NematicidalPropertiesofChitosanNanoformulation

Abstract

Chitosan is the second most abundant bio-polymer available in the world, second only to cellulose. It is found in crustaceous shells, e.g., those of crabs, shrimps, prawns, and fungi, as well as insect exoskeletons.Theuseofnanoformulationsforthemanagement of pests and diseases is receiving increased interest with the advancement of nanotechnology. Here, chitosan nanospheres wereobtainedfromchitosanusingtheionicgelationtechnique.The nanoformulationsobtainedwerecharacterizedusingaparticlesize analyzer, Fourier transform infrared spectroscopy, and a transmission electron microscope. The efficacy of chitosan nanospheres in suppressing the root-knot nematode *Meloidogyne incognita* was studied. The particle size of nanospheres formulated for this study was 380.2 nm, with a polydispersity index (PI) of 0.4 and ~~Zeta~~ zeta potential of 45.7 or 50.9 mV at pH 5.2.The chitosan nanospheres weresphericaland theparticlesdidnotagglomerate.FTIRspectra ofthechitosannanospherespeakedat3334cm^{-1},therebyindicating thestretchingoftheOHandNHgroups.In~~In~~in-vitrostudies,chitosan nanospheresshowedsignificantnematicidalactivityagainst

M. incognita. Under pot culture conditions, chitosan nanospheres (1%~~-~~ active compound chitosan) at 2ml/plant decreased the nematodepopulationinrootsorsoil.Comparedtothecontrol, the number of galls was reduced by 83.68%, the number of egg massesby83.85%,thenumberofadultfemalesby66.56%,andthe numberofsecond-stagejuvenilesby73.20%.Inafieldexperiment, applicationofchitosannanospheres(1%)wasfollowedby~~a~~an18.75% increase in fruit yield compared to the non-treated control.

Keywords

Chitosannanospheres,*Meloidogyneincognita*,particlesize,tomato, biochemistry

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Introduction

Biopolymersarenaturallyoccurringmaterialsformed duringthelifecycleofplants,animals,bacteria,and fungi (Yadav *et al*., 2015). Biopolymers are easily biodegradable because they include oxygen and nitrogen atoms. Through biological processes, the biopolymers are naturally recycled. Worldwide, chitosan is the second most abundant biopolymer, second only to cellulose. Through enzymatic and chemicaldeacetylationprocesses,chitinisconverted tochitosan(Kafetzopoulos*etal*.,1993).Chitosan was first discovered in mushrooms by Henri Braconnotin 1811 (Periayah*et al*., 2016). It is obtained from crustaceous shells, e.g., from the exoskeletons of crabs,shrimps,prawns,fungi,andinsects.Chitosan consistsofN-actetylD-glucosamineandβ-(1-4) D-glucosamine. Glucosamine (GlcN) is a product of the decomposition of chitosan by the chitosanase enzyme (Jung and Park, 2014). Chitosan is considered acationicpolymer,andduetoitsbiocompatibility,non-toxicity,and biodegradability~~bio-degradability~~properties,itisusedin

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agricultural,medical,biotechnological,environmental,and industrialapplications(Naveed*etal.*,2019).Itisalso known to possess antifungal, antibacterial, antiviral, and antinematicidalproperties(Goyetal.,2016).

Tomato (*Solanum*lycopersicum*lycoperiscum*) is the second most-abundant vegetable crop next to potato, with an annual production of 182.3 million tons on 4.85 millionhectares.Thesouthernroot-knotnematodes, *Meloidogyneincognita*,*M.javanica*,and*M.arenaria*, are the most frequent nematode species infecting tomato.Nematodeinfectioncanpredisposetheplant to fungal infection, leading to disease complexes causingconsiderableyieldlosses(KhanandSharma, 2020).GanaieandKhan(2011)studiedtheinteractive effectoftheroot-knotnematode*M.incognita*and the root pathogenic fungi *Fusarium solani* on tomato.Theyconcludedthatinoculationofroot-knot nematode*M.incognita*,followedbyinoculationof *F. solani*, caused more severe damage on tomato thaneitherpathogenalone.Whenchitosanisapplied to soil, it is converted into chitin (a polysaccharide), andthenintochitobiose(adisaccharide).Chitobiose damagestheeggsandcuticlesofyoungnematodes, whichhavechitinintheircomposition(Gortari*etal.*, 2008; Abd El-Aziz and Khalil, 2020). The release of toxic chemical compounds during decomposition hasalethaleffectonthesecond-stagejuveniles of *M. incognita* and on nematode multiplication(Asif*etal.*,2017).Duringchitinhydrolysis, ammonia concentrationsincrease,causingdeathinnematodes (Spiegel *et al.*,1987). Chitosan induces signaling molecules in plants, such as the specific cellular receptorthatistransducedbysecondarymessengers. These secondary messengers include Reactive OxygenSpecies(ROS),H_2O_2,Ca2+,nitricoxide,and phytohormones(Hidangmayum*etal.*,2019).Inseveral studies,chitosanhasbeenusedasananocarrierfor encapsulateddrugsoractivecompounds,delivering themintoaspecificplaceandprovidingcontrolled release(YanatandSchroen,2021).

