MANAGEMENT OF ROOT ROT OF ASHWAGANDHA (*Withania somnifera*)

THESIS

Submitted to the
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola
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By

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DECLARATION OF STUDENT

I hereby declare that the experimental work and its interpretation of the Thesis entitled **"MANAGEMENT OF ROOT ROT OF ASHWAGANDHA (*Withania somnifera*)"** or part thereof has neither been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis / publication of any University or scientific organization. The source of materials used and all assistance received during the course of investigation have been duly acknowledged.

Place: Akola **(Borade Atul Subhash)**

Date: / / 2017 Enrolment No. NN-3206

CERTIFICATE

This is to certify that thesis entitled **"MANAGEMENT OF ROOT ROT OF ASHWAGANDHA (*Withania somnifera*)"** submitted in partial fulfilment of the requirement for the degree of "**Master of Science in Agriculture (Plant Pathology)"** of Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola is a record of bonafide research work carried out by **Borade Atul Subhash** under my guidance and supervision.

The subject of the thesis has been approved by the Student's Advisory Committee.

Place: Akola
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%	-	Per cent
/	-	Per
^{0}C	-	Degree celcius
@	-	At the rate
C.D.	-	Critical difference
Cfu	-	Colony forming unit
cm	-	Centimetre
CMA	-	Corn meal agar
DAS	-	Days after sowing
e.g.	-	Exampli gratia (For example)
et al.	-	Et alia (and others)
etc.	-	Et cetra
Fig.	-	Figure
g	-	Gram
h	-	Hours
ha	-	Hectare
HCl	-	Hydrochloric acid
HCN	-	Hydrocynic acid
i.e.	-	That is
J.	-	Journal
Kg	-	Kilogram
m	-	meter
ml	-	milliliter
mm	-	millimeter
NA	-	Nutrient Agar

PCNB	-	Pentachloronitro benzene
PDA	-	Potato Dextrose Agar
ppm	-	Parts per million
SA	-	Soil application
SE (m) ±	-	Standard error of mean
ST	-	Seed treatment
t/ha	-	Tonnes per hectare
UV	-	Ultraviolet light
viz.	-	Videlicet (Namely)
µ	-	Micron
µg	-	Microgram

(E) Thesis Abstract

a) Title of thesis	**:**	**"MANAGEMENT OF ROOT ROT OF ASHWAGANDHA (*Withania somnifera*)"**
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ABSTRACT

The knowledge about the importance of medicinal and aromatic plants is being recognized as gifted several modern drugs to civilized man in the form of Ayurveda, Sidda, Unani, Naturopathy, Homeopathy etc. During the recent days, commercial cultivation of medicinal and aromatic plants increased. Among these, Ashwagandha is widely used medicinal plant in various medicinal preparations. Alkaloid present in roots of Ashwagandha is mostly used in various therapies but increased pathological problems resulting in yield reduction and

deterioration of phytochemical constituents. Therefore studies were initiated under the title “Management of root rot of Ashwaganha (*withania somnifera)*.

Fusarium solani causing root rot disease is one of the serious pathogen because it attacks on root and deteriorate the quality of alkaloid. The attack of pathogen was increased with increasing rainfall and humidity. It causes mortality of plants up to 48.94 per cent. The attack of *Fusarium solani* also resulted in less root and shoot length, minimum shoot and root length, minimum fresh and dry root weight and minimum alkaloid per cent. Per cent plant stand also reduced due to the attack of the pathogen. Per cent root rot incidence increases at each succeeding growth stage of plant from sowing to harvesting.

Among various treatments, minimum disease attack was observed in fungicide seed treatment with Mancozeb 63% + Carbendazim 12%. This combined fungicidal seed treatment also increases the germination (plant stand), fresh and dry root weight, shoot and root length and alkaloid content.

In case of bio agents, *Trichoderma viride* was more efficient than *Pseudomonas fluorescens* in arresting the growth of pathogen. But some environmental parameters *viz.,* temperature, rainfall are responsible for reducing the capacity of the biocontrol agent’s.

CHAPTER I

INTRODUCTION

1.1 Background Information

Long before the development of modem medicines, man in ancient times, was entirely dependent on herbal medicines for health care. India was the leader in health care through Ayurveda. The knowledge about the importance of medicinal plants is being lost because of rapid progress of allopathic medicines and modernization.

Indian system of medicines strengthened over centuries through practice and oral tradition makes use of many medicinal herbs. These include large number of native preparations established in Ayurveda. These medicinal herbs include Ashwagandha as an important medicinal plant. Ashwagandha mostly grown on dried region of India. In Maharashtra, especially in Vidarbha region. Ashwagandha belongs to genus *Withania* and family *Solanaceae.* Only two species of Ashwagandha are found in India, such as *Withania coagulans* and *Withania somnifera.*

Ashwagandha *(Withania somnifera),* also known as Indian ginseng or Indian winter cherry. The roots of Ashwagandha have been employed in Indian traditional systems of Medicine, Ayurveda and Unani.

Medicinal plants are those plants rich in secondary metabolites and potential source of drugs; include alkaloids, glycosides, coumarins, flavonoids, steroids etc.

1.2 Importance and need of the study

Roots of Ashwagandha contains many secondary metabolites i.e. alkaloids such as withanine, somnifereine, pseudomwithanine etc. The total alkaloid content of Indian roots varies between 0.13 to 0.31 per cent. Along with alkaloids, roots of ashwagandha also contain starch, reducing sugars, glycosides, aspartic acids, glycine, tyrosine, and praline. These

constituents of roots are also affected by occurrence of root rot at seedling stage (Nigam and Kancjalkar, 1995).

Root rot caused by *Fusarium solani* has been considered among the most deleterious disease, which causes great losses in many parts of the world.

Due to soil borne nature and wider host range of *Fusarium* spp., it leads to heavy economic crop loss at every year. The infection of *Fusarium* spp. is increases with increased rainfall and relative humidity in environment. In water stagnated areas, *Fusarium* spp. attacks on the delicate roots of plant which later show the symptoms like yellowing of leaves, drying of plant, decayed root system and wilting, whitish fungus growth also observed on infected portion of stem at ground level (Gupta and Shrivastava, 1976).

Initial symptoms were withering and drooping of the plants while at later stages, plants showed severe wilting leading to death and decay of underground parts. The root of infected plant showed pulpiness with brownish colour. White cottony growth of the fungus was observed at the basal part of infected plants near ground level. The plant in the nurseries also showed symptoms of yellowing, drooping and decay at seedling stage leading to 30-40% mortality. Further investigations to characterize the infecting fungus led to identification of *Fusarium solani* as the causative organism (Gupta *et al*.2004).

The disease becomes serious under high temperature and humid conditions. Spread of disease is more in August and September. Damage increases with increase in rainfall.

1.3 Objective

In view of above specific problems of medicinal plants, regarding the attack of fungal pathogen, it is felt necessary to undertake the study with following objective.

1. To manage the root rot of Ashwagandha with bioagents and fungicides.

1.4 Scope and Limitation

Fusarium solani causes root rot in *Withania somnifera* leading to enormous yield losses. Due to soil borne nature and wider host range of *Fusarium* spp., it leads to heavy economic crop loss at every year. The infection of *Fusarium solani* is increases with increased rainfall and relative humidity in environment.

Application of chemical fungicide and bioagents may prove to be a helpful tool for managing root rot disease in *Withania somnifera* .

The present investigation entitled “Management of root rot of ashwagandha (*Withania somnifera*)” was conducted during *semi rabi* 2016, to investigate incidence of root rot diseases of Ashwagandha.

1.5 Hypothesis

Ashwagandha is an important medicinal plant. In India Ashwagandha production is less as compared to other medicinal plants because of fungi like *Fusarium solani* can cause root rot disease in Ashwagandha. The root rot infected plant has fully disturbed by these fungi and finally whole plant is rotted and becoming farmers get less yield. The demand of Ashwagandha is increasing day by day but farmers does not produce the more yield due to this pathological problems. Hence the gap between demands of Ashwagandha of pharmaceuticals companies and production of this crop is drastically increased.

Therefore, the attempts are required to investigate the pathological problem in regard to occurrence of major diseases, its causal pathogen with some remedial measures to minimize the disease.

CHAPTER II

REVIEW OF LITERATURE

2.1 Symptoms and pathogen

Matuo and Synder (1972) recorded that *Fusarium solani* f. sp. *pisi*, a widespread soil born pathogen, causing foot and root rot of pea, branch blight of mulberry trees and root rot of ginseng. The round or irregular, light brown lesion appear initially on roots and may spread, destroying all roots, above ground stems and leaves are then killed. If diseased roots are dug and left above ground, white mycelia of the *Fusarium* appear on lesion surface in a few days.

Gupta and Shrivastava (1976) observed that the plants infected with *Fusarium solani* showed yellowing and withering of upright foliage. The whole plant wilted and dried up later on. When pulled up, the root system was found to be partially or fully decayed. The infection was extended up to lower portion of the stem at ground level and a dark brown discolouration of the affected portion of the stem was observed. Whitish fungus growth was present on the affected portion of the stem at ground level.

Lahoz *et al*. (1996) observed that the unusual symptoms of crown and root rot on oriental tobacco plants. After testing pathogenicity, the pathogen observed was *Fusarium solani*. In host, the fungus infected all the plant species tested including tobacco, melon, soybean, and pea.

Gupta *et al*. (2004) reported the symptoms of root rot and wilt of Ashwagandha in field. The plants also showed symptoms of yellowing, dropping and decay at seedling stage leading to 30-50% mortality. The root of infected plant showed pulpiness with brownish colour. White cottony growth of fungus was observed at the basal part of infected plants near ground level.

2.2 Pathogenicity test

Ramakrishna *et al*. (1996) isolated *Fusarium solani* from fruiting annona plant suffering from wilt, yellowing of foliage followed by leaf drop. Two plants each of *A. squamosa* and *A. reticulate* were inoculated and the resulting symptoms observed were similar confirming that *F. solani* was the causal organism.

Booth and Waterston (1998) stated that *Fusarium solani* causes diseases to wide host range (among 66 families) and distributed worldwide in soil. *Fusarium solani* causes diseases like root rot of beans, damping off of seedlings, foot rot of legumes which transmitted through soil at the depth of 40 cm, persisting in the soil as chlamydospores.

Panka and Sadowski (1999) tested the pathogenicity of 29 selected *Fusarium solani* isolated from different agricultural plants under laboratory and pot conditions at two temperature range i.e. 9-11°C and 19-21°C. The results indicated that *F. solani* were not specializing in infecting the plants and it causes diseases to wide range of host plants.

Rathnamma *et al*. (1999) reported that *Fusarium solani* produces the symptoms like pale green leaves which later turned yellow followed by drying, death of plant. The isolation of the pathogen was carried out by tissue isolation method and identification was carried out on the basis of colony character, morphology of vegetative mycelium and micro and macro-conidia.

Zapata *et al*. (2001) isolated *Fusarium solani* from affected egg plants. Pathogenicity tests were carried out separately for each fungus while concluded that *F. solani* was the casual agent of root rot.

2.3 Germination percentage

Shanmugaiah *et al*. (2009) reported that the seed treatment with *T. viride* and *P. fluorescens* shows higher germination due to production of IAA which plays crucial role in plant growth promotion. These

biocontrol agents also produce plant growth regulator which associated with growth stimulation of cotton plants.

Nasreen Sultana and Ghaffar (2010) recorded that in *in vitro* conditions Carbendazim and Mancozeb significantly increased germination by 84-88 per cent as compare to control. In case of root infection, Carbendazim found more effective. In case of microbial antagonists, all the antagonists significantly reduced seed infection caused by *Fusarium* spp. without any significant effect on seed germination. *Trichoderma viride* was found most effective to control the infection.

Andrabi *et al*. (2011) observed that the fungicides applied as seed treatment reduced disease incidence significantly and seed treatment with Carbendazim increased seed germination (71.24%), though it was at par with Carbendazim + Mancozeb (62.21%) and Mancozeb (61.46%).

Khan *et al*. (2014) observed that the application of *Trichoderma* spp. reduced the inhibitory effect of the pathogens on seed germination, resulting in a significant increase in seed germination with *T. hamatum* (46% and 35%), *T*. *harzianum* (42% and 37%), *T. viride* (41% and 37%), and Carbendazim (40% and 36%) in the first and second years, respectively.

