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Biochemical Influence of Cyanophos Insecticide on Radish Plant II. Effect on Some Metabolic Aspects During the Growth Period

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Abstract: In a field experiment, effect of the organophosphorus insecticide cyanophos on the photosynthetic pigments and the metabolism of carbohydrate and nitrogen were declared in the leaves of radish plant (*Raphanus sativus* L.). Three sprays with different concentrations (0.0, 0.025, 0.037 and 0.05% v/v) were applied at age intervals of 12, 24 and 36 days, respectively. Data were recorded after 3 and 12 days after each spray. The results revealed that there was a slight significant effect on the total pigments. The spray by insecticide attenuated most prominently the chlorophyll a/b ratio. For the carbohydrate components, after 3 days there was reduction in the monosaccharide specially with high concentrations of insecticide, the contrast after 12 days of spray, the monosaccharides content was enhanced by all the test concentrations used except the high concentration 0.05%. For the sucrose, there was fluctuations in its amount, better accumulation was after 1^{st} and 3^{rd} spray regardless of the intervals. The total soluble sugar was highly accumulated by low concentration 0.025%. The increased of the insoluble sugar occur after 3 days, was converted after frequency of spraying after 12 days, by all concentrations used. The same trend was observed with the total carbohydrate. Regarding of the nitrogen fractions, the amino acid and the peptide amounts remarkably dropped with the high insecticide concentrations 0.037 and 0.05% and/or frequency of spraying after 3 and 12 days. For the total soluble nitrogen there was initial increase at 0.025% and 0.037% then decrease after 12 day, of the 2^{nd} and 3^{rd} spray. The insoluble and the total nitrogen, were increased in all treated plants compared to the control

Key words: Insecticide, photosynthetic pigments, carbohydrate metabolism,nitrogen metabolism,radish plant.

INTRODUCTION

The increasing use of agricultural chemicals may cause deleterious effects in the environment. The complete impact of these compounds on health of agricultural workers, consumers, animals and plants is still largely unknown[4,17]. Organophosphorus insecticides are extremely persistent and form the largest and most diverse group of insecticide providing insects control. Any organophosphorus insecticide released into the environment, is detoxified and degraded rapidly[21]. The acute toxicity of organophosphorus insecticide for insect and mammals is mainly due to the blocking of the cholinesterase enzyme by its phosphorylation[20]. Cyanophos (Cyanox) with chemical abstracts named 0-(4-cyanophenyl) 0, 0-dimethyl phosphorothioate is used at 25-50 g (a.i.)/hl to control aphidide, coccidae, Lepidoptera and margarodidae in cotton, fruits and vegetables. It is known to react with DNA generally as alkylating agent[18]. Our knowledge of the adverse effect of these pesticides on important plant enzyme and quality parameters of vegetables is quite meager. The pesticides may affect some biochemical composition of plant. Organophosphorus compounds used in agriculture usually alter the chemical composition and nutritive value of plant product. Despite the simplicity in structure and simple mode of action, the cyanophos (phosphorothioate) is typical xenobiotic[14] and has an adverse effect on the plant. The common mechanism of its toxic action is inhibition of biological pathways such as photosynthesis and mitochondrial electron transport[11]. Plant productivity depends on the conversion of light energy into stable chemical energy[11]. If the photosynthetic apparatus is inhibited by environmental contaminates, changes in plant cell physiology, growth and biomass yield are inevitable. As well it has been shown that inhibition of photosynthesis is a reliable assay of the potential toxicity and xenobiotic contaminants towards plants[11]. Integrated potential management (IPM) field school demonstrated, the agricultural, economic and health logic of spraying less often and using fewer and better selected pesticides only when needed. This policy recommendation is according to Workshop of environmental health[9]. To increase our knowledge of the adverse effects of environmental hazards on biological pathways, many

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Table 1a: Changes of photosynthetic pigments components in leaves of radish plant after spraying with different Concentrations of cyanophos during the growth period (Average of 3 replicates in mg/g fresh weight) ± S.E.

