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Management of *Cylindrosporium mappiae* a causal agent of Leaf Spot Disease of *Nothapodytes nimmoniana* an Anti Cancer Drug Yielding Tree

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A B S T R A C T

The experiment was carried out during 2015-16 in the department of Forest Biology and Tree improvement at College of Forestry, Sirsi. In order to know the *in vitro* and *in vivo* efficacy of different biocontrol agents, plant extracts, fungicides and combi-products, both laboratory and nursery experiments were conducted. The study revealed that, *Trichoderma harzianum* (IOF strain) *Azadiractha indica* Tebuconazole250EC, Trifloxistrobin 25% + Tebuconazole 50%, 75% WC, Hexaconazole 4% + Zineb 68%, 72% WP and Hexaconazole 5% + Captan 70%, 75% WP were found effective under *in vitro* conditions in inhibiting the leaf spot pathogen. The combi product Trifloxistrobin 25% + Tebuconazole 50%, 75% WP has recorded the lowest disease index and found effective under nursery conditions. Thus the integrated use of biofungicides, plant extracts and synthetic fungicides have a greater role in effectively managing leaf spot disease of *Nothapodytes nimmoniana* which can be suggested to the growers.

Introduction

Recently, several tree species of the Western Ghats are gaining international importance due to their newly identified pharmacological and curative properties and as well as due to the threat posed to them by abiotic and biotic factors. One such medicinal tree is *Nothapodytes nimmoniana* (Syn: *Mappia foetida)* which belongs to Icacinacceae family also called as white pear family. This species is easily recognized in field during blooming season by its foetid odour; hence, the name foetida (commonly referred to as stinking tree) (Kingsbury *et al.,* 1991).

It is feared that as a consequence of its over exploitation and habitat loss, the natural populations of *N. nimmoniana* species have declined by 50 to 80 per cent in the last one decade (Hombe gowda *et al.*, 2002). Hence, it is now designated as an endangered species in the northern Western Ghats and threatened in the remaining parts of western ghats

(Ramesha *et al.*, 2008). Among several plant species known to contain the camptothecin, by far the highest concentration of about 0.3 per cent (w/w) has been reported from *Nothapodytes nimmoniana*. In India, the excessive harvesting of *N. nimmoniana* from their natural populations has driven the resources to severe depletion. Estimated 20 per cent of the natural population of *N. nimmoniana* has been lost from southern India in past two decades, presumably due to illicit felling of trees (Suhas *et al.*, 2006). Besides, illicit felling other factors like biotic pressure from insect pest and plant pathogens causing severe diseases are the possible reason for the depletion of the tree species. Of late *N. nimmoniana* is being reported to suffer from fungal leaf spot disease causing cent per cent defoliation. This disease has been found to be another threat and is reported to be due to the soil borne pathogen *Cylindrosporium mappiae* and the pathogen not only affects the leaves but also most economic parts of the tree, twigs and barks also (Nagaraja and Patil, 2009).

The leaf spot disease that defoliates the trees in midsummer and permit re-foliation late in the growing season is especially harmful for tree health because stored carbohydrates are used inappropriately. The studies on screening the Clones of *Nothapodytes nimmoniana* against Leaf Spot Disease has been studied (Shwetha *et al*., 2020). However, no work has been conducted on leaf spot disease management of *Nothapodytes nimmoniana.* Hence, an experiment was planned both *in vitro* and *in vivo* to evaluate the potential of biocontrol agents, plant extracts and chemicals against leaf spot disease.

Materials and Methods

The experiment was conducted at Department of Forest Biology & Tree Improvement, College of Forestry, Sirsi during 2015-16. Following biocontrol agents, plant extracts and chemical fungicides and their combi products have been used to test the efficacy under *in vitro* conditions against leaf spot disease of *Nothapodytes nimmoniana.*

Fungicides

All the fungicides used for *in vitro* studies were triazoles except the mancozeb and carbendazium. These triazoles are systemic and modern group of fungicides, which are used at 0.05 and 0.1 per cent concentration with four replications. However mancozeb and carbendazium were also used to compare with the new group of fungicides at 0.1 and 0.2 per cent concentration without the statistical analysis.