Rawal *et al*. (2014) showed that among the fourteen treatments evaluated in the field, the minimum disease incidence with ST Mancozeb 63% + Carbendazim 12% (SAAF) 0.2% +ST *T. viride* +SA Neem cake manure resulted in maximum germination (89.2%) and plant stand (232/plot) in Ashwagandha.

2.4 Disease incidence

Bhatti and Kraft (1992) observed that the root rot or wilt increased with increased inoculum levels of *Fusarium oxysporum* f. sp. *pisi.* and *Fusarium oxysporum* f. sp. *ciceris* and wilt severity did not increases with increased inoculums level of 10^4 or 10^5 microconidia and macroconidia per mililitre. However, wilt symptoms were less severe at 10, 15 and 20^0C

than 30^0C. Severity of root rot caused by *Fusarium* spp. was positively correlated with increasing temperatures and inoculums densities.

Maharashi (2006) observed that *Fusarium solani* and *Rhizoctonia bataticola* (*Macrophomina phaseolina*) causing seedling mortality and later manifesting wilt/ root rot syndrome in Ashwagandha (*Withania somnifera*).

Andrabi *et al*. (2011) recorded that the fungicides i.e. Carbendazim applied as seed treatment reduced disease incidence (6.40%) and Carbendazim + Mancozeb (8.56%) significantly.

2.5 Root and shoot length

Shankar and Jeyarajan (1996) stated that the seed treatment with *Trichoderma* spp. increases root length, shoot length and yield over control and also Carbendazim treatment increasing shoot length.

Marnoranjitham *et al*. (2000) recorded that the application of *T. viride* and *P. fluorescens* either individually or in combination highly reduced the pre and post emergence damping-off and increased the root length, shoot length and dry matter production of chilli seedlings.

Shanmugaiah (2009) reported that the seed treatment with biocontrol agent's *viz*., *T. viride* and *P. fluorescens* was responsible for higher shoot length 12.42 cm, 10.83 cm and root length 20.28 cm, 15.79 cm respectively, due to production of IAA, MG-6, UV-10, MNT-7 and other plant growth regulators.

Maitlo *et al*. (2015) tested six fungicides such as Bavistin D.F, Topsin-M, Alliette, Ridomil gold, Mancozeb and Copper oxychloride at 0.5, 1.5 and 2.5 (g) concentrations against *F. solani* in potted date palm seedlings under greenhouse conditions. The highest shoot length was recorded by using Bavistin D. F. (19.3, 20, 20.3 cm) and the lowest in plants treated with Mancozeb (9.8. 10.2, 12.5 cm) as compared to control.

The maximum root length was recorded using Bavistin D.F. (9.2, 9.9, 10.5 cm) while Mancozeb (4.2, 4.5, 5.1 cm) were appeared as least effective.

2.6 Fresh and dry root weight

Singh *et al*. (2004) recorded that among different antagonist, *Trichoderma viride* treated plants were taller had more number of leaves, good health and more fresh and dry weight as Carbendazim treated plants.

Shanmugaigh *et al*. (2009) reported that, seed treatment with *Trichoderma viride* and *Pseudomonas fluorescens* shows significant increases in fresh weight (11.750 g, 9.520 g) and dry weight (3.867 g, 2.647 g) respectively over control due to production of IAA and other plant growth regulators.

Anju Tanwar *et al.* (2013) reported that the application of *T. viride* alone increase fresh root weight (5.93 g) and dry root weight (2.51 g) in Broccoli than used in combination with *P. fluorescens,* fresh root weight (4.58 g) and dry root weight (1.37 g).

2.7 Disease control

2.7.1 Chemical Control

Sharma and Jain (1984) tested the fungicides and plant extract for the control of *Fusarium* rot of tomato fruits. Carbendazim was found to be most effective fungicide in inhibiting the mycelial growth of *Fusarium solani*.

Kapoor and Kumar (1991) reported that the efficacy of the three systemic (Benomyl Carbendazim and Thiophanate methyl) and three non-systemic (Captafol, Captan and Thiram) fungicides against two isolates each of *Fusarium solani* and *Fusarium oxysporum* obtained from tomato using poisoned food technique. Carbendazim and Benomyl were most toxic. *F. solani* isolates KHFs-41 was most sensitive and it required 3-5 times more dosages of non-systemic fungicides.

Ramaswamy and Kumar (1997) reported that the pre flowering application of Carbendazim has been found helpful in minimizing the root and foot rot incidence.

Singh *et al*. (2000) observed that the test fungicides like Carbendazim, Contaf and Indofil M-45 could inhibit the growth of *Fusarium* spp. completely up to 7 days of incubation. As the fungus failed to grow, it failed to sporulate in solid and liquid media treated with fungicides at recommended doses. It also reported that Ziram, Mancozeb and Carbendazim were most effective against *Fusarium* spp. causing seedling blight and root rot in Neem.

Patil *et al*. (2001) recorded that among systemic fungicides, Carbendazim were effective in inhibiting the growth and sporulation of *Fusarium solani* 94.74 per cent.

Sharma *et al*. (2004) tested seven systemic and non-systemic fungicides under *in vitro* conditions. Among fungicides, Carbendazim could effectively control the mycelial growth of *Fusarium solani* under different concentrations.

Mallesh and Narendrappa (2009) tested different non systemic and systemic fungicides against root rot of sage caused by *F. solani* and *R. solani.* Among the non systemic fungicide Mancozeb was found to be effective with 100 per cent inhibition of myclial growth at all concentration against *F. solani* and *R*. *solani* and among systemic fungicide Carbendazim recorded 100 per cent inhibition.

Nasreen Sultana and Ghaffar (2010) observed that the complete inhibition of colony growth of *Fusarium* spp. was observed where Carbendazim was used, whereas Mancozeb completely inhibit the colony growth at higher concentration (1000 ppm). Combination of Carbendazim + Mancozeb gave 100 per cent inhibition of mycelial growth of *Fusarium* spp. at 0.2 and 0.3% concentration.

Prasad *et al*. (2011) recorded that the application of Carbendazim (Bavistin) at 0.1% when applied individually was also found almost complete control as is evident from low disease incidence (1.04%) and severity (0.52%), considered to be second best treatment. Apparent infection rate (0.0) was observed in Carbendazim + Mancozeb and Carbendazim followed by Copper oxychloride (0.006) and Carboxin (0.009).

Taskin-Un-Nisha (2011) reported that the systemic fungicides at different concentrations significantly inhibit the mycelial growth of *F. oxysporum*. However, the Hexaconozole at highest concentration (1000 ppm) caused highest reduction of mycelial growth (8.80 mm) followed by Carbendazim (9.40 mm), Bitertanol (18.60 mm) and Myclobutanil (20 mm) at the same concentration. It was also observed from the study that amongst the non-systemic fungicides, Mancozeb was found most effective (14.20mm) in reducing mycelial growth of the fungi followed by Captan (20.00 mm) and Zineb (22.00 mm).

Kapadiya *et al*. (2013) recorded that the effectiveness of Carbendazim + Mancozeb against *F. solani* has been reported. Carbendazim 12% + Mancozeb 63% were proved the most effective and gave cent per cent growth inhibition of test fungus at lowest concentration of 250 ppm than Carbendazim and Mancozeb.

Singh (2013) reported that the out of six fungicides, the best two fungicides Bavistin (Carbendazim) and Topsin-M (Thiophanate methyl) were taken at different concentration (0.1%, 0.2% and 0.4%) and evaluated against *F. solani* causing the pre and post emergence mortality. Maximum disease control in pre (68.75%) and post (70.95%) emergence was observed in seed treatment with Bavistin whereas the figures of pre and post emergence mortality were 65.00 and 67.54 per cent.

Bhaliya and Jadeja (2014) tested different contact, systemic and combination of fungicides in *in vitro* against *Fusarium solani*. Out of six systemic fungicides, Carbendazim found best with 98.68% mycelial growth

inhibition. Carbendazim showed complete inhibition of mycelial growth of the test fungus at 500 ppm concentration followed by same fungicide with 250 ppm (99.46%) and 100 ppm (98.12%). Among the fungicides combination, Carbendazim + Mancozeb gave 100 per cent growth inhibition at all concentration.

Maitlo *et al*. (2015) reported that among six fungicides Bavistin D.F and Topsin M were the most effective fungicides in reducing the infection of the *F. solani* inoculated date palm plantlets. The plantlets treated with Bavistin D.F at 0.5, 1.5 and 2.5 g/l concentrations showed 15, 10 and 5% followed by Topsin M (20, 15 and 10%), Alliette (25, 20 and 15%), Ridomil gold (30, 25 and 20%), Mancozeb (40, 30 and 25%) and Copper oxychloride (50, 45 and 35%) respectively as compared to control (95%).

Khanzada *et al.* (2016) tested ten different fungicides, plant extracts and bio control agents were tested *in vitro* by food poisoned method against *Fusarium solani*. All fungicides were tested at three concentration that are 100, 1000 and 10000 ppm. All fungicides showed varied effects against *F. solani*. However, Carbendazim was highly effective at low as well as at medium and high concentrations, which reduced 100 per cent mycelial growth.

2.7.2 Biological control

Vyas (1994) recorded that the application of *Trichoderma viride* with Carbendazim to soybean seeds reduces the infection of dry root rot. Yield of the treated plot was significantly higher as compared to check.

Wuike *et al.* (1995) observed that the soil application of *Trichoderma viride* had significant reduction root or stem rot as compared to control. Carbendazim seed treatment was at par with *Trichoderma*. *T. viride* was effective in controlling the root/stem rot of sesame when incorporated in soil at sowing. Biological control will be cheaper and safer as compared to Carbendazim.

Raghuchander *et al*. (1997) observed that the *Trichoderma viride* and *Pseudomonas fluorescens* were equally effective against *Fusarium oxysporum* under laboratory conditions and field trials during *rabi* and *kharif* were effectively reduced *Fusarium* with incidence (4.5 and 4.1%) respectively and produced the highest yield (14.95 and 15.6 t/ha) respectively.

Larkin and Fravel (1998) treated tomato seedlings with potential biocontrol agents in the greenhouse and transplanted into pathogen infested field soil. Organism tested included non pathogenic strains of *Fusarium* spp., Trichoderma spp., *Phaseolina* spp. and others. Specific non pathogenic isolates of *Fusarium oxysporum* and *Fusarium solani* collected from a *Fusarium* wilt suppressive soil were the most effective antagonists, providing significant and consistent disease control (50-80%) in several reported test. These isolated were effectively control *Fusarium* wilt diseases in other crops.

Jayashree K. *et al*. (2000) observed that *Pseudomonas fluorescens* strains Pf-1 effectively inhibited the mycelial growth of pathogen which causes root rot in black gram and sesame. Application of Pf-1 as seed treatment @ 10 g/kg seed followed by soil application (2.5 kg/ha) against root rot effectively supported higher plant growth, better native *Rhizobium* nodulation and yield. Sclerotial number and root rot incidence were also greatly reduced.

Zaidi and Singh (2004) observed that *Trichoderma* spp., *Pseudomonas fluorescens* were two most widely used biocontrol agents due to their antifungal, anti nematode, plant growth promoting activities. Use of biocontrol agents noticed was their relatively short shelf life and inconsistent field performance. To overcome these problems, biocontrol agents were grow on fresh cow dung, FYM and poultry manures which provide excellent growth. *Trichoderma* and *Pseudomonas fluorescens* grow on colonized compost not only provide better protection against disease but also promote plant growth better than non colonized compost.

Joshi and Raut (2005) studied biological control agent's i.e. *Trichoderma* antagonist of *Fusarium solani*. Maximum inhibition in colony area of *F. solani* was achieved with *T. viride* (90%). In other experiment *T. viride* and *Pseudomonas fluorescens* isolates Pf1 and Pf2 also proved to be effective antagonistic of *F. solani*.

Mallesh and Narendrappa (2009) concluded that biological control for soil borne pathogens were ideal, cheap, long lasting and ecofriendly. According to this experiment, all the species of *Trichoderma* showed more hyphal inhibition compared to bacterial antagonists. This can be attributed to higher competitive ability of *Trichoderma* spp. against *Fusarium* spp. Maximum inhibition of mycelial growth was observed in *T. virens* (94.8%) and *T. viride* (94.4%) while least per cent mycelial inhibition noticed in *Pseudomonas* isolates against *Fusarium solani.*

Beevi and Qadri (2010) reported that the reduction in root rot disease by *Trichoderma* spp. might be due to higher antagonistic potential like antibiosis, parasitism and production of lytic enzymes. Also studied that, the treatment *T. harzianum* + *T. viride* + Effective microorganism recorded higher survival of plant (73.30%) under sick soil condition.

