LIST OF PUBLICATIONS


6. Carbaryl toxicity in germinating seeds of *Vigna sinensis*:


Effect of methyl parathion in germinating wheat

As a replacement for persistent chlorinated hydrocarbons, organophosphorus pesticides are finding increased use for the protection of crops. However, at certain concentrations, the organophosphorus pesticides can suppress seed germination and seedling growth1-3. They are found to interact with normal metabolism of plants1,2. Methyl parathion, a widely used organophosphorus insecticide, can inhibit growth and respiration of plants4. So far little is known about the effect of methyl parathion on physiological and biochemical processes of germinating seed. The present paper describes the toxic effect of methyl parathion on seedling growth, protein and DNA content, peroxidase and different hydrolases in roots of four-day old wheat seedlings. Attempts were also made to prevent methyl parathion toxicity on seedling growth by gibberellic acid (GA3) treatment.

Wheat seeds, after surface-sterilization with 0.1% HgCl2, were imbibed for 4 hr and then spread in Petri-dishes lined with blotting paper containing methyl parathion. Some plates contained GA3 along with methyl parathion. They were germinated in the dark at 20°C for four days prior to measurements of growth and biochemical analysis. Wheat roots were homogenized in 10 mM Tris-HCl buffer (pH 7.0) and centrifuged at 12,000 g for 20 min. From the supernatant the acid phosphatase activity was assayed according to the method of Mitchell et al5. Peroxidase was extracted from root by homogenizing in cold 0.02 M phosphate buffer (pH 6.1) and centrifuged at 10,000 g for 20 min. Peroxidase was assayed from the supernatant following the method of Chance and Maehly6 with colorimetric determination of the change in colour intensity of oxidised catechol at 420 nm. Protease was extracted7 and assayed8 by the method described earlier. Roots were homogenized in 0.25 M sucrose and 0.003 M EDTA (pH 7.5) and then centrifuged. From the supernatant ATPase was estimated according to the method described elsewhere9. Protein and DNA were extracted according to the method described earlier DNA content was estimated by the method of Burton10. Protein content was measured according to the method of Lowry et al11.

The elongation of wheat seedling was markedly inhibited by methyl parathion. The effect was more pronounced in case of root. The pesticide also inhibited the fresh weight of the seedling (Table 1). It was found that GA3 was able to overcome the toxic effect of the pesticide on shoot completely but in case of root it was only partially able to reverse the harmful consequence of methyl parathion (Table 2).

As compared to the control, the activity

<table>
<thead>
<tr>
<th>Condition</th>
<th>Root length (cm)</th>
<th>Inhibition (%)</th>
<th>Shoot length (cm)</th>
<th>Inhibition (%)</th>
<th>Fresh weight of seedling (mg)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.69±0.149</td>
<td>—</td>
<td>3.58±0.105</td>
<td>—</td>
<td>111.0 ±3.09</td>
<td>—</td>
</tr>
<tr>
<td>+50 ppm methyl parathion</td>
<td>3.05±0.152*</td>
<td>54.4</td>
<td>2.28±0.089*</td>
<td>45.4</td>
<td>64.25±1.75*</td>
<td>42.1</td>
</tr>
<tr>
<td>+100 ppm methyl parathion</td>
<td>1.74±0.20*</td>
<td>74.0</td>
<td>2.06±0.04*</td>
<td>58.4</td>
<td>43.73±1.56*</td>
<td>60.6</td>
</tr>
<tr>
<td>+200 ppm methyl parathion</td>
<td>0.85±0.043*</td>
<td>87.7</td>
<td>1.76±0.040*</td>
<td>50.8</td>
<td>31.25±1.72*</td>
<td>71.3</td>
</tr>
</tbody>
</table>

Results have been expressed as mean ± S. D. of four sets of experiments *P<0.001
TABLE 2: Effect of gibberellic acid applied in combination with methyl parathion on the elongation of wheat seedlings (4 days old)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Root length (cm)</th>
<th>Shoot length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.54 ± 0.155</td>
<td>3.61 ± 0.089</td>
</tr>
<tr>
<td>+50 ppm methyl parathion</td>
<td>3.65 ± 16*</td>
<td>3.01 ± 0.055*</td>
</tr>
<tr>
<td>+50 ppm methyl parathion + 10-7 M GA,</td>
<td>3.70 ± 0.157*</td>
<td>3.6 ± 0.185*</td>
</tr>
<tr>
<td>+50 ppm methyl parathion + 10-8 M GA,</td>
<td>3.72 ± 0.118*</td>
<td>3.58 ± 0.072*</td>
</tr>
</tbody>
</table>

Results have been expressed as mean ± S.D. of four sets of experiments
*P<0.001

TABLE 3: Effect of methyl parathion on the activities of acid phosphatase, protease, ATPase and peroxidase in roots of wheat seedlings (4 days old)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Specific activities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acid phosphatase (µg p-nitrophenol /mg protein/hr)</td>
</tr>
<tr>
<td>Control</td>
<td>0.93 ± 0.046</td>
</tr>
<tr>
<td>+50 ppm methyl parathion</td>
<td>0.6966 ± 0.039*</td>
</tr>
</tbody>
</table>

Results have been expressed as mean ± S.D. of four sets of experiments
*P<0.01

of acid phosphatase and ATPase were inhibited but the activity of peroxidase and protease were stimulated (Table 3). Inhibition of phosphatase activity by phenyl urea and phenyl carbamate herbicides were shown by Geike et al. The inhibition of ATPase activity by menazon and disulfoton (organophosphorus insecticides) was reported earlier. Cell division and cell elongation with accompanying synthesis of food matter go on rapidly in growing tissue which demands the release of energy. Inhibition of ATPase activity in treated root signifies a decreased ability of the treated root to mobilize energy from the breakdown of ATP which results in impairment of the above processes during germination. Increase in protease activity in treated root may lead to accelerated rate of protein breakdown during toxic condition. The increase in peroxidase activity was supposed to be a protective reaction of the plant against the organophosphorus insecticide. Though the exact function of peroxidase is not known, it is generally thought to be a part of the enzyme responsible for auxin destruction in vivo. The growth retardants (CCC and Amo-1618) stimulated the peroxidase activity in young barley seedling. Rao et al. mentioned about the accumulation of peroxidase activity in slow growing tissue and dwarf plants which explains the growth inhibiting activity of the enzyme. The decrease in protein and DNA content in wheat root by methyl parathion as evident from Table 4 may be due to the decline in protein and DNA synthesis. The inhibition of protein and DNA synthesis by organophosphorus pesticide malathion in germinating roots of Vigna sinensis seed are known. The reduction in DNA synthesis in corn roots after treatment with organochlorine insecticide lindane was shown by Anderegg et al.

