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EFFICACY OF ORGANIC INPUTS AND BIO-AGENTS AGAINST *COLLETOTRICHUM TRUNCATUM* CAUSING ANTHRACNOSE OF BLACK GRAM (*VIGNA MUNGO* L. HEPPER)

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ABSTRACT

An *in vitro* experiment was conducted to assess the efficacy of organic inputs and bio-agents against *Colletotrichum truncatum* causing anthracnose disease in black gram. All the organic inputs and biocontrol agents showed inhibitory effect on mycelial growth of pathogen. Among the five organic inputs, at the 3 per cent concentration, *Vermiwash* was found superior with 66.48 per cent growth inhibition and at 5 per cent concentration, *Panchgavya* was found superior with 80.22 per cent growth inhibition. Among all the bio-agents tested, *T. harzianum* and *T. asperellum* recorded cent per cent mycelial growth inhibition while *T. viride* showed least growth inhibition of *C. truncatum.*

Key words : Black gram, *Colletotrichum*, Anthracnose, Bioagents, Organic inputs.

Introduction

The black gram (*Vigna mungo* L. Hepper) commonly known as urdbean, is an annual semi erect to spreading herb belonging to the family Leguminosae. Black gram improves soil fertility by fixing atmospheric nitrogen. India is a leading producer of black gram accounting for more than 70 per cent of global output. The production of black gram in India stands for 22.76 lakh tonnes from an area of 38.48 lakh ha (Anonymous, 2021). In Gujarat, black gram is cultivated over 18.00 thousand hectares with the production of 13.00 thousand tonnes.

Black gram is usually cultivated during *kharif* season and its productivity is mainly hampered by insect pest and diseases. Among diseases, powdery mildew (*Erysiphe polygoni*), leaf spots (*Cercospora* sp. *Alternaria* sp.), anthracnose (*Colletotrichum* spp.), mosaic and leaf crinkle are the major ones affecting black gram (Agarwal, 1991). The occurrence of anthracnose disease in black gram is commonly observed in most of the cultivated areas. Anthracnose continues to be one of the major constraints in black gram cultivation. Anthracnose pathogen (*Colletotrichum* spp.) attacks all aerial parts of plants at all stages of crop development leading to 24 to 67 per cent losses (Deeksha and Tripathi, 2002). Various approaches have been explored to manage the anthracnose disease, however foliar spray with fungicides appears to be more effective. Further, the overreliance on chemicals has resulted in the problem of resistance, creation of environmental pollution and human health risk hence use of bio-agents and organic inputs would help to minimize ill effects of fungicides use. Thus the present study was carried out to evaluate the efficacy of organic inputs and bio-control agents against anthracnose pathogen under *in-vitro* conditions.

Materials and Methods

Isolation of anthracnose pathogen

The anthracnose pathogen was isolated by following standard tissue isolation procedure (Tuite, 1969). The infected leaf exhibiting typical symptoms of anthracnose cut into small bits and surface sterilized for 30 seconds in 1per cent sodium hypochlorite solution and washed thoroughly in sterile distilled water for thrice. Blot dried pieces were aseptically transferred to Potato Dextrose Agar (PDA) plates and incubated at 28±1^0 C for growth.

The culture, thus obtained was purified by single spore isolation method.

Identification of pathogen

The cultural and morphological characterization of pathogen was carried out by culturing on PDA medium. The cultural characters, such as the colour of the colony, growth pattern and acervulus were examined and confirmed. Slide was prepared from 15 days old culture of *C. truncatum* and observed under light microscope for morphological characters such as conidia colour, shape, setae and acervulus production (Singh, 1978; Brayford and Samuels, 1993; Subhani, 2015). The pathogen was further confirmed through ITS rDNA region amplification and sequencing using Universal primer pair (ITS1 and ITS4). A BLAST search for similarities of pathogen showed 96.43 per cent similarity with *Colletotrichum truncatum* (GenBank Accession No: 0N345250).

The colony diameter was measured after growth in control plate reached 90 mm diameter. Per cent inhibition of mycelial growth of the fungus was calculated by using the formula given by Vincent (1947) as indicated below.

