Antifungal Efficacy of Zinc Oxide Nanoparticles Against Anthracnose andSouthernBlightdiseaseingreen gram

Abstract

Nanotechnology has rapidly advanced in the 21st century, with significant developmentsin the synthesis and application of nanoparticles. ZnNps (Zinc nanoparticles) were synthesizedusing extracts from mehandi (*Lawsonia inermis*), neem (*Azadirachta indica*), marigold (*Tagetuserecta*)andginger(*Zingiberofficinalis*).ZnNpsweresynthesizedafteranalyzingthecompatability of theplant extracts with the test pathogens.

This study investigates the antifungal activity of green synthesized zinc nanoparticles (ZnNps) inthelaboratoryagainst*Colletotrichumlindemuthianum*,thecausativeagentofanthracnoseinmungbeans and *Sclerotium rolfsii*causing southern blight in mung bean using the poisoned foodtechnique. Various concentrations (100ppm, 250ppm and 500ppm) of zinc nanoparticles derivedfrom neem,mehandi, marigold and ginger were evaluated alongside corresponding plant extractsand Zinc nitrate hexahydrate at 250ppm. Results indicated significant fungistatic activity, withMehandi ZnNps at 500ppm exhibiting the highest efficacy, demonstrating minimal mycelialgrowth (28.00mm)andagrowthinhibitionrateof68.88%followedbyMarigoldandNeemZnNpsagainst*Colletotrichum*.Asfor*Sclerotium*,NeemZnNpsat500ppmexhibitingthehighestefficacy,demonstrating minimal mycelial growth (15.00 mm) and a growth inhibition rate of 83.33%followed by ginger and Mehandi. All tested ZnNps concentrations outperformed their respectiveplant extracts and Zinc nitrate hexahydrate, confirming the potential of green synthesized ZnNpsaseffectiveagents.Thisstudyprovidesaneco-friendlyalternativetochemicalfungicides,addressing the increasing need for sustainable agricultural practices to combat fungal diseases inmung beans.These findings underscore the potential of green synthesized ZnNps as effectiveecofriendlyfungicides.

Keywords: Anthracnose,*Colletotrichumlindemuthianum*,Zincnanoparticles,*Invitro*,Southernblight,*Sclerotium rolfsii*

Introduction

GreengramorMungbean(*Vignaradiata*)belongstothefamilyLeguminaceae.Numerousfungaldiseases namely anthracnose, southern blight, powdery mildew, cercospora leaf spot

etcfrequentlyhindertheabilityoftheaffectedplantstogerminate,killthemor

significantlylowertheircapacityforproduction.Anthracnosecausedby*Colletotrichumlindemuthianum* andSouthernblightcausedby*Sclerotiumrolfsii*causessignificantproblemingreengram.Inanthracnose,th esymptomsmanifestedasspherical,black,shrunkenlesionsonpods,seeds,stems and leaves, that contained flesh-coloured spores and leaves wilt. Padder *et al*. (2017).[10]*Colletotrichum* causes early defoliation, early flower and pod fall, degradation of the seeds and insevere cases, plant death. (Campa *et al*. 2014) [2]. As in southern blight caused by *Sclerotium rolfsii*the fungal hyphae developed upward on the surface of stems and covered the lesion with a cottony,white mass of mycelium. The infected stems' exterior and interior white mycelium spread to thesurrounding soilsurface. Numerous uniformlysized, roundish,white immature sclerotia wereformedbythefungus,whichmaturedtoadarkbrownorblackcolour.Thefungusencircledthestem,exp andedinto the cortexand finally killedtheplant Kwon*et al.* (2002).[6]

Nanoparticlesareextremelysmallparticles,whosesizesareexpressedinnanometers(1nm=10^{-9}meter) . Various nanoparticles can be used against seedborne pathogens and soil borne pathogensongreengram.Zincnanoparticleswerealsousedagainst *Colletotrichum sp,* which causesanthracnose diseases inFabaceae family (Mosquera *et al.* 2020)[8].Zincnanoparticleswereeffectivelyusedagainst*Sclerotiumrolfsii*,whichcausessouthernbligh tonmungbeancropKamel(2024) [5]. This study was conducted to analyze the efficacy of zinc oxide nanoparticles against*Colletotrichumlindemuthianum*causinganthracnoseongreengramand*Sclerotiumrolfsii*causi ngsouthernblight on green gram.

