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Effect of Malathion on Seed Germination and Photosynthetic Pigments in Wheat (*Triticum aestivum* L.)

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ABSTRACT

Background: Malathion belongs to organophosphates class of synthetic pesticide. It is widely used as an effective insecticide. The main purpose of this study is to evaluate the significant effect of malathion at different concentrations towards seed germination and estimate the content of various photosynthetic pigments in *Triticum aestivum* L.

Methods: Two different methods have been applied for this study, first moist sterile Whatman No. 2 filter paper in petri dish and second with sterile soil in plastic ice-cream cups. Qualitative and quantitative estimations of the photosynthetic pigments such as chlorophyll, xanthophyll and carotenoid etc. were performed by thin layer chromatography and biospectrophotometer respectively.

Results: The percentage germination and growth of seedling in term of shoot length were enhanced in lower concentrations (2ppm to 10ppm) as compared to control on filter paper. However soil morphogenic response significantly declined with increasing concentration. Photosynthetic pigments significantly reduced with increasing concentrations of malathion as compared to control.

Conclusion: We observed that malathion at 100 ppm shown adverse effect on germination and growth, but still growing the seedling.

Keywords: Malathion, Seed germination, Photosynthetic pigments, Thin layer chromatography, *Triticum aestivum*

1. INTRODUCTION

Malathion is an important member of organophosphates (OP), which is commonly known as carbophos, maldison and mercaptothion, and chemically known as [S-(1, 2-dicarbethoxyethyl)-O, O-dimethyl dithio-phosphate]. Malathion is a non-systemic, wide-spectrum insecticide that has been registered for use in the United States since 1956. Malathion is used in agriculture, residential gardens, public recreation areas, and in public health pest control programs [1]. Malathion may be found in some cosmetic items like special shampoos for the treating lice. When applied in accordance with the rate of application and safety precautions specified on the label, malathion can be used to kill mosquitoes without posing unreasonable risks to human health or the environment. However, at high doses, malathion, like other organophosphates, can over stimulate the nervous system causing nausea, dizziness, or confusion. Severe high-dose poisoning with any organophosphate can cause convulsions, respiratory paralysis, and death [2]. People who apply products containing malathion may be exposed if they do not wear the proper protective equipment. Consumption of food that has been treated with malathion may also result in detrimental effects. [3&4].

The higher concentration (300 ppm) of malathion has been shown to toxic effect on seeds germination, starch, amino acid and protein content. A person who was exposed to enough amount of malathion to responsible many health problem like vomited, weakness, shortness of breath, a slowed heart rate, headache and diarrhea [1&5]. Pets may be exposed to malathion if they get into contact by accident or eat plants that have just been sprayed. The

nervous system is very similar in people and other animals, so animals poisoned by malathion may show signs similar to those observed in people [6]. Bacteria may break down malathion in soil and sunlight can also break down malathion in the air. Less amount of malathion mixed with water and can move quickly through soil. Because of these properties, malathion can be found in surface waters such as streams, and sometimes it is found in well water. The time it takes for malathion to break down to half of the original amount in soil is about 17 days, depending on the soil type. This length of time is known as the half-life. In water, malathion has a half-life between 2 to 18 days, depending on conditions like temperature and pH. malathion vapor may also move long distances in air or fog. [7]

Cereals are one of the most traditional forms of carbohydrate and minerals intake amongst the Indian population. *Triticum aestivum* commonly known as wheat is one of the most important cereal crops cultivated since ancient times in India, with Uttar Pradesh followed by Punjab being the highest producer of wheat as a cereal crop. It is a carbohydrate and vitamin rich staple food containing about 23 % total carbohydrate content on dry weight basis, and 339 Calories per 100 gm. Apart from being an important source of carbohydrate in the human diet, wheat plants are also a source of fodder for cattle's. Some legume crop (*Lens culinaris*) was cultivated with wheat; legume plants play a key role in maintaining the fertility of the soil by fixing atmospheric nitrogen, thereby increasing nitrogen content in the soil. Its effect on crop plants such as *Triticum* was studied and employed to give a better understanding of its mode of action and toxicity towards seed germination and photosynthetic pigments [8&9].

