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VARIATION IN PHOTOSYNTHETIC PIGMENTS OF LEAVES INFESTED WITH LEAF MINER (*LIRIOMYZA TRIFOLII*) (BURGESS) IN CASTOR AND TOMATO

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Abstract

Leaf miner causes impeccably harmful effects on tomato and castor which is a dangerous pest in India. Due to hazard caused by *Liriomyza trifolii* (Family Agromyzidae and order Diptera) on tomato and castor in Telangana region, it's important to understand the infection and loss caused on those crop plants. The discoverable stage of the pest where observations could easily be made is, the juvenile (maggot) phase. The areas where the larval stage of the pest could be identified are the upper and lower surfaces of leaves. At the Central Research Institute for Dryland Agriculture (CRIDA), Hyderabad, Telanagana, an attempt was made in this study to examine some biochemical changes in leaf miner infecting tomatoes and castor crops. Systemically infected tomato and castor leaves showed characteristic leaf miner infection, both infected and healthy leaves were collected from ICAR-CRIDA. The castor and tomato leaves collected from fields are used for different biochemical analysis. i.e. chlorophylls, proteins, sugars, starch estimation. Among infected plants, the total amount of chlorophyll, starch, and sugar was found to be less than among healthy ones. The overall leaf protein was found to be lower in leaf miner infected leaves that may be due to leaf miner infestation.

***Keywords*:** *Liriomyza trifolii,* Leaf miner, Chlorophyll, Tomato, Protein, Sugars and Starch

Introduction

Most of the Indian farmers are involved in cultivating the high yielding crop i.e., tomato *(Solanum lycopersicum* = *Lycopersicon esculentum*), which belongs to family solanaceae. Average crop growth area of tomato is 4.6 million hectares in the world producing 128 million tonnes (Anonymous, 2006) in India. States such as Telangana, Bihar, Karnataka, Uttar Pradesh, Uttarakhand, Orissa, Andhra Pradesh, Maharashtra, Madhya Pradesh and Assam contribute major percentage of yields in tomato production. Many pests attack tomato crop from the time of first emergence of plant till harvest. Leaf miner in tomato can cause loss of foliage up to 90%, if pest populations are left unchecked. Leaf miner can also be observed in shaded and older leaves, where the photosynthetic production becomes less (Santos *et al*., 2011).

Leaf miners damage the crops by puncturing the leaf surface to feed on exuding sap and laying eggs on the leaf tissue (Knodel-Montz *et al*., 1985). The larvae tunnel in the leaf tissue damages and disfigures mines when the eggs hatch.This reduces the quality of high value horticultural crops and, moreover, decreases plant photosynthetic efficiency (Foster and Sanchez 1988; Kox *et al*., 2005). Total crop damage during outbreaks can result in severe infestations from both adult puncturing and larval mining (Spencer, 1973; 1990) and many export markets.

Castor (*Ricinus communis* L.) is an essential non-edible oilseed crop of the spurge (Euphorbiaceae) family and is believed to have originated in Abyssinia. It is found primarily in tropical areas around the world and spreads to moderate areas in the North and South hemisphere, including the American and African tropical regions (Banderjee *et al*., 1990). Castor plant is infected by several pests including the leaf miner *L. trifolii* (Mc Keown *et al*., 2012) and (Salihu*et al*. 2012), Sharma *et al* 1980 indicated the presence of holes induced by *L. trifolii* female when oviposits. Anjani (2005) showed the leaf miner *L. trifolii* can damage the mesophilic tissue and turn the parenchyma tissue into a white strip which affects photosynthesis process and destroys leaves of castor. The objective of the study was to estimate photosynthetic pigments of leaves infested with leaf miner (*L. trifolii*) in castor and tomato.

