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Original Article

Foliar application of Chitosan modulates the morphological and biochemical characteristics of tomato

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Abstract

Chitosan is a very important linear polysaccharide used in agricultural and horticultural practices primarily for plant defense and yield increase in recent decade. This proposed research study was conducted at the Ornamental Nursery Horticulture Department, The University of Agriculture Peshawar to investigate the response of foliar application of chitosan on vegetative growth and quality characteristics of tomato during the year 2016 using experimental design Complete Randomized Design (CRD) with one factor repeated three times. Tomato plants were sprayed with different chitosan concentration (0, 30, 60, 90, 120 mg/1000ml) with 25 days interval under plastic tunnel condition. The analysis of results showed significant effect of chitosan concentration on all growth and quality attributes of tomato. The results of foliar application of chitosan on different growth (number of leaves plant^{-1}, number of branches plant^{-1} and plant height) and quality traits of tomato (chlorophyll content, , dry matter content, total nitrogen content and total phosphorus content) were found significantly higher @ 90 mg/1000ml than other concentration of chitosan. On the basis of above results, it is concluded that tomato plant could be sprayed with chitoson @ 90 mg/1000ml for achieving maximum growth and quality of tomato under plastic tunnel condition.

Keywords: Chitin, Chitosan, Phytoalexins, Natural defense, Adaptability

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Introduction

Tomato (*Lycopersicon esculentum* Mill.), a subtropical plant, originated in Western South America, is an important worldwide consumed crop that includes more than 3,000 species. In particular, the section of the *Lycopersicon* genus *Solanum* consists of 13 species or subspecies: the cultivated tomato, *Solanum lycopersicum*, which is the only domesticated species, and 12 wild species (such as *S. chmielewskii*, *S. habrochaites*, *S. pennellii*, and *S. pimpinellifolium*) (Anonymous, 2008). Tomato is the most popular and widely consumed vegetable crop grown in outdoor fields, greenhouses and net houses of world including Pakistan. Tomato fruits are mostly used as a salads or cooked in sauces, soup and meat or fish dishes. It can be processed into purées, juices and ketchup. It grow best in relatively cool, dry climate for achieving better yield and quality. However, it is adapted to a wide range of climatic conditions from temperate to hot and humid tropical. It require average monthly temperature of 20-25°C (Haque et al., 1999) and start fruit setting abundantly when the night temperature is in the range of 15-20°C and the day temperature at about 22-25°C (Kalloo, 1985).

Lycopene is a powerful natural antioxidant that exhibits the highest physical quenching rate constant with singlet oxygen among dietary carotenoids (Di Mascio et al., 1989; Stahl and Sies, 1996; Agarwal and Rao, 2000), thus reducing the risk of several important pathologies of our times, such as cardiovascular diseases and some cancer typologies (Clinton, 1998; Rao, 2006).Recent studies suggest that tomatoes contain the antioxidant lycopene, the most common form of carotenoid, which markedly reduces the risk of prostate cancer (Kucuk,. 2001). Because the mineral composition`of tomato depends on the amount and type of nutrients taken from the growth medium, such as soil, it is necessary that adequate amount of nutrients should be available for the production and nutrient content of tomatoes. While inadequate amount of nutrient availability can show deficiency symptom and influence the yield and quality of tomato (Sainju et al.,1999).

Among the promising approaches for inducing plant disease resistance and reducing damage from fungal pathogens and stimulate the immunity of plant is chitosan. Chitosan is a natural polysaccharides, which consists of a copolymer of N-ace tyl-D-glucosamine and D-glucosamine residues, linked by β- 1,4 glycosidic bonds (Khin et al., 2006) .Chitosan is derived from chitin, a polysaccharide found in the exoskeleton of shellfish such as shrimp, lobster, and or crabs and cell walls of fungi (Wojdyla, 2001). Very few efforts were done to study the effect of chitosan on plant growth and its productivity which applied mainly as antimicrobial to protect plants from soil pathogenics or to increase the storability of fruits such as strawberry (Vargas et al., 2006). The results of application of chitosan revealed that chitosan can increase the immunity of plant (Patkowska et al., 2006), antimicrobial effect of chitosan on pathogenics and microparasitic fungi (Abou Sereih et al., 2007; Palma-Guerrero et al., 2008) promote root system (Gornik et al., 2008), increase the plant health, the photosynthetic pigments and consequently the plant production (Chibu and Shibayama, 1999; Khan et al., 2002; Gornik et al., 2008). Few efforts were done to study the effect of chitosan on plant growth, development and productivity, which is mainly, attributed stimulation of plants immunity against microorganisms like bacteria and (ChunYan,2003; Patkowska,2006; Górnik, 2008). Moreover, plants treated with chitosan may be less prone to stress evoked by unfavorable conditions,such as drought, salinity, low or high temperature (Lizárraga-Paulín, 2011; Pongprayoon, 2013). The objectives of this study was to evaluate the response of appropriate concentration of chitosan as foliar application on growth and quality characteristics of tomato under tunnel condition.

