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Exploitation of Phytochemicals from Plants Extracts and its Effect on Growth of *Colletotrichum capsici* (Syd.) Butler and Bisby Causing Anthracnose of Chilli (*Capsicum annuum* L.)

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Abstract: The crude leaf extract of *Andrographis paniculata*, *Azadirachta indica*, *Cissus quadrangularis*, *Datura metel*, *Hibiscus rosa-sinensis*, *Ocimum basilicum*, *Polygala elata*, *Solanum xanthocarpum* and bulb extract of *Allium sativum* were screened to determine their effect on the colony diameter, sporulation and disease incidence of anthracnose of chilli (*Capsicum annuum* L.) caused by *Colletotrichum capsici*. The potentially active compound also assessed in effective plant extracts showing strong fungitoxicity effect against the causal pathogen. All the plant extracts were inhibited the colony growth and sporulation of *Colletotrichum capsici*. The leaf extracts of *Polygala elata* and *Datura metel* exhibited the strong fungicidal activity against the pathogen. The leaf extract of *P. elata* recorded lowest mycelial growth of 2.33 cm, followed by *D. metel, A. indica* and bulb extract of *A. sativum* were recorded the mycelial growth of 2.67, 2.97 and 3.37 cm, respectively. The group of compounds present those are likely to be responsible for fungitoxicity to be alkaloids, flavonoids, glycosides, saponins and tannins.

Key words: Chilli, plant extracts, phytochemicals, *Colletotrichum capsici,* anthracnose

INTRODUCTION

Chilli (*Capsicum annuum* L.) belongs to the family Solanaceae, is also known as red pepper and it is important spice cum vegetable crop in India. It is grown throughout the year and used as green and dried stages their pungency and colour, it is an integral part of Indian diet and used at tine in every Indian home. Indian chilli is being exported to over 90 countries and has become a good foreign exchange earner. India ranks second next to China in the vegetable production in the world (Vitkar *et al*., 2007). In India chilli is being grown in area of 7.69 lakh hectares with a production of 12.39 lakh tonnes and a productivity of 1.61 t ha^{-1} (Basavaraj, 2008). The important chilli growing states are Andhra Pradesh, Karnataka and Tamil Nadu. The pathogen *Colletotrichum capsici* inciting anthracnose disease in chilli has been found to be crop wherever it is grown. The seed borne nature of *C. capsici* may be transmitted from mother plant, which were present throughout the storage period which cause severe seed rot, seedling decay, twig blight, fruit rot and affect the seed germination of chilli and *C. capsici* able to survive up to the next crop season in the infected seeds (Ramesh, 2007). Anthracnose disease caused by the fungus *C. capsici* is the most destructive disease of chilli, which cause pre and post emergence damping off, leaf spots, premature fruit drop, mummification of unripe green fruits and fruit rot, which contribute 50-100% loss in India (Amusa *et al*., 2004). Anthracnose caused the healthy green fruits lost 31% and red ripe fruits lost 46% ascorbic acid after 14 days of pathogenesis (Ramesh, 2007), 25% loss of capsaicin content (Prasad *et al*., 2000). Chemical is not advisable to disease management because of the ill effects associated with the chemical control like residual toxicity, the development of resistance by the pathogen, environmental pollution, besides repeated application involving more expenditure on plant production. So, we try to use some plant extracts for controlling this disease. The use of plant extract for the control of fruit rot of chilli is cheep, locally available, non toxic and biodegradable.

MATERIALS AND METHODS

This study was carried out in the Department of Plant Pathology, Agricultural College and Research Institute, Tamil Nadu state, India during June 2008 to March 2009.

Pathogen isolation: Chilli fruit with anthracnose lesions were collected from farmer field of Madurai area of Tamil Nadu state. The section of 3-5 mm^2 were cut from the margin of the infected lesions and sterilized for one minute

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in 1.0% sodium hypochlorite and rinsed with several changes of Sterile Distilled Water (SDW). The sterile pieces were blotted on sterilized Petri plates containing solidified Potato Dextrose Agar (PDA) in aseptic conditions. The plates were incubated at ambient temperature (28±2°C) for five days after incubation. The tip of hyphal growth radiating from the infected tissue was transferred onto PDA. The fungus was purified again by single hyphal tip method and culture was confirmed by microscopic examination (Riker and Riker, 1936).

