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Evaluation of Antagonistic Potential of *Trichoderma asperellum* (*in-vitro*) against Fungal Diseases of Important Medicinal Plants

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ABSTRACT: Now-a-days plant products are increasingly being used by humans for food and health care benefits. At the same time, we are concerned for the health of plants especially medicinal and aromatic plants as their health can yield products that are healthy for us. A survey was carried out on fungal diseases of economically important five medicinal plants *viz*. Ashwagandha (*Withania somnifera*), Sarpagandha (*Rauvolfia serpentina*), Tulsi (*Ocimum sanctum*), Mint (*Mentha arvensis*) and Mandukparni (*Centella asiatica*) in MAP germplasm blocks at Dr. RPCAU, Pusa and its neighbouring areas at different time intervals. During the survey, eleven fungal diseases namely leaf spot/ blight (*Alternaria alternata*), wilt (*Fusarium solani*) and downy mildew (*Peronospora* sp.) of Ashwagandha, leaf spot (*Colletotrichum truncatum*) and leaf blight (*Alternaria* sp.) of Sarpagandha, leaf spot (*Colletotrichum gloeosporioides*) and leaf blight (*Alternaria* sp.) of Tulsi, leaf spot (*Curvularia lunata*) and leaf blight (*Alternaria alternata*) of Mint and leaf spots (*Cochliobolus* sp. and *Alternaria* sp.) of Mandukparni were recorded. We attempted to control it in vitro by biocontrol agent *Trichoderma asperellum* and for this purpose we employed dual culture plate method.*In vitro* evaluation of native *Trichoderma asperellum* showed effective inhibition against the pathogens. Maximum inhibition percent was recorded against *Fusarium solani i.e.* 56.36 per cent followed by *Cochliobolus* sp. *i.e.* 55.38 per cent. According to the research results, *Trichoderma asperellum* had the potential to effectively manage the plant pathogens and this antagonistic nature can help in increasing the yield and quality of medicinal plants. This work may encourage other researchers to study these diseases further and their integrated management using biocontrol agents and plant extracts by properly testing their efficacy in the agrifields.

Keywords: Medicinal plants, fungal pathogens, biocontrol, *Trichoderma asperellum.*

INTRODUCTION

Products originated from plants are increasingly being used by humans for food and health care benefits. At the same time, we are concerned for the health of plants especially medicinal and aromatic plants as their health can yield products that are healthy for us. Diseases and pests cause deterioration of the quantity and quality of economic product and loss of genetic resources. Many diseases of medicinal plants have been reported in different locations of the country. In Bihar, research work on fungal diseases of medicinal plants is limited, so we surveyed different locations in Samastipur district and identified major fungal diseases of important medicinal plants *viz.* leaf blight (*Alternaria alternata*), wilt (*Fusarium solani*) and downy mildew (*Peronospora* sp.) of Ashwagandha (*Withania somnifera*), leaf spot (*Colletotrichum truncatum*) and leaf blight (*Alternaria* sp.) of Sarpagandha (*Rauvolfia serpentina*), leaf spot (*Colletotrichum gloeosporioides*) and leaf blight (*Alternaria* sp.) of Tulsi (*Ocimum sanctum*), leaf spot (*Curvularia lunata*) and leaf blight (*Alternaria alternata*) of Mint (*Mentha arvensis*) and leaf spots (*Cochliobolus* sp. and *Alternaria* sp.) of Mandukparni (*Centella asiatica*).

Hence, these diseases need to be managed and generally chemical fungicides are recommended which are continuously polluting the environment and killing non-targeted microflora and fauna (Alabouvette and Couteadier, 1992). Due to these harmful effects, we are

compelled towards eco-friendly management of diseases. Biological control by *Trichoderma* sp. is one of the most effective and eco-friendly ways as different *Trichoderma* sp. produce various enzymes that play a significant role in activities such as cell wall degradation, abiotic and biotic stress tolerance, hyphal growth and antagonistic potential against plant pathogens. Many *Trichoderma* species have shown potential antagonistic activity against various soil borne fungi such as *Sclerotium rolfsii* (Dutta and Das, 2002; Das *et al*., 2006), *Rhizoctonia solani*, *Fusarium* spp., *Aspergillus niger* (Dutta *et al*., 1999), *Sclerotinia sclerotiorum* (Dutta *et al*., 2008) and others. Their success depends on the development of rhizospheric competent strains in different agro-climatic zones of the country. Therefore, in our research work, we have isolated different pathogens from different medicinal plants and have used *Trichoderma asperellum* (local strain) for *in-vitro* management of the above mentioned diseases.