Thus, methyl parathion expressed its toxic effect in germinating wheat seedling
### TABLE 4: Effect of methyl parathion on protein and DNA content in roots of wheat seedlings (4 days old)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Protein content (mg/gm of fresh tissue)</th>
<th>DNA content (mg/gm of fresh tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.642 ± 0.484</td>
<td>0.361 ± 0.026</td>
</tr>
<tr>
<td>+50 ppm methyl parathion</td>
<td>6.491 ± 0.312*</td>
<td>0.240 ± 0.014*</td>
</tr>
</tbody>
</table>

Results have been expressed as mean ± S. D. of four sets of experiments.
*P < 0.001

by decreasing the vital macromolecules of plant cells and altering different hydrolytic enzymes.

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Received: 2 July, 1985.
Revised: 8 October, 1985.

Effect of lindane in germinating wheat

The organochlorine insecticide lindane can inhibit growth and respiration of different plants. Lindane can cause cessation of mitotic division and induce different cytological abnormalities in plant roots. It can also inhibit DNA synthesis in corn roots. So far little is known about the effect of lindane on different biochemical processes during seed germination. The present paper describes the toxic effect of lindane on different hydrolases, transaminase and peroxidase in germinating wheat seedlings (4-day old).

Wheat seeds were soaked for 4 hr and then spread in Petri-dishes lined with blotting paper containing different concentrations of lindane and allowed to germinate in the dark at 20°C prior to measurements of growth and biochemical analysis.

Roots were ground in 10 mM Tris-Cl buffer (pH 7.0) and the homogenate centrifuged at 12000 g for 20 min. From the supernatant, the activity of acid phosphatase and acid pyrophosphatase were assayed. Glutamate oxaloacetate transaminase (GOT) was extracted by homogenizing in 0.1 M potassium phosphate buffer (pH 7.4) containing 2 mM EDTA and 100 mM 2-mercaptoethanol and the homogenate centrifuged at 20,000 g for 20 min. From the supernatant, GOT activity was assayed.

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Peroxidase was extracted from roots and assayed by the method described earlier. DNase was extracted from roots and assayed. RNase of roots was extracted by homogenizing in 50 mM Tris-Cl buffer (pH 7.5) and the homogenate centrifuged at 20,000 g (15 min) and from the supernatant RNase was assayed in a reaction mixture described by Riley. The reaction was stopped by uranyl acetate (0.1 M) in 5N PCA and after centrifugation the supernatant was read at 260 nm. Protease was extracted from roots and assayed as described previously. ATPase from roots was extracted by homogenizing in 0.25 M sucrose and 0.003 M EDTA (pH 7.5) and then centrifuged and from the supernatant ATPase activity was estimated. Protein content was measured according to the method of Lowry et al.

The elongation of wheat seedling was markedly inhibited by lindane. The growth inhibition was more pronounced in root (Table 1). Lindane stimulated the activity of acid phosphatase and acid pyrophosphatase in root (Table 2). The increase of acid phosphatase and acid pyrophosphatase activity during malathion (organophosphorus insecticide) toxicity was reported. The accumulation of acid phosphatase was shown during growth inhibition in many plants. It was observed that lindane caused an inhibition of the activity of peroxidase.

### TABLE 1 : Effect of lindane on the elongation of wheat seedlings (4-day old)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Root length (cm)</th>
<th>Inhibition (%)</th>
<th>Shoot length (cm)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.69 ± 0.145</td>
<td>-</td>
<td>3.72 ± 0.125</td>
<td>-</td>
</tr>
<tr>
<td>+5 ppm lindane</td>
<td>3.84 ± 0.166*</td>
<td>42.6</td>
<td>3.18 ± 0.064*</td>
<td>14.5</td>
</tr>
<tr>
<td>+10 ppm lindane</td>
<td>1.73 ± 0.071*</td>
<td>74.1</td>
<td>2.74 ± 0.057*</td>
<td>26.3</td>
</tr>
<tr>
<td>+15 ppm lindane</td>
<td>1.27 ± 0.064*</td>
<td>81.0</td>
<td>2.22 ± 0.044*</td>
<td>40.3</td>
</tr>
<tr>
<td>+20 ppm lindane</td>
<td>1.02 ± 0.085*</td>
<td>84.7</td>
<td>1.91 ± 0.102*</td>
<td>48.6</td>
</tr>
</tbody>
</table>

Results have been expressed as mean ± S.D. of four sets of experiments

*P < 0.001

mM 2-mercaptoethanol and the homogenate centrifuged at 20,000 g for 30 min and from the supernatant GOT activity was assayed.
TABLE 2: Effect of lindane on the activities of acid phosphatase, acid pyrophosphatase, transaminase, protease and peroxidase in roots of wheat seedlings (4-day old)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Specific activities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acid phosphatase (µg p-nitrophenol/µg protein/hr)</td>
</tr>
<tr>
<td>Control</td>
<td>0.916 ± 0.054</td>
</tr>
<tr>
<td>+10 ppm lindane</td>
<td>1.200 ± 0.062*</td>
</tr>
</tbody>
</table>

Results have been expressed as mean ± S.D. of four sets of experiments
*P<0.001, **P<0.05

protease and GOT (Table 2). Peroxidase is known for its role in growth and development. Inhibition of peroxidase activity may be due to the interaction of lindane with the growth processes during germination. Trifluralin, a nitroaniline herbicide, affects peroxidase activity of maize and horseradish seedlings. The inhibition of protease activity in treated root signifies the breakdown of protein to amino acids is impaired in treated condition. The aminotransferases, essential enzymes during germination, are required for the interconversion of amino acids and the production of metabolic intermediates during germination. Thus the low activity of GOT during treatment will prevent normal root growth. Moreover the low activity of GOT may lead to a decline in protein synthesis due to poor availability of amino acids. Declere et al. reported inhibition of GOT activity in wheat seed by triazine herbicide.

Lindane treatment enhanced the activity of nucleases in germinating wheat root (Table 3). Thus, the content of RNA and DNA in treated root may be lower owing to the rapid degradation of nucleic acids. Anderegg et al. reported that corn roots exposed to lindane for 48 and 96 hr had lower quantities of DNA than untreated root while the RNA content was lowered after 48- and 120-hr exposure.

