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Effect of Malathion on the Concentration of Primary Metabolites from *Vigna radiata* and *Trigonella foenum-graecum*

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ABSTRACT
In the present study, the effect of the pesticide, Malathion, on the concentration of primary metabolites from young plants was studied. Primary metabolites are of prime importance and they are essentially required for the healthy growth of plants. The primary metabolites chosen for this study were protein and chlorophyll. Malathion is a wide-spectrum organophosphorus pesticide which is used to control sucking and chewing pests in agricultural field crops. Green gram (Vigna radiata) and common fenugreek (Trigonella foenum-graecum) were selected for the study as both plants are a part of the regular diet of the Indian population[1] and they are important leguminous crops. However, seedlings of green gram and common fenugreek are prone to attack by various pests. V. radiata is attacked by whiteflies which are vectors of viruses and caterpillars which feed voraciously on leaves of young plants. T. foenum-graecum is commonly infested with black aphids. Therefore Malathion 50 EC is applied in the form of an aerial spray on the seedlings of green gram and common fenugreek to control the growth of these pests. Three different concentrations of Malathion 50 EC were prepared and sprayed on young plants of V. radiata and T. foenum-graecum. After an interval of 5 and 10 days after application, sample of plant parts were collected, and proteins were estimated by Folin-Lowry method and chlorophyll was estimated using Holden's protocol[2].
Keywords: *Primary metabolites, Protein, Chlorophyll, Malathion, Vigna radiata, Trigonella foenum-graecum*

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INTRODUCTION

Pulses are one of the most traditional forms of protein intake amongst the Indian population [3]. *Vigna radiata (*common name - Green gram) is one of the most important pulse crops grown cultivated since ancient times in India, with Maharashtra being the highest producer of green gram as a pulse crop. It is a protein rich staple food containing about 25 % protein content on a dry weight basis [1]. Thus, the most vital primary metabolite synthesized in *V. radiata* is protein. Apart from being an important source of protein in the human diet, green gram plants are leguminous in nature. They play a key role in maintaining the fertility of the soil by fixing atmospheric nitrogen, thereby increasing nitrogen content in the soil. Therefore, it is used as a leguminous plant grown in crop rotation to replenish soil fertility. Thus, the functioning and health of this plant is vital, and another primary metabolite which helps in growth is chlorophyll.

Trigonella foenum-graecum **(**common name - Fenugreek), locally known as *methi*, is also another important leguminous crop. It provides nitrogen content to the soil in which it grows. The seeds and leaves of fenugreek are aromatic. They are consumed as they have a high nutritional value being rich in protein, vitamins A and C. It is also grown as a fodder for animals. Thus, even for fenugreek, it is imperative that primary metabolites like proteins and chlorophyll are synthesized in adequate concentrations. The protein content in the leaves depends upon the stage of growth and gets reduced during the flowering stage [4]. Therefore to estimate the protein content in the plant, young fenugreek plants were grown.

Malathion 50 EC [S-(1, 2-dicarbethoxyethyl)-O, O-dimethyl dithio-phosphate], also known as carbophos, maldison and mercaptothion is a non-systemic, wide-spectrum organophosphorus pesticide used in agricultural settings. Malathion is suited for the control of sucking and chewing insects of fruits and vegetables, mosquitoes, flies, household insects, animal parasites (ectoparasites). Organochlorine

pesticides are banned in many countries including India therefore organophosphate pesticides including Malathion are largely used for public health and agricultural purposes [5].

Both of the legumes, green gram and common fenugreek, are susceptible to many pests. *T. foenum-graecum* gets severely infested by black aphids (*Aphis cracivora*) which cause adverse effects on the plant [6]. To control the population of black aphids, Malathion 50 EC is sprayed at 625 ml per hectare. *V. radiata* gets infested by whiteflies (*Bemesia tabaci*). The whitefly is a vector of the mungbean yellow mosaic virus (MYMV). The young plants show yellow mosaic spots and chlorosis, causing the plant tissue to degenerate. Thus, to control the infestation of whiteflies, Malathion 50 EC (2.0 ml/L) is sprayed [1]. Green gram can also be infested by the tobacco caterpillar (*Spodoptera litura*) which feeds gregariously on the leaf surface of young plants, leaving behind only the skeleton and also making irregular holes in the leaf. In order to destroy these pests, Malathion 50 EC (2.0 ml/L) can be sprayed on the foliage [1]. Thus, this study will attempt to observe the effect of Malathion on the concentration of proteins and chlorophyll in *V. radiata* and *T. foenum-graecum*.

