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Management of *Pythium deliense*-induced damping-off in acid lime seedlings using fungicides, essential oils and biocontrol agents

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This study was undertaken to assess the most effective treatments,including fungicides, essential oils, and bio-agents for the control of damping-off in acid lime under laboratory conditions, as well as their effectiveness under nursery conditions. The poisoned food technique was used to assess the *in vitro* efficacy of various fungicides and essential oils against *Pythium deliense*. The fungicides Cymoxanil 8% + Mancozeb 64% WP, Metalaxyl 8 % + Mancozeb 64% WP and Copper Hydroxide 53.8% DF were found to be the most effective, completely inhibiting the growth of the test pathogen. Among the essential oils, Citronella oil, Lemongrass oil, Tea tree oil and Garlic oil were also found to completely inhibit the growth of the pathogen. Utilizing dual culture technique, *Trichoderma asperellum* emerged as the most promising among the tested bio-agents in inhibiting radial mycelial growth of *Pythium deliense*. The effect of these best fungicides, essential oils and bio-agent treatments on the growth parameters and damping-off in acid lime seedlings was studied under nursery conditions. Pre- and post-emergence damping off (mortality of seedlings) observed across all treatments ranged 4.0 to 11.00% and 6.00 to 15.00% respectively. Cymoxanil 8% + Mancozeb 64% WP exhibited the lowest pre-emergence (4.00%) and post-emergence mortality (6.00%), alongside the highest percentage reduction in pre-emergence (66.66%) and post-emergence seedling mortality (62.50%), followed by the combi product Metalaxyl 8% + Mancozeb 64% WP.

Keywords : Acid lime, *Citrus aurantifolia*, damping-off, evaluation, *Pythium deliense*

INTRODUCTION

Acid lime (*Citrus aurantifolia* Swingle) is economically propagated by seeds in India because of its high degree of nucellar embryony, which guarantees true-to-type plants, uniformity, regular bearing, etc. Its propagation method is also the simplest and least expensive. Seed propagation is still widely practiced by nursery growers across the country. Biotic stresses can result in poor germination and pre and post-emergence damping off in acid lime seeds and seedlings. In most farmer's nurseries, acid lime seedlings suffer pre-emergence and post-emergence damping off, which results in seedling decline and poses a serious risk to seedling raising.

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Damping off of acid lime is one of the most dangerous and prevalent acid lime diseases in Maharashtra's Vidarbha region. Every year, substantial economic losses occur due to damping-off disease. Damping-off of seedlings in nursery beds is a widespread problem in the citrus industry. More than 20% seedlings mortality has been observed in Central India due to the disease in nursery beds. Damping off is a disease caused by a variety of pathogens that kill or weaken seeds or seedlings before or after germination. Damping off caused by *Pythium* spp., *Fusarium solani, S. rolfsii* and *Phytophthora* spp. is probably the worst enemy to acid lime seedlings in nursery (Naqvi, 1999).

Pythium is one of the most important genera of soil-borne plant pathogens, found in almost every agricultural soil. It attacks the roots of thousands of hosts, reducing crop yield and quality

(Schroeder *et al*. 2013). They can live saprophytically, but under specific conditions, they can become pathogenic and cause pre- or post-emergence damping off of seeds and seedlings from a variety of plants. *Pythium* species have a diverse host range, and disease development is heavily influenced by environmental factors. *Pythium irregulare* and *P. ultimum*, as well as *Pythium aphanidermatum* and *P. myriotylum,* have been documented to induce damping off in a variety of crops, including fruits and vegetables (Adhikari *et al*. 2024)

An increasing number of strategies have been developed to control damping off disease of acid lime by suppressing or killing pathogens. To date, chemical control is the most widely used and most effective of these strategies. Biological control and non-conventional approaches are alternative strategies for combating plant diseases. It is eco-friendly, inexpensive and sustainable in protecting plants.

MATERIAL AND METHODS

In vitro evaluation of fungicides

Commercially available newer single and pre-mixed ten chemical fungicides (Table 1) were evaluated by employing poisoned food technique (Nene and Thapliyal, 1993) against *Pythium deliense*.