Nidhi Bharti *et al*. (2013) reported that the *P. fluorescens* were found to be effective in reducing the severity by 60 per cent. The reduction in disease severity in PGPR treated plants could be attributed to their direct antagonism and their capability to produce siderophores not only useful in iron acquisition but also their known role to interfere in the establishing a pathogenic relationship of *Fusarium* with host plant. *P. fluorescens* may prove to be a helpful tool for managing root rot disease in *Withania somnifera.*

Singh (2013) tested the different bio-control agent's for their efficacy *in vitro* against *Fusarium solani* by dual culture technique. There was much variation in the efficacy of antagonists as the inhibition varied from 51.00 to 72.18 per cent. *Trichoderma harzianum* stood at first place in

order of effectivity with 72.18 per cent inhibition followed by *T. viride* and *P. flourescens* where the figures were 67.70 and 63.80 per cent, respectively.

Bashar and Chakma (2014) tested various soil fungi inhibited the radial growth of the test fungi in varied degrees in dual culture experiments on agar plates. In case of *F. solani, T. harzianum* showed highest inhibition of radial growth (78.60%) followed by *A. niger* (56.25%) and *T. viride* (50.00%). Least inhibition of radial growth was noticed with *Aspergillus terreus* (23.53%). High antagonistic activity of the *Trichoderma* spp. observed against the test fungi might be due to fast growing nature, rapid sporulation and toxic metabolite producing capacity.

Zape *et al*. (2014) tested ten antagonists tested under *in vitro* condition. All the antagonists were found to be significantly effective over control in inhibiting the mycelial growth of *F*. *solani*. These antagonists inhibited 62.22 to 87.03 per cent mycelial growth of the fungus. Inhibition of radial growth of *Fusarium solani* was maximum with *T. viride* (Akola isolate), *T. hamatum*, *T. viride* (Parbhani isolate), *A. niger*, *T. harzianum*, *T. koningii*, *P. fluorescens*, *G. virens* (Parbhani and Akola isolate) and *T. lignorum*. *P. fluorescens*.

EL-Morsi *et al*. (2015) reported that *Bacillus megaterium* and *T. viride* was the better biocontrol agents for controlling root rot/ wilt diseases and improved growth of date palm offshoots than the other tested biological control agents in field cultivated in El-Kharga and El-Khala. *T. viride* was most effective BCA for decreasing root rot and wilt severity 18.83, 25.36 per cent compared with 75.38, 86.36 per cent disease severity in control in both locations respectively.

Hafiza Asma Shafique *et al*. (2016) reported that the soil application of biocontrol agent's *viz*., *T. viride*, *T. harzianum*, *P. fluorescens* and *Bacillus subtilis* effectively reduced root rot caused by soil borne pathogens in several crops. *Trichoderma* spp. is known to produce large quantities of fungi toxic metabolites. Also found that endophytic *T. viride*

was effective in suppressing the *F. solani*, *F. oxysporum* and root-knot nematode on okra used alone or with *Pseudomonas aeruginosa*.

Jetawat and Mathur (2016) reported that the seed treatments with integration of fungicides, Neem cake manure, Neem oil and *T. viride* agent evaluated as seed treatments individually as well as in different combination of seed treatment and soil application of Neem cake was found effective integrated treatment (ST SAAF + Neem cake manure + *T. viride*) and soil application of Neem cake manure @ 500 g/plot showed minimum per cent root rot (11.8%) and maximum per cent germination and maximum yield (345 g/plot) of Ashwagandha as compared to their individual applications over the control.

2.8 Alkaloid per cent

Nigam and Kandalkar (1995) reported that the pharmacological activity of roots of Ashwagandha was attributed to presence of several alkaloids and withanoids. Also reported that the total alkaloid content of Indian roots was varied between 0.13 and 0.31 per cent.

Karthikeyan *et al*. (2009) reported that the effect of different plant growth promoting rhizobacteria like *Azospirillum brasilense* and *Pseudomonas fluorescens* on growth parameters and the production of terpenoid indole alkaloids are investigated in two varieties ‘rosea’ and ‘alba’ of *Catharanthus roseus*. The maximum ajmalicine content recorded in the combined inoculation of *Azospirillum brasilense* + *Pseudomonas fluorescens* in ‘rosea’ variety on 90 DAP (0.700 mg). Also concluded that, the seed priming and seedling treatments of native PGPRs can be used as a good tool in the enhancement of biomass yield and alkaloid contents in medicinal plant cultivation.

Rawal *et al*. (2014) showed that among the fourteen treatments evaluated in the field, the maximum alkaloid content (0.74%) with ST Mancozeb 63% + Carbendazim 12% (SAAF) 0.2% + ST *T. viride* + SA Neem cake manure in Ashwagandha.

CHAPTER III

MATERIAL AND METHODS

The present investigation is carried out during the year 2016-17 at Nagarjun Research Station and Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. The mean annual rainfall of Akola is 700 mm distributed over a period of three months with a prominent peak from July to August. The months of April and May are hottest with maximum temperature i.e. 42^0C and minimum 27.6^0C. Akola is situated at 20.42^0C latitude, longitude of 77.02^0C and MSL 307.415 m. The details of material used and methodology adopted during the studies as below.

Fungal diseases of medicinal plants cause severe reduction in yield and qualitative losses. The present investigation deals with recording of root rot of Ashwagandha caused by *Fusarium solani*. The effect of some fungicides and antagonist were also accessed against *Fusarium solani*. For this research, local variety of Ashwagandha was used.

3.1 MATERIAL

Following materials were used for experimentation.

3.1.1 Glassware's

The glassware's used during the course of research work were of Borosil make. The glassware's *viz.,* Petri plates, test tubes, conical flasks of 250 ml, 500 ml and 1000 ml, funnel, beakers, pipettes, measuring cylinder, slides, cover slips and glass rods were used.

3.1.2 Equipments used

Standard laboratory equipments *viz*., autoclave, BOD incubator, laminar air flow, research microscope, stereoscopic binocular microscope, refrigerator, hot air oven, digital weighing balance, Bunsen burner, water distillation unit etc. were used.

3.1.3 Other materials used

Blotter paper, non adsorbent cotton, muslin cloth, cork borer, inoculating needle, forceps, potato dextrose, agar-agar, mortar and pestle, sterilized soil, pots etc. were used during research programme.

3.2 METHODS

3.2.1 Sterilization of glassware's and other materials

For laboratory studies, standard pure chemical and glassware's such as Petri plates, slides, cover slips, beakers, conical flasks, test tubes etc. were used. Glassware's were cleaned by washing with detergent, dried and sterilized in hot air oven at 180^0C for 1 hr before use. Distilled water and media were sterilized in autoclave ay 1.04 kg/cm^2 for 15 min. Soil was mixed with 10% formalin solution and then covered with the polythene for 5 days or soil sterilized in an autoclave at 30 lbs PSI.

3.2.2 Collection of diseased samples

Infected roots of Ashwagandha plants were collected from field of Department of Plant Pathology, Dr. P. D. K. V., Akola and examined in laboratory for isolation of disease causing pathogen.

3.2.3 Preparation of culture media (PDA)

Peeled potato = 200 g

Dextrose = 20 g

Agar-agar = 20 g

Distilled water = 1000 ml

Healthy peeled potatoes 200 g were cut into pieces and boiled in 500 ml sterilized distilled water in sauce pan for 30 minutes. Extract was strained through muslin cloth and quantity was measured. In remaining 500 ml water, 20 g agar-agar and 20 g dextrose were dissolved by heating. Both were mixed and volume was made up to 1 litre. The

medium was filtered through muslin cloth and poured into conical flask and test tubes, then plugged with non absorbent cotton and autoclaved at 1.04 kg/cm^2 for 20 min. Autoclaved test tubes were kept in slanting position to obtain slants for maintenance of cultures.

3.2.4 Isolation and maintenance of pure cultures

For isolation of pathogen, potato dextrose agar (PDA) medium was used. Approximately 20 ml autoclaved PDA was poured in each sterilized Petri plates and allow solidifying. The diseased part of root was clean properly. The diseased portion cut into small bits along with healthy portion with sterile blade and transferred into sterile Petri plate containing 0.01% Sodium hypochlorite solution for surface sterilization.

After, a minute bit were transferred to sterile water and washed with 3 changes of sterilized water to remove the traces of Sodium hypochlorite. Bits were blot dried by keeping them on sterilized filter paper to absorb the excess water. Three bits each was aseptically transferred to a solidified PDA medium in sterile Petri plate at equal distance and kept for incubation at room temperature (27^0+1^0C). All the operations were carried out aseptically in laminar air flow chamber. Growth of organism was observed regularly. The fungus observed around the infected bits was transferred on PDA in plates. The slides were prepared and examined under research microscope for identification.

3.2.5 Purification of pathogen

This method was followed for maintaining pure culture, since *Fusarium solani* is known to be heterokaryotic in nature. Hyphal tip isolation was done on water agar plates. Dilute spore suspension (8-10 spores/ml) was prepared in sterile distilled water. One ml of such suspension was spread uniformly on two per cent water agar plates and the excess of which was aseptically drained. Single spore was then marked under the microscopic field with ink on the glass surface of the plate and it was allowed to germinate. Such plates were incubated at 27±1°C and

periodically observed for germination of spores under the microscope. Hyphae coming from each end cell of the single spore was traced and marked with the ink. Then tip of hypha was cut and transferred to PDA slants with the help of cork borer under aseptic conditions and incubated at temperature of 27±1°C for 10 days. Later, mycelial bits of the fungus were placed in the center of Petri plates containing potato dextrose agar medium and incubated at 27±1°C for 10 days. No saltation or sectoring was observed in the culture and it was concluded that, it was a pure culture of the fungus. Such culture was used for further studies.

3.2.6 Identification of the pathogen

Identification of *Fusarium solani* was made on the basis of morphological characters confirmed with CMI publications (Booth and Waterston, 1998).

$$\text{Per cent disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

3.2.7 Preparation of mass inoculum

Sorghum sand medium was used for developing inoculum. It was prepared by mixing 100 g sorghum and 50 g dry sand with 50 ml distilled water in 500 ml flask and autoclaved at 1.05 kg/cm^2 for 30 minutes for two consecutive days. Autoclaved grains were then inoculated with pure culture of *Fusarium solani* separately under aseptic condition (Laminar air flow chamber). The inoculated flasks were incubated at room temperature for 2 weeks. This mass inoculum of *Fusarium solani* was used for experiment.

3.2.8 Preparation of sick soil

The field soil 7 parts + 3 parts sand was sterilized for two successive days. Full growth of fungus was then added to the sterilized soil in 1:9 proportions (inoculum + soil). The earthen pots of 30 cm diameter were filled with above mixture (Pot inoculation method).

3.2.9 Pathogenecity test

The seeds of Ashwagandha were obtained from Nagarjun Medicinal Plant Garden, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola for testing pathogenicity. In each pot 10 seeds were sown.

Degree of susceptibility of plants was determined on the basis of percentage of plants showing root rot symptoms in artificially developed sick soil pots by incorporating the inoculum.

3.2.10 Field trial

A field experiment in randomized block design (RBD) was carried out at field of Department of Plant Pathology, Dr. P. D. K. V., Akola with the application of seven different treatments and replicated thrice against the root rot of Ashwagandha caused by *Fusarium solani*.

Seeds of Ashwagandha of local variety treated with bioagent and fungicides were sown. The experiment consisted of seven treatments and replicated thrice. Mortality of plant was noted.

