Plant Pathology Journal

ISSN 1812-5387

science alert

ANSInet
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http://ansinet.com

Plant Pathology Journal 13 (2): 87-99, 2014
ISSN 1812-5387 / DOI: 10.3923/ppj.2014.87.99

Antifungal Activity of Plant Products for the Management of Fruit Rot Infection in Chillies

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Abstract: The efficacy of 13 plant products were evaluated against chilli fruit rot and dieback incited by *Colletotrichum capsici*. Among them bulbs of *A. sativum* (20%) and *A. cepa* (60%) and seed extract *A. indica* 60% gave complete inhibition of the pathogen and were on par with carbendazim (0.1%) leaf extracts of *D. metel*, *E. globulus* and *P. juliflora* at 60% concentration totally inhibited the mycelial growth of *C. capsici*. Conidial germination of *C. capsici* was completely inhibited by *A. sativum* 20%, *E. globulus*, *D. metel* 60% and *P. juliflora* 60%. Plant products at their respective MIC's totally inhibited the sporulation of *C. capsici*. Production of cellulolytic and pectinolytic enzymes the pathogen was also inhibited maximum with fungicide carbendazim (0.1%), followed by *A. sativum* and *E. globulus*. The same treatments recorded maximum seed germination (17.74%), seedling vigour and maximum control of disease incidence. Pots treated with *A. sativum* (20% conc.) recorded higher yield and fruit characters such as increased fruit length (43.53 cm) and fruit weight (36.19 g).

Key words: Plant products, *A. sativum*, *E. globulus*, *P. juliflora*, disease incidence, *Colletotrichum capsici*

INTRODUCTION

Chilli (*Capsicum annum* L.) is considered an important tropical and subtropical crop on the basis of high consumption, nutritional and cash values to the farmers and consumers both in developing and developed countries. Among them, India is the largest consumer and exporter of chillies in the international market and exports dry chilli, chilli powder and oleoresins to over 90 countries (Singhal, 1999). It is grown in several parts of India. Although, India has a layer area, its productivity is low when compared to other countries. The prominent reason is due to the existence of dieback of fruit rot incited by *Colletotrichum capsici* a serious menance in chilli cultivation. 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 cast 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).

In the light of the above experiments were planned and conducted to study the effect of different plant products as source of antifungal principle against *C. capsici*.

MATERIALS AND METHODS

Plant material: Chilli variety K1 (susceptible to fruit rot and dieback) was used for pot culture studies in the entire period of investigation.

Isolation and maintenance of the pathogen: *C. capsici* was isolated from infected chilli seeds collected from orchard, Faculty of Agriculture, Annamalai University. The seed were collected from infected fruit were surface sterilized with 0.1% sodium hypochlorite solution for 3 min and washed with three changes of sterile water. The seeds were placed in petridishes containing PDA medium and incubated at 25±28°C for seven days. The fungus was subsequently purified by single hyphal tip method (Ainsworth, 1961). The pure culture was obtained and preserved in refrigerator and subcultured once in a month.

Identification of the pathogen: The morphological characters of *C. capsici* such as acevulus, setae and conidia were studied. These characters were compared with that of *C. capsici* described earlier by Singh *et al*. (1973), Ahmed (1982) and Singh (1995).

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Table 1: Plant species used in the study

Scientific name	Common name	Family
Bulb		
Allium sativum	Garlic	Liliaceae
Allium cepa var. *aggregatum* L.	Onion	Liliaceae
Seed		
Azadirachta indica A. Juss.	Neem	Meliaceae
Zingiber officinale L.	Ginger	Zingiberaceae
Leaves		
Datura metel	Umathi	Solanaceae
Pongamia glabra Vent.	Pongam	Papillionaceae
Lawsonia inermis L.	Henna/Maruthani	Lythraceae
Vitex negundo	Nochi	Verbanaceae
Prosophis juliflora	Velikaruvai	Leguminosae
Acalypha indica	Kuppaimeni	Euphorbiaceae
Tectona grandis	Velikaruvai	Leguminosa
Eucalyptus globulus Labill	Eucalyptus	Euphorbiaceae
Bougainvillea spectabilis	Bougainvilla	Nyctaginacae

Plant products used for screening against *C. capsici*: Thirteen plant products were evaluated for their antifungal effect. The selected plant products were taken for further studies. The plant products used for screening are given in Table 1. Carbendazim 50 WP at 0.1% concentration was used as a standard fungicide against *C. capsici* for comparison.