Oat meal agar was prepared and distributed at the rate of 100 ml in each 250 ml conical flask; autoclaved at 1.05 kg/cm^2 for 15 minutes. Requisite quantity of each of the chemical fungicides as per concentration was added in sterilized melted Oat meal agar separately so as to obtain desired concentration. Flask containing poisoned medium was shaken well to have even and uniform distribution of fungicides. About 20 ml of poisoned Oat meal agar was poured in each pre sterilized Petri plate and allowed to solidify. These Petri plates were inoculated by test pathogen. Five mm discs of 7 days old fungal culture were cut with sterilized cork borer and transferred to the centre of plates in inverted position containing poisoned medium. Suitable controls without fungicides were maintained by placing fungal discs in untreated plates. The whole procedure was carried out under aseptic conditions. Experiment conducted in Completely Randomized Design (CRD) with three replications. The Petri plates were then incubated in an incubator at 25±2°C for 10 days.

The diameter of the colony of the fungal pathogen on medium was measured after 10 days of incubation and per cent inhibition was calculated by using the formula given by Vincent (1947).

In vitro evaluation of essential oils

Eight essential oils were evaluated by employing poisoned food technique (Nene and Thapliyal, 1993) against *Pythium deliense*. Oat meal agar was prepared and distributed at the rate of 100 ml in 250 ml conical flask; autoclaved at 1.05 kg/cm^2 for 15 minutes. The pure grade essential oil in the selected concentrations was individually dispersed as an emulsion using Tween 80 (0.05%) and added to autoclaved Oat meal agar immediately before pouring it into Petri dishes. Flask containing poisoned medium was shaken well to have even and uniform distribution of essential oils. About twenty ml of poisoned Oat meal agar was poured in each pre sterilized Petri plate and allowed to solidifying. These Petri plates were inoculated by test pathogen. 5 mm discs of 7 days old fungus cultures were cut with sterilized cork borer and transferred to the center of plates in inverted position containing poisoned medium. To prevent test oils from leaking, Petri dishes were sealed with parafilm. Suitable controls without essential oils were maintained by placing fungal discs in untreated plates. The whole procedure was carried out under aseptic conditions. The experiment was conducted in a Completely Randomized Design (CRD) with three replications. The Petri plates were then incubated in an incubator at 25±2°C for 10 days.

The diameter of the colony of the respective fungal pathogens on medium was measured after 10 days of incubation and per cent inhibition was calculated by using the formula suggested by (Vincent, 1947).

In vitro evaluation of bio-agents

The dual culture technique (Dennis and Webster,

1971) was used to test the antifungal activity of bio-agents**.** The different bio-agents were tested against *Pythium deliense*. The pathogens and bio-agents were grown on different media for a week at 25±2°C. Five culture discs (each 5 mm) one disc of the test pathogen and four discs of bio-agents were cut out with sterilized corkborer and pathogen disc was placed at centre while four discs of bio-agents were placed at periphery on autoclaved and cooled solid surface of Oat meal agar medium in Petri plates. For bacterial antagonists, streaking was done in parallel lines on both the sides of the pathogen discs (at a distance of 15 mm) and test pathogen disc was kept in the centre. Five replications of each treatment were maintained and incubated at 25±2°C. Oat meal agar plates inoculated alone with pure culture disc of the test pathogen were maintained as control. The experiment was conducted in Completely Randomized Design (CRD) with five treatments and a control and five replications.

Linear mycelial growth of test pathogen was tested after 24 hours, continued till untreated control plates were fully covered with mycelial growth of test pathogen and averaged finally. Per cent inhibition of the test pathogen with test bio-agents, over control was calculated by applying formula suggested by (Vincent, 1947).

In vivo effect of different treatments on damping off of acid lime

The experiment was carried out at AICRP on Fruits scheme to evaluate the efficacy of best effective fungicides, essential oils and bio-agents against damping off disease of acid lime in nursery conditions. The experiment consists of following seed treatments:

Matured fruits of acid lime (Local Kagzi lime) were harvested from the mother trees and the seeds were extracted carefully for avoiding any injury to the embryo. The best treatments identified under laboratory conditions were evaluated under nursery conditions. The seeds were treated with different fungicides, essential oils and bio-agents. For each treatment about 100 seeds were taken on a vessel. The respective fungicides, essential oils and bio-agents were mixed with the seeds thoroughly to put a layer of fungicidal chemicals, essential oils and bio-agents cover over the seed coat. Then the seeds were dried in open air for twenty minutes. Untreated seed served as control.