Spraying	Insecticide %	Chl A	Difference (%)	Chl B	Difference (%)	Chl A+B	Difference (%)	Chl A/B	Difference (%)	Carotenoid	Difference (%)	Total Pigments	Difference (%)
		Days after each spray (3 days)											
First	Control	3.13 ±0.07		2.17 ±0.18		5.30 ±0.22		1.44 ±0.08		0.28 ±0.02		5.58 ±0.21	
	0.025	2.78 ±0.68	-11.18	2.15 ±0.36	-0.29	4.93 ±1.04	-6.98	1.29 ±0.09	-10.42	0.15 ±0.01	-46.34	5.08 ±1.03	-8.96
	0.037	1.96 ±0.24	-37.38	1.40 ±0.15^	-35.48	3.36 ±0.39^	-36.6	1.40 ±0.03	-2.78	0.04 ±0.01	-85.71	3.40 ±0.4^	-39.07
	0.050	1.87 ±0.58	-40.26	1.79 ±0.49	-17.51	3.66 ±1.04	-30.94	1.04 ±0.10	-27.78	0.08 ±0.00	-71.43	3.74 ±1.01	-32.97
Second	Control	4.53 ±0.33		2.63 ±0.72		7.16 ±1.06		1.72 ±0.58		1.13 ±0.30		8.29 ±1.34	
	0.025	4.22 ±0.53	-6.84	2.84 ±0.08	7.99	7.06 ±0.45	-1.40	1.49 ±0.22	-13.37	1.37 ±0.04	21.56	8.43 ±0.46	1.69
	0.037	3.51 ±0.12	-22.52	2.50 ±0.21	-4.94	6.01 ±0.16	-16.06	1.40 ±0.33	-18.60	0.60 ±0.04^	-46.76	6.61 ±0.12^	-20.27
	0.050	2.18 ±0.14^	-51.88	1.54 ±0.32^	-41.45	3.72 ±0.44^	-48.05	1.42 ±0.33	-17.44	0.62 ±0.15^	-44.99	4.34 ±0.48^	-47.65
Third	Control	2.04 ±0.21		1.49 ±0.10		3.53 ±0.31		1.37 ±0.04		0.35 ±0.12		3.88 ±0.42	
	0.025	2.18 ±0.27	6.86	1.55 ±0.13	4.03	3.73 ±0.40	5.67	1.41 ±0.07	2.92	0.27 ±0.04	-24.36	4.00 ±0.42	3.09
	0.037	2.16 ±0.16	5.88	1.66 ±0.07	11.41	3.82 ±0.23	8.22	1.30 ±0.05	-5.11	0.35 ±0.07	0.00	4.17 ±0.26	7.47
	0.050	2.59 ±0.16	26.96	1.94 ±0.04	30.20	4.53 ±0.15	28.33	1.34 ±0.10	-2.19	0.48 ±0.04	35.98	5.01 ±0.17	29.12

^ significant difference ($P < 0.01$) in comparison with the corresponding control.

researches must be focused on the mechanism of toxic action of the pesticide on the plant. In the former published paper, the changes of some enzymes activity in radish plant in response to cyanophos was investigated. In this study the effect of repeated spray cyanophos with different concentrations on the photosynthetic pigments, chlorophyll a, b and carotenoid and on the carbohydrate and nitrogen components in radish plant was examined, the samples of the leaves were taken at two intervals 3 and 12 days after each spray.

MATERIALS AND METHODS

Radish seeds (*Raphanus sativus* L.) were kindly supplied by the Agricultural Research Center, Giza. A homogenous batch of seeds was selected for uniformity of size, shape and viability, sown in the experimental field station of the Faculty of Agriculture, Cairo University, following the usual procedure of water irregation. Twelve days after sowing, the growing plants were sprayed with 45 ml/plant of cyanophos insecticide solutions containing 0.0%, 0.025%, 0.037% and 0.05%.

