Evaluating theefficacyofneemoilandmicroalgaeagainstleafspotcaused by*Alternariaspp*incowpea(VignaunguiculataL.)

ABSTRACT

The present investigation aimed to evaluate the efficacy of neem oil and microalgae againstleaf spot caused by Alternaria spp. in cowpea (Vigna unguiculata L.). The studywasconducted during the Zaid season of 2022 at the Central Research Field of the Department ofPlant Pathology, SHUATS, Prayagraj, Uttar Pradesh. In a Randomized Block Design. Withthree replication and seven treatments The analysis of variance (ANOVA) technique wasapplied for drawing conclusion from data. Leaf spot disease caused by *Alternaria spp*. is asignificant threat to cowpea crops, leading to yield losses and reduced plant vigor. An in vivostudy was carried out to assess the effectiveness of microalgae and neem oil in differentconcentration of micro algae with neem oil of 1% . to evaluate their effect on plant growthparameters and Disease Intensity (%). Among the treatments investigated, the combination ofmicroalgae and neem oil@1%.(T4) demonstrated promising results. Notably, the T4 treatmentsignificantly 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 ina 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 indisease intensity, with a notable decrease of 24.47% compared to untreated plants. Thissuggests that the combined application of microalgae and neem oil effectively suppressed leafspot disease caused by Alternaria spp. in cowpea, thereby enhancing plant health andproductivity. Overall, these findings highlight the potential of using eco-friendly alternativessuch as microalgae and neem oil for the management of leaf spot disease in cowpeacultivation. Further research is warranted to optimize application methods and concentrationstomaximizethebenefitsofthesebiocontrolagentsinsustainableagriculturepractices

Keywords:[Alternaria,Microalgae,Neemoil,Leafspot,DiseaseIntensity,Zaid,Foliar}

1.INTRODUCTION

Cowpea(*Vignaunguiculata*L.Walp.)isalsoknownasblack-eyedpeaorsouthernpeaetc. andhasmultipleuseslikefood,feed,forage,fodder,greenmanuringandvegetable. Cowpeaseedis a nutritiouscomponent inthe humandiet,andcheap livestockfeedaswell. Therearesymbioticassociationswithnitrogen-fixingbacteriabyrootnodulesand phosphorus-absorbingarbuscularmycorrhizal fungiincowpearoots**(Goncalves*etal*., 2016)**.Itsfreshleavesareusedasvegetables,thehaulms(cowpeapodwalls,stems,and leaves)areusedaslivestockfodder,providingdietarynutrientsforanimals,andas additionalincomeforthefarmers**(KebedeandBekeko,2020)**.Themajorpathogenic groupsassociatedwithcowpeadiseases,include:fungi,bacteria,viruses,andnematodes **(EmechebeandLagoke,2002).**Fungiarethemainphytopathogensthatcauseeconomic lossesincowpeacrop.Diseasescausedbyfungiwiththegreatesteconomicimpacton cowpeacroparecausedbyMacrophominaphaseolina,Fusariumspp,Rhizoctoniasolani, Curvulariaspp.,Trichodermaspp,Alternariaspp, Aspergillusspp. AndPenicilliumspp. **(Alvesetal.,2019)**.Amongthefoliarfungaldiseasesleafspotincitedby Alternariaspp. causes quantitative and qualitative losses to cow pea crop.

Microalgaehavebecomethefocusofextensiveresearchefforts,aimedatfindingnovel compoundsthatmightleadtotherapeuticallyusefulagents**(Jena*etal.,2019*).**Microalgae havemeanwhilebeenfoundtoproduceantibiotics,alargenumberofmicroalgalextracts andorextracellularproductshaveprovenantibacterial,antifungal,antiprotozoalandanti-plasmodial**(Ghasemi*et al*.,2004)**.Thereissignificantroleofmicroalgaein plantprotection and improvementfor sustainableagriculturaltechnology**(Hamedetal.,2018).**

Neemhasbeenknownforagesasaninsecticidalplantandrecentlyisclassifiedas therapeuticplant**(Naginiandsubapriya,2005).**TheNeemoilcontainsatleast100 biologicallyactivecompounds.Owingtothepresenceofantimicrobialsubstancesin differentpartsoftheplant.Thepossiblefungicidalrole,ofplantpartsandextracts,hasbeen studied against soilbornepathogen**(Paule*tal*.,2002).**Given theimportanceofcowpea asa foodandfoddercropandthechallengesposedbyfungaldiseases,thereisaneedfor researchaimedatevaluatingtheefficacyofalternativeapproachessuchasmicroalgaeand neemoilinmitigating diseaseimpactwhilepromotingsustainableagricultural practices.This studyaimstoinvestigatethepotentialofmicroalgaeandneemoilinmanagingleafspot diseasecausedbyAlternariaspp.incowpeacultivation,therebycontributingtothe development of eco-friendly and effective disease management strategies in agriculture.

