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Effect of Neem Oil in Combination with Propiconazole and Organic Amendments on Anthracnose (*Colletotrichum lindemuthianum*) of Cowpea (*Vigna unguiculata* L.)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The cowpea (*Vigna unguiculata*) is an annual herbaceous legume from the genus *Vigna*. Anthracnoses (*Colletotrichum lindemuthianum*) is a serious disease in cowpea growing areas and occurs as pre-harvest as well as post-harvest brown to black spots appear on the leaves. Neem oil at different concentrations (0.5%, 1%,1.5%, and 2%) along with Propiconazole were tested @ 0.1% *in vivo* during *Kharif* 2023 for their efficacy against disease incidence, plant growth and yield parameters of Cowpea at the Central Research Field of the Sam Higginbottom University of

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Agriculture, Technology and Sciences, Prayagraj. The results of the present investigation revealed that it significantly reduced the disease incidence. Among the treatments the minimum disease intensity (%) at 75 DAS was recorded in T5 – Neem oil (2 ml/L) + Propiconazole 25 EC (0.1%) + VC@10gm/kg +SMC@10gm/kg (Foliar spray) was found to be significantly most effective treatment against Anthracnose (*Colletotrichum lindemuthianum*) on Cowpea and recorded minimum disease intensity (%) at 30, 45, 60 and 75 DAS, maximum plant height (cm), maximum number of branches, maximum yield (t/ha) and highest B:C ratio. The findings of the present experiment are limited to one crop season under Prayagraj agro-climatic conditions, as such to validate the present findings; more such experiments should be carried out in the future.

Keywords: Anthracnose (Colletotrichum lindemuthianum); cowpea; disease intensity (%); neem oil.

1. INTRODUCTION

"Botanically speaking *Vigna unguiculata* (L.) Walp, commonly called Cowpea; is an annual herbaceous legume from the genus *Vigna*. Its tolerance for sandy soil and low rainfall have made it an important crop in the semiarid regions across Africa and Asia. It requires very few inputs, as the plant's root nodules can fix atmospheric nitrogen, making it a valuable crop for resource-poor farmers and well-suited to intercropping with other crops. The whole plant is used as forage for animals, with its use as cattle feed likely responsible for its name. Its many varieties differ substantially in the shape of the fruit. It is one of the several species of the widely cultivated genus *Vigna*. Cowpea is a diploid species with a somatic chromosome number 2n=22. It is one of the most important pulse crops native to West Africa" [1].

"Cowpea is called a poor man's meat or vegetable meat due to its high amount of protein. The young leaves, pods, and peas contain vitamins and minerals, which are used for human consumption and animal feed. It is a most versatile pulse crop because of its smothering nature, drought tolerant characters, soil restoring properties, and multi-purpose uses. As a pulse crop, cowpea fits well into most of the cropping systems. It is cultivated for its seed (green or dried), pods, and leaves, which are consumed in a fresh form as a green vegetable, while snacks and main meal dishes are prepared from the dried grain" [2].

"Cowpea plays a major role in human nutrition, fruit contains high nutritive value constituting a high amount of Carbohydrate 60.03 g, protein 23.52 g, dietary fiber 10.6 g, magnesium 184 mg, sodium 16 mg, Vitamin C 1.5 mg, 424 mg Phosphorous, Iron 8.27 mg, Calcium 110 mg, Potassium 1152 mg, Vitamin A 3 µg, Folate (B9) 633 µg and many other nutrients out of 100 g of edible portion" [3].

"Cowpea diseases caused by various pathogens (fungi, bacteria, viruses, nematodes, and parasitic plants) constitute one of the important biotic constraints to cowpea production in all regions where the crop is cultivated. These diseases can infect cowpeas at different stages such as during emergence, vegetative, and reproductive stages causing substantial plant damage hence leading to yield loss or complete production failure" [4].