Periodically and percent root rot was calculated, the various treatments include –

Experiment details:

1. Location	:	Field of Department of Plant Pathology, Dr .P. D. K. V., Akola.
2. Design	:	Randomized Block Design (RBD)
3. Number of treatment	:	7
4. Number of replication	:	3
5. Plant to plant distance	:	5 cm
6. Row to row distance	:	30 cm
7. Plot size	:	2.4×1.8 m^2
8. Crop	:	Ashwagandha (*Withania somnifera)*
9. Season	:	Semi *rabi* (2016)
10. Date of sowing	:	10 August 2016

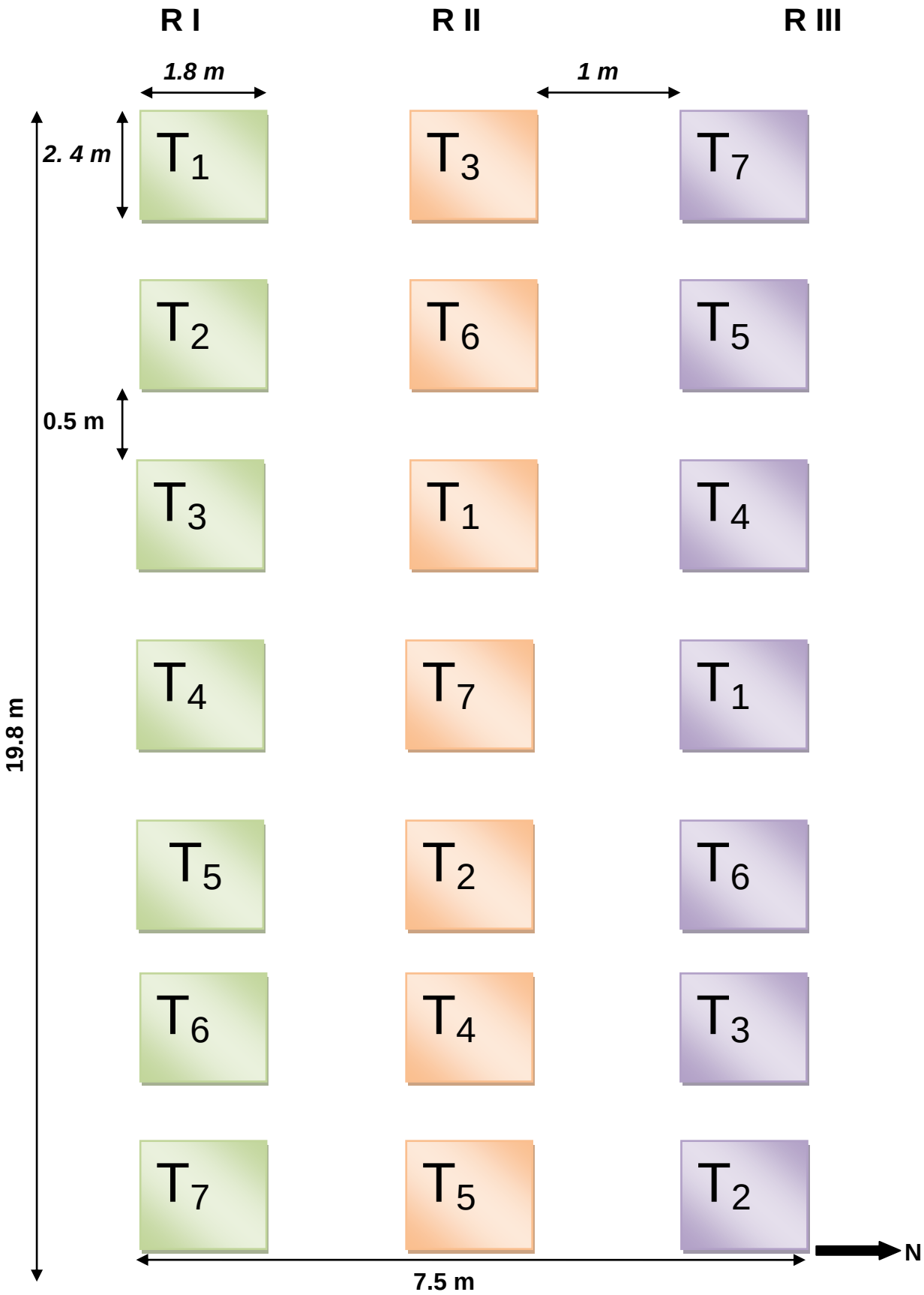


Fig. 1. Plan of Layout

Plate 1. Ashwagandha - Experimental Plot

Treatment details:

Sr. No.	Treatment No	Treatment Details
1.	T_1	Seed treatment with Carbendazim @(1.25g/kg)
2.	T_2	Seed treatment with Mancozeb @(2.5g/kg)
3.	T_3	Seed treatment with Mancozeb 63% + Carbendazim 12% (SAAF) 75WP @(2.5g/kg)
4.	T_4	Seed treatment with *Trichoderma viride* @(4g/kg)
5.	T_5	Seed treatment with *Pseudomonas fluorescens* @(10 g/kg) of seed
6.	T_6	Seed treatment with *Pseudomonas fluorescens* @(5g/kg) + *Trichoderma viride* @(2g/kg)
7.	T_7	Control

3.2.11 Observations

In field observations, the observation was recorded on germination at 30 DAS.

The disease incidence of root rot of ashwagandha was recorded at 30, 60, 90 and 120 DAS (Harvesting stage).

Also check the shoot length at 60, 90 and 120 DAS (Harvesting stage) and root length after harvesting. Five plants were selected at random from each plot and labelled for data recording. The shoot length from the ground level to the growing tip of the plants was measured. The mean length of five plants was calculated and expressed in centimeters. The length of roots in five labeled plants was measured from collar region to the tip of root and the average was calculated and expressed in centimeters.

The roots from the uprooted plants were separated from the stem and weighed. The fresh root weight per plot was recorded and final plant count was recorded and expressed in gram per plant.

After recording the fresh root weight the roots were sun dried for 7 days and weighed. The dry root weight per plot was recorded and final plant count was recorded and expressed in gram per plant.

The berries were also separated and dried. Later, the seeds are extracted from the dried berries, cleaned and weighed separately.

Percent alkaloid content in roots was estimated by following procedure.

3.2.12 Method for estimation of total alkaloids in Ashwagandha root powder

Procedure:

1. 0.5 g of Ashwagandha root powder was taken in a stopper test tube.
2. 5 ml chloroform and 2-3 drops ammonia was added in test tube.
3. Tubes was mixed well and kept for overnight.
4. Tube was shaken and filtered through cotton wool in a small beaker.
5. The residue was washed with chloroform thrice.
6. The extract was dried on water bath.
7. 10 ml ethyl alcohol was added and mixed the content with clean glass rod.
8. The liquid portion was evaporated which confines the removal of ammonia.
9. 10 ml standard acid 0.01 N H_2SO_4 was added in the beaker then warmed slightly to dissolve the alkaloid and then cooled the solution.
10. 2-3 drops of phenolphthalein indicator were added and titrated the acid with standard 0.01 N NaOH.
11. The volume of NaOH was noted.

12. Same time, blank was also run.

13. The per cent total alkaloid was calculated by using following formula.

Formula:

$$\text{Total alkaloid content (\%w/w)} = \frac{\text{TV (blank) -TV (sample)}}{\text{TV (blank)}} \text{ X} \frac{1}{\text{Wt of sample}}$$

Where; TV = Titer value

(Swami, 2011)

CHAPTER IV

RESULTS AND DISCUSSION

The root rot of Ashwagandha is caused by a soil borne pathogen *Fusarium solani* is a disease of worldwide occurrence. At present it is confined to limited pockets in the Vidarbha region. The climatic conditions of the region are conducive for perpetuation and spread of fungal diseases of many crops. Under such circumstances, in near future, the disease may pose a major threat to the Ashwagandha cultivation in this region.

In lieu of this, the present study was taken up to initiate the work on isolation, pathogenicity and evaluation of fungicides, and bio-control agents against the pathogen under *in vivo* conditions. The results of the experiments conducted on these lines are presented in this chapter.

4.1 Collection of diseased sample

Infected samples of Ashwagandha were collected from the field of Dr. P. D. K. V., Akola.

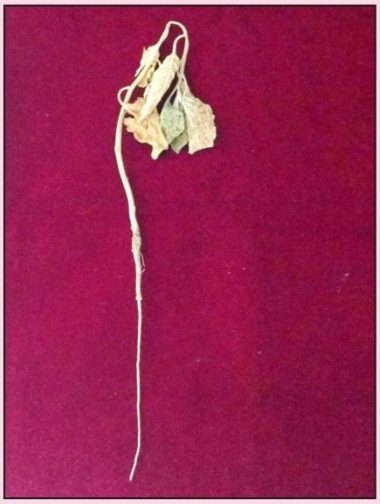
The symptoms of root rot were observed in the field of Ashwagandha (*Withania somnifera*) during the month of September to December 2016. The initial symptoms were yellowing and withering of leaves starting from lower most leaves. While at later stages, plant showed severe wilting, drying, and leading to death and decay. White cottony growth of the fungus was observed at the basal part of infected seedling near ground level. The disease samples were brought to laboratory for isolation.

Isolation of fungal pathogen from diseased root samples was made on potato dextrose agar medium by tissue isolation technique.

4.2 Purification and identification

The identification and purification of *Fusarium solani* was carried out. The causal organism of root rot of Ashwagandha is *Fusarium solani* which is the most common *Fusarium* spp. The species is quite easily

Healthy plant



Infected plant

Healthy root

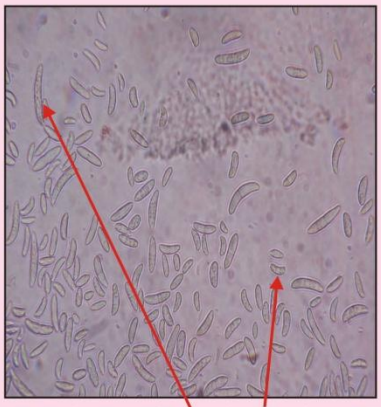
Infected root

Plate 2. Difference between healthy and infected plant of Ashwagandha

Isolation of *Fusarium solani* by tissue isolation method

Growth of *Fusarium solani* on PDA media

Slant of *Fusarium solani*



Conidia (Macro & Micro)

Clamydospore of *Fusarium solani*

Plate 3. Isolation and morphology of *Fusarium solani*

recognized based upon its cream colour, long macroconidia and microconidia. Colonies of *Fusarium solani* growing rapidly upto 4.5 cm in four days. Hyphae of *F. solani* are septate and hyaline. Conidiophores are simple or branched monophialides (phialides with a single opening). Macroconidia are moderately curved, stout, thick walled, usually 3-4 septate, measure 4-6 x 65 pm long, and are borne on short conidiophores that soon form sporodochia. Microconidia borne from long monophialides, are one to three celled, 2-5 x 8-16 pm long and occur in false heads only.

Clamydospores are hyaline, globuse, smooth to rough-walled, borne singly or in pairs on separate branches or intercalary (Booth and Waterson, 1996).

4.3 Pathogenicity and symptoms

For root rot diseases, seven days old fungal inoculums of *Fusarium solani* multiplied separately on sorghum grains were incorporated in the pot containing sterilized soil and one pot of without inoculum as a control. After that, 10 seeds are sown in each pot and observations were recorded. The plants in inoculated pot shows the same symptoms of disease which previously recorded in field. After reisolation, the pathogenicity was proved.

Rathnamma *et al.* (1999) reported that, *Fusarium solani* produces the symptoms like pale green leaves which later turned yellow followed by drying, death of plant. The isolation of the pathogen was carried out by tissue isolation method and identification was carried out on the basis of colony character, morphology of vegetative mycelium and micro and macro-conidia.

Similar results were recorded by Lahoz *et al.* (1996), Gupta and Shrivastava (1976) and Gupta *et al*. (2004).

4.4 Field experiment

The field experiment was carried out at field of Department of Plant Pathology, Dr. P. D. K. V., Akola in Randomized Block Design with seven treatments and three replication.

Mass culture of *Fusarium solani*

Pathogenicity test of *Fusarim solani*

Plate 4. Mass culture and pathogenicity test of *Fusarim solani*

4.4.1 Per cent plant stand at 30 DAS and at harvesting stage

The effects of various treatments like fungicides and bioagents on germination of Ashwagandha plants are given as follows;

Table 1. Per cent plant stand at 30 DAS and at harvesting stage

Sr. No.	Treatments	Per cent plant stand (30 DAS)	Per cent plant stand at harvesting stage
T_1	Carbendazim	84.98 (68.10)*	71.47 (57.71)
T_2	Mancozeb	78.97 (63.66)	63.36 (52.75)
T_3	Mancozeb 63% + Carbendazim 12% (SAAF)	93.99 (76.96)	81.08 (64.22)
T_4	*Trichoderma viride*	80.98 (64.11)	66.86 (54.86)
T_5	*Pseudomonas fluorescens*	71.97 (59.73)	55.25 (48.01)
T_6	*Pseudomonas fluorescens* + *Trichoderma viride*	76.97 (61.26)	60.96 (51.33)
T_7	Control	68.36 (55.53)	51.05 (45.60)
	'F' test	Sig.	Sig.
	SE(m)±	0.94	0.55
	CD at 5%	2.89	1.71

*Figures in parenthesis are arc sine transformed values

Data presented in Table 1 and graphically represented in fig 2. The highest plant stand at 30 DAS was obtained in treatment T_3 (Mancozeb 63% + Carbendazim 12%) is 93.99%. It was followed by T_1 (Carbendazim), T_4 (*T. viride*) and T_2 (Mancozeb) is 84.98%, 80.98%, 78.97% respectively. The least plant stand was observed in (T_7) control plot is 68.36%. The results obtained from table 1 were statistically significant.

