Assessingtheeffectivenessofneemoilandmicroalgaeinthe managementofcowpea(*Vignaunguiculata*L.)leafspotcausedby *Alternariaspp.*

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ABSTRACT

The current study aimed to assess the effectiveness of neem oil and microalgae in controlling leaf spot caused *by Alternariaspp*. in cowpea (*Vignaunguiculata*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 Designwiththreereplications andseventreatments.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 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 ofbranches(10.83) at60DAS. Moreover, an increased numberof 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 of eco-friendly alternatives like microalgae and neem oil for managing leaf spot disease in cowpeacultivation. Further research in this area is recommended for sustainable agriculturalpractices.

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

1.INTRODUCTION

Cowpea(*Vignaunguiculata*L.Walp.)isalsoknownasblack-eyedpeaorsouthernpeaetc. andhasmultipleuseslikefood,feed,forage,fodder,greenmanuringandvegetable. Cowpeaseedisanutritiouscomponentinthehumandiet,andcheaplivestock feedas well. Therearesymbioticassociationswithnitrogen-fixingbacteriabyrootnodulesand phosphorus-absorbingarbuscular*mycorrhizal*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 cowpeacroparecausedby*Macrophominaphaseolina*,*Fusariumspp*,*Rhizoctoniasolani*, *Curvulariaspp*.,*Trichodermaspp*,*Alternariaspp*,*Aspergillusspp*.and*Penicilliumspp*. **(Alvesetal.,2019)**.Amongthefoliarfungaldiseasesleafspotincitedby*Alternariaspp*. causesquantitativeandqualitativelossestocowpeacrop.

Microalgaehavebecomethefocusofextensiveresearchefforts,aimedatfindingnovel compoundsthatmightleadtotherapeuticallyusefulagents**(Jena*etal.,2019*).**Microalgae havemeanwhilebeenfoundtoproduceantibiotics,alargenumberofmicroalgalextracts andorextracellularproductshaveprovenantibacterial,antifungal,antiprotozoalandanti-plasmodial**(Ghasemi*etal*.,2004)**.Thereissignificantroleofmicroalgae inplantprotection andimprovementforsustainableagriculturaltechnology**(Hamedetal.,2018).**

Neemhasbeenknownforagesasaninsecticidalplantandrecentlyisclassifiedas therapeuticplant**(NaginiandSubapriya,2005).**TheNeemoilcontainsatleast100 biologicallyactivecompounds.Owingtothepresenceofantimicrobialsubstancesin different partsoftheplant.The possiblefungicidal role,ofplantpartsandextracts,hasbeen studiedagainstsoilbornepathogen**(Paul*etal*.,2002).**Giventheimportanceofcowpeaas afoodandfoddercropandthechallengesposedbyfungaldiseases,thereisaneedfor researchaimedatevaluatingtheefficacyofalternativeapproachessuchasmicroalgaeand neemoilinmitigatingdiseaseimpactwhilepromoting sustainableagriculturalpractices.This studyaimstoinvestigatethepotentialofmicroalgaeandneemoilinmanagingleafspot diseasecausedby*Alternariaspp*.incowpeacultivation,therebycontributingtothe developmentofeco-friendlyandeffectivediseasemanagementstrategiesinagriculture.

2.MATERIALAND METHODS

Thepresentexperiment"Effectofmicroalgaeandneemoilagainstleafspotcausedby *Alternariaspp*.ofCowpea(*Vignaunguiculata*L.)"wasconductedatCentralResearchField (CRF)ofSamHigginbottomUniversityofAgriculture,TechnologyandSciences(SHUATS), Prayagrajduringthe*Zaid*seasonofMarch2022.

