Phyto-extracts for managing Alternaria leaf spot on mung bean

ABSTRACT

The present study was investigated to evaluate the effect of selected phyto-extracts for the management of Alternaria leaf spot on mung bean caused by *Alternaria alternata* (Fr.) Keissler, under field conditions. Three replications of mung bean were planted in a randomized block design. At the research plot of the Central Research Field, Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj during *Zaid* season of 2023. The effect of neem leaf extract, onion bulb extract, ginger rhizome extract, garlic bulb extract, turmeric rhizome extractand Mancozeb were compared and tested the plant height (cm), pod length (cm), disease intensity (%) and B:C ratio recorded at 45, 60 and 75 DAS interval. The effect of foliar spray of T_4–garlic bulb extract @ 10% was found the best for managing Alternaria leaf spot of mung bean under field conditions as minimum disease intensity(34.4%), maximum plant height (65.4cm), pod length significantly increased (8.4cm)and highest B:C ratio(1:2.05).Followed by minimum disease intensity(35.5%),plant height (60.9cm), pod length (7.8 cm) and highest B:C ratio(1:1.97) was recorded inT_1–neem leaf extract @ 10%followed by T_5–turmeric rhizome extract @ 10% when compared to (Treated check) T_6–Mancozeb @0.2% and (Untreated check)T_0–control. Overall results revealed that T_4–garlic bulb extract @10% is significantly effective against *A. alternata* (Fr.) Kessler.

Keywords: Alternaria alternata (Fr.) Keissler, garlic bulb extract, neem leaf extract, turmeric rhizome extract, ginger rhizome extract, onion bulb extract.

1. INTRODUCTION

Mung bean [*Vigna radiata* (L.) Wilczek] is an important summer food legume crop of short duration and cultivated in the tropical and sub-tropical countries of the world. In India, this crop is cultivated in allthree seasons *viz*., *Kharif*,*Rabi* and *Zaid*. Its seed are a good source of dietary proteins,vitamins andminerals forhumansespeciallyformarginal and vegetarian people. It is an ideal crop for small holding farmers duetoitslow input requirement, nitrogen fixation capabilityand short duration.These qualities makeit highly suitable for crop rotation [1,2]. Worldwide, the total cultivated area and production of mung bean are 7 million ha and 5 million tonnes respectively.The yield potential of this crop ranges from 2.5 to 3.0 t ha^{-1}; however, the global average productivity is 0.5 t ha^{-1}. South Asiacontributes 90% of total mung bean production worldwide, and India is the largest producer in South Asia with production of about 1.5 to 2.0 million tonnes from about 3 to 4 million hectare annually, with average productivityof 0.5tha^{-1}[3,4].

Abiotic and biotic stresses caused significant decline in legume yield in South Asia and South East Asia. Among biotic stresses, fungal diseases are responsible for reducing yield up to 40–60% in mungbean [5]. Fungal pathogens can infectmungbean plants at different stages, such as during emergence, seedling, vegetative and reproductive stages and cause substantial damage leading to yield loss or complete failure of production. Species of the genera *Fusarium* (wilt), *Rhizoctonia* (wet root rot), and *Macrophomina* (dry root rot) infect mungbean plants during seed/seedlings stages (seed-borne or soil borne), while species of

the genera *Colletotrichum* (anthracnose), *Alternaria* and *Cercospora* (leaf spot), *Erysiphe*/*Podospheara* (*Sphaerotheca*) (powdery mildew) affect plants during vegetative and reproductive stages [6].[7]reviewed the status of web blight in mungbean and recently,[8]published a review on yellow mosaic in mungbean and urd bean from India.

Mung bean is an important pulse crop in many Asian countries including Pakistan, where the diet is mostly cereal based. Mung bean similar to other pulses, is a good source of protein, vitamins, minerals and calories [9].A wide variety of fungus isolates has been identified that infect the mung bean crop causing dry root rot, charcoal rot and cercospora leaf spot [1]. The most prominent fungal disease of mung bean is the leaf spot disease. The symptoms include the formation of spots of different kind and shape on leaves (sometimes on stems and fruits; depending upon the type of the fungus). The genus *Alternaria* has its various species that are known to cause a significant damage of crops in the field as well as at late harvesting stage. It is well known pathogen affecting pulses, cereals fruits and vegetables that result in severe agricultural losses [10,26,27,28].