"Amongst predominantly associated pathogens, *Colletotrichum lindemuthianum* (Sacc. and Magnus) regularly occurs in tropical and sub-tropical areas, especially under cool and humid conditions. The term Anthracnose means "like coal" and was first used by Fabre and Dunal to describe a disease of grapes in which blackening of tissue was a characteristic feature. In Nigeria, the disease is one of the major fungal diseases of cowpea crops. The fungus overwinters in the previous crop debris and can also be seed-borne as dormant mycelia within the seed coat or as spores between the cotyledons; from where it initiates infection of hypocotyls and young leaves in the field" [5]. "Anthracnose characterized by sunken, black lesion is one of the major fungal diseases of cowpea which constrain its economic production" [6]. "In affected cowpea plants, up to 50 percent yield reduction occurs. Its causal agent has been the subject of much scientific debate. It has been variously advanced and reported as a form of *C. lindemuthianum"* [7].

Pathogen is known to infect the crop at every stage of plant growth from seedling to maturity, depending upon the availability of favorable environmental conditions as required by the pathogen for its growth, spread, and disease development. Seed borne nature and cosmopolitan distribution of the pathogen makes its management difficult especially when farmers use their seed for cultivation year after year. The disease has been reported to infect the majority of the widely grown bean cultivars leading up to 100 percent yield loss in the susceptible ones making it one of the major limiting factors for its profitable cultivation. The use of chemicals for seed treatment has ill effects on soil health as well as on the environment. Among all the available management practices, a highly efficient, simple, and economic approach is the use of genetically improved cultivars [8].

2. MATERIALS AND METHODS

The present study was conducted at Central Research Field of Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, during the *kharif* season of 2023. The details of materials which are used, experimental procedures, and statistical analysis by RBD, as per the method "Analysis of Variance (ANOVA) technique" followed for the estimation of various growth parameters and yield during investigation are presented in the Table 1.

The variety Kashi Kanchan was selected for sowing. This is dwarf and bush type (height 50-60 cm), photo-insensitive, early flowering (40-45 days after sowing), and early picking (50-55 days after sowing) variety suitable for growing in both spring-summer and rainy seasons. Pods are about 30-35 cm long, dark green, soft, fleshy and free from parchment. The cultivar gives a green pod yield of about 150-175 q/ ha [9,10].

Isolation of the fungal organism: "Diseased samples were collected from different areas during the season and pathogens were isolated 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 so that the sample contained a 50 percent healthy portion and a 50 percent diseased portion. The surface of the pieces was sterilized by using 1 percent sodium hypochlorite solution for 30 seconds to 1 minute, then finally washed well with the three changes of sterilized distilled water to remove excess water then pieces were 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. Five pieces of PDA media on each plate. Inoculated Petri plates are kept in an incubator at 25°±2°C and examined at frequent intervals to check the growth of the target fungal pathogen" (Desai and Prasad, 1955).

Disease intensity: Percent disease intensity was recorded at certain day intervals after an incidence of anthracnose of cowpea in several crops [11].

The disease severity/incidence was assessed by visual assessment of the test plants with typical symptoms of the anthracnose disease, using the descriptive scale of 1-10 as outlined by [6]:

$$\text{Disease intencity}(\%) = \frac{\text{No. of diseased plants}}{\text{Total No. of plants assessed} \times \text{Maximum disease Scale}}$$

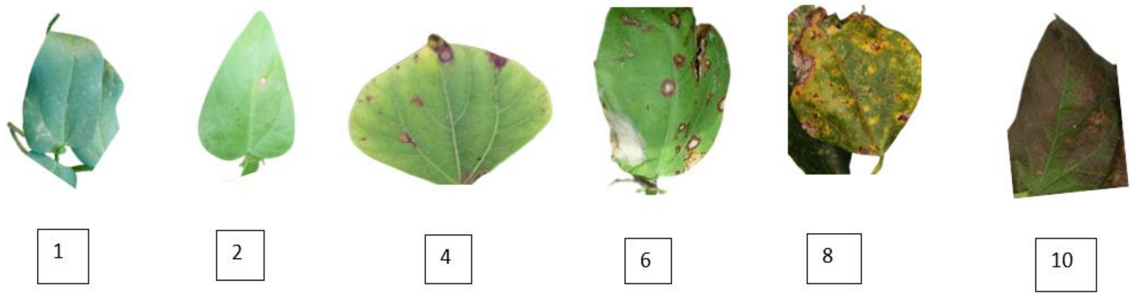
Table 1. Details of treatment combinations