Spraying with cyanophos was repeated at 24 and 36 days from sowing date Three and twelve days after each spray, certain homogenous healthy plants from each treatment were picked up and the leaves were used for estimating some metabolic aspects. Group of fresh leaves were taken for photosynthetic pigments estimation, chlorophyll a, b and carotenoids were determined by the spectrophotometric method, recommended by Lichtenthaler[10]. The amounts are calculated as mg/g fresh weight. Another group of the leaves of the plants were oven dried for 48 hours at 80°C, then ground to fine powder to estimate the carbohydrate and the nitrogen components which were recommended by Naguib[12,13]. The data were analyzed using statistical analysis systems of multifactor analysis (SPSS, ver. 16) to clear the effect of time,

Table 1b: Changes of photosynthetic pigments components in leaves of radish plant after spraying with different concentrations of cyanophos during the growth period (Average of 3 replicates in mg/g fresh weight) ± S.E.

Spraying	Insecticide %	Chl A	Difference (%)	Chl B	Difference (%)	Chl A+B	Difference (%)	Chl A/B	Difference (%)	Carotenoid	Difference (%)	Total Pigments	Difference (%)
		Days after each spray (12 days)											
First	Control	2.97 ±0.80		1.84 ±1.02		4.81 ±1.68		1.61 ±0.22		0.28 ±0.02		5.09 ±1.69	
	0.025	4.32 ±1.59B	45.46	2.82 ±0.95	53.26	7.14 ±2.43B	48.44	1.53 ±0.24^	-4.97	0.29 ±0.20	3.57	7.63 ±2.50	49.90
	0.037	2.37 ±0.92	-20.2	1.48 ±0.36	-19.57	3.85 ±1.22	-19.96	1.60 ±0.35^	-0.62	0.27 ±0.08	-3.57	4.12 ±1.27	-19.06
	0.050	2.22 ±0.10	-25.25	1.58 ±0.14	-14.13	3.80 ±0.11	-21.00	1.41 ±0.19^	-12.42	0.19 ±0.07	-32.14	3.99 ±0.13	-21.61
Second	Control	1.95 ±0.18		1.65 ±0.27		3.60 ±0.44		1.18 ±0.09		1.36 ±0.11		4.96 ±0.55	
	0.025	2.41 ±0.64B	23.59	1.93 ±0.36	16.97	4.34 ±0.93B	20.56	1.25 ±0.22	5.93	1.16 ±0.33	-14.71	5.50 ±1.26	10.89
	0.037	2.36 ±0.31	21.03	1.77 ±0.33	7.27	4.13 ±0.63	14.72	1.33 ±0.06	12.71	0.36 ±0.19	-73.53	4.49 ±0.78	-9.48
	0.050	1.88 ±0.30	-3.59	1.74 ±0.31	5.46	3.62 ±0.59	0.56	1.08 ±0.09	-8.47	1.22 ±0.40	-10.29	4.84 ±0.97	-2.42
Third	Control	1.24 ±0.07		1.20 ±0.06		2.44 ±0.13		1.03 ±0.02		0.07 ±0.02		2.51 ±0.12	
	0.025	1.27 ±0.04	2.42	1.16 ±0.03	-3.33	2.43 ±0.07	-0.41	1.09 ±0.3	5.83	0.02 ±0.00	-71.43	2.45 ±0.07	-2.39
	0.037	1.18 ±0.17	-4.84	1.07 ±0.16	-10.83	2.25 ±0.33	-7.79	1.10 ±0.05	6.80	0.01 ±0.00	-85.71	2.26 ±0.33	-9.96
	0.050	1.13 ±0.07	-8.87	1.02 ±0.06	-15.00	2.15 ±0.13	-11.89	1.11 ±0.10	7.77	0.01 ±0.00	-85.71	2.16 ±0.12	-13.94

^ significant difference ($P < 0.01$) in comparison with the corresponding control.
B significant difference at the corresponding time at different concentrations.

Table 2: Multifactor analysis to clear the effect of time, concentrations of insecticide, spraying and their interaction on the pigments (mg/g fresh weight) components in leaves of radish plant.

