

Control of *Erysiphe pisi* Causing Powdery Mildew of Pea (*Pisum sativum*) by Cashewnut (*Anacardium occidentale*) Shell Extract

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The effect of methanolic extract of cashewnut (*Anacardium occidentale*) shell extract was seen on conidial germination of *Erysiphe pisi* and powdery mildew development in pea (*Pisum sativum*). Maximum conidial germination inhibition of *E. pisi* on glass slides was observed at 300 ppm. Similar effect on floated pea leaves was observed after 48 h at the same concentration. Conidial germination on intact untreated pea leaves was also assessed on II and IV nodal leaves while IV and II nodal leaves were treated with the extract and vice versa. There was tremendous reduction in conidial germination on all the nodal leaves. The disease intensity of pea powdery mildew was significantly reduced by methanolic extract of cashewnut shells. Maximum reduction was observed with 200 ppm where 39% disease intensity was recorded in comparison to 96.53% in the control. The phenolic acid content of pea leaves following treatments with this extract varied and no definite pattern was observed. Out of several phenolic compounds, namely, gallic, ferulic, chlorogenic, and cinnamic acids, only gallic acid was found to be present consistently in all the treatments with varied amounts.

KEYWORDS : *Anacardium occidentale*, *Erysiphe pisi*, Induced resistance, *Pisum sativum*

Erysiphe pisi, the causal agent of powdery mildew of pea (*Pisum sativum*), is a destructive pathogen causing infection on all the above ground parts of pea plants (Singh, 2000). Various management practices are adopted to control this pathogen at farmers fields. However, with the increase in awareness regarding the hazardous effect of synthetic fungicides, attempts are being made to go “back to nature” by managing the plant diseases with ecologically acceptable management practices. Among them use of botanicals becomes an integral part of current research. Several plant products have been reported to be antifungal (Singh *et al.*, 1980; Singh *et al.*, 1990; Lyon *et al.*, 1995; Suheyla *et al.*, 1996), either by inducing resistance in hosts against pathogen attack or by inhibiting the growth of the pathogens directly. Disease intensity of powdery mildew of pea is reported to be lowered by plant products like Neemazal, a product of neem (*Azadirachta indica*) under field conditions (Prithiviraj *et al.*, 1998) and the mechanism was reported to be the induced resistance in the host (Singh and Prithiviraj, 1997). Similarly, extract of *Reynoutria sachalinensis* that induces resistance in the host by activating the phenyl propanoid pathway has also shown satisfactory results against powdery mildews (Daaye *et al.*, 2000). Interestingly, ajoene a constituent of garlic (*Allium sativum*) has also shown control of some powdery mildews in greenhouse (Singh *et al.*, 1995). Recently, methanolic extract of cashewnut shells was also found

inhibitory to spore germination of some fungi (Sahani *et al.*, 2002).

Phenolic compounds are the products of phenyl propanoid pathway. Some of which occur constitutively and are thought to function as preformed inhibitors associated with non-host resistance (Millar and Higgins, 1970; Stoessl, 1983). Others are formed in response to the ingress of pathogens, and their appearance is considered as part of an active defense response (Nicholson and Hammer-schmidt, 1992). In the background of the above informations, the present investigation was taken up to assess the efficacy of methanolic extract of cashewnut (*Anacardium occidentale*) shells against *E. pisi* and possible involvement of phenolic compounds in controlling the disease by inducing resistance in pea plants.

Materials and Methods

Extraction of *A. occidentale* shells with methanol. Shells of *A. occidentale* were dried in oven at 60°C for 24 h and finally crushed into powder. The powder (2 kg) was extracted in methanol (2.5 l) in Soxhlet apparatus at 55 ± 2°C for 8 h. Methanol extract was filtered and solvent was distilled in rotary evaporator. Crude methanol extract of cashewnut shells (120 g) thus obtained was stored at 4°C for further bioassay.

