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Effect of Commonly used Pesticides on the Biochemical Profile of a Few Vegetables Grown in Kanniyakumari District of Tamil Nadu

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ABSTRACT

The present investigation was carried out to find out the effect of commonly used pesticides on the morphological, biochemical and phytochemical parameters of vegetable crops like *Amaranthus gangeticus* L. *Solanum melongena* L. and *Lycopersicon esculentum* Mill. which are commonly grown in Kanyakumari District of Tamil Nadu. The experiments were conducted using control group of the above said vegetables exposed to biopesticide flower extract of *Tagetes minuta*, and an experimental group in which the above said vegetables plants were exposed to three commonly used pesticides – Monocrotrophos, Cypermethrin and DDT at varying concentrations during pest infestations. The harvested useful parts from experimental and control vegetable crops were evaluated biochemically and phytochemically. The above said vegetables procured from local market was also screened. Results from this evaluation indicated that crop exposed to chemical pesticides exhibited a variation in the biomolecules and phytochemicals evaluated. Protein, Total Chlorophyll, carbohydrate, Proline, Total Phenolic content and Total Flavanoid content of control plants treated with *Tagetes minuta* flower extract were normal values. While the experimental plants exposed to pesticides showed a decrease in the level of chlorophyll and carbohydrate content, while there was an increase in stress amino acid (Proline) and the Total protein content. However, the Total Phenol content and Total Flavonoid content increased with increasing concentration of pesticides, but at still higher levels of pesticides there was a decrease in these biomolecules. Phytochemically, secondary metabolites like terpenoids, alkaloids, phenolic compounds, steroids, flavonoids, saponins, catechins showed increased accumulation in the experimental plants exposed to pesticides at

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lower levels of pesticides. The various biological parameters evaluated characters showed variation in the control and experimental plants, which is probably because of the ill effects of pesticides in the experimental group of vegetables and the good effects of biopesticide used in the control group vegetables. This work is only basic study which needs further sustainable study into the topic to bring out the impact of chemical pesticides by assessing their residue level in the experimental plants.

Keys Words

Vegetables, Biochemical, Monocrotrophos, Cypermethrin, DDT, Proteins, Total Chlorophyll, Proline, Total Phenolic Content, Total Flavonoid Content.

INTRODUCTION

Amaranthus (red amaranth) is a unique source of betalains, β-xanthins, β-cyanins that have potential free radical detoxifying ability. Red to purple colored betalains are β-cyanins and yellow colored betalains are β-xanthins (Stintzing and Carle 2007). Brinjal or eggplant (*Solanum . melongena* Linn.), is a very important common vegetable in India. It is often described as a poor person's vegetable because it is popular amongst small-scale farmers and low income consumers. It is featured in the dishes of virtually every household in India, regardless of food preferences, income levels and social status. Low in calories and high in nutrition, the vegetable has very high water content and is a very good source of fibre, calcium, phosphorus, foliate, and vitamins B and C (Solovchenko and Schmitz-Eiberger, 2003). Amaranths, is known as a good source of protein-rich leafy vegetables and an inexpensive source of valuable nutrients, such as dietary fiber, mineral, vitamins, and antioxidant as said by Caselato Sousa, and Amaya-Farfán, 2012. It is also used in Ayurveda medicine for curing diabetes, hyper-tension and obesity. In addition, dried brinjal shoots are used as fuel in rural areas. Pesticides constitute the key control tactics for management of pests and diseases and the productivity of crops depends on their effective control. Together with high yielding crop varieties and fertilizers, pesticides have helped the Indian farmers in achieving a substantial increase in agricultural productivity. The area under plant protection has been continuously increasing in India. The average per hectare consumption of pesticides in Indian agriculture was only 1.2 gha^{-1} in 1953 – 54, it increased to 377 gha^{-1} in 1985 – 86 and further to 431 gha^{-1} in 1992 – 93. However, thereafter the fertilizer consumption has gradually declined to 288 gha^{-1} in 1999 – 2000. Excessive and indiscriminate

use of pesticides not only increases the cost of production but also results in many human health problems and environmental pollution. According to WHO estimates, one million cases of pesticide poisoning occur every year and consequently there are 20,000 deaths (Wasim Aktar *et al.*, 2009). The most damaging ecological disturbance of ecological disturbance of injudicious us of pesticides is the existence of high concentration of pesticide residues in food chain, including cereals, pulses, vegetables, fruits, milk and milk products (including mother's milk) fishes, poultry, meat products and water. India is the second largest producer of vegetables in the world next only to China with an estimated annual production of about 73 million tones from an area of about 4.5 million hectares at an average yield of 11.3 tonnes per hectare. India shares about 13 percent of the world output of vegetables from about two percent of the cropped area in the country fruits and vegetables crops receive considerably high quantity of pesticides and with a cropped area of three the effect of chemical pesticides. They consume 13 percent of the total pesticide in the country and its use is more harmful in vegetables. These problems have necessitated the search for safer and effective methods of pest control including behavioral (use of attractants. E.g – Pheromone traps), bio-control and botanical pesticides. Their use, however, has limitation and chemical control continuous to be the preferred strategy in practice. This use of pesticides is so common that the term pesticide is often treated as synonymous with plant protection product, although it is in fact a broader term, as pesticides are also used for non-agricultural purposes. Cypermethrin (RS)-alpha cyano-3-phenoxybenzyl(JRS) cis ;transe (2,2 dichlorovinyl) 2-2 dimethylcyclopropane carboxylate) is fourth generation synthestic compound primarily used as an insecticides. Its acts as a fast acting neurotoxin insects. Monocrotophos is an organophosphate insecticide. It is acutely toxic to birds and humans, so it has been banned in the U.S., the E.U. and many other countries, however it is still available in India. Dichlorodiphenyltrichloroethane, commonly known as DDT, is a colorless, tasteless, and almost odorless crystalline chemical compound. In insects, DDT opens sodium ion channels in neurons, causing them to fire spontaneously, which leads to spasms and eventual death. It was widely used a s a pesticides since its synthesis in 1940, however due to its potential environmental hazardous effect it is banned in many countries around the wo9rld, but in India it comes in the form of different names as pesticides.

MATERIALS AND METHODS

Three vegetable commonly grown in Kanniyakumari district of Tamil Nadu were selected for the present study. They are *Amaranths gangenticus. Solanum melongena* L. and *Lycopersicon esculentum* L., This investigation was carried out to find out the harmful effect of commonly used pesticides on the various biomolecules of the selected vegetable plants. For this experiment authenticated seeds of the selected vegetables were procured from the Tamil Nadu Agricultural station and grown in a field. During a period of six months the vegetables were exposed to three commonly used pesticides viz:- Cypermethrin, Monotrophos and DDT (Dichloro Diphenyl Trichloroethane) periodically at concentration ranging from 0.01-1% (1ml in 100ml water)for the former two pesticides, but for the latter it was 0.01 -1 mgs/100ml. A set of these vegetable plants were also used as control which were exposed to bio-pesticide (flower extract of *Tagetes minuta).*

Biochemical Analysis

Biochemical analysis of the selected vegetables were done to evaluate the level of biomolecules like Proteins, Total Chlorophyll, Carbohydrates, Proline, Total Phenolic Content (TPC) and Total Flavanoid Content (TFC).

