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Enhancing the Leaf Quality of Mulberry by Foliar application of Chitosan

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ABSTRACT
Chitosan is an important bio-stimulator for many crops due to its ability to promote the plant defense mechanism. A study to assess the effect of chitosan as foliar spray in enhancing the mulberry leaf quality on three mulberry different varieties (V1, MR2 and G4) was taken up. In this study, five different concentrations of chitosan viz., 25, 50, 75, 100 and 125 ppm were applied thrice at 15 days interval starting from 25 days after pruning (DAP) in early hours of the day. The result showed that foliar application of chitosan had statistically significant effect in almost all the traits studied. Mulberry plants treated with chitosan 75 ppm of V1 had maximum photosynthetic pigments viz., Chlorophyll-a (3.12 mg g^{-1}), chlorophyll-b (0.85 mg g^{-1}), total chlorophyll (3.23 mg g^{-1}) and carotenoids (1.02 mg g^{-1}); higher moisture content (75.82 %) and moisture retention capacity (72.65) compared to all other treatments. Correspondingly, Chitosan 75 ppm of V1 had more accumulation of primary metabolites as soluble protein (31.21 mg g^{-1}) and total carbohydrate (20.61 mg g^{-1}); high amount of macronutrients as nitrogen (4.73 %), phosphorus (0.51 %) and potassium (3.51 %) over the control. The same trend was observed in all three varieties in increasing the mulberry leaf quality. Therefore, the application of chitosan at chitosan 75 ppm on foliage of the plant could be an ideal way to increase the mulberry leaf quality.

Keywords
Chitosan, Biostimulator, Mulberry varieties, Primary metabolites and

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INTRODUCTION

Worldwide, about 92.20 per cent of silk is obtained from mulberry silkworm, *Bombyx mori* L. which is reared exclusively only by feeding the leaves of mulberry, *Morus* spp (Shah *et al*, 2019). It is estimated that, in sericulture approximately 60-65 per cent of total cost of cocoon production goes for the mulberry leaf production alone (Miyashita, 1986). Nourishing a better quality mulberry leaves to silkworm is one of the pre-requisite for producing a good quality cocoons; hence, cultivation of mulberry with proper nutrient management practices helps to enhance mulberry growth as well as improve its quality. Because of this reason, many of the sericulture farmers are applying excessive amount of chemical fertilizers to improve crop nutrition resulting in the depletion of nutrients in the soil as well as crop. This may affect the silkworm growth and development. To overcome this difficulty, use of natural bio-stimulators is the most logical way which improves the nutritional status of mulberry leaf. In the last few years, there has been growing interest in the use of chitosan for enhancing the growth of many economic crops (Dhargalkar and Pereira, 2005). Chitosan is a linear amino polysaccharides obtained by deacetylation of chitin, an abundant by-product in the sea food processing industry and obtained as one of the important by-products in the silk reeling industries. For the present experiment, chitosan was extracted from dried mulberry silkworm pupae by chemical method Battampara *et al* (2020).

Many of the researchers reported that chitosan has been widely used as substance to increasing the chlorophyll content, photosynthesis, chloroplast enlargement (Limpanavech *et al*, 2008) and escalating nitrogen fixing nodes of leguminous plant species (Dzung and Thang, 2004). A positive effect of chitosan was observed as increasing mineral nutrient uptake in coffee seedlings (Dzunga *et al*, 2011) and *Majorana hortensis* (El-Khateeb *et al*, 2017). Chitosan possesses antioxidant activity (Chen *et al*, 2009) , act as anti-transpirant compound (Karimi *et al*, 2012), acts as a plant growth regulator and considered to elicit the induction of plant defense mechanisms in many plants (Ben-Shalom *et al*, 2003) and Photchanachai *et al* (2006). By keeping this in mind, a study was undertaken to assess the impact of chitosan on the biochemical constituents of mulberry.

MATERIALS AND METHODS

The field experiments were carried out at Department of Sericulture, Forest College and Research Institute, Mettupalyam during two successive seasons to investigate the effect of chitosan on quality of mulberry leaves. For evaluation of chitosan effect on mulberry, three main commercial varieties viz., V1, MR2 and G4 were used due to their various genetic background and characteristics. For instance, V1 has high

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yielding ruling variety under irrigated condition (Saratchandra *et al*, 2011) ; MR2 has resistance to drought under moderate irrigate condition (Venkatesh, 2017) and G4 has susceptible to high temperature stress condition (Kumar and Vijayalakshmi, 2018). These mulberry varieties were grown under conventional management practices during the experimental period (Dandin and Giridhar, 2014).

