Bioactivity of leaf extracts of Alphonso mango against cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae).

Hesham A. Srour

Plant Protection Research Institute, Agricultural research Center, Dokki, Giza, Egypt
hamsor98@hotmail.com

Abstract: The contact toxicity of methanolic extract of Alphonso mango leaves was studied by treating 4th instar larvae of the cotton leaf worm *Spodoptera littoralis* with various concentrations (50, 100, 200, 300, 400, and 500 µg/cm^2) of the extract for 24 hours. Also, the impact of the same concentrations of Alphonso extract on the total protein, carbohydrate, lipid content and seven enzyme activity of the body tissue were studied. Results showed that extract exhibit concentration-dependant contact toxicity against *S. littoralis* larvae with LC_{50} of 325 µg/ cm^2. While Alphonso leaf extract did not affect total protein, carbohydrate and lipid content in treated larvae, it significantly reduced activity of amylase, invertase and trehalase and significantly elevated activities of non-specific esterases, acetylcholinesterase (AChE), glutathione-S-transferase (GST) and peroxidase. The alteration of such parameters provides some insights about the mode of action of the allelochemicals in the methanol extracts in insect body and suggests that strong oxidizing agent(s) could be responsible for the toxic effect of the methanolic extract of Alphonso mango leaves.

[Hesham A. Srour. **Bioactivity of leaf extracts of Alphonso mango against cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae)**]. *Nat Sci* 2014;12(1):43-48]. (ISSN: 1545-0740). http://www.sciencepub.net. 7

Key words: ***Mangifera indica*****,** *Spodoptera littoralis***,** Toxicity, Protein, Lipids, Carbohydrates, Enzyme

1.Introduction

Synthetic chemicals commonly used to control insect pests not only pollute the environment, but they are also harmful to human health. Thus there has developed a world-wide interest in exploring plant chemical components as potential sources of commercial pest control agents or as lead compounds with a novel and safe mode of action (**Isman, 1999**). Plants synthesized natural compounds that can affect insects in several different ways: they may disrupt major metabolic pathways and cause rapid death, act as deterrents, phagostimulants, and antifeedants or modify oviposition (**Kessler and Baldwin, 2001**). In Egypt, mango, *Mangifera indica* L, is considered one of the most economic crops, where many local cultivars such as Alphonso, Baladi, and Ewaisi, are successfully grown (**El-Zohgbi and Mostafa. 2002**). Studies that conducted in different Egyptian localities showed that different mango cultivars express varying levels of susceptibility to insect infestations (**Salem, 1994, and Salem, et al., 2006&2007**). Long-term observation in several mango orchards located at Giza Governorate, Egypt indicated that Alphonso mango trees were completely free of *Iceria seychellarum* infestation even in tree closely adjacent to heavily infested Baladi mango trees (**Monzer et al., 2006&2007 and Salem, et al., 2006$ 2007**). **Monzer et al., (2006)** showed that methanolic extract of Alphonso mango leaves had toxic effect on *I. seychellarum*. However, the exact mode of action of toxicants(s) in mango leaves is mostly unknown.

In the present study, toxic effect of methanol extract from Alphonso mango leaves was tested using cotton leaf worm *Spodoptera littoralis* as a model insect species. The effects of such extract on certain biochemical; and enzymatic parameters were also explored in order to have some insights about the physiological responses of insects toward Alphonso leaf extracts.

2. Materials and Methods:

Sample Collection:

Mango leaves of Alphonso cultivar were collected from a Fisher mango orchard located in El-Saff, Giza Governorate. Fresh mature leaves were hand-plucked from three trees packed in plastic bags, hermetically sealed, labeled, and transported in icebox to the laboratory. Healthy leaves were maintained at –20°C until extracted (within one week).