2. MATERIALS AND METHODS

2.1. Chemicals and Seeds

Malathion (commercial formulation) was obtained from Davani crop science, Hapur, Utter Pradesh, India. For this study chemicals and reagents such as $CaCO_3$, $MgSO_4$, and acetone were used analytical grade and were purchased from authorized venders. Seeds of wheat (*Triticum aestivum*) were purchased from Agricultural seed store, Pusa complex, Delhi, India.

2.2. Soil and Filter Paper

Soil was collected from Delhi Technological University ground; most of organic matter was firstly removed and then dug around one cm deep. Whatman No.2 filter papers were used in the present study.

2.3. Sterilization

1 kg soil was sterilized by autoclaving at 121^{o}C for 40 min at 1.05 Kg/cm^2 (115 lb psi) pressure for three times every alternate day. Distilled water, glass wares and filter papers were sterilized by autoclaving at 121^{o}C for 20 min at 115 lb psi. Forceps, needle, petri-plates and plastic ice-cream cups were cleaned with 90% ethanol before use. The seeds were surface sterilized using liquid detergent teepol and then were thoroughly washed under running tap water for 10 min. The seeds were treated with 1% Citric acid solution, an antioxidant kept in flask and placed on rotary shaker for 10 min at 100 rpm and washed with distilled water. Subsequently, these seeds were surface sterilized with 0.1% (w/v) freshly prepared $HgCl_2$ solution for 5 min with constant shaking and was finally washed 4-5 times with

autoclaved distilled water. All materials like autoclaved distilled water, tissue paper, lamp, etc. except seeds, were exposed to ultra violet light in a laminar flow cabinet for 30 min prior to their use. Surface of laminar flow and working platform were cleaned with 90% ethanol before the start of seeds germination experiment.

2.4. Germination Test on Filter Paper

Sterile Whatman No. 2 filter papers, 10 cm round shape were placed on sterile petri-discs, 9 cm in diameter and covered by sterile petri-plate (10 cm diameter). The filter papers were moistened with sterile distilled water and then different concentrations (2 ppm to 100 ppm) from stock of pesticide along with control (without pesticide) were added. 10 seeds were placed on filter paper at equal distances. 1ml sterile distilled water was added in each petri-disc every day in morning to maintain moisture. The experiment was performed in the replicates of 10 with 10 seeds in each concentration. Rest of germination conditions was same to soil in cups experiment. The seedling was observed and measured after five days of experiment.

2.5. Germination Test on Soil

First of all 100 g of sterilized soil was filled in all the plastic cups with 8.0 cm diameter. Different concentrations (2 ppm to 100 ppm) from stock of pesticide were mixed thoroughly on plastic sheet along with control. Pesticide stock was prepared in organic solvent i.e. acetone. The sides and bottom of plastic ice-cream cups were punched with needle to facilitate air and also help in remove extra water if inside. 10 seeds per cups were propagated at 1 cm depth at equal distance with help of forceps. After the seeds propagated, 10 ml of sterile distilled water was dispensed with pipette for moisture. 10 plastic cups of each concentration were set aside at room temperature (28±2 ^{0}C) in air conditioned room under natural light. 2 ml sterile distilled water was added with pipette in each cup every day at evening time to maintain water content. After five days, the experiments were terminated and growth parameter of seedling such as morphogenesis, rhizogenesis and leaves surface area etc. were measured and calculated.

2.6. Analytical

2.6.1. Growth of seedling

Observations were recorded after 6 days of sowing of seeds. After recording the final data, the *in vivo* raised shoots were excised and preserved at 4 ^{0}C for further work like photosynthesis pigments and enzyme study. The data were recorded in terms of (i) Percentage of seed germination, (ii) Average shoot length in cm, (iii) Average root length in cm, (iv) Average No. roots per shoot, (v) Average No. of leaf per shoot.

