



Assessing the Effectiveness of Neem oil and Microalgae in the Management of Cowpea (*Vigna unguiculata* L.) Leaf Spot Caused by *Alternaria* spp.

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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 current study aimed to assess the effectiveness of neem oil and microalgae in controlling leaf spot caused by *Alternaria* spp. in cowpea (*Vigna unguiculata* L.). The research was conducted during the Zaid season of 2022 at the Central Research Field of the Department of Plant Pathology, SHUATS, Prayagraj, Uttar Pradesh, using a Randomized Block Design with three replications and seven treatments. Analysis of variance (ANOVA) was utilized to draw conclusions from the data. Leaf spot disease caused by *Alternaria* spp poses a significant threat to cowpea crops, resulting in yield losses and decreased plant vigor. An in vivo study was carried out to evaluate the impact of different concentrations of microalgae combined with neem oil at 1% on plant growth parameters and Disease Intensity (%). The application of microalgae @540 grams in various splits and neem oil @1% was done. Among all the treatments, T4 (Microalgae 48gms @5

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DAS 96gms@15,45 DAS + Neem oil @1% showed promising results. Notably, the T4 treatment significantly increased plant height (84.63 cm) and the number of branches(10.83) at60DAS. Moreover, an increased number of nodules per plant (21.44) was observed at 75 DAS, indicating improved root health and nitrogen fixation. Additionally, the treatment led to longer pod length (34.44 cm) in three pickings, suggesting enhanced yield potential. Furthermore, the combined application of microalgae and neem oil resulted in a significant reduction in disease intensity, with a notable decrease of 24.47% compared to untreated plants and those treated with chemicals (Bavistin). This indicates that the use of microalgae and neem oil together effectively suppressed leaf spot disease caused by *Alternaria spp.* in cowpea, thereby improving plant health and productivity. In conclusion, these findings underscore the potential use of eco-friendly alternatives like microalgae and neem oil for managing leaf spot disease in cowpea cultivation. Further research in this area is recommended for sustainable agricultural practices.

Keywords: *Alternaria*; *micro algae*; *neem oil*; *leaf spot*; *disease intensity*; *zaid: foliar*.

1. INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) is also known as black-eyed pea or southern pea etc. and has multiple uses like food, feed, forage, fodder, green manuring and vegetable. Cowpea seed is a nutritious component in the human diet, and cheap livestock feed as well. There are symbiotic associations with nitrogen-fixing bacteria by root nodules and phosphorus-absorbing Arbuscular Mycorrhizal fungi in cowpea roots [1]. Its fresh leaves are used as vegetables, the haulms (cowpea pod walls, stems, and leaves) are used as livestock fodder, providing dietary nutrients for animals, and as additional income for the farmers [2]. The major pathogenic groups associated with cow pea diseases, include: fungi, bacteria, viruses, and nematodes (Emechebe and Lagoke, 2002). Fungi are the main phytopathogens that cause economic losses in cow pea crop. Diseases caused by fungi with the greatest economic impact on cowpea crop are caused by *Macrophomina phaseolina*, *Fusarium spp.*, *Rhizoctonia solani*, *Curvularia spp.*, *Trichoderma spp.*, *Alternaria spp.*, *Aspergillus spp.* and *Penicillium spp.* [3] Among the foliar fungal diseases leaf spot incited by *Alternaria spp.* causes quantitative and qualitative losses to cow pea crop.

Microalgae have become the focus of extensive research efforts, aimed at finding novel compounds that might lead to therapeutically useful agents [4]. Microalgae have meanwhile been found to produce antibiotics, a large number of microalgal extracts and or extracellular products have proven antibacterial, antifungal, antiprotozoal and anti- plasmodial [5]. There is significant role of micro algae in plant

protection and improvement for sustainable agricultural technology [6].

