



P-ISSN: 2349-8528

E-ISSN: 2321-4902

www.chemijournal.com

IJCS 2021; 9(1): 1819-1823

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Received: 16-10-2020

Accepted: 29-11-2020

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Chitosan and salicylic acid: Its role in growth and biochemical characteristics of floral malformation in mango (*Mangifera indica* L.)

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DOI: <https://doi.org/10.22271/chemi.2021.v9.i1z.11487>

Abstract

Mango (*Mangifera indica* L.) is the fifth largest cultivated fruit crop globally with the yields of approximately 58.4 million tonnes, second only to banana among the tropical fruit species (FAOSTAT, 2019). Mango malformation (MMD) is an ambiguous disease of mango with the tremendous economic importance throughout the mango growing regions. The experiment was laid out in Randomized Block Design (RBD) with three replications. Seven different concentrations of chitosan and salicylic acid viz. (Control, 0.25% CT, 0.50% CT, 0.75% CT, 0.20% SA, 0.40% SA and 0.60% SA) were sprayed at three different stages i.e. prior to panicle emergence, pre blooming and full bloom in the month of February to March on two mango cultivars Amrapali (moderately susceptible) and Dashehari (Moderately resistant). The data revealed that chitosan 0.50% shows maximum length of healthy panicle (12.47 cm), malformed panicle (9.45cm), total chlorophyll content (4.90 mg g⁻¹ fr. wt) and phenolic content (296.92) over control. On the contrary, highest weight of healthy panicle (32.33g) and malformed panicle (71.50g) was recorded in 0.60% SA and 0.20% SA treatment. However, maximum leaf area (77.85 cm²) and MDA content (8.56 n molg⁻¹fr. wt.) was at 0.40% salicylic acid treatment over control. Among both the varieties maximum growth characteristics were recorded maximum in Amrapali than Dashehari. However total chlorophyll and phenolic contents was recorded highest in Dashehari as compared to Amrapali. Therefore, it was concluded that protective application of either chitosan or salicylic acid prior to panicle emergence, pre blooming and full bloom reduced the negative effect of mango malformation by increasing growth characteristics and elevate the defense induced antioxidant activities to confer resistance to floral malformation.

Keywords: Amrapali, Dashehari, mango malformation, chitosan, salicylic acid

1. Introduction

Mango (*Mangifera indica* L.) is the most popular tropical fruit crop in the World. It is known as king of fruit in India. India obtains first place in the field of the production of the mango but the production per hectare is so low that it keeps India much lower position in respect of other countries of the world (Pal *et al.*, 2017) [18]. In spite of fertile soil and favorable environmental condition, the cause of lower production is floral mango malformation. Mango malformation is a serious constraint to mango growing counties in subtropical conditions, which not only negatively affect plant health, but also reduces the yield directly (Zagzog *et al.*, 2017) [27]. This mysterious malady is credited to be acarological, fungal, viral and physiological. However, recent reports focused that *Fusarium* species are associated with mango malformation (Freeman, 2014; Katoch *et al.*, 2019) [6, 11]. Although, disease management through application of fungicides, insecticides, plant growth regulators, nutrients and pruning of malformed parts have been reported previously, but limited success has been found in controlling this disease. Furthermore, the increasing input cost, public concern about residues in food and harmful effects on the environment and human health are major challenges (Kumar, 2018) [13]. Hence, there is a need for developing novel management strategies which are practically effective, cheaper and environmentally safe.

Chitosan (CT), a naturally-occurring compound that have potential in agriculture with regard to controlling plant diseases. These molecules were shown to display toxicity and inhibit fungal growth and development (Malerba and Cerana, 2016) [15]. They were reported to be active against viruses, bacteria and other pests (Hadrami *et al.*, 2010) [4].

Fragments from chitosan is known to have eliciting activities leading to a variety of defense responses in host plants in response to microbial infections, including the accumulation of phytoalexins, pathogen-related (PR) proteins and proteinase inhibitors, lignin synthesis, and callose formation (Hadrami *et al.*, 2010) [4]. Based on these and other proprieties that help strengthen host plant defenses, interest has been growing in using them in agricultural systems to reduce the negative impact of diseases on yield and quality of crops (Orzali *et al.*, 2017) [17].