2.6.2. Photosynthetic pigments

Chlorophyll pigments were estimated by using slightly modified Holden protocol (1960). 0.2g of plant samples from each concentration were weighed and homogenized individually using mortar-pestle in the presence of excess of acetone: water (70% : 30%) until all the color was released from the tissue. Few amount of CaCO3 was added to prevent pheophytin formation and this was then centrifuged at 5000 rpm for 10 minutes at room temperature. The

clear supernatant was collected and then made up to a known volume (10 ml). The test tubes were wrapped with black paper to protect chlorophyll degradation. The Biospectrophotometer (Eppendorf) was adjusted at wavelength of 663 nm, 645 nm, 665 nm, 434 nm, 480 nm, 486 nm for chlorophyll a, chlorophyll b, pheophytin 'a' pheophytin 'b', for carotenoid and xanthophyll respectively. The optical density was measured and the content of chlorophyll- a and chlorophyll- b (in mg/l) was calculated using optical density and standard formula [10]. For examples total chlorophyll, add value of chlorophyll a, chlorophyll b, and amount of carotenoid = (4×OD×total volume of sample)/ weight of fresh leaf.

2.6.3. Thin layer chromatography

200 mg of fresh green wheat with similar amount of anhydrous magnesium sulfate was grounded in a mortar and pestle in presence of acetone for 5-7 minutes. The light green solid was transferred into a micro-centrifuge tube with the help of acetone. This heterogeneous mixture was agitated to ensure complete mixing of the acetone and the semi-solid. This mixture was allowed to stand for 10 minutes and the green acetone solution was removed by pipette and transferred to a micro-centrifuge tube, then centrifuged at 5000 rpm for 10 minutes at room temperature (25 ^{0}C). The clear supernatant was collected (1 ml). Similar protocol follows with frozen wheat leaf. The 20 x 20 cm^2 plates were prepared by spreading a uniform layer of 0.25 mm thickness silica gel G using TLC kit in our lab. These plates were allowed to dry at RT and further activated at 120 ^{o}C for half an hour. The acetone extract was spotted in the standard manner and the plate was eluted in a closed TLC chamber saturated with the organic solvents as mobile phase (cyclohexane: petroleum ether: acetone: n-hexane **::** 5:2:2:1). The intensity of chromatogram was observed in normal light and retention factor was calculated.

2.6.4. Statistical analysis

All the experiments have been repeated with nine replicas and initially the experimental setup was performed with three replicas for every method and each concentration for average length of shoots, root. Average numbers of secondary roots per shoot have been represented as mean values along with standard error (mean ± SE).

3. RESULTS AND DISCUSSION

3.1 Effect of Malathion on Seeds of Wheat on Filter Paper

The wheat seeds were grown on the filter paper supplemented with 2, 5, 25, 50 and 100 ppm malathion. A maximum of 100% germination was observed with an average shoot length of 10.41±0.7 per seedling and roots per shoots were reported. It was also observed that the percentage germination and shoot lengths increased at lower concentration (5 ppm & 10 ppm) as compared to the controls (Fig. 1, Table 1). The morphogenic response in term of shoot length and percentage germination decreased with an increasing concentration (25 ppm to 100 ppm) of malathion. Higher level of malathion (100 ppm) proved toxic as seen a single leaf seedling with small and thick roots. However, seedling could survive on this concentration (100 ppm). The morphogenic response was variable at different concentrations (Table 1; Fig. 1).

Table 1: Effect of malathion on the germination of wheat seeds tested by filter paper (petri dish) method.