Neem has been known for ages as an insecticidal plant and recently is classified as therapeutic plant [7]. The Neem oil contains at least 100 biologically active compounds. Owing to the presence of antimicrobial substances in different parts of the plant. The possible fungicidal role, of plant parts and extracts, has been studied against soil borne pathogen [8]. Given the importance of cowpea as a food and fodder crop and the challenges posed by fungal diseases, there is a need for research aimed at evaluating the efficacy of alternative approaches such as microalgae and neem oil in mitigating disease impact while promoting sustainable agricultural practices. This study aims to investigate the potential use of microalgae and neem oil in managing leaf spot disease caused by *Alternaria spp.* in cowpea cultivation, thereby contributing to the development of eco-friendly and effective disease management strategies in agriculture.

2. MATERIALS AND METHODS

The present experiment “Effect of micro algae and neem oil against leaf spot caused by *Alternaria spp.* of Cowpea (*Vigna unguiculata* L.)” was conducted at Central Research Field (CRF) of Sam Higginbottom University of Agriculture, Technology and Sciences (SHUATS), Prayagraj during the *Zaid* season of March 2022.

2.1 Systematic Position of *Alternaria spp.* [9]

Kingdom: Fungi
Division: Eumycota
Sub-division: Deuteromycotina

Class: Hyphomycetes
Order: Moniliales
Family: Dematiaceae
Genus: *Alternaria* spp

Microalgae applied in three intervals on 5 Days after sowing (DAS) @12, 24, 36, 48 and 60 grams and 15 and 45 DAS @ 24,48,72,96 and 120 grams. Madhavi et al.,[10]. The application of micro algae At 5, 15, and 30 DAS different doses for 3 three replications mixed with 5 liters of water for each plot and that micro algae slurry applied along with irrigation. Foliar spray of Neem oil @ 1 % Sarathambalet al. [11] followed by three sprays at 15 days of interval crop 15 days interval @ 30, 45, 60 DAS and Bavistin (Carbendazim) of 0.2% Sharma et al. [12] was sprayed in 15 days interval @30, 45, 60 DAS. Microalgae was manufactured by Phycoline Technologies Pvt Ltd and bought from Biotik™ OGL.

2.2 Collection of Disease Sample

The infected leaves exhibiting typical symptoms were fetched from the standing cow pea crop and brought to the laboratory for further investigation. The spotted area on the leaf surface was identified and cut into small pieces of about 4-6mm. These pieces were washed with freshwater followed by running tap water. 20 ml (approx.) of sterilized molten warm PDA media was poured into sterile distilled plates aseptically. The leaf pieces were further surface sterilized with 0.1% mercuric chloride (HgCl₂) for 30 seconds followed by washing thrice with distilled water and allowed to dry. Later, these dried pieces were carefully placed on the molten media (PDA) in inverted position to ensure that the spot area contacts with the media. And these plates were incubated at 25±2 °C for 2-3 days. After, the fungal mycelial was examined under the microscope and used for further studies.



Fig.1.Pure culture in petri dishes

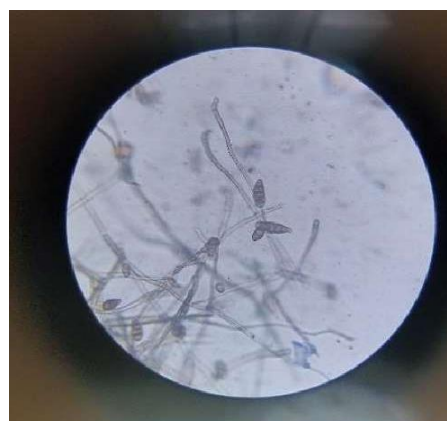


Fig.2. Microscopic view of *alternaria*



Fig.3.Pure culture in slants

2.3 Morphology and Symptomology of the Pathogen

The mycelium of the pathogen was septate. Conidiophores were simple, septate, olive brown and varied in length with solitary terminal conidia or chains (Fig. 2). Initially, the symptoms appeared as a small oval dark brown necrotic sunken spot (2-18 mm dia.) found at the leaf tips and central part of leaf, these later on forms as large necrotic patches.

Symptoms begin as semi-circular, water-soaked lesions at the leaf margins. Lesions enlarge towards the centre of the leaf, eventually becoming necrotic. Sporulation is visible with the naked eye on the leaf surface as black velvet mass. Occasionally circular lesions are observed in the centre of the leaf. Lesions begin as small brown spots, surrounded by a yellow chlorotic halo. The lesions enlarge and become water-soaked and a black mass of conidia is visible on the brown, necrotic tissue surfaces.



