Eco-friendly Management of Anthracnose of Chilli (Capsicum annuum L.) caused by Colletotrichum capsici (Syd.) Butler and Bisby

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A B S T R A C T

A field experiment was conducted to evaluate the effect of bioagents and botanicals in vivo during kharif, 2017 to manage anthracnose caused by Colletotrichum capsici on chilli (Capsicum annuum L.). The anthracnose of chilli is one of the most devastating diseases. The various factors viz. nutritional, physical and toxicological factor showed wide variation on growth and sporulation of C. capsici. The effect of treatments on anthracnose disease management of chilli in field condition revealed that all the treatments showed significant reduction in the intensity and over control. Average disease intensity and its average reduction over control recorded with all the treatments were ranged from 19.84 to 31.18 per cent and 52.97 to 26.09 per cent, respectively. Antagonistic ability of Trichoderma viride and Pseudomonas fluorescens was tested by dual culture test against pathogen. Among them Trichoderma viride was found effective in inhibiting the growth of C. capsici. The toxicological factors like Eucalyptus oil, Neem oil and Garlic bulb extract were found effective.

Keywords: Bio-agents, Colletotrichum capsici, Plant extracts/plant products

Introduction

Chilli (Capsicum annuum L.) is an important vegetable as well as spice crop, cultivated worldwide. It is not only used in many cuisines but also found to have many medicinal properties. The genus Capsicum comprises about 20-25 species, out of which C. annuum, C. baccatum, C. chinense, C. frutescens and C. pubescens are cultivated. Capsicum annuum is widely cultivated variety, second being C. frutescens. Commonly used term is Chilli, which refers to hot types of Capsicum. Though it was originated in the American tropics, it is widely propagated (Sahitya et al., 2014).

Chilli is an important commercial crop grown in India. India emerged as leading producer and exporter of chilli contributing one fourth of world’s production. The strong spicy taste comes due to the presence of active alkaloid compounds capsaicin, capsanthin, capsorubin. Chilli contains steam volatile oils, carotenoids, fatty oils, vitamins, mineral elements etc. Chilli is an important commercial crop grown in India. Andhra Pradesh, Orissa, Maharashtra, West Bengal,
Uttar Pradesh, Karnataka, Rajasthan and Tamil Nadu are found to be important states growing chilli in India (Sahitya et al., 2014).

Chilli anthracnose was first reported in India on from the Coimbatore of Madras Presidency (Sydow, 1913). The disease has been identified in all the chilli producing regions of the world and has become a serious constraint to chilli production. Different species of Colletotrichum, namely C. capsici, C. gloeosporioides, C. acutatum are known to cause anthracnose in chilli in India. Anthracnose disease appears as small circular spots that coalesce to form large elliptical spots on fruits and leaves. Under severe conditions, defoliation of affected plants occurs.

Among all the diseases, anthracnose disease is the major constraint to chilli production worldwide resulting in high yield losses. This fungal disease caused by Colletotrichum species drastically reduces the quality and yield of fruit resulting in low returns to farmers. 10-80% of marketable yield is reduced in Thailand, about 13% in Korea. This die back/fruit rot/anthracnose disease is seen on mature fruits resulting in both pre harvest and post-harvest fruit loss. In India, in severe cases, pre harvest and post-harvest losses comprise up more than 50%. Significant yield losses were reported from Punjab and Haryana (20-60%) and Assam (12-30%) (Sahitya et al., 2014). Among the fungal diseases, anthracnose caused by Colletotrichum spp. is considered to be the major constrain to increase chilli production. It occurs every year with varying intensities and inflicts considerable quantitative and qualitative losses of the crop in the fields as well as in the storage. Anthracnose is mainly a problem on mature leaves and fruits, causing severe losses due to both pre-harvest and post-harvest fruit decay (Hadden and Black, 1989; Bosland and Votava, 2003). Four species of Colletotrichum; C. capsici, C. gloeosporioides, C. acutatum and C. coccodes have been reported as causal agents of pepper anthracnose in many countries. The major species are C. capsici and C. gloeosporioides (Hadden and Black, 1987).

