MALATHION-INDUCED EFFECTS ON GROWTH AND NITROGEN METABOLIZING ENZYMES IN GERMINATING WHEAT

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ABSTRACT

Malathion inhibited growth of wheat shoots when seeds were grown in nitrate-containing medium. The pesticide stimulated nitrate reductase, glutamine synthetase and glutamate dehydrogenase activities in wheat shoots growing in nitrate medium, and led to an increase in the level of soluble protein and nitrite content in the shoots. Nitrite content did not, however, change much at concentrations above 100 ppm of malathion. Stimulation of glutamate dehydrogenase was highest at 400 ppm malathion while a decline in glutamine synthetase was observed at the same concentration.

INTRODUCTION

THE process of nitrate assimilation is considered to be of prime importance to plant growth as this anion is the main source of inorganic nitrogen for crop plants. The assimilation of nitrate in plant tissues involves reductive reactions forming ammonium ions, which are promptly incorporated into amino acids[1,2]. The limiting enzyme of the reduction process is considered to be nitrate reductase[3]. The principal route of ammonia assimilation was previously thought to be via glutamate dehydrogenase[4] but ample evidence[2,5] now suggests that the glutamine synthetase/glutamate synthase pathway is the primary route for ammonia assimilation. Various investigators have reported changes in nitrogen metabolism of various plants after exposure of seedlings to organophosphate insecticides by quantitating amino acids, and total and protein nitrogen in roots and leaves[6-8]. Information on the effect of organophosphate insecticides on enzyme systems concerned with inorganic nitrogen metabolism in germinating seeds is scanty. It has been reported from our laboratory that malathion (*O,O*-dimethylphosphorodithioate of diethylmercaptosuccinate), an organophosphate insecticide, can retard root growth and affect associated metabolic processes in germinating seeds[9,10]. The present investigation deals with the effect of malathion on growth, nitrite and soluble protein content and some of the enzymes of nitrogen metabolism in 4-day-old germinating wheat shoots.

MATERIALS AND METHODS

NADH, ATP, bovine serum albumin and Tris were purchased from Sigma Chemical Company, USA. All other chemicals used were of analytical grade. Malathion (99.9%) was obtained as gift from Cynamid India Ltd, Bombay. Wheat seeds of Sonalika variety were collected from the Calcutta University Seed Farm, Baruipur, West Bengal.

Wheat seeds after surface sterilization with 0.1% mercuric chloride, were allowed to imbibe water for 4 h. Malathion was dissolved in acetone and the solution added to petri dishes containing distilled water over a filter paper to achieve different concentrations of pesticide (50, 100, 200 and 400 ppm). In a control plate, only acetone was added to distilled water. Acetone was allowed to evaporate from all the plates. Wheat seeds were then spread over the filter paper in the petri dishes and allowed to germinate at 20°C in light. After three days of germination seeds from all the plates were transferred separately to standard nutrient medium[11] containing 30 mM KNO_3. Medium to which seeds germinated in presence of pesticide also contained the pesticide at the same concentration. Shoots were excised the next day, washed in cold distilled water, and ground in a buffer medium containing 25 mM potassium phosphate (pH 7.5), 5 mM EDTA and 5 mM L-cysteine hydrochloride. The homogenate was centrifuged at 20,000 *g* for 20 min. The supernatant was used as the source of nitrate reductase and glutamate dehydrogenase, and to estimate nitrite

and soluble protein. Nitrate reductase was measured following the method of Evans and Nason[12] as modified by Hageman and Flesher[13]. Glutamate dehydrogenase was estimated by the method of King[14]. Soluble protein was determined by the method of Lowry *et al*[15] after precipitation with 50% TCA. The nitrite content was also determined[13]. For glutamine synthetase, shoots were homogenized in 0.05 M Tris–Cl buffer (pH 7.5) containing 1 mM 2-mercaptoethanol and 2 mM disodium EDTA, the homogenate centrifuged at 20,000 *g* for 15 min, the supernatant used for the assay[16].