Fig. A. Symptoms on leaf



Fig. B. Symptoms on leaf



Fig. C. Symptoms on leaf

Fig. 4 (A-C). Symptoms of Alternaria leaf spot disease

Chart 1. Category Infection with their numerical values

Category infected (%)	Numerical value	Leaf area
I	0	Disease free
II	1	0.1-10.0
III	2	10.1-25.0
IV	3	25.1-50.0
V	4	50.1-75.0

Disease incidence (%) was calculated by using the formula mentioned below Number of infected plants

Disease Intensity (%) = Sum of all disease ratings/ Total number of ratings × Maximum disease grade × 100 [13]

Disease intensity: - The disease intensity was assessed by using 0 to 5 rating scale[14]

$$PDI = \sum (n \times v) \div N \times G$$

Where,

Σ=Summation;
 n=Number of leaves in each category;
 v=Numerical value of each category;
 N=Total Number of leaves examined;
 G=Maximum numerical value.

3. RESULTS AND DISCUSSION

The data presented in table 1, 2,3,4,5 reveals the action of Microalgae and Neem oil on disease Intensity (30, 45, 60 DAS) of *Alternaria* spp of cowpea under field condition. The results

shows that T4 (Microalgae 48gms @5 DAS 96gms@15,45 DAS + Neem oil @1% (24.47) treatment was effective and reduced the percentage of leaf spot caused by *Alternaria* spp. And increased the plant growth

parameters(height of plant, No of branches, length of pod, and no of nodules) in cowpea crop. Minimum growth was observed in T4 (Microalgae 48gms @5 DAS 96gms@15,45 DAS + neem oil @1%).

3.1 Effect of Selected Treatments on Plant Height (cm) of Cowpea at 30, 45 and 60 DAS

At 30, 45, and 60 (DAS), the findings presented in Table 1 and illustrated in Graph 1 demonstrate a significant growth in cowpea plant height across all treatments. Notably, treatment T4, involving the application of microalgae with neem oil, exhibited the noticeable height in T4(84.63), followed by T5 (78.47), T3 (76.67), T1 (73.10), and T2 (72.27) when compared against both the chemical treatment T6, utilizing Bavistin (86.97), and the untreated control (T0) (67.70) under field conditions.

3.2 Effect of Selected Treatments on Number of Branches of Cowpea at 30, 45 and 60 DAS

At 30, 45, and 60 (DAS), the findings presented in Table 2 and illustrated in Graph 2 demonstrate a significant increase in cowpea plant branches across all treatments. Notably, treatment T4, involving the application of microalgae with neem oil, exhibited the noticeable increase in number of branches in T4 (10.83), followed by T5 (9.46),

T3 (9.06), T2 (8.93), and T2 (72.27) when compared against both the chemical treatment T6, utilizing Bavistin (86.97), and the untreated control (T0) (67.70) under field conditions.

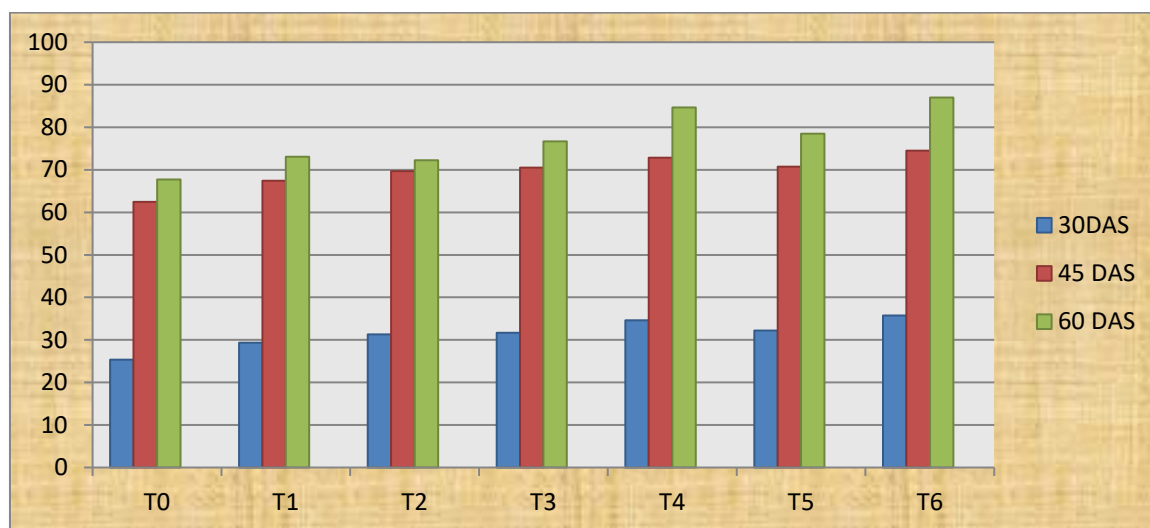
3.3 Effect of Selected Treatments on Length of Pods (cm) on 3 Pickings

