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Effect of Deltamethrin and a Neem Based Pesticide Achook on Some Biochemical Parameters in Tissues Liver, Ovary and Muscle of Zebrafish Danio rerio (Cyprinidae)

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Abstract

The present study is aimed to evaluate the changes in total protein, total free amino acid content and nucleic acids (DNA and RNA) in the liver, ovary and muscle of Zebrafish after exposure to 96 h LC_5, LC_{10} and LC_{20} of Deltamethrin and neem based pesticide Achook. It was found that the protein content was reduced to 45, 68, 65% (after Deltamethrin exposure) and 54, 81, 85% (after Achook exposure) as compared to the controls (100%) after 16 days in the liver, ovary and muscle, respectively at LC_{20} exposure. The total free amino acid contents in liver were enhanced to 146 and 142% of controls (100%) after exposure to LC_{20} of Deltamethrin and Achook respectively. However, in the ovary of treated fishes the total free amino acid content was significantly ($P<0.05$) reduced. In addition, DNA content was reduced to 26, 40 and 38% of controls (100%) in liver, ovary and muscle respectively after 16 days exposure to LC_{20} of Deltamethrin. The reduction in DNA content from the control was 37, 46 and 44% in liver, ovary and muscle respectively due to Achook at the same concentration and exposure period as that of Deltamethrin. Similarly, RNA contents were also reduced in the liver, ovary and muscle significantly after Deltamethrin and Achook exposure. It was observed that all the changes were concentration as well as time dependent. This study will reflect the potential role of these biochemical parameters for assessment of aquatic pollution as far as the natural pesticides are concerned.

Keywords: *Danio rerio, achook, deltamethrin, toxicity; biochemical constituents.*

Introduction

The pollution has become a major challenge for existence of mankind on the earth. The degradation of aquatic system is a worldwide phenomenon originated from the intense population and from the corresponding increase in agriculture practices as well as industrial and domestic activities. Pesticides are major cause of concern for aquatic environment because of their toxicity, persistency and tendency to concentrate in organisms as they move up the food chain, increase their toxicity to fish, birds and other wildlife and, in turn to man[1]. While the pesticides are instrumental in achieving significant increase in crop productivity, it has to be considered that they cause serious ecological hazards to the non-target animals especially the fish, which forms an important part of food chain for various animals including human beings. Because of their beneficial qualities, synthetic pyrethroids, such as Deltamethrin, have attracted farmers and health departments to use them in pest control. However, these compounds are generally found to be highly toxic to fish and zooplankton communities[2]. Deltamethrin is a type-II pyrethroid compound that is more potentially toxic to fish and least toxic to mammals contaminating aquatic ecosystems as potential toxic pollutants, causes severe morphological alterations in the gill and liver of fingerlings of Nile tilapia, *Oreochromis niloticus*[3].

An attempt to preserve and protect the environment as well as human health as a best alternative different parts of the neem tree (*Azadirachta indica*) have been studied and reported to contain compounds including nimbin, azadirachtin and meliantroil etc. which have a variety of properties ranging from insecticidal to antiviral from ancient times[4]. Azadirachtin, a scientific gold mine is a valuable natural pesticide, has very low toxicity for vertebrates[5]. The extract of *A. indica* has been used successfully in aquaculture to control fish predators. Although neem extract is less toxic to *Prochilodus lineatus* than other synthetic insecticides used in fish-farming it does cause functional and morphological changes in this fish species[6]. Recently, some neem based formulations were found toxic to the adult, embryo and fingerlings of Zebrafish as well as affected its reproductive ability[7-9]. Also, Ansari and Ansari[10] observed that Dimethoate was toxic to the adult, embryos and fingerlings of Zebrafish and caused a significant reduction in fecundity, viability, hatchability and survival of fingerlings.

Pesticides produce many physiological and biochemical changes in fish and other aquatic species by influencing the activities of several enzymes[11]. It has been shown that the liver is the prime location for detoxifying pesticides in fishes muscle glycogen and protein responses appear particularly suitable for measuring stressful levels of pollutants and have long been used as indicators of stress in fish[12]. Doses of pesticides that are not high enough to kill fish are associated with subtle changes in behavior and physiology that impair both survival and reproduction. Hence, a need was felt to investigate a comparative effect of sub-lethal exposure of Deltamethrin (synthetic pyrethroid) and Achook (neem pesticide) to different biochemical parameters in the liver, ovary and muscle of Zebrafish (*Danio rerio*). The Zebrafish was selected as the test species for toxicological studies according to the recommendation of the International Organization for Standardization and the Organization for Economic Co-operation and Development[13]. The present study, therefore, is an addition to the long sought understanding of toxic effect of pyrethroid and neem based pesticide on the tissues (liver, ovary and muscle) of Zebrafish.

