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Zygosporium masonii: a new fungal antagonist against *Colletotrichum capsici* incitant of anthracnose on bellpeppers

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The antagonistic activity of *Zygosporium masonii* was studied against *Colletotrichum capsici,* pathogen responsible for Anthracnose disease in bell pepper, by dual culture and poisoned food technique *in-vitro*. Seed vigour index and pot experiments were also conducted by treating *Capsicum* seeds with culture filtrate of *Z. masonii* under greenhouse conditions. Formation of clear inhibition zone in dual culture and decrease in mycelial growth of pathogen were observed when treated with volatiles and non volatile compounds from the antagonist. *Z.masonii* treated seeds showed significant increase in seed germination, shoot length, root length and dry weight of the plant. The experiments showed that *Z .masonii* is a potential antagonist to control anthracnose and can be used as a biocontrol agent (BCA).

Key words: *Zygosporium masonii, Colletotrichum capsici*, Bell pepper, Anthracnose, Seed vigour.

Introduction

The Bell pepper (*Capsicum frutescence* L.) belongs to family *Solanaceae*, is one of the major spice crop cultivated in an area of about 3,284 hectares in Karnataka state, India (Anon, 1995). The capsicum fruit is used as a fresh or processed vegetable adding flavor, color and is rich source of Vitamin C. Several fungal diseases affect capsicum crop among which anthracnose is most severe causing fruit loss. It is observed that about 10-80% yield loss results both in the field as well as in storage (Poonpolgul and Kumphai, 2007).

Anthracnose of bellpepper is caused by the fungus *Colletrichum capsici* (sydow) Butler and Bisby (1918) which infects virtually on all parts of the pepper plant at all stages of plant growth. The most characteristic feature of anthracnose is formation of acervuli in concentric ring, forming fruit lesions (Fig. 1). Although anthracnose disease can be controlled by applying

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commercially available fungicides like bavistin (carbendazim), dithane M-45, captan, maneb (manganese ethylenebisidithiocarbamate).

Use of chemicals for control of plant disease is one of the most commonly used strategy usually what farmers followed, but nowadays people are more aware of various side effects caused by pesticide residues present in food and water. Also, relaying wholly on chemicals may be very expensive for farmers too, and also may lead to environmental pollution. Keeping all these undesirable problems in view, there is needed to incorporate an alternative approach or strategy for control of plant disease.

Use of antagonist for disease control offers an alternative to the use of chemical pesticides. These biological control methods which make the use of microorganisms are highly specific, eco-friendly and the most important cost effective approach.

In the present study an attempt was made to control anthracnose of bellpepper using biocontrol agents.

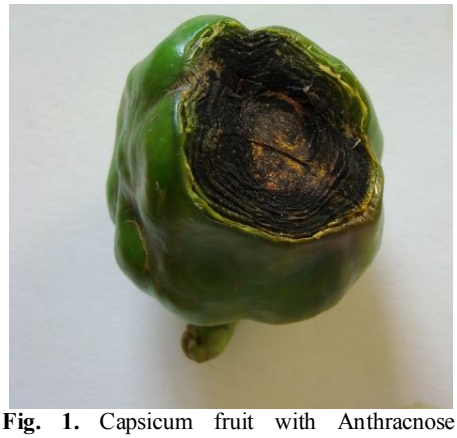

Fig. 1. Capsicum fruit with Anthracnose symptom- formation of acervuli in concentric rings.

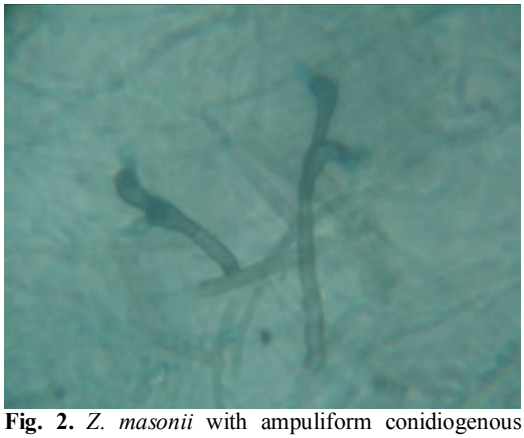

Fig. 2. *Z. masonii* with ampuliform conidiogenous cells.

