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In vitro Evaluation of Fungicides and Bio-Control Agents against *Colletotrichum truncatum* in Soybean in Dehradun Valley

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ABSTRACT

A study was conducted for evaluation of the effectiveness of different concentration of four different fungicides and three different bio- agent against anthracnose of soybean caused by Colletotrichum truncatum (Andrus and Moore) under in vitro condition. Among the fungicide, carbendazim was found to be most effective and recorded highest inhibition in growth (98%) which was at par with hexaconazole (97%) over control after 7 days of incubation, whereas mancozeb (42.67%) and chlorothionil (33.67%) were appreciably ineffective for disease inhibition over control after 7 days of incubation. It was also revealed that carbendazim and hexaconazol had significant inhibitory effect on appressorium formation which has direct impact on pathogen penetration and disease development on the host surface. Three bio-agents evaluated for the experiment were T.viride, T.harzianum, and T.aesperellum. Among all three bio-agent T .harzianum, showed highest inhibition in growth (67%) followed by T.viride (58%) and T.aesperellum (50%).

Key words: *Soybean, Anthracnose, Collectotrichum truncatum, Fungicide and Bio-agent.*

INTRODUCTION

Soybean (*Glycine max* L. Merill) is leading oilseed crop of the world's having appreciable nutrient content along with highest protein among all legumes. In India, Soybean is relatively new crop which was introduced during 1960s only and then onwards it became one of most recognized and highly desirable pulse and oil seed crop. In fact in recent year soybean oil has preference over other cooking oil among Indian household. Presently, India represents itself at fifth position in term of the area and production of soybean all over the world just behind USA, Brazil, Argentina, and China. In spite of diversity of product and emerging popularity of soybean related food in India, there has been very poor productivity of soybean in India (1.1 t/ha) as compared to other courtiers (world average 2.2 t/ha)[1]. India has only 4% share in world soybean production although it has 10% of the world soybean area under production.

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It has been reported that there are various biotic and abiotic factors which causes constrain in soybean production in India. Soybean is affected by various pathogens, limiting its productivity as well as deteriorating its quality. Soybean anthracnose caused by *Colletotrichum truncatum* (Schw) Andrus and Moore is an imported disease reported to cause estimated yield losses of 16-26% in the United States, 30-50% in Thailand, and up to 100% in Brazil and in India[7]. Anthracnose of soybean was first reported in Korea in 1917, and now it is reported from all regions of world wherever soybean is commercially cultivated. Anthracnose is reported to have increased incidences in wet, warm and humid areas where main source of inoculum is from infected seeds and crop residue. Initial symptom of disease appears as lesions on cotyledon which later on spread on stems and leaves. However, the most destructive effect of disease is during reproductive phase of plant when the effected pod become twisted and aborted before maturity. At this stage disease management is most crucial for the successful cultivation of crop otherwise it can result in reduced production of crop causing significant economic loss[4].

MATERIAL AND METHODS

The present study was conducted in laboratory of Uttaranchal College of agricultural sciences, Uttaranchal University, Dehradun and the protocol of the present study was as follows.

Fungal Pathogen:

Pure culture of *Colletotrichum truncatum* has been obtained from Indian Type Culture Collection (ITCC), Division of Plant Pathology, Indian Agriculture Research Institute, New Delhi with the catalogue number 7074. The pathogen was transferred to PDA (Potato dextrose agar) and OMA (Oat meal agar) medium in Petri plates and the plates were incubated at 28 ± 1°C for growth. Sub-cultures were made from the periphery of the mycelial growth, which appeared after 6 -7 days.

Pathogenicity tests:

The pathogenicity of the isolated fungus *Colletotrichum truncatum* causing soybean anthracnose was proved by Koch's postulate under *in-vitro* condition.