Materialand method

The efficacy of zinc nanoparticles synthesized from neem (*Azadirachta indica)*, mehandi(*Lawsonia inermis*), marigold (*Tagetus erectus*) and ginger (*Zingiber officinalis*) were assessedagainst *Colletotrichum lindemuthianum*and *Sclerotium rolfsii*using Poisoned food techniquewherevaryingconcentrationsofZnNpswereincorporatedintothegrowthmediumtoassessth eireffect on fungal growth (Nene and Thapliyal, 1993) [9] by utilizing Potato dextrose agar mediumasthebaseculture.Thenanoparticlesweresynthesizedfromneem,mehandi,marigoldandginge rplant extracts after testing compatibility with plant extracts and the test pathogens.The plantextractsofneem,mehandi,marigoldandgingershowssignificantfungistaticagainsttherespectivef ungus.Hencethesefourplantextractswereselectedfornanoparticlessynthesis.Zincnanoparticlessynth esizedfromneem,mehandiandmarigoldplantextractswereevaluatedagainst*Colletotrichum lindemuthianum*at 100, 250 and 500 ppm. Neem plant extracts at 250 ppm,mehandiplantextractsat250ppm,marigoldplantextractsat250ppmandZincnitratehexahydrateat 250 ppm were also evaluated against *Colletotrichum lindemuthianum*in order to find out

thepotentialofantifungal activity of zinc nanoparticles against thefungus.

Zincnanoparticlessynthesizedfromneem,mehandiandgingerplantextractswereevaluatedagainst*Sclerotiumrolfsii*at100,250and500ppm.Thesynthesizednanoparticlesfromplant extracts were analysed by uv visible spectroscopy and Fesem analysis.Neem plant extractsat 250 ppm, mehandi plant extracts at 250ppm, ginger plant extracts at 250ppm and Zinc nitratehexahydrate at 250 ppm were also evaluated against *Sclerotium rolfsii*in order to find out thepotentialofantifungal activity ofzincnanoparticles against thefungus.

The nanoparticles were synthesized from the plant extracts by the mixing of plant extractsand the precursor Zinc nitrate hexahydrate at 1:3 ratio and magnetic stirred at 80˚C temperatureand 60-minute incubation period. Soon the colour change occurred and the synthesis was furtheranalysed by the characterization using uv spectroscopy and Fesem analysis. The PDA mediumcontaining appropriate quantity of zinc nanoparticles or plant extracts or zinc nitrate hexahydrateat 250ppm were poured aseptically into Petri dishes (90 mm in diameter), where it solidifies atroom temperature. All of the plates were inoculated aseptically with a 5 mm culture disc madefrom anactivelygrowingpurecultureof apathogen.The disc waspositionedinthemiddleofthepetriplateonPDA,andtheplatesthenbeincubatedatatemperatureof27 ± 2°C.Threereplicationof treatment were made. Petri plates with basic PDA containing the pathogen's culture disc werecontinued to be used as the untreated control. Thus, replications with14 treatments and by usingdesignCRD for*Colletotrichum* and *Sclerotium*.

Mechanismofnanoparticles

The ions released by the nanoparticles interfered with the cell wall membrane of the fungusand it will cause the death of the fungus at later stage. (Martínez-Camacho *et al*. (2010) [7]. Theoxidation of proteins in cells of the fungus were mediated by ROS (Reactive Oxygen Species) butthe degradation of DNA is a ROS-independent phenomenon caused by the intracellular release ofions.Chatterjee*et al*. (2014)[3].

