EVALUATION OF SEED BIOPRIMING AGAINST TOMATO (*Solanum lycopersicum* L.) DISEASES

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Abstract

EVALUATION OF SEED BIOPRIMING AGAINST TOMATO (*Solanum lycopersicum* L.) DISEASES

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ABSTRACT

Tomato [*Solanum lycopersicum* L.] is most widely cultivated vegetable crop in the world. Major diseases of tomato viz., damping-off, early blight and fusarial wilt were observed as they caused losses in the field of Navsari district. Considering the present investigation was carried out.

Field survey was conducted in Navsari district during Kharif-Rabi 2015-16 for damping-off, early blight and fusarial wilt. Among them early blight was found with maximum per cent disease intensity (44.91%).

Plant showing typical symptoms of damping-off, early blight and fusarial wilt diseases in field condition were brought to the laboratory for isolation. The pathogenicity test was proved by artificial inoculation methods. *viz.*, soil inoculation (for damping-off) spore spray (for early blight), root dip (for fusarial wilt). The pathogenicity test revealed symptoms similar to those observed under field conditions. Further the diseased sample were subjected to isolation and cultures obtained were purified. The cultural and

morphological characters revealed the damping-off, early blight and fusarial wilt caused by *P. aphanidermatum, A. solani, F. oxysporum f. sp. lycopersici*, respectively.

Occurrence of seed borne pathogen was carried out by two different methods. *viz*., blotter paper and Potato Dextrose Agar (PDA). In blotter paper method *Rhizopus* sp. was found dominant fungus in sterilized and unsterilized both conditon.

In PDA method the disease incidence was not recorded by *Rhizopus* sp., *A. niger, A. flavus, F. oxysporum* and *A. solani* in sterilized seeds while higher 36.50% incidence of *Rhizopus* sp. was found in unsterilized seeds.

In vitro biopriming of tomato seeds with *T. harzianum* @ 10g/kg seed (10^8 cfu/g) recorded highest seed germination (94.00%) and lower seed infection (6.28%).

In vivo biopriming of tomato seeds revealed plant growth promoting activity was highest in seeds treated with *P. fluorescens* applied at imbibition @10g/kg seed (10^8 cfu/g), higher seed germination (71.82%), maximum shoot length (6.30 cm), root length (9.72 cm) and seedling height (15.66 cm) which was found significantly superior over the rest and reduce disease of tomato. Also reduced per cent diseases incidence of damping-off in seeds treated with *P. fluorescens* applied at imbibition (18.44%), reduced per cent diseases intensity of early blight in seeds treated with *T. harzianum* applied at imbibition (15.67%) and reduced per cent diseases incidence of fusarial wilt in seeds treated *T. harzianum* applied at imbibition (14.73%).

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C E R T I F I C A T E

This is to certify that the thesis entitled "EVALUATION OF SEED BIOPRIMING AGAINST TOMATO (***Solanum lycopersicum*** **L.)** DISEASES" submitted by **Mr. HASAMUKH KANUBHAI GHINAIYA** in partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **PLANT PATHOLOGY N. M. College of Agriculture, NAVSARI AGRICULTURAL UNIVERSITY** is a record of bonafied research work carried out by him under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma or other similar title.

Place: Navsari **(J. R. PANDYA)**

Date: June 2017 **Major Advisor**

DECLARATION

This is to declare that the whole of the research work reported in the thesis in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **PLANT PATHOLOGY** by the undersigned is the result of investigations done by me under direct guidance and supervision of **Dr. J. R. Pandya,** Assistant Professor, Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari and no part of the work has been submitted for any other degree so far.

Place: Navsari

Date: June 2017 **(H. K. GHINAIYA)**

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A C K N O W L E D G E M E N TS

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Place: Navsari

Date: June 2017 **(H. K. GHINAIYA)**

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Introduction

I. INTRODUCTION

Tomato [*Solanum lycopersicum* L.] is one of the most widely cultivated vegetable crop in the world. It is a solanaceous fruit vegetable believed to have its origin in Tropical America (Thompson and Kelly, 1957). It is known as Love apple, *Tomate*, *Tomat*, *Tomatar*, *Rangam* and *Tomati* in different parts of the world. It is also popularly called as 'Poor man's orange'. It is grown extensively and marketed throughout the world. It ranks second largest vegetable crop after potato (Vanita *et al*., 2011).

It is a traditional vegetable crop commercially cultivated throughout the world over an area 4.02 million hectares with a production 152.9 million tones and productivity 37.83 tonnes/ha. Tomato crop grown in almost all kinds of ecological condition. In India, tomato is cultivated as one of the leading vegetable crop, covering an area of 8.82 lakh hectares with total production of 187.35 lakh tonnes having productivity of 21.20 MT/ha (Anon., 2014). The major tomato producing states are Bihar, Karnataka, Odisha, Maharashtra, Himachal Pradesh, West Bengal, Tamil Nadu, Uttar Pradesh and Gujarat (Chadha, 2002), among them Karnataka is the highest tomato producer state in India. In Gujarat, tomato is one of the major vegetable crop cultivated in an area of 38.80 million hectares with the total production of 978.40 million tonnes/ha with the productivity of 25.00 tonnes/ha (Anon., 2014). It is cultivated in almost all 33 districts of Gujarat of Gandhinagar, Mehsana, Sabarkantha, Patan, Vadodara, Surat, Navsari, Valsad, and Dang. Tomato is a major diet, used almost every day either as whole or mixed with other vegetables.

Tomato is an attractive fruit vegetable in market. Attractive red color of fruit is due to the presence of lycopene and pigment carotene imparts yellow color to fruits (Kalloo, 1985). It is an important source of nutrients as it has high nutritive value of Vitamin A, B, C, E and other important nutrients *viz*., protein, carbohydrates, fiber, fat, biotin, malic acid, citric acid, oxalic acid *etc*. The 100 g edible fruit of tomato contains 94.7 g water, 1.0 g protein, 0.1 g fat, 1.6 g fibre, 1.9 g carbohydrates, 0.51 g organic acid, 19.1 mg vitamins and 224.5 mg minerals. Tomato is generally grown throughout the year (Rai *et al*., 2002). Reducing sugars are the main carbohydrates present in tomato fruit and free sugars such as fructose, glucose and α- present in fruit represent more than 60 per cent of the solid.

Due to its nutritive value and low price, it can be consumed as fresh ripe fruit and is one of the most popular salad vegetables. It is taken either raw as salad or cooked vegetable. Tomato can be processed into soup, juice, ketchup, paste, pickles, powder, sauces and many other products. Hence, there is a great demand for tomato in the market as fresh fruit. It has many other uses also. The tomato seeds contain 24 per cent oil and semi-drying oil is used as salad oil and in the manufacture of margarine. Tomato contain variety of colours and flavour to the foods, and also rich in medicinal values. The pulp juice is easily digestible also act as promoter of gastric secretion and blood purifier. It stimulates the torpid liver and useful in chronic dyspepsia. It is a good appetizer and removes the constipation and has a pleasing taste. It is an important cash crop for medium scale commercial vegetable growers. Organic acids such as citric, malic, formic and acetic acids known as health acids are present in fresh tomato

fruits (Kalloo, 1985).

Tomato is commercially grown across the global, there is no place where the plant is free from disease. Tomato is affected by several fungal, bacterial and viral diseases like damping-off (*Pythium aphanidermatum*), early blight (*Alternaria solani*), late blight (*Phytophthora infestans*), buckeye rot (*Phytophthora nicotianae var Parasitica*), fusarial wilt (*Fusarium oxysporum f. sp. lycopersici*), septoria leaf spot (*Septoria lycopersici*), powdery mildew (*Leveillula taurica*), anthracnose (*Colletotrichum phomoides*), bacterial wilt (*Ralstonia solanacearum*), bacterial leaf spot (*Xanthomonas campestris* pv. *vesicatoria*), bacterial canker (*Clavibacter michiganesis* pv. *michiganensis*), tomato mosaic virus (TOMV), tomato leaf curl virus (TLCV), tomato spotted wilt virus (TSWV), tomato bunch top virus (TBTV), tomato big bud (TBB) etc.

Biological control of fungal diseases of crop is an eco-friendly and a potential component of integrated disease management. Biological control of soil borne pathogen received less attention, owing to poor establishment of the introduced bioagent and resulting variation in disease suppression due to lack of effective delivery system. It does not lead to development of resistance in plant pathogen, no phytotoxic effects, does not create any pollution problem as it is eco-friendly, promotes plant growth, induces resistance in host and hence increase soil fertility. It significantly minimizes losses due to crop disease and reduces cost of production, increased yield, quality and profit.

Successful use of biocontrol agents for the control of various diseases caused by pathogens *viz.*, *Rhizoctonia Solani, Sclerotium rolfsii, Fusarium oxysporium, Pythium*

aphanidermatum, Phytophthora infestans etc. in several crop have been reported (Cook & Baker, 1983).

Biological control through seed treatment by biopriming was found an attractive as well as efficient method for introducing the antagonists into the soil-plant environment. The efficacy of certain biological control agents can be enhanced by incorporating them into seed biopriming processes, a variety of procedures that enhance seed germination and vigour. Seed priming reduces quantities of seed exudates and the number of surface wounds, both of which reduce seed infection by pathogen (Osburn and Schroth, 1988). Biopriming, in which specific biological control agents are incorporated into the seed priming process, can be very effective in suppressing disease. (Harman and taylor, 1988; Harman *et al*., 1989).

Seed Biopriming is a technique of seed treatment that integrates biological (inoculation of seed with beneficial organism to protect seed) and physiological aspects (seed hydration) of disease control. It is an ecological approach using selected funal antagonists against the soil and seed borne pathogen. Biological seed treatment may provide an alternative to chemical control. Seed biopriming were used commercially in many horticulture crops, as a tool to increase speed and uniformity of germination and improve final stand. Biopriming has great promise for enhancing the efficacy, shelf life, and consistent performance of biological control agents (Callan *et al*., 1997).

With the line of above fact, present work has been carried out with following objectives and different aspects.

Objectives

1. To know the present status of tomato diseases particularly at seedling stage.
2. To study seed borne fungal flora in tomato.
3. To know the effect of seed biopriming against tomato diseases.

Aspects

1. Survey and collection of diseased samples
2. Isolation of pathogens
3. Pathogenicity
4. Identification of the pathogens
5. Occurrence of seed borne pathogens in popular local cultivars of tomato
6. Evaluation of seed bio-priming against tomato diseases *in vitro* and *in vivo*

Review Of Literature

II. REVIEW OF LITERATURE

2.1 Survey

Survey is most important aspect to know the status of diseases surrounding the area; several scientists have carried out survey on tomato disease who recorded/ noticed/ observed the per cent disease incidence/ intensity/severity (Table 2.1).

2.2 Isolation of pathogens

Isolation is the process of separation of pathogen which cause disease and for that so many workers have worked on this aspect. The following reviews are supporting the present aspect:

2.2.1 Damping-off

Rhizoctonia solani isolated from infected tomato plant showed severe symptoms of collar necrosis. [Curtis *et al.* (2007), Jiskani *et al.* (2007) and Prabha *et al.* (2014)].

Pythium sp. (*P. aphanidermatum and P. ultimate*) were isolated from infected tomato seedlings. [Suleiman (2010), Chavan *et al.* (2012), Anita *et al.* (2012) and Shinde (2016)].

2.2.2 Early Blight

Alternaria solani isolated from the infected leaves of tomato [Alhussaen (2012), Chanthini *et al.* (2012), Chaudhari (2016)].

2.2.3 Fusarial Wilt

Fusarium spp. (especially *F. oxysporum*) isolated from the infected tomato plant showing typical symptoms *viz.*, yellowing of leaves and wilting of plants [Ignjatov *et al.* (2012), Toju *et al.* (2012), Chopada (2014), Ramaiah and Garampalli (2015) and Patel (2015)]

Table 2.1: Survey of tomato diseases under field condition

Sr. No.	Year & Region	Name of Diseases	Name of Causal Organism	Disease Incidence /intensity /severity (%)	Source & Year
1.	2004 and 2005 Thane and Raigad, Dapoli, M.S.	Early blight	*Alternaria Solani*	20.78 to 42.30 and 35.12 to 55.75	Kamble *et al.*(2009)
	October 2006 to June 2008 in Chittagong			leaf 27.00 & fruit 33.00	Hossain *et al.*(2010)
	2008 and 2009 Konkan region			26.33 to 50.77	Munde *et al.*(2013)
	2010 and 2011 Varanasi			73.56	Sunil kumar and Srivastava (2013)
	2011 and 2012 Jammu & Kashmir			21.66 to 34.13 and 10.48 to 18.56	Sunita Rani *et al.* (2015)
	2015 Navsari			33.33 to 56.67	Patel (2015)
2.	2004 Chittoor	Damping off	*Pythium aphanidermatum*	4.5-33.1	Reddy *et al.*(2004)
	2011 Karnataka			5.50 to 45.50	Kulkarni (2004)
	2015 Himachal Pradesh			4.0 - 32.0	Dohroo *et al.*(2015)
	2016 Navsari			18.50	Shinde (2016)
3.	2008 Tamil Nadu	Fusarial Wilt	*Fusarium oxysporum*	Max.75	Anitha *et al.*(2009)
	2012 Tamil Nadu			19 to 45	Manikandan *et al.* (2014)

2.3 Pathogenicity

2.3.1 Damping-off

Yadav and Joshi (2012) proved pathogenicity of different sixs isolates of *P. aphanidermatum* on tobacco cultivar Anand-19 causing damping-off disease, using soil inoculation method and hydrophonic method and they was found cent per cent damping-off incidence in Tobacco cultivar Anand-19.

Gilardi *et al.* (2013) proved pathogenicity of *P. aphanidermatum* causing damping-off of Beet (*Beta vulgaris subsp. vulgaris*) by artificially inoculating sterilized pot mixture with the fungus and they found seventy per cent of plant infected seven days of sowing.

Prabha *et al.* (2014) proved *R. solani* which causes damping off disease was isolated from the infected soil sample and *S. sclerotiorum* which causes stem rot was isolated from infected stem of the tomato plants. It is then identified in lab and maintained in Potato Dextrose Agar (PDA) slants. The culture was streaked in Starch Casein Potato Dextrose Agar (SCPDA) and incubated for 5 days, after which the mycelial plug of the fungi was streaked perpendicular to the actinomycete strain and incubated for 5 days. The results were observed for 14 days.

