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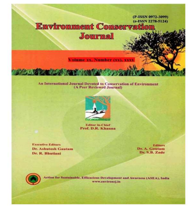

Efficacy of chitosan on growth and yield traits of mulberry, *Morus sp.*

K. Thanga roja ✉

Department of Sericulture, Forest College & Research Institute Tamil Nadu Agricultural University Mettupalayam

K.A. Murugesh

Department of Sericulture, Forest College & Research Institute Tamil Nadu Agricultural University Mettupalayam

P. Mangammal

Department of Sericulture, Forest College & Research Institute Tamil Nadu Agricultural University Mettupalayam.

R. Shanmugam

Department of Sericulture, Forest College & Research Institute Tamil Nadu Agricultural University Mettupalayam

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ABSTRACT

Chitosan is biopolymer of glucosamine residues, nontoxic, biodegradable and friendly to environment and it also helps to enhance crop production due to their bioactivities. The present experiment was conducted to assess the efficacy of chitosan on the growth and yield related attributes of mulberry, *Morus* sp. with various concentrations such as 25, 50, 75, 100 and 125 ppm. The chitosan was sprayed thrice on 15, 30 and 45 days after pruning (DAP) on the foliage of three different mulberry varieties namely V1, MR2 and G4. The result revealed that, irrespective of varieties, the foliar application of chitosan at 75 ppm significantly increased the biometric as well as yield related attributes of mulberry over other concentrations. Among the three varieties, V1 showed more response to chitosan application than G4 and MR2. At 75 ppm, chitosan showed marked effect on VI variety of mulberry and significantly increased shoot length (170.67 cm), number of shoots per plant (9.03), number of leaves per shoot (29.48), leaf area (220.26 cm^2) and leaf area index (2.72) were observed when compared with control. The application of chitosan at 75 ppm significantly enhanced the yield traits namely weight of 100 leaves (448.10 g), leaf shoot ratio (1.45) and leaf yield (14.01 MT/ha/harvest) in V1.

Introduction

Mulberry is one of the most important economic crops cultivated exclusive for silkworm rearing and it is the only host plant for *Bombyx mori* L. The mulberry contributes by 38.20 per cent in successful production of cocoon (Miyashita, 1986). Mulberry being a perennial crop and pruned frequently needs to be maintained properly with the application of recommended quantity of organic and inorganic fertilizers. Many-a-times, the poor soil health status of mulberry garden and improper management practices, lead to significant reduction in the quality of leaf. Under such circumstances, the leaf quality and biomass production can be improved by the application of soluble nutrients or elicitors on the leaves. In the last decade, there was global realization on the important role of chitosan as plant growth promoter due to its non-toxicity, biodegradability and biocompatibility for better growth and yield of crops (Elieh-Ali-Komi and Hamblin, 2016). Chitosan is a natural, co-polymer of glucosamine and N-acetyl –D-glucosamine obtained from the shells of marine organisms as shrimp, crab and prawn; insects, mushrooms and cell wall of microorganisms. The chitosan has been used as a fertilizer to increase the plant productivity (Nitar *et al.,* 2004), to protect plants against microorganisms (Faruok *et al.,* 2000), against oxidative stress (Guan *et al.,* 2004) and to stimulate plant growth (Farouk *et al*., 2008 and 2011). Recently, few researchers have reported that

Corresponding author E-mail: *rojathanga@gmail.com*
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chitosan enhanced plant growth of cucumber (Farouk *et al.,* 2008), sweet pepper (Farouk *et al*., 2011), raddish (Ghoname *et al*., 2010), strawberry (Abdal-Mawgoud *et al*., 2010), bean (Sheikha and Al-Malki, 2011), tomato and egg plant (Sultana *et al.,* 2017) by increasing the activity of enzymes involved in nitrogen metabolism. To the best of our knowledge, there is no previous report regarding the effect of foliar application of chitosan as an elicitor on growth of mulberry. Considering the above facts, the present work was undertaken to study the effect of chitosan on growth and yield attributes of different mulberry varieties.

