Efficacy of Neem Oil and Selected Plant Extracts against Anthracnose of Soybean Caused by *Colletotrichum truncatum* (Schw.) Andrus and Moore

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Soybean [*Glycine max* (L.) Merill.] is one of the most important an Asiatic oil seed crop. Soybean comes under the leguminous crop is having symbiotic association with *Rhizobium*. It is a significant commercial crop that yields oil and is beneficial for both oil extraction and the production of numerous items that are used in human and animal nutrition. The present study was investigated on the effect of neem oil, Eucalyptus leaf extract, Parthenium leaf extract, Lantana leaf extract, Darek leaf extract, Ficus leaf extract and Mancozeb were compared and tested against anthracnose leaf spot [*Colletotrichum truncatum* (Schw.) Andrus and Moore] of soybean under field conditions at the research plot of the Central Research Field, Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj during the *Kharif* season of 2023. Among the treatments, T_3 – Eucalyptus leaf extract exhibited the minimum disease intensity (%) 50

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DAS (10.66 %), 70 DAS (11.50 %) and 90 DAS (12.60) Significantly increased in T_3 – Eucalyptus leaf extract @ 10%, followed by T_1 – Parthenium leaf extract @ 10% as compared to other treatments including (Treated check)T_6 -Mancozeb @ 0.2 and (Untreated check) T_0 – Control.

Keywords: Soybean; Colletotrichum truncatum (Schw.); darek leaf extract; eucalyptus leaf extract; ficus leaf extract; lantana leaf extract; mancozeb; neem oil and parthenium leaf extract.

1. INTRODUCTION

Soybean [*Glycine max* (L.) Merill.] is one of the most important an Asiatic oil seed crop. Soybean comes under the leguminous crop is having symbiotic association with Rhizobium. It is a significant commercial crop that yields oil and is beneficial for both oil extraction and the production of numerous items that are used in human and animal nutrition. As a result, it has spread throughout the entire world, including Europe. Originating in the temperate northern plains of China circa 1100 BC. Although the USA is a major producer of soybeans and their products, India depends heavily on its economy. Soybean a legume crop and is the second largest after groundnut oilseed in India. It is growing in diverse agro-climatic conditions. It is an important rainfed agricultural oil seed crop in the tropical and subtropical regions.

"Soybean ranks first among the oilseeds in the world and contributes for nearly 25 per cent of the world's total oil and fats production. Currently, soybean is cultivated over an area of 11.00 million ha and the production is 11.53 million tonnes registering a productivity of 10.45 q/ha" [1]. The USA leads in terms of area and production of soybean, while India ranks fourth in area and fifth in production in the world. USA, Argentina, Brazil, China and India are the major producers of soybean accounting for 90% of world production.

Major states in the nation that grow soybeans are Madhya Pradesh, Maharashtra, Karnataka, and Andhra Pradesh. Profitable soybean production depends critically on the health of the bean plants [2]. The most prevalent species found on soybeans is *Colletotrichum truncatum*, which can affect soybeans at any point throughout their growth, but especially from bloom to pod fullness**.**

Nutritionally soybean is very good source of protein, near about 42 per cent protein, 20 per cent and 31.3 per cent carbohydrates content occur in soybean seed. The protein quality of soybean is equal to the meat, fish and egg and it is very much nutritious produces a lot of products as like soya milk, soya cake, soya peat, cattle feed etc. As well as it is used for the making bread, biscuits, cake, flour, etc. It is also called as poor man's meat because highest content of protein and other valuable essential components such as Vitamin C, A, E, B1, B2, B3, B5, B6, B12 and Folic acid etc.

Soybean plant is highly able to fix the atmospheric nitrogen in to the soil nitrogen and it is the key that influence plant nutrients in crop production. Nitrogen requirement of the plant is very much high but chemically supplying of the nitrogen is very costly, but biologically it is fixed by different bio-inoculants and it is beneficially and eco-friendly for the crops. It will increase root nodulation, branch number, dry matter pod number as well as crop yield. Not only root nodules but also leaves residual effect for crop succeeding equal to the 35 to 40 kg nitrogen per hectare (Sharma *et al.,* 2019).

