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Some Macromolecular Abnormalities Developed by the Interaction of Malathion and Permethrin and Subsequent Refeeding in *Tribolium castaneum* Larvae

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The effects of sublethal treatments of malathion and malathion + permethrin combinations on activities of acetylcholine esterase (ChE), carbohydrate-metabolizing enzymes (amylase; lactate dehydrogenase, LDH), protein-metabolizing enzymes (alanine and aspartate aminotransferase, ALAT, ASAT), as well as acid and basic phosphatases (AcP and AkP, respectively), and Kreb's cycle enzymes (isocitrate dehydrogenase, ICDH) were studied on sixth instar larvae of the red flour beetle, *Tribolium castaneum*. In addition, the levels of lipid, cholesterol, glucose, glycogen, proteins, free amino acids (FAA), urea, and nucleic acids (DNA and RNA) were determined. Malathion (400 ppm) increased the activity of LDH (53%), as well as the concentrations of FAA (31%) and urea (39%). Malathion treatments of 400 ppm decreased the glucose (20%) and glycogen (24%) content but did not affect other enzymes and biochemical components. Permethrin (200 ppm) and malathion (20 ppm) mixtures increased the activities of ChE (708%) and LDH (55%), and raised the concentrations of FAA (26%), urea (24%), glucose (23%), lipid (14%), cholesterol (21%), DNA (24%), and RNA (8%). The decrease in AcP activity and in glycogen concentration observed with malathion was sustained by permethrin in the mixture. Permethrin + malathion mixtures also depressed the levels of AkP (30%), ICDH (24%), and glycogen (36%). The intensity of effects commensurated with the dose and duration of insecticide exposure. Refeeding showed tendencies towards normalization of various biochemical parameters.

Key words: acetylcholine esterase, protein, carbohydrate-metabolizing enzymes

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INTRODUCTION

Malathion is an organophosphate insecticide that has received wide acceptance for use on harvested grains. It is often used with stored grain crops because of its low mammalian toxicity [1,2]. Many new synthetic pyrethroids have also attracted much attention [3,4] because of their reasonable cost, the lack of insect resistance, and low mammalian toxicity [5–7]. These and the other favorable properties of synthetic pyrethroids may well herald a new era in pest control.

The introduction of these insecticides necessitated studies of their biochemical effects in insects [8]. These insecticides exert their toxic action directly or indirectly at the enzyme or membrane levels. Little information is available on the biochemical effects of these insecticides in grain insects. Speirs and Zettler [9] have studied the toxicity of OP* compounds and pyrethrins in malathion resistant *T. castaneum*, while Binns [10] studied the toxicity of malathion along with triphenyl phosphate to resistant and susceptible strains of *T. castaneum*. Larvae were shown to have a higher tolerance to malathion than the adults in all strains. A few biochemical studies have described enhanced protein synthesis in *Triatoma infestans* [11]; elevation of enzymes [12,13], induction of detoxication enzymes in insects [14] after several chlorinated insecticide treatments, and bioresmethrin-elevated lipid levels in *Locusta migratoria* [15]. None of these studies has provided any information on the biochemical changes after exposure to malathion and permethrin in grain insects.

We have reported the effects of permethrin and malathion on DNA, RNA, and total protein content [16], of cypermethrin [17], permethrin, and deltamethrin on several enzymes and some biochemical components [18] in *T. castaneum* larvae. Generally, the previous biochemical studies show enhanced carbohydrate metabolism after permethrin and deltamethrin application [18], and enhanced lipid and protein metabolism after cypermethrin [17] treatment. Recently a comparative study of effect of permethrin (1 and 2 ppm) and malathion (150 and 300 ppm), individually and in combination, has been made on adults of *T. castaneum* [19]. Permethrin treatment resulted in enhancement of carbohydrate metabolism and slowing down of protein metabolism. Mixing the two insecticides resulted in antagonistic action with reference to various enzymatic activities. Glucose and glycogen contents were at first mobilized for energy supply under insecticidal stress conditions followed by lipid and cholesterol mobilization.

The induction of amylase, ChE, AcP, AkP, and LDH and inhibition of ALAT, ASAT, ICDH, and trehalase after treatment with 2 ppm permethrin may constitute a toxic response in the adult beetle. Extensive enzymatic inhibition (e.g., amylase, AkP, ALAT, ASAT, ICDH, and trehalase) and a several-fold increase in ChE, AcP, and LDH activities after 300 ppm malathion treatment suggest that this may be another toxic compound for *Tribolium*, which, though having a different mechanism of action, produces similar types of gross biochemical changes. These insecticides, however, when administered in combination, min-

*Abbreviations: AcP = acid phosphatase; AkP = alkaline phosphatase; ASAT = aspartate - 2 oxoglutarate aminotransferase; ALAT = alanine - 2 oxoglutarate aminotransferase; ChE = cholinesterase; FAA = free amino acids; ICDH = isocitrate dehydrogenase; LDH = lactate dehydrogenase; OP = organophosphate.

