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RESEARCH ARTICLE

Estimation of biochemicals constituents in the yellow vein mosaic virus infected leaves of okra [*Abelmoschus esculentus* (L.) Moench] after sprays of insecticides and botanicals

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ABSTRACT

Chlorophyll 'a', chlorophyll 'b' and total chlorophyll contents were found increased in the plants sprayed with Dimethoate 30% EC (1 ml/l), Imidacloprid 17.8% SL (1 ml/3 L), Azadirachtin 1500 ppm (5 ml/l) and Karanj oil (2%) and two sprays of these chemicals followed by one spray of botanical *i.e.* Azadirachtin 1500 ppm (5 ml/l) and Karanj oil (2%). Total sugar contents were found increased in the plants treated with insecticide and botanicals, while total phenol was decreased in yellow vein mosaic virus (YVMV) infected leaves of okra.

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INTRODUCTION

Okra [*Abelmoschus esculentus* (L.) Moench.] commonly referred to as lady's finger belongs to the family Malvaceae, is an annual herb primarily valued for its edible pods. Several species of genus *Ablemoschus* are grown in many parts of the world. Okra originated in tropical Africa (Purseglove, 1987) is an important vegetable throughout the tropical and subtropical regions of the world. It is extensively cultivated in *Kharif* throughout India and also during summer seasons for its tender fleshy fruits. Yellow vein mosaic virus (YVMV) is a devastating viral disease transmitted through white fly (*Bemesia tabaci*) in okra. In India, the occurrence of this disease was first reported by Kulkarni (1924) in Bombay province. It has been reported that when plants infected at 20, 35 and 50 days after germination, the losses are upto an extent of 98, 83 and 49 per cent, respectively (Shastry and Singh, 1974). Therefore, in this study efforts have been made to estimate the chlorophyll a, b and total chlorophyll, sugar and phenol in the YVMV infected leaves of okra with chemicals and botanicals.

MATERIAL AND METHODS

Estimation of total chlorophyll, chlorophyll 'a' and 'b' :

Materials:

Dilute analytical grade acetone to 80% acetone (prechilled).

Procedure:

Acetone reagent method was used for detecting chlorophyll content in okra leaves 100 mg sample from middle part of leaves collected separately from healthy and diseased plants. The leaves were crushed in pestle and mortar using 10 ml of 80 per cent acetone for extraction of chlorophyll pigments. Centrifuged the homogenized extract at 5000 rpm for 5 minute and transferred the supernatant to a 100 ml. volumetric flask. Ground the residue with 100 ml of 80% acetone, again centrifuged and transferred the supernatant to the same volumetric flask. The procedure was repeated until the residue was colourless. Washed the pestle and mortar thoroughly with 80% acetone and collected the clear washing in the same volumetric flask. The volume of supernatant was made to 100

ml by adding 80 per cent acetone. An optical density of each pigment extract was taken at 645 and 663 nm on spectronic 20 spectrophotometer. A blank reading was taken by using 80 per cent acetone separately. The amount of chlorophyll 'a', 'b' and total chlorophyll per cent in the extract were calculated, mg chlorophyll per g tissue using the following equations :

$$\text{mg chlorophyll a / g tissue} = 12.7(A_{663}) - 2.69(A_{645}) \times \frac{V}{1000 \times W}$$

$$\text{mg chlorophyll b / g tissue} = 22.9(A_{645}) - 4.68(A_{663}) \times \frac{V}{1000 \times W}$$

$$\text{mg total chlorophyll tissue} = 20.2(A_{645}) + 8.02(A_{663}) \times \frac{V}{1000 \times W}$$

where,

A = absorbance at specific wavelengths

V = Final volume of chlorophyll extract in 80% aceton

W = Fresh weight of tissue extracted

Estimation of total sugar :

Materials **:**

- 2.5 N – HCl
- Anthrone reagent: - Dissolved 200 mg anthrone in 100 ml of ice cold 95% H_2SO_4. Prepared fresh before use.
- Standard glucose :-
- Stock: - Dissolved 100 mg dextrose in 100 ml water.
- Working standard: - 10 ml of stock diluted to 100 ml with distilled water. Store refrigerated after adding a few drop of toluene.

Preparation of standards curve **:**

Prepared the standard by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 m of the working standard. 'O' serves as blank make up the volume to 1 ml in all tubes. 4 ml of Enthrone reagent was added and was heated for eight minutes in a boiling water bath.