Conidial germination. The effect of methanol extract of cashewnut shells was assayed against conidial germina-

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tion of *E. pisi* on glass slides as well as on floated pea leaves on sterile distilled water in petri plates. Various concentrations (150, 200, 300 ppm) of the extract were prepared in sterilized distilled water and a drop of each concentration was placed on glass slides. *E. pisi* conidia from infected pea plants were tapped on the drops of the extract and mixed thoroughly with the help of a needle. The glass slides were placed in moist chambers prepared by placing moist filter paper on the lower surface of the lid and on inner surface of the base of Petri plates and incubated at 25°C for 24 h. Conidia tapped only on sterile distilled water for germination served as control. The germination was observed under light microscope after staining the conidia with lactophenol - cotton blue.

Twenty ml of the extract from each concentration (150, 200, 300 ppm) of the extract was taken in each Petri plate and freshly excised healthy pea leaves (3) were floated on extract solutions by keeping the adaxial surface up. Conidia were tapped as described above in such a way that each leaf received approximately 200–300 conidia. Pea leaves floated on sterilized-distilled water only (Control) also received same number of conidia. All the plates were incubated at 25 ± 2°C. Conidial germination was recorded periodically after 6, 12, 24, and 48 h after removing the chlorophyll from pea leaves according to the method described by Carver and Adaiabe (1990). The whole experiment was conducted in triplicate.

Conidial germination following treatment with cashewnut shell extracts on intact nodal leaves. Conidial germination of *E. pisi* on intact pea leaves following treatment with extract was conducted on plants grown in plastic pots in glasshouse conditions. Ten seeds of pea (var. Arkel) were sown in pots (15 cm dia.) and kept in glasshouse at 25 ± 2°C. Different concentrations (150, 200, 300 ppm) of shell extract were sprayed on either II or IV nodal leaves of 20-day-old pea plants. When II nodal leaves were sprayed with extract, IV nodal leaves of same plants were left untreated and vice-versa. Conidia of *E. pisi* were inoculated on II or IV nodal leaves of treated plants in such a way that the extract-treated leaves in both conditions did not receive conidia. This was done by covering the respective leaves with plastic sheets. Control plants where only distilled water was sprayed instead of extract received similar treatments. Pea leaves inoculated with *E. pisi* conidia were excised after 24 and 48 h of treatments and conidial germination was observed after their processing according to the method of Carver and Adaiabe (1990). Each treatment was replicated thrice on 25 plants and average percent conidial germination was calculated.

Field experiment. Field experiments were carried out at the Vegetable Research Farm of Banaras Hindu Univer-

sity, Varanasi, India during December–March 2000–2001. The field was ploughed three times and fertilizers (@ 20 kg nitrogen, 50 kg phosphorus, 50 kg potash per hectare) were applied after first ploughing. Field was then labeled to maintain optimum moisture for seed germination. Pea (cv. Arkel) seeds were sown in plots (2 m × 2 m) 10–15 cm apart during second week of December. The row-to-row distance was 45 cm. Each treatment had three replications in randomized block design.

Different concentrations of methanol extract of cashewnut shells (150, 200, 300 ppm) were prepared in sterile distilled water in which 0.1% Tween 20 solution was added and sprayed on the plants prior to the occurrence of first symptoms of powdery mildew. The disease intensity was recorded after 10–12 days of treatment and the final estimation was calculated by 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; 4 = > 51% leaf area infected

Extraction of phenolic acids. First and second nodal leaves from pea plants were collected randomly from each treatment periodically at 6, 12, 24, 48 and 72 h. Samples (2 g) from each treatment were prepared from such leaves. Fresh leaves (2 g) from each treatment were extracted separately by macerating in a pestle - mortar. Phenolic acids from treated nodal leaves (either I/II or III/IV) of pea were extracted periodically at 6, 12, 24, 48 and 72 h. Randomly collected pea leaves (2 g) from each treatment was extracted in 10 ml ethanol : water (80 : 20) by macerating the leaves in a pestle - mortar. The finally crushed leaves suspended in solvent samples were collected in screw - capped tubes and the suspension was subjected to ultrasonication (Branson Sonifier, USA) for 15 min at 4°C followed by centrifugation at 7,500 rpm for 15 min. The clear greenish supernatant was subjected to charcoal treatment to remove pigments of each sample and was then transferred to glass tubes. The residue was re-extracted twice and supernatant was pooled prior to evaporation under vacuo (Buchi Rotavapor Re Type). Dried samples were resuspended in 1.0 ml HPLC grade methanol by vortexing and stored at 4°C for further analysis.