MATERIAL AND METHODS

Preparation of different concentrations of Malathion

Three different concentrations of Malathion were prepared. The stock solution was Malathion 50 EC (emulsifiable concentrate-50% of Malathion). The concentrations were prepared by taking 1ml, 2ml, and 3ml of the liquid stock solution in 1 litre (1000ml) of distilled water. These concentrations were stored in spray bottles which are ready for application.

Growth of Test Plants

Seeds of *Trigonella foenum-graecum* and *Vigna radiata* were collected. They were moistened and kept overnight at around 25°C to allow sprouting to occur. Fertile, sandy, loamy and well drained soil suitable for the growth of the plants was obtained. A total of 8 potted plants were grown: 4 of common fenugreek and 4 of green gram. Each pot was sprinkled with 10-15 seeds of the respective plant (equally spaced) and allowed to grow up to seedling stage (10-11 days). Since 3 different concentrations of Malathion were prepared, 3 pots were used as test and 1 pot as control. These young gram bean and fenugreek plants are now ready to be sprayed with the pesticide.

Treatment of Plants by Malathion

Out of the 4 seedlings of *V. radiata*, 3 were treated with 8-10 sprays of Malathion-1ml/L, 2ml/L, and 3 ml/L respectively. The fourth seedling was not sprayed with any pesticide (control). Similarly, 3 seedlings of *T. foenum-graecum* were treated with 1ml/L, 2ml/L and 3ml/L of Malathion respectively, and the fourth used as control. The plants were kept at temperatures of 20°C-23°C which is suitable for the growth of the seedlings.

Collection of Plant Material

5 days after the seedlings were sprayed with Malathion 50 EC, leaves and stems from the test and control pots of *Vigna radiata* and *Trigonella foenum-graecum* were collected, washed thoroughly with distilled water, shade dried, and stored in clear plastic bags which were tightly sealed and labeled. The plant samples were taken to the laboratory for the estimation of primary metabolites (protein and chlorophyll). This same procedure was also carried out 10 days after the first application of Malathion. To avoid bias, the plant parts were taken randomly from the test pots and weighed equally (about 1000mg).

Estimation of Proteins

Protein Extraction

Each of the plant parts were homogenized over ice using mortar and pestle, in 10% cold Tri Chloro Acetic acid (TCA) (around 2gm in 5ml TCA) and were centrifuged at 5000 rpm for 10 minutes. Supernatant was discarded and pellets were saved. Pellets were again suspended in 5 ml of 10% cold TCA and re-centrifuged for 10 minutes. Supernatant was discarded again and the pellet was dissolved in 10 ml of 0.1 N NaOH. 0.1 ml and 0.2 ml of this solution was used for protein estimation.

Quantitative Estimation of Proteins

Total protein content was estimated using the protocol [7]. A stock solution of 1 % Bovine Serum Albumin (BSA) (1 mg/ml) was prepared in 1 N NaOH; five concentrations (0.2, 0.4, 0.6, 0.8 and 1 mg/ml) from the working standard solution were taken in series of test tubes. In another set of test tubes 0.1 ml and 0.2 ml of the sample extracts were taken and the volume was raised up to 1 ml in all the test tubes. To each test sample, 5ml of freshly prepared alkaline solution (prepared by mixing 50 ml of 2% Na_2CO_3 in 0.1 N NaOH and 1 ml of 0.5% $CuSO_4$. $5H_2O$ in 1% sodium potassium Tartrate) was added at room temperature and left undisturbed for a period of 10 min. Subsequently, to each of these mixture tubes 0.5 ml of 2N Folin-Ciocalteau reagent (diluted with equal volume of distilled water just before use) was rapidly added and incubated at room temperature (about 25°C) for 30 minutes until the blue colour

developed. The colorimeter was adjusted at wavelength of 700 nm and set at zero absorbance using the blank before taking the readings of the standard and the test samples respectively.

Estimation of Chlorophyll

Chlorophyll was estimated using the protocol of Holden[2]. Each of the plant samples was weighed approximately 2 mg and homogenized over ice using mortar and pestle in the presence of 5 ml of 80% acetone until all the color was released from the tissue. CaCO3 was added to prevent pheophytin formation and this was then centrifuged at 5000 rpm for 10 minutes at room temperature [8]. The clear supernatant was collected. The colorimeter was adjusted at wavelength of 663 nm for chlorophyll 'a' and 645 nm for chlorophyll 'b' set at zero absorbance using 80% acetone as blank before taking the readings of the samples respectively. The optical density was measured and the chlorophyll contents in the original extract was estimated using the formula:

Total chlorophyll (mg/L) = $20.20A_{645}$ + $08.02\ A_{663}$ [9].

