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Eco-friendly Management of Anthracnose Disease of French Bean Caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.)

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ABSTRACT

The present investigation entitled, "Eco-friendly Management of Anthracnose Disease of French bean Caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.)" was carried out at Veer Chandra Singh Garhwali, Uttarakhand University of Horticulture and Forestry, Bharsar, Pauri Garhwal during year 2022-2023. French bean is also known as common bean or green bean and is a rich source of proteins, carbohydrates, fibres, vitamins and minerals for humans. Anthracnose is one of the most destructive diseases of French bean caused by *Colletotrichum lindemuthianum* which can lead up to 100% crop loss. In this experiment the efficacy of different treatments such as essential oils, animal products and plant leaf extract were tested in *in vitro* and *in vivo* condition against *Colletotrichum lindemuthianum.* Out of the seven treatments screened *in vitro* by poisoned food technique for their inhibitory effect on mycelium growth of *Colletotrichum lindemuthianum* showed that minimum mycelial growth was recorded in Eucalyptus oil (00 mm) with (100%) inhibitory effect followed by Buttermilk (21.00 mm) with (72.37%) inhibition. In *in vivo* efficacy of treatments, prophylactic spray of Eucalyptus oil @ 5% gave the minimum per cent disease incidence and per cent disease index (31.00%) and (24.76%) and afterwards came Buttermilk @ 15% with per cent disease incidence and index (33.53%) and (26.33%) respectively. The yield was maximum from the plots treated with Eucalyptus oil @ 5% (1.96 kg/plot) and (242.79 q/ha). All the treatments used were efficient against the disease and gave better control. The treatments used were eco-friendly, easily accessible and non-toxic.

Keywords Essential oils, Animal products, Leaf extracts, *Colletotrichum lindemuthianum, In-vitro, In-vivo,* Prophylactic, Per cent disease incidence, Per cent disease index.

INTRODUCTION

French bean is an annual herbaceous plant that is planted all over the world for its unripe fruit or dry edible seeds. *Phaseolus vulgaris* is a native of America; it is said to have originated in Central or South America. Beans are an important source of dietary protein and are eaten as leaves, green pods, fresh grains, and dried grains (FAO 2008). French bean production is dominated by China (1,200,000 tonnes), with Spain being in fourth place (250,000 tonnes). In India, green bean and dry bean cultivation cover 150 and 9000 thousand hectares, produce 420 and 2900 thousand tonnes, and yield 2.8 and 0.32 tonnes per hectare,

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respectively. According to Horticulture Statistics at a Glance, 2017, Uttarakhand has a 5.70-hectare bean cultivation area with a yield of 39.98 million tonnes. According to a study done by Fernandez *et al.* (2000), an epidemic caused by an infection of a cultivar that is vulnerable to the disease can cause a crop loss of 100%. The anthracnose fungus can spread through seeds and infected agricultural debris. The broad or fava bean (*Vicia faba*), cowpea (*Vigna unguinculata*), scarlet runner bean (*Phaseolus coccineus*), and lima bean (*Phaseolus lunatus*) are other commonly cultivated legumes vulnerable to this disease (Bush 2019). After repeated use of synthetic chemicals that render the crop unfit for food, accumulation of dangerous chemical residues in grain, water, and soil has also been recorded (Falandysz 2000, Voorrips *et al.* 2004, Shovan *et al.* 2008). According to Wilson *et al.* (1997), fungicide food residues are more likely to cause cancer than insecticide and herbicide residues. The continuous use of chemical fungicides also pollutes the land and the atmosphere. In accordance to certain reports, natural compounds such as essential oils, animal products, and plant extracts are superior to synthetic fungicides because they are usually biodegradable, environmentally safe, less expensive and simpler to obtain than synthetic insecticides (Amadioha 2000, Maulana 2000, Tripathi and Dubey 2004). Essential oils, animal products, and plant extracts do not cause any toxic residues to remain on the plant after application, and they are non-carcinogenic and have no negative effects on crops or human health (Edwards 2010). Essential oils, animal products, and plant extracts are helpful for treating diseases without harming the environment or people's health and are easily accessible. For ecologically friendly management and maintaining a sustainable agroecosystem the essential oils, animal products, and plant extracts can be used.

