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RESEARCH PAPER

Optimization of chitosan level to alleviate the drastic effects of heat stress in cucumber (*Cucumis sativus* L.)

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Key Message: This study reveals the potential of chitosan to alleviate the drastic effects of heat stress on cucumber genotypes and optimization of chitosan for its effective and economical use.

Abstract: Heat stress is a major concern during cucumber production. To explore its production potential, chitosan could play a vital role in alleviation of heat stress. This study was planned to evaluate the potential of chitosan in cucumber growth where it is more effective under high temperature with two factorial under Completely Randomized Design (CRD). Four genotypes were previously screened out; two were tolerant (L3466, Desi-cucumber) and two were sensitive (Suyo Long and Poinsett). These genotypes were grown in the growth room having a normal temperature (28°C/22°C day/night). One day before heat treatment (40 °C/32 °C day/night), chitosan foliar spray with different concentrations (0, 50, 100, 150, 200, 250, 300 ppm) was applied. It was found that 200 ppm of chitosan level revealed significantly better results on growth index (seedling length of shoot and root, the mass of fresh and dry seedlings and the number of leaves per seedling) and physiological index (chlorophyll contents and electrolyte leakage). It was also found that heat sensitive genotype Suyo Long gave the maximum seedling electrolyte leakage (74.03%) followed by Poinsett (69.35%) at control (0 ppm chitosan). The lowest seedling electrolyte leakage (28.85%) was noted in Desi-cucumber followed by L3466 (30.16%) with foliar application of 200 ppm of chitosan. So, chitosan spray at 200 ppm explored the maximum potential to alleviate the effect of heat stress in cucumber genotypes.

Keywords: Abiotic stress, Chitosan, Cucumber, Heat stress, Optimization

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Introduction

Cucumber (*Cucumis sativus* L.) has been playing a vital role in food security. Its world production is 80.6 MT (Food and Agricultural Organization Statistics [FAOSTAT], 2016). Although, it is warm season crop yet it is sensitive to heat stress (Zhang et al., 2012). Its annual production is 71.7 MT globally (Khater, 2017). Cucumber is native to subtropical and temperate zones and its best growth and development is observed at 15-32 °C. However, high temperature above threshold level deteriorates cucumber yield and quality (Zhao et al., 2011). Characterization or screening of various genotypes of the species against heat stress is inevitable at seedling level (Shaheen et al., 2016; Sita et al., 2017) as heat stress is the main hindrance in agriculture production (Schauberger et al., 2017).

Agriculture and global warming are directly correlated. The global temperature increased by 0.5 °C in the last hundred years. The global temperature would elevate by 1.8 °C to 4°C during the next century (Intergovernmental Panel on Climate Change [IPCC], 2014). The current climatic model predicts that environmental temperature would increase by 1.1-6.4 °C if the CO_2 level doubles (Kim et al., 2007). Global warming is becoming a sever issue (Haworth et al., 2018). The high temperature is a principal cause of unwanted alterations in the growth, development, and physiology of plants (Shaked et al., 2004). The reproductive phase is more sensitive as compared to the vegetative phase because there are high losses at this stage (Meehl & Tebaldi, 2004). Heat stress proved to be the damaging effects during the pollination process in chilies (Shaked et al., 2004), comparable results were observed in bell pepper (Thuy & Kenji, 2015), bean plant growth, development and yield (Omae et al., 2012), and tomato yield and quality (Golam et al., 2012). When plants suffer from heat stress, they enable the development of reactive oxygen species that ultimately produce the oxidative stress (Hasanuzzaman et al., 2012). It causes alterations in the expression of genes that control heat tolerance potential (Shinozaki & Yamaguchi-Shinozaki, 2007). In response, the plants contest for the endurance of heat stress, such as adjustment by changes in gene expression which becomes a cause of heat tolerance to some extent (Moreno & Orellana, 2011). To avoid such situations in plants, foliar applications of

