Available online at www.easletters.com

ISSN No: 2349-2864

Entomology and Applied Science Letters, 2015, 2, 2:1-6

Disruptive effects of pomegranate *Punica granatum* Linn. (Lythraceae) extracts on the feeding, digestion and morphology of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae)

Ben Hamouda Amel[1,2], Mechi Atika[1], Zarrad Khaoula[1], Laarif Asma[1] and Chaieb Ikbal[1]

[1]Sousse University, UR13AGR09, Regional Centre of Research on Horticulture and Organic Agriculture, Chott Mariem, Tunisia.
[2] Olive Tree Institute, 40, Rue Ibn Khouldoun 4061 Sousse, Tunisia.
Correspondence: ben.hamouda@yahoo.fr

(Received: 21/05/15) *(Accepted:18/07/15)*

ABSTRACT

The present study was carried out to investigate the feending deterrence, nutritional indices and morphological abnormalities of Spodoptera littoralis in response to methanol, ethanol and aqueous extracts of Punica granatum peel. The peel powder was extracted on ethanol, methanol and water and the dose of 2% was applied on the 3rd instars' larvae maintained on artificial diet or Emex spinosa leaves. Feeding deterrence, nutritional and morphological abnormalities were evaluated. Pomegranate peel extracts showed promissing for the control of African Cotton Leafworm for disrupting of feeding, development and for causing deformities involved in vital activities like feeding, walking or flying, making the insect vulnerable to several sorts of mortality agents or prevent them from causing damage to the crop.

Keywords: Pomegranate, extract, feeding, ingestion, morphology, leafworm

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisd.) considered as one of the most series pest for many different crops in Asia, Africa and Europe [1, 2]. The intensive use of conventional pesticides led to several important problems, i.e. environmental pollution, destruction of the natural enemies and insect resistance to different insecticides. Therefore, there is a great need to develop alternative or additional techniques, which would allow a rational use of pesticides and provides adequate crop protection for sustainable food, feed and fiber protection. Among the most promising alternative to the conventional insecticides, is the use of natural products.

The study of natural products is an old science that has been evolving and changing through the years [3]. From eighteenth century onward, all the myths and magic of the use of natural products has been set aside and their use has increased their rationality [4].

The chemical ecology is the study of the interactions between living organisms mediated by chemical compounds, and has been one of the newest concepts incorporated to the natural products chemistry [5].

Plants have developed defense mechanisms against environmental aggressions; one of the most important is the defense of vegetables against parasites and predators. It is postulated that most of the defensive mechanisms of plants have a chemical character and their existence is due to secondary metabolites [3, 6, 7].

Natural products found in plants have an important role in pest control. Many research studies have been focused on plants' secondary metabolites that affect specific insect pests' processes such as oviposition, reproduction, fertility, and feeding behavior; these abnormalities are related to physiological changes resulting from modifications of the endocrine system, which controls growth and ecdysis [8, 9].

The use of insecticides obtained from plants has many advantages in pest control; in general, they are less persistent and can be used without modifying the natural balance of the ecosystem, respecting the sustainability principles [10].

Pomegranate (*Punica granatum* Linn., Lythraceae) is cultivated in Central Asia and the drier parts of Southern Asia [11], as well as in Mediterranean, tropical and subtropical areas [12]. It was introduced into Latin America, California and Arizona [13]. From the medical point of view, pomegranate is of a great interest to research in pharmaceutical and new drug development fields because of its distinctive bioactivities [14-24]. For pest control, aqueous extract of *P. granatum* fruit rind was toxic against tapeworms [25] and extracts of bark exhibited molluscicidal activity [26, 27]. Also, the fruit rind was effective on some parasitological parameters of *Schistosoma mansoni* [28]. With regard to insect pests, available literature reported the insecticidal effects of *P. granatum* extracts and its disruptive effects on growth and development [29-36]. Also, *P. granatum* peel extracts affected the adult performance and transaminase activity in *S. gregaria* [37, 38].