Nanospheres are nanostructures formed by a densepolymericmatrix.Thedrugsaredispersed in the matrix-type structure of the nanospheres. Nanospheres are prepared using several polymers like cellulose, chitosan, and ~~Poly~~ poly (d, l- lactic acid). Thesizerangeofnanospheresisbetween10and 200 nm in diameter. Nanospheres are amorphousor crystalline in nature (Singh and Sharma, 2010). Embeddingthedesiredcompoundsinnanospheres protects the drugs from enzymatic and chemical degradation.Chitosannanoparticlesareusedas nanopesticides and carriers of fungicides, insecticides, herbicides, plant hormones, elicitors,

andnucleicacids(Zhang*etal.*,2003;DeiLam*etal.*,2006).

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2006). Because of their small size and high contact area,chitosannanoparticlescaneasilypenetrateand permeate into the membrane of phytopathogens or planttissues,resultinginincreasedcontrolordefense response activity.

Alfy *et al.* (2020) reported that a chitosan nanoparticle at 2000 ppm was the most efficient biopolymerincontrollingtheroot-knotnematode, *M. incognita*, in tomato. Nanochitosan at 2000 ppm decreased the egg hatching rate by up to 95.3%and caused 97.2% juvenile mortality after 72 hr of exposure.Itincreasedplantgrowthparametersand, compared to the control, decreased the nematode population in soil by 78% and in the roots by 98%. Nematodemanagementremainschallengingdueto the limited availability of nematicides.

Human health concerns and environmental considerations call for environmentally-friendly alternatives that can be integrated into existing practices. With this background in mind, a studywas undertaken to assess the efficacy of chitosan nanospheres against root knot nematode, *M. incognita*. Our objectives were to formulate and characterizeofchitosannanospheresanddetermine theirefficacyon*M.incognita*underin vitro, field,in-vitro,fieldand pot culture conditions.

MaterialsandMethods

Preparationofchitosannanospheres

Commercial-grade high-molecular-weight chitosan was used in the study (obtained from PanvoPvt. Ltd.,pvt-ltd.Chennai).Chitosannanospherewerepreparedusing theionicgelationmethod(YoussefandMasry,2018). 1gofcrudechitosanwasdissolvedin1%glacialacetic acidandstirredfor24hrinamagneticstirrerat500 rpm(SpinitTMDigitalMagneticstirrer).After24hrof stirring,onepart0.7mg/mlSodiumTripolyphosphate (TPP)wasaddeddropwiseto3partsof1%chitosan acidicsolutionandagainallowedfor2hr,stirring at 500 rpm. The pH of the prepared solution was checkedusingapHmeterandlatersonicatedin an Ultraprobesonicator (Ultra Sonics instruments) with an amplitude of 35%, pulse of 10 seconds, and temperature of 35ºC, for seven minutes.

In a second experiment, 5 ml of surfactant 2% Tween80wasslowlyaddedto3partsof1%chitosan acidic solution at 45ºC. The mix was stirred for 2 hr in a magnetic stirrer at 500 rpm. One part of TPP wasaddedtothreepartsof1%chitosan-aceticacid solution. The sample was then homogenized in a pressurizedhomogenizer(Homeostatinstrument)for 5 minutes and ultrasonicated for 15 min.

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Characterizationofchitosan *nanospheres*

Theparticlesizeandpolydispersityindex(PI)ofthe synthesized chitosan nanoparticles were measured inaparticlesizeanalyzer(HORIBASZ-100,Japan). Their stability was determined using zeta potential. Thesizeandshapeofthechitosannanospheresweре characterized using transmission electron microscopy (TEM).Thefunctionalgroupsofbiomoleculespresent in the chitosan nanospheres were identified using Fourier infrared spectroscopy (FTIR).