imize each other's toxic effects on the enzymes of *Tribolium*, and therefore cannot be used effectively for the control of this pest. In this paper, we report the effects of mixtures of permethrin and malathion administered for different time periods at various concentrations on the biochemical milieu of *Tribolium* larvae. A few enzymes, representing different aspects of intermediary metabolism of carbohydrates (amylase, LDH, and ICDH) and protein (ASAT and ALAT) along with ChE and phosphatases (AcP and AkP), and several intermediates (glucose, amino acids, cholesterol, and urea) and the macromolecules (lipid, glycogen, proteins, DNA, RNA), were selected to assess the changes induced by insecticides in larvae. The object was to understand the biochemical basis of effects of these insecticides and their possible interaction. The effect of feeding insects previously exposed to insecticides on insecticide-free food to assess the possible recovery after insecticidal exposure has also been investigated.

MATERIALS AND METHODS

Treatment of Insects

The details of rearing and other maintenance conditions of red flour beetle, *T. castaneum* (Herbst.), and the insecticides used have already been described [19–21].

Lethal doses of 200 and 400 ppm malathion (57% w/w emulsifiable concentrate) and 200 + 10 ppm of malathion + permethrin (25% w/w emulsifiable concentrate) were applied on glass Petri dishes (130 cm^2) [17]. Each concentration (1.3 ml) was applied to the bottom of a Petri dish with a micropipette and then spread uniformally by rotating the plate. One hundred (sixth instar; 28 ± 1 days post-oviposition) larvae of both sexes in each dish were exposed to different insecticide treatments, in the absence of food, as follows: i) malathion at 200 and 400 ppm for 48 h, ii) malathion + permethrin at 200 + 10 ppm and 400 + 20 ppm for 48 h, iii) malathion + permethrin at 200 + 10 ppm and 400 + 20 ppm for 120 h and subsequently fed with whole meal flour free from insecticides *ad libitum* for another 120 h. Controls were treated with acetone only. Four replicates were used in each experiment. Larvae that died naturally or due to insecticides treatments during the course of experiments were not included for various biochemical tests.

Biochemical Analyses

One hundred larvae (175–180 mg), in each replicate, were homogenized in 4 ml of cold isotonic sodium chloride solution (0.89%, w/v). The homogenate was centrifuged at 4,900*g* for 45 min at 4°C. The supernatant was used for the estimation of enzymatic activities of AcP, AkP, LDH, ICDH, ChE, ASAT, ALAT, and amylase, and concentrations of soluble proteins, FAA, glucose, and urea contents.

For lipid and cholesterol analyses 30 larvae (60–65 mg) in each replicate were crushed in 2 ml of 98% ethanol and left overnight before centrifugation at 1,300*g* for 15 min. The ethanol extract was used for the estimation of ethanol soluble lipid and cholesterol contents. Glycogen content were determined from saline extract of eight larvae (16–18 mg). Nucleic acid content was estimated from 30 larvae (60–65 mg) according to Shakoori and Ahmad [23]. The pellet left after

the extraction of nucleic acids was used for the estimation of total protein content.

The various enzymatic activities have been expressed as units per mg weight, while biochemical components have been expressed both in terms of content/mg weight and content/larva. The definitions used for various enzyme units are as follows: IU, international units, the amount of enzyme, which under defined assay conditions, will catalyze the conversion of 1 μmol of substrate per minute; RU, Rappaport units, the amount of enzyme which will liberate 1 μmol of acetic acid from acetylcholine in 30 min at 25°C at pH 7.8 under the conditions of this test; SU, Somogyi units, the amount of enzyme digesting 5 mg of starch in the experimental conditions used here; Sigma units, the amount of enzyme in 1 ml extract that will produce 1 nmol of NADPH in 1 h at 25°C, pH 7.5, under the conditions of this test. Details of methodology employed for estimation of enzymatic activities and concentrations of various biochemical components have been given previously (see [19] and [22]).

RESULTS

Effect of Malathion

Table 1 shows effects of malathion on enzymes and other macromolecules of *T. castaneum* larvae. Sublethal concentrations of 200 and 400 ppm malathion

TABLE 1. Effect of Malathion on Some Biochemical Components of *Tribolium castaneum* Larvae Exposed for 48 h*

