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Wheat (Triticum aestivum L.) : A study on Efficacy of Malathion on Germination of Seed and Photosynthetic Pigments

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Abstract : Malathion is an important member of synthetic pesticide belonging to organophosphate class. It is still widely used worldwide as an effective pesticide. The chief aim of this research is to study the efficciancy of different concentration of malathion on germination of seed and to estimate the content of various photosynthetic pigments in wheat. The study was carried out by two separate method in petri-dish and in sterile soil in plastic sweetmeat container. Estimation of photosynthetic pigments were performed by T.L.C. and biospectrophotometer. Percentage germination and shoot length were enhanced at lower concentration (2ppm,-10ppm), but morphogenic response in soil declined significantly with increasing concentration of malathion resulting in much reduced photosynthetic pigments content.

(Keywords : Triticum aestivam L., Malathion, Seed-germination, chlorophyll, xanthophyll, carotenoid, Thin layer chromatography).

Introduction

Malathion is an organophotosphate insecticide[1] which acts as an acetylcholinestirase inhibitor[2-5]. It is commonly known as carbophos, maladison, mereceptonthion[6] etc and chemically known as Diethyl-2 [dimethoxyphos phorothioyl) sulfanyl] btanediate. It is a pesticide wide used in agriculture, residential, landscaping, public recreation areas and in public health pest control programs such as mosquito cradication[7], controlling west Nile virus combating Mediterranean fruit fly[8] treatment of lice[9], treatment of pedicolosis[10], It applied judiciously with proper safety precautions, it can be used without posing unreasonable risks to the flora and fauna. Malathion has low toxicity; however, absorption or, ingestion into the human body readily results in its metabolism to malaoxon, which is substantially more toxic (sixty one times more toxic than malathion). It is cleared from the body quickly in three to five days[11].According to the US-EPA there is currently no reliable information on detrimental health effects of chronic exposure to malathion.[12] Acute exposure to extremely high levels of malathion (over 300 ppm) will cuse bodywide symptoms, whose intensity depends on the severity of exposure. These symptoms include skin, an eye irritation, cramps, nausea, diarrhea, excessive sweating, sezure and even death[13-14] Pets and domestic animals exposed to malathion also show similar symptoms[15]. Malathion and its metabolites mixed with water may move quickly through soil and are found in surface water like steams, ponds, rivers, tanks and sometimes in ground water, the half life period of Malathion in soil is about 17 days and in water 2-18 days, depending upon temperature and pH. Its vapours may also move long distances in air,particulary moist air fog etc. Cereals are one of the most customary source of carbohydrates and minerals intake amongst Asian population. Wheat is one the most common cereals crops cultivated since olden times in India, with Uttar Pradesh followed by Punjab being the highest producer. It is a carbohydrate and rich staple food containing approximately 23% total carbohydrate content on dry weight basis and has 340 calories per 100g. wheat stalks are also used as cattle's fodder. A few leguminous plants such as Glycine max (soyabean), arachis hypogaea (peanut) and Glycyrrhizan glabra (liquorice) are also cultivated wit wheat Legum plants play key role in enhancing the fertility of soil through nitrogen fixation. Effect of malathion on wheat as well as legums are also studied for understanding their mode of action towards germination of seeds and photosynthetic pigments content.[15-16]

Experimental

2. MATERIALS & METHODS

2.1. Chemical and seeds

Malathion (commercial Formulation) was obtained from Hindustan Insecticide Ltd. Gujrat, India, A.R. grade chemical and other reagens viz calcium carbonate, magnesium sulphate, acetone etc are used. Seeds of triticum aestivum L, were obtained from Rajendra Agriculture University Pusa, Samastipur, Bihar.

2.2 Soil and filter paper

Samples of soil were collected from Gaya College, Gaya grounds most of the organic matters were thoroughly removed and then soil from one inch deep trench were collected Whatsman number-2 filter papers were employed in the present research work.