Materials and Methods

Study area

Research work was carried out at Entomology Laboratory, Indian Council of Agricultural Research ICAR-CRIDA. The tomato variety, US 440 and castor variety, arun were sown during *Kharif*-2014 season, liberated from the research field. The primary step to be followed was to collect both healthy and *L. trifolii* infected leaves, wash them with distilled water. Then, the leaves were dried on blotting paper and kept in hot air oven at 70-110^{o}C. The processed samples of leaf were ground with pestle and motor. For the calculation the leaf samples were used as follows.

Estimation of Chlorophyll by Dimethyl Sulphoxide (DMSO) Method

Fresh tomato and castor leaf samples were collected at CRIDA field at morning hours, samples were collected based on leaf miner damage. In a healthy leaf there was no visible scar, whereas total infected leaf showed damage by the leaf miner. Tomato and castor leaf samples (50 mg) were taken and dissolved in 10 ml of DMSO in a separately filled container. The bottle was labelled with the respective grade

and the bottle cap was sealed with parafilm. Same protocol for all samples was observed. To avoid light exposure, all samples were covered with black cloth and incubated at room temperature for 24 hours under dark conditions. After the incubation period. Samples were interpreted using a SYNCO spectrophotometer (Porra *et al*., 1989). At 645 nm respectively, and 663 nm. Readings were taken against blank.

Calculation of chlorophyll a, chlorophyll b and total chlorophyll was done by using the formula. (Sadasivam and Manikam, 2008)

Total chlorophyll: [(20.2xO.D 645)+(8.02 + O.D 663)]*v/1000*w

Chlorophyll a: [(12.7xO.D 663)-(2.69 + O.D 645)]*v/1000*w

Chlorophyll b: [(22.9 x O.D 645) - (4.68 + O.D 663)]*v/1000*w

Here

v=volume of DMSO, w= weight of the sample, O.D stands for optical density.

The result given as mg of chlorophyll /g fresh weight of leaf. (1950). For this analysis,

Estimation of Total Sugars and Starch

The method for estimation of sugars and starch are same for both the samples (healthy and infected) used in this study. The method of Dubois *et al*. (1951*)* was followed to estimate the total amount of sugars in the leaves and starch by method described by Mc Cready *et al*. (1950). For this analysis. Take 50 mg of healthy and infected leaves, Take 50 mg of healthy and polluted leaves, thoroughly wash with tap water followed by distilled water and dry between filter paper folds. The leaf samples were cut into bits and macerated with 5 ml of 80% ethanol. The macerates were centrifuged at 5000 rpm for 15 min and pellet was washed thrice with 80% ethanol. Supernatants were pooled and made with 80% ethanol up to known volume. In the water bath, samples were heated at 85 °C, until the alcohol evaporated. Estimation of sugars was performed using the supernatants despite subsequent evaporation of the alcohol. Starch extraction and estimation were performed from the pellet.

Estimation of Total Sugars

The aliquots of 20 ml of healthy and infected supernatant sample, collected as mentioned Above were taken separately into test tubes Each tube was quickly added one ml of distilled water and 4 ml of cold anthrone reagent, shaken well, and incubated on ice bath for 10 minutes, and cooled to room temperature. The blank was prepared by taking 1 ml of distilled water and 4 ml of reactive cold anthrone. Absorbance in spectrophotometer read at 625 nm. The volume of total sugars was calculated using a standard D-glucose prepared curve.

Estimation of starch

Pellet which was obtained from the above process was used for starch extraction. The obtained pellet was solubilized in 5 ml of 52% PCA (perchloric acid) and boiled for 10 min at 80 °C. The solution was filtered through glass wool and weighed by filtrate and made with PCA up to 10 ml. Healthy and infected sample extracts (20ml) were taken separately, adding 3 ml of distilled water and 5 ml of anthrone reagent and incubating in ice bath for 10 min. and Sample absorbance was recorded on a spectrophotometer at 625 nm. Standard Glucose curve is used to measure the starch amount.

