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Application of phylloplane fungi to manage the Leaf spot of *Rauwolfia serpentina* caused by *Alternaria alternata*

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ABSTRACT- Phylloplane fungi presented on the leaf surface were screened and selected for the assessment of their potential against *Alternaria alternata* causing leaf spot disease of *Rauwolfia serpentina* (Sarapgandha). Among the antagonists, Trichoderma harzianum ISO-2 showed minimum conidial germination. Effect of foliar spray of phylloplane fungi in lesion development was also studied. In the mist chamber studies, seven treatments comprising the application of antagonists in the presence of pathogen proved effective in causing percent disease reduction by 30-50 % in comparison with the control. In the field trials, *Penicillium sublateritium* showed the maximum fresh and dry root weight. *P. sublateritium* also showed significant percentage disease reduction in comparison to control.

Key-Words- *Alternaria alternata*, Foliar spray, Leaf spot, Phylloplane fungi, *Rauwolfia serpentina*

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INTRODUCTION

Medicinal plants play an important role in the development of potential therapeutic agents. They contain a variety of different nutritious and therapeutic constituents: vitamins, minerals, trace elements as well as active ingredients with a variety of medicinal actions. These include volatile oils, tannins, mucilage, alkaloids, bitters and flavonoids.

Rauwolfia serpentina (L). Benth. Ex Kurz. (Apocynaceae family) commonly known as *sarpagandha* is an important medicinal plant of Indian subcontinent and South East Asian countries. It is an erect, evergreen perennial under shrub and contains a number of bioactive chemicals, including ajmaline, deserpidine, rescinnamine, serpentinine, and yohimbine. Reserpine is an alkaloid first isolated from *R. serpentina* and was widely used as an antihypertensive drug (Fabricant and Fransworth, 2001; Harisaranraj*et al*., 2009; Dey and De, 2010).

R. serpentina is also used for the treatment of various central nervous system disorders associated with psychosis, schizophrenia, insanity, insomnia, epilepsy and acts as hypnotics (Pakrashi and Akkhari, 1968; Meena *et al*., 2009). Extracts of the roots are valued for the treatment of intestinal disorders, particularly diarrhoea and dysentery. The juice of the leaves has been used as a remedy for opacity of the cornea and also to prevent inflammation (Anisuzzaman *et al*., 2007).

Leaf spot in *R. serpentina* was reported by (Puni and Harsh, 2009) and the causal organism was identified as *A. alternata*. The disease appears as minute yellow spots which gradually increase in size, turns dark and the leaves fall off. The disease was found in nurseries in Dehradun, Udham Singh Nagar, Rishikesh and Naini Tal.

Application of fungicides is still the effective method to control these diseases, but the widespread use of the chemical fungicides has become a subject of research

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concern due to their harmful effect on non-target organisms as well as their possible carcinogenicity (Ziedan and Farrag, 2011). Due to health risks and pollution hazards by the use of chemical fungicides in plant disease control, it is considered appropriate to minimize their use.

Biological control of plant pathogens through antagonistic microorganisms is eco-friendly and a sustainable approach than the use of fungicides (Prakasam and Sharma, 2012). The possibility of controlling the pathogenic fungi by antagonistic microorganisms has been explored by various workers (Blakeman, 1985; Mercier and Reelender, 1987; Pandey *et al*., 1993). Biological control based on myco-parasitism and hyper-parasitism between some organisms provides an alternative to chemical control. Biological control is ab increasingly important alternative to chemicals in crop protection. Perello *et al*. (2006), Go-sawmi and Islam (2002) reported, the antagonistic effects of saprophytic microorganisms.

The aim of the present study was to evaluate the effectiveness of antagonistic phylloplane fungi and their metabolites, against *A. alternata* causing leaf spot of *R. serpentina*.

