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Oligo chitosan: An ecofriendly agent to control soil borne pathogens of chickpea (*Cicer arietinum* L.)

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Abstract
Chickpea production is limited by many diseases and insect-pests. Major losses in chickpea yield are due to wilt (*Fusarium oxysporum* f. sp. *ciceri*), dry root rot (*Rhizoctonia bataticola*) and collar rot (*Sclerotium rolfsii*) and these altogether are designated as 'Chickpea wilt complex'. Fungicidal property of Chitosan has been reported by many workers. Present study was carried out to test the efficacy of oligo chitosan against the major soil borne pathogens of chickpea. Oligo-chitosan alone @ 50, 75, 100, 125 and 150 ppm and in combination with *Trichoderma viride* along with the combi fungicide carbendazim 12% + mancozeb 63% @ 0.1% were evaluated by *in vitro* by poison food technique, similarly these were evaluated as seed treatment under glass house conditions against the three pathogens. The fungicidal treatment carbendazim 12% + mancozeb 63% @ 0.1% was found significantly superior to all the treatments to inhibit the mycelial growth of pathogen under *in vitro* conditions with minimum disease incidence in pot culture. It was followed by the treatment oligo-choitosan @ 100 ppm + *T. viride* (90.81, 91.70 and 93.33% inhibition in *F. oxysporum* f. sp. *ciceri*, *R. bataticola and S. rolfsii*, respectively) *in vitro* with minimum disease incidence of wilt (13.33%), dry root rot (16.67%) and color rot (16.67%) under glasshouse conditions in pot. Thus oligo-chitosan in combination with T. viride was found most effective in controlling all the three soil borne pathogens of chickpea both under *in vitro* and glasshouse condition.

Keywords: *Cicer arietinum* L., soil borne pathogens, oligo-chitosan, eco-freindly management

1. Introduction
Chickpea (*Cicer arietinum* L.) is an important pulse crop in India. During 2020-21 global production of chickpea was 15 m tonnes, out of which 73% was contributed by India. India ranks 1st in area and production. However, in terms of productivity India lags behind several countries. Chickpea production is constrained by many diseases and insect-pests. Soil borne diseases such as Fusarium wilt, collar rot, dry root rot, etc., are more prevalent in Central and Peninsular India, whereas foliar diseases such as Ascochyta blight, Botrytis grey mold, etc., are important in northern, northern-western and eastern India. Major losses in chickpea yield are due to wilt (*Fusarium oxysporum* f. sp. *ciceri*), dry root rot (*Rhizoctonia bataticola*) and collar rot (*Sclerotium rolfsii*) and these are designated as 'Chickpea wilt complex'. Fungicides have direct and indirect effect on ecosystem, hazard may occur during use and due to its residues. Fungicides have direct impact on human being, impact on environment *viz.*, surface water and ground water contamination, soil fertility losses, effect on soil micro flora, contamination of air and effects on non-target vegetation and organisms.
To overcome these adverse effects, there is need of eco-friendly and safe alternative to manage the disease. Chitosan molecules are known for the induction of the host plant resistance. Treatment of the chitosan to the plant tissues strengthens natural defense mechanism and improves the physiological properties in the plant (Ghauth *et al.*, 1994) [9]. Chitosan has direct activity against most of the pathogens and due to its antifungal activity, it is helpful agent to manage many pathogens of economically important crops. As it is a natural compound, there is no harm to environment. Chitosan is an organic natural biopolymer modified from chitin, which is the main structural component of squid pens, cell wall of some fungi and shrimp and crab shells (Boonlertnirun *et al.*, 2010) [3]. Chitin is the second most abundant polymer in nature after cellulose (Cohen-Kupiec and Chet, 1998) [6]. It has been shown to modulate plant disease, phytoalexin production and reactive oxygen species (ROS) generation (Lee *et al.*, 1999) [14], induce cell wall lignification (Pospieszny and Zielinska 1997) [18].

Kumar (2000) [13] stated that Chitosan fungal shows toxicity and inhibit fungal growth and it is environmentally safe and non-toxic to higher organisms. Considering the antifungal activities of chitosan during the present investigation the irradiated Chitosan i.e. oligo-chitosan was evaluated against three soil borne pathogens of chickpea.