Plant extract preparation: Leaf of *Andrographis paniculata*, *Azadirachta indica*, *Cissus quadrangularis*, *Datura metel*, *Hibiscus rosa-sinensis*, *Ocimum basilicum*, *Polygala elata*, *Solanum xanthocarpum* and bulb extract of *Allium sativum* were collected freshly. The collected plant materials were washed with fresh tap water and then with alcohol and finally in repeated changes with SDW. These were separately ground with sterile water at the rate of one mL g^{-1} of the material in a pestle and mortar. The extract was filtered through two layers of muslin cloth, subsequently filtered through Whatman No.1 filter paper and finally passed through membrane filter to eliminate bacteria. This formed the standard plant extract solution (100%). This extract was further diluted to 10% concentration by adding requisite quantity of medium (Shovan *et al*., 2008).

Fungitoxic effect of plant extracts: Efficacy of plant extracts against the mycelial growth of *Colletotrichum capsici* was assessed by following poisoned food technique (Schmitz, 1930). The media amended with plant extracts at the concentration of 10% were inoculated with mycelial disc of 9 mm size of 10 days old culture of *C. capsici.* The Petri plates were incubated at room temperature (28±2°C). The medium without incorporating the plant extract served as control. The mycelial growth measured after 10 days. The percent inhibition of mycelial growth was calculated. The treatments consist of three replications arranged in completely randomized design. The diameter of the fungal colony was measured using a rule along two diagonal lines drawn on the reverse side of each Petri Plate 10 days after inoculation. The sporulation was determined by adding 10 mL of SDW to each plate and gently scraping with a sterile glass rod to dislodge the spores. The spore suspensions obtained were filtered through sterile cheese cloth into a sterile 50 mL glass beaker and homogenized by manual shaking. The spores were then counted using a haemocytometer. The percentage of reduction by the extracts was computed using the following formula:

$$\text{Reduction over control (\%)} = \frac{(C-T)}{C} \times 100$$

where, T is colony diameter or sporulation in treatment medium and C is colony diameter or sporulation in untreated medium (control).

Similarly, the media without agar (PDA) amended with plant extracts at the concentration of 10% were inoculated with mycelial disc of 9 mm size of 10 days old culture *C. capsici.* The conical flasks were incubated at room ambient (28±2°C). The medium (PDA broth) without incorporating the plant extract served as control. The Carbendazim (0.1%) was used for comparison in both solid and liquid, respectively. The treatments consist of three replications arranged in completely randomized design. The dry mycelial mat was measured after 10 days after inoculation. The mycelial mat was removed by filtering through pre weighted in Whatman No.1 filter paper and dried in a hot air oven at 60°C till a constant weight was obtained. Three replications were maintained and % reduction in mycelial dry weight was calculated.

Glass house experiment: To study the effective plant extracts against the pathogen *in vitro* viz., leaf extracts of *P. elata* (10%), *D. metal* (10%), *A. indica* (10%) and bulb extract of *A. sativum* (10%) were tested against fruit rot disease in pot culture experiment. The Carbendazim (0.1%) was used for comparison. Chilli plants of susceptible local variety K2 were raised in mud pot in the glass house, each pot contained 3 kg of pot mixture. The plant extracts were sprayed at 20 days after fruit set (105 days old plants). A spore suspension of *C. capsici* was prepared and sprayed on ripe fruits on the plants *in situ* five days after spraying of plant extracts. Two days after pathogen inoculation, a second spray of 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. The treatments consist of four replications arranged in completely randomized design and each replication contains five pots. The experiments were conducted in two seasons viz., July to October and December to March. The% Disease Index (PDI) was calculated using Mckinney (1923) the infection index:

$$\text{PDI} = \frac{\text{Sum of numerical ratings}}{\text{Total No. of fruits observed}} \times \frac{100}{\text{Maximum category value}}$$

Phytochemical analysis: Phytochemical analysis of leaves and bulb extracts of plants that showed antifungal activity against *C. capsici* was carried out using 10% concentrations. The plant extracts used were those of

Polygala elata, Datura metel, Azadirachta indica and *Allium sativum*. The extracts were evaluated for the presence of alkaloids, flavonoids, glycosides, saponins, steroids and tannins as follows.