Bioefficacyofchitosannanospheres against ~~root~~ root-knot nematode, *M.incognita*

Pure cultures of root-knot nematode, *M. incognita*, wereobtainedatthe DepartmentofNematology,TNAU, Coimbatore. Concentrations of 100 ppm, 500 ppm, 1000ppm,and5000ppmwerepreparedfromthe1% chitosannanospheresuspensionsinsterile(100ml) water. Each concentration was sonicated in a water bath sonicator (POWERSONIC 510) for 20 minutes before testing them against *M. incognita*.

Egghatchingtest

Egg masses of *M. incognita* were sterilized in 0.5% sodiumhypochloritesolutionfor1minute.Theculture wasmaintainedatthe DepartmentofNematology,~~Glass~~ glass house, TNAU, CBE. Egg masses were collected fromnematode-infestedroots.Theexperimentwas repeated 3 times.2 ml of each concentration suspension was placed in a 5-cm diameter petri dish. One egg mass of M. incognita was placedinto the liquid in the petri dish. The experiment was conducted in a randomized complete block design~~,~~ with five treatments and four replications. Numbers of hatched juveniles were observed at different time intervals,*viz.*,24hr.,48hr.,and72hr.

Juvenilemortality test

Freshly hatched second-stage juveniles (J2) were used for juvenilemortalitystudies.Foreachdifferentconcentration of the chitosan nanospheres, 1ml of the suspension wasplacedina5-cmdiameterpetridish,towhich100 infectivejuveniles(J2)wereadded(the1mlsuspension containingnematodes~~were~~was counted withthehelpofa countingdishunderastereozoommicroscope).Thefive treatmentswerereplicatedfourtimesandarrangedin arandomizedcompleteblockdesign.All100juveniles

wereexamined,andthenumberofdeadjuvenileswere was recordedafter24hr.,48hr.,and72hr.

Efficacy of chitosan nanospheres on Root root knotnematodes inpotsi na greenhouse (in-vitro conditions)

Agreenhouseexperimentinpots(3kgofsoil;potsize 20.7x11x16.4cm)wasconductedtostudytheeffect of different concentrations of chitosan nanospheres against *M. incognita*. Seedlings purchased from the vegetable nursery at Thondamuthur (15 days old; Shivam hybrid), Coimbatore-03, were used for pot cultureexperiments.The15-day-oldseedlingswere transplantedintoasterilizedpotmixture,wherethey wereinoculatedwithinfectivejuvenilenematodesat a rate of 2/gm of soil. One week after inoculation, chitosannanosphereswereappliedasasoildrench. On the 45th day after nematode inoculation, plants were uprooted, and plant shoot lengths and root weightsweredetermined.Thenematodepopulations were counted based on the gall numbers (Heald *et al.*, 1989) and egg masses on the roots. In total, 10 treatmentsandthreereplicationswerearrangedina randomized complete block design.

Thetreatmentsincluded:

1. Chitosannanospheres(1%)1ml/plant.
2. Chitosannanospheres(1%)2ml/plant.
3. Chitosannanospheres(1%)3ml/plant.
4. Chitosan(1%)1ml/plant.
5. Chitosan(1%)2ml/plant.
6. Chitosan(1%)3ml/plant.
7. Velumprime1ml/plant-34.6%Fluopyran
8. Purpureocilliumlilacinum1g/plant.
9. Chitosannanospheres(1%)3mlwithoutnematodes.
10. Untreatedcontrol.

Effect of chitosan nanospheres against rootknotnematode,*M.incognita*,under field conditions

A *M. incognita*-infested field was selected near Karadimadai Village, Coimbatore, India. Seven treatmentswereappliedassoildrenchestooneweek-oldtomatoseedlings.Onthe45thdayafterplanting, asecondsetoftreatmentswerewasapplied.Treatme nts were applied to three replicate plots with 40 plants each, arranged in a randomized block design. Plant growth parameters and nematode population density in soilandrootswereobserved90daysafterapplication. Fruityieldsweremeasuredforeachtreatment.

