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Research Article
Effect of Nano-chitosan on Vegetative Growth, Fruiting and Resistance of Malformation of Mango

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Abstract
Background and Objective: Mango malformation causes about 37% annually losses of mango all over the world. This study investigated the effect of nano-chitosan on vegetative growth, fruit set, yield, fruit quality and resistance of malformation of two different mango cultivars in sensitivity to malformation; Ewais (sensitive) and Zebda (resistant). **Materials and Methods:** This investigation carried out during the two consecutive seasons 2015/16 on mango trees (*Mangifera indica* L.) cvs. Zebda and Ewais, grown in a private orchard at Wadi El-Muluk, Sharkia governorate, Egypt to evaluate the effect of foliar nano-chitosan at 15 February before flowering on vegetative growth, fruit set, yield and fruit quality as well as resistance of malformation. A complete block design (two seasons combined) had used. Two way ANOVA used to compare the samples of different coating treatments. **Results:** Ewais cv. displayed the highest of their tested attributes in most cases as well as fruits number/tree and fruits yield/tree compared with Zebda cv. On the contrary, the content of carotene in leaves and fruit characteristics, as well as resistance to malformation percentage Zebda cv. was highly significant compared with Ewais cv. The obtained results, foliar nano-chitosan treatments increased fruits yield as number of fruit or weight/tree and decreased malformation percentage as well as improving studied attributes especially nano-chitosan 5 mL L^{-1} treatment. **Conclusion:** It is concluded that by using the nano-chitosan as a foliar spray on mango trees with concentration of 5 mL L^{-1} to improve the vegetative growth and fruit quality. Physical and chemical properties increase yield of the trees and showed more resistance of malformation.

Key words: Nano particles, chitosan, malformation, *Mangifera indica*, vegetative growth, ball mill

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most important tropical fruits in the world[1] and one of the most popular fruits in Egypt. The total area of the Egyptian mangoes reached 240804 fed[2].

Mango malformation is the fatal disorder of mango trees, which not only negatively affect plant health, but also reduces yield in many cultivars all over the world. Floral malformation reducing the yield directly because malformed panicles seldom produce fruit rather presents an ugly look as they persist long on the tree[3]. It is an intricate disorder, which linked directly with vegetative growth behavior; causing about 37% annually losses[4]. The seriousness of malformation varies from region to region and with the cultivar[5]. It is widely reported in most of the mango growing countries of the world i.e. India, Egypt, South Africa, Brazil, Sudan, USA, Mexico, Bangladesh and Pakistan. The prevalence of this disorder since long and causing heavy yield losses has attracted many agencies and governments of various mango-growing countries to find out the causes to control this enigmatic malady. A lot of research had done on vegetative growth and malformation of inflorescence but the phenomenon is yet least understood due to much complicated growth systems in mango[6]. Understanding physiology of floral malformation and its relationship with vegetative growth of mango tree is very important as vegetative growth and floral malformation of mango trees vary greatly depending upon variety[7].

Chitosan is a partially deactivated polymer of N acetyl glucosamine obtained through alkaline deactivation of chitin. It consists of a β-(1, 4)-linked-D-glucosamine residue with the amine groups randomly acetylated[8]. The amine and -OH groups endow chitosan with many special properties, making it applicable in many areas and easily available for chemical reactions. Chitosan is safe, non-toxic and can interact with poly anions to form complexes and gels[9,10].

The main objective of this study was to investigate the effect of nano-chitosan on vegetative growth, fruit set, yield, fruit quality and resistance of malformation of two different mango cultivars in sensitivity to malformation; Ewais (sensitive) and Zebda (resistant).

MATERIALS AND METHODS

This investigation was carried out on two mangoes (*Mangifera indica* L.) cultivars Zebda (resistant) and Ewais (sensitive to malformation) in 2015 and 2016 seasons on mango trees grown in sandy soil under drip irrigation at a private orchard at Wadi El-Muluk, Sharkia governorate, Egypt. Trees were 20 years old, planted at 4×6 m apart, grafted on succary rootstocks grown under the common agricultural practices adopted in the area. Eighteen healthy trees, similar in vigor and size, selected to evaluate the effect of foliar spray with nano-chitosan before flowering at 15 February on vegetative growth, fruit set, yield and fruit quality as well as resistance of malformation on mango cultivars Ewais and Zebda.