Lindane caused an inhibition of ATPase activity in treated root. Inhibition of ATPase activity by organophosphorus pesticide was shown earlier. Cell division and cell elongation with accompanying synthesis of food matters go on rapidly in growing tissue which demands release of energy. Inhibition of ATPase activity in lindane-treated root signifies a decreased ability of the treated root to mobilize energy from the breakdown of ATP which resulted in impairment of the above processes during germination.

TABLE 3: Effect of lindane on the activities of ATPase and nucleases in roots of wheat seedlings (4-day old)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Specific activities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ATPase (µmoles Pi/mg protein/hr)</td>
</tr>
<tr>
<td>Control</td>
<td>21.88 ± 1.59</td>
</tr>
<tr>
<td>+10 ppm lindane</td>
<td>13.20 ± 0.98*</td>
</tr>
</tbody>
</table>

Results have been expressed as mean ± S.D. of four sets of experiments
*P<0.001, **P<0.01
germination. Charnetski et al. reported a cessation of mitotic division in the apical meristems of lindane-treated pea roots resulting in enlarged cells with multilobed nuclei.

From the present study, it was found that lindane interferes with some basic metabolic processes associated with germination.

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Received 8 July, 1985.
Revised: 8 October, 1985.

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. A few reports are available about the interaction of organophosphorus insecticides with the normal metabolism of plants. Various organophosphorus insecticides could alter the nitrogen metabolism in plants. Menazon and disulfoton, the two organophosphorus pesticides, suppressed germination and seedling growth as a consequence of impaired respiration, starch and protein degradation in germinating seeds. Kasturi et al. reported that organophosphorus pesticide treated pea seedlings have very few or 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 l-thyl 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. 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 Cyanamid 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 Smillie and Krotkov. Contents of protein, RNA and DNA were measured. To determine acid soluble inorganic phosphate, roots were homogenized in ice-cold 0.2 N perchloric acid. 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. 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 manezon and disulphoton was known. Increase in DNA 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 concomitant 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, a 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>
<thead>
<tr>
<th>Measurement</th>
<th>Units</th>
<th>None</th>
<th>+ Malathion 200 ppm</th>
<th>% Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Root length</td>
<td>cm</td>
<td>6.6 ± 0.30</td>
<td>4.87 ± 0.26*</td>
<td>73.12</td>
</tr>
<tr>
<td>II Protein</td>
<td>mg/g of fresh tissue</td>
<td>9.74 ± 0.47</td>
<td>8.37 ± 0.41**</td>
<td>85.93</td>
</tr>
<tr>
<td>III RNA</td>
<td>do-</td>
<td>1.39 ± 0.13</td>
<td>2.10 ± 0.20*</td>
<td>151.08</td>
</tr>
<tr>
<td>IV DNA</td>
<td>do-</td>
<td>0.34 ± 0.02</td>
<td>0.19 ± 0.02*</td>
<td>55.88</td>
</tr>
<tr>
<td>V Inorganic phosphate</td>
<td>( \mu ) mol ( P_i )/g of fresh tissue</td>
<td>1.98 ± 0.10</td>
<td>1.21 ± 0.06*</td>
<td>61.11</td>
</tr>
<tr>
<td>VI Acid phosphatase</td>
<td>( \mu ) g ( p )-nitrophenol/( \mu ) g protein/hr</td>
<td>0.91 ± 0.05</td>
<td>1.25 ± 0.06*</td>
<td>137.36</td>
</tr>
<tr>
<td>VII Alkaline phosphatase</td>
<td>( \mu ) g ( p )-nitrophenol/100 mg protein/hr</td>
<td>35.82 ± 1.48</td>
<td>55.13 ± 2.61*</td>
<td>153.91</td>
</tr>
<tr>
<td>VIII ATPase</td>
<td>( \mu ) mol ( P_i )/mg protein/hr</td>
<td>0.48 ± 0.17</td>
<td>47.26 ± 2.56*</td>
<td>220.02</td>
</tr>
<tr>
<td>IX Phytase</td>
<td>do-</td>
<td>2.84 ± 0.11</td>
<td>1.59 ± 0.04*</td>
<td>55.98</td>
</tr>
<tr>
<td>X Protease</td>
<td>( \Delta )OD/mg protein/hr</td>
<td>1.95 ± 0.07</td>
<td>1.38 ± 0.12*</td>
<td>70.77</td>
</tr>
<tr>
<td>XI RNase</td>
<td>( \Delta )OD/mg protein/hr</td>
<td>6.46 ± 0.33</td>
<td>10.36 ± 0.83*</td>
<td>160.37</td>
</tr>
<tr>
<td>XII DNase</td>
<td>do-</td>
<td>2.08 ± 0.07</td>
<td>4.47 ± 0.40*</td>
<td>214.90</td>
</tr>
</tbody>
</table>

Values are mean ± SD of four sets of experiments, *denotes the level of significance \( *P < 0.001, **P < 0.001 \)
phytase activity in treated condition can thus account for the reduction of root growth in germinating wheat seeds Hall and Hodges\textsuperscript{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\textsuperscript{22}. Malathion also activated APase in wheat roots. Increase in APase activity of plasma membrane during malathion treatment in germinating Vigna sinensis roots was reported earlier\textsuperscript{22}.

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.

ACKNOWLEDGEMENT

This work was partially supported by a research grant from DST, New Delhi.

4 October 1985, Revised 6 December 1985


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Biochemical Changes in Germinating Seeds of *Vigna sinensis* during Carbaryl Toxicity

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*Received 24 September 1985, revised 18 March 1986*

Carbaryl at different concentrations inhibited seedling growth of germinating *V. sinensis* seeds (3 day old). Above 50 ppm of carbaryl treatment the nucleic acids contents were lowered in germinating seedlings in a dose dependent manner. But the protein content of the seedlings was increased at concentrations above 75 ppm of carbaryl treatment also in a dose dependent manner. The decline in nucleic acids contents was correlated with the increase in nuclease activities in treated seedlings. The accumulation of protein induced by carbaryl is shown to be due to inhibition of protease activity.

The carbamate insecticide carbaryl (1-naphthyl methyl carbamate) is extensively used on a variety of agricultural crops. It is more often used as a replacement of organochlorine insecticide. Earlier reports indicated that carbaryl at certain concentration affected the plant growth and cellular respiration. Roots grown in carbaryl treated sand were shorter and thicker. A reduction of 28 to 54% in root tips respiration was observed in carbaryl treated plants. Carbaryl also altered the phenolics and sugars in brinjal plants. So far little is known about the toxic effects of carbaryl on different biochemical processes in germinating seedling. Studies of the plant-pesticide interaction at physiological and biochemical levels have recently been a topic of interest. The present study reports changes in growth, protein and nucleic acids of germinating seedling of *V. sinensis* seeds exposed to deleterious concentrations of carbaryl.