It was cooled rapidly and read the green to dark green colour at 630 nm. Standard graph by plotting concentration of standard on the X-axis versus absorbance on the Y-axis was drawn.

The amount of total sugar was estimated by enthrone reagent (Dubois *et al.*, 1956). 100 mg samples from middle part of fruits was weighed separately from healthy and diseased plants. Tissue samples were crushed in pestle and mortar using 10 ml of 80% ethanol for extracting total sugar. Transferred the homogenized to test tube and centrifuged at 5000 rpm for 10 minutes and supernatant was collected in test tubes and took 0.5 ml aliquots in other test tubes for analysis. Increased the volume to 1 ml in all tubes by adding distilled water. Then added 4 ml of anthrone reagent. The tube was placed in boiling water bath for eight minutes and aluminum foil was placed on the open end to prevent evaporation. It was cooled in running water and the absorbance of the blue green solution was recorded at 630 nm on spectronic – 20 spectrophotometer – reagent blank containing 2 ml water and 4 ml enthrone reagent was used to adjust absorbance at zero. The quantity of sugar from each sample was calculated from the standard curve prepared from different concentrations. Amount of carbohydrate present in 100 mg of the samples :

$$= \frac{\text{mg of glucose}}{\text{volume of test samples}} \times 100$$

Estimation of total phenol :

Materials:

- 80% ethanol
- Folin – Ciocalteau reagent
- Na_2CO_3, 20%
- Standard (100 mg catechol in 100 ml water)
- Diluted 10 times for working standard.

Procedure:

The total phenol was estimated by Folin Ciocalteau method of Bray and Thrope (1954). Total phenolic compound from healthy and diseased fruits of okra were extracted from 100 mg of fresh fruit tissue in 5 ml of 80% ethanol. Centrifuged and pooled out the supernatant. Supernatant were evaporated to dryness by heating in boiling water bath. The residue was dissolved in a known volume of distilled water (5 ml). 0.2 ml aliquat were pipetted out into test tubes. The volume was made 3 ml in each tube with distilled water and 0.5 ml of folin – Ciocateau reagent was added. After 3 minutes, 2 ml of 20% Na_2CO_3 solution was added to each tube.

The solution was mixed thoroughly. The tubes were placed in a boiling water bath for exactly one minute, cooled and its absorbance was measured at 650 nm on a spectrophotometer. A blank containing all the reagents mixes fruit extract was used to adjust the absorbance to zero. A standard curve was prepared using catechol. Total phenols in different samples were calculated by the help of the standard curve and the amount was expressed as mg/g fresh weight.

RESULTS AND DISCUSSION

The result of the present investigation as well as relevant discussion have been presented under following heads :

Effect of insecticides and botanicals on the chlorophyll content of okra leaves :

For biochemical analysis, one susceptible variety *i.e.* Nirmal-101 (Nisha) was grown in Randomized Block Design, replicated thrice at Horticulture farm, Rajasthan College of Agriculture, Udaipur. Plots were sprayed with Dimethoate 30% EC (1 ml/l), Imidacloprid 17.8% SL (1ml/3l) and Azadirachtin 1500 ppm (5ml/l), Karanj oil (2%) alone and insecticides were also used in combination with each botanical *i.e.* Azadirachtin

1500 ppm (5ml/l), Karanj oil (2%). Two sprays were given at vegetative stage at 15 days intervals followed by one spray with botanicals. Samples were collected seven days after spraying, for estimation of chlorophyll 'a' chlorophyll 'b' and total chlorophyll contents in the leaves. The acetone reagent method was used for quantitative determination. The observation was recorded on spectrophotometer (model, Hitachi U-2000) at 645 nm and 663 nm wave lengths. The experiment was repeated with three replications in each treatment. The amount of the pigment in the samples was calculated and the average results are presented in Table 1.

Table 1 indicates that total chlorophyll contents increased in all the treatments (T_1–T_8). Maximum increased in total chlorophyll (63.37%) contents was found in the plants treated with Imidacloprid and Azadirachtin whereas chlorophyll 'a' and chlorophyll 'b' were also increased. Similarly, chlorophyll 'a', chlorophyll 'b' and total chlorophyll (54.45%) was found to be increased in the samples treated with Imidacloprid + Karanj oil. Hence, it is clear (Table 1 and Fig. 1) that chlorophyll 'a', chlorophyll 'b' and total chlorophyll contents increased in the okra plants when sprayed with Dimethoate 30% @ (1ml/l), Imidacloprid 17.80 SL (1 ml/3l) Azadirachtin 1500 ppm (5 ml/l), Karanj oil (2%) alone. Maximum total chlorophyll content was recorded in imidacloprid used with Azadirachtin (0.513mg/g) followed by Imidacloprid + Karanj oil (0.485mg/g). Similar trend was observed when Dimethoate used along with Azadirachtin (0.477mg/g) followed by Dimethoate +Karanj oil (0.422mg/g). The results were superior when insecticides were used along with botanicals and increased the total chlorophyll content in the leaves.