Material and methods

Isolation and Identification of fungal cultures- *Colletotrichum capsici* were isolated from fruits of bell pepper (*Capsicum frutescence* L.) showing anthracnose symptoms from major capsicum growing areas of Karnataka state, India. All the isolates were tested for pathogeneicity to capsicum using Koch's postulate. The isolate showing maximum virulence were chosen for further experiments. The antagonists was isolated from rhizosphere soil, identified and maintained on potato dextrose agar (PDA) under refrigerated conditions.

Antagonism in-vitro by Dual culture method

The fungal antagonist was tested against the pathogen *C. capsici* by dual culture technique. Petri plates containing sterile 20 ml potato dextrose agar (PDA) medium were taken, to which mycelial discs of 5 mm diameter from 7 day old actively growing cultures of antagonist and pathogen were placed 4 cm apart. These Petri plates were incubated at 27±1ºC for seven days and radial growth was measured. The percent inhibition of pathogen with antagonist (dual culture) was calculated over control.

Effect of volatile compounds from Z. masonii on radial growth of C. capsici

The method given by Dennis and Webster (1971) were followed, to test the volatile compounds from *Z. masonii* against the radial growth of *C. capsici*. The two bottom portion of petri plates containing PDA were inoculated with a 5 mm disc of *C. capsici* and *Z. masonii* respectively and both inoculated bottom plates were placed facing each other and sealed with cellophane adhesive tape.

The petri plate containing PDA without antagonist served as control. The observations on the radial growth of the test fungus were recorded after 7 days of incubation at 27±1ºC. The colony diameter of the test fungus in the treated plate is compared to control plate that calculated as percent growth inhibition.

Effect of non-volatile (culture filtrate) compounds from antagonist(s) on the radial growth of C. capsici

The biocontrol agent was grown in Potato dextrose broth (PDB) at 27±1ºC with intermittent shaking at 150 rpm. The metabolites were collected after 12 days, filtered and autoclaved. The sterilized filtrate was amended with PDA to make 5%, 10%, 25% and 50% concentrations in the petri plates. The solidified agar plates in triplicates were inoculated at the centre with 5 mm diameter mycelial disc of pathogen and incubated at 27±1ºC for 7 days. The Plates without filtrate served as control. The colony diameter was measured and percent inhibition of radial growth was calculated.

Effect of biocontrol agent on seed germination and seedling vigour

Capsicum seed were treated with pure cultures of *Z. massoni* (1X10 ^{8} cfu/ml) prepared in sterile distilled water shaken well and kept for 24 hour. Seeds treated with recommended chemical and seeds without any treatment served as control. After 24 hour, seeds were 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±1ºC. On 15th day, the germinated seeds were counted and the percent germination was calculated.

Seedling vigour index

The vigour index was calculated according to the method suggested by Abdul-Baki and Anderson (1975). 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 ten seedlings was calculated and expressed as mean seedling length. The vigour index (VI) was calculated by using the formula as follows:-

VI= (Mean root length + Mean shoot length) X Percent germination

Greenhouse experiment

Treated capsicum seeds were tested under greenhouse 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 twenty five seeds were sown in plastic pots each with 4 replicates and two controls were taken, seeds treated with sterile distilled water and chemical fungicide bavistin (2 g/kg seeds). 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. Seeded pots were maintained at 27 ºC to 30 ºC 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

Identification of Antagonist: It was observed that among the fungal isolates tested the *Zygosporium masonii* showed good activity aginst the test pathogen by forming a clear inhibition zone.

The genus *Zygosporium* is hypomycetous microfungi possessing darkly pigmented, incurved vesicular cells that give rise to ampuliform conidiogenous cells. *Z. masonii* is characterized by the presence of vesicular conidiophores in stacked chains of 1-6, each with 2 conidiogenous cells, and a cylindrical sterile

cell at the apex. Conidia is ovoid, hyaline and smooth (Fig. 2) (Mason 1941; Hughes 1951).

It is observed that *Z. masonii* inhibited the radial growth of the pathogen by 8.70% when compared to control and formed a zone of inhibition measuring 1 cm between the two cultures (Fig. 3).