MATERIALS AND METHODS:

Isolation of pathogenic fungi

For the isolation of pure culture of fungal pathogen, a portion of leaf containing brown spot on the leaves of *R. serpentine* in the month of august during rainy season were collected from the nursery of non-wood forest products division (NWFP), F.R.I. Dehradun, Uttarakhand. Leaves were surface sterilized with 0.1% mercuric chloride for 1 min, followed by rinsing with three changes of sterilized distilled water and was placed on potato dextrose agar medium in petri plates. The plates were incubated in a B.O.D. incubator at 25±1°C for mycelial growth. Based on the morphological characters; the organism was identified with the help of standard monographs (Ellis, 1971).

Isolation of phylloplane fungi

Phylloplane fungi were isolated from healthy leaves of *R. serpentine* was also collected from non-wood forest products division (NWFP), F.R.I. Dehradun, Uttarakhand through leaf washing technique (Dickinson, 1967; Aneja, 2003) and identified with the help of standard monographs (Ellis, 1971) and expertise available. To study their antagonistic properties, pure cultures were maintained on potato dextrose agar medium at 4°C in a refrigerator.

Conidial germination of pathogen in the culture filtrates of antagonists

Sterilized PDB broth was prepared and taken in 150 ml flasks (50 ml PDB) and inoculated with the discs of antagonistic fungi viz., *Trichoderma harzianum* ISO-1, *T. harzianum* ISO-2, *T. piluliferum, Aspergillus niger,* and *Penicillium sublateritium* and incubated at 25±1°C for 7 days. The broth culture was filtered by Whatman No.1 filter paper. The culture filtrate was divided into two parts. One part was left as such (with cell) and the second part was filtered by bacterial syringe filter (0.45 µm) to make the culture cell free. A humid chamber for conidial germination was prepared by using germination paper which was cut according to the size of the Petri plate (14 cm) and moistened with distilled water and then sterilized in an autoclave.

Six sets of cavity slides were taken, cleaned with rectified spirit, followed by sterilized distilled water and placed in Petri plates. In the first and second set of cavity slides the wells of cavity slides, were filled with sterilized distilled water (20 µl) in which the conidia from the 7 day's old culture of the test pathogen were introduced and mixed with the sterilized needle. Slides were then incubated in a humid chamber. In the third and fourth set of cavity slides, 20 µl of (with cell) culture filtrate of the antagonist fungi was placed. Conidia of the test pathogen were introduced over the culture filtrate and the slides were incubated in a humid chamber. Similarly fifth and sixth sets were also

prepared in which 20 µl of (cell free) culture filtrate of the antagonist fungi was placed and conidia of test pathogen was placed over the culture filtrate and the slides were incubated in a humid chamber. First, third and fifth sets were incubated in light at room temperature. Simultaneously second, fourth and sixth sets were incubated in dark conditions at room temperature by covering them with brown germination paper.

Observations for conidial germination were started after 6 hours and continued till 48 hours. Conidial germination and characteristics of the germ tube were determined and compared to that of the control (Dhingra & Sinclair, 1985).

The percentage of conidial germination was determined by counting total number of conidia in five microscopic views under a light microscope and their average was taken for determining percent germination by the formula:

Germinated conidia (%) = Germinated conidia/Total number of conidia × 100

Where, Germinated conidia= Number of germinated conidia observed in five microscopic field

Total = No. of conidia in five microscopic field

In vivo assessment of screened phylloplane fungi against pathogen (*A. alternata*)

Interactions on the Leaf surface (Mist chamber)

The experiments were conducted at Forest Pathology Division mist chamber, at Forest Research Institute, Dehradun, Uttarakhand. The experiments were conducted in nursery polybags.

A comparative study of prophylactic treatments (fungicides) copper oxychloride and antagonists*T. harzianum* ISO-1, *T. harzianum* ISO-2, *T. piluliferum*, *A. niger* and *P. sublateritium* was made against *A. alternate* isolated from diseased plants of *Rauwolfia serpentina*. The experiments were laid out with fourteen treatments in three replications all in CRBD inside the mist chamber, Forest Pathology Division. 10 ml of conidial suspension of test pathogen/antagonist were applied to 4 plants per replication.

