

# Evaluating the efficacy of neem oil and microalgae against leaf spot caused by *Alternaria* spp. in cowpea (*Vigna unguiculata* L.)

## ABSTRACT

The present investigation aimed to evaluate the efficacy of neem oil and microalgae against leaf spot caused by *Alternaria* spp. in cowpea (*Vigna unguiculata* L.). The study was conducted during the Zaid season of 2022 at the Central Research Field of the Department of Plant Pathology, SHUATS, Prayagraj, Uttar Pradesh, in a Randomized Block Design. ~~With~~with three replication and seven treatments. The analysis of variance (ANOVA) technique was applied for drawing conclusion from data. Leaf spot disease caused by *Alternaria* spp. is a significant threat to cowpea crops, leading to yield losses and reduced plant vigor. An in vivo study was carried out to assess the effectiveness of ~~microalgae and neem oil~~ in different concentration of micro algae with neem oil ~~of @ 1%~~ to evaluate their effect on plant growth parameters and ~~Disease disease Intensity intensity~~ (%). Among the treatments investigated, the combination of microalgae ~~(at what concentration?)~~ and neem oil @ 1% (T4) demonstrated promising results. Notably, the T4 treatment significantly increased plant height (84.63 cm) and the number of branches (10.83) at 60(DAS). Additionally, an enhanced number of nodules per plant (21.44) was observed at 75DAS, indicating improved root health and nitrogen fixation. Moreover, the treatment resulted in a longer pod length (34.67 cm) during the second picking, indicating enhanced yield potential. Furthermore, the application of microalgae and neem oil showed a significant reduction in disease intensity, with a notable decrease of 24.47% compared to untreated plants. This suggests that the combined application of microalgae and neem oil effectively suppressed leaf spot disease caused by *Alternaria* spp. in cowpea, thereby enhancing plant health and productivity. Overall, these findings highlight the potential of using eco-friendly alternatives such as microalgae and neem oil for the management of leaf spot disease in cowpea cultivation. Further research is warranted to optimize these application methods and concentration to maximize the benefits of these biocontrol agents in sustainable agriculture practices.

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17 **Keywords:** [*Alternaria*, *Microalgae*, *Neem oil*, *Leaf spot*, *Disease Intensity*, *Zaid*, *Foliar*]

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## 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 manure 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 (Goncalves et al., 2016). 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 (Kebede and Bekeko, 2020). The major pathogenic groups associated with cowpea diseases, include: fungi, bacteria, viruses, and nematodes (Emechebe and Lagoke, 2002). Fungi are the main phytopathogen that cause economic losses in cowpea 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. (Alves et al., 2019). Among the foliar fungal diseases, leaf spot is caused 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 (Jena et al., 2019). Microalgae have meanwhile been found to produce antibiotics, a large number of microalgal extracts and other extracellular products have proven antibacterial, antifungal, antiprotozoal and antiplasmodial (Ghasemier et al., 2004). There is significant role of microalgae in plant protection and improvement for sustainable agricultural technology (Hamed et al., 2018).

Neem has been known for ages as an insecticidal plant and recently is classified as a therapeutic plant (Nagini and Subapriya Subapriya, 2005). 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 (Paule et al., 2002). 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 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. MATERIAL AND METHODS

The present experiment "Effect of microalgae 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-March 2022.

**Systematic position of *Alternaria* spp. (Ainsworth et al., 1973)**

Kingdom: Fungi  
Division: Eumycota  
Sub-division: Deuteromycotina  
Class: Hyphomycetes  
Order: Moniliales  
Family: Dematiaceae  
Genus: *Alternaria* spp.

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71 Microalgae was applied in three intervals on 5 days after

72 sowing (DAS) (@ 12, 24, 36, 48 and 60 grams) (

71 days after

7273 sowing) and 15 and 45 DAS (@ 24, 48, 72, 96 and 120 grams). At 5 DAS the microalgae with

7374 different doses for 3 three replications mixed with 5 litres of water for each plot and that

7475 microalgae slurry applied along with irrigation. Microalgae was manufactured by Phycoline

7576 Technologies Pvt Ltd and bought from Biotik TMOGL. Foliar spray of Neemoil@ 1% was

7677 done after the first appearance of Alternaria leaf spot disease symptoms followed by two

7778 sprays at 15 days of

interval. 78

### 79 Collection of disease sample:

80

81 The infected leaves exhibiting typical symptoms ~~are~~ were fetched from the standing Cowpea crop

82 and brought to the laboratory for further investigation.

