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Antifungal Activity of Nor-securinine Against Some Phytopathogenic Fungi

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Crude extracts and active principles from medicinal plants have shown potential role in controlling plant diseases in glasshouses as well as in fields as one of the safest and ecofriendly methods. The effect of nor-securinine (an alkaloid) isolated from *Phyllanthus amarus* has been seen against spore germination of some fungi (*Alternaria brassicae*, *A. solani*, *Curvularia pennisetti*, *Curvularia* sp., *Erysiphe pisi*, *Helminthosporium frumentacei*) as well as pea powdery mildew (*Erysiphe pisi*) under glasshouse conditions. The sensitivity of fungi to nor-securinine varied considerably. Nor-securinine was effective against most of the fungi. *H. frumentacei* was more sensitive even at the lowest concentration (1,000 μg/ml). Likewise conidia of *E. pisi* were also inhibited in partially or completely appressorium formation. Pre-inoculation treatment showed greater efficacy than post-inoculation in inhibiting powdery mildew development on pea plants in a glasshouse. Maximum inhibition occurred at 2000 μg/ml.

KEYWORDS: **Antifungal activity, *Erysiphe pisi*, Nor-securinine, *Phyllanthus amarus***

Fungal diseases of plants have always been one of the major constraints in crop production causing severe losses every year. Since the very beginning of their appearance researchers have succeeded in controlling some devastating diseases by synthetic fungicides. As several synthetic fungicides are highly effective in controlling plant diseases, their negative effect on human and animal health and also on the agroecosystem was gradually realized which entailed serious research in developing alternative environmentally acceptable (environment-friendly) methods. These efforts included biological control, genetic engineering, use of systemic acquired resistance (SAR) with the help of biotic and abiotic agents (Lyon *et al*., 1995), and biodegradable natural products especially from medicinal plants (Prithiviraj *et al*., 1996).

Several workers have used crude plant extracts *in vitro*, in glasshouse and under field conditions against several plant pathogens with considerable success (Chakravorty and Pariya 1977; Asthana *et al*., 1982; Vollekova *et al*., 2001). Also various compounds isolated from the plants were shown to be effective against some plant pathogenic fungi *in vitro* (Maillard *et al*., 1987, 1989; Kobayashi *et al*., 1987; Singh *et al*., 1988, 1990; Prithiviraj *et al*., 1997a, b; Singh *et al*., 2001; Ameer Basha, 2002; Maurya *et al*., 2001, 2002), in glasshouse (Reamers *et al*., 1993; Singh *et al*., 1995) and also in the field (Prithiviraj *et al*., 1996, 1998; Sarma *et al*., 1998, 1999). The use of plant products under field conditions is rare because of their presence in small amounts in plants which is usually cost-prohibitive Neemazal, a preparation from neem (*Azadirachta indica*) and ajoene, a constituent of garlic (*Allium sativum*), have recently been used successfully against powdery mildew (*Erysiphe pisi*) of pea under field conditions (Singh *et al*., 1995; Prithiviraj *et al*., 1998).

Several alkaloids are known to affect biological functions at a very low concentration showing antimicrobial activity (Atta-Ur Rahman *et al*., 1997; Mahajan *et al*., 1982; McCarthy *et al*., 1992; Singh *et al*., 1994, 1999, 2000; Srivastava *et al*., 1994). (−)-Amarbellisine, a lycorine type alkaloid from *Amaryllis belladonna* L. showed potent antimicrobial efficacy (Evidente *et al*., 2004). Powdery mildew is one of the serious diseases of pea (*Pisum sativvm*). Several workers (Prithiviraj, 1997; Singh *et al*., 2000) reported the effects of a number of plant products against pea powdery mildew as well as other diseases as a unique alternative to synthetic fungicides. The present study deals with the effect of nor-securinine against spore germination of some fungi and also its effect on conidial germination and development of *Erysiphe pisi* conidia on excised pea leaves and on potted plants. The results are presented here.