MATERIALS AND METHODS

Preparation of pomegranate peel extracts.
Pomegranates, *P. granatum* cv. Kalaii were obtained from local market. The fruits were washed and the peels were manually removed, dried at room temperature (20 to 25°C) and powdered to get 0.5 mm size. About 100 g of the powder was extracted by stirring using a magnetic stirrer with 300 ml of ethanol, methanol and water for 24 h each at 25°C.

The extract was sieved through Whatman filter paper to remove peel particles. After filtration, the ethanol and methanol extracts were let to evaporate at room temperature during 48 h and the aqueous extract was evaporated under vacuum at -100°C.

Insect rearing
Insects were obtained from a culture of *S. littoralis* maintained under standard conditions of temperature (28 ± 2°C), photoperiod (Light 12: Dark 12) and relative humidity (60-70%). Larvae were reared on an artificial diet based on 800 ml water, 20 g agar, 150 g chickpea powder, 40 g beer yeast, 5 g ascorbic acid, 1 g benzoic acid and 1 g nipagin.

Feeding bioassay with leaf discs
Antifeedant activity of the fruit peel extracts of pomegranate against larvae was investigated using leaf discs no-choice assay. Test solutions were prepared with dilution of 100 mg of crude residue of each extract in 5 ml of distilled water. Leaf discs (14.1 mm in diameter) were prepared from devil's thorn (*Emex spinosa*) leaves using a cork borer. Each disc was dipped in test solution for 1 min. Control leaf discs has dipped in distilled water for the same period. All discs were left at room temperature for 5 min to let the solvent evaporate.

Each disc was weighed before being presented to the larvae, and reweighed and replaced by a new weighed disc every day.

The feeding trial was conducted for third and fourth instars larvae, separately, with 10 replicates. The Antifeeding index (AFI %) was calculated using the formula of Simmonds *et al.* [39]:

$$\mathbf{AFI\ \% = (C - T) / (C + T) \times 100}$$

With **C:** Consumption of the control discs.
T: Consumption of treated discs.
The following criteria were adopted to categorize the antifeedant index according to Liu *et al.* [40]:
FDI% < 20%: (-) No feeding deterrence,
50% > FDI% ≥ 20%: (+) Weak feeding deterrence,
70% > FDI% ≥ 50%: (++) Moderate feeding deterrence,
FDI% ≥ 70%: (+++) Strong feeding deterrence.

Feeding bioassay with artificial diet
Thirty 3rd instars larvae were separately placed in glass Petri dishes (1 cm high and 9 cm in diameter) and provided with 1g of appropriate artificial diet added with 2% of peel extracts. Control diet does not contain extracts. Each diet was weighed before being presented to the larvae, and reweighed and replaced by a new weighed diet every day. The antifeeding index and nutritional parameters were recorded.

Effect of extracts on nutritional parameters

The effect of aqueous, ethanol and methanol extracts of fruit peel of *P. granatum* on nutritional parameters was investigated on third instar larvae reared on artificiel diet. They were weighed and individually placed in Petri dishes. Then, they were fed with 1g of diets containing 2% of extracts (n=10 for each treatment) and allowed to feed for 6 days. Every 2 days, the larvae, their faeces and uneaten food were weighed. At the end of experiments, the nutritional indices, namely relative consumption rate (RCR), relative growth rate (RGR), efficiency of conversion of ingested food (ECI), efficiency of conversion of digested food (ECD) and approximate digestibility (AD) were calculated as follows:

$RCR = I/BaT$
$RGR = \Delta B/BaT$
$ECI = (\Delta B/I) \times 100$
$ECD = [\Delta B/ (I - F)] \times 100$
$AD = [(I - F)/I] \times 100$

where
I = weight of food consumed;
Ba = arithmetic mean of insect weight during the experiment = [(PF-PI)/log (PF/PI)];
PF = larvae final weight (mg);
PI = larvae starting weight (mg);
T = feeding period in days;
ΔB = change in body weight;
F = weight of faeces produced during the feeding period [41, 42].

Abnormalities

To check for abnormalities during the treatment, larvae treated with each extract were observed for morphological aberrations until adulthood.

Statistical analysis

The experiment results were statistically analyzed by the mean of one-way analysis of variance ANOVA and when results were statistically significant at p = 0.05, Student-Newman-Keuls test was used.