For the management of anthracnose of Chilli, now a day’s increasing use of chemical has challenged both public health and environment hazards. Thus emphasis for using botanicals such as plant extract and bioagents for the management of the plant disease which is less costly and environment friendly.

**Materials and Methods**

The experiment was conducted in the research laboratory of Department of Plant Pathology and Central Research Farm, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad. The experiment was laid out Randomized Block Design with three replications and Eight Treatments. Three spraying of all the treatments were undertaken at 15 days interval, starting first spray at appearance of anthracnose symptoms. One plot per replication was maintained as the control. Five plants per treatment per replication were selected randomly and tagged; three leaves (bottom, middle and top) from main branch on each observation plant were selected for recording observations. Observations on foliage anthracnose disease intensity were recorded applying standard 0-9 grade disease rating scale (Mayee and Datar, 1986) one day before each spraying and last observation was recorded.

**Standard disease rating scale (0-9 scale) for assessing PDI of anthracnose of chilli**

0-No symptoms on plant.; 1- Small spots on leaves, less than 1 per cent of leaf area diseased; 3- Medium six spots on leaves covering 1-10 per cent infected area; 5- Spots...
big; coalescing covering 11-25 per cent of
leaf area.; 7- Spots large; coalescing covering
26-50 per cent of leaf area; 9- Spots on leaves
covering above 51 per cent of leaf area.

Collection, Isolation and purification

The anthracnose disease samples (just after
initiation of the disease) were collected in
polythene bags from various plants from
research plot of the Department of Plant
Pathology, Sam Higginbottom University of
Agriculture, Technology and Sciences,
Allahabad (U.P.). The chilli leaves exhibiting
disease symptoms were brought to the
laboratory for isolation. The anthracnose
infected parts were cut into small pieces by
sterilized stainless steel blade and surface
sterilized with 0.1% mercuric chloride for one
minute followed by three washing with
sterilized water. Anthracnose infected pieces
were placed in petriplates containing 20 ml of
solidified potato dextrose agar (PDA) medium
mixed with streptomycin sulphate to avoid
bacterial contamination. Plates were kept for
incubation at 28±2˚C in an incubator. Fungal
colonies appeared within 5-7 days, they were
sub cultured in PDA slants and purified.

Efficacy of bioagents on the radial growth
of C. capsici in vitro

Pseudomonas fluorescens: P. fluorescens
were tested for their antagonistic ability
against C. capsici by dual culture method on
PDA medium and allowed to solidify.5mm
mycelial disc were cut from young growing
ing edge of the fungus from seven days old
culture and placed at one side of petriplates.
The Pseudomonas fluorescens whose
inhibition ability need to be tested were streak
parallel to the fungus roughly at a distance of
15-20 mm and incubated at 28±2˚C for seven
days and percentage of inhibition of the
fungus was calculated.

Trichoderma viride

The Trichoderma viride were screened for
their antagonistic ability by dual culture
method on PDA medium. An amount of 20
ml PDA was poured in 90 mm sterilized
petriplates. A 5 mm disc of C. capsici was
taken from the margin of young vigourously
growing culture and placed at the one end in
petriplates and at the other end, four days old
pure culture of the Trichoderma viride were
inoculated roughly at a distance of 15-20 mm
and placed in incubator at 28±2˚C. Three
replications for each treatment were
maintained. The observations of per cent zone
inhibition between the antagonists and test
fungus were recorded after 7 days of
incubation period and the per cent growth
inhibition was calculated.

Per cent inhibition of colony = \( \frac{C - T}{C} \times 100 \)

(Dennis and Webster, 1971).

Where,

C = Colony diameter in control
T = Colony diameter in treatment

Effect of different botanicals against C.
capsici in vitro

To test the antifungal activity of some
botanicals was studied in vitro by poisoned
food technique (Nene and Thapliyal, 1971). Different Botanicals named as Neem oil,
Eucalyptus oil, Garlic Bulb, Tulsi, Datura
were used. The plant materials (100 gm) were
blended with 100 ml water till they become
soften and pulpy, then extract was filtered.
After that each plant extracts were dispensed
in 100 ml melted PDA in conical flasks,
separately (garlic bulb extract, Tulsi leaves
extract, Datura leaf extract, neem oil and
Eucalyptus oil each@5%). Trace amount of
streptomycin sulphate was added to prevent
bacterial contamination and then poured into
90 mm petriplates.
The plain PDA plates served as control. A 5 mm disc of seven days old culture of fungus was placed on the center of the medium and kept in incubator at 28±2˚C.

