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A New Technique For Development of Biocontrol Formulation and its Use to Control Root Diseases of *Azadirachta Indica* A.juss

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ABSTRACT

A study was carried out to develop biocontrol formulations and their delivery system which are eco-friendy and economical feasible at nursery level at Tropical Forest Research Institute, Jabalpur (M.P.). Eight antagonistic fungi have been tested i.e Trichoderma harzianum, Trichoderm polysporum, Trichoderm virens, Trichoderm longibrachiatum, Trichoderm koningii, Trichoderm viride, Aspergillus terreus and Penicillium herquei against fungus. Trichoderma virens (85.94) has better effectiveness followed by Aspergillus terreus (85.59) and Penicillium herquie (85.12). The highest survival percentage was maintained in soil drenching with bavistin and soil mixing with Trichoderma virens (Tv) (93.33% at par), followed by seed treatment with bavistin and seed pelleting with Trichoderma virens (Tv) (86.67% at par) and seed pelleting with (Aspergillus terreus) At (80.00%).

KEYWORDS

Introduction

Pre-and post- emergence damping- off diseases are common in forest nurseries in moist as well as dry climates and most of the species being raised are affected, if congenial climatic conditions exist, causing severe losses to emergent crop which some times have been reported to the extent of 70% morality due to post-emergence damping-off alone (Harsh and Gupta 1993). *Fusarium* species are known to cause pre -and post-emergence damping-off and seedling wilt in a wide range of tree species.

Considering great importance of nurseries and the extent of damage caused by *Fusarium* species, particularly *Fusarium solani,* it is imperative to control these diseases in forest nurseries. Traditional control methods, such as use of chemical fungicides and management practices have not been found to deliver desired results unless augmented by technologies which will lead to more rapid, long term and effective solution of problems of diseases in forest nurseries and plantations. Disease control or management approach should be safe, economical, efficient, environmentally acceptable and have permanent effect. Today there is a growing movement in most of the countries to reduce the amount of chemicals being released into environment and their persistence and accumulation over a period of time in the environment especially in the soil and aquatic ecosystems, and to reduce their inherent hazards to plant and animal life and even human beings. Therefore, it is of utmost importance and timely needed that biological control should be accepted in place of chemical methods of control.

Neem (*Azadirachta indica*) is an important multipurpose evergreen (deciduous in drier areas) tree native to the Indian sub continent but cultivated through out South east Asia, Australia, Africa, many countries in central and South America, the Caribbean, Puerto Rico and the Virgin Islands. It is extensively planted along the road side, in village community land, near wells and in many deserted areas of the villages. It has capability to establish itself under the protection of thorny vegetation and in unoccupied land near mature trees.

Neem trees is described important culturally and medicinally because of amoebicidal, antiallergic, antidermatic, antifeedant, antifungul, antiginguitic, antienzemic, antiinflammatory, antipyrrhoeic, antiscabic, antiviral, bactericide, cardiac, diuretic, insecticidal, larvicidal, nematicidal, piscicidal, spermicidal and other biological activities of its derivatives.

Seedling wilt in *A. indica* caused by *Fusarium moniliforme* has been reported by Harsh & Gupta (1993) from Seoni, M.P. and also by *Fusarium* solani by Jamaluddin *et. al.* (1997).

Materials and Methods

Antagonistic activity of *Aspergillus terreus, Penicillium herquei* and *Trichoderma* species,. and their different isolates was tested in *in-vitro* against for *Fusarium solani* responsible for root diseases in forest nurseries of the *Azadirachta indica*. During this investigation antagonistic activity was carried out through presumptive test Broadbent *et al* (1971) and biculture test. Antagonist efficacy for each antagonist against individual Fusarium *avenaceum* was worked out according to the formulae.

Antagonistic efficacy = b+c-a, where

a=% of area of test fungus (*Fusarium solani*) with antagonist in the same Petri plate (cm^2), b=% of area of antagonist (at four corners) and c=% area of inhibition zone between antagonist and test fungus sp.