Materials and Methods

Isolation and maintenance of fungi. The test fungi were isolated from their respective hosts collected from the experimental farm of the Banaras Hindu University (Varanasi, India) on potato dextrose agar (PDA: peeled potato 250 g, dextrose 20 g, agar 15 g, distilled water 1 *l*) medium. The cultures were further purified by single spore isolation technique and maintained by periodic

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transfer on the same medium for further experiments. Seven to ten day old cultures were used in the experiments. *Erysiphe pisi* was multiplied on pea plants grown in plastics pots (90 mm in dia.) in glasshouse for the experiments.

Extraction and purification of nor-securinine from *Phyllanthus amarus*. The plant *Phyllanthus amarus* (Family: Euphorbiaceae) is distributed throughout India as a weed in cultivated and wastelands. The earlier literature indicated that *Phyllanthus amarus* has potent biological efficacy in animal system. It is widely used as anti-hepatotoxic agent in Indian system of medicine (Manske, 1973). Preliminary test of crude extract done *in vitro* showed high efficacy against the test pathogens, indicating that the extract has spore germination inhibitory compounds. Keeping this view, the isolation and purification of extract were done by column chromatography to isolate active ingredients. Two major alkaloids, nor-securinine and ent-norsecurinine were isolated from this plant. Antimicrobial efficacy of ent-norsecurinine has been already reported by (Mitul *et al*., 2002). The present experiment deals with the isolation of the compound and its antifungal activity.

The dried and powdered whole plant (2.1 kg) was successively extracted with petroleum ether (60~80°C) and methanol in a soxhlet extractor. The solvents were distilled off on water bath, which gave greenish brown syrup of methanol extract (40 g). The methanol extract was chromatographed over a silica gel column eluting with solvents of increasing polarity. TLC monitored the collected eluants at every stage for their homogeneity. The similar eluants collected from $CHCl_3$-MeOH (4 : 1) were mixed together which on crystallisation from methanol furnished a mixture of two alkaloids having Rf values 0.50 and 0.55 (C_6H_6-$CHCl_3$-MeOH, 4.2 : 1). Preparative TLC as oil separated the nor-securinine. The nor-securinine could not be crystallised by different solvents and polymerized readily on keeping. It was then taken in methanol and ethereal HCl was added to it. After work up, it gave nor-securinine hydrochoride as oil. The nor-securinine was regenerated by addition of ammonium hydroxide and extraction with $CHCl_3$, as an oil, which further did not crystallise with any solvent. It exhibited $[\alpha]_D^{20}$ −268° (c, 0.42, MeOH). Its molecular formula was determined as $C_{12}H_{13}NO_2$ (M^+ = m/z 203) from the mass spectrum. It exhibited UVλ max (MeOH): 255 and 257 nm and IR vmax (KBr): 1,800, 1,769 and 1,638 cm^{-1}. The peaks in IR together with ^{1}H-NMR fully supported for α, β-unsaturated-γ-lactone unit. In ^{1}H-NMR it showed a sextet at δ 6.4~7.0 (2H, H-14 and H-15), a triplet at δ 3.63 (1H, H-7), a singlet at δ 5.7 (1H, H-12) for ABX pattern. The high field multiplet at δ 1.4~2.2 accounted for H-8α and H-8β together with C-3 and C-4 methylene protons.

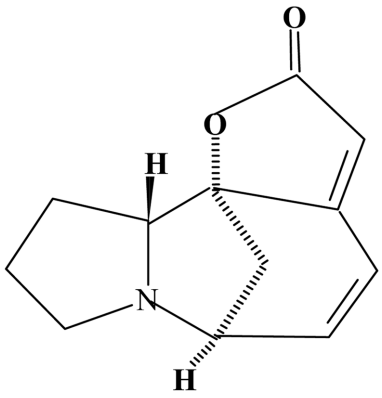

Fig. 1. Structural formula of Nor-securinine.