In this study two different concentrations were used to confirm the minimal concentrations for effective management of the test pathogen under *invitro* conditions

Fungal antagonists *viz. Trichoderma harzianum* (Local), *Trichoderma koengii* (Local), *Trichoderma viride* (Dept. of Plant Pathology, UASD), *Trichoderma koengii* (Dept. of Plant Pathology, UASD) and *Trichoderma harzianum* (IOF strain) were used for the *in vitro* evaluation. The five mm disc of test fungus *Cylindrosporium mappiae* was inoculated at the centre of the petri dish and two 5 mm discs of antagonistic fungi was inoculated on either side of test fungus. The work was carried out using laminar airflow to avoid contamination. Three replications for each antagonist were maintained and were incubated at 30 ± 1^{0}c. A control was maintained only with test fungus. Observations were recorded on the zone of inhibition produced by the antagonistic organisms.

Assay for bacterial antagonist

Bacterial antagonists *viz. Pseudomonas fluorescens* (IOF strain) and *Bacillus subtilis* (IOF strain) was sub cultured using kings'B and nutrient agar media respectively.

Freshly sub cultured bacterial antagonists were streaked at the centre of the petri dish. At two points on either side of the bacterium, 5 mm disc of test fungus *Cylindrosporium mappiae* was inoculated. Three replications were maintained and incubated at

30±1^{0}C. Observations were recorded on the zone of inhibition produced by the antagonistic organisms.

Preparation of plant extracts

For the preparation of extracts the leaf samples were washed with sterilized distilled water. 50 ml sterilized water was added to 25 grams of material and crushed by using mixer grinder. The mixer grinder was scrubbed to clean after each grinding and it was followed by three serial washes using sterilized distilled water. The extract was filtered through Whatman No. 1 filter paper and the filtrate thus obtained was treated as 50 per cent solution (1:2). To obtain concentrations of 5 and 10 per cent, 10 and 20 ml of 50 per cent stock extract respectively were added to separate conical flasks of 250 ml containing 45 ml of sterilized PDA in laminar air flow (Sarvamangala *et al.,* 1993).

Recording of observations

Observations on extent of growth of mycelium in different treatments were recorded and per cent inhibition of growth was calculated using the formula developed by Vincent (1947).

$$I = \frac{(C - T)}{C} \times 100$$

Where,

I= Inhibition C= Rate of growth in control T= Rate of growth in treatment

Management of leaf spot disease of *N. nimmoniana* under nursery conditions

Highly effective biocontrol agents, plant extracts, fungicides and combi-products found under *in vitro* studies, were used for field evaluation under nursery conditions. Concentrations of biocontrol agents, plant extracts, fungicides and combi-products were decided as per the laboratory observations. For biocontrol agents a suspension of 10^7 cfu/ml was used as spray.

The chemicals were measured accurately just before spraying and mixed thoroughly with water. The first spray was given when disease pressure was moderate, subsequent sprays were given in monthly interval.

Recording of observations

Observations were recorded on per cent disease index and growth parameters such as height of seedlings and number of leaves. The per cent disease index was calculated before spray and also at 30, 60 and 90 days after spray. At 120th day after spray also the per cent disease index was recorded to test the efficacy of various fungi toxicants.

Per cent disease index

Per cent disease index was calculated by using the formula given by Wheeler (1969).

$$PDI = \frac{\text{Sum of numerical ratings}}{\text{Total No. of leaves observed} \times \text{Maximum grade used}} \times 100$$

Seedling height

Height was measured at 120th day after first spray, from the base to the growing tip of the seedling and expressed in centimeter (cm).

Number of leaves

Number of fully opened leaves was counted in all the seedlings at 120th day after first spray in each replication and average was reported as number of leaves per seedling.

Results and Discussion

In vitro management using bio agents

The results of *in vitro* management of leaf spot of *N. nimmoniana* using fungal and bacterial biocontrol agents are presented in Table 1.

Results revealed that, the significant variation was found with respect to zone of inhibition by the different bio agent over untreated control. The growth of inhibition of the pathogen varied between 51.48 to 99.81 per cent. However, no inhibition was recorded in untreated control.

Among the different fungal bio agents T_5 (*Trichoderma harzianum*) exhibited maximum zone of inhibition (99.81 %) followed by T_3 (*Trichoderma viride*) which has exhibited 89.97 per cent inhibition and found on par with T_2 (*Trichoderma koengii*) which inhibited 88.59 per cent and T_4 (*Trichoderma koengii*) 88.10 per cent. The least growth of inhibition of 84.03 per cent was recorded in T_1 (*Trichoderma harzianum*).

Among the bacterial antagonists *Pseudomonas fluorescens* (T_6) has shown maximum growth inhibition of 70.74 per cent followed by *Bacillus subtilus* (T_7) which recorded lower per cent inhibition of 51.48 per cent. It is always essential and important to evaluate the fungi toxicants (biocontrol agents, plant extracts and chemical fungicides) under *in vitro* condition before it is used for field trials. The laboratory studies will help in analysing the effective fungitoxicant and concentrations for its application under *in vivo* studies.