***In-vitro* condition**

Healthy soybean pods were collected from the fields crop of soybean which was 25 days old. Collected pods were washed under tap water and then surface sterilized with 70% ethyl alcohol. *Colletotrichum truncatum* was cultured on PDA for 10 days under 12 hours dark light condition at 25°C. Then 0.7 cm agar plug containing mycelia of *Colletotrichum truncatum* was placed on the collected soybean pod. Pods inoculated with sterilised water served as control for the experiment. Inoculated pod were kept in moistened polythene bags to maintain humidity and incubated at 28 ±2°C and observed daily for the disease symptoms. The pathogen was re-isolated from the infected pod and compared with the original culture.

***In- vitro* evaluation of fungicides**:

For the present experiment four fungicide viz., carbendazim (0.1%), chlorothalonil (0.2%), hexaconazole (0.1%), and mancozeb (0.1%) were evaluated *in vitro* applying "poison food technique". The experiment was laid out in CRD having three replication for each treatment and in each treatment there were three plates per replication. At the very outset of an experiment, an appropriate amount of each fungicide was calculated and thoroughly mixed with auto-claved and cooled (40-45^{0}C) PDA in conical flasks to obtain desired concentrations. The fungicide amended PDA was then poured (15 – 20 ml/plates) in sterilized Petri plates (90 mm dia.) under aseptic conditions. On solidification of PDA in Petri plates, all treatment plates were inoculated aseptically by placing in the centre with 5.0 mm uniform mycelial disc obtained from 7 days old culture of *Colletotrichum truncatum*. Petri plates containing plain PDA without any fungicide were inoculated with 5.0 mm disc of the test pathogen and maintained as suitable untreated control. Petri plated for all the treatment (inoculated) and

control were then incubated at 24 to 28^{0}C in BOD incubator till the control plates were fully covered with mycelial growth of the test pathogen. Observations on per cent growth inhibition of the test pathogen over control was worked out[8] as follows

Percent inhibition (I) = R_1-R_2 / R_1 x100

Where,

R_1 = Radial growth of pathogen (mm) toward opposite in control

R_2 =Radial growth (mm) of test pathogen

***In –vitro* evaluation of bio-agents**

Three bio-agents *Trichoderma viride*, *Trichoderma harzianum* and *Trichoderm aaesperellum* were also evaluated *in-vitro* applying dual culture technique[3]. The fungal pathogen was grown on PDA for 7 days. After 7 days with the help of a sterile cork borer, a disc of fungal growth from culture plate of *Colletotrichum truncatum* was taken and placed at an equidistance of antagonists on fresh PDA plate, and then, similarly with the help of sterile cork borer, a disc of *Trichoderma spp*. was taken and placed the other side of PDA plate. The plates were kept for incubation at 30°C for 7 days in BOD incubator. Visual observations on the inhibition of growth of pathogen were recorded after 7 days of Incubation in comparison with the PDA plate simultaneously inoculated with only the fungal pathogen.

Observations on radial mycelial growth of *C.truncatum were* recorded in each treatment and per cent growth inhibition of the test pathogen over control was worked out[8] as follows.

Percent inhibition (I) = R_1-R_2 / R_1 x100

Where,

R_1 = Growth of test fungus (mm) in control plate,

R_2 = Growth of test fungus (mm) in treatment plates.

Statistical analysis

The data were statistically analyzed with the help of analysis of variance (ANOVA). Three replications for each treatment were laid out in a completely randomized design

RESULTS AND DISCUSSION

The pathogen *colletotrichum truncatum* used for the present studies was ordered from ITCC (Indian type of culture collection) IARI (New Delhi). The pathogen was sub-cultured in PDA (potato dextrose agar). The growth was observed after 7 days. The fungus produced profuse black mycelial growth on PDA media. Mycelium is hyaline, septate and irregularly branched. The findings of the present study as well as relevant discussion have been presented under the following heads:

***In-vitro* evaluation of fungicides:**

Results (Table no: 1) indicated that, all the four fungicide tested significantly inhibited the mycelial growth of *C. truncatum* over untreated control. Among the four fungicides tested, carbendazim recorded highest inhibition (98%) of mycelial growth of the test pathogen which was at par with hexaconazole (97%), wheras mancozeb (42.67%), and chlorothalonil (33.67%) were not effective. Fungicide chlorothionil was found comparatively least effective than other fungicides. Similar result were reported by[2] for anthracnose of French bean caused by *Colletotrichum lindemuthia*

