In vitro Study on the Effect of Some Plant Extracts, Cow urine, Cow dung, Cow milk and Honey against Colletotrichum capsici

Roseline Salam, Ph. Sobita Devi, Bireswar Sinha*, Rahee Bui, K. Dinesh and W. Tampakleima Chanu

Department of Plant Pathology, College of agriculture, Central Agricultural University, Imphal-795004, India

*Corresponding author

Abstract

Anthracnose of chilli caused by Colletotrichum capsici is a widespread problem limiting the profitable cultivation of chilli in Manipur. An experiment on antifungal efficacy of four plant extracts viz., Garlic (Allium sativum), Neem (Azadirachta indica), Citronella (Cymbopogon spp), Ginger (Zingiber officinale) and three Cow urine, Cow dung, Cow milk viz., Cow urine, Cow dung, Cow milk and honey was done through poisoned food technique by setting three concentrations in in vitro conditions against C. capsici. The results revealed that among the plant extracts, garlic had shown 70.19 per cent inhibition at 5 per cent concentration and among the Cow urine, Cow dung, Cow milk and honey, cow milk had shown 69.80 per cent inhibition at 15 per cent concentration. Present study suggests that Garlic and cow milk could be tried for the eco-friendly management of the disease. Citronella extract and cow dung found to be less effective.

Keywords

Chilli, Cow urine, Cow dung, Cow milk, honey, plant extracts

Introduction

Chilli (Capsicum annuum L.) belongs to the Solanaceae family and is considered valuable cash crop around the world and also in Manipur. India ranks first among the chilli growing countries in the world. India is largest producer with 36% share in global production (Sahitya et al., 2014). The chilli suffers from various diseases and chilli anthracnose is one of the most important among them. It is the most important disease of chilli in tropics and subtropics worldwide. The disease drastically reduces the yield, deteriorates the fruit quality, and hence results in low returns to farmers. In severe cases, the crop loss may exceed 50%. Species of the genus Colletotrichum such as C. capsici, C. gloeosporioides, C. acutatum etc. have been identified as pathogens causing chilli anthracnose. Out of these, C. capsicii is the major pathogen causing anthracnose disease (Gomathi and Kannabiran, 2000; Kaur et al., 2006; Susheela, 2012).

Many fungicides such as mancozeb, captan, bavistin, thiram, copper oxychloride, cosan, benlate and ziram are employed in order to control anthracnose disease. The resistance to these fungicides has been noticed in most fungal pathogens including C. capsici. Moreover, the residues of these fungicides remain in the harvested produce. Therefore,
search for alternative disease control strategies are of immense interest. Natural products are promising in terms of their low cost, potential efficacy as well as no or negligible side effects. Plants and their derivatives have been extensively studied for the control of phytopathogenic fungi. Several studies have been carried out on inhibitory potential of many botanical extracts against phytopathogenic fungi including species of *Colletotrichum* (Kumaran *et al.*, 2003; Rahman *et al.*, 2011; Bajpai and Kang, 2012; Ajith *et al.*, 2012). Hence, the present study was carried out to study the antifungal activity of some plant extracts, Cow urine, Cow dung, Cow milk and honey against *C. capsici* under *in vitro*. 

**Materials and Methods**

**Poisoned food technique**

Four locally available botanicals namely Garlic (*Allium sativum*), Neem (*Azadirachta indica*), Citronella (*Cymbopogon sp.*), Ginger (*Zingiber officinale*) and also cow urine, cow dung, cow milk and honey were evaluated for their efficacy on the growth of the fungus at three concentrations *in vitro*. Different plant parts were used for the inhibitory effect against *Colletotrichum capsici* namely Garlic clove, Neem leaves, Citronella leaves and Ginger rhizomes.

**Preparation of plant extraction**

The plant parts were collected, washed in running tap water, surface sterilized with 1% sodium hypochloride and then with sterile distilled water. These were air dried over a blotting paper. The air dried plant parts were crushed separately in sterilized mortar and pestle with sterile distilled water at ratio 1:1 (w/v). These extracts were filtered through 2 fold muslin cloth and filtrate was centrifuged at 1500 rpm for 15 minutes and the supernatants were collected. These extracts thus prepared were considered as 100% concentration. Three concentrations were taken for each treatment. For the plant extracts, different concentrations taken were 1.25, 2.5 and 5% and for cow’s product and honey concentrations taken were 5, 10 and 15%. Required quantity of each treatment was added to the sterilized melted PDA medium in the conical flask under aseptic condition and were shaken for well mixing of the two. Each mixture was then poured into the sterilized petriplate under aseptic condition and allowed to solidify. Medium without any treatment and natural product served as control.