The experiment was conducted in plastic tray of 60 x 40 x 12 cm size with proper hole for well drainage and were placed at one feet height. For the experiment the potting media was prepared with sand: soil: FYM (1:1:1). Then the broth culture of *Pythium deliense* was incorporated into the soilat the rate of 40 ml per tray. The seeds were sown in the tray. The observation on per cent germination, pre-emergence and post-emergence damping off, percent reduction in pre and post-emergence damping off and final plant stand were calculated as analysed by Upadhyay and Mukhopadhyay (1986).

After 25 days of seed sowing, the germination percentage of the seeds and percentage increase over control was determined using the formula below -

$$\% \text{ Germination} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

$$\% \text{ Increase Germination over control} = \frac{\text{Number of seeds germinated in treatment - Number of seeds germinated in control}}{\text{Number of seeds germinated in control}} \times 100$$

Pre-emergence damping off and Post emergence damping off was calculated by using following formula -

$$\text{Pre-emergence damping off (\%)} = \frac{\text{Number of diseased seeds decayed before germination}}{\text{Total number of seeds sown}} \times 100$$

The percentage damping-off (Pre and Post) reduction over control was computed using the following formula :

$$\text{Post-emergence damping off (\%)} = \frac{\text{Number of diseased seedlings toppled due to damping-off}}{\text{Total number of emerged seedlings}} \times 100$$

The percentage damping-off (Pre and Post) reduction over control was computed using the

following formula:

$$\% \text{ Reduction in damping off} = \frac{\text{Number of diseased seeds/seedlings in control} - \text{Total number of seeds/seedlings in treatment}}{\text{Number of diseased seeds/seedlings in control}} \times 100$$

Experiment was carried out in Randomized Block Design (RBD) and each treatment and replicated thrice. The data of each character were subjected to analysis of variance (ANOVA) for different treatments. Fisher's protected critical difference (CD) test was used to indicate the difference between the treatments at the probability level of p= 0.01 and p=0.05 following the procedure described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

In vitro evaluation of fungicides

The colony's radial growth data were noted and the percentage of growth inhibition was computed.The results (Table 2) showed that all of the tested fungicides, at their respective concentrations, significantly suppressed mycelial growth of *P. deliense* compared to the untreated control. Mycelial growth inhibition varied from 13.33% to 100%. Cymoxanil 8% + Mancozeb 64% WP, Metalaxyl 8% + Mancozeb 64% WP and Copper Hydroxide 53.8% DF all exhibited complete (100%) inhibition of mycelial growth of the tested pathogen. Metalaxyl 35% WS and Benalaxyl-M 4% + mancozeb 65% WP were the next best fungicides resulted with growth inhibition rates of 92.96% and 88.88%, respectively. Metalaxyl-M 3.3% + Chlorothalonil 33.1% SC and Fosetyl-Al 80% WP recorded 83.33% and 77.77% growth inhibition. Thiophanate Methyl 45% + Pyraclostrobin 5% FS and Mandipropamid 23.4% SC was shown to be the least effective fungicide, with only 13.33% and 22.22% of the tested fungus demonstrating growth inhibition, respectively.

It can be concluded that the tested fungicides differ greatly in their effectiveness in control of *P. deliense*. Several researchers have recommended these fungicides for the control of oomycetes fungi particularlyseveral species of *Pythium* and *Phytophthora* (Gade *et al*. 2005; Kaur *et al*. 2009; Thakre and Soni, 2018).

The present findings agree with Trivedi *et al*. (2021) who found that combination formulation of metalaxyl and mancozeb (8% + 64% WP) at three doses (2,3 and 4 ml/L) reduced disease severity and increased productivity when treating ginger soft rot caused by *Pythium aphanidermatum*. Sole application of metalaxyl 35% WS and mancozeb 75% WP was found less effective as compared to combined formulation. Naqvi (2001) also demonstrated the inhibitory effects of fosetyl-Al, metalaxyl 4% + mancozeb 64%, and metalaxyl on *Phytophthora* spp. in citrus. According to Kaur *et al*. (2009), the most promising fungicides for citrus foot rot under field conditions were Ridomil Gold 68 WP, Ridomil MZ 72 WP, and Curzate M8. Boughalleb *et al*. (2006) found that Ridomil Gold MZ 68 (Metalaxyl + mancozeb) and Curzate M (cymoxanil + mancozeb) inhibited the growth of *Phytophthora cactorum.* Fosetyl-Al (aluminum tris-O-ethyl phosphonate) and metalaxyl-M + mancozeb are registered to prevent oomycetes, which cause diseases such as damping off and root and lower stem rots in agricultural and horticulture crops (Turkolmez and Dervis, 2017). However, there were no previous investigations on its effectiveness against *P. deliense* associated with acid lime damping-off.