Salicylic acid (SA), chemically known as 2-hydroxy benzoic acid is a member of phenolic compounds containing an aromatic ring bearing a hydroxyl group. It is synthesized naturally by the plants. It can be synthesized from phenylalanine and converted to SA either from free benzoic acid, benzoyl glucose or o-coumaric acid as precursors depending on the plant species (Lefevere *et al.*, 2020). [14] It is involved in various developmental processes of the plant such as vegetative bud formation, flower stimulation, stomatal closure, thermogenesis, photosynthesis, respiration and senescence regulation (Raskin, 1992; Rivas-San Vicente & Plasencia, 2011) [20, 21]. It is an important signal molecule that regulates various aspects of plant responses to biotic and abiotic stresses through extensive signalling cross-talk with other growth hormones. The SA concentrations increase after a pathogen attack, inducing the expression of pathogen-related genes and initiating the development of systemic acquired resistance and hypersensitive response (Dempsey *et al.*, 1999; Durrant and Dong, 2004; Kumar, 2018) [2, 3, 13]. Hence, the present experiment was conducted in mango CVs. Amrapali and Dashehari to study the effect of chitosan and salicylic acid on growth dynamics and biochemical changes.

2. Material and Methods

The field experiment was carried on mango (*Mangifera indica* L.) cultivars 'Amrapali' and 'Dashehari' in the Horticulture Research Station, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India during February and March 2019. The experiment was carried out on seven years old trees planted at 2.5 m × 3 m (Amrapali) and 5.0 m × 5.0 m (Dashehari). The spray was done at three different stages i.e. (i) panicle emergence (ii) pre-blooming (iii) full bloom. Various treatments comprised of control (water spray), 0.25% CT, 0.50% CT, 0.75% CT, 0.20% SA, 0.40% SA and 0.60% SA. Each treatment included 3 replicates. After three successive foliar applications of chitosan and salicylic acid the mango panicles and leaves (5-6-months old leaves) samples were collected for measurement of growth parameters and physiobiochemical analysis.

2.1 Length of healthy and malformed panicles

Length of healthy and malformed panicles was measured with the help of scale and the average length was expressed as square centimeter (cm).

2.2 Fresh weight of healthy and malformed panicles

Fresh weight of healthy and malformed panicles was taken with the help of electronic balance and average fresh weight of healthy and malformed panicles were expressed in grams (g).

2.3 Leaf area

The leaf area was measured with the help of scale and average leaf area of the plant in each treatment was calculated and expressed as a square centimeter (cm²).

2.4 Estimation of total chlorophyll content

The method of Hiscox and Israelstam (1979) [9] was used to estimate total chlorophyll content. 50 mg of fresh leaf tissues were taken in test tubes and 10 ml of dimethyl sulfoxide (DMSO) was added. These test tubes were incubated at 65 °C for three hours in hot air oven. After, three hours of incubation absorbance of DMSO containing total chlorophyll content at 649.1 and 665.1 nm by using a multiple wavelength spectrophotometer. Pure DMSO was used as blank.

2.5 Determination of total phenolic content

Total phenolic content in fresh leaves of mango was estimated according to the method given by Kaur and Kapoor, (2001) [12] with some modifications. The total phenolic content was calculated in the test sample by using a standard curve.

2.6 Determination of MDA content

The MDA complex in fresh leaves was estimated by a method presented by Heath and Packer (1968) [8] after full bloom.

2.7 Statistical analysis

ANOVA analysis for the data conducted at p= 0.05 and the means of treatments compared by Statistic 10, Software.

3. Results and Discussion

The findings that were obtained from the execution of the experiment were recorded and are thoroughly discussed below:

3.1 Length of healthy and malformed panicle

The data presented in Table 1 show that there was significant effect of chitosan and salicylic acid treatments on the length of healthy panicles. Among all treatments the maximum length of healthy panicles (12.47cm) was found in 0.50% chitosan treatment and minimum (8.93cm) in control. Among both the varieties highest length of healthy panicles was recorded in Amrapali (11.13cm), while lowest in Dashehari (10.25 cm). The earlier studies also revealed that the malformed panicles were significantly shorter and broader than the normal ones (Ram, 1989) [25]. The decrease in length of malformed panicles might have attributed to abnormal metabolic reactions in the plant that have resulted in reduction of internodal distance leading to condensed rosette like appearance of malformed panicles.