osmo-protectants, osmolytes, phytohormones, polyamines, signaling molecules, and major nutrients or trace elements have been successfully applied (Waraich et al., 2012). The seedling stage is mostly affected by heat stress in different vegetables (Rasheed et al., 2011: Chen et al., 2016). Different genotypes of different crops have their specific optimum temperature range within that range, the performance of their physiological and morphological process is maximum. Outside these optimum temperatures, the crops fail to grow. Above the threshold level of the temperature range, plants fail to achieve their normal functions (Fahad et al., 2017). Heat stress causes photosynthetic acclimation and influence developmental patterns. Research work on cucumber exposed that heat stress caused physiological damage to membrane lipids and caused in lipid peroxidation (Li et al., 2007).

Chitosan is a linear polysaccharide that exists naturally in various crustaceans e.g. shells of crabs, shrimps, krill, within insect's exoskeleton and in some micro-organisms e.g. fungi, algae, yeast in the form of chitin (Wojdyla, 2001). It is commercially prepared by reacting crustacean shells with alkali, normally NaOH is used. It is a plant growth enhancer and it functions like plant growth regulators (PGRs) (Uthairatankij et al., 2007). It has been used as a natural seed treatment (Orzali et al., 2018). Its coating on fruits and vegetables enhanced the shelf life and quality (Shah & Hashmi, 2020; Cui et al., 2020) but a study verified that its foliar application is more effective than seed treatment (Janmohammadi et al., 2014). It is an environmental friendly bio-pesticide that enhances ability of plants to survive against fungal infections. As biodegradable materials, chitosan is found in abundance and as decomposed molecules of chitin in soil and water (Linden et al., 2000). Chitosan has found to be much effective in alleviation of drought and heat stress in root cuttings of grapevine (Gornik et al., 2008), it enhanced growth and yield in lentils under drought stress (Janmohammadi et al., 2014), increased plant height, leaf number, relative growth and yield of okra (Mondal et al., 2012). It substantially enhanced yield of strawberry plant (Mukta et al., 2017). Its efficiency depends on its molecular weight and concentration as well as degree of deacetylation in alleviation of abiotic stress (Cho et al., 2008). Chitosan has auspicious future scope in the progress of sustainable agriculture, food production and alleviation of food security (Malerba & Cerana, 2016). It has been found to be effective in horticultural crop production (Hidangmayum et al., 2019) as it enhances plant growth by improving plant defense mechanisms (Akter et al., 2018). The aim of present research was to mitigate the heat stress in cucumber. To explore morphological and physiological changes in cucumber plant due to exogenous application of chitosan and its effects on growth under hot environment.

Materials and Methods

Seeds of the selected four genotypes i.e. two heat tolerant (L3466, Desi-cucumber) and two heat sensitive (Suyo Long and Poinsett) in previous experiment (Ali et al., 2019) were sown in plastic pot at (28 °C/22 °C day/night) in growth room having automated heating and cooling units. 14 hours' day (lights turned on) 10 hours (lights turned off). Sand was used as a growing medium. Half strength Hoagland's solution was used as nutrition. Growth room was lightened up by 10000 lux light intensity to provide a suitable environment for photosynthesis with 65% humidity level. When seedlings were one-month old, temperature was raised daily by 2 °C to avoid osmotic shock, until the highest temperature (40 °C/32 °C day/night) was achieved. To enhance the dissolution of chitosan in water, it was dissolved in water by adding 0.1 molar $C_2H_4O_2$ (at room temperature, 12 hours with stirring). One-week after application of high temperature (40/32 °C with 14 hours' day/10 hours' night), various chitosan levels were sprayed on foliage of seedlings. While the plants which were considered as a control sprayed with distilled water without chitosan. One day before heat treatment, chitosan levels (0, 50, 100, 150, 200, 250, 300 ppm) were applied as a foliar spray. Then one week after chitosan applications, the following parameters were studied.