Estimation of Total Proteins

The Lowry *et al*. (1951) process estimated total leaf protein content for tomatoes and castors. Healthy and infected tomato and castor leaves were taken, washed thoroughly and blotted to dry in between the filter paperfolds. Leaves were cut separately and homogenized in a 4°C morter. Use a grinding buffer (0.1 M Tris HCl; pH 8.3; 0.5 M Sucrose and 0.5 per cent 2Mercaptoethanol) 2 ml/gm, The homogeneous was squeezed through the muslin cloth and centrifuged for 10 min at 10,000 rpm. Supernatants were separately obtained by adding 20 percent Trichloroacetic acid (TCA) to each sample and held at 4 ° C for 2 hours until further use. The TCA precipitate collection was obtained for 10 min with centrifugation at 10,000 rpm. The pellet was treated twice with 5 percent TCA and three times with ice cold solvent ether. The final protein pellet was dried under vacuum and solubilized in a minimum known amount of 0.1 N NaOH solution. Insoluble content was extracted at 8000 rpm for 10 min by centrifugation. 20µl of protein samples were then taken from healthy and infected samples and 5 ml of freshly prepared alkaline copper sulphate reagent was added to each sample. Samples were well mixed, and solution should stand at room temperature for 10 min. 0.5 ml of Folin phenol reagent was applied to each sample after 10 min of incubation and thoroughly mixed. During 30 minutes the samples were incubated and the absorbance was measured at 660 nm. The total leaf protein content (mg / g fresh weight) was determined using normal bovine serum albumin (BSA).

Results and Discussion

The healthy and infected samples were collected from fields of tomato and castor at CRIDA. The chlorophyll, sugars, starch, protein content levels in infected and healthy samples were analysed by biochemical methods. The chlorophyll content in healthy samples i.e chlorophyll a was 7.7mg/g which is 3 folds more than infected leaves in tomato, 4.2 mg/g. On the other hand increased levels of chlorophyll b (5.2 mg/g) were observed in infected samples when compared to healthy samples (5.3 mg/g). The damage was more in *L. trifolii* infected tomato leaves. Where as in castor the chlorophyll a content in healthy leaves was 7.2mg/g which is 4 folds more than infected leaves (4.6 mg/g) on the other hand, 3.5 folds difference was observed in chlorophyll b infected samples (1.0 mg/g) when compared to healthy leaf samples of castor (4.5mg/g).

Chlorophyll a/b ratio in healthy leaves of tomato was 1.45 mg/g and the total chlorophyll is 16.9 mg/g. The chlorophyll a/b ratio in tomato infected leaves was 0.81mg/g and the total chlorophyll was 12.3mg/g. Where as in castor, chlorophyll a/b ratio in healthy leaves was 1.6 mg/g and total chlorophyll is 16.3mg/g. The chlorophyll a/b ratio in infected leaves was 1.0 mg/g and the total chlorophyll is 12.5 mg/g. In comparison, the leaf miner affect was severe in castor when compared to tomato leaves.

Total sugars content in healthy leaves of tomato was 1.7mg/g and starch was 1.13mg/g. Total sugar content in infected leaves was 0.99 mg/g and starch0.98 mg/g, which decreased to 0.71 folds in sugar content and 0.14 folds in

starch content. Whereas in castor the total sugars in healthy leaves was 2.3mg/g and starch in healthy leaves was 2.03mg/g. Total sugars in infected leaves was 0.60mg/g and starch content in infected leaves was 1.18mg/g which decreased to 1.71 folds in sugars and 0.85 folds in starch. The sugar and starch content was less in infected leaves of tomato and castor when compared to healthy leaves.

The protein content in healthy leaves in tomato was 1.53 mg/g which is 0.5 folds more than infected leaves i.e. 0.98 mg/g. Whereas in castor, protein content in healthy leaves was 2.10 mg/g which is 0.5 folds more than infected leaves i.e. 1.58 mg/g. The protein content decreased in infected leaves of tomato and castor than in healthy one.