### 82 Isolation of the pathogen from the leaves

83 from infected plants were identified and selected. The spotted area on the leaf surface ~~is~~ was

84 identified and cut into small pieces of about 4-6 mm. ~~These~~ these pieces ~~are~~ were washed with

85 fresh water followed by running tap water. 20 ml (approx.) of sterilized molten warm PDA

86 media was poured into sterile distilled plates aseptically. The leaf pieces ~~are~~ were further surface

87 sterilized with 0.1% mercuric chloride (HgCl<sub>2</sub>) for 30 seconds followed by washing thrice with

88 distilled water and allowed to dry. Later, these dried pieces were carefully placed on the

89 molten media (PDA) in inverted position to ensure that the spot area contacts with the

90 ~~media~~ medium. And these plates ~~are~~ were incubated at 25 ± 2 °C for 2-3 days. ~~After,~~ ~~the~~ obtained fungal

91 mycelial ~~thus~~ obtained growth was examined under the microscope and used for

92 further studies. 92

93



Figure 1 Pure culture in petridishes



Figure 2 Microscopic view of ~~alternaria~~ Alternaria



Figure 3 Pure culture in slants

94

### 95 Morphology and symptomology of the pathogen

96 The mycelium of the pathogen ~~is~~ was septate. Conidiophores ~~are~~ were simple, septate, olive-brown

97 and ~~varied~~ in length with solitary terminal conidia or chains (Fig. 4). Initially, the symptoms

98 appeared as a small, oval, dark brown necrotic sunken spot (2-18 mm dia.) found at the leaf tips

99 and central part of leaf, these later on forms as large necrotic

100 patches. 100

101 Symptoms begin as semi-circular, water-soaked lesions at the leaf margins. Lesions large

102 toward the centre of the leaf, eventually becoming necrotic. Sporulation is visible with the

103 naked eye on the leaf surface as a black velvet mass. Occasionally circular lesions are

104 observed in the centre of the leaf. Lesions begin as small brown spots, surrounded by a

105 yellow chlorotic halo. The lesions enlarge and become water-soaked and a black mass of

106 conidia is visible on the brown, necrotic tissue surfaces.

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Figure A,-  
Symptomsonleaf



FigureB



FigureC

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

122 **Diseaseincidence(%)**wascalculatedbyusingtheformulamentionedbelowNumberof  
123 infected  
plants125

126 **DiseaseIntensity(%)** =  $\frac{\text{Sumofalldiseaseratings}}{\text{Totalnumber of ratings} \times \text{Maximumdiseasegrade}} \times 100$  (Wheeler 1969)

127

128 **Diseaseintensity:-**The diseaseintensitywasassessedbyusing0to5ratingscale  
129 (Abbas2022)

130  
131 **Chart 1. Category Infection with their numerical values**

Categoryinfected(%)	Numericalvalue	Leafarea
<b>I</b>	<b>0</b>	<b>Diseasefree</b>
<b>II</b>	<b>1</b>	<b>0.1-10.0</b>
<b>III</b>	<b>2</b>	<b>10.1-25.0</b>
<b>IV</b>	<b>3</b>	<b>25.1-50.0</b>
<b>V</b>	<b>4</b>	<b>50.1-75.0</b>
<b>VI</b>	<b>5</b>	<b>&gt;75</b>

$$PDI = \frac{\sum (n \times v)}{N \times G}$$

where,

$\Sigma$ =Summation;

n=Numberof leavesin eachcategory;

v=Numericalvalueofeachcategory;

N=TotalNumberof leavesexamined;

G=Maximumnumericalvalue.

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### 3.RESULTSANDDISCUSSION

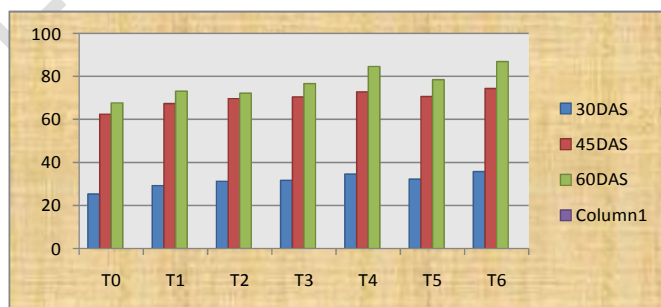
The data presented in table 1,2,3,4,5 reveals the action of Microalgae and Neem oil on disease Intensity (30, 45,60 DAS) of Alternariaspp of cowpea under field condition. The results shows that T4 (Microalgae + Neem oil@1% (24.47) treatment was effective and reduced the percentage of leaf spot caused by Alternaria spp. Andincreased the plant growth parameters(height of plant, No of branches, length of pod, and no of nodules) incowpeacrop.MinimumgrowthwasobservedinT4(Microalgae+ neemoil).

#### Effectofselectedtreatmentsonplantheight(cm)ofcowpeaat30,45and60DAS:

At 30, 45, and 60 (DAS), the findings presented in Table 1 and illustrated in graph 1 demonstrate a significantgrowthincowpeaplantheightacrossalltreatments.Notably,treatmentT4,involvingtheapplicationofmicroalgae 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 untreatedcontrol(T0)(67.70)underfieldconditions.