Material and Methods

Zebrafish, *Danio rerio* were reported from Uttar Pradesh[14]. They were collected from the local ponds, stocked and acclimatized for a time period of 10-15 days in the laboratory conditions in glass aquaria containing dechlorinated water. The water of the aquarium was aerated continuously through stone diffusers connected to a mechanical air compressor. Water temperature ranged between 25±2^{o}C and the pH was maintained between 6.6 and 8.5. Fish were fed twice daily alternately with raw chopped goat liver and brine shrimps. The diet was supplemented with *Drosophila* flies once daily.

For the present study, matured adult fishes were exposed to different concentrations *viz.* LC_5, LC_{10} and $LCss_{20}$ of Deltamethrin and Achook for 16 days continuously. Three replicates of ten fishes for each concentration of the pesticides were used. In these aquaria water was replaced daily with fresh treatment of pesticides. Each experiment was accompanied by its respective control.

After the expiry of the exposure periods (4, 8, 12 and 16 days), required number of exposed fishes were taken out, their liver, ovary and muscle were removed and processed for the estimation of nucleic acid, total protein and total free amino acids in the liver, ovary and muscle of *D. rerio*. Nucleic acid was estimated according to the method of Schneider[15] using diphenylamine reagent for DNA and orcinol reagent for RNA. Total protein was estimated according to the method of Lowry *et al.*[16] using Folin-phenol reagent whereas total free amino acids were estimated by the method of Spies[17] using ninhydrin reagent. The values were expressed as µg/mg wet tissue. The data so obtained was analyzed statistically by two-way Analysis of variance (ANOVA) to test the significance by StatPlus® software purchased from analystsoft Vancouver, Canada.

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Results and Discussion

During the present investigation we observed significant (P<0.05) alterations in DNA, RNA, total protein and free amino acid contents in the liver, ovary and muscle of *D. rerio* exposed to Deltamethrin and Achook at different concentrations and exposure periods. It was observed that after 8 days of exposure to LC_{20} of Deltamethrin in the ovarian protein was reduced to 76% but after Achook treatment the protein content reduced to 91% from the control (100%). This shows that Achook is less toxic than Deltamethrin as far as ovary is concerned. The total protein content were reduced to 45, 68, 65 and 54, 81, 85% of controls (100%) after exposure of fish to LC_{20} of Deltamethrin and Achook for 16 days in the liver, ovary and muscle, respectively (table 1). The total free amino acid contents in liver were enhanced to 146 and 142% of controls (100%) after the exposure to LC_{20} of Deltamethrin and Achook respectively. This shows that after 16 days the effects of these pesticides are almost similar (table 2). From table 2 it is also evident that in the liver and muscle LC_5 and LC_{10} of Deltamethrin causes greater increase in total free amino acid content than Achook. Here too, the enhancement was concentration-dependent. In the ovary of treated fishes the total free amino acid content was significantly (P<0.05) reduced. In the ovary the total free amino acid content reduced to 71% after LC_{10} treatment of Deltamethrin while similar reduction *i.e.* 71% of control (100%) was observed after LC_{20} exposure of Achook. This again proves that Deltamethrin is more toxic than Achook. In addition, DNA content was reduced to 26, 40 and 38% of controls (100%) in liver, ovary and muscle respectively after 16 days exposure to LC_{20} of Deltamethrin. The reduction in DNA content from the control was 37, 46 and 44% in liver, ovary and muscle respectively due to Achook at the same concentration and exposure period as that of Deltamethrin (table 3). RNA contents also reduced to 63, 55, 69% (Deltamethrin) and 79, 64, 72% (Achook) of controls after 16 days in liver, ovary and muscle respectively at LC_{20} exposure (table 4). There was a concentration-dependent inhibition in the DNA, RNA and total protein contents. On the other hand, the total free amino acid content was significantly enhanced (P<0.05) in liver and muscle of treated fishes exposed to sub-lethal concentrations of both pesticides. The results showed that Deltamethrin is more toxic than Achook because the reduction in DNA, RNA and total protein contents observed was more as compared to Achook treated fishes.

