

Original Research Article

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***In Vitro* Evaluation of Bio-Control Agents, Biopesticides and Botanicals against *Colletotrichum truncatum* Causing Anthracnose of Horse Gram (*Macrotyloma uniflorum*)**

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ABSTRACT

The experiment was conducted under *in vitro* to observe the effect of bio-agents, biopesticides and botanicals against *Colletotrichum truncatum*. Antagonistic activity of bioagents viz., *Trichoderma viride*-H (Hydrabad strain), *T. harzianum*-H (Hydrabad strain) and *Pseudomonas fluorescens* (TNAU strain) against *C. truncatum* was tested using dual-culture technique. Maximum inhibition of mycelial growth was observed with *T. viride*-H (58.81%) followed by *P. fluorescens*-TNAU (46.03%) and *T. harzianum*-H (35.47%). *In vitro* evaluation of neem based biopesticides was carried out by poisoned food technique. For this three neem based biopesticides viz., Neemban, Neembenicide and Achook were tested at 0.05, 0.1 and 0.5 per cent concentrations for inhibitory activity on mycelial growth. Achook was found most effective and resulted in maximum inhibition of 52.34, 57.03 and 66.40 per cent followed by Neembenicide which showed 29.66, 45.29 and 59.37 per cent inhibition at 0.05, 0.1 and 0.5 per cent concentrations, respectively. Eight locally available plants which grow in abundance viz., basuti (*Adhatoda vasica*), bhang (*Cannabis sativa*), kali basuti (*Chromolaena odorata*), barein (*Acorus calamus*), butter-cup (*Ranunculus bulbosus*), safeda (*Eucalyptus globulus*), curry plant (*Murraya koenigii*) and pine needles (*Pinus longifolia*) were used and aqueous extracts of different concentrations (20, 40, 60, 80 and 100%) were tested for their efficacy against the pathogen. An increased inhibitory effect of mycelial growth was observed with an increase in concentration from 20 to 100 per cent. *Cannabis sativa* at 100 per cent was found best (81.23%) followed by *Chromolaena odorata* (80.46%) and *Adhatoda vasica* (67.96%). At 20 per cent *Eucalyptus globulus* was found to be most effective (35.14%) followed by *Pinus longifolia* (19.51%) and *Acorus calamus* (17.14%). At 40 (49.24%), 60 (60.13%) and 80 (70.28%) per cent concentration *Chromolaena odorata* was found to be effective.

Keywords

Bio-control agents,
Botanicals,
Biopesticides,
Colletotrichum truncatum, Horse
Gram.

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Introduction

Pulses are important food crops due to their high protein and essential amino acid content. Like many leguminous crops, pulses play a key role in crop rotation due to their ability to fix nitrogen. Majority of population being vegetarian, pulses constitute an integral part

of Indian diet. However, pulses are subjected to the attack of a variety of diseases and insect pests. Horse gram (*Macrotyloma uniflorum*), commonly known as *Kulthi* in north India, is an important edible legume, consumed throughout the country. It is primarily a crop

of the dry and upland areas of the peninsular and eastern states of India. In India horse gram is extensively grown in Karnataka, Andhra Pradesh, Tamil Nadu, Madhya Pradesh and parts of Maharashtra, Bihar, Orissa and to some extent in the hilly slopes of Himachal Pradesh and Uttar Pradesh. Horse gram requires an average temperature of 20–30°C and does not tolerate frost. It is drought-resistant and can be grown with rainfall as low as 380 mm. It is mostly grown in areas with less than 900 mm annual rainfall. Among diseases, leaf spotting fungi are a major constraint in realization of full genetic potential of a pulse crop. Horse gram too, suffers from both biotic and abiotic constraints. During the cropping season, wet and humid environment conditions predispose the crop to the attack of many pathogens. The main diseases of horse gram in India are anthracnose (*Colletotrichum truncatum*), leaf spot (*Cercospora dolichi*), rust (*Uromyces appendiculatus*), root rot (*Pellicularia filamentosa*), dry root rot (*Macrophomina phaseolina*) and horse gram yellow mosaic virus (HgYMV).