**Table1:-Effectofselectedtreatmentsonplantheight(cm)ofcowpeaat30,45and60DAS:**

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



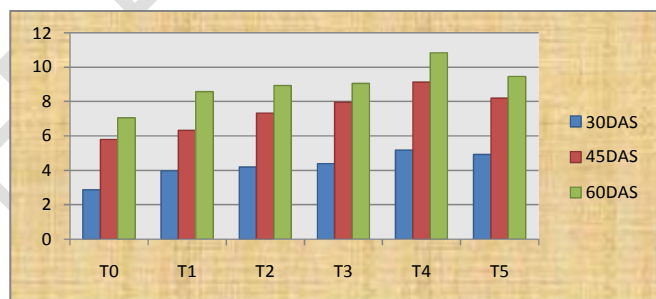
**Graph 1.Graphicalrepresentationofplantheight**

## 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 T1 (7.2.27) when compared against both the chemical treatment T6, utilizing Bavistin (86.97), and the untreated control (T0) (67.70) under field conditions

**Table 2 Effect of selected Treatments on Number of branches of cowpea at 30, 45 and 60 DAS:**

S.NO	Treatment details	30DAS	45DAS	60DAS	Mean
T0	Control	2.87	5.80	7.06	5.24
T1	Microalgae + Neemoil	3.97	6.33	8.58	6.29
T2	Microalgae + Neemoil	4.20	7.33	8.93	6.82
T3	Microalgae + Neemoil	4.40	7.96	9.06	7.14
T4	Microalgae + Neemoil	5.17	9.13	10.83	8.37
T5	Microalgae + Neemoil	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**

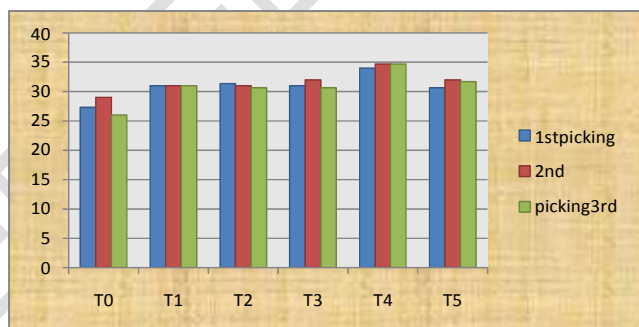
## 3 Effect of selected Treatments on Length of pods (cm) on 3 pickings:

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.

**Table 3 Effect of selected Treatments on Length of pods (cm) on 3 pickings:**

S.NO	Treatment details	1st picking	2nd picking	3rd picking	Mean
T0	Control	27.33	29.00	26.00	27.44
T1	Microalgae + Neemoil	31.00	31.00	31.00	31
T2	Microalgae + Neemoil	31.33	31.00	30.67	31
T3	Microalgae + Neemoil	31.00	32.00	30.67	31.22
T4	Microalgae + Neemoil	34.00	34.67	34.67	34.44
T5	Microalgae + Neemoil	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



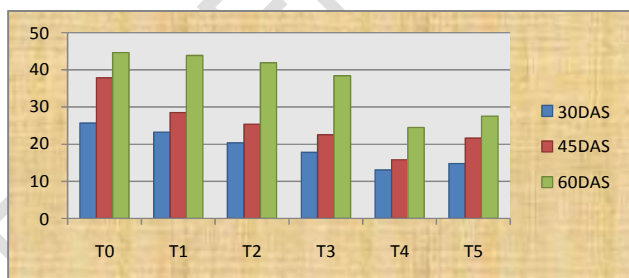
**Graph 3. of selected Treatments on Length of pods (cm) on 3 pickings:**

**4 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 graph 4 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 both the chemical treatment T6, utilizing Bavistin (14.99), and the untreated control (T0) (36.07) under field conditions.

**Table 4** Effect of selected treatments on Plant Disease Intensity

S.NO	Treatment details	30DAS	45DAS	60DAS	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 + Neem oil	20.33	25.40	41.93	29.22
T3	Microalgae + Neem oil	17.77	22.50	38.43	26.23
T4	Microalgae + Neem oil	13.07	15.76	24.47	17.76
T5	Microalgae + Neem oil	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 4.** Effect of selected treatments on Plant Disease Intensity

The above results are in agreement with the findings of Kumar et al., (2018) where he studied the efficacy of microalgae as biofertilizer of onion plants. Moreover, a field experiment was carried out by Ramjagadhesi et al. (2011) 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. (2020) 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. (2022), neem extract was used as a

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botanical treatment, bavistin (2 g/litre of water) was used as a chemical treatment, and Trichoderma harzianum (107 cfu/ml-1) was used as a biocontrol 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 biocontrol agents.

#### 4. CONCLUSION

Concluded that as per the results of this study, plant height (cm), number of branches 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 such trails should be carried out in future to promote sustainable agricultural practices.

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