On the other hand the length of malformed panicles was recorded maximum (9.45cm) at 0.50% CT treatment and minimum (6.21 cm) in control. Similarly, Zagzog *et al.*, (2017) found that foliar application of nano-chitosan (5 mL/L) treatment significantly (LSD = 0.05) increased panicle length in mango cultivar i.e. Ewais and Zebda at 7 and 28 days than control. The studies revealed that the variety Amrapali indicated the maximum length of malformed panicles (8.711cm) as compared to Dashehari (7.572 cm). The interaction between varieties and treatments was found nonsignificant for both lengths of healthy and malformed panicles in the present investigation. The interaction between cultivars × nano-chitosan treatments was non-significant in panicle length at 21 and 28 days for mango cultivar i.e. Ewais and Zebda (Zagzog *et al.*, (2017) [27]. Presence of mangiferin like substances responsible for reduced malformed panicle length as compared to healthy ones (Tiwari, 2011) [26]. 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.

Table 1: Effect of foliar application of chitosan and salicylic acid on length of healthy and malformed panicle (cm) in two cultivars of mango

Treatment Variety	Length of healthy panicle (cm)			Length of malformed panicle (cm)		
	Amrapali	Dashehari	Mean	Amrapali	Dashehari	Mean
Control (Water Spray)	9.42±1.13	8.44±1.26	8.93	6.79±0.40	5.63±0.44	6.21
Chitosan 0.25%	10.94±1.95	10.78±0.69	10.86	8.59±1.22	6.94±1.01	7.77
Chitosan 0.50%	13.45±0.69	11.50±1.20	12.47	10.36±1.46	8.54±0.39	9.45
Chitosan 0.75%	11.56±1.64	10.83±1.01	11.20	9.55±0.25	8.50±0.58	9.02
Salicylic Acid 0.20%	10.34±0.58	10.00±0.33	10.17	8.32±1.53	7.70±0.51	8.01
Salicylic Acid 0.40%	12.18±0.49	10.39±1.00	11.29	9.58±0.81	8.70±0.70	9.14
Salicylic Acid 0.60%	10.00±0.88	9.83±0.72	9.92	7.75±1.11	6.99±1.09	7.37
Mean	11.13	10.25	10.69	8.711	7.572	6.33
	Treatment (T)	Variety (V)	T × V	Treatment (T)	Variety (V)	T × V
SE(m)	0.439	0.235	0.621	0.362	0.193	0.512
C.D.(0.05)	1.284	0.686	NS	1.058	0.565	NS

3.2 The weight of healthy and malformed panicle

The assessment of fresh weight of healthy panicle was estimated to study the effect of chitosan and salicylic acid on floral malformation. Highest fresh weight of healthy panicles was recorded in 0.60% SA acid treatment (32.33 g). The assessment of fresh weight of healthy panicles under various treatments from Table 2 showed that the salicylic acid 0.60% was found to be associated with the highest (32.33 g) fresh weight of the healthy panicles whereas, lowest in control (22.17g). Dashehari displays maximum fresh weight of healthy panicle (27.73 g) as compared to Amrapali (25.27 g). The varieties and treatment interaction was highly significant ($p < 0.05$). In interaction maximum weight of healthy panicle (34.67g) was measured in Dashehari with 0.25% chitosan treatment, whereas minimum in (19.00 g) Amrapali with control treatment. The highest weight might be due to higher rate of synthesis of photosynthates like nitrogen, protein and carbohydrates which ultimately increase the mass and weight. The association of floral malformation with accumulation of excessive amount of acid hydrolysable polysaccharide may

also be the reason for such results. Ram (1991) [25] also reported that mango malformation disturbs the natural orientation of the shoots and panicles and causes excessive and abnormal growth in them.