After solidification, each plate was inoculated with 5mm diameter mycelium disc cut from the periphery of actively grown culture on PDA. The plates were then incubated at 25±1°C in inverted position. Observations on the fungal growth were taken till the control plates were fully covered by the test fungus. Each treatment was replicated 3 times with three plates in each replication. Per cent inhibition (PI) on growth was calculated by following the method described by Vincent (1927) as given below:

\[
PI = \frac{(C-T)}{C} \times 100
\]

Where,

\(C\) = linear growth of the fungus in control

\(T\) = linear growth of the fungus in treatment

**Results and Discussion**

**Effect of plant extracts on the growth of *C. capsici* in vitro**

Four botanicals namely Garlic, Neem, Citronella and Ginger were tried at three different concentrations *viz.*, 1.25%, 2.5% and 5% against *C. capsici* in vitro and the results are presented in table 1 and figure 1 and 3.
**Table 1** Effect of plant extracts at different concentrations on the growth of *C. capsici*

| Treatment | Plant part used | Concentration (%) | Colony diameter (in cm)* | Inhibition over control (%)* |
|-----------|----------------|------------------|--------------------------|------------------------------|
| Garlic    | bulb           | 1.25             | 4.93                     | 41.95                        |
|           |                | 2.5              | 4.80                     | 43.52                        |
|           |                | 5                | 2.53                     | 70.19                        |
| Neem      | leaves         | 1.25             | 5.16                     | 39.21                        |
|           |                | 2.5              | 4.66                     | 45.09                        |
|           |                | 5                | 4.50                     | 47.05                        |
| Citronella| leaves         | 1.25             | 6.93                     | 18.42                        |
|           |                | 2.5              | 6.66                     | 21.56                        |
|           |                | 5                | 6.10                     | 28.23                        |
| Ginger    | rhizome        | 1.25             | 6.00                     | 29.40                        |
|           |                | 2.5              | 5.60                     | 34.11                        |
|           |                | 5                | 5.30                     | 37.64                        |
| Control (untreated) |  | 8.5              | 0.00                     | 0.00                          |

*Mean of three replications

**Table 2** Effect of Cow urine, Cow dung, Cow milk and honey at different concentrations on the growth of *C. capsici*

| Treatment | Concentration (%) | Colony diameter (in cm)* | Inhibition over control (%)* |
|-----------|------------------|--------------------------|------------------------------|
| Cow urine | 5                | 3.40                     | 59.99                        |
|           | 10               | 3.20                     | 62.34                        |
|           | 15               | 3.04                     | 64.30                        |
| Cow dung  | 5                | 7.70                     | 9.40                         |
|           | 10               | 7.60                     | 10.58                        |
|           | 15               | 7.56                     | 10.97                        |
| Cow milk  | 5                | 4.33                     | 49.01                        |
|           | 10               | 2.86                     | 66.26                        |
|           | 15               | 2.56                     | 69.80                        |
| Honey     | 5                | 4.43                     | 47.83                        |
|           | 10               | 4.23                     | 50.19                        |
|           | 15               | 4.16                     | 50.97                        |
| Control (untreated) |  | 8.5              | 0.00                     | 0.00                          |
| SE(d)     |                  |                           | 1.26                         |
| CD(0.05)  |                  |                           | 2.60                         |

*Mean of three replications*
**Fig. 1** *In vitro* efficacy of plant extracts on the growth of *C. capsici*

**Fig. 2** *In vitro* efficacy of Cow urine, Cow dung, Cow milk on the growth of *C. capsici* at three different concentrations
Fig. 3 Effect of Garlic, neem, citronella and ginger extracts on growth of *C. capsici* at three concentrations
Fig. 4 Effect of Cow urine, cow milk, cow dung and honey on growth of *C. capsici* at three concentrations

1. Control
2. 15%
3. 10%
4. 5%
Effect of Cow urine, Cow dung, Cow milk and honey on the growth of *C. capsici* in *vitro*

The effect of three Cow urine, Cow dung, Cow milk and honey were studied *in vitro* at three concentrations of *viz.*, 5, 10, and 15% against *C. capsici* and the results are presented in table 2 and figure 2 and 4. The table shows that maximum per cent inhibition of the fungal growth was observed at 15% cow milk (69.8%) followed by 10% cow milk (66.26%), 15% cow urine (64.30%), 5% cow urine (59.99%), 5% cow milk (49.01%), 15% honey (50.97%), 10% honey (50.19%), 5% honey (47.83%), 15% cow dung (10.97%), 10% cow dung (10.58%) and least inhibition was observed at 5% cow dung with 9.40%. Table also indicated that among the different treatments, cow milk and cow urine were found very effective in inhibiting the growth of the fungus in all the three concentrations tried. Less effectiveness was observed in cow dung treatment. Present findings are in support of the findings of Rahman et al., (2005), Ushakiran et al., (2006), Pardhi and Raut (2011). Rahman et al., (2005) also reported Garlic extracts to be the best for controlling pre-dominant fungi of chilli followed by Neem and Ginger. Kambar et al., (2013) reported the inhibitory effect of cow urine against *Colletotrichum capsici* isolated from anthracnose of chilli (*Capsicum annuum* L.). Sci. Technol. Arts Res. J., 2(4): 91-93.