		Malathion treatment	
Parameters	Control (n = 4)	200 ppm (n = 4)	400 ppm (n = 4)
ChE (RU/mg)	2.49 ± 0.35[a]	2.57 ± 0.17	2.64 ± 0.34
Amylase (SU/mg)	3.93 ± 0.06	4.19 ± 0.14	4.05 ± 0.34
AcP (IU/mg)	9.36 ± 0.19	7.96 ± 0.37[b]	7.69 ± 0.16[d]
AkP (IU/mg)	2.16 ± 0.13	1.97 ± 0.04	2.01 ± 0.05
ASAT (IU/mg)	36.53 ± 0.34	36.97 ± 0.59	37.87 ± 0.33
ALAT (IU/mg)	166.15 ± 2.35	166.93 ± 0.45	163.59 ± 3.24
LDH (IU/mg)	21.34 ± 2.42	20.86 ± 2.87	32.64 ± 3.68[b]
ICDH (Sig.U/mg)	32.39 ± 0.48	33.38 ± 0.59	31.84 ± 0.50
Soluble protein (μg/mg)	120.18 ± 0.60	113.94 ± 1.66	136.25 ± 14.14
Total protein (μg/mg)	322.56 ± 7.43	328.46 ± 5.72	330.13 ± 8.19
FAA (μg/mg)	0.322 ± 0.003	0.308 ± 0.003	0.423 ± 0.04[b]
Urea (μg/mg)	0.18 ± 0.04	0.24 ± 0.03[b]	0.25 ± 0.03[b]
Glucose (μg/mg)	0.086 ± 0.003	0.064 ± 0.001[b]	0.071 ± 0.003[b]
Glycogen (μg/mg)	181.32 ± 10.98	145.66 ± 9.66[b]	141.73 ± 8.87[b]
Lipids (μg/mg)	321.18 ± 9.15	326.38 ± 18.07	339.13 ± 15.50
Cholesterol (μg/mg)	0.15 ± 0.011	0.15 ± 0.01	0.15 ± 0.004
DNA (μg/mg)	1.09 ± 0.12	1.13 ± 0.15	1.15 ± 0.11
RNA (μg/mg)	10.15 ± 0.57	10.75 ± 0.61	10.81 ± 0.44

*For statistical significance, the treated values have been compared with their respective controls. Superscripts are used in Tables 2–5.
[a]Mean ± S.E.M.
[b]Student's t test, $P < 0.05$.
[c]Student's t test, $P < 0.01$.
[d]Student's t test, $P < 0.001$.

resulted in 15% and 18% decreases in AcP activity, respectively, while all other enzymes, viz., amylase, ChE, AkP, ASAT, ALAT, and ICDH remained unaltered. The LDH activity was unaffected at 200 ppm but increased by 53% at 400 ppm.

DNA, RNA, cholesterol, lipids, soluble proteins, and total proteins (content/mg wet weight) remained unaffected by malathion. Glycogen and glucose content of the insect decreased 20 and 22%, and 26 and 17%, respectively, after exposure to 200 ppm and 400 ppm of malathion. The urea content, however, increased 33% and 39% after insecticide treatment. The FAA content was not significantly altered at 200 ppm concentration but showed 31% increase at the higher concentration. When interpreted on larval weight basis, the components showed similar trends as described above except for the urea content, which remained unaltered and for soluble protein content that increased by 10% after insecticide exposure.

Effect of Permethrin

Effects of permethrin alone on *T. castaneum* larvae have already been described elsewhere [18] and are listed for comparison purposes in Tables 5 and 7.

Effect of Malathion + Permethrin Combination

Biochemical effects induced by malathion + permethrin mixtures (200 + 10 ppm and 400 + 20 ppm) are shown in Tables 2, 5, and 7. These combinations resulted in significant decreases in activities of AcP (11 and 10%), AkP (17 and 30%), and ICDH (21 and 24%), and increases of LDH (36 and 55%)

TABLE 2. Effect of Malathion + Permethrin, in Combination on Some Biochemical Components of *Tribolium castaneum* Larvae Exposed for 48 h*

Parameters	Control (n = 4)	Malathion + permethrin treatment: 200 + 10 ppm (n = 4)	Malathion + permethrin treatment: 400 + 20 ppm (n = 4)
ChE (RU/mg)	2.39 ± 0.58[a]	18.51 ± 1.69[d]	19.30 ± 1.45[d]
Amylase (SU/mg)	2.47 ± 0.18	2.60 ± 0.11	2.82 ± 0.21
AcP (IU/mg)	10.41 ± 0.33	9.27 ± 0.16[b]	9.37 ± 0.17[b]
AkP (IU/mg)	2.09 ± 0.07	1.74 ± 0.12[b]	1.46 ± 0.03[d]
ASAT (IU/mg)	36.05 ± 1.71	38.63 ± 0.86	38.44 ± 1.41
ALAT (IU/mg)	162.78 ± 8.82	147.66 ± 3.33	173.57 ± 6.36
LDH (IU/mg)	20.35 ± 2.48	27.59 ± 1.87	31.50 ± 2.19[b]
ICDH (Sig.U/mg)	38.93 ± 6.37	30.56 ± 0.59[d]	29.58 ± 0.65[d]
Soluble protein (μg/mg)	124.41 ± 10.14	136.27 ± 7.09	134.29 ± 10.64
Total protein (μg/mg)	318.45 ± 6.35	322.58 ± 7.76	303.66 ± 8.81
FAA (μg/mg)	0.31 ± 0.01	0.40 ± 0.01[c]	0.39 ± 0.07[d]
Urea (μg/mg)	0.21 ± 0.01	0.27 ± 0.05[b]	0.26 ± 0.04[b]
Glucose (μg/mg)	0.088 ± 0.003	0.107 ± 0.005[b]	0.108 ± 0.008[b]
Glycogen (μg/mg)	179.85 ± 11.77	132.45 ± 10.10[b]	124.81 ± 10.52[b]
Lipids (μg/mg)	317.71 ± 29.80	410.67 ± 24.69[b]	363.51 ± 24.24[b]
Cholesterol (μg/mg)	0.148 ± 0.024	0.155 ± 0.001	0.179 ± 0.013[b]
DNA (μg/mg)	1.19 ± 0.02	1.43 ± 0.05[c]	1.48 ± 0.08[b]
RNA (μg/mg)	9.36 ± 0.18	10.38 ± 0.29[b]	10.08 ± 0.18[b]

