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Botanicals for control of anthracnose of bell peppers

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A B S T R A C T

Botanicals or plant extracts from *Cathranthus roseus, Coleus aromaticus , Manilkara zapota* and *Azadirachta indica* was studied by poisoned food technique, seed germination and under green house experiments for control of *Colletotrichum capsici*, fungal pathogen responsible for anthracnose disease in bell peppers (*Capsicum frutescence* L.). The results showed that all the selected plants have potential to inhibit the radial mycelial growth of *C. capsici in-vitro*. The plant extract from *C. aromaticus* at 50% concentration showed 42% radial mycelial growth inhibition in poisoned food technique, whereas 15% increased seed germination and 26% reduced disease incidence is recoded when compared to untreated control. In pot experiments, there is an increase in height and weight of the plants by 22% and 44% respectively in seeds treated with *C. aromaticus* when compared to chemical fungicide Bavistin and also 13% reduction in disease incidence is observed when compared to untreated control. From the results it is evident that *C. aromaticus* were effective in not only controlling the disease but also increase the height and weight of the plants and hence are highly recommended for plant disease control.

Keywords**:** *Coleus aromaticus, Colletotrichum capsici, Capscium frutescence,* bavistin, poisoned food technique

Introduction

Capsicum (*Capsicum frutescence* L.) is a fruit vegetable belongs to the family Solanaceae and is believed to have originated from South America. The crop is cultivated in many regions of the world, including India, China, Europe, Dominican Republic, Hiti, Hawaii, Mexicao, Europe, Philippines and the US. In Karnataka state (India), capsicum is cultivated in an area of about 3,284 ha with annual production of 22,331 tons (Anon. 1995). Capsicum is one of the important high value crops due to its nutritional and medicinal properties. It is rich in vitamin 'C', 'B' and 'E'. Anthracnose of capsicum is one of the most important disease caused by *C. capsici* which is major economic constraints for capsicum production worldwide. The disease is seed borne as pathogen can survive in and on seeds as fruiting bodies (acervuli) and micro-sclerotia, with favorable environmental conditions, may be transmitted to cause diseases in developing seedlings or plants. Hence the effective control measures should aim on treating seeds, to eliminate or prevent the *Colletotrichum* infection. Due to increase concern over human health, plants, animals and harmful effects to our environment, use of synthetic fungicides for control is not acceptable. It was reported that 0.1% of the synthetic fungicides used for crop protections reach the target pathogen, leaving 99.9% remaining to get enter into environment causing hazardous effects to non-target organisms (Pimental 1995). In order to overcome such problems alternative methods which are safe, eco-friendly and economically feasible are used for pathogen control. One such control measures is the use of botanicals or plant extracts which may be toxic to a spe-

cific pathogen and harmless to humans and surrounding environment. Botanicals are easily available, cost effective or economically feasible and are easy to prepare, even farmers can prepare it easily. Plant extracts not only help in control of plant pathogens but also stimulate the growth of host plant and this is may be due to the presence of some growth hormones such as IAA and IBA (Datar 1999). Hence, botanicals (plant extracts) obtained from plants which are active against plant pathogens are used for present research work, as most of the plants or botanicals are not evaluated for control of anthracnose in capsicum plants.

Materials and Methods

Collection and identification of plants having antimicrobial properties

Plants were collected from fields, local nurseries and different geographical locations during their growing season. For the present research work four plants/trees were selected based on their antimicrobial properties, availability, easy application and by reviewing the previous studies carried out by several workers for crop management, they are *Catharanthus roseus* (L.) G. Donn*, Coleus aromaticus* (Benth), *Manilkara zapota* (L.) P. Royen and *Azadirachata indica* A. Juss.

Preparation of plant extracts

The aqueous leaf extract of *Cathranthus roseus, Coleus aromaticus , Manilkara zapota* and *Azadirachta indica* were prepared by taking 100 g fresh leaf samples, thoroughly washed with sterile distilled water, blot dried and macerated with 100 ml distilled water (w/v) using blender (Warring International, New Hartford, CT, USA) for 10 min. The macerate was first filtered through double layered muslin cloth and then centrifuged at 4000 g for 30 minutes. The supernatant was filtered through Whatmann No.1 filter paper and sterilized at 120°C for 30 min, which served as the mother extract.