Among all the treatments maximum fresh weight of malformed panicles (71.50 g) was reported in control whereas, minimum in 0.50% chitosan (51.67 g). Among both the varieties higher weight of malformed panicle was reported in Amrapali (76.48 g) as compared to Dashehari (45.00 g). The varieties × treatment interaction was non-significant ($p < 0.05$). Both the varieties evaluated for this trait showed that variety Amrapali has found to be more susceptible to the malformation as indicated by the maximum fresh weight of malformed panicles (76.48 g) which may be due to genetic constitution of the plant. It is reported by Pandey *et al.* (1977) [19] that acid hydrolysable polysaccharides and total carbohydrates remained at higher level in leaves, stems and panicles of malformed shoots as compared to healthy ones that is found to be in close proximity with the findings.

Table 2: Effect of foliar application of chitosan and salicylic acid on weight of healthy and malformed panicle (g) in two cultivars of mango

Treatment Variety	Weight of healthy panicle (g)			Weight of malformed panicle (g)		
	Amrapali	Dashehari	Mean	Amrapali	Dashehari	Mean
Control (Water Spray)	19.00± 2.65	25.33± 1.15	22.17	89.33± 1.53	53.67± 10.21	71.50
Chitosan 0.25%	21.67± 3.21	34.67± 3.51	28.17	72.33± 5.51	46.00± 8.72	59.17
Chitosan 0.50%	26.00± 3.00	31.33± 2.52	28.67	63.67± 7.23	39.67± 11.68	51.67
Chitosan 0.75%	26.35± 1.75	25.33± 2.52	25.84	77.00± 10.82	44.00± 15.10	60.50
Salicylic Acid 0.20%	26.55± 3.21	23.67± 3.21	25.11	84.67± 2.52	44.33± 10.69	64.50
Salicylic Acid 0.40%	22.33± 3.06	24.08± 0.68	23.21	70.00± 7.00	37.33± 3.79	53.67
Salicylic Acid 0.60%	35.00± 3.61	29.67± 1.53	32.33	78.33± 9.29	50.00± 5.00	64.17
Mean	25.27	27.73	26.50	76.48	45.00	60.74
	Treatment (T)	Variety (V)	T × V	Treatment (T)	Variety (V)	T × V
SE(m)	0.61	1.14	1.61	3.63	1.94	5.13
C.D.(0.05)	1.78	3.33	4.70	10.60	5.67	NS

3.3 Leaf area

The leaf area was estimated to study the effect of chitosan and salicylic acid on floral malformation. The application of chitosan and salicylic acid showed significant ($p < 0.05$) increase in leaf area. The results show that amongst all the treatments maximum leaf area was measured at 0.50% CT treatment (78.50 cm²) followed by 0.40% SA treatment (77.85 cm²). The varieties and treatment interaction was non-significant with respect to leaf area. SA (2mM) treatment increases leaf area by the cell division and expansion of meristematic cells thus enhancing the leaf area in mango (El-Hosieny, 2015) [5]. Similarly, Cabrera *et al.*, 2010 [11] reported that 0.40% chitosan significantly increases leaf area over control on in high bush blueberry plants. Our results indicate

a foliar stimulating activity of chitosan which is in agreement with previously published results.

3.4 Total chlorophyll content

Foliar application of chitosan and salicylic acid significantly ($p < 0.05$) enhanced the concentrations of total chlorophyll content in Amrapali and Dashehari (Table 3). Maximum total chlorophyll content was found with 0.50% CT treatment (4.90 mg/g fr.wt.) over control (2.96 mg/g fr.wt.). Among both the varieties Dashehari has highest total chlorophyll content (4.39 mg/g fr.wt.) compared to Amrapali (3.69 mg/g fr.wt.). The varieties*treatment interaction was highly significant ($p < 0.05$). In the interaction between varieties and treatment total chlorophyll content (5.36± 0.06 mg/g fr.wt) recorded in