Estimation of proteins

The method of Lowry *et al.,* 1951 was used to estimate protein levels in the vegetables analysed.

Estimation of carbohydrates

The total carbohydrate content was estimated by the method of Hedge and Hofreiter, 1962. 1. Glucose stock standard: 100 mg of glucose was dissolved in 100 ml of water in a standard flask. 2. Working standard: 10 ml of the stock was diluted to 100 ml. 1.0 ml of this solution contains 100µg of glucose. 3. Anthrone reagent: 0.2% anthrone was dissolved in ice cold concentrated sulphuric acid. Prepared fresh before use 4. 2.5 N HCl. Procedure weighed 100mg of the sample into a boiling tube, hydrolysed by keeping it in a boiling water bath for three hours with 5.0 ml of 2.5 N HCl and cooled to room temperature. Neutralized it with solid sodium carbonate until the effervescence cease made up the volume to 100 ml and centrifuged, collected the supernatant and take 0.2 to 1.0 ml for analysis. Add 4.0 ml of anthrone reagent, heat for eight minutes in a boiling water bath, cooled rapidly and read the green to dark green colour at 630 nm. Calculation A standard graph was drawn by taking the concentration of glucose on X

axis and spectrophotometer reading on Y axis. From the graph the concentration of glucose in the sample was calculated.

Estimation of Total Chlorophyll

The method of Amon, 1949 was done on the useful parts of selected plants in both experimental group of plants exposed to pesticides and the control group of plants to estimate Total Chlorophyll content.

Proline Estimation

Proline was extracted from plant samples by homogenization in 3% (w/v) sulphosalicylic acid and was quantified by the ninhydrin-based colorimetric assay as described by Bates *et al*. (1973). Simply, 1 g of fresh samples were homogenized in 5ml of 3% sulphosalycylic acid using mortar and pestle and centrifuged at 4000 rpm for 30 min at room temperature. Supernatant of 1 ml was added in 1 ml of acid ninhydrin (1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6M phosphoric acid) and 1ml of glacial acetic acid and kept at 100 °C for an hour in oven. The reaction was completed in an ice bath and mixed with 2ml toluene and placed at room temperature until the separation of two layers. The upper coloured layers were taken for absorbance at 520 nm using toluene as blank. Proline content was calculated using a standard curve.

Total Phenol Content (TPC)

Fresh leaf tissues of 0.5 g was homogenized with acetone (80%) and centrifuged at 10,000×*g* for 10 min at 4°C. One hundred microliters of the supernatant was diluted with 2 mL of water and 1 mL of Folin–Ciocalteaus phenol reagent and shaken vigorously. Then 5 mL of 20% Na_2CO_3 was added and the volume made up to 10 mL with distilled water. The absorbance was read at 750 nm and results expressed as mg g^{-1} of fresh leaf (Julkenen-Titto, 1985).

Total Flavonoid Content (TFC)

The method of Chang *et al*. (2002) was used for flavonoid extraction in the control and experimental vegetables. 1g of fresh tissue was extracted in 8 ml methanol and 2 ml distilled water using a chilled pestle and mortar. The extract was filtered with Whatman No. 1 filter paper and exposed to air for evaporation. To the partially, evaporated extract 6 drops of 2 M sulphuric acid was added to it followed by the addition of chloroform to the mixture in 3:1 ratio. The chloroform layer was separated out using a separating funnel and was then the extract was

exposed to air for 12 hrs. After complete evaporation, the residue was dissolved in minimal amount of methanol and used for the estimation of flavonoids. Absorbance was read at 670 nm and the concentration of flavonoids was calculated from the standard graph.

Data analysis

The data are expressed as mean of three independent replicates and were analysed by using One-Way Analysis of Variance (ANOVA). Least significance difference (LSD) at 5% probability level was used to detect the significance of differences among treatment means. The difference was considered to be significant if p value was smaller than or equal to 0.05 ($p \leq 0.05$).

RESULTS AND DISCUSSION

The results of the present investigation revealed that the biomolecules analysed varied in the experimental plants with varying concentration of pesticide exposure The experimental vegetables exposed to varying concentrations of Cypermethrin, Monotrophos and DDT showed an increase in protein content initially but as the concentration of pesticide exposure increased above 0.5% the level of protein in the useful parts showed a decline (Fig1-3). The protein content was maximum at 0.5 % pesticide concentration and lowest at 1% concentration in all the vegetables analysed. When *Lycoperscicon esculentum* was exposed to Cypermethrin the protein content in its fruits showed a maximum of 1.69 ± 0.08mg/gm F.wt and 3.65 ± 0.21 at 0.5 % and 1% concentration of the pesticide (Fig 1: Table 3). This vegetable crop when exposed to Monotrophos and DDT there was a decline in protein levels in the fruits when compared to fruits from Cypermethrin exposed plants (Fig 2). When fruits of Tomato (*Lycoperscicon esculentum*) was exposed to DDT there was a drastic decrease in protein levels with increase in the concentration of DDT used (Fig 2). Compared to Cypermithrin and Monotrophos, DDT exposure reduced the level of protein in Tomato fruits from 0 .01% of DDT. A similar trend in the level of protein was also seen in the fruits of *Solanum melongena* analysed (Fig 2 Table 2). In *Solanum melongena* 0.79 ± 0.11 mg/gm F.wt. of protein content was observed in fruits obtained from plants exposed to 1% Monotrophos. However when *Amaranthus gangeticus* was exposed to the selected pesticides, an increase in protein content was observed in the plant exposed to 0.01 to 0.25% of pesticides. In the presence of DDT, the protein level in *Amaranthus gangeticus* showed drastic decrease in protein levels at 0.25% of the pesticide (Fig 3: Table 1). At 0.75% of DDT,

very low level of about 0.39 ± 0.67 mg/gm F.wt. of protein was calculated from this plant tissue, and higher levels of this pesticide, the plant failed to develop (Table 1).