Use of bioagents is one of the most successful, non-chemical and eco-friendly approach in the disease management. The growth inhibition of the *Cylindrosporium mappiae* by biocontrol agents were compared with untreated control. Among the different fungal bio agents T_5 (*Trichoderma harzianum*, IOF strain) has exhibited maximum zone of inhibition (99.81 %) followed by T_3 (*Trichoderma viride*, Department of Plant Pathology, UASD strain) which has exhibited 89.97 per cent and found on par with T_2 (*Trichoderma koengii*, Local strain) which has inhibited 88.59 per cent and T_4 (*Trichoderma koengii* Department of Plant Pathology, UASD strain) inhibited 88.10 per cent. The least growth of inhibition of 84.03 per cent was recorded in T_1 (*Trichoderma harzianum*, Local strain that, has inhibited 84.03 per cent. The bacterial biocontrol agent *Pseudomonas fluorescens*, IOF strain (T_6) has shown maximum growth inhibition of 70.74 per cent and (T_7) *Bacillus subtilus*; IOF starin) which has recorded 51.48 per cent. Whereas, all the biocontrol agents were found superior to the untreated control.

The inhibition of the *Cylindrosporium mappiae* by these biocontrol agents may be attributed to mechanisms such as parasitism, competition and antibiosis (by production of antibiotics, enzymes and toxins etc) (Arras and Arru, 1997). It has been suggested that biocontrol agents which act by antibiosis rather than by competition would be the most effective method to inhibit the pathogen (Dennis and Webster, 1971; Ryther *et al.*, 1989; Kakde and Chavan, 2011; Jat and Agalave, 2013).

The studies conducted by Singh *et al.*, (2010) have reported that, the *Trichoderma* spp. were observed as the most effective antagonists against root rot caused by *Phytophthora cinnamomi* among the associated fungal flora of *Cedrus deodara* rhizosphere. It is also mentioned that volatile compounds released by the antagonists inhibited the growth of the pathogen (Thakur *et al.*, 2013).

Chambers and Scott (1995) also found that, coiling and parallel growth formation of apprisorium by *Trichoderma* spp. inhibited the growth of *Phytophthora cinnamomi*. Aryanatha and Guest (2006) observed antibiosis as the main mode of action although mycoparasitism, indicated by parallel hyphal growth, hyphal coiling, appresorium formation and direct penetration with one isolate of *Trichoderma* against *P. cinnamomi*.

In vitro management using plant extracts

Efficacy of plant extracts against leaf spot pathogen of *N. nimmoniana* was studied under *in vitro* conditions and the results are presented in Table 2. Various plant extracts were used at five and 10 per cent concentration to check the growth of inhibition of test pathogen. The significant difference was

recorded in all the plant extracts at both the concentrations.

Among the various treatments, the extracts of *Azadiractha indica* (T_3) has exhibited the maximum growth of the pathogen to an extent of 98.40 per cent at five per cent concentration, followed by (T_5) *Calotropis gigantia* (52.67 %) inhibition, (T_1) *Vitex negundo* (49.55%) inhibition, (T_6) *Pongamia glabra* (52.78 %) inhibition. The lowest inhibition (27.65 %) was recorded in *Oscimum sanctum* (T_4) and in (T_2) *Chromolaena odorata* (26.51 %).

When the same treatments were tried at 10 per cent concentration, the cent per cent inhibition of the pathogen was observed in *Azadiractha indica* (T_3). This is followed by *Calotropis gigantia* (T_5) (57.77 %), which was on par with *Vitex negundo* (T_1) which has shown 53.29 per cent inhibition and *Pongamia glabra* (T_6) which has inhibited 52.78 per cent of growth of the pathogen.

