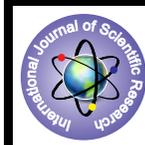


Studies on *Colletotrichum capsici* - a Plant Pathogenic Fungus: Selection of Culture Medium for Growth And Determination of Minimum Inhibitory Concentration of Fungicide



Botany

KEYWORDS : *Colletotrichum capsici*, PDA, disc diffusion method, fungicides, MIC

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ABSTRACT

The present study investigated the growth of *Colletotrichum capsici* in four different growth medium Potato Dextrose Agar (PDA), Czapek Dox Agar (CDA), Sabouraud Dextrose Agar (SDA) and Peptone Yeast Extract Dextrose Agar (PYDA). Among the different media tested, PDA supported the maximum growth significantly compared to all other media. The two fungicides Indofil M-45 and Carbendazim were evaluated for MIC (minimum inhibitory concentration) activity against the fungi. The effect of Indofil M-45 and Carbendazim on the fungi was evaluated by the disc diffusion method and the inhibition zones showed that the diameter of the colony of the *Colletotrichum capsici* was reduced. The higher concentration gave the maximum effect which decreased with dilutions. Carbendazim showed maximum efficacy even at a very low concentration of 10^{-9} mg/ml as compared to that of Indofil at 10^{-3} mg/ml. The two fungicides Indofil M-45 and Carbendazim were found to inhibit the growth of the fungi. However, Carbendazim was more effective than Indofil.

Introduction

Colletotrichum capsici (Sydow) E. J. Butler & Bisby is an important pathogen with a worldwide distribution and is involved in diseases of economically important hosts such as pepper (Than et al., 2008) and papaya (Tapia-Tussell et al., 2008). Many species of the fungal genus *Colletotrichum* are ubiquitous and polyphagous. Some are considered saprophytes, while others cause anthracnose and fruit rots. Description of the infection process and symptoms has been reported (Adikaram et al., 1983; and Mah, 1985). Anthracnose caused by *C. capsici* is the most destructive disease of pepper (Amusa et al., 2004), and causes losses by pre- and post-emergence damping off, leaf spots, premature fruit drop, mummification of unripe green pepper fruits and fruit rots (Agrios, 1988). Between 50 - 100% fruit loss has been reported in India, North America and tropical Africa due to anthracnose infection (Tindall, 1983; Amusa et al., 2004).

The MIC was determined as the lowest concentration of the antifungal drug preventing growth of macroscopically visible colonies on drug containing plates when there was visible growth on the drug-free control plates (Therese, 2006). Chemicals can have a wide range of effects on our health. Depending on how the chemical will be used, many kinds of toxicity tests may be required. Disk diffusion method is simpler and faster than broth-based methods (Espinel-Ingroff and Rezusta, 2002).

Keeping the information in mind, we have investigated that the growth of *C. capsici* in four different growth medium. Moreover, two fungicides Indofil M-45 and Carbendazim were evaluated for MIC (minimum inhibitory concentration) activity against this fungus.

Material and methods

C. capsici (MTCC No. 2071) were collected from Microbial Type Culture Collection (Institute of Microbial Technology, Chandigarh, India), and were subcultured on Malt extract medium and incubated at 28°C. Mycelial growth were estimated by day wise measuring colony diameters of *C. capsici* from 3rd day to 20th day at $25 \pm 2^\circ\text{C}$ in four media i.e., Czapek Dox Agar (CDA), Sabouraud Dextrose Agar media (SDA), Peptone Yeast Dextrose Agar (PYDA) and Potato Dextrose Agar (PDA). Different solid media mentioned above were used for assessing the growth of isolates of *C. capsici*. The mycelial growth diameter as well as morphological character of mycelia in different media was recorded. The different colony characters were recorded in each medium by visual observation and measured day wise from 3 days growth up to 20th day's growth condition. Three replicates were made for each treatment. Means were separated by using Tukey test.

Agar plates were inoculated with mycelia taken from the margin of a colony of the fungus grown on Malt extract medium, for 7 days. After seven days, black sporodochia with conidia and setae were formed on the surface of petriplates. The growth of the isolate was evaluated against Indofil M-45 (Indofil Chemicals Company, India) and Carbendazim (Insecticides India Limited, India) at different concentrations using serial dilution method. The MIC was determinate against the tested pathogenic fungi.

An activity was performed by standard method, Kirby-Bauer disk-diffusion method (Bauer et al., 1959) on agar plates and the MIC was calculated using dilution method. Dilution susceptibility testing methods were used to determine the MIC of fungicide. A serial dilution procedure that will allow obtaining dilutions of the fungicide at dilution factors of 10^{-1} , 10^{-2} , 10^{-3} and so on. Indofil M-45 and Carbendazim (purity 100%) were accurately weighed and dissolved in sterile deionized water to give appropriate dilutions of 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} , 10^{-10} , 10^{-11} and 10^{-12} to yield the required concentrations. The dilutions were aliquot in 5 ml volumes and frozen at -20°C .