2.3 Sterilisation

1 kg soil was autoclaved at 125^0C for 45 minutes at 1.05 kg/cm^2 (115 lb psi) pressure for three times every alternate days for three cycles to achieve sterilization. Distilled water, glassware and filter papers were sterilized by autoclaving at 125^0C for 25 minutes at 115 lb psi, Forceps, needle, petri-dishes and plastic sweetmeat containers were cleaned with propanol-2, before using. Wheat seeds were surface sterilized using liquid detergent teepol and then treated thoroughly washing under running tap water for 15 minutes. The seeds were then treated with 1% present citric acid solution, an antioxidant dept in flask and placed on a rotary shaker for 15 minutes at 100 rpm and then thoroughly washed with sterile water subsequently, these seeds were surface sterilized 0.1 % (w/v) mercuric chloride solution for 5 minutes with continuous shaking and were finally washed severals times with sterile water. All other materials such as autoclaved distilled water, tissue paper, lamp etc. excluding seeds were irradiated with ultraviolet light in a Laminar flow cabinet for 30 minutes prior to their use. Surface for Laminar flow cabinet and working platform were cleaned with propanol-2 preceding seeds germination study.

2.4 Test germination filter paper

Sterile Whatman Number-2 filter papers, 10 cm circumference were placed on sterile petri discs, 9 cm in diameter and covered by sterile petri plate, 10 cm diameter, Filter papers were moistened with sterile distilled water and with different concentration stock malathion solution (2ppm-100ppm), along with blank (without pesticide). 10 seeds were placed on moist filter paper at equal distance, I ml sterile distilled water was added in each petri disc everyday at 8 AM to maintain moisture. The experiment was carried out in the replicates of 10 seeds in each concentrations of pesticide. Other conditions in every petri disc remained identical. Three seeding were observed each day and measured after six days.

2.5 Test germination of soil

Sweetmeat plastic containers with 8.0 cm diameter are filled with 100g of sterilized soil. Now stock solutions of different concentrations (from 2ppm to 100 ppm of pesticide) were mixed with soils in separate container were mixed thoroughly on clean polythene sheet with control having nil pesticide. Pesticide stock solutions were prepared in acetone. Bottom and sides of the plastic container were performed in order to facilitate the passage of air and to drain out excess water. With the help of sterilized forceps, ten seeds per container were planted at one cm depth and at equal distances. After the seeds germinated ten ml of sterilized distilled water was added with the help of a pipette a batch of ten plastic containers of separate concentration were placed at room temperature in an air conditioned room under natural sun light. Two ml of sterilized distilled water was dispensed with the help of pipette in each container everyday in the evening 06:00 pm to maintained proper moisture in soil. After the passage of six days, plastic containers were removed from the air conditioned room and various parameter governing growth of seedling viz. morphogenesis, rhizogenesis, surface area of leaves etc. were measured and determined.

2.6 Analytical study

2.6.1 Growth of seeding

On the sixth days after planting of seeds experiment observations were recorded and thereafter the in vivo shoots were excised and preserved at 4^0C for further study such as quantitaitive estimation of photosynthetic pigments and other enzymes. Five types of experimental data were recorded namely.

(a) Percentage of seed germination.
(b) Average shoot length in cm.
(c) Average root length in cm.
(d) Average number of roots per shoot.
(e) Average number of leaves

2.6.2 Estimation of Photosynthetic pigments

Chlorophyll 'a' and chlorophyll 'b' were estimated by using little modified Holden protocol (1960). For this 0.2 g seedling samples from every concentration were weighed and homogenized with the help of a small electric grinder in excess of aqueous acetone (30% 70%) till the complete extraction of green pigment from the tissue. Small quantity of calcium carbonate was added to prevent undesirable formation of phenophytin. The extract was centrifuged at high speed (5000 rpm) for ten minutes at room temperature and the superannuate liquid was removed and its volume was made up to ten ml with aqueous acetone. The experimental test tubes were thoroughly wrapped with opaque black paper to prevent photodegradaton of chlorophyll. The biospectrophotometer (Colvistic) was adjusted at 665 nm, 663 nm, 486 nm, 480 nm 434 nm for phenophytin 'a', chlorophyll 'a' chlorophyll 'b', xanthophylls carotenoid and phenophytin 'b' respectively. The optical density were also measured and amount of chlorophyll 'a' and chlorophyll 'b' were calculated with the help of optical density and standard formula[19]. For used total chlorophyll the algebraic sum of amount of chlorophyll 'a' and chlorophyll 'b' was similarly the amount of carotenoid is equal to the ratio of four times the product of optical density and total volume of sample to the weight of fresh leaves.