Preparation of plant extracts

Water extract: For the preparation of plant extracts (bulbs, leaf, rhizome, seed) 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 *C. capsici*:** 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 plate^{-1}. 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 *C. capsici* 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 germintaion of conidia of *C. capsici

Cavity slide method: Antifungal activity of aqueous extracts of plant products on spore germination of *C. capsici* 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* evlauation of antifungal activity of plant products on the sporulation of *C. capsici*:** Antifungal activity of plant products on the sporulation of *C. capsici* 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 plate^{-1}. 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 storage on the fungitoxic nature of selected plant products on growth of *C. capsici*: The aqueous extracts of plant products were taken in sterilized bottles and stored at room temperature for 9 days. One set of extracts was kept in closed bottles and another set in open bottles. The antifungal activity of extracts was tested at 1, 3, 5, 7 and 9th day of storage.

Effect of plant products on the *in vitro* production of cellulolytic and pectinolytic enzymes of *C. capsici*

Preparation of enzyme source: Czapeak'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 flakshs. 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 were 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 C_1 (Norkrans, 1950): The reactor 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 (C_x) (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:

T_0 = Flow time at zero time (sec)
T_1 = Flow time at one interval (sec)
T_2 = 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 mililitter 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 transeliminase (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.

Polygalacturonase-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.

Effect of plant products on the *in vitro* production of toxin by *C. capsici*: Czapek's broth was prepared, distributed in 40 and 20 mL quantities in 250 mL Erlenmeyer flask and autoclaved. The extracts were added separately at the rate of 10 and 30 mL so as to get the final concentration of 20 and 60. Carbendazim at 0.1% concentration used for comparison. Broth without any extract served as control. Three replications were maintained. The flasks were

inoculated each with nine mm culture disc and incubated for 15 days at room temperature (28±1°C). After the incubation period was over the culture filtrates were filtered through Whatman No. 1 filter paper and filtrate was retained for assay.

Seed germination bioassay: Seed germination inhibition of toxin of *C. capsici* was studied by following Ludwig (1957) method. Seeds of paddy, blackgram and sorghum were used in the study.

The seeds were first soaked in sterile distilled water, surface sterilized with 0.1% mercuric chloride and washed repeatedly with sterile distilled water. The seeds were soaked separately in culture filtrates at respective concentration for 6 h. Twenty seeds were than transferred to sterile Petri plates lined with sterile blotting paper into which 5 mL of the respective culture filtrates were added. Petri dishes with sterile water served as control. After three days of incubation the seed germination was recorded.

Effect of seed treatment with individual plant products on seed germination, growth and vigour of chilli seedlings: Water extracts of selected plant products at 20 and 60% concentration were prepared as described earlier. Chilli (var K1) seeds were soaked in their for six hours. Seeds soaked in distilled water served as control. Carbendazim 0.1% concentration was employed as standard seed treatment fungicide for comparison. By following roll towel method (ISTA, 1976) the seed germination, seedling growth and vigour were observed.

Roll towel method (ISTA, 1976): The germination paper used was soaked in water for 2 to 4 h to moist in evenly and to remove water soluble toxic substances of present. The treated seeds were placed equidistantly between two sheets of paper towel (27×20 cm) rolled carefully ensuring no pressure on seed, wrapped in polythene sheet to reduce surface evaporation and kept in germination chamber an upright position. Each treatment was replicated thrice. They where incubated at room temperature for 7 days and the following observation were made.

Evaluation of plant products against fruit rot of chilli caused by *C. capsici*

Pot culture trial: The pot culture experiment was carried out in the glass house at Department of Plant Pathology, Faculty of Agriculture, Annamalai University. The pot culture experiment consisted of 6 treatments. Each treatment was replicated thrice. The details of the pot culture experiment are given below:

T_1 = *Allium sativum* 20%
T_2 = *Datura metel* 20%
T_3 = *Eucalyptus globulus* 60%
T_4 = *Prosophis juliflora* 60%
T_5 = Carbendazim 0.1%
T_6 = Control

Variety: Certified seeds of K1 were collected from Regional Research Station, Kovilpati are used.

Characters of K1:

- A pure line selection from an Assam type chilli B 72A
- Tall and compact plant with long and shiny red fruits
- The crop duration is 210 days

Seedling: The raised nursery bed with 2 m breadth and 5 m length was prepared. The seeds were sown in line. The nursery was watered regularly. Fifty five days old seedlings were transplanted to pots. Irrigation and fertilization were done as per schedule.

