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Research Paper

Anti-fungal activities of soybean leaf surface fungal diffusates and spores against *Alternaria alternata* the causal agent of leaf spots

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ABSTRACT

The effect of non-volatile and volatile growth products and spore concentrations of ten dominant phylloplane fungi of soybean was assessed on growth and spore germination *in vitro* and on brown spot severity *in vivo* of pathogenic *Alternaria alternata*. Differential response in growth of the test pathogen was observed with culture filtrates of leaf surface fungi on solid and in liquid media. A total of 42 fungal taxa were isolated by the plate dilution method. Growth products of *Acrophialophora fusispora*, *Fusarium semitectum*, and *Drechslera bicolor* caused appreciable 33.32 %, 40.27 %, and 24.50%, respectively, decline in radial growth, while that of *Trichoderma*, *Pseudokoningii*, and *D. bicolor* inhibited dry weight increase of the pathogen. Stimulatory effect on growth of the test pathogen was obtained with culture filtrates of *Aspergillus niger*, *Myrothecium verrucaria*, and *F. semitectum* in liquid media. Antagonistic interaction was generally noted on spore germination, germ tube length, and number of germ tubes produced by the pathogen under the staling products of phylloplane fungi utilized. Volatiles emanated from cultures of phylloplane fungi caused a decline in the growth of the test pathogen, with superior results shown by *D. bicolor* and *Curvularia Lunata*. Brown spot severity caused by the pathogen on the leaf surface tended to reduce with increasing concentration of spores of the antagonists. Pathogenicity of *A. alternata* was shown to decline when spores of antagonists were applied 24 h before that of the pathogen. Quantitatively appreciable decline in growth of *A. alternate* in descending order was obtained with *M. verrucaria, C. lunata* and *F. semitectum* i.e. 43.56%, 40.64% and 40.27%, respectively. With the fungal diffusates significant reduction in brown spot disease severity, i.e. 60 % was obtained with spores of *Aspergillus flavus*, *F. semitectum*, and *Penicillium Citrinum*.

***Key words:** Alternaria alternata*, Biomass, Leaf spot pathogen, Pathogenicity, Soybean

INTRODUCTION

Suppression of activities of pathogens to control foliar diseases through microbial antagonism utilizing innocuous components of the serial mycoflora has been examined in several reviews (Baker and Cook 1974, Corke and Risbeth 1981, Fokkema 1981, Balkeman and Fokkema 1982, Dubos 1987). First report of *Colletotrichum musicola* causing soybean anthrax use in Brazil by Boufleur *et al.* (2020), fungi causing leaf spot diseases of soybean reported by Borah and Bishakha (2022), Hosseini *et al.* (2023), Lin and Kelly (2018). Successful control of foliar diseases employing resident micro-organisms belonging to the same ecological niche has been demonstrated in some studies (Fokkema 1973 & 1976, Pace and Campbell 1974, Mishra and Tewari 1976, Hodges and Madson 1979, Rai and Singh 1980, Melgarejo *et al.* 1985). Biological control offers an environmentally friendly and sustainable diseases, reducing the reliance on chemical pesticides and improving crop health. Different parts of the world *Alternaria Alternata* causing various diseases like Alternaria leaf spot of *Brassica* (Hara *et al.*, 2016, Al-lami *et al.* 2019), Brown spot on *Solanum* (Choi *et al.* 2023), coriander in South Africa (Mangwende *et al.* 2018), ornamental plants in Italy (Matic *et al.* 2020), potato plant affected by leaf spot disease in Korea (Park and Kim 2024), leaf spot in Celtis (Wang *et al.* 2023), causing allergens (Wojcicka and Siwak 2014), needle blight disease in Pinus (Zhang

et al. 2023), potato foliar disease in China (Zhang and *et al.* 2014), essential oil and antifungal activity reported by Nazzaro *et al*. (2017). Productivity and profitability of soybeans are influenced by site-specific nutrient management under mid-hill conditions of Himachal Pradesh, studied by Singh (2020). Although great variations have been reported in the measure of activity of antagonists *in vitro* and on leaf surface, systematic laboratory screening is still a desirable means of detecting potential antagonists.In the present investigation's antifungal activities of cultures -filtrates, volatiles, and spore concentrations of phylloplane fungi against *Alternaria alternata* were compared *in vitro* and on leaf surface in order to obtain suitable saprophytic antagonists.