The formulation were prepared in bagasse, which was collected from juice centres, chopped, in to small bits and pieces (1-2 cm long), surface washed and dried in an oven at 80^0C for 12 hrs. The dried bagasse pieces were powdered by a Willey Mill Grinder and sieved with a 22 mesh screen. This powdered (25 g) was packed in autoclaveable polypropylene bags (23.5 x 17.5 cm) and to make it sufficiently moist, 50 ml water was added. The bags were sealed with a hot impulse and autoclaved. After the bags cooled down to room temperature 5 ml of spores suspension of antagonist prepared in sterilized water was inoculated through a hypodermic syringe in each bag. The bags were closed with a cello tape at the inoculation point and incubated for 15 days at room temp. (25 ± 2^0C), at 12 hrs alternate day-night cycles. The bags were shacked twice daily to spread the inoculum uniformly for first four days. The contents inside the bags was fully colonized by the inoculants in 15 days showing extensive sporulation. The contents were either taken out of the bags and dried in a laminar flow, packed in polythene bags and tight sealed for storage or the prepared packets left as such.

The culture of *Fusarium solani* causing root disease was multiplied on CMC media (Capillini and Peterson 1968) in 250 ml flask. Two 5 mm discs to 10 days old fungus was placed in the flask containing 200 ml autoclaved CMC media. The fun-

gus was allowed to grow for 15 days. The flasks were gently shacked twice daily. This culture was used in pathogenecity test and field trials of biocontrol formulations preparation. CFUs were counted with help of a Haemocytometer slide.

Experiments were conducted in field to control disease using biocontrol formulations and chemical fungicicde bavistin (carbrndazim) for comparison with nine treatments and three replications. In these treatments soil mixing and seed pelleting were given with biocontrol formulation and bavistin. Observations were recorded periodically regarding systoms of disease, growth behavior, survival and disease percentage. Seedling Vigour Index (SVI) and Seedling Vigour Index in Nursery (SVIN) were calculated by using formulae given by Abdul-babki and Alderson (1973).

Seedling Vigour Index (SVI) = (root length + Shoot length) X germination percentage

Seedling Vigour Index in Nursery (SVIN) = (root length + Shoot length) X Survival percentage

Statistical analyses were made to facilitate the results.

Results and Discussion

Eight antagonistic fungi have been identified, which are tested against each *Fusarium solani* separately. The results of presumptive tests have been presented in Table 1. In presumptive tests the percent area covered by the isolate in Petri plate have been taken into consideration for categorizing effective antagonist. The perusal of results indicate that on the basis of area covered by the antagonist in Petri plates, *Trichoderma virens* (29.19) followed by *Penicullium herquie* (29.67) and *Aspergillus terreus* (27.55) were the most effective antagonists against *Fusarium solani.* Therefore, these antagonists were screened for further test (biculture test). The results of biculture test have been tabulated in table No.2. It is evident that *Trichoderma virens* (85.94) has better effectiveness followed by *Aspergillus terreus* (85.59) and *Penicullium herquie* (85.12). The reduction in the growth of test fungus, area of inhibition zone between antagonist and the test fungus and area of antagonist are the deciding factors for the efficacy of antagonists under study. Versatility of antagonism to various species of the pathogen on different hosts is also a standard criterion for selection of a potential antagonist (Philipp and Cruger, 1979).

The results of biocontrol formulations on disease and growth parameters have been presented in table No.3. All treatments were significantly superior to control for survival percentage of seedlings, except soil mixing with *Aspergillus terreus* (*At*.) The highest survival percentage was maintained in soil drenching with bavistin and soil mixing with *Trichoderma virens* (Tv) (93.33% at par), followed by seed treatment with bavistin and seed pelleting with *Trichoderma virens* (Tv) (86.67% at par) and seed pelleting with (*Aspergillus terreus*) *At* (80.00%). Ahmed and Ahmed (1977) while estimating the losses, due to downy mildew disease in lucern reported decrease green fodder and dry matter. Sankar and Jeyarajan (1996) reported that *Trichoderma harzianum* and *Trichoderma viride* significantly reduced the root rot incidence of *Seasamum indicum* caused by *Macrophomina phaeolinus* to 10.1 % and 12.8 % respectively compared to 60 % in the control plots. Ojha, (2001) reported that bio-control formulation of *Trichoderma virens* is effective to control root diseases caused by *Fusarium* sp. of *Azadirachta indica, Moringa pterygosperma* and *Tectona grandis*.