Dashehari at 0.50% chitosan treatment, whereas minimum (2.77 ± 0.04 mg/g fr.wt.) in Amrapali with control treatment. Chlorophyll is an important pigment of photosynthetic reaction centers and light-harvesting complexes exclusively required for light-harvesting pigment-protein complexes of PS I and PS II. An appropriate ratio of chlorophyll a/b is critical in the regulation of photosynthesis. Changes in chlorophyll content and photosynthetic rate in cultivars of mango during reproductive phase limit the crop yield (Pandey and Tyagi, 1999) [19]. Therefore, foliar spray of nano-chitosan (5 ml L^{-1}) before flowering was highly significant for total chlorophyll

and carotene to induce resistance against mango malformation (Zagzog *et al.*, 2017) [27]. It is noteworthy that our results concerning photosynthetic pigments (Table 3) substantiate this assumption as pre-treatment of chitosan and salicylic acid could help in maintaining higher chlorophyll content in Amrapali and Dashehari. SA application significantly increased the photosynthetic pigments. However, the application of salicylic acid in low concentration (0.5 mM) increases the chlorophyll content in plants whereas high concentration (5 mM) decreases chlorophyll content in barley leaves (Habibi and Vaziri, 2017) [7].

Table 3: Effect of foliar application of chitosan and salicylic acid on leaf area (cm^2) and total chlorophyll content (mg g^{-1} fr. wt) in two cultivars of mango

Treatment Variety	Leaf area (cm^2)			Total Chlorophyll content (mg g^{-1} fr. wt)		
	Amrapali	Dashehari	Mean	Amrapali	Dashehari	Mean
Control (Water Spray)	68.21 ± 4.33	66.20 ± 2.31	67.21	2.77 ± 0.04	3.14 ± 0.04	2.96
Chitosan 0.25%	71.44 ± 5.01	68.80 ± 1.11	70.12	3.63 ± 0.12	4.24 ± 0.03	3.93
Chitosan 0.50%	79.63 ± 3.89	77.37 ± 3.74	78.50	4.44 ± 0.14	5.36 ± 0.06	4.90
Chitosan 0.75%	73.46 ± 2.51	70.70 ± 2.77	72.08	3.87 ± 0.03	4.55 ± 0.06	4.21
Salicylic Acid 0.20%	74.80 ± 3.50	73.77 ± 1.91	74.29	3.30 ± 0.09	4.23 ± 0.07	3.77
Salicylic Acid 0.40%	80.33 ± 4.11	75.37 ± 2.80	77.85	4.15 ± 0.09	4.84 ± 0.03	4.49
Salicylic Acid 0.60%	73.30 ± 3.10	67.83 ± 4.49	70.57	3.68 ± 0.06	4.40 ± 0.13	4.04
Mean	74.45	71.43	72.94	3.69	4.39	4.04
	Treatment (T)	Variety (V)	T × V	Treatment (T)	Variety (V)	T × V
SE(m)	1.44	0.77	2.03	0.029	0.016	0.041
C.D.(0.05)	4.20	2.24	NS	0.086	0.046	0.121

3.5 Phenol content

Phenol and MDA contents were significantly ($p < 0.05$) increased by the foliar application of salicylic acid and chitosan (Table 4). The results showed that amongst all the treatments highest phenol content ($296.92 \mu\text{g}^{-1}$ g fr. wt.) was recorded in 0.50% chitosan treatment, minimum ($178.57 \mu\text{g}^{-1}$ g fr. wt.) in control. Among both the varieties highest phenol content was measured in Dashehari ($289.51 \mu\text{g}^{-1}$ g fr. wt.) than Amrapali ($195.40 \mu\text{g}^{-1}$ g fr. wt.). Mango plants are a rich source of various phenolic compounds. The major polyphenols in the mango in terms of antioxidative capacity and/or quantity are mangiferin, catechins, quercetin, kaempferol, rhamnetin, anthocyanins, gallic and ellagic acids, propyl and methyl gallate, benzoic acid, and protocatechuic acid (Rymbai and Rajesh, 2011) [22]. It is also well known that these phenolic compounds are produced in response to attack and infection of pathogens, and their appearance is considered as part of an active defense system (Nicholson and Hammerschmidt, 1992) [16]. Salimgandomi and Shabrangi, (2016) reported that the spray of chitosan at 50 and 100 μM concentrations on peppermint (*Mentha piperita* L) shoots and the soil surface, increases phenolic compounds, flavonoids and antioxidant activities. Mango plants are a rich source of various phenolic compounds. The major polyphenols in the mango in terms of antioxidative capacity and/or quantity are mangiferin, catechins, quercetin, kaempferol, rhamnetin, anthocyanins, gallic and ellagic acids, propyl and methyl

gallate, benzoic acid, and protocatechuic acid (Rymbai and Rajesh, 2011) [22]. It is also well known that these phenolic compounds are produced in response to attack and infection of pathogens, and their appearance is considered as part of an active defense system (Nicholson and Hammerschmidt, 1992) [16]. The spray of chitosan at 50 and 100 μM concentrations on peppermint (*Mentha piperita* L) shoots and the soil surface, increases phenolic compounds, flavonoids and antioxidant activities (Salimgandomi and Shabrangi, 2016) [23].