Lai *et al.* (2015) tested pathogenicity at 25^0C, *Pythium ultimate* was the most pathogenic species, causing 97.00 per cent seed rot and 46.40 per cent damping-off and *Pythium aphanidermatum* was the second most pathogenic species, resulting in 88.50 per cent seed rot and 41.80 per cent damping-off in soybean.

2.3.2 Early Blight

Chanthini *et al.* (2012) proved the pathogenicity of *A.*

solani on tomato plants by spore suspension method. Spores from actively growing colony of the fungi were collected using sterile distilled water. The spore suspension was adjusted 10^4spores/ml. The 45 days old healthy tomato plants were challenged with the fungal spores. 10 ml of each inoculum was sprayed to three plants by using hand atomizer. They were covered with polypropylene bags with scattered holes (for exchange of air). Plants were watered (sterile distilled water) every alternate day for a period of another 10 days. After 10 day, the infected leaves were collected and the fungi reisolated and identified.

Gupta *et al*. (2014) proved Pathogenicity test was carried out by spraying uninjured 15-20 cm long plants to runoff with spore suspension, containing 3×10^5 spores/ml followed by 48 hours of incubation in moist chamber after which the plants were returned to the greenhouse bench.

Bashir *et al.* (2014) proved pathogenicity test was confirmed by growing the tomato plants in pathogen infected soil as well as spray of fungal spores on to the leaves of young tomato plants. Spore suspension (50 ml) was mixed well with sterilized sieved 500 g of soil. This soil was filled in each plastic pots followed by sowing of 5 seeds per pot. Plants were regularly monitored for disease development.

2.3.3 Fusarial wilt

Chopada (2014) proved pathogenicity test of *F. oxysporum* f. sp. *lycopersici* (tomato wilt) conducted through root dip inoculation technique.

Joshi *et al*. (2012) observed nursery preparation for the pot experiment was performed by surface sterilized seeds of chilli, sterilized with 2% sodium hypochlorite for 2 min, washed with

distilled water and sown into plastic trays filled with steam sterilized soil. The seeds were germinated at 27°C and 70% relative humidity for 14 hours light period (500 lux). All forty eight isolates identified as *F. oxysporum,* were evaluated for their ability to cause disease, and non-pathogenic characteristic on three leaf stage chilli plantlets.

Nirmaladevi *et al.* (2012) proved the pathogenicity test of *F. oxysporum* f. sp. *lycopersici* in twenty days old tomato seedlings inoculated by standard root deep method and severity of the disease was assessed from two weeks of inoculation up to 45 days.

2.4 Identification of the pathogens

2.4.1 Damping-off

Prabha *et al.* (2014) identified *R. solani* and *S. sclerotiorum* by physiochemical and biochemical methods. Physiochemical methods consist of macroscopic and microscopic methods. The microscopic characterization was done by cover slip culture method. The mycelial structure, colour and arrangement of the spore were observed through microscope.

Mehrotra and Agrawal (2004) described the morphological characteristic of *P. aphanermatum* and observed that the fungus bears sporangia which are inflated, lobulated and may be branched or unbranched having zoospores which measure 7 x 12 microns. Oogonia are spherical, terminal measuring 22 to 27 microns. The oospores are aplerotic, single, measuring 17-19 microns and the antheridia are monoclinous or diclinous and may be intercalary or terminal.

Gaur and Chauhan (2007) studied the morphological characteristics of *P. aphanidermatum* are found that the mycelium is much branched, hyaline, coenocytic with a diameter of 2.5-8.0um

in length and 20.0um in width with rich protoplasm.

2.4.2 Early blight

Arunakumara (2006) observed that conidia of *A. solani* were solitary straight or slightly flexuous, oblong or ellipsoidal tapering to a beak, smooth, 150-300 um in length, 13-20 jam thick in the broadest part with 8-10 transverse and 1 -4 longitudinal septa.

Alhussaen (2012) observed that conidiophores of *A. solani* formed singly or in groups or flexuous brown to olivaceous brown. The conidia were solitary straight or slightly flexuous, sometimes branched and 35-75 in length, 10-20 in width, 2-7 transverse septa and 1-4 longitudinal septa. The mycelial width was 0.8-1.5.

Chanthini *et al.* (2012) identified *A. solani* from tomato plants, research was done by comparing its cultural and morphological characteristics (spore shape and color, hyphae). The fungus showed greenish black pigmentation with a radial growth of 31.5 mm.

Gupta *et al.* (2014) identified *A. alternata* cultures on PDA under fluorescent lights were at first fluffy and off-white, but become dusky neutral gray with an off-white border within 48 hours. Identified based on morphological characters. Conidiophores were brown, straight, bearing light brown conidia formed in long chains and were obclavate and muriform, often with a short conical or cylindrical, pale beak, less than one third of the length of the conidium. Conidia had 3-7 transverse septa and usually several longitudinal or oblique septa.

Bashir *et al.* (2014) identified *A. metachromatica* colony color was olivaceous green to black; reverse greenish black,

exudates and odor not present. Conidial chains with several short lateral branches of 5-7 conidia; Conidiophores abundant, branched and short. Simple chain of 8-10 conidia; secondary conidiophores not conspicuously long, 20-30 x 3-4 µm, 1-2 geniculate. Mature conidia reached in size of 25-45 x 8-12 µm, with 4-7 transverse and 1-2 longitudinal septa.

2.4.3 Fusarial wilt

Ignjatov *et al.* (2012) identified *F. oxysporum* on tomato plants from growth characteristics on potato-dextrose medium, fungus isolated from stem of diseased tomato plants formed a hyaline, branching mycelium that was white to gray. On Czapek agar isolates formed colonies and mycelium appeared as aerial, grey to light purple in color depending on the isolate. All observed isolates formed macro conidia as elliptical, gradually pointed or curved edges (pointed end). Macro conidia varied in size from 30-60 µm to 3.0-5.0 µm. Most often they were short and had three septa.

Bakar *et al.* (2013) used phenotype based identification of the fusarium on tomato, isolates with emphasis for characterization of the shape and sizes of the macro conidia and micro conidia, number of septa and shapes of the apical and basal cells of the macro conidia, conidiogenous cells, growth rate, presence of chlamydospore, colony color, growth and pigmentation on PDA media.

Joshi *et al.* (2013) identified as *F. oxysporum* on the basis of macro conidia characteristics which were thin walled generally 3-5 septate, fusoid falcate macro conidia with somewhat hooked apex and pedicillate base. The macro conidia of *F. oxysporum* were found in the rage of 5.30 to 28.40 µm and the micro conidia were

found in the rage of 2.30 to 11.80 µm.

Chopada *et al.* (2014) identified *F. oxysporum* f. sp. *lycopersici* on tomato plants on the basis of morphological and cultural characteristics, initially the fungus produced dirty white cottony mycelium, which turned into occur yellowish to dull pink colour. Chlamydospores formed when culture becomes old gradually in all the cases, mycelium was delicate dirty white to pink, mostly with purple tinge and are sparse to abundant. Macro conidia were straight, spindle as well as sickle shaped. The size of macro conidia ranged from 15.46-21.8 x 4.91-5.45 µm. The micro conidia were hyaline, round to oval in shape. The size of micro conidia ranged from 3.57-14.28 x 2.68-4.46 µm. Chlamydospores were round, oval, terminal and intercalary in all the isolates.

Hernandez *et al.* (2014) the identification of *F. oxysporum* was made using the molecular technique of Chain Reaction (PCR) coincided with the results of tests pathogenic.

Ramaiah and Garampalli (2015) identified *F. oxysporum* f. sp. *lycopersici* on tomato plants on the basis of morphological characters of micro and macro conidia and with clamidospores were observed.

2.5 Occurrence of seed borne pathogens

The aspect is used to know the association of different pathogens on tomato seeds. The following collected reviews which supporting the present research work.

F. oxysporum f.sp. *lycopersici, A. solani, Fusarium pallidoroseum, Myrothecium verrucaria, Phoma* sp., *Hainesia lythri,* Tomato mosaic virus, Tobacco mosaic virus and *Clavibacter michiganensis sub.* sp. *michiganensis* were detected on analyzed tomato seeds.

Abdalla (2000) assayed tomato seeds for the presence of seed borne bacterial pathogens. *Pseudomonas corrugata and Xanthomonas campestris* pv.*vesicatoria* [*X. vesicatoria*] were detected in 14.7 per cent and 12 per cent of the seed samples tested, respectively.

Al-kassam and Monawar (2000) isolated fungi and reported to be pathogenic to the seeds of different crops causing diseases such as seed rot, damping off, root rot, fruit rot, wilt and foliar diseases. Some of the seed borne fungi are also known to cause seed rot, decrease seed germination and cause pre and post damping off and seedling death.

Zakaria (2010) studied eighty seed samples of tomato for seed borne fungal pathogens, a total of ten genera of fifteen species of fungi were recorded and found *Fusarium moniliforme* was the most prevalent.

Chamling *et al.* (2011) found *Aspergilus niger*, *Aspergilus flavus*, *Alternaria alternata, Fusarium moniliforme*, *Fusarium* sp., *Cladosporium* sp., *Penicillium* sp. and *Cunnninghamella echinulata* were detected on tomato seeds.

Sowley and Kodua (2012) found *Aspergilus flavus*, *Aspergilus* niger, *Penicillium* spp. and *Rhizopus stolonifer* from tomato seeds stored by farmers by agar plate method and also found *A. flavus* in seeds tested by the blotter method for sterilized and unsterilized seeds were 56.70 per cent and 56.10 per cent respectively and 3.20 per cent and 3.50 per cent, respectively for *Penicillium* sp.

Patel (2015) carried out experiment on seed borne nature of fungi by using standard blotter method which revealed that highest incidence of *A. flavus* and *A. niger* is recorded in sterilized

and unsterilized seeds, respectively while *Penicillium* sp. had the least in both.

2.6 Evaluation of seed bio-priming against various pathogen

Manoranntham *et al*. (2000) revealed soil application of *Trichoderma viride* and *Pseudomonas fluorescens* effectively checked the pre-emergence and post-emergence damping off of tomato caused by *Pythium aphanidermatum* under pot culture experiments. Talc based formulation of antagonists significantly reduced the soil population of *Pythium* and increased the shoot length, root length and dry matter production of tomato seedlings.

El-Mohamed *et al*. (2004) recorded, when the okra seeds were primed and coated with Plant Guard, Rhizo-N, *Trichoderma harzianum* or *Bacillus subtilis* in Egypt. The reduction in disease incidence at pre- and post-emergence was 81.30, 75.0, 68.10, 60.0 per cent and 70.10, 60.50, 57.80, 57.90 per cent, respectively.

Zaidi and Singh (2004) carried out the experiment on seed bio-priming. They apply *T. harzianum* on seed of soybean, chickpea and tomato. They found that population of bioagent on surface of bioprimed seeds increased by almost 10000 folds in 48 hours after incubation. Higher germination of seeds than non-bioprimed seeds was also observed in all tested crops.

El-Mohamed *et al*. (2004) carried out experiment under field conditions. Biopriming of okra seeds with Plant Guard, Rhizo-N, *T. harzianum* and *B. subtilis*. Treatments reduced pre-emergence (20 days after sowing) damping off by more than 68.40 per cent and post-emergence at 45 and 60 days after sowing by more than 64.40 per cent and 59.30 per cent, respectively.

Raj *et al*. (2004) revealed that due to *Pseudomonas fluorescens* resulted in enhancement of germination, seedling vigour, plant height, leaf area, tillering capacity, seed weight, (measured for 1000 seeds) yield and induces resistance against downy mildew at Mysore.

Pandey and Pandey (2005) observed that coating the tomato seeds with bioagent *T. viride* was most effective against *F. solani* and *Sclerotium rolfsii* with 56.70 and 80.80 per cent seed germination, respectively. Whereas, *T. virens* was the best antagonist against *Rhizoctonia solani* (71.70%) and *Macrophomina phaseolina* (75.8%) under pot condition.

Verma and Dohroo (2005) tested six bioagents *viz., T. viride, T. harzianum, Paecilomyces lilacinus, T. virens, Laetisaria arvalis* and *P. fluorescens* against *F. oxysporum* f. *sp. pisi* under pot condition on pea. Among them, seed coating with *T. harzianum* and *T. viride* were found most effective with 93.83 per cent disease control.

Mohamedy *et al.* (2006) reported that biopriming of cowpea seed with *T. harzianum* reduced root rot caused by *F. solani* (60.80%), *R. solani* (60.80%) and *M. phaseolina* (75.0%) at pre emergence stage.

EI-Mougy and Abdel-Kader (2008) evaluated the effect of biopriming of faba bean seeds against root rot pathogen *(R. solani, F. solani and S. rolfsii)*. They noticed that bio primed faba bean seeds showed a highly significant effect causing complete reduction of root rot incidence at both pre and post emergence stages of plant growth as compared to the control.

Morsy *et al*. (2009) evaluated the efficiency of *T. viride* and *B. subtilis* as biocontrol agent against *F. solani* on tomato

plants and resulted that seed coating with *T. viride* or *B. subtilus*, significantly increased survival rate ranging between 70-73 per cent in the *F. solani* infected soil.

Sharma *et al.* (2009) reported that biopriming of cumin seeds with *T. harzianum* increased the germination of seeds while *T. viride* showed good shoot-root ratio in pot condition against cumin wilt.

Srivastava *et al.* (2010) Evaluation of arbuscular mycorrhizal fungus, *P. fluorescens* and *T. harzianum* against *F. oxysporum* f. sp. *lycopersici* for the management of tomato wilt in U.S.

Someshwar and Sitansu (2010) used bacterial bioagent *P. fluorescens* for seed bio priming and found better performance, stimulating the germination of seeds of chili, tomato and brinjal over some fungal bioagents *viz.*, *T. viride* (strain AN-10) and *T. harzianum* (AN- 13) which was equivalent to T. *harzianum* (strain WB- 1) in inducing germination of the seeds. The highest germination of seeds was obtained when seeds were bio primed with mycelial form of inoculum of *T. harzianum* AN-5 and WB-1.