Anthrachnose and leaf spot are the major diseases of the crop under high rainfall conditions of Himachal Pradesh. No precise estimates of losses due to these diseases are known. About nine species of *Colletotrichum* have been recorded on legume crops worldwide, including *C. capsici*, *C. coccodes*, *C. crassipes*, *C. dematium*, *C. destructivum*, *C. gloeosporioides*, *C. lindemuthianum*, *C. trifolli* and *C. truncatum* (Lenne 1992). Legume crops such as bean (*Phaseolus vulgaris* L.), cowpea (*Vigna unguiculata* L. Walp), soybean (*Glycine max* (L) Merr.), peanut (*Arachis hypogea* L.), lentil (*Lens culinaris* Medik.) and alfalfa (*Medicago sativa* L.) have been reported as hosts of *Colletotrichum* species (Bailey and Jeger 1992). *C. truncatum* is a highly unspecialized pathogen, which attacks many grain and

forage legumes including soybean (*Glycine max*), lentil (*Lens culinaris*), urdbean (*Vigna mungo*), mungbean (*V. radiata*), cowpea (*V. unguiculata*), horse gram (*M. uniflorum*), pea (*Pisum sativum*) and various weed hosts (Lenne and Sonoda 1978; Sharma and Kaushal 1999). Isolates of *C. truncatum* vary considerably in colony characteristics, size of fruiting structures and pathogenicity. Additionally, *C. truncatum* population from different hosts often exhibit different host preferences. Conidia of *C. truncatum* are described as hyaline and one celled, with slightly falcate shape and a size of 17.0-31.5 x 3.0-4.5µm (length x width). Dark brown to black setae are generally produced in abundance (Tiffany and Gilman 1954; Sutton 1992). Anthracnose caused by *C. truncatum* is one of the most important seed-borne fungal pathogens of horse gram. The disease causes a significant reduction of seed germination, seed quality thereby limiting its potential yield. Anthracnose may also attack more mature plants during the later part of the growing season. The crop is vulnerable to attack at all growth stages depending upon the conditions favourable for initiation and development of the disease.

Materials and Methods

Bio-control agents

Antagonistic activity of bioagents viz., *Trichoderma viride*-H (Hydrabad strain) and *T. harzianum*-H (Hydrabad strain) against *C. truncatum* was tested using dual-culture technique (Huang and Hoes 1976). Two mm dia. culture discs of each of the pathogen and biocontrol agent were picked from the margin of 7 day old cultures with a sterilized cork borer and transfer under aseptic conditions to PDA plates in such a way that the distance between the pathogen and bioagent was about 5 cm. In case of bacterial antagonist *Pseudomonas fluorescens* (TNAU-

Coimbatore strain) disc of 2 mm dia of the pathogen was placed at the centre of PDA plate and the bacterium was streaked around the disc (Laha *et al.*, 1992). The disc of the pathogen grown alone on the PDA plates served as control. The cultures were incubated at 25±1°C. Each treatment was replicated thrice. Data on mycelial growth were recorded when control Petriplate was fully covered. Per cent inhibition of mycelial growth was calculated by Vincent (1947) formula:

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

I = % inhibition

C = growth in control

T = growth in treatment

Biopesticides

In vitro evaluation of neem based biopesticides was carried out by poisoned food technique. For this desired quantity of biopesticide *viz.*, neemban, neembenicide and ahook were measured and different concentrations (0.05%, 0.1% and 0.5%) were made. Then double strength PDA medium was prepared and supplemented with the equal amount of different concentrations of biopesticides in the flasks under aseptic conditions.

Twenty ml of this mixture was poured in sterilized Petriplates and inoculated with disc of 2 mm dia. taken from the periphery of 7 day old culture of *C. truncatum* and incubated at 25±1°C. Each treatment was replicated thrice. A control was also maintained for comparison where no biopesticide was added. Data on mycelial growth were recorded when control Petriplate was fully covered. Per cent inhibition on mycelial growth was calculated (Vincent 1947).

Botanicals

Eight locally available plants which grow in abundance *viz.*, basuti (*Adhatoda vasica*), bhang (*Cannabis sativa*), kali basuti (*Chromolaena odorata*), barein (*Acorus calamus*), butter-cup (*Ranunculus bulbosus*), safeda (*Eucalyptus globulus*), curry plant (*Murraya koenigii*) and pine needles (*Pinus longifolia*) were used for their efficacy against the pathogen.

Preparation of extracts

These plants were selected to estimate the antimicrobial activity against *C. truncatum* by poisoned food technique (Nene and Thapliyal 1965). Two hundred grams of fresh leaves from each plant were washed well and grounded in 200 ml of distilled water by using mixer and grinder. The macerate was filtered through double layered cheesecloth and centrifuged at 3500 rpm for 20 min. The supernatant was filtered through Whatman No. 41 filter paper. The supernatant (pure stock, 100%) was filter sterilized with filter syringes (0.2 µ pore size) under aseptic conditions and further dilutions were made to different concentrations of 20, 40, 60, 80 and 100 per cent.