At 30, 45, and 60 (DAS), the findings presented in Table 3 and illustrated in Graph 3 demonstrate

a significant increase in length of pods across all treatments. Notably, treatment T4, involving the application of microalgae with neem oil, exhibited the noticeable increase in length of pods in T4 (34.44), followed by T5 (31.44), T3 (31.22), T2 (31), and T1 (31) when compared against both the chemical treatment T6, utilizing Bavistin (35.66), and the untreated control (T0) (27.44) under field conditions.

Table1. Effect of selected treatments on plant height (cm) of cow pea at 30,45 and 60DAS

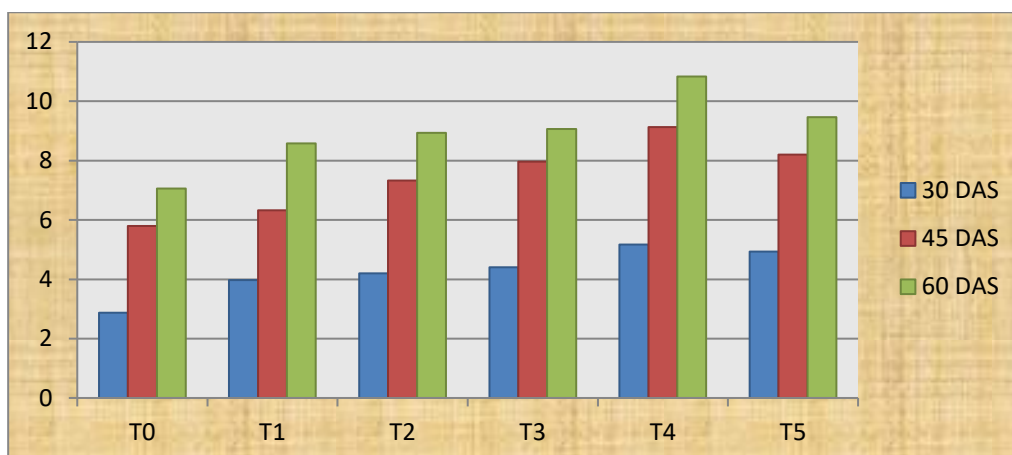
S.NO	Treatment details	30DAS	45DAS	60DAS	Mean
T0	Control	25.33	62.47	67.70	51.83
T1	Microalgae + Neem oil	29.33	67.40	73.10	56.61
T2	Microalgae + Neem oil	31.30	69.67	72.27	57.74
T3	Microalgae + Neem oil	31.67	70.50	76.67	59.61
T4	Microalgae + Neem oil	34.60	72.83	84.63	64.02
T5	Microalgae + Neem oil	32.23	70.73	78.47	60.47
T6	Bavistin	35.77	74.47	86.97	65.73
	S.E(d)±	1.09	1.39	1.93	1.47
	C.D(0.05)	2.37	3.02	4.21	3.2



Graph 1. Graphical representation of plant height

Table2. Effect of selected Treatments on Number of branches of cowpea at 30,45 and 60DAS