MATERIALS AND METHODS

Isolation of antagonists

Isolation of strains of fungi was conducted from the phylloplane by plate dilution method at different growth stages of soybean (*Glycine max* L.) cv. T 49 as a part of preliminary screening. Amongst a heterogeneous population,ten strains were selected for the present studies, which were the most dominant phylloplane residents throughout the progression and development of the leaf spot disease. Strains of fungi selected were *Aspergillus niger* van Tieghem, *Aspergillus flavus* Link ex Fries, Acrophialophor *fusispora* Samson, *Curvularia Lunata* Boedijn, *Drechslera bicolor* Subram and Jain, *Epicoccum purpurascens* Ehrenb ex. Schlecht, *Fusarium semitectum,* Berk and Rev, *Myrothecium verrucaria* Alb. and Schw. Fr. Ditm, *Penicillium citrinum* Thom and *Trichoderma pseudokoningii* Rifai. All the isolated fungi were identified on the basis of morphological characters.

Preparation of culture filtrates

A 250 ml Erlenmeyer flask containing 100 ml liquid Czapek-Dox + 0.05% yeast extract was taken, and each flask was inoculated with two 5 m mycelial disks of the respective fungus. The flasks were incubated for 10 days at 25 ± 2^0 under a bank of fluorescent tubes, and cultures were filtered through Whatman filter paper No. 44 and finally through a sintered glass (G_4). The present study was performed in a randomized block design (RBD) with five replications. The ANOVA for the weather parameter and disease severity was statistically performed with the software package of SPSS (18), and multiple regression and correlation were performed at 0.05 and 0.01 levels of significance. The pH value of the culture filtrates was recorded, and the effects of culture filtrates on the growth and spore germination of the test fungus *Alternaria alternata* Fr. Keissler was studied as under:

In- vitro Studies

Colony diameter

The 5 ml culture filtrates of each fungus were incorporated in 15 ml autoclaved, cooled Czapek agar (pH 6.5) in 5 replicates of 90 mm Petri dishes. An equal volume of inoculated liquid media was substituted for culture filtrates in control sets in each experiment.Petri dishes were inoculated with a 5 mm agar block of fungal inoculum. The incubation was done at $25+2^0$ under a bank of NUV light. The measurement of colony diameter was taken 48 h after incubation and was continued for 7 days.

Hyphal dry weight

The 10 ml culture filtrates of each phylloplane fungus were incorporated (pH adjusted at 6.5) in Erlenmeyer flasks containing 40 ml liquid Czepak-Dox medium (pH 6.5). Five replicates were prepared for each experiment. Each flask was inoculated with one 5 mm mycelial block cut from the edge of a developing fungal colony. After completion of incubation as described earlier for 10 days mycelial mats were obtained over a filter paper, dried at 60^0C °C for 48 h, and weighed. Control sets were prepared by the incorporation of an equal volume of uninoculated culture media instead of culture filtrate.

Spore germination test

Conidial suspension of the test organism was prepared by flushing the spores from the culture of the fungus on the nutrient with distilled water. The fragments of mycelium were removed by filtration through folds of muslin cloth. The harvested spores were washed five times in distilled water. One drop (0.01 ml) of culture filtrate of each fungus was dispensed in the glass cavity slides in 3 replications, containing nearly 100 spores of the pathogen. The measurements of spore germination, number of germ tubes and length of germ tubes were taken after 24 hours.

Antifungal activity of volatile metabolites

Petri dishes containing 15 ml Czapek-Dox-agar medium were inoculated with a 5 mm block

of phylloplane fungi separately in 90 mm Petri dishes with 5 replications. All sets were incubated as described earlier for 5 days. After incubation, the lid of each petri dish was replaced by the bottom of another Petri dish containing 20 ml of Czapek-Dox-agar pre-inoculated with a 5 mm block of *A. alternate* and sealed with gum tape and were re-incubated at 25±2 °C. The control sets were virgin nutrient Petri dishes covered with inoculated plates of *A. alternata.* The observations of radial growth of *A. alternate* were taken after incubation for 24h and were continued for 5 days. The inhibition in radial growth was calculated and represented as a percentage.

Interaction of leaf surface

The propagule suspensions were maintained at 2×10^3, $4 \times 10^3$and 6×10^3spores / ml in the case of phylloplane fungi and 4×10^3 spores/ml for *A. alternate* using a hemocytometer.