HPLC analysis. Quantitative analysis of the samples was performed as per the method of Singh *et al.* (2002). The HPLC system (Shimadzu Corporation, Kyoto, Japan) was equipped with two Shimadzu LC-10 ATVP recipro-

cating pumps, a variable Shimadzu SPD-10 AVP UV-VIS detector and a Rheodyne Model 7725 injector with a loop size of 20 μ l. Peak area was calculated with Winchrom integrator. Reverse phase chromatographic analysis was carried out in isocratic conditions using C-18 reverse phase column (250 \times 4.6 mm id, particle size 5 μ m Luna 5 μ C-18 (2), Phenomenex, USA) at 25°C. Running conditions included injection volume 5 μ l, mobile phase methanol : 0.4% acetic acid (80 : 20 v/v), flow rate 1 ml/min, and detection at 290 nm. Samples were filtered through membrane filter (pore size 0.45 μ m, E-Merck, Germany) prior to injection in sample loop. Tannic, gallic, vanillic, caffeic, ferulic, o-coumaric, chlorogenic, cinnamic and synapinic acids were used as internal and external standards. Phenolic acids present in the samples were identified by comparing chromatographic peaks with the retention time (Rt) of individual standards and further confirmed by co-injection with isolated standards.

Results

Methanol extract of cashewnut shells reduced conidial germination of *E. pisi* and the reduction was proportional to the increase in concentration. Maximum reduction of conidial germination was 11% at 300 ppm as compared to control (42%) after 24 h of treatment. Similarly, conidial germination on detached pea leaves was also affected. Reduction was maximum at 300 ppm after 48 h (6% at 300 ppm) as compared to 27% in control (Table 1).

Table 1. Effect of methanolic extract of *Anacardium occidentale* on conidial germination of *Erysiphe pisi* on glass slides and detached pea leaves

Concentration of methanolic extract	On glass slides		% conidial germination on detached pea leaves		
	Period (h)				
	24	6	12	24	48
Control	42 \pm 3.3	10 \pm 1.6	23 \pm 1.2	28 \pm 2.4	27 \pm 1.7
150 ppm	34 \pm 2.8	12 \pm 2.1	15 \pm 1.7	13 \pm 1.3	17 \pm 1.8
200 ppm	21 \pm 2.9	10 \pm 2.0	14 \pm 1.5	9 \pm 1.4	11 \pm 1.3
300 ppm	11 \pm 1.7	7 \pm 1.4	9 \pm 1.1	9 \pm 1.7	6 \pm 0.8

Table 2. Effect of methanolic extract of *Anacardium occidentale* on conidial germination of *Erysiphe pisi* on different nodal leaves of pea

Treatment	% conidial germination on I & II nodal leaves		% conidial germination on III & IV nodal leaves	
	III & IV nodal leaves treated		I & II nodal leaves treated	
	Time of observation (h)			
	24	48	24	48
<i>E. pisi</i>	22.00 \pm 2.4	26.00 \pm 1.7	25.00 \pm 2.8	31.00 \pm 3.2
150 ppm	2.00 \pm 1.6	10.00 \pm 0.8	6.00 \pm 0.7	20.00 \pm 2.1
200 ppm	9.00 \pm 0.9	8.66 \pm 1.6	2.00 \pm 0.3	16.00 \pm 1.9
300 ppm	6.00 \pm 1.2	13.00 \pm 1.3	3.00 \pm 0.5	14.00 \pm 1.2

Treatment with extract on III and IV (upper) nodal leaves inhibited conidial germination on I and II (lower) nodal leaves as compared to control after 24 and 48 h. Similarly, conidial germination on III and IV nodal leaves (upper leaves) when I and II nodal (lower) leaves were treated, was also inhibited. Maximum reduction in spore germination was observed at 300 ppm of the extract. Germination on III and IV nodal leaves was (3% and 14% at 300 ppm after 24 and 48 h, respectively) as compared to 25 and 31% in control at the same time. On intact pea leaves conidial germination was also reduced after 24 h following application of the extract. After 24 h, the germination on I and II nodal leaves was lower (6% at 300 ppm) as compared to control (22%) when II and IV nodal leaves were treated. Similarly, the germination on III and IV nodal leaves was inhibited after 24 h when I and II nodal leaves were treated (Table 2).