Various insecticides affect the biochemical compositions of fishes. The liver is the target organ which is greatly affected by pesticidal contaminations due to detoxification of the xenobiotics. Cytoplasmic granulation in the liver of the Indian snake head, *Channa punctatus* following acute exposure to sub-lethal concentration on dimethoate was reported by Anees[18]. Since, fish have a very little amount of carbohydrate the next alternative source of energy during stress is protein to meet the increased energy demand. In this study, depletion of protein content in liver, muscle and ovary may have due to their degradation and possible utilization of degraded products for metabolic purposes. According to Kumar and Ansari[19] Zebrafish exposed to long term sub-lethal concentrations of malathion, failed to spawn and showed skeletal deformities.

Acute toxicity data for deltamethrin in fish have been published as a report of the World Health Organization[20] and classified as highly toxic to fish, being in the range of $LC_{50}<100$ µg/l. Effect of deltamethrin on physiological changes in freshwater catfish, *Heteropneustes fossilis* has been reported[21]. However, several studies have shown that plant toxins at low concentrations are very toxic to all groups of aquatic fauna. Recently, Ogbuewu *et al.*[22] studied toxicological effects of leaf meal of ethno-medicinal plant neem on serum biochemistry of crossbred New Zealand white typed rabbit bucks and reported reduction in the serum cholesterol and glucose value. This reduction could be attributed to the presence of bio-active compounds contained in neem leaves which has the ability to block the energy metabolic pathway thus making it difficult for the animals to meet their energy requirements[23]. Hussein *et al.*[24] recorded that the decrease of the total protein in *Oreochromis niloticus* treated with atrazine herbicide. Swietla and Zuk[25] recorded that herbicides act as inhibitor of the synthesis of nucleic acids and proteins. Also, Katherine *et al.*[26] recorded a significant reduction in protein after the exposure of herbicides in Atlantic salmon. Therefore, present results are in agreement with the observations of the previous workers.

Supporting our observation, Anita Susan *et al.*[27] reported a decrease in protein content in the liver, muscle, brain and gill of the two carps, *Labeo rohita* and *Cirrhinus mrigala* exposed to sub-lethal concentration of fenvalerate. Also, toxicological impact of lindane on the ovary in air-breathing catfish, *Heteropneustes fossilis* has been reported[28]. Decreased protein content may be attributed to stress mediated immobilization of these compounds to fulfill on increased element for energy by the fish to cope with environmental condition exposed by the toxicant. In this study, increment in free amino acids content may be due to the breakdown of protein for energy requirement and impaired incorporation of amino acids in protein synthesis[29].

Nucleic acids play a major role in growth and development. The amount of DNA, the carrier of genetic information, remains stable under changing environmental situations and has been used as an indicator of biomass. It is also known that the DNA functions as primer in DNA and RNA polymerase reactions and the inhibition in the DNA content caused inhibition of both DNA and RNA synthesis[30]. The inhibition of DNA synthesis affects both protein and amino acid contents by decreasing the content of RNA. Therefore, it is possible that the enzyme necessary for DNA synthesis might have been inhibited by these pesticides. On compilation of the result, it appears that the disruption of DNA synthesis might have affected RNA synthesis and consequently protein synthesis.

Conclusion

The present investigation indicates that chronic exposure of low concentrations of Deltamethrin and Achook can cause significant alterations in DNA, RNA, total protein and free amino acid contents of liver, ovary and muscle of fish *D. rerio*. Thus, the present study provides evidence that both the pesticides cause the alterations in the physiological and biochemical changes in vital tissues which influences the survival of Zebrafish. It means that the "safe" neem based products are not so safe to the Zebrafish and these chemicals should be used in sustainable way in areas near water bodies. This implies that one should take the necessary precaution in the application of insecticides even the natural one to protect the life of fish and other aquatic fauna. It is suggested that these types of toxicological studies are highly required to monitor the aquatic system and asses the toxic effect of pesticides on aquatic organisms particularly fish.