The least growth inhibition of 33.37 and 32.25 per cent was observed in *Chromolaena odorata* (T_2) and in *Oscimum sanctum* (T_4) respectively, and were on par with each other. However, no growth inhibition was observed in untreated control. Plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumer in contrast to synthetic pesticides. Extracts of many higher plants have been reported to exhibit antifungal properties under lab conditions (Mohana *et al.,* 2008 and Tapwal *et al.,* 2012). Among the plant extracts used under *in vitro* studies the maximum zone of inhibition was recorded in *Azadiractha indica* (98.40 %) at 5 per cent concentration and 100 per cent inhibition at 10 per cent concentration followed by, *Calotropis gigantia* which has shown 52.67 per cent inhibition, *Vitex negundo* shown 49.55 per cent inhibition, *Pongamia glabra* has exhibited 52.78 per cent inhibition. The lowest inhibition of the pathogen was recorded in *Oscimum sanctum* (27.65 %) and in *Chromolaena odorata* (26.51 %). However, these plant extracts were found effective and significantly superior over untreated control. Many of such plant extracts show antifungal activity against wide array of fungi (Wilson *et al.,* 1997). In the present investigation, *Azadirachta indica* inhibited cent per cent of pathogen growth among the six botanicals used. In all other botanicals used considerable amount of inhibition of pathogen growth was noticed. The phenolics and phenolic acids, coumarins and pyrones, flavonoids, isoflavonoids, steroids and steroidal alkaloids and other miscellaneous compounds present in the extracts could be the reason for inhibition of pathogen *in vitro* (Mitra *et al.,* 1984). In case of *A. indica* the highest inhibition may be due to the alkaloids such as azadirachtin and nimbicidin, which might have acted on toxic elements in the suppression of the pathogen. These results are in conformity with Choi *et al.,* (2004) and Tapwal *et al.,* (2012).

In vitro management using chemical fungicides

Efficacy of fungicides was tested against leaf spot pathogen of *N. nimmoniana* under *in vitro* condition and results are presented in Table 3. Among the different treatments tested at 0.05 per cent T_3 (Tebuconazole 250 EC) has recorded maximum inhibition 99.63 per cent, which was found on par with (T_4) Difenconazole 250 EC (98.86 %), followed by (T_1) Propiconazole 25 % EC which has inhibited the pathogen to an extent of 96.91 per cent (T_2) Hexaconazole 5 % EC which has inhibited the pathogen to an extent of 93.30 per cent. Similar studies when conducted at 0.1 per cent concentration where in T_3 (Tebuconazole 250 EC) and (T_4) Difenconazole 250 EC has recorded cent per cent inhibition followed by (T_1) Propiconazole 25 % EC (99.44 %) and found significantly superior over (T_2) Hexaconazole 5 % EC (98.33 %) and untreated control.

Further the studies were conducted by evaluating two more fungicides *viz.* Mancozeb (T_1) and Carbendazium (T_2) at 0.1 and 0.2 per cent concentration and the growth inhibition of the pathogen was recorded. Among these two fungicides Mancozeb (T_1) has recorded 76.70 and 89.36 per

cent inhibition at 0.1 and 0.2 per cent concentration respectively. Whereas, Carbendazium (T_2) has recorded 75.49 and 88.66 per cent inhibition at 0.1 and 0.2 concentration respectively. These two molecules were found numerically inferior to the triazoles fungicides. Thus, all the triazole fungicides have greater influence in inhibition of *Cylindrosporium mappiae* compared to untreated control and the other group of fungicides.

The efficacy of fungicides is not a constant phenomenon because it is influenced by many biological and environmental factors that directly influence the metabolic activities of fungal cells. Sometimes critical concentrations are not usually effective for a long time as the fungus can be adopted on molecules of the fungicides therefore; *in vitro* tests are very important to measure and rank the fungitoxicity of fungicides against a particular pathogen.

All the fungicides showed variable response in inhibiting the colony growth of the phytopathogen, according to their nature and specificity at different concentrations.

All the triazoles used in the *in vitro* studies at 0.05 and 0.1 per cent concentration were found to be superior compared to untreated control. However, earlier recommended fungicides *viz*. Carbendazium and Mancozeb were not on par with triazole fungicides. The fungicides Tebuconazole 250 EC, Difenconazole 250 EC, Propiconazole 25 % EC Hexaconazole 5 % EC were found more effective. Cent per cent inhibition was recorded in Tebuconazole 250 EC and Difenconazole 250 EC which is found on par with Propiconazole 25 % EC (99.44 %) and Hexaconazole 5 % EC (98.33 %) at 0.1 per cent concentration. The ergosterol body inhibiting (EBI) fungicides such as Tebuconazole, Difenconazole, Propiconazole and Hexaconazole are proved to be effective in management of most foliar diseases in field crops (Sunkad, 2012). Fungicides belonging to triazoles group inhibit biosynthesis of ergosterol which plays an important role in structure of cell membrane of fungi (Dahmen *et al.,* 1989). These triazoles fungicides have systemic character and can be applied to green plants safely (Sudini *et al.,* 1999 and Chijamo and Daiho, 2014).