Treatment	Details	Application	References
T0	Control	-	-
T1	Neem oil (0.5 ml/l)	FS	Moharam et al. [12].
T2	Neem oil (0.5 ml/l) + Propiconazole 25EC (0.1%) + VC@10g/kg + SMC@10g/kg	FS+FS+ST+ST	Moharam et al. [12], Dabbas et al.(2015) and Sahoo et al. (2020).
T3	Neem oil (1 ml/l) + Propiconazole 25 EC (0.1%) + VC@10g/kg + SMC@10g/kg	FS+FS+ST+ST	Moharam et al. [12], Dabbas et al.(2015) and Sahoo et al. (2020).
T4	Neem oil (1.5 ml/l) + Propiconazole 25 EC (0.1%) + VC@10g/kg + SMC@10g/kg	FS+FS+ST+ST	Moharam et al. [12], Dabbas et al.(2015) and Sahoo et al. (2020).
T5	Neem oil (2 ml/l) + Propiconazole 25 EC (0.1%) + VC@10g/kg + SMC@10g/kg	FS+FS+ST+ST	Moharam et al. [12], Dabbas et al.(2015) and Sahoo et al. (2020).
T6	Propiconazole 25 EC (0.1%)	FS	Dabbas et al. (2015).

F.S – Foliar spray, S.T- Soil treatment, VC – Vermicompost, SMC- Spent mushroom compost

Table 2. Disease intensity scale

Scale	Symptoms
1	No infections
2	1-25% of seedlings with anthracnose disease
4	26-50% of seedlings with anthracnose disease
6	51-75% of seedlings with anthracnose disease
8	76-100% of seedlings with anthracnose disease
10	Stem breakage, girdling, or death of seedling due to anthracnose disease



Disease Rating scale

3. RESULTS AND DISCUSSION

A field study was carried out to assess the effect of selected organics and fungicides against anthracnose (*Colletotrichum lindemuthianum*) on Cowpea with two sprays taken up at 45 and 60 DAS during *kharif* 2023 - 2024, The results presented in Table 1 and depicted in Fig. 1 reveals that the percent disease index was significant at 30, 45, 60 and 75 DAS.

Disease intensity (%) at 30 DAS (Days after sowing): The data presented in Table 3 and depicted in Fig. 1 revealed that minimum disease intensity (%) was recorded in T5- Neem oil (2 ml/l) + Propiconazole 25 EC (0.1%) + VC@10g/kg+SMC@10g/kg (17.7%), followed by T4 - Neem oil (1.5 ml/l) + Propiconazole 25 EC (0.1%) + VC@10g/kg +SMC@10g/kg (18%), T6 - Propiconazole 25 EC (0.1%) (18.6%), T3 - Neem oil (1ml/l) + Propiconazole 25 EC (0.1%) + VC@10g/kg +SMC@10g/kg (19.5%), T2 - Neem oil (0.5 ml/l) + Propiconazole 25 EC (0.1%) + VC@10g/kg +SMC@10g/kg (20.3%), T1 - Neem oil (0.5 ml/) (21.4%) and untreated check T0- (23.1%).

Comparing the CD value, (0.03) all the treatments were significant over the untreated check.

Disease intensity (%) at 45 DAS; The data presented in Table 3 and depicted in Fig. 1 reveals that minimum disease intensity (%) was recorded in T5- Neem oil (2 ml/l) + Propiconazole 25 EC (0.1%) + VC@10g/kg +SMC@10g/kg (19.8%), followed by T4 - Neem oil (1.5 ml/l).