"A variety of pathogens, bacteria, viruses, nematodes, mycoplasma, including fungi, attack soybean. So far, 29 fungal, 6 bacterial, 18 viral, 6 nematodes and 3 mycoplasma diseases have been recorded on this crop" (Sinclair and Dhingra, 1975; Sharma *et al.,* 2016). "Soybean cultivation is often subjected to several biotic stresses of which diseases like Anthracnose (*Colletotrichum truncatum*), Fusarium wilt (*Fusarium solani*), Alternaria blight (*Alternaria tenuis*) are important. Among them, anthracnose/ pod blight of soybean caused by *Colletotrichum truncatum.*, rust, which is caused by *Phakopsora pachyrhizi*, *Rhizoctonia* aerial blight, target leaf spot, which is caused by *Rhizoctonia solani*, Myrothecium leaf spot, which is caused by *Myrothecium roridum*, charcoal rot, which is caused by *Macrophomina phaseolina, Sclerotinia Xanthomonas axonopodis* pv. Glycines and *Xanthomonas campestris* pv. Glycines causes purple seed stain; Soybean mosaic virus (SMV); Soybean chlorotic mottle virus (SBCMV) causes soybean mosaic; and Bean Yellow Mosaic virus (BMW) causes bean yellow mosaic. In India it was considered the most serious disease" (Khare and Chako, 1983). The disease causes reduction of seed germination, seed quality and

yield losses occur in warm (20-25 ^{0}C) and humid region of sub-tropics and tropics. In India, soybean anthracnose caused 16-25% of yield loss [3].

Soybean crop is susceptible for anthracnose at all stages of development. Pre- and post-emergence damping – off occurs when infected seeds are planted. On emerging seedlings, dark brown, sunken lesions develop on the cotyledons. These lesions can extend along the stem when conditions favor diseases development. Causing one or both cotyledons to become water soaked, shrivel. Early season infection of pods or pedicels can result in fewer and smaller seed or no seed development, at advanced stages of diseases development, near soybean maturity, black fungal fruiting bodies called acervulli that produce minute black spines are abundant and randomly distributed on infected tissue [4].

2. MATERIALS AND METHODS

From the field-grown soybean seeds of the JS-335 variety plants, leaves and pods displaying characteristic symptoms of the anthracnose disease of soybeans were collected separately. This field experiment was conducted during the Kharif season 2023 at the Central Research Field, Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj.

Isolation of fungal organism: Diseased samples collected from different areas during the season and isolation of pathogen was carried out in the laboratory. Collected diseased samples were 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 such a way that the sample contained a 50 per cent healthy portion

as well as a 50 per cent diseased portion. The surface of the pieces was sterilized by using 1 per cent sodium hypochlorite solution for 30 seconds to 1 minute, then finally wash well with the three changes of sterilized distilled water 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 (PDA) medium under the aseptic conditions in the laminar airflow chamber (Plate 3). 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 [5].

Evaluation of treatments under field conditions: The efficacy of fungicide and plant-extracts against *C. truncatum* was carried out in field conditions.

Preparation of plant-extracts: "Hundred grams of fresh healthy plant parts (leaves) collected from field was washed with distilled water and air dried and crushed in 100 ml of distilled water (w/v). The crushed product was filtered through double layer, muslin cloth and further filtrated through Whatsman No. 1 filter paper using funnel and volumetric flasks (100 ml cap.). The prepared solution was 100 per cent concentration, which was further diluted to required concentrations of 10 per cent" [6]. (Plate 1). All the treatments were given as foliar sprays. Plant-extracts were sprayed @ 1 and 10 ml/litre of water, mancozeb @ 2 ml/ litre of water.

Disease intensity (%): The Percent disease intensity of 5 randomly selected plants was calculated at 50, 70 and 90 DAS. Disease intensity (%) formula is given by Wheeler [7]. It was calculated by using the following formula:

Plate 1. Preparation of plant-extracts for treatments

$$Disease\ intensity\ (\%) = \frac{Sum\ of\ all\ disease\ ratings}{Total\ number\ of\ ratings \times Maximum\ disease\ grade} \times 100$$

List 1. Rating Scale for Anthracnose of Soybean [*Glycine max* (L.) Merill] [8]