Experimental Treatments

The chitosan was prepared from dried mulberry silkworm pupae by chemical method (Battampara *et al*, 2020) with degree of deacetylation of 68.56 per cent. The chitosan solution was prepared by dissolving proper amount of chitosan in 2 per cent lactic acid and five concentrations such as 25, 50, 75, 100 and 125 ppm were obtained. The distilled water spray and untreated plots were also maintained as controls. Foliar application of chitosan at concentrations was taken up thrice with 15 days interval using Knapsack sprayer during in the hours of the day starting from 25 DAP.

Experimental design and laboratory analysis

The field experiment was carried out in Randomized Block Design (RBD) with three replications per treatment. Each plot having the size of 40 sq.m. area with the plant spacing of 90 X 90 cm was used for the experiments. After application of chitosan, fresh and healthy mulberry leaves were collected at 65-70 DAP from the labelled plants. Collected leaf samples were dried under shade followed by oven dried at 60°C till constant weight was attained. Bio-chemical constituents of mulberry leaves were estimated by various standard methods. Chlorophyll-a, Chlorophyll-b and photosynthetic pigments were determined according to the methods described by Goodwine (1965). Total protein was measured using Folin-phenol reagent (Lowry *et al*, 1951). Anthrone reagent was used to estimate the total carbohydrate (Plummer, 1971). The moisture content and moisture retention capacity was determined following the procedure described by Gowda and Sudhakar (2002). Nitrogen was determined according to Pregle (1945). Phosphorus was determined according to the method of Jackson (1967). Potassium was determined by the method described by Black (1965).

Statistical analysis

Collected data were statistically analyzed according to the technique of analysis variance (ANOVA). The least significant difference (L.S. D) method was used to compare the difference between the means of treatment values described by Gomez and Gomez (1984). All statistical analyses were performed using analysis of variance technique by means of SPSS Computer Software.

RESULTS AND DISCUSSION

The application of chitosan on three different mulberry varieties showed advantageous effect on its quality of leaves. The result of laboratory analysis on biochemical constituents of mulberry varieties are elaborately presented hereunder.

Influence of chitosan on photosynthetic pigments of mulberry

The results of experiments on photosynthetic pigments are summarized in Table 1, Figure 1 and Figure 2. The result presented in Table 1 clearly showed that the total chlorophyll and carotenoids content of three mulberry varieties were increased due to foliar application of chitosan. In respect of varieties, the highest values of total chlorophyll and carotenoids content of 2.86 mg g^{-1} and 0.92 mg g^{-1}, respectively were obtained in V1 followed by G4 (2.27 mg g^{-1} and 0.61 mg g^{-1}, respectively) and MR2 (1.80 mg g^{-1} and 0.45 mg g^{-1}, respectively). Among the different concentrations, chitosan 75 ppm recorded maximum total chlorophyll and carotenoids of 2.41 mg $g^{-1}$and 0.63 mg g^{-1},respectively. This was followed by chitosan 100 ppm (2.30 mg $g^{-1}$and 0.58 mg g^{-1}, respectively) and chitosan 50 ppm (2.25 mg $g^{-1}$and 0.56 mg g^{-1}, respectively), which were found to be significantly on par with each other. The minimum was observed on control (1.49 mg $g^{-1}$and 0.43 mg g^{-1}, respectively), which was found to be on par with water spray (1.52 mg $g^{-1}$and 0.47 mg g^{-1}, respectively).

With regards to the interaction of different concentrations and varieties, higher total chlorophyll and carotenoids of 3.23 mg g^{-1} and 1.02 mg g^{-1}, respectively were observed on chitosan 75 ppm of V1. The next better treatment was chitosan 100 ppm of V1 (3.12 mg $g^{-1}$and 0.97 mg g^{-1}, respectively), which was found to be par with chitosan 50 ppm of same variety (3.07 mg $g^{-1}$and 0.95 mg g^{-1}, respectively). The lower values were noticed in water spray (1.28 mg $g^{-1}$and 0.39 mg g^{-1}, respectively) and control (1.25 mg $g^{-1}$and 0.35 mg g^{-1}, respectively) of MR2 which were found to be on par. These results are in agreement with Khan *et al* (2002), who reported that application of chitosan increases the photosynthetic pigments on the leaves of maize and soybean.