2.1Systematic positionof*Alternariaspp*.(Ainsworthetal.1973)

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

Microalgaewasappliedinthreeintervalson5Daysaftersowing(DAS)@12,24,36,48and 60gramsand15and45DAS@24,48,72,96and120grams.**Madhavi*etal*,.(2021).**The applicationofmicroalgaeAt5,15,and30DASdifferentdosesfor3threereplications mixedwith5litersofwaterforeachplotandthatmicroalgaeslurryappliedalongwith irrigation.FoliarsprayofNeemoil@1%**Sarathambal*etal*.(2009)**followedbythree spraysat15daysofintervalcrop15daysinterval@30,45,60DASandBavistin (Carbendazim)of0.2%**Sharma*etal*.(2022)**wassparyedin15daysinterval@30,45,60 DAS.MicroalgaewasmanufacturedbyPhycolineTechnologiesPvtLtdandboughtfrom BiotikTMOGL.

2.2Collectionofdiseasesample:

Theinfectedleavesexhibitingtypicalsymptomswerefetchedfromthestandingcowpea cropandbroughttothelaboratoryforfurtherinvestigation.Thespottedareaontheleaf surface wasidentifiedandcut into small piecesofabout4-6mm.Thesepieceswerewashed withfreshwaterfollowedbyrunningtap water.20ml(approx.)of sterilizedmoltenwarmPDA mediawaspouredintosteriledistilledplatesaseptically.Theleafpieceswerefurthersurface sterilizedwith0.1%mercuricchloride($HgCl_2$) for30secondsfollowedbywashingthricewith distilledwaterandallowedtodry.Later,thesedriedpieceswerecarefullyplacedonthe moltenmedia(PDA)ininvestedpositiontoensurethatthespotareacontactswiththe media.Andtheseplateswereincubatedat25±2°Cfor2-3days.After,thefungalmycelial wasexaminedunderthemicroscopeandusedforfurtherstudies.

Figure1Purecultureinpetri dishes

Figure2Microscopicview of alternaria

Figure3Pureculturein slants

2.3Morphologyandsymptomologyofthepathogen

Themyceliumofthepathogenwasseptate.Conidiophoresweresimple,septate,olive-brownandvariedinlengthwithsolitaryterminalconidiaorchains(Fig.2).Initially,the symptomsappearedasasmallovaldarkbrownnecroticsunkenspot(2-18mmdia.)found attheleaf tipsandcentral partof leaf,theselater onforms as largenecroticpatches. Symptomsbeginassemi-circular,water-soakedlesions attheleafmargins. Lesionsenlarge towardsthecentreoftheleaf,eventuallybecomingnecrotic.Sporulationisvisiblewiththe nakedeyeontheleafsurfaceasblackvelvetmass.Occasionallycircularlesionsare observedinthecentreoftheleaf.Lesionsbeginassmallbrownspots,surroundedbya yellowchlorotichalo.Thelesionsenlargeandbecomewater-soakedandablackmassof conidiaisvisibleonthebrown,necrotictissuesurfaces.

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

FigureA,-Symptomson leaf

FigureB

FigureC

Diseaseincidence(%)wascalculatedbyusingtheformulamentionedbelowNumberof infectedplants

DiseaseIntensity(%)=Sumofalldisease ratings ×100(**Wheeler1969)**

Totalnumberofratings×Maximumdisease grade

Diseaseintensity:-Thediseaseintensitywasassessedbyusing0to5ratingscale (**Abbas2022)**

Chart 1. Category Infection with their numerical values

Category infected(%)	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

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

Where,

Σ=Summation;

n=Numberofleaves ineachcategory;

v=Numericalvalueofeach category;

N=TotalNumberofleaves examined;

G=Maximumnumericalvalue.