2. Materials and Methods

2.1 Isolation of soil borne pathogens of chickpea

Chickpea plants showing typical symptoms of wilt, root rot and collar rot were collected from fields separately. Standard tissue isolation procedure was followed to isolate the pathogen. The infected tissues along with healthy portions were surface sterilized with 0.1% sodium hypo chloride solution for 30 seconds and such bits were transferred to petri plates containing sterile water successively for three times and then bits were transferred into petri plates containing 15 ml of potato dextrose agar medium and incubated at 28 °C for 7 days in BOD incubator. After a week of incubation, the well-developed mycelial growth free from any contamination was obtained.

2.2 Identification of isolated pathogens

The isolated pathogens, parameters were identified on the basis of symptomatology (visual and microscopic), morphological, cultural characteristics and pathogenicity test.

2.3 Pathogenicity

The isolated and purified pathogens from diseased samples were tested for their pathogenicity. The pathogenicity of the three pathogens namely *Fusarium oxysporum* f. sp.*cicieri*, *Sclerotium rolfsii*, and *Rhizoctonia bataticola* was tested under pot conditions by soil inoculation technique (sick soil).

2.4 Mass multiplication and preparation of inoculum of pathogens

The isolated pathogens were cultured on sand maize meal medium for mass multiplication. Mass multiplication of the pathogens was done by placing 90 g of sand, 10 g of maize meal and 20 ml of distilled water in each of the 250 ml conical flask. The flasks were autoclave. The medium in the flasks at 1.21 kg/cm^2 for 20 minutes. After sterilization each flask was inoculated with a bit of actively growing fungal culture and incubated at 25 °C for 15 days. Fungal soil mixture was prepared by hand mixing contents of each flask with the required quantity of autoclaved field soil under hygienic condition. Mass cultured inoculum was mixed @ 10% in each pot containing autoclaved soil and the pots were incubated to allow the maximum visible growth of the test fungus.

2.5 Testing of Efficacy of Oligo chitosan against soil borne pathogens

The efficacy of oligochitosan was evaluated at various concentrations *in vitro* by poison food technique method.

Under glass house condition the seeds of susceptible genotype JG 62 were treated with oligo-chitosan at different concentrations and sown in pots filled with inoculated soil. Separate uninoculated pots served as untreated control. Pot culture experiment was conducted in CRD with three replication. Following treatments were followed under *in vitro* and glass house condition.

Treatment details

Treatment details			
T1	Oligo chitosan @ 50 ppm	T5	Oligo chitosan @150 ppm
T2	Oligo chitosan @ 75 ppm	T6	Oligo chitosan @100 ppm + *Trichoderma viride*
T3	Oligo chitosan @100 ppm	T7	Carbendazim 12% WP + Mancozeb 63% WP @ 0.1%
T4	Oligo chitosan @125 ppm	T8	Untreated control

The observations on mycelial growth of fungi in each treatment under *in vitro* condition as well as disease incidence under glasshouse condition in various treatments was recorded.

3. Result and Discussion

3.1 Effect of oligo chitosan against *Fusarium oxysporum* f.sp. *ciceri* under *in vitro* and glass house conditions

The data obtained from *in vitro* and glasshouse studies is presented in Table 1 and 2. From the data it is revealed that the percent inhibition of *Fusarium oxysporum* f.sp. *ciceri* (FOC) by various treatments was ranged from 77.41% to 100%. Among the treatments of oligo chitosan, the combination treatment of oligo chitosan @ 100 ppm + *Trichoderma viride* was found significantly superior which inhibited 90.81 percent mycelial growth of FOC over untreated control. This was followed by oligo chitosan @ 150 ppm (89.93%), oligo chitosan at 125ppm (87.70%), oligo chitosan @ 100 ppm (82.15%) and oligo chitosan @ 75ppm (79.63%). However, the combi fungicide treatment carbendazim 12% WP + mancozeb 63% WP @ 0.1% was found significantly superior over rest of the treatments showing inhibition 100%. The least percent inhibition of the pathogen was found in the treatment of oligo chitosan at 50 ppm (77.41%). Under glass house condition same trend of results was observed. Minimum percent disease incidence was recorded with treatment oligo chitosan @ 100 ppm + *Trichoderma viride* (13.33%) and maximum disease incidence was observed in treatment oligo chitosan @ 50 ppm (63.33%).