Alkaloids: One milliliter of 1% HCl was added to 3 mL of the water extract of each of the five plants which showed antifungal activity against *C. capsici* in separate test tubes. Each extract was then treated with two drops of Mayer's reagent. A creamy white precipitate indicates the presence of alkaloids (Hassan *et al*., 2004).

Flavonoid (Shinoda test): One gram of $MgSO_4$ powder and two drops of concentrated HCl was added to 3 mL of each water extract. A red coloration indicates the presence of flavonoids (Hassan *et al*., 2004).

Glycosides: One milliliter of the water extracts of individual plants in a test tube, 10 mL of 50% H_2SO_4 was added. The mixture was heated in boiling water for 15 min. Fehling's solution (2 mL) was added and the mixture was boiled. A brick red precipitate indicates the presence of glycosides (Isaac and Chinwe, 2001).

Saponins (frothing test): Two milliliters of the extracts in separate test tubes were vigorously shaken for two minutes. An observation of frothing in the extract indicates the presence of saponins (Isaac and Chinwe, 2001).

Steroids (Liebermann-Burchard Reaction): One milliliter of concentrated H_2SO_4 was added to 1 mL of each extract. Absence of red colouration indicates the presence of steroids (Hassan *et al*., 2004).

Tannins: Two drops of 5% $FeCl_3$ were added to 1 mL of each extract. A dirty-green precipitate shows the presence of tannins (Hassan *et al*., 2004).

Statistical 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 colony diameter of *C. capsici* was significantly lower on PDA amended with leaf extracts *P. elata, D. metel* and *A. indica* (Table 1). The results of the *in vitro* screening of plant extracts against mycelial growth of chilli fruit rot pathogen *C. capsici* revealed that 10% leaf extract of *P. elata* recorded lowest mycelial growth of 2.33 cm, this was followed by *D. metel, A. indica and* bulb extract of *A. sativum* recorded the mycelial growth of 2.67, 2.97 and 3.37 cm respectively as against 8.50 cm in the control in respect of solid medium *in vitro*. The 10% leaf extract of *P. elata* recorded lowest mycelial dry weight of 119.07 mg, this was followed by leaf extracts of *D. metel, A. indica* and bulb extract of *A. sativum* were recorded mycelial dry weight of 201.67, 236.00 and 290.67 mg, respectively as against 621.00 mg in control in liquid medium. In both solid and liquid medium Carbendazim were kept as comparison which recorded 2.27 cm and 118.33 mg in solid and liquid medium, respectively. The leaf extracts of *P. elata* most significantly reduced the spore germination and recorded 21.67% spore germination accounting 76.45% spore germination inhibition over control. The both the plant extract *P. elata* and Carbendazim showed on bar with each other *in vitro*. This was followed by *D. metel* and *A. indica* recorded 29.00 and 34.33% spore germination with 68.43, 62.68% spore germination inhibition over control, respectively (Table 2). The results of the pot culture experiment conducted to assess the efficacy of the selected plant extracts were found effective against the pathogen under pot culture conditions revealed that the leaf extracts of *P. elata* (10%) and *D. metel* (10%) were

Table 1: Effects of certain plant extracts on colony diameter and mycelia dry weight of *Colletotrichum capsici* the causal pathogen of anthracnose of chilli