The experiment included six treatments as follows:

- Zebda trees sprayed with mineral oil 1.5% (Control)
- Zebda trees sprayed with nano-chitosan 2.5 mL L^{-1}
- Zebda trees sprayed with nano-chitosan 5 mL L^{-1}
- Ewais trees sprayed with mineral oil 1.5% (Control)
- Ewais trees sprayed with nano-chitosan 2.5 mL L^{-1}
- Ewais trees sprayed with nano-chitosan 5 mL L^{-1}

Each treatment included 3 replicates. The replicate represented as one tree of every cultivar.

Chitosan nano crystallite powder synthesized by high-energy ball milling. Powder mixture conducted in a 0.4 mini lab planetary ball mill (model DECO-PBM-V-0.4L, Changsha Deco Equipment Co., Ltd., China) to 40 h using ball to powder mass ratio of 8:1 by prof. Dr. Osama M. Hemeda at Central lab., department of physics, faculty of science, Tanta University, Egypt. The microstructure of the sintered samples examined using High Resolution Transmission Electron Microscope (HRTEM) model JOEL EM 2-100. Transmission electron microscope (TEM) imaging showed a spherical, smooth and almost homogenous structure for nanoparticles. In the present study, TEM images (Fig. 1) have shown the morphological properties and surface appearance of nanoparticles, which have nearly spherical shape, smooth surface and size range of about 50-35 nm, which confirm the result of XRD. The size of chitosan nanoparticles, as evident from the TEM images found to be 50 nm. The TEM analysis of chitosan nanoparticles showed uniform size distribution in nanometer range.

Recorded data

Panicle properties and malformation

Panicle length: It measured after 7, 21 and 28 days from full bloom.

Sex ratio at full bloom time: It is calculated as total number of flowers and proportion of male to hermaphrodite flower. The floral malformation calculated as a percentage.

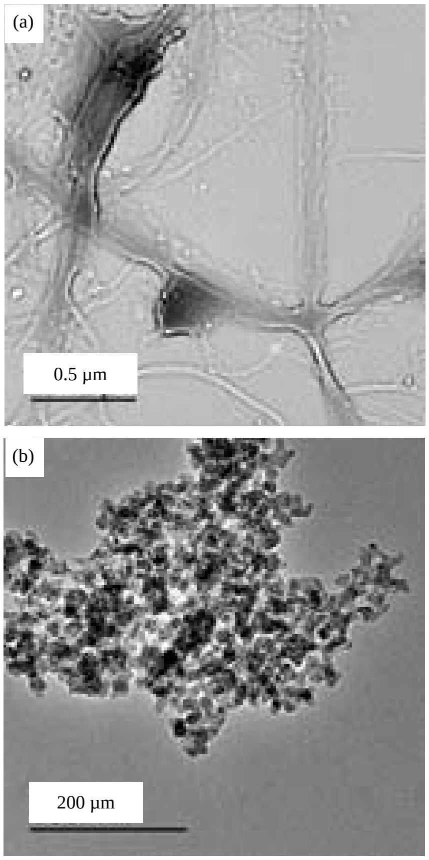

Fig. 1(a-b): TEM micrograph for chitosan, (a) Before milling and (b) After milling

Fruit retention and yield/tree: The number of fruits/panicle counted after 48 days of full bloom and directly pre-harvest. At harvest time, the number of fruits per panicle and per tree counted for each treatment. Tree yield as kg/tree estimated by multiplying the fruits/tree×the average of fruit weight (g)[11].

Leaf physical characteristics and pigments contents: To determine plant growth effects, samples of mature leaves grown on unfruitful shoots randomly taken at harvest date length and width of leaf (cm) measured. In addition, leaf disk samples obtained at harvest to determined leaf tissue contents (mg g^{-1} fresh weight) of chlorophyll a, chlorophyll b, total chlorophyll (a+b) and carotenoids[12].

Leaf mineral contents: Another leaves samples were washed, dried ground and digested using sulphuric acid and hydrogen peroxide[13]. The N, P, K and Zn percentages were determined in the digested solution.

Fruit physical and chemical characteristics: At harvest time, samples of five firm ripe (commercial stage) fruits were taken from each replicate to study fruit length (mm), width (mm), fruit shape index (length/width), total soluble solids (TSS %) by Atago brix N1 Hand-Held Refractometer, 0.0-32.0% Brix, fruit acidity (%) and vitamin C (mg/100 mL juice)[14].

