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In vitro Evaluation of Botanicals against *Colletotrichum capsici* inciting Fruit Rot of Chilli

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A B S T R A C T

The anthracnose of chilli caused by *Colletotrichum capsici,* is a serious widespread disease in India and a limiting factor for profitable cultivation and seed production of chilli. Present experiment was aimed at studying the efficacy of various botanical extract on the mycelial growth, germination and seedling vigour of *C. capsici*. All the twenty botanicals at 10% concentration that were tested were found effective in inhibiting the mycelial growth of the pathogen with per cent inhibition ranging from 6.67 to 60.19%. Among different botanicals tested, *Aegle marmelos*, *Eucalyptus globules*, *Polyalthia longifolia*, *Allium sativum*, *Zingiber officinale*, *Allium cepa*, *Carica papaya* and *Curcuma longa* were found superior as compared to other treatments and control. *A. marmelos* recorded the highest inhibition of 60.19% with a radial growth of 3.58 cm. The least inhibition was recorded from *Duranta repens* (7.78%) and *Bougainvillea spectabilis* (6.67%) with a radial growth of 8.3 and 8.4 cm. The most promising botanicals (eight) were selected for further evaluation on the seed germination and seedling vigour index. Maximum per cent seed germination and seedling vigour index was observed in seeds treated with extract of garlic with germination per cent of 94.67% and vigour index of 747.73. Among all treatments, maximum root/shoot length and vigour index was found by *A. sativum* and *A. marmelos* treated seeds.

Introduction

Chilli (*Capsicum annuum L.*) one of the most important commercial crop of India belongs to the Solanaceae family which represents a diverse plant group. *Capsicum* contains approximately 20-27 species, five of which are domesticated *viz*., *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, and *C. pubescens*, and are cultivated in different parts of the world. Among the five species of cultivated *Capsicum*, *C. annuum* is one of the most common cultivated crops worldwide (Tong and Bosland, 1999) followed by *C. frutescens* (Bosland and Votava, 2003). It comprises numerous chemicals including steam-volatile oils, fatty oils, capsaicinoids, carotenoids, vitamins, protein, fibre and mineral elements (Bosland and Votava, 2003). The world area and production of chilli is around 1.5 million ha and 7 million tonnes, respectively (Rao, 2014). In Nagaland the

area, production and productivity of green chilles is estimated at 5.82 thousand hectares, 41.90 thousand tonnes and 7.20 tonnes/hectare whereas that of dry chillies is estimated at 0.80 thousand 3 hectares, 1.00 thousand tonnes and 1.25 tonnes/hectares respectively (Anon, 2015). Chilli suffers from various diseases and chilli anthracnose is one of the most important among them. Anthracnose disease caused by the fungus *Colletotricum 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 causes the healthy green fruits and red ripe fruits lose 31% and 46% ascorbic acid after 14 days of pathogenesis (Ramesh, 2007) and 25% loss of capsaicin content (Prasad *et al.,* 2000). The above reasons prompted the present study to test the efficacy of botanicals *in vitro.*

Materials and Methods

Fruit rot caused by *C. capsici and* chilli fruit showing characteristic symptoms of the pathogen were collected and brought to the Department laboratory for isolation. The pathogen, *C. capsici* was isolated on Potato Dextrose Agar medium (PDA). The infected fruits were surface sterilized with 0.4% sodium hypochlorite for 2 minutes to remove the non-causal micro-organisms.

The specimen were then taken and cut into small bits through the infected spots and was transferred into Petri plates containing the medium and were incubated for 3 days until the mycelial growth was observed. Thereafter, the growing tips of the mycelia of the fungi were then transferred to PDA slants and incubated at 25±2˚C till conidial formation for obtaining pure cultures of the pathogen.

Characteristics of the pathogen

The pathogen obtained was studied based on colony characters and their morphological characters. The stock culture was maintained on PDA slants in the refrigerator at 5˚C.

The pathogen isolated was observed under the microscope for identification. Under observation, the characteristics of the pathogen were recorded as follows:

Conidia- The conidia were found to be falcate, fusiform, single celled and hyaline with a central oil globule.

Conidiophores- The conidiophores are hyaline to faintly brown, cylindrical in shape and either septate or aseptate.