RESULTS

Antifeeding effect of extracts

The antifeeding effect of *P. granatum* extracts at 2% were assessed on third instar larvae of *S. littoralis* maintained on *E. spinosa* leaves after 5 days of treatment. The results have shown a higher significant antifeedant index when larvae reared on ethanol extract (92.96 % ± 2.92) and methanol extract (77.63 % ± 3.78). However, aqueous extract had a weaker effect (66.33 % ± 9.04) (Table 1). For larvae maintained on artificial diet, the antifeedant index reached only 42.74 % ± 5.77 when larvae feed with aqueous extract. As well, ethanol and methanol extracts induced a weak effect of 36.56 % ± 7.38 and 38.01 % ± 6.70 respectively (Table 1).

Table 1. Antifeedant activity of crude extracts of pomegranate peel extracts against third instar larvae of *S. littoralis* maintained on *E. spinosa* and artificial diet.

Plant extract	Larvae maintained on *E. Spinosa*	Larvae maintained on artificiel diet
Ethanol	92.96 ± 2.92 (+++)	36.56 ± 7.38 (+)
Methanol	77.63 ± 3.78 (+++)	38.01 ± 6.70 (+)
Aqueous	66.33 ± 9.04 (++)	42.74 ± 5.77 (+)

Effects of extracts on nutritional parameters

Nutritional analysis revealed that all extracts affected significantly the nutritional parameters. A significant increase of RCR and AD for all extracts was observed (Tab. 2). Conversely, RGR, ECI and ECD show a decrease compared to larvae control.

The larvae that fed on methanol extract, had the highest value of RCR (4.02 ± 0.80) ($F = 8.14$; $df = 3$; $P < 0.0001$) and the lowest aqueous extract (2.49 ± 0.45).The lowest and highest RGR values belonged to the larvae reared on aqueous and ethanol, respectively ($F = 11.04$; $df = 3$; $P < 0.0001$).

The lowest ECI (22.54 ± 6.13) ($F = 8.95$; $df = 3$; $P < 0.0001$) and ECD (24.22 ± 6.84) ($F = 15.91$; $df = 3$; $P < 0.0001$) was observed on methanol and the highest values were on aqueous extract. The larvae that fed on ethanol

extract, had the highest AD (94.11 ± 1.63) and the larvae reared on methanol extract had the lowest AD (93.51 ± 1.49) ($F = 34.53$; $df = 3$; $P < 0.0001$).

Table 2. Nutritional parameters (Mean ± SE) of the third instar larvae of *S. littoralis* maintained on artificial diet treated with 2% of three pomegranate peel extracts.

Extract	RCR ± SE (mg/mg/day)	RGR ± SE (mg/mg/day)	ECI ± SE (%)	ECD ± SE (%)	AD ± SE (%)
Control	1.70 ± 0.25 ^{a}	0.90 ± 0.01 ^{a}	55.84 ± 7.38 ^{a}	80.71 ± 16.23 ^{a}	72.12 ± 6.34 ^{a}
Ethanol	3.20 ± 0.86 ^{bc}	0.79 ± 0.05 ^{b}	32.05 ± 12.76 ^{b}	34.23 ± 13.51 ^{b}	94.11 ± 1.63 ^{b}
Methanol	4.02 ± 0.80 ^{c}	0.78 ± 0.02 ^{b}	22.54 ± 6.13 ^{b}	24.22 ± 6.84 ^{b}	93.51 ± 1.49 ^{b}
Aqueous	2.49 ± 0.45 ^{ab}	0.74 ± 0.04 ^{b}	32.85 ± 7.34 ^{b}	35.16 ± 7.76 ^{b}	93.59 ± 1.29 ^{b}

Anatomical Abnormalities

Pomegranate peel extracts affected moulting and induced a wide range of anatomical abnormalities during the larval and pupal stages in *S. littoralis*. For the insects survivors maintained on leaf discs treated with different extracts, the expansion of the larval period compared to the control treatment was observed (Fig.1).