Sample	Germination (%)	Shoot length (cm)	Root length (cm)	Roots per shoot
Control	98	10.39±0.6	6.67±0.44	5.21±0.8
2ppm	100	10.41±0.7	6.25±0.4	5.4±0.65
5ppm	98.5	11.8±0.4	5.81±0.66	5.2±0.50
10ppm	99	12.68±0.55	4.70±0.7	4.66±0.6
25ppm	96	8.87±0.2	4.25±0.9	4.35±0.3
50ppm	88.2	7.87±0.3	4.85±0.48	4.25±0.4
100ppm	80	5.4±0.85	3.75±0.22	4.75±0.5

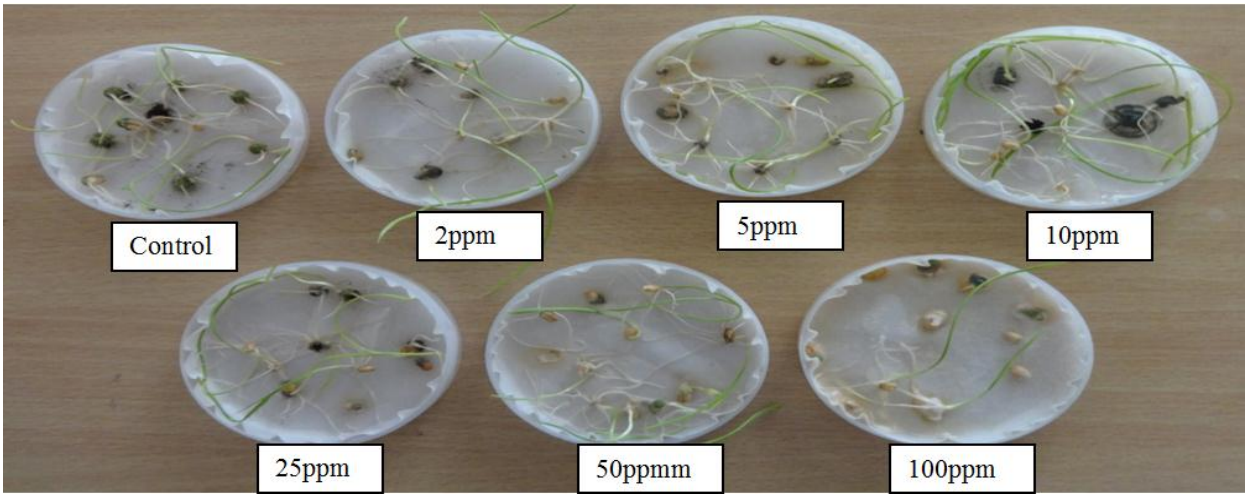

Fig.1. Seed germination on filter paper observed after 6 days at different concentrations of malathion

3.2 Effect of Malathion on Seeds of Wheat in Soil

The effect of malathion at different levels (2 ppm to 100 ppm) on the germination and growth of wheat seeds in soil was observed. The inhibitory effects towards seed germination and growth of seedling gradually increased with increase in concentration of malathion. A maximum of 97.5% seeds germinated with an average of 24.06 cm length of seedling on 2 ppm (Table 2). However, in control (without malathion) the percentage germination of seeds was less compared to the seeds grown on 2 ppm. Interestingly, the low toxicity towards seed germination was observed in higher concentration of malathion (50 &100 ppm). A low percentage of seed germination and survival was also observed at 100 ppm (Table 1, Fig 1). We observed that the malathion is less toxic in higher concentration as compare of organ-chlorine pesticides i.e. Lindane (17),because at lower concentrations most of seedlings survived and germination was around 64%.

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

Sample	Germination (%)	Shoot length (cm)	Root length (cm)	Roots per shoot
Control	97	13.41±0.55	11.98±0.6	5.74±0.084
2ppm	97.5	12.81±0.4	11.25±0.4	5.18±0.65
5ppm	96	11.88±0.7	11.11±0.8	4.83±0.55
10ppm	96	9.15±0.3	9.70±0.74	4.66±0.63
25ppm	96.5	9.0±0.2	9.45±0.9	4.35±0.37
50ppm	90.8	6.87±0.35	6.85±0.28	3.85±0.45
100ppm	64	5.08±0.6	0575±0.82	3.75±0.58