Setae- The setae was observed to be dark brown in colour which are paler at the apex, rigid and tapering towards the apex.

Colour- The colour of the isolate in PDA medium was observed to be light grey to dark grey in colour.

Microsclerotia- The presence of small microsclerotia arranged in concentric rings in the colony was found to be irregular in shape under the microscope.

Source of seed

The seeds were of local variety obtained from the local market at Medziphema, Nagaland.

Composition of media used in the experiment

Potato Dextrose Agar (PDA)

Peeled potato 200 g
Dextrose 20 g
Agar-agar 20 g
Distilled water 1000 ml

Czapek Dox medium

The formulation used as developed by Thom and Church (1926) is as follows:

Sucrose 30.0 g
Sodium Nitrate 2.0 g
Dipotassium Phosphate 1.0 g
Magnesium Sulfate 0.5 g
Potassium Chloride 0.5 g
Ferrous Sulfate 0.01 mg
Agar-agar 15.0 g
Distilled water 1000 ml

Preparation of botanicals

The plant parts (100g each) used were initially washed, air dried and individually crushed in mortar and pestle after which it was transferred into a conical flask. It was soaked in ethanol 95% (100 ml) @ 1:1 w/v and was incubated at 60˚C for 4-5 days for the ethanol to evaporate It was taken out when the ethanol was evaporated and 100 ml of sterile distilled water was added to the conical flask containing the extract. The macerate was then filtered through sterile Whattman filter paper No. 41 and the filtrates were considered as standard extract (100%).

Evaluation of botanicals against *Colletotrichum capsici*

The efficacy of botanical extracts in relation to the growth of pathogens was determined by the method of Schmitz (1930). An appropriate amount of leaf extract was added to sterilize warm Czapek Dox medium and thoroughly mixed just before plating to form 10% concentration.

Twenty ml of this mixture was immediately poured into a sterilized Petri plate of 90 mm diameter in three replications and allowed to solidify. A 10 mm culture disc of *C. capsici* from PDA culture was removed and placed onto the centre of the medium. The plates were incubated at 28±2˚C for 10 days. Czapek Dox medium without plant extract served as the control.

The radial growth of the colony was measured on the 10th day when the mycelium fully covered the control plates. The per cent inhibition of the growth was calculated. The per cent inhibition of the growth of the colony was calculated as per Vincent (1947) and expressed by using the formula:

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

Where,

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

Evaluation of botanicals on seed germination and seedling vigour index

The test was carried out following the method of international rules for seed health testing (ISTA, 1996). Based on the above experiment, selected botanicals which showed the maximum radial inhibition on the test pathogen were used. The seeds were soaked in the selected botanicals at the required concentration (10%) for 1 hour. For control, the seeds were soaked in distilled water.

Three pieces of blotting paper of 90 mm size were moistened with distilled water and placed in 90 mm sterilized Petri dishes after draining excess water. After 1 hour, the treated seeds were placed on the Petri dishes at the rate of 25 seeds per plate at equal distance in each Petri dish. The plates were incubated at room temperature (28˚C) under alternate cycles of 12 hours NUV light and darkness. Data was recorded on seedling germination, root length, shoot length and total length at 15 DAS.

The germinated seeds were counted and the percent germination was computed by using the formula:

$$\text{Per cent germination} = \frac{\text{Number of germinated seeds}}{\text{Number of seeds sown}} \times 100$$

Length of shoot was measured from the collar region to the tip of the longest leaf and expressed as cm. Root length of the seedlings was measured from the base of the stem to the tip of the longest root and expressed as cm. the vigour index was calculated by using the formula:

Vigour Index = (Mean root length + Mean shoot length) × Per cent germination

Experimental design

The experiment was done in a Completely Randomized Design (CRD) and each treatment was replicated three times. The treatment combination for evaluation of botanicals (Table 1) on radial growth of *C. capsici* was laid as follows:

T1: *C. capsici* + Bael
T2: *C. capsici* + Blue gum
T3: *C. capsici* + Bougainvillea
T4: *C. capsici* + False ashoka
T5: *C. capsici* + Garlic
T6: *C. capsici* + Ginger
T7: *C. capsici* + Golden dewdrop
T8: *C. capsici* + Hibiscus
T9: *C. capsici* + Holy basil
T10: *C. capsici* + Neem
T11: *C. capsici* + Onion
T12: *C. capsici* + Papaya
T13: *C. capsici* + Periwinkle
T14: *C. capsici* + Satavari
T15: *C. capsici* + Shrub verbena
T16: *C. capsici* + Sweet basil
T17: *C. capsici* + Thai nightshade
T18: *C. capsici* + Turmeric
T19: *C. capsici* + Veld grape
T20: *C. capsici* + Yellow nightshade
T0: *C. capsici* (Control)

The treatment combination for evaluation of botanicals on seed germination and seedling vigour index are as follows:

T1: Seed treatment with bael
T2: Seed treatment with blue gum
T3: Seed treatment with false ashoka
T4: Seed treatment with garlic
T5: Seed treatment with ginger
T6: Seed treatment with onion
T7: Seed treatment with papaya
T8: Seed treatment with turmeric
T0: Seed treatment with distilled water (Control)

Results and Discussion

Evaluation of botanicals on *Colletotrichum capsici*

The selected twenty plants were used for preliminary screening at 10 % concentration each using the method followed by Schimtz (1930). The average growth of the pathogen was recorded on the 10^{th} day when the mycelium fully covered the control plates.

Effect of the treatments with botanicals on the radial growth and per cent inhibition of the isolated test pathogen *C. capsici* were recorded and are presented in Table 2. The results revealed that all the treatments inhibited the radial growth of the pathogen ranging from 60.19 % to 6.67 % compared to the non-treated control (00.00 %). Amongst the botanicals that were tested, T_1 (bael) was found most effective against *C. capsici* which showed a radial growth of 3.58 cm and per cent inhibition of 60.19 %. The findings are in accordance with the report of Anand and Bhaskaran (2009), who reported that the leaf extracts of *Abrus precatorius* and *Aegle marmelos*, demonstrated the highest inhibition

of growth against two pathogens studied viz., *C. capsici* and *Alternaria alternata*. The reason of bael being the most effective treatment in the conducted experiment may be due to the presence of an essential oil terpenoid which is known to be effective against fungi (Gurjar *et al.*, 2012).

These treatments were further followed by T_5 (garlic) showing a radial growth of 4.4 cm which was statistically at par with T_{18} (turmeric) with a mean colony diameter of 4.4 cm and an inhibition of 51.30 and 50.93%. The observations are in tune with the works of Ushakiran *et al.*, (2006) who also reported that *Allium sativum* showed a radial growth of 4.5 cm with an inhibition of 50.33% at 10% concentration and a radial growth of 5.1 cm and inhibition of 43.67% at 5% concentration, respectively. The antifungal activity of garlic against the pathogen has also been reported by other researchers such as Rajamanickam *et al.*, (2012) and Sundramoorthy *et al.*, (2014). The efficacy of turmeric against *C. capsici* has also been previously reported by Anand and Bhaskaran (2009), Jagtap *et al.*, (2013) and Rahman *et al.*, (2011) who reported that *Curcuma longa* (leaf) also possesses high ability to inhibit conidial germination and germ tube formation of *C. capsici.* The antimicrobial activity of garlic and turmeric is due to the presence of an allicin, a sulfoxide and curcumin which is a terpenoid known to be effective against fungi, bacteria and protozoa (Gurjar, 2012). Singh *et al.*, (1990) also reported that a compound ajoene, derived from garlic inhibited *Colletotrichum* spp. Among the botanicals evaluated, golden dewdrop (T7) and bougainvillea (T3) showed the least effectiveness against the studied pathogen with a radial growth of 8.3 cm and 8.4 cm with minimum inhibition of 7.78% and 6.67%.