However the level of Total Chlorophyll showed a decrease in all the experimental vegetables exposed to varying concentration of pesticides (Tables 1-3). When exposed to Cypermethrin, *Solanum melongena* showed maximum level of total chlorophyll at 0.01 % cypermethrin which came to 3.029 ± 0.36 mg/gm F.wt, while in the leaves of *Lycoperscicon esculentum* and *Amaranthus gangeticus* at the same concentration of pesticide, the total chlorophyll content was 3.149 ± 0.46 and 2.665 ± 0.56 mg/gm F. wt. respectively (Fig 4 & 6). The lowest level of Total Chlorophyll was observed when the vegetable crops were exposed to 1% of cypermethrin (Fig 4). The Total Chlorophyll level in *Solanum melongena* exposed to 0.01 % Monotrophos was 2.836 ± 0.19 mg/gm F.wt. and 2.532 ± 0.32 mg/gm F.wt. in *Amaranthus gangeticus*(Fig 6). Lowest Total chlorophyll content was calculated in the leaves of *Solanum melongena* and *Lycoperscicon esculentum* when exposed to 1% Monotrophos (Fig.5 & 4), while *Amaranthus gangeticus* failed to develop from seedling stage at this concentration of Monotrophos pesticide.

DDT pesticide drastically affected the Total Chlorophyll content of all the experimental vegetable crops (Table 1-3). The highest Total Chlorophyll content was recorded in the leaves of *Lycoperscicon esculentum* (2.298 ± 0.29 mg/gm F.wt.), followed by *Solanum melongena* (2.196 ± 0.73 mg/gm F.wt.) and *Amaranthus gangeticus* (1.767 ± 0.78 mg/gm F.wt). (Fig 5 & 6). The lowest Total Chlorophyll levels were recorded on exposure to 1% of the pesticides in the leaves *Lycoperscicon esculentum* and *Solanum melongena* (Fig 5 & 4). In *Amaranthus gangeticus* at 0.5% the lowest Total Chlorophyll content was calculated which came to 0.457 ± 0.09 mg/gm (Fig.6). Higher concentration of DDT above 0.75 % caused failure of fruit production in *Lycoperscicon esculentum* and *Solanum melongena.* But in *Amaranthus gangeticus* concentration of DDT above 0.5% caused an inhibition of growth of the plant (Table 1).

The stress amino acid Proline in the leaves of the selected vegetable crops showed concomitant increase in its level with increase to exposure to the pesticides (Tables 1-3). In *Solanum melongena* a higher accumulation of this amino acid (618.5 ± 0.42 µg/ g D.wt.) was observed at 1% exposure of DDT, when compared with the other selected vegetable crops. This

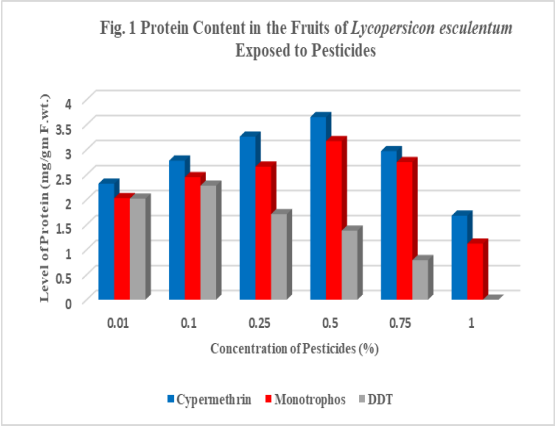
was followed by *Lycoperscicon esculentum* having 575.6 ± 0.03µg/ g D.wt at the same concentration of the pesticide (Fig 7). At 0.01 % Cypermethrin *Solanum melongena* leaves showed 375.3 ± 0.35µg/ g D.wt, while it was 323.4 ± 0.74 µg/ g D.wt in *Lycoperscicon esculentum* and 465.5 ± 0.24 µg/ g D.wt in *Amaranthus gangeticus*. Maximum level of Proline was observed when these vegetable crops were exposed to 1% Cypermethrin (Fig7-9). Around 647.7 ± 1.11 µg/ g D. wt was the level of stress aminoacid Proline in the leaves of *Solanum melongena* when the crop was exposed to 1% Monotrophos, it was 627.3 ± 1.33 µg/ g D.wt and 706.3 ± 1.06 µg/ g D.wt in leaves of *Lycoperscicon esculentum* and *Amaranthus gangeticus* respectively (Fig 7 & 9). At low levels of DDT (0.01-0.75%) *Solanum melongena*, the proline content varied from 406.5 ± 0.13 to 618.5 ± 0.42 µg/ g D.wt (Table 2: Fig 8). 373.4 ± 0.32 µg/ g D. wt of proline was calculated as the minimum amount of proline in the leaves of *Lycoperscicon esculentum*, while the maximum came around 575.6 ± 0.03 µg/ g D.wt at 0.75 % of the pesticide (Fig7). Increase in the concentration of DDT above 0.5% inhibited plant growth in *Amaranthus gangeticus*, while in the other experimental plants increase in DDT levels above 0.75% inhibited plant growth (Table 1-3: Fig. 9).

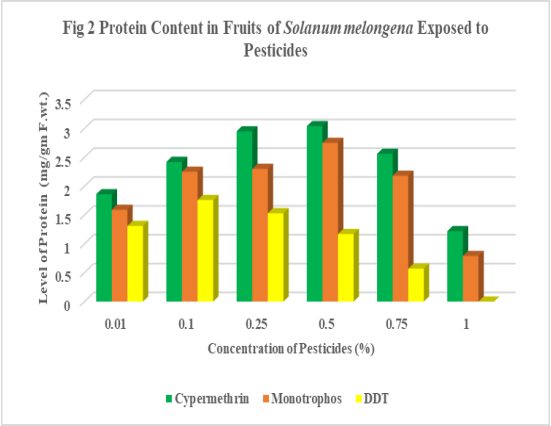
Total phenolic content in the experimental vegetables showed gradual increase with increase in the concentration from 0.01% to 0.75%, then a decrease in Total phenolic content was calculated (Tables1-3). In *Amaranthus gangeticus* it ranged from 28.3 ± 0.74 to 137.2 ± 0.45 mg/GAE/gm F.wt, in *Solanum melongena* the lowest Total Phenolic content was 36.6 ± 0.33 mg/GAE/gm F.wt at 0.01% and highest at 0.75% cypermethrin which came to 153.2 ± 0.45 mg/GAE/gm F.wt , while in *Lycoperscicon esculentum* it ranged from 31.2 ± 0.14 to 98.59 ± 0.08 mg/GAE/gm F.wt (Fig: 10-12). In *Amaranthus gangeticus* it the highest value was 80.08 ± 0.06 mg/GAE/gm F.wt, in *Solanum melongena* it was 90.19 ± 0.11 mg/GAE/gm F.wt and it came to 89.12 ± 0.23 mg/GAE/gm F.wt in *Lycoperscicon esculentum* (Fig10-12). However the exposure of the experimental vegetable crops to DDT drastically decreased the level of Total Phenols accumulated after 0.5% of DDT used (Table 1-3). In *Solanum melongena* the minimum Total phenolic content was 25.1 ± 0.23 mg/GAE/gm F.wt observed when the plant was exposed to 0.01% of DDT and higher concentration of DDT above 0.5 % decreased the secondary metabolite content in the useful part (Fig 11), while in *Lycoperscicon esculentum* 17.33 ± 0.84 mg/GAE/gm F.wt of total phenolic content was calculated at 0.01% DDT, and 62.8 ± 0.14

mg/GAE/gm F.wt at 0.5% of this pesticide was observed (Fig 10). In *Amaranthus gangeticus* the maximum Total Phenol accumulation was observed when the crop was exposed to 0.01% DDT and was 11.3± 0.46 mg/GAE/gm F. wt and at 0.5% of the pesticide it came to 66.7 ± 0.19 mg/GAE/gm F.wt and higher concentration of DDT above 0.5 % inhibited plant growth (Fig 12).

