

Effect of Combined Application of *Polygala elata* extract and *Eucalyptus obliqua* Oil on Chilli (*Capsicum annuum* L.) Fruit Rot Disease Incidence

S. Rajamanickam* and K. Sethuraman

Department of Plant Pathology, Tamil Nadu Agricultural University, Madurai - 625 104

Antifungal activity of plant extracts from forty different plant species and essential oils from five plants belonging to diverse taxonomic group were ascertained *in vitro* through mycelial growth and spore germination assays against *Colletotrichum capsici* isolated from anthracnose of chilli (*Capsicum annuum* L.). Poisoned food technique was performed to investigate antifungal effect of plant extracts and essential oil *in vitro*. The extract of *Polygala elata* and essential oil from *Eucalyptus oblique* arrested growth and sporulation of *C. capsici* effectively. The leaf extract of *P. elata* recorded the lowest mycelial growth of 2.33 mm accounting 72.79 per cent inhibition and was followed by *Datura metal* and the maximum growth of test fungus was documented in *Eclipta alba*. The extent of inhibition of the fungus by other extracts ranged between 6.24 to 65.35 per cent. Further, the combination of these two products suppressed the fruit rot disease incidence in chilli under field.

Key words: Polygala elata, Eucalyptus oblique, Plant extracts, Colletotrichum capsici, Control

Chilli (*Capsicum annuum* L.) belonging to the family solanaceae is one of the important spice cum vegetable crops in India. The crop is grown over an area of 7.69 lakh hectares with a production of 12.39 lakh tonnes. India is the largest consumer and exporter of chilli in the international market as dry chilli, chilli powder and olioresins to over 90 countries. The major constraint to chilli production in India is fruit rot disease, caused by *Colletotrichum capsici* (Syd.) Butler and Bisby. The yield losses of staggering dimension have been reported in various countries including India. The loss tolls up to 80-90 % under congenial conditions (Patil *et al.*, 1993; Pandey and Pandey, 2003).

A larger number of chemicals have been deployed in market for the management of this disease. However, the inherent disadvantages of continuous usage of chemicals in ecosystem have warned for the search of alternatives. In the desperate attempt aiming towards reduction of synthetic chemical pesticides in managing disease, a long step has been marched in searching alternative arsenals. However, of the many alternatives used, plant products and essential oils have brought considerable changes in the direction of managing diseases. A number of plant species have been reported to possess natural substances that are toxic to many fungi causing plant diseases (Kazmi et al., 1993; Jeyalakshmi and Seetharaman, 1998; Amadioha, 2000; Sateesh et al., 2004). Extensive research on exploitation of these towards management is being taken as they are safe for environment and human beings. With this background, in the present study, plant species

*Corresponding author's e-mail: rajamanickam.path@gmail.com

were exploited for the effective management of fruit rot incidence in glasshouse and field conditions.

Materials and Methods

Pathogen and plant materials

The chilly plant exhibiting characteristic symptoms of anthracnose disease were used for isolation of Colletotrichum capsaici. The pathogen was isolated through tissue segment method using PDA medium (Rangaswami, 1958) and pure culture was obtained by single spore isolation technique (Choi et al., 1999). The identity of pathogen was confirmed through morphological and cultural examination of ten days old test pathogen using MZ-16 Leica image analyser. Forty plant extracts and five essential oils were obtained from various plant species belonging to diverse taxonomic group. The plant species were collected from botanical garden, Tamil Nadu Agricultural University, Coimbatore. India. Chilli cv K2, which is susceptible to anthracnose disease was obtained from Agricultural Research Station, Kovilpatti, Tamil Nadu Agricultural University (TNAU), Tamil Nadu, India.

Preparation of plant extracts and essential oils

The plant materials (leaf, bulb or rhizome) were washed separately with fresh water and finally with sterilized water. They were ground in a pestle and mortar with sterile water at the rate of one ml/g. The extract was obtained by squeezing the macerate with cotton wool. It was strained through muslin cloth, finally through Whatman No.1 filter paper and passed through Zeitz filter to free it from bacterial contaminants. This formed a standard plant extract solution (100 %). This extract was further diluted with sterilized distilled water to the required concentrations (Shekhawat and Prasada, 1971). Further, essential oils were obtained from five plants according to Clevenger *et al.* (1928).