Three replications were maintained for each treatment and observations of radial growth were recorded after second day at regular interval of two days up to 7 days. The observations recorded at 7th day were used for computation.

Results and Discussion

**Efficacy of Bioagents and Botanicals against C. capsici in vitro**

Studies on antagonistic ability tested, exhibited significant mycelial growth inhibition of C. capsici. However, it was significantly highest with *Trichoderma viride* (74.22%), followed by *Pseudomonas fluorescens* (57.56%).

Results (Table 1) revealed that the Eucalyptus oil was found most effective with least mycelial growth (0.00 mm) and significantly highest mycelial inhibition (100%) of the test pathogen. All the plant extract showed their inhibitory influence on the growth of C. capsici.

The radial growth of C. capsici was minimum in Garlic Bulb extract, Datura leaf extract and Tulsi leaves extract as compared to control. Thus all the extracts of plants of different species adversely affect the growth of C. capsici. The inhibitory effect of volatile oil of Eucalyptus on mycelial growth and sporulation of C. capsici was also reported by Ramezani *et al.*, (2002), which supports the present findings. Bioagents *viz.*, *T. viride* and *P. fluorescens* were reported as efficient antagonists against many *Colletotrichum* spp by several earlier workers (Tiwari *et al.*, 2008; Pardhi and Raut, 2011).

**Disease intensity**

The results (Table 2) of the effect of treatments on anthracnose disease management of chilli in field condition revealed that all the treatments showed significant reduction in the intensity and over untreated control during kharif, 2017. After first spraying, the disease intensity ranged from 14.66 (*Trichoderma viride*) to 26.87 (Datura leaf extract) as against 27.26 per cent in the unsprayed control, and all the treatments were found significantly superior over unsprayed control. After second spraying, the disease intensity recorded was comparatively over that of observed after first spraying and was ranged from 20.26 (*Trichoderma viride*) to 30.14 (Tulsi Leaves extract) as against 32.79 per cent in unsprayed control, and all the treatments were found significantly superior over unsprayed control. After Third spraying, the disease intensity recorded ranged from 24.60 (*Trichoderma viride*) to 38.14 (Datura leaf extract) as against 68.53 percent in unsprayed control, and all the treatments were found significantly superior over unsprayed control.

Average disease intensity and its average reduction over unsprayed control with all the spray treatments were ranged from 19.84 (*Trichoderma viride*) to 31.18 (Datura leaf extract) per cent and 26.09 (Datura leaf extract) to 52.97 (*Trichoderma viride*) per cent, respectively.

The *Trichoderma viride* as a bioagent and Garlic Bulb Extract as a botanical could also effectively manage anthracnose of chilli (Kamble *et al.*, 2015). The phytoextracts *viz.*, Garlic bulb, Tulsi leaves extract were reported antifungal fungistatic against many *Colletotrichum* spp, earlier by several workers (Jayalakshmi *et al.*, 1998; Shinde and Gawai, 2014) (Fig. 1–3).
Table.1 Efficacy of bioagents and botanicals against *C. capsici* in vitro

<table>
<thead>
<tr>
<th>Tr. No.</th>
<th>Treatments</th>
<th>Radial growth of pathogen (mm)</th>
<th>Percent Growth inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>Control</td>
<td>75</td>
<td>0.00</td>
</tr>
<tr>
<td>T1</td>
<td><em>Trichoderma viride</em></td>
<td>19.33</td>
<td>74.22</td>
</tr>
<tr>
<td>T2</td>
<td><em>Pseudomonas fluorescens</em></td>
<td>31.83</td>
<td>57.56</td>
</tr>
<tr>
<td>T3</td>
<td>Garlic bulb extract</td>
<td>33.66</td>
<td>55.12</td>
</tr>
<tr>
<td>T4</td>
<td>Datura leaf extract</td>
<td>36.66</td>
<td>51.12</td>
</tr>
<tr>
<td>T5</td>
<td>Neem oil</td>
<td>29.00</td>
<td>61.33</td>
</tr>
<tr>
<td>T6</td>
<td>Tulsi leaves extract</td>
<td>40.00</td>
<td>46.66</td>
</tr>
<tr>
<td>T7</td>
<td>Eucalyptus oil</td>
<td>0.00</td>
<td>100</td>
</tr>
</tbody>
</table>