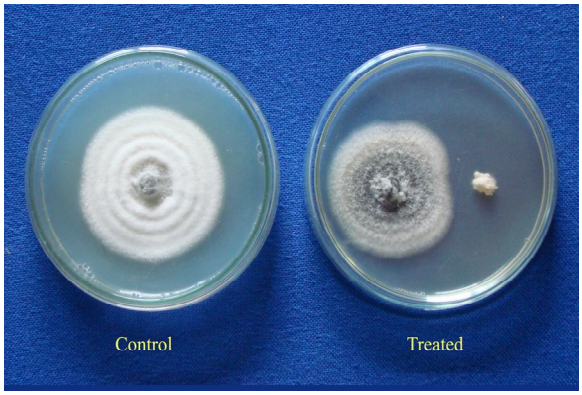

Fig. 3. Effect of fungal biocontrol agent *Z.masonii* on growth of *C. capsici in* vitro by dual culture technique.

Effect of volatile compounds

After 48, 96, and 144 of incubation it is observed that radial growth of pathogen was inhibited by 16%, 4%, 22% respectively and at 168 hours of incubation there was 41% inhibition when compared to control (Fig. 4).

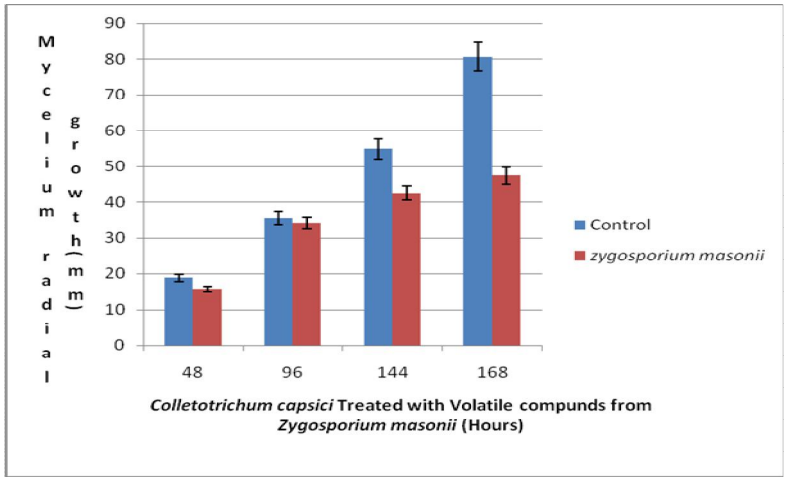

Fig. 4. Effect of volatiles from *Z. masonii* on Radial growth of *C. capsici*

Effect of Non-volatile compunds

Culture filtrate (non-volatiles) at 5% concentration inhibit the radial growth of *C.capsici* by 29%. Whereas, 56% growth inhibition was recorded (Fig. 5) at higher concentrations (50%) of culture filtrate.

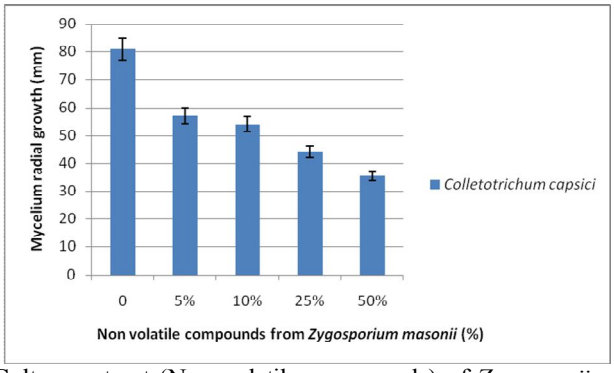

Fig. 5. Effect of Culture extract (Non-volatile compounds) of *Z. masonii* on radial growth of *C. capsici*

Effect of Biocontrol agent (BCA) on seed germination and seedling vigour

On 15th day after plating, highest percentage germination was observed (Table 1) in seeds treated with Bavistin which were recorded 37.45% and culture filtrate showed 0.6% increase germination when compared to untreated seeds. Vigour index of seeds is 396.39 in control, is raised to 729.27 (45.64%) and 428.06 (7.39% more) incase of seeds treated with bavistin and BCA *Z. masonii* respectively. Whereas seeds treated with chemical fungicide bavistin and *Z. masonii* culture filtrate reduced per cent disease incidence by 61.41% and 22.12% respectively when compared to untreated control.