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(A)

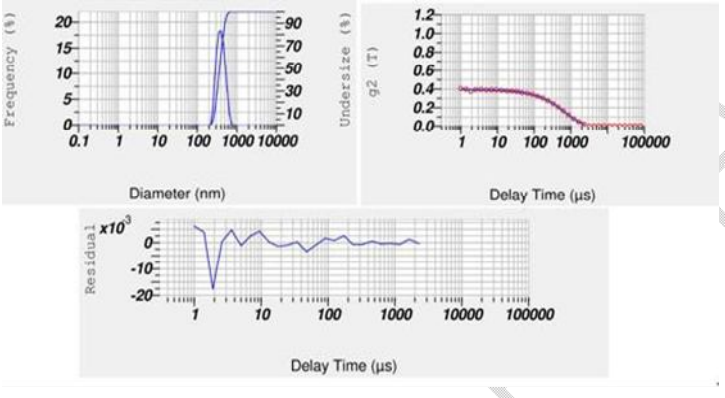
Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	380.2 nm	90.3 nm	375.8 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	380.2 nm	90.3 nm	375.8 nm

Cumulant Operations

Z-Average : 312.6 nm

PI : 0.417



(B

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	45.7 mV	0.000355 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean) : 45.7 mV

Electrophoretic Mobility mean : 0.000355 cm²/Vs

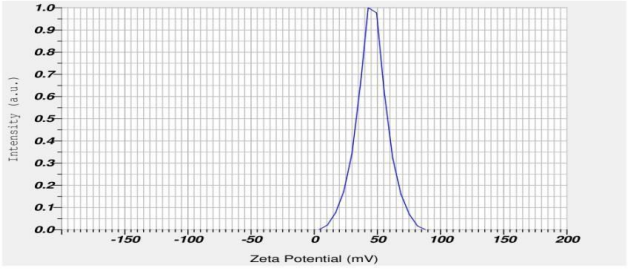


Figure 1:(A)Particlesizeof chitosannanospheres.(B)Zetapotential ofchitosannanospheres.

Thefollowingtreatmentswereused:
T1- Chitosan nanospheres 1% (5ml/plant).
T2- Chitosan nanospheres 2% (5ml/plant).
T3- Chitosan 1% (5ml/plant).
T4-Chitosan2%(5ml/plant).
T5-Velum prime (34.46% fluopyran)- 500 ml/acre.-0.0005 metric
T6-Carbofuran3G1kga.i/ha.
T7- Untreated control.

Statisticalanalysis

The data obtained from the above-mentioned experiments were subjected to statistical analysis followingthemethodformulatedbyPanseandSukhatme(1967).TheobtaineddatawereruninAggresssoftware withsingle-factoranalysistoobtaintheresults.

Results

Formulationandcharacterizationof chitosan nanospheres

1gchitosanwasdissolvedin1%aceticacidinthe presenceofTween80andTPP.Thepreparedsolution washomogenizedinapressurizedhomogenizerfor5 minutes.Thehomogenizedsolutionyieldedasizeof 380.2nmwithaPIof0.4(Fig.1).Thepresentstudy provedthatsynthesizedchitosannanospheresusing sodium tripolyphosphate and Tween 80 were highly stable,asmeasuredbytheirzetapotentialvalueof +49.7mV(Fig.1b).Thezetapotentialofthechitosan nano formulation obtained in this study was well above +30 mV, indicating high stability.

TransmissionElectronMicroscope(TEM)

TEMmicrographywasusedtostudythemorphology, shape, and size of the synthesized chitosan nanospheres.Thenanospherewerepredominately spherical,withnoagglomerates.Theaveragesizeof each nanosphere was 89.0 nm (Fig. 2).

FTIR (Fourier Transform Infra-Red Spectroscopy)

The chitosan nanospheres obtained with the above method were analyzed using Fourier Transform Infra–red Spectroscopy (FTIR), which was used to studythechemicalinteractionbetweenchitosanand

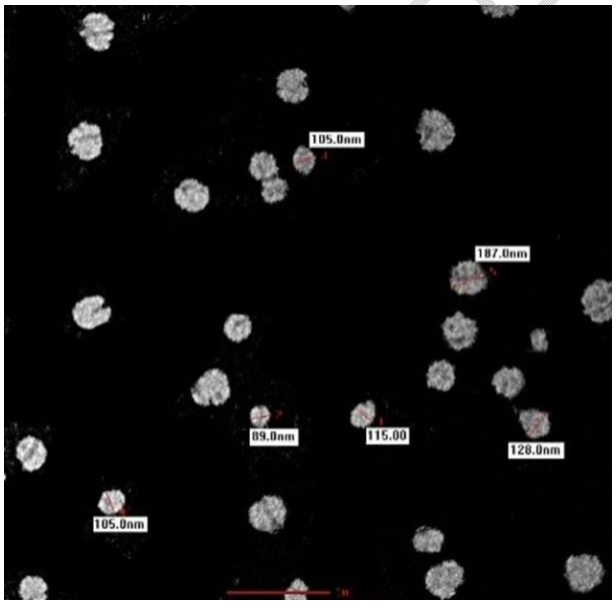


Figure2:TransmissionElectronMicroscope(TEM)micrographofchitosannanospheres.

