Eco Friendly Management of Anthracnose of Black Gram

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Received:- 06 August 2025/ Revised:- 11 August 2025/ Accepted:- 18 August 2025/ Published: 31-08-2025

Abstract— *With an objective to find out the non-chemical alternative to manage the Colletotrichum lindemuthianum (L), infecting anthracnose disease in black gram [Vigna mungo (L.) Hepper] in vitro condition during the year 2021-23. Different antagonist bio-agents viz. Trichoderma viride, T. harzianum, Pseudomonas fluorescens and Bacillus subtilis were evaluated by dual culture technique as well as efficacy of different organic inputs and phytoextracts viz. panchagavya, jivamrutha, cow urine, vermiwash, neem leaf extract, ginger rhizome extract, garlic bulb extract, datura leaf extract were tested in vitro by using poisoned food technique against C. lindemuthianum. The result indicated that among the in vitro tested fungal and bacterial antagonist, T. viride showed maximum growth inhibition of 60.48 per cent which was followed by T. harzianum (50.19%). Among eight organic inputs and phytoextracts, the highest mean growth inhibition of 60.99 per cent was recorded with panchagavya which was followed by jivamrutha (57.17%) whereas in pot condition vermiwash at 10 per cent gave highest per cent disease control (57.20%) followed by garlic bulb extract at 10 per cent (51.19%).*

Keywords— Black Gram, Anthracnose, Bio-Agents, Organic Inputs, Phytoextracts.

I. 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 is locally known as Urad dal (Hindi), Minumulu (Telgu), Ulundu Paruppu (Tamil), Uddina bele (Kannada), Masakalai dala (Bengali), Biri dali (Oriya), Adad dal (Gujrat), Kali dal, Udid (Marathi) in India. It has been growing in India, Pakistan, Bangladesh, Sri Lanka, Thailand, Vietnam, Indonesia, South China and Malaysia since ancient times. India is said to be the origin of black gram (Piper and Morse, 1914). India as the primary Urd bean origin centre with Central Asia as a secondary location (Vavilov, 1926). Black gram has high nutritional value containing, fat (1.4%), protein (24%), carbohydrate (59.6%), calcium (154 mg), phosphorus (385 mg), iron (9.1 mg), thiamine (0.4 mg), riboflavin (0.37 mg), niacin (2 mg) and beta carotene (38 mg) per 100 g seeds (Gopalan *et al.* 1971). It is a nutritive fodder for animals, especially milch animals. The leaves and stems are the most common sources of fodder, but seeds, pods and pod husks are also used. Black gram crop is itself a mini-fertilizer factory, as it has unique characteristics of maintaining and restoring soil fertility through fixing atmospheric nitrogen through symbiotic association with *Rhizobium* bacteria, which are present in the root nodules. Black gram can fix atmospheric nitrogen to the tune of 30 kg nitrogen per hectare per year. Black gram can be used as green manure and a cover crop. The crop is suitable for intercropping with different crops such as sorghum, cotton, pearl millet, green gram, maize, groundnut and soybean for increasing production and maintaining soil fertility (Parashar, 2006). India is the world's leading producer of black gram, accounting for more than 70 per cent of global output, followed by Myanmar and Pakistan (Anon., 2020). In India, the black gram area increased by 386 per cent in *Kharif* 2020-21, from 1.88 lakh ha in 2019-20 to 8.77 lakh ha in 2020-21. Madhya Pradesh (4.45 lakh ha), Maharashtra (1.79 lakh ha), Rajasthan (0.71 lakh ha), Karnataka (0.58 lakh ha), Telangana (0.11 lakh ha) and Andhra Pradesh (0.04 lakh ha) are the major *kharif* growing states (Anon., 2020). In Gujarat, black gram is primarily grown in the *kharif* season in the Kutch, Banaskantha, Saurashtra, Mahesana and Panchmahal districts, with adequate but erratic rainfall. During the summer, however, it is grown extensively in the districts of Kheda, Vadodara and Panchmahal (Anon., 2020).

