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Research Article

In vitro antifungal activity of S*alicornia* and A*vicennia* against *Macrophomina phaseolina* the incitant of dry root rot of black gram

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Abstract

The efficacy of *Salicornia* and *Avicennia* plant products were evaluated against *Macrophomina phaseolina* the incitant of dry root rot of black gram. Among them of *A.marina* (leaves) (20%) and *A. marina (*stem*)* (60%), *A. marina* (pneumatophores) 60% and *Salicornia brachiata* (60%) gave complete inhibition of the pathogen and were on par with carbendazim (0.1%). Conidial germination of *M. phaseolina* was completely inhibited by *A. marina* (leaves) (20%) and *A .marina* (stem) (60%) *A.marina* (pneumatophores) 60%. Plant products at their respective MIC = s totally inhibited the sporulation of *M. phaseolina*. Production of cellulolytic and pectinolytic enzymes the pathogen was also inhibited maximum with fungicide carbendazim (0.1%), followed by *A. marina* (leaves) (20%) and *A.marina* (stem) (60%) *A. marina* (pneumatophores) 60% and *Salicornia* (60%).

Keywords: *Salicornia, Avicennia,* Plant products, Carbendazim and Enzymes.

Introduction

Macrophomina phaseolina (Tassi) Goid. is an important cosmopolitan soil borne phytopathogen causing dry root rot of blackgram, (*Vigna mungo* L.) which if uncared will become a serious one. The pathogen is both seed and soil borne in nature. Control of the disease with seed and soil treatment chemicals have not been economical and successful. The pathogens are getting resistant to chemicals and moreover these chemicals add to the problem of pollution and their entry upset our food chains. Hence, many eco-friendly, inexpensive, and easily available control measures have become a challenge. The indiscriminate use of fungicides may lead to toxic residues, development of fungicide resistance environmental pollution and carcinogenic chilli (Rajavel, 2000) products. In this context, a search for naturally occurring substances with potential biological activity continues in an increasing scale. Plant products are gaining importance in crop protection in view of their selective properties, low cost and safer alternative means to the ecosystem. The medicinal use of plant materials for the treatment of microbial infections has been shown to depend on the presence of antimicrobial chemicals. Likewise antimicrobial compounds present in healthy plant tissues have been regarded as an important factor in disease resistance (Annapurna *et al*., 1983).

Materials and Methods

Plant collection

Avicennia marina plant parts *viz*., leaves, stem and pneumatophores and *S. brachiata* were collected from Pichavaram mangrove wetlands were air dried under shade with occasional shifting and finely powdered using electric blender.

Isolation and maintenance of the pathogen

M. phaseolina, was isolated from infected blackgram, collected from 10 different locations of Tamilnadu. The fungus was subsequently purified by single hyphal tip method. The pure culture was obtained and preserved in refrigerator and sub cultured once in a month.

Identification of the pathogen

The morphological characters of the pathogens were studied and compared and confirmed as per the earlier works (Ainsworth, 1961).

Plant Products used for screening against the pathogen

Salicornia and *Avicennia* were evaluated for their antifungal effect. Carbendazim 50 WP at 0.1% concentration was used as a standard fungicide against pathogens for comparison.

Preparation of plant extracts

Cold water extract

For the preparation of plant extracts (leaves, stem and pneumatophores) and evaluation the method suggested by Ezhilan *et al*. (1994) was followed.

Hot water extract

The plant material were chopped into small bits and plunged in required quantity of water (1:1 w/v) taken in a beaker and heated over water bath at 80°C for 10 min. The materials were then crushed with pestle and mortar and strained with a cheese cloth. It gave the standard plant hot water extract (100%). After extraction, they were subjected to low centrifugation (2000 rpm for 20 min) and the clear supernatant was used.

***In vitro* evaluation of antifungal effect of plant products on the growth of pathogens**

The PDA medium was prepared in 250 mL Erlenmeyer flasks and sterilized. Aqueous extracts of 5, 10, 20, 30 and 40 mL were added to 45, 40, 30, 20 and 10 mL of aliquots, respectively in flasks so as to get the final concentration of 10, 20, 40, 60 and 80% of the extracts in the medium. Carbendazim 0.1% concentration in the medium was used for comparison. PDA medium without any extract served as control. The medium was poured in to 90 mm Petri plates at the rate of 15 mL. The fungal culture disc of nine mm size obtained from a seven days old culture were taken and inoculated in the centre of Petri plates aseptically and incubated for seven days. The diameter of the colony was measured when the mycelium fully covered the Petri plates of any one of the treatments.
The % inhibition of growth was calculated as per Vincent (1947) for each treatment and expressed:

$$\text{Percent inhibition} = \frac{C\text{-}T}{C} \times 100$$

Where,

C = Diameter of growth in control
T = Diameter of growth the treatment

***In vitro* evaluation of antifungal effects of plant products on the growth of pathogens in liquid medium**

Potato dextrose broth was prepared in 250 mL Erlenmeyer flasks and autoclaved. Aqueous extracts of 5, 10, 20, 30 and 40 mL were added to 45, 40, 30, 20 and 10 mL of broth in flasks so as to get the final concentration of 10, 20, 40, 60 and 80% of the extracts in broth. All the flasks were inoculated with 9 mm culture disc and incubated 28±1°C for 10 days. Carbendazim 0.1% was added to the broth for comparison. Broth without any extract served as control. Three replications were maintained for each treatment. After the incubation period the mycelial mat was harvested on a previously weighed filter study and dried at 105°C for 48 h in hot air oven cooled in desiccator and the mycelial weight was recorded and expressed in mg 50 mL^{-1} broth.

***In vitro* effect of antifungal effect of plant products on the germination of conidia of pathogen**

Cavity slide method

Antifungal activity of aqueous extracts of plant products on spore germination of Pathogen were assayed by cavity slide method. Test extracts 0.05 and 0.05 mL of spore suspension (2000-3000 spores mL^{-1}) of the test fungus estimated using a haemocytometer

were mixed in a cavity slide and incubated for 24 h in Petri dish glass bridge moist chamber at 25±2°C. Cavity slide with sterile distilled water were treated as control. Three replications were kept for each treatment. Observations were taken from 20 microscopic fields for each slide and total number of conidia germinated in each microscopic fields was recorded and percent germination was calculated.

***In vitro* evaluation of antifungal activity of plant products on the sporulation of pathogen**

Antifungal activity of plant products on the sporulation of pathogens was determined by the method of Bera and Saha (1983). Aqueous plant extracts containing medium was poured into Petri dishes plates aseptically at 15 mL. Fungal disc of 9 mm size was inoculated in the centre of Petri plates and incubated at 28±1°C for seven days. Three replications were maintained for each treatment. Suitable control was maintained. A single mycelial disc from the growing point was removed with a aid of a sterile cork borer and transferred to 1 mL sterile distilled water and shaken vigorously. A drop of spore suspension was placed on a glass slide and number of spores produced was recorded.

Effect of plant products on the *in vitro* production of cellulolytic and pectinolytic enzymes of pathogen
Preparation of enzyme source

Czapek's broth devoid of sucrose and supplemented with 0.3% carboxy methyl cellulose for cellulolytic enzymes or 3% pectin for pectinolytic enzymes was prepared. The medium was distributed in 40 and 20 mL quantities in 250 mL Erlenmeyer flasks. After autoclaving, the plant products were added separately at the rate of 10 and 30 mL, respectively so as to get 20 and 60% final concentration. Carbendazim at 0.1% concentration in the medium was used for comparison. Broth without any extract served as control. Three replications were maintained for each treatment. The flasks were inoculated with 9 mm culture disc and incubated at 28±2°C. After 15 days of incubation, the mycelial mat was harvested. The filtrates were centrifuged at 2100 rpm for 30 min to remove the spores and the solution was retained for assay.

Cellulolytic enzymes
Cellulose C1 (Norkrans, 1950)

The reaction mixture consisted of 1.0 mL of cellulose suspension (the concentration of which was adjusted approximately to 0.85 absorbance at 620 nm), 4.0 mL of 0.2 M sodium acetate acetic acid buffer at pH 5.6 and 5 mL of culture filtrate. The absorbance of the mixture was determined immediately at 620 nm in the calorimeter and incubated at room temperature (28±1°C). At the end of 24 h the absorbance was again measured and the enzyme activity was expressed as units (1 unit = 0.01 absorbance at 620 nm), calculated as to difference in absorbance.