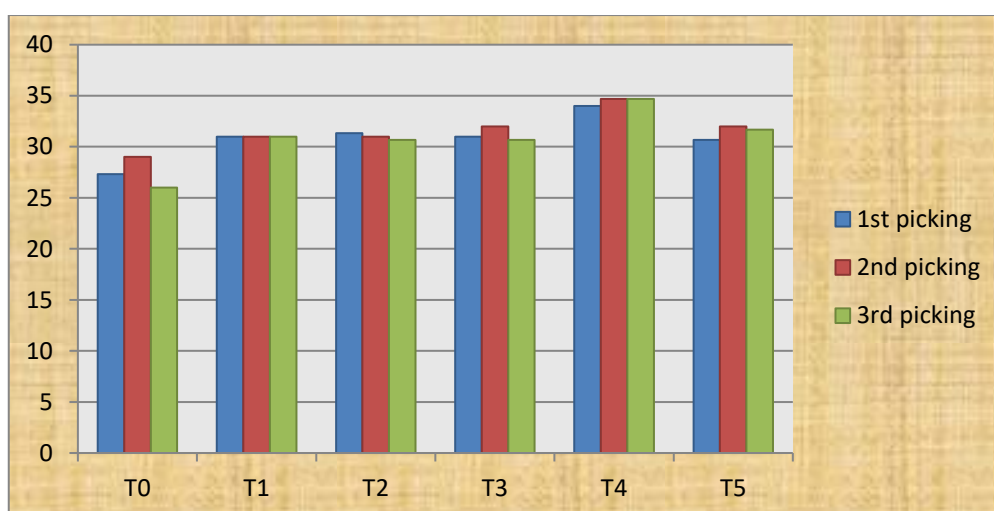
S.NO	Treatment details	30DAS	45DAS	60DAS	Mean
T0	Control	2.87	5.80	7.06	5.24
T1	Microalgae + Neem oil	3.97	6.33	8.58	6.29
T2	Microalgae + Neem oil	4.20	7.33	8.93	6.82
T3	Microalgae + Neem oil	4.40	7.96	9.06	7.14
T4	Microalgae + Neem oil	5.17	9.13	10.83	8.37
T5	Microalgae + Neem oil	4.93	8.20	9.46	7.53
T6	Bavistin	5.56	9.46	11.00	8.67
	S.E(d)±	0.27	0.56	0.47	0.43
	C.D (0.05)	0.60	1.21	0.89	0.9



Graph 2. Graphical representation of number of branches

Table3. Effect of selected Treatments on Length of pods(cm) on 3 pickings

S.NO	Treatment details	1stpicking	2ndpicking	3rdpicking	Mean
T0	Control	27.33	29.00	26.00	27.44
T1	Microalgae + Neem oil	31.00	31.00	31.00	31
T2	Microalgae + Neem oil	31.33	31.00	30.67	31
T3	Microalgae + Neem oil	31.00	32.00	30.67	31.22
T4	Microalgae + Neem oil	34.00	34.67	34.67	34.44
T5	Microalgae + Neem oil	30.67	32.00	31.67	31.44
T6	Bavistin	35.67	37.33	34.00	35.66
	S.E(d)±	1.35	1.46	1.28	1.36
	C.D (0.05)	2.94	2.95	2.786	2.89



Graph 3. Graphical representation of length of pods in three pickings

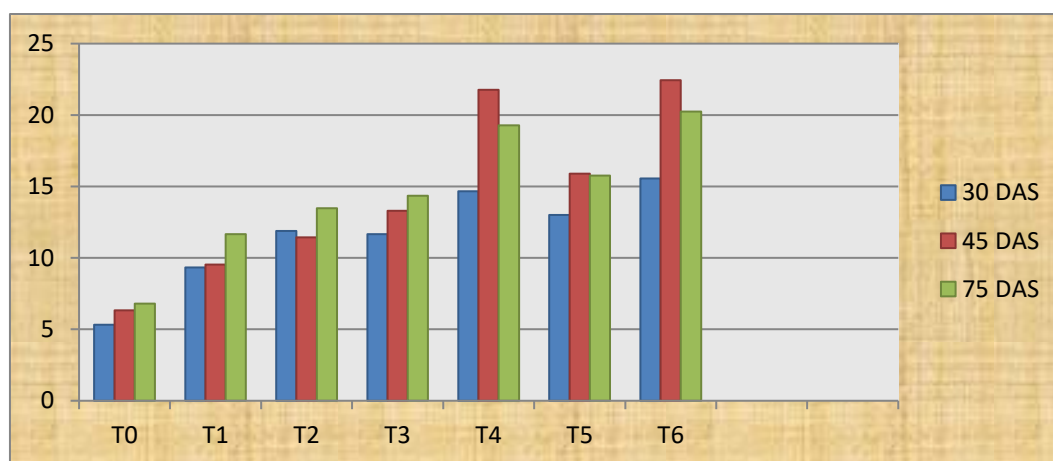
3.4 Effect of Selected Treatments on Number of Nodules of Cowpea at 30, 45, 60 DAS

