RESEARCH ARTICLE Legume Research- An International Journal, Volume 48 Issue 12: 2070-2076 (December 2025)

Assessing the Effects of Chitosan on Groundnut (*Arachis hypogaea* L.) Growth and Productivity

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10.18805/LR-5266

ABSTRACT

Background: Groundnut, also known as peanut is a vital crop worldwide, valued for its oil and protein-rich seeds. However, globally the production of groundnut is constrained by a number of biotic and abiotic factors, which significantly reduce yield. Among these, seed borne pathogen plays a major role.In order to manage plant disease and increase the yield, chitosan was used in this study since it is a natural polymer derived from chitin found in crustacean shells.

Methods: Groundnut seeds were treated with chitosan 1.5 and 2.0% along with bio-control agents and carbendazim.Treated seeds were sown in field and observations *viz.,* disease incidence (%), field emergence (%), plant height, flowering characters, yield attributing pod and seed characters were recorded.

Result: The results revealed that no disease incidence was recorded in chitosan treated seeds. Seeds treated with chitosan 2% increased the field emergence and plant height up to 13 and 17 per cent over control, respectively. Chitosan 2% treated seeds-initiated flowers 4 days earlier than the control seeds and also quickly attained the 50% flowering. Apart from this, seeds treated with chitosan 2% increased the pod yield and seed yield ha^{-1} up to 27 and 29 per cent, respectively. In between the biocontrol agents, *Bacillus subtilis* showed an increased yield and yield attributing parameters.

Key words: *Bacillus subtilis*, Chitosan, Groundnut, Growth and yield attributing characters, Seed treatment, *Trichoderma asperellum*.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.), often called peanut, earthnut, monkey-nut, pindar, or poor man's nut, is an annual legume crop produced primarily for its edible seeds. Its place of origin is Brazil and it belongs to the Leguminosae family. It has abundant dietary fibres, vitamins and minerals such as copper, magnesium, potassium, biotin, niacin, folate, thiamine and the antioxidant vitamin E (Hassan *et al.*, 2015). In India, it is cultivated in 6.02 million ha, with a production of 10.24 metric tonnes and a productivity of 1703 kg/ha (INDIASTAT, 2022). One of the significant challenges faced by groundnut farmers is low yield and reduced quality due to various diseases. Among the diseases, seed-borne fungal diseases like collar rot, dry root rot and yellow mould cause the greatest loss. These diseases cause severe seedling mortality, resulting in patchy crop stands, reducing yields from 25 to 40% and compromisenut quality. Most of the groundnut cultivars are prone to these diseases and plant mortality caused by collar rot varies from 9 to 30 per cent (Le *et al.,* 2019). These diseases often necessitate the use of chemical pesticides, but their widespread usage can have detrimental effects on the environment, including soil and water pollution, harm to organisms that aren't the target, the subsequent development of pathogen fungicide resistance andpose health risks to humans.Chitosan is a biologically active substance that helps to protect agricultural plants against pests and diseases. In recent times, efforts have been made to produce chitosan from chitin obtained from fungal cell walls, which represent the second most abundant source of chitin after marine invertebrates (Shahrajabian *et al.*, 2021; Ma *et al.*, 2022).It is harmless to higher organisms and safe for the environment (Kumar, 2000). Chitosan and its oligomers have become viable sources for a variety of applications because they may be used to make biodegradable fungicides to control plant growth and preserve seeds (Alburquenque *et al.*, 2010). Chitosan can be used alone or in combination with other polymers as an antibacterial agent due to its strong antioxidant and antimicrobial properties (Qin and Li, 2020). Vasudevan *et al.* (2002) reported that chitosan formulation application may improve root and shoot length and grain production in rice. With this context, the main objective of the present study was to

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How to cite this article: Malarkodi, K., Divya, V., Natarajan, K., Navamaniraj, K.N., Ganesan, K., Bhaskaran, M., Vigneshwari, R., Selvi, D.T. and Kavitha, S. (2025). Assessing the Effects of Chitosan on Groundnut (*Arachis hypogaea* L.) Growth and Productivity. *Legume Research.* **48(12):** 2070-2076. doi: 10.18805/LR-5266.