sodium tripolyphosphate molecules. The peak at 3334 cm^{-1}showed a stretching of the OH and NH groups. Peaks at 2925 $cm^{-1}$and 2856 cm^{-1} show the stretching of the CH group. A peak of 2285 cm^{-1}representedN=C=Ostretching.Apeakat2114cm^{-1} showed N=C=S stretching, and a peak at 1635 cm^{-1}explained the stretching of C=N. The peak at 1412 cm^{-1}showedbendingoftheO-Hgroup,andthepeak at 1004 cm^{-1}revealed the stretching of the C-F group (Fig. 3).

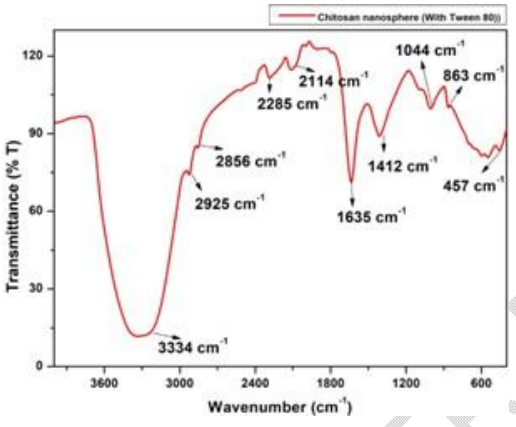


Figure3:FT-IRspectroscopyof1%chitosannanospheresformulation.

Table1.Effectofchitosannanospheresontheegghatchingof*M.incognita*

Treatments	Numberofhatchedjuveniles(Meanoffourreplications)		
	24h	48h	72h
	Meanand transformed value	Meanand transformed value	Meanand transformed value
Chitosannanospheres at100 ppm	12.50^{b}(3.31)	16.50^{b} (4.84)	41.50^{b}(6.29)
Chitosannanospheres at 500 ppm	10.50^{b}(3.09)	14.16^{b} (3.61)	26.50^{b}(4.79)
Chitosannanospheres at1000 ppm	0.00^{a} (0.70)	0.00^{a} (0.70)	0.00^{a} (0.70)
Chitosannanospheres at 5000 ppm	0.00^{a} (0.70)	0.00^{a} (0.70)	0.00^{a} (0.70)
Control-tapwater	38.83^{c}	61.66^{c}	84.80^{c}
	(6.21)	(7.85)	(17.27)
SEd	0.72	0.46	1.12
CD(p=0.01%)	2.29	1.48	3.88

*Figuresinparenthesesaresquarerootransformedvalue.Inacolumn,meansfollowedbycommondifferent from each other at 1% level by DMRT.

Bio-efficacy of chitosan nanospheres against ~~Root~~root knot nematode *M.incognita* under in-vitro conditions

Different concentrations of chitosan nanospheres were used to assess the efficacy of chitosan nanospheres on the hatching of M. incognita eggs. ~~To assess the efficacy of chitosan nanospheres on the hatching of *M. incognita* eggs, different concentrations of chitosan nanospheres were reused.~~ Among the concentrations tested, chitosan nanospheres at 1000 ppm and 5000 ppm completely inhibited egg hatching compared to the control. Exposure of *M. incognita* egg masses to chitosan nanospheres at 500 ppm concentration resulted in a 68.75% decrease in egg hatching compared to the untreated control 72hr after treatment (Table1).

At this concentration, hatching of eggs was observed, but all the hatched juveniles from the eggs were found dead. A scanning electron microgram (x1000 magnification) of treated egg masses revealed degradation of the gelatinous matrix (Fig.4). The first-stage juveniles found within the treated eggs were deformed.

