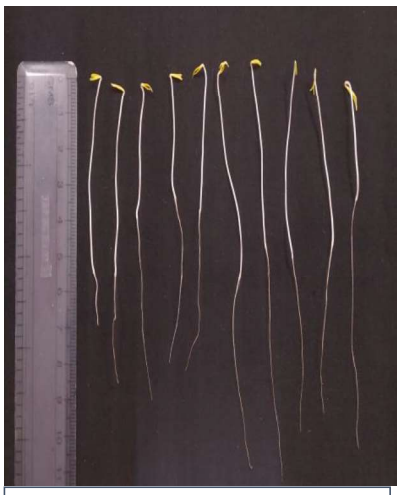
**Plate 23: Standardization of effective concentration of suspensions of endophytes and duration for seed biopriming in tomato**



EF 1 ($10^8$ cfu per ml ) 12 hrs

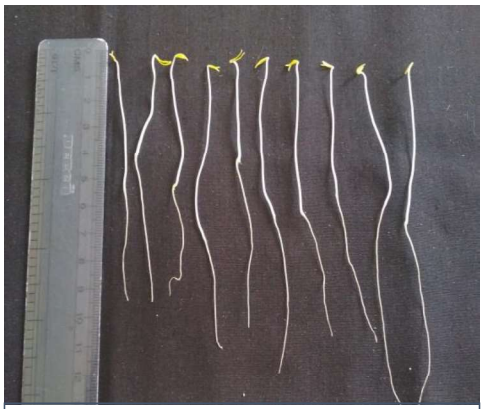


EF 3 ($10^8$ cfu per ml ) 12 hrs



EB 2 ($10^7$ cfu per ml ) 12 hrs

EB 3 ($10^7$ cfu per ml ) 12 hrs



EB 5 ($10^7$ cfu per ml ) 12 hrs

**Plate 24: Standardization of effective concentration of suspensions of endophytes and duration for seed biopriming in chilli**

endophyte EB3 at $10^6$ cfu per ml was found next in efficacy with seed germination of 86.33 per cent and this was followed by treatment of biopriming with EF3 at $10^8$ cfu per ml in which seed germination of 85.0 per cent was recorded. Seed biopriming with bacterial endophyte EB2 at $10^8$ cfu per ml was found least effective with germination of 61.67 per cent.

**Table 30: Standardization of effective concentration of effective endophytes for biopriming in capsicum**

| S. No. | Biopriming treatments | Seed germination (%) | Seedling length (cm) | Seedling dry weight (mg) | Seedling Vigour Index-L | Seedling Vigour Index-M |
|---|---|---|---|---|---|---|
| 1. | EF 1 $10^6$ cfu per ml | 78.67 (62.51) | 13.28 | 15.47 | 1045.78 | 1216.67 |
| 2. | EF 1 $10^7$ cfu per ml | 67.67 (55.33) | 9.59 | 23.83 | 649.17 | 1610.57 |
| 3. | EF 1 $10^8$ cfu per ml | 83.33 (65.91) | 9.86 | 33.40 | 822.11 | 2780.40 |
| 4. | EF 3 $10^6$ cfu per ml | 71.33 (57.63) | 10.91 | 24.57 | 778.32 | 1752.23 |
| 5. | EF 3 $10^7$ cfu per ml | 68.33 (55.74) | 10.82 | 17.23 | 739.48 | 1176.67 |
| 6. | EF 3 $10^8$ cfu per ml | 85.00 (67.19) | 10.78 | 28.10 | 916.30 | 2388.77 |
| 7. | EB 2 $10^6$ cfu per ml | 73.33 (58.91) | 8.81 | 16.27 | 646.75 | 1193.83 |
| 8. | EB 2 $10^7$ cfu per ml | 73.67 (66.15) | 10.43 | 22.93 | 872.04 | 1917.63 |
| 9. | EB 2 $10^8$ cfu per ml | 61.67 (51.73) | 10.42 | 20.53 | 642.65 | 1265.50 |
| 10. | EB 3 $10^6$ cfu per ml | 76.67 (61.11) | 10.52 | 18.60 | 806.66 | 1426.97 |
| 11. | EB 3 $10^7$ cfu per ml | 86.33 (68.33) | 9.54 | 22.67 | 823.18 | 1954.80 |
| 12. | EB 3 $10^8$ cfu per ml | 72.67 (58.47) | 11.14 | 19.27 | 808.91 | 1399.50 |
| 13. | EB 5 $10^6$ cfu per ml | 83.67 (66.17) | 8.63 | 18.60 | 721.60 | 1556.07 |
| 14. | EB 5 $10^7$ cfu per ml | 89.00 (70.75) | 11.79 | 33.63 | 1048.36 | 2993.50 |
| 15. | EB 5 $10^8$ cfu per ml | 83.00 (65.63) | 9.64 | 18.73 | 799.96 | 1554.97 |
| 16. | Hydropriming | 67.67 (55.33) | 9.45 | 22.33 | 639.74 | 1512.93 |
| 17. | Control | 52.00 (46.13) | 10.37 | 12.10 | 539.24 | 629.20 |
| | **CD $_{(0.05)}$** | **2.87** | **0.81** | **2.62** | **75.57** | **209.75** |

Figures in the parenthesis are arc sine transformed values

Seed biopriming with fungal endophyte EF1 at concentration of $10^8$ cfu per ml resulted in maximum seedling length of 13.28 cm followed by seed biopriming at $10^7$ cfu per ml with bacterial endophyte EB5 with seedling length of 11.79 cm (Plate 28). Seed biopriming with bacterial endophyte EB5 at $10^6$ cfu per ml was found least effective with seedling length of 8.63 cm. Seedling dry weight was found maximum (33.63 mg) in biopriming with bacterial endophyte EB5 at $10^7$ cfu per ml concentration followed by

biopriming with fungal endophyte EF1 at $10^6$ cfu per ml concentration (33.40 mg). SVI-L was found maximum (1048.36) in seed biopriming with bacterial endophyte EB5 at $10^8$ cfu per ml concentration followed by fungal endophyte EF1 at $10^7$ cfu per ml concentration (1045.78). SVI-M was found maximum (2993.50) in seed biopriming with bacterial endophyte EB5 at $10^7$ cfu per ml concentration followed by fungal endophyte EF1 at $10^6$ cfu per ml concentration (2780.40).

**Standardization of duration of seed biopriming with endophyte suspension**

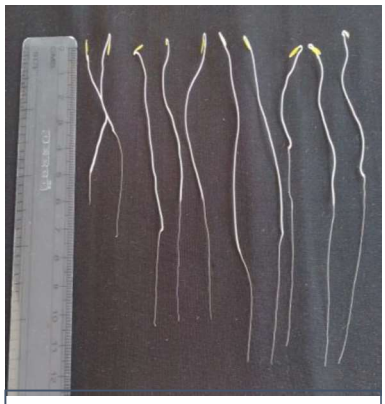
Biopriming of tomato seeds with endophytes for 12 hrs duration was found most effective in improving seed health attributes in comparison to effect of biopriming treatments for 6 and 8 hrs. Three durations of biopriming, and their interactions had statistically significant differences in the seed germination percentage, seedling length, seedling dry weight and seedling vigour indexes in different biopriming treatments.

**Table 31: Effect of duration of seed biopriming with endophytes on seed germination and seedling length in tomato**

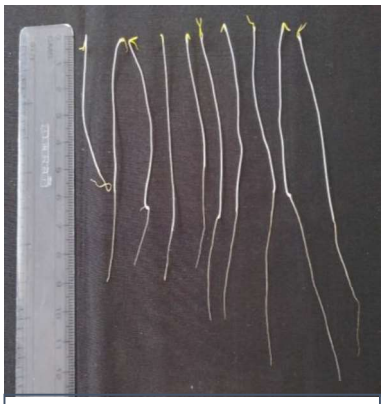
| S. No. | Treatments | Seed germination (%) | | | | Seedling length (cm) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 6h | 8h | 12h | Mean | 6h | 8h | 12h | Mean |
| **1.** | EF1 | 75.00 (59.97) | 79.33 (62.95) | 92.33 (73.98) | **82.22 (65.64)** | 13.00 | 15.21 | 17.80 | **15.34** |
| **2.** | EF3 | 73.67 (59.11) | 77.33 (61.56) | 84.67 (66.93) | **78.56 (62.53)** | 11.19 | 10.15 | 13.6 | **11.65** |
| **3.** | EB2 | 63.67 (52.93) | 66.00 (54.31) | 77.00 (61.34) | **68.89 (56.19)** | 10.84 | 9.74 | 11.44 | **10.68** |
| **4.** | EB3 | 72.00 (58.05) | 85.67 (67.76) | 88.33 (70.08) | **82 (65.29)** | 11.80 | 12.59 | 12.41 | **12.27** |
| **5.** | EB5 | 92.33 (73.95) | 86.33 (68.29) | 90.33 (72.17) | **89.67 (71.47)** | 11.43 | 10.77 | 15.13 | **12.44** |
| **6.** | Hydropriming | 75.67 (60.43) | 79.00 (62.75) | 86.33 (68.29) | **80.33 (63.83)** | 10.69 | 11.28 | 11.29 | **11.09** |
| **7.** | Control | 62.00 (51.92) | 62.00 (51.92) | 62.00 (51.92) | **62.00 (51.92)** | 10.90 | 10.90 | 10.90 | **10.90** |
| | Mean | **73.48 (59.49)** | **76.52 (61.36)** | **83.00 (66.39)** | | **11.41** | **11.52** | **13.23** | |
| | **CD $_{(0.05)}$** | | | | | | | | |
| | **Treatment (T)** | **1.73** | | | | **0.89** | | | |
| | **Duration (D)** | **1.14** | | | | **0.58** | | | |
| | **T X D** | **3.00** | | | | **1.55** | | | |

Figures in the parenthesis are arc sine transformed values

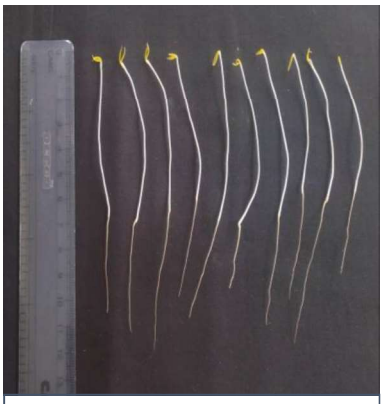
Tomato seeds bioprimed for 12 hrs with suspensions of fungal endophyte EF1 at $10^8$ cfu per ml concentration resulted in maximum germination of 92.33 per cent followed by



EF 1 ($10^8$ cfu per ml ) 12 hrs

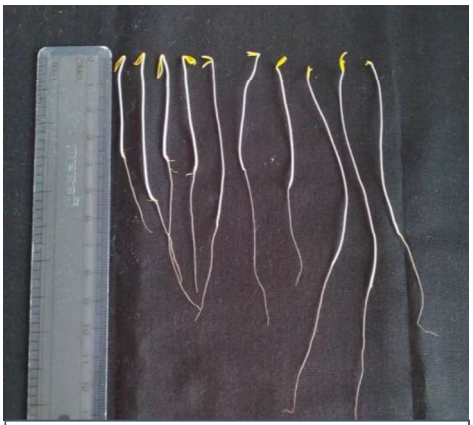


EF 3 ($10^8$ cfu per ml ) 12 hrs



EB 2 ($10^7$ cfu per ml ) 12 hrs

EB 3 ($10^7$ cfu per ml ) 12 hrs



EB 5 ($10^7$ cfu per ml ) 12 hrs

**Plate 25: Standardization of effective concentration of suspensions of endophytes and duration for seed biopriming in capsicum**

**Table 32: Effect of duration of seed biopriming with endophytes on seedling dry weight and seedling vigour in tomato**

| S. No. | Treatments | Seedling dry weight (mg) | | | | Seedling vigour Index-L | | | | Seedling vigour Index-M | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 6h | 8h | 12h | Mean | 6h | 8h | 12h | Mean | 6h | 8h | 12h | Mean |
| **1.** | EF1 | 8.33 | 32.53 | 21.93 | **20.93** | 978.75 | 1206.22 | 1644.19 | **1276.39** | 627.00 | 2583.46 | 2027.13 | **1745.87** |
| **2.** | EF3 | 9.47 | 15.70 | 15.63 | **13.60** | 825.92 | 785.23 | 1151.74 | **920.96** | 696.60 | 1217.40 | 1322.40 | **1078.80** |
| **3.** | EB2 | 12.47 | 13.27 | 11.77 | **12.50** | 686.83 | 643.06 | 881.28 | **737.05** | 793.07 | 874.63 | 905.47 | **857.72** |
| **4.** | EB3 | 12.33 | 14.10 | 14.33 | **13.59** | 849.61 | 1078.81 | 1096.53 | **1008.32** | 888.00 | 1208.93 | 1266.17 | **1121.03** |
| **5.** | EB5 | 14.33 | 14.03 | 24.03 | **17.47** | 1054.97 | 929.81 | 1366.50 | **1117.09** | 1321.33 | 1214.03 | 2158.50 | **1564.62** |
| **6.** | Hydropriming | 9.03 | 12.50 | 13.83 | **11.79** | 808.87 | 777.43 | 975.18 | **853.83** | 684.83 | 989.33 | 1193.10 | **955.76** |
| **7.** | Control | 8.60 | 8.60 | 8.60 | **8.60** | 675.80 | 675.80 | 675.80 | **675.80** | 533.20 | 533.20 | 533.20 | **533.20** |
| | **Mean** | **10.62** | **15.82** | **15.73** | | **840.11** | **887.19** | **1113.03** | | **792.01** | **1231.57** | **1343.71** | |
| | **$CD_{(0.05)}$**<br>**Treatment (T)**<br>**Duration (D)**<br>**T X D** | **1.97**<br>**1.29**<br>**3.42** | | | | **74.70**<br>**48.91**<br>**129.39** | | | | **166.18**<br>**108.75**<br>**287.72** | | | |

seed biopriming with bacterial endophyte EB5 at $10^7$ cfu per ml with germination of 90.33 per cent for 12 hrs duration of seed biopriming (Table 31, Plate 23). Seed biopriming for 6 hrs with bacterial endophyte EB2 at $10^7$ cfu per ml was found least effective with seed germination of 63.67 per cent in comparison to non-primed seeds with seed germination of 62 per cent. It means that as the duration of seed biopriming was increased from 6 to 12 hrs, the seed germination also improved.

Seed biopriming duration also has positive impact on seedling growth and maximum seedling length (17.80 cm) was recorded in biopriming treatment for 12 hrs with fungal endophyte EF1 at $10^8$ cfu per ml followed by seed biopriming with bacterial endophyte EB5 at $10^7$ cfu per ml for 12 hrs (15.13 cm). Least seedling growth of tomato seedlings was observed in biopriming treatment with bacterial endophyte EB2 at $10^7$ cfu per ml for 8 hrs (9.74 cm). Seeds bioprimed for 8 hrs with fungal endophyte EF1 at $10^8$ cfu per ml had accumulated maximum biomass (32.53 mg) followed by 24.03 mg of dry weight of seedling in seed biopriming for 12 hrs with bacterial endophyte EB5. Seedling dry weight found was least (8.6 mg) in non-primed seeds (Table 32). Biopriming of tomato seeds for 12 hrs with fungal endophyte EF1 at $10^8$ cfu per ml resulted in SVI-L of 1644.19 which was 73.69 per cent higher than control. SVI-M was observed maximum (2583.46) in seed biopriming for 8 hrs with fungal endophyte EF1 at $10^8$ cfu per ml in comparison to non-primed seeds with value of 533.20.

Biopriming of chilli seeds for 12 hrs with fungal endophyte EF1 at concentration of $10^8$ cfu perml resulted in maximum seed germination of 95.0 per cent followed by 92.0 per cent germination in seed biopriming with bacterial endophyte EB5 at $10^7$ cfu per ml for similar duration (Table 33, Plate 24). Seed biopriming for 6 hrs with bacterial endophyte EB2 at $10^7$ cfu per ml concentration was found least effective with seed germination of 66.67 per cent in comparison to non-primed seeds with seed germination of 65.0 per cent. Maximum seedling length of 11.88 cm was recorded in biopriming with fungal endophyte EF1 at $10^8$ cfu per ml concentration for 8 hrs followed by seedling growth of 11.5 cm in seed biopriming with bacterial endophyte EB2 at $10^7$ cfu per ml for similar duration.

Biopriming of chilli seeds for 12 hrs with fungal endophyte EF1 at $10^8$ cfu per ml concentration resulted in maximum biomass (45.47 mg) followed by seed biopriming for 8 hrs with bacterial endophyte EB5 at $10^7$ cfu per ml which has biomass of 44.97 mg. Seedling

dry weight was least (20.1 mg) in non-primed seeds of chilli. Biopriming of chilli seeds for 12 hrs with bacterial endophyte EB5 at $10^7$ cfu per ml resulted in maximum (1129.83) SVI-L which was 37.87 per cent higher in comparison to control.

**Table 33: Effect of duration of seed biopriming with endophytes on seed germination and seedling length in chilli**

| S. No. | Treatments | Seed germination (%) | | | | Seedling length (cm) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 6h | 8h | 12h | Mean | 6h | 8h | 12h | Mean |
| **1.** | EF1 | 73.33 (58.89) | 83.00 (65.67) | 95.00 (77.09) | **83.78 (67.22)** | 10.45 | 11.88 | 10.85 | **11.06** |
| **2.** | EF3 | 67.33 (55.13) | 75.00 (60.02) | 82.67 (65.39) | **75.00 (60.18)** | 8.67 | 10.55 | 9.42 | **9.54** |
| **3.** | EB2 | 66.67 (52.92) | 83.33 (65.91) | 80.00 (63.52) | **76.67 (60.78)** | 9.73 | 11.5 | 9.84 | **10.36** |
| **4.** | EB3 | 81.67 (64.65) | 84.67 (67.01) | 84.33 (66.68) | **83.56 (66.11)** | 10.26 | 9.37 | 10.61 | **10.08** |
| **5.** | EB5 | 85.00 (67.19) | 87.67 (69.46) | 92.00 (73.56) | **88.22 (70.07)** | 8.84 | 9.46 | 12.28 | **10.19** |
| **6.** | Hydropriming | 66.00 (53.11) | 67.00 (54.93) | 70.67 (57.19) | **67.89 (55.08)** | 8.26 | 9.69 | 10.94 | **9.63** |
| **7.** | Control | 65.00 (53.71) | 65.00 (53.71) | 65.00 (53.71) | **65.00 (53.71)** | 10.80 | 10.80 | 10.80 | **10.80** |
| | Mean | **72.14 (57.94)** | **77.95 (62.39)** | **81.38 (65.31)** | | **9.57** | **10.45** | **10.68** | |
| | **CD $_{(0.05)}$** | | | | | | | | |
| | **Treatment (T)** | **1.58** | | | | **0.60** | | | |
| | **Duration (D)** | **1.04** | | | | **0.39** | | | |
| | **T X D** | **2.74** | | | | **1.04** | | | |

Figures in the parenthesis are arc sine transformed values

SVI-M was found maximum (4318.33) in seed biopriming for 12 hrs duration in fungal endophyte EF1 at $10^8$ cfu per ml in comparison to non-primed seeds with value of 1306.50 (Table 34).

**Table 34: Effect of duration of seed biopriming with endophytes on seedling dry weight and seedling vigour in chilli**

| S. No. | Treatments | Seedling dry weight (mg) | | | | Seedling vigour Index-L | | | | Seedling vigour Index-M | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 6h | 8h | 12h | Mean | 6h | 8h | 12h | Mean | 6h | 8h | 12h | Mean |
| **1.** | EF1 | 35.07 | 35.87 | 45.47 | **38.80** | 766.51 | 985.23 | 1030.37 | **927.38** | 2571.20 | 2979.47 | 4318.33 | **3289.67** |
| **2.** | EF3 | 23.47 | 27.07 | 35.80 | **28.78** | 583.70 | 790.86 | 775.89 | **716.82** | 1578.53 | 2024.87 | 2956.97 | **2186.89** |
| **3.** | EB2 | 32.03 | 36.17 | 37.90 | **35.37** | 619.82 | 958.67 | 787.22 | **788.57** | 2040.04 | 3013.37 | 3030.50 | **2694.64** |
| **4.** | EB3 | 26.20 | 28.83 | 32.53 | **29.19** | 837.78 | 792.94 | 895.82 | **842.18** | 2137.87 | 2438.33 | 2742.63 | **2588.67** |
| **5.** | EB5 | 40.60 | 44.97 | 46.63 | **44.07** | 751.97 | 829.81 | 1129.83 | **903.87** | 3451.00 | 3941.90 | 4289.13 | **3675.66** |
| **6.** | Hydropriming | 21.77 | 23.73 | 25.60 | **23.70** | 529.20 | 649.72 | 773.05 | **650.66** | 1393.07 | 1590.27 | 1809.60 | **1597.64** |
| **7.** | Control | 20.10 | 20.10 | 20.10 | **20.10** | 702.00 | 702.00 | 702.00 | **702.00** | 1306.50 | 1306.50 | 1306.50 | **1306.50** |
| | Mean | **28.46** | **30.96** | **34.86** | | **684.43** | **815.61** | **870.59** | | **2068.31** | **2470.67** | **2921.95** | |
| | **$CD_{(0.05)}$**<br>**Treatment (T)**<br>**Duration (D)**<br>**T X D** | **1.58**<br>**1.04**<br>**2.75** | | | | **52.31**<br>**34.25**<br>**90.60** | | | | **128.42**<br>**84.07**<br>**222.44** | | | |

**Table 35: Effect of duration of seed biopriming with endophytes on seed germination and seedling length in capsicum**

| S. No. | Treatments | Seed germination (%) | | | | Seedling length (cm) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 6h | 8h | 12h | Mean | 6h | 8h | 12h | Mean |
| **1.** | EF1 | 86.00 (68.02) | 87.67 (69.47) | 91.67 (73.28) | **88.44 (70.26)** | 10.57 | 11.4a | 13.53 | **11.84** |
| **2.** | EF3 | 73.00 (58.69) | 74.33 (59.58) | 82.67 (65.41) | **76.67 (61.23)** | 7.80 | 11.83 | 10.44 | **10.02** |
| **3.** | EB2 | 73.33 (58.91) | 75.00 (59.97) | 85.33 (67.51) | **77.89 (62.13)** | 8.47 | 10.43 | 11.42 | **10.11** |
| **4.** | EB3 | 66.00 (54.31) | 76.67 (61.11) | 82.33 (65.16) | **75 (60.19)** | 8.83 | 10.86 | 9.47 | **9.72** |
| **5.** | EB5 | 83.67 (66.15) | 83.67 (66.15) | 86.00 (68.01) | **84.44 (66.77)** | 8.96 | 9.64 | 8.79 | **9.13** |
| **6.** | Hydropriming | 67.67 (66.15) | 63.67 (52.91) | 73.33 (58.89) | **68.22 (55.71)** | 9.45 | 10.32 | 11.32 | **10.37** |
| **7.** | Control | 52.00 (46.13) | 52.00 (46.13) | 52.00 (46.13) | **52.00 (46.13)** | 6.80 | 6.80 | 6.80 | **6.80** |
| | **Mean** | **71.67 (58.22)** | **73.29 (59.33)** | **79.05 (63.48)** | | **8.70** | **10.18** | **10.26** | |
| | **CD (0.05)** | | | | | | | | |
| | **Treatment (T)** | **1.45** | | | | **0.43** | | | |
| | **Duration (D)** | **0.95** | | | | **0.28** | | | |
| | **T X D** | **2.52** | | | | **0.75** | | | |

Figures in the parenthesis are arc sine transformed values

Seeds of capsicum bioprimed for 12 hrs with fungal endophyte EF1 at $10^8$ cfu per ml concentration resulted in maximum seed germination of 91.67 per cent followed by the treatment of seed biopriming for 8 hrs with fungal endophyte EF1 at $10^7$ cfu per ml concentration and with bacterial endophyte EB5 for 12 hrs at $10^7$ cfu per ml concentration with 87.67 and 86.0 per cent germination, respectively (Table 35, Plate 26). Seed germination was recorded least (52.0 %) in non-primed seeds. Maximum seedling length of 11.83 cm was recorded in biopriming treatment with fungal endophyte EF3 for 8 hrs at $10^8$ cfu per ml followed by seed biopriming with bacterial endophyte EB3 at $10^7$ cfu per ml for similar duration with seedling length of 10.86 cm.

**Table 36: Effect of duration of seed biopriming with endophytes on seedling dry weight and seedling vigour in capsicum**

| S. No. | Treatments | Seedling dry weight (mg) | | | | Seedling vigour Index-L | | | | Seedling vigour Index-M | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 6h | 8h | 12h | Mean | 6h | 8h | 12h | Mean | 6h | 8h | 12h | Mean |
| **1.** | EF1 | 27.20 | 21.50 | 26.23 | **24.98** | 909.93 | 999.13 | 1240.41 | **1049.82** | 2338.20 | 1883.90 | 2406.87 | **2209.66** |
| **2.** | EF3 | 28.03 | 28.20 | 24.83 | **27.02** | 569.17 | 880.69 | 862.53 | **770.79** | 2044.40 | 2095.17 | 2052.63 | **2064.07** |
| **3.** | EB2 | 22.27 | 14.20 | 20.53 | **19.00** | 621.75 | 782.16 | 975.42 | **793.11** | 1632.17 | 1065.23 | 1754.67 | **1484.02** |
| **4.** | EB3 | 24.00 | 18.77 | 20.27 | **21.01** | 582.80 | 831.66 | 779.40 | **731.29** | 1584.10 | 1438.30 | 1666.00 | **1562.80** |
| **5.** | EB5 | 17.27 | 24.77 | 24.42 | **22.15** | 749.22 | 806.61 | 756.13 | **770.66** | 1445.30 | 2074.30 | 2088.36 | **1869.32** |
| **6.** | Hydropriming | 22.33 | 21.20 | 24.37 | **22.87** | 639.74 | 657.12 | 830.29 | **709.05** | 1512.93 | 1393.36 | 1789.57 | **1565.29** |
| **7.** | Control | 15.40 | 15.40 | 15.40 | **15.40** | 353.60 | 353.60 | 353.60 | **353.60** | 800.80 | 800.80 | 800.80 | **800.8 0** |
| | **Mean** | **22.36** | **20.68** | **22.28** | | **632.32** | **758.71** | **828.26** | | **1622.56** | **1535.87** | **1794.13** | |
| | **CD $_{(0.05)}$**<br>**Treatment (T)**<br>**Duration (D)**<br>**T X D** | **1.52**<br>**0.99**<br>**2.63** | | | | **39.44**<br>**25.82**<br>**68.32** | | | | **132.09**<br>**86.48**<br>**228.80** | | | |

Least seedling length of 8.26 cm was observed in non-primed capsicum seeds. Seeds bioprimed for 6 hrs with fungal endophyte EF1 at $10^8$ cfu per ml concentration resulted in maximum biomass of 27.20 mg followed by seed biopriming for 8 hrs with bacterial endophyte EB5 at $10^7$ cfu per ml which resulted in biomass of 24.77 mg. Seedling dry weight was found least (15.4 mg) in non-primed seeds. Biopriming of capsicum seeds for 12 hrs with fungal endophyte EF1 at $10^8$ cfu per ml concentration resulted in maximum (1240.41) SVI-L which was 71.49 per cent higher in comparison to control. SVI-M was observed maximum (2406.87) in seed biopriming for 12 hrs with fungal endophyte EF1 at $10^8$ cfu per ml which was 66.73 per cent higher in comparison to non-primed seeds (Table 36).