Material and Methods

i) Mulberry garden

Field experiments were conducted at Department of Sericulture, Forest College and Research Institute, Mettupalayam (Latitude 11.20^{o}N; Longitude 76.56^{o}N) with foliar application of various concentrations of chitosan on three different mulberry varieties. The well-established three years old mulberry garden having varieties *viz.,* V1, MR2 and G4, planted with the spacing of 90 X 90 cm was used for the study. Package of practices recommended for irrigated condition of mulberry garden were followed throughout the study period (Dandin and Giridhar, 2014).

ii) Preparation of chitosan solution

The chitosan was extracted from mulberry silkworm pupae with chemical method (Battampara *et al*., 2020) and diluted in 2 per cent lactic acid. Then, it was dissolved in required quantity of distilled water to arrive at different concentrations *viz.,* 25 ppm (25mg/l), 50 ppm (50mg/l), 75 ppm (75mg/l), 100 ppm (100 mg/l) and 125 ppm (125 mg/l). The plots sprayed with distilled water and untreated control was maintained for comparison. The chitosan was uniformly applied on the mulberry foliage with knapsack sprayer on 15, 30 and 45 days after pruning (DAP).

iii) Observations and statistical analysis

The observations on growth traits *viz.,* shoot length, number of shoots per plant, internodal length, number of leaves per shoot, leaf area, leaf area index, and yield traits *viz.,* weight of 100 leaves, leaf shoot ratio and leaf yield were recorded on 70 DAP. The experiments were conducted in Randomized Block Design (RBD) with three replications. The collected data were analyzed statistically as described by Gomez and Gomez (1984). Treatment means and interaction effects were compared using critical difference at 5% level of significance.

Results and Discussion

The application of chitosan on mulberry foliage had greater beneficial influence on growth of mulberry. The result on growth and yield attributes of mulberry have been analysed statistically that are presented in detail hereunder.

A. Shoot attributes of mulberry

The foliar application of chitosan elucidated positive significant effect on shoot attributes of mulberry (Table 1). Among the various concentrations, significantly higher shoot length (154.37 cm) and number of shoots per plant (8.35) was recorded in chitosan 75 ppm over the control, which was followed by 100 ppm. Here, the concentrations namely 125 and 50 ppm did not differ statistically. This is due to optimum level chitosan concentration leads to enhance the stomatal mechanism at a right time and increases the leaf metabolism in plants (Hidangmayum *et al.,* 2019).

Regarding different mulberry varieties, V1 recorded longest shoot length (154.37 cm) and more number of leaves per shoot (8.39) followed by G4 (148.90 cm and 7.51, respectively) and MR2 (119.16 cm and 7.20, respectively), which were found to differ significantly from each other.

In interaction between different concentrations and varieties, significantly maximum shoot length (170.67 cm) and high number of leaves per shoot (9.03) was recorded in the V1 variety at chitosan 75 ppm, which was statistically superior to all other treatments. At the same time, minimum shoot length of 109.12 cm and less number of shoots per plant of 6.12 were observed in MR2 at control. The stimulating effect of chitosan on plant growth in the present study might be attributed to an increase in the 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 enzyme activities as reported by Guan *et al.,* (2009). The present observation is supported by the results Abu-Muriefah (2013), who found that the application of chitosan at 200 mgL^{-1} significantly increased the plant growth (45.20 cm) and number of branches

Table 1: Impact of chitosan on shoot attributes of mulberry, *Morus* sp.

Treatments	Shoot length (cm)				No. of shoots per plant			
	V1	MR2	G4	Mean	V1	MR2	G4	Mean
Chitosan 25 ppm	154.44	118.67	145.56	**139.56**	8.37	7.35	7.51	**7.74**
Chitosan 50 ppm	163.54	124.67	153.78	**147.33**	8.89	7.58	7.65	**8.04**
Chitosan 75 ppm	170.67	128.78	163.67	**154.37**	9.03	7.81	8.20	**8.35**
Chitosan 100 ppm	166.11	125.56	158.11	**149.93**	8.75	7.68	8.00	**8.14**
Chitosan 125 ppm	159.42	115.54	148.67	**141.21**	8.62	7.72	7.56	**7.97**
Water spray	146.56	111.78	138.76	**132.37**	7.56	6.16	6.86	**6.86**
Control	146.32	109.12	136.76	**130.73**	7.52	6.12	6.82	**6.82**
Mean	**158.15**	**119.16**	**148.90**	**142.07**	**8.39**	**7.20**	**7.51**	**7.70**
CD (P=0.05)	**T**	**4.39****			**0.11****			
	V	**2.53****			**0.10****			
	T x V	**7.33***			**0.13***			

