In Vitro Evaluation Of Fungicides And Organic Preparations Against Colletotrichum Gloeosporioides Causing Anthracnose Of Black Pepper (Piper Nigrum L.)
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Abstract
Seven commercially available fungicides- propiconazole (0.1%), fluzilazole (0.1%), azoxystrobin (0.15%), tebuconazole (0.1%), contact fungicide copper hydroxide (0.25%), combination fungicides such as captan + hexaconazole (0.1%), mancozeb + carbendazim (0.1%) and indigenous organic preparations like jeevamruth, fish amino acid, compost tea and vermiwash were evaluated for in vitro suppression of the mycelial growth of Colletotrichum gloeosporioides by poisoned food technique. Among fungicides maximum inhibition was obtained with tebuconazole (0.1 %) and combination fungicide mancozeb+ carbendazim(0.1 %) which gave 100 % growth inhibition over the control. Among the organic preparations, jeevamruth gave maximum growth inhibition followed by fish amino acid.

Keywords – Fungicides, organic preparations, Colletotrichum gloeosporioides, poisoned food technique.

I. INTRODUCTION
Black pepper (Piper nigrum L.) is famed as “King of Spices”, owing to its economic importance. It is a good source of many antioxidant substances like Vitamin A, Vitamin C, carotenes, flavanoids and lycopene which helps the body to remove harmful free radicals and protect from diseases. India is one of the leading producers of black pepper in the world and Kerala contributes about 80-90% of total production in the country. But, over recent years, the production and productivity of pepper has come down drastically due to incidence of various pests and diseases, high cost of production and subsequent neglect of crop by the farmers. Of these, diseases affecting pepper play a significant role. Anthracnose caused by Colletotrichum gloeosporioides (Penz)Penz and Sac. is gaining importance in recent years[1]. Infection occurs in leaves, spikes and berries. Damage on berries has been reported to cause 100 % yield loss[2]. Spraying of fungicides like carbendazim 0.1 % and combination fungicide (carbendazim+ mancozeb 0.1 %) have been reported to be effective in significantly reducing the disease[3]. Use of indigenous organic preparations is emerging as a potential non chemical alternative for disease management. The present study was aimed to evaluate effectiveness of various fungicides and indigenous organic preparations in inhibiting the mycelial growth of Colletotrichum gloeosporioides under in vitro conditions.

II. MATERIALS AND METHODS
A. Isolation and identification of causal agent
Black pepper leaves infected with anthracnose was collected from various pepper growing regions of the State. The infected leaves were first washed under running water and cut into small bits. The bits were then surface sterilized with 0.1 % mercuric chloride (HgCl₂) followed by washing in three changes of sterile water. These bits were then transferred aseptically to sterile petriplates containing...
potato dextrose agar (PDA) medium. The petriplates were sealed with parafilm and incubated at room temperature for fungal growth. The culture was purified by transferring the growing tip of the fungal mycelium to PDA slant [4]. The causal agent was identified based on morphological and cultural characteristics.

**B. In vitro evaluation of fungicides**

The suppression of *C. gloeosporioides* using new generation fungicides was tested by poisoned food technique [5]. Seven commercially available fungicides- propiconazole (0.1%), fluzilazole (0.1%), azoxystrobin (0.15 %), tebuconazole (0.1%), contact fungicide copper hydroxide (0.25 %), combination fungicides such as captan +hexaconazole (0.1 %), mancozeb + carbendazim (0.1 %) were tested at three different concentrations – recommended dose, higher and lower dose. For each fungicide to be tested, 50 ml sterile water was taken in three 250 ml conical flasks and in another three conical flasks 50 ml PDA medium was taken and autoclaved. The required concentrations of fungicide were added to sterile water, which was in turn added to PDA medium. The amended medium was then added to the sterile petriplates and 5 mm mycelial disc of pathogen was inoculated at the centre. The unamended PDA medium with pathogen at centre serves as control. Observations were recorded as radial growth of pathogen when growth in the control plate was completed. The percentage inhibition of pathogen over control was calculated using the formula [6],

\[ I = \frac{C - T}{C} \times 100 \]

where:
- **I** = Percentage growth inhibition
- **C** = Growth of pathogen in control (cm)
- **T** = Growth of pathogen in treatment (cm)