Cellulase (Cx) (Hussain and Diamond, 1960)

The activity of cellulase (endo glucanase) can be estimated by loss in viscosity of the cellulosic substrate using Ostwald-Fenske viscometer (150 size with efflux time of 20 sec for double distilled water). Carboxy methyl cellulose of 0.5% concentration was prepared in sodium acetate-acetic acid buffer at pH 48 and pipetted out 4 mL of CMC, 1 mL of the buffer and 2 mL of enzyme substrate and transferred into Ostwald-Fenske viscometer and kept in water bath at 30±1°C. The contents were mixed and the efflux time at fixed interval (2 h) was determined. The percent loss in viscosity was calculated through the following equation:

$$\text{Loss in viscosity (\%)} = \frac{T_0 - T_1}{T_0 - T_w} \times 100$$

Where,

T0 - Flow time at zero time (sec)
T1 - Flow time at one interval (sec)
Tw - Flow time of double distilled water (sec)

Pectinolytic enzymes (Mahadevan and Sridhar, 1986)

Polygalactouronase (PG)

The PG enzyme activity was measured by the loss in viscosity of sodium polypectate in sodium acetate-acetic acid buffer at pH 5.2. One milliliter of buffer and immediately transferred to Ostwald-Fenske viscometer (size 150) placed in a water bath at 30±1°C. Viscosity losses were measured and percent loss in viscosity was calculated as detailed earlier. Culture filtrates without test compound served as control.

Pectin Trans Eliminase (PTE)

The activity of PTE was determined by viscosity loss of 1% citrus pectin. The reaction mixture consisted of 4 mL of 1% citrus pectin in 0.2 mL of culture filtrate. The pH of the reaction mixture was adjusted to 8.6 and immediately transferred to ostwalol. Fenske viscometer (size 150) and the loss in viscosity was determined as detailed above.

Poly Galacturonase-Tran-Eliminase (PGTE)

The activity of enzyme PGTE was determined by the viscosity loss of sodium polypectate dissolved in boric acid borax buffer at pH 8.6, 1 mL of buffer (at pH 8.6) and 2 mL of culture filtrate was added and immediately transferred to Oswald-Fenske viscometer (size 150) and loss in viscosity was determined as detailed earlier.

Results

The isolates recorded percent disease incidence that ranged from 22.61 and 42.36. Among the isolates TR & RI Aduthurai - Thanjavur district recorded 42.36 per cent disease infection under artificially inoculated condition and it was followed by Papanasam isolated from Thanjavur district (40.50 per cent). The virulent cultures of TR & RI and Papanasam isolates exhibited brown to black lesions on the stem after inoculation. The virulent culture of TR & RI isolate was used in all experiments in the subsequent studies. The Annamalai Nagar isolate collected from Cuddalore district is considered as moderate virulent culture which recorded lesser disease incidence of 22.39 per cent (Table - 1).

The color of the *M.phaseolina* colony and topography of all the isolates were documented, the virulent isolates were collected from TR & RI - Thanjavur, expressed as white color colony. The next best virulent isolate of Papanasam (Thanjavur dt) recorded as grey color colony. The isolate of Needamangalam and Mannarkudi expressed as whitish grey color colony. The remaining isolates *viz*., Annamalai university, KVK Pallur, Sirkali, Veddaranyam, Musiri, and Kulithalai recorded as Blackish white, Whitish grey, White, Black, Greyish white and Black colonies respectively. The topography of all isolates was aerial (Table - 2).

A. marina (leaves) at 20% concentration, *A. marina* (stem & pneumatophores) and *Salicornia* at 60% concentration recorded the complete inhibition and they were on par with carbendazim at 0.1% when compared to control, all the treatments gave appreciable reduction in mycelial growth of the fungus (Table - 3).

A. marina (leaves) at 20% concentration, *A. marina* (stem & pneumatophores) and *Salicornia* at 60% concentration recorded the complete inhibition and they were on par with carbendazim at 0.1% when compared to control, all the treatments gave appreciable reduction in mycelial dry weight of the fungus (Table 4).

A. marina (leaves) at 20% concentration, recorded the complete inhibition and they were on par with carbendazim at 0.1% when compared to control, all the treatments gave appreciable reduction in **mycelial growth** of the fungus (Table - 5).

A. marina (leaves) at 20% concentration recorded the complete inhibition and they were on par with carbendazim at 0.1% when compared to control, all the treatments gave appreciable reduction in mycelial dry weight of the fungus (Table - 6).

A. marina leaf extract at 20% concentration completely inhibited the spore germination while *Salicornia* needed 40% concentration for complete inhibition of spore germination of *M. phaseolina*. *A. marina* (stem and pneumatophores) at 60%concentration completely inhibited the spore germination of *M.phaseolina* they were on par with carbendazim. All treatments differed significantly over control (Table - 7).