Submitted: 28-10-2023 **Accepted:** 19-04-2024 **Online:** 24-07-2024

studythe influence of chitosan on growth, flowering and yield attributing pod and seed characters of groundnut.

MATERIALS AND METHODS

Chitosan antifungal activitytest and its effect on seed quality

Different concentrations of chitosan *viz.,* 0.01, 0.05, 0.1, 0.5, 1.0, 1.5 and 2.0% along with biocontrol agents*Trichoderma asperellum* and *Bacillus subtilis* were tested against *Aspergillus niger* and *A. flavus* under *in vitro* and groundnut seeds were treated with above chitosan concentrations and tested for its effect on seed quality parameters. The results revealed that chitosan 2% showed greater inhibition against *Aspergillus niger* and *A. flavus* followed by chitosan 1.5%. With respect to seed quality parameters, seeds treated with chitosan 2% increased the seed quality parameters *viz.,* speed of germination, germination (%), root and shoot length, dry matter production and vigour index followed by seeds treated with chitosan 1.5%. From this experiment, best treatments *viz.,* chitosan 1.5 and 2% were selected for further study.

Effect of chitosan on growth and yield parameters of groundnut

Genetically pure seeds of groundnut cv. CO 7 procured from the Regional Research Station, Virudhachalam (Tamil Nadu), India were used as base material for this study. The best concentration of chitosan against *Aspergillus* spp. *viz.,* 1.5 and 2% were chosen for this experiment along with biocontrol agents and carbendazim. This experiment was carried out at the Agricultural College and Research Institute, Eachangkottai, Thanjavur (Tamil Nadu) during 2022-2023. Experiment was conducted by adopting randomized block design (RBD) with four replications in each by following recommended package of practices.

Treatment details

T_1 - Seeds treated with Chitosan 1.5%.
T_2 - Seeds treated with Chitosan 2%.
T_3 - Seeds treated with *Trichoderma asperellum* (4 g kg^{-1}).
T_4 - Seeds treated with *Bacillus subtilis* (10 g kg^{-1}).
T_5 - Seeds treated with Carbendazim (2 g kg^{-1}).
T_6 - Control (Untreated seeds).

Disease incidence (%)

The number of plants infected by collar rot and afla root on 25 days after sowing were counted, the disease incidence was computed and expressed in percentage (Chaudhary *et al.*, 2003).

$$\text{Disease incidence (\%)} = \frac{\text{No of diseased plants}}{\text{Total number of plants}} \times 100$$

Field emergence (%)

After 10 days of sowing, the number of seedlings that had germinated was counted.The following formula was used to compute the field emergence and expressed in percentage (Maurya *et al.*, 2014).

$$\text{Field emergence (\%)} = \frac{\text{No. of seedings emerged}}{\text{Total number of seeds sown}} \times 100$$

Growth parameter

Plant height (cm)

The height of each plant was measured at 15, 30 and 45 days after sowing by measuring from the base of the stem to the top of the terminal leaf. The average measurements were expressed in centimetre.

Flowering parameters

Number of days to flower initiation

In each plant, number of days required for initiation of first flower bud was recorded. The average measurements were then reported in whole number.

Number of days to 50% flowering

Number of days taken for 50% of the plants to initiate their first flowering was recorded in each treatment and replication. The mean values were reported in whole number.

Number of flowers $plant^{-1}$

The total number of flowers produced by a plant in ten randomly selected plants in each treatment and replication from days to first flower initiation to 75 days after sowing were recorded. The mean values were computed and expressed in whole number.

Number of pegs $plant^{-1}$

The plants that were used to record the number of flowers $plant^{-1}$ were used expressed in whole number.

Yield attributing pod characters

Pod set (%)

The plants that were used to record the number of pegs $plant^{-1}$were used to record the peg to pod set percentage. It was computed using the following formula and expressed in percentage.

$$\text{Pod set (\%)} = \frac{\text{Total number of pods plant}^{-1}}{\text{Total number of pegs plant}^{-1}} \times 100$$

Number of filled pods $plant^{-1}$

The plants that were used to record the peg to pod set percentage were used to count the numberof filled pods $plant^{-1}$. The mean values were computed and expressed in whole number.