Plant extracts and essential oils on spore germination of C. capsaici

One drop of 10% plant extract or 1% and 0.5% essential oil was placed in a cavity slide and allowed to air dry. A drop of the spore suspension (5 x 10⁵ spores/ml) of *C. capsici* prepared in sterile distilled water was added to dried plant extract or oil and thoroughly mixed. The cavity slide was incubated in a Petri dish glass bridge moist chamber. Three replications were prepared for each treatment. The spore germination was recorded after 48 h and the per cent inhibition was calculated. The spore suspension in sterile distilled water served as the control.

Plant extracts and essential oils on dry mycelial weight of C. capsaici

Forty different plant extracts and five essential oils at ten and one per cent, respectively were screened against mycelial growth of *C. capsici* using PDA broth. The known quantity of plant extracts or essential oil were mixed with PDA broth so as to get the desired concentration in 250 ml conical flasks and mycelial disc of 9 mm from 15 days old culture was inoculated. The medium without incorporating the plant extract or essential oil served as control. The dry mycelial weight was recorded after 10 days. The percent inhibition of mycelial growth was calculated.

Plant extracts and essential oils on radial growth of C. capsaici

Poisoned food technique (Dhingra and Sinclair, 1985) was followed to evaluate the efficacy of plant extracts and essential oils in inhibiting the mycelial growth of C. capsici. Forty different plant extracts and ten essential oils at ten and one per cent respectively were used to test the efficacy against radial growth of test fungus. Sterile PDA medium was amended with either plant extract or essential oils of known quantity so as to get the desired concentration of the test material and 20 ml of amended PDA was poured in sterile Petri plate. The 9 mm culture disc from 15 days old culture was inoculated in each plate. The PDA medium without amended with plant extracts or oils served as control. Three replications were maintained in the experiment. The diameter of the colony was measured when maximum growth in control plates was occurred. The per cent inhibition was calculated by using the formula of Vincent (1947).

Evaluation of plant extracts and essential oils against fruit rot of chilli under glass house conditions

The highly susceptible chilli cv K2 was raised in mud pot containing 3 kg of pot mixture in the glass house. The 105 (20 days after fruit set) days old seedlings were sprayed with effective plant extract and essential oil individually and in combination. The chemical carbendazim was used for comparison. The treatment composed of T1- *Polygala elata* (10

%), T2- Eucalyptus (1 %), T3- Polygala elata (10 %) + Eucalyptus (1%), T4- Carbendazim (0.1 %), T5-Control. The spore suspension of *C. capsici* was prepared and sprayed on ripe fruits on the plants in situ five days after spraying of essential oils or plant extracts. Two days after pathogen inoculation, a second spray of essential oils or plant extracts was given as per the treatment. Water congestion was provided both 24 h prior and after inoculation by covering the plants with polythene bags and spraying with sterile distilled water inside. The plants inoculated with the pathogen alone served as control. Four replications were maintained and each replication contains five pots with each pot contains three plants. The plants were maintained in the glass house. The intensity of fruit rot diseases were recorded 15 days after the last spray. The per cent disease index (PDI) was calculated by using Mc Kinney (1923) formula. The results were expressed as per cent disease reduction.

Evaluation of plant extracts and essential oils for the management of fruit rot disease under field conditions

A field experiment was conducted to test the efficacy of plant extracts and essential oils against chilli fruit rot disease on variety K2 in the farmer's holdings at Gopalapuram, Dindigul district, Tamil Nadu, India. This area receives an annual rainfall of 800–1200 mm with a mean annual temperature of 30 °C (maximum36 °C, minimum 25 °C). The experiment was laid out in a randomized block design with four replications with a plot size of 5 X 4m (20 m²). Regular agronomic practices were followed as per the crop production guide of TNAU, Coimbatore, India. The treatments were similar as in glass house conditions. A total of four sprays were given at 20 days interval right from one month old stage. Disease intensity was assessed and expressed as explained earlier.