Comparative study on the antimicrobial properties of plant extracts

Poisoned food technique

The sterilized plant extract (mother extract) was amended with PDA to make 5%, 10%, 25% and 50% concentration in the Petri plates. The solidified agar plates in triplicates were inoculated at the centre with 5 mm diameter mycelial disc of pathogen *C. capsici* and incubated at 27±1ºC for seven days (Nene and Thapliyal 1979). The plates without extract will serve as control. The colony diameter was measured and per cent inhibition of radial growth was calculated by using the formula given by Vincent (1927).

$$I = \frac{C-T}{C} \times 100$$

Where, I- per cent inhibition; C- Colony diameter in control; T- Colony diameter in treatment.

Effect of plant extracts on seed germination and seedling vigour

Capsicum seeds (100g) were treated with aqueous plant extract (100ml) of *Cathranthus*

roseus, Coleus aromaticus, M. zapota and *A. indica*, shaken well and kept for 24h. The two control set of experiments i.e., seeds treated with recommended chemical and seeds without any treatment were also carried out. After 24h, seeds will be blot-dried and the germination test was carried out by paper towel method.

Germination test

400 seeds in four replicates of 100 seeds each were placed in between two wet paper towels specially made for germination test; the papers were rolled and kept for incubation at 27±1°C. On 15th day, the germinated seeds were counted and the per cent germination was computed by using the formula

$$\text{Per cent germination} = \frac{\text{No. of seeds germinated}}{\text{No. of seeds sown}} \times 100$$

Seedling vigour index

The vigour index was calculated according to the method suggested by Abdul-Baki and Anderson (1973). Ten normal seedlings were taken out carefully at random from each treatment and the root length and shoot length were measured. An Average length of ten10 seedlings was calculated and expressed as mean seedling length. The vigour index was calculated by using the formula

VI= (Mean root length + Mean shoot length) × Per cent germination.

Efficacy of plant extracts under green house conditions (pot experiment) for incidence of anthracnose disease

Treated capsicum seeds were tested under green house condition to evaluate the antagonistic activity against the expression of anthracnose and for establishment on capsicum plant *in vivo*. Capsicum seeds were treated as mentioned above and 25 seeds were sown in plastic pots each with four replicates. The diameter of plastic pot is 22 cm with a holding capacity of 3 kg soil. Each pot is filled with 2 kg of sterilized soil without any fertilizers and two controls were taken, seeds treated with sterile distilled water and chemical fungicide Bavistin (2g/kg seeds). Seeded pots were maintained at 28 to 30ºC and at 95% relative humidity. The pots were observed at the end of vegetative growth and results were taken in the form of growth parameters like plant height and dry weight of plants in both treated and untreated controls. Plants were measured from the base to the tip and dry weight was determined by drying the plants in an oven at 65ºC (Dubey *et al*. 2007).

Results

Poisoned food technique

It was observed that all the aqueous plant extracts tested showed antifungal activity against the growth of *C. capsici in vitro*. At 50% concentration aqueous plant extract of *Coleus aromaticus* treated PDA plates showed 42.59% inhibition of radial mycelial growth of *C. capsici* when compared to untreated control (Table 1). Whereas, least antifungal activity was exhibited by *Vinca rosea* which exhibited 8.44% at 50% concentration.

Effect of botanicals on seed germination, seed vigour and per cent disease incidence

Per cent seed germination

All aqueous plant extract treated seeds showed significant increase in seed germination when compared to untreated control. Increase in seed germination (15.76%) is observed in seeds treated with aqueous plant extracts of *Coleus aromaticus* . Whereas, seeds treated with *Manilkara zapota, Azadiracta indica* and *Vinca rosea* showed 11.08%, 6.03% and 3.57% increase seed germination respectively when compared to untreated control seeds (Table 2). Also it was observed that an increase (17.10%) in seed germination of seeds treated with *Coleus aromaticus* when compared to chemical fungicide Bavistin (methyl benzimidazol-2-ylcarbamate) treated capsicum seeds.