Source of variance	P: Chl a	Chl b	Chl a/b ratio	Chl a+b	Carotenoid	Total Pigments
Concentrations	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$
Spraying	$P < 0.001$	$P < 0.01$	$P > 0.05$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Conc x Spraying	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$
Time	$P < 0.001$	$P < 0.01$	$P > 0.05$	$P < 0.001$	$P > 0.05$	$P > 0.05$
Conc x Time	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$
Spraying x Time	$P < 0.05$	$P > 0.05$	$P < 0.05$	$P > 0.05$	$P < 0.001$	$P > 0.05$
Time x Spraying x Conc	$P < 0.05$	$P > 0.05$	$P < 0.05$	$P < 0.05$	$P > 0.05$	$P < 0.05$

$P > 0.05$, insignificant; $P < 0.01$, significant; $P < 0.001$, highly significant.

Table 3a: Changes of Carbohydrate components in leaves of radish plant after spraying with different concentrations of cyanophos during the growth period (Average of 3 replicates in mg/g dry weight) ± S.E.

Spraying	Insecticide %	Days after each spray (3 days) mono-saccharides	Difference %	sucrose	Difference %	Total soluble sugars	Difference %	Total insol-uble sugars	Difference %	Total carboh-ydrate	Difference %
First	Control	10.74 ±0.49		18.47 ±0.56		29.21 ±0.03		43.26 ±0.54		72.47 ±0.56	
	0.025	9.95 ±0.15	-7.36	24.60 ±0.55^	33.19	34.55 ±0.06	18.28	55.30 ±0.20	27.83	89.85 ±0.14	23.98
	0.037	7.42 ±1.02^	-30.91	19.04 ±0.35	3.09	26.46 ±1.37	-9.45	69.60 ±0.25	60.89	96.06 ±1.12	32.55
	0.050	5.84 ±0.61^	-45.62	17.94 ±.0.35	-2.87	23.78 ±0.80	-18.59	84.94 ±.49	96.34	108.72 ±1.09	50.02
Second	Control	9.12 ±0.66^		16.31 ±0.00		25.43 ±0.66		107.70 ±3.97		133.13 ±3.31	
	0.025	11.78 ±0.66^	29.17	18.88 ±1.21	15.76	30.66 ±1.87^	20.57	183.30 ±1.93^	70.20	213.96 ±3.79^	60.71
	0.037	4.18 ±0.22^	-54.17	17.22 ±0.14	5.58	21.4 ±0.08^	-15.85	149.00 ±0.10	38.35	170.40 ±4.91	28.00
	0.050	2.28 ±0.44^	-75.00	15.38 ±0.54	-5.70	17.66 ±0.1^	-30.56	115.20 ±4.54	7.40	132.86 ±4.63	-0.20
Third	Control	7.79 ±0.33		13.75 ±0.94		21.54 ±0.62		115.60 ±3.63		137.14 ±3.83	
	0.025	6.27 ±0.11	-19.51	15.61 ±0.40	13.53	21.88 ±0.17	1.58	115.90 ±1.63	0.23	137.78 ±1.93	0.47
	0.037	1.26 ±0.07^	-83.83	15.38 ±0.81	11.86	16.64 ±0.74^	-22.75	124.80 ±2.89	7.96	141.44 ±2.14	3.14
	0.050	1.90 ±0.44^	-75.61	11.89 ±0.14	-13.53	13.79 ±0.30^	-35.98	169.50 ±9.59^	46.63	183.29 ±9.28	33.65

^ significant difference ($P < 0.01$) in comparison with the corresponding control.

concentrations of insecticide and the frequency of spraying, as well as their interaction on photosynthetic pigments, carbohydrates and nitrogen fractions in radish plant during the growth period.

