Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.6946

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **6 (5):** 51-56 (2018)

International Journal of Pure & Applied Bioscience

Research Article

OPEN ACCESS

In vitro Study of Biocontrol Agents, Fungicides and Botanicals Against *Colletotrichum capsici* Causing Anthracnose of Capsicum (*Capsicum annuumm* L.)

Vani M.* and Y. M. Somashekhara

Dept. of Plant Pathology, GKVK Agricultural University, Bengaluru, Karnataka

*Corresponding Author E-mail: vanimsagri@gmail.com

Received: 12.07.2018 | Revised: 8.08.2018 | Accepted: 19.08.2018

ABSTRACT

Capsicum (Capsicum annuum L.) being an important vegetable and high value crop cultivated in the protected condition, is being affected by several post-harvest fungal disease anthracnose caused by Colletotrichum capsici (Syd.) Butler and Bisby. Ten fungal, eight bacterial bio-agents and ten fungicides were evaluated in vitro by using dual culture and poisoned food technique respectively for studying their effect on the inhibition of mycelial growth of C.capsici. Among these bio-agents T. harzianum-58-IIHR showed maximum inhibition and B. subtilis P-37 inhibited radial growth of pathogen significantly over the control. Among ten fungicides viz., Propiconazole (100 %), Difenconazole (97.92 %) and Tebuconazole (100 %) were found effective at 250ppm, 500ppm and 1000 ppm fallowed by Nativo (58.19 %). Ten botanicals were evaluated in vitro by using poisoned food technique, among these botanicals Simarouba leaf extract recorded maximum per cent inhibition of the fungus, was 32.16, 36.47, and 42.75 per cent at 10, 20 and 30 per cent respectively followed by Subabul, Pongamia, and Neem leaf extract.

Key words: *Anthracnose, Bio-agents, Botanicals, Colletotrichum capsici, Fungicides*

INTRODUCTION

Capsicum (*Capsicum annuum* L.), commonly known as sweet pepper or Shimla mirch is native to Mexico belongs to family Solanaceae. Bell pepper has attained a status of high value crop in recent years and occupies a pride place among vegetables because of delicacy and pleasant flavour coupled with rich content of ascorbic acid. It is rich in minerals like iron, potassium, calcium, magnesium, phosphorous, sodium and selenium. Anthracnose is one of the serious diseases which affects fruits in particular and it is caused by *C. capsici* (Syd.) Butler and Bisby. The disease is characterized by the production of symptoms on fruits and causes severe damage to mature fruits in the field. Moreover it causes both pre and post harvest fruit decay during storage. Anthracnose infection on fruits causes lesions and fruits become unmarketable.

Cite this article: Vani, M. and Somashekhara, Y. M., *In vitro* Study of Biocontrol Agents, Fungicides and Botanicals Against *Colletotrichum capsici* Causing Anthracnose of Capsicum (*Capsicum annuumm* L.), *Int. J. Pure App. Biosci.* **6(5):** 51-56 (2018). doi: http://dx.doi.org/10.18782/2320-7051.6946

Several management strategies are used for the control of *Colletotrichum* diseases such as cultural control, the use of resistant cultivars, biological control, and the use of fungicides. Manandhar *et al*.[4], found that fungicide spraying is the most common and practical method to control anthracnose. Bio-control agents are fast replacing synthetic pesticides owing to their safety aspects as against chemical fungicides. Therefore, in the present investigation, inhibition of mycelial growth of *C. capsici* exposed to different concentrations of some fungicides, botanicals and bioagents were studied *in vitro*. The objectives of the study were to evaluate different fungicides, botanicals and bio-agents under lab conditions to find out the most effective one for final use. The results of these studies will be helpful to the growers to adopt the most suitable control strategy.