Different concentrations of 1% chitosan nanospheres, *viz*., 100 ppm, 500 ppm, 1000 ppm, and 5000 ppm, were tested against infective juveniles (J2). Among the above-mentioned concentrations, chitosan nanospheres at 5000 ppm caused 100% juvenile mortality within 24hr (Table2). At the 1000-ppm concentration, the infective juvenile mortality

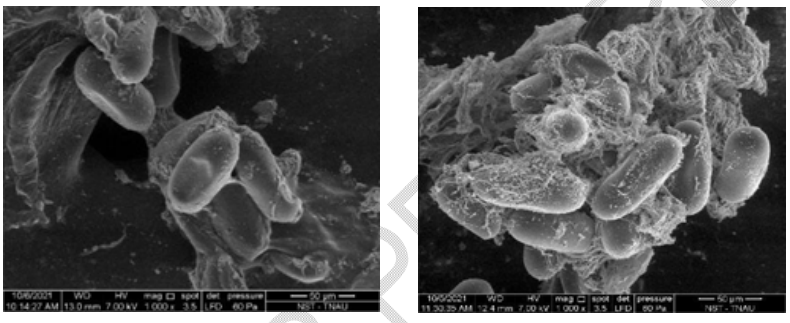


Figure4: Scanning Electron Microscope images of chitosan nanospheres treated eggs (left side) and chitosan nanospheres untreated eggs (right side).

Table2. Effect of chitosan nanospheres on *M.incognita* juvenile mortality

Treatments	Juvenile mortality in percentage (Mean of four replications)		
	24h	48h	72h
Chitosan nanospheres at 100 ppm	13.00^{b}(5.40)	21.5^{c}(3.47)	26.5^{c}(5.10)
Chitosan nanospheres at 500 ppm	27.25^{b} (5.14)	31.25^{b}(5.67)	28.25^{c}(5.26)
Chitosan nanospheres at 1000 ppm	29.00^{b} (5.85)	36.00^{b}(5.96)	46.25^{b}(6.70)
Chitosan nanospheres at 5000 ppm	100.00^{a}(10.02)	10.00^{a} (10.02)	100.00^{a}(10.02)
Control	0.00^{c}(0.707)	0.00^{d} (0.70)	0.00^{d} (0.70)
SEd	1.52	0.72	0.55
CD(p=0.01%)	4.48	2.14	1.64

*Figuresinparenthesesaresquareroottransformedvalue.Inacolumn,meansfollowedbycommonalphabetare significantlydifferentfromeachotherat1%levelbyDMRT.

Table3.Effectchitosannanospheresagainstrootknotnematode,*M.incognita*under pot culture conditions

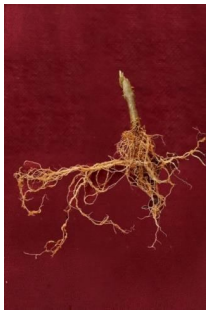
Treatments	ications			
	Numberof galls/5gof roots	Numberof eggmasses/ 5gofroots	Numberof females/5gof roots	Numberof J2/100ccofsoil
	Meanand transformed value	Mean and transformed value	Mean and transformed value	Mean and transformed value
Chitosannanospheres	13.30[a]	11.67[bcd]	17.67[cd]	48.60[bc]
1ml/plant+*M.incognita*	(3.51)	(3.32)	(4.25)	(12.06)
Chitosannanospheres	10.00[a]	6.00[b]	14.60[b]	28.30[ab]
2ml/plant+*M.incognita*	(3.12)	(2.53)	(3.87)	(5.36)
Chitosannanospheres	15.00[a]	7.33[bc]	17.60[cd]	40.30[bc]
3ml/plant+*M.incognita*	(3.87)	(2.73)	(4.25)	(6.38)
Chitosan1ml/plant+	18.00[a]	19.00[c]	22.30[f]	62.00[bcd]
M.incognita	(4.28)	(4.41)	(4.76)	(7.90)
Chitosan 2ml/plant +	17.00[a]	10.00[bcd]	16.67[c]	46.70[bc]
M.incognita	(4.25)	(3.22)	(4.13)	(6.86)
Chitosan 3ml/plant +	17.60[a]	10.66[bcd]	17.90[d]	44.70[bc]
M.incognita	(3.34)	(3.30)	(4.19)	(6.66)
Velumprime0.5ml/plant+ *M.incognita*	25.67[a](4.87)	15.67[de] (3.95)	19.30[e] (4.44)	32.67[bc](5.75)
*P. lilacinum*1g/plant+ *M.incognita*	17.67[a](4.25)	13.39[cde](1.3 7)	18.67[e] (4.44)	50.60[bc](7.14)
Chitosannanospheres without nematode Inoculums	0.00[a] (0.70)	0.00[a] (0.70)	0.00[a] (0.70)	0.00[a] (0.70)
Untreatedcontrol	61.30[e](7.83)	37.16[f] (6.04)	43.67[g] (6.63)	105.60[cd](10.2 1)
SEd	0.96	0.47	0.06	2.26
CD(p=0.01%)	2.73	1.36	0.18	6.44