All chemicals used in different determinations obtained from El-Gomhouria for Trading Chemicals and Medical Appliances, El-Sawah, El-Amiria, Cairo, Egypt.

Statistical analysis: Data subjected to the analysis of variance (ANOVA) and a complete block design (two seasons combined) were used[15]. Two way ANOVA used to compare the samples of different coating treatments. Means were tested using LSD, at 5%[16]. Using Duncan's multiple-range test had done by Co-Stat program version 3.

RESULTS

Panicle properties and malformation: Panicle length at 7, 21 and 28 days and sex ratio in Ewais cv. was highly significant compared with Zebda cv., while Zebda cv. was significantly more resistant to malformation percentage than Ewais cv. (LSD = 0.05) as shown in Table 1.

About 5 mL L^{-1} nano-chitosan treatment significantly (LSD = 0.05) increased panicle length at 7 and 28 days than 2.5 mL L^{-1}. As well as, all nano-chitosan treatments increased panicle length at 7 and 28 days than control. Control and 2.5 mL L^{-1} nano-chitosan significantly (LSD = 0.05) increased sex ratio than 5 mL L^{-1} nano-chitosan treatment. Nano-chitosan treatments 2.5 and 5 mL L^{-1} decreased malformation percentage with 38-40% compared with control, respectively.

The interaction between cultivars×nano-chitosan treatments was non-significant in panicle length at 21 and 28 days. The significantly (LSD = 0.05) highest value of panicle length at 7 days was Ewais treated with 5 mL L^{-1} nano-chitosan, while the lowest values were Zebda treated with 2.5 mL L^{-1} nano-chitosan. The significantly highest sex ratio was Ewais×control while the lowest was Zebda×5 mL L^{-1} nano-chitosan and control. In addition, Ewais×control treatment recorded significantly the (LSD = 0.05) highest malformation percentage, while Zebda×2.5 and 5 mL L^{-1} nano-chitosan recoded significantly the lowest malformation percentage.

Table 1: Means of panicle length, sex ratio and malformation percentage of Zebda and Ewais mango cultivars was affected by different concentrations of nano-chitosan

Treatments	Characters				
	Panicle length (cm) at 7 days	Panicle length (cm) at 21 days	Panicle length (cm) at 28 days	Sex ratio	Malformation (%)
A. cultivars					
Zebda	3.7	5.9	12.4	28.5	17.2
Ewais	8.2	11.2	19.5	40.2	29.0
F-test	**	**	**	**	**
B. Nano-chitosan					
2.5 mL L^{-1}	5.5^{b}	8.8^{a}	15.0^{b}	35.3^{a}	19.4^{b}
5 mL L^{-1}	8.4^{a}	8.9^{a}	20.0^{a}	32.4^{b}	18.5^{b}
Control	3.8^{c}	8.0^{a}	12.8^{c}	35.4^{a}	31.4^{a}
F-test	**	NS	**	*	**
LSD at 5%	0.6	-	0.6	2.3	2.4
C. Interaction A×B					
Zebda					
2.5 mL L^{-1}	2.8^{e}	5.5^{a}	11.5^{a}	31.6^{c}	13.0^{d}
5 mL L^{-1}	4.7^{c}	6.8^{a}	16.3^{a}	25.8^{d}	15.3^{d}
Control	3.5^{de}	5.5^{a}	9.3^{a}	27.9^{d}	23.1^{bc}
Ewais					
2.5 mL L^{-1}	8.2^{b}	12.0^{a}	18.5^{a}	39.0^{b}	25.7^{b}
5 mL L^{-1}	12.2^{a}	11.0^{a}	23.7^{a}	38.9^{b}	21.6^{c}
Control	4.2^{cd}	10.5^{a}	16.3^{a}	42.8^{a}	39.6^{a}
F-test	**	NS	NS	*	**
LSD at 5%	0.8	--	--	3.3	3.3

The data in the table expressed as a means of 2015 and 2016 seasons. Means in each column followed by the same letter(s) did not differ at <0.50 according to Duncan's multiple-range test. The data subjected to ANOVA and a complete block design was used. *Significant differences, **High significant differences