Plant products on the production of cellulolytic enzymes by *M. phaseolina*: All the treatments significantly reduced the production of cellulolytic enzymes of *M. phaseolina* when compared to control. The fungicide carbendazim (0.1%) recorded maximum inhibition. The plant products *A. marina (leaves, stem and pneumatophore)*, and *Salicornia brachiata* inhibited the cellulolytic enzyme production of *M. phaseolina* in a decreasing order of merit (Table - 8).

Plant products on the production of pectinolytic enzymes by *M. phaseolina*: All the treatments significantly reduced the production of pectinolytic enzymes of *M. phaseolina* when compared to control. The fungicide carbendazim (0.1%) recorded maximum inhibition. The plant products *A. marina (leaves, stem, and pneumatophore)*, and *Salicornia brachiata* inhibited the pectinolytic enzyme production of *M. phaseolina* in a decreasing order of merit (Table - 9).

Table - 1: Black gram root rot incidence by different isolates of *M. phaseolina* in Tamil Nadu

S.No	Name of the Districts	Isolates of *M.phaseolina*	Percent Disease Incidence
1.	Tiruvarur	Needamangalam	22.61(28.39)
		Mannarkudi	26.38(30.90)
2.	Thanjavur	TR&RI-Aduthurai	42.36(40.60)
		Papanasam	40.50(38.29)
3.	Cuddalore	FAC-AnnamalaiUniversity	22.39(21.24)
		KVK Pallur	38.20(39.69)
4.	Nagapattinam	Sirkali	38.26(38.21)
		Veddaranyam	28.37(26.18)
5.	Trichy	Musiri	31.63(30.22)
		Kulithalai	32.89(31.99)

Table - 2: Cultural characters of different isolates of *M. phaseolina*

S.No	Isolates of *M.phaseolina*	Color	Topography	Sclerotial size(µm)
1.	TR-1	Whitish grey	Aerial	<19 µm
2.	TR2	Greyish white	Aerial	<20 µm
3.	TH-3	White	Aerial	>25 µm
4.	TH-4	Grey	Aerial	>24 µm
5.	CU-5	Blackish white	Aerial	<20 µm
6.	CU-6	Whitish grey	Aerial	>23 µm
7.	NP-7	Whitish grey	Aerial	>22 µm
8.	NP-8	Black	Aerial	<18 µm
9.	TR-9	Greyish white	Aerial	Medium 22
10	TR-10	Black	Aerial	Medium 21

Table - 3: Effect of cold water extracts of selected plant products against **mycelial growth** of *M.phaseolina* , (solid medium)

Sources	% concentration/ Mycelial growth (mm)				% decrease over control			
	20	**40**	**60**	**80**	**20**	**40**	**60**	**80**
Avicennia marina(leaves)	0.00	0.00	0.00	0.00	100.00	100.00	100.00	100.00
A.marina(stem)	62.40	56.50	0.00	0.00	30.67	37.22	100.00	100.00
A.marina(Pneumatophores)	62.40	56.50	0.00	0.00	30.67	37.22	100.00	100.00
Salicornia brachiata	62.25	27.50	0.00	0.00	30.83	69.44	100.00	100.00
Carbendazim(0.1%)	-	-	-	-	-	-	-	-
Control	90.00	89.00	89.00	90.00	-	-	-	-

Table - 4: Effect of cold water extracts of selected plant products against mycelial dry weight of *M.phaseolina*, (liquid medium)

Sources	% of Concentraion/ Mycelial dry wt. (mg)				% decrease over control			
	20	40	60	80	20	40	60	80
Avicennia marina(leaves)	0.00	0.00	0.00	0.00	100.00	100.00	100	100
A.marina(stem)	200.50	170.00	0.00	0.00	62.87	68.52	100	100
A.marina(Pneumatophores)	150.50	0.00	0.00	0.00	72.13	100.00	100	100
Salicornia brachiata	200.00	75.00	0.00	0.00	62.96	86.11	100	100
Carbendazim(0.1%)	-	-	-	-	-	-	-	-
Control	540.00	538.00	539.00	537.00	-	-	-	-

Table - 5: Effect of hot water extracts of selected plant products against mycelial growth of *M. phaseolina* (solid medium)