*Figuresinparenthesesaresquareroottransformedvalue.Inacolumn,meansfollowedbycommonalphabetare significantlydifferentfromeachotherat1%levelbyDMRT.

was lower, with a maximum of 46.25% mortality observed after 72 hr of exposure.

The effect of chitosan nanospheres on root knot nematode *M. incognita* intomatounderpotcultureandfield conditions

~~In vitro~~ results revealed that chitosan nanospheres reduced nematode populations in roots and soil. Applicationofchitosannanospheresat2ml/plant decreasedrootgallsby83.68%.Applicationofchitosan nanoformulationat2ml/plantdecreasedthenumber ofeggmassesby83.85%.Thechitosannanospherefor mulationat2ml/plantregisteredthelowestnumberof adultfemales,withthehighestpercentreductionof66.56 % (Table 3). The highest reduction of infective juveniles (73.20%)wasrecordedwiththe2ml/plantformulation (Fig.5).Infieldexperiments,thechitosannanosphere formulation (2%) at 5ml/plant decreased galls by 92.47%. Furthermore, the fruit yield was higher by 18.75% in plots treated with chitosan nanospheres (Table 4).

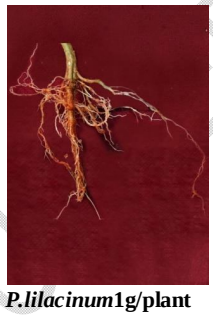


Chitosannanospheres1ml/plant

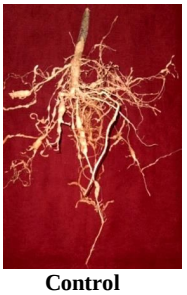
Chitosannanospheres2ml/plant

Chitosannanospheres3ml/plant

Velumprime@0.5ml/plant



***P.lilacinum*1g/plant**



Control

Figure5:Efficacyofdifferentdosesofchitosannanospheres(1%)on*M.incognita*underpot culture conditions.

Table4.Effectofchitosannanospheresagainstrootknotnematode,*M.incognita*underfieldconditions

Treatments	Meanofthreereplications					
	Numberof galls/5gof roots[1]	Numberofegg masses/5gof roots[1]	Numberof females/5gof roots[1]	Numberof J2/100ccof soil[1]	Fruitweight (kg)/ plot[2]	Fruitweight (tonne)/ha[2]
	Meanand transformed value	Meanand transformed value	Meanand transformed value	Meanand transformed value	Mean andtransformed value	
Chitosan nanospheres(1%)-5ml/plant	5.61^{ab} (2.29)	3.34^{b} (1.82)	18.31^{b} (4.26)	50.63^{c} (7.11)	95^{a} (18.75)	14.25
Chitosan nanospheres(2%)-5ml/plant	3.31^{a} (1.81)	0.99^{ab} (1.14)	17.38^{b} (4.16)	35.00^{b} (5.90)	89.5^{b} (11.87)	13.42
Chitosan(1%)-5ml/plant	11.25^{b} (3.10)	3.62^{b} (1.93)	20.90^{b} (4.54)	58.54^{e} (7.61)	86^{bc} (7.5)	12.90
Chitosan(2%)-5ml/plant	4.91^{ab} (2.30)	1.88^{ab} (1.52)	17.79^{b} (4.19)	48.75^{b} (6.92)	83^{cd}(3.75)	12.45
Velum prime500 ml/acre	2.83^{a} (1.60)	0.09^{a} (0.75)	11.42^{a} (3.35)	30.45^{a} (5.47)	99^{a} (23.75)	14.85
Carbofuran3G-1kg ai/ha	7.32^{b} (2.95)	1.43^{ab} (1.35)	23.20^{b} (4.78)	54.23^{d} (7.34)	89^{b} (11.25)	13.35
Untreatedcontrol	44.00^{c} (0.62)	22.3^{c} (4.77)	57.25^{c} (7.57)	165.80^{f} (12.73)	80^{d}	12.00
SEd	0.46	0.37	0.37	0.10	2.28	
CD(p=0.05%)	1.00	0.80	0.80	0.22	4.90	

*[1]and[2]-Figuresinparenthesesaresquarerootransformedvalueandincreasedovercontrolrespectively.Inacolumn,meansfollowedbycommonalphabet aresignificantlydifferentfromeachotherat1%levelbyDMRT.