Table 2: Means of retention fruit, fruit weight, fruit number/tree and fruit yield/tree of Zebda and Ewais mango cultivars affected by different concentrations of nano-chitosan

Treatments	Characters				
	Retention fruit (No.) at 48 days	Retention fruit (No.) at harvest	Fruit weight (g)	Fruit number/tree	Fruit yield/tree (kg)
A. cultivars					
Zebda	13.22	1.17	455.8	73.5	33.1
Ewais	21.22	1.06	269.4	169.1	45.9
F-Test	**	NS	**	**	**
B. Nano-chitosan					
2.5 mL L^{-1}	22.2^{a}	1.50^{a}	377.4^{a}	122.7^{b}	41.9^{b}
5 mL L^{-1}	19.5^{a}	1.08^{b}	335.9^{b}	139.0^{a}	44.1^{a}
Control	10.0^{b}	0.75^{c}	374.5^{a}	102.3^{c}	32.6^{c}
F-test	**	**	**	**	**
LSD at 5%	2.8	0.28	9.9	3.6	1.9
C. Interaction A×B					
Zebda					
2.5 mL L^{-1}	17.8^{a}	1.50^{a}	484.0^{b}	81.7^{d}	39.5^{c}
5 mL L^{-1}	15.0^{a}	1.00^{a}	382.5^{c}	82.0^{d}	31.4^{e}
Control	6.8^{a}	1.00^{a}	500.8^{a}	56.8^{e}	28.5^{f}
Ewais					
2.5 mL L^{-1}	26.5^{a}	1.50^{a}	270.8^{e}	163.7^{b}	44.3^{b}
5 mL L^{-1}	24.0^{a}	1.17^{a}	289.3^{d}	196.0^{a}	56.7^{a}
Control	13.2^{a}	0.50^{a}	248.2^{f}	147.7^{c}	36.7^{d}
F-Test	NS	NS	**	**	**
LSD at 5%	-	-	14	5.2	2.6

The data in the table expressed as a means of 2015 and 2016 seasons. Means in each column followed by the same letter(s) did not differ at <0.50 according to Duncan's multiple-range test. The data subjected to ANOVA and a complete block design was used. *Significant differences, **High significant differences

Fruit retention and yield/tree: The fruit retention at 48 days was significantly (LSD = 0.05) high in Ewais cv. than Zebda, without significant differences between them at harvest as shown in the data of Table 2. The weight of Zebda fruits was significantly (LSD = 0.05) higher than Ewais fruits, so, the fruits number/tree in Ewais cv. was significantly more than Zebda. The fruit yield/tree in Ewais was highly significant than Zebda.

Table 3: Means of leaf length and width, chlorophyll a, b, total and carotene pigments content of Zebda and Ewais mango cultivars affected by different concentrations of nano-chitosan

	Characters					
Treatments	Leaf length (cm)	Leaf width (cm)	Chlorophyll a (mg g^{-1} fresh weight)	Chlorophyll b (mg g^{-1} fresh weight)	Total chlorophyll (mg g^{-1} fresh weight)	Carotene (mg g^{-1} fresh weight)
A. cultivars						
Zebda	22.2	3.0	1.284	1.811	3.096	2.605
Ewais	33.3	5.3	1.425	1.936	3.362	2.499
F-Test	**	**	**	**	**	**
B. Nano-chitosan						
2.5 mL L^{-1}	27.6^a	4.4^a	1.569^b	2.141^b	3.711^b	2.765^b
5 mL L^{-1}	28.0^a	4.1^a	1.638^a	2.254^a	3.892^a	2.855^a
Control	27.8^a	4.0^a	0.857^c	1.226^c	2.083^c	2.038^c
F-test	NS	NS	**	**	**	**
LSD at 5%	-	-	0.0013	0.0012	0.0013	0.0013
C. Interaction A×B						
Zebda						
2.5 mL L^{-1}	21.5^a	3.2^a	1.482^d	2.069^d	3.551^d	2.707^d
5 mL L^{-1}	23.3^a	3.2^a	1.551^c	2.149^c	3.700^c	2.775^c
Control	21.8^a	2.7^a	0.820^f	1.216^f	2.036^f	2.334^e
Ewais						
2.5 mL L^{-1}	33.7^a	5.7^a	1.657^b	2.214^b	3.871^b	2.822^b
5 mL L^{-1}	32.7^a	5.0^a	1.725^a	2.360^a	4.085^a	2.935^a
Control	33.7^a	5.3^a	0.894^e	1.236^e	2.130^e	1.741^f
F-Test	NS	NS	**	**	**	**
LSD at 5%	-	-	0.0018	0.0018	0.0018	0.0018