Sources	% of Concentration/ Mycelial growth (mm)				% decrease over Control			
	20	40	60	80	20	40	60	80
Avicennia marina (leaves)	0.00	0.00	0.00	0.00	100.00	100.00	100.00	100.00
A.marina(stem)	75.00	63.25	50.25	38.25	16.67	29.72	44.17	57.50
A.marina (Pneumatophores)	70.50	60.25	40.00	25.50	21.67	33.05	55.56	71.67
Salicornia brachiata	73.25	61.50	45.20	30.25	18.61	31.67	49.78	66.69
Carbendazim(0.1%)	-	-	-	-	-	-	-	-
Control	89.00	90.00	90.00	90.00	-	-	-	-

Table - 6: Effect of hot water extracts of selected plant products against mycelial dry weight of *M.phaseolina,* (liquid medium)

Sources	% of Concentraion/ Mycelial dry wt. (mg)				% decrease over control			
	20	40	60	80	20	40	60	80
Avicennia marina(leaves)	0.00	0.00	0.00	0.00	100.00	100.00	100.00	100.00
A.marina(stem)	300.50	220.50	100.00	20.00	44.35	59.17	81.48	96.30
A.marina(Pneumatophores)	168.00	90.50	40.00	0.00	68.89	83.24	92.59	100.00
Salicornia brachiata	250.50	120.00	85.50	10.00	53.61	77.78	84.17	98.15
Carbendazim(0.1%)	-	-	-	-	-	-	-	-
Control	540.00	540.00	540.00	540.00	-	-	-	-

Table - 7: Effect of plant products on the conidial germination and sporulation of *M. phaseolina*

Sources	Conidial Germination and Percentage Concentration				Sporulation
	20	40	60	80	
Avicennia marina (leaves)	0	0	0	0	-
A.marina (stem)	48.50(44.14)	11.20(19.54)	0	0	-
A.marina (Pneumatophores)	42.75(40.82)	30.50(33.52)	0	0	-
Salicornia brachiata	60.25(50.91)	0	0	0	-
Carbendazim (0.1%)	-	-	-	-	-
Control	-	-	-	-	+++

(-) = no sporulation, (+++) = heavy sporulation

Table - 8: Effect of plant products on the *in vitro* production of cellulolytic enzymes by *M. phaseolina*

Sources	Concentration (%)	C. activity	Decrease over control percentage (%)	C. activity (percentage loss in viscocity)	Decrease over control (%)
Avicennia marina(leaves)	20	0.42	90.02	8.68(17.12)	87.86
A.marina(stem)	60	0.47	88.84	9.10(17.56)	87.27
A.marina(Pneumatophores)	60	0.44	89.55	8.85(17.33)	87.62
Salicornia brachiata	60	0.43	89.79	8.90(17.36)	87.55
Carbendazim(0.1%)	0.1	0.41	90.26	8.41(16.85)	88.24
Control	-	4.21	-	71.50(57.74)	-

Table - 9: Effect of plant products on the *in vitro* production of Pectinolytic enzymes by *M.phaseolina*

Sources	Concentration (%)	PG activity (% loss in velocity)	Decrease over control (%)	PTE activity(% loss in viscocity)	Decrease over control (%)	PMG activity(% loss in viscocity)	Decrease over control (%)
Avicennia marina(leaves)	20	8.70	87.82	7.7	89.98	7.82	88.83
A.marina(stem)	60	9.00	87.39	8.5	87.94	8.35	88.07
A.marina(Pneumatophores)	60	8.80	87.68	7.9	88.79	8.0	88.57
Salicornia brachiata	60	8.90	87.54	8.1	88.51	8.2	88.29
Carbendazim(0.1%)	0.1	8.10	88.66	7.6	89.22	7.75	88.93
Control	-	71.40	-	70.5	-	70.0	-

Discussion

Blackgram is being affected by a number of diseases caused by fungi, bacteria and viruses. Among them, root rot caused by *M. phaseolina* is a serious malady in major malady in blackgram growing areas. Indiscriminate use of synthetic chemicals has led to several problems like residual toxicity, environmental pollution and induced resistance in pathogens. So, pathologist are now being directed towards the use of eco-friendly technologies for disease management, among which biological method forms one of the important components.