There are few reports on biopriming of seeds with endophytes, However, work on potential of endophytes in managing soil-borne disease has been carried out by various workers. Sundaramoorthy and Balabaskar (2012) reported that two endophytic *Bacillus subtilis* isolates, from coconut and cotton, and three rhizospheric isolates of *Pseudomonas fluorescens* enhanced germination of tomato seeds by about 88-93 per cent which was 80.0 per cent higher than the untreated control after 10 days of incubation. Abdallah et al. (2020) reported four nonpathogenic, effective and putative endophytic bacterial isolates, from *Withania somnifera* fruits (S7, S8 and S9), and stems (S15) against *Fusarium oxysporum* in tomato. While, antagonistic potential of isolates of *Bacillus cereus* BT8 has been reported against *Fusarium oxysporum* and *Rhizoctonia solani*, *Bacillus amyloliquefaciens* (CEIZ-11) has been found effective against *Pythium aphanidermatum* and *Stenotrophomonas maltophilia* (S37) found effective against *Fusarium oxysporum* in datura (Gao et al. 2015; Melnick et al. 2008; Abdallah et al. 2020).

Fungal endophyte *Fusarium oxysporum* EF119 was also reported to have antagonistic effect against *Pythium ultimum* causing damping-off disease in capsicum. Strains of endophytic bacteria *Streptomyces* sp. (*S. somaliensis*, *S. cyaneus* and *S. griseus*) and *Serratia* strains isolated from the different host plants were reported to possess antagonistic activity against fungal pathogens like *Rhizoctonia solani, Fusarium oxysporum, Pythium* sp, *Phytophthora parasitica*, *Botrytis cinerea* (Liu et al. 2010).

## 10. Seed biopriming with effective endophytes under field conditions

Effective seed biopriming treatments of endophytes were further evaluated against damping-off disease of tomato, chilli and capsicum. Seeds of tomato, chilli and capsicum

were bioprimed with effective fungal endophytes at $10^8$ cfu per ml concentration and with bacterial endophytes at $10^7$ cfu per ml concentration for 12 hrs. Bioprimed seeds were then sown in nursery beds to check their efficacy against damping-off disease.

Seed biopriming with fungal endophyte EF1 and bacterial endophyte EB5 was found most effective against damping-off disease in tomato, chilli and capsicum. Among all the endophytes evaluated against damping-off disease, fungal endophyte EF1 was found most effective with 54.09 per cent reduction in seedling mortality in comparison to control. In tomato, seed biopriming with bacterial endophyte EB5 was found next in efficacy with seedling mortality of 27.08 per cent followed by bacterial endophyte EB3 with seedling mortality of 30.99 per cent (Table 37, Plate 26).

Bacterial endophyte EB2 was found least effective with seed germination of 45.27 per cent. Bacterial endophyte EB5 was also found effective in improving the plant growth with average shoot length of 22.75 cm with per cent increase of 28.04 per cent and root length of 8.23 cm with per cent increase of 22.96 per cent in comparison to control in tomato seedlings (Plate 30). Fungal endophyte EF1 was found next in efficacy with average shoot length of 21.88 cm and root length of 8.09 cm. Bacterial endophyte EB2 was found least effective with shoot length of 18.32 cm and root length of 6.48 cm in comparison to control.

In chilli, seed biopriming with fungal endophyte EF1 was found most effective with least seedling mortality with 50.0 per cent reduction in comparison to control. Seed biopriming with bacterial endophyte EB5 was found next in efficacy with seedling mortality of 22.48 per cent (Table 37, Plate 27). Biopriming with fungal endophyte EF1 was found most effective with seed germination of 81.50 per cent which was 45.26 per cent higher than control. Bacterial endophyte EB5 with seed germination of 74.79 per cent followed by EF3 with seed germination of 58.33 per cent.

Efficacy of bacterial endophyte EB2 was found least effective with seed germination of 49.66 per cent. Seed biopriming with bacterial endophyte EF1 was found most effective with average shoot length of 18.95 cm and root length of 8.78 cm, respectively which were 33.93 and 58.09 per cent higher in comparison to control (Plate 27). Bacterial endophyte EB5 was found next in efficacy with average shoot length of 17.93 cm and root length of 5.86 cm. Bacterial endophyte EB3 was found least effective with average shoot length of 15.59 cm and root length of 4.22 cm in comparison to control.

**General layout of field experiment in tomato**

**Seed biopriming with Fungal endophyte EF1 in Tomato**

**Control**

**Plate 26: Seed biopriming with effective endophytes under field conditions**

Biopriming with EB5

Biopriming with EF1

Tomato

Biopriming with EF1

Biopriming with EB5

Chilli

Biopriming with EF3

Biopriming with EF1

Capsicum

**Plate 27: Effect of seed biopriming with endophytes on seedling length of tomato, chilli and capsicum**

**Table 37: Effect of seed biopriming with effective endophytes on damping-off and plant growth in tomato, chilli and capsicum under field conditions**

| S. No. | Treatments (Seed biopriming) | Tomato | | | | Chilli | | | | Capsicum | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Disease incidence (%) | Seed germination (%) | Shoot length (cm) | Root length (cm) | Disease incidence (%) | Seed germination (%) | Shoot length (cm) | Root length (cm) | Disease incidence (%) | Seed germination (%) | Shoot length (cm) | Root length (cm) |
| 1. | EF 1 | 15.21 (22.90) | 89.75 (71.58) | 21.88 | 8.09 | 16.33 (23.83) | 81.50 (64.53) | 18.95 | 8.78 | 14.40 (22.29) | 77.16 (61.46) | 22.93 | 8.59 |
| 2. | EF 3 | 36.73 (37.30) | 64.22 (53.28) | 19.98 | 7.78 | 23.23 (28.81) | 58.33 (49.79) | 17.56 | 5.57 | 19.27 (26.03) | 52.58 (46.48) | 22.05 | 7.28 |
| 3. | EB 2 | 32.19 (34.56) | 45.27 (42.29) | 18.32 | 6.48 | 29.82 (33.10) | 49.66 (44.81) | 14.53 | 4.59 | 22.65 (28.42) | 46.97 (43.26) | 18.94 | 7.14 |
| 4. | EB 3 | 30.99 (33.82) | 68.46 (55.86) | 20.21 | 6.85 | 26.66 (31.08) | 53.59 (47.06) | 15.59 | 4.22 | 24.61 (29.74) | 38.31 (38.24) | 20.88 | 5.58 |
| 5. | EB 5 | 27.08 (31.34) | 82.13 (65.00) | 22.75 | 8.23 | 22.48 (28.30) | 74.79 (59.87) | 17.93 | 5.86 | 19.32 (26.07) | 53.92 (47.25) | 21.70 | 7.37 |
| 6. | Control | 33.13 (35.13) | 42.38 (40.62) | 16.37 | 6.34 | 32.66 (34.85) | 44.61 (41.90) | 12.52 | 3.68 | 30.20 (33.33) | 36.00 (36.87) | 15.26 | 4.21 |
| | **CD $_{(0.05)}$** | **2.15** | **3.32** | **1.65** | **1.02** | **1.06** | **1.02** | **0.57** | **0.74** | **1.08** | **1.49** | **1.36** | **1.03** |

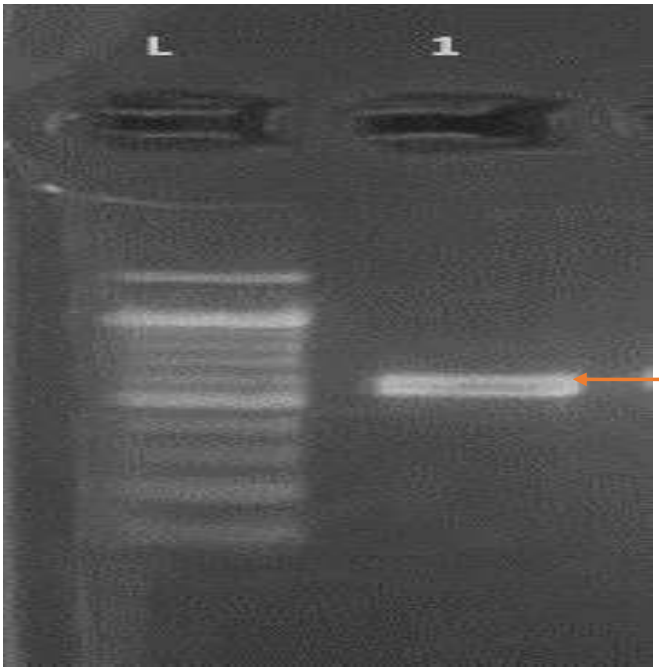
Figures in the parenthesis are arc sine transformed values.

Similarly, biopriming of capsicum seeds with bacterial endophyte EF1 was also found most effective with 52.32 per cent reduction in seedling mortality followed by 19.27 per cent seedling mortality in seed biopriming with fungal endophyte EF3 (Table 37, Plate 26). Seed biopriming with bacterial endophyte EB5 was found next in efficacy with seedling mortality of 19.32 per cent. Seed biopriming with fungal endophyte EF1 was found most effective with seed germination of 77.16 per cent which was 53.34 per cent higher than control. Bacterial endophyte EB5 was found next in efficacy with seed germination of 53.92 per cent followed by EF3 with seed germination of 52.58 per cent. Efficacy of bacterial endophyte EB2 was found least with seed germination of 46.97 per cent. Fungal endophyte EF1 was found most effective with average shoot length of 22.93 cm and root length of 8.59 cm in capsicum which was 33.44 and 50.98 per cent higher in comparison to control, respectively (Plate 27). Bacterial endophyte EB3 was found least effective with root length of 5.58 cm.

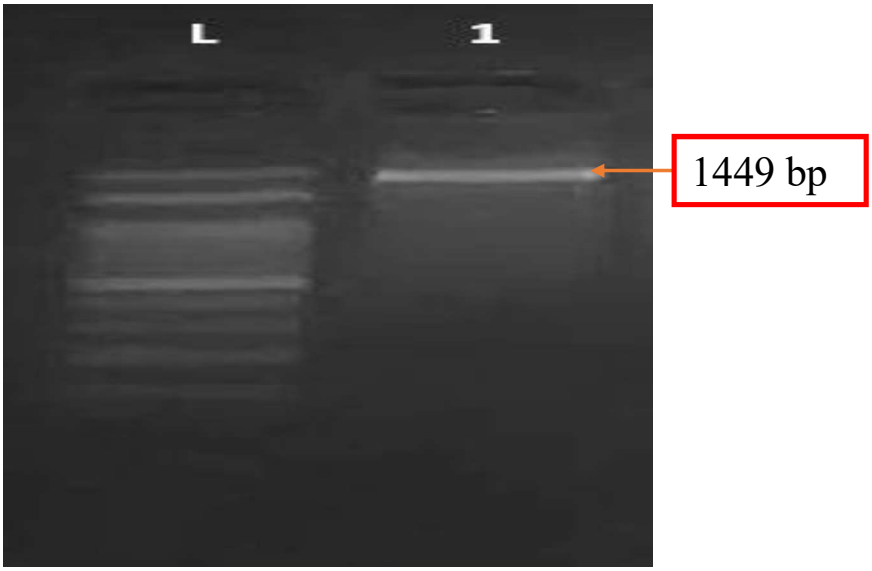
Rashad et al. (2022) also reported that endophyte *Bacillus subtilis* SR22 resulted in 51.0 per cent reduction of *Rhizoctonia solani* in tomato. Role of *Stenotrophomonas* species has been reported in producing plant growth hormone and also accumulation of other factors resulting in plant growth promotion (Suckstroff and Berg 2003; Mehnaz et al. 2010). Khanna et al. (2022) reported that as a result of seed biopriming with single isolate and also due to consortium biopriming bacterial endophyte CRBE7 and CRBE3 possess antagonistic activity against *Fusarium oxysporum* in chickpea. *Penicillium dipodomyicola* has been isolated as endophytic fungi from leaves of *Paliocourea tetraphylla* along with other species of *Penicillium* like *Penicillium griseofulvum, Penicillium implicatum*, *Penicillium goetzii* which have been reported for their endophytic existence in leaves of various medicinal plants (Nicoletti et al. 2014). Seed bio-priming with endophytic PGPR consortium (*Azotobacter chroococcum* + *Azospirillum lipoferum*) has been found to significantly increase several yield attributes in barley and chickpea (Meena et al. 2010; Mirshekari et al. 2012).

## 11. Molecular characterization of effective endophytes

Molecular characterization of fungal endophyte EF1 and bacterial endophyte EB5 which were most effective endophytes under field conditions against damping-off disease of tomato, chilli and capsicum, was carried out based upon ITS (Internal Transcribed Spacer) sequences and 16S rRNA sequences, respectively. The fungal endophyte (EF1), isolated from




**PCR amplification of EF1 using ITS1 and ITS4 primers which amplifies 626 bp amplicon against the DNA ladder L of 1kb**




**PCR amplification of EB5 using 27F and 1492R primers which amplifies 1449 bp amplicon against the DNA ladder L of 1.5 kb**

**Plate 28: Molecular characterization of fungal endophyte EF1 and bacterial endophyte EB5**

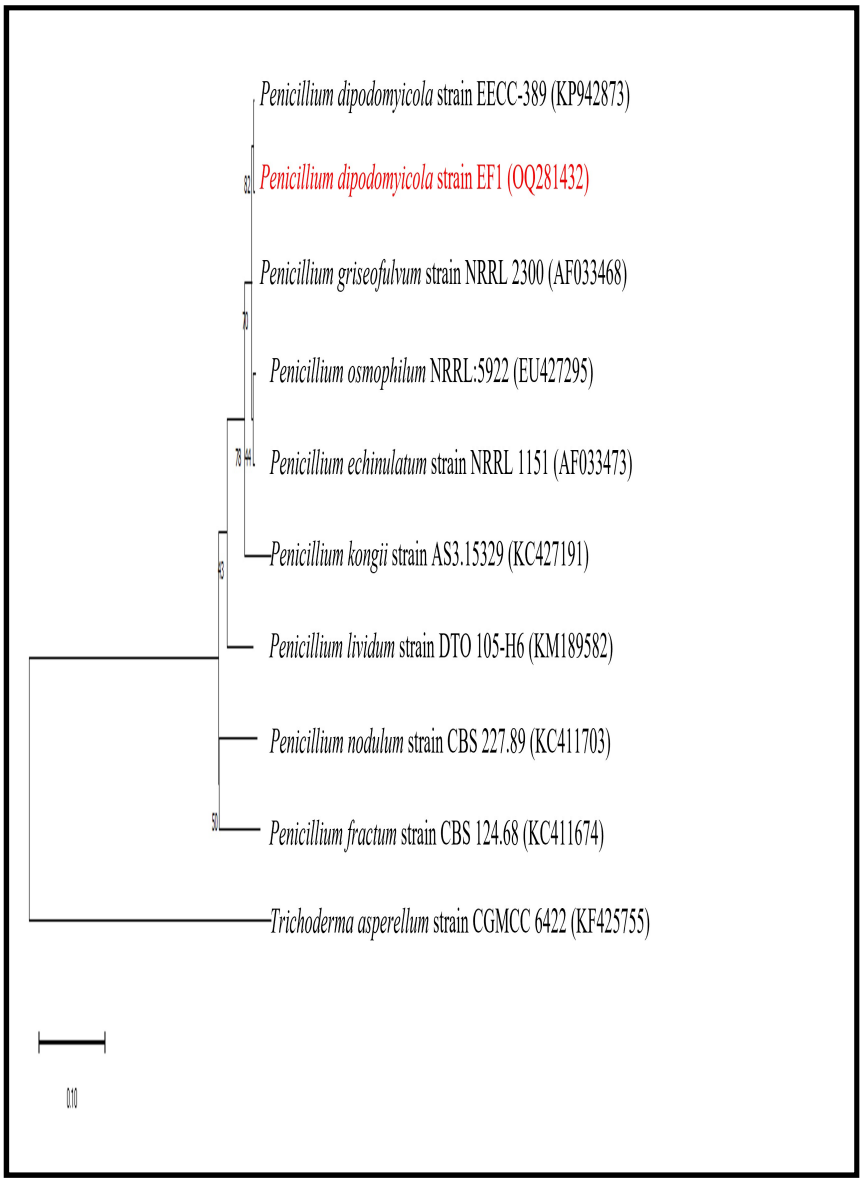



**Fig 1: Phylogenetic tree based on the 18S rRNA sequences showing relationship between representative fungal genera and closely associated neighbors**

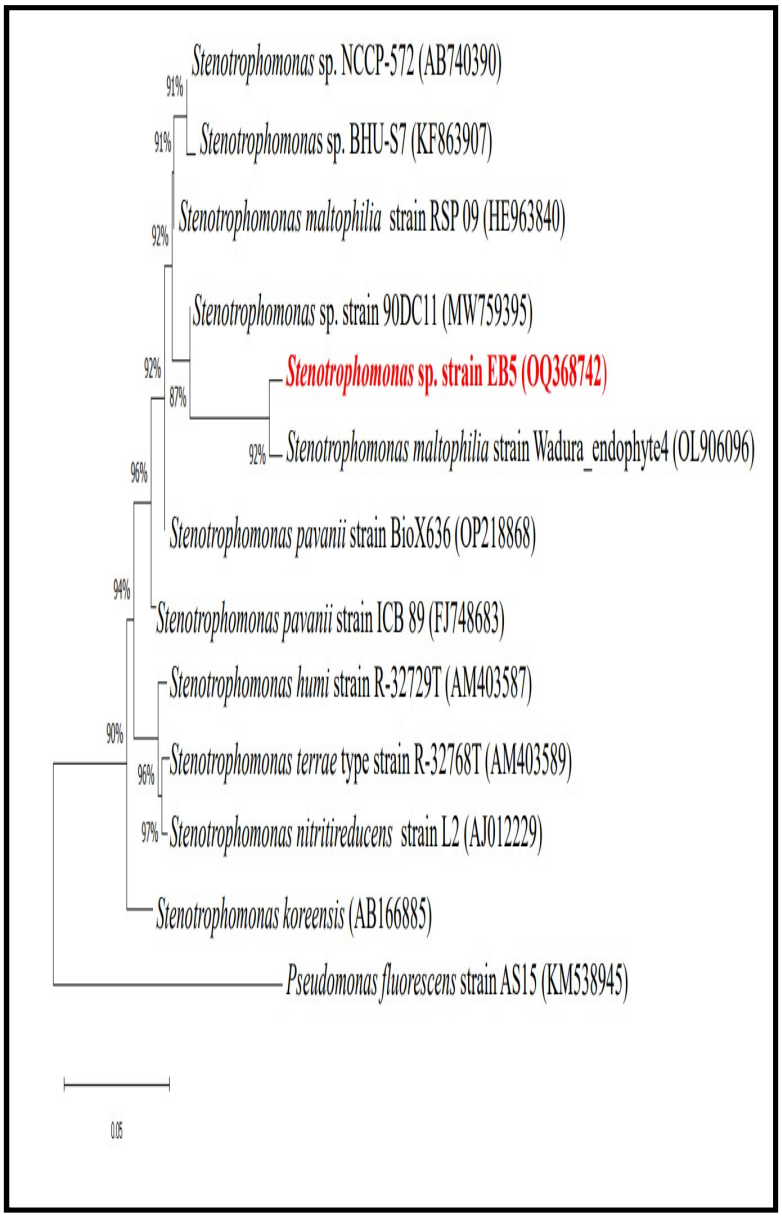



**Fig 2: Phylogenetic tree based on the 16S rRNA sequences showing relationship between representative bacterial genera and closely associated neighbors**

leaves of *Mentha* sp. was identified as *Penicillium dipodomyicola* using ITS sequencing analysis. The nucleotide sequence of 626 bp was phylogenetically characterized using BLAST tool of NCBI (Plate 28). Phylogenetic tree was constructed using maximum likelihood method based on Tamura-Nei model with 1000 replicates as bootstrap value (Fig 1). The constructed phylogenetic tree indicated that the fungal endophyte (*Penicillium dipodomyicola* strain EF1) shared maximum homology with *Penicillium dipodomyicola*. The partial nucleotide sequence for 18S rRNA of fungal endophyte EF1 was submitted to Genbank under accession number OQ281432. The bacterial endophyte (EB5), isolated from leaves of *Ocimum* sp. was identified as *Stenotrophomas* sp. using 16S rDNA sequencing. The nucleotide sequence of 1449 bp (Plate 28) was phylogenetically characterized using BLAST (Basic Local Alignment Search Tool) of NCBI (National Centre for Biotechnology Information). Phylogenetic tree was constructed using neighbour joining method with 1000 replicates as bootstrap value (Fig 2). The constructed phylogenetic tree indicated that the bacterial strain EB5 shared maximum homology with *Stenotrophomona*s sp. The partial nucleotide sequence for 16S rDNA of bacterial strain EB5 was later submitted to Genbank under accession number OQ368742.

## 12. Conjoint application of seed biopriming with effective biocontrol agents and soil amendments

Biopriming of tomato, chilli and capsicum seeds was done with effective fungal biocontrol agents (*Trichoderma harzianum*, *T virens*, *T. atroviride*) at concentration of $10^7$ cfu per ml (tomato and chilli) and $10^8$ cfu per ml (capsicum) for 8 hrs in each case under artificially inoculated conditions. However, biopriming of seeds with effective bacterial biocontrol agents (*Bacillus licheniformis*, *Pseudomonas fluorescens*) was carried out at $10^8$ cfu per ml only for 8 hrs. These bioprimed seeds were then sown in sick pots which were amended with (NSKE (5%), compost tea and vermiwash) after 7 days of sowing at one week interval for evaluating their efficacy against damping-off disease.

In tomato, treatment combination of *Trichoderma harzianum* and NSKE was found most effective and reduced the seedling mortality by 55.9 per cent with increase in the seed germination by 60.77 per cent in comparison to control. Treatment combination of *Trichoderma virens* and vermiwash was found next in efficacy with 55.0 per cent reduction in seedling mortality. Treatment combination of *Trichoderma virens* and NSKE was also

found equally effective to the earlier one in efficacy with seedling mortality of 10.73 per cent (Table 38, Plate 29).

**Table 38: Conjoint application of seed biopriming with effective biocontrol agents and soil amendments in tomato under artificially inoculated conditions**

| S. No. | Treatments (Seed biopriming) | *Seed germination (%) | Shoot length (cm) | Root length (cm) | **Disease incidence (%) |
|---|---|---|---|---|---|
| 1. | Seed biopriming with *Trichoderma harzianum* + soil amended with NSKE (5%) | 81.33 (64.41) | 25.55 | 9.12 | 10.31 (3.36) |
| 2. | Seed biopriming with *Trichoderma harzianum* + soil amended with compost tea | 59.00 (50.19) | 20.63 | 7.93 | 14.47 (3.93) |
| 3. | Seed biopriming with *Trichoderma harzianum* + soil amended with vermiwash | 79.13 (62.82) | 25.44 | 4.84 | 13.30 (3.72) |
| 4. | Seed biopriming with *Trichoderma virens* + in soil amended with NSKE (5%) | 77.42 (61.63) | 23.84 | 4.67 | 10.73 (3.42) |
| 5. | Seed biopriming with *Trichoderma virens* + soil amended with compost tea | 53.15 (46.81) | 16.58 | 4.27 | 16.57 (4.19) |
| 6. | Seed biopriming with *Trichoderma virens* + soil amended with vermiwash | 78.55 (62.41) | 17.52 | 4.57 | 10.52 (3.39) |
| 7. | Seed biopriming with *Trichoderma atroviride* + soil amended with NSKE (5%) | 71.88 (57.98) | 23.63[c] | 5.78 | 10.62 (3.41) |
| 8. | Seed biopriming with *Trichoderma atroviride* + soil amended with compost tea | 48.17 (43.95) | 17.55 | 4.44 | 17.27 (4.27) |
| 9. | Seed biopriming with *Trichoderma atroviride* + soil amended with vermiwash | 51.23 (45.71) | 23.71 | 4.66 | 16.33 (4.16) |
| 10. | Seed biopriming with *Bacillus licheniformis* + soil amended with NSKE (5%) | 43.00 (40.98) | 25.39 | 4.80 | 17.27 (4.27) |
| 11. | Seed biopriming with *Bacillus licheniformis* + soil amended with compost tea | 41.20 (39.93) | 17.41 | 4.14 | 15.63 (4.08) |
| 12. | Seed biopriming with *Bacillus licheniformis* + soil amended with vermiwash | 44.50 (41.84) | 18.19 | 4.41 | 14.33 (3.92) |
| 13. | Seed biopriming with *Pseudomonas fluorescens*+ soil amended with NSKE (5%) | 74.70 (71.96) | 17.98 | 3.85 | 11.17 (4.02) |
| 14. | Seed biopriming with *Pseudomonas fluorescens* + soil amended with compost tea | 36.17 (36.96) | 17.55 | 4.15 | 14.35 (3.92) |
| 15. | Seed biopriming with *Pseudomonas fluorescens* + soil amended with vermiwash | 43.17 (41.07) | 15.19 | 4.36 | 11.20 (3.49) |
| 16. | Control | 31.90 (34.38) | 14.67 | 3.64 | 23.38 (4.94) |
| | **$CD_{(0.05)}$** | **1.57** | **1.58** | **0.94** | **0.21** |

***** Figures are arc sine transformed values

** Figures are square root transformed values

Treatment combination of *Trichoderma harzianum* and NSKE was found most effective with seed germination of 81.33 per cent followed by treatment combination of *Trichoderma harzianum* and vermiwash with germination of 79.13 per cent. Average shoot length of tomato seedlings was found maximum (25.55 cm) in treatment combination of *Trichoderma harzianum* and NSKE followed by average shoot length of 25.44 cm in

treatment combination of *Trichoderma harzianum* and vermiwash. Root length of tomato seedlings was recorded maximum (9.12 cm) in treatment combination of seed biopriming with *Trichoderma harzianum* and soil amended with NSKE followed by treatment combination of *Trichoderma harzianum* and compost tea with root length of 7.93 cm (Table 38).