One of the protons in the C-5 methylene exhibited absorption at δ 2.2~2.8, while C-2-H and the other C-5-H exhibited multiplet at δ 3.0~3.4. These data were in perfect agreement with the reported data of nor-securinine. It was thus identified as nor-securinine (Fig. 1) (Manske, 1973). After purification and characterization of nor-securinine their biological efficacy was tested on some plant pathogenic and saprophytic fungi.

Assay of alkaloid. Stock solution of alkaloid nor-securinine (2,000 μg/ml) was prepared by dissolving 20 mg of the chemical in 20 *ml* of distilled water. Required concentrations (1,000, 1,500, 2,000 μg/*ml*) were prepared from the stock solution by diluting with distilled water. However, test concentrations were determined on the basis of their biological efficacy. It has been found that higher concentration of the active chemical (viz., 2,500, 3,000 μg/*ml*) showed strong fungicidal and phytotoxic activity and 100% inhibition was observed among all the test fungi, thus 1,000, 1,000 and 2,000 μg/*ml* concentrations were used to observe their biological efficacy against phytopathogens. One drop (30~40 μ*l*) from each concentration of the chemical was placed on grease-free glass slides. With a sterile inoculation needle, fungal spores (200~300) were picked up from 7-10-day-old growing cultures and mixed in the solution. The slides were later placed in moist chambers made by placing two sterile moist filter papers on upper surface of the lid and base of Petri plates. The plates containing the slides of spores were incubated at 25 ± 2°C for 24 h. Germination was observed after staining with cotton blue prepared in lacto phenol under binocular light microscope (Nikon, Japan). Spores mixed in sterile distilled water served as control. All the experiments were conducted in triplicate. Randomized block design (RBD) for statistical significance was used for the analysis of data.

Germination and development of *Erysiphe pisi* conidia on detached pea leaves. Second nodal leaves from 20-

day-old pea plants were excised with the help of sharp scissors and spread on filter paper towels. The leaves were then inoculated by *Erysiphe pisi* conidia on adaxial surface by tapping heavily infected pea leaves so as to get 200~300 spores per mm² of the leaf area. These leaves were then floated on sterilized distilled water in Petri plates which served as control.

Pre-inoculation treatment. Different dilutions of the chemical (1,000, 1,500 and 2,000 μg/m*l*) were sprayed thoroughly with the help of a hand atomizer 24 h prior to seeding on the excised pea leaves of 20-day old plants and then placed on filter paper towels. These leaves were later floated on distilled water keeping adaxial surface upwards. After 24 h of spraying, the leaves were then seeded with *E. pisi* conidia by tapping heavily infected leaves on the adaxial surface.

Post-inoculation treatment. For post-inoculation treatment, the experiments were conducted as stated above except that the spores were tapped first and after 24 h the chemical was sprayed by a hand atomizer on the seeded leaves which were then again floated on distilled water in Petri plates. All the pre- and post-inoculation treated leaves including control were incubated for 24 and 48 h at 25 ± 2°C. After incubation, the leaves were fixed and stained by the method of Carver and Adaigbe (1990). A pad of filter paper was placed in a Petri plate containing the fixative (Ethyl alcohol-acetic acid, 3 : 1). The leaves were placed on the filter paper with adaxial side up to minimize the disturbance of conidia on the leaf surface. The leaves were fixed for 48 h to remove the chlorophyll completely. They were then placed on filter paper pads soaked with lactophenol for another 24 h to soften the leaf tissue and mounted in lactophenol-cotton blue for staining the conidia. Observations regarding unipolar and bipolar germination, number of germ tubes and number of appressorium formation were made under light microscope. All the experiments were conducted in triplicate and subjected to RBD for statistical analysis.