Inoue and Kinoshita (2017) reported that photosynthesis or water use efficiency is largely dependent on stomatal regulation. The maximum photosynthetic rate (27.39 $\pm$ 0.65 μmol m^{-2} s^{-1}) was observed on V1 among five different mulberry cultivars as reported by Kumar *et al* (2012). Additionally, El-Tantawy (2009) reported that chitosan application increased photosynthetic pigments thereby increases the photosynthetic process on leaves. It was also supported by Farouk and Amany (2012), Sheikha and Al-Malki (2011), who observed higher chlorophyll contents on cucumber, radish and cowpea and bean through the foliar application of chitosan at 0.5 g/l.

Influence of chitosan on photosynthetic pigments (mg g^{-1}) of mulberry, *Morus* sp.

Result of different treatments and varieties on chlorophyll-a and chlorophyll-b are presented in Figure 1 and Figure 2. As found in the above results, significantly higher values of chlorophyll-a and chlorophyll-b of 3.12 mg g^{-1} and 0.85 mg g^{-1}, respectively were noticed on chitosan 75 ppm of V1. The lowest values were notices in control (1.04 mg $g^{-1}$and 0.37 mg g^{-1}, respectively) of MR2. The present result more or less falls in line with the findings of Dzunga *et al* (2011), who reported that increased chlorophyll-a content of 1.18 mg g^{-1} compared to control (0.70mg g^{-1}) in coffee.

Table 1: Influence of chitosan on photosynthetic pigments (mg g^{-1}) ofmulberry, *Morus* sp.

Treatments	Total chlorophyll				Carotenoids			
	V1	MR2	G4	Mean	V1	MR2	G4	Mean
Chitosan 25 ppm	2.90	1.84	2.32	**2.08**	0.90	0.43	0.59	**0.51**
Chitosan 50 ppm	3.07	2.01	2.49	**2.25**	0.95	0.48	0.64	**0.56**
Chitosan 75 ppm	3.23	2.17	2.65	**2.41**	1.02	0.55	0.71	**0.63**
Chitosan 100 ppm	3.12	2.06	2.54	**2.30**	0.97	0.50	0.66	**0.58**
Chitosan 125 ppm	3.02[b]	1.96	2.44	**2.20**	0.92	0.45	0.61	**0.53**
Water spray	2.36	1.28	1.75	**1.52**	0.86	0.39	0.55	**0.47**
Control	2.31	1.25	1.73	**1.49**	0.82	0.35	0.51	**0.43**
Mean	**2.86**	**1.80**	**2.27**	**2.04**	**0.92**	**0.45**	**0.61**	**0.53**
CD (P=0.05) T	0.09**				0.02*			
CD (P=0.05) V	0.05**				0.03*			
CD (P=0.05) T x V	0.15*				0.06*			

*Significant; ** Highly significant. Each value is the mean of three replications and pooled mean of two crops

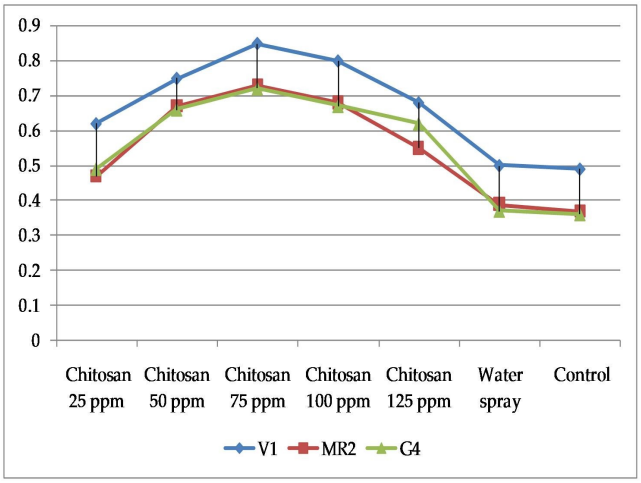

Fig. 1: Influence of chitosan onchlorophyll-a of mulberry, *Morus* sp.