A wide variety of research based on epidemiological studies have revealed that *Alternaria alternata* spores are themost commonly recognized fungal spore in the environment [11].Alternaria black rot, Alternaria leaf spot and Alternaria brown spot are the most significant phenotypes of plant diseases caused by *A. alternata* spores [12]. Alternaria leaf spot damage is more prominent on aerial parts and approximately upto 80% losses were recorded.Natural plant extracts modulate plant growth and are involved in plant defense responses, including limiting pathogen development [13]. The action of natural compounds such as terpenoids, phenolics, and alkaloids are not specific, and their effects on pathogens are versatile[14]. Thus the present study aimed to evaluate the effect of selected phyto-extracts for the management of Alternaria leaf spot on mung bean. Keeping the above in view the study was undertaken during *Zaid* season 2023, at the Central Research Field, Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology And Sciences, Prayagraj.

2. MATERIAL AND METHODS

The experiment was carried out at the Central Research Field, Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology And Sciences, Prayagraj during *Zaid* season 2023.The study was laid-out with Randomized Block Design (RBD) with three replications.Three sprays of all treatments were given at an interval of 15 days. Treatments were imposed after appearance of the first disease symptoms. Observations on plant height (cm) and disease intensity (%) of Alternaria leaf spot of mung bean were recorded at 15 days interval, pod length (cm) and B:C ratiodata were obtained after the harvest on physiological maturity.The treatments comprised of application of selected phyto-extracts *viz*.,neem, garlic, ginger, onion, turmeric @10% andmancozeb @0.2% (treated check) and control (untreated). The crop was sprayed three times at 45, 60, and 75 DAS of interval. The disease intensity of Alternaria leaf spot was recorded after ten days of spray. Plant disease intensity (PDI)was calculated after each spray by using 0-5 disease rating scale on the basis of percentage area of foliage infected by the pathogen.

2.1 Isolation and identification of the pathogen

Diseased samples collected from different areas during the season and isolation of pathogenwas carried out in the laboratory. Firstly collected diseased samples washed thoroughly under the tap water and then cut into small pieces 2-4 mm in size with the help of a sterilized blade in sucha way that the sample contained a 50 per cent healthy portion as well as a 50 per cent diseasedportion. The surface of the pieces was sterilized by using 1

per cent Sodium hypochlorite solutionfor 30 seconds to 1 minute, then finally wash well with the three changes of sterilized distilledwater and to remove excess water then pieces was placed on blotter paper. With the help of a sterilized inoculating needle place the sample pieces on petri plates containing potato dextrose agar medium under the aseptic conditions in the laminar airflow chamber. Five pieces on PDA media on each plate. Inoculated petri plates kept in an incubator at 25°±2°C and examine at frequent intervals to check the growth of the target fungal pathogen.

Identification of the target pathogen (*Alternariaalternata*). The fungal colony culture was initially white, cottony, and abundant in aerial mycelium, but it progressively turned grey. Withaerial mycelium, the old culture appeared entirely grey, and clear concentric rings formed on thePDA medium. Conidiophores ranged simple to branched, and appeared singly. Conidia was seen in lengthy chains on conidiophores, with strong walls, beaked edges, and transverse and longitudinal septation and golden to brown in appearance. The fungus was identified as *A.alternata* based on colony characteristics and morphological characteristics of conidiophores and conidia [15,16].

2.1.1Purification and maintenance of the pathogen

The culture obtained was purified once after the pathogen identity confirmed. Periodic sub culturing on PDA slants was done to keep the pure culture. Throughout the investigation, this pure culture employed. The cultures of the fungus was sub-cultured on PDA slants and kept in laboratory at 28±1 °C for 15 days. Further, these cultures was sub-cultured once in a month and used for future purpose [17].

2.2Preparation of Phyto-extracts

Phyto-extract was prepared from, leaves of neem (*Azadirachtaindica*), bulb of garlic (*Alliumsativum*), rhizome of ginger (*Zingiberofficinale*), bulb of onion (*Alliumcepa*), rhizome of turmeric (*Curcumalonga*) washing with running tap water followed by sterile distilled water, air dry at 27^0C and ground to obtained extracts of each plant species the extraction was done by means of pestle and mortar. Water extract was obtained by adding one gm of tissue in one ml of water (1:1w/v) and filtered through double layers of muslin cloth. This forms the standard solution (100%). The phyto-extracts was sprayed at the rate of 10% prepared from standard solution [18].All the treatments was given as foliar sprays. Phyto-extracts was sprayed @ 10 ml/liter of water, mancozeb @ 2 ml/ liter of water.