2.MATERIAL ANDMETHODS

Thepresentexperiment“Effectofmicroalgaeandneemoilagainstleafspotcausedby Alternariaspp.ofCowpea(VignaunguiculataL.)”wasconductedatCentralResearchField (CRF)ofSamHigginbottomUniversityofAgriculture,TechnologyandSciences(SHUATS), Prayagraj during the Zaid season of march 2022.

SystematicpositionofAlternariaspp.(Ainsworthetal.1973)

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

Microalgaewasapplied inthreeintervalson5DAS(12,24,36,48and60grams) (daysafter sowing)and15and45 DAS (24,48,72,96and120grams).At5 DAS themicroalgaewith differentdosesfor 3 threereplications mixedwith5litresofwaterforeachplotandthat microalgaeslurryappliedalong with irrigation.Microalgaewas manufacturedbyPhycoline TechnologiesPvtLtd andboughtfromBiotikTMOGL.Foliar sprayofNeemoil@ 1% was done after the firstappearanceofAlternaria leafspotdiseasesymptomsfollowedbytwo sprays at 15 days of interval.

Collectionofdiseasesample:

The infectedleavesexhibitingtypicalsymptomsare fetched fromthe standingCowpea crop andbroughttothelaboratoryforfurtherinvestigation.Isolationofthepathogentheleaves frominfectedplantswereidentifiedandselected.Thespottedareaontheleafsurfaceis identifiedandcutintosmallpiecesofabout4-6mm,thesepiecesarewashedwith freshwaterfollowedbyrunningtapwater.20ml(approx.)ofsterilizedmoltenwarmPDA mediawaspouredintosteriledistilledplatesaseptically.Theleafpiecesarefurthersurface sterilizedwith0.1%mercuricchloride($HgCl_2$)for30secondsfollowedbywashingthricewith distilledwaterandallowedtodry.Later,thesedriedpieceswerecarefullyplacedonthe moltenmedia(PDA)ininvestedpositiontoensurethatthespotareacontactswiththe media.Andtheseplatesareincubatedat25±2°Cfor2-3days.After,theobtainedfungal mycelial growth was examined under the microscope and used for further studies.

Figure 1 Pure culture in petridishes

Figure2Microscopicviewof alternaria

Figure 3 Pure culture inslants

Morphologyand symptomologyofthepathogen

Themyceliumofthepathogenisseptate.Conidiophoresaresimple,septate,olive-brown andvaryinlengthwithsolitaryterminalconidiaorchains(Fig.4).Initially,thesymptoms appearasasmallovaldarkbrownnecroticsunkenspot(2-18mmdia.)foundattheleaftips and central part of leaf, these later on forms as large necrotic patches.

Symptomsbeginassemi-circular,water-soakedlesionsattheleafmargins.Lesionsenlarge towardsthecentreoftheleaf,eventuallybecomingnecrotic.Sporulationisvisiblewiththe nakedeyeontheleafsurfaceasablackvelvetmass.Occasionallycirularlesionsare observedinthecentreoftheleaf.Lesionsbeginassmallbrownspots,surroundedbya yellowchlorotichalo.Thelesionsenlargeandbecomewater-soakedandablackmassof conidia is visible onthe brown,necrotictissuesurfaces.

FigureA,- Symptomsonleaf

FigureB

FigureC

- Fig 4(A-C):Symptoms of Alternaria leaf spot disease
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Diseaseincidence(%)wascalculatedbyusingtheformulamentionedbelowNumberof infected plants

DiseaseIntensity(%) =Sumofalldiseaseratings / Totalnumber of ratings× Maximumdiseasegrade ×100(**Wheeler 1969)**

Diseaseintensity:-Thediseaseintensitywasassessedbyusing0to5ratingscale (**Abbas2022)**

Chart 1. Category Infection with their numerical values

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

PDI=∑ $(n \times v)$÷N×G

where,

Σ=Summation;

n=Numberof leavesin eachcategory;

v=Numericalvalueofeachcategory;

N=TotalNumberof leavesexamined;

G=Maximumnumericalvalue.