These results are in consistent with increased photosynthetic pigments due to foliar application of chitosan at 250 ppm on tomato as reported by Farouk and Amany (2012) and Dawa *et al* (2019).

Influence of chitosan on moisture content and moisture retention capacity of mulberry

The present study indicated that chitosan application increased the moisture content and moisture retention capacity of different mulberry varieties (Table 2). Among the different mulberry varieties, V1 was found to be significantly superior in moisture content (72.10 %) and moisture retention capacity (69.86 %) of mulberry leaves than other varieties. This was strengthened by the report of Shivashankar (2015) who observed that V1 has more thickness of upper cuticle cum epidermis and more number of stomata compared to other genotypes, which resulted in increased moisture content.

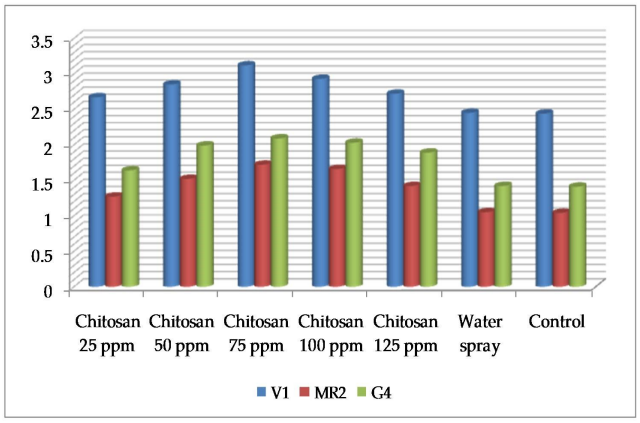

Fig. 2: Influence of chitosan onchlorophyll-b of mulberry, *Morus* sp.

In the present experiments, by the way of foliar application of chitosan, the increment in moisture content and moisture retention capacity ranged from 3.37 to 13.57 per cent and 2.90 to 14.27 per cent, respectively over the control. Here, chitosan 75 ppm recorded the highest moisture content and moisture retention capacity of 72.38 and 67.59 per cent, respectively, which was followed by chitosan 100 ppm (71.17 % and 65.11 %, respectively) and chitosan 50 ppm (68.35 % and 62.88 %, respectively). The lower values were observed in control (63.73 % and 59.46 %).

In respect of interaction effect, maximum moisture content (75.86 %) and moisture retention capacity (72.65 %) were

noticed in chitosan 75 ppm in V1 variety and minimum in the control of same variety (55.29 % and 50.16 %, respectively). Enhancement of moisture and moisture retention capacity by the application of chitosan could be due to increased chloroplast size which may affect the aeration via stomatal conductance and transpiration rates (Dzung and Thang, 2004). It was supported by the findings of Limpanavech *et al* (2008), who used chitosan to increase the chloroplast diameter of 10.43 μm at 50 ppm in older leaves of dendrobium orchid. Hence, change in chloroplast size and enlargement of chloroplast might be one of the factors that led to an increase in the moisture content and moisture retention capacity of leaves, so same trend was observed in the present experiment.

Table 2: Influence of chitosan on moisture content (%) and moisture retention capacity (%) of mulberry, *morus* sp.

Treatments		Moisture				Moisture retention capacity			
		V1	MR2	G4	Mean	V1	MR2	G4	Mean
Chitosan 25 ppm		71.95	56.26	69.42	**65.88**	68.21	53.89	60.52	**60.87**
Chitosan 50 ppm		73.09	60.38	71.58	**68.35**	70.24	54.71	63.69	**62.88**
Chitosan 75 ppm		75.82	66.01	75.30	**72.38**	72.65	62.58	67.54	**67.59**
Chitosan 100 ppm		73.98	64.91	74.63	**71.17**	71.89	57.97	65.46	**65.11**
Chitosan 125 ppm		72.67	58.24	73.89	**68.27**	70.85	55.24	62.66	**62.92**
Water spray		68.63	55.76	67.59	**63.99**	67.78	50.69	59.92	**59.46**
Control		68.53	55.29	67.38	**63.73**	67.42	50.16	59.87	**59.15**
Mean		**72.10**	**59.55**	**71.40**	**67.68**	**69.86**	**55.03**	**62.81**	**62.57**
CD (P=0.05)	T	0.56*				0.22*			
	V	0.46*				0.35**			
	T x V	1.09*				0.75*			

*Significant; ** Highly significant. Each value is the mean of three replications and pooled mean of two crops.