Interaction between the pathogen and the antagonistic fungi were studied on the leaf surfaces by measuring the lesion development produced by *A. alternata*. Healthy leaves of *R. serpentina* were inoculated with conidial suspension (10^5spores/ml) of each of the pathogen and the antagonistic fungus was also sprayed onto the leaves. the leaves were separately inoculated with 10 ml spore suspension of the pathogen without the antagonists (as control). Inoculated leaves were covered with sterile polythene bags to maintain humidity. After 15 days, the lesion size of the treated and control leaves were measured. Percent disease index was calculated by the formula:

$$\text{PDI} = \frac{\text{Sum of numerical rating}}{\text{Total number of leaves examined}} \times \frac{100}{\text{Maximum grade value}}$$

Interactions on the leaf surface (field assay)

The field experiments were conducted during 2012-2013 under rainfed conditions at Non Wood Forest Product Division, Nursery, F.R.I., Dehradun in order to evaluate the efficacy of biocontrol agents in managing leaf spot of *R. serpentina*. A total of seven treatments consisting of one treated with fungicide (copper oxychloride), five antagonists viz., *T. harzianum* ISO-1, *T. harzianum* ISO-2, *T. piluliferum*, *A. niger* and *P. sublateritium* and one inoculated control i.e. (pathogen), respectively were applied in the field.

The experiment was conducted in a plot size 10 ft.×10 ft. All the treatments were replicated thrice following complete randomized block design. Three months old plants of the host species were transferred from the nursery bed to experimental site and were allowed to establish. 10ml of conidial suspension of test pathogen/antagonist were applied to 4 plants per replication. The leaves were separately inoculated with 10 ml spore suspension of the pathogen without the antagonists (as control). Inoculated leaves were covered with sterile polythene bags to maintain humidity. After 15 days, the lesion size of the treated and

control leaves were measured. Observations recorded after the termination of the experiments in mist chamber and field trials (After 60 days):

1. Shoot height using a meter scale
2. Collar diameter using a digital Vernier Calliper
3. Fresh and dry shoot and root weight using an electronic top pan balance
4. Percent disease index (PDI)
5. Percentage disease reduction

Statistical analysis

Experiments were performed in triplicates and the data were analyzed by using ANOVA procedures of GENSTAT software to determine any significant differences among parameters analyzed at 5% level of significance.

RESULTS

A pathogen causing leaf spot in *R. serpentina* was identified as *Alternaria alternate* Keissler. The antagonists were identified on the basis of their cultural and microscopic characteristics as *Trichoderma harzianum* Rifai ISO-1 and *T. harzianum* ISO-2, *T. piluliferum* Webster and Rifai, *Aspergillus niger*van Tieghem, *P. sublateritium* Biourge.

Conidial germination of pathogen in the culture filtrates of antagonists

When the effects of the culture filtrates of antagonists against *A. alternata* were examined under the light condition, it was observed that the minimum conidial germination was shown in the culture filtrate of *T. harzianum* ISO-2 (12.86%) followed by *P. sublateritium* (18.79%). Conidial germination of *A. alternate* in the culture filtrate of *T. harzianum* ISO-1 (20.82%) was found to be at par with *A. niger* (21.61%) and *T. piluliferum* (21.06%) (Table 1a).When the interaction between antagonists and treatments were examined minimum conidial germination was observed in the culture filtrate of *T. harzianum* ISO-2 (0.00, 0.00) 'with cell' and 'cell free' and maximum in control (38.57) (sterilized distilled water). were at par and significant in comparison to inoculated The minimum conidial germination of *A. alternate* was shown by *T.harzianum* ISO-2 (10.71%) followed by *T. harzianum* ISO-1 (14.02%) under the dark condition. Conidial germination of *A. alternata* in the culture filtrate of *P. sublateritium* (15.96%), *A. niger* (16.62%) and *T. piluliferum* (17.61%) were found to be at par with each other (Table 1 b). Minimum conidial germination was observed in the culture filtrate of *T. harzianum* ISO-2 (0.00, 0.47) 'with cell' and 'cell free' respectively and maximum was in control (31.67) (sterilized distilled water) when the interaction between antagonists and treatments were studied.