MATERIALS AND METHODS

The present investigation on "Eco-friendly Management of Anthracnose Disease of French bean Caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.)". *In vitro* study was conducted in the Department of Plant Pathology Laboratory and *in vivo* study was carried out at Vegetable Research and Demonstration block of College of Horticulture, VCSG UUHF, Bharsar, Pauri Garhwal, Uttarakhand during the year 2022-2023. The details of the experimental material used and the methodology adopted are described below.

Methodology

In laminar air flow cabinet isolation and cultural studies was carried under aseptic conditions using spirit and flame for sterilizing inoculating needles and forceps tips. The working surface of laminar air flow cabinet was surface sterilized by swabbing with 2% formaldehyde solution or ethanol or spirit. Sterilization of all the glassware's was done in an electronic oven at 180°C for 3 hours. Sterilization of media was done in an electric vertical autoclave which uses moist heat or steam for sterilization at 121°C with a pressure of 15 lbs for 20 minutes. Hands was sterilized with ethanol or spirit and forceps, inoculation loop was sterilized by heating in the flame. Any glassware which was coming from outside was flamed before and after use.

Preparation of medium

Potato dextrose agar (PDA) medium was prepared by peeled potato, dextrose and agar-agar dissolving in 1000 ml of water. The solution was heated completely to dissolve the solid components of the medium and then sterilized by autoclaving.

Collection, isolation and identification of the pathogen

Infected leaves of French bean showing symptoms of Anthracnose were collected from experimental site, Vegetable Research and demonstration block, College of Horticulture VCSG, UUHF Bharsar, Pauri Garhwal, Uttarakhand and used for isolation of the pathogen. The sample were transferred to laboratory to isolate and identify the pathogen. The sample collected were washed with tap water and air dried and infected lesions with healthy part were cut into small pieces of about 2 cm each and then surface sterilized by immersing in 0.1% sodium Hypo chloride solution for 30 seconds. These pieces were thoroughly washed in at least three changes of sterilized distilled water to remove the residue of Sodium Hypo chloride and

then aseptically the leaf pieces are transferred to petri plates containing sterile Potato Dextrose Agar medium. After that incubation is done at 25±2°C temperature and observed daily for mycelial growth of the fungus. After one-week profuse growth of the fungus was observed.

The morphological characters of the fungus were studied to confirm the identity of the isolated pathogen. The slides were made in water and cotton blue stain, after that fungal growth of the pathogen was examined through the compound microscope at 40X magnification.

Pathogenicity test

Pathogenicity test was carried out in the Laboratory of Department of Plant Pathology according to Koch's postulates. Plants of French bean were grown in pots filled with sterilized soil in aseptic condition. The conidia of the test pathogens were taken from freshly prepared ten days old culture and then it was suspended in distilled water to obtain 10 conidia per ml. Different inoculation methods were used to inoculate the pathogen into the plant such as foliar spray of pathogen suspension, wound inoculation on stem, wound inoculation on branch and soil inoculation. Through these treatments *Colletotrichum lindemuthianum* takes 7-10 days for showing the symptoms. After that re-isolation was done from diseased tissues of artificially infected plants using PDA plate technique. Then the isolate obtained was compared with the original culture for confirmation of same pathogenic isolates which were inoculated.

Preparation of aqueous solution

Essential oils such as neem oil and eucalyptus oil are taken in less concentration and the appropriate amount was mixed in sterilized molten PDA to make the desired concentration (V/V) for experiment.

Animal products such as Cow urine and Buttermilk was collected in a sterilized beaker and then let set for few hours to days. Then can be used in an appropriate concentration to make the stock solution with PDA.

Healthy and fresh leaves of each plant material i.e., Eucalyptus, Datura, and Marigold, were washed thoroughly in cold running tap water and then air dried separately. Known weight of plant materials were ground using mortar and pestle by adding equal amount of distilled water 1:1 (W/V). The material was homogenized for 5 minutes, then filtered with muslin cloth followed by Filtration through Whatman's No. 1 filter paper and filtrates was considered as standard extract (100%) or stock solution. The appropriate amount of plant extract was mixed in sterilized molten PDA to make desired concentration (V/V) for experiment.