Growth parameters

Numbers of leaves were calculated manually for plants of each variety and the average was calculated. Shoot length was measured using a foot ruler (four plants per treatment). The stem was spread out on the ruler to ensure a straight orientation and average was calculated. Root length was measured using a foot ruler, cutting the root and separating them from the stem. Roots were measured of the longest root hairs. Finally, average was calculated. Mass of fresh seedlings was measured by the digital weighing balance in grams and average was calculated. After measuring the mass of fresh seedlings, these were enclosed in a paper bag and labeled. These bags were kept in the plant drying oven (Memmert-110, Schawabach, Germany). Samples were placed at 72 °C for twenty hours. After the prescribed period, bags were taken out and dry weight was recorded by weighing balance in grams.

Physiological parameters

Chlorophyll contents (SPAD value)

Chlorophyll contents (SPAD value) of leaves of randomly selected 4 plants per pot were measured by the help of SPAD mater (CCM-200plus Bio- Scientific USA).

Electrolyte leakage (EL) (%)

Electrolyte leakage of leaf cells was used as an assessment of the cell membrane stability (CMS). For this purpose, randomly selected 4 plants per pot were used for measuring electrolyte leakage following the method of Farkhondeh et al. (2012) with a few modifications. After washing the leaves with deionized water, 0.3 g of leaf samples were placed in tubes which had 15 mL of deionized water and incubated for two hours at 25 °C. After that electrical conductivity of the solution (L_1) was

determined. Samples were then autoclaved at 120 °C for twenty minutes and the final conductivity (L_2) was calculated after equilibration at twenty-five degrees Celsius. Leaf electrolyte leakage (EL) was measured by the following equation:

$$EL\ (\%) = \frac{L1}{L2} \times 100$$

Statistical analysis

It was two factor factorial experiment (different genotypes and chitosan levels) with four replications under completely randomized design (CRD). Analysis of variance technique was employed by Fisher's analysis and significance among treatment means were compared by using Tuckey Honestly Significant Difference Test (HSD) at $P \leq 0.05$.

Results

Influence of chitosan on morphological attributes

Shoot length

Chitosan significantly ($P \leq 0.05$) enhanced the shoot length (cm) of cucumber genotypes as compared to non-treated plants (control) (Table 1). The genotype L3466 revealed maximum shoot length (15.25 ± 0.48) when chitosan was applied at 200 ppm, followed by Desi-cucumber (heat tolerant genotype) at the same chitosan level. The minimum shoot length (6.40 ± 0.90) was observed in Poinsett (a heat sensitive genotype) at 50 ppm of chitosan application, followed by Suyo Long (6.95± 0.80) when 150 ppm of chitosan was applied. Furthermore, it was noted that chitosan, when sprayed at 50, 100 and 150 ppm, didn't affect shoot length of seedling of cucumber genotypes and was not significantly different with chitosan non-treated plants irrespective of genotypes. On overall basis, regardless of chitosan application genotypes performed in descending order regarding shoot length i.e. L3466 (13.20), Desi-cucumber (10.64), Suyo Long (8.21) and least in Poinsett (7.70). There was non-significant interaction when the combined response of genotype vs chitosan foliar application (G x CHT) was observed (Table 1). This indicates that effect of chitosan in shoot length is consistent with the genotypes. Chitosan application significantly ($P \leq 0.05$) enhanced root length (cm) of the cucumber genotypes over control (Table 2).

Root length

It was observed that chitosan significantly ($P \leq 0.05$) enhanced root length (cm) of cucumber genotypes as compared to non-treated plants (control) (Table 1). Combined data analysis showed that the interaction of genotype and chitosan application (G X CHT) was significant (Table 1). The genotype L3466 revealed maximum root length (15.33 ± 0.50) when chitosan was applied at 200 ppm, followed by Desi-cucumber (12.43 ± 0.50) at 200 ppm of chitosan application. The minimum root length (7.00 ± 1.00) was revealed in when chitosan was foliarly applied at 100 ppm, followed by Poinsett (7.60 ± 0.39) at the dose 300 ppm of chitosan by recording root length of 7.60 ± 0.39. Furthermore, it was noted that chitosan, when sprayed at 50 and 250 ppm, didn't affect root length and was not significantly different with chitosan non-treated plants irrespective of genotypes. On overall basis, regardless of chitosan application genotypes performed in descending order regarding root length i.e. L3466 (11.83 ± 0.56), Desi-cucumber (10.20), Suyo Long (8.87) and least in Poinsett (8.10) (Table 3).