RESULTS AND DISCUSSION

Analysis of Photosynthetic Pigments: Presented data given in Tables 1 (a, b) revealed a significant difference between the treated and untreated plants in chlorophyll a, b and carotenoid level during the growth period. There was a fluctuation in results which remarkably appeared in the treated plants at high concentrations of 0.037, 0.05% regardless of the time from spraying. The a/b ratio was attenuated mostly in treated radish leaves then increased after 3 days of the 3rd spray. This result adds further support to the changes in photosynthetic activity and/or alteration in the chloroplast.

Analysis of Carbohydrate Components: Generally there were a different responses according to the concentrations and frequency of spraying of the insecticide Tables 3 (a, b) showed that after 3 days of spraying. The monosaccharides content decreased than control except at low concentration 0.025% of 2nd spray. The decrease of monosaccharides was remarkably enhanced by increasing the insecticide concentration and the frequency of spraying $P<0.01$. Conversely after 12 days the monosaccharides content increased in all treated plants except at 0.05%. Such result is in agreement with Habiba *et al.*[5]; Ismail *et al.*[6] who worked on potatoes and tomatoes, respectively. In the meantime sucrose content increased by 0.025, 0.037% in the treated leaves as compared to untreated regardless to the time of spraying. The dropping of sucrose level by high concentration 0.05% was recovered after 12 day from spray Table (3b). Regarding the total soluble sugar (T.S.S.) Table (3a) showed that the low concentration

Table 3b: Changes of Carbohydrate components in leaves of radish plant after spraying with different concentrations of cyanophos during the growth period (Average of 3 replicates in mg/g dry weight) ± S.E.

Spraying	Insec-ticide %	Days after each spray (12 days) mono-saccha-rides	Difference %	sucrose	Difference %	Total soluble sugars	Difference %	Total insoluble sugars	Difference %	Total carboh-ydrate	Difference %
First	Control	3.52 ±0.28B		14.22 ±0.67B		17.74 ±0.95B		39.71 ±0.77		57.45 ±1.71	
	0.025	10.26 ±0.22^B	191.48	14.22 ±0.14	0.00	24.48 ±0.36^B	37.99	73.06 ±1.46^B	83.98	97.54 ±1.81^	69.78
	0.037	8.34 ±0.34	136.93	15.51 ±0.00	9.07	23.85 ±0.34^	34.44	89.99 ±0.00^B	126.62	113.84 ±0.33^	98.15
	0.050	4.07 ±0.59^B	15.63	26.80 ±0.40^	88.47	30.87 ±0.19^	74.01	131.80 ±5.70^B	231.91	162.67 ±5.89^	183.15
Second	Control	4.18 ±0.22B		25.16 ±0.81		29.34 ±0.59B		151.60 ±3.59B		180.94 ±3.00	
	0.025	6.00 ±0.39B	34.35	32.39 ±0.40	28.74	38.29 ±0.80^B	30.50	75.82 ±5.73^B	-49.99	114.11 ±1.52^	-36.93
	0.037	4.75 ±0.33	13.64	14.91 ±0.54^	-40.74	19.66 ±0.21^	-32.99	141.50 ±0.00	-6.66	161.16 ±0.21	-10.93
	0.050	3.61 ±0.33	-13.64	19.11 ±.54	-24.05	22.72 ±0.87^B	-22.56	133.90 ±6.83	-11.68	156.62 ±7.70	-13.44
Third	Control	0.57 ±0.11B		9.44 ±0.07		10.01 ±0.04B		129.90 ±3.77		139.91 ±3.73	
	0.025	2.57 ±0.11B	350.88	13.40 ±0.20^	41.95	15.97 ±0.09^B	59.54	108.30 ±6.46	-16.63	124.27 ±6.37	-11.18
	0.037	3.04 ±0.44^	433.33	11.61 ±0.25	20.76	14.65 ±0.19^	46.35	124.70 ±2.48	-4.00	139.35 ±2.66	-0.40
	0.050	0.38 ±0.00	-33.33	11.18 ±0.00	18.43	11.56 ±0.00	15.48	128.20 ±2.69	-1.34	139.76 ±2.69	-0.11

^ significant difference ($P < 0.01$) in comparison with the corresponding control.
B significant difference at the corresponding time at different concentrations.