***In -vitro* evaluation of bioagents**

Result (Table: 2) reported that in the present experiment among the three evaluated bioagents, *T. harzianum* was found to be most effective and recorded 67 per cent inhibition of the test pathogen of soybean followed by *T. viride* (58%) and *T. aesprillium*. Similar results were reported by several researchers who also confirmed that *T.viride* and *T.*

harzianum and *T. aesprillium* are effective bioagents having significant antagonists against *Colletotrichum* species in various crops[6,5].

Table 1: *In-vitro* evaluation of fungicides on radial growth of *C. truncatm*

Treat.no.	Treatments	Concentaration(%)used	Inhibition (%)
T_1	Carbendazim	0.1%	98.00
T_2	Chlorothionil	0.2%	33.667
T_3	Hexaconazole	0.1%	97.00
T_4	Mancozeb	0.1%	42.667
T_5	Control		0.00
	S.Em±		1.067
	CD at 5%		3.534

Table 2: *In-vitro* evaluation of effect of bio-agents on radial growth of *C. truncatum*

Treat.No	Treatments	Inhibition (%)
T_1	*Trichoderma viride*	58
T_2	*Trichoderma harzianum*	67
T_3	*Trichoderma asperillium*	50
T_4	Control	
	SE(m) CD at 5%	1.732 6.11

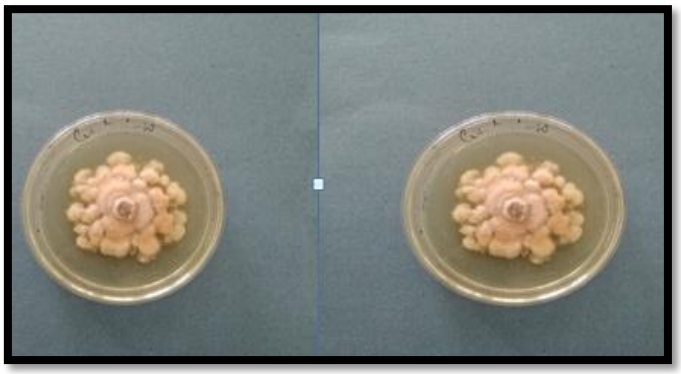
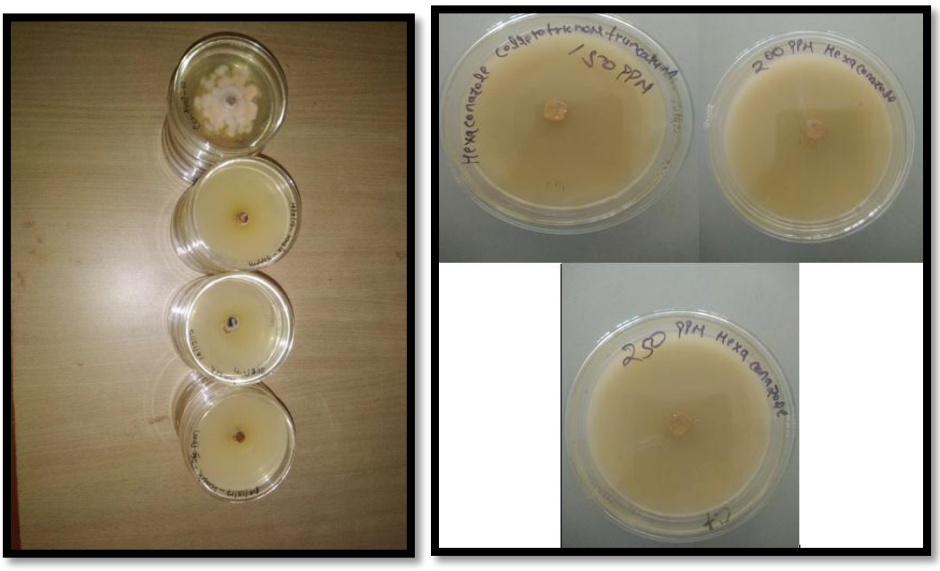