The data in the table expressed as a means of 2015 and 2016 seasons. Means in each column followed by the same letter(s) did not differ at <0.50 according to Duncan's multiple-range test. The data subjected to ANOVA and a complete block design was used. *Significant differences. **High significant differences

Both nano-chitosan treatments significantly (LSD = 0.05) increased retention fruit at 48 h than control. The fruit retention at harvest was high in 2.5 mL L^{-1} nano-chitosan, then 5 mL L^{-1} and the lowest retention value was the control with significant differences between them. The fruit weight was significantly high in 2.5 mL L^{-1} nano-chitosan and control in comparison with 5 m L^{-1}. On the contrary, the fruit number/tree and fruit yield/tree were significantly the highest in 5 mL L^{-1} nano-chitosan, then 2.5 mL L^{-1} and control. Nano-chitosan treatments 5 and 2.5 mL L^{-1} increased fruit yield/tree with 35.28 and 28.53% compared with control, respectively.

The interaction between cultivars×nano-chitosan treatments was non-significant in fruit retention at 48 days and at harvest. The interaction between control treatment×Zebda cv. gave significantly the highest values in fruit weight, while the lowest values came from the interaction between control treatment×Ewais cv. Nano-chitosan 5 mL L^{-1} treatment×Ewais cv. showed significantly the highest fruit number and fruit yield/tree, while the interaction between control treatment×Zebda cv. was the lowest values.

Leaf physical characteristics and pigments content: The leaf length and width was significantly larger in Ewais than in Zebda cv. as shown in Table 3. Chlorophyll a, b and total was significantly (LSD = 0.05) higher in Ewais than in Zebda cv. on the contrary the carotene content.

The tested nano-chitosan treatments did not show any significant effects on length and width of leaf. Meanwhile, it was highly (LSD = 0.05) significant in leaf content of pigments chlorophyll a, b total chlorophyll and carotene. The nano-chitosan 5 mL L^{-1} treatment recorded the highest values.

The interaction between cultivars×treatments was insignificant in length and width of leaf. While, the interaction between Ewais×5 mL L^{-1} nano-chitosan recorded significantly the highest values of chlorophyll a, b, total chlorophyll and carotene but the interaction between Zebda×control recorded the least values in chlorophyll pigments and Ewais×control recorded the least carotene value.

Leaf mineral contents: Ewais cv. trees displayed significantly (LSD = 0.05) higher leaf content of N, K and Zn compared with Zebda cv. (Table 4). The effect of cultivar on leaf content of phosphorus was insignificant.

Nano-chitosan treatment 5 mL L^{-1} recorded significantly the highest N, K and Zn leaf content compared with other treatments, while, recorded the lowest phosphorus content.

The interaction between cultivar×treatments recorded significantly the highest values in N, K and Zn from Ewais cv.×5 mL L^{-1} nano-chitosan, while Zebda cv.×control treatment recoded the highest values of phosphorus content.

Table 4: Means of leaf mineral contents of Zebda and Ewais mango cultivars affected by different concentrations of nano-chitosan

	Characters			
Treatments	N (%)	P (%)	K (%)	Zn (ppm)
A. cultivars				
Zebda	2.18	0.27	1.7	27.9
Ewais	2.33	0.25	1.85	29.1
F-Test	**	NS	**	**
B. Nano-chitosan				
2.5 mL L^{-1}	2.26[b]	0.26[b]	1.78[b]	27.4[c]
5 mL L^{-1}	2.31[a]	0.24[c]	1.85[a]	29.9[a]
Control	2.19[c]	0.27[a]	1.69[c]	28.1[b]
F-Test	**	*	**	**
LSD at 5%	0.04	0.001	0.001	0.1
C. Interaction A×B				
Zebda				
2.5 mL L^{-1}	2.20[c]	0.25[d]	1.66[f]	26.5[f]
5 mL L^{-1}	2.25[bc]	0.23[e]	1.77[c]	29.6[b]
Control	2.07[d]	0.32[a]	1.67[e]	27.5[e]
Ewais				
2.5 mL L^{-1}	2.32[a]	0.28[b]	1.90[b]	28.4[d]
5 mL L^{-1}	2.36[a]	0.25[c]	1.93[a]	30.1[a]
Control	2.31[ab]	0.23[e]	1.71[d]	28.7[c]
F-Test	*	**	**	**
LSD at 5%	0.06	0.002	0.002	0.13