Data analysis

The data were statistically analyzed using the IRRISTAT version 92 developed by the International Rice Research Institute (IRRI) Biometrics unit, the Phillippines (Gomez and Gomez, 1984). Prior to statistical analysis of variance (ANOVA) the percentage values of the disease indices were arcsine transformed. Data were subjected to analysis of variance (ANOVA) at two significant levels (P < 0.05 and P < 0.01) and means were compared by Duncan's Multiple Range Test (DMRT).

Results and Discussion

The increasing social and economic implications caused by fungi mean there is a constant striving to produce safer food crops and to develop new antifungal agents. In general, essential oils are considered as nonphytotoxic compounds and potentially effective in food and agriculture industries against pathogenic fungi (Pandey *et al.*, 1982; Bajpai and Kang, 2010). In recent years, interests have been generated in the development of safer antifungal agents such as plant based essential oils and extracts

to control phytopathogens in agriculture (Bajpai and Kang, 2010).

In vitro evaluation of plant extracts and essential oils provides useful and preliminary information regarding efficacy against pathogen within a shortest

period of time and therefore, serves as a guide for field testing. In this study, poisoned food and spore germination methods were employed for evaluation. The poisoned food technique of fungicide testing, *i.e.* comparison of the radial growth rates of the fungus on

Table 1. Influence of	plant extracts on	growth and spore	germination of (C. capsaici
-----------------------	-------------------	------------------	------------------	-------------

Diant	Mycelial	Inhibition	Dry mycelial	Inhibition	Germinated	Inhibition
Plant	growth*	(%)	weight*	(%)	spores*	(%)
Apply the indian	0 771	20.25	474.07	23 56	11.45 ^{r-t}	18 20
Асагурна шиса	0.77	20.35	4/4.07	20.000	(19.94)	
Achyranthes aspera	6 27 ^{f-i}	26 24	457 00 ^{h-l}	26.41	10.62 ^{0-q}	24.14
Achyranines aspera	0.27	20.24	-07.00		(19.17)	
Aegle marmelos	5.77 ^{ef}	32.12	441.67 ^{g-j}	28.88	9.59 ^{ki}	31.50
					(18.19)	
Adathoda vasica	7.70 ^{0-q}	9.41	566.67 ^{u-x}	8.75	12.57**	10.21
					(20.94)	
Aloe vera	6.40 ^{h-k}	24.71	479.67 ^{j-n}	22.76	(18.07)	25.71
					(10.97) 9 16 ^{ijk}	
Alpinia calcarata	5.73 ^e	32.59	420.33 ^{f-h}	32.31	(17.76)	34.56
					5.02°	
Allium sativum	3.37°	60.35	290.67°	53.19	(13.05)	64.12
					7.02 ^f	
Andrographis paniculata	4.43 ^d	47.88	332.33 ^d	46.48	(15.49)	49.87
				04 74	10.80 ^{pq}	00.05
Aristolochia indica	6.80 ^{j-l}	20.00	486.00 ^{k-o}	21.74	(19.34)	22.85
				62.00	4.20 ^c	70.00
Azadirachta indica	2.97 ^{bc}	65.06	236.00 ^b	62.00	(11.92)	70.00
0	7 500 0		500.00av	14 26	11.84 ^{t-w}	15 45
Carum roxburghiannum	7.53 ^{⊪q}	11.41	୦୪∠.୪୪୩	14.20	(20.29)	10.40
	E E2e	24.04	202 22ef	36.06	8.55 ^h	38.95
Cissus quanurangulus	5.55°	54.94	393.33		(17.14)	
Coleus forskholi	7 / 3 n-p	12 59	528 67 ^{p-u}	14.86	11.45 ^t	18.20
Colcus Torsknon	7.40	12.00	520.07		(19.94)	
Cvanodon dactvlon	6.27 ^{fghi}	26.24	438.67 ^{g-i}	29.36	10.10 ^{mn}	27.84
	• -= -				(18.68)	
Cvmbopagan martinii	7.37 ^{m-p}	13.29	553.33 ^{₅-x}	10.90	11.96 ^{uw}	14.56
					(20.40)	
Cyperus rotundus	6.87 ^{j-m}	19.18	557.00 ^{t-x}	10.31	10.99⊶	21.52
					(19.52)	
Datura metal	2.67 ^{ab}	68.59	201.67 ^ь	67.53	4.54 [°]	67.56
					(12.40) 12.57xy	
Eclipta alba	7.97 ^q	6.24	580.33×	6.55	(20.94)	10.21
					(20.94) 11 32 ^{rs}	
Eucalyptus globules	6.80 ^{j-l}	20.00	493.00 ^{Lp}	20.61	(19.82)	19.16
					11.70 ^{stu}	
Euphorbia hirta	7.43 ^{n-p}	12.12	523.67°-t	15.67	(20.17)	16.45
				20.00	8.81 ^{h-j}	07 40
Hibiscus rosasinensis	5.50 ^e	34.94	378.67°	39.02	(17.41)	37.10
latrona auroca	7 1 Ol-n	16 47		16.00	11.66 ^{s-u}	46.75
Jauopa cuicas	7.10"	10.47	010.0/113	10.96	(20.13)	10.75