Seed biopriming with bio-inoculant is reported to improve the crop plant's defense ability by stimulating the activity of defense enzymes (Song et al. 2017). Oxidative enzymes namely PPO, PO, PAL and TPC were triggered as a result of activation of ISR (Induced Systemic Resistance) which contribute in protection against the biotic and abiotic stresses (Nakkeeran et al. 2006; Jetiyanon 2007). In tomato, there was a significant increase of 78.23 per cent in the polyphenol oxidase (PPO) enzyme activity in treatment combination of *Trichoderma harzianum* and vermiwash followed by treatment combination of *Trichoderma harzianum* and NSKE with enzyme activity of 19.27 in comparison to non-primed seedlings with enzyme activity of 4.23. Similarly, Peroxidase (PO) enzyme activity was also found maximum (1.07) in treatment combination of *Trichoderma virens* and soil amended with NSKE in comparison to non-primed seedlings with enzyme activity of 0.30 (Table 41).

Another important component of plant resistance *i.e.* Total phenolic content (TPC) was also found maximum (458.17) in treatment combination of *Pseudomonas fluorescens* and NSKE followed by treatment combination of *Trichoderma harzianum* and NSKE with value of 404.45. Phenylalanine ammonia lyase (PAL) activity which also determine the production of secondary compounds which have role in defense activity against the plant pathogens was also found maximum (1612) in treatment combination of *Trichoderma harzianum* and NSKE followed by treatment combination of *Trichoderma harzianum* and vermiwash which had enzyme activity of 1045.11 in comparison to non-primed seeds with enzyme activity of 612.17 (Table 42).

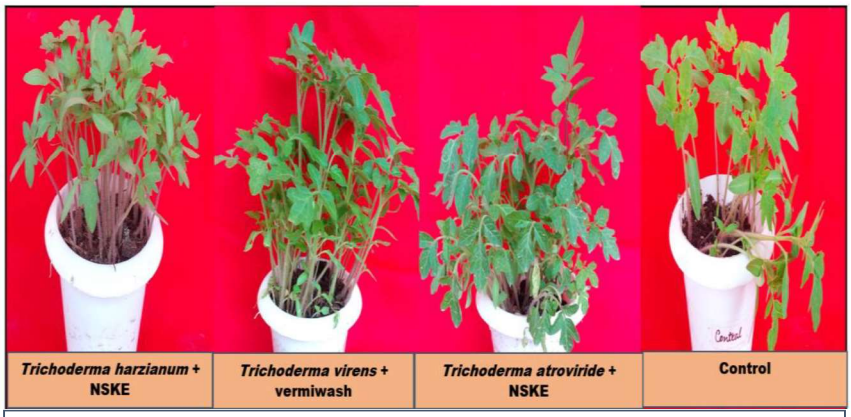
In chilli, treatment combination of *Trichoderma virens* and vermiwash was found most effective against the damping-off disease in chilli with 61.97 per cent reduction in seedling mortality followed by treatment combination of *Trichoderma harzianum* and NSKE with reduction of 53.23 per cent. Treatment combination of *Trichoderma virens* and compost tea resulted in reduction of 50.30 per cent in seedling mortality. Treatment combination of *Trichoderma harzianum* and NSKE with an increase of 40.82 per cent in seed germination was found most effective followed by treatment combination of *Trichoderma harzianum* and vermiwash with increase of 37.47 per cent in seed germination (Table 39, Plate 29).

Treatment combination of *Trichoderma atroviride* and NSKE was found next in efficacy with seed germination of 74.34 per cent. Shoot length of chilli seedlings was found maximum (17.25 cm) in treatment combination of *Trichoderma harzianum* and NSKE followed by treatment combination of *Trichoderma harzianum* and compost tea with shoot length of 15.04 cm (Table 39).

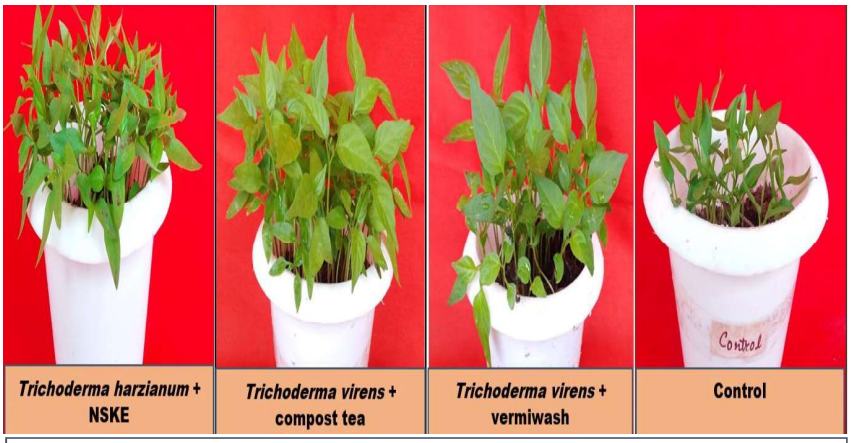
**Table 39: Conjoint application of seed biopriming with effective biocontrol agents and soil amendments in chilli**

| S. No. | Treatments (Seed biopriming) | *Seed germination (%) | Shoot length (cm) | Root length (cm) | **Disease incidence (%) |
|---|---|---|---|---|---|
| 1. | Seed biopriming with *Trichoderma harzianum* + soil amended with NSKE (5%) | 78.58 (62.43) | 17.25 | 7.37 | 10.30 (3.58) |
| 2. | Seed biopriming with *Trichoderma harzianum* + soil amended with compost tea | 70.16 (56.89) | 15.04 | 6.49 | 14.31 (3.85) |
| 3. | Seed biopriming with *Trichoderma harzianum* + soil amended with vermiwash | 74.37 (59.59) | 11.98 | 6.56 | 14.15 (3.83) |
| 4. | Seed biopriming with *Trichoderma virens* + in soil amended with NSKE (5%) | 63.48 (52.83) | 13.26 | 8.12 | 14.35 (3.85) |
| 5. | Seed biopriming with *Trichoderma virens* + soil amended with compost tea | 53.87 (47.22) | 10.29 | 5.10 | 13.07 (3.68) |
| 6. | Seed biopriming with *Trichoderma virens* + soil amended with vermiwash | 65.08 (53.78) | 10.46 | 4.42 | 10.00 (3.24) |
| 7. | Seed biopriming with *Trichoderma atroviride* + soil amended with NSKE (5%) | 74.37 (59.59) | 12.41 | 4.44 | 15.88 (4.05) |
| 8. | Seed biopriming with *Trichoderma atroviride* + soil amended with compost tea | 63.48 (52.83) | 12.13 | 4.70 | 18.38 (4.34) |
| 9. | Seed biopriming with *Trichoderma atroviride* + soil amended with vermiwash | 62.26 (52.11) | 11.26 | 5.23 | 18.25 (4.33) |
| 10. | Seed biopriming with *Bacillus licheniformis* + soil amended with NSKE (5%) | 51.83 (46.05) | 12.64 | 4.75 | 15.75 (4.03) |
| 11. | Seed biopriming with *Bacillus licheniformis* + soil amended with compost tea | 50.83 (45.48) | 12.66 | 4.25 | 21.50 (4.69) |
| 12. | Seed biopriming with *Bacillus licheniformis* + soil amended with vermiwash | 61.75 (51.82) | 10.28 | 3.52 | 18.30 (4.34) |
| 13. | Seed biopriming with *Pseudomonas fluorescens*+ soil amended with NSKE (5%) | 62.41 (52.19) | 9.91 | 3.73 | 17.49 (4.24) |
| 14. | Seed biopriming with *Pseudomonas fluorescens* + soil amended with compost tea | 65.08 (53.78) | 12.37 | 3.81 | 20.75 (4.61) |
| 15. | Seed biopriming with *Pseudomonas fluorescens* + soil amended with vermiwash | 63.08 (52.60) | 9.18 | 3.63 | 17.83 (4.28) |
| 16. | Control | 46.50 (42.99) | 8.64 | 3.47 | 26.30 (5.18) |
| | **CD $_{(0.05)}$** | **2.39** | **1.50** | **1.36** | **0.21** |

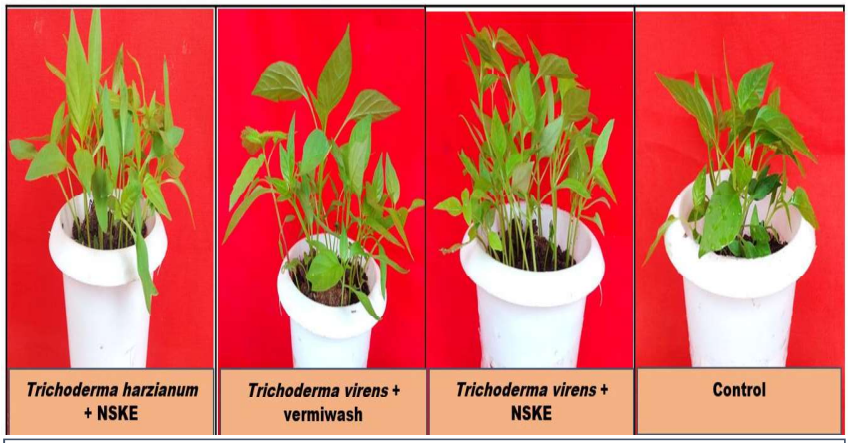
* Figures in the parenthesis are arc sine transformed values.
** Figures in the parenthesis are square root transformed values




**Tomato**




**Chilli**




**Capsicum**

**Plate 29: Conjoint application of seed biopriming with biocontrol agents and soil amendments**

Root length of chilli seedlings was also recorded maximum (8.12 cm) in treatment combination of *Trichoderma virens* and NSKE which was 57.27 per cent higher than control followed by treatment combination of *Trichoderma harzianum* and NSKE with root length of 7.37 cm Treatment combination of *Trichoderma harzianum* and vermiwash was found next in efficacy with root length of 6.56 cm (Table 39).

**Table 40: Conjoint application of seed biopriming with effective biocontrol agents and soil amendments in capsicum**

| S. No. | Treatments (Seed biopriming) | *Seed germination (%) | Shoot length (cm) | Root length (cm) | **Disease incidence (%) |
|---|---|---|---|---|---|
| 1. | Seed biopriming with *Trichoderma harzianum* + soil amended with NSKE (5%) | 72.83 (58.57) | 15.63 | 7.84 | 10.00 (3.24) |
| 2. | Seed biopriming with *Trichoderma harzianum* + soil amended with compost tea | 53.98 (47.27) | 12.41 | 5.22 | 11.31 (3.44) |
| 3. | Seed biopriming with *Trichoderma harzianum* + soil amended with vermiwash | 57.82 (49.49) | 12.96 | 6.46 | 10.95 (3.38) |
| 4. | Seed biopriming with *Trichoderma virens* + in soil amended with NSKE (5%) | 55.48 (48.13) | 13.71 | 5.76 | 8.55 (3.01) |
| 5. | Seed biopriming with *Trichoderma virens* + soil amended with compost tea | 51.00 (45.56) | 11.27 | 4.55 | 11.20 (3.42) |
| 6. | Seed biopriming with *Trichoderma virens* + soil amended with vermiwash | 53.98 (47.27) | 12.55 | 5.22 | 10.65 (3.34) |
| 7. | Seed biopriming with *Trichoderma atroviride* + soil amended with NSKE (5%) | 51.08 (45.60) | 12.30 | 5.50 | 13.90 (3.79) |
| 8. | Seed biopriming with *Trichoderma atroviride* + soil amended with compost tea | 40.92 (39.75) | 9.78 | 4.55 | 16.22 (4.09) |
| 9. | Seed biopriming with *Trichoderma atroviride* + soil amended with vermiwash | 49.28 (44.57) | 10.74 | 4.41 | 14.90 (3.92) |
| 10. | Seed biopriming with *Bacillus licheniformis* + soil amended with NSKE (5%) | 50.17 (45.08) | 9.44 | 5.08 | 14.25 (3.84) |
| 11. | Seed biopriming with *Bacillus licheniformis* + soil amended with compost tea | 41.00 (39.79) | 7.67 | 4.55 | 14.23 (3.84) |
| 12. | Seed biopriming with *Bacillus licheniformis* + soil amended with vermiwash | 46.74 (43.11) | 8.80 | 4.45 | 12.90 (3.36) |
| 13. | Seed biopriming with *Pseudomonas fluorescens*+ soil amended with NSKE (5%) | 40.67 (39.61) | 8.54 | 4.22 | 13.05 (3.68) |
| 14. | Seed biopriming with *Pseudomonas fluorescens* + soil amended with compost tea | 25.91 (30.58) | 7.56 | 4.43 | 14.82 (3.91) |
| 15. | Seed biopriming with *Pseudomonas fluorescens* + soil amended with vermiwash | 33.27 (35.21) | 7.81 | 3.73 | 14.20 (3.83) |
| 16. | Control | 25.80 (30.51) | 6.67 | 3.43 | 20.10 (4.54) |
| | **$CD_{(0.05)}$** | **2.45** | **1.39** | **0.89** | **0.24** |

*Figures in the parenthesis are arc sine transformed values

** Figures in the parenthesis are square root transformed values

PPO activity was found most significant (19.6) in treatment combination of *Trichoderma harzianum* and NSKE with 82.80 per cent increase in comparison to control. Treatment combination of *Trichoderma virens* and NSKE was next in efficacy with enzyme activity of 18.5 in comparison to non-primed seedlings with enzyme activity of 3.37 (Table 41). PO activity was found maximum (0.91) in treatment combination of *Trichoderma atroviride* and NSKE followed by treatment combination of *Trichoderma harzianum* and NSKE with enzyme activity of 0.75 in comparison to non-primed seeds with enzyme activity of 0.10 (Table 41).

Total phenolic content was also found maximum (452.08) in treatment combination of *Pseudomonas fluorescens* and NSKE followed by treatment combination of *Trichoderma harzianum* and NSKE with enzyme activity of 443.34 in comparison to non-primed seeds with a value of 255.53. The PAL activity was found maximum (1675.48) in treatment combination of *Trichoderma harzianum* and NSKE followed by treatment combination of *Pseudomonas fluorescens* and NSKE with enzyme activity of 1111.50 in comparison to non-primed seeds which had enzyme activity of 244.30 (Table 42).

Among all the biopriming treatments evaluated against damping-off disease in capsicum, treatment combination of *Trichoderma virens* and NSKE was found most effective with 57.46 per cent reduction in seedling mortality followed by treatment combination of *Trichoderma harzianum* and NSKE with 50.24 per cent reduction. Treatment combination of *Trichoderma virens* and vermiwash was found next in efficacy with 47.01 per cent reduction in seedling mortality, respectively. Treatment combination of *Trichoderma harzianum* and NSKE also significantly improved the seed germination by 64.57 per cent followed by treatment combination of *Trichoderma harzianum* and vermiwash with 55.38 per cent increase in seed germination in comparison to control. Treatment combination of *Trichoderma virens* and NSKE with seed germination of 55.48 per cent was found next in efficacy. Treatment combination of *Trichoderma harzianum* and NSKE followed by treatment combination of *Trichoderma virens* and NSKE also increased the seedling length to 15.63 and 13.71 cm, respectively in comparison to control (Table 40, Plate 29).

Root length of capsicum seedlings was recorded maximum (7.84 cm) in treatment combination of *Trichoderma harzianum* and NSKE followed by treatment combination of *Trichoderma harzianum* and vermiwash with root length of 6.46 cm. Treatment combination of *Trichoderma virens* and NSKE was found next in efficacy with root length of 5.76 cm.

PPO activity was found significantly higher (14.8) in treatment combination of *Trichoderma harzianum* and NSKE followed by treatment combination of *Pseudomonas fluorescens* and NSKE with enzyme activity of 14.37 and both were found 72.77 and 71.95 per cent higher, respectively than control (Table 40).

Treatment combination of *Trichoderma harzianum* with NSKE and *Trichoderma virens* with NSKE were found next in efficacy with activity of 0.94 and 366.07, respectively (Table 41). PAL activity was found maximum (1743.64) in treatment combination of *Trichoderma atroviride* and NSKE followed by treatment combination of *Trichoderma harzianum* and NSKE with enzyme activity of 1563.30 (Table 42).

There are several research reports which testify that seed biopriming with biocontrol agents namely *Trichoderma* sp. and *Pseudomonas* sp. resulted in increased levels of enzyme activity (PPO, PAL, PO, TPC) and which ultimately helped in reduction of the soil-borne pathogens and also improved the seedling growth. Singh et al. (2020b) reported that tomato seeds primed with *Trichoderma asperellum* and *Ochrobactrum* sp. had higher contents of Total phenol with increase in activity of PAL, PO and PPO in comparison to control against *Fusarium oxysporum*. Rajput et al. (2020) reported that seed biopriming with *Trichoderma pseudokoniningii* along with soil application of vermiwash in comparison to control against *Sclerotium rolfsii* in tomato resulted in 16 per cent higher shoot length (24.46 cm), root length. They also reported that the seed bioprimed with *Trichoderma pseudokoniningii* had high activity of PAL with significant increase in total phenolic content.

Gowthamy and Manonmani (2018) reported that seed biopriming with PGPR *Bacillus amyloliquefaciens* (6 %) significantly improved the seed germination to 93.0 per cent with increase in shoot length (16.89 cm) and root length (5.78 cm). Hyder et al. (2021) reported that chilli seedlings inoculated with bacterial isolates (*Bacillus megaterium, Bacillus subtilis*) resulted in significant increase in PPO, PO and PAL activities. Kamali et al. (2020) reported that application of Mahua cake extracts (10 %) followed by neem cake extract (10 %) resulted in mycelial reduction of *Pythium aphanidermatum* under *in vitro* conditions. Elshahawy and El Mohamedy (2019) reported that combined inoculation of five *Trichoderma* isolates increased the survival rate of tomato plants by 87.5 % against *Rhizoctonia solani* and *Pythium aphanidermatum* and these isolates also stimulated the systemic defense responses

in tomato plants by elevating the level of defense enzymes (peroxidase and polyphenoloxidase). Several other studies also highlight and corroborate the result findings of the present studies (Ramamoorthy et al. 2002; Ananthi et al. 2017; Jayapala et al. 2019).

## 12. Efficacy of integration of seed biopriming with effective bioinoculants (BCAs, plant extracts, endophytes) and soil solarization

Different effective treatments of seed biopriming *i.e.,* biocontrol agents (*Trichoderma harzianum* and *T. virens*), plant extracts (*Roylea elegans* and *Melia azedarach*), endophytes (*Penicillium dipodomyicola* and *Stenotrophomonas* sp.) were integrated with soil amendments namely neem cake and vermicompost in 13 different combination and were evaluated against damping-off disease under solarized and un-solarized beds (Plate 30a). Results of this experiment indicate that a negative correlation between seed germination and seedling mortality was observed in tomato, chilli and capsicum as a result of integration of seed biopriming treatments with bioinoculants and soil solarization (Fig 3). The efficacy of treatments in managing damping-off disease in solarized beds was significantly superior over the unsolarized beds (Plate 30b, Plate 30c).

In tomato, treatment combination T9 (seed biopriming with fungal endophyte *Penicillium dipodomyicola* and soil application of neem cake) in solarized plots was found most effective against damping-off disease with 49.93 per cent reduction in seedling mortality followed by treatment combination T5 (seed biopriming with *Roylea elegans* and soil application with neem cake) with 43.59 per cent reduction and T6 (seed biopriming with *R. elegans* and soil application with vermicompost) where 42.44 per cent reduction in seedling mortality was recorded (Table 43, Plate 30b). Treatment combination T8 (seed biopriming with *Melia azedarach* and soil application with vermicompost) was found least effective in managing damping-off disease in comparison to control. In solarized beds, treatment combination T9 (seed biopriming with *P. dipodomyicola* and soil application with neem cake) was found most effective with seed germination of 90.85 per cent followed by treatment combination T1 (seed biopriming with *P. dipodomyicola* and soil application with vermicompost) with seed germination of 86.74 per cent. In-unsolarized beds, treatment combination T9 (seed biopriming with fungal endophyte *Penicillium dipodomyicola* and soil application of neem cake) was found most effective with 20.27 per cent reduction in seedling mortality. The treatment combination T9 was found significant in solarized beds in comparison to un-solarized beds (Table 43).

Soil solarization of beds with polythene sheet (25 µm) for 30 days

Solarized



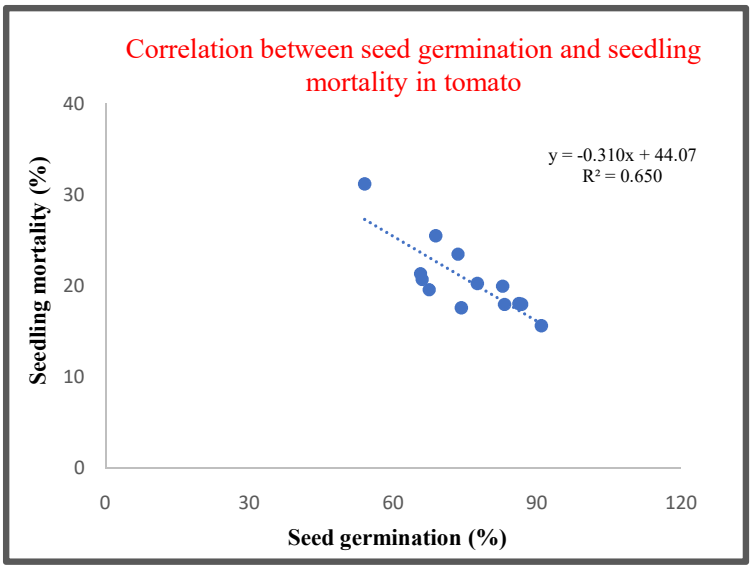
Un-solarized

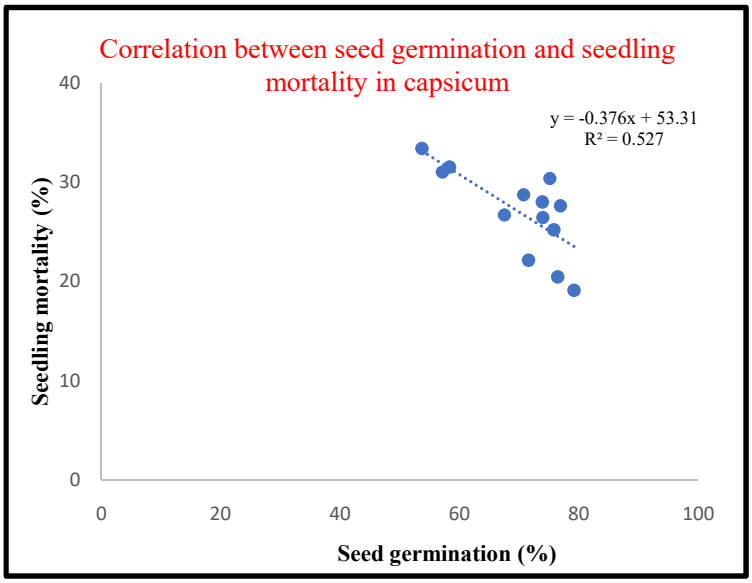
Solarized



Un-solarized

**Plate 30a: Efficacy of integration of seed biopriming with effective bioinoculants and soil solarization**



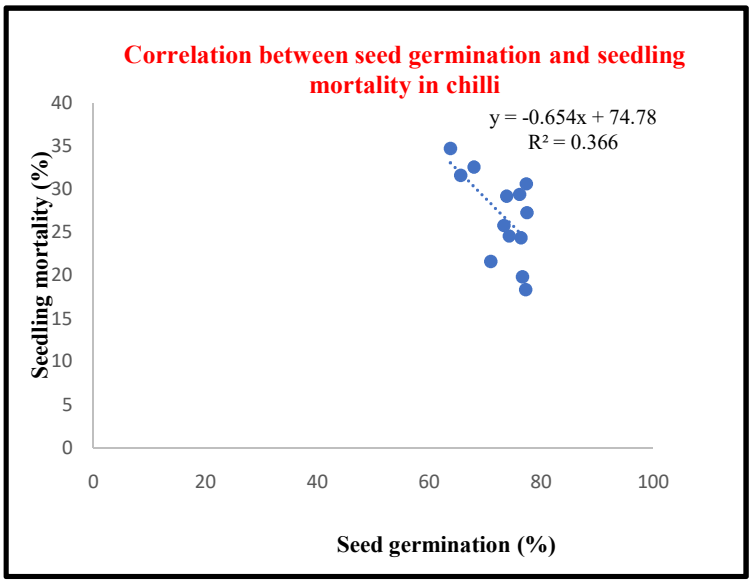




**Fig 3: Correlation between seed germination and seedling mortality in integrated experiment carried out with bio-inoculants and soil solarization**

Treatment combination T1 (seed biopriming with *T. harzianum* and soil application with neem cake) was found next in efficacy in solarized beds with seed germination of 86.15 per cent in tomato. In un-solarized beds, treatment combination T9 (seed biopriming with *P. dipodomyicola* and soil application with neem cake) was found most effective with 82.97 per cent germination followed by treatment combination T10 (seed biopriming with *P. dipodomyicola* and soil application with vermicompost) with seed germination of 79.60 per cent. Treatment combination T9 (seed biopriming with *P. dipodomyicola* and soil application with neem cake) also significantly improved plant growth characters in solarized beds. Treatment combination T9 (seed biopriming with *P. dipodomyicola* and soil application with neem cake) was most effective in improving plant growth with average shoot length of 24.16 cm. Treatment combination T12 (seed biopriming with *Stenotrophomonas* sp. and soil application with vermicompost) was found least effective with average shoot length of 12.43 cm and root length of 5.48 cm in solarized beds (Table 43).