Kumar *et al.* (2010) found that combined application of *T. harzianum* and P. *fluorescens* as seed biopriming resulted in significant growth of seedling (18.38 cm after 30 days of sowing) in sweet pepper.

Patel *et al.* (2011) evaluated efficacy of different isolates of *T. viride* and *T. harzianum* against *F. udum* under *in vitro*. Among the different isolates, *T. harzianum* sardarkrushinagar isolated found to be most effective *in vitro* and was selected for the control of pigeon pea wilt disease caused by *F. udum* under pot condition. The seed treatment with talk based formulation of *T.*

harzianum showed seed germination of 75.00 per cent and wilt incidence of 39.73 and 29.91 per cent in 2 and 4 g per kg seed treatment, respectively.

Nazir *et al.* (2011) *T. viride* and *T. harzianum* showed significant reduction in the radial growth of *P. aphanidermatum* and *Thanatephorus cucumeris* and increased seed germination, plant height, leaf number and fresh weight of tomato and chilli compared to the control.

Ananthi *et al.* (2013) revealed chilli (*Capsicum annuum* L.) seed to standardise bio-priming with the bio-control agents *T. viride* or *P. fluorescens* in order to improve seed germination and seedling vigour.

Eutesari *et al.* (2013) reported that three fungal bio control agents including *T. harzianum, T. virens, T. atroviride* and a bacteria; *P. fluorescens* were applied on soybean seed and their impact on seedling were evaluated under greenhouse conditions. Soybean seed germination, seedling growth and chlorophyll amount were investigated. There are improved seed factors such as germination rate and seedling growth indices including root length, seedling length, and dry weight of root. Also they increased seedlings and leaf area per plant and total chlorophyll amount.

Kumar *et al.* (2014) evaluated two bioagents *viz.*, *T. harzianum* and *T. viride* for their efficacy on colony growth by dual culture plate method showed that the two bio-agents suppressed the colony growth of *F. oxysporum* f. sp. *ciceri* at Uttar Pradesh.

Rai and Basu (2014) studied on seeds of eight varieties of okra *viz.*, Lalu, Arka Anamika, Ramya, Satsira, Lady Luck, Debpusa Jhar, Japani Jhar and Barsha Laxmi were bio-primed with *T. viride* and *P. fluorescens*, and sown in the field. *T. viride* was

improved plant length as 108.21cm and 112.25 cm for variety Arka Anamika, maximum pod length i.e. 19.01 and 19.21 as well as in pod diameter as 16.64 mm and 16.85 mm for Lalu variety in first and second year, respectively.

Jogani and John (2014) revealed three different application methods of *T. harzianum viz.*, seed treatment, soil application and combination with fungicides were against wilt disease of tomato caused by *F. oxysporum* f. sp. *lycopersici*. Among the different seed treatment methods (bio-priming during and after imbibitions, seed coating, wheat bran based (WBB)) and talc based formulations, tested maximum seed germination (78.33%) and disease control (66.53%) were observed in bio-priming during imbibitions. Among the delivery of different formulations (bagasse + *T. harzianum* 10 and 5%, WBB, talc based, farm yard manure based) of *T. harzianum* in soil significantly lowered disease incidence (17.67%) and maximum disease control (77.82%) was recorded in WBB formulation which was statistically at par with bagasse + *T. harzianum* 10% (20.00 and 74.89%).

Materials And Methods

III. MATERIALS AND METHODS

Present research work entitled as **"Evaluation of seed biopriming against tomato (*Solanum lycopersicum L.*) diseases."** *In vitro* experiments were carried out at the Department of Plant Pathology, N. M. College of Agriculture, NAU, Navsari during 2016-2017. The field aspects were carried out at College farm Navsari, various farmers field of Navsari district.

All the materials used and methods pursued for its execution is furnished here under.

3.1 General laboratory procedures

3.1.1 Glassware cleaning

Borosil brand glasswares were used for all the laboratory experiments. The glasswares were subjected to cleaning by dipping them for 24 hours in cleaning solution i.e. chromic acid solution followed by thoroughly washing with distilled water and dried before use.

Preparation of chromic acid solution:

- 60 g potassium dichromate($K_2Cr_2O_7$) dissolved in 300 ml of distilled water
- Cooled at room temperature
- Added 400 ml concentrated H_2SO_4 with constant stirring

3.1.2 Sterilization

The thoroughly cleaned glasswares were sterilized at 180°C temperature for hours in hot air oven. Culture media, water, solutions, cotton, blotter papers and autoclavable plastic bags etc. were autoclaved at 15 lbs. p.s.i for 20 minutes in autoclave.

3.1.3 Preparation of growth medium

Potato dextrose agar (PDA) medium was used for obtaining pure cultures of plant pathogens.

Composition of PDA:

Peeled potatoes : 200 g
Dextrose : 20 g
Agar agar : 20 g
Distilled water : 1000 ml

Preparation of PDA

- The clean and peeled potatoes (200 g) were cut into small pieces.
- These pieces were boiled in 500 ml distilled water and the extract was collected by filtering through muslin cloth.
- Dextrose (20 g) and Agar agar (20 g) were dissolved in another 500 ml of hot water.
- Both the solutions were mixed in one container and the final volume of one liter was maintained by adding distilled water.
- Desired quantity of the medium dispensed into conical flasks, plugged with non-absorbent cotton and finally wrapped with aluminum foil.
- Further, they were sterilized in an autoclave at 121°C temperature (15 lbs. p.s.i.) for 20 minutes.

3.2 Survey

Survey was conducted in *Kharif* and *Rabi* season of 2015-16. Random roving survey farmers' field of Navsari district was

conducted and observations mentioned were recorded by selecting 10 plants randomly by diagonal walk method. The per cent disease incidence (PDI) was calculated by using the formula given by Singh *et al.*, (1987)

$$\text{Per cent disease incidence} = \frac{\text{Number of diseased seedlings}}{\text{Total Number of seedlings}} \times 100$$

3.3 Isolation of the pathogens

Isolation of pathogens subjected from the infected leaves, seedlings and plants which shows typical symptoms. The isolation made from fresh samples. Small pieces of diseased tissues were used.

The pieces were washed with distilled water and surface sterilized with 0.1 per cent mercuric chloride ($HgCl_2$) solution for 30 seconds, followed by three subsequent washing with sterilized distilled water in aseptic condition. The sterilized pieces were transferred aseptically under laminar air flow system on sterilized Petri plates containing 20 ml Potato Dextrose Agar (PDA) medium and incubated at room temperature (27 ± 2 ^{0}C) for 7 days.

3.4 Maintenance of pure cultures

The cultures of obtained pathogens were maintained in refrigerator and sub-cultured periodically at an interval of 30 days during the course of this study.

3.5 Pathogenicity

3.5.1 Damping off

To prove the Kotch' postulate of *Pythium aphanidermatum* the pure culture obtained as mentioned earlier was used. The pure culture of the above isolates was used to prove pathogenicity of *Pythium aphanidermatum* on tomato seedlings.

The pathogenicity test was carried out by soil inoculation methods. Susceptible variety GT-2 was selected for pathogenicity.

To prove the pathogenicity of the fungus by soil inoculation technique, tomato seedling available from Regional Horticulture Station, NAU, Navsari were used for pathogenicity. The seed of GT-2 variety was seeded in 30cm diameter pots. Each pot was filled with sterilized soil with compost and inoculated with pure culture of *Pythium aphanidermatum* (15ml/pot) two days prior to seed sown. Ten seeds were seeded in each pot. The pots were irrigated frequently to maintain the soil humidity. Observation on germination count was recorded, un germinated seeds were consider as pre-emergence mortality while damped-off seedlings after germination were considered as post-emergence mortality.

3.5.2 Early Blight

The fungi isolated from diseased tomato seedling, leaves and plants, were subjected to pathogenicity for the conformation of ability of pathogen. Spores from actively grown colony of the fungi were collected in sterilized distilled water. The spore suspension was adjusted to 10^4 spores/ml. The 45 day old healthy tomato plants grown were challenged with the fungal spores. Ten milliliter of each inoculum was sprayed to three plants using hand atomizer and covered with polypropylene bags with scattered holes (for exchange of air). Plants were watered (sterilized distilled water) every alternate day for a period of another 10 days. After 10 days the infected leaves were collected and the fungi was re-isolated and identified. Un-inoculated plants served as control. (Chanthini *et al.* 2012)

3.5.3 Fusarial Wilt

To prove the pathogenicity of the fungus by seedling root dip inoculation technique was used, the method was followed as described by Nirmaladevi and Srinivas (2012). Earthen pots were filled with sterilized soil autoclaved at 121^0C temperature for 1 hours for three consecutive days. The seeds of Tomato (GT-2) were surface sterilized with 0.1 per cent mercuric chloride solution for 30 seconds followed by three washings with sterilized distilled water. The sterilized seeds were then sown in an earthen pot to get healthy seedlings.

Inoculum was prepared from one week old pure culture of fusarium grown on PDA medium. Inoculum was transferred in 500 ml of distilled water. The roots of seedlings (20 days old) were washed with distilled water and then they were trimmed with a sterilized scissor and submerged in fusarium suspension for about 30 minutes. Such seedlings were utilized for transplanting in autoclaved soil filled in sterilized earthen pots. One pot containing 5 seedlings were kept. The observations on the disease development and incidence of wilt were regularly recorded after inoculation. Seedlings subject to re-isolation for confirming the pathogenicity.

3.6 Identification of pathogens

To identify the pathogens, cultural and morphological characters of the pathogens were recorded in laboratory under binocular microscope and compared with those given in standard literature.

3.6.1 Cultural and morphological characters

3.6.1.1 Cultural characters

- All the pathogens were grown on PDA in Petri dishes.

- Plates were inoculated in the center with mycelial disc taken from stock slants.
- The plates then were incubated at room temperature (27 ± 2^0C).
- Each pathogen was examined properly and regularly.
- Colony diameter and characters were also observed and recorded.

3.6.1.2 Morphological characters

- Mycelia were lifted off the surface of the plate, using a needle, and transferred to a glass microscope slide in each case.
- A drop of distilled water was added to the mycelium and the mycelium was gently tested out using needles.
- A coverslip was placed over the water droplet and slide was examined under a light microscope.
- Recorded the size and shape of conidia and conidiophores.

3.7 Occurrence of seed borne pathogens in popular local cultivars of tomato

Tomato seed samples collected and subjected to plating by using the Blotter method as recommended by Mathur and Kongsdal (2003). The petri-dishes with seeds arranged in seed trays and incubated it for 7 – 10 days at fixed temperature of 22°C. Each seed sample at the end of the incubation examined thoroughly under microscope. Whatsoever pathogens found associated with seeds that carefully examined and identified based on ‘habit

characters'. Slides of the respective pathogens prepared and examined using compound microscope.

3.7.1 Observations

Data on the incidence and severity of pathogenic associated microflora collected by examination of incubated seeds under microscope as recommended by Mathur and Kongsdal (2003).The percent severity of microbewas determined by using the following mathematical formulae.

$$S\% = \frac{Tn}{TSp} \times 100$$

Where, Tn = Total number of seeds infected (infested)

TSp = Total number of seeds examined

S = Severity of fungi

3.8. Evaluation of seed bio-priming against tomato diseases *in vitro*

3.8.1 Experimental details

1. Design : Completely Randomized Deign (CRD)
2. Location : P. G. research laboratory department of Plant Pathology N. M. C. A., N. A. U. Navsari.
3. Method : Standard blotter Paper method
4. Treatment : 12 (Twelve) (Table 3.8.1.1)
5. Repetition : 3 (Three)
6. Soaking duration : 1hour
7. Concentration : 10 g or ml/kg seed

Table 3.8.1.2 Details of treatment

Treatment	Applications
T_1	*Trichoderma viride* applied at imbibition
T_2	*T. viride* applied after imbibition
T_3	*Trichoderma harzianum* applied at imbibition
T_4	*T. harzianum* applied after imbibition
T_5	*Pseudomonas fluorescens* applied at imbibition
T_6	*P. fluorescens* applied after imbibition
T_7	*Bacillus subtilis* applied at imbibition
T_8	*B. subtilis* applied after imbibition
T_9	*Paecilomyces lilacenus* applied at imbibition
T_{10}	*P. lilacenus* applied after imbibition
T_{11}	Hydro priming (Water only)
T_{12}	Control (Without any treatment)

3.8.2 Methodology

Detection of fungi associated with bio-primed tomato seeds at imbibition and after imbibition carried out by taking 100 seeds through standard blotter paper method. These were incubated for 7-10 days in room temperature at 27±2º C. Developing fungal growth on each of the seed observed regularly and identified by microscopic observations. Isolation carried out by inoculating the detected fungus by standard agar plate method **(**Shinde 2016) (Plate I).

3.8.3 Observations

Total number of normal seedling were observed 10 days after sowing and per cent germination was calculated by using following formula (Elwakil and Ghoneem, 1991).

$$\text{Germination Percentage (\%)} = \frac{\text{Total no. of germinated seed}}{\text{Total no. of seed sown}} \times 100$$

$$\text{Per cent infection (\%)} = \frac{\text{No. of infected seeds}}{\text{Total Number of seeds}} \times 100$$

3.9 Evaluation of Seed bio-priming against tomato diseases *in vivo*

3.9.1 Experimental details

1. Design : Randomized Block Design (RBD)
2. Location : Main Sugarcane Research Station, N.A.U., Navsari (For seedling preparation and to maintain seedlings) and College farm N. A. U.

Different bioagent applied at imbibition and applied after imbibition as seed treatment.

Plate I Seed biopriming *in vitro*.

Navsari. (For transplanting to flowering stage)

3. Treatment : 12 (Twelve) (Table 3.8.1.1)
4. Replication : 3 (Three)
5. Soaking duration : 1hour
6. Concentration : 10 g or ml/kg seed

3.9.2 Methodology

For the application of bio-agents was followed as mentioned below suggested by Jogani and John (2014).