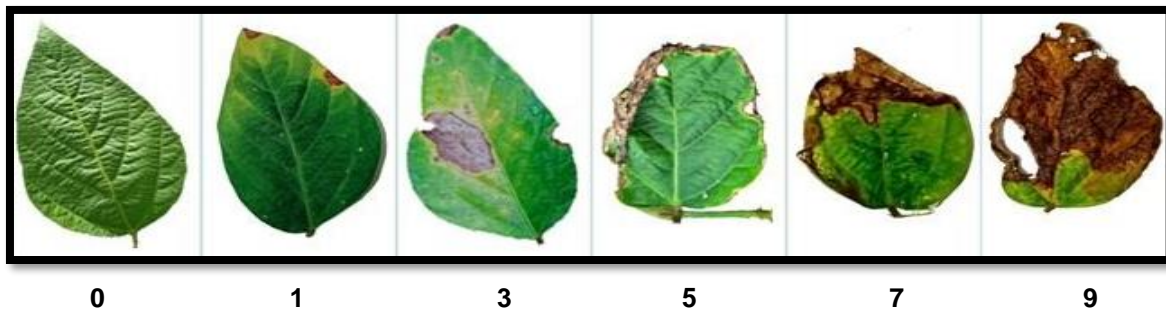| Scale	Description
0	No of lesions / discoloration
1	1% area covered with lesions/ spots / discoloration
3	1. 1–10% area covered with lesions/ spots / discoloration
5	10. 1—25% area covered with lesions / spots / discoloration
7	25. 1—50% area covered with lesions / spots / discoloration
9	> 50% area covered with lesions / spots / discoloration

0	1	3	5	7	9

Plate 2. Disease rating scale

List 2. Experimental Details

Name of the selected crop	Soybean
Variety	JS-335
Spacing	15 cm
Row to Row	30 cm
Plant to plant	30 cm x 15 cm
Experimental design	RBD
Number of treatments	7
Number of replications	3
Total number of plots	21
Plot size	2 ×1 = 2 m^2
Size of bunds	0.2 m
Main irrigation channel	1m
Width of sub irrigation channel	0.5 m
Total net cultivated area	42 m^2
Date of sowing	15 Sept.2023

List 3. Treatment Details

The treatment details are given below under the following headings:

| S.No.	Treatments	Treatment details	
		Seed treatment	Foliar spray
1	T_0	Control (untreated)	Water spray
2	T_1	Mancozeb (S.T)	Neem oil @1% + Parthenium leaf extract @10%
3	T_2	Mancozeb (S.T)	Neem oil @1% + Lantana leaf extract @10%
4	T_3	Mancozeb (S.T)	Neem oil @1% + Eucalyptus leaf extract @10%
5	T_4	Mancozeb (S.T)	Neem oil @1% + Darek leaf extract @10%
6	T_5	Mancozeb (S.T)	Neem oil @1% + Ficus leaf extract @10%
7	T_6	Mancozeb 0.2% (F.S)	
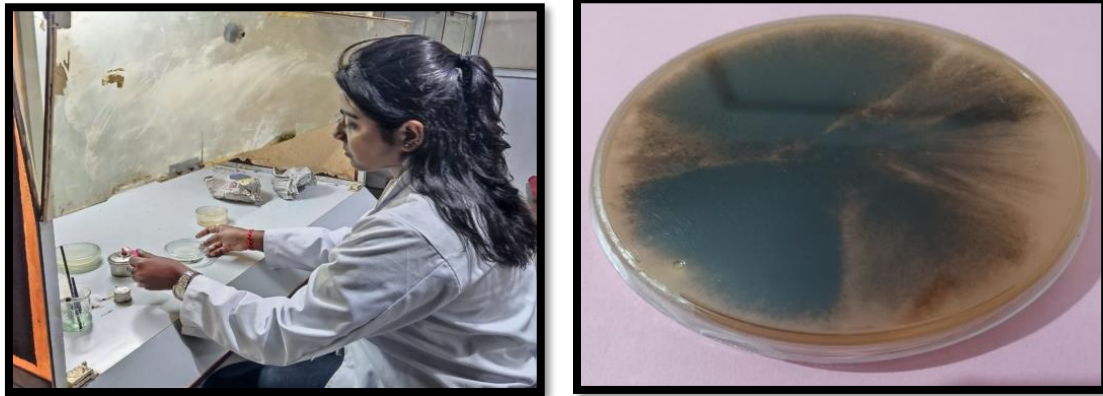
Plate 3 Isolation and pure culture of the pathogen