Also maximum plant stand at harvesting stage was recorded in treatment T_3 (Mancozeb 63% + Carbendazim 12%) is 81.08%. It was followed by T_1 (Carbendazim), T_4 (*T. viride*) and T_2 (Mancozeb) is 71.47%,

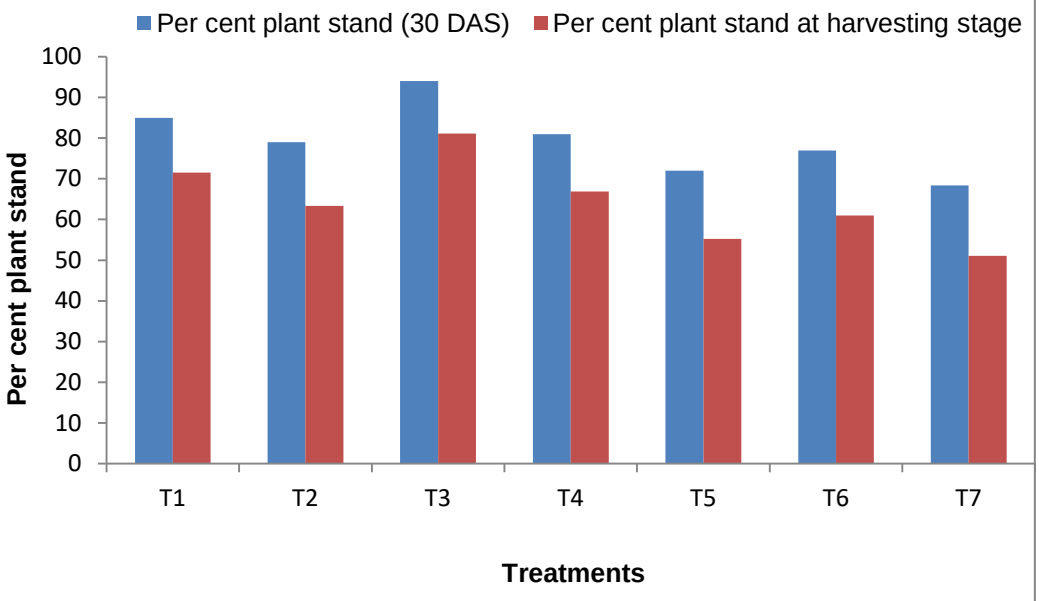


Fig. 2. Per cent plant stand at 30 DAS and at harvesting stage

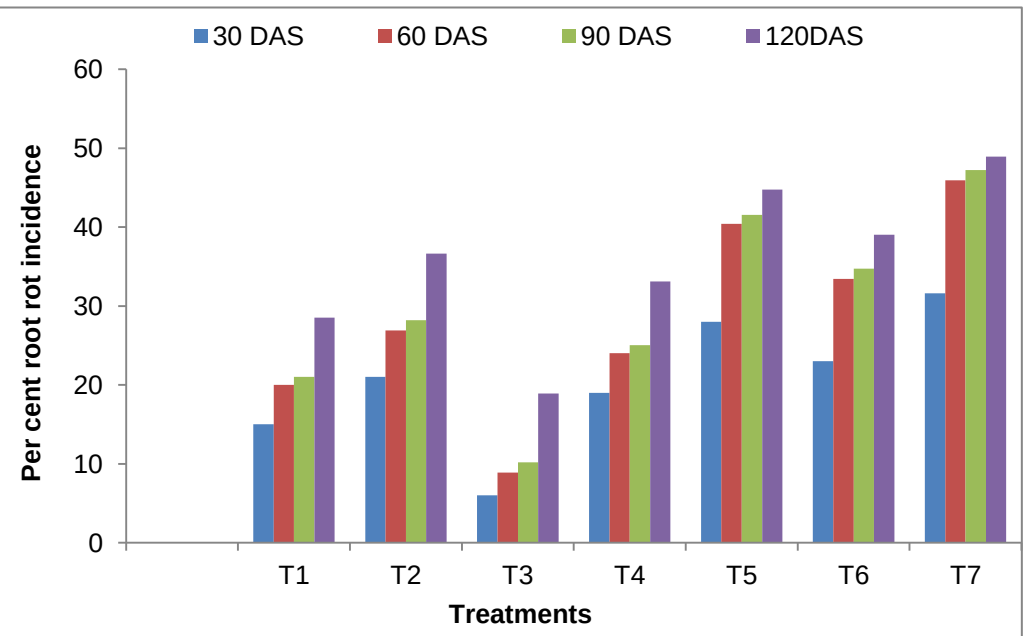


Fig. 3. Root rot incidence at different growth stages of ashwagandha plant

66.86%, 63.36% respectively. The least plant stand was observed in (T_7) control plot is 51.05%. The results obtained from table 1 were statistically significant.

The Table 1 shows that, the plant stand reduced at harvesting stage, in case of fungicide like Mancozeb 63% + Carbendazim 12% plant stand was reduced from 93.99% to 81.08% and in biocontrol agents, plant stand in *T. viride* was reduced from 80.98% to 66.86%.

Kredics *et al.* (2003) recorded that, some environmental parameters *viz.,* temperature, humidity and rainfall were responsible for the reducing the efficacy of fungicides and biocontrol agent's. Most *Trichoderma* species were mesophilic and cannot protect germinating seeds from soil borne disease during cold, autumn and spring conditions. Water potential also influences the linear mycelial growth, secretion and enzymatic activities of *Trichoderma* strains at different temperature.

From above table, it is observed that plant stand in seed treatment with *T. viride* and was higher as compare to control at different growth stages of plant.

Shanmugaigh *et al.* (2009) reported that, the seed treatment with *T. viride* and *P. fluorescens* shows higher germination due to production of IAA which plays crucial role in plant growth promotion. This biocontrol agent's also produce plant growth regulator which associated with growth stimulation of plants. It was recorded that the fungal and bacterial population were increased in rhizosphere which prevent the population of harmful strain of pathogen resulted in greater plant population.

Similar results were recorded by Andrabi *et al.* (2011), Khan *et al.* (2014), and Rawal *et al*. (2014).

4.4.2 Disease incidence at different growth stages of Ashwagandha

The incidence of *Fusarium solani* causing root rot disease of Ashwagandha was observed in field condition at 30, 60, 90, and 120 DAS.

Data presented in Table 2 and graphically represented in fig 3, revealed that, the minimum root rot incidence was recorded in T_3 (Mancozeb 63% + Carbendazim 12%) is 6%. It was followed by T_1

Table2. Root rot incidence at different growth stages of Ashwagandha

Sr. No.	Treatments	Days after sowing							
		30 DAS	Per cent reduction over control	60 DAS	Per cent reduction over control	90 DAS	Per cent reduction over control	Harvesting Stage (120DAS)	Per cent reduction over control
T_1	Carbendazim	15.01 (22.78)*	52.54	20.01 (26.57)	56.44	21.02 (27.28)	55.50	28.53 (32.28)	47.71
T_2	Mancozeb	21.02 (27.28)	33.54	26.92 (31.25)	41.40	28.22 (32.09)	40.26	36.63 (37.24)	25.14
T_3	Mancozeb 63% + Carbendazim 12% (SAAF)	06.00 (14.14)	81.03	8.9 (17.35)	80.62	10.20 (18.62)	78.40	18.91 (25.77)	61.34
T_4	*Trichoderma viride*	19.01 (25.85)	39.89	24.02 (29.33)	47.71	25.02 (30.00)	47.03	33.13 (35.14)	32.31
T_5	*Pseudomonas fluorescens*	28.02 (31.96)	11.41	40.43 (39.48)	11.99	41.54 (40.12)	12.06	44.74 (41.98)	08.60
T_6	*Pseudomonas fluorescens* + *Trichoderma viride*	23.02 (28.67)	27.22	33.43 (35.32)	27.23	34.73 (36.10)	26.48	39.03 (38.66)	20.24
T_7	Control	31.63 (34.22)	-	45.94 (42.66)	-	47.24 (43.41)	-	48.94 (44.39)	-
	'F' test	Sig.		Sig.		Sig.		Sig.	
	SE(m)±	0.43		0.67		0.66		0.69	
	CD at 5%	1.33		2.08		2.04		2.14	

*Figures in parenthesis are arc sine transformed values

(Carbendazim), T_4 (*T. viride*) and T_2 (Mancozeb) is 15.01%, 19.01%, 21.02% respectively. Maximum root rot incidence was recorded in control treatment i.e. 31.63%.

At 60 DAS, minimum root rot incidence was observed in fungicidal treated seed with (Mancozeb 63% + Carbendazim 12%) 8.9%. It was followed by T_1 (Carbendazim), T_4 (*T. viride*) and T_2 (Mancozeb) is 20.01%, 24.02%, 26.92% respectively. Maximum root rot incidence was recorded in control treatment i.e. 45.94%.

At 90 DAS, fungicide treatment with (Mancozeb 63% + Carbendazim 12%) was again found effective with minimum (10.20%) root rot incidence followed by T_1 (Carbendazim), T_4 (*T. viride*) and T_2 (Mancozeb) is 21.02%, 25.02%, 28.22% respectively. Maximum root rot incidence was recorded in control treatment i.e. 47.24%.

At harvesting stage (120 DAS), fungicide treatment with (Mancozeb 63% + Carbendazim 12%) was found effective with minimum (18.91%) root rot incidence followed by T_1 (Carbendazim) 28.53%, T_4 (*T. viride*) 33.13%, and T_2 (Mancozeb) 36.53%. Maximum root rot incidence was again recorded in control treatment i.e. 48.64%.

Gupta and Misra (2004) reported that *F. solani* causing root rot of Ashwagandha leads to 30-50% mortality of plants. Andrabi *et al.* (2011) reported that seed treatment with Carbendazim + Mancozeb reduced disease incidence (8.56%) significantly.

Similar results were recorded by Chavan *et al.* (2009) in Patchouli.

4.4.3 Shoot and root length (cm) at different growth stages of Ashwagandha

Shoot length (cm)

Data presented in Table 3 and graphically represented in fig 4(a). At 60 DAS, maximum shoot length of plant was recorded in fungicidal seed treatment with Mancozeb 63% + Carbendazim 12% (37.39 cm), followed by seed treatment like Carbendazim (35.19 cm), followed by seed treatment

with bioagent *T. viride* (36.13 cm). Minimum shoot length was recorded in control treatment (33.52 cm).

Table3. Shoot and root length (cm) at different growth stages of Ashwagandha

Sr. No.	Treatments	Shoot length (cm)			Root length(cm) Harvesting stage (120DAS)
		60 DAS	90 DAS	Harvesting stage (120 DAS)	
T_1	Carbendazim	35.19	42.12	46.53	23.00
T_2	Mancozeb	35.72	41.06	46.06	20.80
T_3	Mancozeb 63% + Carbendazim 12% (SAAF)	37.39	42.46	47.41	26.33
T_4	*Trichoderma viride*	36.13	41.72	46.32	21.93
T_5	*Pseudomonas fluorescens*	34.72	39.99	44.73	19.55
T_6	*Pseudomonas fluorescens* + *Trichoderma viride*	35.39	40.92	45.46	19.86
T_7	Control	33.52	39.93	43.00	18.33
	'F' test	Sig.	Sig.	Sig.	Sig.
	SE(m)±	0.61	0.55	0.28	0.95
	CD at 5 %	1.88	1.71	0.86	2.99

At 90 DAS, seed treatment with fungicides like Mancozeb 63% + Carbendazim 12% again found effective with maximum shoot length (42.46 cm), followed by seed treatment with Carbendazim (42.12 cm) followed by antagonist seed treatment with *T. viride* (41.72 cm). Minimum shoot length was recorded in control treatment (39.93 cm).

