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Foliar application of nano fertilizers to enhance growth and cocoon yield of mulberry silkworm, *Bombyx mori*



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ABSTRACT

The objective of the current study was to boost the growth of the mulberry silkworm, Bombyx mori, and increase cocoon yield by feeding the larvae on mulberry leaves sprayed with various doses of nano fertilizers, specifically nano NPK (19:19:19) at concentrations of 2, 4 and 6 g/l, as well as nano urea at concentrations of 2, 4 and 6 ml/l. The application of nano fertilizers to the leaves, followed by their consumption by silkworms from hatching until the spinning stage, resulted in notable improvements in various economic parameters related to larval development, cocoon production, and silk quality. Among the different doses of nano fertilizers assessed, it was observed that nano NPK (19:19:19) at a concentration of 6 g/l exhibited favorable outcomes in terms of reduced larval duration, increased mature larval weight, improved effective rate rearing, enhanced silk productivity, cocoon yield, greater cocoon weight, higher shell weight, improved cocoon shell ratio, increased pupal weight, greater silk gland weight, longer cocoon filament length, finer denier and the highest fibroin protein, while concurrently having the lowest sericin protein content.

Keywords: Silkworm, cocoon yield, nano fertilizer, mulberry leaves, economic parameter, improvement and nano NPK.

INTRODUCTION

The Mulberry silkworm, Bombyx mori is an economically important insect in the silk industry due to its silk-secreting ability. The nutritional requirements of these silkworms are exclusively met by the mulberry plant, Morus spp. The quality of mulberry leaves profoundly influences the growth of silkworm larvae and the subsequent silk production [10]. Approximately 70 percent of silk proteins are sourced from mulberry leaves, underscoring the significance of providing silkworms with ample quantities of high-quality mulberry leaves to ensure successful cocoon production [18]. To enhance mulberry yield, the application of manures and fertilizers is considered crucial. However, a substantial portion of added fertilizers, particularly those containing nitrogen and phosphorus, often becomes inaccessible to plants shortly after application due to factors like leaching, volatilization, and fixation [14]. In such scenarios, the direct application of soluble nutrients to mulberry leaves through foliar spraying can enrich the leaves and offer immediate nutrient availability to silkworms, benefiting their growth and silk production.

The practice of supplementing nutrients to enhance mulberry leaves and subsequently feeding these enriched leaves to silkworms has emerged as a modern technique to elevate the economic value of cocoons [16] Therefore, it is crucial that nutrients applied through foliar nutrition remain easily

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DOI: https://doi.org/10.58321/AATCCReview.2023.11.04.322 © 2023 by the authors. The license of AATCC Review. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/). accessible during later leaf development stages and are costeffective [18]. Impressively, applying nutrient solutions via spraying on mulberry leaves, which are then consumed by immature silkworms, has shown significant improvements in larval weight, silk gland weight, and growth rate. These improvements subsequently lead to enhanced economic parameters such as cocoon weight, shell weight, and shell ratio in nutrient-treated silkworm batches [16].

In recent years, there has been a growing interest in the utilization of nanomaterials for nutrient elements in the field of fertilizers, giving rise to the term "nanofertilizers" [2]. Conventional fertilizers have limitations in terms of nutrient absorption efficiency, resulting in substantial losses and negative environmental impacts. Therefore, the adoption of nano fertilizers holds promise in reducing nutrient loss and potentially decreasing the quantity of fertilizers required for application. However, studies focusing on the use of nanomaterials as fertilizers have primarily concentrated on micronutrients, specifically zinc, copper, manganese, and iron. The role of NPK fertilizer and secondary nutrients in enhancing leaf yield, as well as larval and cocoon characteristics, has been elucidated by [17] and [11]. Taking these factors into consideration, the present study aims to investigate the impact of nano fertilizers applied to mulberry through foliar spray on the growth and cocoon yield of mulberry silkworms.