In tomato, treatment combination T5 (seed biopriming with *R. elegans* and neem cake) was found most effective with least seedling mortality of 22.18 per cent in un-solarized beds followed by treatment combination T9 (seed biopriming with *P. dipodomyicola* and soil application with neem cake) with seedling mortality of 24.03 per cent (Plate 30b). Among all the treatment combinations in unsolarized beds, treatment combination T7 (seed biopriming with *M. azedarach* and soil application with neem cake) was found most effective with shoot length of 13 cm followed by treatment combination T6 (seed biopriming with *R. elegans* and soil application with vermicompost) with shoot length of 10.92 cm. Treatment combination T1 (seed biopriming with *T. harzianum* and soil application with neem cake) was found most effective with root length of 8.60 cm followed by treatment combination T2 (seed biopriming with *T. harzianum* and soil application with vermicompost) with root length of 6.56 cm (Table 43).

Efficacy of different treatments in managing damping-off disease was found significantly better in solarized beds in comparison to the unsolarized beds in chilli. Among the solarized beds, treatment combination T9 (seed biopriming with *Penicillium dipodomyicola* and soil application of neem cake) was found most effective against damping-off disease with 47.21 per cent reduction in seedling mortality followed by treatment combination T10 (seed biopriming with *Penicillium dipodomyicola* and soil application of

**Table 41: Biochemical estimation of Polyphenol oxidase and Peroxidase enzyme activity in bioprimed plants**

| S. No. | Treatments (Seed biopriming) | PPO Activity (OD/min/mg FW) | | | PO Activity (U/min/g FW) | | |
|---|---|---|---|---|---|---|---|
| | | Tomato | Chilli | Capsicum | Tomato | Chilli | Capsicum |
| **1** | Seed biopriming with *Trichoderma harzianum* + soil amended with NSKE (5%) | 19.27 | 19.60 | 14.80 | 0.77 | 0.75 | 0.94 |
| **2** | Seed biopriming with *Trichoderma harzianum* + soil amended with compost tea | 15.70 | 12.27 | 9.50 | 0.46 | 0.32 | 0.40 |
| **3** | Seed biopriming with *Trichoderma harzianum* + soil amended with vermiwash | 19.43 | 13.90 | 13.13 | 0.53 | 0.53 | 0.78 |
| **4** | Seed biopriming with *Trichoderma virens* + in soil amended with NSKE (5%) | 17.4b | 18.50 | 10.00 | 1.07 | 0.58 | 0.75 |
| **5** | Seed biopriming with *Trichoderma virens* + soil amended with compost tea | 13.60 | 10.40 | 8.40 | 0.35 | 0.43 | 0.40 |
| **6** | Seed biopriming with *Trichoderma virens* + soil amended with vermiwash | 13.00 | 13.80 | 7.63 | 0.65 | 0.75 | 0.15 |
| **7** | Seed biopriming with *Trichoderma atroviride* + soil amended with NSKE (5%) | 11.40 | 6.37 | 6.97 | 0.54 | 0.91 | 0.27 |
| **8** | Seed biopriming with *Trichoderma atroviride* + soil amended with compost tea | 12.10 | 4.37 | 3.63 | 0.50 | 0.18 | 0.49 |
| **9** | Seed biopriming with *Trichoderma atroviride* + soil amended with vermiwash | 10.73 | 4.90 | 5.70 | 0.69 | 0.43 | 0.09 |
| **10** | Seed biopriming with *Bacillus licheniformis* + soil amended with NSKE (5%) | 19.20 | 10.49 | 7.20 | 0.53 | 0.48 | 0.14 |
| **11** | Seed biopriming with *Bacillus licheniformis* + soil amended with compost tea | 19.17 | 8.44 | 5.93 | 0.39 | 0.40 | 0.20 |
| **12** | Seed biopriming with *Bacillus licheniformis* + soil amended with vermiwash | 17.70 | 9.20 | 8.37 | 0.43 | 0.37 | 0.43 |
| **13** | Seed biopriming with *Pseudomonas fluorescens*+ soil amended with NSKE (5%) | 15.93 | 11.59 | 14.37 | 0.77 | 0.62 | 1.09 |
| **14** | Seed biopriming with *Pseudomonas fluorescens* + soil amended with compost tea | 11.95 | 8.73 | 9.70 | 0.72 | 0.37 | 0.41 |
| **15** | Seed biopriming with *Pseudomonas fluorescens* + soil amended with vermiwash | 14.33 | 10.83 | 12.80 | 0.51 | 0.42 | 0.42 |
| **16** | Control | 4.23 | 3.37 | 4.03 | 0.30 | 0.10 | 0.09 |
| | **$CD_{(0.05)}$** | **1.67** | **1.01** | **1.07** | **0.20** | **0.11** | **0.13** |

**Table 42. Biochemical estimation of Total phenolic content and Phenylalanine ammonia lyase enzyme activity in bioprimed plants**

| S. No. | Treatments (Seed biopriming) | TPC Activity | | | PAL Activity | | |
|---|---|---|---|---|---|---|---|
| | | Tomato | Chilli | Capsicum | Tomato | Chilli | Capsicum |
| **1** | Seed biopriming with *Trichoderma harzianum* + soil amended with NSKE (5%) | 404.45 | 443.34 | 336.64 | 1612.00 | 1675.48 | 1563.30 |
| **2** | Seed biopriming with *Trichoderma harzianum* + soil amended with compost tea | 212.38 | 332.01 | 294.27 | 912.00 | 743.11 | 934.29 |
| **3** | Seed biopriming with *Trichoderma harzianum* + soil amended with vermiwash | 304.16 | 344.32 | 316.70 | 1045.11 | 811.33 | 1128.00 |
| **4** | Seed biopriming with *Trichoderma virens* + in soil amended with NSKE (5%) | 281.01 | 223.27 | 366.07 | 812.00 | 821.17 | 1085.61 |
| **5** | Seed biopriming with *Trichoderma virens* + soil amended with compost tea | 332.28 | 133.08 | 143.55 | 377.77 | 775.51 | 829.17 |
| **6** | Seed biopriming with *Trichoderma virens* + soil amended with vermiwash | 159.20 | 153.92 | 346.83 | 676.81 | 811.33 | 869.00 |
| **7** | Seed biopriming with *Trichoderma atroviride* + soil amended with NSKE (5%) | 226.39 | 163.94 | 313.12 | 446.30 | 576.19 | 743.64 |
| **8** | Seed biopriming with *Trichoderma atroviride* + soil amended with compost tea | 116.28 | 140.55 | 157.71 | 543.94 | 411.00 | 983.50 |
| **9** | Seed biopriming with *Trichoderma atroviride* + soil amended with vermiwash | 287.89 | 151.32 | 214.02 | 677.47 | 543.63 | 1127.67 |
| **10** | Seed biopriming with *Bacillus licheniformis* + soil amended with NSKE (5%) | 255.56 | 336.51 | 215.51 | 417.75 | 950.35 | 955.40 |
| **11** | Seed biopriming with *Bacillus licheniformis* + soil amended with compost tea | 247.79 | 122.05 | 153.33 | 404.20 | 892.50 | 846.67 |
| **12** | Seed biopriming with *Bacillus licheniformis* + soil amended with vermiwash | 254.79 | 196.62 | 250.33 | 494.33 | 920.17 | 894.11 |
| **13** | Seed biopriming with *Pseudomonas fluorescens*+ soil amended with NSKE (5%) | 458.17 | 452.08 | 376.73 | 424.00 | 1111.50 | 1192.00 |
| **14** | Seed biopriming with *Pseudomonas fluorescens* + soil amended with compost tea | 178.58 | 382.24 | 298.69 | 711.00 | 775.52 | 872.00 |
| **15** | Seed biopriming with *Pseudomonas fluorescens* + soil amended with vermiwash | 401.68 | 387.69 | 374.26 | 547.72 | 911.17 | 915.48 |
| **16** | Control | 126.21 | 255.53 | 123.46 | 612.17 | 244.30 | 648.00 |
| | **$CD_{(0.05)}$** | **4.99** | **4.06** | **3.44** | **3.19** | **3.55** | **4.19** |

vermicompost) and T1 (seed biopriming with *T. harzianum* and soil application with neem cake) with 42.88 and 37.84 per cent reduction in seedling mortality, respectively (Table 44, Plate 30c). Treatment combination T8 (seed biopriming with *Melia azedarach* and soil application with vermicompost) was found least effective against damping-off disease with seedling mortality of 32.55 per cent in comparison to control (Table 44).

In chilli, treatment combination T3 (seed biopriming with *T. virens* and soil application with neem cake) was found most effective in solarized beds with seed germination of 77.47 per cent in solarized beds followed by treatment combination T6 (seed biopriming with *R. elegans* and soil application with vermicompost) with seed germination of 77.33 per cent. Treatment combination T9 (seed biopriming with *P. dipodomyicola* and soil application with neem cake) was found next in efficacy in solarized beds with seed germination of 77.23 per cent. Treatment combination T7 (seed biopriming with *M. azedarach* and soil application with neem cake) was found least effective with seed germination of 65.62 per cent in comparison to control. However, treatment combination T8 (seed biopriming with *M. azedarach* and soil application with vermicompost) was found most effective with shoot length of 18.77 cm with significant increase in shoot length of chilli seedlings followed by T9 (seed biopriming with *P. dipodomyicola* and soil application with neem cake) with shoot length of 17.39 cm. Treatment combination T9 (seed biopriming with *P. dipodomyicola* and soil application with neem cake) was found most effective with root length of 10.84 cm followed by treatment combination T5 (seed biopriming with *R. elegans* and soil application with neem cake) with root length of 10.06 cm (Table 44).

In chilli, treatment combination T10 (seed biopriming with *P. dipodomyicola* and soil application with vermicompost) was found most effective in un-solarized beds with least seedling mortality of 30.56 per cent followed by treatment combination T12 (seed biopriming with *Stenotrophomonas* sp. and soil application with vermicompost) with seedling mortality of 32.45 per cent. Among all the treatment in unsolarized beds, treatment combination T9 (seed biopriming with *P. dipodomyicola* and soil application with neem cake) was found most effective with average shoot length of 15.92 cm followed by treatment combination T8 (seed biopriming with *M. azedarach* and soil application with vermicompost) with shoot length of 14.58 cm. Treatment combination T9 (seed biopriming with *P. dipodomyicola* and soil application with neem cake) was found most effective with root length of 10.63 cm

***Roylea elegans*** **+ Neem cake**

***Penicillium dipodomyicola*** **+ Neem cake**

***Roylea elegans*****+ Vermicompost**

**Unsolarized plot**

**Solarized plot with primed seedlings**

**Solarized plot with non-primed seedlings**

**Plate 30b: Efficacy of integration of seed biopriming with effective bio-inoculants and soil solarization**

***Penicillium dipodomyicola* + Neem cake**

***Penicillium dipodomyicola* + Vermicompost**

***Stenotrophomonas sp.* + Neem cake**

***Stenotrophomonas sp.* + Vermicompost**

***Stenotrophomonas sp.* + Vermicompost**

**Control**

**Plate 30c: Efficacy of integration of seed biopriming with effective bioinoculants and soil solarization**

followed by treatment combination T5 (seed biopriming with *R. elegans* and soil application with neem cake) with root length of 8.9 cm in unsolarized beds (Table 44).

In capsicum, treatment combination T9 (seed biopriming with *Penicillium dipodomyicola* and soil application of neem cake) in solarized plots was found most effective against damping-off disease with 42.89 per cent reduction of seedling mortality in comparison to control. Treatment combination T10 (seed biopriming with *Penicillium dipodomyicola* and soil application of vermicompost) followed by T1 (seed biopriming with *T. harzianum* and soil application with neem cake) were next in efficacy with 38.79 and 24.59 per cent reduction in seedling mortality, respectively (Table 45, Plate 30c).

Treatment combination T7 (seed biopriming with *Melia azedarach* and soil application with neem cake) was found least effective with seedling mortality of 31.50 per cent in comparison to control. Treatment combination T9 (seed biopriming with *P. dipodomyicola* and soil application with neem cake) was also found most effective with higher seed germination of 79.17 per cent in solarized beds followed by treatment combination T12 (seed biopriming with *Stenotrophomonas* sp and soil application with vermicompost) with seed germination of 76.87 per cent. Treatment combination T10 (seed biopriming with *P. dipodomyicola* and soil application with vermicompost) was found next in efficacy in solarized beds with seed germination of 76.43 per cent (Table 45).

Treatment combination T8 (seed biopriming with *M. azedarach* and soil application with vermicompost) also significantly improved average shoot length to 18.92 cm followed by T3 (seed biopriming with *T. virens* and soil application with neem cake) where average shoot length of 15.72 cm was recorded. Treatment combination T11 (seed biopriming with *Stenotrophomonas* sp. and soil application with neem cake) was found least effective with shoot length of 11.18 cm in solarized beds. Treatment combination T5 (seed biopriming with *R. elegans* and soil application with vermicompost) was found most effective with root length of 10.98 cm followed by treatment combination T8 (seed biopriming with *Melia azedarach* and soil application with vermicompost) where average root length of 10.31 cm was recorded (Table 45).

**Table 43: Efficacy of integration of seed biopriming with effective bio-inoculants (BCAs, plant extracts, endophytes) and soil solarization in tomato**

| Treatments (Seed biopriming) | Seed germination (%) | | | Shoot length (cm) | | | Root length (cm) | | | Disease incidence (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Solarized beds | Un-solarized beds | Mean | Solarized beds | Un-solarized beds | Mean | Solarized beds | Un-solarized beds | Mean | Solarized beds | Un-solarized beds | Mean |
| T1 *Trichoderma harzianum*+ Neem cake | 86.15 (68.12) | 77.01 (61.33) | **81.58 (64.73)** | 20.49 | 10.87 | **15.69** | 10.83 | 8.60 | **9.72** | 18.07 (25.14) | 30.45 (33.48) | **24.26 (29.31)** |
| T2 *Trichoderma harzianum* + Vermicompost | 82.78 (65.46) | 73.92 (59.27) | **78.35 (62.36)** | 14.32 | 9.55 | **11.94** | 8.64 | 6.56 | **7.59** | 19.98 (26.54) | 33.37 (35.27) | **26.68 (30.91)** |
| T3 *Trichoderma virens* + Neem cake | 65.65 (54.12) | 64.34 (53.31) | **64.99 (53.72)** | 15.26 | 8.62 | **11.94** | 7.61 | 6.25 | **6.93** | 21.35 (27.51) | 28.19 (32.05) | **24.77 (29.78)** |
| T4 *Trichoderma virens*+ Vermicompost | 67.47 (55.21) | 61.33 (51.53) | **64.40 (53.37)** | 17.78 | 8.30 | **13.04** | 8.28 | 5.24 | **6.76** | 19.60 (26.27) | 29.61 (32.95) | **24.61 (29.61)** |
| T5 *Roylea elegans* + Neem cake | 74.17 (59.43) | 71.07 (57.44) | **72.62 (58.43)** | 15.35 | 9.87 | **12.61** | 6.50 | 5.23 | **5.87** | 17.61 (24.79) | 22.18 (28.08) | **19.89 (26.44)** |
| T6 *Roylea elegans* + Vermicompost | 83.17 (65.79) | 73.90 (59.26) | **78.53 (62.53)** | 18.53 | 10.92 | **14.72** | 9.13 | 4.82 | **6.97** | 17.97 (25.07) | 25.55 (30.31) | **21.76 (27.69)** |
| T7 *Melia azedarach* + Neem cake | 65.98 (54.30) | 61.33 (51.53) | **63.66 (52.92)** | 16.22 | 13.00 | **14.61** | 6.63 | 5.33 | **5.98** | 20.72 (27.06) | 27.23 (31.44) | **23.98 (29.25)** |
| T8 *Melia azedarach* + Vermicompost | 68.83 (56.04) | 63.91 (53.06) | **66.37 (54.55)** | 15.11 | 10.72 | **12.91** | 5.58 | 5.22 | **5.39** | 25.52 (30.33) | 27.17 (31.39) | **26.34 (30.86)** |
| T9 *Penicillium dipodomyicola* + Neem cake | 90.85 (72.41) | 82.97 (65.61) | **86.91 (69.01)** | 24.16 | 9.64 | **16.90** | 11.48 | 6.45 | **8.96** | 15.63 (23.27) | 24.03 (29.31) | **19.83 (26.29)** |
| T10 *Penicillium dipodomyicola* + Vermicompost | 86.74 (68.64) | 79.60 (63.13) | **83.17 (65.86)** | 13.90 | 10.67 | **12.29** | 6.53 | 5.53 | **6.03** | 18.00 (25.09) | 25.57 (30.36) | **21.78 (27.73)** |
| T11 *Stenotrophomonas* sp. + Neem cake | 73.45 (58.99) | 71.41 (57.66) | **72.43 (58.32)** | 14.83 | 8.88 | **11.86** | 9.45 | 5.56 | **7.50** | 23.50 (28.98) | 36.45 (37.12) | **29.97 (33.05)** |
| T12 *Stenotrophomonas* sp. + Vermicompost | 77.50 (61.66) | 69.76 (56.62) | **73.63 (59.14)** | 12.43 | 9.41 | **10.92** | 5.48 | 5.48 | **5.48** | 20.28 (26.75) | 38.88 (38.56) | **29.58 (32.66)** |
| T13 Control | 54.00 (47.28) | 44.57 (41.86) | **49.28 (44.57)** | 9.64 | 7.56 | **8.60** | 4.17 | 3.78 | **4.04** | 31.22 (33.95) | 43.16 (41.05) | **31.19 (37.50)** |
| **Mean** | **75.13 (60.57)** | **68.86 (56.28)** | | **16.00** | **9.84** | | **7.72** | **5.73** | | **20.73 (26.98)** | **30.14 (33.18)** | |
| **$CD_{(0.05)}$** | | | | | | | | | | | | |
| **Treatment (T)** | **1.49** | | | **1.22** | | | **0.48** | | | **0.93** | | |
| **Solarization (S)** | **0.58** | | | **0.48** | | | **0.19** | | | **0.37** | | |
| **TxS** | **2.10** | | | **1.72** | | | **0.68** | | | **1.32** | | |

Figures in the parenthesis are arc sine transformed values

**Table 44: Efficacy of integration of seed biopriming with effective bio-inoculants (BCAs, plant extracts, endophytes) and soil solarization in chilli**

| Treatments (Seed biopriming) | Seed germination (%) | | | Shoot length (cm) | | | Root length (cm) | | | Disease incidence (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Solarized beds | Un-solarized beds | Mean | Solarized beds | Un-solarized beds | Mean | Solarized beds | Un-solarized beds | Mean | Solarized beds | Un-solarized beds | Mean |
| T1 *Trichoderma harzianum*+ Neem cake | 71.00 (57.39) | 68.02 (55.55) | **69.51 (56.47)** | 14.75 | 13.09 | **13.92** | 9.87 | 8.51 | **9.19** | 21.58 (27.67) | 39.75 (39.07) | **30.67 (33.37)** |
| T2 *Trichoderma harzianum* + Vermicompost | 74.27 (59.49) | 68.00 (55.53) | **71.13 (57.51)** | 14.32 | 12.18 | **13.25** | 8.47 | 5.24 | **6.86** | 24.53 (29.68) | 35.37 (36.48) | **29.95 (33.08)** |
| T3 *Trichoderma virens* + Neem cake | 77.47 (61.64) | 65.35 (53.92) | **71.41 (57.78)** | 16.47 | 11.48 | **13.97** | 7.50 | 7.44 | **7.48** | 27.26 (31.46) | 37.31 (37.63) | **32.29 (34.54)** |
| T4 *Trichoderma virens*+ Vermicompost | 76.13 (60.74) | 66.48 (54.61) | **71.31 (57.67)** | 13.92 | 12.19 | **13.05** | 8.98 | 8.77 | **8.87** | 29.37 (32.79) | 38.90 (38.57) | **34.13 (35.69)** |
| T5 *Roylea elegans* + Neem cake | 73.83 (59.21) | 68.66 (55.94) | **71.25 (57.58)** | 13.52 | 12.38 | **12.95** | 10.06 | 8.90 | **9.48** | 29.17 (32.67) | 41.99 (40.38) | **35.58 (36.52)** |
| T6 *Roylea elegans* + Vermicompost | 77.33 (61.55) | 64.51 (53.42) | **70.92 (57.48)** | 13.49 | 11.08 | **12.29** | 8.96 | 8.60 | **8.78** | 30.61 (33.58) | 44.27 (41.69) | **37.44 (37.64)** |
| T7 *Melia azedarach* + Neem cake | 65.62 (54.08) | 65.53 (54.02) | **65.57 (54.05)** | 13.01 | 11.69 | **12.35** | 7.87 | 6.25 | **7.06** | 31.59 (34.18) | 46.50 (42.98) | **39.05 (38.58)** |
| T8 *Melia azedarach* + Vermicompost | 68.00 (55.53) | 63.51 (52.82) | **65.75 (54.17)** | 18.77 | 14.58 | **16.68** | 9.32 | 8.20 | **8.78** | 32.55 (34.79) | 48.77 (44.28) | **40.66 (39.52)** |
| T9 *Penicillium dipodomyicola* + Neem cake | 77.23 (61.48) | 74.00 (59.32) | **75.62 (60.40)** | 17.39 | 15.92 | **16.66** | 10.84 | 10.63 | **10.74** | 18.33 (25.34) | 33.91 (35.60) | **26.12 (30.47)** |
| T10 *Penicillium dipodomyicola* + Vermicompost | 76.67 (61.10) | 74.67 (59.76) | **75.67 (60.43)** | 16.12 | 12.48 | **14.30** | 8.12 | 5.21 | **6.65** | 19.83 (26.43) | 30.56 (33.55) | **25.19 (29.99)** |
| T11 *Stenotrophomonas* sp. + Neem cake | 73.33 (58.92) | 68.17 (55.63) | **70.75 (57.28)** | 11.19 | 9.88 | **10.53** | 9.56 | 8.10 | **8.83** | 25.77 (30.49) | 36.04 (36.88) | **30.91 (33.69)** |
| T12 *Stenotrophomonas* sp. + Vermicompost | 76.40 (60.92) | 64.27 (53.27) | **70.33 (57.09)** | 11.71 | 11.45 | **11.58** | 9.57 | 7.52 | **8.54** | 24.33 (29.54) | 32.45 (34.71) | **28.39 (32.12)** |
| T13 Control | 63.77 (52.98) | 62.67 (52.32) | **63.22 (52.65)** | 10.53 | 9.18 | **9.86** | 7.50 | 4.58 | **6.04** | 34.72 (36.09) | 54.90 (47.79) | **44.81 (41.94)** |
| **Mean** | **73.16 (58.85)** | **67.22 (55.08)** | | **14.24** | **12.12** | | **8.97** | **7.54** | | **26.89 (31.13)** | **40.06 (39.19)** | |
| **$CD_{(0.05)}$** | | | | | | | | | | | | |
| **Treatment (T)** | **1.06** | | | **1.15** | | | **0.72** | | | **0.82** | | |
| **Solarization (S)** | **0.42** | | | **0.45** | | | **0.28** | | | **0.32** | | |
| **TxS** | **1.49** | | | **1.62** | | | **1.01** | | | **1.15** | | |

Figures in the parenthesis are arc sine transformed values

**Table 45: Efficacy of integration of seed biopriming with effective bio-inoculants (BCAs, plant extracts, endophytes) and soil solarization in capsicum**

| Treatments (Seed biopriming) | Seed germination (%) | | | Shoot length (cm) | | | Root length (cm) | | | Disease incidence (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Solarized beds | Un-solarized beds | Mean | Solarized beds | Un-solarized beds | Mean | Solarized beds | Un-solarized beds | Mean | Solarized beds | Un-solarized beds | Mean |
| T1 *Trichoderma harzianum*+ Neem cake | 75.78 (60.49) | 71.02 (57.41) | **73.40 (58.96)** | 15.45 | 13.78 | **14.62** | 8.81 | 8.35 | **8.58** | 25.17 (30.09) | 30.83 (33.71) | **27.99 (31.91)** |
| T2 *Trichoderma harzianum* + Vermicompost | 74.85 (59.22) | 71.1 (59.61) | **74.15 (59.42)** | 14.67 | 10.81 | **12.74** | 8.55 | 5.94 | **7.25** | 27.98 (31.92) | 49.58 (44.71) | **38.78 (38.32)** |
| T3 *Trichoderma virens* + Neem cake | 73.92 (59.27) | 71.22 (57.53) | **72.57 (58.40)** | 15.72 | 11.78 | **13.75** | 7.28 | 7.48 | **7.38** | 26.41 (30.91) | 46.05 (42.72) | **36.23 (36.82)** |
| T4 *Trichoderma virens*+ Vermicompost | 70.72 (57.23) | 50.36 (45.19) | **60.54 (51.20)** | 13.69 | 10.72 | **12.20** | 9.41 | 8.58 | **8.99** | 28.70 (32.38) | 34.42 (35.91) | **31.56 (34.14)** |
| T5 *Roylea elegans* + Neem cake | 67.50 (55.22) | 64.07 (53.16) | **65.79 (54.19)** | 13.38 | 11.79 | **12.59** | 10.98 | 8.11 | **9.55** | 26.67 (31.07) | 39.87 (39.14) | **33.27 (35.11)** |
| T6 *Roylea elegans* + Vermicompost | 75.10 (60.04) | 61.17 (51. 43) | **68.13 (55.74)** | 12.12 | 10.82 | **11.47** | 8.24 | 7.94 | **8.09** | 30.36 (33.42) | 31.44 (34.09) | **30.89 (33.75)** |
| T7 *Melia azedarach* + Neem cake | 58.33 (49.78) | 58.15 (49.67) | **58.24 (49.73)** | 13.37 | 11.68 | **12.53** | 7.31 | 5.65 | **6.48** | 31.50 (34.13) | 41.59 (40.14) | **36.55 (37.14)** |
| T8 *Melia azedarach* + Vermicompost | 57.11 (49.07) | 55.25 (47.99) | **56.18 (48.53)** | 18.92 | 13.56 | **16.24** | 10.31 | 8.62 | **9.46** | 31.00 (33.82) | 37.34 (37.65) | **34.17 (35.73)** |
| T9 *Penicillium dipodomyicola* + Neem cake | 79.17 (62.82) | 57.04 (49.03) | **68.10 (55.93)** | 14.89 | 14.72 | **14.81** | 8.73 | 7.43 | **8.08** | 19.06 (25.88) | 26.50 (30.97) | **22.78 (28.42)** |
| T10 *Penicillium dipodomyicola* + Vermicompost | 76.43 (60.94) | 69.44 (56.42) | **72.94 (58.68)** | 13.48 | 12.70 | **13.09** | 8.99 | 5.22 | **7.11** | 20.43 (26.87) | 26.02 (30.66) | **23.22 (28.76)** |
| T11 *Stenotrophomonas* sp. + Neem cake | 71.55 (57.74) | 61.34 (51.54) | **66.44 (54.64)** | 11.18 | 10.89 | **11.03** | 9.44 | 9.91 | **9.32** | 26.56 (30.92) | 32.86 (34.96) | **29.71 (32.94)** |
| T12 *Stenotrophomonas* sp. + Vermicompost | 76.87 (61.25) | 70.21 (56.90) | **73.54 (59.07)** | 11.82 | 11.14 | **11.49** | 8.73 | 8.41 | **8.57** | 27.63 (31.70) | 32.50 (34.74) | **30.07 (33.22)** |
| T13 Control | 53.67 (47.09) | 50.78 (45.43) | **52.26 (46.26)** | 9.51 | 8.56 | **9.04** | 7.34 | 5.22 | **6.28** | 33.38 (35.28) | 42.55 (40.69) | **37.96 (37.99)** |
| **Mean** | **68.93 (56.27)** | **63.73 (53.07)** | | **13.69** | **11.85** | | **8.78** | **7.39** | | **27.29 (35.41)** | **36.27 (36.93)** | |
| **$CD_{(0.05)}$** | | | | | | | | | | | | |
| **Treatment (T)** | **0.77** | | | **0.88** | | | **0.75** | | | **2.01** | | |
| **Solarization (S)** | **0.30** | | | **0.34** | | | **0.29** | | | **0.79** | | |
| **TxS** | **1.09** | | | **1.24** | | | **1.07** | | | **2.85** | | |

Figures in the parenthesis are arc sine transformed values

Treatment combination T3 (seed biopriming with *T. virens* and soil application with neem cake) was found most effective with seed germination of 71.22 per cent followed by treatment combination T2 (seed biopriming with *T. harzianum* and soil application with vermicompost) where seed germination of 71.1 per cent in unsolarized beds was recorded which are statistically at par. Among all the treatment combinations evaluated in unsolarized beds, treatment combination T9 (seed biopriming with *P. dipodomyicola* and soil application with neem cake) was found most effective with shoot length of 14.72 cm followed by treatment combination T1 (seed biopriming with *T. harzianum* and soil application with neem cake) where average shoot length of 13.78 cm was recorded. Treatment combination T11 (seed biopriming with *Stenotrophomonas* sp and soil application with neem cake) was found most effective in un-solarized beds with increasing root length of 9.91 cm followed by treatment combination T8 (seed biopriming with *Melia azedarach* and soil application with vermicompost) where root length of 8.62 cm was recorded (Table 45).