Plant product appears to be a good reservoir of effective chemotherapeutant and would constitute an inexhaustible source of harmless pesticides. Application of plant products in plant disease management is emerging as an acceptable method and many workers have used plant products against several pathogenic fungi (Natarajan and Lalithakumar, 1987; Mohan and Ramakrishnan, 1991; Babu *et al*., 2000). In the present study *A.marina* and *S.brachiata* and its plant parts were screened for their fungitoxicity, if any against *M.phaseolina.*

Morphological characters of *M. phaseolina* such as color of the mycelium, topography and *Scelerotial* production were studied and they were in agreement with those as described by (Nakkeeran *et al.*, 1992). The difference in inhibitory effect of various plant products may be due to qualitative and quantitative differences in the antifungal principles present in them. The presence of antibiotic like substances in plant products was reported by Nene and Thapliyal (1965), Blakeman and Atkinson (1979) and Adeleye and Ikolin (1989). There are several reports on the antifungal activity of *A. sativum*, *E. globulus*, *P. juliflora* and *A. sativum* bulb extract inhibited the growth of *Fusarium oxysporum* f. sp. *Lycopersici* (Tariq and Magee, 1990; Raja, 1995); *F. solani* f. sp. *phaseoli* (Russel and Mussa, 1977); *Venturia inequalis* (Gilliver, 1947); *F. moniliforme* (Gohil and Vala, 1996); *Macrophomina phaseolina* (Rani, 1999) and *C. capsici* (Krishnakumar, 2002) etc. Singh *et al*. (1997) reported that extract of *A. sativum* inhibited

the growth and spore germination of *C. capsici*. The extracts of *E. globulus* were effective against *Rhizoctonia solani* (Ezhilan, 1998) and *Pythium phanidermatum* (Thiruvudainambi, 1993). Leaf extract of *S. toruvum, D. metel* and *P. juliflora* were found to inhibit the mycelial growth of *C. capsici* (Gomathi and Kannabiran, 2000). Sunderraj *et al*. (1996) recorded inhibition of mycelial growth of *R. solani* by *A. sativum* and *P. juliflora* at 10% concentration.

Dubey (1981) stated that effectively of plant extracts depend upon the nature and amount of active principle contained. Stoll and Seebeck (1951) reported that *A. sativum* bulb contained allicin (dialkyl thiosulphate) which was responsible for the fungi toxicity. Allicin, a major constituent of *A. sativum* containing sulphur showed strong toxic properties against several bacterial and fungi (Skinner, 1955). El-Sayeed *et al*. (1985) found that steroidal alkaloids like saponins in *Eucalyptus* sp. were responsible for its toxicity.

Among the cold and hot water extracts cold water extracts were found to be superior over hot water extracts. The slight loss in inhibitory effect of plant extracts might be due to its inability to withstand the increase in temperature. All treatments significantly inhibited the sporulation and conidial germination of *M.phaseolina* when compared to control. Carbendazim 0.1% concentration completely inhibited the sporulation and conidial germination.

Gomathi and Kannabiran (2000) found that leaf extracts of *Solanum torvum, D. metel* and *P. juliflora* effectively inhibited the conidial germination of *C. capsici* and *Gloeosporum piperatum*. Mathan *et al*. (2008) reported that reduction in spore germination and growth rate of the test fungi due to treatment of natural of product may be due to the presence of inhibitory substances. These are in agreement with our findings.

All the treatments significantly reduced the production of both the enzymes of *M.phaseolina* when compared to control. Umalkar *et al*. (1976) reported that the inhibitory effect of extracts of *Acacia nilotica* on the enzyme production of some pathogenic fungi may be due to phenolic compound present in them. Aqueous extracts of garlic bulb inhibited the cellulolytic and pectinolytic enzyme activity of *P. aphanidermatum, Fusarium oxysporum* f. sp. *lycopersici* and *M. phaseolina* (Raja, 1995). The enzymes of *C. capsici* were inhibited by *Datura metel* leaf extract (Asha and Kannabiran, 2001). These findings lend support to the present study. The inhibitory effect of *D. metel* leaf extract might be due to the phenolic compound or some other compounds present in them (Umalkar *et al*., 1976).

The present investigation clearly indicated that foliar application of four selected plant products as well as fungicide carbendazim at 0.1% concentration checked the fruit rot disease incidence significantly. The use of plant products as foliar spray in disease management were made by several researchers (Salama *et al*., 1988; Singh *et al*., 1990;Babu *et al*., 2000).

In the present study the results obtained from the invitro studies conducted for indicated that among the plant products, *A. marina (leaves, stem and pneumatophore)* and *S.brachiata* have the potential efficiency in the protection of blackgram plant against *M.phaseolina*. The future of using plant products is promising because they are less expensive and less hazardous to the environment.

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