***Significant, ** Highly Significant; Each value is mean of three replications and pooled mean of two crops**

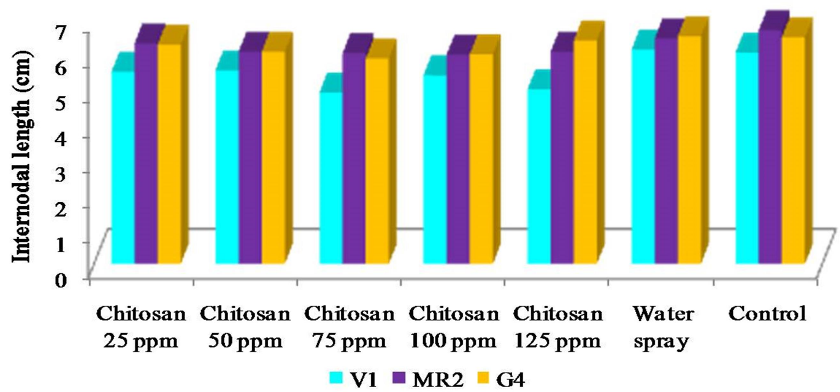

Figure 1: Impact of chitosan on internodal length of mulberry, *Morus* sp.

(9.50) on bean as compared to rest of the treatments. Our observations are in line with the findings of Lu *et al*., (2002), who observed that application of chitosan significantly increased the plant height and number of branches in rice. Further, the findings of Farouk *et al.* (2008) and Ghoname *et al.* (2010), who reported an increased vegetative growth and improvement in fruit quality due to the foliar application of chitosan in the crops like pepper, radish and cucumber were also strengthen the present observations.

As shown in Figure 1, the data indicated in different chitosan treatments and different mulberry varieties reduced the internodal length as compared with control. Among different concentrations evaluated, minimum internodal length of 5.56 cm was found in 75 ppm. This was followed by 100 ppm (5.75 cm), which was found to be statistically at par with 125 ppm (5.79 cm). Irrespective of the concentrations tested, lowest internodal length of 5.41 cm was observed in V1 variety. The next better variety was G4 (6.15 cm) which was found to be on par with MR2 (6.17 cm). In case of interaction, the reduced internodal length of 4.87 cm was registered in V1 variety at chitosan 75 ppm, whereas, MR2 showed higher internodal length of 6.64 cm in untreated plants. The findings of Chandrkrachang *et al.* (2003) and Boonlertnirun *et al.* (2005), who recorded reduced internodal length in the crops like chilli, celery and cucumber due to application of chitosan, support the present observations.

B. Leaf attributes of mulberry

The application of chitosan pronounced positive response in the leaf attributes of mulberry (Table 2 and Fig 2). The data in table 2 showed that leaf area increased by 51.29 per cent and number of leaves per shoot improved by 32.71 per cent over the

Table 2: Impact of chitosan on leaf attributes of mulberry, *Morus* sp.

Treatments		No of leaves per shoot				Leaf area (cm^2)			
		V1	MR2	G4	Mean	V1	MR2	G4	Mean
Chitosan 25 ppm		24.64	21.74	23.37	**23.25**	167.19	140.69	154.80	**154.23**
Chitosan 50 ppm		27.14	22.67	25.52	**25.11**	182.42	151.50	169.22	**167.71**
Chitosan 75 ppm		29.48	25.69	28.04	**27.74**	220.26	159.76	195.06	**190.69**
Chitosan 100 ppm		27.74	24.01	26.55	**26.10**	209.54	155.92	186.34	**183.93**
Chitosan 125 ppm		26.45	23.31	23.41	**24.39**	179.23	153.04	163.99	**165.42**
Water spray		22.85	18.36	21.45	**20.89**	135.51	117.01	133.31	**128.61**
Control		22.04	18.16	21.23	**20.48**	132.43	114.93	130.23	**125.86**
Mean		**25.76**	**21.99**	**24.22**	**23.99**	**182.36**	**141.84**	**161.85**	**162.01**
CD (P=0.05)	T	**0.44****				**4.32****			
	V	**0.75****				**2.44****			
	T x V	**1.02***				**7.40***			

*Significant, ** Highly Significant; Each value is mean of three replications and pooled mean of two crops

Table 3. Impact of chitosan on yield attributes of mulberry, *Morus* sp.