Interactions on the leaf surface (mist chamber)

The test antagonists conidial suspension when applied in the presence of pathogen conidial suspension, they reduced the development of the lesion caused by the pathogen on leaves. The criteria of maximum root biomass were used in deciding the best biocontrol treatment because the roots of *R.serpentina* are used in medicinal preparations other growth parameters were also taken into consideration. There was a significant increase in the fresh and dry root weight of *R. serpentina* when treated with antagonists in the presence of pathogen in comparison to the inoculated control (T2) (Table 2). Plants treated with *T. harzianum* ISO-2 (T11) (5.72g) in the presence of pathogens could significantly increase the fresh root weight of the plants over inoculated control and was at par with *T. piluliferum* (T12). Treatment with *A. niger* (T13) was at par with *P. sublateritium* (T14). Treatments with Copper oxychloride (T9) and *T. harzianum* ISO-1 (T10) were at par when compared to inoculated control (T2) (1.98g).

Dry root weight was observed maximum in the treatment with *T. harzianum* ISO-2 (T11) (3.14g) which was at par with *T. piluliferum* (T12), *A. niger* (T13) and *P. sublateritium* (T14) in the presence of pathogen. Treatments with Copper oxychloride (T9) and *T. harzianum* ISO-1 (T10) control (T2) (0.37g).

The conidial suspension of all the test antagonists, either in the absence or in the presence of pathogens reduced the development of lesions (leaf spot) caused by the pathogen on leaves. Percentage disease index (P.D.I.) shown by the plants treated with antagonists in the presence of pathogens treatment with *T. harzianum* ISO-1 (T10) showed minimum P.D.I. followed by *T. harzianum* ISO-2 (T11) which was at par with *A. niger* (T13). Treatments with *T. piluliferum* (T12), *P. sublateritium* (T14) and Copper oxychloride (T9) showed less P.D.I. when compared to inoculated control (T2) (56.63).The percentage disease reduction was significantly superior in treatment with *T. harzianum* ISO-1 (T10) (51.72%) in the presence of pathogen.

Table 1: Percent conidial germination of *A.alternata* in Light condition

Treatments	Antagonists					Mean
	***T. harzianum* ISO-1**	***T. harzianum* ISO-2**	***T. piluliferum***	***A. niger***	***P. sublateritium***	
Control (sterlized distilled water)	38.57±3.21	38.57±3.21	38.57±3.21	38.57±3.21	38.57±3.21	38.57
With cell	11.83±1.62	0.00	7.87±0.55	11.43±3.78	8.87±1.28	8.00
Cell free	12.07±0.90	0.00	16.73±1.27	14.83±2.95	8.93±0.90	10.51
Mean	20.82	12.86	21.06	21.61	18.79	
	A		**T**		**A x T**	
SEM±	0.78		0.60		1.35	
CD at 5%	2.25		1.74		3.91	

A-Antagonist; T-Treatment

Table 1: Percent conidial germination of *A. alternata* in dark condition

Treatments	Antagonists					Mean
	***T. harzianum* ISO-1**	***T. harzianum* ISO-2**	***T. piluliferum***	***A. niger***	***P. sublateritium***	
Control (sterilized distilled water)	31.67±2.01	31.67±2.01	31.67±2.01	31.67±2.01	31.67±2.01	31.67
With cell	1.30±0.17	0.00	5.80±1.99	7.20±3.46	8.27±1.87	4.51
Cell free	9.10±1.03	0.47±0.41	15.37±2.56	11.00±2.51	7.93±2.05	8.77
Mean	14.02	10.71	17.61	16.62	15.96	
	A		**T**		**A x T**	
SEM±	0.65		0.50		1.13	
CD at 5%	1.89		1.46		3.28	