The Total Flavanoid Content (TFC) in the experimental crops showed a decrease when the exposure of pesticides was increased above 0.5%. In *Lycoperscicon esculentum* the level of TFC ranged from 83.4 ± 0.08mg/gm F.wt. at 0.01% cypermethrin exposure to 137.6 ± 0.21mg/gm F.wt. at 0.5% of the above pesticide (Fig 15:Table 3). Highest level of Total Flavanoid was 143.7 ± 0.17 mg/gm F.wt.at 0.5 % and lowest level of 97.4 ± 0.04 mg/gm F.wt at 0.01 % cypermethrin was observed in *Solanum melongena*, while in *Amaranthus gangeticus* 95.59 ± 0.08mg/gm F.wt was lowest at 1% Cypermethrin and highest of 156.6 ± 0.21mg/gm F.wt at 0.5% of the pesticide(Table1,2 : Fig:13 & 14). When the experimental vegetable crop *Amaranthus gangeticus* was exposed to pesticide Monotrophos at concentration ranging from 0.01 to 1% the level Total Flavanoid Content was 107.65 ± 0.52 and 97.59 ± 0.45 mg/gm F.wt. (Fig 13). The highest TFC of 139.5 ± 0.56 mg/gm F.wt. and lowest of 81.12 ± 0.63 mg/gm F.wt. in *Solanum melongena*, while in *Lycoperscicon esculentum* the TFC values ranged from 88.59 ± 0.49 at 1 % to129.4 ± 0.39mg/gm F.wt.at 0.5 % monotrophos (Fig 14& 15). About 147.87 ± 0.98 mg/gm F.wt. was observed in *Amaranthus gangeticus* at 0.5 % Monotrophos and lowest of 97.59 ± 0.45 mg/gm F.wt at 1% of the pesticide(Fig 13). The level of TFC when the experimental vegetable crops were exposed to DDT at concentrations ranging from 0.01 to 1 % of pesticide was seen decreasing in the parts analysed when the pesticide level was above 0.25 %. In *Amaranthus gangeticus* 81.76 ± 0.46 mg/gm F.wt. was the lowest TFC observed on exposure to 0.01 % DDT, while in *Solanum melongena* and *Lycoperscicon esculentum* it was 78.51 ± 0.23 and 73.67 ± 0.84 mg/gm F.wt. respectively (Fig 14 & 15). The maximum TFC level was calculated from parts of these experimental vegetable crops when the crops were exposed to 0.25 %DDT which came to 146.06 ± 0.43, 137.93 ± 0.38 and 123.06 ± 0.51 mg/gm F.wt. in *Amaranthus gangeticus*, *Solanum melongena* and *Lycoperscicon esculentum* respectively (Fig :13-15).

Control plants showed a proline level of 454.6 ± 1.35 µg/gm d.wt, in *Solanum melongena*, in *Amaranths gangeticus* it was 317.8 ± 0.84 µg/gm d. wt and in *Lycopersicon esculentum* the proline level was 366.7 ± 0.23 µg/gm d. wt. (Table 4). The level of Total Phenolic content and Total Flavonoid content was 101.1 ± 0.04 mg/gm F.wt. and 87.4 ± 0.08 in *Solanum melongena*, and *Lycopersicon esculentum* respectively, while in *Amaranths gangeticus* it was 118.3 ± 0.84 mg/gm F. wt.(Table 4). In *Amaranths gangeticus* the level of Total Phenolic Content was 27.4 ± 0.45 mg/GAE/gm F.wt, while in *Solanum melongena* it was 36.2 ± 0.45 mg/GAE/gm F.wt and 18.4 ± 0.45 mg/GAE/gm F.wt in *Lycopersicon esculentum* (Table 4). The protein content was 1.43 ± 0.11, 1.62 ± 0.57 and 2.12 ± 0.43 mg/gm f.wt. in *Amaranths gangeticus*, *Solanum melongena*, and *Lycopersicon esculentum* respectively. The total chlorophyll levels ranged from 2.453 ± 0.38, 2.976 ± 0.12 and 3.038 ± 0.69mg/gm f.wt. in *Amaranths gangeticus*, *Solanum melongena*, and *Lycopersicon esculentum* respectively (Table 4).





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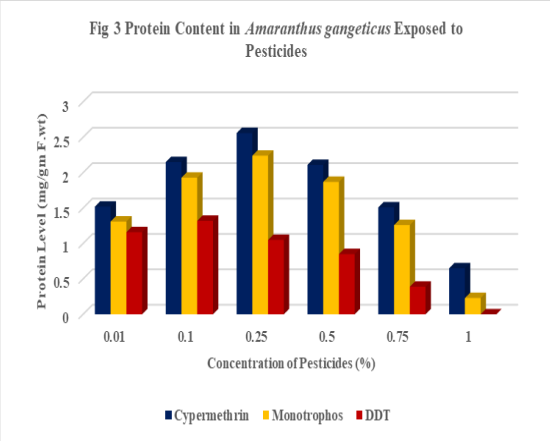