Double strength PDA medium was prepared and then supplemented with the equal amount of different concentrations of plant extracts in the flasks under aseptic conditions. Twenty ml mixture of medium and extract was poured into sterilized Petriplate and inoculated with a disc of 2 mm dia. taken from the periphery of 7 days old culture of *C. truncatum* and incubated at 25±1°C. Each treatment was replicated thrice. A control was also maintained for comparison where no plant extract was added. Data on mycelial growth were recorded when control Petriplate was fully covered. Per cent inhibition on mycelial growth was calculated (Vincent 1947).

Results and Discussion

In vitro evaluation of bio-control agents

Results of evaluation of antagonistic effects of biocontrol agents *viz.*, *Trichoderma viride*-H, *T. harzianum*-H and *Pseudomonas fluorescens*-TNAU against *C. truncatum*, recorded as inhibition of mycelial growth in dual culture are presented in Table 1 and Figure 1. Maximum inhibition of mycelial growth was observed with *T. viride*-H (58.81%) followed by *P. fluorescens*-TNAU (46.03%) and *T. harzianum*-H (35.47%) (Plate 10).

Pollution problems in the environment and the toxic effect of synthetic chemicals on non-target organisms have promoted investigations on exploiting biological control agents as one of the component of disease control. As many species of fungi and other microbes have been reported to be antagonists of *Colletotrichum*, only a few of them have been studied extensively. To reduce the harmful effects of pesticides in the ecosystem the use of biocontrol agents in plant disease management has been experimented. All the three bio-control agents used in the present study showed antagonistic activity under *in vitro* conditions by inhibition of mycelial growth of the pathogen. Bankole (1990) observed an isolate of *T. viride* TH-31 was found to hyperparasitize a number of plant pathogenic fungi including *Colletotrichum*. Gawade *et al.*, (2009) evaluated two biocontrol agents *in vivo* against anthracnose of soybean. Both biocontrol agents (*T. viride* and *Verticillium lecanii*) were found significantly superior to unsprayed control.

In vitro evaluation of biopesticides

Three neem based biopesticides *viz.*, Neemban, Neembenicide and Ahook were tested at 0.05, 0.1 and 0.5 per cent

concentrations using poisoned food technique (Plate 11). Data on per cent growth inhibition is presented in Table 2 and Figure 2. The data revealed that all biopesticides were found significantly effective against the *C. truncatum*. The per cent inhibition was increased with the increasing concentration from 0.05 to 0.5 per cent of all the biopesticides. However, Ahook was found most effective and resulted in maximum inhibition of 52.34, 57.03 and 66.40 per cent followed by Neembenicide which showed 29.66, 45.29 and 59.37 per cent inhibition at 0.05, 0.1 and 0.5 per cent concentrations, respectively. Minimum inhibition was recorded in Neemban 25.78, 41.40 and 57.01 per cent at 0.05, 0.1 and 0.5 per cent concentrations, respectively.

However, Neemban (57.01%) and Neembenicide (59.37%) at 0.5 per cent concentration were at par with each other. During the past three decades, Neem has dominated the international literature on botanicals (NRC, USA 1992). In the present investigation, the results of evaluation of Neem based biopesticides showed that all the biopesticides inhibited mycelial growth of *C. truncatum* as compared to control.

In vitro evaluation of botanicals

The data with respect to effect of eight plant extracts on mycelial growth of *C. truncatum* are presented in Figure 3 and Table 3. *Cannabis sativa* at 100 per cent was found best (81.23%) followed by *Chromolaena odorata* (80.46%) and *Adhatoda vasica* (67.96%) (Plate 12). At 20 per cent *Eucalyptus globulus* was found to be most effective (35.14%) followed by *Pinus longifolia* (19.51%) and *Acorus calamus* (17.14%). At 40 (49.24%), 60 (60.13%) and 80 (70.28%) per cent concentration *Chromolaena odorata* was found to be effective. Minimum mycelial inhibition was

observed in *Murraya koenigii* at all the concentrations. *Murraya koenigii* (14.01%) and *Acorus calamus* (17.14%) were statistically at par at 20 per cent concentration. *Eucalyptus globulus* (46.07%) and *Chromolaena odorata* (49.24%) at 40 per cent concentration were found to be

statistically at par. *Adhatoda vasica* (43.74%) and *Cannabis sativa* (45.30%) were statistically at par at 60 per cent concentration. Similarly, *Pinus longifolia* (60.95%) and *Eucalyptus globulus* (64.82%) were statistically at par with each other at 100 per cent concentration.

