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Effect of chitosan on plant growth and mycorrhization in *Zea mays*

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In recent years, use of chitosan as soil amendment material has increased and has been among the effective approach to promote plant growth and pest control. Arbuscular Mycorrhizal Fungi (AMF) is a natural bio-fertilizer which helps plant to absorb water and nutrient more efficiently. In the present study different concentrations of chitosan (i.e 0.1, 0.5, 1.0, 5.0 and 10 mg/ml) were used on *Zea mays* along with AMF *Rhizophagus irregularis*. The chitosan treatment was given with an interval of 14 days for a period of 120 days. A positive response in shoot biomass has been observed in plants colonized with Arbuscular Mycorrhizae (AM) treated with chitosan. Chitosan was found to have no effect on mycorrhization until the concentration of chitosan was increased to 10 mg/ml, where mycorrhization decreased.

Key words : Arbuscular mycorrhizal fungi, chitosan, spore viability, root colonization, spore density, *Zea mays*.

INTRODUCTION

Nowadays to uplift the agricultural production and to fulfil the country's needs, use of chemicals in the form of pesticides and fertilizer has increased. These pesticides and chemical fertilizer spraying can provide positive response by reducing pest, weed and also by providing variety of nutrients required for the growth of the plant, but it can cause soil infertility, contamination in the environment and have tremendous risk to human health. (Aktar *et al*., 2009; Zhang *et al*., 2018). To maintain the soil fertility, use of natural environmentally friendly product (e.g., chitosan) has attracted much attention.

Chitosan is a naturally occurring bio-polymer obtained from alkaline deacetylation of chitin present in exoskeleton of crustaceans like shrimp, lobster and crab and is also found in fungi and insects and is soluble in nearly all diluted acids with the pH < 6.5. It is biodegradable, environmentally approachable for agriculture and non-toxic to human health. Chitosan has various eliciting activities such as ligninsynthesis, callose formation and phytoalexin production, resulting in defensive responses against microbial infections in host plants (Cabrera *et al*., 2010). It has been found that chitosan could control several pre- and post-harvest diseases of many crop plants and has positive effect on enriching biodiversity in the rhizosphere (Oliul and Taehyun, 2017).

Arbuscular mycorrhizal fungal association is the most ubiquitous root associated plant symbiosis which reduces several abiotic stresses (such as drought, salinity, heavy metal and temperature) and increases the nutrition and water uptake which causes increase in plant yield (Syib'li *et al*., 2013). AMF symbiosis has shown increased accumulation of chlorophyll and can also enhance the biosynthesis of valuable phytochemicals in edible plants, proving that AMF has a significant potential for improving crop yield (Begum *et al*., 2019). The present study aims to find out the influence of chitosan on mycorrhization and plant growth of *Zea mays.*

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MATERIALS AND METHODS

Chitosan solution preparation

Chitosan was prepared using Shrimp shells by Hossain and Iqbal (2014). Chitosan solution was prepared by dissolving 1 g of Chitosan in 1 L of 0.05% ascorbic acid under constant stirring for two days. From this stock solution (1 mg/ml), various dilutions were prepared using 0.05% ascorbic acid. The final pH of the solution was adjusted to 6.5 with NaOH.

Plant materials and treatment

Maize seeds were surface sterilized with sodium hypochlorite germinated and planted in 3:1 ratio of soil: sand (total 5 kg) in pots. After the seedlings turned 14 days, *Rhizophagus. irregularis* was inoculated by adding 0.1g of pure inoculum containing approximately 2000 spores. After 10 days of addition of AM inoculum, plants were treated with chitosan solution, which was applied every two weeks for a period of 120 days. For control, plants colonized by AMF were taken without addition of chitosan. The experiments were conducted in replicates of three.