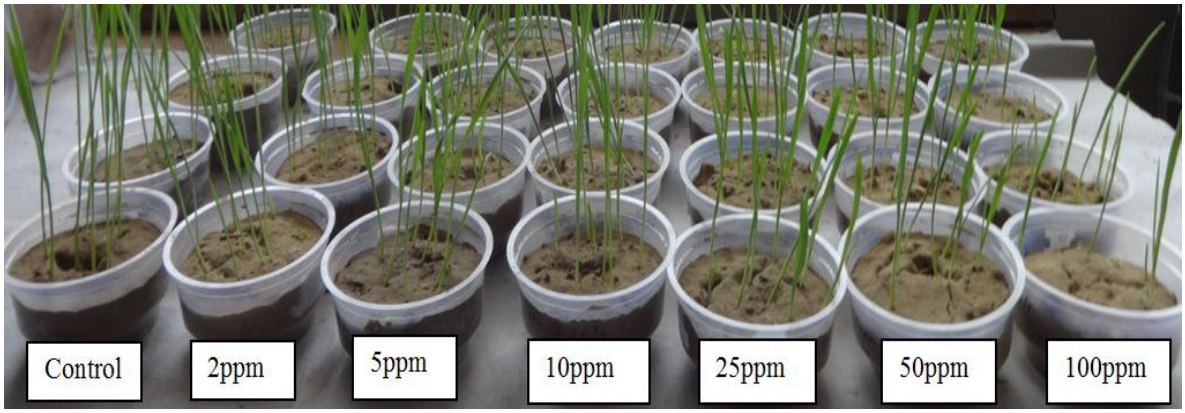

Fig.2. Seeds germinated at different concentrations of malathion after 6 days.

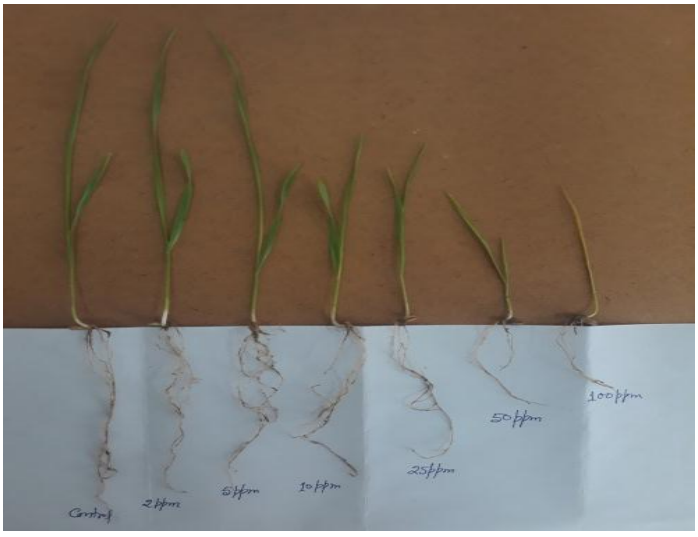

Fig.3. Seedling at different concentrations of malathion after 6 days.

3.3 Estimation of Photosynthetic Pigments in Green Leaf

In the present investigation, 6 days old seedling, which was cultivated by soil method, was evaluated qualitatively and quantitatively for the analysis of various photosynthetic pigments, total soluble chlorophyll and carotenoid. As we know chlorophyll is the most valuable primary metabolite of a plant. It is the center point for the capture of sunlight and is responsible for photosynthesis. Maximum amount of total chlorophyll was found in control i.e. 11.58 mg/g. and minimum in 100 ppm sample i.e. 2.16 mg/g. The effects of malathion on amount of total chlorophyll and carotenoid gradually decrease with increase in concentration (Table 3).