Experiments on detached pea leaves for disease development. This experiment was also conducted as stated above except leaves were floated on 2 percent sucrose solution in petri plate instead of sterilized distilled water. All the pre- and post-inoculation treated leaves including control were incubated for 24 and 48 h at 25 ± 2°C. Three leaves per treatment were taken and all the experiments were conducted in triplicate. Disease development was observed at each 48 h interval on each pea leaves in all cases for 14 days. Disease intensity was calculated by taking percent leaf area infected. All the experiments were conducted in triplicates.

Effect of nor-securinine on disease development of *Erysiphe pisi* on pea plants in pots in glasshouse. To check the effectiveness of the chemical under glasshouse conditions, the experiments were conducted in earthen pots (20 cm dia.) The pots were filled with garden soil. Six seeds of pea were sown in each pot and kept in the glasshouse at 25 ± 2°C. Each replicate consisted of three pots. The lesion development was seen on 20-25-day-old pea (*Pisum sativum*) plants. Two treatments of the chemicals were given as pre- and post-treatments. Firstly, healthy plants were selected for pre- and post-inoculations along with the control. Then for pre-inoculation, chemical was sprayed with the help of a hand atomizer on selected plants. After 24 h the plants were seeded with *E. pisi* conidia by tapping heavily infected leaves on the adaxial surface of the leaves. For post-inoculation treatment, the plants were seeded with the conidia of *E. pisi* on adaxial surface of the leaves. After interval of 24 h different concentrations of chemicals were sprayed on the surface of leaves using a hand atomizer. The control experiments included only pea plants seeded with conidia of *E. pisi*. Disease development assessed was at each 48 h interval on each pea leaves in all cases for 26 days. Disease intensity was calculated by taking percent leaf area infected. All the experiments were conducted in triplicate.

Disease intensity, on a 0-4 scale, was calculated using the following formula-

$$\text{Disease intensity (\%)} = \frac{\text{Sum of rating (0-4 scale)}}{\text{Maximum possible score} \times \text{No. of leaves observed}} \times 100.$$

The rating was done as: 0 = No powdery mildew symptoms, 1 = 1~10% leaf area infected, 2 = 11~25% leaf area infected, 3 = 26~50% leaf area infected and 4 = > 51% leaf area infected.

Results and Discussion

The effect of nor-securinine on spore germination of some pathogenic fungi is shown at Table 1. The sensitivity of different fungi varied considerably. Spore germination of *Alternaria brassicae*, *Curvularia penniseti*, *Curvularia* sp., *Erysiphe pisi* and *Helminthosporium frumentacei* was completely inhibited at 2,000 μg/*ml* while only (39%) germination was observed in *A. solani*. Similar effect was seen at 1,500 μg/*ml* in *H. frumentacei*. Spore germination of *A. brassicae* (4.0%), *C. penniseti* (2.7%) and *E. pisi* (4.7%) was extremely low at 1,500 μg/*ml* while *A. solani* (66%) and *C. species* (58%) showed poor germination as compared to control at this concentration. Among all the tested fungi, *C. species* (81%) and *E. pisi* (13%) were less sensitive at minimum concentration (1,000 μg/*ml*) but

Table 1. Effect of nor-securinine on spore germination of some fungi

Fungus	Host	Spore germination (%)				
		C	1,000 μg/*ml*	1,500 μg/*ml*	2,000 μg/*ml*	CD
Alternaria brassicae	*Brassica campestris*	84	16[a]	4.0[b]	0.0[b]	8.2
Alternaria solani	*Solanum tuberosum*	96	82[a]	66[b]	39[c]	12
Curvularia penniseti	*Pennisetum typhoides*	92	36[a]	2.7[b]	0.0[b]	14
Curvularia sp.	*Imperata cylendrica*	92	81	58[a]	0.0[a]	14
Erysiphe pisi	*Pisum sativum*	18	13	4.7[a]	0.0[a]	7.3
Helminthosporium frumentacei	*Echinoclova frumentacum*	90	51[a]	0.0[b]	0.0[b]	9.0

Numbers with same superscript letters in rows are non-significant ($p \leq 0.01$). C = Control, CD = Critical difference.
Critical difference (CD): Critical difference is used to find out those differences between the treatment means, which are significant. If any difference is equal to or greater than the critical difference, it will be declared as significant.

spore germination of other fungi such as *A. brassicae* (16%), *C. pennisetti* (36%), and *H. frumentacei* (51%), was significantly inhibited. *A. solani* was found to be most resistant as its spores germinated even at 2,000 μg/*ml*. *A. brassicae*, *C. pennisetti* and *H. frumentacei* were more sensitive than *A. solani*.