Material and Methods

The experimental trial was conducted at Ornamental nursery, Horticulture Department The University of Agriculture Peshawar, Pakistan during the year 2016 using experimental design Complete Randomized design (CRD) having one factor repeated three times. The aim of this study was to investigate the performance of chitosan on morphological and biochemical attributes of tomato. The seeds were sown in the plastic tubes containing leaf mold for the best germination of seeds. After 3 weeks of germination the seedlings having 2-3 leaves and proper uniform sizes were transplanted in plastic tunnel with planting geometry of 100×50cm respectively. The experiment consisted of four chitosan spray i.e Water application (control), ii) Chitosan @ 30mg/1000ml, iii) Chitosan @ 60mg/1000ml (iv) Chitosan @ 90mg/1000ml (v) Chitosan @ 120mg/1000ml. Chitosan of different application were sprayed with an interval of 25 days. Foliar sprays were done in the morning. The land was prepared one month before transplantation of seedlings and FYM @ 20 tons.hac^{-1} were applied to whole plot of $6m^2$ and the whole plot were divided into sub plots of each having an area of $2m^2$. All cultural practices were done uniformly for all treatments such as weeding, hoeing and irrigation.

Preparation of chitosan solution

The different concentrations of chitosan (30mg, 60mg, 90mg, 120 mg/1000ml) were dissolved in 1 liter distilled water and sprayed on tomato plant in each replication.

Growth and quality attributes

The number of leaves , number of branches, number of flowers $plant^{-1}$ of randomly selected five plant were calculated manually.Plant height (cm) of randomly selected five plants were recorded by using measuring tape Young but fully expanded leaves of selected plants were picked for leaf tissue analysis..The mineral content of leaves especially total nitrogen (N) was determined by the micro Kjeldahl method. A portion 0.05 g of the dry matter was taken and subject to acid digestion with 4 mL of digester mixture (1 L of concentrated sulfuric acid + 25 g of potassium sulfate + 10 g of red mercury oxide + 25 mL of copper sulfate saturated solution); subsequently, the result of the digestion was subjected to a distillation process with 25 mL of 50% sodium hydroxide. The distillation was captured in 30 mL of 2.2% boric acid and three drops of bromocresol green and methyl red, then titrated with 0.025 N sulfuric acid. The phosphorus (P) was determined by a spectrophotometric method, with an Ammonium molybdate reagent and Aminonaphthol sulphonic acid solution. The reading was performed with a UV-Vis spectrophotometer model Helios Epsilon at a wavelength of 640 nm. (AOAC, 1990)

Statistical analysis

An appropriate procedure for Complete Randomized Design (CRD) using statistical software Statistix 8 (Statistix® 8 Analytical Software, 2003) were used for analyzing experimental data and their means were compared using LSD test when F value were significant at 5% level of significance (Basit et al., 2019)

Results

Number of leaves per plant

The analysis of data indicated that maximum number of leaves per plant (65.33) was noted with foliar application of chitosan at 90mg/1000ml followed by number of leaves per plant (59.66, 43.66) with foliar application of chitosan (30,120 mgL^{-1}). While the lowest number of leaves per plant (40.66) were noticed over without chitosan application (Figure 1).