In the present study, cymoxanil 8% + mancozeb 64% WP showed 100% growth inhibition of *P. deliense*. It is a combination product available in the market that has both protective and early curative action properties. Mancozeb acts by its contact action and fungitoxic when exposed to air. It is converted to an isothiocyanate, which inactivates the sulfhydryl (SH) groups found in fungal enzymes. Metals are sometimes transferred between mancozeb and fungal enzymes, disrupting fungal enzyme function. Other chemical is cymoxanil, has contact and locally systemic activity. It inhibits sporulation in fungi and reduces spore viability.

The fungicides thiophanate methyl 45% + pyraclostrobin 5% FS inhibited *P. deliense* the least (13.33%). This finding is comparable to that of Biljana(2022), who documented that the fungicide thiophanate methyl 70 % WP (0.1%) has no efficacy in suppressing the *Pythium debarianum* infection.

In vitro evaluation of essential oils

Plant essential oils were tested *in vitro* against *Pythium deliense* utilizing the poisoned food technique. Among the essential oils at 0.5% concentration, Citronella oil, Lemongrass oil, Tea tree oil, and Garlic oil showed no growth (0.00 mm) of *Pythium deliense*, proving to be greatly superior. Lime oil (15.00 mm) and neem oil (85.00 mm) came next in order of merit. Karanj oil exhibited slight inhibitory activity at 1% concentration, whereas eucalyptus oil showed no inhibitory effect(Table 3).

Essential oils suppressed *Pythium deliense* growth at 1% concentration, with radial mycelial growth ranging from 00.00 mm (Citronella oil, Lemongrass oil, Tea tree oil, Lime oil and Garlic oil) to 90.00 mm (Eucalyptus oil), compared to 90mm in the untreated control. At 1% concentration Citronella oil, Lemongrass oil, Tea tree oil, Lime oil and Garlic oil, were shown to be the most efficient in preventing the pathogen with the least mycelial growth (0 mm). Following this, Karanj oil (75.00 mm) and Neem oil (80mm) were applied. Eucalyptus oil was shown to be ineffective, with maximum mycelial growth of 90.00 mm observed in this oil (Table 3). Citronella oil, Lemongrass oil, Tea tree oil and Garlic oil limit growth (100%) completely at concentrations of 0.5 and 1% respectively. Lime oil inhibited pathogen growth by 83.33% at 0.5% concentration but completely suppressed it at 1% concentration. However, even at higher (1%) concentrations, eucalyptus oil did not inhibit mycelial growth.

The antifungal properties of essential oils were dose-dependent, with larger dosages demonstrating stronger antifungal activity. Many plant-derived essential oils and extracts exhibit antimicrobial properties against various plant diseases. Therefore, various essential oils were evaluated in current study for their efficacy against *Pythium deliense* under *in vitro* conditions, and interesting results were found, including Citronella oil, Lemongrass oil, Tea tree oil, Lime oil and Garlic oil, which completely inhibited mycelial growth (100%) at tested dosages. Other researchers have previously proven the inhibitory effect of essential oils on a variety of soil-borne and foliar diseases (Shrivastava *et al*. 2011; Amini *et al*. 2012; Abdel-Kader *et al*.2012; Dianez*et al*. 2018).