Fresh mass

Analysis of variance exposed a significant ($P \leq 0.05$) influence of chitosan application on the mass of fresh seedlings (Table 1). The interaction (G x CHT) was non-significant (Table 1). Desi-cucumber gave the maximum mass of fresh seedlings of (17.23 ± 0.52) when chitosan was applied at 200 ppm followed by L3466 with the mass of fresh seedlings (14.80 ± 0.38) with chitosan application of 200 ppm. The lowest mass of fresh seedlings (3.09 ± 0.13) was noted in Poinsett with foliar application of chitosan at 150 ppm (Table 4). On overall basis, regardless of chitosan application genotypes performed in descending order regarding i.e. fresh mass Desi-cucumber (13. 85), L3466 (6.29), Poinsett (4.62) and least in Suyo Long (4.41). Significant ($P \leq 0.05$) results were observed for the mass of dry seedlings (g) when the foliar application was done on cucumber genotypes under consideration.

Dry mass

Chitosan significantly ($P \leq 0.05$) enhanced dry mass of seedlings (g) of cucumber genotypes as compared to non-treated plants (control) (Table 1). The interaction (G x CHT) was non-significant between the mass of dry seedlings and chitosan application (Table 1). Maximum mass of dry seedlings (1.02 ± 0.05) was observed in Desi-cucumber at 200 ppm followed by same genotypes (0.87 ± 0.07) at 150 ppm. The lowest mass of dry seedlings (0.16 ± 0.03) was recorded in Suyo Long with foliar application of chitosan without chitosan application, followed by Poinsett genotype (0.23 ± 0.05) when no chitosan was applied. On overall basis, regardless of chitosan application genotypes performed in descending order regarding dry mass i.e. Desi-cucumber (0.82), L3466 (0.49), Poinsett (0.36) and least in Suyo Long (0.26) (Table 5).

Number of leaves

There was a significant difference ($P \leq 0.05$) in cucumber genotypes under consideration regarding number of leaves per plant (Table 1). There was non-significant interaction between the various concentration of chitosan and genotypes (Table 1). It was revealed that the maximum number of leaves per plant (8.13 ± 0.52) were found in Desi-cucumber, after that it was seen in L3466 (8.00 ± 0.41) both at 200 ppm also responded

efficiently to foliar application of chitosan. While the minimum number of leaves per plant was noted in heat sensitive genotypes Poinsett (4.13 ± 0.28), followed by Suyo Long (4.44 ± 0.18) both at control (without chitosan application). On overall basis, regardless of chitosan application genotypes performed in descending order regarding number of leaves i.e. L3466 (7.55), Desi-cucumber (7.05), Suyo Long (5.90) and least in Poinsett (5.53) (Table 6).

Influence of chitosan on physiological attributes

Chlorophyll contents

Chitosan significantly ($P \leq 0.05$) improved chlorophyll contents (SPAD units) of cucumber genotypes as compared to non-treated plants. (Table 1). Combined data analysis revealed that interaction (G x CHT) was non-significant for the of chitosan vs genotypes response (Table 1). L3466 gave the maximum chlorophyll contents (37.25 ± 1.32) with chitosan having a concentration of 200 ppm, followed by Desi-cucumber at the same chitosan concentration. The lowest chlorophyll contents (11.31 ± 0.33) were observed in non-treated plants of Suyo Long (heat sensitive genotype), followed by Poinsett (18.81 ± 0.45). Furthermore, So, a non-significant difference was found in treated vs non-treated plants regarding chlorophyll at 50 ppm. On overall basis, regardless of chitosan application genotypes performed in descending order regarding i.e. chlorophyll contents Desi-cucumber (33.70), L3466 (32. 45), Poinsett (25.67) and least in Suyo Long (21.37) (Table 7).