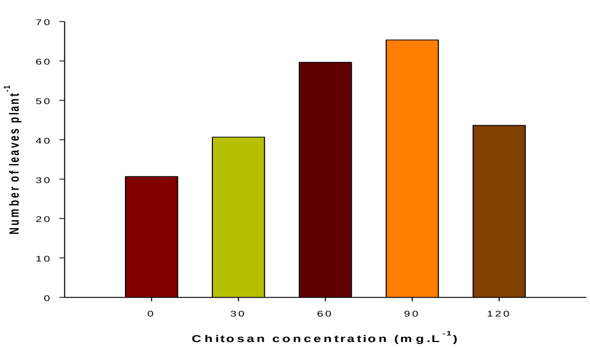

Fig 1. Number of leaves of plant^{-1} as affected by foliar application of chitosan concentration

Total chlorophyll content (SPAD)

Plant height exhibited a significant ($P \leq 0.05$) difference in all treatments (Fig 2).The foliar application of chitosan treated plants @ 90mgL^{-1} had higher total chlorophyll content (35.23 SPAD) followed by total chlorophyll content (33.86,24.96 SPAD) with foliar application of chitosan (120,60 mg/1000ml).While least total chlorophyll content (21.36 SPAD) were noticed in untreated plants.

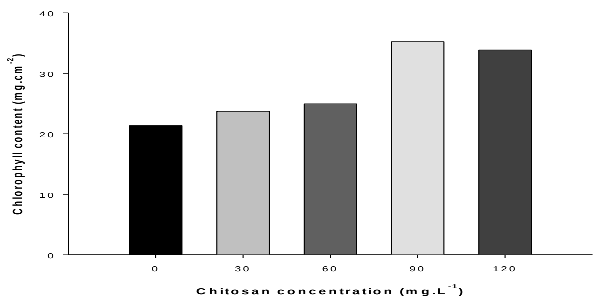

Fig 2: Chlorophyll content of tomato plant as affected by foliar application of chitosan concentration

Number of branches per plant

Number of branches per plant were significantly affected by chitosan application (Fig 3). Maximum number of branches plant^{-1} (12) were observed with foliar application of chitosan at 90mgL^{-1} which was statistically at par with number of branches plant^{-1} (9.66, 9) with foliar application of chitosan (60,120 mgL^{-1}).The untreated plant produced minimum number of branches plant^{-1} (9.66).

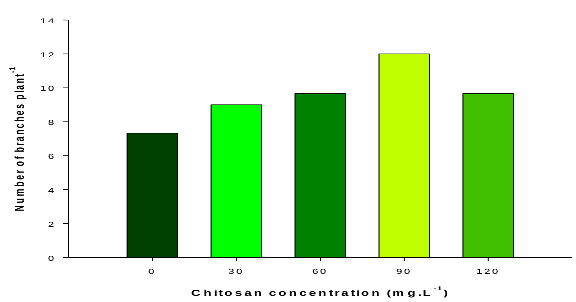

Fig 3. No. of branches plant^{-1} as affected by foliar application of chitosan concentration

Plant height (cm)

Plant height of tomato plant significantly affected by foliar application of chitosan (Fig 4). Foliar application of chitosan @ 90mg/1000ml produced taller plants (37cm) that was at par with plant height (32, 21.33cm) recorded in plant sprayed with chitosan @ 60 and 30mg/1000ml.While shorter plants (15.33cm) were noticed in untreated plot.

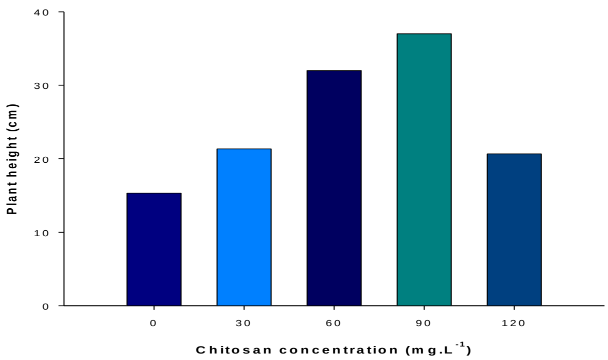

Fig 4. Plant height of tomato plant as affected by foliar application of chitosan concentration