3.2 Effect of oligo chitosan against *Rhizoctonia bataticola* under *in vitro* and glass house conditions.

The results showed in table 1 and 2, Plate 1(b) depicted that, the percent inhibition of *Rhizoctonia bataticola* by various treatments ranged from 28.88% to 100%. The combination treatment of oligo chitosan @ 100 ppm + *Trichoderma viride* was found significantly superior over all the treatments of oligo chitosan which inhibited 91.70% percent mycelial growth over untreated control. This was followed by oligo chitosan @ 150 ppm (75.40%), oligo chitosan @ 125ppm (68.81%), oligo chitosan @ 100 ppm (41.85%) and oligo chitosan @ 75 ppm (34.59%) mycelial growth inhibition. The combi fungicide treatment carbendazim 12% WP + mancozeb 63% WP @ 0.1% was found significantly superior over rest of the treatments (100% inhibition). The least percent inhibition of the pathogen was in the treatment of oligo chitosan at 50 ppm (28.88%). Under glass house condition similar results were obtained. Minimum percent disease incidence was recorded in pot applied with treatment oligo chitosan @ 100 ppm + *Trichoderma viride* (16.67%) while maximum (80.00%) was in treatment oligo chitosan @50 ppm.

3.3 Effect of oligo chitosan against *Sclerotium rolfsii* under *in vitro* and glass house conditions.

The percent inhibition of pathogen *Sclerotium rolfsii* by various treatments ranged from 73.41% to 98.88%. The combination treatment of oligo chitosan @ 100 ppm + *Trichoderma viride* was found significantly superior over all the treatments of oligo chitosan which recorded 93.33% percent mycelial inhibition over untreated control. This was followed by oligo chitosan @150 ppm (90.74), oligo chitosan @ 125ppm (86.73%), oligo chitosan @ 100 ppm (82.22%) and oligo chitosan @ 75ppm (80.07%). However, the combi fungicide treatment carbendazim 12% WP + mancozeb 63% WP @ 0.1% was found significantly superior over rest of the treatments showed inhibition of fungus 100%. The least percent inhibition of the pathogen was in the treatment of oligo chitosan @ 50 ppm (73.41%) (Table 1 and 2, Plate 1(c)). Similarly under glass house condition same minimum percent disease incidence was recorded in pot applied with oligo chitosan @ 100 ppm + *Trichoderma viride* (16.67%) and maximum disease incidence was observed in treatment oligo chitosan @ 50 ppm (66.67%).

Anusuya and Sathiyabama, (2016) [2] reported that the Chitosan increases chitinase and chitosanase activity in turmeric plants and provided enhanced resistance against *Pythium aphanidermatum* infection. Lopez *et al.* (2004) [15] evaluated chitosan for growth inhibition of *Fusarium oxysporum*, *Penicillium digitatum* and *Rhizopus stolonifera in vitro.* The sporulation of all the fungi was inhibited at a concentration of 3% whereas, *Fusarium oxysporum* was inhibited at a lower concentration 1.5%. Elmer and Lamondia (1994) showed a linear decrease in growth of *R. solani*as the chitosan concentration gradually increased from 0.5 to 6.0 mgl^{-1}. Hernandez-Lauzardo *et al.* (2008) [10] reported that the low molecular weight chitosan was more effective for inhibition of mycelial growth of *Rhizoctonia stolonifera*. Saharan *et al.* (2013) [19] found that Chitosan nanoparticle showed antifungal activities against phytopathogenic fungi namely *Alternaria alternata*, *Macrophomina phaseolina and Rhizoctonia solani*and the maximum growth inhibitory effects (87.6%) was on *in vitro* mycelial growth of *M. phaseolina*at 0.1% concentration.