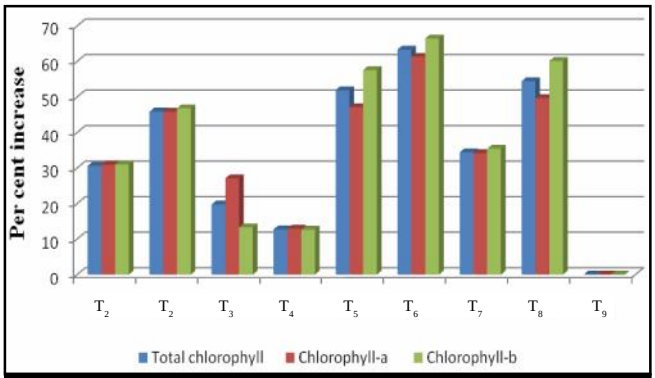

Fig. 1 : Effect of different insecticides and botanicals on the chlorophyll content in okra leaves

Effect of insecticides and botanical on total sugar and phenols:

Total sugar and phenol contents were estimated in the fresh fruit tissue of okra plants sprayed with different insecticides and botanicals and their combinations. The Folin – Ciocalteau reagent method developed by Bray and Thrope (1954) was used for estimation of phenol whereas total sugar contents were estimated according to anthrone reagent method developed by Dubois *et al*., 1956. Three samples from each treatment were analyzed for estimation of total sugar and phenol contents and the average results are presented in Table 2.

Results of Table 2 and Fig. 2 show that total sugar contents increased in the plants sprayed with insecticides and botanicals. It is apparently clear from the results that total sugar contents were found increased as compared to untreated control. The maximum increased in total sugar content(63.23%) was recorded in plant sprayed with Imidacloprid 17.8% SL (1 ml/3l) + Azadirachtin 1500 ppm (5ml/l) followed by the plant sprayed with Imidacloprid 17.80 SL (1ml/3l + Karanj oil 2%. Minimum total sugar (150.0 mg/g) content was found in plant sprayed with Karanj oil 2%.

Further, phenol content was decreased in treatment (T_1–T_8) as compared to untreated control plant. Maximum decrease

Table 1 : Effect of yellow vein mosaic virus disease of okra on chlorophyll content after spray of insecticides and botanicals

Sr. No.	Treatments	P.D.I.	Total chlorophyll (mg/g)	Per cent increase over control	Chlorophyll-a (mg/g)	Per cent increase over control	Chlorophyll-b (mg/g)	Per cent increase over control
1.	Dimethoate 30% EC (1ml/l)	15.3	0.410	30.57	0.203	30.96	0.207	31.01
2.	Imidacloprid (1ml/3l)	09.8	0.458	45.85	0.226	45.80	0.232	46.83
3.	Azadirachtin (5ml/l)	20.6	0.376	19.74	0.197	27.09	0.179	13.29
4.	Karanj oil (2%)	27.2	0.354	12.73	0.175	12.90	0.178	12.65
5.	Dimethoate + Azadirachtin	08.9	0.477	51.91	0.228	47.09	0.249	57.59
6.	Imidacloprid + Azadirachtin	07.6	0.513	63.37	0.250	61.29	0.263	66.45
7.	Dimethoate + Karanj oil	13.4	0.422	34.39	0.208	34.19	0.214	35.44
8.	Imidacloprid + Karanj oil	08.3	0.485	54.45	0.232	49.67	0.253	60.12
9.	Control	40.9	0.314	–	0.155	–	0.158	–
	SEm±		0.013		0.007		0.006	
	CD at 5%		0.038		0.2		0.018	
	CV (%)		5.20		5.56		4.95	

of phenol (48.87%) was found in plant sprayed with Imidacloprid 17.80 SL (1ml/3l) + Azadirachtin 1500 ppm (5ml/l) followed by Imidacloprid 17.80 (1ml/3 L) + Karanj oil 2% (44.63%). The minimum decrease in phenol was found in plant sprayed with Karanj oil 2%.