Table.1 List of the botanicals used in the experiment

Sl. No.	Common name	Scientific name	Parts used
1.	Bael	*Aegle marmelos*	Leaves
2.	Blue gum	*Eucalyptus globules*	Leaves
3.	Bougainvillea	*Bougainvillea spectabilis*	Leaves
4.	False ashoka	*Polyalthia longifolia*	Leaves
5.	Garlic	*Allium sativum*	Cloves
6.	Ginger	*Zingiber officinale*	Rhizome
7.	Golden dewdrop	*Duranta repens*	Leaves
8.	Hibiscus	*Hibiscus rosa sinensis*	Leaves
9.	Holy basil	*Ocimum sanctum*	Bulb
10.	Neem	*Azadirachta indica*	Leaves
11.	Onion	*Allium cepa*	Bulb
12.	Papaya	*Carica papaya*	Leaves
13.	Periwinkle	*Catheranthus roseus*	Leaves
14.	Satavari	*Asparagus racemosus*	Leaves
15.	Shrub verbena	*Lantana camara*	Leaves
16.	Sweet basil	*Ocimum basilicum*	Leaves
17.	Thai nightshade	*Solanum trilobatum*	Leaves
18.	Turmeric	*Curcuma longa*	Rhizome
19.	Veld grape	*Cissus quadrangularis*	Whole
20.	Yellow nightshade	*Solanum xanthocarpum*	Leaves

Table.2 Evaluation of botanicals against *Colletotrichum capsici*

Treatment	Botanicals @ 10%	Mean colony diameter (cm)	Per cent inhibiton
T_1	Bael	3.6	60.19 (50.88)
T_2	Blue gum	5.8	35.74 (36.72)
T_3	Bougainvillea	8.4	6.67 (14.94)
T_4	False ashoka	5.4	39.81 (39.12)
T_5	Garlic	4.4	51.30 (45.74)
T_6	Ginger	5.7	36.67 (37.27)
T_7	Golden dewdrop	8.3	7.78 (16.20)
T_8	Hibiscus	5.8	35.37 (36.50)
T_9	Holy basil	7.9	12.78 (20.94)
T_{10}	Neem	6.4	28.70 (32.39)
T_{11}	Onion	5.5	39.07 (38.69)
T_{12}	Papaya	5.1	43.70 (41.38)
T_{13}	Periwinkle	7.4	17.41 (24.66)
T_{14}	Satavari	8.3	8.15 (16.58)
T_{15}	Shrub verbena	6.3	30.19 (33.33)
T_{16}	Sweet basil	7.4	18.15 (25.21)
T_{17}	Thai nightshade	7.4	17.41 (24.66)
T_{18}	Turmeric	4.4	50.93 (45.53)
T_{19}	Veld grape	6.1	24.44 (29.63)
T_{20}	Yellow nightshade	7.4	18.15 (25.21)
T_0	Control	9	00.00
S. Em ±		0.03	0.25
CD (p=0.05)		0.09	0.72

Note: Figure in the table are mean values and those in parenthesis are angular transformed value

Table.3 Effect of botanicals on seed germination and seedling vigour

Treatment	Plant extracts (10%)	Percent germination (%)	Root length (cm)	Shoot length (cm)	Vigour index
T_1	Bael	92.00 (73.92)	5.6	2.5	738.67
T_2	Blue gum	93.33 (75.55)	4.8	2.4	672.53
T_3	False ashoka	88.00 (69.73)	4.6	2.3	607.20
T_4	Garlic	94.67 (76.83)	5.4	2.5	747.73
T_5	Ginger	90.67 (72.29)	4.7	2.4	646.53
T_6	Onion	85.33 (67.81)	4.4	2.2	558.93
T_7	Papaya	90.67 (72.82)	4.5	1.9	578.00
T_8	Turmeric	89.33 (71.54)	4.7	2.4	635.07
T_0	Control	78.67 (62.53)	4.0	1.5	430.40
S. Em ±		1.62	0.11	0.02	15.91
CD (p=0.05)		4.61	0.30	0.07	45.40

Note: Figure in the table are mean values and those in parenthesis are angular transformed value

Inhibition of plant pathogenic fungi by many antifungal compounds of plant origin has been supported by previous works of Hemmanavar (2008), Ranasingh *et al.*, (2011), Geat (2014), and Harsha *et al.*, (2004).