Preparation of pathogen inoculum: The spore suspension of the isolate was prepared in sterile distilled water from 10 days old culture grown on Czepek's agar slants. The spore suspensions were diluted with sterile distilled water in order to contain approximately 2000 to 3000 spores mL^{-1}.

Inoculation of plants: Chilli plants 105 days old were used for inoculation. The plants were sprayed with sterile water before inoculation. The conidial suspension was sprayed over plant parts by using an atomizer early in the late evening. Control plants were sprayed with sterile distilled water.

Spraying of plant products: First spraying was done 10 days after inoculation and subsequent spraying done at 15 days intervals. The disease intensity was assessed on seventh day after each spraying. Carbendazim at 0.1% concentration was used for comparison.

Observation: The following observation were taken:

- Percent disease incidence
- Yield pot^{-1}
- Fruit length
- Fruit weight

Disease index: The fruits were grouped into '0' to '4' grade based on the scale followed by Bansal and Grover (1969):

Grade	Percent disease infection
0	No disease
1	1-5% disease
2	6-25% disease
3	26-50% disease
4	51-100% disease

$$PDI = \frac{\text{Sum of individual ratings}}{\text{No. of fruits assessed}} \times \frac{100}{\text{Maximum disease grade}}$$

Yield: The ripe fruits of chillies were harvested on 150th, 160th and 170th days after transplanting. The weight of fresh fruits was recorded and the yield was expressed in g pot^{-1}. The effect of plant products on fruit length and fruit weight was also studied.

RESULTS

Isolation of *Colletotrichum capsici*: The pathogen was isolated from diseased fruits of chilli and identified as *C. capsici* on the basis of morphological studies. The pathogen was purified and maintained in pure culture on PDA slants.

***Acervulus*:** *Acervulus* was circular to saucer shaped with dark brown to black setae. The acervulus was 59-115 μm in diameter.

***Setae*:** *Setae* were dark brown to black in colour erect and tapering towards the apex. Length of setae measure 70.1-180.6 μm.

Conidia: Conidia were sickle shaped, single celled, hyaline, smooth walked with a central oil globule. Width of conidia measured 16.8×3.6 μm.

Study of pathogenicity: Pathogenicity of the fungus was proved by inoculation of the culture to red healthy fruits of chilli. Symptoms developed within 16 days of inoculation. The presence of typical small circular spots enlarged in long axis of fruits and in these lesions numerous black acervuli which looked like pin heads were found in concentric rings.

Effect of cold water extracts of plant products on the mycelial growth of *C. capsici*: Water extracts of 13 plant species at various concentrations viz., 10, 20, 40, 60 and 80% on the mycelial growth of *C. capsici* showed a complete inhibition of mycelial growth of *C. capsici* was observed with carbendazim at 0.1% concentration which showed 100% reduction over control. Among the various plant species tested bulb extracts of *A. sativum* at 20% and *A. cepa* at 60% concentration totally inhibited the mycelial growth of *C. capsici* and was on par with carbendazim. Seed extract of *Azadirachta indica* at 60% concentration also totally inhibited the mycelial growth of *C. capsici*.

In case of leaf extracts *Datura melel*, *Eucalyptus globulus* and *Prosoptis juliflora* at 60%, *Acalypha indica*, *Vitex negundo* and *Tectona grandis* at 80% concentration totally inhibited the mycelial growth of *C. capsici* and were on par with carbendazim.

The extracts of *Bougainvilla spectabilis*, *Lawsonia inermis*, *Pongamia glabra* and *Zingiber officinale* were not at all effective against the test fungus (Table 2).

Effect of hot water extracts of selected plant products on mycelial growth of *C. capsici*: The effect of hot water extract of four selected plant products at 10, 20, 40, 60 and 80% concentration and of the fungicide carbendazim at

Table 2: Screening of cold water extracts of selected plant products against mycelial growth of *Colletotrichum capsici* (Solid medium)