Inoculation

Leaves showing apparently no symptom of disease were selected, detached, and collected in new polyethylene bags. Fully expanded leaves were rubbed gently with a moist cotton swab, and inoculation was carried out as under:

(i) Leaves inoculated with phylloplane fungi and pathogenic form simultaneously.

(ii) Leaves inoculated with phylloplane fungi 24 hours after inoculation with the pathogenic form.

(iii) Leaves inoculated with phylloplane fungi 24h before inoculation with pathogenic form.

Inoculation was done on six points on the upper surface of the leaf lamina using one drop (0.01 ml) spore suspension of each of the phylloplane fungi and that of the pathogenic form in five replications for each dilution. Control leaves were inoculated with spores of *A. alternate* and sterile distilled water in place of phylloplane fungi. The inoculated leaves were placed in a Petri dish moist chamber covered with polyethylene bags. Brown spot development on leaves was usually evaluated after 7 days using a quantitative ordinal scale (Horsfall and Barratt 1945) from 1 to 5, where 1= no symptoms, 2=light yellow lesions, 3=yellowish brown lesions, 4 = light brown lesions, and 5 = extensive brown lesions. The data from six inoculation sites on each leaf was rated, and the rating from five leaves was averaged.

RESULTS AND DISCUSSION

Non-volatile metabolites

Colony diameter

The radial growth of *A. alternata*was inhibited during incubation from 48h extending up to 7 days, with generally all the metabolites contributors having the largest decline caused by the filtrates of *A. fusispora, F. semitectum* and *D. bicolor* (Table 1 and Table 1A). However, the degree of inhibition in growth with respect to the length of incubation showed a decreasing trend. The effect of culture filtrates on growth was apparently most pronounced on the third and fourth day of incubation, but did not nullify at the time of final assessment. Species such as*A. niger*and *P. citrinum* consistently reduced the growth of the pathogen over the entire length of incubation studied.

Hyphal dry weight

Table 2 shows that metabolites from *T. pseudokoningii* and *D. bicolor* caused an appreciable degree of inhibition in the growth of the test fungus in liquid medium.On the contrary, metabolites of *A. flavus* and *F. purpurascens* were least effective while those of *A. niger, F. citrinum, M. verrucaria, C. lunata* and *T. semitectum* were promontory on the test fungus.

Spore germination

Effect of culture filtrates on the percentage inhibition /promotion of germination, germ tube length and number of germ tubes produced by spores of *A. alternate* is presented in Table 3. A good degree of inhibition on all three parameters investigated for spores was observed with culture filtrates of *A. niger. T. pseudo koningii, A. flavus, A. fusispora* and *M. verrucaria* whereas culture filtrates of *P. citrinum* showed a stimulatory effect on germ tube number.

Volatile metabolites

Table 4 and Table 4A show the effect of the volatile extract of phylloplane fungi on the inhibition of radial growth of *A. alternate* after 7 days of incubation. Volatiles emanated from *D. bicolor* and *P. citrinum* caused an appreciable decline. A perusal of observations on the growth behavior of *A. alternate* at the phylloplane fungi employed revealed the most pronounced decline in growth 72 hours after incubation, which was gradually nullified with the lengthening of incubation.

Table 1. Effect of culture filtrates of some phylloplane fungi on growth rate and on radial growth at different incubations of *A. alternata*

Metabolite contributor	Growth rate (mm-day	Incubation (h)					
		48	72	96	120	144	168
		Colony diameter (mm)					
Aspergillus flavus	4.14	19.66±0.33	24.66±1.45	28.33±0.88	31.33±0.66	36.33±0.88	40.33±0.88
Aspergillus niger	7.01	17.66±0.88	24.00±7.08	27.66±1.45	37.33±2.66	44.33±4.10	52.66±3.71
Acrophialophora fusispora	3.68	13.66±0.88	18.00±0.57	22.33±1.20	26.66±1.20	28.330.88	32.00±1.15
Curvularia lunata	3.54	19.66±0.33	22.33±0.88	28.00±1.15	30.00±1.15	34.66±0.33	37.33±0.66
Drechslera bicolor	3.74	15.33±0.88	18.00±1.15	21.66±0.88	25.00±1.15	30.33±0.33	34.00±1.00
Epicocoum purpurascens	4.21	19.66±0.33	28.33±0.33	28.33±0.33	31.00±0.57	34.33±0.33	40.66±0.88
Fusarium semitectum	5.54	14.66±0.88	17.33±1.20	22.33±1.20	30.00±1.15	34.66±0.88	42.33±1.45
Myrothecium verrucaria	5.33	14.33±1.20	21.66±0.88	30.33±0.33	34.00±1.00	37.66±0.33	41.00±1.00
Penicillum citrinum	7.73	15.00±1.73	20.00±2.89	32.33±1.43	41.66±0.88	44.66±0.88	53.66±1.85
Trichoderma pseudokoningii	5.26	15.00±0.57	19.66±0.88	22.33±0.88	28.33±0.88	12.00±0.57	41.33±0.66