Where A_{645}= Absorbance at 645 nm and A_{663}= Absorbance at 663 nm

RESULTS AND DISCUSSION

Estimation of Protein

All the plant samples from Day 5 and Day 10 were subjected to protein estimation. The absorbance values were obtained using the colorimeter at 700nm. The values were marked against the Standard Graph prepared from the absorbance values obtained from using 1 % BSA. From the graph, the concentration of the unknown samples was calculated by extrapolating the points on the x-axis[3]. The average concentration from using 0.1ml and 0.2 ml of extract was calculated, & tabulated in **Fig. 1.**

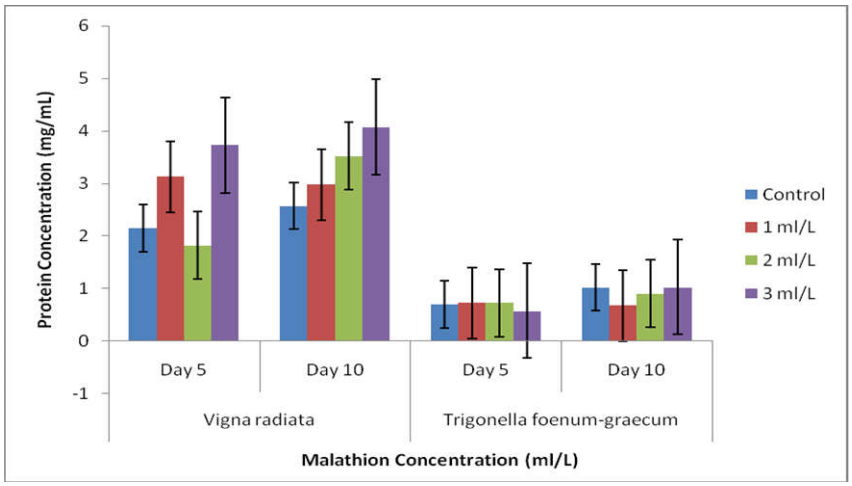

Fig. 1- Estimation of Protein Concentration in the plant extracts of test samples *Vigna radiata* and *Trigonella foenum-graecum* after exposure to various concentrations of Malathion. The protein concentrations were estimated in two time slots: 5 days after exposure and 10 days after exposure. Average of two estimations taken per sample (n=2) with error bars representing standard error of mean (SEM) (Control=No Malathion exposed to the plant)

Thus from, Fig.1, we can see that, 5 days after application of the pesticide, at 1ml/L concentration of Malathion, protein content in the Green gram plant increases as compared to control. At 2ml/L concentration, the protein content decreased and then increased at 3ml/L. During Day 10, the protein content in the control, 2ml/L, and 3ml/L increased whereas that in 1ml/L decreased. *Trigonella foenum-graecum* has lesser protein content on comparison with that of *Vigna radiata*. On Day 5, Malathion seemed to have very little effect on the protein concentration of the fenugreek plants, except at 3ml/L concentration of Malathion, where the protein content decreased to 0.575. On Day 10, however, with increasing concentration of Malathion, the protein content increased in the fenugreek plants, as well as in the control.

Estimation of Chlorophyll

Similarly, all the plant samples from Day 5 and Day 10 were subjected to chlorophyll estimation and the absorbance values were obtained using the colorimeter at 663 nm for chlorophyll 'a' and 645 nm for chlorophyll 'b'. The total chlorophyll content was calculated by using the formula given by Arnon [19] and tabulated in **Fig. 2**.