	*Mycelial growth and percentage concentration (mm)					Decrease over control and concentration percentage				
Sources	10	20	40	60	80	10	20	40	60	80
Allium sativum	50.60	0.00	0.00	0.00	0.00	43.78	100.00	100.00	100.00	100.00
Allium cepa	75.30	62.40	56.50	0.00	0.00	16.33	30.67	37.22	100.00	100.00
Azadirachta indica	50.00	40.75	24.50	0.00	0.00	44.44	54.72	72.78	100.00	100.00
Acalypha indica	81.50	77.40	72.00	68.50	0.00	9.44	14.00	20.00	23.89	100.00
Bougainvillea spectabilis	85.50	80.00	77.00	65.00	51.00	5.00	11.11	14.44	27.78	43.33
Datura metel	62.20	48.50	35.40	0.00	0.00	30.89	46.11	60.67	100.00	100.00
Eucalyptus globulus	75.30	44.50	25.00	0.00	0.00	16.33	50.56	72.22	100.00	100.00
Lawsonia inermis	81.00	76.51	65.00	51.25	40.00	10.00	14.99	27.78	43.05	55.56
Pongamia glabra	81.00	76.00	68.00	56.00	43.00	9.44	15.56	24.44	37.78	52.20
Prosophis juliflora	80.00	62.25	27.50	0.00	0.00	11.11	30.83	69.44	100.00	100.00
Vitex negundo	72.00	70.00	60.50	45.00	0.00	20.00	22.22	32.78	50.00	100.00
Tectona grandis	85.00	83.20	78.00	40.25	0.00	5.56	7.56	13.33	55.28	100.00
Zingiber officinale	85.50	80.00	71.00	69.00	55.25	5.00	11.11	21.11	23.33	38.61
Carbendazim (0.1%)	0.00	-	-	-	-	100.00	-	-	-	-
Control	90.00	90.00	90.00	90.00	90.00	-	-	-	-	-

*Mean of three replications factors S.E.C.D. (p = 0.05), Main treatment: 1.329, 3.852, Sub treatment: 0.643, 1.782, MT×ST: 2.490, 6.901, ST×MT: 2.593, 7.275

Table 3: Effect of hot water extracts of selected plant products on the mycelial growth of *C. capsici*

	*Mycelial growth and percentage concentration (mm)					Decrease over control and percentage concentration				
Sources	10	20	40	60	80	10	20	40	60	80
Allium sativum	60.5	0.00	0.00	0.00	0.00	32.78	100.00	100.00	100.00	100.00
Datura metel	88.0	75.00	63.25	50.25	38.25	2.22	16.67	29.72	44.17	57.50
Eucalyptus globulus	86.5	70.50	60.25	40.00	25.50	3.89	21.67	33.05	55.56	71.67
Prosophis juliflora	88.0	73.25	61.50	45.20	30.25	2.22	18.61	31.67	49.78	66.69
Carbendazim (0.1 %)	0.0	-	-	-	-	100.00	-	-	-	-
Control	90.0	90.00	90.00	90.00	90.00	-	-	-	-	-

*Mean of three replications factors S.E.C.D. (p = 0.05), Main treatment: 0.1800.567, Sub treatment: 0.1270.362, MT×ST: 0.3120.886, ST×MT: 0.3320.974

Table 4: Effect of cold water extracts of selected plant products on the mycelial dry weight of *C. capsici* (Liquid medium)

	*Mycelial growth and percentage concentration (mm)					Decrease over control and percentage concentration				
Sources	10	20	40	60	80	10	20	40	60	80
Allium sativum	47.00	0.00	0.0	0	0.00	91.30	100.00	100.00	100	100
Datura metel	350.00	200.50	170.0	0	0.00	35.19	62.87	68.52	100	100
Eucalyptus globulus	180.50	150.50	0.0	0	0.00	66.57	72.13	100.00	100	100
Prosophis juliflora	240.00	200.00	75.0	0	0.00	55.56	62.96	86.11	100	100
Carbendazim (0.1 %)	0.00	-	-	-	-	100.00	-	-	-	-
Control	540.00	540	540.0	540	54.00	-	-	-	-	-

*Mean of three replications factors S.E.C.D. (p = 0.05), Main treatment: 0.4941.558, Sub treatment: 0.3871.101, MT×ST: 0.9492.697, ST×MT: 0.9822.868

Table 5: Effect of hot water extracts of selected plant products on the mycelial dry weight of *C. capsici* (Liquid medium)

	*Mycelial growth and percentage concentration (mm)					Decrease over control and percentage concentration				
Sources	10	20	40	60	80	10	20	40	60	80
Allium sativum	90.50	0.00	0.00	0.00	0.00	85.09	100.00	100.00	100.00	100.00
Datura metel	400.00	300.50	220.50	100.00	20.00	25.93	44.35	59.17	81.48	96.30
Eucalyptus globulus	205.00	168.00	90.50	40.00	0.00	62.04	68.89	83.24	92.59	100.00
Prosophis juliflora	300.00	250.50	120.00	85.50	10.00	44.44	53.61	77.78	84.17	98.15
Carbendazim (0.1 %)	0.00	-	-	-	-	100.00	-	-	-	-
Control	540.00	540	540	540	54.00	-	-	-	-	-