E. pisi showed sensitivity towards nor-securinine. There was a marked reduction in the germination of *E. pisi* conidia on excised pea leaves (Table 2). In preinoculation treatment, significant reduction in germination was observed (60, 38 and 25% at 1,000, 1,500 and 2,000 μg/*ml* while post-inoculation treatment yielded 88, 58, and 28% germination at the same concentrations, respectively. Maximum inhibition was recorded at 2,000 μg/*ml* when the leaves were pre-treated. Mostly unipolar conidial germination with one germ tube and bipolar with two germ tubes (one at each pole) occurred in all treatments. Unipolar germination with three germ tubes was completely inhibited in pre as well as post inoculation treatments at 2,000 μg/*ml*, whereas unipolar germination with one and two germ tubes was significantly reduced in preinoculation as compared to postinoculation treatment at 1,000, 1,500 and 2,000 μg/*ml*. Similarly, bipolar germination with three germ tubes at each pole was completely inhibited at 2,000 μg/*ml* in pre as well as postinoculation treatments. Whereas bipolar germination with one and two germ tubes at each pole was significantly reduced in both pre- as well as postinoculation treatments but pre-inoculation treatment at the same concentration showed greater efficacy (Table 2).

Table 2. Effect of nor-securinine on mode of germination of *Erysiphe pisi* conidia during pre-and post-inoculation treatments on detached leaves of *Pisum sativum*

Concentration (μg/*ml*)	Germ tube formation (%)						Total (%)
	Unipolar			Bipolar			
	G1	G2	G3	G1	G2	G3	
Control	46	14	3.3	11	8.3	5.0	88
Pre-inoculation							
1,000	30[a]	9.0[a]	4.0	9.0	6.3	2.0[a]	66[a]
1,500	23[b]	7.3[a]	1.7	3.7[a]	1.3[a]	1.0[a]	38[b]
2,000	18[b]	5.0[a]	0.0[a]	1.7[a]	0.3[a]	0.0[a]	25[c]
Post-inoculation							
1,000	46	14	4.3	12	7.3	4.0	88
1,500	36[a]	9.3	3.0	5.0[a]	4.0[a]	2.0[a]	58[a]
2,000	20[b]	5.7[a]	0.0[a]	2.0[a]	0.7[a]	0.0[a]	28[b]
CD	10	5.8	3.2	6.0	3.5	2.5	12

Numbers with same superscript letters in rows are non-significant ($p \leq 0.01$). C = Control, CD = Critical difference, G1 = One germ tube, G2 = Two germ tubes, G3 = Three germ tubes.
Critical difference (CD): Critical difference is used to find out those differences between the treatment means, which are significant. If any difference is equal to or greater than the critical difference, it will be declared as significant.

The number of appressoria was also affected significantly by nor-securinine. There was a marked reduction in appressorium formation in the germ tubes of *E. pisi* conidia on excised pea leaves. Maximum inhibition was recorded at 2,000 μg/*ml*. The appressorium formation was significantly reduced to 25, 14 and 11% at 1,000, 1,500 and 2,000 μg/*ml*, respectively, in preinoculation while in postinoculation treatment 37, 20 and 12% at 1,000, 1,500 and 2,000 μg/*ml*, respectively, was observed. Appressorium formation in bipolar germination having three germ tubes at each pole was completely inhibited at 2,000 μg/*ml* in pre as well as postinoculation treatment, while significant reduction in appressorium formation in bipolar germination with two germ tubes at each pole in pre as well as postinoculation treatment. The number of appressoria in unipolar germination was not affected significantly in most of the cases, except that unipolar germination with two germ tubes was significantly reduced to 3 and 2.3% at 1,500 and 2,000 μg/*ml*, respectively, in preinoculation treatment while 3% at 2,000 μg/*ml* in postinoculation treatment was observed (Table 3).

