The Bioscan 10(1): 1901-1904, 2015
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

# INTEGRATED MANAGEMENT OF ANTHRACNOSE OF CHILLI CAUSED BY *COLLETOTRICHUM CAPSICI* IN WEST BENGAL CONDITION

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**KEYWORDS**
Fungicides
Bioagents
Botanicals
*Colletotrichum capsici*
Per cent disease index

**Received on :**
03.09.2015

**Accepted on :**
20.11.2016

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**ABSTRACT**
Different botanicals, fungicides and bioagents were evaluated in vitro and field condition against *Colletotrichum capsici*. Amongst fungicides it was found that at 150 ig/ml concentration 100% inhibition in mycelial growth was recorded in Tebuconazole, Mancozeb and Trifloxystrobin + Tebuconazole and minimum inhibition in mycelial growth was recorded in Carbendazim(6.71%). Two isolates each of three bioagents*viz*., *Trichoderma harzianum*, *T. viride* and *Pseudomonas fluorescens* were evaluated for their antagonistic properties against *C. capsici*. *T. harzianum* isolate Th-2 was found most effective giving 77.78% inhibition on the mycelial growth followed by *T. harzianum* isolate Th-1 inhibiting 74.00%. Among four botanical oils Garlic, Neem, Polyalthia and Citronella were tested for their *in vitro* efficacy against *C. capsici* at concentrations of 0.05%, 0.1% and 0.2%. Garlic oil at all concentrations and neem oil at 0.1% gave cent per cent inhibition of mycelial growth of the fungus followed by Polyalthia at 0.20% (84.45%). Under field condition, significant minimum disease severity of 4.85% was found in plot receiving seed treatment with garlic oil @ 0.5 ml/kg seed followed by three sprays of Trifloxystrobin + Tebuconazole (50%) @1g/litre water.

## INTRODUCTION

Chilli is a significant source of income making in India, the world's single largest producer and exporter to the USA, Canada, UK, Saudi Arabia, Singapore, Malaysia, Germany and many other countries across the world (Ashwini and Srividya, 2014). The sustainability of chilli-based agriculture is threatened by a number of factors. Anthracnose disease is a major problem in India and one of the most significant economic constraints to chilli production worldwide, especially in tropical and subtropical regions (Than *et al*., 2008). Hegde *et al* (2002) determined the *in vitro* efficacy of 3 triazole fungicides, *i.e.* hexaconazole (0.1%), propiconazole (0.1%) and triadimefon (0.1%), against the fruit rot pathogen (*Colletotrichum capsici*) of chilli, by poison food technique. The radial growth of the fungus in Petri plates was measured and the results were expressed as percentage inhibition of mycelial growth over control. Significant inhibition of mycelial growth was recorded with the 3 fungicides (85.29, 80.97 and 79.67%, respectively). Rajesh *et al*. (2010) reported that in poison food technique bitter temru fruits preparation checked the growth of all the four fruit rot pathogens of chilli viz., *Colletotrichum capsici, Alternaria alternata, Fusarium oxysporum* and *Aspergillus niger* at 2,000 ppm concentration (93.00, 87.13, 83.50 and 92.40%, respectively) followed by datura leaves at same concentration (83.77, 82.23, 80.00 and 91.00%, respectively). Mandeep *et al*. (2006) evaluated *Trichoderma harzianum, T. viride, T. viride E* and *T. virens (Gliocladium virens)* for their antagonistic potential against *Colletotrichum capsici* (causing fruit rot of chilli, *Capsicum annuum*) using dual culture technique. All the 4 antagonistic fungi caused significant inhibition of mycelial growth of the pathogen. The maximum inhibition was obtained with *T. harzianum* (77.78%), followed by *T. harianuu* Th1 (74%), whereas it was the least with *T. fluorescenes* PS1 (31.44%). All the biological control agents had negative effect on spore germination of the pathogen. Mesta *et al*. (2009) reported that a field experiment was conducted during *kharif* 2006 and 2007 at Agricultural Research Station, Devihosur, with seven treatments and three replications including seedling dip and spray of *Pseudomonas fluorescence* @ 10g/l in various combinations with chemicals, viz., carbendazium and hexaconazole. Seed treatment with Carbendazim (0.2%) + seedling dip in *P. fluorescence* at 45 and 60 DAT + 2 spray Hexaconazole 75 and 90 DAT recorded least disease incidence for fruit rot (20.60 PDI), highest yield (8.0 q/ha) higher net returns (Rs25,140/ha). Fruit rot is not only responsible for decreasing the yield but also the quality of the fruit. Keeping in view the importance of chilli fruit and its economic value and the seriousness of the disease the present investigation was carried out in order to evaluate the efficacy of different fungicides , bio pesticides and botanicals both in vitro and in field condition so that the disease could be effectively controlled.