A-Antagonist; T-Treatment

Table 2: Effect of different treatments on the growth parameters of *Rauwolfia serpentina*in mist chamber

Treatments	Parameters							
	Collar diameter (mm)	**Plant Height (cm)**	**Fresh Shoot Weight (g)**	**Dry Shoot Weight (g)**	**Fresh Root Weight (g)**	**Dry Root Weight (g)**	**Disease Incidence (%)**	**Disease reduction (%)**
T1 (Control)	1.36±0.03	24.17±3.37	4.35±0.57	1.95±0.43	3.29±0.28	1.77±0.37	1.66±1.92	54.97
T2 (Pathogen)	0.90±0.14	18.35±1.56	1.84±0.52	0.47±0.12	1.98±1.01	0.38±0.07	56.63±2.73	–
T3 (*T. harzianum* ISO-1)	1.31±0.02	33.30±3.09	2.60±1.16	1.47±0.52	3.20±1.64	1.89±1.07	0.83±1.66	55.83
T4 (*T. harzianum* ISO-2)	1.26±0.03	32.58±3.05	4.57±2.43	2.40±1.13	2.26±0.60	1.25±0.34	1.66±1.92	54.97
T5 (*T. piluliferum*)	1.36±0.05	30.25±2.94	4.44±1.36	2.24±0.91	2.80±0.65	1.67±0.49	3.25±2.58	53.38
T6 (*A. niger*)	1.35±0.05	31.57±2.94	2.82±2.51	1.10±0.40	4.41±1.60	1.85±0.74	2.50±1.66	54.13
T7 (*P. sublateritium*)	1.50±0.05	30.85±4.51	4.88±2.07	2.34±0.40	5.13±1.37	2.36±1.89	0.83±1.66	55.83
T8 (Copperoxychloride)	1.32±0.03	26.52±2.82	3.10±1.52	1.45±0.68	1.88±0.72	1.12±0.58	1.66±1.92	54.97
T9 (Pathogen + Copperoxychloride)	1.27±0.02	29.65±3.72	4.25±0.38	1.77±0.09	2.62±0.09	1.80±0.26	18.30±4.31	38.33
T10(Pathogen + *T. harzianum* ISO -1)	1.43±0.05	31.02±2.50	7.45±3.02	3.50±0.83	2.72±0.55	1.55±0.24	4.91±1.83	51.72
T11 (Pathogen + *T.harzianum* ISO-2)	1.40±0.07	30.50±5.04	3.70±1.23	1.73±0.74	5.72±2.16	3.14±1.37	6.08±3.31	50.55
T12(Pathogen + *T. piluliferum*)	1.31±0.05	31.82±4.44	4.13±2.12	2.13±0.68	4.69±2.81	2.91±1.98	9.07±3.26	47.56
T13 (Pathogen + *A. niger*)	1.29±0.03	31.35±5.47	5.10±2.06	2.44±1.32	4.30±1.75	2.41±1.15	5.83±1.66	50.80
T14 (Pathogen + *P. sublateritium*)	1.29±0.04	34.10±1.60	4.34±1.62	2.05±0.71	4.28±1.79	2.14±0.64	11.64±4.27	44.99
SEM±	0.02	1.81	0.89	0.36	0.71	0.49	1.32	
CD at 5%	0.08	5.18	2.55	1.03	2.05	1.42	3.78	

Interactions on the leaf surface (Field assay)

After the mist chamber studies, field trials were conducted. Based on the mist chamber experimentations, the treatments (antagonists and copper oxychloride) in the presence of pathogens were found effective when growth parameters in respect to the plant parts responsible for medicinal properties possessed by *R. serpentina*, were taken into consideration in comparison to the treatments (antagonists and copper oxychloride) during the absence of pathogen.