**F- test**

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| S.E. ± | 0.52               |

**C.D. (P=0.05)**

1.11

Table.2 Efficacy of various treatments against anthracnose disease intensity in vivo condition

<table>
<thead>
<tr>
<th>TR No.</th>
<th>Treatments</th>
<th>Conc. (%)</th>
<th>Percent Disease Intensity (PDI)</th>
<th>Avg.PDI</th>
<th>Avg. PDC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>45 DAT</td>
<td>60 DAT</td>
<td>75 DAT</td>
</tr>
<tr>
<td>T1</td>
<td><em>Trichoderma viride</em></td>
<td>2%</td>
<td>14.66</td>
<td>20.26</td>
<td>24.60</td>
</tr>
<tr>
<td>T2</td>
<td><em>Pseudomonas fluorescens</em></td>
<td>2%</td>
<td>15.38</td>
<td>26.07</td>
<td>32.30</td>
</tr>
<tr>
<td>T3</td>
<td>Garlic bulb extract</td>
<td>5%</td>
<td>16.74</td>
<td>27.07</td>
<td>35.35</td>
</tr>
<tr>
<td>T4</td>
<td>Datura leaf extract</td>
<td>5%</td>
<td>26.87</td>
<td>28.54</td>
<td>38.14</td>
</tr>
<tr>
<td>T5</td>
<td>Neem oil</td>
<td>5%</td>
<td>16.37</td>
<td>26.73</td>
<td>33.70</td>
</tr>
<tr>
<td>T6</td>
<td>Tulsi Leaves extract</td>
<td>5%</td>
<td>25.14</td>
<td>30.14</td>
<td>37.49</td>
</tr>
<tr>
<td>T7</td>
<td>Eucalyptus oil</td>
<td>5%</td>
<td>15.21</td>
<td>22.55</td>
<td>31.03</td>
</tr>
<tr>
<td>T0</td>
<td>Control</td>
<td>-</td>
<td>27.26</td>
<td>32.79</td>
<td>68.53</td>
</tr>
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</table>

**F test**

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| S.E. ± | 1.01   | 1.15 | 1.35 | 2.02 |

**CD(P=0.05)**

2.18 | 2.47 | 2.91 | 4.35 |
**Fig.1** Efficacy of bioagents on the radial growth of *C. capsici* by dual culture technique

![Control, Trichoderma viride, Pseudomonas fluorescens](image)

**Fig.2** Efficacy of botanicals on the radial growth of *C. capsici* by poison food technique

![Control, Eucalyptus oil, Neem oil, Garlic bulb extract, Datura leaf extract, Tulsi leaves extract](image)

**Fig.3** Effects of various treatments on anthracnose disease average percent intensity and reduction

![Average % intensity and reduction](image)
From present study, it was concluded that the severity of anthracnose of chilli disease can significantly be reduced by the use of bioagent *Trichoderma viride* and botanicals viz. Garlic bulb extract, Eucalyptus oil and Neem oil at least three times foliar spray after initiation of disease symptoms in order to have a higher profitable yield and higher economic return without health risk as well as environmental pollution. Whereas, in lab experiment Eucalyptus oil and *Trichoderma viride, Pseudomonas fluorescens*, Neem oil were found to be most effective. Recently there has been great interest in essential oils and biocontrol agents for controlling plant pathogens. The present study shows that botanical oils possess antifungal activity and can be exploited for effective management of plant diseases. Therefore, the farmers may be advised to take an integrated approach, which should be raised a profitable production without polluting the environment and adding toxins in the food chain.

References


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