## MATERIALS AND METHODS

### Collection and Isolation of *Colletotrichum capsici*

Chilli fruits showing typical fruit rot symptoms with sunken necrotic tissues and concentric rings of acervuli were collected. Infected fruit bits were surface sterilized in 0.1% mercuric chloride for 30 seconds and repeatedly washed with sterilized distilled water to remove traces of mercury and then transferred to water agar media and incubated at 27 ± 1ºC. Fungal mycelium developed from the infected tissue in water agar media was finally transferred to PDA slants and incubated at 27 ± 1ºC to obtain pure culture of *Colletotrichum capsici*.

### *In vitro* effect of fungicides

The evaluation of eight different commercial fungicides, *viz.*, Bavistin® 50 WP (Carbendazim), Nativo®75 WP (Trifloxystrobin + Tebuconazole ), Folicur®25.9 EC (Tebuconazole), Flint® 50 WG (Trifloxystrobin), Amistar® 23 SC (Azoxystrobin), Kocide® 77 WP (Copper hydroxide), Indofil M-45® 75 WP (Mancozeb) and Saaf® 75 WP (Carbendazim + Mancozeb) at four different concentrations *i.*e., 50 µg/ml, 100 µg/ml, 150 µg/ml and 200 µg/ml against *Colletotrichum capsici* was done by poisoned food technique according to Nene and Thapliyal (1993). The per cent inhibition was calculated by following the method described by Vincent (1947) as given below:

$$I \quad \frac{C \quad T}{C} \quad 100 \quad 0, \text{ Where,}$$

I = per cent inhibition, C = linear growth of the fungus in control (cm) and T = linear growth of the fungus in treatment (cm).

### *In vitro* effect of biocontrol agents

Two isolates each of three bioagents *viz.*, *Trichoderma harzianum*, *T. viride* and *Pseudomonas fluorescens* were evaluated for their antagonistic properties against *C. capsici* through dual culture technique (Faheem *et al.*, 2010). Per cent inhibition of growth of the test fungus was calculated by using the formula of Vincent (1947) as described above.

### *In vitro* effect of botanical oils

Four botanical oils *viz.*, Garlic (*Allium sativum*), Neem (*Azadirachta indica*), Polyalthia (*Polyalthia longifolia*) and Citronella(*Cymbopogon nardus*) were tested for their efficacy against *C. capsici* of at concentrations 0.05%, 0.1% and 0.2%.The poisoned food technique was followed to evaluate the efficacy of essential oils in laboratory against the test fungus. Required amount of oil extracts were dissolved in 5% tween 80 (5g of tween 80 + 95g of xylene) and thoroughly mixed with melted PDA to provide concentrations of 0.05, 0.1 and 0.2 % with three replications each of different botanical oils. The actively growing periphery of seven days old culture of *C. capsici* was aseptically transferred to the centre of each petri plates containing the poisoned solid media. Suitable control was maintained by growing the cultures on PDA without botanical oils. Inoculated plates were incubated at 27 ± 1º C for twelve days and colony diameter was recorded.Per cent inhibition of growth of the test fungus was calculated by using the formula of Vincent (1947)as described above.