3.6 MDA content

The MDA content was recorded maximum (8.56 n molg^{-1} fr. wt) in 0.40% salicylic acid treatment and minimum (and 3.06 n molg^{-1} fr. wt) was in control. Among both the varieties Amrapali (6.00 n molg^{-1} fr. wt) has maximum MDA content than Dashehari (5.35 n molg^{-1} fr. wt). The varieties and treatment interaction was nonsignificant ($p < 0.05$) with reference to MDA content. MDA is usually measured to assess the extent of damage caused by biotic and abiotic stresses. In the present study, it was observed that foliar application of chitosan and salicylic acid significantly ($p < 0.05$) reduced MDA content in mango leaves and membrane damage (Table 4). Membrane damage might be caused by high H_2O_2 levels, which could accelerate the Haber-Weiss reaction, resulting in hydroxyl radical formation and thus lipid peroxidation (Soliman *et al.*, 2018) [24].

Table 4: Effect of foliar application of chitosan and salicylic acid on phenolic (μg^{-1} g fr. wt.) and malondialdehyde (n molg^{-1} fr. wt) contents in two cultivars of mango

Treatment Variety	Phenolic content (μg^{-1} g fr. wt.)			MDA content (n molg^{-1} fr. wt.)		
	Amrapali	Dashehari	Mean	Amrapali	Dashehari	Mean
Control (Water Spray)	137.22 ± 16.78	219.92 ± 2.13	178.57	2.17 ± 0.21	3.94 ± 0.12	3.06
Chitosan 0.25%	183.04 ± 21.20	229.65 ± 4.56	206.34	3.73 ± 0.95	4.69 ± 0.64	4.21
Chitosan 0.50%	239.37 ± 15.30	354.47 ± 2.65	296.92	7.52 ± 0.71	6.64 ± 0.03	7.08
Chitosan 0.75%	213.84 ± 2.30	291.85 ± 3.65	252.85	5.36 ± 0.10	6.20 ± 0.05	5.78
Salicylic Acid 0.20%	203.10 ± 5.47	321.03 ± 4.26	262.07	6.68 ± 0.01	5.51 ± 0.20	6.10
Salicylic Acid 0.40%	231.27 ± 4.72	342.31 ± 1.82	286.79	11.22 ± 4.36	5.90 ± 0.05	8.56

Salicylic Acid 0.60%	159.96± 4.59	267.34±0.93	213.65	5.34± 0.14	4.56± 0.38	4.95
Mean	195.40	289.51	242.45	6.00	5.35	5.68
	Treatment (T)	Variety (V)	T × V	Treatment (T)	Variety (V)	T × V
SE(m)	3.592	1.920	5.079	0.48	0.26	0.68
C.D.(0.05)	10.498	5.612	14.847	1.41	NS	2.01

4. Conclusion

From the present study, it may be concluded the growth dynamics of mango plants to the foliar application of chitosan and salicylic acid were studied in terms of the length of healthy panicle, length of malformed panicle, weight of healthy panicle, weight of malformed panicle and leaf area at full bloom stage. The total chlorophyll content, phenolic content and MDA contents, increases the plant defense response against floral malformation. This study will help the researcher to uncover the critical areas of chitosan on mango characteristics and malformation resistance that many researchers were not able to explore. Thus, chitosan is a new eco-friendly, chipper and effective management strategy for mango malformation.

5. Acknowledgements

Authors are highly thankful to the facilities and funds provided by G.B Pant University of Agriculture and Technology, Pantnagar, and National fellowship for higher studies of ST students (NFST), Ministry of Tribal Affairs, Govt. of India.

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