Propiconazole 25 EC (0.1%) + VC@10g/kg +SMC@10g/kg (20.3%), T6 - Propiconazole 25 EC (0.1%) (22.3%) , T3 - Neem oil (1ml/l) + Propiconazole 25 EC (0.1%) + VC@10g/kg +SMC@10g/kg (23.4%), T2 - Neem oil (0.5 ml/l) + Propiconazole 25 EC (0.1%) + VC@10g/kg+SMC@10g/kg (24.3%), T1 - Neem oil (0.5 ml/) (25.6%) and untreated check T0- (27.4%).

Comparing the CD value, (0.49) all the treatments were significant to the untreated check.

Disease intensity at 60 DAS: The data presented in Table 3 and depicted in Fig. 1 revealed that at 60 DAS significantly that minimum disease intensity (%) was recorded in T5- Neem oil (2 ml/l) + Propiconazole 25 EC (0.1%) + VC@10g/kg +SMC@10g/kg (25.8%), followed by T4 - Neem oil (1.5 ml/l) + Propiconazole 25 EC (0.1%) + VC@10g/kg +SMC@10g/kg (27.0%), T6 - Propiconazole 25 EC (0.1%) (27.9%), T3 - Neem oil (1ml/l) + Propiconazole 25 EC (0.1%) + VC@10g/kg +SMC@10g/kg (29.2%), T2 - Neem oil (0.5 ml/l) + Propiconazole 25 EC (0.1%) + VC@10g/kg +SMC@10g/kg (30.4%), T1 - Neem oil (0.5 ml/) (32.1%) and untreated check T0- (34.0%).

Compared with the CD value, (0.98) all the treatments were found statistically significant with the T_0 – control. However, (T_1, T_2, T_3, T_5) was found statistically significant, whereas, (T6 and T4) were found statistically non-significant with each other.

Disease intensity at 75 DAS: The data presented in Table 3 and depicted in Fig. 1 revealed that disease intensity (%) at 75 DAS significantly that minimum disease intensity (%) was recorded in T5- Neem oil (2 ml/l) + Propiconazole 25 EC (0.1%) + VC@10g/kg +SMC@10g/kg (26.7%), followed by T4 - Neem oil (1.5 ml/l) + Propiconazole 25 EC (0.1%) + VC@10g/kg +SMC@10g/kg (27.1%), T6 - Propiconazole 25 EC (0.1%) (28.0%), T3 - Neem oil (1ml/l) + Propiconazole 25 EC (0.1%) + VC@10g/kg +SMC@10g/kg (29.4%), T2 - Neem oil (0.5 ml/l) + Propiconazole 25 EC (0.1%) + VC@10g/kg +SMC@10g/kg (30.6), T1 - Neem oil (0.5 ml/) (32.3%) and untreated check T_0-(34.6%).

Comparing the CD value, (0.35) all the treatments were significant to the untreated check.

Table 3. Effect of selective treatments on disease intensity of anthracnose of Cowpea at different day intervals

Treatment combination		Disease Intensity (%)			
		30 DAS	45 DAS	60 DAS	75 DAS
T0	Control	23.13	27.40	34.03	34.60
T1	Neem oil (0.5 ml/l) [FS]	21.40	25.66	32.10	32.33
T2	Neem oil (0.5 ml/l) + Propiconazole 25EC (0.1%) [FS] + VC@10g/kg + SMC@10g/kg [ST]	20.30	24.36	30.46	30.66
T3	Neem oil (1 ml/l) + Propiconazole 25 EC(0.1%) [FS] + VC@10g/kg + SMC@10g/kg [ST]	19.50	23.40	29.23	29.40
T4	Neem oil (1.5 ml/l) + Propiconazole 25 EC (0.1%) [FS] + VC@10g/kg + SMC@10g/kg [ST]	18.00	20.36	27.06[a]	27.13
T5	Neem oil (2 ml/l) + Propiconazole 25 EC (0.1%) [FS] + VC@10g/kg +SMC@10g/kg [ST]	17.70	19.86	25.83	26.70
T6	Propiconazole 25 EC (0.1%) [FS]	18.60	22.30	27.90[a]	28.06
	C.D. at 5%	0.03	0.49	0.98	0.35