Plant species	Colony diameter (cm)*	Reduction over control (%)	Mycelial dry weight (mg)	Reduction over control (%)
Allium sativum	3.37^{c}	60.35	290.67^{e}	53.19
Andrographis paniculata	4.43^{d}	47.88	332.33^{f}	46.48
Azadirachta indica	2.97^{b}	65.06	236.00^{d}	62.00
Cissus quadrangularis	5.53^{e}	34.94	393.33^{h}	36.06
Datura metel	2.67^{b}	68.59	201.67^{c}	67.53
Hibiscus rosa-sinensis	5.50^{e}	34.94	378.67^{g}	39.02
Ocimum basilicum	5.77^{f}	32.12	413.00^{i}	33.49
Polygala elata	2.33^{a}	72.79	119.07^{b}	80.73
Solanum xanthocarpum	5.67^{ef}	33.29	422.67^{j}	31.94
Carbendazim	2.27^{a}	73.29	118.33^{a}	80.94
Control	8.50^{g}	-	621.00^{k}	-
SED	0.09		0.10	
CD (0.05%)	0.20		0.21	

*Values are mean of three replications. Values in a column followed by same letters are not significantly different according to Duncan's multiple rang test at p = 0.05

Table 2: Effects of certain plant extracts on sporulation of *Colletotrichum capsici*

Medicinal plant	Spore germination (%)*	Reduction over control (%)
Allium sativum	37.33 (37.66)^{e}	59.42
Andrographis paniculata	49.67 (44.81)^{g}	46.01
Azadirachta indica	34.33 (35.87)^{d}	62.68
Cissus quadrangularis	59.00 (50.19)^{i}	35.87
Datura metel	29.00 (32.58)^{c}	68.43
Hibiscus rosa-sinensis	64.33 (53.33)^{j}	30.08
Ocimum basilicum	45.33 (42.32)^{f}	50.73
Polygala elata	21.67 (27.74)^{b}	76.45
Solanum xanthocarpum	56.67 (48.83)^{h}	38.40
Carbendazim	0.00 (2.56)^{a}	100.00
Control	92.00 (73.63)^{k}	-
SED	0.04	
CD (0.05%)	0.09	

*Mean of three replications, Data in parentheses are arc sine transformed values. Values in a column followed by same letters are not significantly different according to Duncan's multiple rang test at p = 0.05

Table 3: Evaluation of certain plant extracts against fruit rot of chilli under glass house

Treatments	Disease incidence	Reduction over control (%)	PDI*	Reduction over control (%)
Polygala elata (10%)	21.33 (27.49)^{b}	70.91	20.00 (26.56)^{b}	72.63
Datura metel (10%)	22.67 (28.29)^{c}	69.09	20.27 (26.65)^{c}	72.26
Azadirachta indica (10%)	37.33 (37.66)^{d}	49.09	32.27 (34.61)^{d}	55.84
Allium sativum (10%)	41.33 (40.01)^{e}	43.64	39.43 (39.07)^{e}	45.63
Carbendazim (0.1%)	14.67 (22.48)^{a}	79.99	14.13 (22.05)^{a}	80.66
Control	73.33 (58.93)^{f}	-	73.07 (58.75)^{f}	-
SED	0.04		0.12	
CD (0.05%)	0.08		0.26	

Data are pooled data of two experiments *Mean of four replications, Data in parentheses are arc sine transformed values, Values in a column followed by same letters are not significantly different according to Duncan's multiple rang test at p = 0.05

Table 4: Phytochemical analysis for the presence of chemical constituents of some plant extracts

Plant extracts	Alkaloids	Steroids	Glycosides	Flavonoids	Saponins	Tannins
Polygala elata	+	+	-	-	-	-
Datura metel	+	+	-	-	-	+
Azadirachta indica	+	-	+	+	+	+
Allium sativum	-	-	-	-	+	+

+: Present, -: Absent

found to be effective in reducing the fruit rot incidence and fruit rot intensity accounted 72.63 and 72.26% reduction over control. Both the extracts were on par with each other (Table 3). Phytochemical analysis revealed the presence of glycosides, saponins, tannins, alkaloids and flavonoids in the plant extracts (Table 4). The leaf extracts of *P. elata* showed the presence of alkaloids and steroids. Similarly *D. metel* showed the presence of alkaloids, steroids and tannins. The leaf extracts showed strong fungitoxicity due to the presence of alkaloids and steroids against *C. capsici* revealed that these plant species possessed very high antifungal activity. The leaf extract of *A. indica* showed the presence of alkaloids, Glycosides, Saponins, and tannins and bulb extract of *A. sativum* showed the presence of saponins and tannins.