Lantana camera	7.33 ^{m-p}	13.76	522.67°-t	15.83	12.24 (20.65)	12.55
Lowsonia albai	7.47 ^{n-q}	12.18	561.00 ^{t-x}	9.66	11.96 ^w (20.40)	14.59
Mentha arvensis	5.93 ^{e-h}	30.24	448.33 ^{g-k}	27.81	9.80l ^m (18.39)	30.00
Moringa oleifera	7.07 ^{I-n}	16.82	512.00 ^{m-pqr}	17.55	11.41 ^{rs} (19.90)	18.52
Ocimum sanctum	5.83 ^{e-g}	31.41	494.67 ^{1-q}	20.34	9.17 ^{ik} (17.77)	34.52
Ocimum basilicum	5.77 ^{ef}	32.12	413.00 ^{e-g}	33.49	9.38 [⊌] (17.98)	33.00
Phyllanthus niruri	7.96 ^q	6.35	576.00 ^{wx}	6.92	12.94 ^{yz} (21.26)	7.59
Polygala elata	2.33ª	72.79	119.07ª	80.73	3.48 ^b (10.83)	75.15
Pongamia glabra	5.63°	33.76	569.67 ^{v-x}	8.27	13.32 ^z (21.58)	4.86
Sanchus oleraceus	7.83 ^{pq}	7.88	581.67×	6.33	12.52 ^{xy} (20.89)	10.59
Solanum trilobatum	5.57°	34.47	415.67 ^{e-g}	33.06	7.67 ⁹ (16.21)	45.21
Solanum xanthocarpurm	5.67°	33.29	422.67 ^{f-h}	31.94	8.94 ^{hij} (17.54)	36.15
Tylophora asthmatica	7.17 ^{I-n}	15.65	500.67 ^{m-q}	19.38	11.35 ^{rs} (19.85)	18.95
Vettivera ezinoides	7.26⊦∘	14.59	498.33 ^{m-q}	19.75	11.54 ^{s-u} (20.02)	17.56
Vitex negundo	7.53 ^{n-q}	11.41	527.33 ^{p-t}	15.08	12.31 [⊷] × (20.71)	12.10
Vinca rosea	7.27⊦₀	14.47	539.67 ^w	13.10	11.79 ^{s-u} (20.25)	15.82
Withania somnifera	6.33 ^{g-j}	25.53	476.33 ^{i-m}	23.30	10.26 ^{m-o} (18.83)	26.75
Zingiber officinale	5.56°	34.59	426.00 ^{f-h}	31.40	8.68 ^{hi} (17.27)	38.00
Carbendazim(0.1%)	2.27ª	73.29	118.33ª	80.94	2.92ª (9.92)	79.15
Control	8.50 ^r		621.00 ^y		14.00 ^z (22.16)	100
SEd =	0.26		19.29		0.23	
CD(.05)	0.52		38.36		0.46	