Treatments		Weight of 100 leaves (g)				Leaf shoot ratio			
		V1	MR2	G4	Mean	V1	MR2	G4	Mean
Chitosan 25 ppm		400.60	353.60	377.10	**377.10**	1.23	1.09	1.17	**1.16**
Chitosan 50 ppm		423.30	360.30	412.90	**398.83**	1.32	1.17	1.20	**1.23**
Chitosan 75 ppm		448.10	370.50	435.80	**418.13**	1.45	1.29	1.35	**1.36**
Chitosan 100 ppm		433.90	366.30	422.30	**407.50**	1.34	1.20	1.26	**1.29**
Chitosan 125 ppm		411.30	370.90	393.30	**391.83**	1.26	1.19	1.21	**1.22**
Water spray		399.80	342.50	365.90	**369.40**	1.15	1.03	1.09	**1.09**
Control		398.84	339.10	362.70	**366.88**	1.12	1.02	1.07	**1.07**
Mean		**416.55**	**358.46**	**395.71**	**391.10**	**1.27**	**1.14**	**1.19**	**1.20**
CD (P=0.05)	T	**4.10***				**0.04***			
	V	**5.40***				**0.04***			
	T x V	**11.53***				**0.09***			

*Significant, ** Highly Significant; Each value is mean of three replications and pooled mean of two crops

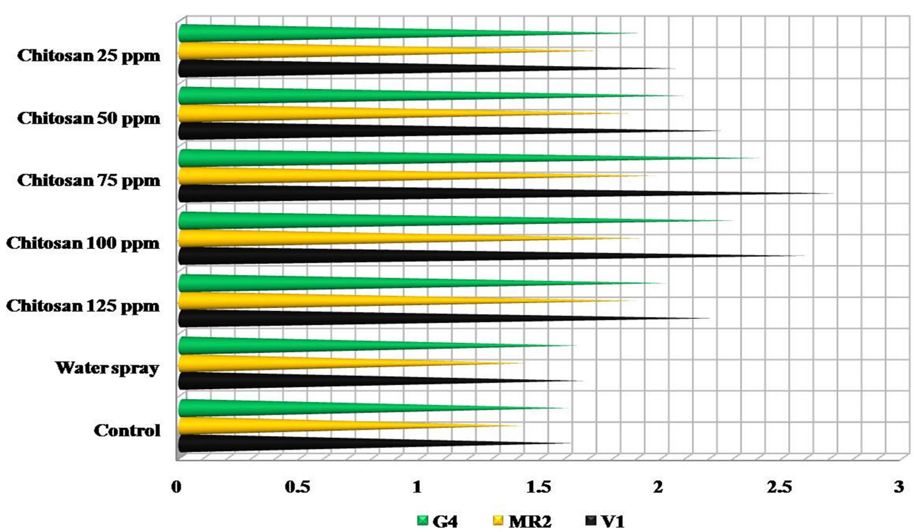

Figure 2: Impact of chitosan on leaf area index of mulberry, *Morus* sp.

control. Among chitosan concentrations, significantly more number of leaves per shoot (27.74) and highest leaf area (195.06 cm^2) was noticed in the plants sprayed with chitosan 75 ppm. The next better treatment was 100 ppm which registered the value of 26.10 and 183.93 cm^2 respectively. This might be due to increased photosynthetic activity in the functional leaves as reported by Gornik *et al.* (2008); Mondal *et al.* (2012) and, Ibraheim and Mohsen (2015). Mondal *et al.* (2012) found more number of leaves per plant (34.00) due to the chitosan application in okra, which supports the present observations. In respect of different mulberry varieties, significantly more number of leaves per shoot (25.76) and higher leaf area (182.36 cm^2) was observed on V1 variety; whereas, less number of leaves per shoot (18.16) and lower leaf area (114.93 cm^2) was noted on MR2 variety. In the interaction effect between different concentrations and varieties, number of leaves per shoot (29.48) and leaf area (220.26 cm^2) was found to be significantly increased in V1 at chitosan 75 ppm, while, less number of leaves per shoot (18.16) and lowest leaf area (114.93 cm^2) was observed in untreated plants of MR2. Our present study shown that chitosan treatments may be increases the leaf metabolism by stomatal mechanism which enhance the leaf area treated mulberry leaves. The results of our experiments are also in agreement with Hassnain *et al.,* (2020), who recorded the higher number of leaves (104.19), leaf area (81.05 cm^2) in tomato, and they attributed this to enhanced activities of enzymes involved in nitrogen metabolism namely glutamine synthetase, protease and nitrate reductase.

Leaf area index of different treatments and different varieties were presented in Figure 2. As found in above results, significantly higher leaf area index of 2.72 was observed on chitosan 75 ppm of V1 compared to others. The present experimental results are also in conformity with Mondal *et al.* (2016), who reported increased number of leaves per plant (53.70) and leaf area $plant^{-1}$ (2216 cm^2) due to the application of chitosan at 50 mg L^{-1} on tomato.