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

Where,

I = Per cent inhibition of mycelial growth

C = Growth of pathogen in control plate (mm)

T = Growth of pathogen in treatment plate (mm)

In-vitro* efficacy of bio-agents against *C. truncatum

Five bio-agents *viz.*, *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma asperellum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were tested against anthracnose pathogen by using Dual culture

Table 1 : *In-vitro* evaluation of organic inputs against *C.truncatum* causing anthracnose of black gram.

Tr. No	Treatment	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)
		3% Conc.		5% Conc.	
T_1	*Panchagavya*	*59.20	34.22	17.80	80.22
T_2	*Jivamrutha*	56.63	37.07	21.10	76.55
T_3	Cow urine	56.66	37.04	60.26	33.04
T_4	*Beejamrutha*	58.73	34.74	58.76	34.71
T_5	Vermiwash	30.16	66.48	18.10	79.88
T_6	Control	90.00	0.00	90.00	0.00
	S. Em. ±	0.79	-	0.71	-
	C.D. at 5%	3.46	-	3.10	-
	C.V.%	3.23	-	3.79	-

*mean of four replications.

In-vitro* evaluation of organic inputs against *C. truncatum

Efficacy of five different organic inputs *viz.*, *Panchagavya*, *Jivamrutha*, Cow urine, *Beejamrutha* and Vermiwash were assayed at 3 and 5 per cent concentrations by following poisoned food technique against *C. truncatum* causing anthracnose in black gram (Nene and Thapliyal, 1993).

The calculated quantities of organic inputs were added in PDA to obtain desired concentration and it was dispensed in sterilized 90 mm Petri plates under aseptic conditions and for comparison, PDA without organic input kept as control. Each Petri plate was aseptically inoculated by placing 5 mm diameter disc cut from the seven days old culture of *C. truncatum*. The plates were incubated in BOD incubator at 28±1°C for seven days.

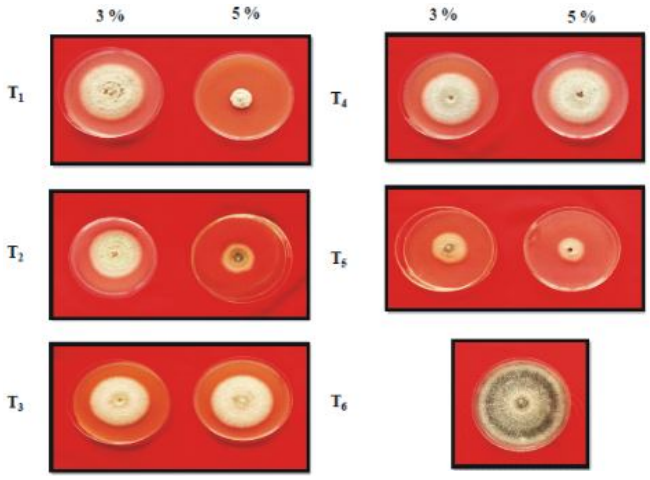

Plate 1 : Efficacy of organic inputs against *C. truncatum.* T_1 : *Panchagavya*, T_2 : *Jivamrutha*, T_3 : Cow urine, T_4 : *Beejamrutha*, T_5 : *Vermiwash*, T_6 : Control.