In vitro management using combi-products

Different combi-products were tried at 0.1 and 0.2 per cent concentrations and the inhibition recorded (Table 4). Among the different combi-products cent per cent inhibition was recorded in T_2 (Taqat; Hexaconazole 5 % + Captan 70 %, 75 %WP), T_3 (Avatar; Hexaconazole 4 % + Zineb 68 %, 72 % WP) and T_4 (Nativo; Trifloxistrobin 25 % + Tebuconazole 50 %,75 %WC) at both 0.1 and 0.2 per cent concentration these treatments were found significantly superior to T_1 (SAAF; Carbendazium 12 % + Mancozeb 63 %, 75 %WP) which inhibited 96.30 and 98.92 per cent respectively. However, all the treatments were found superior *vis- a -vis* untreated control.

Combi-products consist of both systemic and contact fungicides. They are broad spectrum in nature. They offer multiple disease resistance mechanism due to their novel modes of action. Among the combi-products (Hexaconazole 5 % + Captan 70 %, 75 %WP) (Hexaconazole 4 % + Zineb 68 %, 72 % WP) and (Trifloxistrobin 25 % + Tebuconazole 50 %, 75 %WC) were found significantly superior at both the concentration followed by (Carbendazium 12 % + Mancozeb 63 %, 75 %WP). Whereas, all the combiproducts were superior in managing the leaf spot pathogen compared to untreated control. Trifloxistrobin 25 % + Tebuconazole 50 %, 75 %WC is a new combination of fungicide which has inhibited cent per cent growth of the pathogen *in vitro*. It is a systemic broad spectrum fungicide with preventive and curative action which offers not only disease control but in some crops helps in quality improvement. Tebuconazole is a dimethylase inhibitor (DMI) which interferes in process of building the structure of fungal cell wall. Finally it inhibits the reproduction and further growth of the fungus. Trifloxistrobin interferes with respiration in plant pathogenic fungi and also act against electron

transport chain in fungal mitochondrial synthesis (Daren, 2007 and Bag *et al.,* 2016). Another compound (Hexaconazole 5 % + Captan 70 %, 75 %WP) was also inhibited the growth of the pathogen (100 %), it was proved to be effective against leaf spot, rust and powdery mildew disease of crop plants (Pramesh *et al.,* 2016).

In vivo management of the leaf spot disease of *N.nimmoniana*

Per cent disease index

The biocontrol agents, plant extracts, chemical fungicides and combi-products which were found efficient under *in vitro* condition were considered for evaluating against leaf spot of *N. nimmoniana* under *in vivo* condition and the results are presented in Table 5.

The per cent disease index was calculated before spray and also at 30, 60 and 90 days after spray. The disease index ranged from 36.10 to 36.47 per cent before spray in different treatments and found statistically non-significant. The per cent disease index after I spray ranged from 36.10 to 49.27 in various treatments. The least per cent disease index of 36.96 % was recorded in Nativo; Trifloxistrobin 25 % + Tebuconazole 50 %, 75 %WC (T_6) sprayed plants which is significantly superior compared to other treatments and followed by Difenconazole 250 EC (38.26 %) (T_4), Tebuconazole 250 EC (39.20 %) (T_5) *Trichoderma harzianum,* IOF strain (T_1) (41.22 %), *Trichoderma viride,* Department of Plant Pathology, UASD, strain (43.21 %) (T_2) and *Azadiractha indica* (44.24 %) (T_3). All these treatments differed significantly. superior among each other. The per cent disease index after II spray was ranged from 39.23 to 58.66. All the treatments differed significantly with one another. The maximum (58.66 %) was recorded in untreated control. The least per cent disease index of 39.23 per cent was recorded in Nativo; Trifloxistrobin 25 % + Tebuconazole 50 %, 75 %WC (T_6) sprayed plants which is significantly superior compared to other treatments followed by Difenconazole 250 EC (39.23 %) (T_4), Tebuconazole 250 EC (46.21 %) (T_5), *Trichoderma harzianum,* IOF strain (49.20 %) (T_1), *Trichoderma viride,* Department of Plant Pathology, UASD, strain (51.36 %) (T_2) and *Azadiractha indica* (53.21 %) (T_3). All these treatments differed significantly superior among each other.