The inhibitory activity of plant essential oil observed in the study could be attributed to the existence of biologically active anti-fungal chemicals. Essential oils contain a variety of bioactive chemicals, including alkaloids, flavonoids, phenols, quinone saponins, sterols, and tannins, which have antimicrobial properties (Bajpai *et al*. 2009). Furthermore, Devi *et al*. (2010) reported that hydrophobicity is a key property of essential oils. This disrupts the cell structure, resulting in increased porosity. Due to the loss of ions and critical compounds from fungal and bacterial cells finally leads to cell death. According to Bruni *et al*. (2004), essential oils contained antimicrobial compounds such as b-selinene (2.1%), benzaldehyde (3.6%), 1,8-cineole (8.0%), methyl cinnamate (21.6%), and transcinnamaldehyde (27.9%), which effectively inhibit the growth of *P. ultimum* with a MIC of 500 ìg/ml. In addition, Tzortzakis and Economakis (2007) found that lemongrass oil had antifungal action against *Colletotrichum coccodes*, *B. cinerea,Cladosporium herbarium*, *Rhizopus stolonifer* and *A. niger in vitro*. In a study by Amini *et al*. (2012), essential oils from *Thymus kotschyanus, Thymus vulgaris* and *Zataria multiflora* plants were found to effectively inhibit the growth of four pathogenic fungi: *Fusarium graminearum, Rhizoctonia solani* (AG4), *Sclerotinia sclerotiorum* and *Pythium aphani-dermatum*. According to Bi *et al*. (2012) among 14 evaluated commercial essential oils, oregano, palmarosa, and red thyme showed the lowest EC_{50} values (<0.15 μg/ml) for preventing the formation and germination of sporangia and zoospores, as well as mycelial development of *P. capsici.* Jagtap *et al*. (2012) also documented that garlic extract inhibited the most mycelial growth of *P. nicotianae* (47.26%), followed by Neem (38.65%), Onion (32.38%), Ginger (27.26%), and Tulsi (22.81%). Findings also match with the findings of Dianez *et al*. (2018) who demonstrated that in 12 essential oils *viz Syzygium aromaticum, Pelargonium graveolens, Lavandula angustifolia, Cupresus sempervirens, Mentha piperita, Santolina chamaecyparissus, Citrus sinensis, Pogos-temon patchouli, Thymus mastichina, Thymus vulgaris, Eucalyptus*

globulus and *Rosmarinus officinalis* having antifungal efficacy against *Botrytis cinerea, Sclerotinia sclerotiorum, Fusarium oxysporum, Phytophthora parasitica, Pythium aphanidermatum, Alternaria brassicae, Cladobotryum mycophilum,* Also said that oils inhibited the tested fungus in a dose-dependent manner, with higher concentrations increasing inhibition rate.

Present *in vitro* essential oil studies aid in the identification of safe, inexpensive, and easily degradable plant-based compounds that can be used in disease management strategies to reduce the use of hazardous agrochemicals.

In vitro evaluation of bio-agents

Data presented in Table 4 revealed that, bio-agents tested were found effective in the inhibition of mycelial growth of *Pythium deliense*. *Trichoderma asperellum* exhibited the most reduction in *Pythium deliense* mycelial growth (10.17mm) with the highest inhibition (88.70%), whereas *Bacillus subtilis* (60.79 mm) showed the lowest reduction in *P. deliense* mycelial growth with the lowest inhibition (32.45%).The next best bio-agent was *Trichoderma harzianum*, which inhibited growth by 82.96%. Bacterial bio-agent *Pseudomonas fluorescens* exhibited 65.68% growth inhibition. The maximum mycelial growth of *Pythium deliense* was noted in control (90 mm).

The examined species effectively reduced pathogen growth in two ways: direct parasitism and antibiosis. The current data imply that fungal antagonists reduce pathogen growth more effectively than bacterial antagonists. The above findings are consistent with the previous research workers *viz*., El-Mohamedy, (2012) reported that the *T. harzianum* and *T. viride* had larger inhibitory effects on *Pythium ultimum* than those of *P. fluorescens* and *B. subtilis*. *Trichoderma* suppresses infections by competition, antibiosis, inhibition, and parasitism of the mycelium. Myco-parasitism involves the development of lytic enzymes such as cellulase, chitinases, glucanases, proteases, and lipases, which break down cells and use their contents as nutrition. *Trichoderma* spp. produce antibiotics, degrade cell walls, and compete for nutrition to reduce infections (Vinale*et al*. 2006).