Determination of root colonization by staining

Root colonization was determined by using Philips and Hayman method (1970) after 60 and 120 days. Intra-radical structures of *R. irregularis* were stained with Trypan blue. Harvested roots were gently washed with water, cut into 1cm pieces. Root sections were incubated in 10% KOH at 900C for 90 min followed by three washing steps with distilled water. 1N HCl treatment was given for 3 minutes at room temperature and again washed with distilled water.0.05% Trypan blue was added and kept overnight. The excess stain was removed next day and Lactophenol was added to the roots. The roots were observed under compound microscope and percentage of root colonization was calculated by Read *et.al*, (1976).

Spore extraction and quantification

Spore extraction was done by Gerdeman and Nicolson method (1963). After 120 day treatment with chitosan spores were extracted and counted, 50 gm of soil sample was taken and mixed with one litre of water, about 20 ml of diluted liquid detergent added to it and left for half an hour until all the aggregates dispersed to leave a uniform suspension. The detergent washes away the soil particles attached to the spores. The suspension was passed through 150 µm, 75 µm, 45 µm, and 25 µm sieves .The residues in the respective sieves were collected in beakers carefully with approximately 100ml of water. This water which contains spores was filtered on the circular whatman filter paper with grids drawn on them. The spores were taken in four petri-dishes and observed under motic dissecting microscope for AM fungal spores. Spore number was counted by Gaur and Adholeya method (1994). Then the spores were picked up using an injection needle. The spores were mounted on clear glass slides using polyvinyl alcohol lacto phenol Glycerol (PVLG), covered with cover slips and observed under microscope.

Viability of spores treated with chitosan

Approximately 20 spores were collected from the soil treated with different concentrations of Chitosan in Eppendorf tubes with 1ml of distilled water. 1ml of 0.05% MTT 3-(4, 5 dimethylthiazol-yl-2, 5-diphenyl-2H-tetrazolium bromide) was added and kept for 40 hours at room temperature. The spores were taken on the slide and checked for change in the colour of spores. The spores which turned their colour from yellow to bluish/ purplish were considered as viable spores and the spores which turned black or remained unstained were non-viable.

Measured variables: Root fresh weight (g), shoot fresh weight (g), plant height (cm) measured after 120 days.

Statistical Analysis

All statistical analysis were performed using WASP version 2.0, (Web Based Agricultural Statistics Software Package), ICAR, Old-Goa, Goa, India. Mean Values are given with their standard deviation. The significance in differences between the treatments was determined using the analysis of variance (ANOVA) followed by Tukey Kramer test at $p \leq 0.05$.

RESULTS AND DISCUSSION

The effect of chitosan on plant growth and mycorrhization in *Zea mays* was studied. Chitosan was synthesized from the shrimp shells and was used as soil drenching in combination with AM Fungi *Rhizophagus irregularis*. The analysis of shoot fresh weight, root fresh weight, plant height, root colonization, was carried out twice i.e., after 60 days and 120 days. Spore density and viability was checked after 120 days.

The results in Table 1 shows the fresh weight of shoot, root and plant height after 60 and 120 days. It is observed that shoot and root fresh weight considerably increased when the concentration of chitosan increased from 0.1mg/ml to 5mg/ml compared to the control which was mycorrhizal. Similar results were obtained by Fatima El Amerany *et al.* (2020) . Further the root fresh weight was high in mycorrhizal plants (control) compared to the chitosan treatments. The root fresh weight was 33.43±5.42gm in control and 21.09±1.91gm in 10mg/ml chitosan treatment. Based on ANOVA test there was no significant difference observed at P d" 0.05 within the treated plant and control after 120 days. However, plant height showed increase up to the chitosan concentration 5mg/ml. There was not much increase in height in the concentration 10mg/ml.

Spore density in the rhizosphere soil (after 120 days) was less in chitosan treatment with concentration of 10 mg/ml in soil as compared to the control. The root colonization percentage was95.55±7.70, highest in chitosan treatment 0.1mg/ml, but was less compared to the control 97.77±3.85 after 120days.However, these results of spore density and percentage root colonization were not significantly different from each other (Table 2). The colonization was in the form of hyphae, arbuscules, vesicles and intra- radical spores.