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Table -1: Effects of Deltamethrin and Achook on total protein content (µg/mg wet tissue) in tissues of Zebrafish

Tissue	Time days	Treatment and Concentration (µg/l)						
		Control (0.00)	Deltamethrin			Achook		
			LC_5 (0.016µg/l)	LC_{10} (0.025µg/l)	LC_{20} (0.043µg/l)	LC_5 (0.025µg/l)	LC_{10} (0.17µg/l)	LC_{20} (0.35µg/l)
Liver	4	113.73±0.64 (100)	95.23±0.12 (84)	88.80±0.32 (78)	80.30±0.19 (71)	109.90±0.56 (95)	100.23±0.09 (88)	90.24±0.30 (79)
	8	114.02±0.24 (100)	88.67±0.22 (78)	83.03±0.35 (73)	77.13±0.07 (68)	100.23±0.09 (88)	88.80±0.32 (78)	80.14±0.04 (70)
	12	113.47±0.08 (100)	80.19±0.03 (71)	69.53±0.17 (61)	60.50±0.14 (53)	90.43±0.13 (80)	80.30±0.19 (71)	70.10±0.18 (62)
	16	111.94±0.44 (100)	71.48±0.12 (64)	59.44±0.17 (53)	50.39±0.07 (45)	80.40±0.12 (72)	70.13±0.07 (63)	60.58±0.34 (54)
Ovary	4	179.59±0.13 (100)	169.52±0.12 (94)	158.97±0.50 (88)	150.56±0.67 (84)	176.25±0.41 (98)	174.04±0.15 (97)	170.10±0.16 (94)
	8	183.08±0.17 (100)	159.15±0.42 (87)	151.11±0.40 (82)	139.70±0.09 (76)	174.30±0.04 (95)	170.96±0.24 (93)	167.16±0.29 (91)
	12	177.91±0.33 (100)	150.56±0.66 (85)	140.19±0.08 (79)	129.54±0.98 (73)	168.10±0.18 (94)	160.34±0.17 (90)	155.05±0.26 (87)
	16	173.11±0.16 (100)	135.24±0.19 (78)	129.40±0.15 (75)	118.19±0.67 (68)	160.37±0.14 (92)	150.20±0.03 (87)	140.13±0.50 (81)
Muscle	4	183.30±0.20 (100)	160.54±0.26 (87)	144.48±0.15 (79)	130.34±0.28 (71)	173.84±0.31 (95)	170.54±0.77 (93)	168.10±0.34 (91)
	8	179.86±0.34 (100)	153.65±0.08 (85)	136.07±0.33 (75)	125.24±0.22 (69)	170.02±0.04 (94)	165.90±0.97 (92)	160.23±0.28 (89)
	12	178.61±0.04 (100)	147.30±0.12 (82)	131.17±0.81 (73)	120.15±0.56 (67)	165.10±0.33 (92)	161.57±0.73 (90)	155.35±0.80 (87)
	16	175.80±0.44 (100)	140.21±0.15 (79)	124.24±0.20 (70)	115.30±0.18 (65)	158.57±0.56 (90)	156.27±0.10 (88)	150.40±0.76 (85)

Values are means ± SD of six individual observations and significant at $P<0.05$ (two-way ANOVA).

Table -2: Effects of Deltamethrin and Achook on total free amino acid content (µg/mg wet tissue) in tissues of Zebrafish