C. Yield attributes of mulberry

Application of chitosan at various concentrations has brought significant enhancement in different yield related attributes over the control (fig. 3). Among various concentrations, chitosan 75 ppm recorded significantly higher weight of 100 leaves (418.13 g) and leaf shoot ratio (1.35) followed by chitosan 100 ppm with the value of 407.50 g, and 1.29 MT/ha/harvest, respectively. Among the different varieties, V1 registered maximum weight of 100 leaves (416.55 g) and leaf shoot ratio (1.27) as against minimum of 358.46 g and 1.14, respectively in MR2.

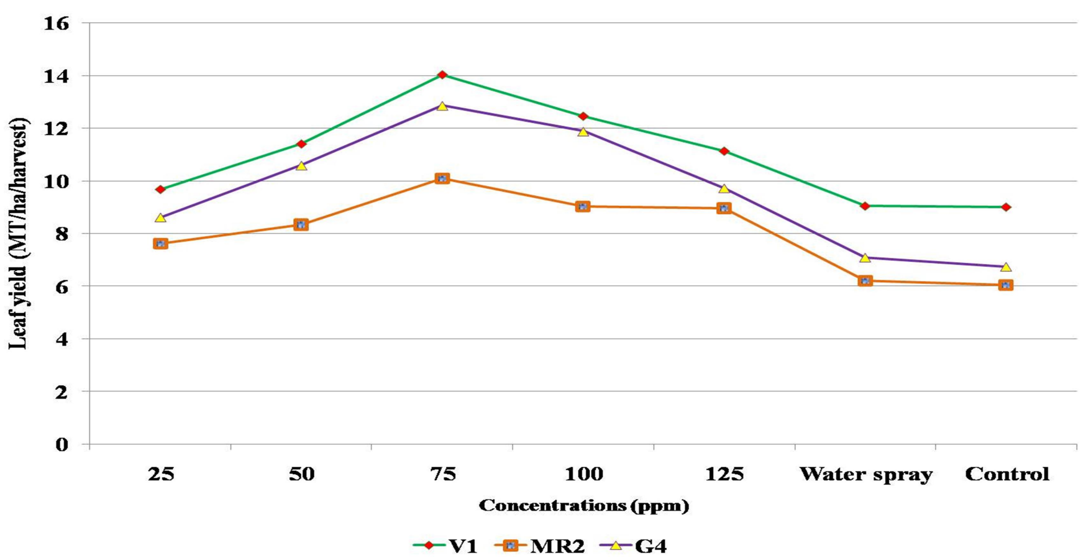

Figure 3: Impact of chitosan on leaf yield of mulberry, *Morus* sp.

The interaction of chitosan concentrations and mulberry varieties showed that V1 at chitosan 75 ppm recorded significantly maximum weight of 100 leaves (448.10 g) and leaf shoot ratio (1.45). G4 variety at chitosan 75 ppm was found to be next better treatment by recording significantly higher weight of 100 leaves (437.80 g) and leaf shoot ratio (1.38) over the control. The untreated plants in MR2 variety registered the lowest weight of 100 leaves (339.10 g) and leaf shoot ratio (1.02). The application of chitosan at 1ml L^{-1} on cucumber recorded the highest yield of 4.86 kg $plant^{-1}$ as compared to control (Shehata *et al.,* 2012).

Results in Figure 3 showed that improvement in leaf yield due to application of chitosan at 75 ppm ranges from 2.48 to 64.74 per cent over the control. At the same as above results, chitosan 75 ppm of V1treated mulberry plants had highest leaf yield of 14.01 MT/ha/harvest and lowest was recorded in untreated plants in MR2 variety of 6.04 MT/ha/harvest. Normally, MR2 has low yielding mulberry variety under moderate irrigate condition compared to V1 because V1 is high yielding ruling variety under normal irrigate condition. Here, V1 response to chitosan more effective compare to other mulberry varieties. Lee *et al.,* (2005) found that chitosan treatment increased the yield and marketability of soybean sprouts. Trials conducted on tomatoes showed that foliar applications of chitosan increased the yield by 20 per cent (Walker *et al.,* 2004). These findings fall more or less in line with the present observations.

Conclusion

It is concluded from the present study that foliar application of chitosan at 75 ppm thrice on 15, 30 and 45 DAP on mulberry significantly enhanced the various growth and yield related attributes. However, chitosan 100 ppm and 125 ppm not much effective compare to optimum level due to increase the closure of stomata long time led to decreases the plant metabolism. From the present study revealed that all the mulberry varieties had good response to chitosan treatments compared to control under unstressed condition. Hence, it could be advocated for large scale field evaluation in order to successfully augmenting the foliage productivity without any damage to the ecosystem.

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