*For statistical details see Table 1.
See Table 1 for explanation of superscripts.

and ChE (674 and 708%) activities without affecting activities of amylase, ASAT, and ALAT.

Total and soluble proteins levels remained unaltered. Glycogen content decreased by about 29% at both doses, while FAA, urea, glucose, lipids, cholesterol, DNA, and RNA increased by 28%, 27%, 22%, 22%, 13%, 22%, and 10%, respectively (see Table 5). When these changes were interpreted on a per insect basis, soluble protein, urea, glucose, lipids, cholesterol, and DNA showed no significant deviation (Table 7). The total proteins (11% and 19%) and glycogen content (35% and 41%) decreased after 200 + 10 and 400 + 20 ppm malathion-permethrin treatment. The RNA content decreased only 8% after high doses of malathion-permethrin, while FAA increased 14% and 8%, respectively, for the weak and strong doses. The results, therefore, showed significant changes in different biochemical components after mixed applications of two insecticides than those used individually (see Tables 5 and 7). This could possibly be attributed to their synergistic activity against various macromolecules of *T. castaneum* larvae.

When the treatment period was extended to 120 h, almost all biochemical parameters except total protein showed significant increases over the control values. Both concentrations of insecticide mixtures resulted in an elevation of activities of amylase (113 and 86%), ChE (60 and 73%), AcP (10 and 11%), AkP (11 and 13%), ASAT (14 and 18%), ALAT (38 and 22%), LDH (28 and 32%), and ICDH (14 and 12%) (Tables 3 and 5).

Glucose (42 and 30%), soluble protein (25 and 19%), FAA (45 and 36%), urea (471 and 614%), lipids (31 and 29%), cholesterol (16 and 22%), DNA (23 and 27%), and RNA (14 and 18%) levels increased, whereas glycogen decreased

TABLE 3. Effect of Malathion + Permethrin in Combination on Some Biochemical Components of *Tribolium castaneum* Larvae Exposed for 120 h*

Parameters	Control (n = 4)	Malathion + permethrin treatment 200 + 10 ppm (n = 4)	400 + 20 ppm (n = 4)
ChE (RU/mg)	2.85 ± 0.11[a]	4.57 ± 0.16[d]	4.92 ± 0.09[d]
Amylase (SU/mg)	2.48 ± 0.30	5.27 ± 0.18[d]	4.61 ± 0.34[c]
AcP (IU/mg)	12.07 ± 0.21	13.22 ± 0.24[d]	13.40 ± 0.24[c]
AkP (IU/mg)	1.54 ± 0.04	1.71 ± 0.03[b]	1.74 ± 0.01[b]
ASAT (IU/mg)	20.66 ± 0.42	23.45 ± 0.51[b]	24.41 ± 0.42[b]
ALAT (IU/mg)	142.99 ± 6.32	197.51 ± 8.87[c]	173.79 ± 5.31[b]
LDH (IU/mg)	18.59 ± 1.17	23.87 ± 1.05[b]	24.45 ± 1.68[b]
ICDH (Sig.U/mg)	64.15 ± 0.39	73.16 ± 2.85[b]	71.64 ± 2.04[b]
Soluble protein (μg/mg)	129.78 ± 3.77	161.60 ± 16.01[b]	154.61 ± 5.82[b]
Total protein (μg/mg)	334.42 ± 11.41	306.60 ± 12.32	301.72 ± 13.09
FAA (μg/mg)	0.49 ± 0.02	0.71 ± 0.08 [b]	0.67 ± 0.04[c]
Urea (μg/mg)	0.14 ± 0.03	0.80 ± 0.02[b]	1.00 ± 0.17[c]
Glucose (μg/mg)	0.053 ± 0.002	0.075 ± 0.007[b]	0.069 ± 0.002[c]
Glycogen (μg/mg)	149.65 ± 10.65	102.93 ± 9.65[b]	95.78 ± 9.81[b]
Lipids (μg/mg)	294.18 ± 28.18	384.64 ± 22.15[b]	378.31 ± 21.39[b]
Cholesterol (μg/mg)	0.15 ± 0.02	0.18 ± 0.02[b]	0.19 ± 0.02[b]
DNA (μg/mg)	1.36 ± 0.02	1.67 ± 0.03[b]	1.72 ± 0.06[b]
RNA (μg/mg)	6.56 ± 0.19	7.49 ± 0.28[b]	7.75 ± 0.19[c]

*For statistical details see Table 1.
See Table 1 for explanation of superscripts.