Number of un-filled pods $plant^{-1}$

The plants that were used to record the numberof filled pods $plant^{-1}$ were used to count the numberof un-filled pods $plant^{-1}$. The mean values were computed and expressed in whole number.

Pod yield ha^{-1} (kg)

Pod yield ha^{-1} was computed based on pod yield m^{-2} and mean values were expressed in kg.

Yield attributing seed characters

Seed yield ha^{-1} (kg)

Seed yield ha^{-1} was calculated based on seed yield m^{-2} and mean expressed in kg.

100-seed weight (g)

100 seeds from each treatment and replication were weighed using electronic balance and mean values were expressed in gram.

Experimental design and data analysis

The experiment was conducted using a Randomised Block design (RBD). Four replicates of each treatment were performed. The data was statistically analysed following the procedure outlined by Panse and Sukhatme (1985). Data were analysed by using OPSTAT software.

RESULTS AND DISCUSSION

Disease incidence (%)

Disease incidencepercentage showed a significant difference among the various seed treatments. No disease incidence was observed in both chitosan concentrations. Seeds treated with carbendazim recorded lowest disease incidence of 3.5 per cent whereas control recorded highest disease incidence of 9.6 per cent. In between the biocontrol agents, *Bacillus subtilis* recorded lowest disease incidence of 4.8 per cent (Table 1).

This result was in conformation with Zohara *et al.* (2019) who found that in cucumber chitosan treatment enhanced the seedling resistance to *Phytophthora capsici* in different concentrations (0, 125, 250 and 500 ppm). Under greenhouse circumstances, Jogaiah *et al.* (2020) observed that plants pretreated with chitosan at 2.5 mg mL^{-1} exhibited a considerable increased disease resistance of 66.6 percent against powdery mildew disease. The induced resistant plants had considerable deposition of lignin, callose and H_2O_2. In particular, defense-responsive enzymes were increased in chitosan-primed plants, including polyphenol oxidase, phenylalanine ammonia-lyase, peroxidase and glucanase. De Genring *et al.* (2023) found that the application of chitosan at a concentration of 0.4% led to a significant reduction in the size of lesions on petunia leaves which is caused by *Botrytis cinerea*, with a decrease of up to 60 per cent compared to the control. Chitosan seed treatment offered 37 per cent protection, foliar spray to seedlings at 2, 7 and 14 days offered wide-ranging protection from 64 to 69 per cent and the combination of seed treatment and foliar spray offered 71 per cent protection against downy mildew disease in pearl millet (Sharathchandra *et al.*, 2004). It might be due to when chitosan is delivered into plant tissues, it frequently agglutinates at the penetration sites and has two key effects. The first step is to isolate the location of the penetration by creating a physical barrier that stops the pathogen from migrating and entering other healthy tissues. This process resembles the abscission zones frequently seen on plants inhibiting the spread of various necrotrophic diseases. Chitosan has the capacity to bind with a variety of substances and start the wound-healing process quickly (Hirano *et al.*, 1999). Plants exposed to chitosan exhibit hypersensitive reactions and programmed cell death (PCD) (Vasil'ev *et al.*, 2009).

Field emergence (%)

Field emergence percentage showed a significant difference among the different seed treatments. Chitosan 2% recorded highest field emergence percentage of 94 followed by chitosan 1.5% (92%) which was on par with each otherwhereas, control seeds recorded lowest emergence of 82 per cent (Table 1). This was confirmed with the results of Saharan *et al.* (2016) who found that in maize, the treatment with chitosan boosted the activity of hydrolytic enzymes such as α-amylase and protease and assisted in the quick mobilisation of food stores and their breakdown, which in turn promoted the emergence and vigour of seedlings. Peanut seeds treated with a low concentration of chitosan increased seedling emergence as well as higher levels of indole acetic acid and gibberellic acid (Zhou *et al.*, 2002).

Table 1: Effect of seed treatments on disease incidence (%), field emergence (%) and plant height (cm) of groundnut cv. CO 7.