Novel endophyte *Stenotrophomonas* sp. have been reported effective against soil-borne pathogens (Nakayama et al. 1999; Wolf et al. 2002; Mishra et al. 2017). However, there is no report where endophyte like *Penicillium dipodomyicola* and *Stenotrophomonas* sp. have been used as seed biopriming agents against damping-off pathogens. The ability of *Trichoderma* spp. to suppress *Pythium aphanidermatum* in tomato seedlings have been reported by various research workers (Khare et al. 2010; Kipngeno et al. 2015). Tiwari et al. (2017) reported that application of antagonists like (*Aspergillus niger*, *Penicillium javanicum* and *Trichoderma hamatum)* with natural plant extracts of *Ocimum sanctum*, *Rauvolfia serpentina* and *Azadirachta indica*) checked growth of *Fusarium* by 57.14-85.71% in eggplant. Efficacy of organic amendments has been reported against soil borne phytopathogens by research workers (Shafique et al. 2016; Bonanomi et al. 2018; Panth et al. 2020). The results of the present investigation corroborate the research findings of various workers regarding the effectiveness of soil solarization against pathogens causing damping-off (Pullman et al. 1979; Cook and Baker 1983; Raj et al. 1997; Raj and Bhardwaj 2000; Joshi et al. 2009; Uddin et al. 2009). Soil solarization has been reported to be effective in reducing the incidence of damping-off disease in solanaceous crops viz. tomato, chilli and brinjal (Pandey and Pandey 2005). Soil solarization for 30 days in nurseries of tomato and chilli resulted in lower incidence of damping-off disease (Rahman et al. 2003; Akhtar et al. 2008; Kadam et al. 2018). Rahman et al. (2003) reported that soil solarization resulted in lowest incidence of damping-off in tomato (3.9 %) and chilli (1.3 %) in comparison to control.

# *Chapter-5*

# SUMMARY AND CONCLUSION

Damping-off disease caused by fungal pathogens namely *Pythium*, *Phytophthora*, *Fusarium*, *Sclerotium* and *Rhizoctonia* is a devastating disease in nursery of solanaceous vegetable crops. Seed and seedling health and protection from pathogens is essential for healthy and high-quality seedling production particularly in transplanted vegetable crops. In Himachal Pradesh, pre-emergence and post-emergence damping-off disease is of major concern in solanaceous vegetable crops. To maintain soil health, biological control methods are gaining importance as an effective alternative over chemical control of disease management. Among different methods of application of biological control agents, seed biopriming is a novel method for disease management. Seed biopriming refers to an approach for seed treatment in which hydration of seed is integrated with bio-inoculation. Seed biopriming is simple, highly effective, economical and eco-friendly approach to counter biotic and abiotic stress conditions being encountered by the seeds. Thus, present study was carried out with the objective to study the potential of different methodologies of seed biopriming in managing damping-off disease in solanaceous vegetable crops.

Symptoms of damping-off disease in nursery stage appeared in two phases as pre-emergence and post-emergence damping-off. In pre-emergence phase, rotting of seeds occur which led to non-uniform and patchy germination in the field. In post-emergence phase, water-soaked brown lesions appeared on the infected seedlings near soil line which ultimately led to collapse and decay of young seedlings.

Pathogens from the symptomatic plants were isolated and purified. The purified pathogens were identified as *Pythium* sp., *Fusarium oxysporum*, *Sclerotium rolfsii* and *Rhizoctonia solani* based upon their cultural and morphological characters. Pathogenicity of isolated pathogens with damping-off disease in tomato, chilli and capsicum was proved by sick soil method. The combined application of inoculum of all the isolated pathogens resulted in higher incidence of pre-emergence (66.67 %) and post-emergence (75.42 %) damping-off in tomato with incubation period of 7 days. Similarly, combined application of inoculum of these pathogens resulted in 72.65 per cent of pre-emergence and 84.33 per cent of post-emergence incidence of damping-off in chilli with incubation period of 6 days. In capsicum,

72.16 per cent of pre-emergence and 81.97 per cent of post-emergence incidence of damping-off was recorded with incubation period of 6 days.

*Trichoderma virens* and *T. harzianum* were found most effective against important damping-off pathogen *Pythium* sp. with mycelial inhibition of 88.70, 83.85 per cent, respectively. *Trichoderma virens*, *T. harzianum* and *T. atroviride* resulted in mycelial inhibition of 86.55, 84.26, 62.77 per cent against *Fusarium oxysporum*. *Trichoderma harzianum* was found most effective among all biocontrol agents with mycelial inhibition of 36.74 per cent followed by *Trichoderma atroviride* and *Bacillus licheniformis* with inhibition of 36.48 and 35.16 per cent respectively, against *Sclerotium rolfsii* which are statistically at par. Similarly, *Trichoderma harzianum* caused maximum (64.40 %) inhibition of *Rhizoctonia solani*. *Trichoderma virens* and *T. atroviride* were recorded next in efficacy against *Rhizoctonia solani* with mycelial inhibition of 53.81 and 53.35, per cent respectively.

Effective concentration and duration of application of suspensions of effective biocontrol agents for seed biopriming was standardized by germination test carried out by paper rolled towel method. In tomato, seed biopriming with spore concentration of $10^7$ cfu per ml was found most effective for *Trichoderma harzianum*, *T. atroviride* and *T. virens* with germination per centage of 76.0, 75.67, 71.67, respectively. *Pseudomonas fluorescens* and *Bacillus licheniformis* were found most effective at spore concentration of $10^8$ cfu per ml with seed germination of 82.0 and 88.33 per cent, respectively. In chilli, seed biopriming with *Bacillus licheniformis* and *Pseudomonas fluorescens* at spore concentration of $10^8$ cfu per ml was found most effective with germination of 92.33 and 90.33 per cent, respectively. *Trichoderma harzianum*, *T. virens* and *T. atroviride*, each applied at spore concentration of $10^7$ cfu per ml were effective with enhanced germination of 84.0, 77.67, 85.33 per cent, respectively. In capsicum, seed biopriming with *Trichoderma harzianum*, *T. atroviride* and *T. virens* at spore concentration of $10^8$ cfu per ml resulted in seed germination of 75.0, 65.0 and 73.0 per cent, respectively. For bacterial biocontrol agents, seed biopriming with *Pseudomonas fluorescens* and *Bacillus licheniformis* was found most effective at $10^8$ cfu per ml concentration with germination of 66.67 and 57.67 per cent, respectively.

Duration of seed biopriming with biocontrol agents had a significant impact on seedling mortality and also on growth of seedlings. Biopriming of tomato seeds with *Trichoderma harzianum*, *T. atroviride* and *T. virens* for 8 hrs at spore concentration of $10^7$

cfu per ml was found most effective with germination of 89.33, 77.67 and 93 per cent, respectively. Similarly, seed biopriming 8 hrs duration with *Pseudomonas fluorescens* and *Bacillus licheniformis* at spore concentration of $10^8$ cfu per ml was found most effective with seed germination of 57.33 and 56.0 per cent, respectively. Similarly, biopriming of chilli seeds with *Trichoderma harzianum*, *T. virens* and *T. atroviride* for 8 hrs each at spore concentration of $10^7$ cfu per ml resulted in maximum seed germination of 90.67, 87.33 and 82.33 per cent, respectively. Bacterial biocontrol agents, *Pseudomonas fluorescens* and *Bacillus licheniformis* were also found effective and treatment for 8 hrs at spore concentration of $10^8$ cfu per ml resulted in seed germination of 83.67 and 88.33 per cent, respectively. In capsicum, seed biopriming with *Trichoderma harzianum* for 8 hrs at $10^7$ cfu per ml was found most effective with germination of 83.67 per cent followed by *Trichoderma virens* and *T. atroviride* with germination of 69.67 and 66.33 per cent, respectively.

Seed biopriming of tomato, chilli and capsicum with effective fungal biocontrol agents (*Trichoderma harzianum*, *T. virens*, *T. atroviride*) at $10^7$ cfu per ml and bacterial biocontrol agents as well as with PGPRs (*Pseudomonas fluorescens*, *Bacillus licheniformis, Azospirillum, Azotobacter, Pseudomonas aeruginosa*) at $10^8$ cfu per ml for 8 hrs was carried out under field conditions. Biopriming of tomato seeds with *Trichoderma harzianum* resulted in 57.88 per cent less seedling mortality and 47.40 per cent higher seed germination. Similarly, in chilli, seed biopriming with *Trichoderma harzianum* resulted in 46.16 per cent reduction in seedling mortality and 30.97 per cent higher seed germination. Seed biopriming with *Trichoderma virens* was found next in efficacy with 54.71 per cent reduction in seedling mortality and 19.54 per cent higher seed germination. However, biopriming of capsicum seeds with *Trichoderma atroviride* resulted in least seedling mortality of 13.85 per cent followed by seed biopriming with *Trichoderma virens* with seedling mortality of 15.77 per cent. Seed biopriming with *Trichoderma harzianum* and *T. virens* was found most effective with 74.73 and 60.69 per cent germination of bioprimed capsicum seeds, respectively.

Extracts of eleven native plants at 10 % v/v concentration were evaluated for their efficacy against damping-off pathogens under *in vitro* conditions. Extract of *Roylea elegans* was found most effective against *Pythium* sp. with mycelial inhibition of 23.89 per cent followed by *Melia azedarach* and *Datura stramonium* with mycelial inhibition of 15.85 and 14.11 per cent, respectively. Extracts of *Melia azedarach* was found most effective against *Fusarium oxysporum* with inhibition of 55.55 per cent followed by *Datura stramonium* with

inhibition of 52.71 per cent. *Datura stramonium* and *Roylea elegans* extract were found most effective against *Sclerotium rolfsii* and *Rhizoctonia solani* with mycelial inhibition of 33.8-42.4 and 40.2-49.8 per cent, respectively.

Effective concentration of plant extracts was standardized for carrying out seed biopriming of tomato, chilli and capsicum seeds. Seed biopriming with 10 per cent concentration of plant extracts was found most effective and significant in tomato, chilli and capsicum. Seed biopriming in tomato with extracts of *Melia azedarach* (10 %) was found most effective with germination of 92.5 per cent. *Roylea elegans* (10 %) was most effective treatment of seed biopriming with germination of 92.33 per cent in chilli. In capsicum, seed biopriming with *Melia azedarach* at 10 % concentration was found most effective with seed germination of 92.0 per cent.

Duration of seed biopriming with effective plant extracts at their standardized concentration was optimized for seed biopriming of tomato, chilli and capsicum. Seed biopriming with plant extracts at 10 % concentration for 12 hrs was found most effective with respect to its effect on seed germination and seed attributes in tomato, chilli and capsicum. Seed biopriming with *Melia azedarach* for 12 hrs at 10 % concentration resulted in highest seed germination of 88.67 per cent followed by *Roylea elegans* (10 %) for similar duration in tomato. In chilli, seed biopriming with *Roylea elegans* for 12 hrs at 10 % concentration was found most effective with germination of 93.33 per cent. Similarly, seed biopriming with *Roylea elegans* for 12 hrs at 10 % concentration was found most effective with germination of 91.0 per cent in capsicum.

Biopriming of tomato, chilli and capsicum seeds with effective plant extracts at 10 % concentration and for 12 hrs was carried out under field conditions to study their potential in managing damping-off disease. In case of tomato seedlings, seed biopriming with *Roylea elegans* was found most effective against damping-off disease with 54.73 per cent less seedling mortality followed by *Melia azedarach* with seedling mortality of 23.27 per cent. However, in chilli seed biopriming with *Melia azedarach* was most effective with least seedling mortality of 17.26 per cent. In capsicum, seed biopriming with *Roylea elegans* was found most effective with seedling mortality of 14.26 per cent followed by *Melia azedarach* with seedling mortality of 19.19 per cent.

Integration of seed biopriming in tomato, chilli and capsicum with effective plant extracts and biocontrol agents was carried out under field conditions against the damping-off disease. Seed biopriming with treatment combination of *Roylea elegans* and *Trichoderma harzianum* was found most effective against damping-off disease with least seedling mortality of 18.27 per cent followed by *Melia azedarach* and *Trichoderma virens* with seedling mortality of 20.12 per cent. In chilli, treatment combination of *Roylea elegans* and *Trichoderma harzianum* was found most effective in managing damping-off disease with seedling mortality of 16.86 per cent. Similarly, treatment combination of *Roylea elegans* and *Trichoderma harzianum* was most effective with 55.19 per cent reduction in capsicum damping-off followed by treatment combination of *Melia azedarach* and *Trichoderma virens* with seedling mortality of 21.68 per cent.

Endophytes are the microorganisms which reside inside the plant tissue without causing any harm to plants and were explored for their biocontrol potential against damping-off disease. Eight endophytes (3 fungal and 5 bacterial endophytes) were isolated from *Mentha* sp., *Ocimum* sp, *Datura stramonium*, *Vinca rosea* and *Hibiscus-rosa-sinensis*. Fungal endophytes were identified as *Penicillium* sp., *Fusarium oxysporum* and *Curvularia* sp. based on their morphological characters. Bacterial endophytes were subjected to biochemical characterization among which, bacterial endophyte EB4 was found gram positive while others were found gram negative. With the exception of bacterial endophyte EB3, all the other bacterial endophytes were oxidase negative. All the tested bacterial endophytes were catalase positive and were also methyl red negative.

*In vitro* evaluation of the isolated endophytes was carried out against the damping-off pathogens. Fungal endophyte EF1 (*Penicillium* sp.) was found as most effective against *Pythium* sp. with mycelial inhibition of 94.44 per cent followed by bacterial endophyte EB5. Among the endophytes tested against *Fusarium oxysporum*, maximum mycelial inhibition (77.7 %) was recorded by fungal endophyte EF1 followed by bacterial endophyte EB2 with 56.96 %. Similarly, fungal endophyte EF1 resulted in 46.37 and 71.85 per cent mycelial inhibition in *Sclerotium rolfsii* and *Rhizoctonia solani*, respectively.

Compatibility between bacterial endophytes and bacterial biocontrol agents was also studied for their potential use in future studies as consortium biopriming. All other biocontrol

agents and bacterial endophytes were compatible with each other except for bacterial endophyte EB5 which was incompatible with *Pseudomonas fluorescens* and *Bacillus* sp.

Concentration of suspensions of endophytes was also standardized for their use in seed biopriming. Seed biopriming treatment with bacterial endophyte EB3 at $10^7$ cfu per ml resulted in 93.3 per cent germination in tomato. Biopriming with bacterial endophyte EB5 at $10^7$ cfu per ml followed by fungal endophyte EF3 (*Curvularia* sp.) at $10^8$ cfu per ml were found next in efficacy with seed germination of 88.67 and 86.0 per cent, respectively. Seed germination of 95.67 % was recorded in biopriming treatment of chilli with fungal endophyte EF1 at $10^8$ cfu per ml. In capsicum, biopriming treatment with bacterial endophyte EB5 resulted in 89.0 per cent seed germination at concentration of $10^7$ cfu per ml.

Duration of seed biopriming with suspensions of endophytes at their standardized concentration was also standardized for better efficiency of seed biopriming with endophytes. Seeds of tomato bioprimed with extracts of fungal endophyte EF1 for 12 hrs at $10^8$ cfu per ml resulted in maximum germination of 92.33 per cent followed by seed biopriming with bacterial endophyte EB5 for 12 hrs at $10^7$ cfu per ml which resulted in 90.33 per cent germination. Similarly, seeds of chilli and capsicum bioprimed with extracts of fungal endophyte EF1 for 12 hrs at $10^8$ cfu per ml concentration had maximum germination of 95.0 and 91.67 per cent, respectively.

Effective fungal endophytes EF1 and EF3 at concentration of $10^8$ cfu per ml and bacterial endophytes EB2, EB3 and EB5 at concentration of $10^7$ cfu per ml bioprimed for 12 hrs were then evaluated under field conditions against damping-off disease. Seed biopriming with fungal endophyte EF1 and bacterial endophyte EB5 was found most efficacious against damping-off disease with least seedling mortality of 15.21 and 27.08 per cent in tomato, 16.33 and 22.48 per cent in chilli, respectively. However, in capsicum, seed biopriming with fungal endophyte EF1 was most effective with seedling mortality of 14.40 per cent followed by seed biopriming with EF3 with seedling mortality of 19.27 per cent.

Most effective endophytes were subjected to molecular characterization based on 18S rRNA and 16S rRNA gene sequencing. The fungal endophyte EF1 (*Penicillium* sp.) isolated from leaves of *Mentha* sp. was identified as *Penicillium dipodomyicola* with nucleotide sequence of 626 bp which has been submitted in NCBI genbank with accession no.

OQ281432. The bacterial endophyte EB5 isolated from leaves of *Ocimum* sp. was identified as *Stenotrophomonas* sp. with nucleotide sequence of 1449 bp and it has been submitted in NCBI genbank with accession no. OQ368742.

Effective treatments in biopriming were also combined with soil amendments. Seeds of tomato, chilli and capsicum bioprimed with effective biocontrol agents (*Trichoderma harzianum*, *T. virens*, *T. atroviride*, *Pseudomonas fluorescens*, *Bacillus licheniformis*) were sown in soil amended with NSKE (5%), compost tea and vermiwash under artificially inoculated conditions to check their efficacy against damping-off disease as well as their role in induction of defense enzyme activity in bioprimed seedlings. In tomato, treatment combination of seed biopriming with *Trichoderma harzianum* and soil amendment with NSKE was found most effective against the damping-off disease with 55.90 per cent reduction in seedling mortality. Total phenolic content was found maximum (458.17) in seedlings in treatment combination of seed biopriming with *Pseudomonas fluorescens* and soil amendment with NSKE. PAL activity was also found maximum (1612.0) in treatment combination of seed biopriming with *Trichoderma harzianum* and soil amendment with NSKE. PPO activity was also found maximum (19.27) in treatment combination of seed biopriming with *Trichoderma harzianum* and soil amendment with NSKE. However, PO activity was found maximum (1.07) in treatment combination of seed biopriming with *Trichoderma virens* and soil amendment with NSKE.

Treatment combination of seed biopriming with *Trichoderma virens* and soil amendment with vermiwash was found most effective against damping-off disease in chilli with 61.98 per cent reduction in seedling mortality followed by treatment combination of seed biopriming with *Trichoderma harzianum* and NSKE which resulted in seedling mortality of 12.30 per cent. PO activity was found higher (0.91) in treatment combinations of seed biopriming with *Trichoderma atroviride* and soil amendment with NSKE followed by treatment combination of seed biopriming with *Trichoderma harzianum* and soil amendment with NSKE (0.75). PPO and PAL activity were found maximum in treatment combination of seed biopriming with *Trichoderma harzianum* and soil amendment with NSKE with values of 19.6, 1675.48, respectively. Total phenolic content was found maximum (452.08) in treatment combination of seed biopriming with *Pseudomonas fluorescens* and soil amendment with NSKE. In capsicum, treatment combination of seed biopriming with *Trichoderma virens* and soil amendment with NSKE was found most effective against

damping-off disease with 57.46 per cent reduction in seedling mortality. PPO activity was also observed maximum in treatment combination of *Trichoderma harzianum* and soil amendment with vermiwash with enzyme activity of 14.8. PO and TPC activity were observed maximum (1.09, 376.73) in treatment combination of seed biopriming with *Pseudomonas fluorescens* and soil amendment with NSKE, respectively. However, PAL activity was found maximum (1743.64) in treatment combination of seed biopriming with *Trichoderma atroviride* and soil amendment with NSKE.

Seed bioprimed with bio-inoculants performed significantly better in solarized beds in comparison to unsolarized beds. Seed biopriming of tomato, chilli and capsicum seeds with effective bioinoculants (BCAs, plant extracts, endophytes) was found more effective in solarized soil against damping-off disease. A negative correlation between seed germination and seedling mortality was observed in tomato, chilli and capsicum due to integration of seed biopriming with soil solarization. In solarized beds of tomato, treatment combination of seed biopriming with fungal endophyte *Penicillium dipodomyicola* and soil application of neem cake was found most effective in managing damping-off disease with 49.93 per cent reduction in seedling mortality followed by treatment combination of seed biopriming with *Roylea elegans* and soil application with neem cake with seedling mortality of 17.61 per cent. In chilli, seed biopriming with *Penicillium dipodomyicola* and soil application of neem cake was found most effective with 47.21 per cent reduction in seedling mortality followed by treatment combination of seed biopriming with *Penicillium dipodomyicola* and soil application of vermicompost with 42.89 per cent reduction in seedling mortality. Treatment combination of seed biopriming with *Penicillium dipodomyicola* and soil application of neem cake was found most effective in managing damping-off disease of capsicum with least seedling mortality of 19.06 per cent followed by treatment combination of seed biopriming with *Penicillium dipodomyicola* and soil application of vermicompost with seedling mortality of 20.43 per cent.

## CONCLUSION

Based on the findings of present study, it can be concluded that seed biopriming with endophyte *Penicillium dipodomyicola* or with *Trichoderma harzianum* followed by their sowing in neem cake amended soil under solarized conditions is effective against damping-off disease in chilli, tomato and capsicum. Soil solarization significantly improved the

efficacy of these treatments. Further, integration of biopriming with *Trichoderma harzianum* and soil amendment of NSKE (5 %) resulted in higher expression of enzyme activity. Seed biopriming with endophytes and biocontrol agents can be utilized as an effective management strategy against damping-off disease in tomato, chilli and capsicum. However, in future research work needs to be carried out on the shelf life of the primed seeds for increasing their efficiency and use by the farmers.