Seedling vigour

Maximum seedling vigour is observed in seeds treated with chemical fungicide Bavistin (729.39) and *M. zapota* (618.25) which is 35.47% and 31.88% more when compared to untreated control (410.30) respectively. Whereas, least vigour index is observed in seeds treated with extracts of *V. rosea* (470.63).

Disease incidence

It is observed that 60.60% and 26.09% reduced disease incidence is exhibited by the seeds treated with Bavistin and aqueous plant extract of *C. aromaticus* respectively when compared to untreated control. Whereas, 21.52% reduction in disease incidence is observed with *M. zapota* treated seeds.

Greenhouse experiment (pot experiment)

All plant extracts tested showed increase in height and weight of the plants and also slightly reduced the disease incidence when compared to Bavistin (methyl benzimidazol-2 -ylcarbamate) and untreated control. A raise in height of plants by 22.74% and 19.74% was observed in seeds treated with *C. aromaticus* when compared to chemical fungicide Bavistin and untreated control respectivley, which is followed by seeds treated with *M. zapota*, which shows 13.91% and 10.56% increase in height of the plants respectively observed after 45 days of sowing in pots (Table 3). Whereas, 44.80% and 46.78% increase in weight of the plants were observed in plants treated with aqueous extract of *C. aromaticus* when compared to bavistin and untreated control, respectively.

Disease incidence

There is slight reduction in per cent disease incidence by 13.23% in seeds treated with aqueous extract of *C. aromaticus.* Whereas, *M. zapota* shows 12.08% reduced disease incidence (Table 3) when compared to untreated control. Whereas, 47.44% reduced disease incidence is observed in seeds treated with chemical fungicide Bavistin when compared to untreated control.

Table 1.
Radial growth of *C. capsici* treated with different concentration of plant extract (non-volatile compounds) after seven days of incubation

Name of the Plant*	Radial mycelium growth (mm)				
	Concentration of culture filtrate (%)				
	0	5	10	25	50
Azadiracta indica	75.42±0.15^{c}	73.52±0.11^{c}	67.12±0.17^{c}	56.06±0.14^{b}	49.64±0.08^{b}
Coleus aromaticus	64.58±0.14^{a}	61.35±0.11^{a}	57.31±0.10^{a}	49.19±0.15^{a}	37.07±0.12^{a}
Manilkara zapota	81.54±0.13^{d}	74.55±0.12^{d}	73.39±0.11^{d}	67.71±0.11^{c}	56.33±0.10^{c}
Vinca rosea	69.37±0.11^{b}	68.59±0.09^{b}	66.35±0.11^{b}	72.15±0.13^{d}	63.51±0.14^{d}
F-value	16661.38	16815.03	15737.45	34870.95	53972.04

*Experiment was performed by maintaining three replicate per treatment. Mean values with different letters are significantly different from each other as indicated by Turkey's HSD (p = 0.05)

Table 2.
Effect of treatments on seed germination, seedling vigour and per cent disease incidence

Treatment*	Per cent germination	Seedling length (cm)	Seedling vigour index	Disease incidence (%)
Azadirachta indica	68.40±1.18^{c}	8.50±0.17^{c}	581.43±12.12^{c}	26.26±1.10^{c}
Coleus aromaticus	76.30±0.40^{e}	6.20±0.27^{a}	473.67±22.95^{b}	23.62±0.13^{b}
Manilkara zapota	72.28±0.37^{d}	8.55±1.23^{c}	618.25±15.00^{d}	25.08±0.18^{b}
Vinca rosea	66.65±0.36^{b}	7.22±0.29^{b}	470.63±18.61^{b}	30.34±1.14^{d}
Bavistin	63.25±0.13^{a}	11.53±0.12^{d}	729.39±7.87^{e}	12.59±0.16^{a}
Control	64.27±0.21^{a}	6.55±0.19^{a}	421.13±12.64^{a}	31.96±0.15^{e}
F-value	1314.28	123.59	142.16	3596.25