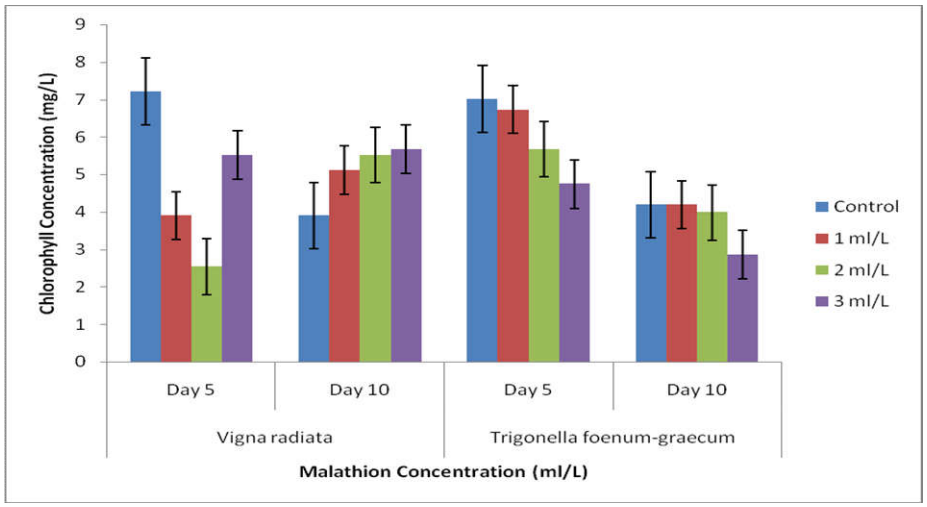

Fig 2.- Estimation of Chlorophyll in the plant extracts of test samples *Vigna radiata* and *Trigonella foenum-graecum* after exposure to various concentrations of Malathion. Chlorophyll content in the plant extract was estimated in two time slots: 5 days after exposure and 10 days after exposure. Average of two estimations taken per sample (n=2) with error bars representing standard error of mean (SEM) (Control= No Malathion exposed to the plant)

In the Green Gram plants, on Day 5, the chlorophyll content in the control was the highest, after which it decreased at 1ml/L and 2 ml/L concentrations of the pesticide, and increased at 3ml/L. On Day 10, however, the concentration of chlorophyll increased in all 3 test plants, whereas it reduced in the control plant. Common fenugreek being a more leafy vegetable than Green Gram (which is more of a pulse crop) has more chlorophyll content. On Day 5, the control plant showed the highest chlorophyll content, after which in all 3 test plants, the chlorophyll content decreased with increase in concentration of Malathion. On Day 10, the chlorophyll content in the control and test plants was lesser than that observed on Day 5, and still, as the concentration of the pesticide increased, chlorophyll content decreased.

DISCUSSION

Malathion, at a concentration of 1 ppm, has been reported to inhibit photosynthesis by reducing chlorophyll production [10]. These organophosphorus pesticides interfere with the production of adenosine triphosphate (ATP) due to their effect on the photophosphorylation in the light reaction [11]. The reduction of the primary metabolite, chlorophyll, in common fenugreek, could also be attributed to the pesticides toxicity at the higher doses and their effects on both physical and chemical characteristics of the soil[12]. Similar results were obtained by Gafer *et al*. 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 and also due to their effects on soil fertility and salinity [12]. This affects the health of the plant and thus the concentration of chlorophyll.

As far as the increase in concentration of chlorophyll in Green gram is concerned, the exact reason as to why this happened is not yet understood. Malathion stimulated the production of chlorophyll in *Chlorella* species [10]. Also, in a study of the effect of Malathion on the photosynthetic pigments of the leaves of Apricot Tree, it was seen that the concentration of chlorophyll a and chlorophyll b increased [13]. There was another study done on the effect of Malathion on the chlorophyll production by zooplankton using a lab mesocosm [14]. It was found that when Malathion was used at a concentration of 100ug/L, there was a continuous increase of chlorophyll production with time (as seen in Green gram on Day 10). Plants usually metabolize Malathion into Malaoxon [15, 16]. It may have an unknown effect on chlorophyll biosynthesis.

Microorganisms such as bacteria may use Malathion as a source of carbon and phosphorus[16]. It could be that the nitrogen fixing bacteria such as *Rhizobia* may utilize Malathion by its powerful carboxyesterase activity which degrades Malathion into thiophophates and carboxylic acid derivatives [17]. Thus, the increase in the amount of nitrogen fixing bacteria would lead to an increase in the production of protein in the plant as nitrogen is available in the soil in the form of nitrates by these bacteria. This may be an explanation for the increase in protein content in both *V. radiata* and *T. foenum-graecum*. Plants can also metabolize Malathion by carboxyesterase catalyzed hydrolysis into phosphorodithioate, thiophosphate, monomethyl phosphate, and Malaoxon [15]. Also, in the study conducted by Ali and Al-Quraishy, the total

protein content in *Armeniaca vulgaris* increased in the presence of Malathion in plant extracts obtained from Malathion treated *Salix alba* [13].

CONCLUSIONS

It is difficult to point out the exact mode of action of a pesticide on the concentration of primary metabolites such as proteins and chlorophyll. The cytological and biochemical changes that occur in the plant depends upon a variety of factors, such as, the type of pesticide, the environmental conditions of the plant, etc. In this study, Malathion does affect the concentration of proteins and chlorophyll in the chosen test plants: *Vigna radiata* and *Trigonella foenum-graecum*. Most insecticides seem to primarily alter the structure and function of plasma membranes which may lead to distortion of cell organelles such as chloroplasts. Malathion is mutagenic and is capable of alkylating DNA which may alter transcription and translation activities, thereby either increasing or decreasing the production of protein[11]. Thus, in order to maintain the health of these legumes, Malathion must be applied at a suitable concentration which will not only succeed in killing the pests, but also, the concentration of primary metabolites of the plant should not drastically change to abnormal levels.

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