The per cent disease index after III spray was ranged from 41.52 to 71.25. All the treatments differed significantly with one another. The maximum disease index of 60.21 % was recorded in untreated control (T7). The least per cent disease index was recorded in Nativo; Trifloxistrobin 25 % + Tebuconazole 50 %, 75 %WC (T_6) sprayed plants (41.52 %) which is significantly superior compared to other treatments followed by Difenconazole 250 EC (49.48 %) (T_4) Tebuconazole 250 EC (50.21 %) (T_5), *Trichoderma harzianum,* IOF strain (53.56 %) (T_1), *Trichoderma viride,* Department of Plant Pathology, UASD, strain (59.21 %) (T_2) and *Azadiractha indica* (60.21 %) (T_3). All these treatments differed significantly superior among each other. After all the three sprays one more observation on per cent disease index was taken at 120^{th} day and the results revealed that, the combi-product Nativo; Trifloxistrobin 25 % + Tebuconazole 50 %, 75 %WC (T_6) has recorded the lowest disease index of 44.23 per cent which was found on par with Tebuconazole 250 EC (53.62 %) (T_5) and Difenconazole 250 EC (51.23 %) (T_4). The per cent disease index was 58.22 and 65.22 per cent in *Trichoderma harzianum* (IOF strain) sprayed seedlings (T_1) and in *Trichoderma viride* (Department of Plant Pathology, UASD starin (T_2)) sprayed plots respectively. Further, these treatments were found on par with treatment sprayed with *Azadiractha indica* (T_3) which has recorded a disease index of 64.22 per cent. All these biocontrol agents, plant extract, fungicides, combi-products were found significantly superior to the untreated control (77.55 %).

Table.1 Biocontrol agents

Treatment	Bioagents	Source
T_1	*Trichoderma harzianum*	Native/Local (sirsi)
T_2	*Trichoderma koengii*	Native/Local (sirsi)
T_3	*Trichoderma viride*	Dept. of Plant pathology, UASD
T_4	*Trichoderma koengii*	Dept. of Plant pathology, UASD
T_5	*Trichoderma harzianum*	Institute of Organic Farming (IOF strain)
T_6	*Pseudomonas fluorescens*	Institute of Organic Farming (IOF strain)
T_7	*Bacillus subtilis*	Institute of Organic Farming (IOF strain)
T_8	Untreated control	-

Design: CRD: Number of treatments: 8 Number of replications: 3
Method used : Dual culture technique (Elad *et al.* 1981).

Table.2 Plant extracts

Treatment	Plant extracts	Concentration (%)
T_1	*Vitex negundo* (Lakki)	5 and 10
T_2	*Chromolaena odorata (Eupatorium)*	5 and 10
T_3	*Azadiractha indica* (Neem leaf extract)	5 and 10
T_4	*Oscimum sacntum* (Tulsi)	5 and 10
T_5	*Calotropis gigantia (Ekke)*	5 and 10
T_6	*Pongamia glabra* (Pongamia)	5 and 10
T_7	Untreated Control	-

Design : CRD Number of treatments: 7: Number of replications: 3
Method used: Poisoned food technique (Nene and Thapliyal, 1979).

Table.3 Fungicides

Treatment	Fungicides		Concentration (%)
	Chemical name	Trade name	
T_1	Propiconazole	Tilt 25 % EC	0.05, 0.1
T_2	Hexaconazole	Contaf 5 % EC	0.05, 0.1
T_3	Mancozeb	Dithane-M-45,75 % WP	0.1, 0.2
T_4	Carbendazium	Bavistin 50 % WP	0.1, 0.2
T_5	Tebuconazole	Folicure 25% EC	0.05, 0.1
T_6	Difenconazole	Score 250 EC	0.05, 0.1
T_7	Untreated control	-	-

Design: CRD Number of treatments: 7 Number of replications: 3
Method used : Poisoned food technique (Nene and Thapliyal, 1979).

Table.4 Combi-products

Treatment	Combi-products		Concentrations (%)
	Chemical name	Trade name	
T_1	(carbendazium 12 % + Mancozeb 63 %)	SAAF75 %WP	0.1, 0.2
T_2	(Hexaconazole 5 % + Captan 70 %)	Taqat 75 %WP	0.1, 0.2
T_3	(Hexaconazole 4 % + Zineb 68 %)	Avatar 72 % WP	0.1, 0.2
T_4	(Trifloxistrobin 25 % + Tebuconazole 50 %)	Nativo 75 %WP	0.1, 0.2
T_5	Untreated Control	-	-

Design: CRD Number of treatment: 5 Number of replications: 4
Method used: Poisoned food technique (Nene and Thapliyal, 1979).

