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RESEARCH NOTE

Eco-friendly management of *Cylindrosporium* leaf spot disease of *Nothapodytes nimmonianaunder in vitro* conditions

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Nothapodytes nimmoniana is one of the important commercialised medicinal tree; exported for its novel alkaloid camptothecin. The use of eco-friendly disease management practices (bioagents and plant extracts) is of paramount importance in order to avoid the residue toxicity in the host plant. Therefore, different bio agents and plant extracts were evaluated under *in vitro* conditions. Among the different fungal bio agents, *Trichoderma harzianum* (IOF strain) has exhibited maximum zone of inhibition (99.81%). Among the bacterial antagonists *Pseudomonas fluorescens* (IOF strain) has shown maximum growth inhibition of 70.74 per cent followed by *Bacillus subtilus;* (IOF strain) inhibited 51.48 per cent. Among the various plant extracts used *Azadiractha indica* has inhibited the maximum growth of the pathogen to an extent of 98.40 per cent and 100 per cent at 5 and 10 per cent concentration, respectively.

Keywords*:* Anti-cancer, Bio agents, Leafspot, Plant extracts

Nothapodytes nimmoniana (Syn: *Mappia foetida*) is an important medicinal tree,which belongs to Icacinacceae family also called as white pear family. It is a small tree locally known as '*Durvasane mara*' or 'Helthare mara' in Kannada. This species is easily recognized in field during blooming season by its foetid odour; hence, the name foetida (commonly referred to as stinking tree). The shrubby under-storey tree is native to warmer regions of South India. It is widely distributed in many parts of the Western Ghats from Satara of northern Western Ghats tosouthwards up to Nilgiris and Annamalais, and it is very common in Uttara Kannada and in districts of Coastal Karnataka. In the northern parts, it occurs above an altitude of approximately 500m, where as in the southern parts, its occurrence starts at 550m msl (Govindachari and Vishwanathan, 1972).Because of overexploitation 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). Besides illicit felling other factors like biotic pressure from insect pest and plant pathogens causing severe diseases is the another 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 percent defoliation. This disease have 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. No studies have been conducted on the leaf spot disease and its management in *N. nimmoniana*. Because of medicinal importance the present study was carried out using bio agents and plant extracts for its *in vitro* management.

Material and methods

The study was carried out during 2015-16 in Department of Forest Biology and Tree improvement at College of Forestry, Sirsi. In order to know the *in vitro* efficacy of different biocontrol agents and plant extracts laboratory experiment was conducted. Biocontrol agents *Trichoderma viride* (Strain-1), and *Trichoderma koengii* (Strain-2) were collected from Department of Plant Pathology, UAS, Dharwad*, Trichoderma harzianum* (Strain-2)*, Pseudomonas fluorescens* (Strain-1) and *Bacillus subtilis* (Strain-1) were collected from Institute of Organic Farming (IOF), UAS, Dharwad, and local strains *Trichoderma harzianum* (Strain-1) and *Trichoderma koengii* (strain-1)were isolated and used in the study. The antagonistic potential of seven bioagents was studied using dual culture technique (Elad *et al.,* 1981). The efficacy of six botanicals was evaluated using poisoned food technique. Observations on extent of growth of mycelium in different treatments were recorded and per cent inhibition of growth was calculated using the zone of inhibition formula (Chijamo and Daiho, 2014).

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

Where, I= Inhibition; C= Rate of growth in control; T= Rate of growth in treatment For evaluation of fungal biocontrol agents, 5 mm mycelial disc of test fungus was inoculated at centre of the petri plate and antagonistic fungus was placed opposite to it at both the ends. In case of evaluation of bacterial antagonist the bacterium was streaked at middle of the petri plates and mycelial disc of pathogen was placed at both sides. The plates were incubated at 30 ± 1^0C and zone of inhibition was recorded (Chijamo and Daiho, 2014).

For preparation of botanicals, fresh plant materials were collected and washed first in tap water and then in distilled water. 100 grams of fresh sample was chopped and then crushed by adding 100ml sterilized distilled water (1:1 w/v). The extract was filtered through muslin cloth. Filtrate thus obtained is used as stock solution. Five and ten ml of stock solution was mixed with 95 and 90ml of sterilized PDA medium respectively, so as to get 5 and 10 per cent concentration. Twenty ml of medium was poured into sterile petri plates, mycelium of 5 mm disc was inoculated at the centre of the petri plate. Then such plates were incubated at 30 ± 1^0C and zone of inhibition was recorded (Chijamo and Daiho, 2014).