Table 1A. ANOVA showing effect of non-volatile metabolites of some phylloplane fungi on % inhibition in radial growth of *A. alternata*at various incubation periods. (Ref. Table 1)

Source of variation	DF	SS	MSS	F Value
Treatments	9	2741.15	304.57	6.17*
Incubation	5	3453.35	690.67	13.99**
Error	45	2220.35	49.34	

Data underwent √x +1/2 transformation before proceeding to ANNOVA

DF = Degree of Freedom SS= Sum of squares

MSS= Mean sum of squares F= Variance ratio

* significant at percent level ** significant at 5% level

Table 2. Effect of culture filtrates of some phylloplane fungi on hyphal dry weight yield of *A. alternata*

Metabolic contributors	Initial pH of culture filtrate	Mycelial dry weight (mg)	Final pH
Aspergillus flavus	4.0	330.00 ± 58.66	7.0
Aspergillus niger	6.5	576.00 ± 12.03	7.0
Acrophialophora fusispora	6.8	273.00 ± 3.33	6.4
Curvularia lunata	26.7	478.00 ± 23.53	7.0
Drechslera bicolor	6.6	180.00 ± 11.56	6.1
Epicocoum purpurascens	6.3	283.33 ± 72.27	6.4
Fusarium semitectum	6.8	463.00 ± 26.06	7.0
Myrothecium verrucaria	6.8	527.00 ± 77.62	6.7
Penicillum citrinum	6.7	522.00 ± 15.91	7.0
Trichoderma pseudokoningii	6.4	147.00 ± 9.29	6.0

Table 3. Effect of Culture filtrates of some phylloplane fungi on radial growth and on spores of *Alternaria alternata*

Metabolite	AF			AN			AF			CL			DB			EP			FS			MV			PC			TP		
Contribution	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Inhibition/ Promotion	60	30	84	67	67	88	60	66	88	32	00	79	79	34	66	34	34	43	34	00	40	40	66	33	34	62	07	62	62	88

AF-*Aspergillus flavus*	FS-*Fusarium Semitectum*
AN-*Aspergillus niger*	MV-*Myrothecium Verrucaria*
AF-*Acrophialophorafusispora*	PC-*Penicilliumcitrinum*
CL-*Curvularialunata*	TP-*Trichoderma pseudokoningii*
DB-*Drechslera bicolor*	
EP- *Epicoccumpurpurascens*	

Note: 1. Spore Germination

2. Number of germ tube

3. Length of Germ Tube

Interaction on leaf surface

Table 5 shows that inhibition in lesion development tended to increase with increasing concentration of spores of the phylloplane fungi. Maximum lesion inhibition was observed at the highest spore concentration (6×10^3 spores/ ml) of composite mycoflora.This was followed individually by *A. flavus, F. semitectum,* and *P. citrinum.* Minimum inhibitory effect was seen with *D. bicolor, E. Purpurascens, A. fusispora* and *T. pseudokonicii.* As regards the effect of protective. Simultaneous and eradicative applications of the antagonist's maximum reduction in lesion development were observed when spores of the antagonists were applied onto the leaves 24 h before those of the pathogen. A smaller reduction was observed when the antagonists were inoculated. 24 h after the inoculation of the pathogen.Simultaneous inoculation of spores brought about an appreciable reduction in lesion development caused by *A. alternata.*

There are several factors involved in the inter-fungal interaction under different environmental conditions and in assay techniques employing different sources.Jain *et al.* (2019) review of plant leaf fungal diseases and their environmental speciation. The metabolic products in the culture filtrates have been demonstrated to possess the antagonistic factors identified as non-volatile antibiotics (Webber and Hedger 1986), antifungal toxins (Briggs *et al.* 1975, Pachneri and Dix 1980, Kellock and Dix 1984) substance causing alteration in the pH of medium (Bartman *et al*. 1981) and nutrient improvement (Park 1961, McBride 1971). There are, however, a few reports of neutral or stimulatory interaction between fungi in culture (Morton and Eggins 1976, Webber and Hedger 1986).