El-Mohamedy *et al.* (2014) [8] studied the effect of combination of chitosan sand biocontrol agents *in vitro* on tomato and they reported that combination of *T. harzianum* and chitosan (1g/l) as root dipping treatment combined with chitosan (0.5 g/ L) as foliar spray reduced FCRR incidence and severity by 66.6 and 47.6%, respectively. However, in their study, only chitosan treatments were least effective. The present findings are in line with these findings. Chittenden and Singh. (2013) reported that the chitosan and *T. harzianum* combination was more effective in controlling sapstain fungi than chitosan or *T. harzianum* alone. This may be due to the antifungal activities of both chitosan and *T. harzianum*. Effect of chitin and chitosan on the growth of tomato root rot fungi was studied by Abdel-El-Kareem (2006) [1]. The inhibitory effect of chitin and chitosan on the growth of tomato root rot fungi, i.e., *F. solani*, *R. solani* and *S. rolfsii* was tested. Chitin has no inhibitory effect on the growth of all tested fungi. On the other hand, all concentrations of chitosan had significant inhibitory effect against tested fungi. Chitosan at 6 g/l completely inhibit the linear growth of all tomato root rot fungi. Chitosan, when applied to plant tissues, often agglutinate around the penetration sites and has two major effects. The first one is the isolation of the penetration site through the formation of a physical barrier preventing the pathogen from spreading and invading other healthy tissues. This phenomenon resembles the abscission zones often observed on leaves preventing several necrotrophic pathogens from spreading further. Chitosan has ability to bind various materials and initiate fast the wound healing process (Hirano *et al.*, 1999) [11]. Chitosan induces programmed-cell death (PCD) and hypersensitive associated responses in plants (Vasil'ev *et al.*, 2009) [20]. Synergistic interaction between chitosan and *trichoderma* has been reported by earlier researchers. Anusuya and Sathiyabama (2016) [2] reported that the Chitosan increases, chitinase and chitosanase activity in turmeric plants provide enhanced resistance against *Pythium aphanidermatum* infection. Kaur *et al.* (2018) reported that the different metal chitosan nanoparticle (CuO-, ZnO, and Ag-CHTNP) prevented Fusarium wilt, the seed borne and soil borne disease caused by *F. oxysporum* on chickpea. Also it was reported that chitosan alone, or in conjugation with other polymers, provided an appropriate medium for growth and reproduction of *Trichiderma viride* (Paneva *et al.*, 2003) [17].

In present studies, oligo-chitosan in combination with bioagent *Trichiderma viride* was found effective to inhibit and reduce soil borne diseases of chickpea. This treatment was as significant as commercial fungicide and may emerge as ecofriendly, sustainable way of plant disease control in future.

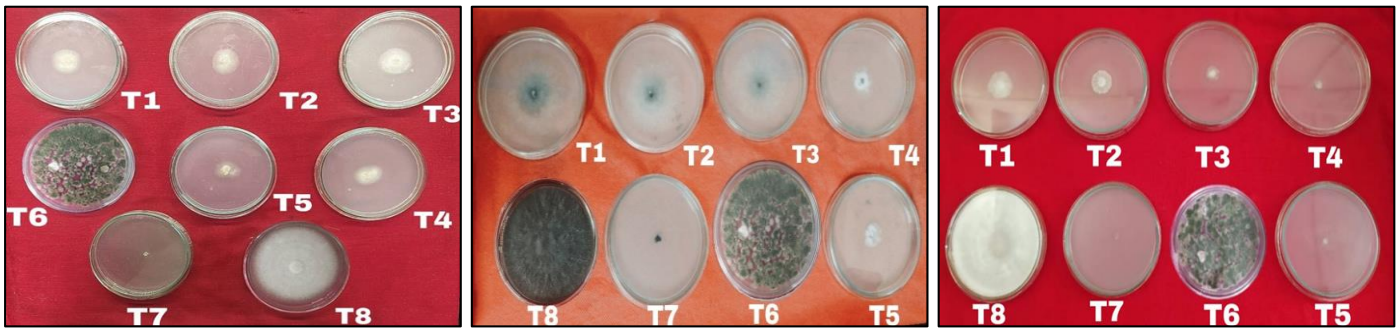
Table 1: Efficacy of oligo chitosan against *Fusarium oxysporum* f. sp. *ciceri*, *Rhizoctonia bataticola* and *Sclerotium rolfsii* under *in vitro* condition.