RESULTS AND DISCUSSION

It is evident from table 1 that malathion at 100, 200 and 400 ppm can significantly retard the elongation of wheat shoot. Fresh weight of shoot was also decreased at these concentrations. But with 50 ppm malathion fresh weight of shoot was significantly increased. Table 1 also indicates that soluble protein was significantly elevated at 100, 200 and 400 ppm malathion. Table 2 shows that malathion at all the concentrations tested induced nitrate reductase activity. Increase in nitrate reductase activity was also reported in *Hordeum vulgare* and *Zea mays* seedlings on atrazine application[17]. Glyphosate[18] (an organophosphate herbicide) and chlorthalonil[19] (a fungicide) also induced nitrate reductase in plant tissues. The nitrite content was higher in malathion-treated wheat shoots (table 2). It did not, however, change much at concentrations above 100 ppm, indicating better utilization of nitrite at higher concentrations of the pesticide. Table 2 also shows that glutamine synthetase activity increased up to 200 ppm malathion, but was lower at 400 ppm. Glutamate dehydrogenase was found to be stimulated at all four concentrations of malathion.

The increase in soluble protein and activity of key enzymes of nitrogen metabolism may be due to either enhanced nitrate absorption from the nutrient medium or higher rate of metabolic processes involving these enzymes under pesticide toxicity. Increased total and protein nitrogen in cabbage root on exposure to organophosphate pesticides has been explained similarly[20]. Rhodes *et al*[21] showed that with rapid increase in intracellular concentration of ammonia glutamate dehydrogenase activity increased while a repression of glutamine synthetase

Table 1 *Effect of malathion on growth and soluble protein content of 4-day-old wheat shoots in nitrate containing medium*

Malathion added (ppm)	Shoot length (cm)	Fresh weight of shoot (mg)	Soluble protein (mg/g fresh tissue)
0	5.17 ± 0.28	35.77 ± 1.7	20.60 ± 1.07
50	4.95 ± 0.17	39.40 ± 1.9††	20.19 ± 1.05
100	4.60 ± 0.26††	31.17 ± 1.5**	23.75 ± 1.18†
200	3.98 ± 0.13*	22.77 ± 1.2*	25.32 ± 1.26**
400	3.14 ± 0.20*	15.35 ± 0.08*	30.35 ± 1.51*

Values are mean ± SD of four sets of experiments; *$P < 0.001$; **$P < 0.01$; †$P < 0.02$; ††$P < 0.05$.

Table 2 *Effect of malathion on nitrite content and nitrate reductase, glutamine synthetase and glutamate dehydrogenase of 4-day-old wheat shoots*

Malathion added (ppm)	Nitrate reductase[a]	Nitrite[b]	Glutamine synthetase[c] ($\times 10^2$)	Glutamate dehydrogenase[d]
0	0.99 ± 0.03	15.6 ± 0.82	1.45 ± 0.06	0.105 ± 0.003
50	4.51 ± 0.21*	25.5 ± 1.25*	2.73 ± 0.11*	0.175 ± 0.003*
100	4.93 ± 0.28*	47.8 ± 1.78*	3.41 ± 0.17*	0.220 ± 0.004*
200	6.13 ± 0.41*	51.5 ± 2.45*	4.85 ± 0.25*	0.315 ± 0.006*
400	7.33 ± 0.56*	53.3 ± 2.15*	3.57 ± 0.16*	0.619 ± 0.024*

Values are mean ± SD of four sets of experiments; *$P < 0.001$; [a]nmoles of nitrite liberated/mg protein/h; [b]nmoles per g fresh tissue; [c]A_{540}/mg protein/h; [d]A_{390}/mg protein/h.

occurred. These changes are favourable for metabolizing increased accumulation of ammonia. The observation in the present study of maximum glutamate dehydrogenase activity and decreased glutamine synthetase activity at 400 ppm of malathion can be explained if one assumes accumulation of ammonia arising from increased nitrate assimilation at higher concentrations of malathion, but any definite conclusion in this regard needs further investigation.

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ANNOUNCEMENTS

SOCIETY OF BIOSCIENCES

Applications with complete biodata are requested from young scientists below the age of 45 years for the award of Zahur Qasim Gold Medal to be given during the Symposium on "Advances in limnology and conservation of endangered fish species" to be held from 23 to 25 October 1989 at Garhwal University, Srinagar. For details contact Dr V. P. Agrawal, Secretary-General, 25/4 Ram Bagh Road, Muzaffarnagar 251 001.

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The congress will include sessions on all aspects of forensic sciences. **Abstracts** of presentations must be sent **before 1 July** and will be considered by the programme committee. The complete text of an accepted presentation must be sent **before 1 August.**

For details contact the congress chairman Prof. P. Chandra Sekharan, President, Forensic Science Society of India, 30A Kamarajar Salai, Madras 600 004.