Biotic and Abiotic stresses cause significant yield reduction in black gram. Among the various fungal diseases, 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 caused by *Colletotrichum* spp. is world's most important seed and soil-borne disease. At least four species of *Colletotrichum* have been found associated with green gram and black gram causing anthracnose in different parts of the world (Saxena and Sinha, 1977). It has been reported to possess high pathogenic variability and more than 100 races of *C. lindemuthianum* have been identified worldwide (Sharma *et al.*, 2007). Anthracnose pathogen (*Colletotrichum* spp.) attacks all aerial parts of plants at all stages of development. Symptoms are black, circular, sunken spots with a dark centre and bright red-orange margins appear on leaves and pods. The cotyledons of seedlings show dark brown to black sunken spots, which may bear pink spore masses of the fungus in wet weather and become blighted due to infection shortly after seed germination. In the event of a severe infection, the affected parts, particularly the leaves, wither. The pathogen perennates on infected seeds and in the soil on diseased plant debris. The secondary infection takes place through airborne conidia. The disease is most common in areas with cool and wet weather and it can result in a yield loss of up to cent per cent. Various researchers have estimated yield losses due to anthracnose between 24 to 67 per cent (Deeksha and Tripathi, 2002), 18.2 to 86.6 per cent (Laxman, 2006) and 21.36 to 60.07 per cent (Kulkarni, 2009).

II. MATERIALS AND METHODS

2.1 Antagonistic effect of different bio-agents against *C. lindemuthianum* by dual culture technique:

Different antagonist bio-agents *viz*. *Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated for their antagonistic activity against *C. lindemuthianum in vitro* by dual culture technique (Dennis and Webster, 1971). The tested bio agents and *C. lindemuthianum* were grown separately on potato dextrose agar (PDA) medium. The mycelial disc (5 mm diameter) of pathogen and fungal antagonists were placed on the same plate 6 cm away from each other. To test for antagonistic bacteria, a 5 mm of the mycelial disc of pathogen cultures were placed on one side of a Petri plate containing PDA medium. A loopful of bacteria was streaked 3 cm away from the disc of *C. lindemuthianum* on the same plate. Paired cultures were incubated at 27±2 °C. The plates inoculated only with test pathogen served as control. Four repetitions were maintained for each antagonist. The antagonistic fungal culture was maintained on PDA culture media and bacterial cultures were maintained on nutrient agar (NA) media. The assay for antagonism was performed on PDA media on Petri plates by the dual culture method. Inhibition zone were measured at 24 hours interval till the colony in the control plate covered with mycelium of pathogen. Per cent Growth Inhibition (PGI) were calculated by using the formula suggested by Vincent (1947).

$$PGI = \frac{C-T}{C} \times 100 \quad (1)$$

Where: -

PGI= Per cent growth inhibition

C= Colony diameter in control (mm)

T= Colony diameter in treatment (mm)