Table.1 Effect of biocontrol agents on mycelial growth of *Colletotrichum truncatum*

Treatment	Mycelial growth (mm)*	Inhibition (%)
<i>T.viride</i> - H	34.00	58.81 (50.06)
<i>T.harzianum</i> - H	53.33	35.47 (36.52)
<i>P.fluorescens</i> - TNAU	44.67	46.03 (42.70)
Control	82.67	-
CD (P=0.05)	6.45	4.48

Figures in parentheses are arc sine transformed values

*Mean of three replications

H- Hyderabad

TNAU- Tamilnadu Agriculture University

Table.2 Effect of neem based biopesticides on mycelial growth of *Colletotrichum truncatum*

Concentration	0.05%		0.10%		0.50%	
	Mycelial growth (mm)*	Growth inhibition (%)	Mycelial growth (mm)*	Growth inhibition (%)	Mycelial growth (mm)*	Growth inhibition (%)
Neemban	63.33	25.78 (30.50)	50.00	41.40 (40.40)	36.67	57.01 (49.01)
Neembenicide	60.00	29.66 (32.96)	46.67	45.29 (42.28)	34.67	59.36 (50.37)
Achook	40.67	52.34 (46.32)	36.67	57.03 (49.03)	28.67	66.40 (54.55)
Control	85.33	-	85.33	-	85.33	-
CD (P=0.05)	2.66	2.48	3.77	3.18	2.17	2.23

Figures in parentheses are arc sine transformed values, *Mean of three replications

Table.3 Effect of botanicals on mycelial growth of *Colletotrichum truncatum*

Treatment	Mycelial growth (mm)* at concentrations (%)					Mycelial growth inhibition (%) at concentrations (%)				
	20	40	60	80	100	20	40	60	80	100
<i>Chromolaena odorata</i>	72.00	43.33	34.00	25.33	16.67	15.56 (23.19)	49.24 (44.55)	60.13 (50.83)	70.28 (56.96)	80.46 (63.75)
<i>Acorus calamus</i>	70.67	62.00	53.33	44.00	32.00	17.19 (24.31)	27.37 (31.49)	37.48 (37.73)	48.39 (44.05)	62.48 (52.21)
<i>Cannabis sativa</i>	73.33	54.67	46.67	25.33	16.00	14.04 (21.96)	35.93 (36.81)	45.30 (42.28)	70.28 (56.96)	81.23 (64.33)
<i>Ranunculus bulbosus</i>	71.33	66.67	53.33	40.00	33.33	16.39 (23.84)	21.91 (27.84)	37.47 (37.71)	53.17 (46.81)	60.93 (51.30)
<i>Eucalyptus globulus</i>	55.33	46.00	40.00	34.00	30.00	35.14 (36.34)	46.07 (42.73)	53.12 (46.78)	60.15 (50.84)	64.82 (53.61)
<i>Murraya koenigii</i>	73.33	72.00	66.67	47.33	38.67	14.01 (22.02)	15.61 (23.26)	21.87 (27.88)	44.50 (41.82)	54.65 (47.65)
<i>Adhatoda vasica</i>	71.33	68.67	48.00	41.33	27.33	16.39 (23.84)	19.51 (26.21)	43.74 (41.39)	51.55 (45.87)	67.96 (55.51)
<i>Pinus longifolia</i>	68.67	57.33	51.33	40.00	33.33	19.51 (26.17)	32.88 (34.88)	39.11 (39.11)	53.10 (46.76)	60.95 (51.30)
Control	85.33	85.33	85.33	85.33	85.33	-	-	-	-	-
CD (P=0.05)	3.36	4.43	3.02	5.44	3.43	3.68	3.26	2.56	4.16	2.89

Fig.1 Effect of biocontrol agents on mycelial growth of *Colletotrichum truncatum*