Treatments	Disease incidence (%)	Field emergence (%)	Plant height (cm)		
			15 DAS	30 DAS	45 DAS
Chitosan 1.5%	0.0 (0.00)	92 (73.47)	26.4	36.6	54.4
Chitosan 2.0%	0.0 (0.00)	94 (75.93)	27.8	38.9	56.2
Trichoderma asperellum (4 g kg^{-1})	5.7 (13.80)	89 (70.70)	24.7	34.9	52.8
Bacillus subtilis (10 g kg^{-1})	4.8 (12.65)	93 (74.76)	25.9	36.2	53.4
Carbendazim (2 g kg^{-1})	3.5 (10.75)	85 (66.90)	23.3	34.1	51.3
Control	9.6 (18.06)	82 (64.99)	20.6	32.4	48.4
Mean	3.9 (9.21)	89 (71.12)	24.8	35.5	52.8
SEd(±)	0.19	2.44	0.83	1.15	1.52
CD (P = 0.05)	0.41	5.25	1.79	2.46	3.26

(Figures in parentheses are angular transformed values. DAS- Days after sowing).

Growth parameter

Plant height (cm)

Seed treatment with chitosan 2% recorded highest plant height of 27.8, 38.9 and 56.2 cm at 15, 30 and 45 DAS, respectively. The lowest plant height of 20.6, 32.4 and 48.4 cm was recorded by control seeds at 15, 30 and 45 DAS, respectively (Table 1).

Mondal *et al.* (2013) observed similar results, showing that foliar application of chitosan increased plant height, number of leaves, length, width and area of leaves in mung bean. Chitosan used as a soil drench or seed treatment increased plant development in the tomato (Algam *et al.*, 2010). The growth-enhancing benefits of chitosan are linked to its bioactive and biocompatible characteristics (Al Hetar *et al.*, 2011) and also some other factors like it leads to an increase in the activity of key enzymes involved in nitrogen metabolism, such as nitrate reductase, glutamine synthetase and protease, which in turn, promotes enhanced photosynthesis and consequently, plant growth (Gornik *et al.*, 2008; Mondal *et al.*, 2012). Additionally, chitosan has the ability to stimulate the synthesis of plant hormones like gibberellins. Furthermore, it fosters growth through certain signalling pathways associated with auxin production, using a tryptophan-independent pathway (Uthairatanakij *et al.*, 2007; El-Bassiony *et al.*, 2014). Ozkurt and Bektas (2022) found chitosan treatment enhances growth-related factors (root and shoot diameters, above and below-ground biomass, number of leaves and branches and plant height), photosynthetic indicators (chlorophyll a and b, total carotenoid content) and other factors in tomato.

Flowering parameters

Earlier flower initiation was observed in chitosan treatment irrespective of concentration. 4 days earlier flower initiation was occurred in chitosan 2% compared with control and also chitosan treatment quickly attained the 50% flowering. Sathiyabama and Manikandan (2018) also reported that copper-chitosan nanoparticles recorded early onset of flowering in finger millet. These results are likely attributed to the presence of growth-promoting hormones, such as GA3 and auxins, the soil alkalizing properties and the supplementation of various macro and micronutrients. These factors might have induced the activation of early flowering genes like *ELF1, ELF2* and *ELF3*.

Table 2: Effect of seed treatments on flowering parameters of groundnut cv. CO 7.

Treatments	Number of days to flower initiation	Number of days to 50% flowering	Number of flowers plant^{-1}	Number of pegs plant^{-1}
Chitosan 1.5%	23	25	105	67
Chitosan 2.0%	22	24	127	68
Trichoderma asperellum (4 g kg^{-1})	24	26	88	55
Bacillus subtilis (10 g kg^{-1})	24	26	98	59
Carbendazim (2 g kg^{-1})	25	27	66	44
Control	26	28	58	39
Mean	24	26	90	55
SEd(±)	1.22	1.28	3.06	2.12
CD (P = 0.05)	2.63	NS	6.58	4.57