*Mean of three replications factors S.E.C.D. (p = 0.05), Main treatment: 0.7122.247, Sub treatment: 0.6551.863, MT×ST: 1.6051.863, ST×MT: 1.6034.654

Table 6: Effect of plant products on the conidial germination and sporulation of *C. capsici*

	Conidial germination and percentage concentration					
Sources	10	20	40	60	80	Sporulation
Allium sativum	15.51 (23.20)	0	0	0	0	-
Datura metel	80.0 (63.49)	48.50 (44.14)	11.20 (19.54)	0	0	-
Eucalyptus globulus	72.50 (58.37)	42.75 (40.82)	0	0	0	-
Prosophis juliflora	85.70 (67.78)	60.25 (50.91)	30.50 (33.52)	0	0	-
Carbendazim (0.1 %)	0	-	-	-	-	-
Control	97.56 (81.17)	-	-	-	-	+++

Figures in parentheses are arcsine transformed values. -: No sporulation , +++: Heavy sporulation, factors S.E.C.D. (p = 0.05), Main treatment: 0.2520.797, Sub treatment: 0.2440.693, MT×ST: 0.5971.698, ST×MT: 0.5911.713

0.1% concentration on the mycelial growth of *C. capsici* (Table 3) revealed that carbendazim (0.1%) recorded complete inhibitory effect against *C. capsici.* Among the four plant products tested, *A. sativum* bulk extract (20%) alone was found to retain its total inhibitory effect while other extracts showed greater loss of toxicity when extracted with hot water of selected plant products.

Effect of cold water extracts on the mycelial dry weight of *C. capsici*: *A. sativum* at 20% concentration and *E. globules* at 80% 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).

Effect of hot water extracts of plant prodcuts on the mycelial dry weight of *C. capsici*: Among the plant products *A. sativum* at 20% concentration and *E. globules* at 80% concentration recorded the complete inhibition and they were on par with carbendazim at 0.1% (Table 5). When compared to control, all the treatments gave appreciable reduction in mycelial dry weight of the fungus.

Effect of plant products on the conidial germination and sporulation of *C. capsici*: *A. sativum* bulb extract at 20% concentration completely inhibited the spore germination while *E. globulus* needed 40% concentration for complete inhibition of spore germination of *C. capsici* (Table 6). *D. metel* and *P. juliflora* at 60%

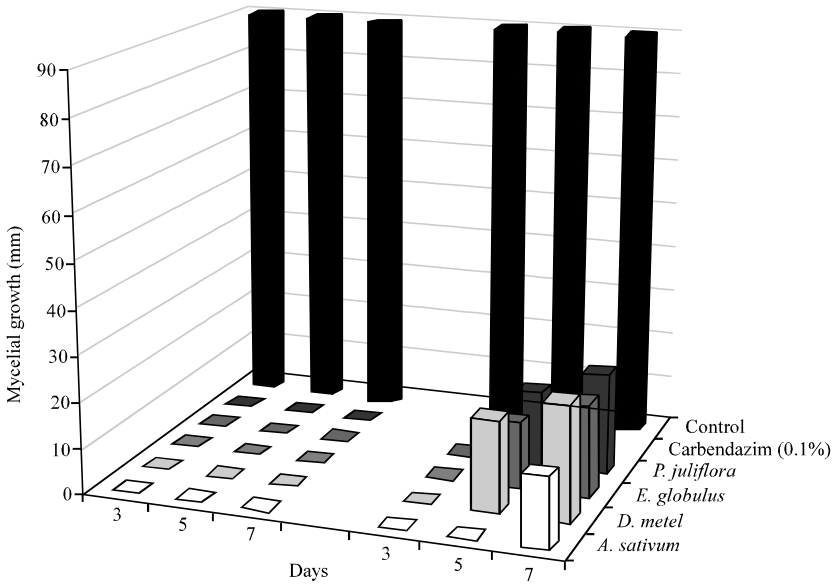

Fig. 1: Effect of storage on the fungitoxic nature of selected plant products on the mycelial growth of *C. capsici*

Table 7: Effect of plant products on the *in vitro* production of cellulolytic enzymes by *C. capsici*