Influence of chitosan on primary metabolites of mulberry

Carbohydrates and protein are the major plant metabolites which influence quality and quantity of leaf yield (Manjula and Kumari, 2017). The result in Table 3 showed that the chitosan application increased the accumulation of soluble protein and total carbohydrate content in the different varieties of mulberry. Regarding the different mulberry varieties, V1 observed high amount of soluble protein (25.14 mg g^{-1}) and total carbohydrate (14.68 mg g^{-1}) compared to G4 and MR2. The present result was supported by Thirumalaisamy *et al* (2009) reported that V1 registered the highest total sugar (12.72%) and total protein (23.72%) content compared to other five mulberry varieties.

Among the various concentrations tested, the increment in soluble protein and total carbohydrate ranged from 15.48 to 63.29 and 26.29 to 55.42 per cent, respectively over the control. The best concentration was 75 ppm, which recorded the highest soluble protein (28.51 mg g^{-1}) and total carbohydrate (19.02 mg g^{-1}) content compared to other treatments. In our experiment, it is observed that the increase in concentration of chitosan over the optimum tends to increase the closure of stomata and reduces CO_2 exchange of leaves. This might lead to the reduction of metabolism in plants (Hidangmayum *et al*, 2019).

In the interaction between different concentrations of chitosan and mulberry varieties, chitosan 75 ppm in V1 recorded the maximum level of soluble protein (31.21 mg g^{-1}) and total carbohydrate (20.61 mg g^{-1}) and the minimum level was in control of MR2 (14.36 mg g^{-1} and 7.30 mg g^{-1}). The present results are in conformity with the findings that, total soluble sugars significantly increased (37.5$\pm$1.1 mg g^{-1}) at 250 ppm as compared to control (30.8$\pm$1.2 mg g^{-1})in cowpea (*Vigna unguiculata*) due to the application with chitosan Farouk and Amany (2012). This increase may be due to up-regulation of different genes involved in sugar transportation and metabolism (Zhang *et al*, 2017). The present findings was also supported by Alizadeh *et al* (2020), who recorded higher soluble protein of 74.08 mg g^{-1} at spraying chitosan of 2g l^{-1} on *Satureja hortensis*. Khateeb *et al* (2018) observed higher total carbohydrate of 5.18 mg g^{-1} at chitosan 100 ppm of *Siliybium marianum*. This also falls in line with the present observations.

Table 3: Influence of chitosan on primary metabolites (mg g^{-1}) of mulberry, *Morus* sp.

Treatments	Soluble protein				Total carbohydrate			
	V1	MR2	G4	Mean	V1	MR2	G4	Mean
Chitosan 25 ppm	23.29	17.66	20.83	**20.59**	12.30	9.53	10.29	**10.71**
Chitosan 50 ppm	25.56	21.56	23.10	**23.41**	16.54	13.77	12.54	**14.28**
Chitosan 75 ppm	31.21	25.58	28.75	**28.51**	20.61	17.84	18.60	**19.02**
Chitosan 100 ppm	29.33	24.70	27.87	**27.30**	18.37	15.60	16.36	**16.78**
Chitosan 125 ppm	26.19	22.93	25.73	**24.95**	14.55	11.78	14.53	**13.62**
Water spray	20.21	14.58	17.75	**17.51**	10.33	7.56	8.35	**8.74**
Control	20.19	14.36	17.83	**17.46**	10.07	7.30	8.06	**8.48**
Mean	**25.14**	**20.20**	**23.12**	**22.82**	**14.68**	**11.91**	**12.67**	**13.09**
CD (P=0.05) T	0.58**				0.59**			
CD (P=0.05) V	0.34*				0.38**			
CD (P=0.05) T x V	1.04*				1.02*			

*Significant; ** Highly significant. Each value is the mean of three replications and pooled mean of two crops

Table 4: Influence of chitosan on macro nutrients (%) of mulberry,Morus sp.