Bovine serum albumin (BSA), RNA, DNA, orcinol and diphenylamine were purchased from Sigma Chemical Co., USA. All other chemicals used were of analytical grade.

**Table 1** — Influence of Various Concentrations of Carbaryl on Seedling Growth, Contents of Protein and Nucleic Acids and Activities of Protease and Nucleases of 3 Day Old Seedlings of *V. sinensis*

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Control</th>
<th>Carbaryl (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
<td>75</td>
</tr>
<tr>
<td>I Length of seedlings</td>
<td>7.0</td>
<td>±0.508</td>
</tr>
<tr>
<td>of seedlings</td>
<td>7.320</td>
<td>±0.035</td>
</tr>
<tr>
<td>Fresh wt</td>
<td>0.372</td>
<td>±0.032</td>
</tr>
<tr>
<td>Protein</td>
<td>±0.38</td>
<td>±0.35</td>
</tr>
<tr>
<td>RNA</td>
<td>±0.17</td>
<td>±0.11</td>
</tr>
<tr>
<td>DNA</td>
<td>±0.12</td>
<td>±0.31</td>
</tr>
<tr>
<td>III Protease</td>
<td>±0.25</td>
<td>±0.27</td>
</tr>
<tr>
<td>RNase</td>
<td>±0.066</td>
<td>±0.084</td>
</tr>
<tr>
<td>DNase</td>
<td>±0.129</td>
<td>±0.131</td>
</tr>
</tbody>
</table>

*Length of seedling expressed in cm, *Fresh weight of seedling expressed in g, *Protein and RNA content expressed as mg/g of fresh weight, *DNA content expressed as mg/g of fresh weight, *Sp activity expressed as AOD_{260nm}/mg protein/hr, *Sp activity expressed as AOD_{280nm}/mg protein/hr

'values *<0.01, **<0.001, ***<0.05
MALATHION EXPOSED SEEDS OF Vigna sinensis (L): CHANGE IN MITOCHONDRIAL RESPIRATION, SWELLING AND CONTRACTION, LEAKAGE OF NAD AND MITOCHONDRIAL MEMBRANE BOUND ATPase AND PYROPHOSPHATASE ACTIVITIES

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(Received August 26, 1985)

Malathion exposure at a concentration above 50 ppm elicited some alteration in the respiratory, swelling and contraction characteristics of mitochondria in the roots of 72 hr germinating seeds of Vigna sinensis (L). Change in these parameters is reflected by seedling growth characteristics of V. sinensis (L). The alteration of mitochondria by malathion (above 50 ppm) is also supported by the experiments which showed a marked leakage of NAD from the intra-mitochondrial pool and a change in the activities of mitochondrial membrane bound ATPase and acid pyrophosphatase activities. Some change of sterol and phospholipid patterns in the associated mitochondrial membrane are also observed in the roots of the 72 hr germinating seeds of V. sinensis (L) under malathion exposure (above 50 ppm).

KEY WORDS malathion, seeds, mitochondria, membrane

INTRODUCTION

Pollution caused by agriculture is one of the greatest national and international environmental quality problems.1,2 Although many aspects of the relationship between pollution and the environment have been extensively studied,1,4 yet systematic studies of the effect of pesticides on germinating seeds are scanty. Malathion is one of the products which is now considered to be a suitable substitute for controlling indane-resistant strains of pests attacking beans and other seeds.5 Our earlier observation revealed that malathion inhibits some lysosomal hydrolases,6 affects cell membrane permeability7 and inhibits plasma membrane protein synthesis8 in the roots of germinating seeds of Vigna sinensis (L).

When cells are injured either lethally or sublethally, changes in intracellular composition and volume changes occur at different rates depending on the injury.9 Changes in swelling and contraction characteristics of mitochondria are also observed when the cells are injured by toxic chemicals.7,8

In view of this, we undertake systematic studies of the effects of malathion on mitochondrial swelling and contraction, leakiness, growth and respiration along with mitochondrial membrane bound ATPase and acid pyrophosphatase activities in the roots of 72 hr germinating seeds of Vigna sinensis (L) in an attempt to further understand the biochemical mechanisms by which malathion inhibits root growth.
MATERIALS AND METHODS

Adenosine triphosphate (ATP) (tris-salt), sodium pyrophosphate, bovine serum albumin were purchased from Sigma Chemical Co., USA. Malathion (O, O-dimethylphosphorothioate of diethylmercapto succinate) was obtained from Cyanamid India Ltd., Bombay, India. All other chemicals used were of analytical grade. Seeds of *Vigna sinensis* (L) were purchased from a local source.

1. **Germination of Seeds and Isolation of Mitochondria**

Seeds of *Vigna sinensis* (L) were surface sterilized by 0.1 percent mercuric chloride for one to two minutes. The seeds were then immediately washed with sterile distilled water till the washings were free from chloride and allowed to soak in distilled water for six hours. The seed coats were then removed and the dehulled seeds were then allowed to germinate at 25°C in several Petri dishes for 72 hours. Experimental plates contained malathion between 50 ppm and 400 ppm. The germination process was carried out by following the method described by Hegarty and Ross. After 72 hours of germination, the roots from the seeds of each plate were excised separately, washed in cold de-ionised water and ground by means of a mortar and pestle in a medium (homogenizing medium) containing 0.25 M sucrose and 10 mM Tris-HCl buffer pH 7.4. The homogenate was then strained through cheese cloth and the supernatant was then centrifuged at 10,000 g for 40 minutes. The supernatant was centrifuged at 10,000 g for 40 minutes. The sediment was resuspended in the homogenizing medium. After a clarification run at 2000 g for ten minutes the mitochondria were sedimented by centrifuging at 10,000 g for 20 minutes. The mitochondria were further purified on a discontinuous sucrose density gradient of 0.75 M sucrose, 1.0 M sucrose and a cushion of 2.4 M sucrose. The mitochondria were collected in 1 M sucrose and sedimented by centrifugation at 15,000 g for 20 minutes.

2. **Isolation of Mitochondrial Membrane**

Mitochondrial membranes of the roots of *Vigna sinensis* (L) from control and experimental plates were isolated by the method as described by Miller et al.