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 in with respect to zone of inhibition by different bioagent over untreated control was recorded. The growth of

inhibition of the pathogen varied between 51.48 to 99.81 per cent. However, no inhibition of pathogen was recorded in untreated control. Among the different fungal bioagents T_5 (*Trichoderma harzianum,* IOF strain) have exhibited maximum zone of inhibition (99.81%) followed by T_3 (*Trichoderma viride,* Department of Plant Pathology, UASD) which has exhibited 89.97 per cent inhibition and found at par with T_2 (*Trichoderma koengii,* Native) which inhibited 88.59 per cent and T_4 (*Trichoderma koengii* Department of Plant Pathology, UASD); 88.10 per cent. The least growth of inhibition of 84.03 per cent was recorded in T_1 (*Trichoderma harzianum,* Native). Among the bacterial antagonists *Pseudomonas fluorescens,* IOF strain (T_6) has shown maximum growth inhibition of 70.74 per cent followed by *Bacillus subtilus;* IOF strain) which has recorded lower per cent inhibition of 51.48 per cent. The inhibition of the *Cylindrosporium mappiae* by these biocontrol agents may be attributed to mechanisms such as parasitism, competition, and antibiosis (Tewari and Dath, 2011). 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 (Kakde and Chavan, 2011 and 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.

Efficacy of plant extracts against *Cylindrosporium* 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 5 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 influence of the pathogen to an extent of 98.40 per cent at five per cent concentration, followed by *Calotropisgigantia* (T5) which has shown 52.67 per cent inhibition, *Vitex negundo* (T_1) that has shown 49.55 per cent inhibition, *Pongamia glabra* (T_6) which has exhibited 52.78 per cent inhibition. The lowest

Table 1. Efficacy of bio agents against *Cylindrosporium* leaf spot pathogen of *N. nimmoniana* under *in vitro* conditions

Treatment	Bioagents	Growth inhibition of pathogen (%)
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	Control (Untreated)	0.00 (0.00)
S.Em±	0.93	
C.D. at 1%	2.81	

*Figures in the parentheses are arcsine transformed values.

Table 2. Efficacy of plant extracts against *Cylindrosporium* leaf spot pathogen of *N. nimmoniana* under *in vitro* conditions

Treatment	Plant extracts	Growth inhibition of pathogen (%)	
		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	*Ocimum sanctum*	27.63 (31.71)	32.25 (34.60)
T_5	*Calotropis gigantia*	52.07 (46.19)	57.77 (49.47)
T_6	*Pongamiaglabra*	47.26 (43.43)	52.78 (46.59)
T_7	Control (Untreated)	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

inhibition (27.65%) of the pathogen was recorded in *Ocimum sanctum* (T_4) and in *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 is found 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 *Ocimum sanctum* (T_4) respectively, which were found on par with each other. The phenolics and phenolic acids, coumarins and pyrones, flavonoids, isoflavonoids, steroids and steroidal alkaloids and other miscellaneous compounds commonly present in the plant extracts could be the reason for inhibition of pathogen under *in vitro* (Reddy *et al.,* 2003). In case of *Azadirachta indica* the highest inhibition may be due to the alkaloids such as azadirachtin and nimbidin, which might have toxic effect on the pathogen. These results are in conformity with Choi *et al.* (2004) and Tapwal *et al.* (2012).

It is revealed that among the biocontrol agents *Trichoderma harzianum* (IOF) and among the plant extracts *Azadirachta indica* have shown promising results in inhibition of the *Cylindrosporium* fungal pathogen under *in vitro* conditions. Thus,the use of eco-friendly management practices (bioagents and plant extracts) could be encouraged to check the *Cylindrosporium* fungal pathogen rather than chemical fungicides for the management of leaf spot disease. In order to avoid the residue toxicity in the host plant use of non-chemical fungitoxicants may be recommended for the management of the *Cylindrosporium* leaf spot disease.

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