At the time of harvesting (120 DAS), maximum shoot length, was observed in fungicidal seed treatment with Mancozeb 63% +

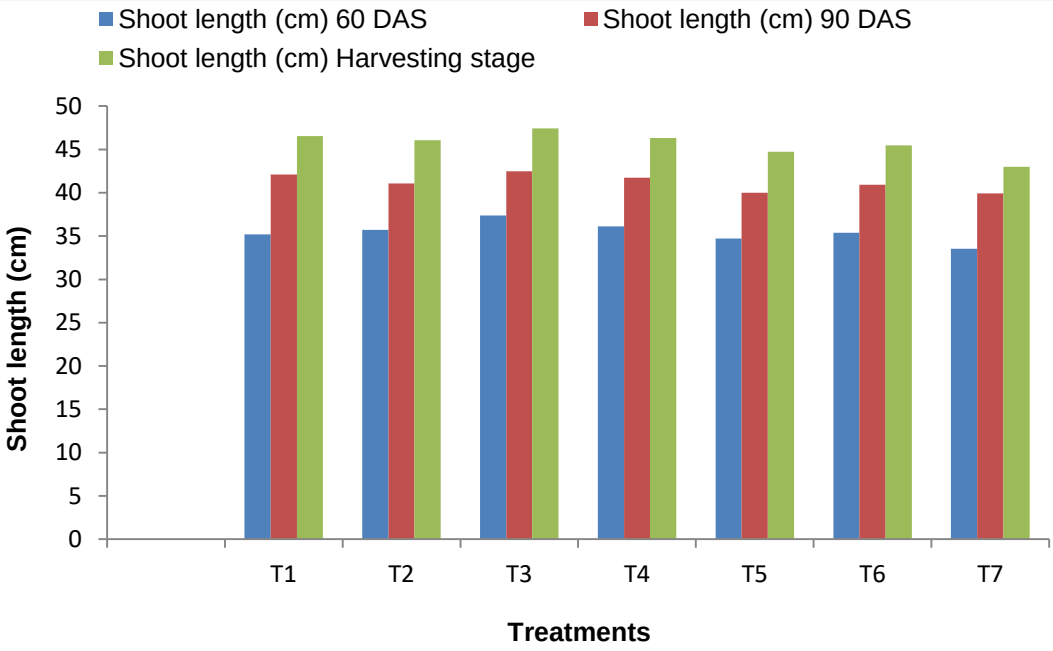


Fig. 4(a) .Shoot length (cm) at different growth stages of Ashwagandha

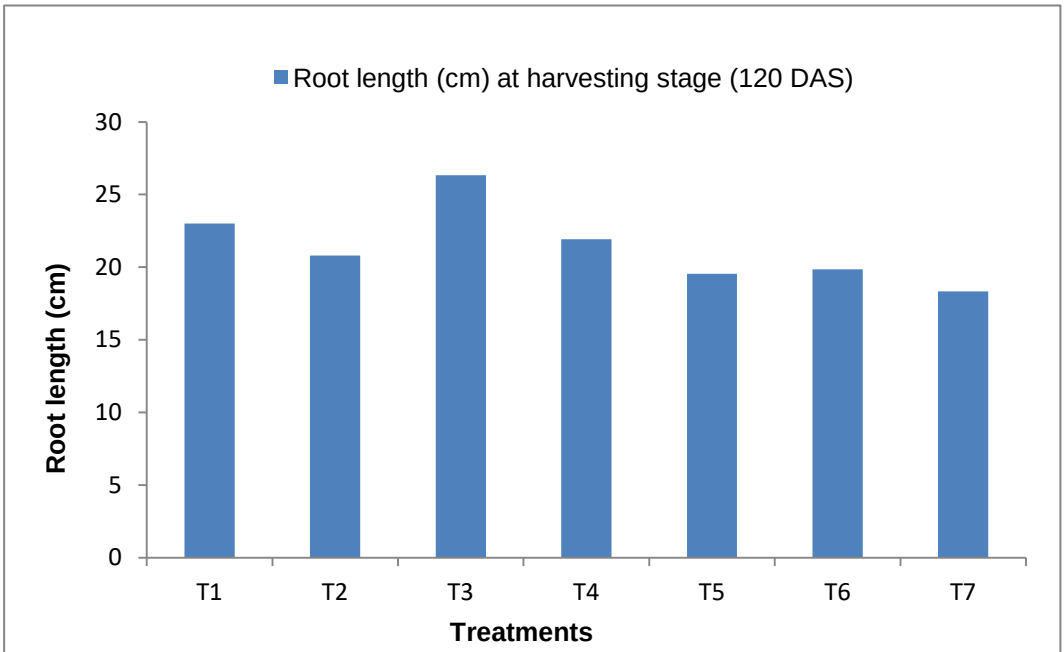


Fig. 4(b) .Root length of Ashwagandha at harvesting stage

Carbendazim 12% (47.41cm) followed by seed treatment with Carbendazim (46.53 cm) followed by antagonist seed treatment with *T*. *viride* (46.32 cm). Minimum shoot length was recorded in control treatment (43.00 cm).

Root length (cm)

Data presented in Table 3 and graphically represented in fig 4(b). At harvesting stage (120 DAS), maximum root length was observed in fungicidal seed treatment with Mancozeb 63% + Carbendazim 12% (26.33 cm), followed by seed treatment with Carbendazim (23.00 cm), followed by seed treatment with bioagent *T. viride* (21.93 cm). Minimum root length was recorded in control treatment (18.33 cm).

Shanmugaiah (2009) reported that, the seed treatment with biocontrol agent's *viz., Trichoderma viride* and *Pseudomonas fluorescens* was responsible for higher shoot and root length due to production of IAA, MG-6, UV-10, MNT-7 and other plant growth regulators.

Maitlo *et al*. (2015) reported that the maximum shoot length and root length was observed in plants treated with Bavistin D.F. while Mancozeb were observed least effective. Similar results were recorded by Marnoranjitham *et al*. (2000) in chilli seedling.

4.4.4 Fresh and dry weight (g) of roots after harvesting of Ashwagandha

Fresh weight (g)

Data presented in Table 4 and graphically represented in fig 5(a). At the time of harvesting, maximum fresh weight per plot was observed in seed treatment with fungicide i.e. Mancozeb 63% + Carbendazim 12% (468 g). It was followed by seed treatment with Carbendazim (406 g), followed by seed treatment with bio agent *T. viride* (369 g). Minimum fresh weight of roots was observed in control treatment (255 g).

Data presented in Table 4 and graphically represented in fig 5(b). Also maximum fresh weight per root was observed in seed treatment with fungicide i.e. Mancozeb 63% + Carbendazim 12% (1.73 g). It was followed by seed treatment with Carbendazim (1.70 g), followed by seed

treatment with bio agent *T. viride* (1.65 g). Minimum fresh weight per root was observed in control treatment (1.50 g).

Dry weight (g)

After harvesting, the fresh roots were collected and dry in clear sunlight condition for 7 days. Further the dried roots were weighted separately. Data presented in Table 4 and graphically represented in fig 5(a). Among this dried root samples the maximum dry weight per plot was obtained in seed treatment with fungicide i.e. Mancozeb 63% + Carbendazim 12% (264.14 g). It was followed by seed treatment with Carbendazim (196 g), followed by seed treatment with antagonist *T. viride* (180 g). Minimum dry weight was observed in control treatment (109.81 g).

Table 4. Fresh and dry weight (g) of roots after harvesting of Ashwagandha

Sr. No.	Treatments	Plant count (Harvesting stage)	Fresh weight of roots per plot	Fresh weight per root	Dry weight of roots per plot	Dry weight per root
T_1	Carbendazim	238	406	1.70	196	0.82
T_2	Mancozeb	211	345	1.63	161.67	0.76
T_3	Mancozeb 63% + Carbendazim 12% (SAAF)	270	468	1.73	246.14	0.91
T_4	*Trichoderma viride*	222.66	369	1.65	180	0.80
T_5	*Pseudomonas fluorescens*	184	296	1.60	130	0.70
T_6	*Pseudomonas fluorescens* + *Trichoderma viride*	203	330	1.62	147	0.72
T_7	Control	170	255	1.50	109.81	0.64
	'F' test	Sig.	Sig.		Sig.	
	SE(m)±	3.11	8.54		5.58	
	CD at 5%	9.59	26.33		17.21	

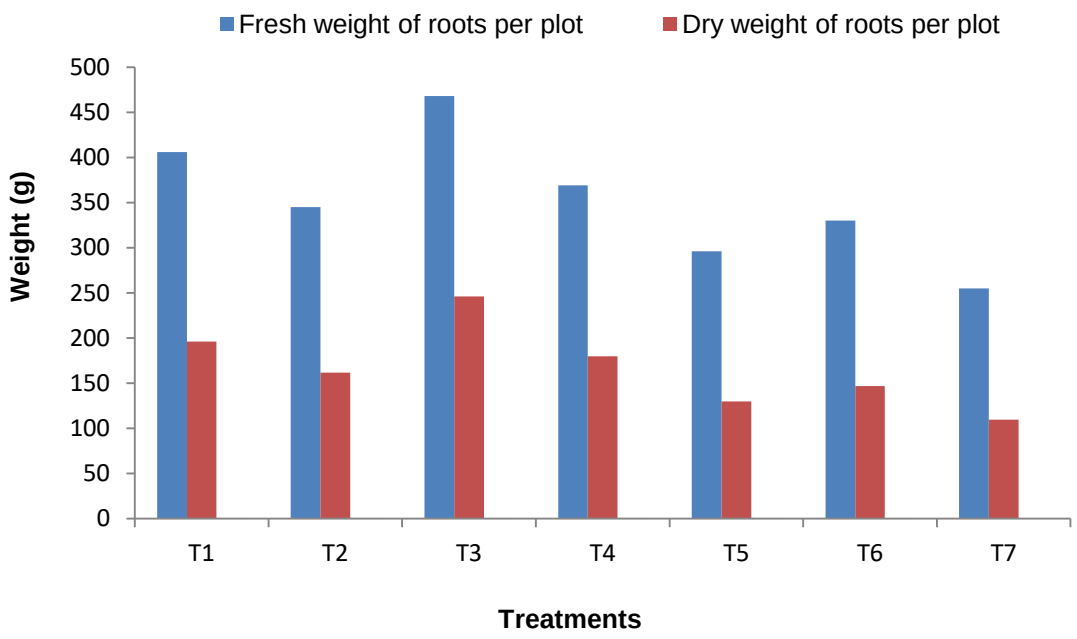


Fig. 5(a). Fresh and dry weight of roots per plot of Ashwagandha

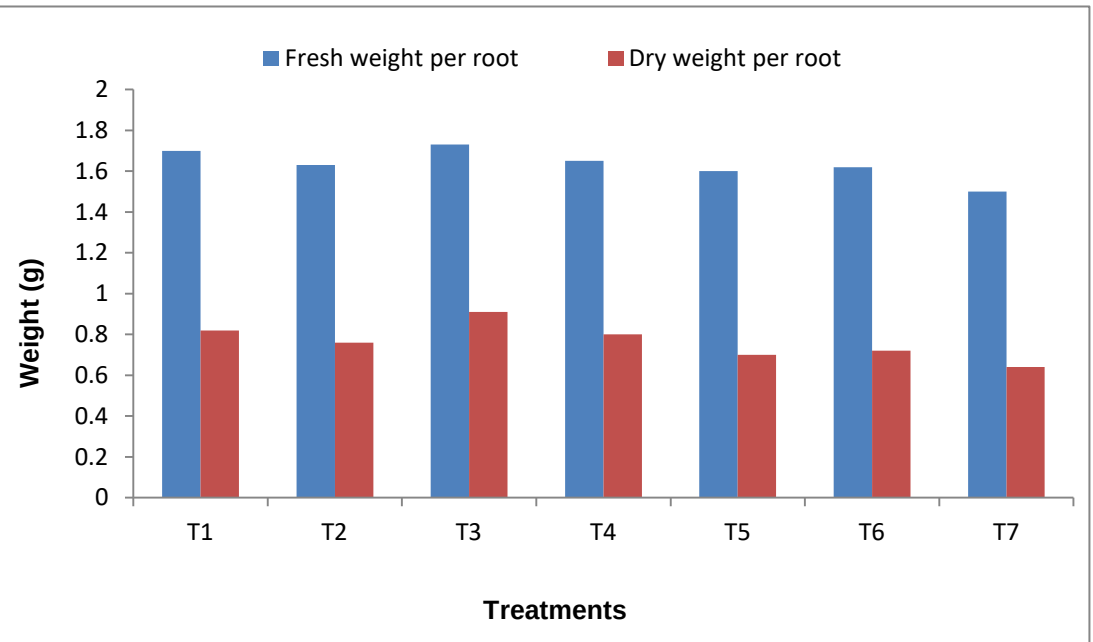


Fig. 5(b) .Fresh and dry weight per root of Ashwagandha

Data presented in Table 4 and graphically represented in fig 5(b). Also maximum dry weight per root was observed in seed treatment with fungicide i.e. Mancozeb 63% + Carbendazim 12% (0.91 g). It was followed by seed treatment with Carbendazim (0.82 g), followed by seed treatment with bio agent *T. viride* (0.80 g). Minimum fresh weight per root was observed in control treatment (0.64 g).