3.9.2.1 Biopriming (At imbibition)

Seed bio-priming is treating seed with bio-agents and incubating under warm and moist condition until just prior to radical emergence. 1 Gram of tomato seeds of variety GT-2 were bio-primed with inoculum produced by using talcum powder as carrier. Ten gram of talc formulation of the bio-agents along with 0.1g gum arabic used as adhesive and mixed in 25 ml of water. (Plate II) Pre-treated tomato seed (surface sterilized with 0.1 % $HgCl_2$ for 2-3 min. followed by three washing with sterilized water) soaked in the slurry at room temperature and then transferred on moist filter paper in petri plate.

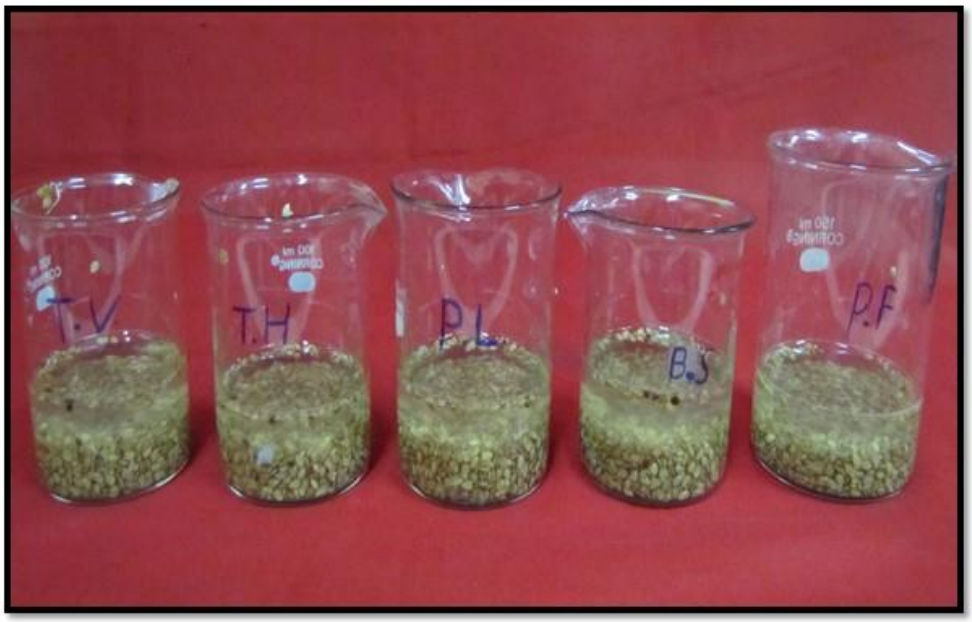
3.9.2.2 Bio-priming (After imbibition)

Seed of tomato imbibed in aerated water (50 g seed per 500 ml water) and then dried at room temperature. Formulation of the bio-agent in talc along with 0.1 g gum arabic dusted on seed (0.01 g per gram of seed) after seed imbibed and dried. (Plate III)

3.9.3 Observations

- Per cent germination of seed

Seed biopriming with bioagent applied at imbibition.



Seed biopriming with bioagent applied after imbibition.

Plate II Bioprimed seeds for *in vivo* experiment.

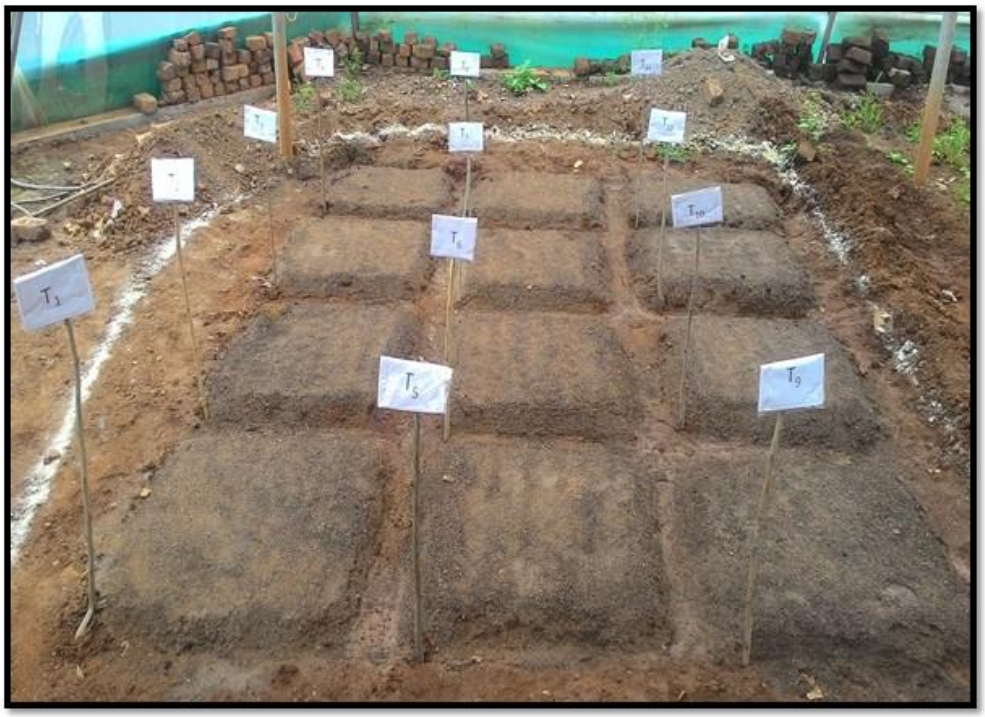


Plate III Preparation of raised bed and tomato (cv. GT-2) seeds were sown at MSRS, NAU, Navsari for seedling preparation.

- Per cent disease incidence
- Seedling height (cm) at 7, 15, & 20 days after germination
- Root & Shoot length (cm) at 7, 15, & 20 days after germination

Results And Discussion

IV. RESULTS AND DISCUSSION

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crop grown in India. Diseases are the major constraint in economic crop production as they impose heavy losses. Totally twenty five diseases have been reported on tomato out of which damping-off, early blight and fusarial wilt were found severe proportion and reported as they causes heavy damage to the crop. Considering the seriousness of the problem and lack of systemic information on these diseases, present investigation was carried out to generate scientific information on various tomato diseases and also to find out efficacy of seed biopriming tomato disease as it may obliging in Integrated Disease Management (IDM) strategy. The experimental results on different aspects *viz*., survey, isolation, pathogenicity, identification, seed borne assessment and evaluation of seed biopriming against tomato disease were discussed here as under.

4.1 Survey

4.1.1 Farmers Field and Regional Horticulture Research Station (RHRS) Survey

Field survey conducted in the Navsari district during *Kharif-Rabi*, 2015-16. Random Roving Survey (RRS) method was adopted and fields of farmers and RHRS farm were selected. Observations were recorded from selected ten plants randomly by diagonal walk method. During the survey three major diseases *viz*., damping-off, early blight, and fusarial wilt were observed. The results obtained were presented below.

4.1.1.1 Damping-off

The damping-off incidence of tomato was recorded ranging from 5.69 to 24.11 per cent in all location and varieties. Mean disease incidence was 16.51%. During the survey, maximum per cent disease incidence (24.11%) was observed at RHRS farm Navsari in GT-2 variety while, the lowest in Gandevi village (5.69%) in Vaishali variety (Table 4.1), (Fig.1).

4.1.1.2 Early Blight

The disease was observed at 60, 75 and 90 days after sowing (DAS) recorded ranging from 23.33 to 56.67 per cent in all locations and varieties. Mean disease intensity was 41.91%. During the survey, maximum per cent disease intensity (56.67%) was observed at Adda in local variety while, the lowest in Gandevi village (23.33%) in Vaishali variety (Table 4.1), (Fig.2).

4.1.1.3 Fusarial Wilt

The disease was observed at 60, 75 and 90 days after sowing (DAS) recorded ranging from 10.00 to 33.33 per cent in all locations and varieties. Mean disease incidence was 20.95%. During the survey, maximum per cent disease incidence (33.33%) was observed at Vansda in Lili badam variety while, the lowest in Limzar village (10.00) in Abhinav variety (Table 4.1), (Fig.3).

The above presented results indicated that all the diseases were found in all the villages of various tehsils of Navsari district. Among the various diseases the early blight was found maximum which was followed by damping-off and fusarial wilt. The obtained results were in conformity with the scientists namely Shinde (2016), Patel (2015), Sharma (2009), Bharat and Sharma (2014), Sahu *et al.* (2013) and El-Mougly *et al.* (2011) as they have proved the various diseases during survey.

Table 4.1 Disease incidence/intensity of tomato diseases at different location of Navsari District (*Kharif-Rabi*, 2015-16)

Variety	Location	Damping off PDI**(%)
Vaishali	Abrama	13.89
Local	Adda	21.05
Lili badam	Vansda	14.11
Vaishali	Gandevi	5.69
GT-2	Dambhar	13.89
Abhinav	Limzar	22.89
GT-2	RHRS Farm	24.11
	Mean	**16.51**

Variety	Location	Early Blight PDI* (%)			
		60 DAS	75 DAS	90 DAS	Average
Vaishali	Abrama	20.00	30.00	50.00	33.33
Local	Adda	40.00	50.00	80.00	56.67
Lili badam	Vansda	20.00	30.00	30.00	26.67
Vaishali	Gandevi	10.00	30.00	30.00	23.33
GT-2	Dambhar	30.00	40.00	60.00	43.33
Abhinav	Limzar	20.00	40.00	50.00	36.67
GT-2	RHRS Farm	30.00	40.00	40.00	36.67
			Mean		**41.91**

Variety	Location	Fusarial Wilt PDI** (%)			
		60 DAS	75 DAS	90 DAS	Average
Vaishali	Abrama	10.00	20.00	20.00	16.66
Local	Adada	30.00	30.00	40.00	33.33
Lili badam	Vansda	10.00	10.00	20.00	13.33
Vaishali	Gandevi	10.00	30.00	30.00	23.33
GT-2	Dambher	20.00	30.00	30.00	26.66
Abhinav	Limzar	10.00	10.00	10.00	10.00
GT-2	RHRS Farm	20.00	20.00	30.00	23.33
				Mean	**20.95**

***** : Per cent Disease Intensity
** : Per cent Disease Incidence
DAS : Day After Sowing

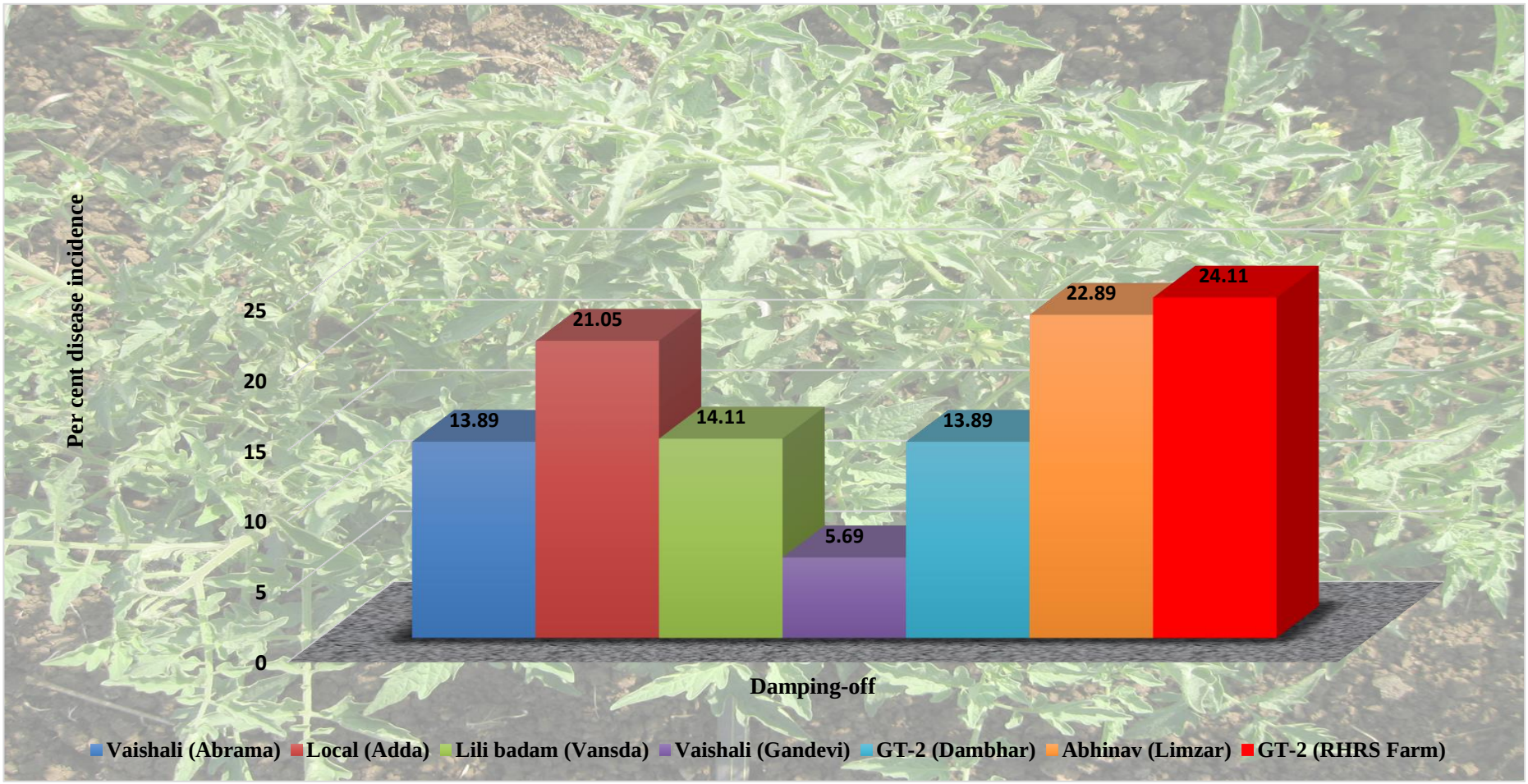


Fig. 1 Disease incidence of tomato Damping-off at different location of Navsari district *(Kharif-Rabi*, 2015-16)