MATERIALS AND METHODS

Efficacy of local biocontrol agent *Trichoderma asperellum* against different pathogens isolated from medicinal plants was evaluated *in vitro* in the Department of Plant Pathology through Dual Culture Technique and their inhibition percentage was recorded.The experiment was conducted in the year 2020-21.

A. Dual culture technique

This technique refers to the culturing of two organisms together in the same medium. It is used to determine the antagonistic potential of any bioagent (fungi or bacteria) against other pathogenic fungi or bacteria. Here, two fungal isolated are inoculated in same petri plate and allowed to grow to their fullest and the growth of the pathogen is then recorded separately in control plates. Mainly three mechanisms are involved in this technique for the control of growth of pathogenic fungi viz. competition, parasitism and antibiosis. If the diameter of fungal mycelium in control is 'C' and treatment is 'T', then the antagonistic efficacy of biocontrol is determined by using the formula C-T/ C× 100.

The local strain *Trichoderma asperellum* was collected from Department of Plant Pathology, Dr. RPCAU and evaluated against different isolated pathogens of medicinal plants by Dual Culture technique. For this process to be carried out 7 mm disc from actively growing cultures of isolated pathogens was placed on one side of the Petri plate and 7 mm disc of *Trichoderma asperellum* was placed at opposite side in the same plate. Each treatment was replicated 3 times and control plates for each pathogen were also maintained. These plates were incubated at 28 $\pm 1^0$ C temperature for 5 days. The colony diameter of different pathogens in dual culture plates and control plates were recorded to calculate the percentage inhibition of growth of each fungus by the *Trichoderma* strain. The percentage inhibition of mycelial growth over control was calculated by the formula given by Bliss (1934).

$$\text{Per cent inhibition over check} = \frac{(C - T)}{C} \times 100$$

Where,

C = growth of pathogen in check

T = growth of pathogen in treatment

B. Statistical analysis

The data that were produced in different studies of current research have been analyzed using Completely Randomized Design (CRD). The percentage, mean, standard deviation were calculated for understanding the efficacy of different parameters present in the objectives of the study. The data which were obtained from the experimental findings were subjected to statistical analysis (Gomez and Gomez, 1984). The calculated values of F were compared with tabulated value at 5 percent probability level for appropriate degree of freedom (Fisher and Yates, 1968; www.OPSTAT.com).

RESULTS AND DISCUSSION

The efficacy of local *Trichoderma* strain was evaluated against the growth of different isolated pathogens from important medicinal plants by Dual Culture technique. Data on radial growth and percent growth inhibition of all fungi were taken after 96 hours, 120 hours and 144 hours of inoculation. The radial growth of pathogens (G in mm) and growth inhibition percent (I) were recorded. Data presented in Table 1 exhibited that *Trichoderma asperellum* showed inhibitory effect and was effective in controlling the growth of pathogens significantly. After 96 hours and 120 hours of inoculation, it showed remarkable inhibition against *Fusarium solani* where the radial growth of the pathogen was 13.10 mm while in control it grew 29.17 mm which means the growth was inhibited by 55.01 per cent followed by *Cochliobolus* sp. where the radial growth recorded was 12.16 mm while in control it was 26.27 mm which indicated that the growth was inhibited by 53.71per cent. Bajwa *et al.*, (2004) evaluated *Trichoderma* isolates against *Fusarium solani* and found *Trichoderma harzianum* to be most effective against the test fungi with reduction in colony growth by 52.4 per cent.