Table 1. Both treated and untreated control tested for seed germination and seedling vigour

Treatments*	**Percent germination (%)**	**Seedling length (mm)**	**Seedling vigour index**	**Disease incidence (%)**
Z. masonii (culture filtrate)	39.82±0.30^{ab}	10.75±0.12^{ab}	428.06±4.32^{b}	25.41±0.22^{b}
Bavistin	63.25±0.13^{c}	11.53±0.18^{c}	729.27±7.87^{c}	12.59±0.16^{a}
Control (H_2O)	39.56±0.26^{a}	10.02±0.14^{a}	396.39±7.05^{a}	32.63±1.11^{c}
F-Value	3419.87	2573.25	3186.92	6891.52

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

Pot experiment

After 45 days of sowing, it is observed that *Zygosporium* treated seeds, there is increase (5.84%) in height of the plants when compare to control. Whereas 12.47% increase in height was observed in seeds treated with bavistin. It is also observed that the dry weight of antagonist treated plants and bavistin treated plants is 13.74% and 13.82% more when compare to untreated control (H2O) respectively (Table 2).

Table 2. Both treated and untreated controls tested for plant height, dry weight and per cent disease incidence of anthracnose recorded 45 days after sowing

Treatments*	Plant height (cm)	Dry weight (g)	Disease incidence (%)
Zygosporium massonii	13.86 ± 0.39^{ab}	0.6127 ± 0.32^{b}	11.45 ± 0.11^{b}
Bavistin	14.91 ± 0.26^{c}	0.6667 ± 0.51^{c}	9.65 ± 0.12^{a}
Control	13.05 ± 0.29^{ab}	0.5285 ± 0.18^{a}	17.63 ± 0.11^{c}
F-value	22.60	388.52	1364.81

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

Discussions

The microfungi *Zygosporium masonii*, which showed significant result while inhibiting the growth of the pathogen, are proven to be a promising biocontrol agent for control of anthracnose disease of bell pepper. As chemical fungicides pose threat to human beings and environment, also develops resistant strains, there is needed of continues search for new biological control agents (BCA). One such trial was carried out in our present research work by introducing *Z. masonii* for control of *C. capsici*. Formation of visible inhibition zone (of 1cm) in dual culture, suggested that *Z. masonii* exert inhibitory effect on the mycelial growth of the test pathogen. Whereas growth of *C. capsici* was inhibited when exposed to the volatile compounds produced in presence of *Z. masonii*. The inhibition started after 24 hours incubation while maximum inhibition of mycelial growth was observed after 168 hours of incubation. Earlier reports also revealed that volatile compounds has antimicrobial properties and is effective against a wide range of plant pathogenic microorganisms eg. *Fusarium* sp., *Rhizoctonia* sp., *Lentinus lepidus, Coriolus versicolor, Curvularia* sp., *Bipolaris* sp., *Colletotrichum acutatum* etc. (Dois and Mori 1994; Yan *et al.* 2001; Zivkovic *et al.* 2010).

Crude extracts (non-volatile compounds) from *Z. massoni* also showed significant result by inhibiting radial growth of mycelium of test pathogen

when compared to volatile compounds. Earlier, it is reported that secondary metabolites such as Zygosporin A, Cytochalasin and Zygosporamide were isolated from *Z .masonii*. (Hayakawa, 1968; Brown and Spudich, 1981; Dong-chan *et al*. 2006). All the above result, secondary metabolites expressed cytotoxic activity and used to treat cancer.

No reports were found, use of secondary metabolites from *Z. masonii* for control of plant pathogens. Secondary metabolites (culture filtrate) from *Z. masonii* not only inhibit the growth of *C. capsici in-vitro*, but also increased the vigour of Capsicum seeds. Pot experiments also revealed that an increase in both height and weight of the plant when compared to control, thus clearly indicating capsicum seeds treated with biocontrol agent *Z. masonii* gave potential not only in controlling the disease but also play a role in promoting capsicum seed germination.

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