In-vitro* evaluation of essential oils, animal products and plant extracts against *Colletotrichum lindemuthianum

Essential oils, animal products and plant extracts were used in different concentration. Essential oils of Neem and Eucalyptus was used in per cent concentration of 3, 4 and 5%, animal product such as Cow urine and Buttermilk and plant extract of Eucalyptus, Datura and Marigold was tested on different per cent concentration like 5, 10 and 15%. All these treatments were tested by food poison technique and mycelium growth was recorded at different concentration at 7 days interval.

Poisoned food technique

The poisoned food technique (Nene and Thapliyal, 1993) was adopted for *in vitro* evaluation of botanicals against the test fungus. For bioassay, concentrations of treatments were prepared by dissolving 3, 4 and 5 ml of essential oils in 97, 96 and 95 ml of PDA and for animal products and plant leaf extracts 5, 10 and 15 ml of stock solution of plant extract in 95, 90, and 85 ml of sterilized PDA respectively to get the final concentration of each treatment separately. After that the flasks were shaken gently to ensure proper mixing of botanicals in PDA.

20 ml of media is poured in petri plate and those plates were inoculates with 5mm disk was inoculate into these plates, these plates were incubated at 25±2°C. The radial colony growth of the fungi was measured at 7 days of incubation. The efficacy of treatments was expressed as per cent inhibition of

mycelial growth over control, calculated by using formula suggested by Vincent (1947).

Observations recorded *in vitro*

Per cent mycelial inhibition

Per cent inhibition in growth was calculated in relation to growth in control using the following formula of Vincent (1947).

$$PGI = C\text{-}T\ /C \times 100$$

Where,

PGI = Per cent inhibition
C = Radial growth of fungus in control
T = Radial growth of fungus in treatment

In-vivo* evaluation of essential oils, animal products and plant extracts against *Colletotrichum lindemuthianum

Field experiment was conducted during March 2023 at Vegetable Research and Demonstration Block, College of Horticulture, VCSG UUHF, Bharsar, Pauri Garhwal, Uttarakhand with seven treatments along with control against *Colletotrichum lindemuthianum.* Randomized Complete Block Design was followed in this experiment. The treatments/spray schedule as initiated at the disease appearance stage and totally three sprays was given at 20 days interval. Spraying was done using manually operated with hand sprayer.

Preparation of aqueous solution

The concentration of essential oils, animal products and plant extracts were prepared by dissolving 50 ml of stock solution of essential oils, and 150ml of stock solution of animal products and plant extracts in 950 and 850 ml of distilled water to make desired concentration (V/V) of 5% and 15% for experiment, respectively.

Observations recorded

Per cent disease incidence

Disease incidence was calculated at 55, 75 and 95 DAS from 5 randomly tagged plants. The given formula was used for calculate the given parameter.

$$\text{Per cent disease incidence} = \frac{\text{No. of infected plants}}{\text{Total number of plants}} \times 100$$

Per cent disease index

Disease index of plants was calculated by using a standard scale of 1-9 given by Pandey *et al.* (2023), from 5 randomly tagged plants.

$$\text{Per cent disease index} = \frac{\text{Sum of all recorded ratings}}{\text{Total number of plant scored} \times \text{Maximum score on scale}} \times 100$$

Rating scale used for recording disease index.

Category	Grade	Infected plant part
I	0	Disease free
II	1	1-10% infection
III	2	11-25% infection
IV	3	26-50% infection
V	4	51-75% infection
VI	5	>75% infection

RESULTS AND DISCUSSION

Isolation, identification and pathogenicity test of the pathogen

At first, the growth was white and fluffy. The mycelium was initially white then turn pale white and separated before turning dark. The morphological characters of the pathogen were studied under the microscope. First the mycelium was white in color and transparent later on with maturity the mycelium turns black and were septate. Conidia has a length that varied from 10.5 to 15.5 m and a breadth of 3.5 to 4.5 m (Rajesha and Mantur 2014).