Discussion

Synthesis and characterization of chitosan nanoformulation and its potential antiemeticantinemetic and antifungaleffectsarediscussed.Pertheobservations of Budi *et al.* (2020), the average particle size of chitosan nanospheres was within the limit of 70 nm, with a Polydispersity polydispersityIndex index of 0.3. In the present study, the particle size of chitosan nanospheres after using Tween 80 and sodium tripolyphosphate was found to be the 380.2 nm, with a PI of 0.4. Karava*et al.* (2020) determined that the optimum size of chitosan nanospheres was 150 nm, with aPI value below 0.6. A PI value between 0 and 0.5 indicates homogeneous particles, and the PI value beyond 0.5 indicates particles in a heterogeneous condition (Danaei *et al.*, 2018). The PI value of the nanoformulation obtained in our study was found to bewithintherangeof0.5,indicatingnanoparticlesof a homogenous nature.

High stability in synthesized chitosan nanospheres is considered to be a desirable characteristic. The presentstudyprovedthatthechitosannanospheres thatweresynthesizedusingsodiumtripolyphosphate andTween80werehighlystable,asmeasured by their zeta potential value of +49.7 mV. The chemical interaction between chitosan and sodium tripolyphosphate molecules was studied using Fourier-transform infrared spectroscopy. The peak at 3334 cm^{-1}showed a stretching of the OH and NH groups. The findings of the present study were similarto the observations of Youssef and Masry (2018), where observations made through transmission electron microscopy and field emission scanning electronmicroscopyrevealedasphericalshapein thechitosannanospheres withsizesof89.0nm-187.0nmandnoagglomerationofparticles.Youssef andMasry(2018)andMohammadpour*etal.*(2011) also observed similar spherical shapes of chitosan nanospheres.

Differentconcentrations,*viz.*,100ppm,500ppm, 1000 ppm, and 5000 ppm, prepared from the 1% chitosan nanospheres solution, were evaluated for their respective nematicidal properties. Exposure of egg masses to chitosan nanospheres at 1000 and 5000 ppm completely inhibited egg hatching and increasedthedeathrateofjuveniles.Alfy*etal.*(2020) reported that chitosan nanospheres concentratedat 2000 ppm inhibited *M. incognita* egg hatching by 95.3% after 72 hr of exposure and caused a 77.5% mortality rate in juveniles. Similar to the treated eggs, the infective juveniles exposed to chitosan internal body parts. This was probably due to the interaction of positively charged chitosan with the negativelychargednematode,leadingtotheleakage ofproteinaceousconstituentsasreportedbyRabea and Badawy (2003).

Adding chitosan to the soil increases the population of chitinolytic microorganisms,populationofchitinolyticmicroorganisms,which produce enzymes that convert chitin (a polysaccharide) to chitobiose (a disaccharide). This destroys the eggs and cuticles of young juveniles, which contain chitin (Abd El-Aziz and Khalil, 2020). Because of chitosan's eliciting activity and ability to generatesystemicresistanceintheplant,aswellas the release of different toxic chemical compounds during decomposition, it is lethal to *M. incognita* second stage juveniles and inhibits nematode multiplication (Asif *et al.*, 2017).

In the present study, the methodology of formulating chitosan nanospheres was standardized. The chitosan nanosphere formulations had both direct and indirect effects on root-knot nematodes. Theydegradedthechitinlayerofnematodeeggsand juvenilesandcauseddeath.Indirectly,theyinduced systemic resistance in plants, thereby reducing nematode infection. As chitosan nanospheres are synthesizedfromabiologicalsource,theformulation is environmentally friendly and does not leave any toxic residues in the ecosystem.

Abbreviations

TPP: Sodium Tripolyphosphate
PSA: Particle Size Analyzer
TEM:TransmissionElectronMicroscope
FTIR:FourierTransformInfra-RedSpectroscopy

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nanospherewerefounddeadwithdeformed

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