Sample extraction:

Leaves were homogenized in a Tempest homogenizer, and were extracted with HPLC grade methanol at room temperature (20ºC) with ratio of 10 ml methanol to 1.0 g fresh weight (FW) according to the method mentioned by **Nicolescu *et al.* (2000)**. Extraction was performed in a Romo shaking apparatus for 48 hrs. The obtained extract was concentrated in a rotator evaporator and allowed to dry at room temperature. Once drying, the residue was weight to calculate the total mass extracted

Cotton leaf worm (*Spodoptera littoralis*)

A colony of the cotton leaf worm, *S. littoralis* was obtained from Central Laboratory of Insecticides, Agriculture Research Center, Dokki, and Giza, Egypt. The insect was reared on castor-oil leaves, *Ricinus communis*, under laboratory conditions at 25 ± 2 °C and 60 ± 5 % R.H.

Contact Toxicity assay:

To study the insecticidal properties of the crude extracts of Alphonso leaves, the thin film procedure adopted from **Pascual-Villalobos and Robledo (1999)** was followed using 4th instar larvae of *S. littoralis*.

Bioassays were conducted in standard Petri dishes (actual measured area of ca. 30 cm^2). Tested extracts was diluted with acetone to give concentrations of 1.5, 3.0, 6.0, 9.0, 12.0 and 15.0 mg extract/ml acetone. One ml acetone solution at the selected concentrations was spread in each dish using one ml pipette, with shaking of the dish to ensure the chemical distribution. This gave concentrations of 50, 100, 200, 300, 400, and 500 µg/cm^2 dish bottom area. Control dishes were received only one ml pure acetone. Acetone was allowed to dry at room temperature for half hour. Actively feeding 4th instar larvae of *S. littoralis* were separated from laboratory mass rearing culture and 10 larvae were transferred to each dish. Five replicates (dishes) with ten larvae/replication were prepared for each concentration as well as control treatment. After 24h at 27±2°C and 70±5% RH, mortality of larvae was recorded.

Biochemical experiments*:*

Sample preparation for biochemical analysis:

Samples for biochemical experiments were collected from both control and treated *S. littoralis* 4th instar larvae, frozen alive and stored in –20ºC four further biochemical tests. Larvae (5 frozen larvae) were crushed and homogenized using liquid nitrogen according to Kazmer ***et al.*** **(1995)**. The Crushed sample was suspended in 1 ml 50 mM phosphate buffer (pH 7.4). All samples were centrifuged at 70000 g for 20 min at 4°C in cooling centrifuge. The resulting supernatant was either assayed immediately or refrigerated at –20 until required. At least, three replicates were done for each test.

Bioassays:

Total soluble protein content was determined according to the method of Bradford **(1976)** using coomassie brilliant blue reagent. Anthrone reagent was used to quantify carbohydrate content according to the method of **Roe (1955)**. Lipid content was quantified by the method of **Zöllner and Krich (1962)** using vanillin reagent.

Amylase, invertase and trehalase activities were assayed using alkaline dinitrosalicylic acid reagent according to the method described by **Ishaaya and Swiriski (1976)**. Starch, sucrose, trehalose was used as substrates for amylase, invertase and trehalase, respectively.

Activity of non-specific esterases was measured with the method of **van Asperen (1962)** using β-naphthyl acetate as a substrate. Acetylcholinesterase (AChE) activity was determined according to a method of **Ellman et al. (1961)**. Peroxidase activity was determined by a direct spectrophotometric method described by **Hammerschmidt and Kuc (1982).** Glutathione-S-transferase activity was measured by the assay described by **Habig et al. (1974).**

Statistical analysis:

The results of toxicity test were presented as percentage, although actual number of insects was used for statistical tests.. Determination of LC50 was done using the software package "LD-Pline", Copyright of Ihab. M. Bakr, Plant Protection Research Institute, Egypt. Biochemical parameter data were analyzed by analysis of variance (Duncan Multiple Range Test at $P<0.05$) using the software package Costat (**Costat, 1992**). Results were recorded as mean ± standard deviation (SD).