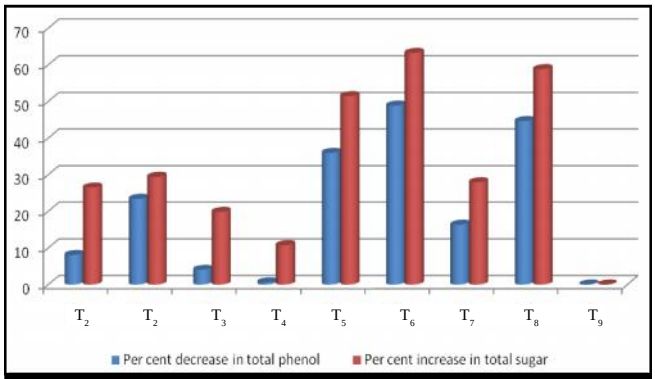

Fig. 2 : Effect of different insecticides and botanicals on total sugar and total phenol content of okra leaves

It is well known fact that systemic insecticides are absorbed and translocated acropettally or basipettally or they are ambimobile within the plant system. Due to profound systemicity these compounds influenced various physiological and biochemical processes, which may result in a beneficial or harmful effect to the host while others, modify the host physiology in such a way that the plant acquires resistance against the disease. This subject has been extensively reviewed by various workers (Mondahar *et al.*, 1971; Sastry and Singh, 1974; Singh and Srivastava, 1974; Bhagat and Yadav, 1977 and Singh *et al.*, 1983). As a consequence of systemic fungicide-plant interaction, the amounts of several physiological and biochemical components of plant such as chlorophylls, total sugar, total phenol etc. have been found altered. Mondahar and Singh (1971) described that both chlorophyll-a and chlorophyll-b were destroyed by yellow vein mosaic of okra. Regupathy and Jayraj (1972) analyzed that diseased leaves contained increased amount of different form of nitrogen, sucrose, moisture, phosphorus and potassium and decreased amount of total carbohydrates, calcium and magnesium as compared to healthy leaves. Sharma *et al*. (1995) reported that BYVMV infection reduced the chemical constituents of okra leaves such as chlorophyll (total 'a' and 'b'), reducing sugar, phosphorus and potassium contents, whereas, total phenols, total sugar, non-reducing sugar, nitrogen and protein contents enhanced. In green fruits, the total sugar, reducing sugar, non-reducing sugar, nitrogen, protein, phosphorus and potassium contents were decreased by YVMV infection.

Conclusion :

In present investigation, the chlorophyll 'a' chlorophyll 'b' and total chlorophyll were found significantly increased in the leaves of okra plants after 15 days of foliar spray with all insecticides and botanicals and their combinations. Tested the sugar contents were also increased. But, total phenol content was decreased. Reviewing the results of these experiments, it is clear that total chlorophyll contents were increased in the treatments (T_1–T_8). Maximum increase in total chlorophyll (63.37%) contents was found in the plants treated with Imidacloprid and Azadirachtin whereas chlorophyll 'a' and chlorophyll 'b' were also increased. Similarly, chlorophyll 'a', chlorophyll 'b' and total chlorophyll (54.45%) were found increased in the samples treated with Imidacloprid + Karanj oil. These insecticides influenced host physiology as well as biochemical contents.

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Table 2 : Effect of yellow vein mosaic virus disease of okra on sugar and phenol contents after spray of insecticides and botanicals

Sr. No.	Cultivars/Treatments	P.D.I.	Total sugar (mg/g)	Per cent increase over control	Phenol (mg/g)	Per cent decrease over control
1.	Dimethoate 30%EC (1ml/l)	15.3	172.00	26.47	30.33	8.09
2.	Imidacloprid (1ml/3l)	09.8	176.00	29.41	25.27	23.42
3.	Azadirachtin (5ml/l)	20.6	163.00	19.85	31.67	4.03
4.	Karanj oil (2%)	27.2	150.67	10.78	32.80	0.60
5.	Dimethoate + Azadirachtin	08.9	206.00	51.47	21.13	35.96
6.	Imidacloprid + Azadirachtin	07.6	222.00	63.23	16.87	48.87
7.	Dimethoate + Karanj oil	13.4	174.00	27.94	27.60	16.36
8.	Imidacloprid + Karanj oil	08.3	216.00	58.82	18.27	44.63
9.	Control	40.9	136.00	–	33.00	–
	SEm±		1.30		1.05	
	CD at 5%		3.89		3.13	
	C.V.(%)		2.11		11.56	

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