The findings of the current study are likewise consistent with Pavitra *et al*. (2022), who observed that *Trichoderma asperellum* inhibited the most mycelial growth of *Pythium aphanidermatum* and *Pythium myriotylum*, followed by *Trichoderma virens*. Singh and Islam (2010) found that all three *Trichoderma harzianum* isolates and one *T. viride* isolate had significant antagonistic potential for the biocontrol of *Phytophthora nicotianae* isolate, in which *T. harzianum* (0034H) was found highly inhibitory to *P. nicotianae* in dual culture, followed by *T. harzianum* (0034M). The bacterial antagonist *Pseudomonas fluorescens* had the least inhibition. Similar results also reported by Apet *et al*. (2018) who tested seven fungal antagonists (*T. viride, T. harzianum, T. hamatum, T. longibrachiatum, T. koningii, T. virens, A. niger*) and one bacterial antagonist (*P. fluorescens*) against *P. aphanidermatum in vitro* using a dual culture technique and demonstrated that *T. viride* was shown to be the most effective, with significantly less mycelial growth (4.52 mm) and the highest mycelial inhibition (94.97%). *T. harzianum* and *T. hamatum* were the second and third best antagonists, with inhibition of 91.26 and 83.43%, respectively. *P. fluorescens* was found to be less efficient, with a colony diameter of 49.24 mm and a 45.28% suppression of the test pathogen.

Bioagents, which are prominent components of soil myco-flora, play a significant role in soil-borne disease management. So, their usage in *Pythium* induce damping off of acid lime seedlings is beneficial to nursery farmers.

In vivo effect of different treatments on damping off of acid lime

The experiment was conducted to evaluate the efficacy of best fungicides, essential oils and bio-agents against damping off disease of acid lime seedling in nursery. Before sowing, seeds were treated with these selected fungicides, essential oils and bio-agents. Untreated seed served as control. Germination percentage, pre-emergence damping-off, and post-emergence damping-off observations were recorded.

Germination %

Result showed that per cent germination in all tested fungicides, essential oils and bio-agents increased significantly compared with control (Table 5). It was observed that the non- treated seeds (control treatment) demonstrated least germination percentage (72) while seed treatment significantly increased the germination percentage. Cymoxanil 8%+ Mancozeb 64% WP (combi product) treated seeds exhibited maximum germination percentage (90.00%), followed by Metalaxyl 8% + Mancozeb 64% WP (87%) and Copper Hydroxide 53.8% DF (85%) treated seeds. The data regarding the effect of essential oils shows that Citronella oil recorded (82%) germination followed by Lemongrass oil (80%). The data regarding the effect of bio-agents on the growth parameter of acid lime seedling indicate that, bio-agent *Trichoderma asperellum* recorded highest seed germination (78%) followed by *Pseudomonas fluorescens* (76%) and these treatments were at par with other seed treatment, Fosetyl-Al 80% WP (79%), Garlic oil (74%) and Tea tree oil (75%).

Due to seed treatment with fungicides, essential oils and bio- agents germination percentage increased, ranged between 2.77% to 25% and the maximum being recorded in Cymoxanil 8%+ Mancozeb 64% WP (combi product) (25%) over control as compared to other treatments.The current study's findings showed that seed treatments with fungicides, essential oils and bio-agents significantly improved acid lime seed germination. This could be because combination of fungicides, essential oils and bio-control agents are toxic to fungi and have acted as seed coat barriers, inhibiting seed respiration and resulting in delayed ageing and higher germination rates. The above findings are in consistency with the earlier workers viz., El–Mohamedy (2012) who reported that when a seed is treated with fungicides, essential oils and bio-agents and planted, the fungicides, essential oils and bio-agents heal the soil around the seed, and the seed germinates in a pathogen-free environment, accelerating its growth. Systemic chemicals, on the other hand, pass through the plant's vascular tissue and keep the seedling free of infection for a specific period of time, assisting in plant establishment and resulting in a lower percentage of symptomatic plants. Hajong *et al*. (2023) also reported that maximal germination in Carbendazim (12%) + Mancozeb (63%) @ 3 g/kg (91%) and among the bioagents *T. asperellum* @ 4 g/kg (88%) followed by *P. fluorescens* @ 4 g/kg (86%) as compared to control (56%) against tomato damping off induce by *P. aphanide-rmatum*. Srivastava *et al*. (2018) demonstrated that three *Trichoderma species, Trichoderma viride, Trichoderma harzianum,* and *Trichoderma koningii* as well as the bacterial bio-agent *Pseudomonas fluorescens*, were equally superior in terms of germination and plant growth when compared to the chemical fungicides.