At 30, 45, and 60 (DAS), the findings presented in Table 4 and illustrated in Graph 4 demonstrate a significant increase in cowpea number of nodules across all treatments. Notably, treatment

T4, involving the application of microalgae with neem oil, exhibited the noticeable increase in number of branches in T4 (19.29), followed by T5 (15.77), T3 (14.36), T2 (13.47), and T1 (11.66) when compared against the chemical treatment T6, utilizing Bavistin (20.25), and the untreated control (T0) (6.81) under field conditions.

Table4. Effect of selected treatments on number of nodules of cowpea at 30,45,60DAS

S.NO	Treatment details	30 DAS	45 DAS	75 DAS	Mean
T0	Control	5.33	6.32	8.78	6.81
T1	Microalgae + Neem oil	9.33	9.54	16.11	11.66
T2	Microalgae + Neem oil	11.89	11.43	17.11	13.47
T3	Microalgae + Neem oil	11.67	13.30	18.11	14.36
T4	Microalgae + Neem oil	14.67	21.77	21.44	19.29
T5	Microalgae + Neem oil	13.00	15.89	18.44	15.77
T6	Bavistin	15.55	22.44	22.78	20.25
	S.E(d)±	0.90	1.32	1.83	1.35
	C.D (0.05)	1.95	6.32	8.78	5.68



Graph 4. Graphical representation of Number of nodules

Table 5. Effect of selected treatments on Plant Disease Intensity

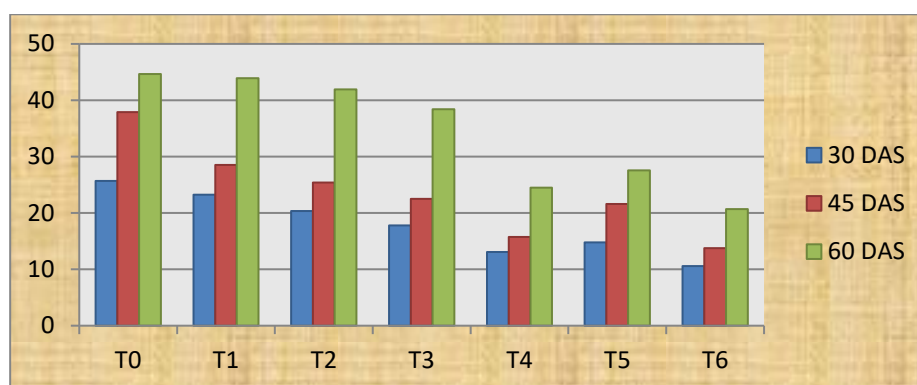
3.5 Effect of Selected Treatments on Plant Disease Intensity of *Alternaria* spp. in Cowpea Crop at 30 ,45, and 60 DAS Intervals

At 30, 45, and 60 (DAS), the findings presented in Table 4 and illustrated in Fig. 4.1 demonstrate a significant reduction in cowpea disease intensity across all treatments. Notably,

treatment T4, involving the application of microalgae with neem oil, exhibited the noticeable decrease in disease intensity (17.76), followed by T5 (21.30), T3 (26.23), T2 (31.88), and T1 (31.88) when compared against the chemical treatment T6, utilizing Bavistin (14.99), and the untreated control (T0) (36.07) under field conditions.