All treatments gave significantly superior values than control for root length except for soil mixing with (*Penicillium herquie*) Ph. The maximum gain in root length was exhibited by soil drenching with bavistin (53.25%), followed by soil mixing with *Trichoderma virens* (Tv) (45.83%), seed treatment with bavistin (36.78%) and seed pelleting with *Trichoderma virens* (Tv) (31.26%) over control.

Shoot length in the treatments with Tv and bavistin were significantly higher than control. The soil mixing with Tv (66.75%) treatment have gained maximum shoot length, followed by soil drenching with bavistin (63.83%), seed treatment with bavistin (51.33%) and seed pelleting with TV (38.56%) over control.

Shoot and root fresh wt. values in all treatments were significantly superior to control, except seed pelleting with *At* for root wt. The maximum root and shoot wt. was gained in soil drenching with bavistin (111.86% and 165.61 %, respectively) followed by soil mixing with Tv (95.15% and 149.431%, respectively) over control.

The root dry wt was gained maximum in soil drenching with bavistin (138.20%) followed by soil mixing and seed pelleting with Tv (135.95% and 115.73%) and seed treatment with bavistin (111.23%) over control. Values for root wt. for these treatments were also significantly superior to control. Shoot wt. (dry) gain was recorded maximum in soil drenching with bavistin (103.57%) followed by seed treatment with bavistin (92.86%), seed pelleting and soil mixing with Tv (45.24%, 44.05%, respectively) and seed pelleting with *Ph* (44.05%) Values for shoot wt (dry) for these treatments were also significantly superior to control. Bhuiyan and Khondaker (2013) also found that *Trichoderma harzianum and Bacillus* strains were effective to control pre and post emergence seedling mortality of cucumber.

SVIN was observed significantly superior over control in all treatments except soil mixing with *At* and seed pelleting with *Ph*. The best SVIN gain was recorded in soil drenching treatment with bavistin (3172.29), followed by soil mixing with Tv (3094.82), seed treatment with bavistin (2662.50) and seed pelleting with Tv (2511.70). Ojha , (2015) reported that seed pelleting and soil mixing with Tv formulations was found superior among the biocontrol formulations in reducing the disease incidence which were at par with the fungicidal treatments. The same trends were also found in SVI and SVIN values.

Table- 1 Antagonistic activity of different antagonistic isolates against *Fusarium solani* using presumptive tests.

S. No.	Isolate No.	Isolate name	% area covered by fungus (cm^2)	% area covered by isolates (cm^2)
1.	08	Trchoderma harzianum	2.55	20.57
2.	09	T. polysporum	2.55	23.84
3.	12	T. virens	2.55	29.19
4.	18	T. longibrachiatum	2.55	19.75
5.	02	T. koningii	3.13	21.48
6.	06	T. viride	3.13	21.25
7.	10	Aspergillus terreus	3.13	27.55
8.	01	Penicillium herquii	3.13	29.67

Table-2 Antagonistic efficacy of selected antagonistic against *Fusarium solani* using biculture tests.

Isolate name	% of area of test fungus without antagonists (control)	% of area of test fungus with antagonists (cm^2) (A)	% of area of antagonists with test fungus (cm^2) (B)	% of area of inhibition zone of test fungus (cm^2) (C)	% of efficacy (B+C-A)
Aspergillus terreus	48.71	6.38	81.28	10.69	85.59 II
Trichoderma virens	48.71	6.06	81.59	10.41	85.94 I
Penicillium herquii	48.71	6.48	80.73	10.87	85.12 III

Table 3 Observations regarding survival and growth parameters of *Azadirachata indica*