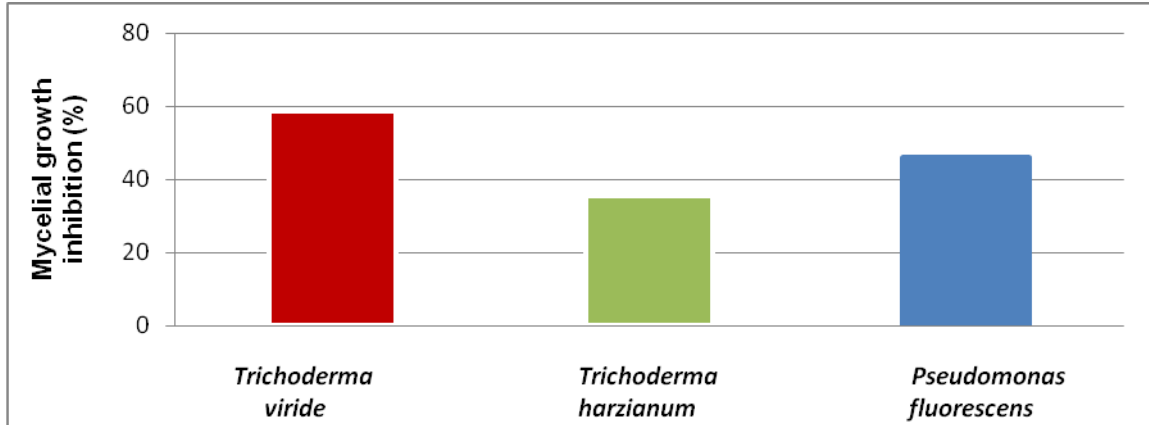


Fig.2 Effect of neem based biopesticides on mycelial growth of *Colletotrichum truncatum*