2.6.3 Thin layer chromatography

200 mg of fresh green shoot with equal amount of unhydrous magnesium sulphate was ground in a small electric grinder with acetone. The resultant green mass was completely transferred into a microcentrifugre tube with the help of acetone and policeman. The resultant heterogenous mixture was continuously agitated for homogenization. The mixture was allowed heterogenous mixture was continuously agitated for homogenization. The mixture was allowed to stand for approximately 10 minutes and the supernatant green liquid was pipetted out and transferred to a microcentrifuge tube. This was centrifuge at high speed (5000 rpm) for ten minute at room temperature 28^0C.Again one ml of supernatant liquid was collected. Similar protocol were followed with frozen wheat leaves. The TLC plate (20×20 cm^2) were prepared by deposition g uniform layer of 0.25 mm thickness silica gel G using the available silicagel kit. The TLC plates were allowed to dry at room temperature and then activated by heating them at 120^0C for 30 minutes. The acetone extract of fresh green leaves was spotted in the standard manner and the plate was eluted in a closed TLC chamber saturate with various organic solvent (cyclohexane : petroleum ether : acetone : n-hexane : : 5:2:2:1) the chromatogram intensity was observed in normal light and the retention factor was calculated.

2.6.4 Statistical Analysis

All the experimental were repeated nine times and at the start the experimental setup were carried out with three replicas for every method and each concentration for determining the average length of shoots and roots. Average number of secondary roots per shoot have been represented in this article as mean values along with standard error. (mean SE)

3. Results and Discussion

3.1 Effect of Malathion on Wheat's seed on filter paper

Seeds of wheat sere grown on the Whatman Number 2. Filter paper incorporated with 2,5,25,50 and 100-ppm malathion solution. A maximum of 100% germination, with shoot length of 10.5 1 ± 0.7 cm per seedling and root length 6.5 0.4 cm per shoot was observed when filter paper was incorporated with 2% malathion solutions. Percentage germination and shoot length increased significantly as compared to control at lower concentrations (5 ppm and 10 ppm) of malatthion. The morphogenic response with respect to shoot length, root length and germination percentage consideradly diminished with an increasing concentration (2.5 ppm to 100 ppm) of malathion solution. Excess level of malathion (100 ppm and above) were found to be highly toxic, only 80% germination with small shoot length and much thicker rot with 4.65 0.65 em roots per shoot. It was amazing to find that seedling could survive even on this highly contaminated filter paper. Morphogenic responses generally show a determinatal effect of increasing concentrations of malathion on germination of wheat seeds (Table-1)

Table-1

Effect of malathion on the germination of wheat seeds tested by filter paper (Petri dish methods)

Sample	Germination (%)	Shoot length (cm)	Root length (cm)	Roots per shoot
Control	98	10.29 ±0.6	6.67±0.44	5.21±0.8
2ppm	100	10.51±0.7	6.35±0.4	5.4±0.65
5ppm	98.5	11.9±0.4	5.81±0.66	5.22±0.50
10ppm	99	12.72±0.55	4.70±0.7	4.66±0.66
25ppm	96	8.87±0.2	4.25±0.9	4.35±
50ppm	88.2	7.87±0.3	4.85±0.48	4.25±0.4
100 ppm	80	5.4±0.85	3.75±0.22	0.65±0.65

3.2 Effet of Malathion on Wheat seeds in soil

The effect of malathion of different concentrations (2 ppm to 100 ppm) on germination and other morphogenic changes in wheat seeds embedded in soil was studied. The detrimental effect seed germination and other morphogenic alteration increased with enhancement of concentration of malathion. However, in control with nil concentration of malathion. Percentage of seed germination and other morphogenic features were lower as compare to soil supplemented with 2% malathion, as depicted in Table-2 a maximum of 97.6% seeds germinated with a mean of 24.09 cm length of seedling was observed at 02% malathion concentration. It was engrowwsing to final low toxicity towards seed germination and other morphogenic changes at higher concentration of malathion (50 ppm and 100 ppm) Table-2. It was concluded that the insecticide under morphogenetic study malathion is considerably less toxic basis at higher concentration as compared to other organochloro pesticides viz. Grammaxane/ Liadane.[20] It was also observed that a majority of seedlings survived at high concentration of malathion land germination was approximately 65%.