*Experiment was performed by maintaining three replicate per treatment. Mean values with different letters are significantly different from each other as indicated by Turkey's HSD (p = 0.05)

Table 3.
Effect of treatments on plant height, dry weight and per cent disease incidence of anthracnose recorded 45 days after sowing

Treatment*	Plant length (cm)	Dry weight (g)	Disease incidence (%)
Azadirachta indica	16.95±0.27^{b}	1.0143±0.64^{b}	16.66±0.11^{b}
Coleus aromaticus	19.30±0.35^{c}	1.2079±1.08^{c}	15.93±0.23^{b}
Manilkara zapota	17.32±0.71^{b}	1.2542±1.01^{c}	16.38±1.17^{b}
Vinca rosea	16.60±1.24^{b}	1.1413±0.78^{c}	17.89±0.38^{c}
Bavistin	14.91±0.26^{a}	0.6667±0.51^{a}	9.65±0.12^{a}
Control	15.49±0.31^{a}	0.6428±1.05^{a}	18.36±0.53^{d}
F-value	67.13	87.43	214.91

*Experiment was performed by maintaining three replicate per treatment. Mean values with different letters are significantly different from each other as indicated by Turkey's HSD (p = 0.05)

Discussion

Botanicals or plant extracts are receiving renewed interest in both scientific community and by farmers as alternatives to chemical fungicides for crop protection. As plant extracts are non-phytotoxic, easy-biodegradability and the most important is cost effective. Plant products have ability to control plant pathogens and have potential to be valued in pest management (Mishra and Dubey 1994). Botanicals contains a wide variety of secondary metabolites such as phenols, flavonoids, quinones, tannins, alkaloids, saponins, essential oils and sterols which plays an important role in plant protection (Tripathi *et al*. 2004). Hence screening of plant extracts for their antifungal activity against the pathogen is essential to minimize the use of chemical fungicides.

In the present research work, we have evaluated four different plant extracts obtained from *Catharanthus roseus*, *Coleus aromaticus*, *M. zapota* and *A. indica*. for the possible presence of fungitoxic activity against *C. capsici* by using poison food technique, seed treatment and pot experiments.

It was evident from the results that aqueous plant extract from *Coleus aromaticus* showed maximum inhibition of radial mycelial growth of the pathogen at 50% concentration when tested using poisoned food technique *in vitro*. It was also observed that difference in antifungal activity (per cent inhibition) of aqueous extracts of all other tested plants against test pathogen. Similar findings were also reported by many workers for antifungal properties of plant extracts against *Colletotrichum* sp. Guerrero-Rodriguez *et al*. (2007) reported 93% growth inhibition of *C. gleosporoides* with methanol-chloroform extracts of *Flourensia cernua*. There are reports that some essential oils of different plant species showed inhibition between 50% and 60% (Meazza *et al*. 2003; Meepagala *et al*. 2002). Dutta *et al*. (2004) reported that crude extract from *Allium sativum* at 20% concentration showed reduced mycelial growth of *R. solani in vitro*. All the plant extracts were further evaluated for their efficacy in seed germination, seedling vigour and per cent disease incidence in capsicum seeds. From the results it was revealed that seeds treated with *Coleus armaticus* showed maximum seed germination and also reduced the per cent disease incidence when compared to all other treatments (except Bavistin). Whereas, seeds treated with *M. zapota* showed highest seedling vigour.

In pot experiments, after 45 days of sowing, seeds treated with plant extracts *C. aromaticus* showed increase in height and dry weight of the plants and also reduced the anthracnose disease incidence when compared to all other plant extract treated and untreated seeds. Among all the plant extracts tested, *Coleus aromaticus* showed good activity in controlling mycelial growth in poisoned food technique, increased the seed germination and reduced the anthracnose per cent disease incidence in pot experiments. Hence it can be used to treat capsicum seeds to prevent seed-borne infection with *C. capsici*.

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