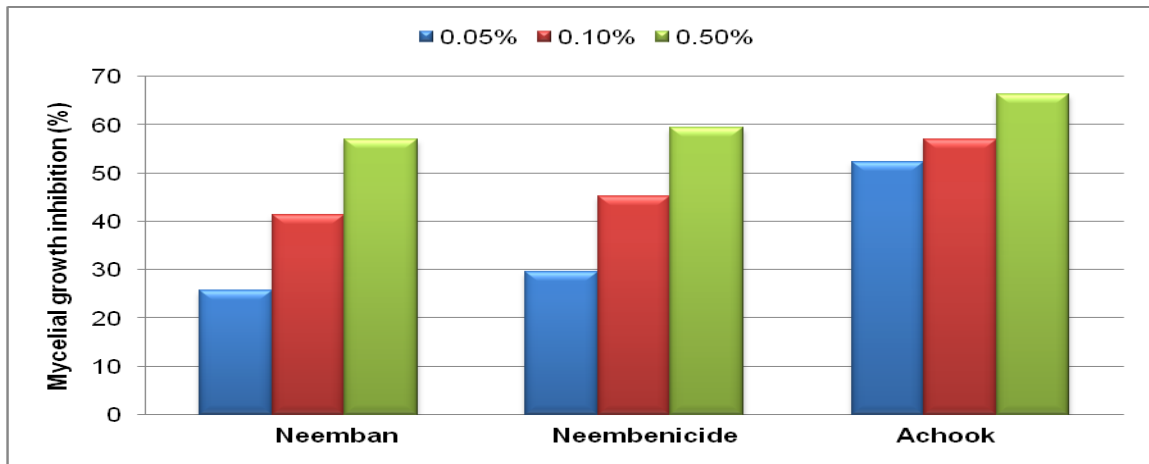


Fig.3 Effect of botanicals on mycelial growth of *Colletotrichum truncatum*

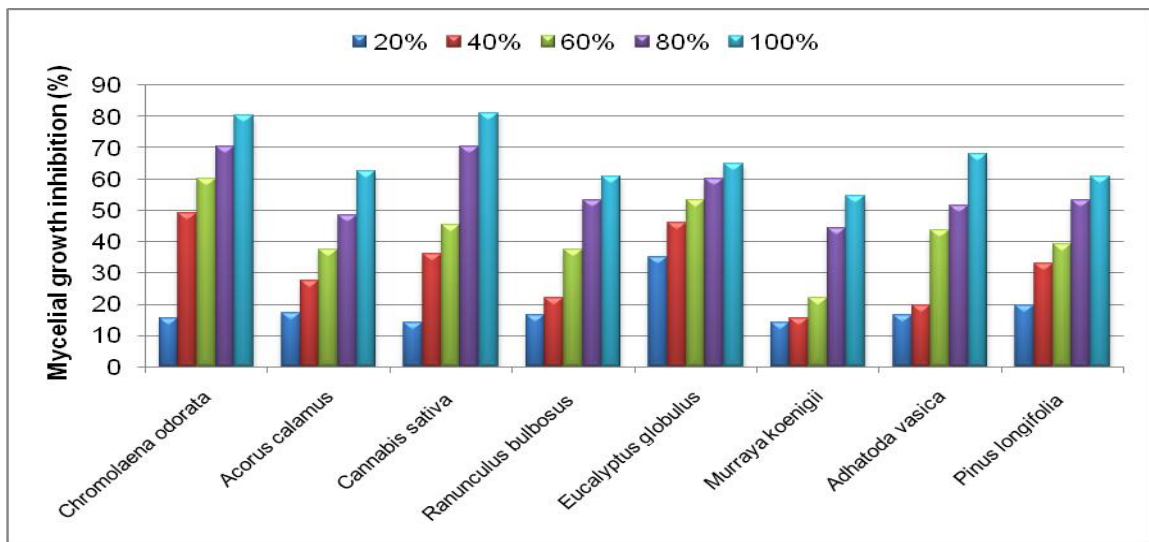
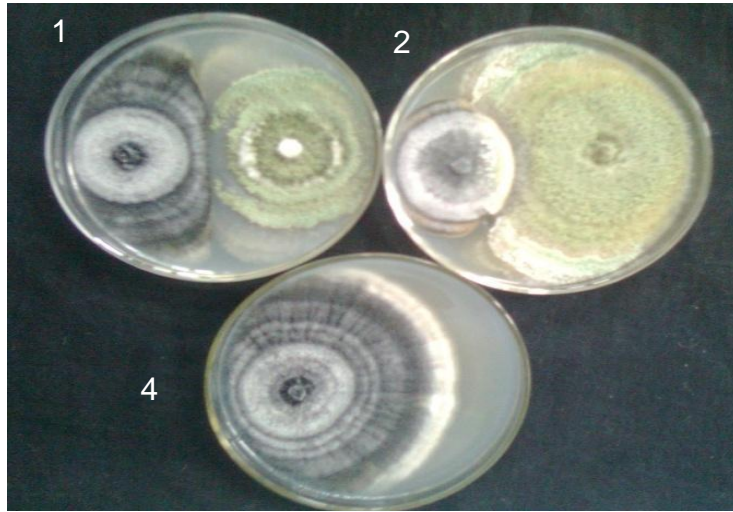
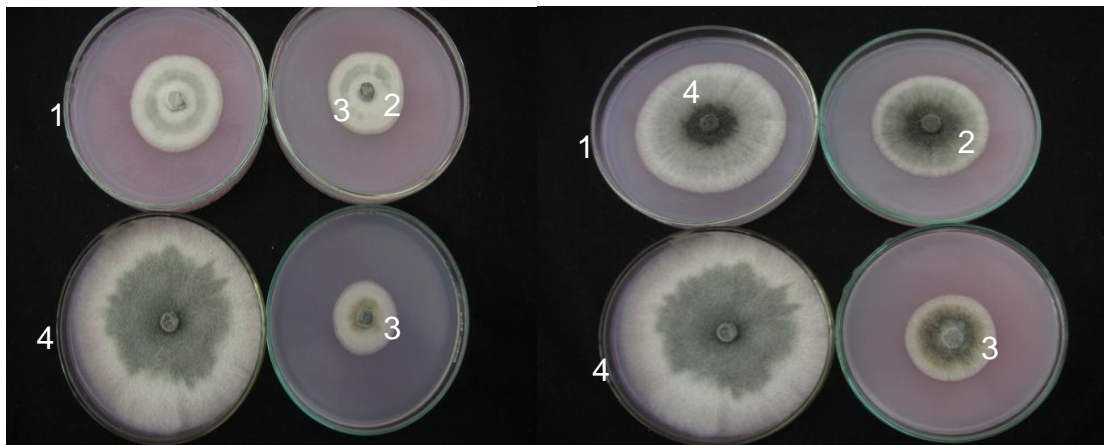


Plate.1 Antagonistic activity of biocontrol agents against *C. truncatum*



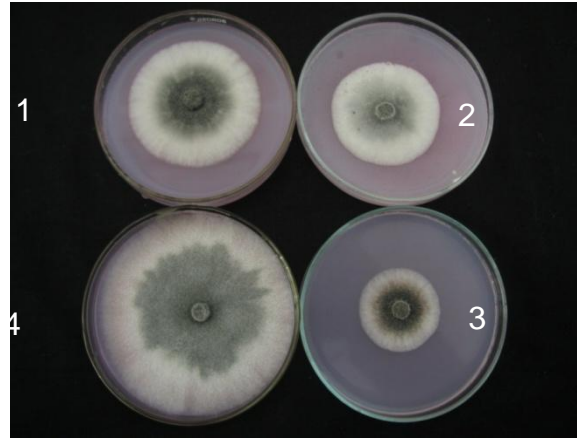
1=*Trichoderma harzianum*- H, 2= *Trichoderma viride*- H 3= *Pseudomonas -fluorescens* –TNAU, 4=Control