The photosynthetic ability of the plant was greatly reduced when chlorophyll containing cells are destroyed. Severely infected leaves may fall, exposing plant stems to wind action, flower buds and developing fruits to scale (Musgrave *et al*., 1975). Several reports showed that *Liriomyza* spp. injury reduced photosynthesis in celery (Trumble *et al*., 1985), in tomato (Johnson *et al*., 1983), in lim bean (Martens and Trumble, 1987),and in chrysanthemum (Parella *et al*., 1985).Similarly, chlorophyll content of leaves is also affected by densely populated larvae. Chlorophyll content decreases in infected plants due to high population density of *L. trifolii* which feeds palisade mesophyll of chrysanthemum (Parrella *et al*., 1995). Previous studies also reported that *L. trifolii* do not affect photosynthetic rates in lower densities of larval infestation (Bueno *et al*., 2007). Likewise, Johson *et al*., (1983) reported that *Liriomyza sativae* decreased photosynthetic rates in tomatoes by 62%, and there is a negative linear correlation between lack of larvae and photosynthesis.

Levantrong *et al*. (2019) observed the physiological changes in tomato fruit during growth and ripening stages. He observed that the changes occurred in pigments content, reduction in sugar and starch levels from formation to fruit ripening that contained low chlorophyll a, high chlorophyll b and high starch content at 26 days old fruit. The reducing sugar content was low at 26 days and increased towards 46 days old fruit and then gradually decreased. Its level decreased in the ripening fruit. Thus difference in the chlorophyll and reduced sugar levels was observed in tomato fruit from growing to ripening stage.

Naidu *et al.* (1983) reported that tomato chlorophyll a/b ratio was decreased at several stages of infection by tomato leaf miner due to decreased chlorophyll a levels. Watson (1964) reported that sugar beet yellows leaves infected with Leaf miner and sprayed with sucrose enhanced the carbohydrate content of the sugar beet leaves and also chlorosis.

Johson (1983) worked on tomato leaf miner and reported that reduction in photosynthesis activity of leaflet. However study by Kamble (2015) reported that chlorophyll a and b was lower in mango younger leaves and later on when infected with leaf miner, the adult leaves showed 4 to 6 folds increase of chlorophyll a and b. Our study showed 3 folds increase in chlorophyll a in tomato infected leaf miner leaves and 3-4 folds increase of chlorophyll b castor infected with leaf miner. Thus we can conclude that the photosynthesis rate was severely decreased in leaves because of the leaf miner infestation in different crops.

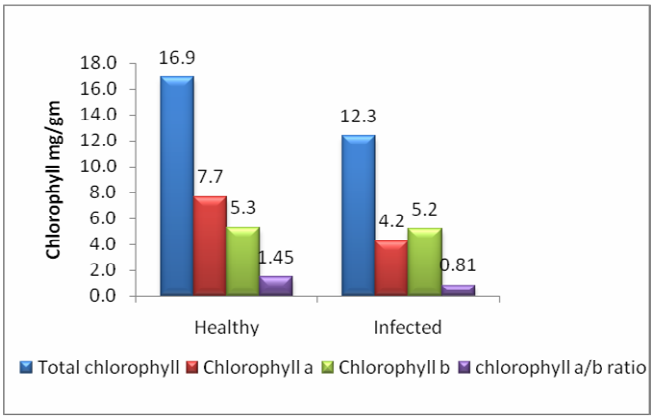

Fig. 1 : Estimation of total chlorophyll, chlorophyll a and chlorophyll b, on tomato