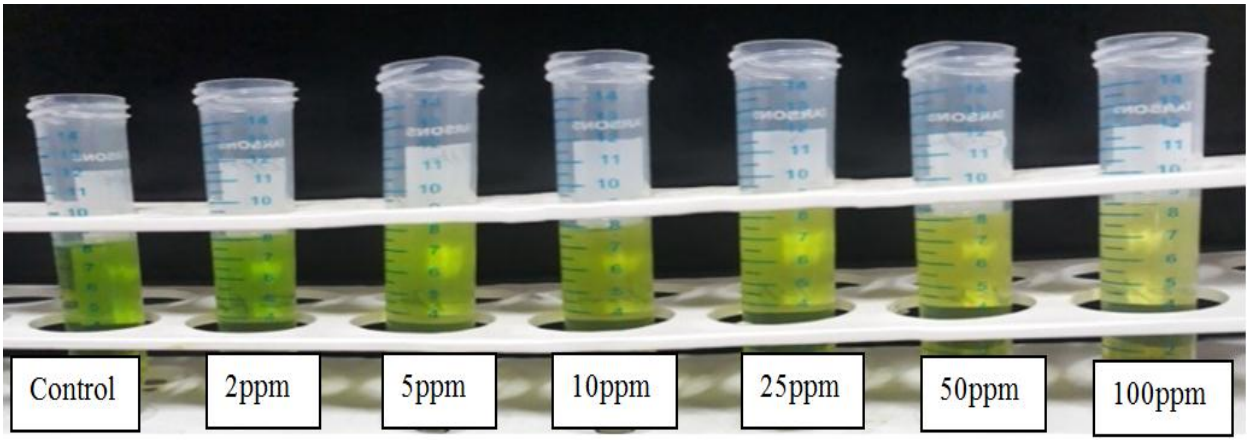

Fig.4. Intensity of photosynthetic pigments decrease with increasing concentration.

Table 3: Total content of photosynthetic pigments in green leaf.

Sample	**Chlorophyll a** (mg/l)	**Chlorophyll b** (mg/l)	**Total Chlorophyll** (mg/l)	**Carotenoid** (mg/l)
Control	8.42	3.16	11.58	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	2.16	0.60

The TLC for photosynthetic pigments was studied by using fresh and frozen leaf samples. The analysis of the various photosynthetic pigments on TLC plates showed the presence of xanthophyll, chlorophyll a, chlorophyll b and carotenoid (Fig. 5, 6) but no spot of pheophytin was observed on TLC plate for frozen sample (Fig. 6). However, TLC spots indicated that pheophytin was very minor spot in TLC plate in sample of fresh leaf (Fig. 5). Pigments pheophytin a and b were observed at *R*f values consistent with literature reports in addition to the

corresponding chlorophyll a and b when fresh or frozen leaves were used to prepare extract (Figure 5, 6). The demetalation of chlorophyll using strong acids has been previously described [11].The elution order using this elution solvent system was carotenoid (*R*f= 0.96), chlorophyll a (*R*f= 0.89), chlorophyll b (*R*f= 0.83), and xanthophyll (*R*f= 0.17).