In the present study, generally all phylloplane fungi employed produced non-volatile substances that caused marked inhibition in the growth of *A. alternate* on poisoned agar media, but the effect was much less marked in liquid media, with some of them even causing stimulation in the Biomass increase.Whether such a variation is connected with the rapid inactivation of antifungal factor or with the ability of fungi to tolerate better in broth media has not been ascertained. The most active species causing inhibition through nonvolatile antibiosis included *F. semitecutum, A. niger* and *T. pseudokonioii.*

The inhibitions and stimulations caused by volatiles were much less marked compared with the chemical antagonism. A slight inhibition in the growth of *A. alternate* or the reversal of the initial stimulatory effect with volatiles emanated from *T. pseudokoninoii* and P. *citrinum* was probably due to the buildup CO_2 analogous to that described by Tamimi and Hutchinson (1975). In the present study, no attempt was made to isolate and identify the composition of volatile compounds, but products implicated in earlier reports may have been responsible for the inhibition of test fungi. Hutchinson (1973) enumerated several antifungal volatiles responsible for the causation of antagonism *in vitro.* Analysis of the species spectrum of the Diaporthe/ Phomopsis complex in European soybean reported by Hosseini *et al.* (2020). The interaction between micro-organisms in the phylloplane has been generally investigated in relation to the influence of saprophytes on the pathogenic population. There is a paucity of information regarding the influence of leaf pathogens on the saprophytic microflora. In a few studies carried out earlier, bacterial and Yeast populations as affected by parasites have been analyzed. The objective of the present investigation has largely been to find out precisely the status of saprophytes on leaf surfaces in relation to pathogens. It is also aimed to determine whether the presence of the pathogen influences quantitatively and qualitatively the population of saprophytes compared with a healthy leaf or not.

The efficacy of inhibiting factors in the culture filtrates and in the volatiles emanated from cultures decreased with the length of the incubation of test fungi in the present study. Brian (1960) suggested two possible reasons for such a variation. Petrovic and Skaltsas (2021) reported that Diaporthe seed decay of soybean (*Glycine max* L. Mere.) is endemic in the United States.Stability analysisin soybean (*Glycine max*. L.) reported by Koraddi *et al.* (2016). In the first place, the antifungal factors present may be of unstable nature, and secondly, the fungi might develop the capacity to annual the toxic effect of stale products. In a similar *in vitro* study on antagonism between species of fungi (Singh *et al*. 1983), found that the degree of antagonism is influenced by the age of culture. Protective application of spores of the antagonist brought a significant reduction in brown spot severity on the leaf surface in the present study, compared with the eradicative and simultaneous applications. Gullino *et al.* (2019) studied a plant pathogen heaven in ready-to-eat salad crops. The comparison of the effect of interactions with the principal leaf surface fungi *in vitro* and *in vivo* has evinced some contradictory results. *P. citrinum,*which was found to be less antagonistic on culture media, was an effective antagonist on the leaf surface. This finding

Table 4. Effect of volatile metabolites of some phylloplane fungi on growth rate and on radial growth at different incubations of *A. alternata*