Electrolyte leakage

A significant ($P \leq 0.05$) influence of various levels of chitosan application was observed on leaf electrolyte leakage (%) of heat tolerant and heat sensitive cucumber genotypes (Table 1). The interaction (G x CHT) was also non-significant (Table 1). Suyo Long gave maximum seedling electrolyte leakage (74.03 ± 2.01), while Poinsett stood second with seedling leaf electrolyte leakage of (69.35 ± 4.99), both without chitosan application. The lowest seedling electrolyte leakage (28.85 ± 3.75) was noted in Desi-cucumber, followed by L3466 (30.16 ± 2.70) both with foliar application of 200 ppm of chitosan. However, there was no significant difference at various levels (0 ppm, 50 ppm and 100 ppm chitosan). Furthermore, it was noted that chitosan, when sprayed at 100 ppm, didn't affect electrolyte leakage of seedling of cucumber genotypes and was not significantly different with chitosan non-treated plants irrespective of genotypes. On overall basis, regardless of chitosan application genotypes performed in descending order regarding electrolyte leakage i.e. Suyo Long (60.55), Poinsett (58.47), Desi-cucumber (42.96) and least in L3466 (40.44) (Table 8).

Table 1 Analysis of variance for influence of various levels of chitosan on shoot length, root length, mass of fresh seedlings, mass of dry seedlings, number of leaves, chlorophyll contents and electrolyte leakage of four cucumber genotypes (2 tolerant and 2 sensitive) under high temperature regime (40°C/32°C day/night)

Parameter	*P* value		
	Genotype(G)	Treatment(CHT)	G x CHT interaction
Shoot length	≤0.001	≤0.001	0.567^{NS}
Root length	≤0.001	≤0.001	≤0.05
Mass of fresh seedlings	≤0.001	≤0.001	0.068^{NS}
Mass of dry seedlings	≤0.001	≤0.001	0.089^{NS}
Number of leaves	≤0.001	≤0.001	0.349^{NS}
Chlorophyll contents	≤0.001	≤0.001	0.180^{NS}
Electrolyte leakage	≤0.001	≤0.001	0.097^{NS}

P >0.05 = Non-significant; *P* ≤0.05 = Significant; *P* ≤0.01 = Highly significant, NS = Non-significant

Table 2 Influence of various levels of chitosan on shoot length (cm) of heat tolerant and heat sensitive cucumber genotypes

	Genotypes				
Chitosan	L3466	Desi-cucumber	Suyo Long	Poinsett	Mean
0 ppm	11.35 ± 0.31	9.26 ± 0.25	7.98 ± 0.30	7.28 ± 0.34	8.97^{b}
50 ppm	11.94 ± 0.57	9.50 ± 0.65	6.95 ± 0.80	6.40 ± 0.90	8.70^{b}
100 ppm	11.94 ± 0.57	10.65 ± 0.24	7.23 ± 0.77	7.15 ± 0.48	9.24^{b}
150 ppm	12.93 ± 0.57	10.75 ± 0.46	6.95 ± 0.80	6.40 ± 0.90	9.26^{b}
200 ppm	15.25 ± 0.48	12.58 ± 0.81	9.98 ± 0.56	9.08 ± 0.54	11.72^{a}
250 ppm	14.50 ± 0.41	10.55 ± 0.30	8.98 ± 0.48	8.33 ± 0.37	10.59^{a}
300 ppm	14.50 ± 0.71	11.20 ± 0.52	9.43 ± 0.59	8.98 ± 0.90	11.03^{a}
Mean	13.20^{a}	10.64^{b}	8.21^{c}	7.70^{c}	

Means sharing different letters in a row or in a column are statistically significant ($P \leq 0.05$). The values after ± indicate standard deviations

Table 3 Influence of various levels of chitosan on root length (cm) of heat tolerant and heat sensitive cucumber genotypes