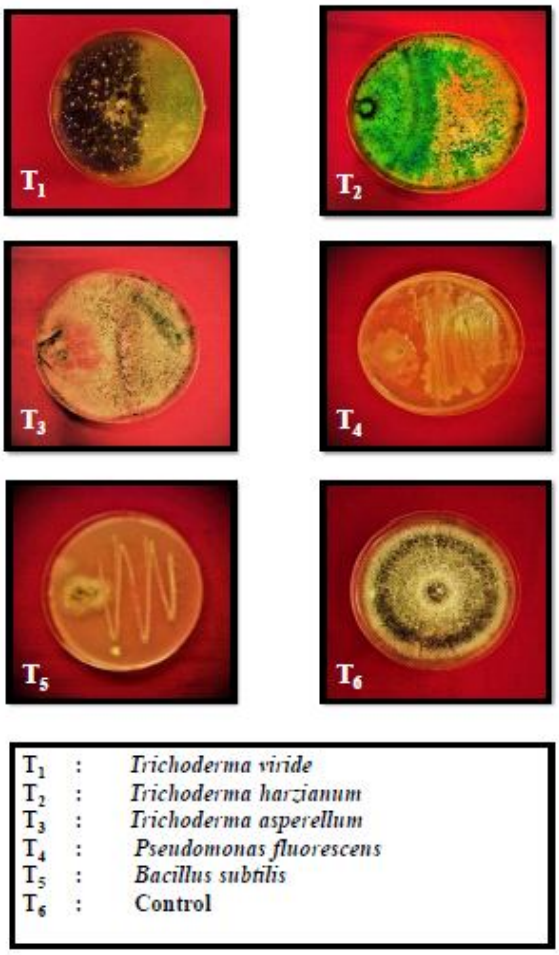

Plate 2 : Efficacy of bio-agents against *C. truncatum*.

technique (Dennis and Webster, 1971a). Four replications were maintained for each treatment. One control plate was maintained by inoculating only pathogen separately. Inoculated and control plates were incubated at 28±1°C. The diameter of the colony of the bio-agents and the pathogen was measured in two directions and average was recorded. The per cent growth inhibition over control was calculated by using the formula given by Vincent (1947).

Results and Discussion

In-vitro efficacy of organic inputs

The data presented in the Table 1, Plate 1 and Fig. 1 revealed that all the organic inputs were effective in inhibiting the mycelial growth of pathogen. At 3 per cent concentration, *Vermiwash* was found superior with 66.48 per cent growth inhibition, which was followed by *Jivamrutha* (37.07%), cow urine (37.04%), *Beejamrutha* (34.74%) and *Panchgavya* (34.22%). At 5 per cent concentration, *Panchagavya* was found superior with 80.22 per cent growth inhibition, which remained at par with *Vermiwash* (79.88%). Next best treatment was *Jivamrutha* with 76.55 per cent growth inhibition followed by *Beejamrutha* (34.71%). Least mycelial growth inhibition was recorded in cow urine (33.04%). The present results are in line with previous findings. Kumar *et al*. (2021) reported that *Panchgavya* was most effective in controlling the mycelial growth of *C. truncatum*. Among the all organic inputs, *jeevamrit* was effective in inhibiting mycelial growth of *C. truncatum* (Chatak, 2020).

In-vitro efficacy of bio-agents

The results, as shown in the Table 2, Plate 2 and Fig. 2 indicated that all the biocontrol agents were effective in inhibiting the mycelial growth of the pathogen. Among the five bio-agents evaluated, *Trichoderma harzianum* and *Trichoderma asperellum* showed cent per cent inhibition of mycelial growth of *C. truncatum* followed by *Pseudomonas fluorescens* (67.26%), which remained at par with *Bacillus subtilis* (64.96%). Least mycelial growth inhibition was recorded in *Trichoderma viride* (54.44%).

The present result on efficacy of biocontrol agents on anthracnose pathogen were in line with the previous workers Ramesh *et al*. (2023), Pushpendra *et al*. (2023), Sushmitha and Zacharia, (2021), Kulkarni and Raja (2019). Ramesh *et al*. (2023) studied the effect of bioagents on growth of *Colletotrichum lindemuthianum* causing anthracnose of soybean and reported that among biocontrol agents *Trichoderma harzianum* showed maximum mycelial growth inhibition (87.29%). Pushpendra *et al*. (2023) reported that *T. viride* and *T. harzianum* were most effective in inhibiting the radial growth of *Colletotrichum capsici* causing anthracnose of chilli under *in vitro* conditions. *Trichoderma harzianum* was found most effective followed by *Pseudomonas fluorescens* in inhibiting mycelial growth of black gram anthracnose pathogen (Sushmitha and