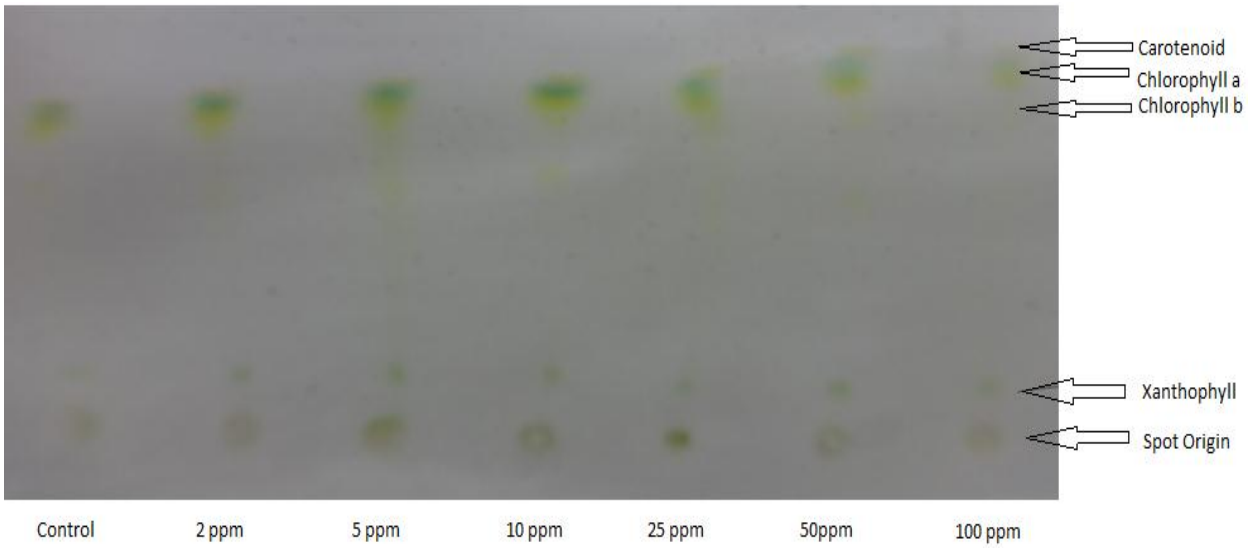

Fig.5. TLC analysis of photosynthetic pigmentsusingfresh green leaf of wheat

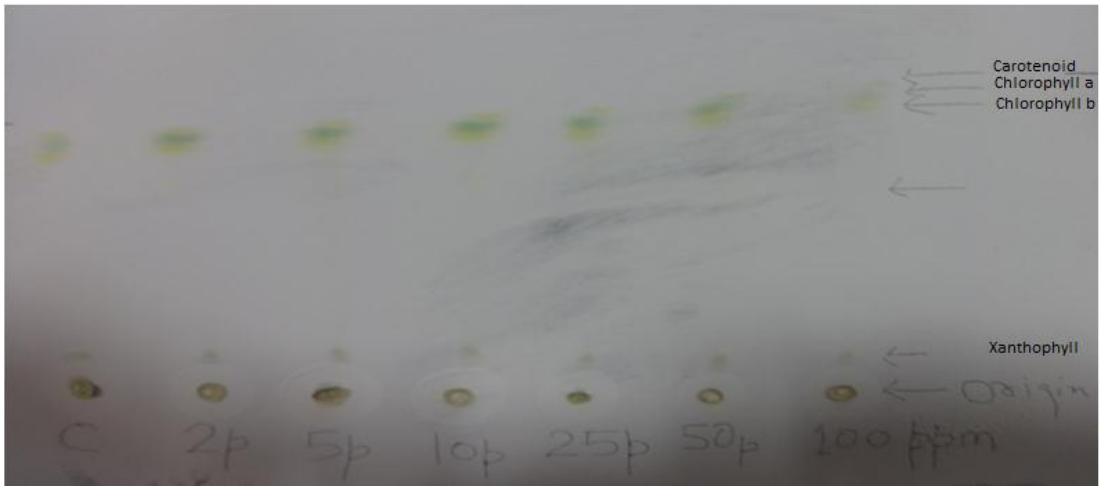

Fig.6. TLC analysis of photosynthetic pigments using frozen green leaf of wheat

On the basis of our result, we found that the low concentration of malathion may be used to promote the seeds germination and growth of seedling. However, it showed adverse effect on photosynthetic pigments. Even a low concentration of 1 ppm has been reported in reducing chlorophyll content [12, 13 &14]. Malathion inhibits the production of adenosine triphosphate (ATP) due to their effect on the photophosphorylation in the light reaction [15]. Similar results were obtained by Gafer *et al.* [3] on some other vegetables using the same pesticides and doses.

Also, pesticides affect crop growth and reduce its yield and quality due to their phytotoxicity. It even has detrimental effects on soil fertility and salinity [16 & 17].

4. CONCLUSION

This study confirmed that, malathion does not show toxicity towards seed germination and growth of seedling at higher concentration of 100 ppm, but shown adverse effect on growth of seedling and content of photosynthetic pigments. At lower levels, it was enhanced the germination and growth of seedling on filter paper. It has shown inhibitory effects towards seed germination and growth of seedling gradually increased with increase in concentration in soil method. Higher concentration of malathion inhibits the production of primary metabolites, like that photosynthetic pigments.

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