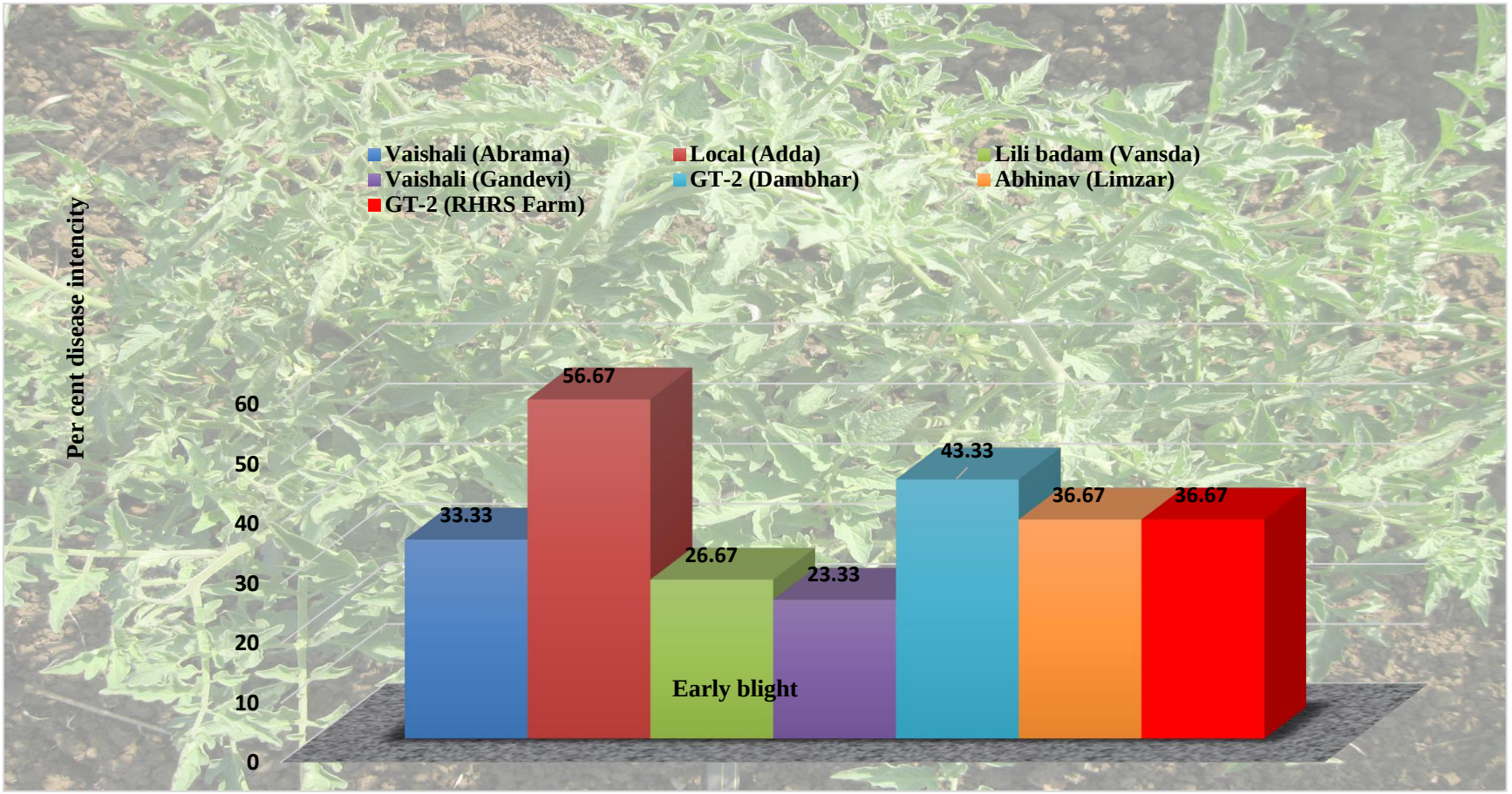


Fig. 2 Disease intensity of tomato early blight at different location of Navsari district *(Kharif-Rabi*, 2015-16)

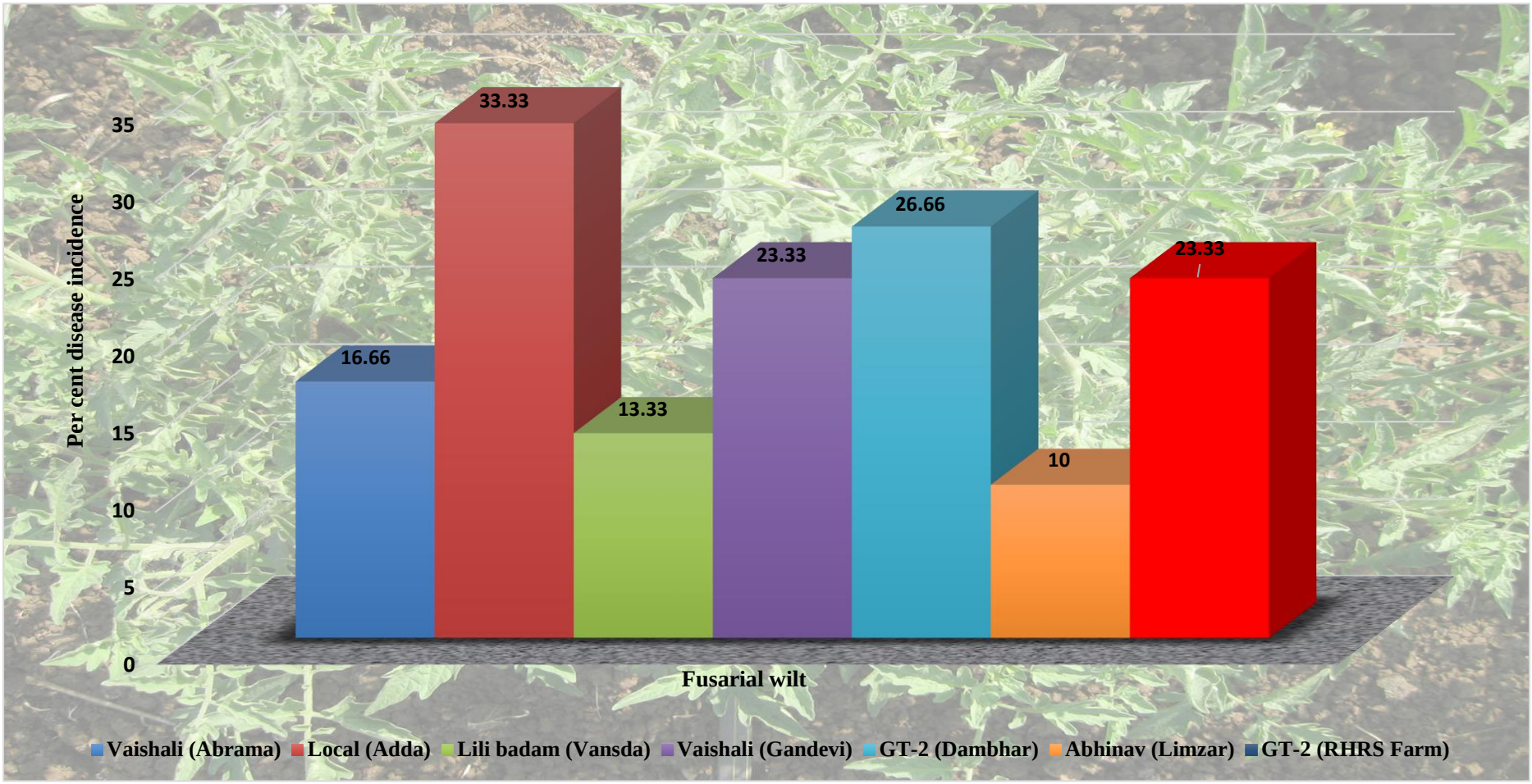


Fig. 3 *D*isease incidence of tomato fusarial wilt at different location of Navsari district *(Kharif-Rabi,* 2015-16)

4.2 Collection and isolation of diseased samples

The samples of infected tomato plants were collected during the year 2015 from the field and RHRS (Regional Horticulture Research Station) farm, Navsari. Diseased samples of seedling, leaves and whole plants were observed and collected in polyethylene bags and properly labeled and brought to the laboratory which was primarily subjected to microscopic examination and isolation was done by using tissue isolation technique, which yielded the pure culture of pathogens. Which was further purified by the hyphal tip isolation technique and maintained on Potato Dextrose Agar (PDA) and incubated at room temperature (27 ± 2^0C) for 7 days.

4.3 Symptomatology

4.3.1 Damping-off

4.3.1.1 Pre-emergence

The young seedlings are killed before they reach the surface of soil. Infect they may be killed even before the hypocotyls has broken the seed coat. The redical and plumle, when they come out the seed, undergo complete rotting.

4.3.1.2 Post-emergence

Toppling over of infected seedling any time after they emerge from the soil until the stem has hardened sufficiently to resist invasion. Infection usually occurs at or ground level. The infected tissue appears soft and water soaked.

4.3.2 Early Blight

The typical early blight symptoms observed in nature dark brown to black spots on leaves and stems with concentric spots on them. The spots are readily recognized by zonation or concentric ring. In the later stage, the fruits are also affected showing sunken,

large, dark black or brown leathery area on green and ripe fruits. The mature fruit is less affected.

4.3.3 Fusarial Wilt

In fusarial wilt yellowing and drooping of lower leaves as well as complete drying of diseased plants when the roots of such infected plants were split opened and examined; the brownish black discoloration of vascular system was observed.

Similar results as found by Chauhan (1979), Kalloo (1985), Rangaswami and Mahadevan (2002), Gevens (2013), Ravishanker (2006), Chopada *et al*. (2014) and Patel (2015).

4.4 Pathogenicity

4.4.1 Damping-off

The result presented in Table 4.2 revealed that inoculation of *Pythium aphanidermatum* in pots by soil inoculation method recorded 24 per cent pre-emergence mortality, while uninoculated pots recorded 5 per cent mortality. Similarly post-emergence mortality in inoculated pots was 80 per cent and in uninoculated it was 10.52 per cent. Germination of the seed started after 7th days of sowing and ungerminated seeds were considered as pre-emergence mortality. Ungerminated seeds were soft and rotted. After 16th day germinated seedlings initially produced small water soaked lesion on collar regions near the soil line and lesion enlarged after three days which covered the newly emerged leaves resulting in gridling and rotting of leaves and stem. Damped-off seedlings were considered as post-emergence mortality.

4.4.2 Early Blight

The results presented in Table 4.2 indicated that the isolated pathogen was capable of causing cent per cent infection by spore spray inoculation method. After 15 to 20 days of inoculation, infected leaves revealed the typical symptoms of early blight. The pathogen was re-isolated from the same infected leaves. No symptoms were observed on Un-inoculated plants.

4.4.3 Fusarial Wilt

The results presented in Table 4.2 indicated that the isolated pathogen was capable of causing cent per cent infection by seedling root dip inoculation method. Inoculated plants showing sudden death of seedling which were similar to damping-off type symptoms and subjected to re- isolation of the same pathogen. Un-inoculated did not develop any symptoms of disease and started normal germination.

The pathogenicity test of all isolates were carried out by three different inoculation methods. Soil inoculation, spore spray method and seedling root dip inoculation, which confirmed the pathogenic nature of isolates, and producing typical symptoms on leaves, seedlings and stems. The symptoms were successfully produced in all inoculation methods.

Similar to those described by Ravishankar (2006), Chopada *et al.* (2014), Chanthini *et al.* (2012), Nirmaladevi and Srinivas (2012), Patel (2015) and Shinde(2016).

4.5 Identification of the pathogens

After the purification of the fungus as described in material and methods, cultural and morphological characters of the fungus on potato dextrose agar were studied for the purpose of identification and compared with those described in the literature.

Table 4.2 Pathogenicity test of pathogen by using standard inoculation method.

Sr. No.	Disease name	Inoculation method	No. of seed plants/seedling used	Per cent infection
1.	Pre-emergence damping-off	Soil inoculation	20.00	25.00
	Post-emergence damping-off	Soil inoculation	20.00	80.00
		Control	20.00	10.52
2.	Early blight	Spore spray method	5.00	100.00
		Control	5.00	0.00
3.	Fusarial wilt	Seedling root dip inoculation	5.00	100.00
		Control	5.00	0.00

4.5.1 Cultural and morphological characteristics of pathogens

4.5.1.1 *Pythium aphanidermatum*

The isolate produced scanty, visible growth surrounding mycelia bits which recorded 2.2 cm radial growth after 12 hours. After 24 hours the redial growth measured 4.6 cm and growth was dense at the centre and scanty towards the periphery. Within 36 hours white cottony growth covered the entire 9 cm Petri plate at room temperature. When petri plate lead was open the whole mycelium stick to media and settle down. Growth of *Pythium aphanidermatum* on potato agar medium was depited in plate IV

Microscopic observation revealed that the fungal hyphae are hyaline, coenocytic with a diameter of 18.17 ± 1.87 µm and branched. The oospore were about 31.62 µm in diameter and produced after seven days of inoculation. On the basis of morphological and cultural character of the pure culture of the fungus, it was tentatively identified as *Pythium sp*. The similar result was found by Mehrotra and Agrawal (2004), Priya (2014) and Shende (2016).

4.5.1.2 *Alternaria solani*

Colonies grew fairly quick on PDA covering whole Petri plate within 10-15 days. Initially the fungus produce greyish mycelial growth with whitish margin which turn in to greenish black pigmentation with a radial growth of 89.00 mm. Septate and light brown in colour. Conidial were light brown to dark brown, muriform with 0-2 transverse septa and 2-7 longitudinal septa, variable in size (85-105 mm) and shape, (Plate V).