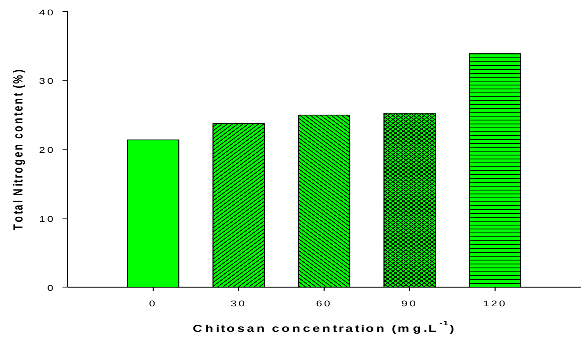

Fig 6. Nitrogen content of tomato leaves as affected by foliar application of chitosan cocentration

Dry matter content (%)

Dry matter (%) of tomato plant varied significantly with foliar application of chitosan (Fig 5). The dry matter content was higher in plant treated with application of chitosan @ 90mg/1000ml (31.03%) as compared to other chitosan application, while untreated plant had lower dry matter content (21.32%).

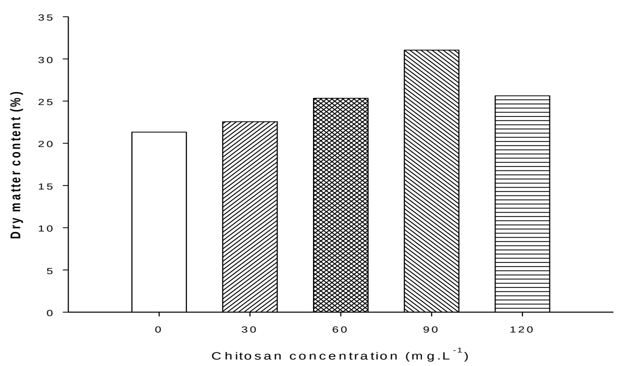

Fig 5. Dry matter content of tomato as affected by foliar application of chitosan concentration

Percent nitrogen content in leaf sample

Nitrogen content in leaf sample was significantly affected by chitosan application (Fig 6).The highest percent nitrogen content (35.23%) was recorded in leaf sample with foliar application of chitosan at 90mg/1000ml which was statistically similar with percent nitrogen content in leaf with chitosan treatment @ 120 mg/1000ml, while lower percent nitrogen content was noted in leaf sample of untreated plant.

Percent phosphorous content in leaf sample

Percent phosphorous content in leaf sample significantly affected with application of chitosan (Fig 7).The percent phosphorous content in leaf sample were significantly increased from 0.60 to 0.82% increasing chitosan application (0 to 90mg/1000ml).

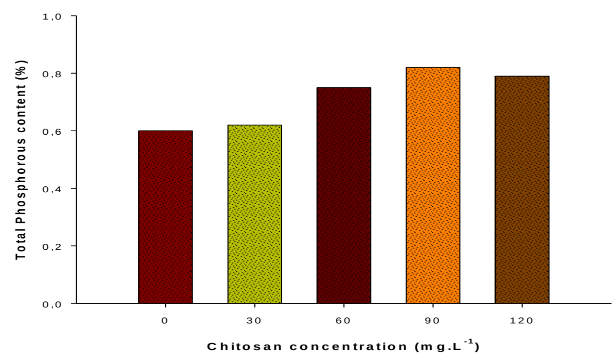

Fig 7. Phosphrous content of leaves as affected by foliar application of chitosan concentration

Discussion

These results indicate tha tapplication of chitosan at early growth stages had tremendous effect on growth and development in tomato. These results are consistent with El-Tantawy (2009), who reported that plant growth and development enhanced by the application of chitosan in tomato. These results indicate that foliar application of chitosan enhanced the biochemical activities.

Our results are consistent with Khan et al. (2002) who reported that application of chitosan increased Pn in leaves of maize and soybean. Again, El-Tantawy (2009) reported that application of chitosan increased photosynthetic pigment thereby the Pn increased. Chitosan has many important bio-molecule that increases plant growth and development (Khan et al., 2002; Chibu and Shibayama, 2003; Gornik et al., 2008). It is reported that chitosan has molecular signals that serve as plant-growth promoters. The stimulating effect of chitosan on plant growth may be attributed due to availability and uptake of water and essential nutrients through adjusting cell osmotic pressure, and reducing the accumulation of harmful free radicals by increasing antioxidants and enzymatic activities (Guan et al., 2009) or may occur due to increment in the main enzymatic activities of nitrogen metabolism (nitrate reductase, glutamine synthetase and protease) and also improved the transportation of nitrogen (N) in the functional leaves which enhance photosynthesis process and improved plant growth and development (Mondal et al., 2012). These obtained results are in accordance with findings of El-Tantawy (2009) on tomato, Abdel-Mawgoud et al. (2010) on strawberry. Spraying tomato plants with chitosan increased significantly all the vegetative growth traits of plant as well as the contents of photosynthetic pigments.