(31 and 36%), and total protein concentrations remained unaffected (Table 3). The concentration of macromolecules and other metabolites, when considered on a per-larva basis (Table 7), showed a trend similar to that of the 48-h experiment. Prolonged exposure for 120 h resulted in increased urea (380% and 496%), and decreased total protein (24% and 26%) and glycogen (43% and 47%), respectively, for weak and strong malathion + permethrin mixtures. The soluble protein, FAA, glucose, glycogen, lipids, cholesterol, DNA, and RNA remained unchanged (Table 7).

Molecular Readjustment After Insecticide-Free Flour Meal Feeding

Larvae that had been previously exposed to malathion + permethrin mixtures for 120 h were given insecticide-free flour meal for an additional 120 h. Feeding on insecticide-free meal did not restore alterations seen at higher insecticide concentration, viz., 400 + 20 ppm. Such parameters include amylase (−82%), ChE (+16%), LDH (−45%), ICDH (+30%), glucose (+50%), glycogen (−20%), soluble protein (+19%), FAA (+66%), urea (+60%), lipids (+19%), cholesterol (20%), DNA (+19%), and RNA (+16%) (Tables 4 and 5). However, at lower dose of 200 + 10 ppm some of these, such as the activities of ASAT, ALAT, AcP, and content of cholesterol, DNA, and RNA returned to normal levels, while others did not (Tables 4 and 5).

When the concentrations of various biochemical components were considered on a per-larva basis the total protein and glycogen levels, which were depleted in the presence of insecticides, recovered to normal levels. Soluble protein (34% and 75%), urea (112% and 69%), glucose (33% and 61%), and lipid (22% and 24%) were increased after feeding for 120 h on insecticide-free

TABLE 4. Effect of Refeeding on Some Biochemical Components of *Tribolium castaneum* Larvae, Exposed to Mixture of Malathion + Permethrin, Administered in Succession for 120 h*

		Malathion + permethrin treatment	
Parameters	Control (n = 4)	200 + 10 ppm (n = 4)	400 + 20 ppm (n = 4)
ChE (RU/mg)	1.99 ± 0.18[a]	1.00 ± 0.07[b]	2.31 ± 0.27
Amylase (SU/mg)	1.90 ± 0.09	0.47 ± 0.06[d]	0.36 ± 0.04[d]
AcP (IU/mg)	17.22 ± 0.09	18.31 ± 0.55	16.50 ± 0.52[c]
AkP (IU/mg)	4.94 ± 0.18	6.14 ± 0.19[c]	6.87 ± 0.11[d]
ASAT (IU/mg)	18.24 ± 1.32	20.80 ± 1.60	22.45 ± 0.51[b]
ALAT (IU/mg)	153.72 ± 5.45	157.72 ± 4.32	220.66 ± 16.32[b]
LDH (IU/mg)	21.77 ± 2.49	8.40 ± 0.64[c]	11.87 ± 0.64[b]
ICDH (Sig.U/mg)	47.80 ± 0.86	58.52 ± 1.68[c]	62.00 ± 1.98[d]
Soluble protein (μg/mg)	123.71 ± 4.90	175.33 ± 7.08[d]	146.66 ± 1.79[c]
Total protein (μg/mg)	316.21 ± 7.48	305.98 ± 8.68	298.03 ± 9.71
FAA (μg/mg)	0.44 ± 0.02	0.56 ± 0.02[c]	0.73 ± 0.07[c]
Urea (μg/mg)	0.20 ± 0.01	0.40 ± 0.06[b]	0.32 ± 0.04[b]
Glucose (μg/mg)	0.016 ± 0.01	0.20 ± 0.01[b]	0.24 ± 0.01[c]
Glycogen (μg/mg)	190.86 ± 12.75	158.12 ± 11.39[b]	152.72 ± 11.85[b]
Lipids (μg/mg)	325.82 ± 21.35	375.67 ± 18.10[b]	387.11 ± 18.46[b]
Cholesterol (μg/mg)	0.15 ± 0.02	0.17 ± 0.03	0.18 ± 0.02[b]
DNA (μg/mg)	1.48 ± 0.02	1.71 ± 0.16	1.76 ± 0.04[b]
RNA (μg/mg)	9.08 ± 0.45	9.96 ± 0.51	10.52 ± 0.18[b]

*For statistical details see Table 1.
See Table 1 for explanation of superscripts.