List 1: Treatmentdetailsfor*Colletotrichum*

Tr. No	Treatments	Tr.No	Treatments
T_1	NeemZnNps@100ppm	T_8	MarigoldZnNps @250ppm
T_2	NeemZnNps@250ppm	T_9	MarigoldZnNps @500ppm
T_3	NeemZnNps@500ppm	T_{10}	Neemextracts @250ppm
T_4	MehandiZnNps@100ppm	T_{11}	Mehandiextracts@ 250ppm
T_5	MehandiZnNps@250ppm	T_{12}	Marigoldextracts @250ppm
T_6	MehandiZnNps@500ppm	T_{13}	ZincNitrateHexahydrate @250ppm
T_7	MarigoldZnNps @100ppm	T_{14}	**Control(Untreated)**

List 2: Treatmentdetailsfor*Sclerotium*

Tr.No	Treatments	Tr.No	Treatments
T_1	NeemZnNps@100ppm	T_8	GingerZnNps @250ppm
T_2	NeemZnNps@250ppm	T_9	GingerZnNps @500ppm
T_3	NeemZnNps@500ppm	T_{10}	Neemextracts @250ppm
T_4	MehandiZnNps@100ppm	T_{11}	Mehandiextracts@250ppm
T_5	MehandiZnNps@250ppm	T_{12}	Gingerextracts@250ppm
T_6	MehandiZnNps@500ppm	T_{13}	ZincNitrateHexahydrate @250ppm
T_7	GingerZnNps @100ppm	T_{14}	**Control(Untreated)**

Observations on radial colony diameter of the test isolates were recorded at an interval of24 hrs and continued till untreated control plates were fully covered with growth of the testpathogen. Per cent growth inhibition of the test pathogens over untreated control was calculatedbyfollowing formula(Vincent,1927)[13].

$$\text{Percentinhibition}(I) = \frac{(C-T) \times 100}{(C)}$$

Where,I=Per centinhibition

C = Growth of test fungus on untreated control platesT = Growth of test fungus pathogen on treated plates**Resultand Discussion**

Afteranalyzingthenanoparticlesbyuvvisiblespectroscopy,itwasfoundresonanceband atmaximumof300nmand400nmofMehandiZnNPs,at400nmofNeemZnNPsandat350nm ofMarigoldZnNPsand300nmofGingerZnNps,respectively.FeSemanalysisofneem,mehandi, gingerandmarigoldnanoparticlesshowedindividualZnNpsaswellasanumberofaggregates.In general,particlesweresphericalinshapeandtheparticlesizeofneemzincnanoparticleswerefound intherangeof9-25nm,MehandiZincnanoparticlesimageswerefoundtobehomogeneousand agglomeratedintherangeof10-45nm,gingerzincnanoparticles30-40nmandMarigoldzinc nanoparticlesin therangeof 25-80 nm.

Incaseof*Colletotrichum*,theresultsindicatedthatsolutionsofzincnanoparticlesderivedfrom Neem, Mehandi and Marigold at concentrations of 100ppm, 250ppm and 500ppm exhibitedsignificantfungistaticeffects.Notably,Mehandizincnanoparticlesat500ppmshowedthegre atestefficacy, resulting in minimal mycelial growth (28.00mm), followed closely by Marigold andNeem at the same concentration (29.00mm and 30.00mm, respectively). In terms of growthinhibitionpercentages,Mehandizincnanoparticlesachievedthehighestinhibitionrateat68.88 %,withMarigoldandNeemfollowingat67.77%and66.66%,respectively.Alltestedconcentrationsof the zinc nanoparticles proved to be more effective than the corresponding plant extracts andzincnitratehexahydrate.Resultsshowsthatallthetreatmentsatallconcentrationweresignificantove rthecontrol ininhibitingthegrowth oftest pathogen. (Table1, Plate1,Fig 1).