Similar result was found by Chanthini *et al*. (2012) and Alhussaen (2012). Conformation was done by comparing its

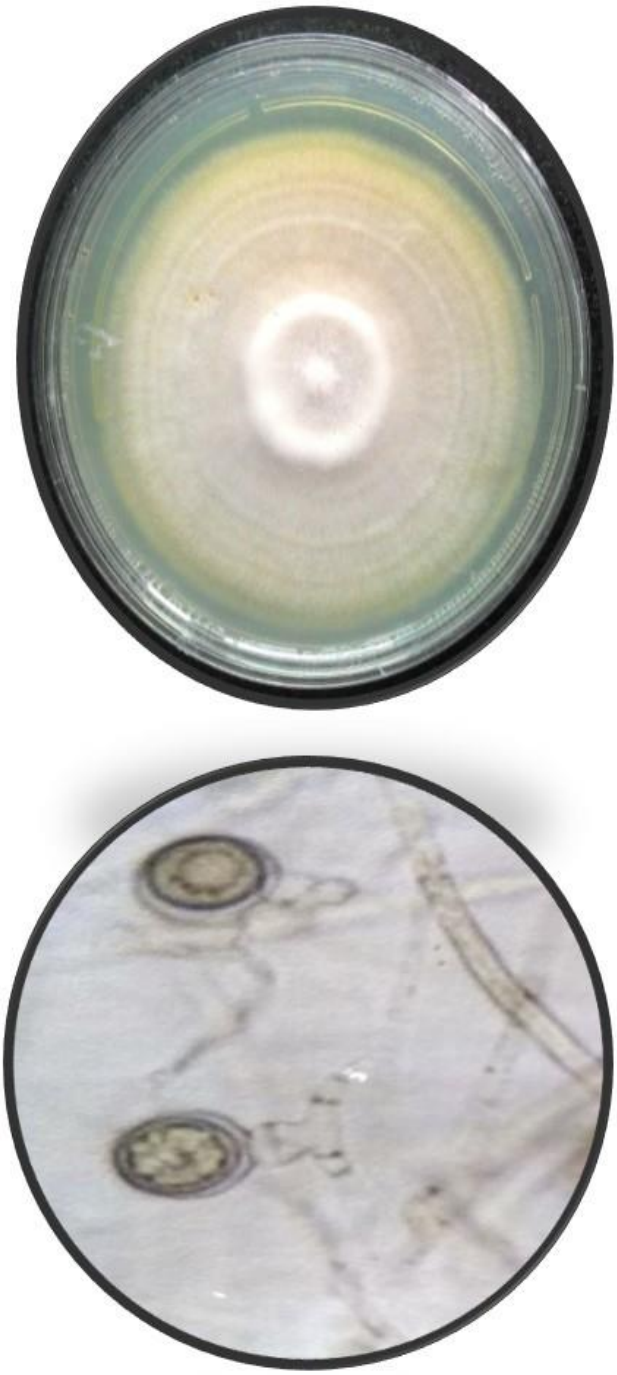


Plate IV Pure culture and microphotograph of *P. aphanidermatum.*

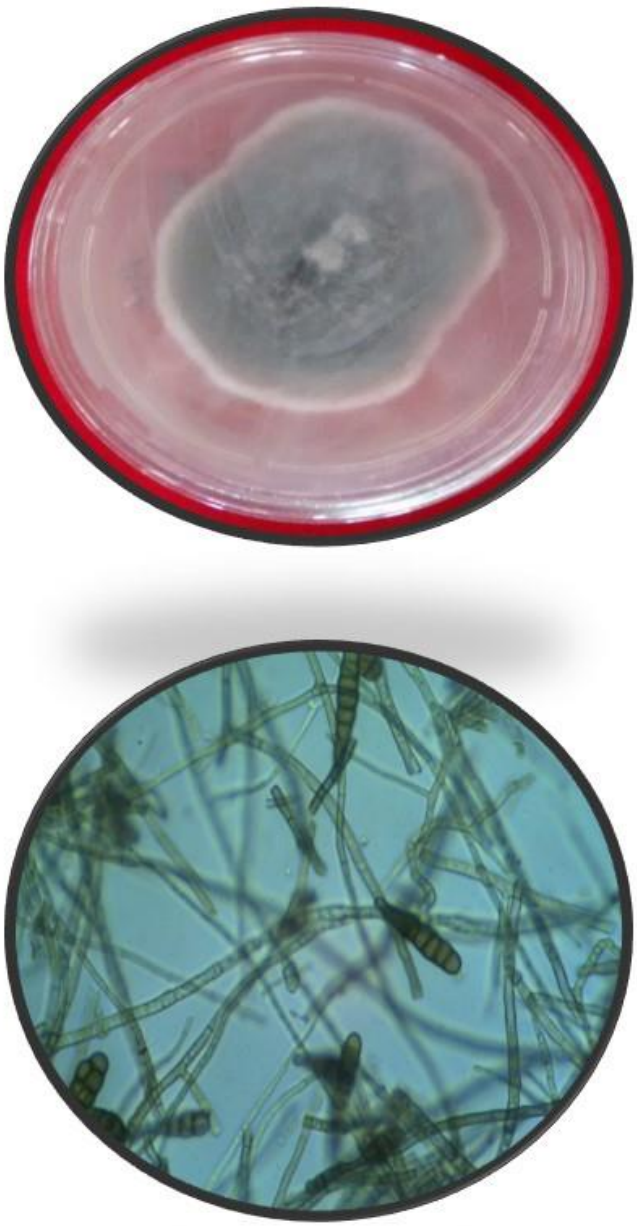


Plate V Pure culture and microphotograph of *A. solani* .

cultural and morphological characteristics (Spore shape and color, hyphae) with published in literature.

4.5.1.3 *Fusarium oxysporum* f. sp. *lycopersici*

Colonies grew fairly fast on PDA covering whole Petri plate within 9-10 days at temperature 27 ± 2°C. Initially the fungus produced dirty white cottony mycelium, which turned into occur yellowish to dull pink colour. Chlamydospores formed when culture becomes old gradually. In all the cases, mycelium was delicate dirty white to pink, mostly with purple tinge and are sparse to abundant. Macro conidia were straight, spindle as well as sickle shaped. The size of macro conidia ranged from 15.46-21.8 x 4.91-5.45 µm. The micro conidia were hyaline, round to oval in shape. The size of micro conidia ranged from 3.57-10.16 x 2.68-4.46 µm, (Plate VI).

Similar result was found by Ignjatov *et al*. (2012), Joshi *et al*. (2013), Chopada *et al.* (2014) and Patel (2015).

4.6 Occurrence of seed borne pathogens in popular local cultivars of tomato

For the study of seed mycoflora in tomato cultivar GT-2 using standard blotter paper method and PDA method, the 400 seeds of tomato were kept in Petri plate in both methods. The results on the occurrence of seed borne pathogens were presented in Table 4.3 and Table 4.4

4.6.1 Blotter paper method

In sterilized seeds the highest incidence of *Rhizopus* spp. (15%) was recorded, the next least incidence is recorded in order of *A. niger* (5.75%), *A. flavus* (4.50%), *F. oxysporum* (2.0%) and *A. solani* (0.0%). (Fig. 4) While in unsterilized seeds highest incidence was recorded by *Rhizopus* spp. (28.5%), the next least

Plate VI Pure culture and microphotograph of *F. oxysporum* f. sp. *lycopersici.*

incidence is recorded in order of *A. niger* (20.0%), *A. flavus* (13.75%), *A. solani* (7.0%) and *F. oxysporum* (5.5%) (Table 4.3).

4.6.2 Potato Dextrose Agar (PDA) plate method

Generally there was a higher incidence of fungi in unsterilized seeds. Highest incidence of *Rhizopus* sp. was recorded in unsterilized seeds. *A. niger* and *A. flavus.* were found in more samples by the PDA method in both sterilized and unsterilized samples (Table 4.4)(Fig. 4).

In sterilized seeds of cultivar GT-2, the incidence of *Rhizopus* sp., *A. niger*, *A. flavus*, *F. oxysporum* and *A. solani* was not observed. While in unsterilized seeds highest incidence of *Rhizopus* spp. (36.50%) was observed, the next least incidence is recorded in order of *A. niger* (27.25%), *A. flavus* (20.25%), *F. oxysporum* (5.50%) and *A. solani* (3.75%).

Similar results described by Patel (2015) the incidence of *Rhizopus* spp. and *Aspergillus* spp. were found on tomato cultivar GT-2 seeds. Similar were described by Sowley and Kodua (2012) and Zakaria (2010).

4.7 Evaluation of seed bio-priming against tomato diseases *in vitro* and *in vivo*

4.7.1 *In vitro*

In vitro study conducted to check out the efficacy of seed biopriming on seed germination and per cent infection by controlling the seed mycoflora was carried out by Standard Blotter Paper method. Result revealed that per cent germination and per cent infection were significantly increased in all the treatment tested over control.

Table 4.3 Severity of fungi in tomato seeds by the Blotter paper method (n=400)

Sr. No.	Name of fungi	Severity (%)	
		Sterilized seed	Unsterilized seed
1	*Alternaria solani*	00.00	7.00
2	*Fusarium oxysporum*	02.00	5.50
3	*Rhizopus*	15.00	28.50
4	*Aspergillus niger*	05.75	20.00
5	*Aspergillus flavus*	04.50	13.75

Table 4.4 Severity of fungi in tomato seeds by the PDA method (n=400)

Sr. No.	Name of fungi	Severity (%)	
		Sterilized seed	Unsterilized seed
1	*Alternaria solani*	0.00	03.75
2	*Fusarium oxysporum*	0.00	50.50
3	*Rhizopus*	0.00	36.50
4	*Aspergillus niger*	0.00	27.25
5	*Aspergillus flavus*	0.00	20.25

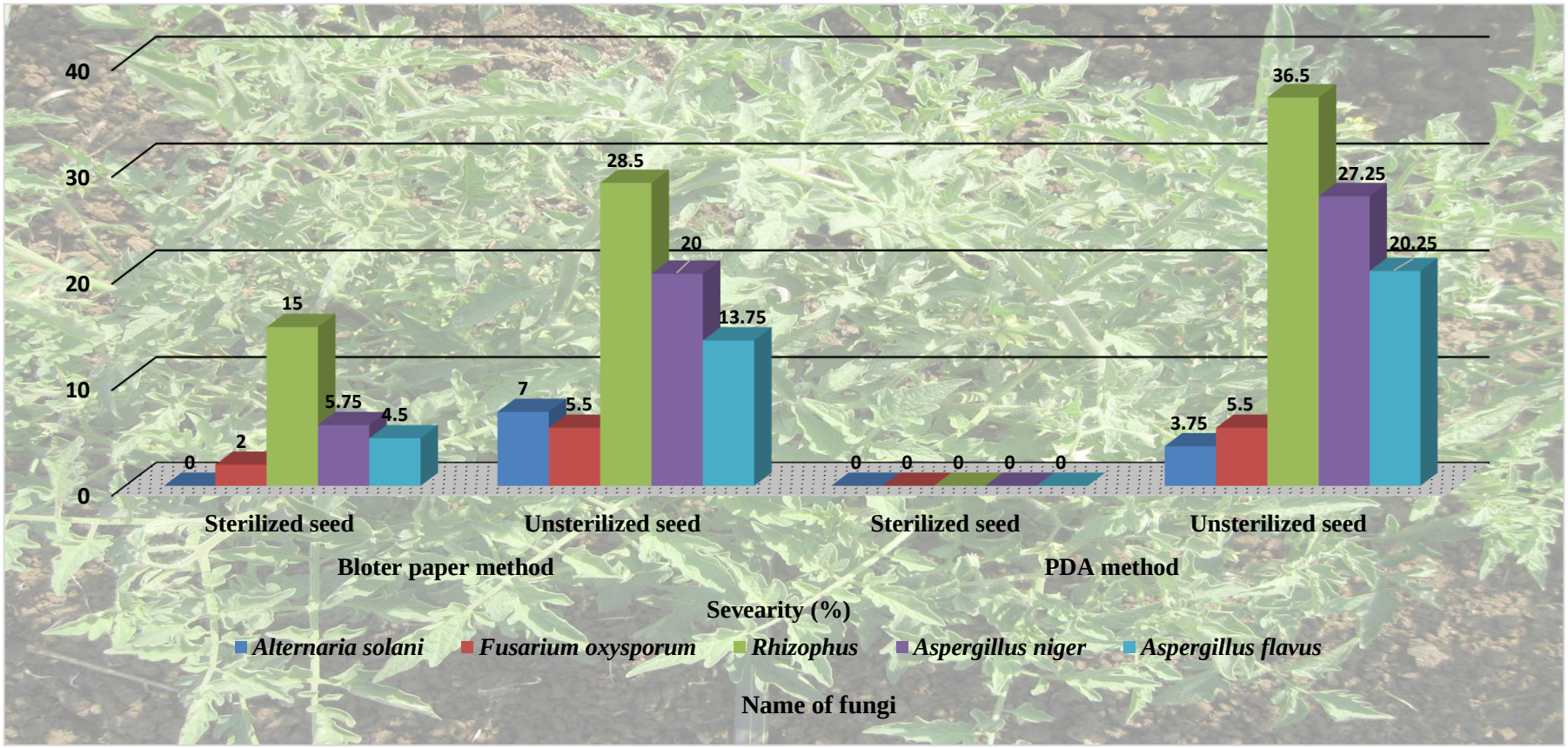


Fig. 4 Severity of fungi in tomato seeds by the Blotter paper method and PDA methods (n=400).

4.7.1.1 Germination (%)

Germination was significantly higher in the seed biopriming with *T. harzianum* (94.00%) than the other treatment tested but it was statistically at par with *T. viride* applied at imbibition (92.33%) and *P. fluorescens* applied at imbibition (91.67%). Next best in order of merit was *P. fluorescens* applied after imbibition (88.00%) *T. harzianum* applied after imbibition (86.00%), *T. viride* applied after imbibition (86.00%), *Bacillus subtilis* applied at imbibition (77.33%), *Paecilomyces lilacenus* applied at imbibition (75.33%), *B. subtilis* applied after imbibition (75.00%), *P. lilacenus* applied after imbibition (71.00%) and Hydro priming (Water only) (64.0%). Lowest seed germination was observed in control (52.33%) (Table 4.5).

4.7.1.2 Infection of seed

Infection of seed was significantly lower in the seed biopriming with *T. harzianum* (6.28%) than the other treatment tested but it was statistically at par with *T. viride* applied at imbibition (7.58%). Next best in order of merit was *P. fluorescens* applied at imbibition (8.30%), *P. fluorescens* applied after imbibition (12.0%) *T. harzianum* applied after imbibition (13.95%), *T. viride* applied after imbibition (13.90%), *Bacillus subtilis* applied at imbibition (22.64%), *Paecilomyces lilacenus* applied at imbibition (24.70%), *B. subtilis* applied after imbibition (25.00%), *P. liliacenus* applied after imbibition (29.08%) and Hydro priming (Water only) (36.11%). Lowest seed germination was observed in control (47.67%) (Table 4.5).