Whatman filter paper (No. 1) was used to prepare discs approximately 6 mm in diameter, which are placed in hot air oven for 30 minutes and autoclaved for sterilization. After sterilization, the discs were loaded with different concentration of two broad spectrum fungicides Indofil M-45 and Carbendazim separately and again kept under refrigeration for 24 hrs.

MIC determination was carried out in Malt Extract Agar Medium as the fungal mycelia grow in the medium as white colony after 7 days of inoculation. Agar plates were inoculated with mycelia taken from the margin of a colony of the fungus grown on Malt extract medium, for 7 days. Sterile Whatman filter paper (No.1) discs were saturated with different concentration of broad spectrum two fungicide Indofil M-45 and Carbendazim and transferred the fungicide impregnated disks on the surface of the solidified medium 0.5cm away from the growing hyphal tip of *C. capsici* in each plate. The plates were then incubated at room temperature for 24 hours. After completion of 24hrs, the plates were inverted and placed in an incubator set to $25 \pm 2^\circ\text{C}$ for 48 hrs and the inhibition zones were measured as described by Barry et al. (1970) and Cruickshank et al. (1975).

Results and Discussion

The results showed that all the four media tested supported the mycelial growth of all the isolates of *C. capsici*. The four

different growth medium were Potato Dextrose Agar (PDA) Medium, Czapek Dox Agar Medium (CDA), Sabou raud Dextrose Agar Medium (SDA) and Peptone Yeast Extract Dextrose Agar Medium (PYDA). Among the different media tested, Potato Dextrose Agar medium supported significantly the maximum growth of all other media (Table 1).

Table 1. Average colony diameter of *Colletotrichum capsici* in different growth media

Media	Average size (cm)
PDA	6.34±0.656a
SDA	0.70±0.05b
PYDA	2.07±0.19b
CDA	4.01±0.50c

C. capsici produced blackish white coloured colonies on PDA and dark white coloured colonies on CDA, SDA and PYDA medium. It has been found that the most significant growth was observed in PDA and CDA by average colony diameter size values. But there was no significant growth was observed in both SDA and PYDA plates.

C. capsici has shown a proper sigmoid growth curve in PDA petriplates whereas in CDA plates the fungi are still in its exponential phase by the end of 20th day. In CDA and SDA petriplates the fungi showing very short exponential phase till 3rd day and then enters into almost stationary phase till 20th day (Fig. 1).

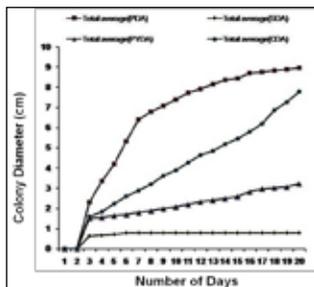


Fig. 1. Growth curve of *Colletotrichum capsici* in different growth media

Carbendazim and Indofil exhibit zone of inhibition of the fungus, *C. capsici* (Fig. 2).

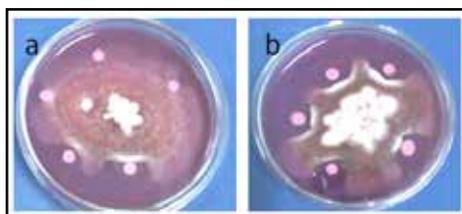


Fig. 2. Growth inhibition of *Colletotrichum capsici* by indofil (a) and carbendazim (b).

The MIC of carbendazim was determined to be 10⁻⁹ mg/ml. The MIC of Indofil M-45 was determined to be 10⁻³ mg/ml (Table 2).

Table 2. Growth inhibition of *Colletotrichum capsici* in different dilution of indofil and carbendazim

Concentration (mg/ml)	Zone of inhibition (in cm) Indofil	Zone of inhibition (in cm) carbendazim
10 ⁻¹	0.6	-
10 ⁻²	0.3	-
10 ⁻³	0.0	2.2
10 ⁻⁴	-	1.5
10 ⁻⁵	-	0.7
10 ⁻⁶	-	0.5
10 ⁻⁷	-	0.3
10 ⁻⁸	-	0.1
10 ⁻⁹	-	0.0

The zone of inhibition by carbendazim ranges from 0.1 to 2.2 cm. The zone of inhibition by Indofil ranges from 0.3 to 2.4 cm.

Carbendazim and Carbendazim + Mancozeb gave 100 % inhibition of mycelial growth of *Fusarium solani* at 0.2 and 0.3% concentrations (Chavan et al., 2009). Root infection BY *Fusarium solani* was completely checked by Benlate and Carbendazim in bitter gourd and was best controlled by Aliette, Topsin-M and Carbendazim in bottle gourd and cucumber (Sultana and Ghaffar, 2010). Our result also shows that Carbendazim inhibit the growth of *C. capsici*.

In conclusion, our results suggest that carbendazim can be used in lower doses for controlling a number of plant pathogens that cause destruction of crops and vegetables with a prospect of potential application in agro-industry.

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