Table 4: Multifactor analysis to clear the effect of time, concentrations of insecticide, spraying and their interaction on the carbohydrate components (mg/g dry weight) in leaves of radish plant.

Source of variance	P Mono- saccharide	Sucrose	Total Soluble sugars	Total Insoluble Sugars	Total Carbohydrate
Concentrations	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.01$
Spraying	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Conc x Spraying	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.01$	$P < 0.001$
Time	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Conc x Time	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Spraying x Time	$P > 0.05$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Time x Spraying x Conc	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

$P > 0.05$, insignificant; $P < 0.01$, significant; $P < 0.001$, highly significant.

Table 5a: Changes of nitrogen components in leaves of radish plant after spraying with different concentrations of cyanophos during the growth period (Average of 3 replicates in mg/g dry weight) ± S.E.

Spraying	Insecticide %	Days after each spray (3 days) Amino acids	Difference %	Peptide	Difference %	Total soluble nitrogen	Difference %	Total insoluble nitrogen	Difference %	Total nitrogen	Difference %
First	Control	2.59 ±0.04		1.55 ±0.00		10.52 ±0.08		32.46 ±0.26		42.98 ±0.18	
	0.025	4.55 ±0.08^	75.68	1.79 ±0.15	15.48	10.87 ±0.15	3.33	32.50 ±0.40	0.12	43.37 ±0.25	0.91
	0.037	4.10 ±0.06^	58.30	1.90 ±0.11^	22.58	12.68 ±0.07	20.53	36.36 ±0.20^	12.02	49.04 ±0.27^	14.10
	0.050	2.51 ±0.04	-3.09	1.55 ±0.14	0.00	7.55 ±0.07^	-28.23	34.35 ±0.09	5.82	41.90 ±0.01	-2.51
Second	Control	2.97 ±0.04		1.62 ±0.00		8.79 ±0.11		35.88 ±0.57		44.67 ±0.46	
	0.025	3.13 ±0.06	5.39	1.67 ±0.19	3.09	9.02 ±0.23	12.74	35.96 ±0.01	0.22	44.98 ±0.30	0.69
	0.037	1.95 ±0.03^	-34.34	0.98 ±0.03^	-39.51	9.91 ±0.00^	12.75	36.10 ±2.81^	0.61	46.01 ±2.81^	3.00
	0.050	2.85 ±0.03	-4.04	0.7 ±0.03	-56.79	6.71 ±1.00^	-23.66	37.58 ±1.25^	4.74	44.29 ±1.20	-0.85
Third	Control	3.90 ±0.06		1.44 ±0.13		8.73 ±0.11		21.88 ±0.66		30.61 ±0.55	
	0.025	1.95 ±0.03^	-50.00	1.30 ±0.00	-9.72	9.56 ±0.20	9.51	26.25 ±0.15^	19.97	35.81 ±3.50	16.99
	0.037	2.90 ±0.003^	-25.64	1.40 ±0.21	-2.78	10.20 ±0.06	16.84	40.14 ±1.13^	83.46	50.34 ±1.19^	64.46
	0.050	2.51 ±0.02^	-35.64	0.70 ±0.08^	-51.39	7.30 ±0.11^	-16.38	40.76 ±0.15^	86.29	48.06 ±0.04^	57.01

^ significant difference (P <0.01) in comparison with the corresponding control.