4.7.2 *In vivo*

A field study for management of tomato disease through biopriming was carried out during 2016 at college farm, N.A.U.,

Table 4.5 Effect of seed biopriming on tomato seed germination and per cent infection *in vitro*

Sr.No.	Treatment	Per cent germination	Per cent infection
T1	*Trichoderma viride* applied at imbibition	92.33	7.58
T2	*T. viride* applied after imbibition	86.00	13.90
T3	*Trichoderma harzianum* applied at imbibition	94.00	6.28
T4	*T. harzianum* applied after imbibition	86.00	13.95
T5	*Pseudomonas fluorescens* applied at imbibition	91.67	8.30
T6	*P. fluorescens* applied after imbibition	88.00	12.00
T7	*Bacillus subtilis* applied at imbibition	77.33	22.64
T8	*B. subtilis* applied after imbibition	75.00	25.00
T9	*Paecilomyces lilacenus* applied at imbibition	75.33	24.70
T10	*P. liliacenus* applied after imbibition	71.00	29.08
T11	Hydro priming (Water only)	64.00	36.11
T12	Control (Without any treatment)	52.33	47.67
	S.Em ± CD at 5 % CV%	1.40 4.09 3.05	0.57 1.66 4.78

Navsari. *In vivo* study conducted to check out the efficacy of seed biopriming on seed germination, Shoot length, Root length and seedling height. Result revealed (Table 4.6) that per cent germination shoot length, root length and seedling height were significantly increased in all the treatment tested over control. (Plate VII)

4.7.2.1 Germination (%)

Per cent germination was significantly higher in the seed biopriming with *P. fluorescens* applied at imbibition (71.82%) than the other treatment tested but it was statistically at par with *T. harzianum* applied at imbibition (70.39%), *T. viride* applied at imbibition (68.32%) and *T. harzianum* applied after imbibition (67.16%). Next best in order of merit was *P. fluorescens* applied after imbibition (63.83%), *Bacillus subtilis* applied at imbibition (62.28%), *T. viride* applied after imbibition (62.04%), *Paecilomyces lilacenus* applied at imbibition (56.82%), *B. subtilis* applied after imbibition (55.65%), *P. liliacenus* applied after imbibition (52.30%) and Hydro priming (Water only) (50.98%). Lower seed germination was observed in control (48.07%) (Table 4.6).

4.7.2.2 Shoot length

Shoot length was significantly longer in all the treatment as compared to the control. Among these, significantly longer shoot was recorded in seed treated with biopriming with *P. fluorescens* applied at imbibition (6.30 cm) than the other treatment tested but it was statistically at par with *T. harzianum* applied at imbibition (05.77 cm), *T. viride* applied at imbibition (02.74 cm) and *T. harzianum* applied after imbibition (05.24 cm). Next best in order of merit was *P. fluorescens* applied after imbibition (04.96 cm), *T.*

General view at Nursery condition

Bioprimed seeds

Control seed

Plate VII Effect of seed biopriming in Nursery condition.

viride applied after imbibition (04.54 cm), *Bacillus subtilis* applied at imbibition (05.07 cm), *Paecilomyces lilacenus* applied at imbibition (04.84 cm), *B. subtilis* applied after imbibition (04.20 cm), *P. liliacenus* applied after imbibition (03.63cm) and Hydro priming (Water only) (02.93 cm). Lower seed germination was observed in control (02.60 cm). (Table 4.6) (Fig. 5)

4.7.2.3 Root length

Root length was significantly longer in all the treatment as compared to the control. Among these, significantly higher root was recorded in seed treated with biopriming with *P. fluorescens* applied at imbibition (09.72 cm) than the other treatment tested but it was statistically at par with *T. harzianum* applied at imbibition (09.41 cm), *T. viride* applied at imbibition (09.09 cm) and *P. fluorescens* applied after imbibition (08.31). Next best in order of merit was *Bacillus subtilis* applied at imbibition (07.74 cm), *Paecilomyces lilacenus* applied at imbibition (07.37 cm), *T. harzianum* applied after imbibition (06.73 cm), *B. subtilis* applied after imbibition (06.48 cm), *T. viride* applied after imbibition (06.44 cm), Hydro priming (Water only) (05.33 cm) and *P. lilacenus* applied after imbibition (04.00 cm). Lower seed germination was observed in control (02.84 cm) (Table 4.6).

4.7.2.4 Seedling height

Seedling height was significantly longer in all the treatment as compared to the control. Among these, significantly longer seedling was recorded in seed treated with biopriming with *P. fluorescens* applied at imbibition (15.66 cm) than the other treatment tested but it was statistically at par with *T. harzianum* applied at imbibition (15.36 cm), *T. viride* applied at imbibition (14.71 cm) and *T. harzianum* applied after imbibition (13.28 cm).

Table 4.6 Effect of seed biopriming on tomato seed germination, Shoot length, Root length and Seedling height *in vivo*

Sr. No.	Treatment	Germination (%)	Shoot length (cm)	Root length (cm)	Seedling height(cm)
T1	*Trichoderma viride* applied at imbibition	68.32* (86.33)**	13.86* (5.74)**	17.55* (9.09)**	22.52* (14.71)**
T2	*T. viride* applied after imbibition	62.04 (78.00)	12.61 (4.54)	14.69 (6.44)	20.01 (11.73)
T3	*Trichoderma harzianum* applied at imbibition	70.39 (88.60)	13.91 (5.77)	17.85 (9.41)	23.06 (15.36)
T4	*T. harzianum* applied after imbibition	67.16 (84.67)	13.23 (5.24)	15.03 (6.73)	21.36 (13.28)
T5	*Pseudomonas fluorescens* applied at imbibition	71.82 (90.00)	14.55 (6.30)	18.17 (9.72)	23.29 (15.66)
T6	*P. fluorescens* applied after imbibition	63.86 (79.40)	12.69 (4.96)	16.72 (8.31)	21.28 (13.20)
T7	*Bacillus subtilis* applied at imbibition	62.28 (78.03)	12.23 (5.07)	16.14 (7.74)	20.83 (12.65)
T8	*B. subtilis* applied after imbibition	55.65 (68.00)	11.84 (4.20)	14.74 (6.48)	19.42 (11.06)
T9	*Paecilomyces lilacenus* applied at imbibition	56.82 (70.00)	11.92 (4.84)	15.66 (7.37)	19.18 (10.80)
T10	*P. liliacenus* applied after imbibition	52.30 (62.33)	10.98 (3.63)	11.53 (4.00)	18.73 (10.34)
T11	Hydro priming (Water only)	50.98 (60.33)	9.87 (2.93)	13.27 (5.33)	18.69 (10.28)
T12	Control (Without any treatment)	48.07 (55.33)	8.71 (2.60)	9.60 (2.84)	17.29 (8.85)
	S.Em ±	2.57	0.61	0.66	0.67
	CD at 5%	7.54	1.79	1.94	1.96
	CV%	7.32	8.69	7.60	5.65

*Figures outside partenthesis are arcsine transformed values
** Figure indicate original values

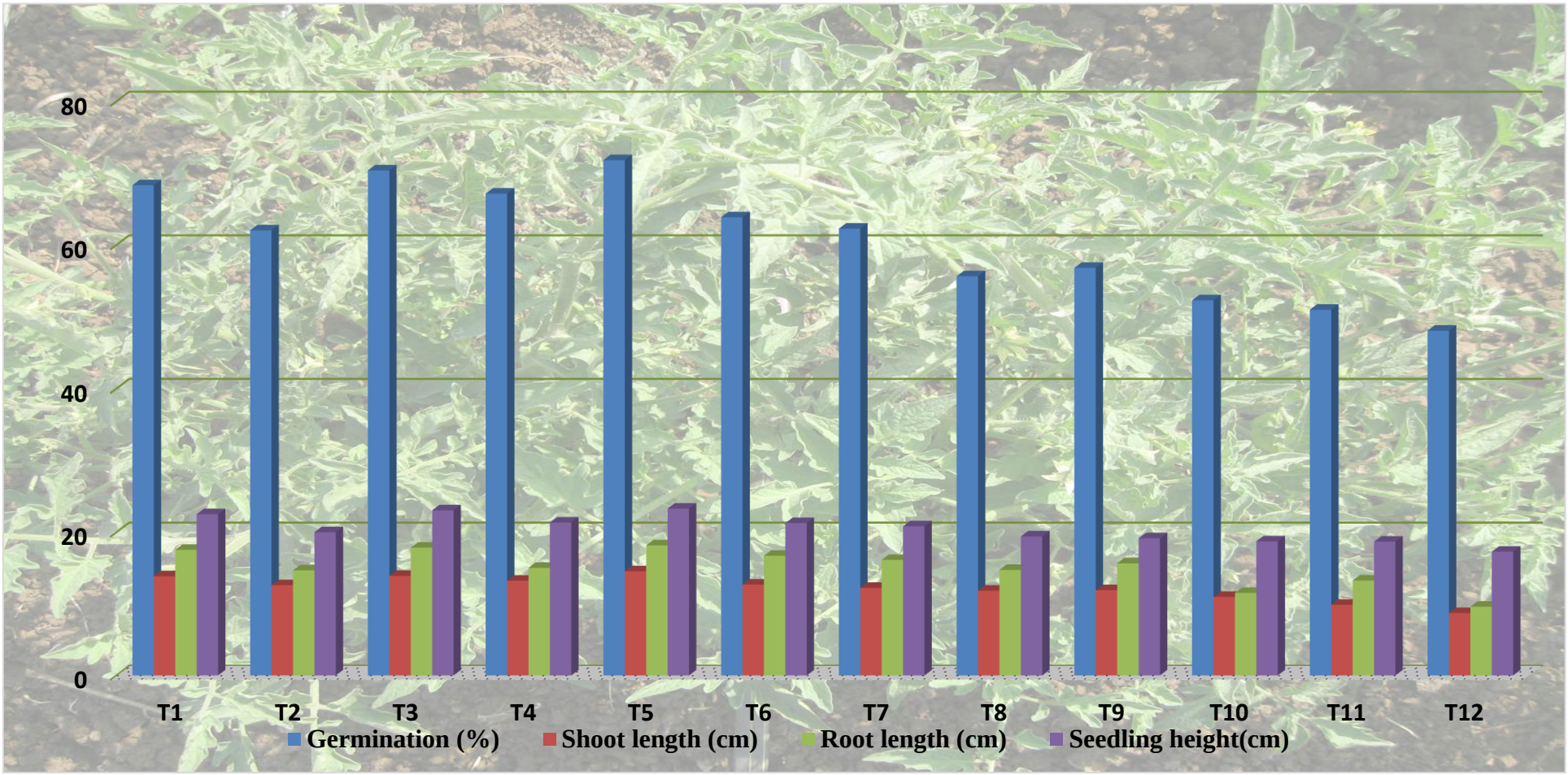


Fig. 5 Effect of seed biopriming on tomato seed germination, Shoot length, Root length and Seedling height *in vivo*.

Next best in order of merit was *P. fluorescens* applied after imbibition (13.20 cm), Bacillus subtilis applied at imbibition (12.65 cm), *T. viride* applied after imbibition (11.73 cm), *B. subtilis* applied after imbibition (11.06 cm), *P. lilacenus* applied at imbibition (10.80 cm), *P. liliacenus* applied after imbibition (10.34 cm) and Hydro priming (Water only) (10.28 cm). Lower seed germination was observed in control (08.85 cm) (Table 4.6).

4.7.2.2 Effect of seed biopriming on tomato diseases *in vivo*

4.7.2.2.1 Damping-off

The disease incidence of damping-off was significantly reduced in all the treatments over control. It was significantly lower in seed biopriming with *P. fluorescens* applied at imbibition (18.44%) (Table 4.7) which was statistically at par with *T. harzianum* applied at imbibition (21.69%). Next best in order to merit was *T. viride* applied at imbibition (22.84%), *T. harzianum* applied after imbibition (23.34%), *P. fluorescens* applied after imbibition (27.68%), *T. viride* applied after imbibition (28.38%), *Bacillus subtilis* applied at imbibition (28.81%), *P. lilacenus* applied at imbibition (32.75%), *Bacillus subtilis* applied after (33.87%) *P. lilacenus* applied after imbibition (34.29%) and Hydro priming (water only) (37.96%). Higher disease incidence was observed in control (42.29%) (Table 4.7).

4.7.2.2.2 Early blight

The disease intensity of early blight was significantly reduced in all the treatments over control. It was significantly lower in seed biopriming with *T. harzianum* applied at imbibition (15.67%) (Table 4.7) which was statistically at par with *T. viride* applied at imbibition (18.11%). Next best in order to merit was *T. viride* applied after imbibition (20.20%), *T. harzianum* applied

after imbibition (20.56%), *P. fluorescens* applied at imbibition (20.69%), *Bacillus subtilis* applied at imbibition (21.30%), *P. fluorescens* applied after imbibition (21.33%), *Paecilomyces lilacenus* applied at imbibition (21.83%), *Bacillus subtilis* applied after imbibition (22.76%), *P. lilacenus* applied after imbibition (23.45%) and Hydro priming (water only) (23.69%). Higher per cent disease incidence was observed in control (26.93%) (Plate VIII) (Table 4.7).