Table 1: Different seed treatments used to screen for *Pythium deliense*

Treatments	Conc. used
Fosetyl-Al 80% WP	2.5g/kg seed
Cymoxanil 8% + Mancozeb 64% WP	2.5g/kg seed
Metalaxyl 8% + Mancozeb 64% WP	2.5g/kg seed
Copper Hydroxide 53.8% DF	2g/kg seed
Citronella oil	10ml/kg seed
Lemongrass oil	10ml/kg seed
Tea tree oil	10ml/kg seed
Garlic oil	10ml/kg seed
Pseudomonas fluorescens	10ml/kg seed
Trichoderma asperellum	4ml/kg seed
Control	

Effect of different seed treatments on pre- and post-emergence damping off of acid lime seedlings

Pre and post-emergence damping off (mortality of seedlings) recorded with all treatments ranged between 4.0 to 11.00 and 6.00 to 15.00% respectively (Table6). Cymoxanil 8%+ Mancozeb 64% WP recorded least pre-emergence (4.00%) andpost-emergence (6.00%) mortality with maximum percent reductioninpre-emergence (66.66%) and post-emergence seedling mortality (62.50%), followed by combi product metalaxyl 4% + mancozeb 64% was at par with 5.00 and 8.00% pre-emergence and post-emergence seedling mortality respectively and achieving second best higher reduction in pre (58.33%) and post (50.00%) emergence seedling mortality.

Table 2: Effect of fungicides on radial mycelial growth of *Pythium deliense* (10 DAI)

Fungicides name	Conc. Used (%)	Mean colony diameter (mm)	%Growth inhibition
Potassium Phosphonate 40% w/w	0.30	30.27	66.36
Fosetyl-Al 80% WP	0.25	20.03	77.77
Metalaxyl 35% WS	0.15	06.33	92.96
Cymoxanil 8% + Mancozeb 64% WP (combi product)	0.25	0.00	100.0
Metalaxyl 8% + Mancozeb 64% WP	0.25	0.00	100.0
Thiophanate Methyl 45% + Pyraclostrobin 5% FS (combi product)	0.20	78.00	13.33
Copper Hydroxide 53.8% DF	0.20	0.00	100.0
Metalaxyl-M 3.3% + Chlorothalonil 33.1% SC(combi product)	0.25	15.00	83.33
Mandipropamid 23.4% SC	0.10	70.00	22.22
Benalaxyl-M 4% + mancozeb 65% WP(combi product)	0.25	10.00	88.88
Control	---	90.00	---
SE (m) ±	-	0.70	-
CD (P=0.01)	-	2.80	-

Table 3 : Effect of essential oils on radial mycelial growth of *Pythium deliense* by poisoned food technique (10 DAI)

Essential Oil	Concn. 0.5 (%)		Concn. 1 (%)	
	Mean colony diameter (mm)	% Growth inhibition	Mean colony diameter (mm)	% Growth inhibition
Eucalyptus oil	90.00	0.00	90.00	0.00
Citronella oil	0.00	100.0	0.00	100.0
Neem oil	85.00	5.55	75.00	16.66
Karanjoil	90.00	0.00	85.00	11.11
Lemongrass oil	0.00	100.0	0.00	100.0
Tea tree oil	0.00	100.0	0.00	100.0
Lime oil	15.00	83.33	0.00	100.0
Garlic oil	0.00	100.0	0.00	100.0
Control	90.00	-	90.00	-
SE (m) ±	0.31		0.39	
CD (P=0.01)	1.25		1.55	

Table 4: Effect of bio-agents on radial mycelial growth of *P. deliense* (10DAI)

SN	Treatments	Mean colony diameter (mm)	%Growth inhibition
1	*Trichoderma harzianum*	15.33	82.96
2	*Trichoderma asperellum*	10.17	88.70
3	*Trichoderma asperelloides*	25.07	72.14
4	*Pseudomonas fluorescens*	30.88	65.68
5	*Bacillus subtilis*	60.79	32.45
6	Control	90	-
	SE (m) ±	0.39	-
	CD (P=0.01)	1.58	-

Table 5 : Effect of different seed treatments on acid lime seed germination

Treatments	Germination (%) (25DAS)	% Increased germinationover control
Fosetyl-Al 80% WP	79.00 (62.72)*	9.72
Cymoxanil 8% + Mancozeb 64% WP	90.00 (71.62)	25.0
Metalaxyl 8% + Mancozeb64% WP	87.00 (69.90)	20.83
Copper Hydroxide 53.8% DF	85.00 (67.24)	16.66
Citronella oil	82.00 (64.90)	13.88
Lemongrass oil	80.00 (63.43)	11.11
Tea tree oil	75.00 (60.01)	4.16
Garlic oil	74.00 (59.34)	2.77
Pseudomonas fluorescens	76.00 (60.67)	5.55
Trichoderma asperellum	78.00 (60.04)	8.33
Control	72.00 (58.05)	-
SE (m) ±	0.36	-
CD (P= 0.05)	1.07	-

*Figures in parentheses are arcsine transformed values.