The data in the table expressed as a means of 2015 and 2016 seasons. Means in each column followed by the same letter(s) did not differ at <0.50 according to Duncan's multiple-range test. The data subjected to ANOVA and a complete block design was used. *Significant differences. **High significant differences

Table 5: Means of fruit length, width, shape, total soluble solids (TSS), acidity and vitamin C of Zebda and Ewais mango cultivars affected by different concentrations of nano-chitosan

	Characters					
Treatments	Fruit length (mm)	Fruit width (mm)	Fruit shape	TSS (%)	Acidity (%)	Vitamin C (mg/100 mL juice)
A. cultivars						
Zebda	129.7	74.0	1.75	18.8	0.86	30.1
Ewais	98.2	67.1	1.46	22.8	1.24	30.6
F-test	**	**	**	**	**	NS
B. Nano-chitosan						
2.5 mL L^{-1}	120.1[a]	72.9[a]	1.65[a]	20.4	0.992[b]	31.6[a]
5 mL L^{-1}	117.0[b]	72.4[a]	1.61[ab]	21.2	0.950[b]	29.1[c]
Control	104.7[c]	66.4[b]	1.57[b]	20.8	1.208[a]	30.3[b]
F-Test	**	**	*	NS	**	**
LSD at 5%	2.5	2.3	0.04	--	0.091	0.92
C. Interaction A×B						
Zebda						
2.5 mL L^{-1}	130.0[b]	76.2[a]	1.71[b]	18.0[a]	0.817[a]	32.2[a]
5 mL L^{-1}	135.1[a]	77.5[a]	1.74[b]	18.7[a]	0.767[a]	27.3[c]
Control	124.0[c]	68.4[b]	1.81[a]	19.8[a]	1.000[a]	30.7[b]
Ewais						
2.5mL L^{-1}	110.1[d]	69.5[b]	1.58[c]	22.8[a]	1.167[a]	31.0[ab]
5 mL L^{-1}	99.0[e]	67.4[bc]	1.47[d]	23.7[a]	1.133[a]	30.8[ab]
Control	85.4 f	64.4[c]	1.33[e]	21.8[a]	1.417[a]	30.0[b]
F-Test	**	*	**	NS	NS	**
LSD at 5%	3.6	3.2	0.06	-	-	1.3

The data in the table expressed as a means of 2015 and 2016 seasons. Means in each column followed by the same letter(s) did not differ at <0.50 according to Duncan's multiple-range test. The data subjected to ANOVA and a complete block design was used. *Significant differences. **High significant differences

Fruit physical and chemical characteristics: Zebda cv. fruits recorded significantly (LSD = 0.05) high values of fruit length, width and shape compared with Ewais cv., while TSS and acidity was higher in Ewais cv. as shown in Table 5. No significant differences obtained between the two cultivars in vitamin C.

Nano-chitosan 2.5 mL L^{-1} treatment gave the highest values of fruit length, width, shape and fruits content of vitamin C, while it recorded the lowest total acidity percentage. No significant differences observed between all treatments in TSS percentage.

The interaction between cultivars×treatments recorded significantly the highest values in fruit length and width from Zebda cv.×5 mL L^{-1} nano-chitosan, while the lowest values came from Ewais cv.×control. The values of fruit shape was significantly the highest in Zebda cv.×control, while the lowest values came from Ewais cv.×control. No significant differences obtained between all interactions in TSS and acidity percentage. The highest values of vitamin C obtained from Zebda cv.×nano-chitosan 2.5 mL L^{-1} treatment, while the lowest values came from Zebda cv.×nano-chitosan 5 mL L^{-1} treatment.