Plate.2 Efficacy of different biopesticides against *C. truncatum*



(i) Achook

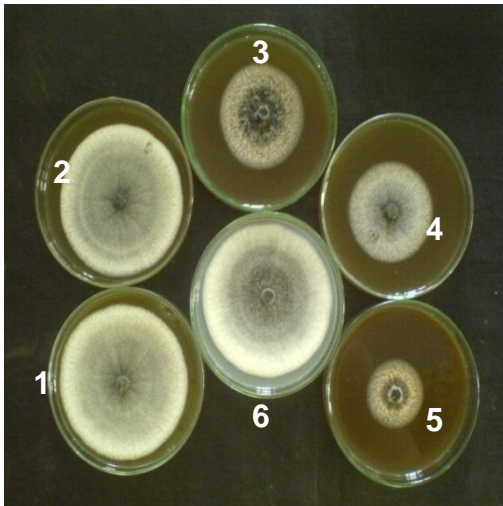
(ii) Neemban



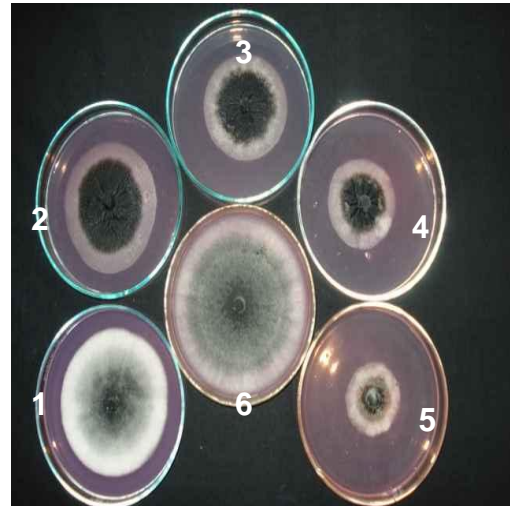
(iii) Neembenicide

1=0.05% 2=0.1% 3=0.5% 4=Control

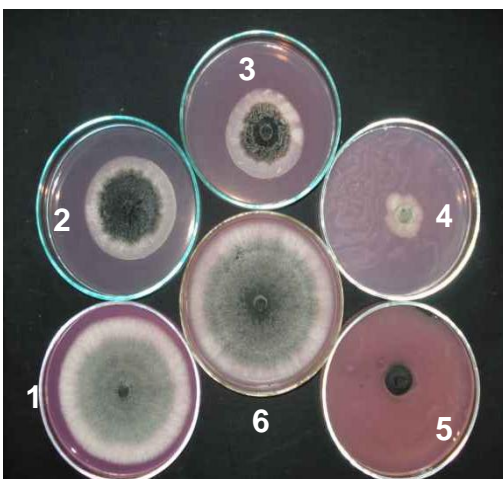
Plate.3 *In vitro* evaluation of botanicals against *C. truncatum*