Table 6: Effect of different seed treatments on pre- and post-emergence damping off of acid lime seedlings

Treatments	Pre- emergence damping off	% reduction in pre-emergence damping off	Post- emergence damping off	% reduction in post-emergence damping off
Fosetyl-Al 80% WP	8.00 (2.81)	33.33	13.00 (3.59)	18.75
Cymoxanil 8% + Mancozeb 64% WP	4.00 (1.99)	66.66	6.00 (2.44)	62.50
Metalaxyl 8% + Mancozeb 64% WP	5.00 (2.23)	58.33	8.00 (2.79)	50.00
Copper Hydroxide 53.8% DF	8.00 (2.81)	33.33	7.00 (2.62)	56.25
Citronella oil	6.00 (2.39)	50.00	11.00 (3.31)	31.25
Lemongrass oil	7.00 (2.60)	41.66	14.00 (3.72)	12.50
Tea tree oil	11.00 (3.30)	8.33	14.00 (3.74)	12.50
Garlic oil	11.00 (3.31)	8.33	15.00 (3.85)	6.25
Pseudomonas fluorescens	10.00 (3.13)	16.66	14.00 (3.73)	12.50
Trichoderma asperellum	9.00 (2.98)	25.00	13.00 (3.59)	18.75
Control	12.00 (3.45)	-	16.00 (3.98)	-
SE (m) ±	0.08	-	0.07	-
CD (P=0.05)	0.25	-	0.23	-

*Figures in parentheses are square root transformed values.

Next best treatments were Copper Hydroxide 53.8% DF, Citronella oil, Lemongrass oil, *Trichoderma asperellum, Pseudomonas fluorescens* recorded at par i.e. 8 and 7, 7 and 11, 6 and 14,10 and 14 and 09 and 13 pre-emergence and post-emergence mortality respectively. Minimum reduction in the pre and post-emergence damping off of acid lime seedling was (8.33%) and (6.25%) respectively was noted in garlic oil treatment. Maximum pre (12) and post-emergence (16) seedling mortality recorded in control treatment.Thus, all of the fungicides, essential oils and bio-control agents used as seed treatment in tray culture experiment were found most effective in improving seed germination and reducing pre-emergence and post-emergence damping off in acid lime.

Some researchers reported comparable results. El-Mohamedy (2012) observed that when seed is treated with fungicides, essential oils and bio-agents and planted, the fungicides, essential oils, and bio-agents allowing the seed to germinate in a pathogen-free environment, increasing its growth and systemic fungicides keep the seedling infection-free for a set length of time, promoting plant establishment and resulting in a smaller percentage of symptomatic plants. These results are also in consonance with Thakare and Soni (2018), who reported that rough lemon seed treatment with Carbendazim, Carbendazim +

Mancozeb and Metalaxyl + Mancozeb effectively reduced pre-emergence damping off (77.70, 67.60 and 57.57%) and post-emergence damping off (71.69, 64.15 and 54.71%) respectively. The present results are similar with the findings of Zagade *et al*. (2012) who reported that drenching with either Metalaxyl or Cymoxanil reduced the plant mortality significantly over control and rest of the fungicides tested. Metalaxyl and Cymoxanil were at par in reducing the percent mortality. The reduction of root rot by *Trichoderma* spp. may be due to high antagonistic potential that includes antibiosis, parasitism and production of lytic enzymes (Jagtap *et al*. 2012).

However, soil-applied pesticides have been shown to lose efficiency after prolonged use, and they are water soluble and run off to groundwater after excessive irrigation or rainfall, contaminating the surrounding ecosystem (Rasool and Thakur, 2023). In such cases, antagonists and natural products, such as essential oil, were used to replace chemicals. However, their field efficacy requires more evaluation with concerted outcomes.

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