After 144 hours of inoculation, maximum inhibition was shown against *Fusarium solani* where the radial growth was recorded to be 15.10 mm with control having 35.10 mm growth indicating the reduction in percent growth by 56.36 per cent. This was followed by inhibition of *Cochliobolus* sp. where the radial growth of pathogen was recorded to be 14.13 mm with control having 31.67 mm growth indicating the reduction in percent growth by 55.38 per cent. *T. asperellum* was

also found effective against *Alternaria alternata* isolated from Ashwagandha (53.06%), *Alternaria* sp. from Sarpagandha (53.51%), *Curvularia lunata* (52.12%). Hasan *et al.*, (2012) investigated antagonistic effect of *Trichoderma harzianum* against *Bipolaris sorokiniana* in wheat and observed that it inhibited the growth by 45.71 per cent. Babychan and Simon (2017) investigated *Trichoderma* isolate MiT-4 to be effective against the radial mycelial growth inhibition of *Fusarium oxysporum* f.sp. *lycopersici* by 58.4 per cent.

The growth inhibition percent for *Alternaria alternata* isolated from Mint and *Alternaria* sp. isolated from Mandukparni were at par with each other *i.e.* the growth of pathogens was inhibited by 47.08 per cent. Rahman *et al.*, (2020) also evaluated five strains of *Trichoderma* against *Alternaria alternata* causing leaf blight in Ashwagandha (*Withania somnifera*) and observed that *Trichoderma harzianum* IMI- 392433 showed maximum inhibition percentage (54.89%) followed by *T. harzianum* IMI-392432 (53.83%), *T. harzianum* IMI- 392434 (48.94%) and *T. virens* IMI-392430 (43.62%).

Minimum growth inhibition was shown against *Colletotrichum truncatum* where the radial growth of pathogen was recorded to be 9.13 mm while growth in control was recorded to be 14.33 mm which means the growth was inhibited by 36.36 per cent.

Table 1: *In vitro* evaluation of *Trichoderma asperellum* (local strain) on radial growth and per cent growth inhibition of isolated pathogens at different time intervals.

Treatments	96hrs (4th day)			120hrs (5th day)			144hrs (6th day)		
	G	C	I	G	C	I	G	C	I
***Alternaria alternata* (A.G)**	9.16	17.30	47.05	10.10	19.57	47.87	10.20	21.73	53.06
Fusarium solani	13.10	29.17	55.01	14.50	32.03	55.47	15.10	35.10	56.36
Colletotrichum truncatum	7.13	10.10	29.40	8.13	12.20	33.36	9.13	14.33	36.36
***Alternaria* sp. (S.G.)**	9.13	17.33	47.31	10.00	19.00	47.36	10.00	21.52	53.51
C. gloeosporioides	9.15	17.20	46.80	10.12	19.33	47.56	11.12	21.60	48.52
***Alternaria* sp.(Tulsi)**	9.16	17.35	47.20	10.21	19.41	47.58	11.2	21.50	47.91
Curvularia lunata	8.20	15.13	46.51	9.00	17.11	46.26	9.16	19.13	52.12
***Alternaria alternata*(Mint)**	10.18	18.27	44.25	11.30	20.50	45.70	11.43	21.60	47.08
***Cochliobolus* sp.**	12.16	26.27	53.71	13.30	29.30	55.11	14.13	31.67	55.38
***Alternaria* sp.(M.P.)**	10.18	18.23	44.15	11.30	20.50	45.70	11.43	21.60	47.08
SE(m)±	**0.08**			**0.09**			**0.07**		
C.D. at 5%	**0.25**			**0.27**			**0.22**		
C.V.	**1.07**			**1.02**			**0.78**		