3. Results:

Table (1) shows that methanolic extract of Alphonso leaves exhibited concentration-dependant contact toxicity against larvae of *S. littoralis* with LC_{50} of 325 µg/ cm^2 (**Table 2**). **Tables (3)** summarize the influence of treatment with methanolic extract of Alphonso leaves for 24 hrs on total soluble protein, lipid and carbohydrate contents in *S. littoralis* 4th instar larvae. Total proteins lipids and carbohydrates contents were not affected significantly by various treatment concentrations, whereas there were significantly and sharp reduction in activities of the three tested carbohydrate hydrolyzing enzymes (**Table 4**). The enzyme activity reached its lowest values in larvae treated with extract concentration of 300 µg/ cm^2, reached 0.41±0.10, 1.10±0.18, and 0.19±0.08 for amylase, invertase and trehalase, respectively compared with 1.30±0.12, 2.35±0.21 and 0.66±0.15 for control.

On the contrary, induction in activities of the assayed detoxification enzymes were obtained after treatment with various concentrations of the methanolic extracts of Alphonso leaves (**Table 5**). Esterase activity using β-NA as substrate increased gradually with increase in concentration, reached its maximum (192.6±2.17 nmole/mg) at concentration of 200 µg/ cm^2, while activities of acetylcholinesterase, Glutahione-S-transferase, and pyroxidase reached their maximum (31.58±2.5, 82.31 ± 5.0 and 0.0371±0.0014, respectively) at concentration of 100 µg/ cm^2

Table 1. Contact toxicity of methanolic extract of Alphonso leaves against *S. littoralis* 4th instar larvae after 24 hrs

Concentration µg/ cm^2	% Mortality*
0	0.0
50	0.0
100	5.4± 6.0a
200	26.5±6.9b
300	48.2±8.3c
400	76.0±8.9d
500	100e

* Values (Mean± SD) followed by different letter are significant different at P<0.05

Table 2: Results of probit analysis for contact toxicity of methanolic extract of Alphonso leaves against *S. littoralis* 4th instar larvae.

Probit analysis	Concentration (µg/cm^2) *
LC_{50}	325
Lower limit	200
Upper limit	360

* 95% confidence limit

Table (3): Effect of treatment with different concentrations of methanol extract of Alphonso leaves on total soluble protein, lipid and carbohydrate contents of *S. littoralis* 4th instar larvae.

Concentration µg/ cm^2	Total soluble proteins mg/g FW	Total carbohydrates mg/g FW	Total lipids mg/g FW
0 (Control	23.1±1.3a	1.5±0.11b	3.4±0.14d
50	19.8±1.0a	1.3 ± 0.07b	3.1 ± 0.11d
100	21.0±1.0a	1.12± 0.13b	3.2± 0.11d
200	18.3±1.3a	1.45± 0.10b	2.9± 0.13d
300	18.9±0. 9a	1.48± 0.09b	3.0± 0.10d
400	19.5±0.24a	1.30± 0.07b	3.0± 0.12d

* Values (Mean± SD) followed by different letter within the same column are significant different at P<0.05
FW = Fresh Weight

Table (4): Effect of treatment with different concentrations of methanol extract of Alphonso leaves on carbohydrate hydrolyzing enzyme activities of *S. littoralis* 4th instar larvae.

Concentration µg/ cm^2	mg glucose librated/mg protein/hour		
	Amylase	Invertase	Trehalase
0 (Control	1.30±0.12a	2.35±0.21a	0.66±0.15a
50	0.82±0.17b	2.11±0.23a	0.49±0.12b
100	0.60±0.14c	1.42±0.21b	0.28±0.14c
200	0.52±0.09d	1.19±0.13c	0.20±0.102d
300	0.41±0.10e	1.10±0.18d	0.19±0.08d
400	0.45±0.16e	1.23±0.04d	0.21±0.13d

* Values (Mean± SD) followed by different letter within the same column are significant different at P<0.05 using Duncan Multiple Range Test

Table (5): Effect of treatment with different concentrations of methanol extract of Alphonso leaves on detoxification and anti-oxidant enzyme activities of *S. littoralis* 4th instar larvae.