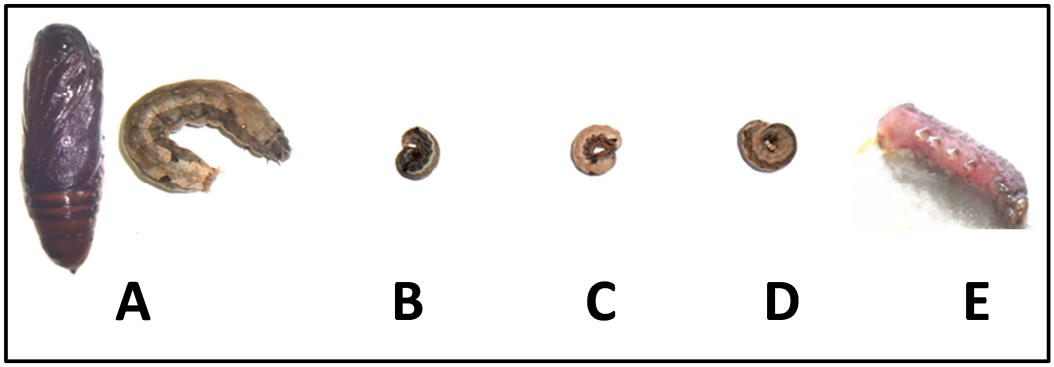

Figure 1. Dwarfing and mortality of larvae of *S. littoralis* fed on leaf discs treated with 2% of three pomegranate peel extracts. A: pupa and larva control treatment; B: ethanol extract; C: methanol extract, D: aqueous extract and E: dead larvae.

Another effect observed in insects maintained on artificial diet treated with different extracts was that some larvae died due to the failure to release the old endocuticle (Fig. 2. A). They exhibited serious disturbances during molting, thus indicating that the extract caused a disturbance in the endocrine system of the larvae, thereby preventing completion of morphogenesis. Some larvae died after a serious tegumental necrosis (Fig. 2. B). Another range of treated larvae developed into deformed pupa, showing the persistence of juveniles characters (Fig.2. C). The treatment causes the appearance of died pupa that had retention of morphological characters of larvae. The three extracts inhibited the normal development of larvae and most of them molted into defective or malformed pupae.

DISCUSSION AND CONCLUSION

This study identified the impact of pomegranate extracts on *S. littoralis*. Besides the high antifeeding effect exerted by ethanol and methanol extracts on larvae maintained on *E. spinosa* leaves, the three extracts affects significantly all gravimetric indices. This explains why the peel of *P. granatum* is rich not only in compounds acting on the taste system of the insect, but also on his digestive system causing the disturbance of digestion of ingested food and consequently inhibit its growth. The decrease of the relative growth rate reflects a decrease of the efficiency of conversion of ingested and digested food. In this respect, Koide *et al.* [43] reported that toxicity caused by *P. granatum* is due to the astringent properties of tannins contained in the peel fruit which stop insect's infestation.

In addition to their antifeedant and anti-digestive activities, the three extracts led to a significant extension of the duration of the development stages. This extension can be explained by the existence of secondary metabolites that interfere with the physiological system of treated insects. The development of larvae maintained on *E. spinosa* leaves treated with the three extracts was delayed. The prolonged larva stage was generally related to the slower growth, the possible existence of toxic allelochemicals in the plant extract or the occurrence of nutritional imbalance.