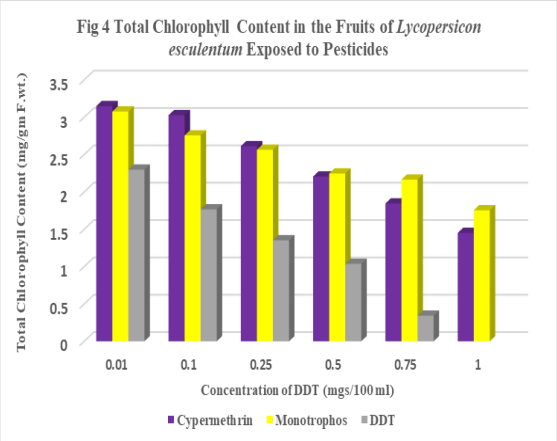

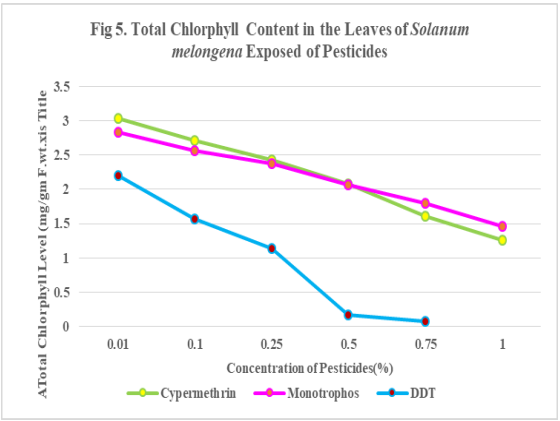

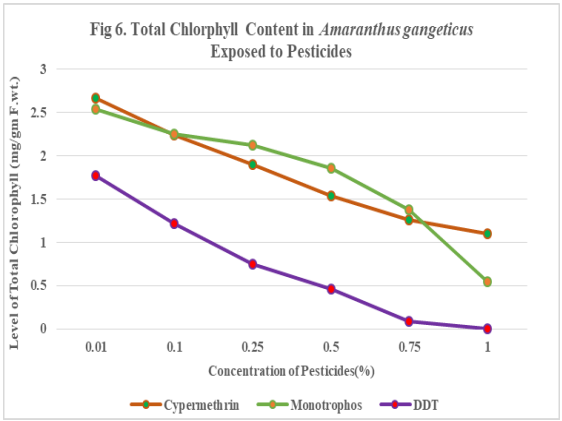

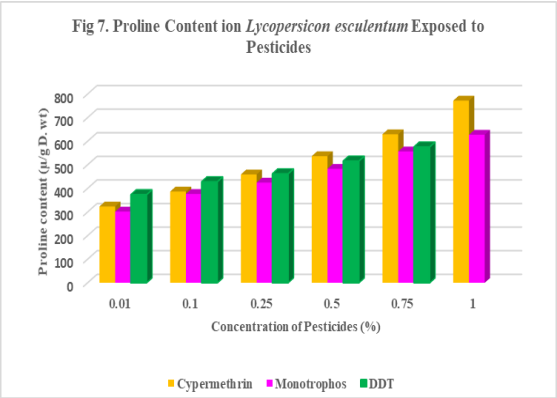

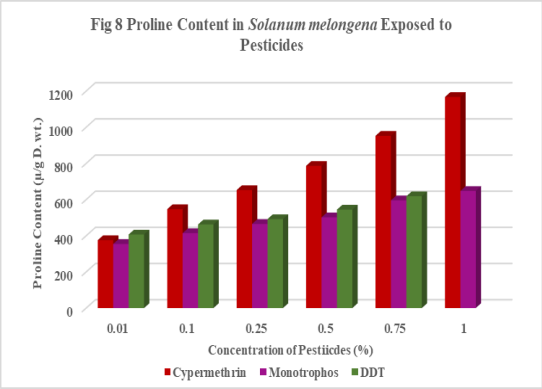

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Fig 9 Proline Content in *Amaranthus gangeticus* Exposed to Pesticides

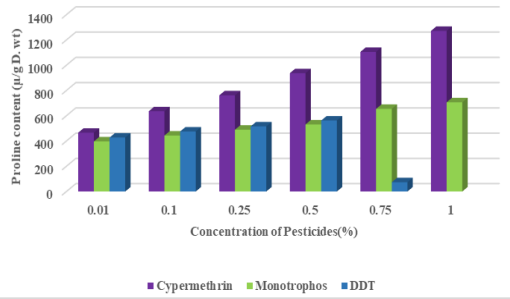

Fig 10. Total Phenol Content in *Lycopersicon esculentum* Exposed to Pesticides

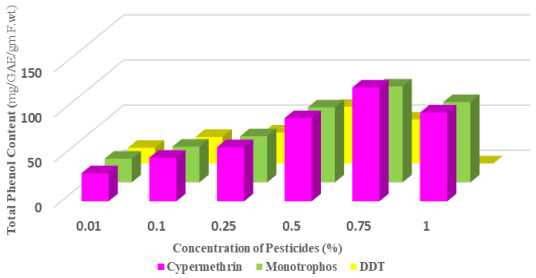

Fig 11. Total Phenol Content in *Solanum melongena* Exposed to Pesticides

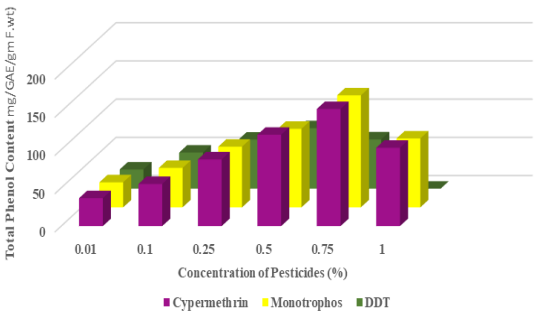

Fig 12. Total Phenol Content in *Amaranthus gangeticus* Exposed to Pesticides

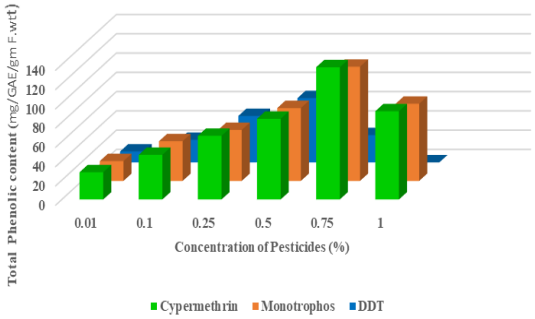

Fig. 13 Total Flavanoid Content in *Amaranthus gangeticus* Exposed to Pesticides

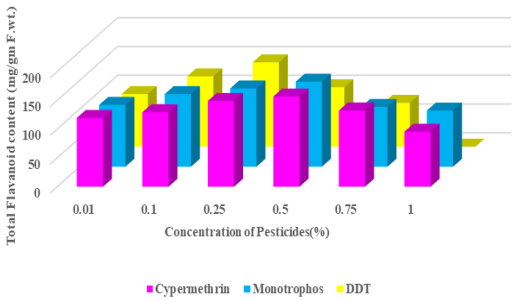

Fig 14 Total Flavanoid Content in*Solanum melongena* Exposed to Pesticides

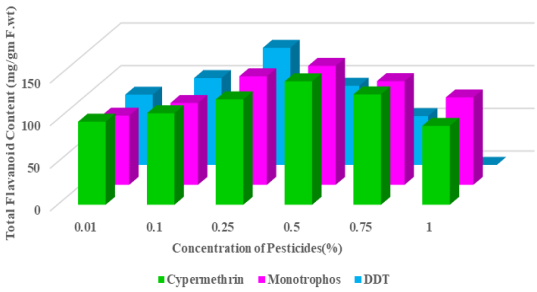

Fig.15 Total Flavanoid Content in *Lycopersicon esculentum* Exposed to Pesticides

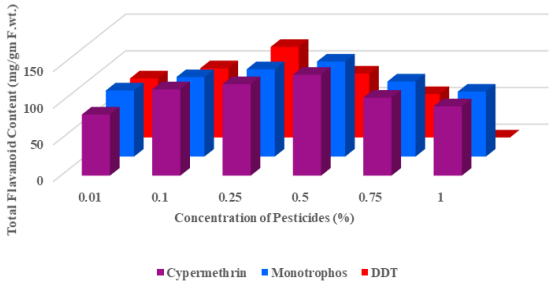

Table 1: Biochemical analysis of useful parts of Amaranthus gangeticus exposed to different pesticides