MATERIAL AND METHODS

An experiment was conducted in a well-established mulberry garden with V-1 mulberry variety grown as per package of practices to know the effect of nanofertilizers on mulberry silkworm growth and cocoon yield. The experiment was laid out in a completely randomized block design with 10 treatments replicated thrice at the Department of Entomology, University of Agricultural Sciences, Dharwad during 2021-22 and 2022-23. Nanofertilizers were sprayed on mulberry as per the treatment details at 35 and 45 days after pruning. Mulberry leaves were harvested from 55th day after pruning and fed to hybrid silkworms (FC1 × FC2) replication and treatment-wise from hatching till spinning stage as per standard package with 200 larvae per replication [4] Observations were recorded on chawki larval duration (hr), chawki larval weight (g/10 larvae), total larval duration (h), mature larval weight (g/10 larvae), effective rate of rearing (%), silk productivity (cg/day), cocoon yield (g/dfl), cocoon weight (g/10 cocoons), cocoon shell weight (g/10 shells), cocoon shell ratio (%), pupal weight (g/10pupae), cocoon filament length (m), denier (d), silk gland weight and silk protein. The data were transformed wherever necessary and analyzed statistically as suggested by [7] Duncan's multiple range test (DMRT) was applied to compare the treatment means.

RESULTS

Chawki larval duration (h): The duration of chawki larval stages remained unaffected by feeding on mulberry sprayed with nanofertilizers. However, when provided with nano NPK (19:19:19) at a concentration of 6 g/l, the larval duration decreased slightly to 162.96 hours, although this reduction was not statistically significant compared to other treatments, including unsprayed mulberry (164.71 hours) (Table 1).

Chawki larval weight (g/10 larvae): The weight of chawki silkworms did not exhibit significant changes when fed with foliar-sprayed nano NPK (19:19:19) and urea. The maximum larval weight of 2.358 g was observed in larvae fed on nano urea at 6 ml/l sprayed leaves, while the minimum weight of 2.145 g was recorded in silkworms fed with water-sprayed mulberry (Table 1).

Total larval duration (h): The application of nano NPK (19:19:19) at 6 g/l on mulberry leaves significantly reduced the total larval duration to 617.96 hours. This reduction was followed by nano NPK (19:19:19) at 4 g/l (621.88 h), nano urea at 6 ml/l (622.58 h), nano urea at 4 ml/l (625.54 h) and nano NPK (19:19:19) at 2 g/l (625.75 h), all of which were statistically similar to nano NPK (19:19:19) at 6 g/l. In contrast, Seri boost at 2.5 ml/l and unsprayed mulberry resulted in longer larval durations with 628.67 and 638.92 h, respectively (Table 1).

Mature larval weight (g/10 larvae): Among the treatments, nano NPK (19:19:19) at 6 g/l demonstrated the most significant enhancement in mature larval weight, reaching 37.493 g per 10 larvae. This was notably higher than feeding silkworms with Seri boost at 2.5 ml/l (34.441 g) and unsprayed mulberry (30.438 g). Additionally, nano urea at 6 ml/l and nano NPK (19:19:19) at 4 g/l showed similar weight improvements (Table 1).

Effective rate of rearing (%): Feeding silkworms with nano NPK (19:19:19) at 6 g/l sprayed leaves resulted in a remarkable increase in the effective rate of rearing, reaching 97.50 percent. In comparison, Seri boost at 2.5 ml/l achieved an effective rate of 91.08 percent. Nano urea at 6 ml/l, nano NPK (19:19:19) at 4 g/l and nano NPK (19:19:19) at 2 g/l exhibited similar rates of 95.67, 95.00 and 93.83, respectively. Conversely, feeding on unsprayed (86.75 %) and water-sprayed mulberry (86.33 %) resulted in lower effective rates of rearing (Table 1).

Silk productivity (cg/day): The highest silk productivity was achieved when silkworms were reared on mulberry leaves sprayed with nano NPK (19:19:19) at 6 g/l (6.082 cg/day), significantly outperforming Seri boost at 2.5 ml/l, which yielded 4.769 cg/day. Nano NPK (19:19:19) at 4 g/l and nano urea at 6 ml/l exhibited similar silk productivity levels. In contrast, silkworms fed on unsprayed mulberry and water-sprayed mulberry exhibited significantly lower silk productivity (Table 1).