The disease intensity (D.I.) on detached leaves was taken up to 12th day with an interval of 2 days. In total four readings were taken. There was a marked reduction in D.I. *in vitro* when plants were subjected to preinoculation treatment, which yielded 0.0, 1.2, 6.0 and 12% at 1,500 μg/*ml* after each 2 day interval from 6th day of inoculation, respectively. Although postinoculation treatment

Table 3. Effect of nor-securinine on appressorium formation in *Erysiphe pisi* conidia during pre-and post-inoculation treatments on detached leaves of *Pisum sativum*

Concentration ($\mu g/ml$)	Appressorium formation (%)						Total (%)
	Unipolar			Bipolar			
	G1A	G2A	G3A	G1A	G2A	G3A	
Control	14	7.0	2.0	2.3	8.3	6.3	39
Pre-inoculation							
1,000	12	3.0	2.3	2.0	3.0[a]	2.3[b]	25[a]
1,500	8.0	3.0[a]	2.0	1.0	0.7[a]	0.0[c]	14[a]
2,000	6.3	2.3[a]	0.0	2.0	0.3[a]	0.0[c]	11[b]
Post-inoculation							
1,000	18	10	3.0	5.0	3.0[a]	3.0[a]	37
1,500	11	3.3	2.3	2.0	2.0[a]	1.0[b]	20[a]
2,000	8.0	3.0[a]	0.0	1.3	1.0[a]	0.0[c]	12[b]
CD	9.2	4.2	2.1	3.0	4.1	0.9	11

Numbers with same superscript letters in rows are non-significant ($p \leq 0.01$). C = Control, C.D = Critical difference, G1 = Appressoria in single germ tube, G2 = Appressoria in two germ tubes, G3 = Appressoria in three germ tubes.

Critical difference (CD): Critical difference is used to find out those differences between the treatment means, which are significant. If any difference is equal to or greater than the critical difference, it will be declared as significant.

yielded 0.0, 2.0, 8.0 and 25% at the same concentration and incubation period, maximum reduction was noticed in preinoculation treatments at 2,000 $\mu g/ml$, such as 0.0, 0.0, 1.2 and 2.0% only after 6th, 8th, 10th and 12th day, respectively while post-inoculation treatment yielded 0.0, 1.0, 4.2 and 11% respectively, at the same concentration and incubation periods (Table 4). In glasshouse, pre-inoculation treatment showed marked reduction in disease intensity (0.0, 0.0, 2.0, 5.0, 7.4, 13, 17, 22, 27 and 31%) at 1,500 $\mu g/ml$ after each 2 day interval from 8th day of inoculation. Although post-inoculation treatment yielded 0.0, 1.0, 3.0, 6.2, 11, 19, 26, 35, 39 and 43% at the same concentration and incubation periods, maximum inhibition was recorded at 2,000 $\mu g/ml$ when the leaves were pre-treated. Disease development completely inhibited for 14 days at 2,000 $\mu g/ml$ in preinoculation treatment whereas it was only 3.0% in post-inoculation treatment. With the increase in days of treatment, efficacy of the chemical was found to be reduced but there was still marked reduction in disease intensity in pre and postinoculation treatments as compared to control. Preinoculation treatment at 2,000 $\mu g/ml$ was found to be more effective for 16 days in reducing the D.I up to 0.5% while postinoculation treatment was also found to be effective for 14 days yielding 4.0% D.I. at the same concentration (Table 5).