* Mean of three replications

Means followed by common letter (s) are not significantly different at 5% level

substrates impregnated with different concentrations of the fungicide, was largely developed for work with wood-rotting fungi (Horsfall, 1956), but it is equally useful with organisms which either do not sporulate at all, or cannot readily be induced to do so in the laboratory (Torgeson, 1967). Hence, in the foregoing study, different plant extracts (10 %) and oils (0.5 and 1 %) were screened against growth of *C. capsaici*. The result indicated that the plant extracts varied in the extent of inhibition exerted by them. However, the least growth of 2.33 mm was observed in *P. elata* accounting 72.79 per cent inhibition and was followed by *Datura metal* (2.67 mm) and maximum growth of test fungus was

documented in *Eclipta alba* (7.97 mm). In case of plant oils at two different concentrations, maximum inhibition was documented in *E. alba* at one per cent with 72.59 % and was followed by *Azardicta indica* at one per cent (69.77 %), whereas the least inhibition was documented in case of *Madhuca longifolia* (23.88 %). In addition to poisoned food technique, in

the foregoing study, for the precision, the influence of plant extracts and oils on dry mycelial weight was assessed using potato dextrose broth. The results were in concurrence with poisoned food method indicating efficacy of *P. elata* and *Azardiricta indica* against growth of *C. capsici* (Table 1 and 2).

Plant oils	Concentration	wycenar	Inhibition	Dry mycelial	Inhibition	Germinated	Inhibition
		growth*	(%)	weight*	(%)	spores*	(%)
Freedow (1997)	4.00	2.33ª	72.59	203.67ª	67.20	3.30ª	74.59
	1.00				07.20	(10.47)	
Eucalyptus obliqua	0.50	4.23 ^b	50.24	333.00 ^b	46.38	6.22 ^b	52.14
	0.50					(14.44)	
	1.00	0.570	00 77	240.33ª	61 20	3.51ª	73.00
Azadiraabta indiaa	1.00	2.37°	09.77		01.23	(10.80)	
Azauliacilla illuica	0.50	4 07h	40.70	373.33°	39.88	6.76 ^b	48.00
	0.50	4.27-	49.70			(15.07)	
Ricinus communis	1.00	5.83 ^{cd}	31.41	478.67°	22.92	9.23 ^{cde}	29.00
						(17.68)	
	0.50	6.03 ^{cde}	29.06	486.00°	21.74	8.97 ^{cd}	31.00
						(17.42)	
	1.00	6.27 ^{de}	26.24	479.00 ^e	22.87	9.52 ^{de}	26.80
Madhuca longifolia						(17.97)	
Madridea longilolla	0.50	6.47 ^e	23.88	492.33°	20.72	9.88 ^e	24.00
						(18.32)	
Pongamia pinnata	1.00	5.67° 5.83∞	33.29 31.41	439.00 ^d 457.00 ^{de}	29.31 26.41	8.58°	34 00
						(17.03)	01.00
						8.83 ^{cd}	32 10
	0.00					(17.28)	02110
Control		8 50 ^f		621 00 ^f		13.00 ^f	
Control		0.00		021.00		(21.13)	
SEd		0.23		17.73		0.35	
CD(.05)		0.47		36.76		0.72	

*Mean of three replications

Means followed by common letter (s) are not significantly different at 5% level

A good fungicide should not only have the fungistatic property but also should be an antisporulant in nature. Keeping in view of this, in present investigation, the efficacy of forty plant extracts and five essential oils were tested against spore germination of C. capsaici. The results of spore germination test revealed among the forty plant extracts tested, Polygala elata exhibited maximum inhibition (75.15 %) and was followed by Azardiricta indica (70.00 %) and the least inhibition was observed in Pongamia glabra (4.86%). Further, among the oils tested, maximum inhibition was observed in E. alba at one per cent concentration accounting 74.59 per cent inhibition and was followed by Azardiricta indica at one per cent concentration with 73.00 per cent inhibition. The results of these study are in agreement with earlier workers who reported the efficacy of plant extracts and essential oils against sporulation of plant pathogens (Akinbode and Ikoteen, 2008; Shovan et al., 2008).