On the basis of studies made in mist chamber, the treatments (antagonists and copper oxychloride) were applied in the presence of the pathogen in the field trials. A comparative study was done with the five antagonistic fungi and fungicide (Copper oxychloride) in the presence of pathogens to test their effects on the growth parameters of *R.serpentina*. In case of field assay plants treated with *P. sublateritium* (E) (4.65g) exhibited maximizes fresh and dry root weight followed by *A. niger* (A), which was at par with *T.harzianum* ISO-2 (C). Treatments with *T. piluliferum* (D) and *T. harzianum* ISO-1 (B) were at par with each other (Table 3).

Minimum P.D.I. was also shown by the treatment with *P. sublateritium* (E) (6.94). Treatment with *A. niger* (A) was at par with *T. harzianum* ISO-1 (B). Treatment with *T. piliuliferum* (D) was also at par with *T. harzianum* ISO-2 (C).

The maximum percentage disease reduction was found in treatment with *P. sublateritium* (E) (38.26%).

Table 3: Effect of different treatments on the growth parameters of *R. serpentina* in field

Treatments	Parameters							
	Collar Diameter (mm)	Plant Height (cm)	Fresh Shoot Weight (g)	Dry Shoot Weight (g)	Fresh Root Weight (g)	Dry Root Weight (g)	Percent Disease Index (%)	% Disease reduction
A (Pathogen + *A. niger*)	1.71±0.31	26.33±4.80	2.43±1.16	0.92±0.30	3.09±1.75	1.32±0.78	13.88±3.97	31.40
B (Pathogen + *T. harzianum* ISO -1)	1.45±0.14	31.10±7.30	2.25±1.12	0.88±0.57	1.86±1.32	0.84±0.55	10.83±4.52	34.40
C (Pathogen + *T. harzianum* ISO-2)	1.47±0.30	27.67±4.90	1.85±0.73	0.81±0.42	3.02±1.83	1.35±0.78	18.33±3.33	26.90
D (Pathogen + *T. piluliferum*)	1.29±0.38	29.47±5.43	2.61±1.11	0.96±0.39	2.06±1.32	1.05±0.86	18.05±2.64	27.20
E (Pathogen + *P. sublateritium*)	1.47±0.11	26.59±3.22	2.91±1.28	1.41±0.62	4.65±2.55	2.03±1.13	6.94±3.32	38.26
F (Pathogen)	0.71±0.26	13.60±2.35	1.09±0.63	0.38±0.20	0.92±0.30	0.37±0.14	45.27±3.61	–
G (Pathogen + Copper oxychloride)	1.36±0.15	19.85±3.45	1.12±0.77	0.61±0.55	1.61±0.73	0.61±0.37	20.55±4.22	24.70
SEM±	0.07	1.37	0.29	0.13	0.45	0.21	1.07	
CD at 5%	0.20	3.86	0.81	0.37	1.27	0.59	3.01	

DISCUSSION

The aerial plant parts surface provides suitable habitat for microorganisms, which are capable of influencing the growth of pathogens (Yadav *et al*., 2011). The saprophytic organisms play an important role in reducing the incidence of foliar diseases of crops in the field (Euveh and Ogbebor, 2008).

From the results of *in vitro* experiment, it was illustrated that five phylloplane fungi were screened against *A. alternata* and they were found to possess varied degree of inhibition viz., *T. harzianum* ISO-1, *T. harzianum* ISO-2, *T. piluliferum*, *A. niger* and *P. sublateritium.* Larkin *et al.*, (1998) analyzed that antibiosis is one of the most important attribute in deciding the competitive saprophytic ability of *Trichoderma* sp. This interaction can result in suppression of the activity of the pathogen and destruction of pathogen propagules. Pathogens antagonism by *Trichoderma* species has been reported (Elad 2000; Howell, 2002; Eziashi *et al*., 2006; Rajendiran *et al*., 2010).