Vegetable used	Biochemical analysis	S. No.	Con. Used	Pesticide (%)		
				Cypermerthin	Monotrophos	DDT
Amaranthus gangeticus	Protein (mg/gm f.wt.)	1	0.01	1.52 ± 0.64	1.31 ± 0.52	1.16 ± 0.46
		2	0.1	2.15 ± 0.32	1.93 ± 0.46	1.32 ± 0.78
		3	0.25	2.56 ± 0.06	2.24 ± 0.17	1.05 ± 0.43
		4	0.5	2.11 ± 0.21	1.87 ± 0.98	0.85 ± 0.29
		5	0.75	1.51 ± 0.45	1.26 ± 0.25	0.39 ± 0.67
		6	1.0	0.65 ± 0.08	0.23 ± 0.06	1.16 ± 0.46
	Total Chlorophyll (mg/gm f.wt.)	1	0.01	2.665 ± 0.56	2.532 ± 0.32	1.767 ± 0.78
		2	0.1	2.243 ± 0.23	2.254 ± 0.54	1.212 ± 0.54
		3	0.25	1.896 ± 0.52	2.116 ± 0.80	0.745 ± 0.12
		4	0.5	1.537 ± 0.46	1.854 ± 0.35	0.457 ± 0.09
		5	0.75	1.262 ± 0.73	1.372 ± 0.79	-
		6	1.0	1.093 ± 1.32	0.543 ± 0.85	-
	Proline (µg/gm D.wt)	1	0.01	465.5 ± 0.24	396.6 ± 0.34	427.3 ± 0.53
		2	0.1	635.4 ± 0.57	443.2 ± 0.61	475.1 ± 0.43
		3	0.25	761.3 ± 0.91	489.6 ± 0.42	515.7 ± 0.86
		4	0.5	936.7 ± 0.43	531.0 ± 0.55	562.1 ± 0.25
		5	0.75	1105.1 ± 0.78	654.2 ± 0.75	-
		6	1.0	1271.4 ± 1.07	706.3 ± 1.06	-
	Phenol (mg/GAE/gm f.wt)	1	0.01	28.3 ± 0.74	20.65 ± 0.52	11.3 ± 0.46
		2	0.1	46.3 ± 0.32	41.25 ± 0.16	23.4 ± 0.68
		3	0.25	66.1 ± 0.06	52.91 ± 0.17	48.2 ± 0.63
		4	0.5	83.7 ± 0.21	75.63 ± 0.58	66.6 ± 0.19
		5	0.75	137.2 ± 0.45	118.5 ± 0.21	28.2± 0.19
		6	1.0	91.59 ± 0.08	80.08 ± 0.06	-
	Flavonoid (mg/gm f.wt)	1	0.01	119.3 ± 0.84	107.6 ± 0.52	91.6 ± 0.86
		2	0.1	129.3. ± 0.14	126.3 ± 0.32	122.3 ± 0.65
		3	0.25	148.91 ± 0.65	136.1 ± 0.24	146.6 ± 0.43
		4	0.5	156.6 ± 0.21	147.8 ± 0.98	103.3 ± 0.16
		5	0.75	132.45 ± 0.06	104.0 ± 0.06	76.34 ± 0.97
		6	1.0	95.59 ± 0.08	97.59 0.45	-

* Mean of 6 replicates ± S.E

Table 2: Biochemical analysis of useful parts of *Solanum melongena* exposed to different pesticides

Vegetable used	Biochemical analysis	S. No.	Con. Used	Pesticide (%)		
				Cypermerthin	Monotrophos	DDT
Solanum melongena	Protein (mg/gm f.wt.)	1	0.01	1.86 ± 0.37	1.59 ± 0.41	1.31 ± 0.23
		2	0.1	2.42 ± 0.72	2.25 ± 0.65	1.76 ± 0.54
		3	0.25	2.95 ± 0.12	2.30 ± 0.22	1.53 ± 0.38
		4	0.5	3.04 ± 0.17	2.75 ± 0.39	1.17 ± 0.87
		5	0.75	2.56 ± 0.45	2.18 ± 0.23	0.57 ± 0.36
		6	1.0	1.22 ± 0.04	0.79 ± 0.11	-
	Total Chlorophyll (mg/gm f.wt.)	1	0.01	3.029 ± 0.36	2.836 ± 0.19	2.196 ± 0.73
		2	0.1	2.706 ± 0.43	2.557 ± 0.59	1.567 ± 0.28
		3	0.25	2.433 ± 0.76	2.375± 0.21	1.134 ± 0.96
		4	0.5	2.081 ± 0.34	2.063 ± 0.57	0.165 ± 0.05
		5	0.75	1.603 ± 0.78	1.797 ± 0.23	0.074 ± 0.65
		6	1.0	1.257 ± 1.03	1.463 ± 0.74	-
	Proline (µg/gm D.wt)	1	0.01	375.3 ± 0.35	354.5 ± 0.48	406.5 ± 0.13
		2	0.1	546.7 ± 0.58	414.2 ± 0.85	462.3 ± 0.56
		3	0.25	652.4 ± 0.54	465.1 ± 0.27	491.8 ± 0.76
		4	0.5	786.1 ± 0.76	501.1 ± 0.54	544.2 ± 0.32
		5	0.75	952.3 ± 0.85	595.3 ± 0.31	618.5 ± 0.42
		6	1.0	1167.5 ± 1.04	647.8 ± 1.11	-
	Phenol (mg/GAE/gm f.wt)	1	0.01	36.6 ± 0.33	32.59 ± 0.67	25.1 ± 0.23
		2	0.1	55.98 ± 0.72	51.61 ± 0.45	47.2 ± 0.54
		3	0.25	87.35 ± 0.12	79.42 ± 0.62	64.8 ± 0.68
		4	0.5	119.5 ± 0.17	102.8 ± 0.39	79.5 ± 0.12
		5	0.75	153.2 ± 0.45	146.6 ± 0.13	64.4 ± 0.27
		6	1.0	102.49 ± 0.04	90.19 ± 0.11	-
	Flavonoid (mg/gm f.wt)	1	0.01	97.4 ± 0.04	81.12 ± 0.63	82.36 ± 0.21
		2	0.1	107.3 ± 0.22	95.98 ± 0.37	101.8 ± 0.71
		3	0.25	133.3 ± 0.18	127.3 ± 0.76	137.3 ± 0.38
		4	0.5	143.7 ± 0.17	139.5 ± 0.56	92.76 ± 0.42
		5	0.75	129.4 ± 0.76	121.6 ± 0.38	57.2 ± 0.32
		6	1.0	92.49 ± 0.04	102.49 ± 0.04	-