Silk gland weight (g/larva): An analysis of the combined data indicated the superiority of nano NPK (19:19:19) at 6 g/l in improving silk gland weight, which reached 1.861 g per larva. This was followed by nano NPK (19:19:19) at 4 g/l (1.710 g/larva) and nano urea at 6 ml/l (1.675 g/larva), both of which were statistically similar. Silkworms reared on Seri boost at 2.5 ml/l sprayed leaves recorded a silk gland weight of 1.390 g. In contrast, unsprayed mulberry resulted in only 1.024 g of silk gland weight (Table 1).

Pupal weight (g/10 pupae): The pupal weight was significantly higher in silkworms when provided with nano NPK (19:19:19) at 6 g/l (13.260 g/ 10 pupae). The next best treatments were nano urea at 6 ml/l (12.778 g/10 pupae) and nano NPK (19:19:19) at 4 g/l (12.748 g/10 pupae), which were statistically similar to nano NPK (19:19:19) at 6 g/l. Seriboost at 2.5 ml/l yielded 11.661 g of pupal weight, while the lowest pupal weight was recorded in silkworms fed with unsprayed (10.562 g/10 pupae) and water-sprayed mulberry (10.530 g/10 pupae) (Table 1).

Cocoon weight (g/10 cocoons): The pooled data revealed the superiority of mulberry leaves sprayed with nano NPK (19:19:19) at 6 g/l in yielding significantly higher cocoon weight, measuring 17.531 g per 10 cocoons. This significantly outperformed Seri boost at 2.5 ml/l, which yielded 15.200 g/cocoons. Nano NPK (19:19:19) at 4 g/l and nano urea at 6 ml/l exhibited similar cocoon weights (Table 2).

Cocoon shell weight (g/10 shells): The best treatment for cocoon shell weight was nano NPK (19:19:19) at 6 g/l, which resulted in a weight of 4.234 g per 10 shells. This was followed by nano NPK (19:19:19) at 4 g/l (4.043 g/10 shells) and nano urea at 6 ml/l (3.990 g/10 shells), both of which were statistically similar. In contrast, silkworms reared from Seri boost at 2.5 ml/l and water-sprayed mulberry produced cocoon shell weights of 3.509 and 3.090 g/10 shells, respectively (Table 2).

Cocoon shell ratio (%): Combining results from two seasons, nano NPK (19:19:19) at 6 g/l recorded the highest cocoon shell ratio at 24.16 percent, followed by nano NPK (19:19:19) at 4 g/l (24.00%) which was statistically similar. Unsprayed and watersprayed treatments resulted in significantly lower cocoon shell ratios of 22.64 percent (Table 2).

Cocoon yield (g/dfl): Applying foliar spray of nano NPK (19:19:19) at 6 g/l to mulberry and subsequently feeding silkworms resulted in a cocoon yield of 769.31 g per disease-free laying (dfl). In comparison, Seri boost at 2.5 ml/l spray yielded 623.34 g/dfl. Nano urea at 6 ml/l and nano NPK (19:19:19) at 4 g/l were the next best treatments, with cocoon yields of 723.91 and 719.67 g/dfl, respectively. Cocoon yield was

significantly lower when silkworms were fed with unsprayed (534.49 g/dfl) and water-sprayed mulberry (530.48 g/dfl) (Table 2).

Cocoon filament length (m): The pooled data showed that nano NPK (19:19:19) at 6 g/l resulted in a significantly longer cocoon filament length, measuring 932.37 meters. Nano NPK (19:19:19) at 4 g/l (907.45 m) and nano urea at 6 ml/l (895.38 m) were next in line and statistically similar in producing longer filaments. In contrast, silkworms reared on water sprayed (702.52 m) and unsprayed leaves (702.08 m) produced shorter cocoon filament lengths (Table 2).

Denier (d): Nano NPK (19:19:19) at 6 g/l (2.73), nano urea at 6 ml/l (2.76), and nano NPK (19:19:19) at 4 g/l (2.82) demonstrated superior silk production with finer denier values. Silkworms reared on Seri boost at 2.5 ml/l fed leaves had a denier value of 3.06. Water-sprayed and unsprayed treatments resulted in coarser silk with denier values of 3.32 and 3.33 d, respectively (Table 2).