Culture filtrates 'with cell' and 'cell free' of antagonists *T. harzianum* ISO-1, *T. harzianum* ISO-2, *T. piluliferum*, *A. niger* and *P. sublateritium* were analyzed *in vitro*, and they were found to inhibit the conidial germination of pathogens *A. alternata*. Odebode (2006) made *in vitro* study in which *Trichoderma* strains effectively inhibited the conidial germination of post harvest pathogenic fungi of fruits viz., *Alternaria* sp. Cell free culture filtrates of *Trichoderma* sp. inhibited the conidial germination of pathogenic fungi. Gveroska and Ziberoski (2012) also observed inhibiting effect of *T. harzianum* on the development of *Alternaria alternata* which can be further applied in biological control of this pathogen.

It was found that minimum conidial germination of pathogens was observed under darker than in light conditions. Moreover the interaction between antagonists and treatments (control, with cell, cell free) were found effective in inhibiting the conidial germination of *A. alternate* under light as well as dark condition.

Application of conidial suspensions in the form of foliar spray in the mist chamber and field trials proved highly effective in reducing the disease severity as reported by several workers (Perello*et al*., 2008 & 2009; Hussein *et al*., 2007; Zegeye*et al*., 2011; Purohit *et al*., 2013). Harish *et al*. (2007) reported that spraying of conidial suspension of *Trichoderma* isolates on rice plant significantly inhibited the growth and conidial germination of *Bipolaris oryzae*. As compared to the inoculated and uninoculated controls, treatments with selected antagonistic fungi were found effective in increasing the root weight of *R. serpentina.*

Treatment with *T. harzianum* ISO-2 followed by *A. niger*, *T. piluliferum*, *P. sublateritium, T. harzianum* ISO-1 and fungicide (Copperoxychloride) were the most efficient in increasing fresh and dry root weight in the presence of pathogen when compared to the treatments in the absence of pathogen. Sastrahidayat *et al.* (1993) found that in greenhouse trials, two *Penicillium* sp. isolates and *Trichoderma* sp. controlled the growth of pathogen *Alternaria porri* on garlic.

Application of bioagents at the same time of inoculation with the pathogen gave higher reduction in disease severity than the application of bioagents three days before inoculation of pathogen. The obtained results are in agreement with those obtained by (Paulitz and Belanger, 2001; Hussein *et al*., 2007).

Trichoderma spp have been reviewed as an effective biocontrol agent for a range of important airborne and soil borne pathogens (Huang *et al*., 2007). Therefore, in *in vivo* studies, foliar spray of conidial suspension was done to ascertain their efficacy against *A. alternata.* In field trials, *P. sublateritium, T. harzianum* ISO-2 and *A. niger* were the most effective antagonists in increasing the fresh and dry (root) weight. In case, of percentage disease reduction *P. sublateritium* was found to be the most effi-

cient.Significant reduction in the lesion development was recorded when phylloplane antagonists conidial suspension was applied to the leaves in the form of foliar spay in the presence of pathogen.

In the present study, reduction of disease incidence was also evaluated under mist chamber and field trials, in most of the experiments, it was observed that *Trichoderma* sp., *P. sublateritium* and *A. niger* were efficient in reducing disease incidence and severity. Significant reduction in lesion development was recorded with the metabolites of the test phylloplane antagonists on leaves.

CONCLUSIONS

This research indicates that phylloplane antagonists can be used as non-chemical alternative against leaf spot of *R. serpentina*. As application of *P. sublateritium*, *T. harzianum* ISO-1 and *A. niger* were found to be efficient in reducing not only the intensity of leaf spot in *R. serpentina* but they are also considered as plant growth promoting biocontrol agents. Therefore, utilization of biocontrol agents for the management of foliar diseases is more effective than use of chemical fungicides.As the excessive use of chemical fungicides leads to the fungicide resistance in pathogens and causes environmental pollution.

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