	Genotypes				
Chitosan	L3466	Desi-cucumber	Suyo Long	Poinsett	Mean
0 ppm	12.76 ± 0.49	9.29 ± 0.50	9.24 ± 0.48	7.82 ± 0.38	9.78^{bc}
50 ppm	10.30 ± 1.08	9.00 ± 0.84	8.63 ± 0.49	8.43 ± 0.64	9.09^{bc}
100 ppm	10.48 ± 0.64	9.08 ± 1.11	8.78 ± 0.38	7.00 ± 1.00	8.83^{c}
150 ppm	12.80 ± 0.84	12.10 ± 0.81	8.35 ± 0.66	8.25 ± 0.83	10.38^{b}
200 ppm	15.33 ± 0.50	12.43 ± 0.50	10.38± 0.50	9.13 ± 0.38	11.81^{a}
250 ppm	11.68 ± 0.61	10.03 ± 0.59	8.33 ± 0.50	8.20 ± 0.80	9.56^{bc}
300 ppm	9.53 ± 0.41	9.45 ± 0.62	8.43 ± 0.55	7.60 ± 0.39	8.75^{c}
Mean	11.83^{a}	10.20^{b}	8.87^{c}	8.10^{c}	

Means sharing different letters in a row or in a column are statistically significant ($P \leq 0.05$). The values after ± indicate standard deviations

Table 4 Influence of various levels of chitosan on fresh mass (g) of heat tolerant and heat sensitive cucumber genotypes

	Genotypes				
Chitosan	L3466	Desi-cucumber	Suyo Long	Poinsett	Mean
0 ppm	5.18 ± 0.23	11.94 ± 0.54	3.17 ± 0.51	3.31 ± 0.46	5.90^{c}
50 ppm	5.18 ± 0.50	12.59 ± 0.55	4.46 ± 0.51	3.90 ± 0.59	6.58^{c}
100 ppm	4.60 ± 0.30	12.96 ± 0.57	3.98 ± 0.41	3.14 ± 0.22	6.17^{c}
150 ppm	5.88 ± 0.41	13.30 ± 0.51	4.00 ± 0.61	3.09 ± 0.13	6.57^{c}
200 ppm	9.36 ± 0.43	17.23 ± 0.52	6.50 ± 0.35	7.60 ± 0.34	10.19^{a}
250 ppm	7.02 ± 0.40	14.80 ± 0.38	4.59 ± 0.37	5.42 ± 0.40	8.06^{b}
300 ppm	6.80 ± 0.43	14.10 ± 0.31	5.39 ± 0.59	3.94 ± 0.77	7.56^{b}
Mean	6.29^{b}	13. 85^{a}	4.41^{c}	4.62^{c}	

Means sharing different letters in a row or in a column are statistically significant ($P \leq 0.05$). The values after ± indicate standard deviations

Table 5 Influence of various levels of chitosan on dry mass (g) of heat tolerant and heat sensitive cucumber genotypes

	Genotypes				
Chitosan	L3466	Desi-cucumber	Suyo Long	Poinsett	Mean
0 ppm	0.39 ± 0.04	0.77 ± 0.05	0.16 ± 0.03	0.23 ± 0.05	0.39^{d}
50 ppm	0.44 ± 0.05	0.77 ± 0.05	0.34 ± 0.03	0.31 ± 0.02	0.44^{cd}
100 ppm	0.45 ± 0.02	0.79 ± 0.05	0.28 ± 0.02	0.35 ± 0.01	0.47^{bc}
150 ppm	0.51 ± 0.02	0.87 ± 0.07	0.24 ± 0.02	0.34 ± 0.02	0.49^{bc}
200 ppm	0.65 ± 0.02	1.02 ± 0.05	0.31 ± 0.02	0.43 ± 0.01	0.60^{a}
250 ppm	0.57 ± 0.05	0.79 ± 0.06	0.34 ± 0.02	0.47 ± 0.05	0.54^{ab}
300 ppm	0.45 ± 0.04	0.77 ± 0.05	0.25 ± 0.03	0.39 ± 0.03	0.47^{bc}
Mean	0.49^{b}	0.82^{a}	0.26^{d}	0.36^{c}	