Methanol extract of cashewnut shells reduced disease intensity of pea powdery mildew to a great extent at all the concentrations (Fig. 1). Maximum reduction was observed with 200 ppm where 39% disease intensity was recorded in comparison to 76% in control. At still higher concentration (500 ppm), the disease intensity was almost similar at it was on 200 ppm, indicating that

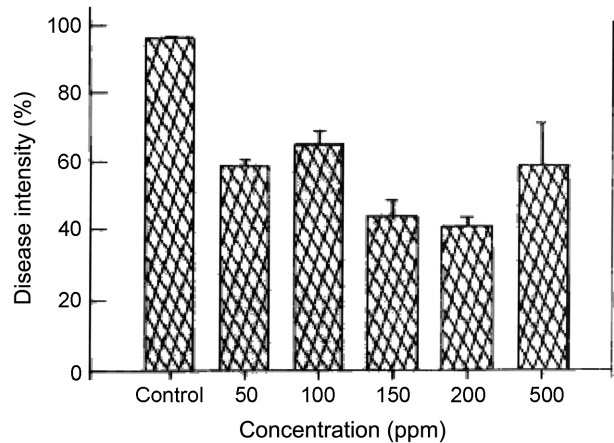


Fig. 1. Effect of methanolic extract of cashewnut shell on disease intensity of *Erysiphe pisi*.

increased concentration had no effect on disease intensity (Fig. 1).

Out of the phenolic compounds detected only gallic acid showed consistent presence with varied amount. Phenolic acid content in pea was recorded periodically both in upper (III and IV) and lower (I and II) leaves after treatment with cashewnut shell extract and also in leaves inoculated with *E. pisi*. Phenolic profile, though inconsistent, was prominent as certain phenolics, e.g. gallic, ferulic, chlorogenic and cinnamic acids were detected regularly in varying quantities. Out of these phenolic acids, only gallic acid showed consistent presence, after 6 h of treatment with the extract, however, the III and IV (upper) nodal leaves showed more accumulation of gallic acid as compared to I and II (lower) nodal leaves. Treatment with 150 and 300 ppm on I and II (lower) nodal leaves followed by subsequent inoculation of *E. pisi* on III and IV (upper)

leaves resulted in maximum accumulation of gallic acid (58.93 and 51.32 $\mu\text{g/g}$ fresh wt.) in III and IV (upper) nodal leaves as compared to control (38.11 $\mu\text{g/g}$) (Table 3). The same treatment also resulted in the maximum accumulation of chlorogenic (2.36 $\mu\text{g/g}$) and cinnamic (0.86 $\mu\text{g/g}$) acids in upper leaves as compared to control and other treatments. (Table 3). After 12 h of treatment with the extract III and IV (upper) nodal leaves showed higher accumulation of gallic, ferulic and cinnamic acids as compared to I and II (lower) nodal leaves (Table 3). Maximum accumulation of gallic and ferulic acids (38.37 and 9.51 $\mu\text{g/g}$ fresh wt, respectively) was observed in T5 in comparison to other treatments (T1-T8) (Table 4). Also, I and II (lower) nodal leaves accumulated maximum ferulic (13.36 $\mu\text{g/g}$) and chlorogenic (0.78 $\mu\text{g/g}$ fresh wt.) acids following treatment with 300 ppm of extract on I and II nodal leaves and subsequent treatment on upper

Table 3. Phenolic acid content in pea leaves after 6 h treatment with methanolic extract of cashewnut shells

Treatment (Concentrations)	Phenolic acid content ($\mu\text{g/g}$ fresh leaf tissues)						
	I and II nodal leaves				III and IV nodal leaves		
	GA	FA	Chl.	Cinn.	GA	Chl.	Cinn.
T1	32.13	–	–	0.26	38.11	–	0.38
T2	39.20	–	0.48	0.56	39.17	1.39	–
T3	23.13	2.15	1.36	1.03	58.93	2.36	0.86
T4	38.70	2.36	–	–	35.55	–	–
T5	33.20	–	–	0.82	51.32	1.76	0.67
T6	21.54	2.35	5.04	1.60	19.41	1.85	–
T7	31.65	1.26	–	–	31.40	0.11	0.02
T8	34.99	–	0.94	0.80	49.71	–	0.08

GA = Gallic, FA = Ferulic, Chl. = Chlorogenic, Cinn = Cinnamic acid.