2.2 Efficacy of different organic inputs and phytoextracts against *C. lindemuthianum in vitro* and in pot condition:

Efficacy of different organic inputs and phytoextracts *viz*. *panchagavya, jivamrutha*, cow urine, vermiwash, neem leaf extract, ginger rhizome extract, garlic bulb extract, datura leaf extract at 5 and 10 per cent, respectively were tested *in vitro* by using poisoned food technique to know their inhibitory effect on the growth of *C. lindemuthianum*. Fresh and healthy, 100 g plant parts *i.e.* leaf, bulb or rhizome, respectively of each phytoextracts were collected, washed thoroughly with tap water and then with sterilized distilled water. Respective plant parts were crushed in mixer grinder by adding 100 ml sterilized distilled water to obtained 1:1 extracts separately. Each phyto-extracts thus obtained were centrifuged and filtered through double layered sterilized muslin cloth in conical flasks and plugged. 100 ml PDA were taken in 250 ml conical flasks, plugged and sterilized by autoclaving at 15 psi for 20 minutes. After cooling to about 45 °C temperatures, 5 and 10 ml of respective concentration were mixed thoroughly in the flask containing PDA individually. From the 100 ml PDA mixed with extracts, 20 ml poured aseptically into sterilized Petri plates and four plates per treatment were kept. The PDA Petri plate were inoculated with 5 mm mycelial disc cut from the periphery of the *C. lindemuthianum.* Culture were grown on PDA medium in the centre with the help of sterilized cork borer. The Petri plates containing PDA media without extract were inoculated with 5 mm mycelial disc cut from the periphery *C. lindemuthianum*. Culture grown on PDA medium were placed in the centre with the help of sterilized cork borer served as a control. The Petri plates were incubated at 27 ± 2°C temperature in an incubator for seven days. The

statistical analysis was done using factorial complete randomized design (FCRD) with four repetitions. Observations regarding per cent disease intensity were recorded on the basis of percent area of leaf surface extension growth from all plants following 0-5 scales (0, No disease; 1, < 5 % leaf area affected; 2, 6 to 10 % leaf area affected; 3, 11 to 25 % leaf area affected; 4, 26 to 50 % leaf area affected; 5, > 50 % leaf area affected) given by (Sharma, 1983). The per cent disease intensity (PDI) were calculated by using following formula given by McKinney (1923). The per cent disease control (PDC) were calculated with the help of the following formula (Mathur *et al.*, 1971).

$$\text{Per cent Disease Intensity (PDI)} = \frac{\text{Sum of individual rating}}{\text{Total no.of samples examined} \times \text{Maximum disease rating scale}} \times 100 \quad (2)$$

$$\text{Per cent Disease Control (PDC)} = \frac{\text{PDI in control} - \text{PDI in treatment}}{\text{PDI in control}} \times 100 \quad (3)$$

III. RESULTS AND DISCUSSION

3.1 Antagonistic effect of different bio-agents against *C. lindemuthianum* by dual culture technique:

The results presented in Table 1 revealed that the significant difference in the growth inhibition of all the antagonist. Among the tested fungal and bacterial antagonist, the fungal antagonist found superior over bacterial antagonist. It was recorded that *Trichoderma viride* showed maximum growth inhibition (60.48%) which was statistically followed by *T. harzianum* (50.19%). In case of bacterial bio-agents *Pseudomonas fluorescens* (28.25%) was potential antagonists followed by *Bacillus subtills* (24.35%).

TABLE 1
EFFICACY OF DIFFERENT BIO-AGENTS AGAINST *C. LINDEMUTHIANUM*

Tr. No.	Bio-agents	Per cent growth inhibition
T_1	*Trichoderma viride*	51.05 (60.48)
T_2	*Trichoderma harzianum*	45.11 (50.19)
T_3	*Pseudomonas fluorescens*	32.11 (28.25)
T_4	*Bacillus subtills*	29.57 (24.35)
T_5	Control	4.05 (0.50)
Mean		32.38 (32.75)
S. Em. ±		0.41
C. D. @ 5%		1.27
C. V. %		2.09

These findings were close enough with the results of Padder *et al.* (2010) that evaluated three bioagents *viz., Trichoderma viride, T. harzianum and Gliocladium virens* under *in vitro* conditions against *C. lindemuthianum* and found that all the three antagonistic were significantly inhibited the mycelial growth of pathogen, maximum being with *T. viride* (69.21%) followed by *T. harzianum* (64.20%). Rathava *et al.* (2017) found that, *T. viride* (68.88%), *T. harzianum* (67.03%) and *T. fasciculatum* (64.44%) significantly inhibited pathogen growth and were appeared as strong and potent antagonists of *C. lindemuthianum.*

3.2 Efficacy of different organic inputs and phytoextracts against *C. lindemuthianum in vitro*:

The results (Table 2) revealed that all the organic inputs and phytoextracts were showed inhibitory effect on growth of *C. lindemuthianum in vitro*. The highest mean growth inhibition of 60.99 per cent was recorded with panchagavya, followed by *Jivamrutha* (57.17%) and vermiwash (56.15%), whereas lowest in datura leaf extract (25.45%) followed by ginger rhizome extract (34.28%).