These findings align with previous research indicating that zinc nanoparticles possessnotableantifungalproperties.Forinstance,Mosquera*etal*.(2020)[8]reportedthatZnOnanoparti cles significantly inhibited *Colletotrichum* growth, while Thienprasert*et al* (2021) [12]foundasubstantialinhibitionrateusingnanoparticlesderivedfrombananapeelextract.Additionally, Saranya*etal*.(2023)[11]highlightedthatzincnanoparticlesfrommustardseedsalsodemonstrated strong inhibitory effects on mycelial growth. Overall, the results underscore thepotentialofgreensynthesizedzincnanoparticlesaseffectiveantifungalagentsagainst*Colletotrichu m lindemuthianum*causing anthracnose in green gram suggesting their applicabilityinagriculturalpracticesfor managing fungal diseases.

In case of *Sclerotium rolfsii*(causing southern blight in mung bean), the results indicated thatsolutionsofzincnanoparticlesderivedfromNeem,MehandiandGingeratconcentrationsof

100ppm, 250ppm and 500ppm exhibited significant fungistatic effects. The results showed that alltreatments significantly inhibited fungalgrowth compared to the untreated control. Among thetreatments, Neem ZnNps at 500ppm exhibited the highest effectiveness, resulting in the lowestmycelialgrowth(15.00mm),followedbyGingerZnNps(19.00mm)andMehandiZnNps(27.00mm) at the same concentration. Lower concentrations also demonstrated antifungal activity,thoughlesseffectively.Intermsofgrowth inhibition percentages, Neem zinc nanoparticlesachievedthe highest inhibition rate at 83.33%,with Ginger zinc nanoparticles andMehandifollowing at 78.88% and 70.00%, respectively. All tested concentrations of the zinc nanoparticlesproved to be more effective than the correspondingplant extracts and zinc nitrate hexahydrate.(Table 2, Platec2, Fig 2). These findings align with theprevious research indicating that zincnanoparticles possess notable antifungal properties. For instance, Ali *et al* [1]. (2020) found that 60mg ZnO-NPs from *Azadirachta indica* inhibited mycelial growth of *Sclerotium rolfsii*by 41%,whilechemicallysynthesizedZnO-NPshadnoeffect.Zaki*etal*[14].(2021)testedvariousconcentrations (20, 40, and 100 µg/mL) of ZnO-NPs against *Rhizoctonia solani*, *Fusarium sp.* and*Macrophominaphaseolina*,observingsignificantgrowthinhibitionacrossallconcentrations.Jomey azdian*et al* [4] (2024) reported that ZnO-NPs synthesized from *Trichoderma harzianum*inhibitedthegrowthof*Fusariumoxysporum*inadose-dependentmanner,withhigherconcentrationsleading to greaterinhibition.

Table1.*Invitro*evaluationofgreen synthesizedZincnanoparticlesagainst *Colletotrichumlindemuthianum*byPoisonedFoodTechnique

Tr.No.	Treatmentatdifferentconcentration	ColonyDiameter*(mm)	%Inhibition
T_1	NeemZnNps@100ppm	40.00	55.55 **(48.18)**
T_2	NeemZnNps@250ppm	35.00	61.11 **(51.41)**
T_3	Neem ZnNps@500ppm	30.00	66.66 **(54.73)**
T_4	Mehandi ZnNps@100ppm	42.00	53.33 **(46.90)**
T_5	Mehandi ZnNps@250ppm	38.00	57.77 **(49.47)**
T_6	Mehandi ZnNps@500ppm	28.00	68.88 **(56.09)**
T_7	MarigoldZnNps @100ppm	41.00	54.44 **(47.54)**
T_8	MarigoldZnNps @250ppm	34.00	62.22 **(52.07)**
T_9	MarigoldZnNps @ 500ppm	29.00	67.77 **(55.40)**
T_{10}	Neemextracts @250ppm	55.00	38.88 **(38.52)**
T_{11}	Mehandi extracts@250ppm	57.00	36.66 **(37.26)**
T_{12}	Marigoldextracts@250ppm	56.00	37.77 **(37.92)**
T_{13}	ZincNitrateHexahydrate @250ppm	47.00	47.77 **(43.72)**
T_{14}	Control(Untreated)	90.00	0.00 **(0.00)**
	S.E.±	**0.55**	**0.58**
	C.D.(P=0.01)	**2.17**	**2.28**