2.3 Recording the disease intensity (%)

After germination, five plants per treatment per replication were randomly selected. Regularly watched for first appearance of disease. The observation on disease intensity was recorded using a progressive 0-5 scale, as showed in (Table 1 and Plate 1). Numerical rating grade was given on the basis of percentage of area infected by pathogen on the leaves[19]as described below:

Table 1: Disease rating scale

Grade	Leaf area covered
0	No symptoms of disease on leaves (Immune)
1	Spots size covering 1-10% of leaf area (Resistant)
2	Spots size covering 11-25% of leaf area (Moderately resistant)

3	Spots size covering 26-50% of leaf area (Moderately susceptible)
4	Spots coalesce to form big patches covering 51-75 % of leaf area (Susceptible)
5	Big spots size covering >75 % of leaf area (Highly susceptible)

2.3.1 Per cent disease intensity (PDI)

$$PDI = \frac{\text{Sum of all numerical ratings}}{\text{(Total number of leaves rating} \times \text{Maximum disease grade)}} \times 100$$

Plate 1: Disease rating scale

2.4 Economics analysis

Cost of cultivation, gross return, net return and benefit cost ratio was worked out to evaluate the economics of each treatment, based on the existing market prices of input and output [20].

2.4.1Cost of cultivation (ha^{-1})

The cost of cultivation for each treatment was work out separately, taking into consideration all the cultural practices followed and costs of inputs used in the cultivation.

2.4.2Gross return (ha^{-1})

The gross return from each treatment was calculated by using the following formula:
Gross return (ha^{-1}) = Yield (q/ha) × Price (Rs./q).

2.4.3Net return (ha^{-1})

The net profit from each treatment was calculated separately by using the following formula:
Net return = Gross return (ha^{-1}) – Cost of cultivation (ha^{-1}).

2.4.4 Benefit Cost ratio

The benefit cost ratio was calculated by using the following formula:

$$\text{Benefit Cost ratio} = \frac{\text{Net return } (ha^{-1})}{\text{Total cost of cultivation } (ha^{-1})}$$

2.5Statistical analysis

The data obtained were statistically analyzed by the methods suggested by [21].The standard error and critical difference (C. D.) was calculated at 5% level ofsignificanceforcomparingtreatment means.

3. RESULTS AND DISCUSSION

Under field conditions, three sprays of all the treatments were taken up at 45, 60 and 75 DAS. The results are presented on Table 2; Fig. 1,2and plate 2.

3.1Disease intensity (%) of Alternaria leaf spot on mung bean

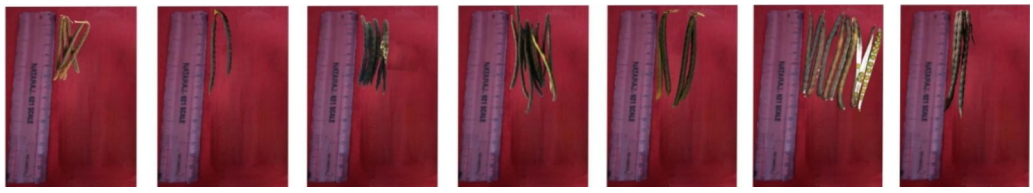
The data presented in the Table 2 and depicted in Fig. 1 reveals that disease intensity(%) of mung beansignificantly decreased in treatment T_4 – Garlic bulb extract (34.4 %) followed by T_1 – Neem leaf extract (35.5 %), T_5 – Turmeric rhizome extract (36.7 %), T_3 – Ginger rhizome extract (38.0 %) and T_2 - Onion bulb extract (39.0 %) as compared to (Treated check) T_6 –Mancozeb (32.8 %) and (Untreated check) T_0 – Control (40.9 %).
Comparing the treatments with CD value (1.07), all the treatments (T_1, T_2, T_3, T_4, T_5, T_6) were found significant over (Untreated check) T_0 – Control. Among the treatments (T_2, T_3) were found non-significant to each other. (T_1andT_4)were found to be significant over all the other treatments.

3.2Plant height (cm) on mung bean

The data presented in the table 2 and depicted in Fig. 1 reveals that plant height (cm) ofmung bean significantly increased in treatment T_4 – Garlic bulb extract (65.4 cm) followed byT_1 – Neem leaf extract (60.9 cm), T_5 – Turmeric rhizome extract (58.8 cm), T_3 – Gingerrhizome extract (57.0 cm) and T_2 - Onion bulb extract (55.2 cm) as compared to (Treated check) T_6 –Mancozeb (69.7 cm) and (Untreated check) T_0 – Control (48.1 cm).
Comparing the treatments with CD value (2.06), all the treatments (T_1, T_2, T_3, T_4, T_5, T_6) were found significant over (Untreated check) T_0 – Control. Among the treatments (T_2, T_3); (T_3,T_5) were found non-significant to each other. (T_1, T_2 and T_4) were found to be significant over all the other treatments.