Fibroin and Sericin (%): Nano NPK (19:19:19) at 6 g/l was the most effective treatment in yielding the highest fibroin, reaching 77.17 percent. This outperformed Seri boost at 2.5 ml/l, which produced silk with 71.82 percent fibroin. Nano urea at 6 ml/l and nano NPK (19:19:19) at 4 g/l were the next best treatments, with fibroin contents of 74.97 and 74.55 percent, respectively, and were on par with nano NPK (19:19:19) at 6 g/l. Unsprayed mulberry produced silk with 68.21 percent fibroin. Additionally, nano NPK (19:19:19) at 6 g/l recorded 22.83 percent sericin content, compared to Seri boost at 2.5 ml/l (28.18 %). Sericin content of 31.80 percent was obtained from unsprayed treatment (Table 2).

DISCUSSION

Results clearly indicated that foliar application of nanofertilizers on mulberry and subsequent feeding of leaves to silkworms had a positive impact on silkworm growth and cocoon yield of hybrid (FC1 X FC2) silkworm. Mulberry leaves sprayed with nano fertilizers *viz.*, nano NPK (19:19:19) at 6 g/l, nano NPK (19:19:19) at 4 g/l, and nano urea at 6 ml/l significantly increased the growth and development of larvae ultimately leading to higher cocoon yield.

In the present study, there was no significant difference in the chawki larval duration and larval weight. While, other larval parameters viz., total larval duration and mature larval weight varied significantly, and higher values were obtained in nano NPK (19:19:19) at 6 g/l. It could be attributed to the fact that the percentage of mulberry leaves consumed and ingested are quite lower in early instar silkworms as compared to later instar. The chawki larval duration did not vary significantly due to the foliar spray of organic formulations and Seri boost [13]. Elemental composition in mulberry leaves due to foliar spray of NPK nutrients might have enhanced the metabolic activities of larvae fulfilling the nutrient requirement both quantitatively and qualitatively thus resulting in reduced larval duration and increased mature larval weight. Reduced weight and prolonged development in silkworms reared on untreated leaves might be due to insufficient essential nutrients in leaves. These observations are in full agreement with [18] who observed reduced larval duration (23.65 days) and increased larval weight (33.87 g/10 larvae) when silkworms were reared on

mulberry leaves sprayed with 100 per cent RDF + foliar nutrition at 2.0 percent (2.79, 1.63 and 2.54 % of NPK, respectively) as compared to feeding untreated leaves.

Increased silk gland weight could be attributed to nano fertilizers mediated activation of tissue metabolism that might have promoted the biological parameters of silk gland in the larvae. Further, higher effective rate of rearing in silkworms fed on nano NPK (19:19:19) at 6 g/l sprayed leaves indicated lesser susceptibility of the worms to the diseases that increased larval survival achieving the highest silk productivity and cocoon yield. These results are supported by [3] who recorded a significantly higher effective rate of rearing (93.98%) and silk productivity (0.62 cg/day) when nano ZnO + nano Cu each @ 500 ppm was used as foliar spray. The significantly higher effective rate of rearing (87.33 %) and cocoon yield (93.93 kg/100 dfls) was noticed upon foliar application 0.5 percent $ZnSO_4$ + 1.0 percent FeSO_4 + 0.1 percent citric acid + 0.2 percent boric acid + 0.5 percent $MnSO_4$ [5]. Further, the highest cocoon yield (79.2 kg/100 dfls) obtained in the treatment which received recommended basal dose (RBD) + three times foliar spray of 2.5 percent urea at 30, 45 and 60 days after pruning [1] support present findings.