G- Radial growth (mm); I- Inhibition per cent; C- Control

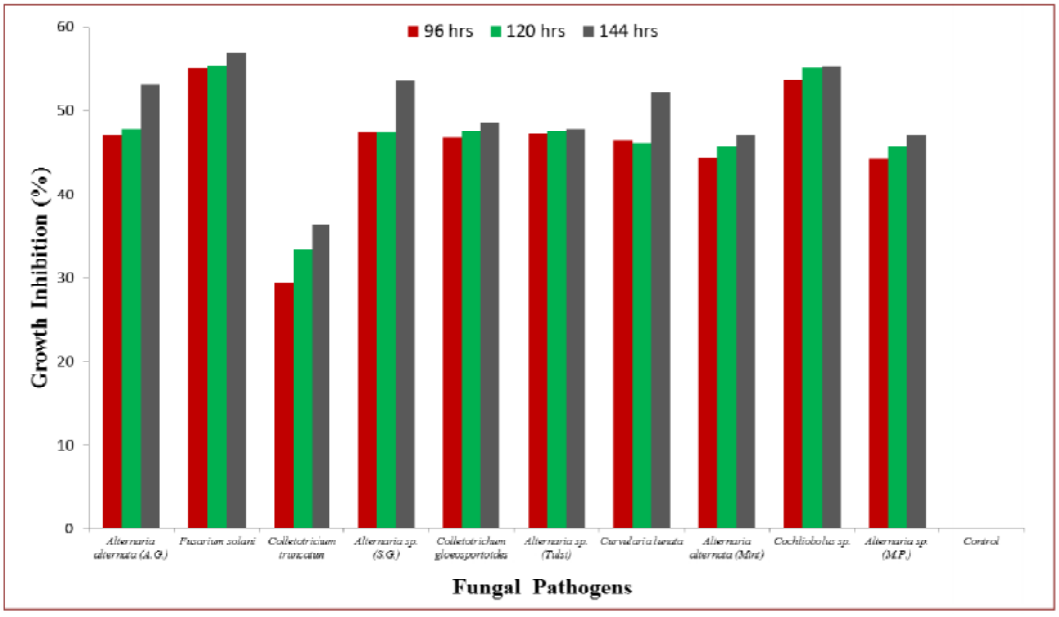


Fig. 1. *In vitro* effect of *Trichoderma asperellum* on per cent growth inhibition of isolated pathogens at different time intervals.

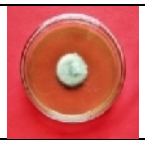
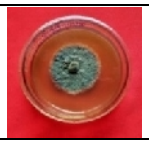
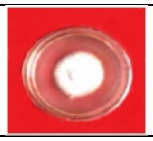
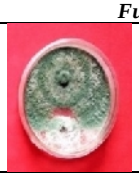
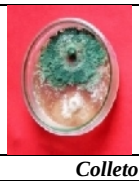
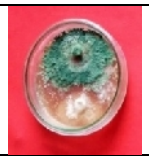
96 hrs	Control	120	Control	144	Control
					
***Alternaria alternata* (Ashwagandha)**					
					
Fusarium solani					
					
***Alternaria* sp. (Sarpagandha)**					
					
Colletotrichum truncatum					
					
Colletotrichum gloeosporioides					
					
***Alternaria* sp. (Tulsi)**					
					
Curvularia lunata					
***Alternaria alternata* (Mint)**					
***Cochliobolus* sp.**					
***Alternaria* sp. (Mandukparni)**					

Plate 1. *In vitro* effect of *Trichoderma asperellum* on radial growth and per cent growth inhibition of isolated pathogens at different time intervals.

CONCLUSION

Based on the results obtained in this work, we can say that some of the minor diseases are emerging to become the major diseases of these plants in Bihar and local *Trichoderma asperellum* strain has a good chance to become a successful biological control agent. The success of biocontrol of plant diseases depend on their effective formulations, their survival and rapid multiplication and colonization after inoculation. Their success depends on the development of rhizospheric competent strains in different agro-climatic zones of the country. Strains of *Trichoderma* sp. against pathogens, their action mechanism, compatibility with components of integrated pest management, quality formulations and success in field are major issues that are needed to be further explored intensively.

FUTURE SCOPE

Biological control has always been a great aspect in plant disease management due to its different benefits both to the plant as well as the environment. It involves biocontrol agents which on interaction with the plant or pathogen reduce the growth of pathogen and limits its negative impact on the plant. Ultimately, extensive studies on biocontrol agents would reveal more information for effective disease management without causing harm to other biosystem.

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Conflict of Interest. None

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