T1 = Healthy, T2 = Treated *Erysiphe pisi* treated, T3 = 150 ppm treated I, II, nodal leaves and *Erysiphe pisi* treated III, IV nodal leaves T4 = 200 ppm treated I, II, nodal leaves and *Erysiphe pisi* treated III, IV nodal leaves, T5 = 300 ppm treated I, II, nodal leaves and *Erysiphe pisi* treated III, IV nodal leaves, T6 = *Erysiphe pisi* treated I, II, nodal leaves and 150 ppm treated III, IV nodal leaves T7 = *Erysiphe pisi* treated I, II, nodal leaves and 200 ppm treated III, IV nodal leaves, T8 = *Erysiphe pisi* treated I, II, nodal leaves and 300 ppm treated III, IV nodal leaves.

Table 4. Phenolic acid content in pea leaves after 12 h treatment with methanolic extract of cashewnut shells

Treatment (Concentrations)	Phenolic acid content ($\mu\text{g/g}$ fresh leaf tissues)						
	I and II nodal leaves				III and IV nodal leaves		
	GA	FA	Chl.	Cinn.	GA	FA	Cinn.
T1	42.19	4.14	0.17	–	31.73	–	0.26
T2	86.01	7.36	–	–	81.98	1.39	–
T3	11.64	1.96	0.45	0.01	22.13	2.13	0.47
T4	10.73	–	–	0.14	23.26	–	–
T5	16.59	13.36	0.78	–	38.37	9.51	0.17
T6	19.19	10.59	–	0.03	17.85	–	0.32
T7	22.66	–	0.14	0.57	24.38	4.34	0.52
T8	10.29	–	0.26	–	25.32	1.29	–

GA = Gallic, FA = Ferulic, Chl. = Chlorogenic, Cinn = Cinnamic acid.

T1 = Healthy, T2 = *Erysiphe pisi* treated, T3 = 100 ppm treated I,II, nodal leaves and *Erysiphe pisi* treated III, IV nodal leaves T4 = 200 ppm treated I,II, nodal leaves and *Erysiphe pisi* treated III, IV nodal leaves, T5 = 300 ppm treated I, II, nodal leaves and *Erysiphe pisi* treated III, IV nodal leaf, T6 = *Erysiphe pisi* treated I, II, nodal leaves and 100 ppm treated III, IV nodal leaves T7 = *Erysiphe pisi* treated I, II, nodal leaves and 200 ppm treated III, IV nodal leaves, T8 = *Erysiphe pisi* treated I, II, nodal leaves and 300 ppm treated III, IV nodal leaves.

Table 5. Phenolic acid content in pea leaves after 24 h treatment with methanolic extract of cashewnut shells

Treatment (Concentrations)	Phenolic acid content ($\mu\text{g/g}$ fresh leaf tissues)			
	I and II nodal leaves		III and IV nodal leaves	
	GA	Cinn.	GA	Cinn.
T1	15.50	0.21	20.13	0.21
T2	15.13	–	23.64	–
T3	36.25	0.47	50.90	0.36
T4	23.36	0.33	14.59	0.04
T5	9.15	–	14.90	0.49
T6	30.55	0.03	28.51	–
T7	16.65	0.27	17.00	0.19
T8	21.11	0.13	4.88	–

GA = Gallic, Cinn = Cinnamic.

T1 = Healthy, T2 = Treated with *Erysiphe pisi*, T3 = 150 ppm treated I, II, nodal leaves and *Erysiphe pisi* treated III, IV nodal leaf T4 = 200 ppm treated I, II, nodal leaves and *Erysiphe pisi* treated III, IV nodal leaves, T5 = 300 ppm treated I, II, nodal leaves and *Erysiphe pisi* treated III, IV nodal leaves, T6 = *Erysiphe pisi* treated I, II, nodal leaves and 100 ppm treated III, IV nodal leaves, T7 = *Erysiphe pisi* treated I, II, nodal leaves and 200 ppm treated III, IV nodal leaves, T8 = *Erysiphe pisi* treated I, II, nodal leaves and 300 ppm treated III, IV nodal leaves.