### Integrated management in field

The field trial was conducted for two consecutive seasons (2013 and 2014) with chilli variety Bullet. The treatment combinations were $T_1$: Seed treatment with. *Trichoderma harzianum* @ 5 g/kg seed, $T_2$ : $T_1$ + two sprays of Trifloxystrobin (25%) + Tebuconazole (50%) @1g/litre water starting from 40 DAT at 10 days interval, $T_3$: $T_1$ + three sprays of Trifloxystrobin (25%) + Tebuconazole (50%) @1g/litre water starting from 40 DAT at 10 days interval, $T_4$: Seed treatment withTrifloxystrobin (25%) + Tebuconazole (50%) @1g/kg seed, $T_5$: $T_4$ + two sprays of Trifloxystrobin (25%) + Tebuconazole (50%) @1g/litre water starting from 40 DAT at 10 days interval, $T_6$: $T_4$ + three sprays of Trifloxystrobin (25%) + Tebuconazole (50%) @1g/litre water starting from 40 DAT at 10 days interval, $T_7$: Seed treatment with garlic oil @0.5 ml/ kg seed, $T_8$:$T_7$ + two sprays of Trifloxystrobin (25%) + Tebuconazole (50%) @1g/litre water starting from 40 DAT at 10 days interval, $T_9$: $T_7$ + three sprays of Trifloxystrobin (25%) + Tebuconazole (50%) @1g/litre water starting from 40 DAT at 10 days interval, $T_{10}$: $T_1$ + spraying of garlic oil @ 0.5 ml/ litre water three times during fruiting, $T_{11}$: $T_7$ + spraying of garlic oil @ 0.5 ml/litre water three times during fruiting and $T_{12}$:Control. The severity of the disease was recorded using 0-9 scale for fruit anthracnose as proposed by Than *et al.* (2008).

Percent Disease Index (PDI) was calculated by using the following formula proposed by Wheeler (1969):-

$$PDI = \frac{\text{(Sum of all numerical ratings)}}{\text{(Total no.of sample observed)} \times \text{(maximum disease grade)}} \times 100$$

### Statistical analyses

Statistical analyses of the data were done with SPSS statistical software version 16.00 (2001).

## RESULTS AND DISCUSSION

### *In vitro* study

It is evident from Table 1 that all the fungicides except Carbendazim efficiently inhibited the linear growth of *C. capsici*. Tebuconazole, Mancozeb and Trifloxystrobin + Tebuconazole each @ 150 µg/ml and Carbendazim + Mancozeb @ 200 µg/ml were significantly superior to all other treatments in inhibiting mycelial growth ofthe fungus by 100 per cent. Out of the eight fungicides tested carbendazim showed the least inhibition of mycelial growth *i.e,* only up to 8.94% even at the concentration of 200 µg/ml. The present results are in accordance with the earlier findings of Ashoka (2005)who reported that Carbendazim + Mancozeb could completely(100%)inhibit the growth of *Colletotrichum gloeosporioides* at all three concentrations (0.025, 0.005 and 0.1%) whereas mancozeb was found to inhibit up to 77.65% of the growth of fungus.

Results summarized in Table 2 indicates that among the biocontrol agents tested *T. harzianum* isolate Th-2 was found most effective giving 77.78% inhibition on the mycelial growth of the fungus followed by *T. harzianum* isolate Th-1 inhibiting 74.00% of the mycelial growth while *P. fluorescens* was least effective. Similarly, Ushakiran *et al.* (2006) observed that in