Meanofthreereplications.**Figuresinparenthesis arearcsinetransformedvalues

Table2.*Invitro*evaluationofgreen synthesizedZincnanoparticlesagainst *Sclerotiumrolfsii*byPoisoned FoodTechnique

Tr.No.	Treatmentatdifferentconcentrations	ColonyDiameter*(mm)	%Inhibition
T_1	NeemZnNps@100ppm	36.00	60.00 **(50.76)**
T_2	NeemZnNps@250ppm	30.00	66.66 **(54.73)**
T_3	Neem ZnNps@500ppm	15.00	83.33 **(65.90)**
T_4	Mehandi ZnNps@100ppm	50.00	44.44 **(41.80)**
T_5	Mehandi ZnNps@250ppm	40.00	55.55 **(48.18)**
T_6	Mehandi ZnNps@500ppm	27.00	70.00 **(56.78)**
T_7	GingerZnNps @100ppm	52.00	42.22 **(40.52)**
T_8	GingerZnNps @250ppm	39.00	56.60 **(48.79)**
T_9	GingerZnNps @500ppm	19.00	78.88 **(62.64)**
T_{10}	Neemextracts @250ppm	70.00	22.22 **(28.12)**
T_{11}	Mehandi extracts@250ppm	60.00	33.33 **(35.26)**
T_{12}	Gingerextracts @250ppm	58.00	35.55 **(36.60)**
T_{13}	ZincNitrateHexahydrate @250ppm	55.00	38.88 **(38.52)**
T_{14}	Control(Untreated)	90.00	0.00 **(0.00)**
	S.E.±	**0.55**	**0.56**
	C.D.(P=0.01)	**2.17**	**2.20**

Meanofthreereplications.**Figures inparenthesisarearcsine transformedvalues

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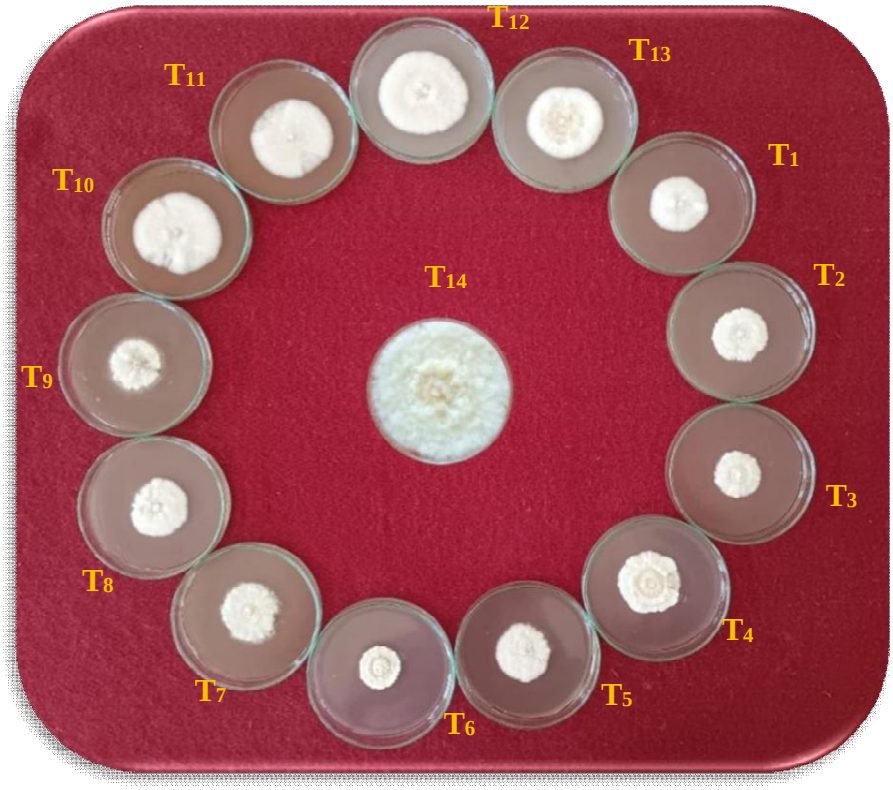