Sources	Concentration (%)	C_1 activity	Decrease over control (%)	Cx activity (% loss in viscosity)	Decrease over control (%)
Allium sativum	20	0.42	90.02	8.68 (17.12)	87.86
Datura metel	60	0.47	88.84	9.10 (17.56)	87.27
Eucalyptus globulus	60	0.44	89.55	8.85 (17.33)	87.62
Prosophis juliflora	60	0.43	89.79	8.90(17.36)	87.55
Carbendazim (0.1 %)	0.1	0.41	90.26	8.41 (16.85)	8824
Control	-	4.21	-	71.50 (57.74)	
S.E.	-	0.008	-	0.215	-
CD (p=0.05)	-	0.0023	-	0.613	-

C_1: 1 unit 0.01 absorbance, C_x: % loss in viscosity, Readings taken at 0th and 120th min, Figures in parentheses are arcsine transformed values

Table 8: Effect of plant products on the *in vitro* production of pectinolytic enzymes by *C. capsici*

Sources	Concentration (%)	PG activity (% loss in velocity)	Decrease over control (%)	PTE activity (% loss in viscosity)	Decrease over control (%)	PMG activity (% loss in viscosity)	Decrease over control (%)
Allium sativum	20	8.70 (17.16)	87.82	7.7 (16.11)	89.98	7.82 (16.22)	88.83
Datura metel	60	9.0 (17.46)	87.39	8.5 (16.95)	87.94	8.35 (16.81)	88.07
Eucalyptus globulus	60	8.8 (17.26)	87.68	7.9 (16.32)	88.79	8.0 (16.43)	88.57
Prosophis juliflora	60	8.9 (17.36)	87.54	8.1 (16.53)	88.51	8.2 (16.43)	88.29
Carbendazim (0.1 %)	0.1	8.1 (16.39)	88.66	7.6 (16.00)	89.22	7.75 (16.18)	88.93
Control	-	71.40 (57.67)	-	70.5 (57.10)	-	70.0 (56.79)	-
S.Ed.	-	0.056	-	0.065	-	0.042	-
CD (p = 0.05)	-	0.160	-	0.184	-	0.120	-

Figures in parentheses are arcsine transformed values

concentration completely inhibited the spore germination of *C. capsici* they were on par with carbendazim. All treatments differed significantly over control. *A. sativum* at 20%, *E. globulus* at 40%, *D. metel* and *P. juliflora* at 60% and carbendazim at 0.1% concentration completely inhibited the sporulation of *C. capsici* when compared to control.

Effect of storage on fungitoxic nature of plant products on the mycelial growth of *C. capsici*: None of the plant products retained the inhibitory effect for 9 days of storage. In lid closed condition, all the extracts retained its toxicity for 7 days only, thereafter the toxicity decreased. In lid opened containers *D. metel*, *E. globules* and *P. juliflora* were found to start losing its toxicity from fifty day of storage while in case of *A. sativum* extract the toxicity was found to start losing its toxicity from seventh day of storage (Fig. 1).

Effect of plant products on the production of cellulolytic and pectinolytic enzymes by *C. capsici*: All the treatments significantly reduced the production of cellulolytic and pectinolytic enzymes of *C. capsici* when compared to control. The fungicide carbendazim (0.1%) recorded maximum inhibition (Table 7 and 8). Among the plant products *A. sativum*, *E. globulus*, *P. juliflora* and *D. metel*

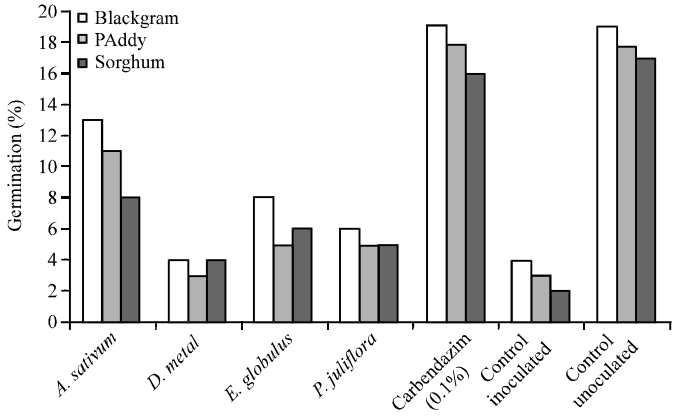

Fig. 2: Effect of toxin production *C. capsici* on seed germination as influenced by plant products