The results of the present investigation indicate that plant alkaloid, nor-securinine, is highly effective against several phytopathogenic and saprophytic fungi including a biotroph. The fungi included in the present study belongs to two different groups but based on the activity of the

Table 4. Effect of nor-securinine on powdery mildew disease intensity on detached leaves of *Pisum sativum* during pre- and post-inoculation treatments

Period of observation (Day)	Control	Disease intensity (%)					
		Pre-inoculation			Post-inoculation		
		1,000 $\mu g/ml$	1,500 $\mu g/ml$	2,000 $\mu g/ml$	1,000 $\mu g/ml$	1,500 $\mu g/ml$	2,000 $\mu g/ml$
6th	0.5	0.0	0.0	0.0	0.2	0.0	0.0
8th	9.2	2.2	1.2	0.0	4.2	2.0	1.0
10th	35	14	6.0	1.2	25	8.0	4.2
12th	84	67	12	2.0	77	25	11

Table 5. Effect of nor-securinine on pea powdery mildew disease intensity in glasshouse during pre-and post-inoculation treatments

Period of observation (Day)	Control	Disease intensity (%)					
		Pre-inoculation			Post-inoculation		
		1,000 $\mu g/ml$	1,500 $\mu g/ml$	2,000 $\mu g/ml$	1,000 $\mu g/ml$	1,500 $\mu g/ml$	2,000 $\mu g/ml$
8th	1.1	0.0	0.0	0.0	1.5	0.0	0.0
10th	4.0	2.0	0.0	0.0	3.1	0.5	0.0
12th	8.5	4.5	1.7	0.0	7.8	3.0	1.0
14th	18	7.7	4.6	0.0	12	6.2	3.0
16th	28	15	7.4	0.5	23	11	4.0
18th	42	23	13	3.1	30	19	6.0
20th	54	31	17	5.0	39	26	9.5
22nd	62	35	21	6.4	53	35	12
24th	68	43	27	8.0	57	39	15
26th	75	48	31	10	64	43	20

chemical it is at present difficult to conclude as to which group of fungi is most susceptible to this chemical as the number of fungi from different groups is very low. Hence, further detailed study on several members of other fungal groups will classify the specificity of efficacy.

Several plant products have shown good efficacy against several fungi both *in vitro* and *in vivo*. Alstovenine, a plant alkaloid from *Alstonia venenata*, was effective against pigmented and nonpigmented spores of several fungi. Conidia of *Erysiphe* sp. were very sensitive but spores of *Fusarium udum* were less sensitive to this alkaloid (Singh *et al*., 1999). In the present investigation similar trend of efficacy was seen. *H. frumentacei* and *Erysiphe* sp. were more sensitive to nor-securinine. It was observed that nor-securinine was highly effective in inhibiting spore germination, appressorium formation and in controlling powdery mildew of pea. Germ tube development and appressorium formation were faster at lower concentration. Singh *et al*. (2001) reported that the number of germ tubes of *E. pisi* determine the degree of infection by the pathogen in pea plants. The number of germ tube and appressorium formation were significantly reduced in both pre as well as post inoculation treatments at the test concentrations proving the efficacy of the nor-securinine. However, pre-inoculation treatment showed greater efficacy than post-inoculation after 24 h of inoculation. Maximum inhibition occurred at 2,000 $\mu g/ml$. Although several alkaloids are already known to be antifungal (Maurya *et al*., 2001; 2002; Ahmed *et al*., 2004: Annapurna *et al*., 2004; Chung *et al*., 2004), such activity of the present chemical is being reported for the first time. As the chemical is highly effective even at low concentration (1,000 $\mu g/ml$), it is quite possible that this chemical may be successful in controlling other plant diseases under field conditions, but for better efficacy the chemical should be sprayed at 15 days of intervals for 2~3 times. Results on the biological efficacy of nor-securine against some of fungi show the possibility of its utilization in field, which may be a substitute of chemical fungicides.

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