S. No	Treatment details	*Fusarium oxysporum* f. sp. *ciceri*		*Rhizoctonia bataticola*		*Sclerotium rolfsii*	
		Mycelial growth (mm)	Percent Inhibition (%)	Mycelial growth (mm)	Percent inhibition (%)	Mycelial growth (mm)	Percent inhibition (%)
T1	Oligo chitosan @ 50 ppm	20.33	77.41 (61.62)	64.00	28.88 (32.51)	23.93	73.41 (58.96)
T2	Oligo chitosan @ 75ppm	18.33	79.63 (63.17)	58.86	34.59 (36.03)	17.93	80.07 (63.49)
T3	Oligo chitosan @ 100 ppm	16.07	82.15 (65.01)	52.33	41.85 (40.31)	16.00	82.22 (65.06)
T4	Oligo chitosan @ 125ppm	11.07	87.70 (69.47)	28.06	68.81 (56.05)	11.93	86.73 (68.65)
T5	Oligo chitosan @ 150 ppm	9.07	89.93 (71.49)	22.13	75.40 (60.27)	08.33	90.74 (72.29)
T6	Oligo chitosan @100	8.27	90.81	7.46	91.70	06.00	93.33

	ppm+*Trichoderma viride*		(72.36)		(73.27)		(75.04)
T7	Carbendazim 12% WP + Mancozeb 63% WP @ 0.1%	00	100 (90)	0.00	100 (90)	00.00	100 (90)
T8	Control	90	0	90	0	90	0
	SE(m) ±		0.11		0.14		0.12
	CD @ 1%		0.46		0.59		0.48

Table 2. Evaluation of oligo chitosan against *Fusarium oxysporum* f. sp. *ciceri*, *Rhizoctonia bataticola* and *Sclerotium rolfsii* under glass house conditions in pot culture.

	Treatment details	***Fusarium oxysporum* f. sp. *ciceri***		***Rhizoctonia bataticola***		***Sclerotium rolfsii***	
		Disease incidence (%)	**Disease control (%)**	**Disease incidence (%)**	**Disease control (%)**	**Disease incidence (%)**	**Disease control (%)**
T1	Oligo chitosan @ 50 ppm	63.33 (52.78)	36.67	80.00 (63.44)	23.33	66.67 (54.78)	33.33
T2	Oligo chitosan @ 75ppm	53.33 (46.92)	46.67	73.33 (59.00)	26.67	53.33 (46.92)	46.67
T3	Oligo chitosan @ 100 ppm	46.67 (43.08)	53.33	63.33 (52.78)	36.67	43.33 (41.15)	56.67
T4	Oligo chitosan @ 125ppm	43.33 (41.15)	56.67	53.33 (46.92)	46.67	36.67 (37.22)	63.33
T5	Oligo chitosan @ 150 ppm	33.33 (35.22)	66.67	33.33 (35.22)	66.67	33.33 (35.22)	66.67
T6	Oligo chitosan @100 ppm + *Trichoderma viride* @ 1%	13.33 (21.15)	86.67	16.67 (23.86)	83.33	16.67 (23.86)	83.33
T7	Carbendazim 12% WP + Mancozeb 63% WP 0.1%	10.00 (18.43)	90.00	13.33 (21.15)	86.67	10 (18.43)	90
T8	Control	100 (90)	0.00	100 (90.00)	0.00	100 (90)	0
	SE(m) ±	1.82		1.98		1.83	
	CD @ 5%	5.46		5.93		5.49	



*a. Fusarium oxysporum*f. sp. *ciceri* *b. Rhizoctonia bataticola* *c. Sclerotium rolfsii*

Plate 1: Efficacy of oligo chitosan against *Fusarium oxysporum* f. sp. *ciceri*, *Rhizoctonia bataticola* and *Sclerotium rolfsii in vitro*

T1: Oligo chitosan @ 50 ppm
T2: Oligo chitosan @ 75ppm
T3: Oligo chitosan @ 100 ppm
T4: Oligo chitosan @ 150 ppm
T5: Oligo chitosan @ 150 ppm
T6: Oligo chitosan @ 100 ppm + *Trichoderma viride*
T7: Carbendazim 12% WP + Mancozeb 63% WP 0.1%
T8: Control

a. Fusarium oxysporum f. sp. *Cicero* *b. Rhizoctonia bataticola* *c. Sclerotium rolfsii*

Plate 2: Efficacy of oligo chitosan against *Fusarium oxysporum* f. sp. *Ciceri*, *Rhizoctonia bataticola* and *Sclerotium rolfsii* of chickpea in pot culture

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