3. **Measurement of Respiration**

Mitochondrial oxygen uptake was determined with a Warburg apparatus using standard manometric techniques. Appropriate amounts of mitochondrial suspension from the control and experimental roots were placed on 6 cm² pieces of filter paper moistened with 0.5 ml of water in the reaction flasks. Carbon dioxide was absorbed by 0.2 ml of 10 percent KOH applied to the centre well. The reaction flasks and manometers were allowed to equilibrate for 30 minutes and oxygen consumption was measured at 25°C. Readings were taken at intervals of 20 minutes during a period of two hours. Respiration was calculated in terms of μl oxygen uptake per mg protein per minute.

4. **Determination of Swelling and Contraction Properties of Mitochondria**

Swelling and contracting properties of mitochondria were determined by measuring the absorbance at 520 nm with a Beckman model DU 2 spectrophotometer fitted with a controlled temperature cell housing. The isolated mitochondrial fractions
were suspended in a solution of 0.4 M sucrose. The procedure for volume changes was to add, at zero time, 0.1 ml of mitochondrial suspension (0.1 to 0.2 mg of protein) to a cuvette containing 2.9 ml of 0.1 M KCl, 0.02 M Tris-HCl buffer pH 7.5. The reaction was run at 25°C. The initial readings were taken at one minute and subsequent readings were taken at five minute intervals. After the mitochondrial swelling terminated, contraction was initiated by the addition of 0.01 ml of succinate to give a final concentration of 8 mM. After the addition of succinate, the readings were taken at five minute intervals until maximum contraction was attained. A decrease in absorbance from the first minute reading indicated swelling, and an increase indicated contraction.

5 Release of NAD from Isolated Mitochondria

The mitochondrial pellet isolated from the roots of *Vigna sinensis* (L) were suspended in 2 ml of 0.3 M mannitol and incubated for 20 minutes at 25°C and then centrifuged at 40,000 g for seven minutes. The amount of NAD was determined from the supernatant layer as well as in the mitochondrial pellet. To the supernatant layer 1 ml of 30 percent trichloroacetic acid (TCA) was added and allowed to stand on ice for 15 minutes before centrifuging to remove the protein precipitate. TCA was then removed from the supernatant layer by ether extractions. The resulting solution was evaporated to dryness in vacuum. The residue was dissolved in water. The mitochondrial pellet obtained after incubation step was suspended in 3 ml of ten percent TCA and was homogenized in a glass homogenizer to release the bound NAD. The remaining steps were as outlined for the supernatant sample. The amount of NAD present in the preparations from both mitochondria and the supernatant layer was calculated using the alcohol dehydrogenase assay.

6 Enzyme Assays

Assays of ATPase and acid pyrophosphatase were performed according to standard methods.

7 Estimation of Protein

Proteins were estimated by following the method of Lowry et al using bovine serum albumin as the standard.

8 Phospholipid and Sterol Estimation of Mitochondrial Membrane

Lipids were extracted and washed according to Folch et al. Lipid phosphorus was determined by the procedure of Bartlett et al. and total sterol was measured by following the method of Stadtman.

RESULTS

1 Effect of Malathion on Seedling Growth and Mitochondrial Respiration

Malathion at concentrations up to 50 ppm, elicited some stimulatory effects on seedling growth and mitochondrial respiration of roots of 72 hr germinating seeds of *Vigna sinensis* (L) (Figure 1). Above 50 ppm malathion treatment both the seedling growth and the respiratory rate of root mitochondria decreased significantly in a dose-dependent manner (Figure 1).
2 Effect of Malathion on Mitochondrial Swelling and Contraction of Vigna sinensis (L)

From Figure 2, it is evident that treatment with malathion changes the swelling and contraction properties of mitochondria isolated from the roots of 72 hr germinating seeds of *Vigna sinensis* (L). After 20 minutes the swelling was found to be completed in the control but in the malathion-treated conditions (above 50 ppm) the swelling time was shifted to 25 minutes.

Figure 2 indicates, the changes in the contraction pattern of mitochondria in the roots of both control and malathion-treated seeds. The contraction property of root mitochondria was found to be significantly retarded under malathion-treated (about 50 ppm) conditions.

3 Rate of NAD Release from the Root Mitochondria of Vigna sinensis (L) under Malathion Exposure

It is evident from Table 1 that mitochondria of the roots of *Vigna sinensis* (L) lose NAD significantly from the inter-mitochondrial pool when treated with malathion (above 50 ppm). Fifty ppm malathion treatment of the seeds does not bring about significant changes on the leakage of NAD from mitochondria as compared to that of the control conditions (Table 1).
FIGURE 2: Swelling and contraction characteristics of mitochondria in the roots of 72-hour germinating seeds of *Vigna sinensis* (L.)

Each point indicates the average of six individual experiments compared to the control data. 

- Control condition (without malathion treatment)
- Malathion (50 ppm)
- Malathion (100 ppm)
- Malathion (200 ppm)
- Malathion (400 ppm)
Rate of NAD release from mitochondria under malathion-treated conditions in the roots of 72 hour germinating seeds of *Vigna unisins* (L).

<table>
<thead>
<tr>
<th>Condition</th>
<th>Released into supernatant (mg mol/mg protein)</th>
<th>Retained by mitochondria (mg mol/mg protein)</th>
<th>Released into supernatant (%)</th>
<th>Retained by mitochondria (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.6 ± 0.11</td>
<td>5.6 ± 0.14</td>
<td>22</td>
<td>78</td>
</tr>
<tr>
<td>+50 ppm malathion</td>
<td>1.7 ± 0.12</td>
<td>5.5 ± 0.16</td>
<td>24</td>
<td>76</td>
</tr>
<tr>
<td>+100 ppm malathion</td>
<td>2.8 ± 0.12ab</td>
<td>4.4 ± 0.11ab</td>
<td>39</td>
<td>61</td>
</tr>
<tr>
<td>+200 ppm malathion</td>
<td>3.8 ± 0.14b</td>
<td>3.4 ± 0.12b</td>
<td>53</td>
<td>47</td>
</tr>
<tr>
<td>+400 ppm malathion</td>
<td>4.8 ± 0.16bc</td>
<td>2.4 ± 0.11bc</td>
<td>67</td>
<td>33</td>
</tr>
</tbody>
</table>

Results have been expressed as the mean ± SD of six sets of experiments. *Experimental details were given in the text. 

Results have been expressed as the mean ± SD of six sets of experiments.