Table 3. Influence of plant extracts and essential oils on yield of chilli under field conditions

Treatments	Yield (kg/ha)*	Per cent increase over control	BC ratio
Polygala elata	1180 ^b	82.95	1:3.76
Eucalyptus obliqua	1031°	59.85	1:3.29
Polygala elata+			
Eucalyptus obliqua	1183⁵	83.41	1:3.77
Carbendazim (0.1%)	1543ª	139.22	1:4.82
Control	645 ^d	-	-
SEd	24.75		
CD(.05)	53.92		

* Mean of four replications

Means followed by common letter (s) are not significantly different at 5% level

The information generated through *in vitro* evaluation of plant extracts and essential oils was employed in designing the ecosafely fungicides for managing the anthracnose disease in chilli. In views of this, the effective plant extract and oil were tested individually and in combination under glass house and field conditions. It was observed that disease spread was reduced at different rates in various treatments. Among the different treatments employed under glass house, the combined application of *P. elata* and Eucalyptus recorded least disease incidence of 19.77 PDI accounting 79.82 per cent reduction over control when compared to individual applications (Fig.1).



- T₁- Polygala elata
- T₂- Eucalyptus obliqua
- T₃- Polygala elata+Eucalyptus obliqua
- T₄- Carbendazim (0.1%)
- T_s- Control

Fig 1. Effect of plant extracts and essential oil against fruit rot disease in chilli under glass house condition

T_1 - Polygala elata	
------------------------	--

- T₂- Eucalyptus obliqua
- T₃- Polygala elata+Eucalyptus obliqua
- T₄- Carbendazim (0.1%)
- T₅- Control

PDI - Per cent Disease Index; PROC - Per cent Reduction over control Vertical bar indicates standard error



Fig 2. Effect of plant extracts and essential oil against fruit rot disease in chilli under field condition

The results of field experiments were consistent with

glass house experimental results. The combination treatment reduced the disease incidence significantly even in field conditions (Fig 2.) and further the increased yield was also recorded in the same treatment. The yield obtained in combined application was 1183 kg/ha which was statistically significant over other treatments. In addition to this cost benefit ratio was also higher in combined application (1:3.77) when compared to individual applications (Table 3). The results obtained in the present investigation clearly evidenced the research outcome of earlier workers who reported the efficacy of plant derived products against wide range of pathogens (Anand and Bhaskaran, 2009). The efficacy of plant products against C. capsici may be attributed to the induction of systemic resistance. The reduction in disease intensity might be due to induction of resistance in the plant system by the plant extract apart from the antifungal activity. Plant products have been considered as one of the major groups of compounds that induce Induced Systemic Resistance (ISR). However, induced resistance is not the only mode of action but a direct action of a plant extract on the pathogen is also involved by producing toxic substances which is necessary for protecting the crop against fruit rot disease (Wilson et al., 1987; Cutler et al., 1996; Al-Mughrabi et al., 2001).