Table.5

Treatment	Fungi-toxicants	Source/trade name	Concentration (%)
T_1	*Trichoderma harzianum*	IOF strain	10^7 cfu/ml
T_2	*Trichoderma viride*	Department of Plant Pathology, UASD	10^7 cfu/ml
T_3	*Azadirachta indica*	Neem Seed kernel Extract	10
T_4	Difenconazole	Score 250 EC	0.1
T_5	Tebuconazole	Folicur	0.1
T_6	(Trifloxistrobin 25 % + Tebuconazole 50 %)	Nativo 25% EC	0.1
T_7	Untreated control	-	-

Design : RBD
Number of treatments : 7
Number of replications : 3
Number of sprays : 3
The first spray was given when the disease pressure was moderate and subsequent at monthly interval

Table.6 Efficacy of bio agents against leaf spot pathogen of *N. nimmoniana* under *in vitro* conditions

Treatment	Bioagents	Growth inhibition (%)
T_1	*Trichoderma harzianum*	84.03 (66.45)*
T_2	*Trichoderma koengii*	88.59 (70.26)
T_3	*Trichoderma viride*	89.77 (71.35)
T_4	*Trichoderma koengii*	89.10 (70.72)
T_5	*Trichoderma harzianum*	99.81 (87.50)
T_6	*Pseudomonas fluorescens*	70.74 (57.25)
T_7	*Bacillus subtilis*	51.48 (45.85)
T_8	Untreated control	0.00 (0.00)
S.Em. ±		**0.93**
C.D. at 1 %		**2.81**

**Figures in the parentheses are arcsine transformed values.*

Table.7 Efficacy of plant extracts against leaf spot pathogen of *N. nimmoniana* under *in vitro* conditions

Treatment	Plant extracts	Growth inhibition (%)	
		5	10
T_1	*Vitex negundo*	49.55 (44.74)*	53.29 (46.89)*
T_2	*Chromolaena odorata*	26.51 (30.99)	33.37 (35.29)
T_3	*Azadiractha indica*	98.40 (82.73)	100.00 (90.00)
T_4	*Oscimum sanctum*	27.63 (31.71)	32.25 (34.60)
T_5	*Calotropis gigantia*	52.07 (46.19)	57.77 (49.47)
T_6	*Pongamia glabra*	47.26 (43.43)	52.78 (46.59)
T_7	Untreated Control	0.00 (0.00)	0.00 (0.00)
S.Em. ±		**0.57**	**1.67**
C.D. at 1 %		**1.74**	**5.12**

**Figures in the parentheses are arcsine transformed values.*

Table.8 Efficacy of fungicides against leaf spot pathogen of *N. nimmoniana* under *in vitro* conditions

Treatment	Fungicides	Growth inhibition (%)		
		0.05	0.1	0.2
T_1	Propiconazole 25 % EC	96.91 (79.88)	99.44 (85.71)	-
T_2	Hexaconazole 5 % EC	93.30 (75.00)	98.38 (82.69)	-
T_3	Mancozeb 75 % WP	-	76.70 (61.14)	89.36 (70.96)
T_4	Carbendazium 50 % WP	-	74.59 (59.73)	88.66 (70.32)
T_5	Tebuconazole 250 EC	99.63 (86.51)	100.00 (90.00)	-
T_6	Difenconazole 250 EC	98.86 (83.87)	100.00 (90.00)	-
T_7	Control	0.00 (0.00)	0.00 (0.00)	-
S.Em. ±		**1.26**	**0.50**	-
C.D. at 1 %		**3.88**	**1.54**	-

*- Not evaluated *Figures in the parentheses are arcsine transformed values.*

Table.9 Efficacy of combi fungicides against leaf spot pathogen of *N. nimmoniana* under *in vitro* conditions

Treatment	Combi -products	Growth inhibition (%)	
		0.1	0.2
T_1	(Carbendazium 12 % + Mancozeb 63 %) 75 %WP	96.30 (78.91)*	98.92 (84.03)*
T_2	(Hexaconazole 5 % + Captan 70 %) 75 %WP	100.00 (90.00)	100.00 (90.00)
T_3	(Hexaconazole 4 % + Zineb 68 %) 72 % WP	100.00 (90.00)	100.00 (90.00)
T_4	(Trifloxistrobin 25 % + Tebuconazole 50 %) 75 %WC	100.00 (90.00)	100.00 (90.00)
T_5	Untreated control	0.00 (0.00)	0.00 (0.00)
S.Em. ±		**0.29**	**0.29**
C.D. at 1 %		**0.90**	**0.88**

**Figures in the parentheses are arcsine transformed values*

Table.10 Management of leaf spot disease of *N. nimmoniana* under nursery condition and its influence on growth parameters