3.3 Pod length (cm) on mung bean

The data presented in the table 2,Fig. 1and depicted in plate 2 reveals that pod length (cm) of mung bean significantly increased in treatment T_4 – Garlic bulb extract (8.4 cm) followed by T_1 – Neem leaf extract (7.8 cm), T_5 – Turmeric rhizome extract (7.4 cm), T_3 – Ginger rhizome extract (7.0 cm) and T_2 - Onion bulb extract (6.8 cm) as compared to (Treated check) T_6 – Mancozeb (9.4 cm) and (Untreated check) T_0 – Control (6.2 cm).
Comparing the treatments with CD value (0.53), all the treatments (T_1, T_2, T_3, T_4, T_5, T_6) were found significant over (Untreated check)T_0 – Control. Among the treatments (T_2, T_3); (T_3, T_5) and (T_5, T_1) were found non-significant to each other. (T_2 and T_4) were found to be significant over all the other treatments.



T_0 T_1 T_2 T_3 T_4 T_5 T_6

Plate 2Effect of treatments on pod length (cm) of mung bean after harvest

3.4Benefit Cost ratio on mung bean

The data presented in the Table 2 and depicted in Fig. 2 reveals that economics analysis of the data over the session that (Treated check) T_6 - Mancozeb recorded higher gross returns Rs. 86,767.00, net returns Rs. 60,587.00 with B:C ratio **1:2.31** followed with T_4 – Garlic bulb extract recorded gross returns Rs. 84,881.00, net returns Rs. 57,051.00 with B:C ratio **1:2.05** as compared to (Untreated check) T_0 – Control gross returns Rs. 64,558.00, net returns Rs. 39,728.00 with B:C ratio **1:1.60**.

Table 2: Effect of treatments on disease intensity (%)of Alternaria leaf spot, Plant height (cm), Pod length (cm) and Benefit Cost ratio on mung bean.

Treatment number	Treatments	Dosage	Disease intensity (%)	Planth eight(cm)	Pod length (cm)	Benefit Cost ratio
T_0	Control (Untreated check)	-	40.9^{a}	48.1^{a}	6.2^{a}	1:1.60
T_1	Neem leaf extract	10 %	35.5^{d}	60.9^{d}	7.8^{d}	1:1.97
T_2	Onion bulb extract	10 %	39.0^{b}	55.2^{b}	6.8^{b}	1:1.84
T_3	Ginger rhizome extract	10 %	38.0^{b}	57.0^{bc}	7.0^{bc}	1:1.89
T_4	Garlic bulb extract	10 %	34.4^{e}	65.4^{e}	8.4^{e}	1:2.05
T_5	Turmeric rhizome extract	10 %	36.7^{c}	58.8^{c}	7.4^{cd}	1:1.90
T_6	Mancozeb (Treated check)	0.2 %	32.8^{f}	69.7^{f}	9.4^{f}	1:2.31
CD at 5%			1.07	2.06	0.53	