3.RESULTSANDDISCUSSION

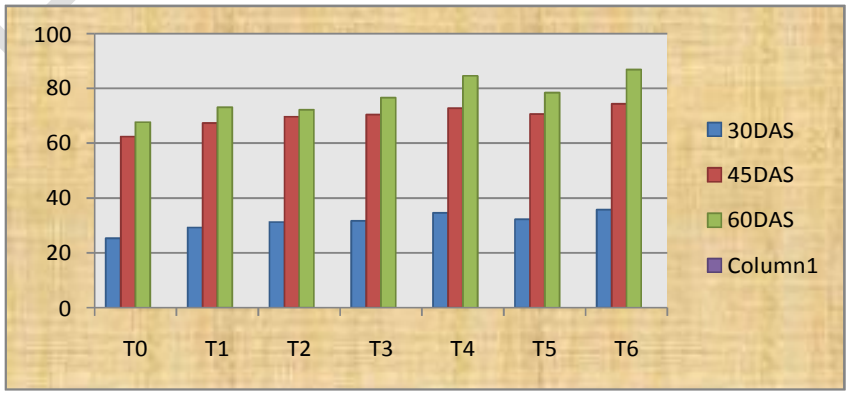
The data presented in table 1,2,3,4,5 reveals the action of Microalgae and Neem oil on disease Intensity (30, 45,60 DAS) of Alternaria spp of cowpea under field condition. The results shows that T4 (Microalgae + Neem oil@1% (24.47) treatment was effective and reduced the percentage of leaf spot caused 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,involvingtheapplicationofmicroalgaewith 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
	S.E(d)±	**1.09**	**1.39**	**1.93**	**1.47**
	C.D(0.05)	**2.37**	**3.02**	**4.21**	**3.2**



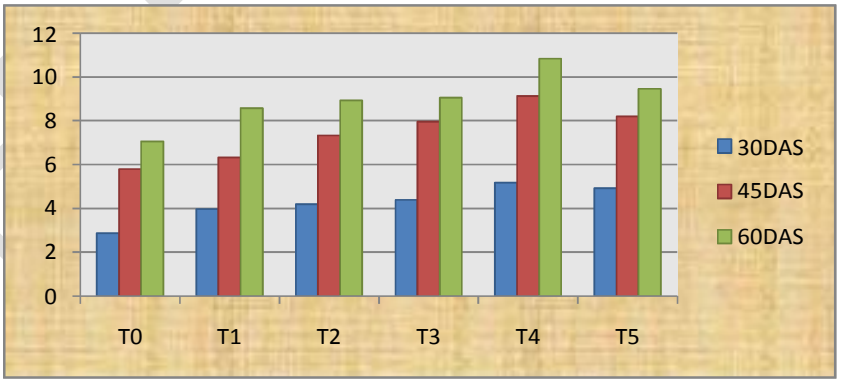
Graph 1.Graphicalrepresentationofplantheight

2 EffectofselectedTreatmentsonNumberofbranchesofcowpeaat30,45and60DAS:

At 30, 45, and 60 (DAS), the findings presented in Table 2 and illustrated in graph 2 demonstrate a significantincrease in cowpea plant branches across all treatments. Notably, treatment T4, involving the application ofmicroalgae with neem oil, exhibited the noticeableincrease in number of branchesin 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, utilizingBavistin(86.97),and theuntreatedcontrol(T0)(67.70)underfieldconditions

Table2EffectofselectedTreatmentsonNumberofbranchesofcowpeaat30,45and60DAS:

S.NO	Treatmentd etails	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.GraphicalrepresentationofNumberofbranches

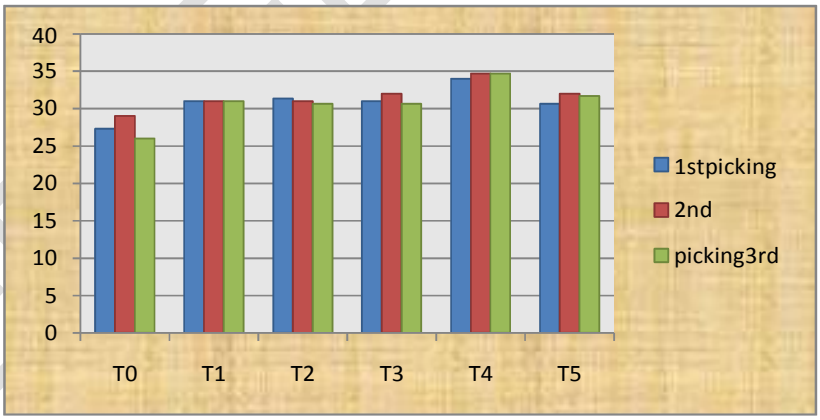
3 EffectofselectedTreatmentsonLengthofpods(cm)on3pickings:

At 30, 45, and 60 (DAS), the findings presented in Table 3 and illustrated in graph 3 demonstrate a significantincrease in length of pods across all treatments. Notably, treatment T4, involving the application of microalgaewithneemoil,exhibitedthenoticeableincreaseinlengthofpodsinT4(34.44),followedbyT5(31.44),T3

(31.22), T2 (31), and T1 (31) when compared against both the chemical treatment T6, utilizing Bavistin (35.66),andtheuntreatedcontrol(T0)(27.44)underfieldconditions.

Table3EffectofselectedTreatmentsonLengthofpods(cm)on3pickings:

S.NO	Treatmentdetails	1stpicking	2ndpicking	3rdpicking	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



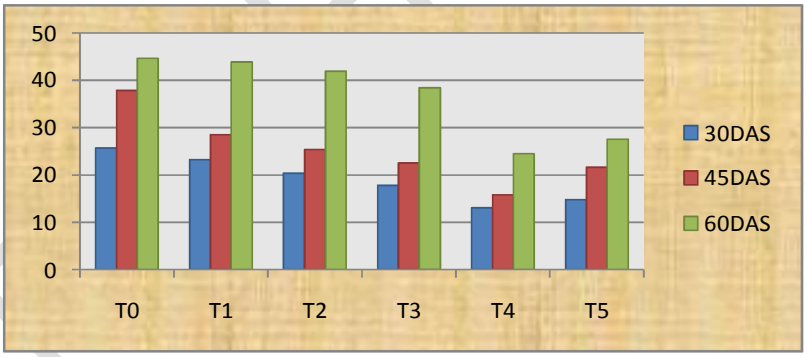
Graph 3.ofselectedTreatmentsonLengthofpods(cm)on3pickings:

4 Effect of selected treatments on Plant Disease Intensity of *Alternaria* spp. in cowpea crop at 30 ,45, and60 DASintervals.

At 30, 45, and 60 (DAS), the findings presented in Table 4 and illustrated in graph 4 demonstrate a significantreduction in cowpea disease intensity across all treatments. Notably, treatment T4, involving the application ofmicroalgae with neem oil, exhibited the noticeabledecrease 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), andtheuntreatedcontrol(T0)(36.07)underfieldconditions.

Table4EffectofselectedtreatmentsonPlantDiseaseIntensity

S.NO	Treatmentdetails	30DAS	45DAS	60DAS	Mean
T0	Control	25.68	37.87	44.67	36.07
T1	Microalgae + Neemoil	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 4.EffectofselectedtreatmentsonPlantDiseaseIntensity

The above results are in agreement with the findingsof Kumar et al., (2018) where he studied the efficacy ofmicro algae as biofertilizer of onion plants. Moreover, a field experiment was carried out by **Ramjagadhesh et al.(2011)** to evaluate the effectiveness of plant oils, plant extracts, and antagonistic microbes against Alternariaalternata-caused onion leaf blight. When the disease first appeared on onion plants, two sprays of 3% neem oilwere applied, and 15 days later, the second spray dramatically reduced the percent disease index (22.22%) whilealso increasing yield. Similarly**Sree *et al.* (2021)** conducted an experiment to observe the effect of bio resourceson *Alternaria alternata*of stevia plants. Among the treatments microalgae has significantly increased the growthparameters (plant height 54cm and 32 branches) and reduced the disease incidence by (15.6%) of Alternariaalternata in stevia. An experiment was carried out by **Dwarakadas et al. (2020)** to handle Alternaria leaf spot incabbage 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 oilconsiderably decreased Alternaria leaf spot in cabbage as compared to other treatments, and it also had thehighestcost-benefitratio.Inanexperimentconductedby**Sharmaetal.(2022)**,neemextractwasusedasa

botanical treatment, bavistin (2 g/litre of water) was used as a chemical treatment, and Trichoderma harzianium(107cfuml-1)wasusedasabiocontrolagent.Thegerminationrate,plantheight,rootandshoot weight,pre-andpostemergence damping-off, and dry root and dry shoot weight of tomato seedlingswere all recorded. thegreatestdecreaseinthe severityofthediseaseandothermetricsincomparisonto biocontrolagents.

4. CONCLUSION

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

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