Evaluation of botanicals on seed germination and seedling vigour index

In the present investigation, eight most promising botanicals *i.e. A. marmelos, Eucalyptus globules, Polyalthia longifolia, A. sativum, Zingiber officinale, A. cepa, Carica papaya, and C. longa*, were selected based on their effectiveness in inhibiting the mycelial growth of *C. capsici* and were further evaluated for its effect on germination and seedling vigour index. The results on the effect of botanicals on germination and seedling vigour index are presented in Table 3. The results revealed that the highest germination was obtained from T4 (garlic) with a germination of 94.67%. This was at par with T2 (blue gum), T1 (bael), T7 (papaya), T5 (ginger), T8 (turmeric) and T3 (false ashoka) showing a germination percentage of 93.33, 92.00, 90.67, 90.67, 89.33, 88.00 respectively. Among the botanicals T6 (onion) with 85.33% recorded the lowest germination. T0 (control) recorded the least germination of 78.67% amongst all the treatments. The results are similar to those found by Sundramoorthy *et al.*, (2014) who reported that among the various plant products tested, *A. sativum* followed by *E. globules* showed maximum germination and seedling growth amongst the botanicals that were tested. Islam *et al.*, (2010) also observed in his experiment that seed treatment with garlic enhanced seed germination. These findings are also similar to the work done by Choudhary *et al.*, (2013) who reported a maximum per cent seed germination (94%) as a result of seed treatment by safeda (*E. tereticornis*). Plant extracts are known to effect seed germination and initial seedling growth parameters (Sahoo *et al.*, 2015).

In the conducted experiment it has been recorded that most of the botanicals increased the germination of the seeds as compared to control which showed the least germination percentage. The reason for increase in germination by use of botanical extract presumed that these botanicals contain some of the micronutrients which are conducive for seed invigoration as reported by Sasthri and Srimathi (2010).

Seed soaking with botanical extract also had a significant effect in root length of chilli. The highest root length was recorded from T_1 (bael) with 5.6 cm which was at par with T_4 (garlic) showing 5.4 cm. This was followed by T_2 (blue gum) which was found to be at par with T_5 (ginger), T_8 (turmeric), T_3 (false ashoka), T_7 (papaya) and T_6 (onion) each showing a root length of 4.8, 4.7, 4.7, 4.6, 4.5 and 4.4 cm. The lowest root length of 4.0 cm was recorded in T0 (control), which might be due to low availability of nutrients in water. The increase in the root length of chilli seedlings might be due to presence of phenols in the botanical extracts which could have promoted the root length.

In the present study the longest shoot length was obtained from T_4 (garlic) with 2.5 cm which was at par with T_1 (bael). This was followed by T_2 (blue gum) which was found to be at par with T_5 (ginger) and T_8 (turmeric) each showing a shoot length of 2.4 cm. These were further followed by T_3 (false ashoka), T_6 (onion) and T_7 (papaya) with 2.3, 2.2 and 1.9 cm. The shortest shoot length was observed in T9 (control) with 1.5 cm.

The increased shoot length due to seed treatment with botanical extracts may be attributed to cell wall extension and increased metabolic activities at low water potential, as

in matripriming as reported by Afzal *et al.*, (2002). Botanicals contain various growth promoting substance and nutrients (Anon, 2002) which could support better seedling performance.

Perusal of the data (Table 3) clearly proves that amongst the seed treated with botanicals @ 10%, T_4 (garlic) gave the highest vigour index at 747.73 which was at par with T_1 (bael) showing a vigour index of 738.67. This was followed by T_2 (blue gum) which was at par with T_5 (ginger), T_8 (turmeric), T_3 (false ashoka), T_7 (papaya) and T_6 (onion) each with a vigour index of 672.53, 646.53, 635.07, 607.20, 578.00 and 558.93 respectively. Among the treatments T_0 (control) recorded the lowest vigour index of 430.40.

Similar findings were reported by Sundramoorthy *et al.,* (2014) who also observed that treatment of chilli seeds with garlic recorded the maximum vigour index. The result are also in accordance with the works of Choudhary *et al.,* (2013) who observed a seedling vigour index of 540.05 by treatment of seeds with safeda leaves extract (*E. terticornis).* Similar results in increasing per cent germination and enhancing growth characters of chilli seedlings by use of different plant extracts were also reported by Sahoo *et al.,* (2015), Alam *et al.,* (2014), Kumar *et al.,* (2014) and Islam and Faruq (2012)

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