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Role of Iron and Zinc in Mitigating Phytotoxic Effects of Pesticide Malathion on Moong Plants (*Phaseolus radiatus*)

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Abstract
Comprehensive research and studies are going on to find out effect and degree of toxicity of pesticides and alternative approaches to minimize the toxic effects of pesticides. The present experiment was hence conducted to study the toxicity of pesticide malathion on growth and metabolic activities of an important pulse crop moong (*Phaseolus radiatus*) and role of micronutrients iron and zinc on this toxicity.

Root length, Shoot length and Germination percentage of moong plants were found to be decreased with increasing doses of pesticide. Decrease in root growth and shoot growth were upto 41% and 79% respectively and germination percentage was recorded as low as 59% at 5% dose of pesticide. When micronutrients, 5.6ppm Fe or 15ppm Zn, were supplied with 2% malathion on moong plants they both showed resistance to the ill effects of pesticides. Decrease in root growth was as low as 1.84% when Fe was supplied along with 2% malathion, while germination percentage was increased when moong seeds were soaked in combined dose of 2% pesticide with Zn.

Chlorophyll, protein content and catalase activity decreased while peroxidase activity increased significantly with increasing doses of malathion. Protein content at higher doses viz. 2% and 5% was 49.11 mg./gm.f.wt. and 21.78 mg./gm.f.wt. as compared to 115.65 mg./gm.f.wt. in control. Both Fe and Zn supplemented with 2% basal dose negated the ill effects of pesticide on protein content significantly. Supplementation of Fe also enhanced the chlorophyll content significantly. Catalase and peroxidase activities were found to increased due to supplementation of Fe whereas decrease in these parameters were observed due to supplementation of Zn with 2% pesticide. Thus it can be inferred that higher dose of malathion can be applied on moong plants if supplemented with 5.6ppm Fe without compromising the plant growth and its antioxidant potential.

Keywords: Malathion, Pesticide, Toxic, Growth, Metabolism, Iron, Zinc.

1. Introduction
Pesticides are substances intended to harm or kill living organisms considered as pests. To control heavy loss in food & other crops caused by pest species during different level (growth, harvest & storage), doses of synthetic pesticides are prescribed. Unfortunately, however, chemical applications in agriculture do not always do just what they are supposed to do. Moreover residual toxic effects, including other effects of many fungicides, herbicides and insecticides on germination & seedling growth have been reported. These concerns include the acute and chronic toxicity of many insecticides to humans, domestic animals and wild life; their phytotoxicity to plants; the development of new pests species after extensive pesticide use; the development of resistance of these chemicals by pest; the persistence of many insecticide in soils and water; and their capacity for global transport and environmental contamination (Edwards 2000) [3].

To a large extent, pesticide residues on plants are the result of direct application or the result of absorption from soil, especially in case of root vegetables. Many of the synthetic pesticides previously used for insect control have now been banned or their use seriously curtailed due to their negative impact on health & environment. The production, trade, use, and release of many synthetic chemicals is now widely recognized as a global threat to the health of human, other living species and the environment as well.

Demands for fruits and vegetables that are attractive and have no blemishes increased manifold, however pressure by consumers for "clean" and uncontaminated foods is also mounted up. This problem is recognized more & more now & large precautions are being taken to avoid unwanted side effects from these chemicals. Comprehensive research and studies are going on to find out effects and degree of toxicity of pesticides and alternative approaches to minimize the toxic effects of pesticides.

The present experiment was hence conducted to study the toxicity of pesticide malathion on growth and metabolic activities of an important pulse crop moong (*Phaseolus radiatus*) and role of micronutrients iron and zinc on this toxicity.

2. Materials and Methods

Petridish experiments were carried out to study the effect of different doses of pesticide on growth & metabolism of Moong plants. 'MALATHION' of organophosphate group was used as chemical pesticide. Its interactive effects with Iron and Zinc were also studied.

Seeds were soaked in controlled nutrient solution and the respective test solution in petriplates with the filter papers liberally moistened with the solution in which the seeds were soaked. Total 22 seeds were soaked. The nutrient solution were again changed after 12 hours & then after 24 hours. No. of seeds germinated were counted for calculation of germination %. Six doses were supplied in the concentration of 0.5%, 1%, 2%, 5% pesticide and 2% pesticide + 5.6ppm Fe and 2% pesticide + 15ppm Zn. Controlled solution was also supplied.