leaves (III and IV) by *E. pisi* (Table 4). After 24 h of treatment with extract presence of gallic acid was consistently recorded in both lower (I and II) and upper (III and IV) nodal leaves. However, it was maximum as compared to control and other treatments when lower leaves were treated with 150 ppm followed by inoculation with *E. pisi* (Table 5). Gallic acid further dominated after 48 h of treatment and maximum quantity (10.46 and 15.79 $\mu\text{g/g}$ in lower and upper leaves, respectively) was recorded in the treatments where I and II nodal leaves were sprayed with 300 ppm of extract and III and IV nodal leaves were inoculated with *E. pisi* only (Table 6).

Discussion

Methanol extract of cashewnut shells affected conidial germination of *E. pisi* on glass slides, detached leaves as well as on intact pea leaves. Cashewnut belongs to the family Anacardiaceae and its nut shells are reported to contain anacardic acid (Biswas and Ray, 1958). Anacardic acid is naturally occurring alkyl substituted salicylic acid (6-alkyl-2-hydroxy benzoic acid) that has been isolated from a number of botanical families, viz., Ginkgoaceae, Myristicaceae as well as Anacardiaceae (Spencer *et al.*, 1980). Anacardic acid is also reported to be antifungal *in vitro* (Prithiviraj *et al.*, 1997) as it inhibited conidial germination in a number of phytopathogenic fungi. Inhibition of conidial germination of *E. pisi* on glass slides by cashewnut shell extract in the present investigation confirms the antifungal property of the extract. The results from the glass slide experiments lead to hypothesize that the methanol extract behaves as contact fungicide but inhibition of conidial germination on floated detached pea leaves confirms inhibitory effect by absorption and translocation of the chemical in upper leaf times.

Similarly, the results of glasshouse experiments are also in conformity with the previous results on glass slides. It is assumed that the translocation of the chemical (s) present in cashewnut shell extract may have occurred that affected the conidial germination on non-treated leaves, indicating systemic nature of the mode of action of the extract.

Induction of phenolic compounds in the early stages of application of extract was a good sign as many of them are antifungal (Singh *et al.*, 2002). However, without a definite pattern of their induction, it is difficult to conclude the exact role of extract in inducing resistance in plants. But, it is easy to interpret that the mode of action of the extract is both contact as well as systemic. The effi-

Table 6. Phenolic acid content in pea leaves after 48 h treatment with methanolic extract of cashewnut shells

Treatment (Concentrations)	Phenolic acid content ($\mu\text{g/g}$ fresh leaf tissues)					
	I and II nodal leaves			III and IV nodal leaves		
	GA	Chl.	Cinn.	GA	Chl.	Cinn.
T1	5.76	0.06	–	5.59	0.09	0.16
T2	10.22	–	–	13.31	0.42	–
T3	2.41	–	0.05	4.23	0.31	0.26
T4	2.24	–	–	2.21	–	0.21
T5	10.46	–	–	15.79	0.76	0.47
T6	5.26	–	0.01	9.93	0.23	–
T7	2.59	–	–	6.36	–	0.06
T8	2.36	0.09	–	4.55	0.17	–

GA = Gallic, Chl. = Chlorogenic, Cinn = Cinnamic acid.

T1 = Healthy, T2 = *Erysiphe pisi* treated, T3 = 150 ppm treated I, II, nodal leaves and *Erysiphe pisi* treated III, IV nodal leaves T4 = 200 ppm treated I, II, nodal leaves and *Erysiphe pisi* treated III, IV nodal leaves, T5 = 300 ppm treated I, II, nodal leaves and *Erysiphe pisi* treated III, IV nodal leaves, T6 = *Erysiphe pisi* treated I, II, nodal leaves and 100 ppm treated III, IV nodal leaves, T7 = *Erysiphe pisi* treated I, II, nodal leaves and 200 ppm treated III, IV nodal leaves, T8 = *Erysiphe pisi* treated I, II, nodal leaves and 300 ppm treated III, IV nodal leaves.

cacy of the extract in controlling pea powdery mildew under glasshouse condition can be exploited for its wider application against several other diseases under field conditions.

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