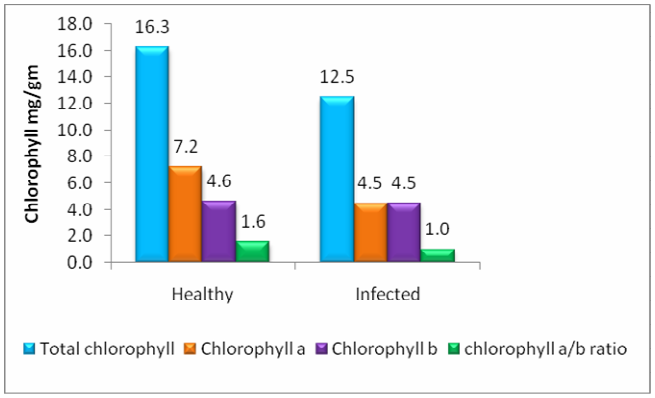

Fig. 2 : Estimation of total chlorophyll, chlorophyll a and chlorophyll b, on castor

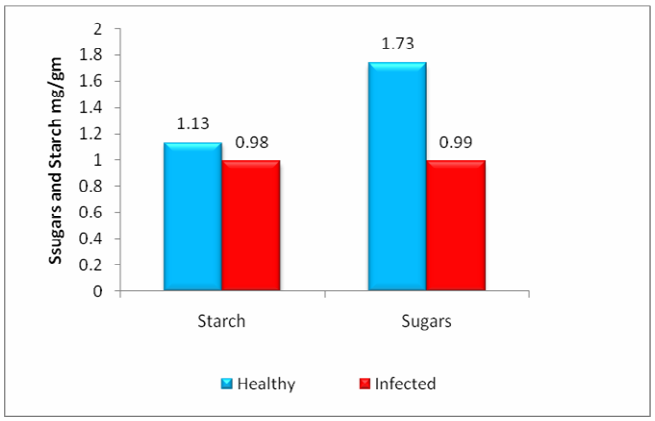

Fig. 3 : Determination of Sugars and Starch in healthy and infected leaves on tomato

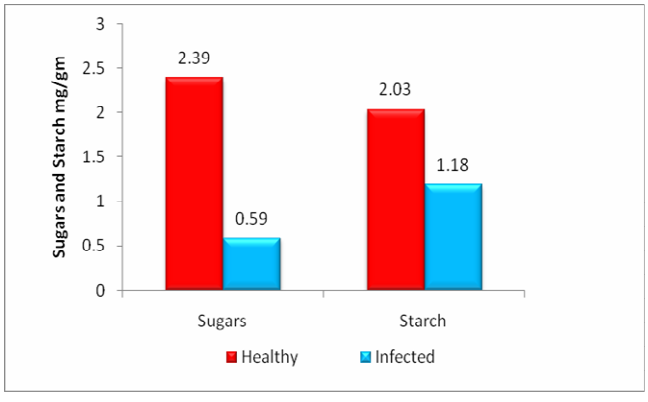

Fig. 4 : Determination of Sugars and Starch in healthy and infected leaves on castor

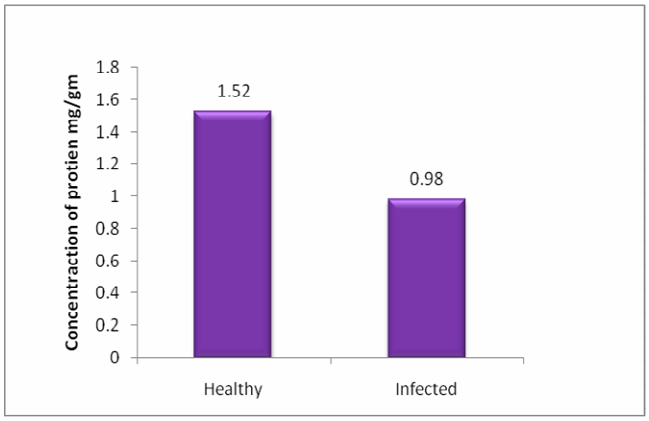

Fig. 5 : Determination of total Proteins in healthy and leaf miner infected leaves on tomato

