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Bio efficacy of different bioagents against *Colletotrichum lindemuthianum* causing anthracnose of cowpea in *in vitro* conditions

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Abstract
Cowpea [*Vigna unguiculata* (L.)] also known as lobia, chowla, southern pea or black-eyed pea belong to the family leguminaceae. The anthracnose caused by *Colletotrichum lindemuthianum* is an important fungal disease of cowpea causing economic losses. In present study, bio control agents *viz.*, *Trichoderma asperellum, T. Harzianum, T. Virens, Aspergillus Niger, Metarrhizium anisopliae, Verticillium lecanii* and *Paecilomyces lilacinus* were tested. Among these highest mycelial growth inhibition over untreated control *C. Lindemuthianum* was obtained with *Trichoderma harzianum* (54.44%), followed by *T. Asperellum* (48.52%). *T. Virens* (39.25%), *Aspergillus Niger* (29.63%) *Metarrhizium anisoplie* (27.41%) and *Paecilomyces lilacinus* (27.41%), *Verticilium licani* (25.18%) was found least effective.

Keywords: Cowpea, *colletotrichum*, *trichoderma*, *metarrhizium*, *verticilium*

Introduction
Cowpea [*Vigna unguiculata* (L.) Walp.], is an important multi utility crop locally known as lobiya, chowla (chowli), southern pea or black eye pea, that is adopted to warm conditions and cultivated in the tropics and sub-tropics for dry grains, green edible pods for vegetable as well as fodder (Gupta *et al*., 2017) [4]. The seeds are important in diets for the provision of protein to rural as well as the urban dwellers as a substitute for the animal protein. (Wakili, 2013) [12]. The crop's haulms are also a valuable source of livestock protein. (Owolade *et al*., 2006) [8]. Especially for the lower-income group population, the cowpea is a better alternative source of protein, minerals, ash, etc., as compared to other legumes. Dry cowpea seed is rich in protein (23-33%), carbohydrates (56-68%) and folic acid. In humid forests of South-western Nigeria, the cakes made from crushed and fried cowpea seeds are very popular and sold as convenience street food in roadsides (Ogu and Owoeye, 2013) [7].
Anthracnose is one of the devastating worldwide fungal diseases that affect the above ground parts. This disease is induced by hemi biotroph deuteromycetous (fungi imperfecti) fungus called *Colletotrichum* (seed borne and soil borne) (Maya and Seal, 2015) [5]. The fungus *Colletotrichum lindemuthianum* is the most destructive disease of cowpea. Field-type cowpeas show various levels of resistance, whereas pole-type vegetable cowpeas are highly susceptible (Pradhan *et al*., 2017) [9]. Some bio-control agents have been reported as a promising disease management tool. (Modi and Tiwari 2020) [6]. Hence the present study was undertaken at College of agriculture, Parbhani to find out the effective biocontrol agent.

Material and Methods
In vitro efficacy of bioagents
A total of seven fungal bioagents as detailed under treatments were evaluated in *in vitro* against *C. lindemuthianum*, applying Dual culture technique (Dennis and Webster, 1971) [3]. Seven days old pure cultures of the test pathogen and test bioagents grown on PDA medium were used for the study. Two 5 mm culture discs, one each of the test pathogen and the test bioagent were cut out with sterilized cork borer and inoculated at equidistance and exactly opposite to each other on autoclaved and solidified PDA medium in Petri plates and plates were incubated at 27±1 °C. PDA plates inoculated alone with pure culture disc (5 mm) of the test pathogen were maintained as control.

Details of the experiment
Design: CRD.
Treatments: Eight.
Replication: Three.