3. RESULTS AND DISCUSSION

Disease intensity (%) of Anthracnose leaf spot on soybean at 50 DAS: The data presented in the Table 1 and depicted in Fig. 1 reveals that disease intensity (%) of soybean significantly decreased in treatment T_3 – Neem oil + Eucalyptus leaf extract (10.66%) followed by T_1 – Neem oil + Parthenium leaf extract (11.50%), T_2 – Neem oil + Lantana leaf extract (12.00%), T_4 – Neem oil + Darek leaf extract (13.00%) and T_5 - Neem oil + Ficus leaf extract (14.63%) as compared to (Treated check) T_6 – Mancozeb (8.73%) and (Untreated check) T_0 – Control (15.50%). Comparing the treatments with CD value (0.21), all the treatments (T_1, T_2, T_3, T_4, T_5, T_6) were found significant over (Untreated check) T_0 – Control.

Disease intensity (%) of Anthracnose leaf spot on soybean at 50, 70 and 90 DAS:

Table 1. Effect of treatments on disease intensity (%) of Anthracnose leaf spot on soybean at 50, 70 and 90 DAS

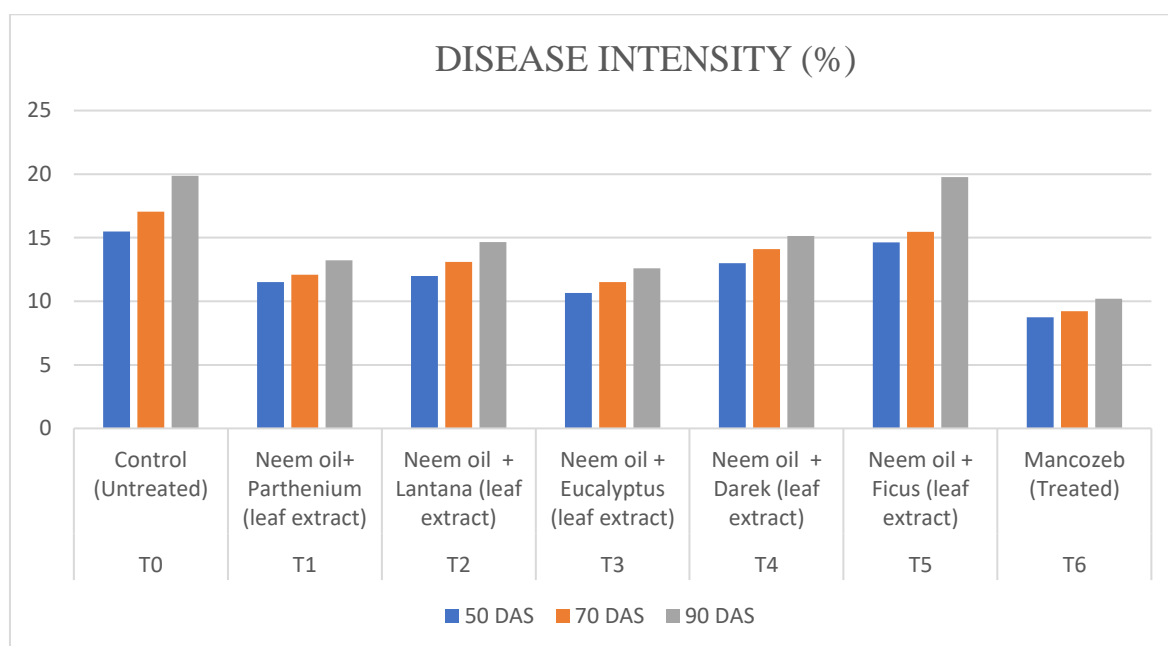| Treatments	Treatment details		Disease intensity (%)		
	Seed treatment	Foliar spray	Before spray of botanicals 50 DAS	10 days after 1st spray of botanicals 70 DAS	10 days after 2nd spray of botanicals 90 DAS
T_0	Control (untreated)	Water spray	15.50[a]	17.06[a]	19.86[a]
T_1	Mancozeb (S.T)	Neem oil @1% + Parthenium leaf extract @10%	11.50[e]	12.10[e]	13.23[e]
T_2	Mancozeb (S.T)	Neem oil @1% + Lantana leaf extract @10%	12.00[d]	13.10[d]	14.66[d]
T_3	Mancozeb (S.T)	Neem oil @1% + Eucalyptus leaf extract @10%	10.66[f]	11.50[f]	12.60[f]
T_4	Mancozeb (S.T)	Neem oil @1% + Darek leaf extract @10%	13.00[c]	14.10[c]	15.13[c]
T_5	Mancozeb (S.T)	Neem oil @1% + Ficus leaf extract @10%	14.63[b]	15.46[b]	19.76[a]
T_6	Mancozeb 0.2% (F.S)		8.73[g]	9.23[g]	10.20[g]
	SE(m) ± 1		**0.07**	**0.98**	**0.99**
	CD at 5%		**0.21**	**0.23**	**0.25**