DISCUSSION

Ewais cv. displayed the higher of their tested attributes in most cases, on the contrary, content of leaf from carotene and fruit characteristics as well as resistance of malformation percentage Zebda cv. was high significantly compared with Ewais cv. However, differences between the two mango cvs in their parameters are varietal differences that go back to genetic composition. In this respect, the growth vigor of a mango cv. is an inherent property ascribing to the genetic make-up of the cultivar[17]. Outweigh of a mango cv. in growth traits especially the area of photosynthetic leaves indicates its higher capacity for accumulating photosynthesis. It is well known that mango cvs; as any other plant cultivars; differ greatly in response of their genetic make up to the environmental factors that affecting developmental processes and ability to thrive benefit from the available growth factors[18].

The cultivars Zebda and Hindi Anshas reported to be rarely affected and Ewais moderately susceptible of floral malformation[19], Zebda resistant to malformation[20]. The extracts of shoots or inflorescence of Zebda strongly retarded the growth of the *F. moniliforme* var. *subglutinans in vitro*. However, no variety in Pakistan have found to be free from the disease[21]. Saddeka cv. followed by Ewais cv. were the most susceptible ones to the disease incidence and Keitt was the lowest affected one[22].

The resistant cultivar had highest activity of PPO as compared to susceptible ones. There was no significant difference in the enzymes catalase and peroxidase activity at early stage of flower differentiation but at flower bud burst stage the catalase activity was enhanced significantly in cv. Elaichi (25.28 unit min^{-1} g^{-1} FW) in comparison to Amrapali (16.20 unit min^{-1} g^{-1} FW), Beauty Mc-lin (18.39 unit min^{-1} g^{-1} FW) and Dashehari (17.50 unit min^{-1} g^{-1} FW). The resistant cultivar had high leaf temperature (30.30°C) and diffusion resistance (476.14 m mol m^{-2} sec^{-1}) during the flowering but the rate of transpiration and relative humidity (RH) were high in susceptible cultivars. Results of the present study clearly indicate that level of mangiferin considered as a potential biochemical indicator for screening mango genotypes to malformation. The mangiferin is a potent stimulator of defense related enzymes for the induction of natural defense system of the plant and considered as a good biochemical indicator for screening mango genotypes for resistance to malformation under northern Indian condition. Negative correlation found between both the enzyme activity, phenolic content at principle initiation and the incidence of oral malformation[23].

Results indicated that, in most cases, nano-chitosan treatments increased fruits yield as number of fruit or weight/tree and decreased malformation percentage as well as improving studies attributes especially nano-chitosan 5 mL L^{-1} treatment. The nano-chitosan treatments increased of yield compared of control treatment may be attributed to the indirect impact of increasing the number of fruit/tree affected by these treatments. The obtained results are in agreement with Mondal *et al.*[24,25]. on mung bean reported that chitosan increased characteristics of growth and yield.

The growth parameters (shoot height, leaf number/plant and plant fresh weight) increased with application of chitosan[26]. Foliar application of chitosan induced the activity levels of defense enzymes such as protease inhibitors (PI), β-1,3-glucanases, peroxidases (PO) and polyphenol oxidases (PPO) in the leaves and rhizomes of turmeric plants. Chitosan treatment to turmeric plants results in high yield and curcumin content. The results suggest that chitosan used as an ecofriendly compound to induce defense responses as well as the growth and curcumin content of turmeric plants, foliar application of edible rape (*Brassica rapa* L.) with chitosan also promoted the plant growth and leaf chlorophyll contents[27]. So, bioactivity assays using *Phaseolus vulgaris* showed that the alginate/chitosan (ALG/CS)-GA_3 nanoparticles were most effective in increasing leaf area and the levels of chlorophylls and carotenoids[28].

CONCLUSION

Using 5 mL L^{-1} nano-chitosan increasing fruit yield of mangoes by 35% compared with untreated fruits. Using 5 mL L^{-1} nano-chitosan decreasing mango malformation disease by 40% compared with untreated fruits. Using 5 mL L^{-1} nano-chitosan enhanced fruit quality of mangoes more than untreated fruits.

SIGNIFICANCE STATEMENTS

This study discovered the effect of nano-chitosan on vegetative growth, fruit set, yield, fruit quality and resistance of malformation. This study will help the researcher to uncover the critical areas of nano-chitosan on mango characteristics and malformation tolerance that many researchers were not able to explore. Thus, a new theory on decreasing malformation disease (one of the most complicated problems of mango) may be arrived at.

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