**TABLE II**

Effect of oligomycin on the mitochondrial membrane bound Na⁺, K⁺, Mg²⁺-ATPase activity of *Vigna unisins* (L).

<table>
<thead>
<tr>
<th>Concentration of oligomycin in the reaction mixture</th>
<th>Specific activity of Na⁺, K⁺, Mg²⁺-ATPase activity (mU/mg protein/hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>3.65 ± 0.26</td>
</tr>
<tr>
<td>0.005 M</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>0.05 M</td>
<td>ND</td>
</tr>
<tr>
<td>0.5 M</td>
<td>ND</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD of four sets of experiments.

---

**TABLE III**

Effect of malathion on the mitochondrial membrane phospholipid and sterol contents in the roots of 72 hour germinating seeds of *Vigna unisins* (L).

<table>
<thead>
<tr>
<th>Condition</th>
<th>mg of phospholipid</th>
<th>mg of sterol</th>
<th>Sterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg of protein</td>
<td>mg of protein</td>
<td>phospholipid</td>
</tr>
<tr>
<td>Control</td>
<td>0.247 ± 0.012</td>
<td>0.019 ± 0.0014</td>
<td>0.202</td>
</tr>
<tr>
<td>+50 ppm malathion</td>
<td>0.231 ± 0.010</td>
<td>0.047 ± 0.0016</td>
<td>0.203</td>
</tr>
<tr>
<td>+100 ppm malathion</td>
<td>0.205 ± 0.014a</td>
<td>0.044 ± 0.0016a</td>
<td>0.213</td>
</tr>
<tr>
<td>+200 ppm malathion</td>
<td>0.136 ± 0.016b</td>
<td>0.040 ± 0.0012b</td>
<td>0.215</td>
</tr>
<tr>
<td>+400 ppm malathion</td>
<td>0.174 ± 0.013c</td>
<td>0.038 ± 0.0012c</td>
<td>0.218</td>
</tr>
</tbody>
</table>

Results have been expressed as the mean ± SD of six sets of experiments.

---

4 Characterization of Mitochondrial Membrane

Results presented in Table II indicate that mitochondrial membrane-bound ATPase is very much sensitive to oligomycin. It is known that oligomycin is a potent inhibitor of mitochondrial ATPase. Again, the sterol to phospholipid ratio of the mitochondrial membrane was determined to be 0.20. From the data presented in Table III, the sterol to phospholipid ratio of the mitochondrial membrane was determined to be 0.20. Taken together, these results lead us to the conclusion that the studied fraction is enriched with mitochondrial membranes.
5  **Effect of Malathion on Mitochondrial Phospholipid and Sterol contents**

It is evident from Table III that above 50 ppm malathion treatment of the seeds, the mitochondrial membrane isolated from the roots of 72 hr germinating seeds of *Vigna sinensis* (L) showed altered levels of phospholipid and sterol contents. The change was marked at 400 ppm malathion treatment of seeds (Table III).

6  **Effect of Malathion on Mitochondrial Membrane Bound ATPase and Pyrophosphatase Activities in the Roots of 72 Hour Germinating Seeds of *Vigna sinensis* (L)**

The activities of the mitochondrial membrane-bound ATPase and pyrophosphatase activities in the roots of 72 hour germinating seeds of *Vigna sinensis* (L) were found to be significantly stimulated when the seeds were treated with malathion, above 50 ppm in a dose-dependent manner (Table IV).

### TABLE IV

<table>
<thead>
<tr>
<th>Condition</th>
<th>Specific activity of Na⁺, K⁺, Mg²⁺-ATPase</th>
<th>Specific activity of acid pyrophosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.65 ± 0.12</td>
<td>25.92 ± 0.57</td>
</tr>
<tr>
<td>+ 50 ppm malathion</td>
<td>3.82 ± 0.21</td>
<td>26.64 ± 0.71</td>
</tr>
<tr>
<td>+ 100 ppm malathion</td>
<td>4.26 ± 0.28a</td>
<td>29.35 ± 0.74a</td>
</tr>
<tr>
<td>+ 200 ppm malathion</td>
<td>5.26 ± 0.24b</td>
<td>32.48 ± 0.85b</td>
</tr>
<tr>
<td>+ 400 ppm malathion</td>
<td>6.84 ± 0.36a</td>
<td>36.85 ± 0.92a</td>
</tr>
</tbody>
</table>

Results have been expressed as the mean ± SD of six sets experiments.

* Specific activity is expressed as μmol P/mg protein/hour

"p < 0.001, highly significant. All other values are not significant.

**DISCUSSION**

This study indicates that malathion has some effect on seedling growth accompanied with alteration of mitochondrial respiration in the roots of the germinating seeds of *Vigna sinensis* (L) (Figure 1). The changes in seedling growth and respiration rate under malathion treatments were found to proceed in a dose-dependent manner. This study agrees with other reports indicating that the altered respiratory rate exerts a profound influence on the subsequent seedling development by some pesticides and toxins. 22-24

The results presented in Figure 2 indicate that mitochondria isolated from the roots of the germinating seeds of *Vigna sinensis* (L), above 50 ppm malathion treatment, elicited change in swelling characteristics (Figure 2) along with a significant loss of NAD from the intra-mitochondrial pool (Table I). These studies indicate a probable internal disorganization in the mitochondria of the roots of *Vigna sinensis* (L) under malathion-treated conditions (above 50 ppm). Poor mitochondrial activity under malathion exposure is also reflected by the altered swelling and contraction properties (Figure 2) and in the enhanced leakage of NAD from the mitochondria (Table I).

The reduced growth characteristics and respiration rate (Figure 1) of the roots by malathion treatment were also accompanied by some changes in the mitochondrial...
membrane bound ATPase (Table IV), whose activity represents a release of energy from ATP for various cellular functions. Acid pyrophosphatase also plays an important role in energy metabolism during germination. These altered mitochondrial membrane-bound enzyme activities showed a reflection of the process of active transport of metabolites and inorganic ions, as well as those processes that convert metabolic energy into mechanical energy.25,26

Malathion caused a change in the phospholipid to protein and also sterol to protein ratios (Table III). It is known that phospholipids are involved in maintaining the structural integrity of mitochondrial membranes.27 Judah et al.28 have found a characteristic change in the phospholipid content during the different phases of swelling and contraction of mitochondria. Masotti et al.27 observed that an alteration in the phospholipid pattern also indicates some change in the conformation of the mitochondrial membrane. Cholesterol has recently been shown to influence the permeability of phospholipid bilayer membranes by modifying the fluidity of the hydrophobic core of cellular and organelle membranes.29 Since malathion and its metabolites are mainly hydrophobic compounds, they can alter the constituents of biomembranes and their fluidity.30 A shift in the sterol to phospholipid ratios and ATPase and pyrophosphatase activities of mitochondrial membrane in the roots of the germinating seeds of Vigna unguicus (L) by malathion exposures could be due to some alteration in the mitochondrial membrane.