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## APPENDIX-I

## ANOVA TABLES

**ANOVA 1: Analysis of variance of pre-emergence damping-off incidence in tomato under artificially inoculated conditions (Table 1)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 4 | 3,934.293 | 983.573 | 576.718 | 0.00000 |
| Error | 15 | 25.582 | 1.705 | | |
| Total | 19 | 3,959.875 | | | |

**ANOVA 2: Analysis of variance of post-emergence damping-off incidence in tomato under artificially inoculated conditions (Table 1)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 4 | 2,306.685 | 576.671 | 509.007 | 0.00000 |
| Error | 15 | 16.994 | 1.133 | | |
| Total | 19 | 2,323.679 | | | |

**ANOVA 3: Analysis of variance of pre-emergence damping-off incidence in chilli under artificially inoculated conditions (Table 2)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 4 | 4,390.422 | 1,097.605 | 1,911.174 | 0.00000 |
| Error | 15 | 8.615 | 0.574 | | |
| Total | 19 | 4,399.037 | | | |

**ANOVA 4: Analysis of variance of post-emergence damping-off incidence in chilli under artificially inoculated conditions (Table 2)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 4 | 3,587.206 | 896.802 | 649.137 | 0.00000 |
| Error | 15 | 24.497 | 1.633 | | |
| Total | 19 | 3,611.703 | | | |

**ANOVA 5: Analysis of variance of pre-emergence damping-off incidence in capsicum under artificially inoculated conditions (Table 3)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 4 | 4115.475 | 1028.869 | 2,653.023 | 0.00000 |
| Error | 15 | 5.817 | 0.388 | | |
| Total | 19 | 4121.292 | | | |

**ANOVA 6: Analysis of variance of post-emergence damping-off incidence in capsicum under artificially inoculated conditions (Table 3)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 4 | 1402,082 | 350.520 | 231.669 | 0.00000 |
| Error | 15 | 22.695 | 1.513 | | |
| Total | 19 | 1424.777 | | | |

**ANOVA 7: Analysis of variance of *in vitro* efficacy of biocontrol agents against *Pythium* sp. (Table 4)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 8 | 26,396.318 | 2,932.924 | 1,148.660 | 0.00000 |
| Error | 18 | 51.067 | 2.553 | | |
| Total | 26 | 26,447.385 | | | |

**ANOVA 8: Analysis of variance of *in vitro* efficacy of biocontrol agents against *Fusarium oxysporum* (Table 4)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 8 | 23,983.657 | 2,664.851 | 445.326 | 0.00000 |
| Error | 18 | 119.681 | 5.984 | | |
| Total | 26 | 24,103.337 | | | |

**ANOVA 9: Analysis of variance of *in vitro* efficacy of biocontrol agents against *Sclerotium rolfsii* (Table 4)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 8 | 4,960.991 | 551.221 | 163.980 | 0.00000 |
| Error | 18 | 67.230 | 3.362 | | |
| Total | 26 | 5,028.221 | | | |

**ANOVA 10: Analysis of variance of *in vitro* efficacy of biocontrol agents against *Rhizoctonia solani* (Table 4)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 8 | 12,575.936 | 1,397.326 | 443.620 | 0.00000 |
| Error | 18 | 62.997 | 3.150 | | |
| Total | 26 | 12,638.933 | | | |

**ANOVA 11: Analysis of variance of standardization of effective concentration of biocontrol agents for seed biopriming in tomato (Seed germination) (Table 5)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 9,075.294 | 567.206 | 104.056 | 0.00000 |
| Error | 34 | 185.333 | 5.451 | | |
| Total | 50 | 9,260.627 | | | |

**ANOVA 12: Analysis of variance of standardization of effective concentration of biocontrol agents for seed biopriming in tomato (Seedling length) (Table 5)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 197.735 | 12.358 | 11.982 | 0.00000 |
| Error | 34 | 35.067 | 1.031 | | |
| Total | 50 | 232.802 | | | |

**ANOVA 13: Analysis of variance of standardization of effective concentration of biocontrol agents for seed biopriming in tomato (Seedling dry weight) (Table 5)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 5,698.162 | 356.135 | 293.746 | 0.00000 |
| Error | 34 | 41.221 | 1.212 | | |
| Total | 50 | 5,739.383 | | | |

**ANOVA 14: Analysis of variance of standardization of effective concentration of biocontrol agents for seed biopriming in tomato (SVI-L) (Table 5)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 3,631,145.053 | 226.946.566 | 22.042 | 0.00000 |
| Error | 34 | 350,065.179 | 10,296.035 | | |
| Total | 50 | 3981,210.231 | | | |

**ANOVA 15: Analysis of variance of standardization of effective concentration of biocontrol agents for seed biopriming in tomato (SVI-M) (Table 5)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 24,232,545.038 | 1,514,534.065 | 145.559 | 0.00000 |
| Error | 34 | 353,767.627 | 10,404.930 | | |
| Total | 50 | 24,586,312.666 | | | |

**ANOVA 16: Analysis of variance of standardization of effective concentration of biocontrol agents for seed biopriming in chilli (Seed germination) (Table 6)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 2,927.255 | 182.953 | 38.556 | 0.00000 |
| Error | 34 | 161.333 | 4.745 | | |
| Total | 50 | 3,088.588 | | | |

**ANOVA 17: Analysis of variance of standardization of effective concentration of biocontrol agents for seed biopriming in chilli (Seedling length) (Table 6)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 115.794 | 7.237 | 6.187 | 0.00000 |
| Error | 34 | 39.774 | 1.170 | | |
| Total | 50 | 155.567 | | | |

**ANOVA 18: Analysis of variance of standardization of effective concentration of biocontrol agents for seed biopriming in chilli (Seedling dry weight) (Table 6)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 1,731.090 | 108.193 | 36.867 | 0.00000 |
| Error | 34 | 99.780 | 2.935 | | |
| Total | 50 | 1830.870 | | | |

**ANOVA 19: Analysis of variance of standardization of effective concentration of biocontrol agents for seed biopriming in chilli (SVI-L) (Table 6)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 1,576,336.601 | 98,521.038 | 11.891 | 0.00000 |
| Error | 34 | 281,703.667 | 8,285.402 | | |
| Total | 50 | 1,858.040.268 | | | |

**ANOVA 20: Analysis of variance of standardization of effective concentration of biocontrol agents for seed biopriming in chilli (SVI-M) (Table 6)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 12,375,709.579 | 773,481.849 | 29.810 | 0.00000 |
| Error | 34 | 882,197.654 | 25,946.990 | | |
| Total | 50 | 13,257,907.233 | | | |

**ANOVA 21: Analysis of variance of standardization of effective concentration of biocontrol agents for seed biopriming in capsicum (Seed germination) (Table 7)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 1792.745 | 112.047 | 37.106 | 0.00000 |
| Error | 34 | 102.667 | 3.020 | | |
| Total | 50 | 1895.412 | | | |

**ANOVA 22: Analysis of variance of standardization of effective concentration of biocontrol agents for seed biopriming in capsicum (Seedling length) (Table 7)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 64.700 | 4.044 | 23.906 | 0.00000 |
| Error | 34 | 5.751 | 0.169 | | |
| Total | 50 | 70.452 | | | |

**ANOVA 23: Analysis of variance of standardization of effective concentration of biocontrol agents for seed biopriming in capsicum (Seedling dry weight) (Table 7)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 1123.092 | 70.193 | 32.894 | 0.00000 |
| Error | 34 | 72.554 | 2.134 | | |
| Total | 50 | 1195.646 | | | |

**ANOVA 24: Analysis of variance of standardization of effective concentration of biocontrol agents for seed biopriming in capsicum (SVI-L) (Table 7)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 522621.289 | 32663.831 | 33.476 | 0.00000 |
| Error | 34 | 33175.149 | 975.740 | | |
| Total | 50 | 555796.438 | | | |

**ANOVA 25: Analysis of variance of standardization of effective concentration of biocontrol agents for seed biopriming in capsicum (SVI-M) (Table 7)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 4,570,400.022 | 285,650.001 | 34.165 | 0.00000 |
| Error | 34 | 284,271.387 | 8,360.923 | | |
| Total | 50 | 4854671.409 | | | |

**ANOVA 26: Analysis of variance of effect of duration of seed biopriming with biocontrol agents on seed germination in tomato (Table 8)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 14045.206 | 2340.868 | 323.409 | 0.00000 |
| Factor B | 2 | 1878.984 | 939.492 | 129.798 | 0.00000 |
| Interaction A x B | 12 | 3689.460 | 307.455 | 42.477 | 0.00000 |
| Error | 42 | 304.000 | 7.238 | | |
| Total | 62 | 19917.651 | | | |

**ANOVA 27: Analysis of variance of effect of duration of seed biopriming with biocontrol agents on seedling length in tomato (Table 8)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 352.097 | 58.683 | 131.138 | 0.00000 |
| Factor B | 2 | 15.652 | 7.826 | 17.489 | 0.00000 |
| Interaction A x B | 12 | 152.058 | 12.671 | 28.317 | 0.00000 |
| Error | 42 | 18.795 | 0.447 | | |
| Total | 62 | 538.602 | | | |

**ANOVA 28: Analysis of variance of effect of duration of seed biopriming with biocontrol agents on seedling dry weight in tomato (Table 9)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 5712.440 | 952.073 | 503.971 | 0.00000 |
| Factor B | 2 | 4522.185 | 2261.092 | 1196.887 | 0.00000 |
| Interaction A x B | 12 | 3986.441 | 332.203 | 175.849 | 0.00000 |
| Error | 42 | 79.344 | 1.889 | | |
| Total | 62 | 14300.409 | | | |

**ANOVA 29: Analysis of variance of effect of duration of seed biopriming with biocontrol agents on SVI-L in tomato (Table 9)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 29247079.103 | 4874513.184 | 292.561 | 0.00000 |
| Factor B | 2 | 34727030.167 | 17363515.084 | 1042.133 | 0.00000 |
| Interaction A x B | 12 | 32940225.192 | 2745018.766 | 164.752 | 0.00000 |
| Error | 42 | 699783.781 | 16661.519 | | |
| Total | 62 | 97614118.244 | | | |

**ANOVA 30: Analysis of variance of effect of duration of seed biopriming with biocontrol agents on SVI-M in tomato (Table 9)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 4368907.116 | 728151.186 | 129.856 | 0.00000 |
| Factor B | 2 | 445005.483 | 222502.742 | 39.680 | 0.00000 |
| Interaction A x B | 12 | 2075104.575 | 172925.381 | 30.839 | 0.00000 |
| Error | 42 | 235509.932 | 5607.379 | | |
| Total | 62 | 7124527.105 | | | |

**ANOVA 31: Analysis of variance of effect of duration of seed biopriming with biocontrol agents on seed germination in chilli (Table 10)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 1,697.079 | 282.847 | 18.370 | 0.00000 |
| Factor B | 2 | 1984.667 | 992.333 | 64.451 | 0.00000 |
| Interaction A x B | 12 | 4568.44 | 380.704 | 24.726 | 0.00000 |
| Error | 42 | 646.67 | 15.397 | | |
| Total | 62 | 8896.857 | | | |

**ANOVA 32: Analysis of variance of effect of duration of seed biopriming with biocontrol agents on seedling length in chilli (Table 10)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 138.508 | 23.085 | 21.086 | 0.00000 |
| Factor B | 2 | 8.039 | 4.020 | 3.672 | 0.00000 |
| Interaction A x B | 12 | 99.664 | 8.305 | 7.586 | 0.00000 |
| Error | 42 | 45.982 | 1.095 | | |
| Total | 62 | 292.193 | | | |

**ANOVA 33: Analysis of variance of effect of duration of seed biopriming with biocontrol agents on seedling dry weight in chilli (Table 11)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 14443.778 | 2407.296 | 81.757 | 0.00000 |
| Factor B | 2 | 2568.222 | 1284.111 | 43.611 | 0.00000 |
| Interaction A x B | 12 | 3932.222 | 327.685 | 11.129 | 0.00000 |
| Error | 42 | 1236.667 | 29.444 | | |
| Total | 62 | 22180.889 | | | |

**ANOVA 34: Analysis of variance of effect of duration of seed biopriming with biocontrol agents on SVI-L in chilli (Table 11)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 1,498,564.882 | 249,760.814 | 24.560 | 0.00000 |
| Factor B | 2 | 400,518.283 | 200,259.142 | 19.692 | 0.00000 |
| Interaction A x B | 12 | 1150902.646 | 95,908.554 | 9.431 | 0.00000 |
| Error | 42 | 427,123.376 | 10,169.604 | | |
| Total | 62 | 3477,109.187 | | | |

**ANOVA 35: Analysis of variance of effect of duration of seed biopriming with biocontrol agents on SVI-M in chilli (Table 11)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 107,700,345.111 | 17,950,057.519 | 76.926 | 0.00000 |
| Factor B | 2 | 38,359,506.889 | 19,179,753.444 | 82.196 | 0.00000 |
| Interaction A x B | 12 | 51,488,503.556 | 4,290,708.630 | 18.388 | 0.00000 |
| Error | 42 | 9,800,328.000 | 233,341.143 | | |
| Total | 62 | 207,348,683.556 | | | |

**ANOVA 36: Analysis of variance of effect of duration of seed biopriming with biocontrol agents on seed germination in capsicum (Table 12)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 2606.857 | 434.476 | 201.265 | 0.00000 |
| Factor B | 2 | 341.365 | 170.683 | 79.066 | 0.00000 |
| Interaction A x B | 12 | 453.524 | 37.794 | 17.507 | 0.00000 |
| Error | 42 | 90.667 | 2.159 | | |
| Total | 62 | 3492.413 | | | |

**ANOVA 37: Analysis of variance of effect of duration of seed biopriming with biocontrol agents on seedling length in capsicum (Table 12)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 27.287 | 4.548 | 11.251 | 0.00000 |
| Factor B | 2 | 5.474 | 2.737 | 6.771 | 0.00000 |
| Interaction A x B | 12 | 29.954 | 2.496 | 6.176 | 0.00000 |
| Error | 42 | 16.976 | 0.404 | | |
| Total | 62 | 79.691 | | | |

**ANOVA 38: Analysis of variance of effect of duration of seed biopriming with biocontrol agents on seedling dry weight in capsicum (Table 13)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 11174.162 | 1862.36 | 391.138 | 0.00000 |
| Factor B | 2 | 428.716 | 214.358 | 45.020 | 0.00000 |
| Interaction A x B | 12 | 5165.743 | 430.479 | 90.410 | 0.00000 |
| Error | 42 | 199.978 | 4.761 | | |
| Total | 62 | 16968.60 | | | |

**ANOVA 39: Analysis of variance of effect of duration of seed biopriming with biocontrol agents on SVI-L in capsicum (Table 13)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 463158.543 | 77193.091 | 36.343 | 0.00000 |
| Factor B | 2 | 101504.079 | 50752.040 | 23.895 | 0.00000 |
| Interaction A x B | 12 | 195122.746 | 16260.229 | 7.655 | 0.00000 |
| Error | 42 | 89207.952 | 2123.999 | | |
| Total | 62 | 848993.320 | | | |

**ANOVA 40: Analysis of variance of effect of duration of seed biopriming with biocontrol agents on SVI-M in capsicum (Table 13)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 60988304.144 | 10164717.36 | 519.665 | 0.00000 |
| Factor B | 2 | 583005.033 | 291502.517 | 14.903 | 0.00000 |
| Interaction A x B | 12 | 18163248.909 | 1513604.076 | 77.382 | 0.00000 |
| Error | 42 | 821524.972 | 19560.118 | | |
| Total | 62 | 80556083.058 | | | |

**ANOVA 41: Analysis of variance of effect of seed biopriming with effective biocontrol agents and bioformulations on disease incidence in tomato under field conditions (Table 14)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 1.486 | 0.495 | 0.521 | 0.00000 |
| Treatment | 8 | 772.051 | 96.506 | 101.538 | 0.00000 |
| Error | 24 | 22.811 | 0.950 | | |
| Total | 35 | | | | |

**ANOVA 42: Analysis of variance of effect of seed biopriming with effective biocontrol agents and bioformulations on seed germination in tomato under field conditions (Table 14)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 17.022 | 5.674 | 1.365 | 0.00000 |
| Treatment | 8 | 4846.823 | 605.853 | 145.739 | 0.00000 |
| Error | 24 | 99.771 | 4.157 | | |
| Total | 35 | | | | |

**ANOVA 43: Analysis of variance of effect of seed biopriming with effective biocontrol agents and bioformulations on shoot length in tomato under field conditions (Table 14)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 0.792 | 0.264 | 1.155 | 0.00000 |
| Treatment | 8 | 217.911 | 27.239 | 119.199 | 0.00000 |
| Error | 24 | 5.484 | 0.229 | | |
| Total | 35 | | | | |

**ANOVA 44: Analysis of variance of effect of seed biopriming with effective biocontrol agents and bioformulations on root length in tomato under field conditions (Table 14)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 0.524 | 0.175 | 0.720 | 0.00000 |
| Treatment | 8 | 31.389 | 3.924 | 16.162 | 0.00000 |
| Error | 24 | 5.827 | 0.243 | | |
| Total | 35 | | | | |

**ANOVA 45: Analysis of variance of effect of seed biopriming with effective biocontrol agents and bioformulations on disease incidence in chilli under field conditions (Table 14)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 107.565 | 35.855 | 1.708 | 0.00000 |
| Treatment | 8 | 2909.277 | 363.660 | 17.324 | 0.00000 |
| Error | 24 | 503.812 | 20.992 | | |
| Total | 35 | | | | |

**ANOVA 46: Analysis of variance of effect of seed biopriming with effective biocontrol agents and bioformulations on seed germination in chilli under field conditions (Table 14)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 6.535 | 2.178 | 0.320 | 0.00000 |
| Treatment | 8 | 1685.738 | 210.717 | 30.998 | 0.00000 |
| Error | 24 | 163.145 | 6.798 | | |
| Total | 35 | | | | |

**ANOVA 47: Analysis of variance of effect of seed biopriming with effective biocontrol agents and bioformulations on shoot length in chilli under field conditions (Table 14)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 6.563 | 2.188 | 1.735 | 0.00000 |
| Treatment | 8 | 105.531 | 13.191 | 10.460 | 0.00000 |
| Error | 24 | 30.268 | 1.261 | | |
| Total | 35 | | | | |

**ANOVA 48: Analysis of variance of effect of seed biopriming with effective biocontrol agents and bioformulations on root length in chilli under field conditions (Table 14)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 0.646 | 0.215 | 0.235 | 0.00000 |
| Treatment | 8 | 100.591 | 12.574 | 13.748 | 0.00000 |
| Error | 24 | 21.951 | 0.915 | | |
| Total | 35 | | | | |

**ANOVA 49: Analysis of variance of effect of seed biopriming with effective biocontrol agents and bioformulations on disease incidence in capsicum under field conditions (Table 14)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 4.357 | 1.452 | 1.232 | 0.00000 |
| Treatment | 8 | 1196.856 | 149.607 | 126.857 | 0.00000 |
| Error | 24 | 28.304 | 1.179 | | |
| Total | 35 | | | | |

**ANOVA 50: Analysis of variance of effect of seed biopriming with effective biocontrol agents and bioformulations on seed germination in capsicum under field conditions (Table 14)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 92.251 | 30.750 | 3.040 | 0.00000 |
| Treatment | 8 | 3025.999 | 378.250 | 37.397 | 0.00000 |
| Error | 24 | 242.747 | 10.114 | | |
| Total | 35 | | | | |

**ANOVA 51: Analysis of variance of effect of seed biopriming with effective biocontrol agents and bioformulations on shoot length in capsicum under field conditions (Table 14)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 0.583 | 0.194 | 0.383 | 0.00000 |
| Treatment | 8 | 151.269 | 18.909 | 37.254 | 0.00000 |
| Error | 24 | 12.181 | 0.508 | | |
| Total | 35 | | | | |

**ANOVA 52: Analysis of variance of effect of seed biopriming with effective biocontrol agents and bioformulations on root length in capsicum under field conditions (Table 14)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 0.265 | 0.088 | 0.179 | 0.00000 |
| Treatment | 8 | 58.138 | 7.267 | 14.707 | 0.00000 |
| Error | 24 | 11.859 | 0.494 | | |
| Total | 35 | | | | |

**ANOVA 53: Analysis of variance of *in vitro* efficacy of plant extracts against *Pythium* sp. (Table 15)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 10 | 1,911.718 | 173.793 | 76.201 | 0.00000 |
| Error | 22 | 54.737 | 2.281 | | |
| Total | 32 | 1,966.455 | | | |

**ANOVA 54: Analysis of variance of *in vitro* efficacy of plant extracts against *Fusarium oxysporum* (Table 15)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 10 | 6,018.119 | 547.102 | 171.530 | 0.00000 |
| Error | 22 | 76.549 | 3.190 | | |
| Total | 32 | 6,094.668 | | | |

**ANOVA 55: Analysis of variance of *in vitro* efficacy of plant extracts against *Sclerotium rolfsii* (Table 15)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 10 | 3,653.374 | 332.125 | 86.243 | 0.00000 |
| Error | 22 | 92.425 | 3.851 | | |
| Total | 32 | 3745.799 | | | |

**ANOVA 56: Analysis of variance of *in vitro* efficacy of plant extracts against *Rhizoctonia solani* (Table 15)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 10 | 4,547.541 | 413.413 | 78.213 | 0.00000 |
| Error | 22 | 126.858 | 5.286 | | |
| Total | 32 | 4,674.399 | | | |

**ANOVA 57: Analysis of variance of standardization of effective concentration of plant extracts for seed biopriming in tomato (Seed germination) (Table 16)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 13 | 4,472.414 | 344.032 | 131.598 | 0.00000 |
| Error | 28 | 73.199 | 2.614 | | |
| Total | 41 | 4,545.614 | | | |

**ANOVA 58: Analysis of variance of standardization of effective concentration of plant extracts for seed biopriming in tomato (Seedling length) (Table 16)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 13 | 153.293 | 11.792 | 19.642 | 0.00000 |
| Error | 28 | 16.810 | 0.600 | | |
| Total | 41 | 170.103 | | | |

**ANOVA 59: Analysis of variance of standardization of effective concentration of plant extracts for seed biopriming in tomato (Seedling dry weight) (Table 16)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 13 | 4,595.732 | 353.518 | 158.590 | 0.00000 |
| Error | 28 | 62.416 | 2.229 | | |
| Total | 41 | 4,658.148 | | | |

**ANOVA 60: Analysis of variance of standardization of effective concentration of plant extracts for seed biopriming in tomato (SVI-L) (Table 16)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 13 | 1782078.705 | 137082.977 | 25.028 | 0.00000 |
| Error | 28 | 153360.748 | 5477.170 | | |
| Total | 41 | 1935439.452 | | | |

**ANOVA 61: Analysis of variance of standardization of effective concentration of plant extracts for seed biopriming in tomato (SVI-M) (Table 16)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 13 | 30199746.835 | 2323057.449 | 161.729 | 0.00000 |
| Error | 28 | 402.189.772 | 14363.920 | | |
| Total | 41 | 30601936.607 | | | |

**ANOVA 62: Analysis of variance of standardization of effective concentration of plant extracts for seed biopriming in chilli (Seed germination) (Table 17)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 13 | 19,190.976 | 1,476.229 | 270.749 | 0.00000 |
| Error | 28 | 152.667 | 5.452 | | |
| Total | 41 | 19,343.643 | | | |

**ANOVA 63: Analysis of variance of standardization of effective concentration of plant extracts for seed biopriming in chilli (Seedling length) (Table 17)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 13 | 98.194 | 7.553 | 4.562 | 0.00000 |
| Error | 28 | 46.362 | 1.656 | | |
| Total | 41 | 144.556 | | | |

**ANOVA 64: Analysis of variance of standardization of effective concentration of plant extracts for seed biopriming in chilli (Seedling dry weight) (Table 17)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 13 | 1,943.226 | 149.479 | 56.544 | 0.00000 |
| Error | 28 | 74.020 | 2.644 | | |
| Total | 41 | 2,017.246 | | | |

**ANOVA 65: Analysis of variance of standardization of effective concentration of plant extracts for seed biopriming in chilli (SVI-L) (Table 17)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 13 | 753,669.438 | 57,974.572 | 5.242 | 0.00000 |
| Error | 28 | 309,653.690 | 11,059.060 | | |
| Total | 41 | 1063,323.128 | | | |

**ANOVA 66: Analysis of variance of standardization of effective concentration of plant extracts for seed biopriming in chilli (SVI-M) (Table 17)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 13 | 12587412.259 | 968262.481 | 52.028 | 0.00000 |
| Error | 28 | 521090.590 | 18610.378 | | |
| Total | 41 | 13108502.849 | | | |

**ANOVA 67: Analysis of variance of standardization of effective concentration of plant extracts for seed biopriming in capsicum (Seed germination) (Table 18)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 13 | 15,806.119 | 1,215.855 | 223.973 | 0.00000 |
| Error | 28 | 152.000 | 5.429 | | |
| Total | 41 | 15,958.119 | | | |

**ANOVA 68: Analysis of variance of standardization of effective concentration of plant extracts for seed biopriming in capsicum (Seedling length) (Table 18)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 13 | 123.271 | 9.482 | 12.923 | 0.00000 |
| Error | 28 | 20.545 | 0.734 | | |
| Total | 41 | 143.816 | | | |

**ANOVA 69: Analysis of variance of standardization of effective concentration of plant extracts for seed biopriming in capsicum (Seedling dry weight) (Table 18)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 13 | 2,717.233 | 209.018 | 66.382 | 0.00000 |
| Error | 28 | 88.163 | 3.149 | | |
| Total | 41 | 2805.396 | | | |

**ANOVA 70: Analysis of variance of standardization of effective concentration of plant extracts for seed biopriming in capsicum (SVI-L) (Table 18)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 13 | 2,206,654.433 | 169,742.649 | 33.243 | 0.00000 |
| Error | 28 | 142,973.075 | 5,106.181 | | |
| Total | 41 | 2,349,627.508 | | | |

**ANOVA 71: Analysis of variance of standardization of effective concentration of plant extracts for seed biopriming in capsicum (SVI-M) (Table 18)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 13 | 21,776,977.048 | 1675,152.081 | 64.039 | 0.00000 |
| Error | 28 | 732,428.242 | 26,158.151 | | |
| Total | 41 | 22,509,405.290 | | | |

**ANOVA 72: Analysis of variance of effect of duration of seed biopriming with plant extracts on seed germination in tomato (Table 19)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 7 | 3,930.667 | 561.524 | 16.590 | 0.00000 |
| Factor B | 2 | 988.861 | 494.431 | 14.608 | 0.00000 |
| Interaction A x B | 14 | 2,074.25 | 148.161 | 4.377 | 0.00000 |
| Error | 48 | 1,624.667 | 33.847 | | |
| Total | | 8,618.444 | | | |

**ANOVA 73: Analysis of variance of effect of duration of seed biopriming with plant extracts on seedling length in tomato (Table 19)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 7 | 72.736 | 10.391 | 48.397 | 0.00000 |
| Factor B | 2 | 93.790 | 46.895 | 218.420 | 0.00000 |
| Interaction A x B | 14 | 57.555 | 4.111 | 19.148 | 0.00000 |
| Error | 48 | 10.306 | 0.215 | | |
| Total | 71 | 234.387 | | | |

**ANOVA 74: Analysis of variance of effect of duration of seed biopriming with plant extracts on seedling dry weight in tomato (Table 20)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 7 | 2,376.214 | 339.459 | 218.571 | 0.00000 |
| Factor B | 2 | 558.665 | 279.332 | 179.857 | 0.00000 |
| Interaction A x B | 14 | 2,265.239 | 161.803 | 104.182 | 0.00000 |
| Error | 48 | 74.548 | 1.553 | | |
| Total | 71 | 5,274.666 | | | |

**ANOVA 75: Analysis of variance of effect of duration of seed biopriming with plant extracts on SVI-L in tomato (Table 20)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 7 | 2,673,201.871 | 381,885.982 | 35.678 | 0.00000 |
| Factor B | 2 | 1,175,473.759 | 587,736.879 | 54.909 | 0.00000 |
| Interaction A x B | 14 | 1,183,661.114 | 84,547.222 | 7.899 | 0.00000 |
| Error | 48 | 513,780.000 | 10,703.750 | | |
| Total | 71 | 5,546,116.744 | | | |