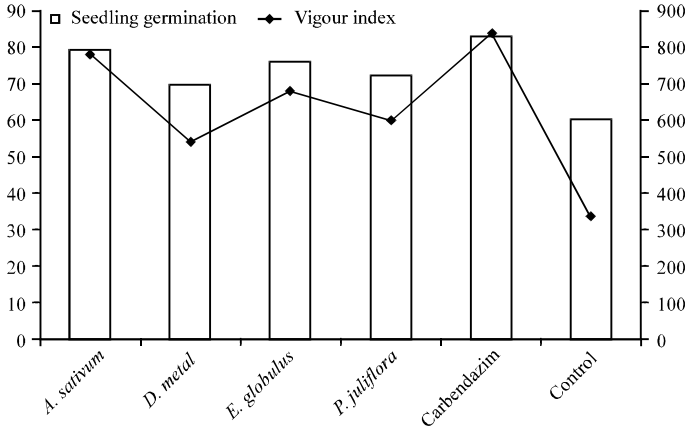

Fig. 3: Effect of plant products on the seed germination and growth of chilli

inhibited the cellulolytic and pectinolytic enzyme production of *C. capsici* in a decreasing order of merit.

Effect of toxin production of *C. capsici* on seed germination (inhibition assay) as influenced by plant products: The culture filtrates of all treatments and control affected, the seed germination of all seeds tested (Fig. 2). Good seed germination was recorded with carbendazim (0.1%) and uninoculated control, follwed by *A. sativum* bulb extract and *E. globulus* extract.

Effect of plant products on the seed germination and growth of seedling of chilli: All the treatments showed stimulatory effect on seed germination and growth of seedlings when compared to control. Among the various plant products tested *A. sativum* followed by *E. globulus* showed maximum seed germination and seedling growth. Highest vigour index was observed in fungicide carbendazim (0.1%) (Fig. 3).

Effect of cold water extract of selected plant products on the incidence of chilli fruit rot under pot culture condition: The results indicated that carbendazim (0.1%) recorded the minimum disease incidence (17.74%). Among the plant products tested, *A. sativum* recorded minimum disease incidence (24.07%) followed by *E. globulus* (27.84%) and *A. sativum* recorded the highest yield with 51.09% increase over control which was on par with carbendazim at 0.1% (53.31%) increase in yield over control (Table 9).

Effect of plant products and the incidence of chilli fruit rot on the yield parameters of chilli under pot culture condition: Among the various plant products tested *A. sativum* extract recorded increased fruit length and fruit weight by 43.53 and 36.19%, respectively over control and was on par with carbendazim at 0.1% concentration (Table 10).

Table 9: Effect of plant products on the incidence of fruit rot of chilli under pot culture conditions

Sources	Concentration (%)	Disease incidence (%)	Decrease over control (%)	Fruit yield (g pot^{-1})	Percent increase over control
Allium sativum	20	24.07 (29.37)	50.29	208.470	51.79
Datura metel	60	30.50 (33.52)	37.00	182.250	44.86
Eucalyptus globulus	60	27.84 (31.84)	42.50	190.500	47.24
Prosophis juliflora	60	29.92 (33.17)	38.21	185.710	45.88
Carbendazim (0.1 %)	0.1	17.74 (24.90)	-	100.500	-
Control	-	48.42 (44.10)	-	100.500	-
SE	-	0.203	-	0.587	-
CD (p = 0.05)	-	0.578	-	1.668	-

Figures in parentheses are arcsine transformed values

Table 10: Effect of plant products on the incidence of fruit rot on the yield parameters of chilli under pot culture conditions

Sources	Concentration (%)	Disease incidence (%)	Decrease over control (%)	Fruit yield (g pot^{-1})	Percent increase over control
Allium sativum	20	8.600	44.18	139.000	36.19
Datura metel	60	7.900	39.24	126.500	29.88
Eucalyptus globulus	60	8.350	42.52	132.000	32.80
Prosophis juliflora	60	8.100	40.74	128.500	30.97
Carbendazim (0.1 %)	0.1	8.650	44.50	140.750	36.98
Control	-	4.800	-	88.700	-
SE	-	0.030	-	0.347	-
CD (p = 0.05)	-	0.084	-	0.988	-

DISCUSSION

Chilli cultivation is being affected by a number of diseases caused by fungi, bacteria and viruses. Among them, fruit rot caused by *C. capsici* is a serious malady in major chilli 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 13 plant species were screened for their fungitoxicity, if any against *C. capsici* the incitant of fruit rot of chilli.

Morphological characters of *C. capsici* such as *Acervulus*, *Setae* and *Conidia* were sickle shaped and hyaline with a central oil globule. The shape and size of all these characters are in agreement with those described by Singh *et al*. (1973), Ahmed (1982) and Singh (1995).