TABLE 5. Percent Increase or Decrease (−) After Treatment of *Tribolium castaneum* Larvae With Malathion (200 and 400 ppm), Permethrin (10 ppm and 20 ppm) and Their Combinations For 48 h and 120 h*

	Malathion (ppm)		Permethrin (ppm)		Malathion + permethrin (200 + 10 ppm) for			Malathion + permethrin (400 + 20 ppm) for		
Parameters	200	400	10	20	48 h	120h	Refeeding for 5 days	48 h	120 h	Refeeding for 5 days
1. ChE	—	—	—	—	674^{d}	60^{d}	(−) 50^{b}	708^{d}	73^{d}	16
2. Amylase	—	—	(−)35^{d}	(−) 42^{d}	—	113^{d}	(−) 75^{d}	—	86^{c}	(−)82^{d}
3. AcP	(−)15^{b}	(−)18^{d}	—	12^{c}	(−) 11^{b}	10^{d}	—	(−) 10^{b}	11^{c}	(−) 4^{c}
4. AkP	—	—	—	—	(−) 17^{b}	11^{b}	24^{c}	(−) 30^{d}	13^{b}	39^{d}
5. ASAT	—	—	—	—	—	14^{b}	—	—	18^{b}	23^{b}
6. ALAT	—	—	—	—	—	38^{c}	—	—	22^{b}	44^{b}
7. LDH	—	53^{b}	83^{d}	150^{d}	36^{b}	28^{b}	(−) 61^{c}	55^{b}	32^{b}	(−)46^{b}
8. ICDH	—	—	—	—	(−) 22^{d}	14^{b}	22^{c}	(−) 24^{d}	12^{b}	30^{d}
9. Soluble protein	—	—	—	(−) 15^{c}	—	25^{b}	42^{d}	—	19^{b}	19^{c}
10. Total protein	—	—	—	—	—	(−) 8	—	—	(−) 10	—
11. FAA	—	31^{b}	—	(−) 9^{c}	29^{c}	45^{b}	27^{c}	26^{d}	37^{c}	66^{c}
12. Urea	33^{b}	39^{b}	—	—	29^{b}	471^{b}	100^{b}	24^{b}	614^{c}	60^{b}
13. Glucose	(−)26^{b}	(−)17^{b}	—	—	22^{b}	42^{b}	25^{b}	23^{b}	30^{c}	50^{c}
14. Glycogen	(−)20^{b}	(−)22^{b}	—	—	(−) 26^{b}	(−) 31^{b}	(−) 17^{b}	(−) 31^{b}	(−) 36^{b}	(−)20^{b}
15. Lipid	—	—	—	—	29^{b}	31^{b}	15^{b}	14^{b}	29^{b}	19^{b}
16. Cholesterol	—	—	—	—	5	20^{b}	—	21^{b}	27^{b}	20^{b}
17. DNA	—	—	10^{c}	21^{d}	20^{c}	23^{b}	—	24^{b}	26^{b}	19^{b}
18. RNA	—	—	—	—	11^{b}	14^{b}	—	8^{b}	18^{c}	16^{b}

*Data for permethrin taken from Saleem and Shakoori [18]. The larvae were also fed for 5 days after exposure to insecticides. No changes are indicated by a dash. For statistical details see Table 1. The statistical significances have been calculated by comparing treated values with their respective controls.
See Table 1 for explanation of superscripts.

flour meal. Nucleic acids, which were unaffected by insecticide treatment, increased 25% and 21%, respectively, after feeding on insecticide-free diet (see Table 7).

Effect of Insecticides on Larval Weight

Table 6 shows the effect of malathion, permethrin, their combinations, and the effects of feeding on insecticide-free flour meal following these treatments on the larval weights. Malathion at 200 and 400 ppm resulted in 2.16% and 2.7% decrease in the larval weight, respectively, while permethrin at 10 and 20 ppm caused 14.28% and 14.77% reduction in the body weight. When malathion was administered in combination with permethrin from 48 h, the body weight loss was 11.97% (200 + 10 ppm) and 14.58% (400 + 20 ppm). After prolonging the insecticide treatment for 120 h, the body weight loss became 16.57% and 17.68%, respectively. The entire insecticide treatment was done in the absence of food. After 120 h of insecticide exposure, the larvae were fed on insecticide-free flour meal for 120 h, the result of which was that the larvae gained weight by 5.66% (200 + 10 ppm) and 4.72% (400 + 20 ppm), as compared with the control larvae.