**Table 1: *In vitro* efficacyof fungicides on the mycelial growth of *Colletotrichum capsici***

| Fungicides | per cent inhibition of mycvelial growth at concentration in µg/ml | | | |
|---|---|---|---|---|
| | 50 | 100 | 150 | 200 |
| Tebuconazole | 55.62 (48.23) | 93.31(75.01) | 100 (90.00) | 100 (90.00) |
| Azoxystrobin | 26.80 (31.18) | 63.94 (53.09) | 68.93 (56.12) | 72.20 (58.18) |
| Trifloxystrobin | 25.79(30.52) | 62.8 (52.42) | 67.20 (55.06) | 72.20 (58.18) |
| Carbendazim | 2.30 ( 8.72) | 4.10 (11.68) | 6.71 (15.01) | 8.94 (17.40) |
| Mancozeb | 27.80 (31.82) | 66.70 (54.76) | 100 (90.00) | 100 (90.00) |
| Copper hydroxide | 38.92 (38.60) | 50.30 (45.17) | 71.11 (57.49) | 78.91 (62.66) |
| Trifloxystrobin + Tebuconazole | 86.14 (68.14) | 92.23 (73.81) | 100 (90.00) | 100 (90.00) |
| Carbendazim + Mancozeb | 26.72 (31.13) | 50.21 (45.12) | 82.23 (65.07) | 100 (90.00) |
| | Fungicide (F) | Concentration (C) | FXC | |
| CD at 5% | 0.84 | 0.6 | 1.68 | |
| SEm ± | 0.3 | 0.21 | 0.6 | |

**Table 2: *In vitro* efficacy of biocontrol agents on the mycelial growth of *C. capsici***

| Biocontrol agents | Isolate | Per cent inhibition of mycelial growth |
|---|---|---|
| *T. harzianum* | Th-1 | 74.00 (59.34) |
| *T. harzianum* | Th-2 | 77.78 (61.88) |
| *T. viride* | Tv-1 | 73.90 (59.28) |
| *T. viride* | Tv-2 | 72.22 (58.19) |
| *P. fluorescence* | Ps-1 | 31.44 (34.11) |
| *P. fluorescence* | Ps-2 | 50.00 (45.00) |
| Control | | 0 (0.00) |
| CD at 5% | | 1.88 |
| S.Em ± | | 0.62 |

dual culture technique with six bio control agents (*T.harzianum, T. hamatum, T. viride, Verticilliumlecanii, Beauberiabassiana* and *Metarhiziumanisopliae*) *T.harzianum, T. hamatum* and *T. viride* could induce maximum percent inhibition on the linear growth of *C. capsici*.It is evident from table 3 that garlic gave complete inhibition of mycelial growth of the fungusat all concentrations. However, neemshowed complete inhibition of mycelial growth of *C. capsici* at 0.1% and o.2%. Citronella oil was least effective among the botanical oils. The present finding is in accordance with the findings of Bhardwaj and Sahu (2014) who reported that plant extracts of Ocimum, ginger, garlic, turmeric and onion extracts inhibited the growth and sporulation of *C. falcutam*

**Table 3: *In vitro* efficacy of botanical oils on the mycelial growth of *C. capsici***

| Botanical oil | Per cent inhibition of mycelial growth Concentration | | |
|---|---|---|---|
| | 0.05% | 0.10% | 0.20% |
| Garlic | 100 (90.00) | 100 (90.00) | 100 (90.00) |
| Neem | 84.45 (66.78) | 100 (90.00) | 100 (90.00) |
| Polyalthia | 73.89 (59.27) | 78.89 (62.65) | 84.45 (66.78) |
| Citronella | 8.27 (16.71) | 8.36 (16.81) | 13.84 (21.84) |
| Solvent | 7.80 (16.22) | 8.00 (16.43) | 8.14 (16.58) |
| | Botanical oil ( B ) | Concentration ( C ) | B x C |
| CD at 5% | 0.62 | 0.48 | 1.08 |
| SEm ± | 0.21 | 0.17 | 0.37 |