The symptoms of *Colletotrichum lindemuthianum* start to appear within 7 days. The symptoms appear on leaves, stem and pods. Symptoms include the appearance of brown and black spot that become necrotic and sometimes are sunken spots with black outer margins on leaf surface and along the veins. The best incubation method was foliar spray inoculation (7.00) days and maximum days were taken in

Table 1. Effect of different inoculation method on symptom development of *Colletotrichum lindemuthianum.*

Treatment details	Incubation period (days) ± SE.(m)	Types of symptoms developed
T_1 Control	0.00±0.00	No symptoms were appeared
T_2 Foliar spray inoculation	7.00*±0.40	Small brown and black spots appear
T_3 Soil inoculation	9.25*±0.47	Spots increase in size and become necrotic
T_4 Wound inoculation on stem	7.75*±0.47	Sunken spots with black margins
T_5 Wound inoculation on branch	7.50*±0.64	Sunken spots with necrotic lesions
SE (d)	**0.64**	
$CD_{(0.05)}$	**1.38**	

*Significant at 5 % level of significance as compared with control.

soil inoculation (9.25) days per the data recorded in (Table 1), also shown by Sewedy *et al.* (2019). The experiment was conducted on common bean and soybean. The fungus was grown separately on autoclaved seed: sand: water (2:1:2 V/V/V) medium in glass bottles for 21 days at 25±2 created the inoculum of *C. lindemuthianum.*

Effect of treatments on mycelial growth of pathogen *Colletotrichum lindemuthianum*

The data presented in (Table 2) shows that among those different treatments the minimum growth and maximum mycelium inhibition was seen in Eucalyptus oil. The mycelium growth was (00 mm) and (100%) inhibition followed by Buttermilk (21.00 mm) growth and (72.37%) inhibition, Neem oil (22.00 mm) growth and inhibition (71.07%). The maximum mycelium growth was seen in Eucalyptus leaf extract (46.33 mm) with minimum inhibition (39.01%). All the treatments tested were significant over control.

Eucalyptus oil plays an important role in damaging mycelium morphology and structure so it does not show any growth. The neem oil is composed of triglycerides and contains many triterpenoid compounds, which are antifungal and responsible for fungal inhibition. Buttermilk have antimicrobial activity hence is a traditional practice for the control of fungal pathogens.

Ashlesha and Paul (2014) found that butter milk controls *Colletotrichum* sp. by inhibiting mycelial growth by 98.22%. Results from Aggarwal *et al.* (2015) showed that Neem oil was found to be most effective which caused 77.83 % inhibition. Lokhande *et al.* (2019) found that Eucalyptus oil had the lowest mycelial growth (0.00 mm) and the highest mycelial inhibition (100%) of the test pathogen and the Neem oil exhibits mycelium suppression (61.33%) and mycelium growth (29.00 mm).

Effect of different treatments per cent mycelium inhibition of *C. linemuthianum*

In-vivo* evaluation of essential oils, animal products and plant extracts against *Colletotrichum lindemuthianum

All of the applied treatments exhibit a minimum percent disease incidence and index when compared

Table 2. Effect of treatments on mycelium growth of the *Colletotrichum lindemuthianum* at different concentration 3, 4, 5% for Eucalyptus oil and Neem oil and 5, 10 and 15% for other treatments.

Treatment details	Mycelium growth on 3 or 5%±SE (m)	Mycelium growth on 4 or 10%±SE (m)	Mycelium growth on 5 or 15%± SE (m)
T_1 (Control)	78.33±0.88	77.00±1.15	76.00± 0.57
T_2 (Eucalyptus oil)	0.00*±0.00	0.00*±0.00	0.00*± 0.00
T_3 (Neem oil)	31.00*±0.57	28.66*±0.88	22.00*± 1.15
T_4 (Cow urine)	41.83*±1.01	39.00*±0.57	34.33*± 0.44
T_5 (Buttermilk)	26.00*±0.57	23.00*±0.86	21.00*± 0.76
T_6 (Eucalyptus leaf extract)	60.50*±1.32	50.83*±0.72	46.33*± 0.60
T_7 (Datura leaf extract)	61.83*±1.01	52.33*±0.60	31.16*± 0.72
T_8 (Marigold leaf extract)	60.66*±0.88	53.16*±0.44	42.16*± 0.60
SE (d)	**1.22**	**1.03**	**0.961**
$CD_{(0.05)}$	**2.62**	**2.21**	**2.055**

*Significant at 5% of significance compared with control.