5 ml of basal nutrient solution was supplied to control and 3 ml each of different concentration of pesticide + 2 ml of nutrient solution was supplied in respective petriplate. In case of combined dose of 2% pesticide with either 5.6ppm Fe or 15ppm Zn, pesticide and Fe/Zn was supplied in the ratio 3:2.

a) Growth of selected plants: Growth parameters observed included root length, shoot length & seed germination.
b) Metabolic parameters: Metabolic parameters such as concentration of chlorophyll, protein and activities of enzymes like catalase and peroxidase were analysed.

Root and shoot length of 3 plants in each petriplate was measured. Observations were taken on alternate days. Total five readings were taken. Average root & shoot length was calculated replicate wise and then treatment wise. Percentage increase/decrease in growth in comparison to control was calculated after final readings.

Chlorophyll was measured by the method of Petering *et al*. (1940) [14]. Protein estimation was done by the method of Lowery *et al*. (1951) [10]. Catalase and peroxidase activity were assayed by the method of Euler & Josephsen (1927) [4] and Luck (1963) [11] respectively.

3. Results and Discussion

Root and shoot length of moong plants were found to be decreased with increasing doses of pesticide and upto 80% decrease in shoot growth was observed at 5% dose of pesticide (Table-1). Resistance to decrease in plant growth was observed when 2% malathion was supplied in combination with 5.6ppm Fe or 15ppm Zn. Fe proved to be more beneficial than Zn in resisting the decrease in plant growth. Percent decrease in root growth was as low as 1.84% at combined dose of 2% malathion + 5.6ppm Fe against a decrease of 27% at 2% malathion dose alone and was better than a decrease of 11% at 1% dose of malathion alone. Also, decrease in shoot growth was only 36% at combined dose of 2% malathion + 5.6ppm Fe against a decrease of 53% at 2% malathion dose alone and was at par with a decrease of 35% at 1% dose of malathion alone (Table-1). Methyl parathion treatment also caused reduction in seedling growth (Thirumaran and Xavier 1987) [20]. Fayez and Kristen (1996) [5] found that low concentration herbicides chlorsulfuron, norflurazon and triallate on *Pisum sativum* and *Vicia faba* inhibited root growth.

Germination percentage also decreased with increasing doses of malathion with germination percentage recorded as low as 59.09% at the highest dose viz. 5% as compared to 95.45% in control (Table-1). Chandrashekhar and Kaveriappa (1994) [2] also found that higher concentration of Malathion & some other pesticides have shown effects on sporulation and germination of aquatic hyphomycetes. Pesticide residue was found to be increased by more than 200% when higher concentration of endosulfan was applied to tomato plants (Sinha *et al*. 2016) [18]. Germination percentage was increased when moong seeds were soaked in combined dose of 2% pesticide with 15ppm Zn.

Chlorophyll content decreased with increasing doses of malathion. 5.6ppm Fe supplied with 2% pesticide enhanced the chlorophyll content significantly, whereas 15ppm Zn supplied instead of 5.6ppm Fe with 2% malathion failed to show any significant increase in chlorophyll content (Table-2) Fayez (2000) [6] found herbicide diuron to be phytotoxic on chlorophyll content of soybean plants. Clomazone application also caused reduction in chlorophyll (a and b) in primary barley (Kana *et al*. 2004) [7]. Reduction in chlorophyll content is probably due to oxidative degradation of chlorophyll and rapid destruction of photosynthetic membranes by Reactive Oxygen Species (ROS) generated from functionally active photosynthetic electron transport (Boger 1996; Kim *et al*. 2001; Kim *et al*. 2004) [1, 8, 9].

Malathion was quite toxic on protein content of moong plants. Protein content at highest dose viz. 5% was 21.78 mg./gm.f.wt. as compared to 115.65 mg./gm.f.wt. in control (Table-2). However 5.6ppm Fe or 15ppm Zn supplemented with 2% basal dose negated the ill effects of pesticide significantly. Protein content was found to be 80.79% mg./gm.f.wt. and 68.12% at 2% malathion + 5.6ppm FE and 2% malathion + 15ppm Zn respectively against 49.11 mg./gm.f.wt.at 2% pesticide dose alone. Singh *et al*. (1984) [16] observed inhibition of total leaf protein in pea due to triazole, metrization or pyrazon herbicides. Thirumaran and Xavier (1987) [20] observed initial increase in protein content but later there was sharp decline due to methyl parathion on black gram seedlings.

Catalase activity decreased significantly with increasing doses of malathion. Even at a low dose of 0.5% pesticide, catalase activity was recorded as 27.50 µmoles H_2O_2 split/100 mg.f.wt. as compared to 37.50 µ moles H_2O_2 split/100 mg.f.wt. in control in moong plants (Table-2). However a combined dose of 2% malathion + 5.6ppm Fe slightly enhanced the enzyme catalase. 15ppm Zn with 2% pesticide showed additional decreasing effect. Catalase activity was found to be significantly descended in tomato plants under dimethoate and cypermethrin stress in comarision to control (Sinha and Tandon 2016) [17]. Vernet and Teisseire (2000) [21] observed that catalase was strongly inhibited by herbicide diuron from lowest concentration used (25 μgL^{-1}) and the shortest duration of exposure (6 hrs).