Concentration µg/ cm^2	β-esterases	Acetylcholine esterase	Glutahione-S-transferase	pyroxidase
	nmole/mg protein	nmole/µg protein/min	mM DCNB /mg protein	ΔA_{420}/min/mg protein
0 (Control	85.9±3.70a	13.87±0.95a	61.91 ± 2.1a	0.0066±0.0005a
50	147.5±4.65b	35.13±1.8b	76.42 ± 2.3b	0.0176±0.0024b
100	164.9±5.12c	31.58±2.5b	85.31 ± 5.0c	0.0371±0.0014c
200	192.6±2.17d	30.83±1.5b	82.65 ± 7.4c	0.0356±0.0013c
300	182.0±2.10d	34.00±0.77b	88.10 ± 4.8c	0.0364±0.00076c
400	182.82±2.16d	33.11±1.7b	85.76 ± 8.6c	0.0354±0.0040c

* Values (Mean± SD) followed by different letter within the same column are significant different at P<0.05 using Duncan Multiple Range Test

4. Discussion:

The present investigation clearly demonstrates that methanol extract of Alphonso leaves is highly toxic against *S. littoralis* 4th instar larvae. The target site(s) of the toxicant(s) in the extract was unknown. Accordingly, this work was extended to investigate the influence of different concentrations of Alphonso leaf extract on *S. littoralis* main energy reserve substances (protein, lipids and carbohydrates) and nine representative enzymes, which can reflect the damage occurred on specific chemical process or specific target tissue. Three enzymes represents hydrolyzing enzymes (amylase, invertase, and trehalase), two represent detoxification enzymes (non-specific esterases, and acetylecholinesterase) and two represent antioxidant enzymes (Glutahione-S- transferase and general pyroxidase). Amylases hydrolyse starch into the monosacccharides, glucose, and fructose and secreted by larva salivary glands and midgut (**Ribeiro *et al.*, 2000**). Invertases cleave sucrose into the monosacccharides, glucose, and fructose and are secreted and worked in the gut of larvae (**Heil *et al.*, 2005**). Trehalase degrades trehalose to glucose for internal energy supply and present in muscles, body wall, malpighian tubules, fat body, midgut and hemolymph of insects (**Wyatt, 1967**). Non-specific esterases catalyze the hydrolysis of esters and are found soluble in hemolymph and membrane-bound (**Maa and Liao, 2000**). They are involved in important physiological processes including detoxification of insecticides and reproduction (**Nascimento and Bicudo, 2002**). Acetylcholinesterase (AchE) regulates nerve impulse transmission across cholinergic synaptises (**Keane and Ryan, 1999**). Pyroxidases is the primary enzymes in insects that dedicated to removal of damaging reactive oxygen species (**Ahmed, 1995**). Glutathione S-transferases (GSTs) play an important role in the protection of different body cell types, against oxidant damage (**Yang *et al.*, 2004**)

The obtained results in this study showed that the amount of the main energy reserve substances (protein, lipids and carbohydrates) in *S. littoralis* larvae was not affected significantly by treatment. This could be attributed to the rapid death of larvae before the toxic substance(s) on the extract could affect the amount of such compounds with detectable amount. However, activities of the carbohydrate hydrolyzing enzymes, amylase, invertase and trehalase were reduced in larvae after treatment with extract of Alphonso leaves, compared with control larvae. This reduction of activity may be due to several reasons. The first hypothesis is the inhibition of the activity of the enzymes themselves. Several studies have already shown that plant extracts inhibit the activity of carbohydrate hydrolyzing enzymes (**Duffey & Stout, 1996** and **Bouayad et al., 2013**). The second hypothesis is that plant secondary metabolites cause cytotoxicity in epithelial cells synthesizing these digestive enzymes (**Jbilou *et a*l., 2008 and Rharrabe *et al*., 2009**).

However, our results showed elevation in activities of the assayed detoxification enzymes in treated larvae. Induction of detoxification metabolic system plays an important role in insect's detoxification mechanism (**Terriere, 1984**). Activity of non-specific esterases and AchE, have been reported to be induced by plant allelochemichals in a number of insects (**Yu, 1983, Caballero et al., 2008 and Bouayad et al., 2013**). The increase in their activity at low concentrations may be due to a general induction of esterase genes in response to specific secondary metabolites in leaves of Alphonso mango

cultivar (**Bouayad et al., 2013**). The recorded elevation in peroxidase and GST activities in this work indicates that methanol extract of Alphonso mango leaves contain toxicants that auto-oxidize producing highly reactive oxygen species and hydrogen peroxide. The increase in peroxidase and GST activities reflect their role in removing both the oxidized molecules and the generated H2O2 molecules, respectively (**Ahmed, 1995** and **Yang *et al*., 2004)**.