Means sharing different letters in a row or in a column are statistically significant ($P \leq 0.05$). The values after ± indicate standard deviations

Table 6 Influence of various levels of chitosan on number of leaves of heat tolerant and heat sensitive cucumber genotypes

	Genotypes				
Chitosan	L3466	Desi-cucumber	Suyo Long	Poinsett	Mean
0 ppm	6.88 ± 0.36	5.13 ± 0.36	4.44 ± 0.18	4.13 ± 0.28	5.14^{c}
50 ppm	7.50 ± 0.20	7.00 ± 0.35	6.38 ± 0.24	5.63 ± 0.38	6.63^{ab}
100 ppm	7.50 ± 0.20	7.13 ± 0.23	5.63 ± 0.31	5.63 ± 0.43	6.47^{b}
150 ppm	7.75 ± 0.48	7.75 ± 0.48	6.75 ± 0.25	7.00 ± 0.41	7.31^{a}
200 ppm	8.00 ± 0.41	8.13 ± 0.52	6.50 ± 0.46	5.63 ± 0.24	7.03^{ab}
250 ppm	7.50 ± 0.29	7.13 ± 0.31	5.88 ± 0.38	5.13 ± 0.38	6.45^{b}
300 ppm	7.75 ± 0.48	7.13 ± 0.38	5.75 ± 0.43	5.50 ± 0.35	6.53^{b}
Mean	7.55^{a}	7.05^{b}	5.90^{c}	5.53^{c}	

Means sharing different letters in a row or in a column are statistically significant ($P \leq 0.05$). The values after ± indicate standard deviations

Table 7 Influence of various levels of chitosan on chlorophyll contents (SPAD units) of heat tolerant and heat sensitive cucumber genotypes

	Genotypes				
Chitosan	L3466	Desi-cucumber	Suyo Long	Poinsett	Mean
0 ppm	23.15 ± 1.79	27.62 ± 0.97	11.31 ± 0.33	18.81 ± 0.45	20.22^{d}
50 ppm	31.25 ± 1.65	33.50 ± 1.56	21.50 ±1.56	26.21 ± 0.75	28.13^{bc}
100 ppm	35.25 ± 0.85	37.25 ± 1.32	25.00 ± 1.47	28.00 ± 2.28	31.38^{ab}
150 ppm	36.25 ± 1.38	31.75 ± 2.69	23.25 ± 2.22	20.50 ± 1.70	27.94^{bc}
200 ppm	36.25 ± 1.11	42.75 ± 2.50	26.75 ± 1.65	32.00 ± 2.20	34.60^{a}
250 ppm	32.00 ± 1.29	31.25 ± 2.18	23.50 ± 1.04	25.50 ± 2.33	28.29^{bc}
300 ppm	33.25 ± 1.32	31.75 ± 1.89	21.00 ± 1.23	24.25 ± 2.02	27.56^{c}
Mean	32. 45^{a}	33.70^{a}	21.37^{c}	25.67^{b}	

Means sharing different letters in a row or in a column are statistically significant ($P \leq 0.05$). The values after ± indicate standard deviations

Table 8 Influence of various levels of chitosan on electrolyte leakage (%) of heat tolerant and heat sensitive cucumber genotypes

	Genotypes				
Chitosan	L3466	Desi-cucumber	Suyo Long	Poinsett	Mean
0 ppm	52.60 ± 3.91	46.50 ± 1.53	74.03± 2.01	69.35± 4.99	60.59^{a}
50 ppm	38.24 ± 6.10	48.35± 4.18	65.15± 3.56	62.98± 5.18	53.68^{ab}
100 ppm	52.14 ± 3.61	51.05± 4.55	64.36± 3.95	58.92± 5.27	56.62^{a}
150 ppm	36.95 ± 2.08	47.36± 4.13	57.19± 2.48	54.20± 2.87	48.93^{bc}
200 ppm	37.40 ± 2.66	44.03± 3.17	52.51± 1.80	54.46± 1.81	47.22^{bcd}
250 ppm	30.16 ± 2.70	28.85± 3.75	52.88± 1.20	52.25± 2.29	41.07^{d}
300 ppm	35.63 ± 3.74	34.60± 1.34	57.78± 2.68	56.57± 3.55	46.14^{cd}
Mean	40.44^{b}	42.96^{b}	60.55^{a}	58.47^{a}	