Table 3. Effect of different treatments on per cent mycelium inhibition of *Collerotrichum lindemuthianum* at different concentration of Eucalyptus oil and Neem oil at 3, 4 and 5% and other treatment at 5, 10 and 15%.

Treatment details	Mycelium inhibition on 3 or 5%± SE (m)	Mycelium inhibition on 4 or 10%± SE (m)	Mycelium inhibition on 5 or 15%± SE. (m)
T_1 (Control)	0.00±0.00 (0.00)	0.00±0.00 (0.00)	0.00±0.00 (0.00)
T_2 (Eucalyptus oil)	100.00*±0.00 (90.00)	100.00*±0.00 (90.00)	100.00*±0.00 (90.00)
T_3 (Neem oil)	60.42*±0.31 (50.99)	63.06*±1.11 (52.28)	71.07*±1.29 (57.44)
T_4 (Cow urine)	46.60*±0.73 (43.03)	49.34*±0.004 (44.60)	54.81*±0.77 (47.74)
T_5 (Buttermilk)	66.81*±0.37 (54.80)	70.13*±0.98 (57.04)	72.37*±0.86 (58.27)
T_6 (Eucalyptus leaf extract)	22.77*±0.94 (28.48)	33.96*±1.07 (36.47)	39.01*±1.17 (38.63)
T_7 (Datura leaf extract)	21.05*±1.24 (27.27)	32.00*±1.10 (34.53)	58.99*±0.88 (50.16)
T_8 (Marigold leaf extract)	22.54*±1.02 (28.32)	30.93*±0.48 (34.25)	44.51*±0.80 (41.83)
SE (d)	**1.03 (0.69)**	**1.09 (0.75)**	**1.20 (0.73)**
$CD_{(0.05)}$	**2.20 (1.49)**	**2.34 (1.62)**	**2.58 (1.56)**

*Significant at 5% of significance compared with control. () value in parentheses is angular transformed.

to the control, and the treatments were effective when compared to the percent disease incidence and index of control.

Effect of different treatments on per cent disease incidence (PDI) 55, 75 and 95 days after sowing (DAS)

As shown in (Table 3) 55 DAS Minimum per cent disease incidence was observed in Eucalyptus oil (21.50%) which was found statistically at par with Buttermilk (22.66%), Neem oil (25.30%) and Datura leaf extract (26.26%).

At 75 DAS Maximum disease incidence was seen in Control (43.16%) while minimum disease incidence was seen in Eucalyptus oil (26.16%) and Buttermilk (27.80%).

At 95 DAS minimum disease incidence was found in Eucalyptus oil (31.00%), followed by Buttermilk (33.53%) and Neem oil (35.43%), and the maximum incidence was found in Control (50.16%). Every treatment was determined to be significant compared to the control. Lokhande *et al.* (2019) in his research observed that Eucalyptus oil shows per cent disease incidence rate of 22.93%, followed by Neem oil treatment at 25.1%

Effect of different treatment on Per cent Disease Index After 55, 75 and 95 DAS at different doses against *Colletotrichum lindemuthianum*

At 55 DAS the data reported in (Table 4) indicates that all treatments were successful in treating the disease. The Eucalyptus oil had the minimum disease index (29.16%), which was followed by Buttermilk (30.33%), Neem oil (32.66%), and Datura (34.86%). The maximum disease index was seen in the Control (40.76%).

At 75 DAS the minimum per cent disease index, was observed in Eucalyptus oil (26.50%), which, statistically, was at par with Buttermilk (28.10%) and Neem oil (32.66%).