Fig. 1. Effect of treatments on disease intensity (%) of Anthracnose leaf spot on soybean at 50, 70 and 90 DAS

Disease intensity (%) of Anthracnose leaf spot on soybean at 70 DAS: The data presented in the Table 1 and depicted in Fig. 1 reveals that disease intensity (%) of soybean significantly decreased in treatment T_3 – Neem oil + Eucalyptus leaf extract (11.50%) followed by T_1 – Neem oil + Parthenium leaf extract (12.10%), T_2 – Neem oil + Lantana leaf extract (13.10%), T_4 – Neem oil + Darek leaf extract (14.10%) and T_5 - Neem oil + Ficus leaf extract (15.46%) as compared to (Treated check) T_6 – Mancozeb (9.23%) and (Untreated check) T_0 – Control (17.06%).

Disease intensity (%) of Anthracnose leaf spot on soybean at 90 DAS: The data presented in the Table 1 and depicted in Fig. 1 reveals that disease intensity (%) of soybean significantly decreased in treatment T_3 – Neem oil + Eucalyptus leaf extract (12.60%) followed by T_1 – Neem oil + Parthenium leaf extract (13.23%), T_2 – Neem oil + Lantana leaf extract (14.66%), T_4 – Neem oil + Darek leaf extract (15.13%) and T_5 - Neem oil + Ficus leaf extract (19.76%) as compared to (Treated check) T_6 – Mancozeb (10.20%) and (Untreated check) T_0 – Control (19.86%). Comparing the treatments with CD value (0.25), 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_6, T_0) were found non-significant to each other. (T_1, T_2, T_3, T_4 and T_5) were

found to be significant over all the other treatments.

Konar and Kushari [9] using Eucalyptus extract on different crop plants. Prasad and Subhashini [10] reported that the allelochemicals greatly inhibited porphyrin synthesis. The study results are consistent with the comparable conclusions made by Patni et al. [11]. The Eucalyptus spray had a substantially lower minimum number of spots (30.73) than all other treatments, with the exception of the Mancozeb spray. Eucalyptus spray also showed minimum number of spots/ $10cm^2$ leaf area (4.16), which was significantly lower than all other treatments but at par with Mancozeb spray (4.25). Eucalyptus spray showed significantly minimum size (5.99mm) of leaf spot in comparison to other treatments except mancozeb spray. Minimum diseases index was observed in Eucalyptus spray (29.05%) followed by Ashok spray (29.31%) and mancozeb spray (29.41%), which were significantly different to all other treatments but at par with each other. Significantly minimum number of spots on pod was observed in Eucalyptus spray (8.26) followed by mancozeb spray (8.60). phyto-extracts of Eucalyptus and neem significantly inhibits the pathogen could lead to better health of the plants which in turn helps in producing minimum disease intensity and leading to a healthier plant.

4. CONCLUSIONS

Colletotrichum truncatum (Schw.) Andrus and Moore, was found associated with Anthracnose leaf spot disease of soybean [*Glycine max* (L.) Merill.]. The disease intensity (%), overall results revealed that T_3 - Eucalyptus leaf extract @10% is significantly effective against *C. truncatum* (Schw.) Andrus and Moore 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 (CRF), Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, during *Kharif* Season 2023, within the agro- climatic conditions of Prayagraj, UP. India. As such, further research and more experimentation over many seasons should be conducted in future to validate the present findings and for further recommendations.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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