Tissue	Time days	Treatment and Concentration (µg/l)						
		Control (0.00)	Deltamethrin			Achook		
			LC_5 (0.016µg/l)	LC_{10} (0.025µg/l)	LC_{20} (0.043µg/l)	LC_5 (0.025µg/l)	LC_{10} (0.17µg/l)	LC_{20} (0.35µg/l)
Liver	4	45.79±0.66 (100)	62.69±0.94 (136)	64.50±0.30 (141)	73.18±0.57 (159)	59.10±0.05 (129)	61.08±0.62 (133)	71.58±0.34 (156)
	8	43.92±0.33 (100)	58.77±0.30 (134)	62.83±0.39 (143)	69.26±0.52 (158)	54.44±0.50 (124)	58.18±0.29 (132)	68.11±0.46 (155)
	12	43.51±0.85 (100)	57.78±0.74 (132)	59.77±0.33 (137)	64.50±0.13 (148)	52.21±0.17 (120)	56.06±0.16 (129)	62.81±0.02 (144)
	16	42.74±0.58 (100)	54.95±0.60 (128)	56.77±0.49 (132)	62.79±0.25 (146)	51.10±0.61 (119)	52.13±0.65 (122)	60.91±0.50 (142)
Ovary	4	57.98±0.15 (100)	48.16±0.45 (83)	45.03±0.54 (78)	40.48±0.33 (70)	55.16±0.45 (95)	51.10±0.61 (88)	48.79±0.66 (84)
	8	56.38±0.34 (100)	45.37±0.09 (80)	43.47±0.30 (77)	38.02±0.50 (67)	52.37±0.01 (93)	48.56±0.37 (86)	43.92±0.33 (79)
	12	55.18±0.03 (100)	43.80±0.30 (79)	41.01±0.39 (74)	34.90±0.88 (63)	45.80±0.30 (83)	43.97±0.30 (80)	40.51±0.85 (73)
	16	54.09±0.16 (100)	41.40±0.13 (76)	38.35±0.07 (71)	32.80±0.22 (60)	42.40±0.13 (78)	39.67±0.93 (73)	38.64±0.58 (71)
Muscl e	4	46.12±0.10 (100)	62.49±0.07 (135)	64.17±0.68 (140)	72.20±0.43 (156)	48.49±0.07 (105)	51.50±0.85 (111)	55.77±0.82 (121)
	8	44.57±0.12 (100)	59.85±0.53 (134)	62.15±0.08 (139)	66.38±0.78 (149)	46.72±0.53 (104)	48.27±0.68 (108)	53.06±0.79 (119)
	12	42.83±0.16 (100)	57.29±0.02 (133)	60.88±0.67 (142)	64.39±0.12 (145)	44.29±0.02 (103)	45.16±0.38 (105)	50.38±0.78 (118)
	16	42.24±0.14 (100)	55.13±0.20 (130)	57.82±0.45 (136)	60.98±0.33 (144)	43.13±0.20 (102)	44.18±0.66 (104)	49.20±0.43 (116)

Values are means ± SD of six individual observations and significant at $P<0.05$ (two-way ANOVA).

Table- 3: Effects of Deltamethrin and Achook on DNA content (µg/mg wet tissue) in tissues of Zebrafish

Tissue	Time (days)	Treatment and Concentration (µg/l)						
		Control (0.00)	Deltamethrin			Achook		
			LC_5 (0.016µg/l)	LC_{10} (0.025µg/l)	LC_{20} (0.043µg/l)	LC_5 (0.025µg/l)	LC_{10} (0.17µg/l)	LC_{20} (0.35µg/l)
Liver	4	35.37±0.14 (100)	24.61±0.18 (70)	24.42±0.54 (69)	16.08±0.72 (45)	29.09±0.27 (82)	26.06±0.03 (74)	21.52±0.17 (61)
	8	34.06±0.06 (100)	22.48±0.16 (66)	22.19±0.38 (65)	12.11±0.67 (36)	26.17±0.72 (77)	25.00±0.23 (73)	20.35± 0.10 (60)
	12	31.70±0.19 (100)	19.36±0.56 (61)	18.36±0.26 (58)	11.02±0.75 (35)	23.75±0.65 (75)	22.40±0.74 (71)	17.52±0.26 (55)
	16	35.07±0.13 (100)	16.35±0.60 (47)	15.78±0.33 (45)	9.29±0.68 (26)	22.72±0.28 (65)	19.22±0.48 (55)	12.95±0.19 (37)
Ovary	4	37.09±0.15 (100)	30.53±0.04 (82)	28.81±0.13 (78)	26.06±0.03 (70)	32.13±0.12 (87)	30.00±0.43 (81)	27.48±0.12 (74)
	8	35.59±0.24 (100)	22.75±0.16 (64)	21.65±0.23 (61)	20.18±0.16 (57)	30.30±0.07 (85)	24.58±0.14 (69)	21.49±0.16 (60)
	12	34.16±0.17 (100)	20.38±0.09 (60)	18.63±0.46 (55)	17.53±0.62 (51)	26.02±0.13 (76)	21.19±0.45 (62)	18.54±0.14 (54)
	16	33.84±0.07 (100)	16.52±0.52 (49)	14.65±0.80 (43)	13.50±0.52 (40)	20.16±0.09 (60)	18.18±0.44 (54)	15.69±0.40 (46)
Muscle	4	37.22±0.16 (100)	29.34±0.30 (79)	24.22±0.11 (65)	19.89±0.06 (53)	31.99±0.38 (86)	28.53±0.14 (77)	27.99±0.35 (75)
	8	35.87±0.15 (100)	25.76±0.17 (72)	20.94±0.24 (58)	18.03±0.35 (50)	29.17±0.72 (81)	24.70±0. 27 (69)	21.76±0.20 (61)
	12	34.86±0.18 (100)	22.25±0.19 (64)	18.49±0.47 (53)	15.44±0.12 (54)	27.05±0.65 (78)	23.52±0.15 (67)	19.49±0.47 (51)
	16	35.70±0.03 (100)	17.84±0.27 (50)	16.59±0.40 (46)	13.68±0.23 (38)	26.74±0.30 (75)	22.99±0.26 (64)	16.39±0.38 (46)