Table 5. Effect of selected treatments on Plant Disease Intensity

S.NO	Treatment details	30 DAS	45 DAS	60 DAS	Mean
T0	Control	25.68	37.87	44.67	36.07
T1	Microalgae + Neem oil	23.23	28.53	43.90	31.88
T2	Microalgae + Neemoil	20.33	25.40	41.93	29.22
T3	Microalgae + Neemoil	17.77	22.50	38.43	26.23
T4	Microalgae + Neemoil	13.07	15.76	24.47	17.76
T5	Microalgae +Neemoil	14.76	21.61	27.54	21.30
T6	Bavistin	10.55	13.76	20.67	14.99
	S.E(d)±	1.51	1.61	1.90	1.67
	C.D (0.05)	3.28	3.50	4.15	3.64



Graph 5. Graphical representation of diseases intensity

The above results are in agreement with the findings of Kumar et al., [15] where he studied the efficacy of micro algae as bio fertilizer of onion plants. Moreover, a field experiment was carried out by Ramjagadhesh et al. [16] to evaluate the effectiveness of plant oils, plant extracts, and antagonistic microbes against *Alternaria alternata*-caused onion leaf blight. When the disease first appeared on onion plants, two sprays of 3% neem oil were applied, and 15 days later, the second spray dramatically reduced the percent disease index (22.22%) while also increasing yield. Similarly Sree et al. (2021) conducted an experiment to observe the effect of bio resources on *Alternaria alternata* of stevia plants. Among the treatments microalgae has significantly increased the growth parameters (plant height 54cm and 32 branches) and reduced the disease incidence by (15.6%) of *Alternaria alternata* in stevia. An experiment was carried out by Dwarakadas et al. [17] to handle *Alternaria* leaf spot in cabbage in vivo. Neem oil, eucalyptus oil, clove oil, *Trichoderma viride*, neem oil + *T. viride*, eucalyptus oil + *T. viride*, and clove oil + *T. viride* were the eight treatments administered in addition to the control. Neem oil considerably decreased *Alternaria* leaf spot in cabbage as compared to other treatments, and it also had the highest cost-benefit ratio. In an experiment conducted by Sharma et al. [12], neem extract was used as a botanical treatment; bavistin (2 g/litre of water) was used as a chemical treatment, and *Trichoderma harzianum* (107cfu ml⁻¹) was used as a bio control agent. The germination rate, plant height, root and shoot weight, pre- and post emergence damping-off, and dry root and dry shoot weight of tomato seedlings were all recorded. the greatest decrease in the severity of the disease and other metrics in comparison to bio control agents. Due to the negative impact of chemical methods on

environment and soil health, non- chemical methods were used as alternative means of managing the disease viz. Microalgae and essential oil treatments (Neem oil) because of their relatively safe status and their wide acceptance by consumers. The micro algae can be used as alternative source for sustainable productivity to replace the Agro chemicals and micro algae improve the soil fertility. Essential oils appeared to be efficient and also show good results in the control of several fungi species, due to their antimicrobial activity. Microalgae produce several macromolecules that are active on plants and bring benefits to their development. Microalgae can produce plant growth hormones, polysaccharides and antimicrobial compounds and metabolites they contribute to plant growth. In this study the microalgae and essential oils like Neem oil, were used which reported significant variation in antifungal activity against *Alternaria spp.* Several researchers had reported that effective control of this disease can be done by the use of microalgae [18-19].

4. CONCLUSION

Concluded that as per the results of this study, plant height (cm), number of branches per plant, Number of nodules per plant and length of pod were significantly increased in the treatment T4 – microalgae + Neem oil and disease intensity (%) of *Alternaria* leaf spot in cowpea at 30, 45 and 60 DAS was significantly decreased in the treatment T4. For a sustainable production micro-Algae and neem oil can be used as the alternative against chemical treatment. Therefore, it can be concluded microalgae is effective against *Alternaria* leaf spot (*Alternaria spp.*) when compared with control treatment, check treatment and other concentrations of microalgae

used in this study. as such to validate the present findings more trails should be carried out in future to promote and promise for improving crop health, productivity, in sustainable agricultural practices.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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