Pathogenicity of the fungus was proved by artificial inoculation of the pure culture to the red fruits of chilli. Typical small circular spots enlarged in long axis of the fruits with black acerouli pin like heads in concentric rings were observed. These symptoms are in agreement with Chowdhery (1957) and Kannan *et al*. (1998).

Among the plant species tested, the cold water extracts of bulbs of *A. sativum* (20%) and *A. cepa* (60%) recorded complete inhibition of mycelial growth of *C. capsici*. Seed extract of *A. indica* at (60%) concentration also recorded complete inhibition of mycelial growth.

Among the leaf extracts, cold water extracts of *D. metel*, *E. globulus*, *P. juliflora* at 60% concentration and *A. indica*, *V. negundo* and *T. grandis* at 80% concentration totally inhibited the mycelial growth of *C. capsici*. The most effective and easily available four plant products viz., *A. sativum*, *E. globulus*, *P. juliflora* and *D. metel* were selected for further studies.

In case of hot water extracts of selected plant products *A. sativum* recorded complete inhibition of mycelial growth at 20% concentration thus proving thermostability. But for other treatments there was considerable loss of toxicity upon hot water extraction. Among the four selected plant products, the cold water extract of *A. sativum* (20%) recorded complete inhibition of mycelial dry weight. From the present findings, it can be concluded that among the13 plant products screened four had no effect on the test fungus. Remaining nine were found to inhibite the mycelial growth to varying degrees. 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 aphanidermatum* (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 fungitoxicity. 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 *C. capsici* when compared to control. Carbendazim 0.1% concentration completely inhibited the sporulation and conidial germination.

A. sativum extract at 20% concentration and *E. globulus* at 40% concentration completely inhibited the conidial germination *D. metel* and *P. juliflora* at 60% concentration completely inhibited the spore germination of *C. capsici*. All the treatments recorded complete inhibition of sporulation of various pathogens (Gillver, 1947; Misra and Dixit, 1979; Jaganathan and Narsimhan, 1988; Wilson *et al.*, 1997). Singh *et al.* (1997) observed that extracts from bulbs of garlic and onion and leaves of *A. indica*, *P. glabra* and *T. erecta* showed complete inhibition of *C. capsici* spore 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 plant products retained their toxicity upto 7 days of storage. Kurucheve *et al.* (1997) reported that in *C. pulcherrimma* inhibitory activity was not lost even after 9 days of storage. Moore and Atkins (1977) observed slight decomposition under storage condition affects the fungicidal effect might be due to the transformation of active compound to inactive a compounds by reaction with atmosphere (Lapis and Dumancas, 1978).

All the treatments significantly reduced the production of both the enzymes of *C. capsici* 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* was 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).

Culture filtrates from all the treatments affected the seed germination of all seeds tested, good seed germination was recorded with carbendazim (0.1%) and uninoculated control. There was remarkable inhibition of seed germination in seeds treated with culture filtrate from control flasks also. This showed that toxin produced by anthracnose fungus was not host specific. Goodman (1959) demonstrated that the toxin produced by *C. fuscum* was non-specific.

Toxins play a major role in the physiology of pathogenesis of all major groups of plant pathogens. The culture filtrate of *C. capsici* inhibited the germination of chilli seeds (Narain and Das, 1970). The suppression of the growth of chilli seedlings might be due to the specific toxin released by the pathogen in the culture media (Jeyalakshmi and Seetharaman, 1998).

All the treatments showed stimulatory effect on the seed germination and growth of seedlings when compared to control. Fungicide carbendazim (0.1%) showed the maximum seed germination and growth of seedlings. Among the various plant products tested, *A. sativum* followed by *E. globulus* showed maximum seed germination and seedling growth. Eswaramoorthi *et al.* (1988) observed stimulatory when paddy seeds were soaked with Pudhina extract. Seeds soaked in *A. sativum*

bulb extract increased the germination, shoot length, root length and vigour of various seedlings (Padmavathi, 1994; Sunderraj *et al*., 1996; Raja, 1995; Sivaprakash, 1998; Santhoshkumar, 2000; Krishnakumar, 2002) and is line with our findings.

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 pot culture experiment conducted for fruit rot incidence indicated that among the plant products, *A. sativum*, *D. metel*, *P. juliflora* and *E. globulu* have the potential efficiency in the protection of chilli plant against *C. capsici*. The future of using plant products is promising because they are less expensive and less hazardous to the environment.

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