3.RESULTSANDDISCUSSION

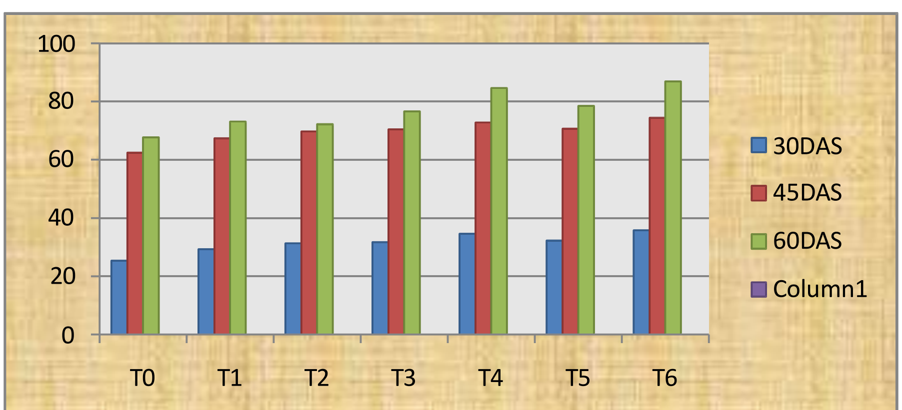
ThedatapresentedintabIe1,2,3,4,5revealstheactionofMicroalgaeandNeemoilondiseaseIntensity(30,45,60 DAS)of*Alternariaspp*ofcowpeaunderfieldcondition.TheresultsshowsthatT4(Microalgae48gms@5DAS 96gms@15,45DAS+Neemoil@1%(24.47)treatmentwaseffectiveandreducedthepercentageofleafspotcausedby *Alternariaspp*.Andincreasedtheplantgrowthparameters(heightofplant,Noofbranches,lengthofpod,andnoof nodules)incowpeacrop.MinimumgrowthwasobservedinT4(Microalgae48gms@5DAS96gms@15,45DAS+neem oil@1%).

1Effectofselectedtreatmentsonplantheight(cm)ofcowpeaat30,45and60DAS:

At30,45, and60(DAS),thefindingspresentedin Table1andillustratedinGraph 1demonstratea significantgrowthin cowpeaplantheightacrossalltreatments.Notably,treatmentT4,involvingtheapplicationofmicroalgaewithneemoil, exhibitedthenoticeableheightinT4(84.63),followedbyT5(78.47),T3(76.67),T1(73.10),andT2(72.27)when comparedagainstboththechemicaltreatmentT6,utilizingBavistin(86.97),andtheuntreatedcontrol(T0)(67.70)under fieldconditions.

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**



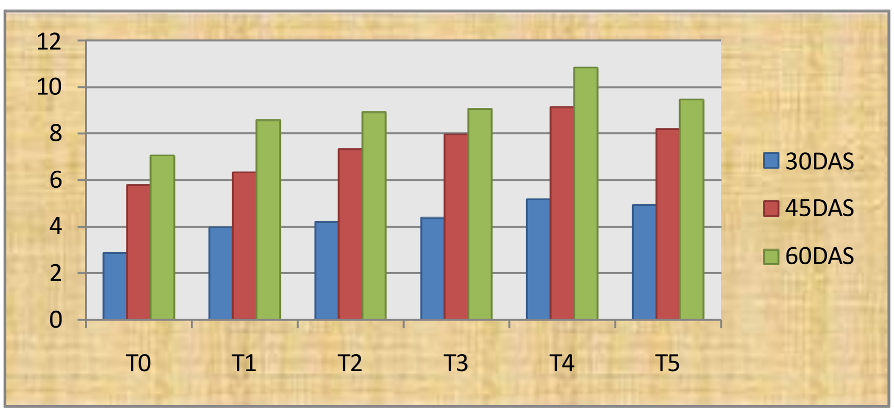
Graph 1. Graphicalrepresentationofplantheight

2EffectofselectedTreatmentsonNumberofbranchesofcowpeaat30,45and60 DAS:

At30,45,and60(DAS),thefindingspresentedinTable2andillustratedinGraph 2demonstrateasignificantincrease incowpeaplantbranchesacrossalltreatments.Notably,treatmentT4,involvingtheapplicationofmicroalgaewithneem oil,exhibitedthenoticeableincreaseinnumberofbranchesinT4(10.83),followedbyT5(9.46),T3(9.06),T2(8.93), andT2(72.27)whencomparedagainstboththechemicaltreatmentT6,utilizingBavistin(86.97),andtheuntreated control(T0)(67.70)underfieldconditions

Table2:-EffectofselectedTreatmentsonNumberofbranchesofcowpeaat30,45and60DAS:

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



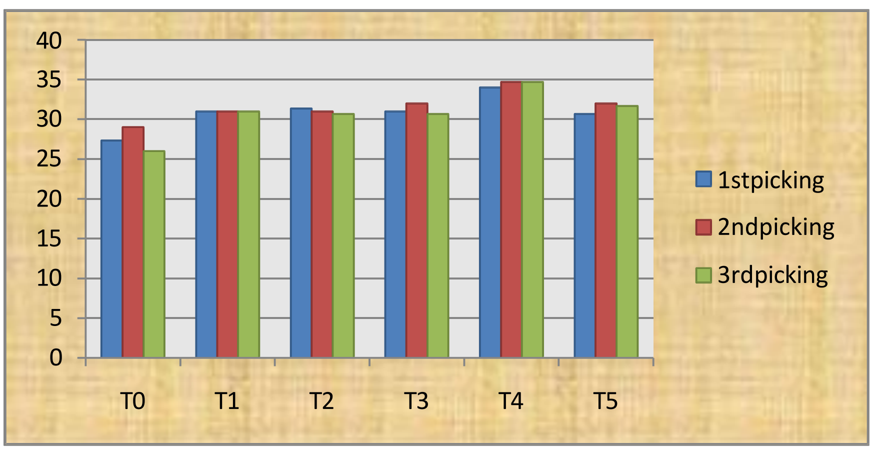
Graph 2GraphicalrepresentationofNumberofbranches

3EffectofselectedTreatmentsonLengthofpods(cm)on3pickings:

At30,45,and60(DAS),thefindingspresentedinTable3andillustratedinGraph 3demonstrateasignificantincrease inlengthofpodsacrossalltreatments.Notably,treatmentT4,involvingtheapplicationofmicroalgaewithneemoil, exhibitedthenoticeableincreaseinlengthofpodsinT4(34.44),followedbyT5(31.44),T3(31.22),T2(31),andT1(31) whencomparedagainstboththechemicaltreatmentT6,utilizingBavistin(35.66),andtheuntreatedcontrol(T0)(27.44) underfieldconditions.

Table3:-EffectofselectedTreatmentsonLengthofpods(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



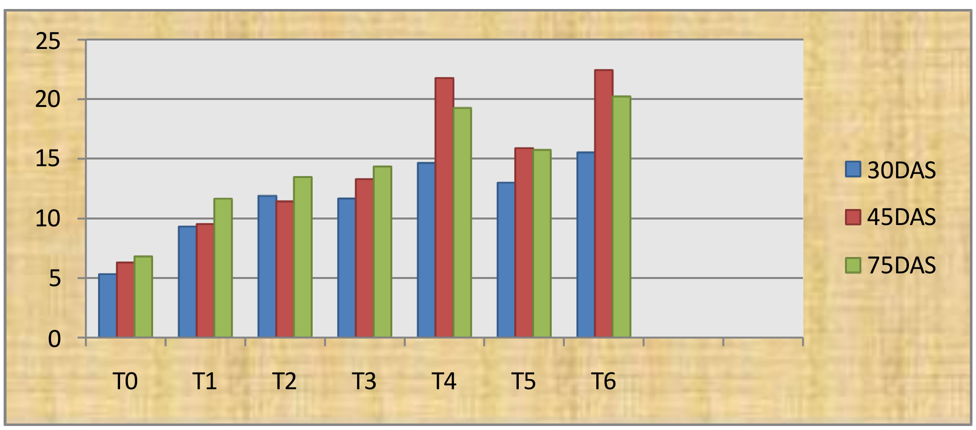
Graph 3GraphicalrepresentationofLengthofpods(cm)on3pickings:

4EffectofselectedTreatmentsonNumberofnodulesofcowpeaat30,45,60DAS

At30,45,and60(DAS),thefindingspresentedinTable4andillustratedinGraph4demonstrateasignificantincrease incowpeanumberofnodulesacrossalltreatments.Notably,treatmentT4,involvingtheapplicationofmicroalgaewith neemoil,exhibitedthenoticeableincreaseinnumberofbranchesinT4(19.29),followedbyT5(15.77),T3(14.36),T2 (13.47),andT1(11.66)whencomparedagainstboththechemicaltreatmentT6,utilizingBavistin(20.25),andthe untreatedcontrol(T0)(6.81)underfieldconditions

Table4:-EffectofselectedTreatmentsonNumberofnodulesofcowpeaat30,45,60DAS

S.NO	Treatmentdetails	30 DAS	45 DAS	75 DAS	Mean
T0	Control	5.33	6.32	8.78	6.81
T1	Microalgae + Neemoil	9.33	9.54	16.11	11.66
T2	Microalgae + Neemoil	11.89	11.43	17.11	13.47
T3	Microalgae + Neemoil	11.67	13.30	18.11	14.36
T4	Microalgae + Neemoil	14.67	21.77	21.44	19.29
T5	Microalgae + Neemoil	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



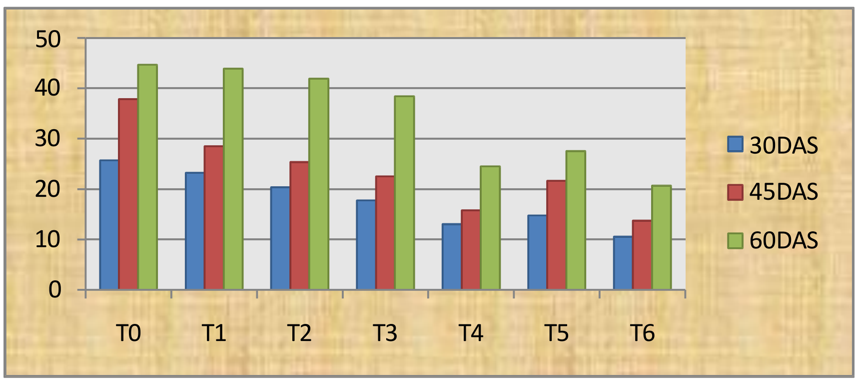
Graph 4GraphicalrepresentationofNumberofnodules

5EffectofselectedtreatmentsonPlantDisease Intensityof*Alternaria*spp.incowpea cropat30 ,45,and 60 DAS intervals.

At30,45, and60(DAS),thefindingspresentedinTable4andillustratedinGraph 4 demonstrateasignificantreduction incowpeadiseaseintensityacrossalltreatments.Notably,treatmentT4,involvingtheapplicationofmicroalgaewith neemoil,exhibitedthenoticeabledecreaseindiseaseintensity(17.76),followedbyT5(21.30),T3(26.23),T2(31.88), andT1(31.88)whencomparedagainstboththechemicaltreatmentT6,utilizingBavistin(14.99),andtheuntreated control(T0)(36.07)underfieldconditions.