**ANOVA 76: Analysis of variance of effect of duration of seed biopriming with plant extracts on SVI-M in tomato (Table 20)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 7 | 10,035,182.65 | 1,433,597.521 | 59.947 | 0.00000 |
| Factor B | 2 | 4,973,578.386 | 2,486,789.193 | 103.987 | 0.00000 |
| Interaction A x B | 14 | 16,893,531.607 | 1,206,680.829 | 50.458 | 0.00000 |
| Error | 48 | 1,147,891.552 | 23,914.407 | | |
| Total | 71 | 33,050,184.195 | | | |

**ANOVA 77: Analysis of variance of effect of duration of seed biopriming with plant extracts on seed germination in chilli (Table 21)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 7 | 2,967.278 | 423.897 | 26.563 | 0.00000 |
| Factor B | 2 | 382.583 | 191.292 | 11.987 | 0.00000 |
| Interaction A x B | 14 | 889.639 | 63.546 | 3.982 | 0.00000 |
| Error | 48 | 766.000 | 15.958 | | |
| Total | 71 | 5005.500 | | | |

**ANOVA 78: Analysis of variance of effect of duration of seed biopriming with plant extracts on seedling length in chilli (Table 21)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 7 | 82.639 | 11.806 | 46.567 | 0.00000 |
| Factor B | 2 | 46.310 | 23.155 | 91.335 | 0.00000 |
| Interaction A x B | 14 | 102.635 | 7.331 | 28.918 | 0.00000 |
| Error | 48 | 12.169 | 0.254 | | |
| Total | 71 | 243.752 | | | |

**ANOVA 79: Analysis of variance of effect of duration of seed biopriming with plant extracts on seedling dry weight in chilli (Table 22)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 7 | 1706.091 | 243.727 | 53.785 | 0.00000 |
| Factor B | 2 | 58.939 | 29.469 | 6.503 | 0.00000 |
| Interaction A x B | 14 | 952.791 | 68.057 | 15.018 | 0.00000 |
| Error | 48 | 217.513 | 4.532 | | |
| Total | 71 | 2935.334 | | | |

**ANOVA 80: Analysis of variance of effect of duration of seed biopriming with plant extracts on SVI-L in chilli (Table 22)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 7 | 1,201,873.345 | 171,696.192 | 71.862 | 0.00000 |
| Factor B | 2 | 581,479.103 | 290,739.551 | 121.686 | 0.00000 |
| Interaction A x B | 14 | 833,368.367 | 59,526.312 | 24.914 | 0.00000 |
| Error | 48 | 114,684.632 | 2,389.263 | | |
| Total | 71 | 2,731,405.447 | | | |

**ANOVA 81: Analysis of variance of effect of duration of seed biopriming with plant extracts on SVI-M in chilli (Table 22)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 7 | 16,481,023.684 | 2,354,431.955 | 57.075 | 0.00000 |
| Factor B | 2 | 1,241,833.092 | 620,916.546 | 15.052 | 0.00000 |
| Interaction A x B | 14 | 6,709,112.840 | 479,222.346 | 11.617 | 0.00000 |
| Error | 48 | 1,980,068.399 | 41,251.425 | | |
| Total | 71 | 26,412,038.016 | | | |

**ANOVA 82: Analysis of variance of effect of duration of seed biopriming with plant extracts on seed germination in capsicum (Table 23)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 7 | 34,894.764 | 4,984.966 | 954.568 | 0.00000 |
| Factor B | 2 | 1,037.694 | 518.847 | 99.354 | 0.00000 |
| Interaction A x B | 14 | 887.194 | 63.371 | 12.135 | 0.00000 |
| Error | 48 | 250.667 | 5.222 | | |
| Total | 71 | 37,070.319 | | | |

**ANOVA 83: Analysis of variance of effect of duration of seed biopriming with plant extracts on seedling length in capsicum (Table 23)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 7 | 51.445 | 7.349 | 8.522 | 0.00000 |
| Factor B | 2 | 17.605 | 8.803 | 10.207 | 0.00000 |
| Interaction A x B | 14 | 75.307 | 5.379 | 6.237 | 0.00000 |
| Error | 48 | 41.395 | 0.862 | | |
| Total | 71 | 185.752 | | | |

**ANOVA 84: Analysis of variance of effect of duration of seed biopriming with plant extracts on seedling dry weight in capsicum (Table 24)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 7 | 3,036.406 | 433.772 | 63.601 | 0.00000 |
| Factor B | 2 | 409.268 | 204.634 | 30.004 | 0.00000 |
| Interaction A x B | 14 | 1,698.620 | 121.33 | 17.790 | 0.00000 |
| Error | 48 | 327.37 | 6.820 | | |
| Total | 71 | 5,471.663 | | | |

**ANOVA 85: Analysis of variance of effect of duration of seed biopriming with plant extracts on SVI-L in capsicum (Table 24)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 7 | 3,649.562.944 | 521,366.135 | 93.340 | 0.00000 |
| Factor B | 2 | 179,399.566 | 89.699.783 | 16.059 | 0.00000 |
| Interaction A x B | 14 | 544,535.151 | 38.895.368 | 6.963 | 0.00000 |
| Error | 48 | 268,111.331 | 5.585.653 | | |
| Total | 71 | 4641608.991 | | | |

**ANOVA 86: Analysis of variance of effect of duration of seed biopriming with plant extracts on SVI-M in capsicum (Table 24)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 7 | 24,153,395.033 | 3,450,485.005 | 101.408 | 0.00000 |
| Factor B | 2 | 2,013,458.601 | 1,006,729.300 | 29.587 | 0.00000 |
| Interaction A x B | 14 | 10,020,366.031 | 715,740.431 | 21.035 | 0.00000 |
| Error | 48 | 1,633,239.414 | 34,025.821 | | |
| Total | 71 | 37,820,459.078 | | | |

**ANOVA 87: Analysis of variance of effect of seed biopriming with effective plant extracts on disease incidence in tomato under field conditions (Table 25)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 1.429 | 0.476 | 0.533 | 0.00000 |
| Treatment | 5 | 1626.042 | 325.208 | 363.506 | 0.00000 |
| Error | 15 | 13.420 | 0.895 | | |
| Total | 23 | 1640.891 | | | |

**ANOVA 88: Analysis of variance of effect of seed biopriming with effective plant extracts on seed germination in tomato under field conditions (Table 25)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 1.892 | 0.631 | 0.389 | 0.00000 |
| Treatment | 5 | 2481.417 | 496.283 | 306.463 | 0.00000 |
| Error | 15 | 24.291 | 1.619 | | |
| Total | 23 | 2507.600 | | | |

**ANOVA 89: Analysis of variance of effect of seed biopriming with effective plant extracts on shoot length in tomato under field conditions (Table 25)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 1.195 | 0.398 | 0.517 | 0.00000 |
| Treatment | 5 | 342.424 | 68.485 | 88.846 | 0.00000 |
| Error | 15 | 11.562 | 0.771 | | |
| Total | 23 | 355.181 | | | |

**ANOVA 90: Analysis of variance of effect of seed biopriming with effective plant extracts on root length in chilli under field conditions (Table 25)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 1.231 | 0.410 | 2.487 | 0.00000 |
| Treatment | 5 | 51.384 | 10.277 | 62.274 | 0.00000 |
| Error | 15 | 2.475 | 0.165 | | |
| Total | 23 | 55.090 | | | |

**ANOVA 91: Analysis of variance of effect of seed biopriming with effective plant extracts on disease incidence in chilli under field conditions (Table 25)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 2.976 | 0.992 | 0.299 | |
| Treatment | 5 | 2497.635 | 499.527 | 150.352 | 0.00000 |
| Error | 15 | 49.836 | 3.322 | | |
| Total | 23 | 2550.447 | | | |

**ANOVA 92: Analysis of variance of effect of seed biopriming with effective plant extracts on seed germination in chilli under field conditions (Table 25)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 16.918 | 5.639 | 3.701 | |
| Treatment | 5 | 2360.853 | 472.171 | 309.878 | 0.00000 |
| Error | 15 | 22.856 | 1.524 | | |
| Total | 23 | 2400.627 | | | |

**ANOVA 93: Analysis of variance of effect of seed biopriming with effective plant extracts on shoot length in chilli under field conditions (Table 25)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 2.776 | 0.925 | 1.373 | 0.00000 |
| Treatment | 5 | 519.512 | 103.902 | 154.213 | 0.00000 |
| Error | 15 | 10.106 | 0.674 | | |
| Total | 23 | 532.395 | | | |

**ANOVA 94: Analysis of variance of effect of seed biopriming with effective plant extracts on root length in chilli under field conditions (Table 25)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 0.981 | 0.327 | 0.435 | 0.00000 |
| Treatment | 5 | 19.531 | 3.906 | 5.195 | 0.00000 |
| Error | 15 | 11.279 | 0.752 | | |
| Total | 23 | 31.791 | | | |

**ANOVA 95: Analysis of variance of effect of seed biopriming with effective plant extracts on disease incidence in capsicum under field conditions (Table 25)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 4.291 | 1.430 | 1.349 | |
| Treatment | 5 | 4806.980 | 961.396 | 907.081 | 0.00000 |
| Error | 15 | 15.898 | 1.060 | | |
| Total | 23 | 4827.169 | | | |

**ANOVA 96: Analysis of variance of effect of seed biopriming with effective plant extracts on seed germination in capsicum under field conditions (Table 25)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 2.217 | 0.739 | 0.420 | 0.00000 |
| Treatment | 5 | 2241.895 | 448.379 | 254.794 | 0.00000 |
| Error | 15 | 26.397 | 1.760 | | |
| Total | 23 | 2270.509 | | | |

**ANOVA 97: Analysis of variance of effect of seed biopriming with effective plant extracts on shoot length in capsicum under field conditions (Table 25)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 6.649 | 2.216 | 1.202 | 0.00000 |
| Treatment | 5 | 259.392 | 51.878 | 28.134 | 0.00000 |
| Error | 15 | 27.660 | 1.844 | | |
| Total | 23 | 293.701 | | | |

**ANOVA 98: Analysis of variance of effect of seed biopriming with effective plant extracts on root length in capsicum under field conditions (Table 25)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 0.125 | 0.042 | 0.890 | 0.00000 |
| Treatment | 5 | 22.119 | 4.424 | 94.743 | 0.00000 |
| Error | 15 | 0.700 | 0.047 | | |
| Total | 23 | 22.945 | | | |

**ANOVA 99: Analysis of variance of effect of conjoint application of seed biopriming with effective biocontrol agents and plant extracts on disease incidence in tomato under field conditions (Table 26)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 2.782 | 0.927 | 0.527 | 0.00000 |
| Treatment | 5 | 767.271 | 153.454 | 87.264 | 0.00000 |
| Error | 15 | 26.378 | 1.759 | | |
| Total | 23 | | | | |

**ANOVA 100: Analysis of variance of effect of conjoint application of seed biopriming with effective biocontrol agents and plant extracts on seed germination in tomato under field conditions (Table 26)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 46.462 | 15.487 | 1.510 | 0.0000 |
| Treatment | 5 | 3787.242 | 757.448 | 73.872 | 0.00000 |
| Error | 15 | 153.803 | 10.254 | | |
| Total | 23 | | | | |

**ANOVA 101: Analysis of variance of effect of conjoint application of seed biopriming with effective biocontrol agents and plant extracts on shoot length in tomato under field conditions (Table 26)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 0.077 | 0.026 | 0.033 | 0.00000 |
| Treatment | 5 | 182.059 | 36.412 | 47.635 | 0.00000 |
| Error | 15 | 11.466 | 0.764 | | |
| Total | 23 | | | | |

**ANOVA 102: Analysis of variance of effect of conjoint application of seed biopriming with effective biocontrol agents and plant extracts on root length in tomato under field conditions (Table 26)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 5.893 | 1.964 | 2.432 | 0.00000 |
| Treatment | 5 | 43.188 | 8.638 | 10.696 | 0.00000 |
| Error | 15 | 12.114 | 0.808 | | |
| Total | 23 | | | | |

**ANOVA 103: Analysis of variance of effect of conjoint application of seed biopriming with effective biocontrol agents and plant extracts on disease incidence in chilli under field conditions (Table 26)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 16.988 | 5.663 | 1.244 | 0.00000 |
| Treatment | 5 | 738.493 | 147.699 | 32.456 | 0.00000 |
| Error | 15 | 68.260 | 4.551 | | |
| Total | 23 | | | | |

**ANOVA 104: Analysis of variance of effect of conjoint application of seed biopriming with effective biocontrol agents and plant extracts on seed germination in chilli under field conditions (Table 26)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 4.085 | 1.362 | 0.565 | 0.00000 |
| Treatment | 5 | 2553.238 | 510.648 | 211.820 | 0.00000 |
| Error | 15 | 36.161 | 2.411 | | |
| Total | 23 | | | | |

**ANOVA 105: Analysis of variance of effect of conjoint application of seed biopriming with effective biocontrol agents and plant extracts on shoot length in chilli under field conditions (Table 26)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 3.245 | 1.082 | 1.390 | 0.00000 |
| Treatment | 5 | 222.919 | 44.584 | 57.277 | 0.00000 |
| Error | 15 | 11.676 | 0.778 | | |
| Total | 23 | | | | |

**ANOVA 106: Analysis of variance of effect of conjoint application of seed biopriming with effective biocontrol agents and plant extracts on root length in chilli under field conditions (Table 26)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 1.501 | 0.500 | 0.830 | 0.00000 |
| Treatment | 5 | 69.561 | 13.912 | 23.089 | 0.00000 |
| Error | 15 | 9.038 | 0.603 | | |
| Total | 23 | | | | |

**ANOVA 107: Analysis of variance of effect of conjoint application of seed biopriming with effective biocontrol agents and plant extracts on disease incidence in capsicum under field conditions (Table 26)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 1.634 | 0.545 | 0.298 | 0.00000 |
| Treatment | 5 | 389.403 | 77.881 | 42.613 | 0.00000 |
| Error | 15 | 27.415 | 1.828 | | |
| Total | 23 | | | | |

**ANOVA 108: Analysis of variance of effect of conjoint application of seed biopriming with effective biocontrol agents and plant extracts on seed germination in capsicum under field conditions (Table 26)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 6.477 | 2.159 | 0.657 | 0.00000 |
| Treatment | 5 | 3629.857 | 725.971 | 220.755 | 0.00000 |
| Error | 15 | 49.329 | 3.289 | | |
| Total | 23 | | | | |

**ANOVA 109: Analysis of variance of effect of conjoint application of seed biopriming with effective biocontrol agents and plant extracts on shoot length in capsicum under field conditions (Table 26)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 7.574 | 2.525 | 0.091 | 0.00000 |
| Treatment | 5 | 252.004 | 50.401 | 1.823 | 0.00000 |
| Error | 15 | 414.817 | 27.654 | | |
| Total | 23 | | | | |

**ANOVA 110: Analysis of variance of effect of conjoint application of seed biopriming with effective biocontrol agents and plant extracts on root length in capsicum under field conditions (Table 26)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 2.383 | 0.794 | 1.617 | 0.00000 |
| Treatment | 5 | 67.460 | 13.492 | 27.457 | 0.00000 |
| Error | 15 | 7.371 | 0.491 | | |
| Total | 23 | | | | |

**ANOVA 111: Analysis of variance of *in vitro* efficacy of native endophytes against *Pythium* sp. (Table 27)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 7 | 20,284.491 | 2,535.561 | 903.574 | 0.00000 |
| Error | 16 | 50.511 | 2.806 | | |
| Total | 23 | 20,335.002 | | | |

**ANOVA 112: Analysis of variance of *in vitro* efficacy of native endophytes against *Fusarium oxysporum* (Table 27)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 7 | 12,647.494 | 1,580.937 | 535.265 | 0.00000 |
| Error | 16 | 53.164 | 2.954 | | |
| Total | 23 | 12,700.658 | | | |

**ANOVA 113: Analysis of variance of *in vitro* efficacy of native endophytes against *Sclerotium rolfsii* (Table 27)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 8 | 5,423.292 | 677.911 | 160.182 | 0.00000 |
| Error | 18 | 76.179 | 4.232 | | |
| Total | 26 | 5,499.470 | | | |

**ANOVA 114: Analysis of variance of *in vitro* efficacy of native endophytes against *Rhizoctonia solani* (Table 27)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 8 | 12,271.462 | 1,533.933 | 310.266 | 0.00000 |
| Error | 18 | 88.991 | 4.944 | | |
| Total | 26 | 12,360.453 | | | |

**ANOVA 115: Analysis of variance of standardization of effective concentration of endophytes for seed biopriming in tomato (Seed germination) (Table 28)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 4,759.294 | 297.456 | 97.245 | 0.00000 |
| Error | 34 | 104.000 | 3.059 | | |
| Total | 50 | 4,863.294 | | | |

**ANOVA 116: Analysis of variance of standardization of effective concentration of endophytes for seed biopriming in tomato (Seedling length) (Table 28)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 68.636 | 4.290 | 6.400 | 0.00000 |
| Error | 34 | 22.789 | 0.670 | | |
| Total | 50 | 91.425 | | | |

**ANOVA 117: Analysis of variance of standardization of effective concentration of endophytes for seed biopriming in tomato (Seedling dry weight) (Table 28)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 1,624.261 | 101.516 | 50.274 | 0.00000 |
| Error | 34 | 68.655 | 2.019 | | |
| Total | 50 | 1,692.916 | | | |

**ANOVA 118: Analysis of variance of standardization of effective concentration of endophytes for seed biopriming in tomato (SVI-L) (Table 28)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 1,108,994.779 | 69,312.174 | 12.910 | 0.00000 |
| Error | 34 | 182,539.899 | 5,368.821 | | |
| Total | 50 | 1,291,534.677 | | | |

**ANOVA 119: Analysis of variance of standardization of effective concentration of endophytes for seed biopriming in tomato (SVI-M) (Table 28)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 12,269,353.104 | 766,834.569 | 55.812 | 0.00000 |
| Error | 34 | 467,149.121 | 13,739.680 | | |
| Total | 50 | 12,736,502.225 | | | |

**ANOVA 120: Analysis of variance of standardization of effective concentration of endophytes for seed biopriming in chilli (Seed germination) (Table 29)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 5092.000 | 318.250 | 78.790 | 0.00000 |
| Error | 34 | 137.333 | 4.039 | | |
| Total | 50 | 5,229.333 | | | |

**ANOVA 121: Analysis of variance of standardization of effective concentration of endophytes for seed biopriming in chilli (Seedling length) (Table 29)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 38.528 | 2.408 | 7.672 | 0.00000 |
| Error | 34 | 10.672 | 0.314 | | |
| Total | 50 | 49.200 | | | |

**ANOVA 122: Analysis of variance of standardization of effective concentration of endophytes for seed biopriming in chilli (Seedling dry weight) (Table 29)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 3,706.206 | 231.638 | 54.273 | 0.00000 |
| Error | 34 | 145.113 | 4.268 | | |
| Total | 50 | 3851.319 | | | |

**ANOVA 123: Analysis of variance of standardization of effective concentration of endophytes for seed biopriming in chilli (SVI-L) (Table 29)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 1,158,085.171 | 72,380.323 | 34.505 | 0.00000 |
| Error | 34 | 71,321.077 | 2,097.679 | | |
| Total | 50 | 1,229,406.248 | | | |

**ANOVA 124: Analysis of variance of standardization of effective concentration of endophytes for seed biopriming in chilli (SVI-M) (Table 29)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 21,038,226.757 | 1,314,889.172 | 45.329 | 0.00000 |
| Error | 34 | 986,254.287 | 29,007.479 | | |
| Total | 50 | 22,024,481.044 | | | |

**ANOVA 125: Analysis of variance of standardization of effective concentration of endophytes for seed biopriming in capsicum (Seed germination) (Table 30)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 4800.039 | 300.002 | 50.329 | 0.00000 |
| Error | 34 | 202.667 | 5.961 | | |
| Total | 50 | 5002.706 | | | |

**ANOVA 126: Analysis of variance of standardization of effective concentration of endophytes for seed biopriming in capsicum (Seedling length) (Table 30)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 60.483 | 3.780 | 15.853 | 0.00000 |
| Error | 34 | 8.107 | 0.238 | | |
| Total | 50 | 68.590 | | | |

**ANOVA 127: Analysis of variance of standardization of effective concentration of endophytes for seed biopriming in capsicum (Seedling dry weight) (Table 30)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 1684.322 | 105.270 | 42.667 | 0.00000 |
| Error | 34 | 83.886 | 2.467 | | |
| Total | 50 | 1768.208 | | | |

**ANOVA 128: Analysis of variance of standardization of effective concentration of endophytes for seed biopriming in capsicum (SVI-L) (Table 30)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 934,877.980 | 58429.874 | 28.410 | 0.00000 |
| Error | 34 | 69.926.105 | 2056.650 | | |
| Total | 50 | 1004804.085 | | | |

**ANOVA 129: Analysis of variance of standardization of effective concentration of endophytes for seed biopriming in capsicum (SVI-M) (Table 30)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 16 | 17385375.395 | 1086585.962 | 69.214 | 0.00000 |
| Error | 34 | 533761.015 | 15,698.853 | | |
| Total | 50 | 17919136.410 | | | |

**ANOVA 130: Analysis of variance of effect of duration of seed biopriming with endophytes on seed germination in tomato (Table 31)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 8216.762 | 1369.460 | 253.753 | 0.00000 |
| Factor B | 2 | 993.524 | 496.762 | 92.047 | 0.00000 |
| Interaction A x B | 12 | 682.476 | 56.873 | 10.538 | 0.00000 |
| Error | 42 | 226.667 | 5.397 | | |
| Total | 62 | 10,119.429 | | | |

**ANOVA 131: Analysis of variance of effect of duration of seed biopriming with endophytes on seedling length in tomato (Table 31)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 137.785 | 22.964 | 26.134 | 0.00000 |
| Factor B | 2 | 43.595 | 21.798 | 24.806 | 0.00000 |
| Interaction A x B | 12 | 49.272 | 4.106 | 4.673 | 0.00000 |
| Error | 42 | 36.906 | 0.879 | | |
| Total | 62 | 267.559 | | | |

**ANOVA 132: Analysis of variance of effect of duration of seed biopriming with endophytes on seedling dry weight in tomato (Table 32)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 1,738.453 | 289.742 | 67.848 | 0.00000 |
| Factor B | 2 | 367.633 | 183.816 | 43.044 | 0.00000 |
| Interaction A x B | 12 | 833.785 | 69.482 | 16.270 | 0.00000 |
| Error | 42 | 179.360 | 4.270 | | |
| Total | 62 | 3119.230 | | | |

**ANOVA 133: Analysis of variance of effect of duration of seed biopriming with endophytes on SVI-L in tomato (Table 32)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 3024206.507 | 504034.418 | 65.487 | 0.00000 |
| Factor B | 2 | 938418.264 | 469209.132 | 60.962 | 0.00000 |
| Interaction A x B | 12 | 571569.393 | 47630.783 | 6.188 | 0.00000 |
| Error | 42 | 323262.874 | 7696.735 | | |
| Total | 62 | 4857457.037 | | | |

**ANOVA 134: Analysis of variance of effect of duration of seed biopriming with endophytes on SVI-M in tomato (Table 32)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 14526399.815 | 2421066.64 | 84.019 | 0.00000 |
| Factor B | 2 | 4043297.038 | 2021648.52 | 70.158 | 0.00000 |
| Interaction A x B | 12 | 5278953.283 | 439912.774 | 15.266 | 0.00000 |
| Error | 42 | 1210257.75 | 28815.661 | | |
| Total | 62 | 25058907.887 | | | |

**ANOVA 135: Analysis of variance of effect of duration of seed biopriming with endophytes on seed germination in chilli (Table 33)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 7058.857 | 1176.476 | 225.970 | 0.00000 |
| Factor B | 2 | 1073.556 | 536.778 | 103.101 | 0.00000 |
| Interaction A x B | 12 | 808.667 | 67.389 | 12.944 | 0.00000 |
| Error | 42 | 218.667 | 5.206 | | |
| Total | 62 | 9159.746 | | | |

**ANOVA 136: Analysis of variance of effect of duration of seed biopriming with endophytes on seedling length in chilli (Table 33)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 16.908 | 2.818 | 7.080 | 0.00000 |
| Factor B | 2 | 14.367 | 7.183 | 18.049 | 0.00000 |
| Interaction A x B | 12 | 33.404 | 2.784 | 6.994 | 0.00000 |
| Error | 42 | 16.716 | 0.398 | | |
| Total | 62 | 81.395 | | | |

**ANOVA 137: Analysis of variance of effect of duration of seed biopriming with endophytes on seedling dry weight in chilli (Table 34)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 3867.144 | 644.524 | 232.945 | 0.00000 |
| Factor B | 2 | 436.974 | 218.487 | 78.966 | 0.00000 |
| Interaction A x B | 12 | 200.860 | 16.738 | 6.050 | 0.00000 |
| Error | 42 | 116.207 | 2.767 | | |
| Total | 62 | 4621.187 | | | |

**ANOVA 138: Analysis of variance of effect of duration of seed biopriming with endophytes on SVI-L in chilli (Table 34)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 603698.812 | 100616.469 | 33.513 | 0.00000 |
| Factor B | 2 | 384242.626 | 192121.313 | 63.991 | 0.00000 |
| Interaction A x B | 12 | 331590.263 | 27632.522 | 9.204 | 0.00000 |
| Error | 42 | 126096.963 | 3002.309 | | |
| Total | 62 | 1445628.664 | | | |

**ANOVA 139: Analysis of variance of effect of duration of seed biopriming with endophytes on SVI-M in chilli (Table 34)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 44495859.631 | 7415976.605 | 409.816 | 0.00000 |
| Factor B | 2 | 7659844.280 | 3829922.140 | 211.646 | 0.00000 |
| Interaction A x B | 12 | 4121836.758 | 343486.397 | 18.981 | 0.00000 |
| Error | 42 | 760027.119 | 18095.884 | | |
| Total | 62 | 57037567.789 | | | |