The inhibition of fungal growth was increase with increase in concentration in all the tested organic inputs and phytoextracts. At 5 % concentration highest mean growth inhibition of 39.64 per cent was recorded with *jivamrutha* which was statistically at par with vermiwash (38.72%) and neem leaf extract (38.67%), whereas lowest growth inhibition were recorded with datura leaf extract (12.95%). At 10 % concentration highest mean growth inhibition of 82.83 per cent was recorded with *panchagavya* which was followed by *jivamrutha* (73.81%) showed at par result with vermiwash (72.82%), whereas lowest growth inhibition were recorded with cow urine (37.7%).

These findings were close enough with the results of Hippe (1991) reported that the accumulation of lipid bodies, thickening of cell walls and undulations of plasmalemma of the cells caused by garlic extract (10%) were similar to those produced by some synthetic fungicides. Garlic extract resulted in serious damage of the mycelium of *R. solani*, *C. lindemuthianum*, and *F. solani* appeared fragmented under standard electron microscope. Sugha (2005) reported that panchagavya was found effective against *Colletotrichum capsici* (97.91%) at 10 per cent concentration. Chatak (2020) studied the effect of organic inputs against anthracnose of black gram caused by *C. truncatum* and found that *jivamrutha* and *beejamrutha* gave highest inhibition.

3.3 Efficacy of different organic inputs and phytoextracts against *C. lindemuthianum* in pot condition

The organic inputs *viz., Panchgavya, jivamrutha* and vermiwash and botanicals *viz*. neem leaf extract and garlic bulb extract that found most effective at 10 % concentration during *in vitro* investigation were selected and evaluated against *C. lindemuthianum* of black gram variety T-9 under pot conditions.

TABLE 2

EFFICACY OF DIFFERENT ORGANIC INPUTS AND PHYTOEXTRACTS AGAINST *C. LINDEMUTHIANUM IN VITRO*

Tr.no.	Treatment	Per cent growth inhibition		
		Concentration (%)		Mean
		5.0	10.0	
T_1	*Panchagavya*	37.17 (36.5)	65.52 (82.83)	51.35 (60.99)
T_2	*Jivamrutha*	39.02 (39.64)	59.22 (73.81)	49.12 (57.17)
T_3	Cow urine	34.53 (32.13)	37.88 (37.7)	36.21 (34.9)
T_4	Vermiwash	38.48 (38.72)	58.58 (72.82)	48.53 (56.15)
T_5	Neem leaf extract	38.45 (38.67)	46.86 (53.24)	42.65 (45.9)
T_6	Ginger rhizome extract	23.37 (15.73)	48.30 (55.75)	35.84 (34.28)
T_7	Garlic bulb extract	31.22 (26.87)	42.08 (44.91)	36.65 (35.63)
T_8	Datura leaf extract	21.09 (12.95)	39.51 (40.48)	30.3 (25.45)
T_9	Control (Water spray)	4.05 (0.5)	4.05 (0.5)	4.05 (0.5)
Mean		29.71 (26.86)	44.67 (51.34)	-
S. Em. ±		**Treatment**	**Concentration**	**Treatment × Concentration**
		0.29	0.15	**0.41**
C. D. @ 5%		0.82	0.41	**1.16**
C. V. %		**1.98**		

The pooled over sprays values (Table 3) revealed that vermiwash @ 10 % gave highest per cent disease control (57.20%) followed by Garlic bulb extract @ 10% (51.19%) and *Panchagavya* @10% (43.82%). The lowest per cent disease control were observed in *Jivamrutha* @ 10 % (35.75%) followed by Neem leaf extract @ 10% (41.68). These findings were close enough with the results of Gurjar *et al*. (2021) concluded that the solely application of Garlic 15% and Vermiwash 15% showed (36.29) disease severity with 43.67% disease control and (39.25) disease severity with 39.08% disease control, respectively.