References

- AL-Mughrabi, K.I., Abujai, T.A., Anfoka, G.H. and Shahrour, W. 2001. Antifungal activity of olive cake extracts. *Phytopathol. Mediterr.*, **40**: 240-244.
- Akinbode, O.A. and Ikotun, T. 2008. Evaluation of some bioagents and botanicals in *in vitro* control of *Colletotrichum destructivum*. African Journal of Biotechnol., **7** (7):868-872.
- Amadioha, A.C. 2000. Controlling rice blast *in vitro* and *in vivo* with extracts of *Azadirachta indica*. Crop Protect., 19: 287-290.
- Anand, T. and Bhaskaran, R. 2009. Exploitation of plant products and bioagents for ecofriendly management of chilli fruit rot disease. *J. of Plant Protec. Res.*, **49**(2): 195-203
- Bajpai, V.K. and Kang, S.C. 2010. Antifungal activity of leaf essential oil and extracts of *Metasequoia glyptostroboides* Miki ex Hu. J. Am. Oil Chem. Soc., 87: 327-336.
- Choi ,Y.W., Hyde, K.D. and Ho, W.H. 1999. Single spore isolation of fungi. *Fungal Diversity*, **3**: 29-38.
- Clevenger, J.F. 1928. Apparatus for the determination of volatile oil. *Journal of American Pharmaceutical Association*, **17**: 345-349.
- Cutler, H.G., Hill, R.A., Ward, B.G., Rohitha, B.H. and Stewart, A. 1996. Antimicrobial, insecticidal and medicinal properties of natural products. Flavours and fragrances. *In: Biotechnologies for Improved Foods and Flavours* G.R. Takeokaed, (Ed.). American Chemical Society, USA, pp: 51-66.
- Dhingra, O.D. and Sinclair, J.B. 1985. Basic Plant Pathology Methods. CRC Press, Inc. Boca Raton, Florida. pp:132-163.
- Gomez, K.A. and Gomez, A.A. 1984. Statistical Procedure for Agricultural Research. John Wiley and Sons, IRRI, 1984, New York. p:680

- Jeyalakshmi, C. and Seetharaman, K. 1998. Biological control of fruit rot and die-back of chilli with plant products and antagonistic microorganisms. *Plant Dis. Res.* 13: 46-48.
- Kazmi, A.R., Niaz, I. and Jilani, G. 1993. Evaluation of some plant extracts for antifungal properties. *Pakistan J. Phytopathol.*, 5:93–97.
- Mckinney, H.H. 1923. A new system of grading plant diseases. *Journal of Agricultural Research*, **26**: 195-218.
- Pandey, K.K. and Pandey, P.K. 2003. Survey and surveillance of vegetable growing areas for prevalence of major diseases. *Veg. Sci.*, **30** (2): 128-134.
- Pandey, D.K., Tripathi, N.N., Tripathi, R.D. and Dixit, S.N. 1982. Fungitoxic and phytotoxic properties of the essential oil *Caesulia axillaris* Roxb. *Angew Bot.*, 56: 259-267.
- Patil, C.V., Korekar, V.B. and Peshney, N.L. 1993. Effect of die-back and fruit rot on the yield of chilli. *PKV Res. J.*, **17** (1): 60–63.
- Rangaswami, G. 1958. An agar blocks technique for isolating soil microorganisms with special reference to Pythiaceous fungi. Science and Culture, 24:85-89

- Sateesh, K., Marimuthu, T., Thayumanavan, B., Nandakumar, R. and Samiyappan, R. 2004. Antimicrobial activity and induction of systemic resistance in rice by leaf extract of *Datura metel* against *Rhizoctonia solani* and *Xanthomonas oryzae* pv. oryzae. Physiol. Mol. Plant Pathol., 65: 91-100.
- Shekhawat, P.S. and Prasada, R. 1971. Antifungal properties of some plant extracts, in inhibition of spore germination. *Indian Phytopathol.*, **24**: 800-802.
- Shovan, R., Bhuiyan, M.K.A., Begum, J.A. and Pervez, Z. 2008. In vitro control of Colletotrichum dematium causing anthracnose of soybean by fungicides, plant extracts and Trichoderma harzianum. International Journal of Sustainable Crop Production, 3: 10-17.
- Torgeson, 1967. Determination and measurement of fungitoxicity. *In: Fungicides* Torgeson, DC (Eds.). Volume I. Academic Press, New York. p: 697.
- Vincent, J.M. 1947. Distortion of fungal hyphae in the presence of certain inhibition. *Nature*, **159**: 850.
- Wilson, C.L., Franklin, J.D. and Otto, B. 1987. Fruit volatiles inhibitory to *Monilinia fructicola* and *Botrytis cinerea*. *Plant Dis.*, **71** (4):316–319.

Received after revision: March 24, 2016; Accepted: June 30, 2016