In summary, this work shows the potential of Alphonso mango trees, commonly found in Egypt as an expected source of bio-insecticide with remarkable bio-insecticidal effect. The alteration of several physiological parameters (digestive detoxification and antioxidant enzymes) provides some insights about the mode of action of the allelochemicals in the methanol extracts in insect body. Therefore, it is recommended that active compounds in Alphonso mango leaves could be isolated identified, and tested as a candidate for preparation of botanical insecticidal formulation.

References:

Ahmed, S., 1995. Oxidative stress from environmental pollutants. Arch. Insect Biochm. Physiol., 29: 135-157.

Bradford, M.M., 1976. "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding". Analytical Biochemistry.5: 248-254.

Bouayad N., Rharrabe K., Ghailani N.N., Jbilou R., Castañera P. and Ortego F. 2013. Insecticidal effects of Moroccan plant extracts on development, energy reserves and enzymatic activities of *Plodia interpunctella*. Spanish Journal of Agricultural Research 11(1), 189-198

Caballero C., López-Olguín J.L., Ruiz M., Ortego F., Castañera P., 2008. Antifeedant activity and effects of terpenoids on detoxication enzymes of the beet armyworm, *Spodoptera exigua* (Hübner). Span J Agric Res 6: 177-184.

Costat, 1992. Software package, Costat, Graphics and Statistics Software for Scientists and Engineers (Cohort Inc), Berkeley, CA, USA.

Duffey S.S, and Stout M.J, 1996. Antinutritive and toxic components of plant defense against insects. Arch Insect Biochem Physiol 32: 3-37.

Ellman GL, 1959. Tissue sulfhydryl groups. Arch Biochem Biophys 82: 70-77.

El-Zohgbi, M. and Mostafa G.A. 2002. Characterization of the geographic origin of mango pulp from Egypt, Brazil and Puerto Rico by ICP-MS. Zagazig-J. Agric. Res., 28(3): 629-639.

Habig, W. H., Pabst, M. J., and Jakoby, W. B. (1974). Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130–7139.

Hammerschmidt, R. and Kuc, J.A., 1982. Lignification as a mechanism for induced systemic resistance in cucumber. Physiol. Pl. Pathol. 20: 61-71.

Heil, M., Buchler, R. and Boland, W., 2005. Quantification of invertase activity in ants under field conditions. J. Chem. Ecol. 31(2):431-7.

Ishaaya, I and Swirski, E., 1976. Trehalase, invertase and amylase activities in the black scale *Saissetia oleae* and their relation to host adaptability. J. Insect Physiol. 22: 1025-1029.

Isman, M. B. (1999) Pesticides based on plant essential oils. Pestic. Outlook, 68–72.

Jbilou R, Amri H, Bouayad N, Ghailani N, Ennabili A, Sayah F, 2008. Insecticidal effects of extracts of seven plant species on larval development, α-amylase activity and offspring production of *Tribolium castaneum* (Herbst) (Insecta: Coleoptera: Tenebrionidae). Bioresour Technol 99: 959-964.

Kazmer D.J., Hopper, K.R., Coutinot, D.M. and Hechel, D.G., 1995. Suitability of random amplified polymorphic DNA for genetic markers in the aphid parasitoid *Aphelinus asychis* Walker. Biological Control. 5: 503-512.

Keane, S and Ryan M.F., 1999. Purification , characterization and inhibition by monoterpens of acetylcholinesterase from wax moth, *Galleria mellonella.* Insect. Biochem. Mol. Biol. 29: 1097-1104.

Kessler, A. and Baldwin, I.T. 2001. Defensive function of herbivore-induced plant volatile emissions in nature. Science 291: 2141-2144.