MATERIALS AND METHODS

Isolation and identification of *Colletotrichum capsici*

Capsicum fruits having fruit rot symptoms were collected from capsicum growing fields. Isolation was done by cutting small pieces from the advancing margin of lesions were then immersed in 0.1% mercuric chloride for thirty seconds, washed three times in sterile distilled water, and blotted dry before being placed on PDA. The mycelium coming out of the tissues were sub-cultured to another petriplate incubated in room temperature. *Colletotrichum capsici* identification was done based on morphological characters such as size and shape of conidia and existence of setae.

Purification of *Colletotrichum capsici* - single spore isolation

Isolated pathogen *Colletotrichum capsici* was purified by single spore isolation. Ten ml of 2% water agar was poured into sterile petridishes and allowed to solidify. Dilute spore suspensionwas prepared in sterilized distilled water from 7 days old culture. One ml of spore suspension wasspread uniformly on agar plate. These plates were incubated at 28±2°C for 12 hrs. The plates were examined under microscope to locate single isolated and germinated conidium and marked with inkon the surface of the dishes. The growing hyphal tip was cut with the help of cork borer under aseptic conditions and with an inoculation needle it was carefully transferred to PDA slants and incubated at 28±2°C. This culture was used for further *in vitro* studies.

***In vitro* evaluation of bio-agents, fungicides and botanicals.**

The study was conducted at Plant Pathology Department, UAS, Bengaluru during 2016-17. Ten fungal and eight bacterial biocontrol agents were tested against test fungus. Both biocontrol agents and test fungus were cultured on potato dextrose agar in order to get fresh and active growth of each fungus. Twenty ml of sterilized and cooled potato dextrose agar was poured into sterile petriplates and allowed to solidify. For evaluation of fungal biocontrol agent. Five mm mycelial discs of test fungus were inoculated at one end of Petriplate and antagonistic fungus was placed opposite to it on the other end. In case of evaluation of bacterial antagonist, the bacterium was streaked at ends of the Petriplates and mycelial discs of the fungus was placed at the center. Three replications were maintained for each treatment. The plates were incubated at 27 ± 1ºC and zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism. The colony diameter of pathogen in control plate was also recorded. The per cent inhibition of the growth of the pathogen was calculated by using the formula suggested by Vincent[9].

The efficacy of 10 systemic fungicides and non-systemic fungicides were assayed at the concentration of 0.25, 0.5 and 0.1, per cent by using "Poison food technique"[3]. Required quantity of individual fungicide was added separately into molten and cool potato dextrose agar so as to get the desired concentration of fungicide. Later 20 ml of the poisoned medium was poured into sterile Petriplates. Mycelial discs of 5 mm size from actively growing culture of the fungus was cut by sterile cork borer and one such disc was placed at the center of each poisoned agar plate. Control was maintained without adding any fungicide to the medium. Each treatment was replicated thrice. Then such plates were

incubated at room temperature for ten days and radial colony growth was measured. The efficacy of a fungicide was expressed as per inhibition of mycelial growth over control that was calculated by using the formula suggested by Vincent[9].

Fresh plant part materials were collected and washed first in tap water and then in distilled water. Hundred grams of fresh sample was chopped and then crushed in a surface sterilized pestle and mortar by adding 100 ml sterile water (1:1 w/v). The extract was filtered through two layer of muslin cloth[2]. Finally filtrate thus obtained was used as stock solution. To study the antifungal mechanism of plant extract, the poisoned food technique was used. The medium was thoroughly shaken for uniform mixing of extract. Twenty ml of poisoned medium was poured into each of the 90 mm sterile petriplates. Each plate was seeded with mycelium of five mm size disc form periphery of actively growing culture were cut out by cork borer and one such disc was placed at the center of each agar plate. Controls were also maintained by growing the pathogen on PDA plates. Then such plates were incubated at 27 ± 1ºC temperature for ten days and radial growth was taken when maximum growth was occurred in the control plates. The efficacy of plant products or botanicals was expressed as per cent of radial growth over the control which was calculated by using the formula suggested by Vincent[9].

$$I = \frac{C - T}{C} \times 100$$

Where, I = per cent inhibition
C = growth in control
T = growth in treatment

Further, angular transformations were made for data and analyzed statistically.