At 95 DAS the minimum per cent disease index in was seen in Eucalyptus oil (24.76%) with perti-

Table 4. Effect of different treatment on per cent disease incidence after 55, 75 and 95 DAS at different doses against *Colletotrichum lindemuthianum.*

Treatments	Dose (%)	Percent disease incidence 55 DAS ±SE (m)	75 DAS ±SE (m)	95 DAS ±SE(m)
T_1 (Control)	-	35.16±0.60 (36.35)	43.16±0.60 (41.05)	50.16±0.60 (45.07)
T_2 (Eucalyptus oil)	5	21.50*±0.76 (27.60)	26.16*±0.60 (30.75)	31.00*±0.57 (33.81)
T_3 (Neem oil)	5	25.30*±0.35 (30.18)	29.56*±0.29 (32.92)	35.43*±0.29 (36.51)
T_4 (Cow urine)	15	28.13*±0.59 (32.01)	35.50*±0.28 (36.55)	42.76*±0.23 (40.82)
T_5 (Buttermilk)	15	22.66*±0.44 (28.41)	27.80*±0.41 (31.80)	33.53*±0.29 (35.37)
T_6 (Eucalyptus leaf extract)	15	32.46*±0.29 (34.72)	41.76*±0.23 (40.24)	48.16*±0.72 (43.93)
T_7 (Datura leaf extract)	15	26.26*±0.63 (30.81)	32.46*±0.29 (34.72)	37.43*±0.29 (37.70)
T_8 (Marigold leaf extract)	15	30.90*±0.49 (33.75)	39.40*±0.30 (38.86)	46.00*±0.57 (42.68)
SE (d)		0.59 (0.38)	0.58 (0.36)	0.59 (0.34)
CD (0.05)		1.28 (0.82)	1.27 (0.77)	1.29 (0.75)

*Significant at 5% of significance compared with control
( ) value in parentheses is angular transformed.

Table 5. Effect of different treatment on per cent disease index after 55, 75 and 95 DAS at different doses against *Colletotrichum lindemuthianum.*

Treatment details	Doses (%)	Per cent disease index 55 DAS ± SE (m)	75 DAS ± SE (m)	95 DAS ± SE(m)
T_1 (Control)	-	40.76±0.23 (39.66)	45.33±0.33 (42.30)	47.66±0.33 (43.64)
T_2 (Eucalyptus oil)	5	29.16*±0.44 (32.67)	26.50*±0.28 (30.97)	24.76*±0.23 (29.83)
T_3 (Neem oil)	5	32.66*±0.33 (34.84)	30.10*±0.49 (33.25)	28.40*±0.30 (32.18)
T_4 (Cow urine)	15	35.06*±0.06 (36.29)	34.16*±0.60 (35.75)	32.56*±0.29 (34.78)
T_5 (Buttermilk)	15	30.33*±0.60 (33.40)	28.10*±0.58 (31.99)	26.33*±0.33 (30.86)
T_6 (Eucalyptus leaf extract)	15	38.66*±0.33 (38.43)	37.33*±0.33 (37.64)	36.50*±0.28 (37.15)
T_7 (Datura leaf extract)	15	34.86*±0.13 (36.17)	32.50*±0.50 (34.74)	30.36*±0.31 (33.42)
T_8 (Marigold leaf extract)	15	37.33*±0.33 (37.64)	35.40*±0.30 (36.49)	34.70*±0.30 (36.07)
SE (d)		0.44 (0.27)	0.64 (0.39)	0.43 (0.27)
CD (0.05)		0.97 (0.59)	1.39 (0.85)	0.95 (0.59)

*Significant at 5% of significance compared with control.
( ) value in parentheses is angular transformed.

nent results in Buttermilk (26.33%) and Neem oil (28.40%). The control reported the maximum per cent disease index (47.66%).

Aggarwal *et al.* (2015) showed in his experiment that in *in-vivo* condition the per cent disease index seen in Neem oil was 48.39%. According to the observations of Modi and Tiwari (2020). The minimum levels of disease index were seen in Eucalyptus oil at 5% (18.33%) and Neem oil at 5% (22.33%). The result recorded by Ashlesha and Paul (2014) the per cent disease index recorded in Buttermilk was 24.20%.

CONCLUSION

Eco-friendly methods produced positive results, efficiently cure disease, and improve the quality of life of their users. The treatments used are non-toxic and do not leave any harmful residue on crop and the crop sprayed by those are not harmful for human consumption. They are easily accessible and give better disease control. Without taking more input they give more output.

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