Anju Tanwar *et al*. (2013) recorded that, the application of *T. viride* alone increases fresh root weight (5.93 g) and dry root weight (2.51 g) in Brocoli. Similar results were recorded by Shanmugaiah *et al.* (2009).

4.4.5 Per cent alkaloid content in roots of Ashwagandha

Alkaloids are special group of secondary compounds and are non toxic when stored but toxic when pathogens are attacked to the plants. Alkaloid was extracted from the root powder of Ashwagandha for comparing the difference in alkaloid percentage among various treatments.

Table 5. Per cent alkaloid content in roots of Ashwagandha

Sr. No.	Treatments	Per cent alkaloid content	Per cent Increase over control	Per cent alkaloid per root	Per cent Increase over control	Per cent alkaloid per plot	Per cent Increase over control
T_1	Carbendazim	0.36 (0.60)*	16.12	0.59	47.50	141.12	107.27
T_2	Mancozeb	0.34 (0.58)	09.67	0.52	30.00	109.93	61.47
T_3	Mancozeb 63% + Carbendazim 12% (SAAF)	0.37 (0.61)	18.81	0.67	67.50	182.14	167.53
T_4	*Trichoderma viride*	0.35 (0.59)	13.44	0.56	40.00	126.00	85.07
T_5	*Pseudomonas fluorescens*	0.32 (0.56)	01.61	0.45	12.50	83.20	22.20
T_6	*Pseudomonas fluorescens* + *Trichoderma viride*	0.33 (0.57)	05.67	0.48	20.00	97.02	42.50
T_7	Control	0.31 (0.55)		0.40		68.08	
	'F' test	Sig.					
	SE(m)±	0.004					
	CD at 5%	0.013					

*Figures in parenthesis are square root transformed values

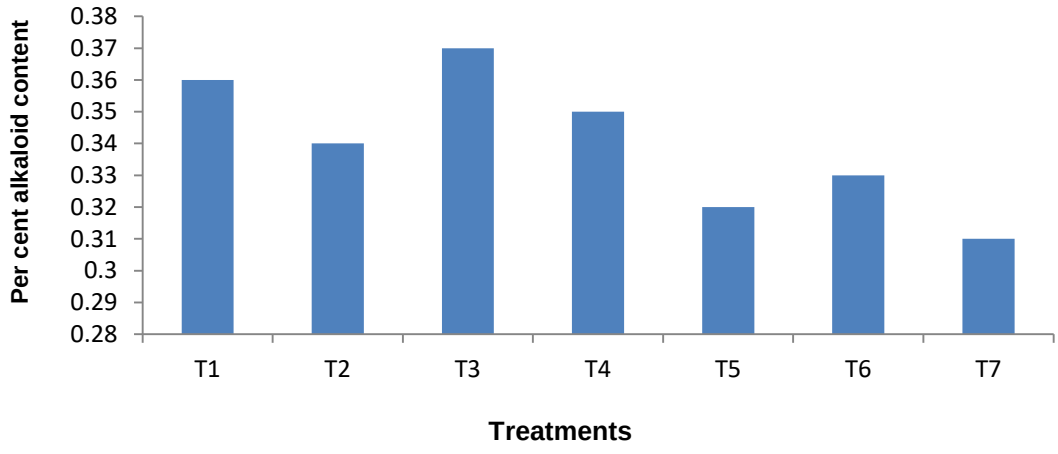


Fig. 6(a). Per cent alkaloid content of Ashwagandha roots

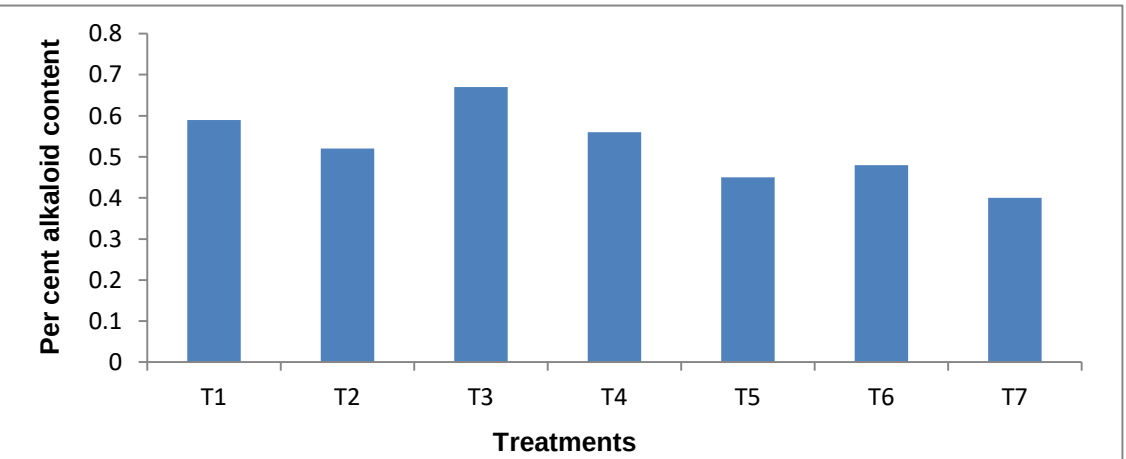


Fig. 6(b). Per cent alkaloid content per root of Ashwagandha

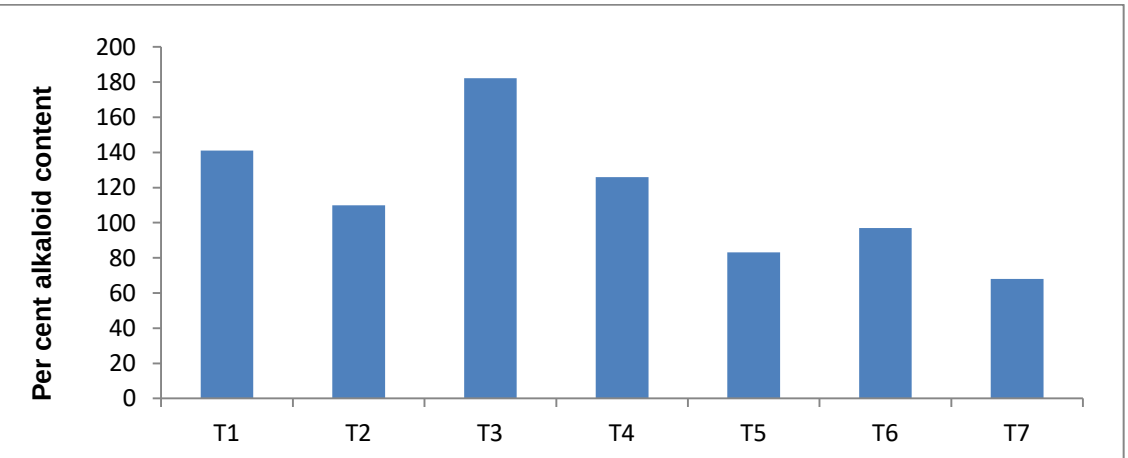


Fig. 6(c) . Per cent alkaloid content per plot of Ashwagandha roots

Plate 5. Alkaloid extraction unit for roots of Ashwagandha

The data was obtained from the Table 5 graphically represented in fig 6(a), revealed that, the maximum alkaloid per cent was recorded in seed treatment with fungicide, Mancozeb 63% + Carbendazim 12% (0.37%). It was followed by seed treatment with Carbendazim (0.36%), followed by seed treatment with bio agent *T. viride* (0.35%). Minimum alkaloid per cent was recorded from control plot (0.33%).

Data presented in Table 5 and graphically represented in fig 6(b&c). Alkaloid per cent was estimated on the basis of dry weight of plant. Alkaloid percent per root and per plot were recorded maximum in fungicide treatment (T_3) and antagonist's treatment (T_4) because dry weight per root and per plot was maximum in chemical treatment (T_3) and antagonist treatment (T_4).

Karthikeyan *et al*. (2009) reported that, the seed priming and seedling treatments of native $PGPR_s$ can be used as a good tool in the enhancement of biomass yield and alkaloid contents in medicinal plant cultivation.

Similar results were recorded by Nigam and Kandalkar (1995) and Rawal *et al*. (2014) in Ashwagandha.

Gurjar *et al*. (2012) reported that, the alkaloid shows antimicrobial effect and serves as plant defense mechanisms against pathogenic microorganisms.

CHAPTER V

SUMMARY AND CONCLUSIONS

Ashwagandha is important medicinal plant with higher alkaloid content in roots. The use of Ashwagandha is increase day by day in Ayurveda and Unani. Ashwagandha is effective on various disorders. But now a day, the diseases have been noticed to tremendously reducing the yield and root quality of Ashwagandha. Hence the present study was carried out under the title "MANAGEMENT OF ROOT ROT OF ASHWAGANDHA (*Withania somnifera*)" in Department of Plant Pathology, Dr. PDKV, Akola.

Ashwagandha was sown and constantly observed at different growth stages at field of Department of Plant Pathology, Dr. PDKV, Akola. Disease causing pathogens were isolated and identified on the basis of morphology. Pathogenicity of isolated pathogen was also proved.

Studies were conducted to know the effect of disease on germination, plant population, shoot and root length, fresh and dry weight of roots and alkaloid content in roots under field and *in vivo* conditions. Management of pathogen also done by using different fungicides and bio agents.

Infection of pathogen was increases with increasing rainfall and humidity. Plant mortality was increases at each succeeding growth stages from sowing to harvesting. The maximum disease incidence was recorded up to 48.94 per cent in field experiments.

For effective management of *Fusarium solani*, Ashwagandha seeds were treated with different fungicides and biocontrol agents.

CONCLUSIONS

From present studies, it was concluded that,

- *Fusarium solani* was predominant fungal pathogen of Ashwagandha causing root rot and resulted in economic loss.

- Maximum germination or plant stand at 30 DAS (93.99%) was recorded in fungicide seed treatment with Mancozeb 63%+ Carbendazim 12%, followed by seed treatment with Carbendazim (84.98%), followed by *T. viride* (80.98%) in field experiment.

- Under *in vivo* condition among fungicides treatments the lowest disease incidence 6%, 8.9%, 10.20% and 18.91% of root rot was recorded in treatment T3 (Seed treatment with Mancozeb 63%+ Carbendazim 12% 75WP @ 2.5g/kg) at 30 DAS, 60 DAS, 90 DAS and 120 DAS respectively.

- Among bio-agent treatments the lowest disease incidence 19.01%, 24.02%, 25.02% and 33.13% of root rot was recorded in treatment T4 (Seed treatment with *T. viride* @ 4g/kg) at 30 DAS, 60 DAS, 90 DAS and 120 DAS respectively.

- Among fungicide the highest shoot length 37.39, 42.46 and 47.41 cm at 60, 90 and 120 DAS respectively and root length 26.33 cm of plant was recorded in seed treatment with Mancozeb 63% + Carbendazim 12% and among antagonist the highest shoot length 36.13, 41.72 and 46.32 cm at 60, 90 and 120 DAS respectively and root length 21.93 cm of plant was recorded in seed treatment *Trichoderma viride* as compared to control.

- The maximum fresh weight (468 g) and dry weight (246.14 g) of plant root was recorded in seed treatment with Mancozeb 63% + Carbendazim 12%.

- Per cent alkaloid content was recorded higher (0.37%) in seed treatment with Mancozeb 63% + Carbendazim 12% as compared to control.

- Finally seed treatment with Mancozeb 63% + Carbendazim 12% @ 2.5 g/kg seed) were found most effective in controlling the root rot of Ashwagandha under field condition.

CHAPTER VI

LITERATURE CITED

Andrabi, M., A. Vaid and V. K. Razdan, 2011. Evaluation of different measures to control wilt causing pathogens in chickpea. J. pl. protect. res. 51(1).

Anju Tanwar, A. Aggarwal, Sunita Kaushish and Sonika Chauhan, 2013. Interactive effect of AM fungi with *Trichoderma viride* and *Pseudomonas fluorescens* on growth and yield of Broccoli. Pl. Protect. Sci. 49(3): 137-145.