* Mean of 6 replicates ± S.E

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Table 3: Biochemical analysis of useful parts of Lycoperscicon esculentum exposed to different pesticides

Vegetable used	Biochemical analysis	S. No.	Con. Used	Pesticide (%)		
				Cypermerthin	Monotrophos	DDT
Lycoperscicon esculentum	Protein (mg/gm f.wt.)	1	0.01	2.32 ± 0.64	2.03 ± 0.83	2.02 ± 0.84
		2	0.1	2.78 ± 0.64	2.46 ± 0.55	2.28 ± 0.69
		3	0.25	3.26 ± 0.06	2.66 ± 0.17	1.71 ± 0.51
		4	0.5	3.65 ± 0.21	3.18 ± 0.38	1.38 ± 0.19
		5	0.75	2.89 ± 0.45	2.79 ± 0.52	0.79 ± 0.09
		6	1.0	1.69 ± 0.08	1.12 ± 0.63	-
	Total Chlorophyll (mg/gm f.wt.)	1	0.01	3.149 ± 0.46	3.076 ± 0.38	2.298 ± 0.29
		2	0.1	3.027 ± 0.77	2.756 ± 0.82	1.767 ± 0.71
		3	0.25	2.612 ± 0.45	2.564 ± 0.67	1.352 ± 0.21
		4	0.5	2.206 ± 0.76	2.247 ± 0.51	1.037 ± 0.34
		5	0.75	1.845 ± 0.54	2.165 ± 0.21	0.343 ± 0.67
		6	1.0	1.451 ± 0.87	1.754 ± 1.00	-
	Proline (µg/gm D.wt)	1	0.01	323.4 ± 0.74	301.7 ± 0.76	373.4 ± 0.32
		2	0.1	386.7 ± 0.83	376.5 ± 0.44	428.2 ± 0.72
		3	0.25	459.2 ± 0.65	424.8 ± 0.87	461.7 ± 0.31
		4	0.5	536.5 ± 0.66	483.1 ± 0.53	515.8 ± 0.14
		5	0.75	629.5 ± 0.86	556.5 ± 0.46	575.6 ± 0.03
		6	1.0	772.1 ± 1.11	627.3 ± 1.33	-
	Phenol (mg/GAE/gm f.wt)	1	0.01	31.2 ± 0.14	26.3 ± 0.63	17.3 ± 0.84
		2	0.1	48.3 ± 0.32	39.6 ± 0.75	29.4 ± 0.69
		3	0.25	60.1 ± 0.06	51.2 ± 0.37	34.2 ± 0.51
		4	0.5	92.4 ± 0.21	83.2 ± 0.98	62.8 ± 0.14
		5	0.75	126.3 ± 0.45	106.45 ± 0.42	48.7 ± 0.49
		6	1.0	98.59 ± 0.08	89.12 ± 0.23	-
	Flavonoid (mg/gm f.wt)	1	0.01	83.4 ± 0.08	90.19 ± 0.11	80.54 ± 0.49
		2	0.1	117.3 ± 0.76	108.3 ± 0.51	94.43 ± 0.44
		3	0.25	124.8± 0.32	119.1 ± 0.42	123.6 ± 0.51
		4	0.5	137.6 ± 0.21	129.4 ± 0.39	87.21 ± 0.11
		5	0.75	102.4 ± 0.42	106.3 ± 0.05	59.32 ± 0.55
		6	1.0	94.59 ± 0.08	88.59 ± 0.49	-

* Mean of 6 replicates ± S.E

Table 4: Molecular Profile of Control Plants

Name of Plant	Protein content (mg/gm f.wt.)	Total Chlorophyll content (mg/gm f.wt.)	Proline content (µ/g D. wt.)	Total phenolic content (mg/GAE/gm F. wt)	Total Flavanoid content (mg/gm f.wt.)
Amaranthus gangeticus	1.43 ± 0.11	2.453 ± 0.38	317.8 ± 0.84	27.4 ± 0.45	118.3 ± 0.84
Solanum melongena	1.62 ± 0.57	2.976 ± 0.12	454.6 ± 1.35	36.2 ± 0.45	101.1 ± 0.04
Lycoperscicon esculentum	2.12 ± 0.43	3.038 ± 0.69	366.7 ± 0.23	18.4 ± 0.45	87.4 ± 0.08

DISCUSSION

The controlled application of a wide variety of pesticides on crop plants is necessary in the tropics to combat pests and vector borne diseases. However, the sporadic and indiscriminate use has been leading to significant consequences not only to public health but also to food quality resulting in an impact load on the environment. Inappropriate application of pesticides affects the whole ecosystem by entering the residucs in food chain and polluting the soil, air, ground and surface water (ICAR, 2006). The present investigation was carried out to find out the effect of commonly used pesticides on a few biochemical parameters of vegetable crops viz – *Amaranthus gangeticus*, *Solanum melongena* and *Lycopersicon esculeutum.* The experiments were conducted using control group (the above said vegetables exposed to biopesticide viz flower extract of *Tagetes minuta* during pest infestations and experimental group in which the above said vegetables plants were exposed to three commonly used pesticides like Monocontrophos, cypermethrin and DDT during pest infestations. After 90-100 days of pesticide application, the plants (experimental and control) were evaluated biochemically.

Biochemically the experimental plants and control plants were evaluated quantitatively for carbohydrates, proteins and pigment chlorophyll the basic building blocks of living organism are indicates of the health status of plants. Total Phenolic compounds, Total Flavanoid Content and stress amino acid Proline was also analysed in to find out the stress level in the experimental plants. In the present investigation it was observed that the chlorophyll and carbohydrate level in the experimental crops decreased with the application of varying levels of pesticides during pets infestation when compared with the control plants exposed to biopesticides during the same

time. Chlorophyll, the pigment molecule which indicates the health status of plant and decrease in pigment level here can be correlated with the performance of photosynthesis in these plants. Abd-Alla and Omar 1993 and Vijayarengan, 2011 has also observed similar observation in Faba bean and *Amaranthus* respectively. The changes in photosynthetic pigments of plants are usually used as a tool for the assessment of stressful conditions (Ashraf and Harris 2013). The analysis of pigments in the present study showed a decrease in photosynthetic pigments in tomato with increasing concentration of pesticides. According to kilic *et al*., 2015, photosynthetic pigment content is an important indicator for determining plant photosynthesis ratio and pigments are inhibited in the presence of stress, thus altering the photosynthesis of plants(Yuan *et al*., 2013). Mallakin *et al*., 2007 ha shown that inhibition of photosynthesis is a reliable assay of the potential toxicity and xenobiotics contaminants towards plants. Gaikwad *et al*., 2016 recommended the dose fixed by agricultural experts with the logic of spraying less often and using fewer and better selected pesticides only when needed can reduce the pesticides stress Concomitant decrease in carbohydrate content is due to low performance of photosynthesis as there was decrease in Total Chlorophyll levels in plants exposed to chemical pesticides.