TABLE 6. Effect of Insecticides and Refeeding on the Weights and the Total Identified Biochemical Components of *Tribolium castaneum* Larvae*

Insecticides	Weight of larvae (mg) (n = 4)	% Decrease (−) or increase (+) with reference to respective control	Identified biochemical components	
			Total (mg)	% of total
Malathion				
Control	1.85 ± 0.06		1.548	83.67
200 ppm	1.81 ± 0.03	− 2.16	1.471	81.27
400 ppm	1.80 ± 0.05	− 2.70	1.482	82.33
Permethrin				
Control	2.03 ± 0.006		1.648	81.18
10 ppm	1.74 ± 0.008[b]	− 14.28	1.383	79.48
20 ppm	1.73 ± 0.006[a]	− 14.77	1.388	80.23
Malathion + Permethrin (48 h)				
Control	1.92 ± 0.02		1.588	82.70
200 + 10 ppm	1.69 ± 0.04[b]	− 11.97	1.484	87.80
400 + 20 ppm	1.64 ± 0.03[b]	− 14.58	1.319	80.42
Malathion + Permethrin (120 h)				
Control	1.81 ± 0.05		1.424	78.67
200 + 10 ppm	1.51 ± 0.07[a]	− 16.57	1.215	80.46
400 + 20 ppm	1.49 ± 0.04[a]	− 17.68	1.173	78.72
Refeeding for 5 days after Malathion + Permethrin treatment				
Control	2.21 ± 0.04		1.790	84.43
200 + 10 ppm	2.24 ± 0.03	+ 5.66	1.910	85.26
400 + 20 ppm	2.22 ± 0.03	+ 4.72	1.890	85.13

*The statistical significance has been calculated by comparing weights of treated larvae with those of their respective controls.
[a]Student's t test, $P < 0.01$.
[b]Student's t test, $P < 0.001$.

DISCUSSION

OP insecticides are known to cause extensive damage at toxic doses to insect blood, such as gross irregularity in the shape of hemocytes, rupturing of hemocytes, nuclear hypertrophy, water loss, inhibition of ChE, depletion of several metabolites including amino acids, etc. [24,25]. However, very little information on the biochemical effects of sublethal doses is available.

Sublethal concentrations of permethrin (10 ppm and 20 ppm) [18] and malathion, whether used alone or in combination, resulted in a significant increase in the activity of LDH. ICDH activity, which was unaffected by each of these insecticides when used individually, was first lowered after 48 h and then increased after 120-h exposure to their mixtures. The results suggest that glycolysis was accelerated and passed on to Kreb's cycle to provide extra energy. Elevated activities of amylase under prolonged treatment with insecticides administered in combinations also endorse utilization of carbohydrates through breakdown of available body starch, although this enzyme remained unchanged in other individual insecticide treatments. Decreased glycogen and increased

glucose levels after insecticide treatment also point towards the same process. Similarly, elevated activities of phosphatases (AcP and AkP) and aminotransferases (ASAT and ALAT) under long-term exposure to the mixture of these insecticides support the contention of production of energy through the breakdown of phosphate bonds of energy rich compounds such as ATP, and amino acids metabolism, respectively. Raised FAA content is presumably linked with higher aminotransferases activities, and elevated soluble protein along with the induction of related enzymes. Similar types of observations have been made by Nohel and Slama [12]. Agosin et al. [11] have also described enhanced protein synthesis in *Triatoma infestans* treated with DDT. The induced activities of various enzymes after insecticide exposure may presumably be attributed to 1) higher concentration of enzymes due to decreased body weight of treated larvae and/or 2) increased synthesis of particular enzyme(s) to furnish a defense mechanism against insecticidal stress conditions. The elevated activities of various enzymes after insecticide treatments have also been reported from other laboratories [13]. Terriere [14] has described induction of several detoxication enzymes in insects. Such increases in enzyme activities have been shown to protect insects from insecticide poisoning.

Although the general pattern of changes induced by malathion and permethrin in larvae of *T. castaneum* are almost the same, as reported earlier for the adult beetles [19], a few points need special mention. 1) A larva, metabolically and synthetically, being a more active organism than its adult, reacts to insecticide treatment in a more drastic way (e.g., ChE). 2) An adult beetle, under the influence of insecticidal stress, generally derives its energy by breakdown of carbohydrates and lipids. But in larvae, proteins and amino acids, in addition to carbohydrates and lipids, are also utilized to produce energy for various metabolic activities. 3) In adult beetles, the mixture of malathion and permethrin produce antagonistic effects at the macromolecular level, while there are several evidences of synergistic effects of these two insecticides in larvae (see Tables 5 and 7).