It appears from the data that shoot fresh weight and plant height showed best result at chitosan concentration of 5mg/ml. Root colonization and spore density was best seen at chitosan concentration 0.1mg/ml.

The viability of spores was tested with MTT after 120 days in all the treatments and the spores were found to be viable in all concentrations of chitosan and control.The colour of the spores which turned bluish/ purplish is an indication of their viability (Marleau *et.al*., 2011).

Therefore, it can be concluded from this experiment that chitosan does not considerably affect AM fungi colonization in root as well as sporulation at concentrations higher than 0.1mg/ml. It is also

Table 1: Shoot fresh weight, Root fresh weight and plant height 60 and 120 DAS

Concentration	Shoot fresh weight (gm)		Root fresh weight (gm)		Plant height (cm)	
	60 Days	120 Day	60 Days	120 Days	60 Days	120 Days
Control	49.61^{c} ±1.60	63.17^{b} ±11.22	28.73^{a} ±4.80	33.43^{*}±5.42	71.87^{*}±3.02	104.20^{c} ±5.34
Chitosan 0.1 mg/ml	51.00^{c} ±6.20	73.33^{ab} ±5.89	27.04^{ab} ±4.65	32.81^{*}±4.97	79.85^{*}±0.43	105.45^{c} ±8.77
Chitosan 0.5 mg/ml	53.11^{bc} ±1.04	75.95^{a} ±5.31	24.82^{abc} ±4.11	23.95^{*}±7.13	80.19^{*}±1.71	109.54^{c} ±5.95
Chitosan 1.0 mg/ml	57.93^{b} ±2.21	77.84^{a} ±3.91	20.513^{c}±1.56	24.96^{*}±8.28	82.41^{*}±2.25	123.71^{ab} ±4.54
Chitosan 5.0 mg/ml	65.17^{a} ±3.75	83.20^{a} ±3.42	20.16^{c} ±2.10	22.53^{*}±3.77	84.62^{*}±3.83	130.73^{a} ±6.66
Chitosan 10.0 mg/ml	65.11^{a} ±1.64	76.73^{a} ±2.04	19.66^{c} ±3.65	21.09^{*}±1.91	75.79^{*}±3.64	114.72^{bc} ±2.55

Table 2: Percent Root colonization, type of colonization & Spore density

Concentration	% Root Colonization (60 DAYS)	Type of Colonization (60 DAYS)	% Root Colonization (120 DAYS)	Type of Colonization (120 DAYS)	Spore density (50 gm soil) (120DAYS)
Control	88.88^{a}±13.87	H,A,V	97.77^{a} ±3.85	H,A,V,I	114.00*±6.24
Chitosan 0.1 mg/ml	82.21^{ab}± 13.33	H,A,V	95.55^{a} ±7.70	H,A,V,I	110.66*±10.06
Chitosan 0.5 mg/ml	75.55^{ab}±3.851	H,A,V	91.11^{a} ±10.183	H,A,V,I	106.00*±6.55
Chitosan 1.0 mg/ml	77.77^{bc}±7.69	H,A,V	93.33^{a} ±6.67	H,A,V,I	104.33*±7.76
Chitosan 5.0 mg/ml	64.44^{bc}±10.18	H,A,V	88.88^{a} ±7.69	H,A,V,I	89.66*±23.62
Chitosan 10.0 mg/ml	46.66^{c} ±20.00	H,A	71.10^{b} ±16.77	H,A,V,I	77.33*±20.23

Legend: H=Hyphal colonization, V=Vesicular colonization, A=Arbuscular colonization, I=Intra-radical spore. Values are mean of three replicates. * Spores 50g^{-1} of soil. ± indicates standard deviation.Data with different letters in the same column are significantly different at P d" 0.05. * indicate not significant

observed that chitosan treatment increased the shoot fresh weight and height of the plant upto 5mg/ml concentration. Similar work was carried by Cabrera *et.al*. (2010), where improvement in plant height was obtained by using chitosan on blueberry plant.

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