The amount of protein was assayed in the experimental plants and control to find out the influence of inorganic pesticides on this biomolecule. In the present investigation, it was noticed that the exposure to chemical pesticides negatively affected the level of protein in the useful parts of vegetable plants. At low concentration of pesticides (0.01-0.05%) though there was increase in this biomolecules, but above 0.05 % pesticide concentration there was decrease in protein levels. The initial increase in protein levels may be due to the fact that the stressed plants start synthesizing more enzymes to counteract metabolize the pesticides which enter the plant system.. Among the pesticides DDT caused the highest decrease in this biomolecule above 0.5% concentration. According to Shakir *et al*., 2015, tomato treated with emamectin also exhibited significant reduction in protein content, however, the effects were less severe as compared with plants exposed to higher concentrations of imidacloprid and cypermethrin.. Pesticides when sprayed onto vegetable plants may interact with plant surfaces, enter the transport system and can introduce effect on metabolism (Lydon and Duke, 1989). Johnson and Aoyanol 2007, also observed that decrease in protein levels in *Amaranths* plants exposed to monocrotophos.

Flavonoids are an important class of low-molecular-weight phenolic compounds - plant secondary metabolites which having a polyphenolic structure, widely found in fruits, vegetables

and certain beverages. Flavonoids have several subgroups, which include chalcones, flavones, flavonols and isoflavones. In plants, flavonoids have long been known to be synthesised in particular sites and are responsible for the colour and aroma of flowers, and in fruits to attract pollinators and consequently fruit dispersion to help in seed and spore germination, and the growth and development of seedlings (Griesbach , 2005). Flavonoids protect plants from different biotic and abiotic stresses and act as unique UV filters, function as signal molecules, allopathic compounds, phytoalexins, detoxifying agents and antimicrobial defensive compounds. Flavonoids have roles against frost hardiness, drought resistance and may play a functional role in plant heat acclimatisation and freezing tolerance (Takahashi and Ohnishi, 2004: Samanta *et al*., 2011). The most abundant flavonoids, flavonols accumulate in their glycosylated form after an inductive light treatment and absorb UV-B light in the 280-320 nm region and are therefore regarded as effective UV filters (Solovchenko and Schmitz-Eiberger, 2003). In the present investigation the level of total flavonoids linearly increased in the experimental vegetable crops with increase in pesticide concentration till 0.5% thereafter it started to decrease. The initial increase in Total Flavanoid Content is due to the protective nature of Flavanoids which can act as antioxidants to debar the toxic effects of pesticides absorbed. The pollutants in the environment are able to induce the intracellular over-production of reactive oxygen species (ROS), thus damaging plant cells. It is known that the reaction of such radicals with macromolecules particularly lipoprotein caused peroxidative damages more rapidly and is evident from membrane lipids destruction (Jan *et al*., 2012b). Aphrodite, 2017 observed an increase in Total Flavanoid content in aromatic herbs exposed to agrochemicals.

The Total Phenolic Content in the experimental vegetables also increased with increase in exposure to pesticides, however at higher concentration of pesticides the level of Total Phenols decreased and the lower levels of Phenols was recorded in vegetable crops exposed to varying levels of DDT. eneral tendency in this study is the reducing levels of phenolic compounds in herbs exposed to pesticides- fertilizers combinations. This could be due to certain suppressive effect exerted by pesticides on the development of plant defense molecules among phenols. Such reducing effects could be due to the presence of herbicides such as Tromissil and Dino as this class of pesticide has shown to reduce the carbon fixation by plants, with potential decreasing effect on the proportion of carbon allocated to the synthesis of secondary compounds (Daniel et al., 1999). Moreover, inorganic fertilizers have been demonstrated to inhibit the synthesis of phenolic compounds in plants at high

concentrations. This could be due to certain suppressive effect exerted by pesticides on the development of plant defense molecules among phenols.

A decrease in Phenolic Content was observed by Aphrodite, 2017 as the general tendency in his study on aromatic herbs exposed to pesticides- fertilizers combinations. HE is of the opinion that this effect is due to certain suppressive effect exerted by pesticides on the development of plant defense molecules such as phenols. Certain pesticides has shown to reduce the carbon fixation by plants, with potential decreasing effect on the proportion of carbon allocated to the synthesis of secondary compounds (Daniel *et al*., 1999). Such decreased carbon fixation is also noticed in the present investigation which can be correlated with decreased Total Phenolic Content on exposure of higher levels of pesticides. Moreover, inorganic fertilizers have been demonstrated to inhibit the synthesis of phenolic compounds in plants at high concentration and their high and preferential use by the farmers could significantly restrict synthesis of the compounds (Nguyen and Niemeyer, 2008).

Proline is an amino acid produced using glutamate and arginine under normal conditions, and glutamate is the main pathway in stress conditions (Chen and Kao,1993). According to Marques *et al*., 2011, amino acid has function of cell protection against denaturation processes when plants are exposed to inadequate situations as mineral, salt and water stresses, because this organic compound is highly soluble in water. Proline is accumulated in cytoplasm of cells present in leaves, stems and roots. In the present investigation this amino acid level in the leaves of the experimental crops showed concomitant increase with increase in the concentration of pesticides exposure to the vegetable crops. Modifications in proline level are intensively described as a biochemical consequence promoted by abiotic stresses, as reported by Lima *et al*., 2004 studying *Oryza sativa* plants induced to salt stress. Lobato *et al*., in 2008, found significant changes in *Glycine max* plants under water deficit. Weinberg *et al*.,1984 found a positive correlation between proline accumulation and increased salt tolerance in sorghum plants. The hydrolysis of proteins in salt stress situations, according to several researchers, may be responsible for this increase in proline contentas noted by Torello and Rice, 1986.

This work is only a basic study which was able to bring out the stress level of everyday vegetables by screening the marker biomolecules. However this work can be taken ahead for substantiating the above information with the GCMS profile analysis of the experimental plants

which can bring out the metabolites of pesticide catabolism in the crops.

CONCLUSION:

The study highlights the deleterious effects of commonly used pesticides Cypermethrin, Monocrotophos, and DDT on the biochemical profile of vegetable crops. Key findings reveal that while low concentrations of these pesticides initially enhance some biochemical markers such as protein and flavonoid content, higher concentrations lead to detrimental effects, including reductions in total chlorophyll, carbohydrates, and secondary metabolites. These changes reflect the stress levels and altered physiological responses in the plants. Conversely, the use of biopesticides like *Tagetes minuta* flower extract demonstrated beneficial effects on these parameters, promoting healthier plant growth and biochemical stability. This research underscores the need for controlled and reduced use of chemical pesticides to mitigate their harmful impact on food quality, environmental health, and human well-being. Future studies involving advanced analytical techniques like GC-MS are recommended to further explore the metabolic pathways affected by pesticide residues.

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