RESULTS AND DISCUSSION

Effect of biocontrol agents on mycelial growth of *C. capsici*

In vitro evaluation of antagonists under dual culture revealed growth inhibition of pathogen (*C. capsici*) by these test antagonists. The maximum inhibition of mycelial growth was observed in *T. harzianum* -58-IIHR, (75.29%), observed in Plate 1. which was followed by *T. harzianum-7* (IIHR), (74.90%), *T. viride*-22-IIHR (74.51%), *T. viride-micro* (IIHR) (73.73%), *T. viride-5* (IIHR) (72.94%) *T. harzianum*-16-(IIHR), (72.59%) *T. harzianum*-41-(IIHR) (71.37%) whereas, *T. viride*-16-(IIHR) (68.63%) Similar results were obtained by Barhate *et al.*[1], who found that among the various bioagents studied, *T. harzianum* recorded highest growth inhibition (83.33 %) of *Colletotrichum capsici* and *T. viride-21* (IIHR), (69.80%) recorded least inhibition against pathogen. Among bacterial bio-agents *B. subtilis* P-37(78.43 %) observed in Plate 2. *B. amyloliquefaciens* P-42 (71.76 %), *B. subtilis* P-21(70.59 %) whereas, *B. subtilis* P-48 (56.47 %) and *Pseudomonas putida* (52.94 %) were recorded least inhibition against pathogen.

Plate. 1 Antagonistic action of *T. harzianum-58* against *C. capsici*

Plate. 2 Antagonistic action of *B. subtillis* (P-37*)* against *C. capsici*

Effect of fungicides on the mycelial growth of *C. capsici*

The studies on *in vitro* evaluation of fungicides against *C. capsici* through poisoned food technique revealed that the recommended doseAt 1000 ppm, Propiconazole, Difenoconazole, Tebuconazole completely inhibited mycelial growth amounting to 100 per cent. Similar results were obtained by Shovan *et al.*[6], who found that among the various fungicides studied, Propiconazole showed complete inhibition of pathogen growth, sporulation of *C. capsici* at 0.1 per cent. Likewise, Shilpa *et al.*[5], said that Difenoconazole and Propiconazole at 0.05 and 0.1 per cent completely inhibited the growth of test fungus *C. capsici*. Highest inhibition was seen in Trifloxystrobin + Tebuconazole amounting to 72.08 per cent, Captan (63.33 %), Benomyl (57.92 %), Mancozeb (40.00 %) and Chlorothalonil (35.00 %). Minimum inhibition was recorded in Copper oxy chloride amounting to 25.83 per cent followed Metalaxyl (28.75 %). Data presented in figure 2. At 500 ppm, complete inhibition of mycelial growth of the pathogen was recorded in Propiconazole and followed by Difenoconazole (97.92 %), Tebuconazole (83.75 %), Trifloxystrobin + Tebuconazole (66.67 %), Captan (62.50 %), Benomyl (44.17 %), and Mancozeb (31.25 %). Minimum inhibition was recorded in Metalaxyl, Copper oxy chloride and Chlorothalonil amounting to 8.75, 17.50 and 22.08 per cent, respectively. At 250 ppm complete inhibition of mycelial growth of the pathogen was recorded in Propiconazole followed by Difenoconazole (87.92 %), Tebuconazole (70.83 %), Trifloxystrobin + Tebuconazole (35.83 %), Benomyl (35.00 %) and Captan (34.58 %). The least inhibition of fungus was recorded in Metalaxyl (9.17%) followed Chlorothalonil (12.92%) and Mancozeb (13.33 %).