Treat-ments	Survival %	Root length (cm)	Gain%	Shoot length (cm).	Gain %	Root fresh wt.(g)	Gain %	Shoot fresh wt (g)	Gain %	Root dry wt(g)	Gain %	Shoot dry wt (g)	Gain %	SVIN
T1	46.67	14.14	0.00	7.52	0.00	3.71	0.00	2.53	0.00	0.89	0.00	0.84	0.00	1023.00
T2	86.67	19.34	36.78	11.38	51.33	6.36	71.43	5.38	112.65	1.88	111.23	1.62	92.86	2662.50
T3	86.67	18.56	31.26	10.42	38.56	6.54	76.28	5.26	117.90	1.92	115.73	1.22	45.24	2511.70
T4	93.33	20.62	45.83	12.54	66.75	7.24	95.15	6.31	149.41	2.10	135.95	1.21	44.05	3094.82
T5	66.67	17.32	22.49	9.32	23.95	5.32	43.40	4.28	69.17	1.22	37.08	1.21	44.05	1776.09
T6	73.33	16.52	16.83	9.24	22.87	5.34	43.93	3.98	57.31	1.42	59.55	1.08	28.57	1888.98
T7	80.00	16.81	18.88	8.26	9.84	4.29	15.63	3.96	56.52	1.12	25.84	1.10	30.95	2005.60
T8	60.00	16.93	19.73	9.39	24.87	5.34	43.93	4.13	63.24	1.23	38.20	1.10	30.95	1599.60
T9	93.33	21.67	53.25	12.32	63.83	7.86	111.86	6.72	165.61	2.12	138.20	1.71	103.57	3172.29
SEM	5.34	0.77		0.58		0.45		0.44		0.15		0.09		239.11
CD@ 5%	17.41	2.51		1.90		1.46		1.42		0.50		0.29		779.68

T1 -(Contril-No treatment) T2 (seed treatment with bavistin i.e seeds were soaked for 5 minutes in concentration of 0.25) T3- seed pelleting with Tv i.e. 2g/ 25 moist seeds, T4- Soil mixing with Tv i.e. 2g/ microplot) , T5- seed pelleting with Ph i.e. 2g/ 25 moist seeds, T6- Soil mixing with Tv i.e. 2g/ microplot

T7- seed pelleting with A ti.e. 2g/ 25 moist seeds, T8- Soil mixing with At i.e. 2g/ microplot, T9- Soil drenching with bavistin i.e. concentration of 0.05)

REFERENCES

1. Abdul-babki and Alderson (1973). Vigour determination in soybean by multiple criteria. ***Crop Science* 3** ;630-633
2. Ahmed, N and Ahmed, O.A. (1977). Possibility of using a bacterial antagonists against fungal diseases of *Piper betle* and *Corchorus* sp. *Mycopath. Mycol. Appl*. **27:**273-279
3. *Bhuiyan, M.K.A and M.A. Khondaker (2013).* Assessing potentiality of rhizosphere bacteria and fungi from soil in antagonism against sclerotia forming pathogens and growth promotion of cucumber seedlings. In 10th international congress of plant pathology, abstracts, volume 43 (supplementary), organized in Beijing, China, August 25-30, pp-42
4. Broadbent P., Baker, K.F. and Waterworth, Y. (1971). Bacteria and actinomycetes antagonistic to fungal root pathogens in Australian soils. ***Aust.J. Biol. Sci***. **24**:925-944
5. Jamaluddin, Harsh, N.S.K. and Nath, V. (1997). Handbook of diseases of tropical tree species, ***TFRI, Pub. No. 8***, 53 p
6. Harsh, N.S.K. and Gupta, B.N. (1993). TFRI Information series No.1 31p
7. Ojha, B.M. (2001). Studies on Biological of root diseases caused by some *Fusarium* species in some multipurpose tree species of forest nurseries. Ph.D thesis, Guru Ghasisdas University, Bilaspur, C.G.
8. Ojha, B.M. (2015). Effect of biocontrol formulations and chemical fungicides (Bavistin) on damping off disease and growth parameters in *Acacia catechu* (KHAIR **).** Indian Journal of Research.Vol.4 issue-12, 2015, pp14-16
9. Philip, W.D. and Cruzer, G. (1979). Porasitimus Von Ampelomyces quisqualis auf Echlen Mchltavpizen an Gurken and Anderm Gertiseartn Z. *Pflanzenkr Pflanzenschutz*. **36:** 129-142pp
10. Sankar , P and Jeyarajan, R. (1996). Seed treatment formulation of *Trichoderma* and *Gliocladium* for biological control of *Macrophomina phaseolina* in seamum. *Indian phytopath*.**49 (2):**148-151