Treatment No.	Treatments	Per cent disease index (PDI)					Plant parameters	
		Before spray	30 DAS*	60 DAS	90 DAS	120th day	Height (cm)	No. of leaves
T_1	*Trichoderma harzianum* (IOF strain)	36.10	41.22	49.20	53.56	58.22	30.50	7.66
T_2	*Trichoderma viride* (Department of Plant Pathology starin)	36.34	43.21	51.36	59.21	65.22	29.50	7.00
T_3	*Azadiractha indica*	36.04	44.24	53.21	60.21	64.22	29.88	7.00
T_4	Difenconazole 250 EC	36.21	38.26	43.87	49.48	51.23	30.76	7.61
T_5	Tebuconazole 250 EC	35.99	39.20	46.21	50.21	53.62	30.56	6.33
T_6	(Trifloxistrobin 25 % + Tebuconazole 50 %) 75 %WC	36.30	36.96	39.23	41.52	44.23	31.28	6.33
T_7	Untreated Control	36.47	49.27	58.66	71.25	77.55	30.15	6.31
S.Em. ±		**0.18**	**0.01**	**0.06**	**0.02**	**3.78**	**0.19**	**0.30**
C.D. at 5 %		**NS**	**0.04**	**0.18**	**0.06**	**11.78**	**0.57**	**NS**
***DAS-Days After Spray**								

Based on the results obtained under *in vitro* condition the most effective fungi toxicants were evaluated under *in vivo* conditions. Among the bio control agents *Trichoderma harzianum*, IOF strain has shown lower PDI (58.22 %) followed by *Trichoderma viride* Department of Plant Pathology, UASD strain (65.22 %). The plant extract of *Azadirachta indica* has resulted in 64.22 per cent disease index. Among the sole fungicides Difenconazole 250 EC (39.23 %) (T_4) and Tebuconazole 250 EC (46.21 %) (T_5) have recorded lower per cent disease index, while the combi-product Nativo; Trifloxistrobin 25 % + Tebuconazole 50 %, 75 %WC (T_6) have recorded 44.23 per cent of disease index (Ali and Sharma, 1996; Arras and Arru, 1997; Mohana *et al.,* 2008; Tapwal *et al.,* 2012; Sunkad, 2012 and Bag *et al.,* 2016).

Plant parameters

The data on plant height and number of leaves as influenced by different treatments is presented in Table 5. The height of *N. nimmoniana* seedlings in various treatments ranged from 29.50 cm to 31.28 cm. The maximum height of 31.28 cm was recorded in Nativo; Trifloxistrobin 25 % + Tebuconazole 50 %, 75 %WC (T_6) followed by *Trichoderma harzianum* (IOF strain) (T_1) (30.05 cm), *Trichoderma viride* (Department of Plant Pathology, UASD strain (T_2)) (29.50 cm), *Azadiractha indica* (30.76 cm) (T_3), Tebuconazole 250 EC (30.56 cm) (T_5) and in untreated control (30.15) (T_7) which were found statistically on par with each other. Thus, except T_6 other treatments do not shown any influence on the height of the seedlings.

Among the various treatments the number of leaves ranged from 6.31 to 7.66. The maximum number of leaves were observed in *Trichoderma harzianum* (IOF strain (T_1)) (7.66) and Difenconazole 250 EC (T_4) (7.61) sprayed seedlings, followed by *Trichoderma viride* Department of Plant Pathology, UASD strain (T_2) (7.00), *Azadiractha indica* (T_3) (7.00), Tebuconazole 250 EC (T_5) (6.33), Nativo; Trifloxistrobin 25 % + Tebuconazole 50 %, 75 %WC (T_6) (6.33) and untreated control (T_7) (6.31). Though, the observations were found numerically different but statistically they were on par with each other (Table 5).

Thus, all the selected fungi toxicants proved their efficacy under *in vivo* conditions and found superior *vis-à-vis* untreated control. Though the fungi toxicants have given the best performance under *in vitro* conditions but failed to give similar trend of effects under nursery conditions. This might be due to the several factors like weather, disease pressure and time & frequency of spray. These results are in line with Sharma *et al.,* (2017) who worked on effect of different fungicidal treatments for control of powdery mildew disease in fieldpea. The results revealed that average tallest plant (41.81 cm) was recorded in Tebuconazole + Trifloxystrobin treated plots and was found significantly longer than any other treatments. Among the plant parameters studied, the plant height was maximum (31.28 cm) in (Trifloxistrobin 25 % + Tebuconazole 50 %). However, no much difference was recorded with respect to height in other treatments.

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