Treatments	Nitrogen				Phosphorus				Potassium			
	V1	MR2	G4	Mean	V1	MR2	G4	Mean	V1	MR2	G4	Mean
Chitosan 25 ppm	3.35	2.92	3.46	**3.24**	0.40	0.25	0.36	**0.34**	2.52	1.78	2.31	**2.17**
Chitosan 50 ppm	3.72	3.08	3.62	**3.47**	0.41	0.29	0.40	**0.37**	2.66	1.82	2.53	**2.34**
Chitosan 75 ppm	4.73	3.93	4.47	**4.38**	0.51	0.39	0.50	**0.47**	3.51	2.67	3.43	**3.20**
Chitosan 100 ppm	4.01	3.40	3.94	**3.78**	0.49	0.34	0.45	**0.43**	3.24	2.40	3.02	**2.89**
Chitosan 125 ppm	3.91	3.23	3.75	**3.63**	0.47	0.31	0.41	**0.40**	2.71	1.87	2.94	**2.51**
Water spray	3.11	2.40	2.94	**2.82**	0.37	0.21	0.32	**0.30**	2.12	1.28	1.79	**1.73**
Control	3.09	2.36	2.90	**2.78**	0.38	0.20	0.31	**0.30**	2.09	1.25	1.84	**1.73**
Mean	**3.70**	**3.05**	**3.58**	**3.44**	**0.43**	**0.29**	**0.39**	**0.37**	**2.69**	**1.85**	**2.55**	**2.37**
CD (P=0.05) T	0.28*				0.02*				0.23*			
CD (P=0.05) V	0.07**				0.03*				0.11*			
CD (P=0.05) T x V	0.41*				0.06*				0.42*			

*Significant; ** Highly significant. Each value is the mean of three replications and pooled mean of two crops.

Influence of chitosan on macronutrients of mulberry

According to results of the present study, macronutrients were significantly altered due to the treatment with chitosan on different mulberry varieties. The findings clearly exhibited that there is significant increase in the macronutrients namely nitrogen, phosphorus and potassium due to the application of chitosan at various concentrations (Table 4). Among different mulberry varieties, V1 variety recorded significantly highest macronutrient content over other two varieties macronutrients. Here, the increment was by 21.31, 48.28 and 45.40 per cent over the MR2 variety, which registered minimum macronutrient content. Among the different concentrations

of chitosan, the plants sprayed with chitosan 75 ppm noticed significantly highest nitrogen (4.38 %), phosphorus (0.47 %) and potassium (3.20 %) content. The next better treatment was chitosan 100 ppm which recorded the value of 3.78, 0.43 and 2.89 per cent, respectively. The lowest macronutrient level was observed in the control (2.78 %, 0.30 % and 1.73 %). The interaction between various concentrations and mulberry varieties showed that more quantity of macronutrients viz., nitrogen, phosphorus and potassium of 4.73, 0.51 and 3.51 per cent, respectively were obtained in the chitosan 75 ppm treated V1 variety, whereas, lesser amount was observed in control (2.36 %,0.20 % and 1.25 %, respectively) in MR2 variety. The present results are in agreement with the findings of Dawa *et al* (2019), who reported the higher nitrogen (2.52 %), phosphorus (0.357 %) and potassium (3.19 %) content of tomato by foliar application of chitosan at 250 ppm. Increase in nitrogen per cent may be brought about by the amino components in chitosan which are easily assimilable by the plants and this explains the uptake of nitrogen content by mulberry. Similar results were also obtained by Shehata *et al* (2012), who registered that high amount of phosphorus content at 4 ml l^{-1} of chitosan in cucumbers.

CONCLUSION

It is clear from the present study the foliar application of chitosan on three mulberry varieties as V1, MR2 and G4 had significant positive impact. Among the different varieties, V1 showed maximum response. Among the different concentrations studied, that chitosan at 75 ppm had registered highest biochemical constituents in mulberry leaf over all other treatments. Hence, the application of chitosan at 75 ppm thrice starting from 25 DAP at an interval of 15 days may be recommended among the farmers for significantly enhancing the mulberry leaf quality.

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