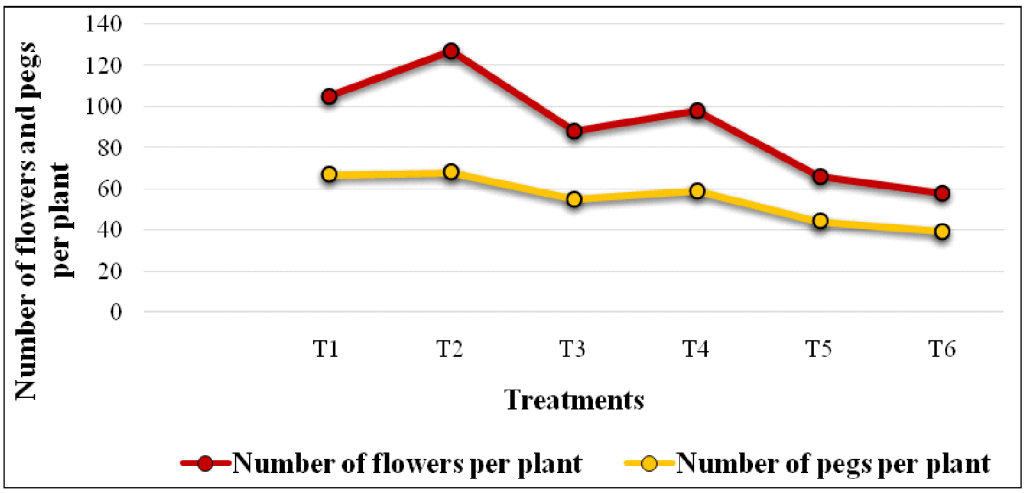

Fig 1: Effect of different seed treatments on number of flowers and pegs plant^{-1} of groundnut cv. CO 7.

Seeds treated with chitosan 2% produced the highest number of flowers (127) followed by chitosan 1.5% (105). Control seeds recorded lowest number of flowers (58) (Table 2). Similarly, seeds treated with chitosan 2% produced the highest number of pegs (68) followed by chitosan 1.5% (67) which was on par with each other. The lowest number of pegs (39) were recorded in control seeds (Fig 1). This was in accordance with Mondal *et al.* (2012), who found that the application of chitosan at concentrations ranging from 25 to 75 mg l^{-1} resulted in the highest numbers of effective flower initiations and flowers per plant in summer tomato. Utsunomiya and Kinai (1994) observed early flowering and increased number of flowers in passion fruit (*Passiflora edulis*) through the application of chitosan as a soil drench.

Yield attributing pod characters

No significant difference was observed in pod set percentage due to different seed treatments. Seeds treated with chitosan 2% produced the highest number of filled pods (30) followed by chitosan 1.5% (25). In between the biocontrol agents, seeds treated with *Bacillus subtilis* produced 21 number of filled pods. Control seeds recorded lowest number of filled pods of 6 Like-wise, seeds treated with chitosan 2% produced the lowest number of un-filled pods $plant^{-1}$ of 6. Highest number of un-filled pods $plant^{-1}$ of 11 was recorded by control seeds (Table 3). Chitosan 2% recorded the highest pod yield ha^{-1} of 4028 kg followedby chitosan 1.5% (3840 kg) which was at par with each other. In between the biocontrol agents, *Bacillus subtilis* recorded the pod yield ha^{-1} of 3560 kg followed by *Trichoderma asperellum* (3298 kg), both remained statistically at par with each other. Control recorded lowest pod yield ha^{-1} of 2920 kg (Table 3, Fig 2).

Chitosan treatment effectively enhanced the yield parameters of groundnut. This might be due to initial seedling quality and vigour. Islam (2016) reported a positive outcome where the application of chitosan led to various advantageous effects in rice cultivation. This included an earlier onset of primary tiller production, an increase in the number of effective tillers, earlier flowering and maturation, resulting in a higher yield. Additionally, chitosan application

Table 3: Effect of seed treatments on yield attributing pod and seed characters of groundnut cv. CO 7.

Treatments	Pod set (%)	Number of filled pods $plant^{-1}$	Number of un-filled pods $plant^{-1}$	Pod yield ha^{-1} (kg)	Seed yield ha^{-1} (kg)	100-seed weight (g)
Chitosan 1.5%	50	25	8	3840	1936	39.85
Chitosan 2.0%	54	30	6	4028	2075	40.29
Trichoderma asperellum (4 g kg^{-1})	49	17	10	3298	1651	38.37
Bacillus subtilis (10 g kg^{-1})	50	21	9	3560	1771	39.28
Carbendazim (2 g kg^{-1})	45	10	10	3157	1565	37.80
Control	44	6	11	2920	1477	37.20
Mean	49	18	9	3467	1746	38.80
SEd(±)	3.56	1.53	0.88	126.22	102.57	1.44
CD (P = 0.05)	NS	3.28	1.89	269.03	218.62	NS