Bashar, M. A. and Mina Chakma, 2014. *In vitro* control of *Fusarium solani* and *F. oxysporum* the causative agent of brinjal wilt. Dhaka Univ. J. Biol. Sci. 23(1): 53-60.

Bhaliya C. M. and K. B. Jadeja, 2014. Efficacy of different fungicides against *fusarium solani* causing coriander root rot. The Bioscan. 9(3): 1225-1227.

Bhatti, M. A. and J. M. Kraft, 1992. Effect's of inoculums density and temperature on root rot and wilt of chickpea. Plant Dis. 76: 50-54.

Beevi, N. D. and S. M. H. Qadri, 2010. Biological control of mulberry root rot disease (*Fusarium* spp.) with antagonistic microorganisms. J. Biopest. 3(1): 90-92.

Booth, C. and J. M. Waterston, 1998. *Fusarium solani*: CMI-Descriptions of Fungi and Bacteria. 3:29.

Chavan, S. C., Y. R. Hegde and S. K. Prashanthi. 2009. Management of wilt of patchouli caused by *Fusarium solani*. J. Mycol. Pl. Pathol. 39: 32-34.

EL-Morsi, M. E. A., M. F. Abdel-Monaim, E. F. S. Ahmad, 2015. Management of root rot and wilt diseases of date palm offshoots using certain biological control agents and its effect on growth parameters in the New Valley Governorate, Egypt. J. Phytopathol. and Pest Management 2(1): 1-11.

Gurjar, M. S., S. Ali, M. Akhtar and K. S. Singh, 2012. Efiicacy of plant extracts in plant disease management. Agr. Sci. 3(3): 425-433.

Gupta, J. H. and V. P. Srivastava, 1976. A new root rot of fennel caused by *Fusarium solani*. Indian J. PI. Pathol. 15: 206.

Gupta, M. L., H. O. Misra, A. Kalra and S. Khanuja, 2004. Root rot and wilt: A new disease of ashwagandha (*Withania somnifera*) caused by *Fusarium solani*. J. Medicinal and Aromatic PI. Sci. 26(3): 285-287.

Hafiza Asma Shafique, V. Sultana, S. Ehteshamul-Haque and M. Athar, 2016. Management of soil-borne diseases of organic vegetables J. Pl. Protec. Res. 56(3): 221-229.

Jayashree, K., V. Shanmugam, T. Raghuchander, A. Ramanathan and R. Samiyappan, 2000. Evaluation of *Pseudomonas fluorescens* (pf-1) against blackgram and sesame root rot disease. J. Biol. Control. 14(2): 55-61.

Jetawat, R. P. S. and K. Mathur, 2016. Management of Ashwagandha root rot disease with fungicides, biocontrol agents and botanicals. J. Applied and Natural Sci. 8(1): 305-309.

Joshi, V. and S. P. Raut, 2005. Promising bioagents in management of *Fusarium solani* causing seedling wilt of Cashew hybrid. Annl. PI. Prot. Sci. 13(1): 149-151.

Kapadiya, I. B., L. F. Akbari, M. R. Siddhapara and S. V. Undhad, 2013. Evaluation of fungicides and herbicides against the root rot of okra. The Bioscan. 8(2): 433-436.

Kapoor, I. J. and B. Kumar, 1991. Relative efficacy of systemic and non systemic fungicides against *Fusarium oxysporum* and *F. solani* affecting tomato. Indian Phytopath. 44(1): 87-93.

Karthikeyan, B., C. A. Jaleel and M. M. Azooz, 2009. Individual and combined effects of *Azospirillum brasilense* and *Pseudomonas fluorescens* on biomass yield and ajmalicine production in *Catharanthus roseus*. Academic J. Pl. Sci. 2(2): 69-73.

Khan, M. R., S. Ashraf, F. Rasool, K. M. Salati, F. A. Mohiddin and Z.Haque, 2014. Field performance of *Trichoderma* species against wilt disease complex of chickpea caused by *Fusarium oxysporum* f. sp*. ciceri* and *Rhizoctonia solani.* Turk. J. Agric. For. 38: 447-454.

Khanzada, M. A., M. Tanveer, S. A. Maitlo, J. Hajano, A. A. Ujjan, R. N. Syed, A. M. Lodhi and A. Q. Rajput, 2016. Comparative efficacy of chemical fungicides, plant extracts and bio-control agents against *Fusarium solani* under laboratory conditions. Pak. J. Phytopathol. 28(2): 133-139.

Kredics, L, A. Zsuzsanna, L. Manczlnger, A. Szekeres, F. Kevel and E. Nagy, 2003. Influence of environmental parameters on *Trichoderma* strains with biocontrol potential. Food Tech. Biotech. 41(1):37-42.

Lahoz, E., R. Nicoletti, R. Contillo and F. Raimo, 1996. *Fusarium solani* associated with crown and root rot of tobacco. Petria. 6(2): 189-193.

Larkin, R. P. and D. R. Fravel, 1998. Efficacy of various fungal and bacterial biocontrol organisms for control of *Fusarium* wilt of tomato. Plant Dis. 32(9): 1022-1028.

Maharshi, R. P. 2006. *Fusarium solani* and *Rhizoctonia bataticola* cause of seedling mortality of Ashwagandha (*Withania somnifera*) and their management. J. Med. And Aromatic Pl. Sci. 28(4): 585-588.

Maitlo, W. A., G. S. Markhand, A. A. Abul-Soad, A. M. Lodhi and M. A. Jatoi, 2015. Evaluation of various fungicides against *Fusarium solani* (mart.) sacc causing sudden decline disease of date palm (*Phoenix dactylifera*) In Sindh (Pakistan). Pak. J. Phytopathol. 27(1): 07-12.

Mallesh, S. B. and T. Narendrappa, 2009. Evaluation of microbial antagonists and organic amendments on root rot of sage (*Salvia officinalis)* by *Fusarium solani* and *Rhizoctonia solani*. Indian J. Pl. Protect. 37(1&2): 119-122.

Marnoranjitham, S. K., V. Prakasam and K. Rajappan, 2000. Biological control of chilli damping-off using talc based formulations of antagonists. Annals Pl. Protec. Sci. (India).

Matuo, T. and W. C. Snyder, 1972. Host virulence and the Hypomyces stage of *Fusarium solani* f.sp. *pisi*. Phytopathol. 62: 731-735.

Nasreen Sultana and A. Ghaffar, 2010. Effect of fungicides, microbial antagonists and oilcakes in the control of *Fusarium solani*. The cause of seed rot, seedling and root infection of bottle gourd, bitter gourd and cucumber. Pak. J. Bot. 42(4): 2921-2934.

Nidhi Bharti, Purvi Agrawal, B. Misra, Arpita Tripathi, R. Singh, Deepamala Maji, H. P. Singh and A. Kalra, 2013. Efficacy of combined applications of antagonist bacteria and chemical resistance inducers for the management of *Fusarium solani* causing root rot in *Withania somnifera.* Biocontrol Sci.Tech. 23(2): 239-244.

Nigam, K. B. and V. S. Kandalkar, 1995. Ashwagandha. Advances in Horticulture- Medicinal and Aromatic Plants. 11: 337-344.

Panka, D. and C. Sadowski, 1999. Pathogenicity of *Fusarium solani* isolates excised from different plants. Phytopath. 18: 81-93.

Patil, S. A., A. M. Parakhia and L. F. Akbari, 2001. Management of root rot (*Fusarium solani*) of cotton. Guj. Agric. Univ. Res. J. 26(2): 44-49.

Prasad, D., K. P. Singh, and S. Bisht. 2011. Fungicidal management of wilt complex of scented geranium. Archives of Phytopathol. and Pl. Protec. 44(15): 1519-1533.

Raghuchander, T., K. Jayashree and R. Samlyappan, 1997. Management of *Fusarium* wilt of banana using antagonistic microorganisms. Pl. Dis. 11: 101-105.

Ramakrishnan, G., K. Sethuraman and N. Chezhiyan, 1996. Wilt of custard apple caused by *Fusarium solani*. South Indian Hort. 44(1/2): 61.

Ramaswamy, G. R. and T. B. A. kumar, 1997. Negative cross resistance in Carbendazim resistant strain of *Fusarium* f. sp*. piperis*. PI. Dis. Res. 12(2): 136-138.

Rathnamma, K., A. N. A. Khan, A. A. Farooqui and M. R. Ravikumar, 1999. Root rot of garden rue (*Ruta graveolens* L.) caused by *Fusarium solani* (Mart.) Sacc. Adv. PI. Sci. Res. 9: 41-48.

Rawal, P., R. P. Singh and Lekha, 2014. Integrated Root Rot Management of Ashwagandha [*Withania somnifera* (L.) Dunal)] VOL.-III, ISSUE-III.

Shankar, K. and R. Jeyarajan, 1996. Biological control of sesamum root rot by seed treatment with *Trichoderma* spp. and *Bacillus subtilis*. Indian J. Mycol. PI. Pathol. 26(2): 217-220.

Shanmugaiah, V., N. Balasubramanian, S. Gomathinayagam, P. T. Manoharan and A. Rajendra, 2009. Effect of single application of *Trichoderma viride* and *Pseudomonas fluorescens* on growth promotion in cotton plant. African J. Agril. Res. 4(11): 1220-1225.

Sharma, N. D. and A. C. Jain, 1984. *In vitro* evaluation of fungicides against some *Fusarium* spp. Pesticide, pp. 37-38.

Sharma, S., R. Pal, P. P. Gupta and J. C. Kaushik, 2004. Management studies on root rot of *Prosopis cineraria* caused by *Fusarium solani*. J. Tropical Forest Sci. 16(1): 71-77.

Singh, F., I. Hooda and G. S. Sidhan, 2004. Biological control of tomato with caused by *Fusarium oxysporum* f.sp*. lycopersici*. J. Mycol. PI. Pathol. 34(2): 568-570.

Singh, M., 2013. Epidemiology and management of root rot disease of Bael (*Aegle marmelos* Correa) caused by *Fusarium solani* (Mart.) Sacc. Thesis (unpub.) M.Sc. (Agri). CCS H. A.U., Hisar.

Singh, I., R. K. Tombisana Devi and P. P. Devi, 2000. Effect of fungicides on growth and sporulation of *Fusarium solani*. Indian Phytopath. 53(3): 327-328.

Swami, D. V., 2011. Standardization of agro-techniques for Ashwagandha (*withania somnifera* Dunal.) in coastal districts of Andhra Pradesh. Thesis (unpub.) M.Sc. (Agri). COA, A. N. G. R. A. U., Rajendranagar, Hyderabad.

Taskeen- Un- Nisa, A. H. Wani, M. Y. Bhat, S. A. Pala and R. A. Mir, 2011. *In vitro* inhibitory effect of fungicides and botanicals on mycelia growth and spore germination of *Fusarium oxysporum*. J. Biopest. 4(1): 53-56.

Vyas, S. C., 1994. Integrated biological and chemical control of dry root rot on soybean. Indian J. Mycol. PI. Pathol. 24(2): 132-134.

Wuike, R. V., A. A. Nanoti, S. G. Bhoyate and R. D. Kitkaru, 1995. Biocontrol of root / stem rot of sesame with the antagonist, *Trichoderma viride*. J. Soils and Crops. 5(2): 145-147.

Zaidi, N. W. and U. S. Singh, 2004. Development of improved technology for mass multiplication and delivery of fungal (*Trichoderma*) and bacterial (*Pseudomonas*) biocontrol agents. J. Mycol. PI. Pathol. 34(3): 732-740.

Zapata, R. L., H. E. Palmucci and V. Blanco-Murray, 2001. *Fusarium solani* causal agent of wilt and root rot of egg plant (*Solarium melongena*) in Argentina. Phytopathol. 36(1): 15-18.

Zape, A. S., R. M. Gade, R. Singh and V. A. Deshmukh, 2014. Efficacy of different antagonist against the *Sclerotium rolfsii*, *Rhizoctonia solani* and *Fusarium solani*. The Bioscan. 9(4): 1431-1434.

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Date: / / 2017

(BORADE ATUL SUBHASH)

APPENDIX

Potato Dextrose Agar

Peeled potato	-	200 g
Dextrose	-	20 g
Agar	-	20 g
Distilled water	-	1000 ml

Potato Dextrose Broth

Peeled potato	-	200 g
Dextrose	-	20 g
Distilled water	-	1000 ml

SAAF = Mancozeb + Carbendazim