The study showed that leaf extracts from *P. elata* (10%) and *D. metel* (10%) showed strong fungitoxicity due to the presence of alkaloids and steroids against *C. capsici* revealed that certain plant species possessed very high antifungal activity, followed by leaf extract of *A. indica* and bulb extract of *A. sativum* effectively inhibited *C. capsici* due to presence of glycosides, flavonoids, saponins and tannins. Similarly Nduagu *et al.* (2008) reported that, the leaf extracts of *Azadirachta indica*, *Citrus limon, Ocimum gratissimum, Vernonia amygdalina* showed the antifungal activity against *C. capsici* due to presence of glycosides, saponins, tannins, alkaloids and flavonoids. The same type result was obtained by Okwu and Morah (2007) reported that, the fruit extract of *Dennettia tripetala* inhibited *Aspergillus niger, Candida albicans* and seed extract of *Garcinia kola* inhibited *Candida albicans.* The alkaloids, saponins, flavonoids, tannins and phenols are responsible for inhibition of microorganism present in the fruits. Similar type of the result was made by Shovan *et al.* (2008) reported that *Allium sativum*, *Allium cepa*, *Zingiber officinale* and *Azadirachta indica* extracts inhibited the radial growth and mycelial dry weight of the *C. dematium.* Akinbode and Ikotun (2008) reported that extract of *Nicotiana tabacum* is known to

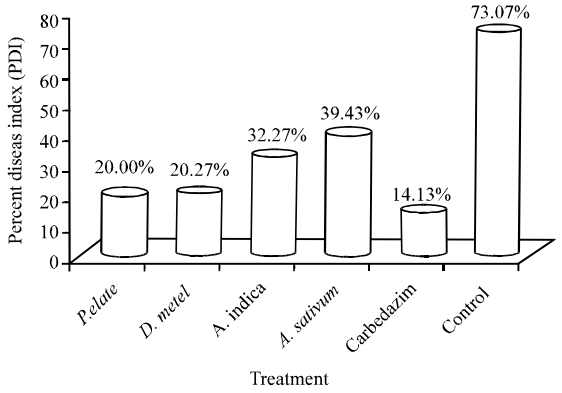

Fig. 1: Evaluation of plant extracts against fruit rot of chilli under glasshouse

have antifungal property against *C. destructivum*. Khewkhom *et al*. (2007) reported that *Cinnamomum zeylanicum, Pimenta dioica* and *Syzygium aromaticum* is known to have antifungal activities against *C. gloeosporioides*. The results of glass house study revealed that the leaf extracts of *Polygala elata* and *Datura metel* effectively controlled the fruit rot disease under pot culture condition (Fig. 1). Similar type of results was observed by Asha and Kannabiran (2001) in which they reported that reduce disease incidence chilli infected plants with in *C. capsici* when treated with extracts of *Datura metel*. Kumar and Yadav (2007) found that the leaf extracts of *Azadirachta indica, Datura stramonium* and clove extract of *Allium sativum* reduced spore germination of *C. capsici* and *C. gloeosporioides*. Similarly, Gomathi and Kannabiran (2000) found that leaf extracts of *Solanum torvum*, *Datura metel* and *Prosopis juliflora* reduced mycelial growth and spore germination of *C. capsici*.

CONCLUSION

The plant extracts of *P. elata*, *D. metel*, *A. indica* and bulb extract of *A. sativum* inhibited the mycelial growth, spore germination and disease incidence of *C. capsici in vitro*. The phytochemicals present those are likely to be responsible for fungitoxicity to be alkaloids, Flavonoids, glycosides, saponins and tannins. Based on the present results the above plant extracts could be suggested as alternative to fungicides.

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