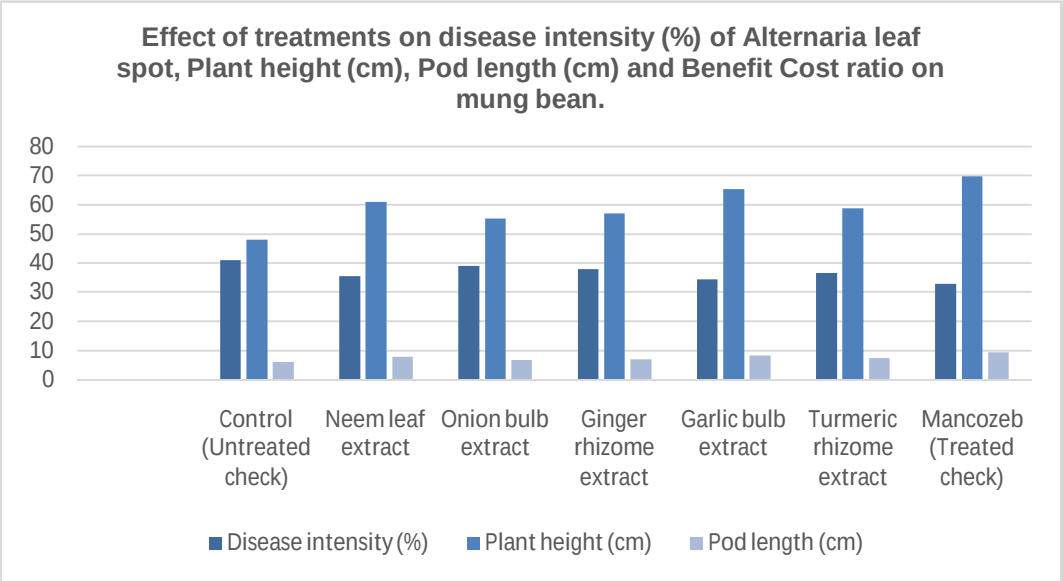


Fig. 1Effect of treatments on disease intensity (%) of Alternaria leaf spot, Plant height (cm), Pod length (cm) and Benefit Cost ratio on mung bean.

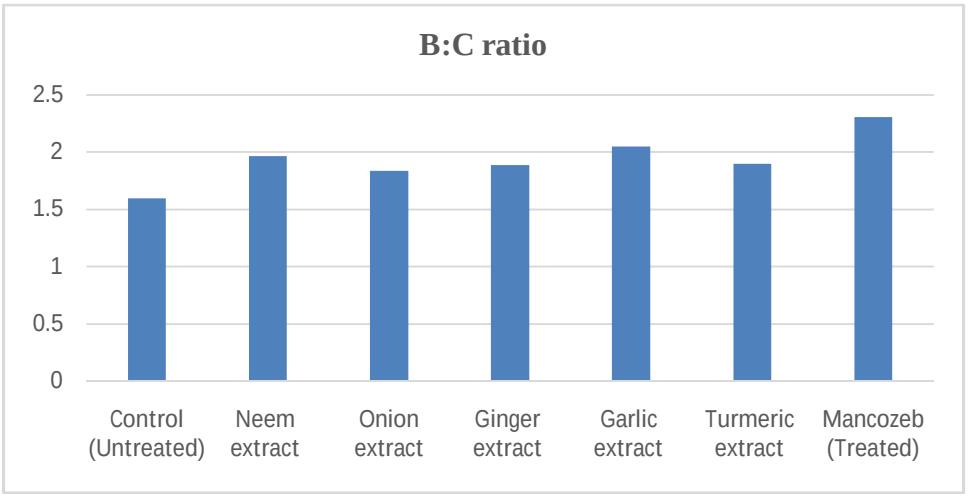


Fig. 2Effect of treatments on Benefit Cost ratio of mung bean

3.5 Discussion

The probable reasons for such findings may be due to the antimicrobial properties of garlic extract and neem leaf extract due to the presence of volatile oil which contains diallyl disulphides, diallyl trisulphides and sulphodioxides derived from allicin or ajoene which disrupts the cellmembrane of the pathogen and restricts the performance of some enzymes that are important to proliferate fungi in nature and inhibits enzyme formation leading to cell death and loss of vigour, ultimately killing the pathogenby this garlic may indirectly influence the plant growth. The research outcomes align with the similar findings was reportedby[22,23,24,25],who revealed that the maximum disease control was found in the

garlic treatment, suggesting that the use of garlic extracts could be a viable approach to control *Alternariaalternata* (Fr.) Keissler. But among all the treatments chemical fungicide (Treated check) has shown the minimum disease control has shown with strong fungicidal effect against pathogen. In order to reduce the pathogen, it may produce some toxic chemical residues, they may have potential harmful effects to non-targeted organism. So, considering the ecosystem, phyto-extracts of garlic and neem significantly inhibits the pathogen could lead to better health of the plants this cascade effect may have contributed to the holistic wellbeing of plants, consequently resulting in producing minimum disease intensity, increased plant height, producing maximum pod length growthand leading to a healthier plant.

4. CONCLUSION

Alternariaalternata (Fr.) Keissler was found associated with Alternaria leaf spot disease of mung bean (*Vigna radiata* L.). The disease intensity (%), plant height (cm), pod length (cm) and B:Cratio, overall results revealed that T_4 - Garlic bulb extract @10% is significantly effective against *A. alternata* (Fr.) Kessler as compared to (Treated check) T_6 - Mancozeb @ 0.2% and (Untreated check) T_0- Control. It is worth mentioning that the conclusions drawn from this study were based on observations carried out under field conditions at Central Research Field, Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology And Sciences, Prayagraj,during *Zaid* Season 2023, within the agro- climatic conditions of Prayagraj, UP. India.

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