CONCLUSION

1) The seedling growth pattern of Vigna unguicus (L) and the respiration rate of root mitochondria of the 72-hour germinating seeds, above 50 ppm malathion exposure, decreased significantly.

2) Malathion (above 50 ppm) elicited a change in swelling and contraction properties of mitochondria along with a marked loss of NAD from the intramitochondrial pool in the roots of the germinating seeds in a dose-dependent manner.

3) Mitochondrial membrane-bound Na+, K+, Mg2+-ATPase and acid pyrophosphatase activities, above 50 ppm malathion exposures, were stimulated significantly in a dose-dependent manner.

Malathion (above 50 ppm) also caused change in phospholipid to protein and sterol to protein ratios.

Taken together, these results lead us to the conclusion that the studied mitochondrial responses elicited by malathion (above 50 ppm) could be due to some alteration in the mitochondrial membrane.

ACKNOWLEDGEMENT

The authors thank Professor A. N. Bhaduri and Dr. (Mrs.) Manya Ray (Department of Pharmacy, Jadavpur University) for their help in this work.

REFERENCES


MALA PI UON EXPOSURE


CARBARYL TOXICITY IN GERMINATING SEEDS OF VIGNA SINENSIS:
EFFECT OF GIBBERELLIC ACID SUPPLEMENTATION

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Department of Biochemistry, University College of Science, 5, Bulliagang, Calcutta, India

ABSTRACT

The toxic effects of carbaryl (a carbamate insecticide) on the activities of different hydrolases of germinating Vigna sinensis seeds have been investigated. The activities of amylase and phytase from cotyledons decreased on exposure to carbaryl. But the ATPase activity extracted from seedlings was stimulated following pesticide application. Gibberellic acid, on simultaneous application with toxic doses of carbaryl, overcame the growth inhibition caused by low concentration of carbaryl. But at higher concentrations of pesticide treatment, it can only partially reverse the deleterious effects of carbaryl on growth and the levels of different hydrolases.

INTRODUCTION

Carbaryl (N-bapthyl methyl carbamate), a widely used carbamate insecticide, can inhibit plant growth and cellular respiration. It also affects the phenolics and sugars in bean plants. Our earlier studies show that carbaryl at toxic concentrations caused poor mobilization of protein and faster depletion of nucleic acids in germinating Vigna sinensis seedlings. The activities of hydrolases of germinating seeds were affected by organochlorine and organophosphorus insecticides. The present communication deals with the toxic effects of carbaryl on some hydrolases of germinating V. sinensis seeds (3-day-old). It was also investigated whether gibberellic acid (GA₃), a plant growth hormone, could play any role in removing the toxic influences of carbaryl on the growth and the activities of the hydrolytic enzymes.

MATERIALS AND METHODS

Tris-ATP, sodium phytate, bovine serum albumin, and GA₃ were purchased from Sigma Chemicals, USA. Carbaryl (99.9%) were obtained as gift from Union Carbide, India. All other chemicals were of analytical grade. V. sinensis seeds were purchased locally.

After surface sterilization with 0.1% mercuric chloride, V. sinensis seeds were imbibed in water for 4 h. The seeds were then allowed to germinate in dark at 28°C in several plates for 3 days. Experimental plates contained carbaryl alone (at concentrations 75, 100 and 150 ppm respectively) and in combination with GA₃ (10⁻⁶ M).

After three days of germination the seedling height, fresh weight and specific activities of different hydrolases were measured. The total amylase was extracted by homogenizing the cotyledons in unbuffered cold distilled water. The homogenate was centrifuged at 2000 g for 15 min and the supernatant dialysed against cold water. Total amylase activity was assayed from the dialysed supernatant according to the method of Bernfeld. ATPase was extracted from seedling 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 and from the supernatant enzyme activity was assayed in a reaction mixture (1 ml) containing 20 mM Tris-CI (pH 7.5), 3 mM ATP and enzyme. P released was determined by the method of Lowry and Lopez. Phytase activity was measured from the cotyledons of the 3-day-old V. sinensis seeds. Cotyledons were homogenized with 10 mM Tris-CI buffer (pH 7.0) and centrifuged at 5000 g for 15 min. The supernatant was assayed for phytase activity. Protein was estimated using bovine serum albumin as standard.

RESULTS AND DISCUSSION

It was observed that both the elongation and the fresh weight of V. sinensis seedling (3-day-old) were inhibited on exposure to different concentrations of carbaryl (table 1). GA₃, when applied in combination with carbaryl, was able to overcome the growth inhibition completely at 75 ppm of carbaryl treatment. But above this concentration of pesticide, the plant hormone was only partially successful in reversing the toxic effects of carbaryl on seedling growth. It has been reported by Chakraborti et al. that different plant hormones...
Table 1 Effect of different concentrations of carbaryl alone and in combination with GA, on the growth and the specific activities of different hydrolases of germinating Vigna sinensis seeds (3-day-old)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Seedling height (cm)</th>
<th>Fresh weight of seedling (mg)</th>
<th>Total amylase (µg maltose/µg protein/h)</th>
<th>ATPase (µmol/mg protein/h)</th>
<th>Phytase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.22 ± 0.43</td>
<td>730 ± 35</td>
<td>5.13 ± 0.27</td>
<td>2.79 ± 0.14</td>
<td>0.223 ± 0.011</td>
</tr>
<tr>
<td>+ 75 ppm carbaryl</td>
<td>5.04 ± 0.36*</td>
<td>610 ± 32***</td>
<td>3.31 ± 0.18*</td>
<td>3.97 ± 0.18*</td>
<td>0.220 ± 0.010</td>
</tr>
<tr>
<td>+ 100 ppm carbaryl</td>
<td>3.83 ± 0.28*</td>
<td>445 ± 24*</td>
<td>2.21 ± 0.14*</td>
<td>4.82 ± 0.28*</td>
<td>0.218 ± 0.013</td>
</tr>
<tr>
<td>+ 150 ppm carbaryl</td>
<td>3.32 ± 0.22*</td>
<td>426 ± 27**</td>
<td>1.95 ± 0.11*</td>
<td>6.46 ± 0.34*</td>
<td>0.194 ± 0.010***</td>
</tr>
<tr>
<td>+ 75 ppm carbaryl + 10^{-6} M GA₃</td>
<td>8.07 ± 0.41**</td>
<td>727 ± 25</td>
<td>4.12 ± 0.20**</td>
<td>3.38 ± 0.17***</td>
<td>0.219 ± 0.016</td>
</tr>
<tr>
<td>+ 100 ppm carbaryl + 10^{-6} M GA₃</td>
<td>4.97 ± 0.29*</td>
<td>489 ± 28*</td>
<td>2.85 ± 0.16*</td>
<td>4.52 ± 0.32*</td>
<td>0.216 ± 0.015</td>
</tr>
<tr>
<td>+ 150 ppm carbaryl + 10^{-6} M GA₃</td>
<td>3.82 ± 0.38*</td>
<td>458 ± 20*</td>
<td>2.43 ± 0.11*</td>
<td>5.84 ± 0.39</td>
<td>0.192 ± 0.011***</td>
</tr>
</tbody>
</table>