Plate1. Growth of *colletotrichum truncatum* on PDA and OMA media



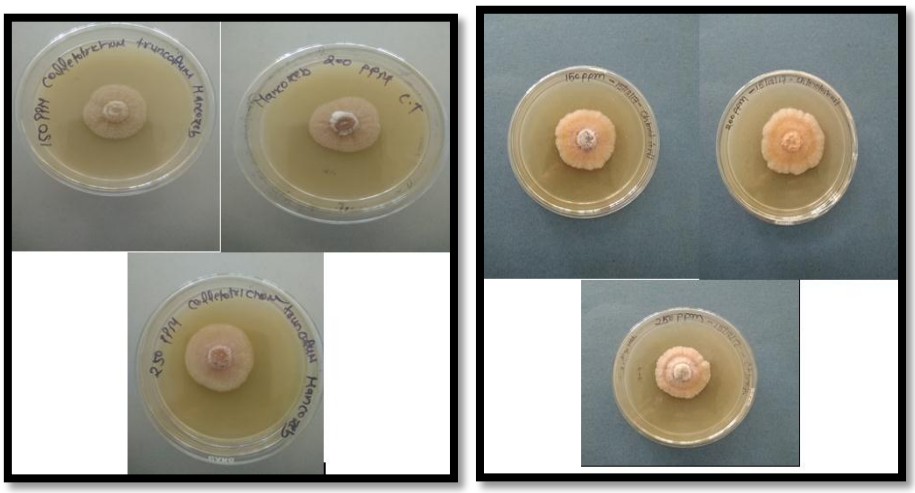


Plate 2: Antifungal activity of different fungicides against *Colletotrichum truncatum.* (different concentration(150,200,250 ppm)

Where, T_1- Carbendazim(different concentration(150,200,250 ppm)
T_2- Hexaconazole
T_3- Mancozeb
T_4 - Chlorothionil

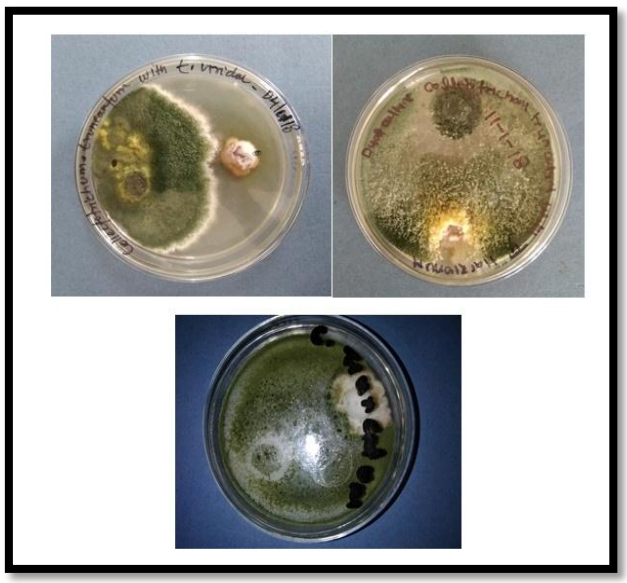


Plate 3: Antifungal activity of *Trichoderma viride, Trichoderma harzianum* and *Trichoderma asperellum* against *Colletotrichum truncatum*

Where, T_1- *Trichoderma viride*
T_2- *Trichoderma harzianum*
T_3- *Trichoderma aperellum*

CONCLUSION

The fungal pathogen *Colletotrichum truncatum* caused anthracnose experiment it was conclude that among the four fungicides tested *in vitro*. Carbendazim (0.1%) found most effective in inhibiting growth of test pathogen, followed by mancozeb (0.1%), hexaconazole (0.1%), and chlorothalonil (0.2%). In biological control the *Trichoderma viride* and *Trichoderma harzianum* found most effective in inhibiting growth of test pathogen followed by *Trichoderma aesprillium*.

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