The foliar spray of nanofertilizers showed better results for cocoon and silk traits of hybrid silkworms. Mineral supplementation for improvement of leaf quality and subsequent feeding to silkworms plays a vital role in silkworm development, cocoon characters and yield. Cocoon weight, cocoon shell weight, pupal weight, cocoon shell ratio, cocoon filament length, denier and silk protein varied significantly among the treatments with higher values for nano NPK (19:19:19) at 6 g/l which might be due to better nutrition as it supplies N, P and K which play a vital role in improving silkworm growth and development which ultimately affect silk production resulting in good quality cocoons. [15] reported that the supply of phosphorus and potassium increases the body weight of the silkworm and significantly enhances cocoon production. The cocoon weight (2.25 g/cocoon) and cocoon shell weight (0.69 g/cocoon) recorded significant increases after feeding the silkworm on N (0.2%), P (0.1%), K (0.3%) and Ca (0.1 %) supplemented mulberry leaves [10]. Application of minerals led to excess amino group availability of fatty tissue in the pupal body and resulted in the heavier pupa [12]. Present findings are in full agreement with the results of [3] who recorded highest cocoon weight (19.64 g/10 cocoons), shell weight (4.65 g/10 shell), cocoon shell ratio (23.67 %), pupal weight (16.39 g/10 pupae), single cocoon filament length (1087.01 m) and finer denier (3.48) in silkworms fed on mulberry leaves that received foliar spray of nano ZnO + nano Cu each at 500 ppm. Increased fibroin protein could be attributed to the fact that the silkworms derive 70 percent of silk protein from mulberry leaves. Hence, increased protein in nano fertilizers sprayed leaves might have increased silk fibroin. Earlier reports by [9, 6, 1, and 8] are in close conformity with present findings.

CONCLUSION

Feeding silkworms on nano fertilizers *viz.*, nano NPK (19:19:19) at 6 g/l, nano NPK (19:19:19) at 4 g/l and nano urea at 6 ml/l sprayed mulberry leaves resulted in improved larval growth and development, along with enhancements in cocoon and silk traits of FC1 X FC2 hybrid silkworm. Hence these nanofertilizers hold promise as valuable recommendations for farmers.

FUTURE SCOPE: Studying the underlying mechanisms by which nanofertilizers influence silkworm physiology and development can provide valuable insights. This might involve studying gene expression, metabolic pathways, and cellular responses and also exploring the potential long-term effects of repeated exposure to nanofertilizers on silkworms' health, reproduction, and subsequent generations can provide a comprehensive understanding of their sustainability and safety.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

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Table 1 Effect of nano	tertilizers on arowth and develo	nment of silkworm Rom	hvx mori (Pooled data c	of 2021-22 and 2022-231
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Treatments	Chawki larval duration (hr)	Chawki larval weight (g/10 larvae)	Total larval duration (hr)	Mature larval weight (g/10 larvae)	Effective rate of rearing (%)	Silk productivity (cg/day)	Silk gland weight (g/larva)	Pupal weight (g/10 pupae)
T ₁ : Nano urea @ 2 ml/l	164.08	2.197	630.75 ^{bc}	33.884 ^{cd}	90.92 (72.54) ^{de}	4.626 ^{de}	1.329 ^d	11.475 ^{de}
T2 : Nano urea @ 4 ml/l	163.63	2.234	625.54 ^{ab}	35.397 ^{bc}	92.83 (74.53) ^{cd}	5.097°	1.512°	12.088 ^{cd}
T ₃ : Nano urea @ 6 ml/l	163.04	2.358	622.58 ^{ab}	36.333 ^{ab}	95.67 (78.04) ^ь	5.613 ^b	1.675 ^b	12.778^{ab}
T4 : Nano NPK (19:19:19) @ 2 g/l	163.92	2.267	625.75 ^{ab}	35.302 ^{bc}	93.83 (75.66) ^{bc}	5.295°	1.496°	12.537 ^{bc}
T ₅ : Nano NPK (19:19:19) @ 4 g/l	163.79	2.277	621.88 ^{ab}	36.087 ^{ab}	95.00 (77.10) ^{ьс}	5.742 ^b	1.710 ^b	12.748 ^{abc}
T ₆ : Nano NPK (19:19:19) @ 6 g/l	162.96	2.321	617.96ª	37.493ª	97.50 (81.14)ª	6.082ª	1.861ª	13.260ª
T ₇ : Urea @ 2.5 %	164.25	2.262	632.83 ^{bc}	32.926 ^d	89.58 (71.20) ^{ef}	4.438 ^e	1.216 ^e	11.211 ^{ef}
Tଃ : Seriboost @ 2.5 ml/l	163.88	2.217	628.67 ^{abc}	34.441 ^{cd}	91.08 (72.71) ^{de}	4.769 ^{de}	1.390 ^d	11.661 ^{de}
T9 : Absolute control	164.21	2.145	638.13°	30.638 ^e	86.33 (68.31) ^g	3.998 ^f	1.040 ^f	10.530 ^g
T ₁₀ : Untreated control	164.71	2.147	638.92°	30.438 ^e	86.75 (68.67) ^{fg}	4.032 ^f	1.024 ^f	10.562 ^{fg}
S.Em (±)	-	-	3.72	0.517	0.96	0.105	0.025	0.221
C.V (%)	1.78	3.356	2.02	2.648	2.23	3.706	3.138	3.293