Values are means ± SD of six individual observations and significant at P<0.05 (two-way ANOVA).

Table -4: Effects of Deltamethrin and Achook on RNA content (µg/mg wet tissue) in tissues of Zebrafish

Tissue	Time (days)	Treatment and Concentration (µg/l)						
		Control (0.00)	Deltamethrin			Achook		
			LC_5 (0.016µg/l)	LC_{10} (0.025µg/l)	LC_{20} (0.043µg/l)	LC_5 (0.025µg/l)	LC_{10} (0.17µg/l)	LC_{20} (0.35µg/l)
Liver	4	37.33±0.27 (100)	35.21±0.18 (94)	33.28±0.31 (89)	30.46±0.34 (82)	36.33±0.27 (97)	35.45±0.10 (95)	32.39±0.29 (87)
	8	35.45±0.27 (100)	33.29±0.19 (93)	30.36±0.27 (85)	27.30±0.24 (77)	34.17±0.19 (96)	33.30±0.24 (94)	30.55±0.03 (86)
	12	34.46±0.28 (100)	31.37±0.60 (90)	28.38±0.25 (82)	24.55±0.17 (71)	32.41±0.15 (94)	30.31±0.18 (88)	27.56±0.14 (80)
	16	32.39±0.29 (100)	28.48±0.17 (88)	25.47±0.29 (79)	20.42±0.32 (63)	29.96±0.13 (92)	27.15±0.19 (84)	25.67±0.45 (79)
Ovary	4	45.34±0.27 (100)	43.50±0.23 (96)	40.24±0.13 (89)	36.57±0.27 (81)	44.15±0.15 (97)	41.46±0.24 (90)	38.42±0.18 (85)
	8	43.50±0.34 (100)	41.33±0.12 (95)	38.41±0.27 (88)	31.53±0.35 (72)	42.02±0.20 (96)	39.06±0.09 (89)	32.71±0.13 (75)
	12	43.35±0.13 (100)	40.38±0.30 (93)	35.37±0.20 (82)	27.53±0.31 (63)	41.05±0.25 (95)	37.18±0.15 (86)	30.57±0.12 (71)
	16	44.34±0.22 (100)	36.30±0.22 (82)	31.46±0.24 (71)	24.32±0.15 (55)	39.19±0.05 (88)	33.17±0.13 (75)	28.43±0.33 (64)
Muscle	4	59.17±0.10 (100)	55.50±0.19 (94)	51.62±0.24 (87)	48.25±0.14 (82)	57.56±0.30 (97)	53.75±0.32 (91)	51.41±0.39 (87)
	8	57.46±0.25 (100)	53.15±0.02 (92)	49.54±0.32 (86)	45.38±0.20 (79)	55.46±0.09 (96)	51.44±0.36 (89)	48.47±0.40 (84)
	12	58.18±0.09 (100)	50.46±0.20 (87)	45.57±0.10 (78)	42.41±0.13 (73)	53.44±0.14 (92)	48.59±0.05 (83)	45.56±0.27 (78)
	16	57.31±0.18 (100)	45.55±0.19 (79)	41.56±0.14 (72)	39.47±0.30 (69)	50.54±0.19 (88)	45.34±0.28 (79)	41.55±0.19 (72)

Values are means ± SD of six individual observations and significant at $P<0.05$ (two-way ANOVA).