Peroxidase activity increased significantly with increasing doses of malathion (Table-2). Increase in peroxidase activity at high concentration of some organophosphorous insecticides was also observed by Srivastava *et al*. (1972) [19]. An increase in peroxidase activity commonly occurs in aging plant tissues, partly as a factor responsible for scavenging hydrogen peroxide (Merzlyak and Hendry 1994) [12]. 5.6ppm Fe when supplied with 2% pesticide showed additional increasing effect. However when 15ppm Zn was supplied instead of Fe it decreased the peroxidase activity (Table-2). Romheld and Marschner (1981) [15] found some role of Fe in peroxidase activity. Nanawati (1973) [13] also showed increase in peroxidase activity with supplementation of iron.

Table 1: Effect of malathion on root length, shoot length and germination% of moong plants (*Phaseolus radiatus*).

Treatment	Root Length (cm)	Growth achieved (cm)	Shoot Length (cm)	Growth achieved (cm)	Germination %
Control	4.13 ± 0.11	5.43 (0.00)	2.28 ± 0.06	3.27 (0.00)	95.45
0.50%	3.88 ± 0.14	5.15 (-5.16)	2.07 ± 0.04	2.72 (-16.82)	88.64
1%	3.56 ± 0.09	4.82 (-11.23)	1.83 ± 0.04	2.10 (-35.78)	81.82
2%	2.98 ± 0.10	3.96 (-27.07)	1.48 ± 0.06	1.53 (-53.21)	70.45
2% + Fe	3.95 ± 0.16	5.33 (-1.84)	1.77 ± 0.04	2.08 (-36.39)	72.73
2% + Zn	3.51 ± 0.11	4.73 (-12.89)	1.60 ± 0.09	1.92 (-41.28)	77.27
5%	2.30 ± 0.14	3.17 (-41.62)	0.76 ± 0.04	0.67 (-79.51)	59.09
CD Value at 5%	0.1066		0.0786		1.8577

Values are given as average value of 5 dates ± SM. Values in bracket under growth achieved column reflects % increase or % decrease in growth achieved with respect to Control

Table 2: Effect of malathion on chlorophyll content, protein content, catalase activity and peroxidase activity of moong plant (*Phaseolus radiatus*).

Treatment	Chlorophyll Content	Protein Content	Catalase Activity	Peroxidase Activity
	(mg./gm.f.wt.)	(mg./gm.f.wt.)	(μ mole H2O2 split / 100 mg f.wt.)	(Δ O.D./100 mg f.wt.)
Control	1.8901 ± 0.0046	115.6539 ± 0.7922	37.50 ± 2.50	0.0535 ± 0.0015
0.50%	1.7663 ± 0.0077	97.4345 ± 2.3765	27.50 ± 2.50	0.0625 ± 0.0025
1%	1.6099 ± 0.0093	83.5718 ± 1.9804	25.00 ± 0.00	0.0720 ± 0.0030
2%	1.4242 ± 0.0124	49.1133 ± 2.3765	20.00 ± 0.00	0.0840 ± 0.0020
2% + Fe	1.6641 ± 0.0077	80.7993 ± 2.3765	22.50 ± 2.50	0.0910 ± 0.0010
2% + Zn	1.4721 ± 0.0108	68.1249 ± 2.3765	17.50 ± 2.50	0.0785 ± 0.0015
5%	1.096 ± 0.0186	21.7841 ± 3.5647	12.50 ± 2.50	0.1065 ± 0.0035
CD Value at 5%	0.0184	3.3671	5.0375	0.0037

Values are given as ± SM.

4. Conclusion

Results showed that malathion caused reduction in plant growth, chlorophyll and protein content of moong plants. Pesticide induced changes in both the antioxidant enzymes was also found. Ill effect of pesticide caused inhibition of catalase whereas peroxidase was increased to work against the adverse effect of pesticide. Results showed beneficial role of iron and zinc when supplemented with pesticide, however iron was found to be more beneficial than zinc. Results indicated that if higher dose viz. 2% of pesticide is supplemented with 5.6ppm Fe then growth and antioxidant potential were at par to the effect at 1% pesticide dose alone in moong plants. Thus it can be inferred that higher dose of malathion can be applied on moong plants if supplemented with 5.6ppm Fe without compromising the plant growth and its antioxidant potential.

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