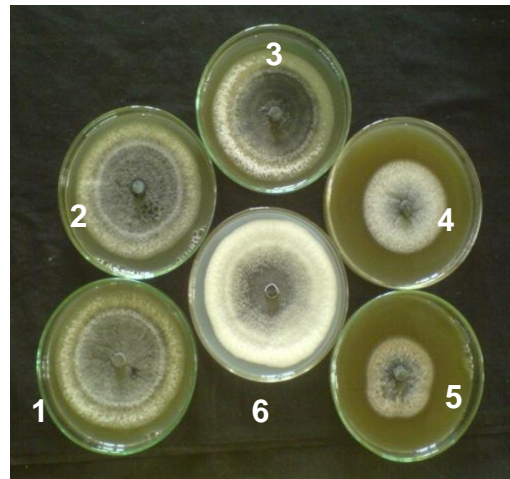
(i) *Adhatoda vasica*



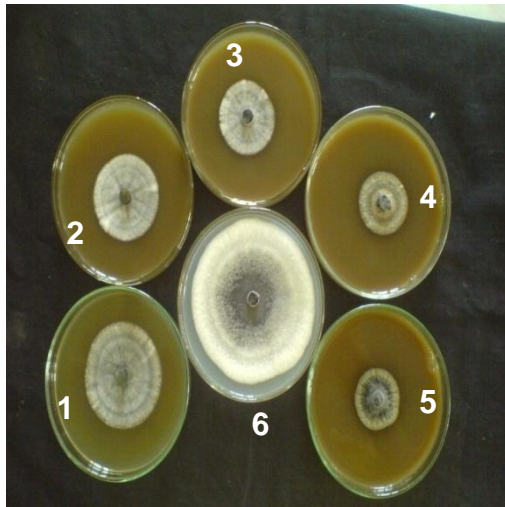
(ii) *Acorus calamus*



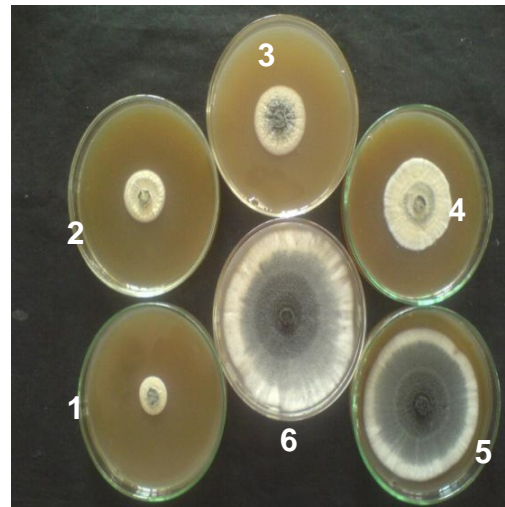
(iii) *Cannabis sativa*



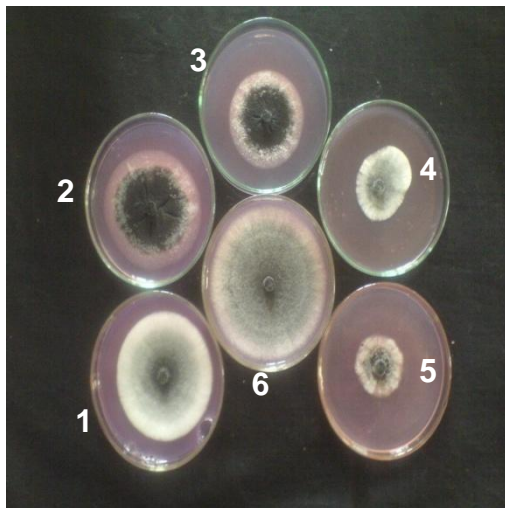
(iv) *Murraya koenigii*



(v) *Eucalyptus globulus*

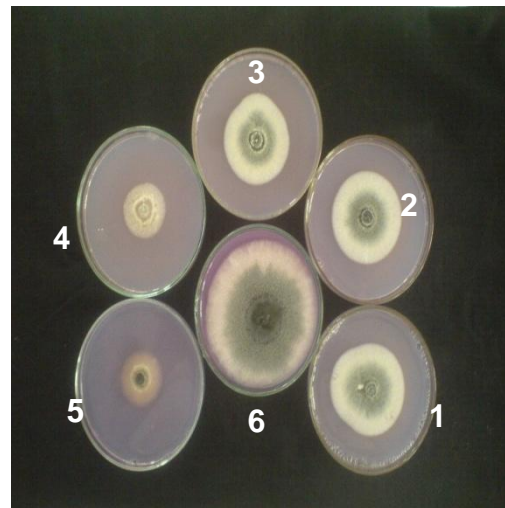


(vi) *Chromolaena odorata*



(vii) *Pinus longifolia*

1= 20% 2=40% 3=60%



(viii) *Ranunculus bulbosus*

4=80% 5=100% 6=Control

The data revealed that the best inhibition of mycelial growth was observed at 100 per cent concentration of all botanicals significantly. An increased inhibitory effect of mycelial growth was observed with an increase in concentration from 20 to 100 per cent. A large number of plants are known to possess antifungal properties against *C. truncatum* as reported by various workers. Kaushal and Paul (1989) studied inhibitory effects of some plant extracts (*Cannabis sativa*, *Pinus longifolia*, *Eupatorium* sp. and *Lantana indica*) on some legume pathogens and found that all the plant extracts inhibited *C. truncatum*.

Laboratory screening of plant extracts in the present study has given encouraging results, indicating their potential use in the management of anthracnose of horse gram.

Among the antagonists tested against the pathogen, *Trichoderma viride*-H was found most effective in inhibiting mycelial growth of *C. truncatum* (58.81%) followed by *Pseudomonas fluorescens*-TNAU (46.03%) and *T. harzianum*-H (35.47%). During the present investigations three neem based biopesticides viz., Neemban, Neembicide and Achook were tested against *C. truncatum* *in vivo* at 0.05, 0.1 and 0.5 per cent

concentrations. Achook was found most effective and resulted in maximum inhibition of 52.34, 57.03 and 66.40 per cent followed by Neembencide which showed 29.66, 45.29 and 59.37 per cent inhibition at 0.05, 0.1 and 0.5 per cent concentrations, respectively. Minimum inhibition was recorded in Neemban 25.78, 41.40 and 57.01 per cent at above mentioned concentrations, respectively. Among all the plant extracts, *Cannabis sativa* at 100 per cent was effective (81.23%) in inhibiting mycelial growth of *C. truncatum* followed by *Chromolaena odorata* (80.46%) and *Adhatoda vasica* (67.96%). At 20% *Eucalyptus globulus* was found to be most effective (35.14%) followed by *Pinus longifolia* (19.51%) and *Acorus calamus* (17.14%). Minimum inhibition was observed in *Murraya koenigii* at all the concentrations

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