The various biochemical components estimated as percentages of wet weight are shown in Table 6. The control larvae on the average contain about 82% of the identified biochemical components with water making up the balance. The weight loss is 2–3% after malathion, 14–15% after permethrin, and 12–15% after 48 h and 17–18% after 120 h of malathion + permethrin treatment. Upon feeding these insects insecticide-free food the larval weight increased 5–6%. This desiccation (as indicated by loss of weight) accounts for the increased content of lipid, cholesterol, DNA, and RNA per unit weight. Increased lipid and cholesterol levels in larvae treated with mixed insecticides presumably indicates interconversion of these macromolecules subsequent to depletion of polysaccharides. Such an inverse interrelationship of lipids against carbohydrates has been described by Singh [15] in *Locusta migratoria* exposed to bioresmethrin treatment. This pyrethroid insecticide caused depletion of hemolymph carbohydrate (hypoglycemia) and elevation in blood lipid (hyperlipemia). Singh [15] suggested that bioresmethrin-induced elevation in hemolymph lipid is owing to the release of hyperlipemic hormone(s). The depletion of hemolymph carbohydrate, however, may be owing to their increased utilization in response to the hyperactivity caused by pyrethroid treatment. In the

TABLE 7. Percent Increase or Decrease (−) in Various Biochemical Components (Content/Larva) After Treatment of *Tribolium castaneum* Larvae With Malathion (200 and 400 ppm), Permethrin (10 ppm and 20 ppm), and Then Combinations for 48 h and 120 h.*

	Malathion (ppm)		Permethrin (ppm)		Malathion + Permethrin				Malathion + permethrin for 120 h + Refeeding for 120 h	
					48 h		120 h			
	200 ppm	400 ppm	10 ppm	20 ppm	20 + 10	400 + 20	200 + 10	400 + 20	200 + 10	400 + 10
Sol. proteins	(−) 7.24	10.3	(−)20.42	(−)27.28	—	—	—	—	49.74	24.14
Total proteins	—	—	(−)12.92	(−)12.28	(−)10.8	(−)18.55	(−) 23.5	(−) 25.72	—	—
FAA	(−) 6.7	(−)28.8	(−)19.34	(−)22.63	13.55	8.47	—	—	34.4	74.19
Urea	—	—	(−)18.33	(−)27.66	—	—	380	496	112	69
Glucose	(−)27.67	(−)20.12	—	—	—	—	—	11.1	33.3	60.6
Glycogen	(−)21.40	(−)23.95	—	(−)31.96	(−)35.17	40.72	(−) 42.6	(−) 47.3	—	—
Lipids	—	—	—	—	—	—	—	—	21.8	24.42
Cholesterol	—	—	—	(−)13.51	—	—	—	—	—	—
DNA	—	—	—	—	—	—	—	—	—	24.6
RNA	—	—	—	—	—	(−) 8.01	—	—	—	21.36

*Data for permethrin taken from Saleem and Shakoori [18]. The larvae were fed for 5 days after exposure to insecticides. Nonsignificant changes are indicated by a dash. All these values are statistically significant values, which have been calculated by comparing the treated groups of insects with their respective controls.

present studies, in which whole insects were used, the glucose content per mg body weight increase after all insecticide treatments. On a per-larva basis, however, malathion treatment reduced the glucose content, while permethrin had no significant effect. The insecticide combinations did not produce any change except at higher concentration and that, too, after 120 h (11% increase). Hence, the results of this present study are not in accordance with the findings of Singh [15].

A five- to six-fold increase in the urea content of larvae after insecticide treatment is probably a reflection of total protein depletion.

Feeding on insecticide-free food after various insecticide treatments tends to normalize several biochemical abnormalities. The glycogen and total protein content returned to normal levels. The soluble proteins and FAA content increased in quantity. The urea content remained 1.7–2.1 times more than the controls, although significantly lower than before feeding of insecticide-free food. Glucose and lipid content increased, which is again an indicator of higher metabolic activity.

The accepted mode of action of OP insecticides is to phosphorylate ChE enzymes [26]. The enzyme is inhibited by dialkyl phosphorylation of the serine hydroxyl group at its activity site. This results in increased levels of acetylcholine at the nerve fibers and eventual failure of nerves to repolarize. However, if an animal survives OP poisoning, ChE activity levels will return to normal [27]. Recovery of ChE activity primarily occurs by synthesis of new ChE and by dephosphorylation of inhibited ChE [28]. Sublethal doses of malathion did not produce any appreciable change in ChE activity of larva, but when it was administered in combination with permethrin, a considerable increase (7-fold) was recorded. In one of our previous reports [19] on adult beetles, however, malathion was shown to produce a 3.95-fold increase in ChE activity, which remained about the same (i.e., 3.9 fold increase) after malathion + permethrin mixture treatments. The degree of this enzyme induction in larvae did not have any relationship with the concentration of enzymes, although exposures for shorter period of time (48 h) had more prominent effect as compared with longer periods (120 h). The continued exposure probably will inhibit the enzyme induction, which may ultimately be completely recovered on longer exposure.

From the results described above, it could be concluded that malathion when administered in combination with permethrin induced many more enzymatic and other macromolecular abnormalities than when used individually [18,19]. The action of insecticide combination in *T. castaneum* larvae could be attributed to their possible synergism at least at the macromolecular level. Whether combined applications of these two insecticides will provide better control of insect pests than those used alone (particularly against sixth instar larvae of *T. castaneum*), or whether the enzymatic and macromolecular abnormalities are part of a defensive mechanism, remain to be seen.

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