Values are mean ± S.D of four sets of experiments, *denotes the level of significance \( P < 0.001, \*\*\*P < 0.05, \*\*\*\*P < 0.01\).  

The activity of total amylase was significantly inhibited in exposure to toxic doses of carbaryl indicating that the breakdown of starch to readily utilisable sugar which is essential for the growth of seedling during germination, is greatly impaired due to the inhibition of amylase activity by carbaryl. So it can be seen that malathion (an organophosphorus pesticide) on seedling growth of germinating V. sinensis. They observed that at low concentration of malathion (100 ppm) treatment, plant hormones effectively overcome the toxic effect of pesticide on the growth. But at higher concentrations of the pesticide, the plant hormones are partly successful in reversing the malathion-induced growth inhibition. Table 1 shows that the activity of total amylase was significantly inhibited at lower concentration (100 ppm) of carbaryl treatment. Inhibition of phytase activity in squash cotyledons by anilide herbicide propachlor was reported by Penner. It is also observed that GA₃ at 10^{-6} M can partially counteract the effects of carbaryl on the enzyme systems studied. The toxic effects of carbaryl on the activities of amylase and ATPase were reversed to certain extent when GA₃ was supplemented with the pesticide. However, GA₃ at the applied concentration, failed to counteract the pesticide-induced inhibition of phytase activity. Reports are available that plant hormones can partly or completely counteract the inhibitory effects of pesticides on the levels of different hydrolases. It was also observed that GA₃ was able to overcome the growth-inhibition caused by 75 ppm carbaryl treatment. But it failed to restore the normal activities of amylase and ATPase in germinating V. sinensis seeds at that concentration of pesticide. GA₃ probably reverses this growth inhibition at 75 ppm by stimulating some other biochemical processes leading to increased cellular growth.

From the foregoing discussion it is apparent that carbaryl at toxic concentration interacted with the normal metabolism of the germinating seed and the effect could somewhat be nullified by using GA₃ along with the pesticide.

13 July 1987

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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. The limiting enzyme of the reduction process is considered to be nitrate reductase. The principal route of ammonia assimilation was previously thought to be via glutamate dehydrogenase, but ample evidence 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. 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. 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, Barasat, 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 containing 30 mM KN03. 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 content. 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, Barasat, 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 containing 30 mM KN03. 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 content.
and soluble protein. Nitrate reductase was measured following the method of Evans and Nason as modified by Hageman and Flesher. Glutamate dehydrogenase was estimated by the method of King. Soluble protein was determined by the method of Lowry et al. after precipitation with 50% TCA. The nitrite content was also determined. 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.

**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 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. Glyphosate (an organophosphate herbicide) and chlorthalonil (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. Rhodes et al. 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

<table>
<thead>
<tr>
<th>Malathion added (ppm)</th>
<th>Shoot length (cm)</th>
<th>Fresh weight of shoot (mg)</th>
<th>Soluble protein (mg/g fresh tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.17 ± 0.28</td>
<td>35.77 ± 1.7</td>
<td>20.60 ± 1.07</td>
</tr>
<tr>
<td>50</td>
<td>4.95 ± 0.17</td>
<td>39.40 ± 1.9**</td>
<td>20.19 ± 1.05</td>
</tr>
<tr>
<td>100</td>
<td>4.60 ± 0.26††</td>
<td>31.17 ± 3.5**</td>
<td>23.75 ± 1.18††</td>
</tr>
<tr>
<td>200</td>
<td>3.98 ± 0.13*</td>
<td>22.77 ± 1.2*</td>
<td>25.32 ± 1.26**</td>
</tr>
<tr>
<td>400</td>
<td>3.14 ± 0.20*</td>
<td>15.35 ± 0.08*</td>
<td>30.35 ± 1.51*</td>
</tr>
</tbody>
</table>

Values are mean ± SD of four sets of experiments, *P < 0.001, †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

<table>
<thead>
<tr>
<th>Malathion added (ppm)</th>
<th>Nitrate reductase (umoles/mg protein/h)</th>
<th>Nitrite (mg/g fresh tissue)</th>
<th>Glutamine synthetase (nmol/mg protein/h)</th>
<th>Glutamate dehydrogenase (umoles/mg protein/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.99 ± 0.03</td>
<td>15.6 ± 0.82</td>
<td>1.45 ± 0.06</td>
<td>0.105 ± 0.003</td>
</tr>
<tr>
<td>50</td>
<td>4.31 ± 0.21*</td>
<td>25.5 ± 1.25*</td>
<td>2.73 ± 0.11*</td>
<td>0.175 ± 0.003</td>
</tr>
<tr>
<td>100</td>
<td>4.93 ± 0.28*</td>
<td>27.8 ± 1.79*</td>
<td>3.41 ± 0.14*</td>
<td>0.220 ± 0.004*</td>
</tr>
<tr>
<td>200</td>
<td>6.13 ± 0.41*</td>
<td>51.5 ± 2.45*</td>
<td>4.85 ± 0.25*</td>
<td>0.315 ± 0.006*</td>
</tr>
<tr>
<td>400</td>
<td>7.33 ± 0.56*</td>
<td>53.3 ± 2.15*</td>
<td>3.57 ± 0.16*</td>
<td>0.619 ± 0.024*</td>
</tr>
</tbody>
</table>

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

6 June 1988, Revised 22 September 1988

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