TABLE 3
EFFICACY OF DIFFERENT ORGANIC INPUTS AND PHYTOEXTRACTS AGAINST *C. LINDEMUTHIANUM* IN POT CONDITION

Tr.no.	Treatments	Con. (%)	After 1st spray		After 2nd spray		After 3rd spray		Pooled PDC
			PDI	PDC	PDI	PDC	PDI	PDC	
T_1	*Panchagavya*	10.0	26.18 (19.47)	40.83 (42.75)	28.06 (22.13)	**41.57 (44.03)**	30.31 (25.47)	42.11 (44.96)	41.45 (43.82)
T_2	*Jivamrutha*	10.0	28.63 (22.96)	35.01 (32.92)	30.31 (25.47)	**36.30 (35.05)**	31.93 (27.97)	38.84 (39.33)	36.72 (35.75)
T_3	Vermiwash	10.0	21.82 (13.82)	47.27 (53.96)	24.42 (17.09)	**49.10 (57.13)**	27.45 (21.25)	51.04 (60.46)	49.14 (57.20)
T_4	Neem leaf extract	10.0	26.91 (20.48)	39.51 (40.48)	28.63 (22.96)	**40.12 (41.52)**	30.85 (26.30)	41.00 (43.04)	40.21 (41.68)
T_5	Garlic bulb extract	10.0	24.47 (17.16)	45.27 (50.47)	25.65 (18.74)	**46.58 (52.76)**	28.61 (22.93)	45.18 (53.31)	45.68 (51.19)
T_6	Control	-	35.55 (33.8)	-	38.53 (38.8)	-	42.4 (45.47)	-	-
Mean			27.26 (21.28)	36.23 (34.93)	29.27 (23.9)	**36.57 (35.5)**	31.92 (27.96)	36.69 (35.7)	42.64 (45.93)
S. Em. ±			0.78	1.89	0.81	**1.55**	0.79	1.53	1.01
C. D. @ 5%			2.33	5.63	2.42	**4.60**	2.34	4.54	2.85
C. V. %			5.75	10.45	5.56	**8.47**	4.94	8.43	9.13

IV. CONCLUSION

The present investigation demonstrated the potential of bio-agents, organic inputs, and phytoextracts as effective non-chemical alternatives for the management of anthracnose disease in black gram caused by *Colletotrichum lindemuthianum*. Among the evaluated antagonists, *Trichoderma viride* proved most effective, showing 60.48% growth inhibition, followed by *T. harzianum* (50.19%), while bacterial bio-agents exhibited comparatively lower efficacy. Similarly, among organic inputs and phytoextracts, *panchagavya* recorded the highest inhibition (60.99%) *in vitro*, followed by *jivamrutha* (57.17%) and vermiwash (56.15%). The results further indicated that the inhibitory effect increased with higher concentrations of the treatments.

Under pot conditions, vermiwash at 10% concentration provided the highest disease control (57.20%), followed by garlic bulb extract (51.19%). These findings highlight the scope of utilizing eco-friendly, sustainable, and easily available bio-agents and organic formulations in integrated disease management strategies for black gram cultivation. The study concludes that *T. viride*, panchagavya, vermiwash, and garlic bulb extract can serve as promising alternatives to chemical fungicides, reducing dependence on synthetic inputs while contributing to sustainable agriculture. Further field validation is recommended to confirm their efficacy under diverse agro-climatic conditions.

CONFLICT OF INTEREST

The authors, affiliated with declare no conflicts of interest

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