BIOCHEMICAL CHANGES INDUCED BY TOXIC CONCENTRATION OF MALATHION IN GERMINATING WHEAT SEEDS

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ABSTRACT

Malathion (O, O-dimethyl phosphorodithioate of diethyl mercaptosuccinate), an organophosphorus insecticide, markedly inhibited the root growth of germinating wheat seeds (4 day old) at 200 ppm. The contents of protein and DNA diminished but the RNA content increased appreciably in malathion (200 ppm) treated roots. The activity of protease was significantly inhibited but the activities of nucleases were stimulated in wheat roots on exposure to 200 ppm of malathion. The phytase activity of endosperm decreased with a decline in the level of inorganic phosphate in roots in malathion exposed condition. The activities of acid and alkaline phosphatases and ATPase increased in roots of four-day-old germinating wheat seeds on application of toxic dose of malathion.

INTRODUCTION

ORGANOPHOSPHORUS pesticides are widely used in agriculture as crop protectants because of their easy degradability inside the plant tissues. Their applications are currently favoured as a replacement of persistent organochlorine insecticides. However, at certain concentrations the organophosphorus insecticides inhibited seed germination and seedling growth[1-4]. A few reports are available about the interaction of organophosphorus insecticides with the normal metabolism of plant[3-5]. Various organophosphorus insecticides could alter the nitrogen metabolism in plants[6,7]. Menazon and disulphoton, the two organophosphorus pesticides, suppressed germination and seedling growth as a consequence of impaired respiration, starch and protein degradation in germinating seeds[3]. Kasturi *et al*[8] reported that organophosphorus pesticide treated pea seedlings have very few and stunted secondary roots compared with untreated control. They suggested that the phytotoxicity of organophosphorus pesticide was mainly due to its inhibitory effect on acetylcholinesterase.

Malathion (O, O-dimethyl phosphorodithioate of diethyl mercaptosuccinate), one of the least toxic organophosphorus compounds available today, is used against aphids, scales and other insects on a wide range of fruits and vegetables. It is presently considered as a suitable substitute for controlling lindane-resistant strains of pests attacking beans and other seeds[9]. Study of the plant-pesticide interaction at the physiological and biochemical levels has recently been a topic of interest. The present study deals with the toxic effect of malathion on growth and some of the biochemical parameters in germinating wheat seeds.

MATERIALS AND METHODS

p-Nitrophenyl phosphate, adenosine triphosphate, yeast RNA, calf thymus DNA, haemoglobin, bovine serum albumin and sodium phytate were purchased from Sigma Chemical Co., USA, Malathion was obtained from Cyanamide India Ltd., Bombay. Wheat seeds of Sonalika variety were used in this study.

Wheat seeds after surface sterilization with 0.1% mercuric chloride were imbibed in water for 4 hr. The seeds were then allowed to germinate in the dark at 20°C in several plates for 4 days. Experimental plates contained malathion at concentration of 200 ppm.

After four days of germination root growth and other measurements were made. The protein and nucleic acids from roots were extracted according to the method of Smille and Krotkov[10]. Contents of protein[11], RNA[12] and DNA[13] were measured. To determine acid soluble inorganic phosphate, roots were homogenized in ice-cold 0.2 N perchloric acid[14] and from the cleared extract P_i was estimated[15]. For the extraction of acid and alkaline phosphatases, roots were homogenized in 10 mM Tris-Cl buffer, pH 7.0 and centrifuged at 12,000 g for 20 min. From the supernatant the acid and alkaline phosphatases were assayed using *p*-nitrophenyl phosphate as substrate[16]. ATPase was extracted from roots by grinding in a medium consisting of 0.25 M sucrose, 0.003 M EDTA (pH 7.5). The homogenate was then centrifuged in the

cold. From the supernatant ATPase was assayed in a reaction mixture (1 ml) containing 20 mM Tris-Cl (pH 7.5), 3 mM ATP and enzyme. P_i released was determined by the method of Lowry and Lopez[15]. Phytase from endosperm was extracted with 10 mM Tris-Cl, pH 7.0 and centrifuged at 5000 g for 15 min. The supernatant was assayed for phytase activity[17]. Protease was extracted by homogenizing the roots in 0.05 M phosphate buffer (pH 7.0) containing 5 mM EDTA and 5 mM L-cysteine. The homogenate was centrifuged at 20,000 g for 20 min. Protease activity was measured from the supernatant using haemoglobin as substrate[18]. The peptides released was determined by the method of Lowry *et al*[11]. For the estimation of RNase, roots were homogenized in 50 mM Tris-Cl, pH 7.5 containing 0.5 M KCl and the homogenate was centrifuged at 20,000 g for 20 min. From the supernatant RNase was assayed in a reaction mixture described by Riley[19]. After incubation for 1 hr at 37°C, the reaction was stopped by 0.1 M uranyl acetate and 5 N PCA. The supernatant after centrifugation was read at 260 nm. DNase from roots was extracted by grinding in 0.05 M sucrose-citrate buffer, pH 6.0 and the homogenate was then centrifuged. The supernatant was assayed for DNase[20].