**ANOVA 140: Analysis of variance of effect of duration of seed biopriming with endophytes on seed germination in capsicum (Table 35)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 7697.111 | 1282.852 | 294.962 | 0.00000 |
| Factor B | 2 | 632.095 | 316.048 | 72.668 | 0.00000 |
| Interaction A x B | 12 | 402.127 | 33.511 | 7.705 | 0.00000 |
| Error | 42 | 182.667 | 4.349 | | |
| Total | 62 | 8914.000 | | | |

**ANOVA 141: Analysis of variance of effect of duration of seed biopriming with endophytes on seedling length in capsicum (Table 35)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 126.090 | 21.015 | 101.828 | 0.00000 |
| Factor B | 2 | 32.292 | 16.146 | 78.237 | 0.00000 |
| Interaction A x B | 12 | 33.148 | 2.762 | 13.385 | 0.00000 |
| Error | 42 | 8.668 | 0.206 | | |
| Total | 62 | 200.198 | | | |

**ANOVA 142: Analysis of variance of effect of duration of seed biopriming with endophytes on seedling dry weight in capsicum (Table 36)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 792.155 | 132.026 | 52.015 | 0.00000 |
| Factor B | 2 | 37.712 | 18.856 | 7.429 | 0.00000 |
| Interaction A x B | 12 | 307.737 | 25.645 | 10.103 | 0.00000 |
| Error | 42 | 106.606 | 2.538 | | |
| Total | 62 | 1244.209 | | | |

**ANOVA 143: Analysis of variance of effect of duration of seed biopriming with endophytes on SVI-L in capsicum (Table 36)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 2259311.966 | 376551.994 | 220.565 | 0.00000 |
| Factor B | 2 | 414408.59 | 207204.295 | 121.370 | 0.00000 |
| Interaction A x B | 12 | 308386.063 | 25698.839 | 15.053 | 0.00000 |
| Error | 42 | 71703.124 | 1707.217 | | |
| Total | 62 | 3053809.743 | | | |

**ANOVA 144: Analysis of variance of effect of duration of seed biopriming with endophytes on SVI-M in capsicum (Table 36)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Factor A | 6 | 11666208.067 | 1944368.011 | 101.554 | 0.00000 |
| Factor B | 2 | 725687.812 | 362843.906 | 18.951 | 0.00000 |
| Interaction A x B | 12 | 1712078.77 | 142673.231 | 7.452 | 0.00000 |
| Error | 42 | 804138.775 | 19146.161 | | |
| Total | 62 | 14908113.424 | | | |

**ANOVA 145: Analysis of variance of effect of seed biopriming with effective endophytes on disease incidence in tomato under field conditions (Table 37)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 9.466 | 3.155 | 0.794 | 0.00000 |
| Treatment | 5 | 4054.569 | 810.914 | 204.121 | 0.00000 |
| Error | 15 | 59.591 | 3.973 | | |
| Total | 23 | 4123.626 | | | |

**ANOVA 146: Analysis of variance of effect of seed biopriming with endophytes on seed germination in tomato under field conditions (Table 37)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 12.935 | 4.312 | 0.582 | 0.63612 |
| Treatment | 5 | 11.426.090 | 2285.218 | 308.257 | 0.00000 |
| Error | 15 | 111.200 | 7.413 | | |
| Total | 23 | 11550.226 | | | |

**ANOVA 147: Analysis of variance of effect of seed biopriming with effective endophytes on shoot length in tomato under field conditions (Table 37)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 16.692 | 5.564 | 1.126 | 0.00000 |
| Treatment | 5 | 258.769 | 51.754 | 10.477 | 0.00000 |
| Error | 15 | 74.095 | 4.940 | | |
| Total | 23 | 349.555 | | | |

**ANOVA 148: Analysis of variance of effect of seed biopriming with effective endophytes on root length in tomato under field conditions (Table 37)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 3.022 | 1.007 | 2.261 | 0.00000 |
| Treatment | 5 | 14.018 | 2.804 | 6.292 | 0.00000 |
| Error | 15 | 6.684 | 0.446 | | |
| Total | 23 | | | | |

**ANOVA 149: Analysis of variance of effect of seed biopriming with effective endophytes on disease incidence in chilli under field conditions (Table 37)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 3.411 | 1.137 | 2.302 | 0.00000 |
| Treatment | 5 | 306.748 | 61.350 | 124.215 | 0.00000 |
| Error | 15 | 7.408 | 0.494 | | |
| Total | 23 | | | | |

**ANOVA 150: Analysis of variance of effect of seed biopriming with effective endophytes on seed germination in chilli under field conditions (Table 37)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 3 | 4.739 | 1.580 | 1.387 | 0.00000 |
| Replication | 5 | 4271.063 | 854.213 | 750.00 | 0.00000 |
| Error | 15 | 17.084 | 1.139 | | |
| Total | 23 | | | | |

**ANOVA 151: Analysis of variance of effect of seed biopriming with effective endophytes on shoot length in chilli under field conditions (Table 37)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 3 | 1.111 | 0.370 | 2.580 | 0.00000 |
| Replication | 5 | 116.670 | 23.334 | 162.565 | 0.00000 |
| Error | 15 | 2.153 | 0.144 | | |
| Total | 23 | | | | |

**ANOVA 152: Analysis of variance of effect of seed biopriming with effective endophytes on root length in chilli under field conditions (Table 37)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 3 | 3.505 | 1.168 | 2.290 | 0.00000 |
| Treatment | 5 | 283.867 | 56.773 | 111.285 | 0.00000 |
| Error | 15 | 7.652 | 0.510 | | |
| Total | 23 | | | | |

**ANOVA 153: Analysis of variance of effect of seed biopriming with effective endophytes on disease incidence in capsicum under field conditions (Table 37)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 3 | 7.016 | 2.339 | 2.088 | 0.00000 |
| Replication | 5 | 585.715 | 117.143 | 104.595 | 0.00000 |
| Error | 15 | 16.799 | 1.120 | | |
| Total | 23 | | | | |

**ANOVA 154: Analysis of variance of effect of seed biopriming with effective endophytes on seed germination in capsicum under field conditions (Table 37)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 3 | 0.192 | 0.064 | 0.024 | 0.00000 |
| Replication | 5 | 4389.571 | 877.914 | 327.542 | 0.00000 |
| Error | 15 | 40.205 | 2.680 | | |
| Total | 23 | | | | |

**ANOVA 155: Analysis of variance of effect of seed biopriming with effective endophytes on shoot length in capsicum under field conditions (Table 37)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 3 | 1.272 | 0.424 | 0.523 | 0.00000 |
| Replication | 5 | 158.163 | 31.633 | 39.035 | 0.00000 |
| Error | 15 | 12.155 | 0.810 | | |
| Total | 23 | | | | |

**ANOVA 156: Analysis of variance of effect of seed biopriming with effective endophytes on root length in capsicum under field conditions (Table 37)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 3 | 2.963 | 0.988 | 2.104 | 0.00000 |
| Replication | 5 | 47.962 | 9.592 | 20.436 | |
| Error | 15 | 7.041 | 0.469 | | |
| Total | 23 | | | | |

**ANOVA 157: Analysis of variance of conjoint application of seed biopriming with effective biocontrol agents and soil amendments in tomato under artificially inoculated conditions (seed germination) (Table 38)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 15 | 12827.090 | 855.139 | 340.814 | 0.00000 |
| Error | 32 | 80.291 | 2.509 | | |
| Total | 47 | | | | |

**ANOVA 158: Analysis of variance of conjoint application of seed biopriming with effective biocontrol agents and soil amendments in tomato under artificially inoculated conditions (shoot length) (Table 38)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 15 | 680.564 | 45.371 | 50.482 | 0.00000 |
| Error | 32 | 28.760 | 0.899 | | |
| Total | 47 | | | | |

**ANOVA 159: Analysis of variance of conjoint application of seed biopriming with effective biocontrol agents and soil amendments in tomato under artificially inoculated conditions (root length) (Table 38)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 15 | 98.676 | 6.578 | 20.515 | 0.00000 |
| Error | 32 | 10.261 | 0.321 | | |
| Total | 47 | | | | |

**ANOVA 160: Analysis of variance of conjoint application of seed biopriming with effective biocontrol agents and soil amendments in tomato under artificially inoculated conditions (disease incidence) (Table 38)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 15 | 535.491 | 35.699 | 33.500 | 0.00000 |
| Error | 32 | 34.101 | 1.066 | | |
| Total | 47 | | | | |

**ANOVA 161: Analysis of variance of conjoint application of seed biopriming with effective biocontrol agents and soil amendments in chilli under artificially inoculated conditions (seed germination) (Table 39)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 15 | 3577.664 | 238.511 | 40.275 | 0.00000 |
| Error | 32 | 189.506 | 5.922 | | |
| Total | 47 | | | | |

**ANOVA 162: Analysis of variance of conjoint application of seed biopriming with effective biocontrol agents and soil amendments in chilli under artificially inoculated conditions (shoot length) (Table 39)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 15 | 214.889 | 14.326 | 17.688 | 0.00000 |
| Error | 32 | 25.918 | 0.810 | | |
| Total | 47 | | | | |

**ANOVA 163: Analysis of variance of conjoint application of seed biopriming with effective biocontrol agents and soil amendments in chilli under artificially inoculated conditions (root length) (Table 39)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 15 | 94.930 | 6.329 | 9.532 | 0.00000 |
| Error | 32 | 21.246 | 0.664 | | |
| Total | 47 | | | | |

**ANOVA 164: Analysis of variance of conjoint application of seed biopriming with effective biocontrol agents and soil amendments in chilli under artificially inoculated conditions (disease incidence) (Table 39)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 15 | 10.104 | 0.674 | 43.425 | 0.00000 |
| Error | 32 | 0.496 | 0.016 | | |
| Total | 47 | | | | |

**ANOVA 165: Analysis of variance of conjoint application of seed biopriming with effective biocontrol agents and soil amendments in capsicum under artificially inoculated conditions (seed germination) (Table 40)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 15 | 6592.064 | 439.471 | 69.307 | 0.00000 |
| Error | 32 | 202.908 | 6.341 | | |
| Total | 47 | | | | |

**ANOVA 166: Analysis of variance of conjoint application of seed biopriming with effective biocontrol agents and soil amendments in capsicum under artificially inoculated conditions (shoot length) (Table 40)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 15 | 303.994 | 20.266 | 28.933 | 0.00000 |
| Error | 32 | 22.415 | 0.700 | | |
| Total | 47 | | | | |

**ANOVA 167: Analysis of variance of conjoint application of seed biopriming with effective biocontrol agents and soil amendments in capsicum under artificially inoculated conditions (root length) (Table 40)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 15 | 51.296 | 3.420 | 11.823 | 0.00000 |
| Error | 32 | 9.256 | 0.289 | | |
| Total | 47 | | | | |

**ANOVA 168: Analysis of variance of conjoint application of seed biopriming with effective biocontrol agents and soil amendments in capsicum under artificially inoculated conditions (disease incidence) (Table 40)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 15 | 6.270 | 0.418 | 20.789 | 0.00000 |
| Error | 32 | 0.643 | 0.020 | | |
| Total | 47 | | | | |

**ANOVA 169: Analysis of variance of biochemical estimation of Polyphenol oxidase enzyme activity in bioprimed tomato plants (Table 41)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 15 | 771.598 | 51.440 | 51.790 | 0.00000 |
| Error | 32 | 31.784 | 0.993 | | |
| Total | 47 | 803.382 | | | |

**ANOVA 170: Analysis of variance of biochemical estimation of Polyphenol oxidase enzyme activity in bioprimed chilli plants (Table 41)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 15 | 958.762 | 63.917 | 176.042 | 0.00000 |
| Error | 32 | 11.619 | 0.363 | | |
| Total | 47 | 970.381 | | | |

**ANOVA 171: Analysis of variance of biochemical estimation of Polyphenol oxidase enzyme activity in bioprimed capsicum plants (Table 41)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 15 | 538.573 | 35.905 | 87.174 | 0.00000 |
| Error | 32 | 13.180 | 0.412 | | |
| Total | 47 | 551.753 | | | |

**ANOVA 172: Analysis of variance of biochemical estimation of Peroxidase enzyme activity in bioprimed tomato plants (Table 41)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 15 | 1.704 | 0.114 | 7.765 | 0.00000 |
| Error | 32 | 0.468 | 0.015 | | |
| Total | 47 | 2.172 | | | |

**ANOVA 173: Analysis of variance of biochemical estimation of Peroxidase enzyme activity in bioprimed chilli plants (Table 41)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 15 | 1.953 | 0.130 | 30.200 | 0.00000 |
| Error | 32 | 0.138 | 0.004 | | |
| Total | 47 | 2.091 | | | |

**ANOVA 174: Analysis of variance of biochemical estimation of Peroxidase enzyme activity in bioprimed capsicum plants (Table 41)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 15 | 4.183 | 0.279 | 47.401 | 0.00000 |
| Error | 32 | 0.188 | 0.006 | | |
| Total | 47 | 4.371 | | | |

**ANOVA 175: Analysis of variance of biochemical estimation of Total phenolic content enzyme activity in bioprimed tomato plants (Table 42)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 15 | 441277.969 | 29418.531 | 3294.637 | 0.00000 |
| Error | 32 | 285.735 | 8.929 | | |
| Total | 47 | 441563.704 | | | |

**ANOVA 176: Analysis of variance of biochemical estimation of Total phenolic content enzyme activity in bioprimed chilli plants (Table 42)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 15 | 620326.892 | 41355.126 | 7004.062 | 0.00000 |
| Error | 32 | 188.942 | 5.904 | | |
| Total | 47 | 620515.834 | | | |

**ANOVA 177: Analysis of variance of biochemical estimation of Total phenolic content enzyme activity in bioprimed capsicum plants (Table 42)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 15 | 352100.963 | 23473.398 | 5549.334 | 0.00000 |
| Error | 32 | 135.358 | 4.230 | | |
| Total | 47 | 352.236.321 | | | |

**ANOVA 178: Analysis of variance of biochemical estimation of Phenylalanine ammonia lyase enzyme activity in bioprimed tomato plants (Table 42)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 15 | 4512550.336 | 300836.689 | 82036.241 | 0.00000 |
| Error | 32 | 117.348 | 3.667 | | |
| Total | 47 | 4512667.684 | | | |

**ANOVA 179: Analysis of variance of biochemical estimation of Phenylalanine ammonia lyase enzyme activity in bioprimed chilli plants (Table 42)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 15 | 4501914.356 | 300.127.624 | 66455.452 | 0.00000 |
| Error | 32 | 144.519 | 4.516 | | |
| Total | 47 | 4502058.875 | | | |

**ANOVA 180: Analysis of variance of biochemical estimation of Phenylalanine ammonia lyase enzyme activity in bioprimed capsicum plants (Table 42)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Treatment | 15 | 3482022.351 | 232134.823 | 6820.433 | 0.00000 |
| Error | 32 | 201.744 | 6.305 | | |
| Total | 47 | 3482224.096 | | | |

**ANOVA 181: Analysis of variance of efficacy of integration of seed biopriming with effective bio-inoculants (BCAs, plant extracts, endophytes) and soil solarization in tomato (seed germination) (Table 43)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 2 | 2.642 | | | |
| Factor A | 12 | 7495.373 | 624.614 | 157.621 | 0.00000 |
| Factor B | 1 | 768.609 | 768.609 | 193.958 | 0.00000 |
| Interaction A x B | 12 | 144.494 | 12.041 | 3.039 | 0.00000 |
| Error | 50 | 198.138 | 3.963 | | |
| Total | 77 | 8408.476 | | | |

**ANOVA 182: Analysis of variance of efficacy of integration of seed biopriming with effective bio-inoculants (BCAs, plant extracts, endophytes) and soil solarization in tomato (shoot length) (Table 43)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 2 | 3.208 | | | |
| Factor A | 12 | 334.833 | 27.903 | 25.462 | 0.00000 |
| Factor B | 1 | 738.831 | 738.831 | 674.204 | 0.00000 |
| Interaction A x B | 12 | 216.810 | 18.067 | 16.487 | 0.00000 |
| Error | 50 | 54.793 | 1.096 | | |
| Total | 77 | 1348.474 | | | |

**ANOVA 183: Analysis of variance of efficacy of integration of seed biopriming with effective bio-inoculants (BCAs, plant extracts, endophytes) and soil solarization in tomato (root length) (Table 43)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 2 | 1.485 | | | |
| Factor A | 13 | 168.333 | 14.028 | 83.381 | 0.00000 |
| Factor B | 1 | 79.607 | 79.607 | 473.181 | 0.00000 |
| Interaction A x B | 12 | 46.364 | 3.864 | 22.966 | 0.00000 |
| Error | 50 | 8.412 | 0.168 | | |
| Total | 77 | 303.201 | | | |

**ANOVA 184: Analysis of variance of efficacy of integration of seed biopriming with effective bio-inoculants (BCAs, plant extracts, endophytes) and soil solarization in tomato (disease incidence) (Table 43)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 2 | 0.140 | | | |
| Factor A | 12 | 1631.945 | 135.995 | 96.766 | 0.00000 |
| Factor B | 1 | 1728.101 | 1728.101 | 1229.612 | 0.00000 |
| Interaction A x B | 12 | 352.244 | 29.354 | 20.886 | 0.00000 |
| Error | 50 | 70.270 | 1.405 | | |
| Total | 77 | 3782.701 | | | |

**ANOVA 185: Analysis of variance of efficacy of integration of seed biopriming with effective bio-inoculants (BCAs, plant extracts, endophytes) and soil solarization in chilli (seed germination) (Table 44)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 2 | 4.894 | | | |
| Factor A | 12 | 367.328 | 30.611 | 37.019 | 0.00000 |
| Factor B | 1 | 25.463 | 25.463 | 30.794 | 0.00000 |
| Interaction A x B | 12 | 389.698 | 32.475 | 39.274 | 0.00000 |
| Error | 50 | 41.344 | 0.827 | | |
| Total | 77 | 828.727 | | | |

**ANOVA 186: Analysis of variance of efficacy of integration of seed biopriming with effective bio-inoculants (BCAs, plant extracts, endophytes) and soil solarization in chilli (shoot length) (Table 44)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 2 | 1.302 | | | |
| Factor A | 12 | 293.355 | 24.446 | 25.203 | 0.00000 |
| Factor B | 1 | 73.973 | 73.973 | 76.261 | 0.00000 |
| Interaction A x B | 12 | 46.849 | 3.904 | 4.025 | 0.00000 |
| Error | 50 | 48.499 | 0.970 | | |
| Total | 77 | 463.978 | | | |

**ANOVA 187: Analysis of variance of efficacy of integration of seed biopriming with effective bio-inoculants (BCAs, plant extracts, endophytes) and soil solarization in chilli (root length) (Table 44)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 2 | 0.662 | | | |
| Factor A | 12 | 127.812 | 10.651 | 28.021 | 0.00000 |
| Factor B | 1 | 19.150 | 19.150 | 50.381 | 0.00000 |
| Interaction A x B | 12 | 42.261 | 3.522 | 9.265 | 0.00000 |
| Error | 50 | 19.005 | 0.380 | | |
| Total | 77 | 208.225 | | | |

**ANOVA 188: Analysis of variance of efficacy of integration of seed biopriming with effective bio-inoculants (BCAs, plant extracts, endophytes) and soil solarization in chilli (disease incidence) (Table 44)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 2 | 2.298 | | | |
| Factor A | 12 | 8624.864 | 718.739 | 272.870 | 0.00000 |
| Factor B | 1 | 675.125 | 675.125 | 256.312 | 0.00000 |
| Interaction A x B | 12 | 482.019 | 40.168 | 15.250 | 0.00000 |
| Error | 50 | 131.700 | 2.634 | | |
| Total | 77 | 9916.005 | | | |

**ANOVA 189: Analysis of variance of efficacy of integration of seed biopriming with effective bio-inoculants (BCAs, plant extracts, endophytes) and soil solarization in capsicum (seed germination) (Table 45)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 2 | 2.751 | | | |
| Factor A | 12 | 2123.428 | 176.952 | 202.032 | 0.00000 |
| Factor B | 1 | 403.961 | 403.961 | 461.215 | 0.00000 |
| Interaction A x B | 12 | 43.546 | 3.629 | 4.143 | 0.00000 |
| Error | 50 | 43.793 | 0.876 | | |
| Total | 77 | 2617.479 | | | |

**ANOVA 190: Analysis of variance of efficacy of integration of seed biopriming with effective bio-inoculants (BCAs, plant extracts, endophytes) and soil solarization in capsicum (shoot length) (Table 45)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 2 | 1.264 | | | |
| Factor A | 12 | 227.326 | 18.944 | 33.265 | 0.00000 |
| Factor B | 1 | 65.980 | 65.980 | 115.860 | 0.00000 |
| Interaction A x B | 12 | 52.638 | 4.386 | 7.703 | 0.00000 |
| Error | 50 | 28.474 | 0.569 | | |
| Total | 77 | 375.682 | | | |

**ANOVA 191: Analysis of variance of efficacy of integration of seed biopriming with effective bio-inoculants (BCAs, plant extracts, endophytes) and soil solarization in capsicum (root length) (Table 45)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 2 | 0.462 | | | |
| Factor A | 12 | 104.541 | 8.712 | 20.707 | 0.00000 |
| Factor B | 1 | 10.765 | 10.765 | 25.587 | 0.00000 |
| Interaction A x B | 12 | 37.044 | 3.087 | 7.338 | 0.00000 |
| Error | 50 | 21.036 | 0.421 | | |
| Total | 77 | 173.847 | | | |

**ANOVA 192: Analysis of variance of efficacy of integration of seed biopriming with effective bio-inoculants (BCAs, plant extracts, endophytes) and soil solarization in capsicum (disease incidence) (Table 45)**

| Source of Variation | DF | Sum of Squares | Mean Squares | F-Calculated | Significance |
|---|---|---|---|---|---|
| Replication | 2 | 3.148 | | | |
| Factor A | 12 | 3083.608 | 256.967 | 439.394 | 0.00000 |
| Factor B | 1 | 205.775 | 205.775 | 351.858 | 0.00000 |
| Interaction A x B | 12 | 117.154 | 9.763 | 16.694 | 0.00000 |
| Error | 50 | 29.241 | 0.585 | | |
| Total | 77 | 3438.927 | | | |

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| | | |
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## ABSTRACT

The present investigation entitled "Potential of seed biopriming in management of damping-off disease in solanaceous vegetable crops" were carried out in the Department of Plant Pathology. Damping-off has been a serious disease in the nursery in solanaceous vegetable crops and *Pythium* sp, *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsii* have been found associated with this disease. Combined application of inoculum of these pathogens resulted in 75.42, 84.33 and 81.97 per cent incidence with incubation period of 6-7 days in tomato, chilli and capsicum, respectively. Among different biocontrol agents evaluated against damping-off pathogens under *in vitro* conditions, *Trichoderma virens*, *T. harzianum* and *T. atroviride* were found most effective with mycelial inhibition of 86.55, 84.26, 62.77 per cent, respectively. In tomato, seed biopriming with *T. harzianum* at $10^7$ cfu per ml was found most effective with seed germination of 76.0 per cent. In chilli and capsicum, seed biopriming with *Bacillus licheniformis* and *T. harzianum* at $10^8$ cfu per ml were found most effective with seed germination of 92.33 and 75.0 per cent, respectively. Biopriming of tomato seeds with *T. virens* and chilli and capsicum seeds with *Trichoderma harzianum* for 8 hrs resulted in maximum seed germination of 93.0, 90.67, 83.67 per cent, respectively. Biopriming of tomato and chilli seeds with *T. harzianum* at $10^7$ cfu per ml for 8 hrs resulted in 57.88, 46.16 per cent reduction in seedling mortality, respectively under field conditions. In capsicum, biopriming with *T. atroviride* at $10^8$ cfu per ml for 8 hrs resulted in 55.51 per cent reduction in seedling mortality. Leaf aqueous extracts (10 %) of *Roylea elegans*, *Melia azedarach* and *Datura stramonium* was found most effective under *in vitro* conditions with mycelial inhibition of 36.78, 36.38, 30.78 per cent, respectively. Seed biopriming with *M. azedarach* in tomato and capsicum resulted in seed germination of 92.5, 92.0 per cent, respectively. However, *R. elegans* was found most effective in chilli with seed germination of 92.33 per cent. Seed biopriming with *M. azedarach* for 12 hrs was found most effective in tomato with seed germination of 88.67 per cent. However, in chilli and capsicum seed biopriming with *R. elegans* for 12 hrs was found most effective with seed germination of 93.33, 91.0 per cent, respectively. Seed biopriming with *R. elegans* (10 %) for 12 hrs was found most effective in tomato and capsicum with least seedling mortality of 18.2, 14.26 per cent, respectively under field conditions. However, in chilli seed biopriming with *M. azedarach* was found most effective with least seedling mortality of 17.26 per cent under field conditions. Endophyte EF1, EB5 and EB3 were found most effective with 72.59, 41.69 and 38.23 per cent mycelial inhibition, respectively. Seed biopriming with EB3 ($10^7$ cfu per ml) was found most effective in tomato with maximum seed germination of 93.3 percent. Further, seed biopriming with EF1 ($10^8$ cfu per ml) and EB5 ($10^7$ cfu per ml) resulted in seed germination of 95.67, 89.0 in chilli and capsicum, respectively. Seed biopriming with EF1 for 12 hrs duration resulted in maximum seed germination of 92.33, 95.0 and 91.67 per cent in tomato, chilli and capsicum, respectively. Seed biopriming with EF1 at $10^8$ cfu per ml for 12 hrs resulted in least seedling mortality of 15.21, 16.33, 14.40, per cent in tomato, chilli and capsicum, respectively under field conditions. Treatment combination of seed biopriming with *T. harzianum* and soil amendment with NSKE was found most effective with 55.90 per cent reduction in seedling mortality in tomato. However, in chili and capsicum treatment combination of *T. virens* and soil amendment with vermiwash was found most effective with 61.98, 57.46 per cent reduction in seedling mortality, respectively. These biopriming treatments also resulted in significant increase in enzyme activity like PAL, PPO, PO and TPC in bioprimed plants. Integration of biopriming along with soil amendments namely neem cake and vermicompost was found more effective in solarized soil. Treatment combination of seed biopriming with *Penicillium dipodomyicola* and soil application of neem cake was found most effective with 49.93, 47.21, 42.89 per cent reduction in seedling mortality in tomato, chilli and capsicum, respectively in solarized nursery beds.

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