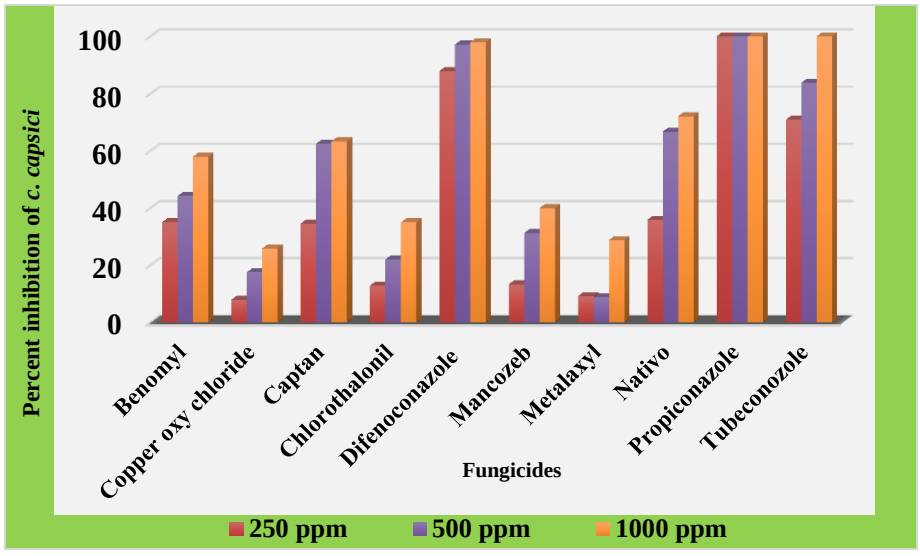

Fig. 1: *In vitro* evaluation of fungicides against *C. capsici*

Effect of botanicals on the mycelial growth of *C. capsici*

At 30 per cent concentration, maximum inhibition of mycelial growth of the pathogen was recorded with Subabul (45.10 %) followed by Simarouba (42.75 %), Pongamia (39.22 %), Lantana (35.29 %). Singh *et al.*[7], studied the different plant extracts, among the them, Pongamia leaf extracts and neem leaf extracts was found to be effective against *C. capsici* reported that extracts completely inhibited the growth and spore germination of *C. capsici*. Minimum inhibition was recorded in Nagadhale (33.33 %), Tulsi (32.94 %), and Lemon grass (28.24 %). Similarly, Simarouba leaf extract recorded maximum per cent inhibition of 32.16 and 36.47 per cent at 10 and 20 per cent, respectively followed by Subabul leaf extract. In Subabul leaf extract, the per cent inhibition was 28.63 and 34.90 per cent at 10 and 20 per cent respectively. Pongamia was inhibited the growth of pathogen was 26.27, and 38.04 per cent at 10 and 20 and 30 per cent respectively. Neem was inhibited the growth of the fungus, the per cent inhibit was 26.27 and 32.94 per cent at 10 and 20 per cent respectively. Minimum inhibition of pathogen was recorded in Nagadhale was 28.24 and 31.76 per cent at 10, and 20 per cent respectively fallowed by Lemon grass in this treatment per cent inhibition of fungus was recorded up to 15.69 and 23.53 per cent at 10 and 20 per cent, respectively. Lantana showed minimum per cent inhibition of 14.51 at 10 per cent concentration, similarly 27.45 per cent at 20 per cent concentration. However, Simarouba, Subabul and Pongamia leaf extract found to be effective against *C. capsici*. Data presented in figure 2.