Plate1:*Invitro*evaluationofbiosynthesizedZincnanoparticlesagainst *Colletotrichumlindemuthianum*bypoisonedfoodtechnique

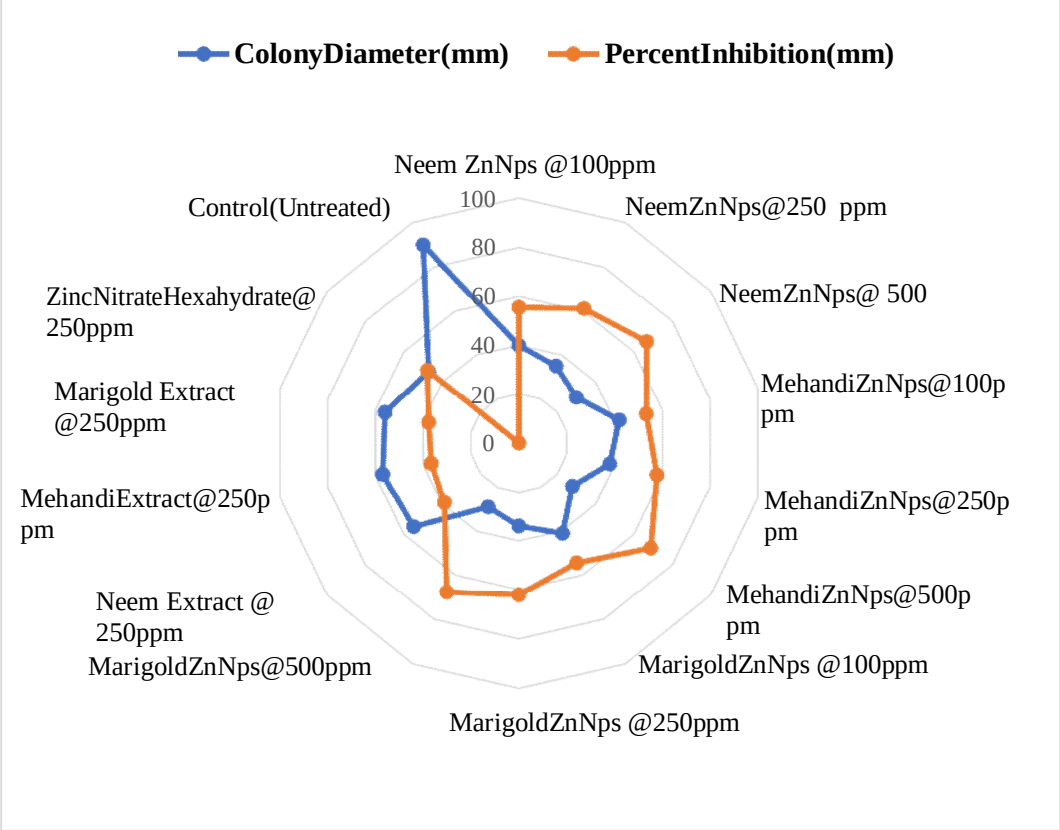

Fig1:*Invitro*evaluation ofbiosynthesizedZincnanoparticlesagainst *Colletotrichumlindemuthianum*bypoisonedfoodtechnique

Plate2:InvitroevaluationofbiosynthesizedZincnanoparticlesagainst *Sclerotiumrolfsii*bypoisonedfoodtechnique