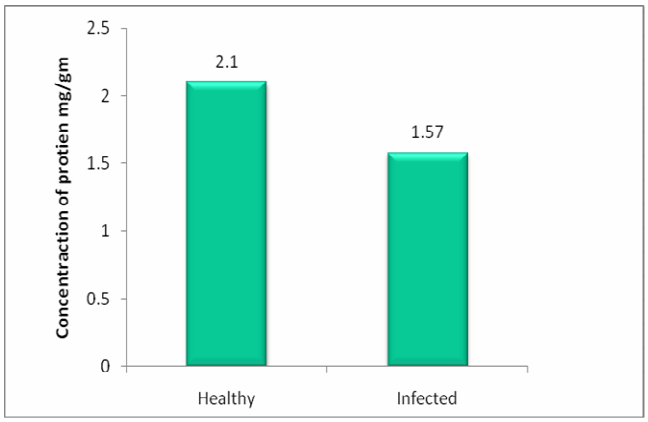

Fig. 6 : Determination of total Proteins in healthy and leaf miner infected leaves on castor

Conclusion

Leaf miner is a gregarious pest which causes overall decrease in the yield of various crops. This pest is responsible for the decrease in chlorophyll content, ultimately effecting photosynthesis of different crop plants.

The biochemical studies of leaf miner infected tomato and castor leaves reported decrease in chlorophyll content, sugars and starch. But on comparative note between infected tomato and castor leaves, the pest showed a more damaging effect on castor than on tomato which showed that the pest had its impact more on castor when compared to tomato.

References

Anjani, K. (2005). Purple-coloured castor (*Ricinus communis* L.). A rare multiple resistant morphotype Directorate of Oilseeds Research, Rajendranagar, Hyderabad 500 030, India current science, 88(2).

Anonymous (2006). Annual Research Report, NRCG, Junagadh.

Banderjee, S.; Mukherjee, A.; Bandyopadhyay, S.K.; Mukherjee, P.K. and Sikdar, S. (1990). Preliminary studies on the anti inflammatory effects of *Ricinus communis*. India J Pharmac; 22: 239-44.

Bueno, A.F.; Zechmann, B.; Hoback, W.W.; Bueno, R.C.O.F. and Fernandes, O.A. (2007). Serpentine leafminer (*Liriomyza trifolii*) on potato (*Solanum tuberosum*): Field observations and plant photosynthetic responses to injury. Ciencia Rural, 37(6): 1510-1517.

Dubois, M.K.; Gilles, J.K.; Hamilton, P.; Rebers, P. and Smith, F. (1951). Nature.168: 167.

Fernandez, S. and Montagne, A. (1990). Boletin De Entomologia Venezolana, 5: 89–99.

Foster, R.E. and Sanchez, C.A. (1988). Effect of *Liriomyza trifolii* (Diptera: Agromyzidae) larval damage qn growth, yield and cosmetic quality of celery in Florida. Journal of Economic Entomology, 81(6): 1721-1725.

Johnson, M.W.; Oatman, E.R.; Wyman, J.A.;Van Steenwyk, R.A. (1980). A technique for monitoring Liriomyza sativae in fresh market tomatoes. Journals of Economic Entomology, 73: 552-555.

Johnson, M.W.; Welter, S.C.; Toscano, N.C.; Ting, I.P. and Trumble, J.T. (1983). Reduction of tomato leaflet photosynthesis rates by mining activity of *Liriomyza sativae* (Diptera: Agromyzidae). Journal of Economic Entomology, 76(5): 1061-1063.

Kamble1, P.N.; Sanjay, P.; Ranjeet, S.M. and Anupreet, T. (2015). Estimation of Chlorophyll Content in Young and Adult Leaves of Some Selected Plants. Universal Journal of Environmental Research and Technology, 5(6): 306-310.

Knodel-Montz, J.J.; Lyons, R.E. and Poe, S.L. (1985). Photoperiod affects chrysanthemum host plant selection by leaf in inners (Diptera: Agromyzidae). Hort Science 20(4): 708-710.