Maa, W.C and Liao, S., 2000. Culture-dependent variation in esterase isozymes and malathion susceptibility of diamondback moth, *Plutella xylostella* L. Zool. Studies. 39(4): 375-386.

Monzer M.A.; Salem M.S., Moussa S.F.M., Nour M.E. and El-Said M.I. (2007). Seasonal fluctuation of the Seychelles fluted scale, *Iceria seychellarum* (Westwood) on four mango cultivars in Egypt. Egypt. J. Agric. Res., 85(1): 77-88.

Monzer M.A.; Salem M.S., El-Said M.I. and Abd El-Ghany A.M. (2006). Resistance of Alphonso mango cultivars to the Margardoid mealybug, *Iceria seychellarum* (Westwood) in relation to

leaf quality. I. Leaf secondary metabolites. Egypt. J. Agric. Res., 84(1): 17-29.

Nascimento A.P. and Bicudo, H.E., 2002. Esterase patterns and phylogenetic relationships of *Drosophila* species in the saltans subgroup (Saltan group). Genetica.114: 41-51.

Nicolescu, G., Giulacu-Purcarea V., Vladimirescu A., Hoanca D., Rosu A. and Chicioroaga S. 2000. Screening of some Romanian plants for their activity against medically important insects. Roum. Arch. Microbiol. Immunol., 59(3): 227-236.

Pascual-Villalobos, M.J. and Robledo A. 1998. Screening for anti-insect activity in Mediterranean plants. Industrial Crops and Products 8: 183–194.

Rharrabe K, Bouayad N, Sayah F, 2009. Effects of ingested 20-hydroxyecdysone on development and midgut epithelial cells of *Plodia interpunctella* (Lepidoptera, Pyralidae). Pest Biochem Physiol 93: 112-119.

Ribeiro, J.M., Rowton, E.D. and Charlab, R., 2000. Salivary amylase activity of the phlebotomine sand fly, *Lutzomyia longipalpis*. Insect. Biochem. Mol. Biol. 30(4):271-7.

Roe J.H., 1955. Determination of sugar in blood and spinal fluid with anthrone reagent. J Biol Chem 212: 335-338.

Salem, H.A. 1994. Factors contributing to the distribution and level of attack of scale insects which infectious some different varieties of mango fruit trees. M.Sc. thesis, Fac. Agric., Zagazig Univ., Egypt.

Salem, M.S.; El-Said M.I.; El-Ghany A.M. and Monzer, M.A. 2006. Susceptibility of five mango cultivars to *Iceria seychellarum* (Westwood) in relation to leaf quality: III. Nutrients and inhibitors. Egypt. J. Agric. Res., 84(3): 697-711

Salem, M.S.; Monzer M.A., Nour M.E. and El-Said M.I.. 2007. Susceptibility of five mango cultivars to Seychelles fluted scale, *Iceria seychellarum* (Westwood) in relation to leaf quality: III. Leaf toughtness and anatomic characteristics. Egypt. J. Agric. Res., 85(1): 89-100.

Terriere L.C, 1984. Induction of detoxication enzymes in insects. Ann Rev Entomol 29: 71-88.

Van Asperen, K., 1962. A study of housefly esterases by means of a sensitive colorimetric method. Journal of Insect Physiology. 8, 401–416.

Wyatt, G.R., 1967. The biochemistry of sugars and polysaccharides in insects. Adv. Insect. Physiol. 4: 287-360.

Yang Y., Trent M.B., He N., Lick S.D., Zimniak P., Awasthi Y.C. and Boor P.J. 2004. Glutathione-S-transferase A4-4 modulates oxidative stress in endothelium: possible role in human atherosclerosis. Atherosclerosis. 173(2):211-21.

Yu S.J. 1983. Induction of detoxifying enzymes by allelochemicals and host plants in the fall armyworm. Pestic Bioch Physiol 19: 330-336

Zöllner N., and Krich K., 1962. The quantitative determination of lipids (micromethod) by means of sulphosphovanillin reaction common to many natural lipids (all known plasma lipids). Z Ges. Exp Med 135: 54-59.

9/10/2013