Means sharing different letters in a row or in a column are statistically significant ($P \leq 0.05$). The values after ± indicate standard deviations

Discussion

Use of antitranspirants have been proved to be an effective technique to mitigate the effects of heat stess in horticultural crops recently (Dash et al., 2020; Tonhati et al., 2020). Chito/chitin oligosaccharides has optimistic influence on triggering the resistance mechanism of plants (Zhao et al., 2019). In this study, Chitosan being an antitranspirant was used with various concentrations (0, 50, 100, 150, 200, 250 and 300 ppm) as a foliar spray on cucumber plants. The foliar application (200 ppm) showed the highest potential to mitigate the stress situation giving relief to plant. This has been reported in previous findings that level of chitosan, its mode of application and timing were consided important in the activation of several biological responses for enhancement of productivity in plants grown under heat stress (Malerba & Cerana, 2016) as it has very effective role in osmotic stress (Pongprayoon et al., 2013) which might be due to drought, salinity, cold stress and heat stress (Guan et al., 2009; Lizarraga-Pauli et al. 2011, Jabeen & Ahmad, 2013). Based on morphological (shoot and root length of seedlings, the mass of fresh and dry seedlings and the number of leaves per plant) and physiological (SPAD value and electrolyte leakage) attributes, chitosan proved to be an important antitranspirant to mitigate the drastic effect of heat stress in cucumber.

Heat stress damages chlorophyll contents in plants which cease the photosynthetic process (Wang et al., 2018). Resutls of this study revealed that chitosan significantly improved the cholorophyll contents. In a previous research study, it was reported that optimum dose of chitosan was found to be effective in alleviation of drought and heat stress in root cuttings in grapevine (Górnik et al., 2008). It was observed that chitosan improved the seedling and root length, fresh and dry mass of seedling (Saharan et al., 2016) with chlorophyll contents and reduced the electrolyte leakage from cell membrane in all levels as compared with no application of chitosan. Because cell membrane of plants is much sensitive to heat stress as it consists of mosaic fluid and proteins. Such kind of effects of chitosan has already been revealed when seedling length, the number of leaves per plant and dry mass of seedlings were increased in summer squash by applying foliar spray of chitosan (Ibraheim & Mohsen, 2015) the increase in shoot length might be due to increase in production of gibberellic acid (Pereira et al., 2017). Increase in root length was observed by Zeng and Luo (2012) when they found an increase in root length in the wheat plant by chitosan foliar spray compared with non-treated plants.

It was found that the chitosan treatment improved the stem length of plants, the number of leaves per plant and mass of the

fresh and dry leaves (Shehata et al., 2012). This fact is also indicated recently that chitosan, when applied as foliar spray on basil plants; enhanced height, inflorescence, numberof branches, leaf area index, fresh and dry mass of seedling, roots and shoots (Pirbalouti et al., 2017). It is proved that chitosan is a plant growth enhancer and acts like a plant growth regulator (Uthairatanakij et al., 2007) because it enhanced the stem length, number of leaves, relative growth rate and yield of okra (Pichyangkura & Chadchawan, 2015) under heat stress regimes. It might act as an osmoprotectant to maintain turgor in order to sustain growth index and cell membrane stability. Hence, chitosan application substantially improved the heat tolerance ability in both heat tolerant and heat sensitive cucumber genotypes. It might be due to the fact that chitosan enhances potential by the stimulation of enzymes, antioxidant and metabolic pathways (Singh, 2016).

Conclusion

It could be concluded that foliar application of various concentrations of chitosan (CHT) under consideration upgraded heat tolerance potential of cucumber genotypes, particularly at 200 ppm level. Chitosan application not only improved the heat tolerance ability of heat tolerant but also enhanced thermo-tolerance capacity in sensitive genotypes.

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