The differences in the performance of the plant extract and the variation in their effectiveness against the pathogen may be due to differences in their antifungal properties present. Kurucheve et al., 1997; Shetty et al., 1989) mentioned that the variation in antifungal potential of plant extracts may be due to qualitative and quantitative differences in antifungal principles. The high growth inhibition of the pathogen may be observed due to the presence of high quantity of active antifungal compounds like tannins, flavonoids, glycosides and alkaloids (Harborne, 1984).

References

Ajith, P.S., Lakshmesha, K.K., Murthy, M.S. and Lakshmidevi, N. (2012). Botanicals for control of anthracnose of bell peppers. *J. Pl. Protection Sci.*, 4(1): 13-19.

Bajpai, V.K., Kang, S.C. (2012). *In vitro* and *in vivo* inhibition of plant pathogenic fungi by essential oil and extracts of *Magnolia liliflora* Desr. *J. Agricultural Sci. Technol.*, 14: 845-856.

Gomathi, V., Kannabiran, B. (2000). Inhibitory effects of leaf extracts of some plants on the anthracnose fungi infecting *Capsicum annuum*. *Indian Phytopathol.*, 53(3): 305-308.

Kambar, Y., Vivek, M.N., Manasa, M., Kekuda, P.T.R. and Nawaz, N.A.S. (2013). Inhibitory effect of cow urine against *Colletotrichum capsici* isolated from anthracnose of chilli (*Capsicum annuum* L.). *Sci. Technol. Arts Res. J.*, 2(4): 91-93.

Kaur, M., Sharma, O.P., Sharma, P.N. (2006). *In vitro* effect of *Trichoderma* species on *Colletotrichum capsici* causing fruit rot of chilli (*Capsicum annuum* L.). *Indian Phytopathol.*, 59(2): 243-245.

Kumaran, R.S., Gomathi, V., Kannabiran, B. (2003). Fungitoxic effects of root extracts of certain plant species on *Colletotrichum capsici* causing anthracnose in *Capsicum annuum*. *Indian Phytopathol.*, 56(1): 114-116.
Kurucheve, V., Gerard, E.J. and Jayaraj, J. (1997). Screening of higher plants for fungitoxicity against Rhizoctonia solani in vitro. Indian J. Phytopathol., 50(2): 235-241.

Mukherjee, A., Khandker, S., Islam, M.R., Shahid, S.B. (2011). Efficacy of some plant extracts on the mycelial growth of Colletotrichum gloeosporioides. J. Bangladesh Agric. Uni., 9(1): 43-47.

Pardhi, S. and Raut. (2011). Disease reaction in chilli cultivars against isolates of Colletotrichum capsici. J. Pl. Dis. Sci., 6: 198-199.

Rahman, D.M.M., Khan, A.A. and Mian, I.H. (2005). Control of seed borne fungi of chilli by seed treatment with fungicides and botanicals. Bangladesh J. Pl. Pathol., 21(2): 63-66.

Sahitya, L., Deepthi, R., Kasim, D., Suneetha, P. and Krishna. (2014). Anthracnose a prevalent disease in capsicum. Res. J. Pharm. Boil. Chem. Sci., 5(3): 1583-1604.

Shetty, S. R., Prakash, H. S. and Shetty, H.S. (1989). Efficacy of certain plant extracts against seedborne infection of Trichoconiella padwickii in paddy (Oryza sativa). Can. J. Bot., 67: 1956-1959.

Susheela, K. (2012). Evaluation of screening methods for anthracnose disease in chilli. Pest Management Hort. Ecosystems, 18(2): 188-193.

Ushakiran. L., Chhetry. G.K. N. and Singh. N.I. (2006). Fruit rot diseases of chilli and their management in agro-climatic conditions of Manipur. J. Mycopathol. 44(2): 257-262.

Vincent J.M. (1927). Distortion of fungal hyphae in presence of certain inhibitors, Nature, 159: 850.

How to cite this article:

Roseline Salam, Ph. Sobita Devi, Bireswar Sinha, Rahee Bui, K. Dinesh and Tampakleima Chanu W. 2018. In vitro Study on the Effect of Some Plant Extracts, Cow urine, Cow dung, Cow milk and Honey against Colletotrichum capsici. Int.J.Curr.Microbiol.App.Sci. 7(06): 2184-2191. doi: https://doi.org/10.20546/ijcemas.2018.706.259