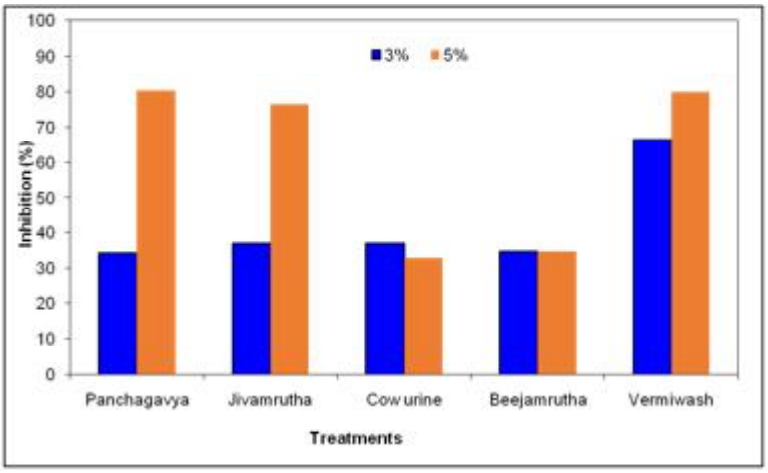

Fig. 1 : *In vitro* efficacy of organic inputs against *Colletotrichum truncatum*.

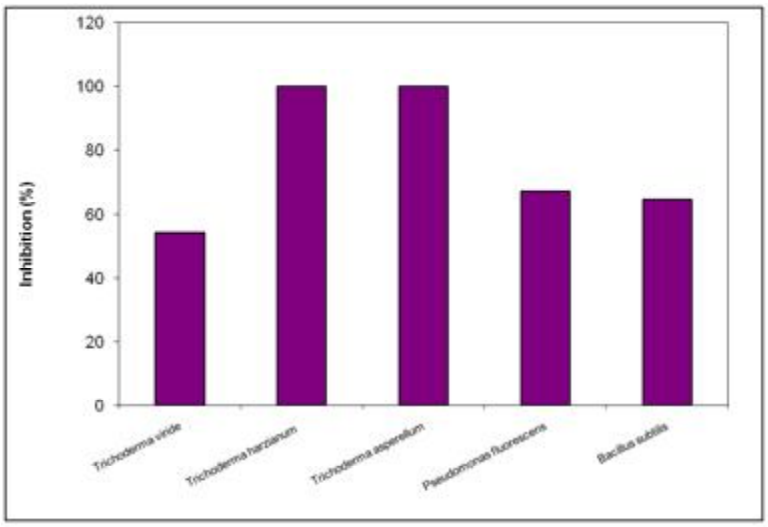

Fig. 2 : *In vitro* efficacy of biocontrol agents against *Colletotrichum truncatum*.

Table 2 : *In-vitro* evaluation of bio-agents against *C.truncatum* causing anthracnose of black gram.

Tr. No	Bio agents	Mycelial growth (mm)	Growth inhibition (%)
T_1	*Trichoderma viride*	*41.00	54.44
T_2	*Trichoderma harzianum*	0.00	100.00
T_3	*Trichoderma asperellum*	0.00	100.00
T_4	*Pseudomonas fluorescens*	29.46	67.26
T_5	*Bacillus subtilis*	31.53	64.96
T_6	Control	90.00	0.00
	S.Em.±	0.69	-
	C.D.at 5%	3.02	-
	C.V.%	5.05	-

*mean of four replications.

Zacharia, 2021).

Conclusion

In this study, five biocontrol agents and five organic inputs were evaluated against *Colletotrichum truncatum* under *in vitro* condition. All the biocontrol agents as well as organic inputs were showed wide range of inhibitory patterns. *T. harzianum* and *Panchagavya* were proved to be most effective inhibiting the pathogen and hence they can be incorporated in integrated disease management strategies to control anthracnose pathogen.

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Author's contribution : Conceptualization and designing of the research work (R. Math).

Execution of lab experiments and data collection, Analysis of data and interpretation Preparation of manual work (M.K.Patel)

Declaration : Authors do not have any conflict of interest.

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