**Table 4: Integrated disease management of chilli anthracnose (pooled data for two years)**

| Treatments | Percent disease index at DAT | | | | Yield |
|---|---|---|---|---|---|
| | 105 | 120 | 135 | 150 | (qn/ha) |
| T1 | 16.74 (24.15) | 17.19 (24.50) | 17.60 (24.80) | 18.14 (25.21) | 31.82 |
| T2 | 13.39 (21.46) | 13.53 (21.59) | 13.74 (21.76) | 14.11 (22.06) | 37.77 |
| T3 | 7.25 (15.61) | 7.83 (16.25) | 8.43 (16.88) | 8.79 (17.25) | 50.61 |
| T4 | 15.42 (23.12) | 15.93 (23.53) | 16.09 (23.65) | 16.46 (23.94) | 33.08 |
| T5 | 11.37 (19.71) | 12.30 (20.53) | 12.67 (20.85) | 13.44 (21.51) | 39.76 |
| T6 | 4.81 (19.71) | 5.14 (13.11) | 5.28 (13.29) | 5.48 (13.54) | 53.02 |
| T7 | 16.17 (23.71) | 16.61 (24.05) | 16.94 (24.31) | 17.47 (24.71) | 31.83 |
| T8 | 10.40 (18.81) | 11.75 (20.05) | 12.32 (20.55) | 12.53 (20.73) | 40.96 |
| T9 | 3.47 (10.73) | 4.19 (11.81) | 4.60 (12.39) | 4.85 (12.72) | 56.95 |
| T10 | 13.70 (21.73) | 14.15 (22.10) | 14.28 (22.20) | 14.71 (22.55) | 35.74 |
| T11 | 14.28 (22.20) | 14.50 (22.39) | 14.67 (22.52) | 14.82 (22.64) | 34.19 |
| T12 | 30.47 (33.50) | 35.88 (36.80) | 38.61 (38.42) | 43.06 (41.01) | 29.41 |
| SEm (±) | 0.17 | 0.18 | 0.18 | 0.18 | 0.81 |
| CD 5% | 0.50 | 0.52 | 0.52 | 0.54 | 1.68 |

Figures in parentheses are angular transformed values

**Integrated disease management under field condition**

Data presented in table 4 showed that the final PDI was found to be least in T9 (seed treatment with garlic oil @0.5 ml/kg seed + three sprays of Trifloxystrobin (25%) + Tebuconazole (50%) @1g/litre water) followed by T6 (seed treatment with Trifloxystrobin(25%) + Tebuconazole (50%) @ 1g/kg seed + three sprays of Trifloxystrobin (25%) + Tebuconazole (50%) @1g/litre water) and T3 (seed treatment with *T. harzianum*@5g/ kg seed + three sprays of Trifloxystrobin (25%) + Tebuconazole(50%) @1g/litre) with PDI of 4.85, 5.48 and 8.79 per cent, respectively which were significantly superior over other treatments. Furthur, the highest yield of 56.95 qn/ ha was observed in seed treatment with garlic oil @0.5 ml/kg seed + three sprays of Trifloxystrobin (25%) + Tebuconazole (50%) @1g/litre water which was followed by seed treatment with garlic oil @ 0.5 ml/kg seed + three sprays of Trifloxystrobin (25%) + Tebuconazole (50%) @1g/litre water (53.02 qn/ha) and seed treatment with*T. harzianum* @ 5g/kg seed + three sprays of Trifloxystrobin (25%) + Tebuconazole (50%) @ 1g/ litre (50.61 qn/ha) and their differences were statistically significant. The present result is supported by Wharton and Dieguez (2004)who observed that effective control of *Colletotrichum* diseases usually involves the use of a combination of cultural control, biological control, chemical control and intrinsic resistance. Similarly,Anand *et al*. (2010)evaluated combined strategy of chilli fruit rot control consisting of reduced fungicidedose and biological control with antagonistic *Pseudomonas fluorescens*(Pf1). Biological control of *C. capsici* with *P. fluorescens*(Pf1) was effective but less so than fungicide alone at the standard dose. However, combination of the biological control agent with a 50% reduction of fungicide dose was as effective as the standard fungicide alone. Yadav *et al*. (2014) tested field efficacy of different beignets for the management of the onion leaf blotch and found all the bio agents effectively control the disease.

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