Spraying tomato plants with chitosan increased the vigor plants, and this phenomenon was probably connected with bigger resistance of tomato roots fungi pathogenesis which had healthier roots (Borkowski et al., 2007). Fig 1 showed that application of chitosan increased the number of leaves that may be attributed to the increment in internodes (Gornik et al., 2008).

Nitrogen content in leaves occur due to presence of amino components in chitosan or higher ability of plant to absorb nitrogen from the soil during chitosan degradation. Our results are supported by El-Tanahy et al. (2012) that chitosan enhance the N, P and K content in leaves of cowpea plant. Dzung et al. (1999) reported that foliar application of chitosan on mungbean significantly increased plant growth and yield attributes such as plant height, leaf area plant^{-1} and number of flowers plant^{-1} grown in greenhouse. Chitosan application improved vegetative growth, leaf content of N and K and yield components (number and weight) of strawberry plants (Abdel-Mawgoud, 2010). Nitrogen content in the leaves of tomato improved with chitosan application due to presence of nitrogen which enhanced the synthesis of protein, nucleic acid and protoplasm formation. That's in role induce cell division and initiate meristematic activity for producing more tissues and organs. Thus, plant growth could be effected by nitrogen amount (Marschner, 1995; Najm et al., 2012). Also, it could be attributed to increase the uptake of nitrogen and its associated role in chlorophyll synthesis which enhance process of photosynthesis and carbon dioxide assimilation (Jasso- Chaverria et al., 2005).These results agreed with those reported by Demir et al. (1996) on spinach, Gabr et al. (2001) on sweet pepper.

Similarly the application of chitosan induced the synthesis of plant hormones such as gibberellins. Furthermore, it enhanced growth by some signaling pathways related to auxin biosynthesis via a tryptophan independent pathway (Uthairatanakij et al., 2007). These results are consistent with Khan et al. (2002) who reported that application of chitosan increased Phosphorous in leaves of maize and soybean. Again, El-Tantawy (2009) reported that application of chitosan increased photosynthetic pigment thereby the phosphorous content increased. In the last few years there has been a growing interest in the use of chitosan, algae and effective microorganism for enhancing the growth of many economic medicinal crops. The activity of these compounds is often attributed to provide mineral nutrients to plants that improves plant productivity (Yakhin et al., 2016) or to increase plant productivity via phytohormones, which influences the plant's ability to control its hormone biosynthesis. Dzunga et al., (2011) on coffee mentioned that chitosan increased the mineral uptake and stimulated seedling growth. On Phaseolus vulgaris, chitosan had a positive effect in enhancing shoot and root length, fresh & dry weights of shoots and leaves area (Sheikha, 2011). On freesia the chitosan resulted in higher chlorophyll content and higher No. of inflorescence shoot and length (Salachna and Zawadzińska, 2014). On mung bean found that branch and leaf number/plant, leaf area/plant, total dry mass/plant, photosynthesis and No. of pods/plant increased significantly with chitosan at 50 ppm (Mondal et al., 2012). On *Ocimum basilicum* stated that foliar application of chitosan at 400ppm increased plant growth (Malekpoor et al., 2016).

Conclusion

Results showed that preharvest foliar spray of chitosan not only enhanced the mineral content in leaves and resistance to cold temperature but also enhanced the growth characteristics of tomato. It was also concluded that tomato plants could be sprayed with chitosan @ 90 mg/1000ml for improving the growth and quality attributes of tomato under plastic tunnel condition.

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Contribution of Authors

Hussain I: Conducted the study and analysed the data
Ahmad S: Conducted the study and analysed the data
Ullah I: Evaluated the results, wrote the manuscript of the study and reviewed the manuscript
Ahmad I: Planned the experimental design of the study
Alam M: Reviewed the manuscript
Khan S: Planned the experimental design of the study, analysed the data and approved the final manuscript
Ayaz S: Planned the experimental design

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