Volatile contributors	Growth rate (mm-day)	Incubation (h)				
		24	48	72	96	120
Aspergillus flavus	9.83	13.00± 0.57	19.33± 0.88	29.00± 0.57	41.66± 0.88	52.33± 2.85
Aspergillus niger	10.00	9.00± 0.57	16.00± 0.57	23.00± 1.15	39.00± 2.08	49.00± 5.57
Acrophialophora fusispora	10.5	9.33± 0.66	15.33± 0.33	27.33± 1.45	39.66± 3.93	51.33± 2.33
Curvularialunata	7.00	12.33± 0.33	16.66± 1.20	24.00± 1.00	40.00± 2.89	39.66± 4.67
Drechslera bicolor	9.66	10.66± 0.33	19.33± 0.66	30.00± 0.57	40.00± 2.89	48.33± 2.02
Epicocoum purpurascens	9.25	10.33± 0.33	18.0± 0.57	27.00± 4.73	32.00± 4.67	47.33± 2.33
Fusarium semitectum	9.33	9.33± 0.33	22.33± 0.33	30.33± 0.33	38.00± 1.52	46.66± 1.66
Myrothecium verrucaria	9.75	10.66± 0.66	18.00± 0.57	29.00± 0.57	36.66± 0.88	47.66± 15.92
Penicillum citrinum	9.41	13.66± 0.33	19.00± 1.15	26.00± 4.73	31.66± 4.41	47.33± 2.33
Trichoderma pseudokoningii	9.25	13.33± 0.57	22.00± 0.57	33.00± 0.57	44.33± 1.20	51.33± 2.40

Table 4A. ANOVA showing effect of volatile metabolites of some phylloplane fungi on percent inhibition in radial growth of *A. alternate* at various incubation periods. (Ref. Table 4)

Source of variation	DF	SS	MSS	F Value
Treatments	9	399.08	44.34	0.82
Incubation	4	1000.18	250.045	4.66**
Error	36	1930.92	53.63	

Data underwent √x +1/2 transformation before proceeding to ANNOVA

DF = Degree of Freedom, SS= Sum of squares

MSS= Mean sum of squares F= Variance ratio

* significant at percent level, ** significant at 5% level

Table 5. Scores of results of disease severity before, simultaneous and after inoculation of spores of *A. alternata*and propagules of some phylloplane fungi on detached leaves of soybean.

Treatment		Disease Index*									
		Protectant fungi									
		Aspergillus flavus	*Aspergillus niger*	*Acrophiolophora fusispora*	*Curvuloria lunata*	*Dreschslera bicolor*	*Epicoccum purpurascens*	*Fusarium semitectum*	*Myrothecium verrucaria*	*Pencillulum citrium*	*Trichoderma pseudokoningil*
Protectant conidia/m Spores concenttations	Pathogen conidia sprayed 24 h before + protectant conidia										
	2×10^3	2.0	2.3	4.0	4.0	5.0	3.6	2.0	3.6	3.0	3.6
	4×10^3	1.6	2.0	3.3	3.0	4.3	3.0	1.3	3.3	2.6	3.0
	6×10^3	1.0	1.63	2.0	2.3	3.6	2.3	1.0	2.3	2.0	2.3
	Simultaneously (pathogen + protectant conidia)										
	2×10^3	2.0	2.3	3.3	2.0	4.0	2.3	1.6	2.0	1.6	2.3
	4×10^3	1.3	1.6	2.6	1.6	3.3	2.0	1.0	1.0	1.0	2.0
	6×10^3	1.0	1.0	1.3	1.0	2.3	1.6	1.0	1.0	1.0	1.6
	Pathogen conidia sprayed 24 h after + protectant conidia										
	2×10^3	1.0	1.0	1.0	1.0	3.3	2.3	1.0	1.0	1.0	1.0
	4×10^3	1.0	1.0	1.0	1.0	2.6	1.6	1.0	1.0	1.0	1.0
	6×10^3	1.0	1.0	1.0	1.0	2.0	1.0	1.0	1.0	1.0	1.0

* Disease index rating scale- Explained as in text

is in contradiction to the reports of Rai and Singh (1980) and highlights the danger of assessing the potential outcome of interactions between organisms in nature based on information obtained only from culture. Differences in antagonistic behavior of leaf surface micro fungi *in vivo* and *in vitro* as obtained in the present investigations, have been reported in some previous studies (Bhatt and Vaughan 1963, Pace and Campbell 1974, Andrews *et al.* 1983). Sharma *et al.* (1979), Singh *et al.* (1983), and Melgarejo *et al.* (1985) found variation in the antagonistic behavior of phylloplane micro fungi on different synthetic media and under different environmental conditions.The effect of nitrogen management on soil fertility and NPK uptake in soybean is suggested by (Rathod *et al.* 2012). Low nutrient status on the leaf surface, neutralization of toxins by the leaf surface chemicals, and evaporation of volatile substances seem to be possible factors responsible for the difference *in vitro* and *in vivo* results.

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