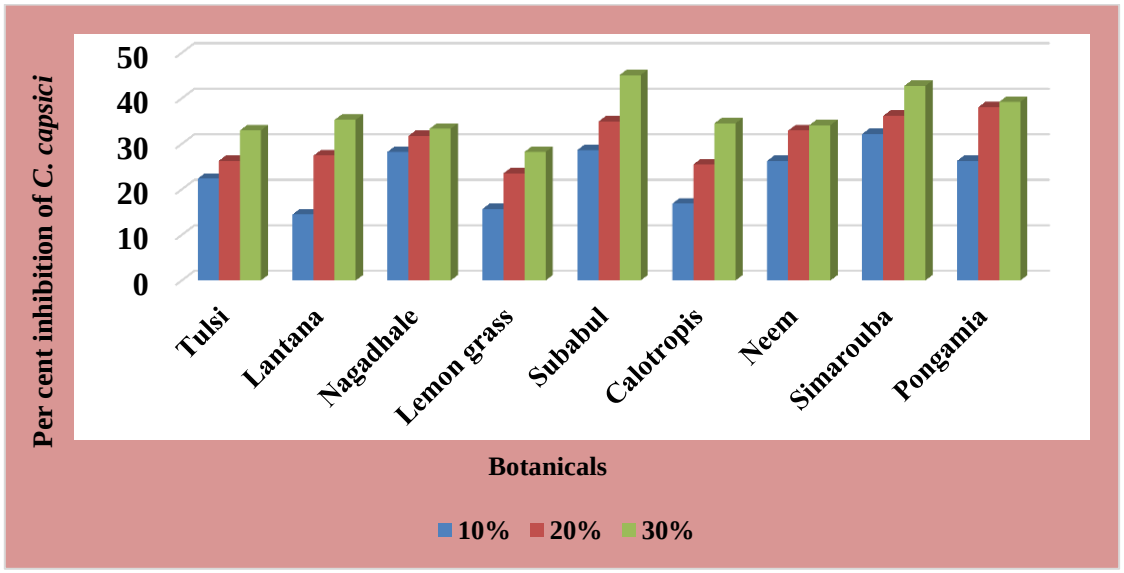

Fig. 2: *In vitro* evaluation of botanicals against *C. capsici*

CONCLUSION

In this study *T. harzianum* -58-IIHR, (75.29%) and *B. subtilis* P-37(78.43 %) were inhibited mycelia growth of pathogen. In fungicides, Propiconazole, Difenoconazole and Tebuconazole (250ppm, 500ppm and 1000ppm) at all concentrations completely inhibiting the mycelial growth of *C. capsici in vitro* and in botanicals Subabul (45.10 %) and Simarouba (42.75 %) were found to be effective.

REFERANCES

1. Barhate, B. G., Bidbag, M. B., Ilhe, B. M. And Shivagaje, A. J., *In vitro* evaluation of fungicides and bioagents against *colletotrichum capsici* causing die-back of capsicum. *J. Pl. Dis. Sci.*, **7(1):** 64–66 (2012)
2. Gerard Ezhilan, J., Chandrasekar, V. And Kurucheve, V., Effect of six selected plant products and oil cakes on the sclerotial production and germination of *Rhizoctonia*

solani. *Indian Phytopathol*., **47:** 183-185 (1994).

3. Grover, R. K. And Moore, J. D., Toxicometric studies of fungicides against brown rot organisms *Sclerotinia fructicola* and *S. laxa*. *Phytopathology*, **52:** 876- 880 (1962).
4. Manandhar, J. B., Hartman, G. L and Wang, T. C., Anthracnose development on pepper fruits inoculated with *Colletotrichum gloeosporioide*s. *Pl. Dis*., **79:** 380–383 (1995).
5. Shilpa. T. C. And Gokulapalan, C., *In vitro* study of fungicides and biocontrol agents against *Colletotrichum capsici* causing anthracnose of chilli (*Capsicum annuum* L.) *Int. J. App. Pure. Sci. Agri.*, **1(5):** 93-98 (2015).
6. Shovan, L. R., Bhuiyan, M. K. A., Begum, J. A. And Pervez, Z., *In vitro* control of *colletotrichum dematium* causing anthracnose of soybean by fungicides, plant extracts and *Trichoderma harzianum. Int. J. Sustain. Crop Prod*., **3(3):** 10-17 (2008).
7. Singh, S. N., Yadav, B. P., Sinha, S. K. And Ojha, K. L., Efficacy of plant extracts in inhibition of radial growth and spore germination of *Colletotrichum capsici*. *J. Appl. Biol.*, **7(1):** 58-61 (1997).
8. Sydow, H., Beitrage Zun Kennt Den Pilzflora De Studliech. *OSTINOLIENS* **1**: ANNMYCO. (Syd.) Butler and Bisby. *Haryana J. Horti. Sci.* **2:** 128-133 (1913).
9. Vincent, J. M., Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, **159:** 850 (1947).