Kox, L.F.F.; Van Den Beld, H.E.; Lindhout, B.I. and DeGhoffau L.J.W. (2005). Identification of economically important *Liriomyza* species by PCR-RFLP analysis. OEPP/ EPPO Bull. 35: 79–85.

Le Van Trong., Le Quy Tuong., Bui Bao Thinh., Nguyen Tuan Khoi and Vu Thi Trong. (2019). Physiological and biochemical changes in tomato fruit (*Solanum lycopersicum* L.) during growth and ripening cultivated in Vietnam.

Lowry, H.; Rose brough, N.J.; Far, A.L and Randall, A.J. (1951). J. Biol. Chem., 193: 256-275.

Martens, B. and Trumble, J.T. (1987). Structural and photosynthetic compensation for leaf miner (Diptera: *Agromyzidae*) injury in lima beans. Environmental Entomology, 16: 374-378.

Mc Cready, R.; Goggle, M.J.; Silviera, V. and Owens, H.S. (1950). Analytical Chem., 29: 1156-1158.

McKeown, W.A. and Bakker, C.J. (2012). Castor oil plant. Evaluation of establishment techniques for castor. Ministry and Agriculture, Food and Rural Affairs.

Musgrave, C.A.; Poe, S.L. and Weems, H.V. (1975). The vegetable leaf miner *Liriomyza sativae* Blanchard (Diptera: *Agromyzidae*) in Florida. Fla. Dep.Agric. Consumer Serv. Div. Plant Industry. Ent. Circ. 162: 4.

Naidu, R.A.; Kriashnan, M.; Ramanujam, P.; Gnanam, A. and Nayudu, M.V. (1984). Physiol. Plant Pathol. 25: 181-190.

Parrella, M.P.; Jones, V.P.; Youngman, R.R. and Lebeck, L.M. (1985). Effect of leaf mining and leaf stippling of *Liriomyza* spp. on photosynthetic rates of chrysanthemum. Annals of the Entomological Society of America, 78: 90-93.

Porra, R.J.; Thompson, W.A. and Kriedemann, P.E. (1989). Determination of accurate extinction coefficients and simultaneous equations for assessing chlorophyll a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochim. Biophys. Acta 975: 384e394.p. 56.

Sadasivam, S. and Manickam, A. (1996). Biochemical methods. New age international (P) Limited, Publishers, II (ed), New Delhi, 152-160.

Salihu, Z.; Bolagi, G. and RewkApuyor B.O. (2012). Castor oil plant: Botany, Ecology and uses. International Journal of Science and Research (IJSR).

Santos, C.S.; Bueno, R.; Vieira, S. and Bueno, A. (2011). Efficacy of insecticides on Tuta absoluta (Meyrick) and other pests in pole tomato. Bio Assay., 6(4): 1-6.

Sharma, R.K.; Durazo, A. and Mayberry, K.S. (1980). Leaf miner control increases summer squash yields. California Agriculture, 34: 21-22.

Spencer, K.A. (1990). Host Specialization in the World Agromyzidae (Diptera). Kluwer Academic Publishers, Dordrecht, the Netherlands. Series Entomologica 45.

Spencer, KA. (1973). *Agromyzidae* (Diptero) of economic importance. Series Entomologica, Junk, The Hague (NL) 9: 418.

Trumble, I.T.; Tmg, I.P. and Bates, L. (1985). Analysis of physiological, growth and yield responses of celery to *Liriomyza trifolii*. Entomologia Expenmentalis et Applicata, 38: 15-21.

Trumble, J.T. (1985). Integrated pest management of *Liriomyza trifolii*: Influence of avermectin, cyromazine and methomyl on the leaf miner ecology in celery Agriculture, Ecosystem and Environment, 12(3): 181-188.

Watson, M.A. (1955). Ann. Appl. Biol., 43: 672-685.

Watson, M.A. and Watson, D.J. (1951). Annal. Appl. Biol., 38: 276-288.