Tr. No.	Treatment details
T_1	: *Trichoderma asperellum*
T_2	: *Trichoderma harzianum*
T_3	: *Trichoderma virens*
T_4	: *Aspergillus niger*
T_5	: *Metarhizhium anisopliae*
T_6	: *Verticillium lecanii*
T_7	: *Paecilomyces lilacinus*
T_0	: Control (untreated)

Observations on radial mycelial growth and colony diameter of *C. lindemuthianum* was recorded at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth. Per cent mycelial growth inhibition with the bioagents, over untreated control was calculated by using formula suggested by (Arora and Upadhyay, 1978) [2].

$$\text{Per cent growth inhibition} = \frac{\text{Colony growth in control plate} - \text{Colony growth in intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

Results and Discussion
Radial mycelial growth
Out of the seven biocontrol agents tested (Table 1) *Trichoderma harzianum* (T_2) was found most effective in controlling the mycelial growth of *C. lindemuthianum* (41.00 mm) which was statistically at par with *T. Asperellum* (T_1) (46.33 mm) and significant over rest of the treatments including untreated control (T_0), which recorded 90.00 mm mycelial growth of *C. lindemuthianum*. The *T. Asperellum* (T_1) was statistically at par with *T. virens* (T_3) (54.67 mm). The *T. Virens* (T_3) was statistically significant over *T. harzianum* (T_2) and *Verticillium lecanii* (T_6) and statistically at par with *T. Asperellum* (T_1) (46.33 mm). The treatment of *Aspergillus Niger* (T_4) was at par with the treatments of *Metarhizium anisopliae* (T_5) (65.33 mm), *Paecilomyces lilacinus* (T_7) (65.33 mm) and *T. virens* (T_3) (54.67 mm). The treatment of *Verticillium lecanii* (T_6) was least effective in controlling the mycelial growth of *C. lindemuthianum* (67.33 mm) which was statistically at par with *Aspergillus Niger* (T_4) (63.33 mm), *Metarhizium anisopliae* (T_5) (65.33 mm) and *Paecilomyces lilacinus* (T_7) (65.33 mm). The untreated control recorded full mycelial growth of *C. lindemuthianum* (90.00 mm).

Per cent inhibition of *C. lindemuthianum*
Out of the seven biocontrol agents tested (Fig 1) *Trichoderma harzianum* (T_2) was found most effective in inhibiting the growth of *C. lindemuthianum* (54.44 %) which was statistically at par with *T. asperellum* (T_1), which recorded 48.52 % inhibition of *C. lindemuthianum* over untreated control (T_0) (00.00%). The *T Asperellum* (T_1) was statistically at par with *T. virens* (T_3) (39.25 %). The least mycelial inhibition (25.18%) of *C. lindemuthianum* was recorded with *Verticillium lecanii* which was statistically at par with *Metarhizium anisopliae* (T_5) (27.41 %), *Paecilomyces lilacinus* (T_7) (27.41 %), *Aspergillus Niger* (T_4) (29.63 %) and T. *virens* (T_3) (39.25 %) (Plate 1).

Table 1: Efficacy of bioagents against *C. Lindemuthianum*

Tr. No.	Bioagents	Radial mycelial growth (mm)	Per cent inhibition
T_1	*Trichoderma asperellum*	46.33	48.52(44.10)
T_2	*Trichoderma harzianum*	41.00	54.44(47.54)
T_3	*Trichoderma virens*	54.67	39.25(38.67)
T_4	*Aspergillus niger*	63.33	29.63(32.86)
T_5	*Metarhizium anisopliae*	65.33	27.41(31.55)
T_6	*Verticillium lecanii*	67.33	25.18(30.06)
T_7	*Paecilomyces lilacinus*	65.33	27.41(31.55)
T_0	Control (untreated)	90.00	00.00(00.00)
	S.E. (m) ±	3.64	2.40
	C.D. at 1%	11.00	7.27

* Figure in parenthesis are angular transformed values.