Figures in parenthesis are arc sine transformed values Figures in the column followed by same letters are not-significant at p=0.05 by DMRT

Table 2. Cocoon and silk traits of mulberry silkworm, Bombyx mori as influenced by nano fertilizers (Pooled data of 2021-22and 2022-23)

	Cocoon	Cocoon			Cocoon		Silk protein	
Treatments	weight (g/10 cocoons)	shell weight (g/10 shells)	Cocoon shell ratio (%)	Cocoon yield (g/dfl)	filament length (m)	Denier	Fibroin (%)	Sericin (%)
T1 : Nano urea @ 2 ml/l	14.940 ^{de}	3.442 ^d	23.07 (28.71) ^{ef}	611.53 ^e	790.76 ^{de}	3.13 ^{bc}	71.57 (57.80) ^{cd}	28.43 (32.22) ^{de}
T2 : Nano urea @ 4 ml/l	15.801 ^{cd}	3.683°	23.33 (28.88) ^{de}	660.38 ^{cd}	839.24 ^{cd}	3.05 ^b	73.29 (58.91) ^{bcd}	26.71 (31.11) ^c
T3 : Nano urea @ 6 ml/l	16.814 ^{ab}	3.990 ^b	23.73 (29.15) ^{bc}	723.91 ^b	895.38 ^{ab}	2.76ª	74.97 (59.98) ^{ab}	25.03 (30.02) ^b

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T4 : Nano NPK	16.221 ^{bc}	2.910	23.50	684.99 ^{bc}	864.30 ^{bc}	3.02 ^b	71.73	28.27
(19:19:19) @ 2 g/l		5.010°	(29.00) ^{cd}				(57.88) ^{cd}	(32.12) ^d
T5 : Nano NPK	16 021ab	4.042h	24.00	710 67h	007 4 Eab	2 023	74.55	25.46
(19:19:19) @ 4 g/l	10.031	4.043	(29.33) ^{ab}	/19.078	907.45 ^{ab}	2.82ª	(59.71) ^{abc}	(30.30) ^{bc}
T ₆ : Nano NPK	17521a	1 72 1a	24.16	760.21a	932.37ª	2.73ª	77.17	22.83
(19:19:19) @ 6 g/l	17.331	4.234	(29.44) ^a	/09.31			(61.46) ^a	(28.54)ª
T7 : Urea @ 2.5 %		2 2 4 2 d	22.94	E07 E1e	770.49 ^e	3.23 ^{cd}	70.18	29.82
	14.509	5.343 ^u	(28.62) ^f	587.51°			(56.90) ^{de}	(33.10) ^e
T ₈ : Seriboost @ 2.5 ml/l	15.200 ^{de}	3.509 ^d	23.10	623.34 ^{de}	806.43 ^{de}	3.06 ^{bc}	71.82	28.18
			(28.73) ^{ef}				(57.95) ^{cd}	(32.06) ^d
T9 : Absolute control	13.651^{f}	3.090 ^e	22.64	530.48 ^f	702.52^{f}	3.32 ^d	68.23	31.78
			(28.41) ^g				(55.69) ^e	(34.31) ^f
T ₁₀ : Untreated control	13.686 ^f	3.100 ^e	22.64	534.49 ^f	702.08 ^f	3.33 ^d	68.21	31.80
			(28.41) ^g				(55.69) ^e	(34.32) ^f
S.Em (±)	0.313	0.056	0.04	13.91	17.18	0.06	0.68	0.29
C.V (%)	3.561	2.691	0.41	3.67	3.66	3.46	2.03	1.63

Figures in parenthesis are arc sine transformed values

Figures in the column followed by same letters are not-significant at p=0.05 by DMRT

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