Table5:-EffectofselectedtreatmentsonPlantDiseaseIntensity

S.NO	Treatmentdetails	30 DAS	45 DAS	60 DAS	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 5GraphicalrepresentationofselectedtreatmentsonPlantDiseaseIntensity

Theaboveresultsareinagreementwiththefindingsof**Kumar et al.,(2020)**wherehestudiedtheefficacyofmicroalgae asbiofertilizerofonionplants.Moreover,afieldexperimentwascarriedoutby**Ramjagadheshetal.(2011)**toevaluate theeffectivenessofplantoils,plantextracts,andantagonisticmicrobesagainst*Alternariaalternata*-causedonionleaf blight.Whenthediseasefirstappearedononionplants,twospraysof3%neemoilwereapplied,and15dayslater,the secondspraydramaticallyreducedthepercentdiseaseindex(22.22%)whilealsoincreasingyield.Similarly**Sree*etal.* (2021)**conducted anexperimenttoobservetheeffectofbioresourceson*Alternariaalternata*ofsteviaplants.Amongthe treatmentsmicroalgaehassignificantlyincreasedthegrowthparameters(plantheight54cmand32branches)and reducedthediseaseincidenceby(15.6%)of*Alternariaalternata*instevia.Anexperimentwascarriedoutby**Dwarakadas etal.(2020)**tohandleAlternarialeafspotincabbageinvivo.Neemoil,eucalyptus oil,cloveoil,*Trichodermaviride*,neem oil+*T.viride*,eucalyptusoil+*T.viride*,andcloveoil+*T.viride*weretheeighttreatmentsadministeredinadditiontothe control.NeemoilconsiderablydecreasedAlternarialeafspotincabbageascomparedtoothertreatments,anditalsohad thehighestcost-benefitratio.Inanexperimentconductedby**Sharmaetal.(2022)**,neemextract wasusedasabotanical treatment,bavistin(2g/litreofwater)wasusedasachemicaltreatment,and*Trichodermaharzianium*(107cfuml-1)was usedasabiocontrolagent.Thegerminationrate,plantheight,rootandshootweight,pre-andpostemergence damping-off,anddryrootanddryshootweightoftomatoseedlingswereallrecorded.thegreatestdecreaseintheseverityofthe diseaseandothermetricsincomparisontobiocontrolagents.Duetothenegativeimpactofchemicalmethodson environmentandsoilhealth,non-chemicalmethodswereusedasalternativemeansofmanagingthediseaseviz. Microalgaeandessentialoiltreatments(Neemoil)becauseoftheirrelativelysafestatusandtheirwideacceptanceby consumers. ThemicroalgaecanbeusedasalternativesourceforsustainableproductivitytoreplacetheAgrochemicals andmicroalgaeimprovethesoilfertility.Essentialoilsappearedtobeefficientandalsoshowgoodresultsinthecontrol ofseveralfungispecies,duetotheirantimicrobial activity.Microalgaeproduceseveralmacromoleculesthatareactiveon plantsandbringbenefitstotheirdevelopment.Microalgaecanproduceplantgrowthhormones,polysaccharidesand antimicrobialcompoundsandmetabolitestheycontributetoplantgrowth.Inthisstudythemicroalgaeandessentialoils likeNeemoil,wereusedwhichreportedsignificantvariationinantifungalactivityagainst*Alternariaspp.*Several researchershadreportedthateffective controlof this diseasecanbedone bythe useof microalgae.

4.CONCLUSION

Concludedthataspertheresultsofthisstudy,plantheight(cm),numberofbranchesperplant,Numberofnodulesper plantandlength ofpodwere significantlyincreasedin thetreatmentT4 –microalgae+Neemoilanddiseaseintensity (%) ofAlternarialeafspotincowpeaat30,45and60DASwassignificantlydecreasedinthetreatmentT4.Forasustainable productionmicro-Algaeandneemoilcanbeusedasthealternativeagainstchemicaltreatment.Therefore,itcanbe concludedmicroalgaeiseffectiveagainstAlternarialeafspot(*Alternaria*spp.)whencomparedwithcontroltreatment, checktreatmentandotherconcentrationsofmicroalgaeusedinthisstudy.assuchtovalidatethepresentfindingsmore trailsshouldbecarriedoutinfuturetopromotesustainableagriculturalpractices.

COMPETINGINTERESTS

Authorshavedeclaredthat nocompeting interests exist.

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