**C. In vitro evaluation of organic preparations**

The organic preparations like compost tea, vermiwash, fish amino acid and jeevamruth were evaluated for the suppression of *C. gloeosporioides* using poisoned food technique [5]. The organic preparations were tested at 3 concentrations : 2.5, 5.0 and 10.0 %. For conducting the study, 40 ml, 45 ml and 47.5 ml of sterile water was taken in three 250 ml conical flasks. In another three 250 ml conical flasks, 50 ml double strength PDA was prepared and sterilized. The organic preparations were first filtered through Whatman No.1 filter paper and further sterilized by passing through bacteria proof filters (0.22 µm). The required amount of organic preparations i.e. 2.5 ml, 5.0 ml and 10.0 ml were added to 40 ml, 45 ml and 47.5 ml of sterile water respectively. The amended medium was then poured into sterile petriplates. 5 mm diameter mycelial disc was taken from seven day old culture of *C. gloeosporioides* and placed at the centre of petriplate. Three replications were maintained for each of the treatments. The unamended medium poured into sterile petriplates with pathogen at the centre served as the control. Observations were recorded as radial growth of pathogen when growth in the control plate was completed. Percentage inhibition of pathogen in the treatment plate over the control was calculated according to the formula,

\[ I = \frac{C - T}{C} \times 100 \]

where:
- **I** = Percentage growth inhibition
- **C** = Growth of pathogen in control (cm)
- **T** = Growth of pathogen in treatment (cm)

**D. Statistical analysis**

All the data were analysed statistically in completely randomized design using computer programme excel.

**III. RESULTS AND DISCUSSION**

**A. Effect of fungicides on mycelial suppression of *C. gloeosporioides***

The studies on the *in vitro* suppression of *C. gloeosporioides* using fungicides revealed that the recommended dose of tebuconazole (0.1 %) and the combination fungicide mancozeb+carbendazim (0.1
%) was significantly better than other treatments which gave 100% inhibition of pathogen over the control. This was followed by propiconazole 0.1% with 93% inhibition and fluzilazole 0.1% with 88.24% inhibition of pathogen over the control. Combination fungicide captan + hexaconazole (0.1%) recorded 86.62% inhibition and azoxystrobin 0.15% gave 86% inhibition of pathogen over control. Least inhibition was recorded with copper hydroxide (0.25%) with an inhibition of 82.42%. This was in confirmation with Pandey et al. (2012) who reported that mancozeb + carbendazim at 100 ppm gave maximum growth inhibition for *C. gloeosporioides*[7]. Results obtained by Patil et al (2009) is in confirmation with this[8]. Ingle et al.(2014) reported that tebuconazole (0.1%) and mancozeb + carbendazim(0.1%) had the highest curative effect against *Colletotrichum*[9]. Efficacy of tebuconazole in inhibiting *Colletotrichum* has been proven by Kumbhar and More (2013)[10].

![Figure 1. In vitro efficacy of fungicides against C. gloeosporioides](image)

B. Effect of organic preparations on mycelial suppression of *C. gloeosporioides*

In *vitro* suppression study of pathogen by the organic preparations revealed that Jeevamruth (10%) showed maximum inhibition of 100% and was significantly better than other treatments. This was followed by vermiwash (10%) and fish amino acid (10%) which gave an inhibition of 79% and 64% of pathogen over the control respectively. A similar pattern was obtained with all the concentrations of organic preparations tested. This was in confirmation with Chadha et al. (2012) who reported that jeevamruth was effective against various plant diseases [11].

Table 1. Effect of organic preparations on mycelial suppression of *C. gloeosporioides*, the incitant of anthracnose of black pepper under *in vitro* conditions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration of preparation</th>
<th>Percentage mycelial suppression*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.5%</td>
<td>5%</td>
</tr>
<tr>
<td>Compost tea</td>
<td>40.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>42.26&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vermiwash</td>
<td>54.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>58.55&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fish amino acid</td>
<td>16.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.66&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Jeevamruth</td>
<td>100.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>CD(0.05)</td>
<td>0.565</td>
<td>0.750</td>
</tr>
<tr>
<td>-----------</td>
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</tbody>
</table>

Values are arc transformed
* Mean of three replications

**IV. CONCLUSION**

In this study, tebuconazole (0.1 %) and combination fungicide mancozeb+ carbendazim(0.1 %) gave complete inhibition of mycelial growth of *C. gloeosporioides* *in vitro*. Jeevamruth (10 %) and fish amino acid (10 %) also inhibited the mycelial growth of the pathogen *in vitro*.

**BIBLIOGRAPHY**