The similar results were reported by earlier workers, Rajesha *et al.* (2010) [10] who reported that the highest mycelial growth inhibition of *C. Lindemithianum* was recorded with *Trichoderma harzianum* (73.54%) followed by *T. viride* (50.90%). Some other researchers also reported similar findings such as Ahamad *et al.* (2018) [1] they reported that *T. Harzianum* was most effective and recorded 67% inhibition of *C. Truncatum.* Sushmitha and Zachariya (2021) [11] they reported that *T. Harzianum* was most effective and recorded 93.59% inhibition of *C. Lindemithianum.*

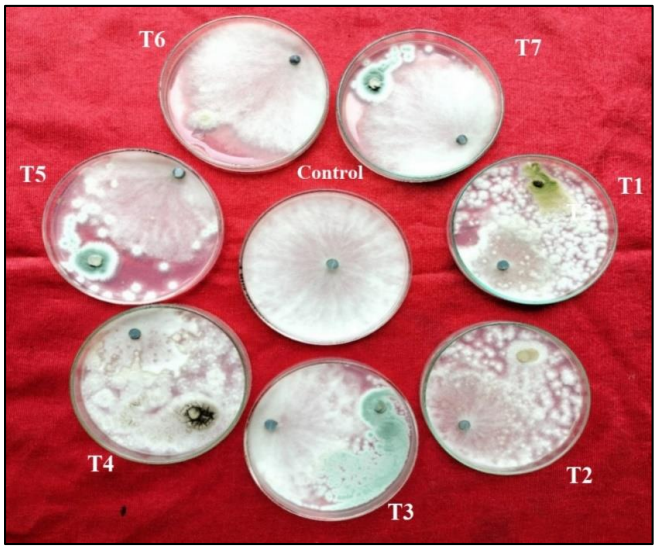

Plate I: Efficacy of bioagents against *Colletotrichum Lindemuthianum*

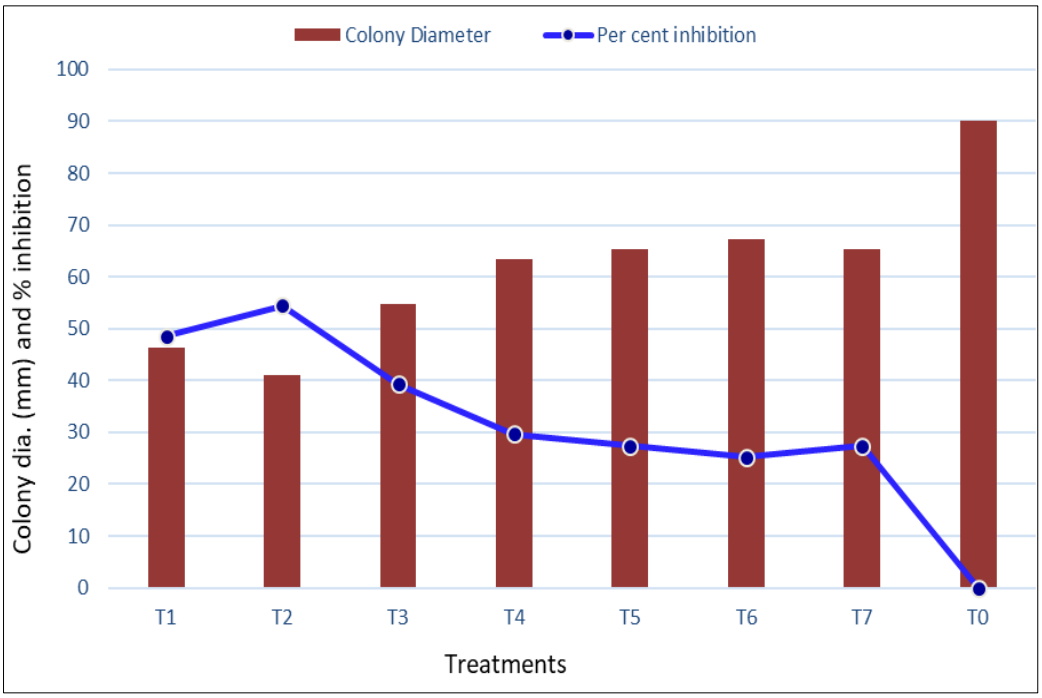

Fig 1: Efficacy of bioagents against *Colletotrichum lindemuthianum*

Conclusion

From the present study, it may be concluded that, in biological control, *Tricoderma harzianum, T. Asperllum, T. Virens* and *A. Niger* were found most effective in inhibiting the mycelial growth of *C. lindemuthianum,*

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