Table-2
Average values of effect of malathion on germination of wheat seeds tested by soil (cup) method.

Sample	Germination (%)	Shoot length (cm)	Root length (cm)	Roots per shoot
Control	97	13.51±0.55	11.95±0.6	3.75±0.084
2 ppm	97.6	12.92±0.4	11.19±0.4	5.16±0.65
5ppm	96	11.79±0.7	11.13±0.8	4.96±0.55
10ppm	96	9.29±0.3	9.72±0.74	4.68±0.65
25ppm	95.4	9.01±0.2	9.56±0.9	4.39±0.37
50ppm	90.7	6.79±0.35	6.96±0.28	3.82±0.45
100ppm	65	5.06±0.6	5.72±0.82	3.78±59

3.3 Estimation of Photosynthetic Pigments in Green leaf of wheat seedling

The present study deals with the estimation of photosynthetic pigment the six days old wheat seedlings excised is the second method (planting in soil). This was evaluated both qualitatively and quantitatively for the determination of plethora of photosynthetic pigments such as chlorophyll 'a' chlorophyll 'b' total soluble chlorophyll and carotenoid. It has been widely known that chlorophyll is the most precious of plat pigment, which is the centre point for the capture of photoradiation and is primarily responsible for photosynthesis. Maximum amount of total chlorophyll was observed in the control (nil malathion) (I 1.61 mg/I) and minimum in 100ppm plant seedling (2.18 g/I). The study has shown that the total chlorophyll and the carotenoid content gradually decreased with increasing concentration of malathion Table-3.

Table-3 Total content of photosynthesis pigments in green leaf.

Sample	Chlorophyll a (mg/I)	chlorophyll b (mg/I)	Total Chlorophyll (mg/I)	Carotenoid (mg/I)
Control	8.42	3.16	11.61	0.211
2ppm	7.62	2.14	9.76	0.186
5ppm	6.18	2.30	8.48	0.154
10ppm	5.58	1.61	7.19	0.125
25ppm	3.47	1.15	4.62	0.093
50ppm	2.54	0.67	3.21	0.84
100ppm	1.45	0.71	2.18	0.60

Photosynthetic pigments were also studied in thin layer chromatography employing fresh as well as frozen leaf samples. On close examination of the resultant chromatogram and the eluted separate fractions of photosynthetic pigments shows the presence of xanthophyll. Chlorophyll 'a', chlorophyll 'b' and carotenoid fig-5,6 but the TLC plate using frozen sample was devoid of any phenophytin fig 6. However, a very minor spot was observed on TLC place in case of fresh leaf (fig.5) Pigments phenophytin 'a' and phenophytin 'b' were observed on the TLC plate with the R_f value consistent with the literature reports in addition to the corresponding chlorophyll 'a' and chlorophyll 'b' both for fresh leaf and frozen leaf extract.

Mostaffa et al have found the similar results using strong acids. The order of elution using a series of eluting so ent system was carotenoid (R_f 0.96) chlorophyll 'a' (R_f 0.88) chlorophyll 'b' (R_f 0.84) and Xanthophyll (R_f 0.18) on the basis of our study we have observed that malathion at lower concentration may be employed for the promotion of seed germination and other favourable morphogenic growth of seedlings. However, it has detrimental effect on seed germination as well as growth of seedlings and decrease in the photosynthetic contents. It has been found that even at 1 ppm malathion concentration the total chlorophyll content gets reduced[16-17]. Suzuki et al have found that malathion has an adverse effect on the production of ATP due to interference the photophosphorylation in the light reaction.[24] Almost identical results were observed by Gafar et al. On the effect of malathion in case of snake cucumber. Other workers namely Bollag et al and Bridlan et al have reported the detrimental effect of this pesticide on the fertility of soil, salinity, germination percentage and other morphogenic features reducing the total yield of the crop.

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