4.7.2.2.3 Fusarial wilt

. Per cent disease incidence of fusarial wilt was significantly reduced in all the treatments over control. It was significantly lower in seed biopriming with *T. harzianum* applied at imbibition (14.73%) (Table 4.7) which was statistically at par with *T. viride* applied at imbibition (17.30%). Next best in order to merit was *P. fluorescens* applied at imbibition (18.24%), *T. harzianum* applied after imbibition (18.62%), *T. viride* applied after imbibition (18.69%), *P. fluorescens* applied after imbibition (19.15%), *Bacillus subtilis* applied at imbibition (20.02%), *Bacillus subtilis* applied after imbibition (20.47%), *P. lilacenus* applied at imbibition (20.50%), *P. liliacenus* applied after imbibition (21.44%) and Hydro priming (water only) (21.82%). Higher per cent disease incidence was observed in control (26.54%). (Table 4.7) (Fig. 5)

First report of biopriming was reported in south Gujarat region on green gram by Deshmukh, during 2012, he found that seed biopriming with *T.harzianum, T, viride* or *P. aeruginosa* @10 g talc base formulation/kg seeds proved very effective not to better seed germination, seedling vigour, plant growth, root growth, root nodules, yield parameters and yield but also to manage

Table: 4.7 Effect of seed biopriming on tomato diseases *in vivo*

Sr. No.	Treatment	Damping-off PDI (%)	Early blight PDI (%)	Fusaruim wilt PDI (%)
T1	*Trichoderma viride* applied at imbibition	22.84 (15.09)	18.11 (9.67)	17.30 (8.85)
T2	*T. viride* applied after imbibition	28.38 (22.85)	20.20 (15.00)	18.69 (10.27)
T3	*Trichoderma harzianum* applied at imbibition	21.69 (13.67)	15.67 (7.30)	14.73 (6.47)
T4	*T. harzianum* applied after imbibition	23.34 (15.70)	20.56 (15.87)	18.62 (10.20)
T5	*Pseudomonas fluorescens* applied at imbibition	18.44 (10.07)	20.69 (12.50)	18.24 (9.80)
T6	*P. fluorescens* applied after imbibition	27.68 (21.67)	21.33 (13.23)	19.15 (10.77)
T7	*Bacillus subtilis* applied at imbibition	28.81 (23.25)	21.30 (13.20)	20.02 (11.72)
T8	*B. subtilis* applied after imbibition	33.87 (31.67)	22.76 (12.13)	20.47 (12.23)
T9	*Paecilomyces lilacenus* applied at imbibition	32.75 (29.30)	21.83 (13.83)	20.50 (12.27)
T10	*P. liliacenus* applied after imbibition	34.29 (31.77)	23.45 (12.33)	21.44 (13.37)
T11	Hydro priming (Water only)	37.96 (37.83)	23.69 (14.47)	21.82 (13.84)
T12	Control (Without any treatment)	42.29 (45.30)	26.93 (20.83)	26.54 (20.33)
	S.Em ±	1.30	1.36	0.94
	CD at 5%	3.82	4.00	2.75
	CV%	7.68	11.05	8.20

*Figures outside partenthesis are arcsine transformed values
** Figure indicate original values

Plate VIII Field view of experiment.

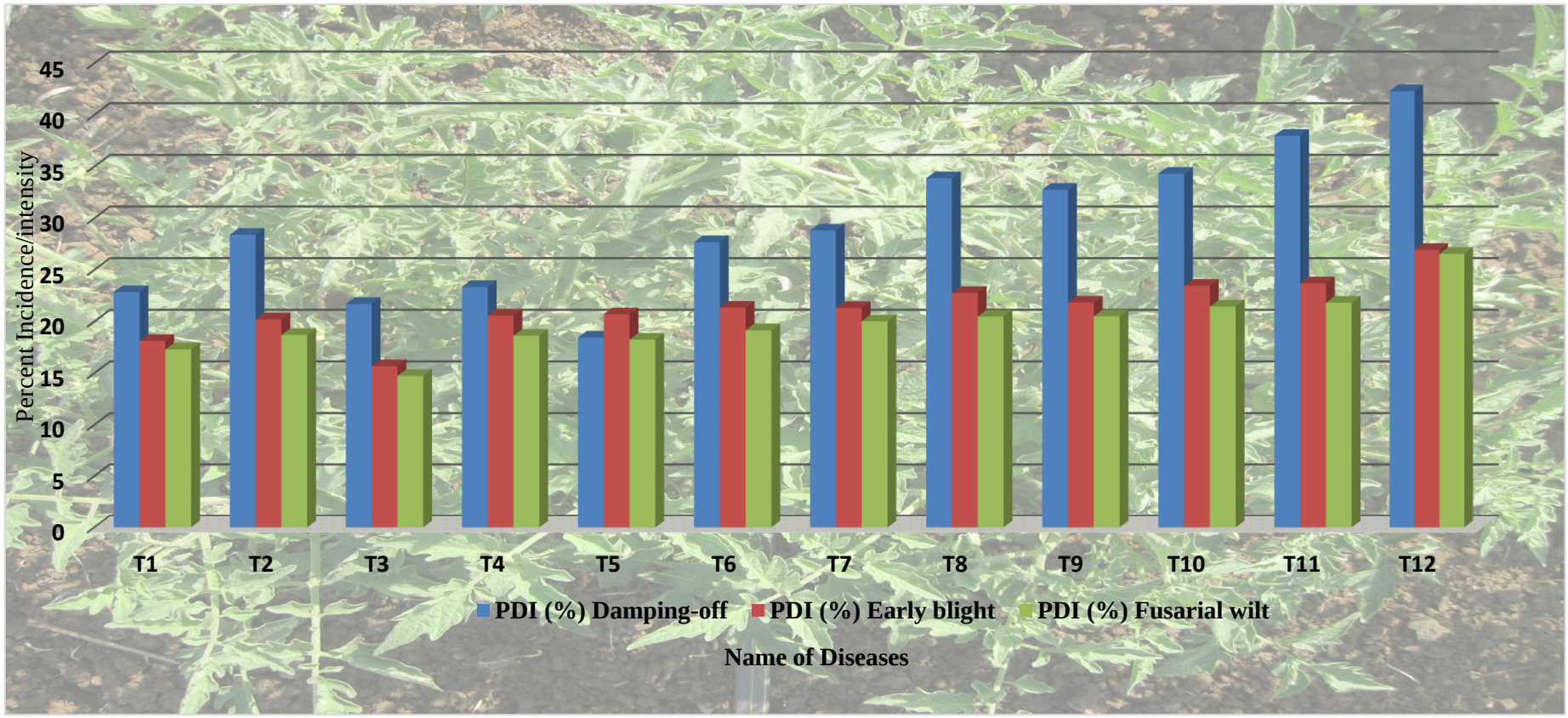
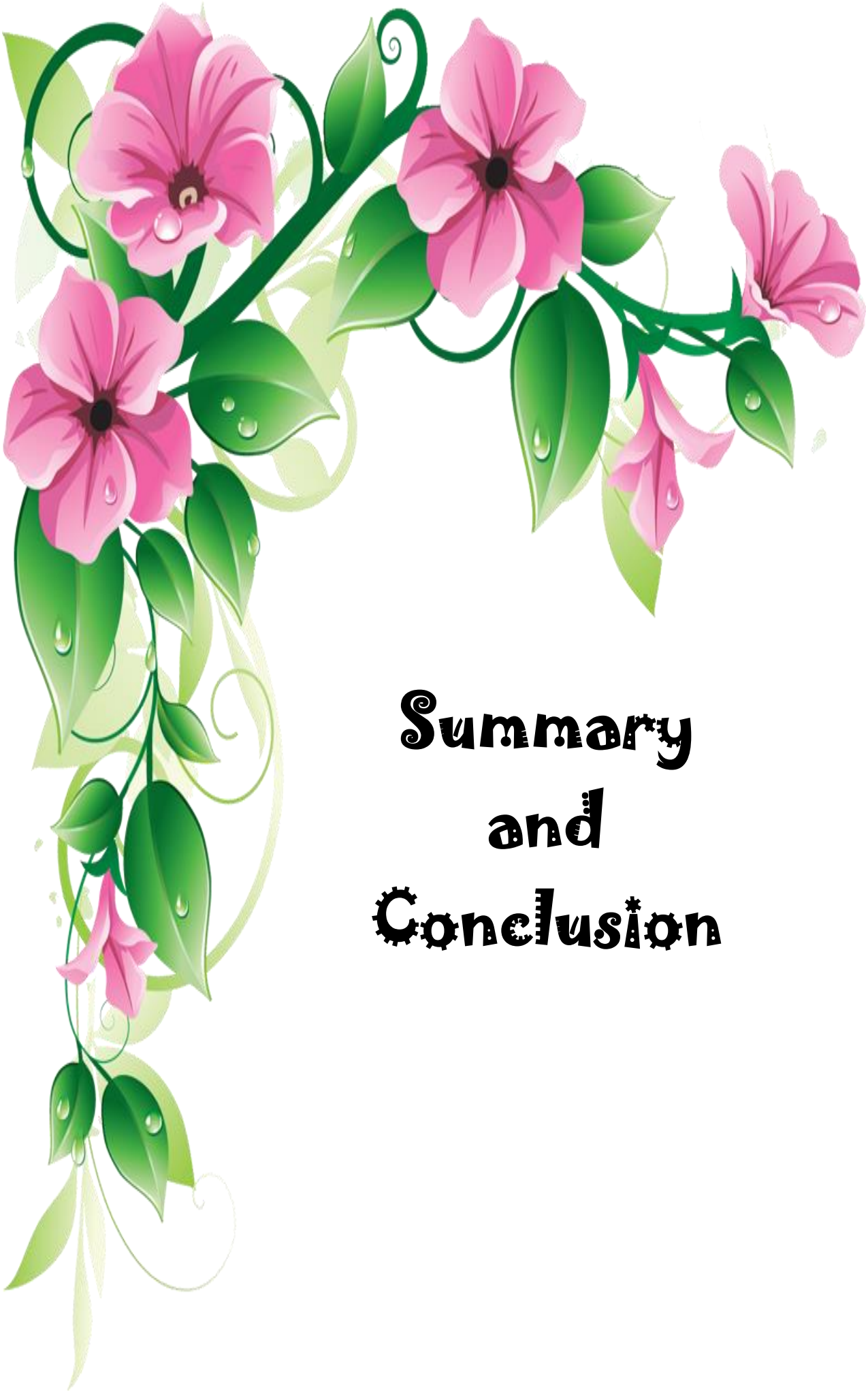


Fig. 6 Effect of seed biopriming on tomato diseases *in vivo*.

significantly leaf spot (*A. alternata*), leaf blight (*M. phaseolina*) and anthracnose (*C. capsici*). The result of the experiment are quite confirmative with result of Jogani and John (2014) found higher seed germination (73.33%) and disease control (66.53%) in tomato against soil-borne pathogen with seed biopriming of *T. harzianum*. Shinde (2016) treated the seeds of tomato with different bioagent among them *T. harzianum* found maximum seed germination, root length, shoot length and per cent root colonization due to seed treatment *in vitro*.



Summary and Conclusion

V. SUMMARY AND CONCLUSION

Tomato [*Solanum lycopersicum* L.] is most widely cultivated vegetable crop in the world. Damping-off **(***Pythium aphanidermatum*), early blight (*A. solani*) and fusarial wilt (*Fusarium oxysporum f.* sp. *lycopersici*) has become a major problem in recent past with a thread to successful and profitable marketing in South Gujarat. Considering this fact present investigation was carried out on various aspects to generate scientific information on the important pathological problems. Which include to know status of tomato diseases, cause of the diseases, and presence of seed borne pathogens and seed bioriming with using bioagent against tomato diseases.

Microscopic examination and isolation was done by using tissue isolation method, which yielded the pure culture of pathogens. The typical Pre-emergence damping-off symptoms observed with the young seedlings are killed before they reach the surface of soil. Infect they may be killed even before the hypocotyls has broken the seed coat. The redical and plumle, when they come out the seed, undergo complete rotting. In post-emergence damping-off toppling over of infected seedling any time after they emerge from the soil until the stem has hardened sufficiently to resist invasion. The infected tissue appears soft and water soaked. In early blight symptoms observed with natural brown concentrate ring on stem, leaf and fruits. In fusarial wilt yellowing and drooping of lower leaves as well as complete drying of diseased plants when the roots of such infected plants were split opened and examined; the brownish black discoloration of vascular system was observed.

Field survey conducted in the Navsari district during *Kharif-Rabi* 2015-16, maximum disease incidence was observed in early blight 41.91%.

The pathogenicity was carried out by three inoculation methods *viz.* soil inoculation, spore spray and root dip method from that confirmed the pathogenic nature of the fungus producing symptoms under artificial inoculation test. These were compared with those produced under natural conditions and found closely identical which have been described. In pathogenicity test 24 per cent pre-emergence mortality was noted in *Pythium aphanidermatum* in pots by soil inoculation method, while post-emergence mortality in inoculated pots was 80 per cent. In pathogenicity test cent per cent infection were noted in early blight and fusarial wilt by spore spray and seedling root dip method, respectively.

The cultural and morphological characters of the pathogens viz., *Pythium aphanidermatum, A. solani and Fusarium oxysporum f.* sp. *lycopersici* were studied and conformed.

In blotter paper method highest incidence was recorded by *Rhizopus sp*. (15%) in sterilized seeds and 28.5% incidence was recorded by *Rhizopus sp*. in unsterilized seeds.

In PDA method incidence was not recorded by *Rhizopus sp., A. niger, A. flavus, F. oxysporum* and *A. solani* in sterilized seeds and 36.50% incidence was recorded by *Rhizopus* sp. in unsterilized seeds.

Significant higher seed germination (94.00%) was recorded in biopriming of tomato seed with *Trichoderma harzianum* @10g/lit containing 10^8 cfu/ml and reduction of

pathogen infections (6.28%) which was statistically at par with *T. viride* applied at imbibition and P. fluorescens applied at imbibition.

Biopriming of tomato seed with different bioagent applied at imbibition and applied after imbibition was carried out during 2016 for management of tomato disease. Five bioagent applied at imbibition and applied after imbibition were tested against tomato disease in field conditions. Significant higher seed germination was found in *P. fluorescens* applied at imbibition (71.82%) with significant higher shoot length (14.55 cm), root length (18.17 cm) and seedling height (23.29 cm) which was statistically at par with imbibition *T. harzianum* applied at imbibition, *T. viride* applied at imbibition and *T. harzianum* applied after imbibition 10g/lit. containing 10^8 cfu/ml and also significant

The disease incidence of damping-off was significantly reduced in seed biopriming with *P. fluorescens* applied at imbibition (18.44%) which was statistically at par with *T. harzianum* applied at imbibition (21.69%). The disease intensity of early blight was significantly reduced in seed biopriming with *T. harzianum* applied at imbibition (15.67%) which was statistically at par with *T. viride* applied at imbibition (18.11%). Per cent disease incidence of fusarial wilt was significantly reduced in seed biopriming with *T. harzianum* applied at imbibition (14.73%) which was statistically at par with *T. viride* applied at imbibition (17.30%).

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This is to certify that I have no objection for supplying to any scientist a copy or any part of this thesis for rendering reference services in a library or documentation center.

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Date: June 2017 **(H. K. GHINAIYA)**