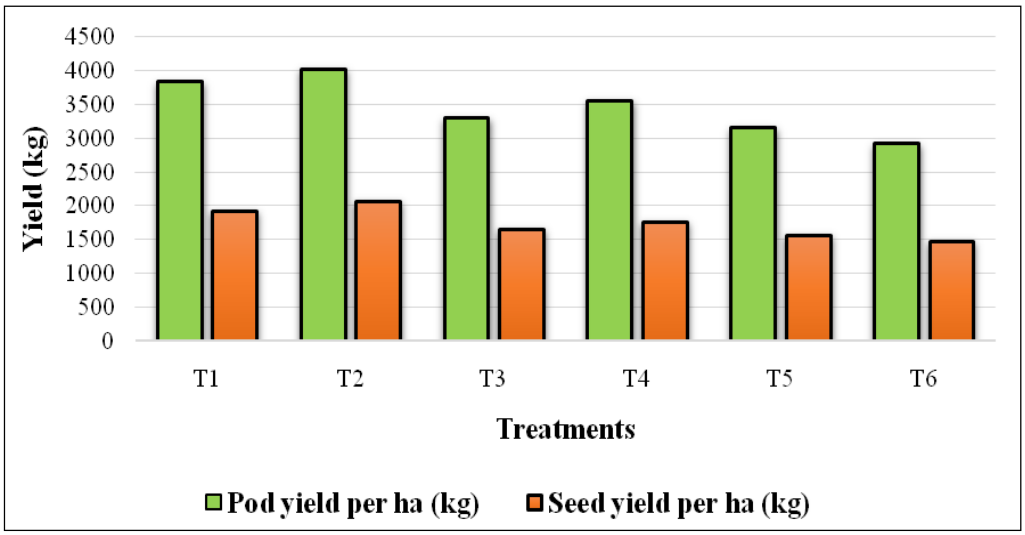

Fig 2: Effect of different seed treatments on pod yield and seed $yield^{-1}$ of groundnut cv. CO 7.

also resulted in increased plant height, a higher number of effective tillers hill^{-1}, greater panicle density m^{-2}, longer panicles and higher yields of both grain and straw compared to the control. The improved seed and biomass yield observed in seeds treated with chitosan can be attributed to early germination and robust growth which results in better crop establishment.Godase *et al.* (2023) found that employing a combination of chitosan treatments, including foliar spraying at a concentration of 200 ppm and seed priming at 2% resulted in increased lablab bean yields when the plants were subjected to water stress conditions.

Yield attributing seed characters:

Due to different seed treatments significant difference was observed in seed yield ha^{-1}. Seeds treated with chitosan 2% recorded the highest seed yield ha^{-1} of 2075 kg followed by chitosan 1.5% (1936 kg) which was on par with each other. The seeds treated with*Bacillus subtilis* recorded the seed yield ha^{-1} of 1771 kg whereas, control seeds recorded the lowest seed yield ha^{-1} of 1477 kg (Table 3, Fig 2). No significant difference was observed in 100-seed weightdue to different seed treatments.

The increase in seed yield in chitosan-treated seeds may be linked to consistent and enhanced germination, vigorous seedling growth, the development of a well-established root system and efficient subsequent growth (El-Tanahy *et al*., 2012).

CONCLUSION

Chitosan unique properties have captured the attention of researchers and farmers alike, as it presents a natural and sustainable solution for improving agricultural productivity. Study revealed that in chitosan treatment, no disease incidence was occurred. Chitosan 2% improved the emergence percentage and plant height up to 13 and 17 per cent over control, respectively and vigorous seedlings were produced in chitosan treatment. 4 days earlier flower initiation was occurred in chitosan 2% compared with control and also chitosan treatment quickly attained the 50% flowering and increased the flower numberplant^{-1}, peg number plant^{-1}. Apart from this, seeds treated with chitosan 2% increased the pod yield and seed yield ha^{-1} up to 27 and 29 per cent, respectively. Hence, seed treatment with chitosan 2% couldimprove the growth and yield of groundnut.

Conflict of interest

All author declare that they have no conflict of interest.

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