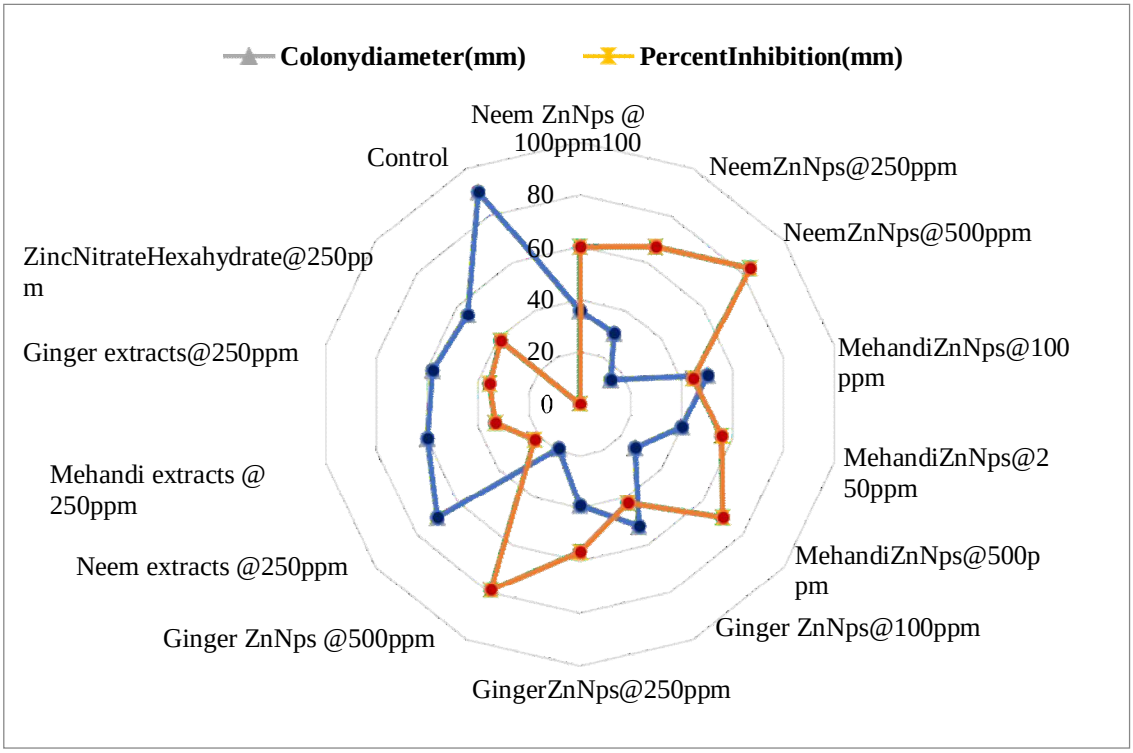

Fig2:InvitroevaluationofbiosynthesizedZincnanoparticlesagainst *Sclerotiumrolfsii*bypoisonedfoodtechnique

Conclusion

The study demonstrated that zinc nanoparticles (ZnNps) derived from Mehandi, Marigoldand Neem significantly inhibit the radial mycelial growth of *Colletotrichum lindemuthianum*, thepathogenresponsibleforanthracnoseinmungbeanandincaseof*Sclerotiumrolfsii*thenanoparticles synthesized from neem, mehandi and ginger were effective. Among the treatments,Mehandi ZnNps at 500 ppm exhibited the highest inhibition rate at 68.88%, followed closely byMarigold and Neem ZnNps at the same concentrationin case of *Colletotrichum* while, for*Sclerotium* Neem ZnNps at 500ppm followed by ginger and mehandi. The effectiveness of thesenanoparticles surpassed that of traditional plant extracts and zinc nitrate hexahydrate, indicatingtheirpotentialasanovelapproachformanagingfungaldiseasesincrops.Thesefindingsunderscoretheimportanceofutilizingzincnanoparticlesasapromisingalternativeforenhancingplantdiseaseresistanceandimprovingagriculturalproductivity.Furtherresearchmayexplorethemechanisms undelying the antifungal activity of ZnNps, their stability under field conditions andtheirenvironmental impact,including potentialeffects onsoil healthand non-target organisms.

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