(0.025%) slightly enhanced the total soluble sugar. Raising the cyanophos concentration significantly lowered the T.S.S. of the leaves . On the other hand, after 12 days Table (3b) all the T.S.S. of the treated radish increased in comparison to control after 1st and 3rd spray. This increment was mostly owing to increase of sucrose content. Ismail *et al*.[6] showed that T.S.S. of treated tomatoes by profenofos increased during the test period. The result is disagreement with Abd ElMageed[1] who reported that there was a significant decrease of T.S.S. in cyanophos treated cotton leaves. Rouchaud *et al*.[16] reported that the soil treatment with 3 insecticides generally increased the free sugar concentration of summer carrots Table (3a) showed also an insignificant increase in the insoluble sugar of the treated radish compared to control P>0.05. After 12 day, this result was converted after 2nd and 3rd sprays. For the total carbohydrate (T.C.) the treated plants had a greater amounts than the control. This response was decreased with repeated spray after 12 days of spray. In this connection, it may be mentioned that this high rate of sucrose and the insoluble sugar and the total carbohydrate in the treated radish which appear markably after 3 days, thought an indication that the insecticide may activate the anabolic process leading to high dry weight gain, i.e. active growth. On the other hand, the lag of sucrose translocation to the root may have its role in sucrose accumulation. In addition, the insecticide acts as a chemical stressor which may interrupt the electron transport activity in PSII, cytochrome b 6/f or PSI or may alter the structure of the chloroplast, or inhibit the calvin cycle[11].

Analysis of the Nitrogen Components: Table (5a) revealed that after 3 days a remarkable stimulation P<0.01 of the amino acid of the treated radish leaves

Table 5b: Changes of nitrogen components in leaves of radish plant after spraying with different concentrations of cyanophos during the growth period (Average of 3 replicates in mg/g dry weight) ± S.E.

Spraying	Insecticide %	Days after each spray (12 days) Amino acids	Difference %	Peptide	Difference %	Total soluble nitrogen	Difference %	Total insoluble nitrogen	Difference %	Total nitrogen	Difference %
First	Control	0.81 ±0.03B		1.07 ±0.08B		2.72 ±0.06		27.62 ±0.30B		30.34 ±0.24B	
	0.025	0.93 ±0.02B	14.81	1.40 ±0.06B	30.84	2.79 ±0.06	2.57	28.82 ±2.11B	4.34	31.61 ±2.05B	4.19
	0.037	1.52 ±0.00B	87.65	1.21 ±0.00B	13.08	4.96 ±0.16^	82.36	54.34 ±1.58^B	96.74	59.30 ±1.74^B	95.45
	0.050	0.81 ±0.08B	0.00	0.98 ±0.03B	-8.41	4.02 ±0.05^	47.79	30.36 ±0.53B	9.92	34.38 ±0.47^B	13.32
Second	Control	1.42 ±0.05B		1.10 ±0.06		4.86 ±0.00		23.82 ±0.00B		28.68 ±0.00B	
	0.025	1.49 ±0.01B	4.93	1.26 ±0.03	14.55	2.64 ±0.08^	-45.68	29.00 ±0.47^B	21.75	31.64 ±0.39B	10.32
	0.037	2.00 ±0.04^	40.85	0.93 ±0.00	-15.45	4.12 ±0.11^	-15.23	57.35 ±0.29^B	140.46	61.47 ±0.40^B	114.33
	0.050	1.43 ±0.06B	0.70	0.65 ±0.05^	-40.91	4.49 ±0.11	-7.61	38.29 ±0.23^B	60.75	42.78 ±0.12^B	49.16
Third	Control	0.67 ±0.06B		1.03 ±0.06B		4.71 ±0.06		18.91 ±0.96B		23.62 ±1.01B	
	0.025	0.91 ±0.03B	35.82	1.15 ±0.03	11.65	3.46 ±0.05^	-26.54	53.22 ±0.00^B	181.44	56.68 ±0.05^B	139.97
	0.037	1.00 ±0.03B	49.25	0.56 ±0.05^B	-45.63	1.96 ±0.16^	-58.39	29.35 ±2.42^B	55.21	31.31 ±2.58^B	32.56
	0.050	0.53 ±0.03B	-20.90	0.61 ±0.08^	-40.78	3.28 ±0.06^	-30.36	26.60 ±1.00^B	40.67	29.88 ±1.05^B	26.50

^ significant difference ($P < 0.01$) in comparison with the corresponding control.
B significant difference at the corresponding time at different concentrations.

Table 6: Multifactor analysis to clear the effect of time, concentrations of insecticide, spraying and their interaction on the nitrogen components (mg/g dry weight) in leaves of radish plant.