RESULTS AND DISCUSSION

It is evident from table 1 that malathion markedly inhibited the root growth of four-day-old germinating wheat seeds at 200 ppm. However, the inhibition (11.1%) of root growth initiated at a concentration of 100 ppm malathion (data not presented). It is seen from the table that the contents of protein and DNA decreased while the RNA content increased in roots on exposure to toxic concentration of malathion (200 ppm). It was further observed that malathion reduced the activity of protease but enhanced the activities of nucleases in wheat roots. Simultaneous decrease of protease activity as well as protein content may be associated with the inhibition of both the degradation and the synthesis of protein by toxic concentration of malathion. The inhibition of protein degradation in germinating seeds by menazon and disulphoton was known[3]. Increase in DNase activity resulted in a decrease in DNA content in treated roots. The decrease in DNA content during treatment may be partially attributed to the declined rate of DNA synthesis. The inhibition of DNA synthesis as well as lowering of DNA content in corn roots by lindane, an organochlorine insecticide, has been reported by Anderegg *et al*[21]. Simultaneous increase in RNase activity and RNA content was observed in malathion-treated roots. This can be explained by assuming that the degradation of RNA by RNase may be rapid but the coincident rate of RNA synthesis is faster in roots during toxic condition. Again it is apparent from table 1 that the specific activity of phytase in endosperm was significantly inhibited (44.02%) with a decline (38.89%) in the level of inorganic phosphate in roots on malathion exposure. Phytin, i.e. calcium or magnesium salt of inositol hexaphosphoric acid, is one of the important phosphorus-containing storage substances. Phytase acts on phytin of endosperm to release inorganic phosphorus needed for metabolic reactions in growing tissues during germination. Inhibition of

Table 1 *Effect of toxic concentration of malathion on the levels of various cell constituents, phosphate and the specific activities of different enzymes in germinating wheat seeds (four day old)*

Measurement		Units	None	+ Malathion 200 ppm	% Control
I	Root length	cm	6.6 ± 0.30	4.87 ± 0.26*	73.12
II	Protein	mg/g of fresh tissue	9.74 ± 0.47	8.37 ± 0.41**	85.93
	RNA	-do-	1.39 ± 0.13	2.10 ± 0.20*	151.08
	DNA	-do-	0.34 ± 0.02	0.19 ± 0.02*	55.88
	Inorganic phosphate	μ mol P_i/g of fresh tissue	1.98 ± 0.10	1.21 ± 0.06*	61.11
III	Acid phosphatase	μ g *p*-nitrophenol/μ g protein/hr	0.91 ± 0.05	1.25 ± 0.06*	137.36
	Alkaline phosphatase	μ g *p*-nitrophenol/mg protein/hr	35.82 ± 1.48	55.13 ± 2.61*	153.91
	ATPase	μ mol P_i/mg protein/hr	21.48 ± 1.17	47.26 ± 2.56*	220.02
	Phytase	-do-	2.84 ± 0.11	1.59 ± 0.04*	55.98
IV	Protease	$\Delta OD_{750/mg\ protein/hr}$	1.95 ± 0.07	1.38 ± 0.12*	70.77
	RNase	$\Delta OD_{260/mg\ protein/hr}$	6.46 ± 0.33	10.36 ± 0.83*	160.37
	DNase	-do-	2.08 ± 0.07	4.47 ± 0.40*	214.90

Values are mean ± S.D. of four sets of experiments; *denotes the level of significance *$P < 0.001$, **$P < 0.01$.

phytase activity in treated condition can thus account for the reduction of root growth in germinating wheat seeds. Hall and Hodges[14] while studying the compositional changes in different phosphorus compounds during germination of oats, had clearly shown that the increase of various phosphorus compounds in seedlings was at the expense of endosperm reserve. Inhibition of phytase activity in the event of malathion treatment indicates decreased utilization of phytin which results in poor mobilization of inorganic phosphorus in the growing axis. The concomitant inhibition of phytase activity from endosperm and a diminution in acid soluble inorganic phosphorus level in roots on exposure to malathion can be explained from the above fact. The present study shows that the activities of different phosphatases were stimulated in wheat roots following treatment of 200 ppm malathion. The increase in the activities of acid and alkaline phosphatases on malathion exposure is not well understood. It was earlier noted that the acid phosphatase accumulates extensively during growth inhibition in many plants[22]. Malathion also activated ATPase in wheat roots. Increase in ATPase activity of plasma membrane during malathion treatment in germinating *Vigna sinensis* roots was reported earlier[4].

Thus, malathion expressed its toxicity by affecting the levels of vital components of plant cells and the activities of different hydrolases in wheat seeds during germination.

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