***FS –** Foliar Spray*
***ST** – Soil Treatment*

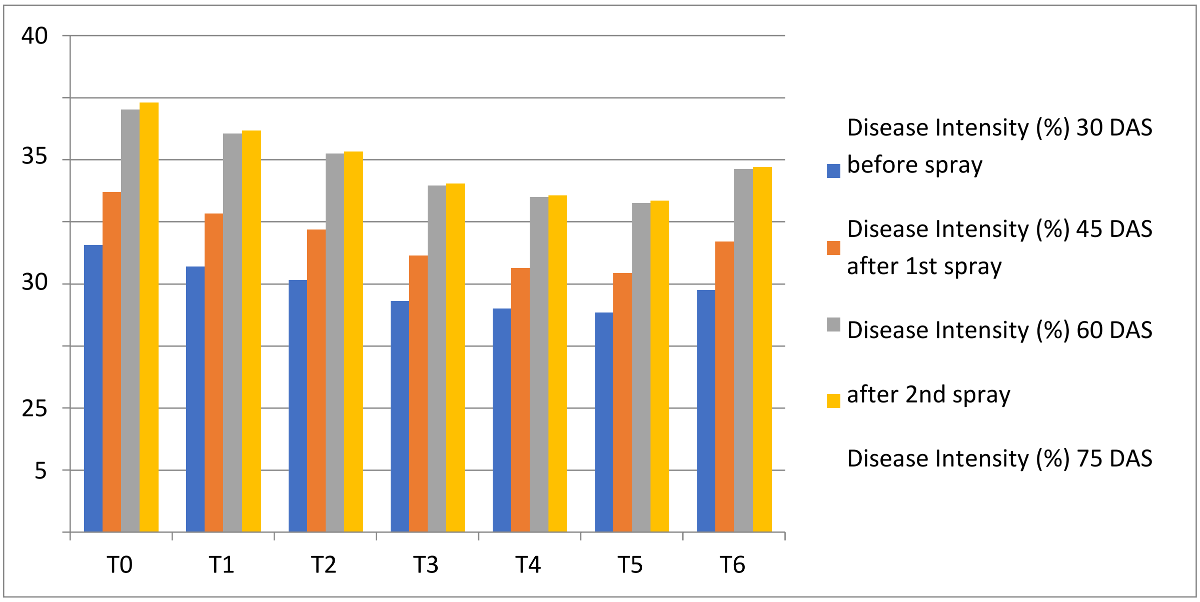


Fig. 1. Effect of selective treatments on disease intensity of anthracnose (*Colletotrichum lindemuthianum*) of Cowpea at different day intervals

The probable reasons for such findings may be due to the initial soil amendment of vermicompost and spent mushroom compost that likely played a crucial role in enhancing the soil's health and microbial activity, which may have suppressed the growth and spread of *Colletotrichum* and promoted healthier crop growth. Following the soil amendment, a foliar spray of neem oil was applied, which may further contribute to disease suppression. Neem oil, rich in phenolic compounds like thymol, may disrupt the fungal cell membrane and result in a significant reduction in per cent disease intensity. Lastly, a foliar spray of Propiconazole 25 EC was applied, which may have inhibited the biosynthesis of ergosterol, which is a critical component of the fungal cell membrane, thus compromising cell integrity and function. The combined effect of soil amendments and strategic foliar applications may have resulted in superior outcomes. similar findings have been reported by Kuck and Gisi [13], Nehal et al. [14], Pathma and Sakthivel [15], Seham et al. [16], and Elawady et al. [17].

4. CONCLUSION

The present study concludes that, in the field conditions, Neem oil (2 ml/L) + Propiconazole 25 EC (0.1%) + VC@10gm/kg +SMC@10gm/kg were found to be significantly most effective treatment against anthracnose (*Colletotrichum lindemuthianum*) on Cowpea and recorded minimum disease intensity (%) at 30, 45, 60 and 75 DAS, maximum plant height (cm), maximum number of branches, maximum yield (t/ha) and highest B:C ratio. The findings of the present experiment are limited to one crop season 2023 to 2024 under Prayagraj agro-climatic conditions, as such to validate the present findings; more such experiments should be carried out in the future.

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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