Source of variance	P Amino acids	Peptides	Total soluble nitrogen	Total Insoluble nitrogen	Total nitrogen
Concentrations	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Spraying	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Conc x Spraying	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Time	P < 0.001	P < 0.01	P < 0.001	P < 0.001	P > 0.05
Conc x Time	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Spraying x Time	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Time x Spraying x Conc	P < 0.001	P > 0.05	P < 0.001	P < 0.001	P < 0.001

$P > 0.05$, insignificant; $P < 0.01$, significant; $P < 0.001$, highly significant.

by low and moderate concentrations. Such result was reversed by the repeated spray. After 12 day the amino acid content was high in the treated plants than the untreated except at 0.05% of the 3rd spray Table (5b). A significant difference at the corresponding time at different concentrations was observed P<0.01 Table (5b).

For the peptide, the low concentration 0.025% enhance the peptide content regardless of the time after spray.In the mean time, a significant decrease in the treated leaves with high concentrations.

The total soluble nitrogen increased after 3 days except by 0.05%. After 12 days the T.S.N. contents of the treated leaves decreased than control after 2nd and 3rd sprays.

This result is in agreement with[1,5] who showed that the total soluble protein decreased by certain insecticide treatment cyanophos and malathion.

For the insoluble nitrogen, a significant increase compared to control regardless to the time of spraying and also a significant differences at the corresponding time at different concentrations. In this respect, cyanophos may enhance the nutrient uptake by roots resulting in higher content of insoluble nitrogen of the treated leaves. Regarding total nitrogen accumulation, there was a significant increase of it in treated radish than the untreated, regardless of the time of spraying. These observations indicated better rate of absorption and metabolization of nitrogen of sprayed plants, if this coupled with the previous observation that the total carbohydrates content was slightly affected or raised in the treated plant after 3 days from spray, one may reach the conclusion that cyanophos also may stimulate photosynthesis. Habiba *et al*.[5] showed that the profenofos residue caused increase in protein content of treated potatoes than control. On the contrary, glucose and protein content decreased in treated tomatoes[7].

In this connection, Abubaker *et al*.[3] mentioned that the use of chemical pesticides demonstrated significantly higher NO_3-N content of pod, seeds, leaves and shoots of beans (*Phaseolus vulgaris* L.). Kerns and Goylor[8] reported that the increase of the concentration of amino acids content in cotton plant by using sulphorophos may alter the biochemistry of the plant.

Singh and Shaner[19] reported that enzymes in different amino acids biosynthesis pathways identified as a target of several pesticides. These suggest that inhibition of the branched chain amino acids pathway causes a unique change in the level of free amino acids in plants. In addition Abd ElMageed[1] reported that after 2 and 7 days from cyanophos application a significant decrease of amino acids was observed. Abdullah *et al*.[2] found that different insecticides showed differences on total amino acids content of cotton leaves. This can be explained, as the total free amino acids pool consists of a mixture of amino acids derived from de-novo synthesis as well as protein degradation, from this pool amino acids are in depletion by their breakdown or utilization in synthesis of protein or other metabolites. Therefore, some insecticides may affect anyone or a combination of the process described above. Singh and Shaner,[19]. Kerns and Goylor[8] found that some pesticides caused a significant changes in total amino acids pools which may have resulted from the effect of pesticides. Also, it can be suggested that a part of these changes in the amino acids contents may be attributed to these reactions between the functional groups of alcohol, acidic and basic amino acids in one side and the organophosphorus insecticide and/or its metabolite in the leaves in the other side. Statistical analysis in Tables (2, 4, 6) clearly demonstrated the effect of the time of spraying , concentrations used and the spray frequency on total photosynthetic pigments which was insignificant and on the carbohydrate and nitrogen fractions which appeared to be highly significant and the same also was for the interactions between different studied factors in addition to full statistical differences between the data according the time of samples estimation. The most significant conclusion which can be derived from this work was that this pesticide can be used with a stringent safety intervals and low-concentration.

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