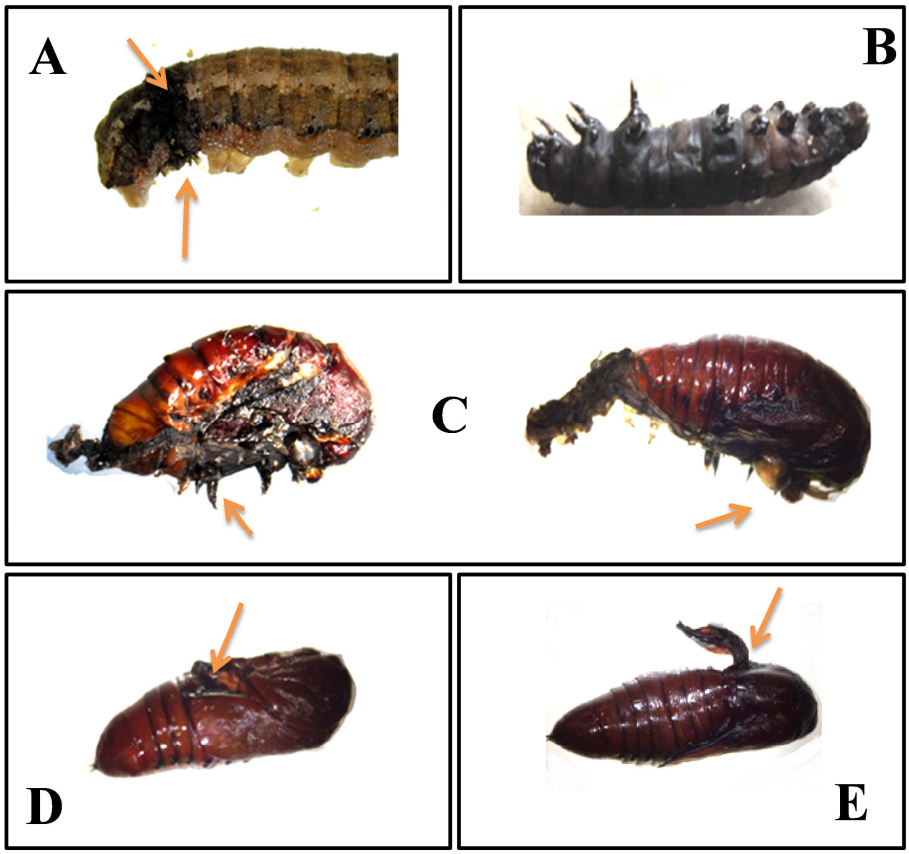

Figure 2. Morphological deformations of *S. littoralis* maintained at artificial diet treated with 2% of three pomegranate peel extracts. A: larva with incomplete molting process. B: dead larvae showing a necrosis in the integument; C: pupa showing the persistence of juvenile characters. D: pupa with morphological features of larvae and E: pupa with head capsule.

We also observed morphological alterations. These abnormalities affect larval and pupal stages. This effect may be attributed to the presence of some compounds in the extract, causing blockage and inhibiting the release of ecdysteroids (molting hormone) responsible for the exchange process of the exoskeleton [44].

These results indicating that this plant material was effective in reducing the normal growth and developmental processes of *S. littoralis*. This was shown previously in *T. castaneum* [35, 45].

P. granatum has shown promising disruptive activity. The peel extracts affected the feeding, ingestion and the morphological development of *S. littoralis*. It would therefore indicate that *P. granatum* peel extracts may be a promising alternative to synthetic insecticides for control this destructive Leafworm.

REFERENCES

[1] Horowitz, A.R., Forer, G. and Ishaaya, I. Insecticide resistance management as a part of an IPM strategy in Israeli cotton fields. In: Constable, G.A. and Forresater, W.W. Challenging the future, Proc. Of the World Cotton Research Conference, I, ed., Csiro, Australia. **1994**, pp 537- 544.
[2] G. Smagghe, D. Degheele, *J Econ Entomol*, **1997,** 90, 278- 282
[3] Torssell, K.B.G. Chemical Ecology, 2nd ed., Swedish Pharmaceutical Press, Stockholm, Sweden. **1983**, pp 42–79.
[4] Migues, I., Magrini, F., Heinzen, H. and Cesio, M.V. Use of plant extracts and wastes from agroindustry as pest management agents. In: Nollet, N.L.M. and Rathore, H.S. Biopesticides Handbook. ed., CRC PressTaylor & Francis Group, London, New York. **2015**, pp 227-245.
[5] N.J. Oldham, W. Boland, *Naturwissenschaften*, **1996,** 83, 248–254.
[6] D. Schlee, *Pharmazie*, **1991,** 46, 19–23.
[7] J.B. Harborne, *Nat Prod Report*, **1993,** 6, 327–348.
[8] J.A. Mordue, A. Blackwell, *J Insect Physiol*, **1993,** 39, 903–924.
[9] J.A. Mordue, A.J. Nisbet, *An Soc Entomol Bras*, **2000,** 29, 615–632.

[10] Smith, C.M. Use of plant resistance in insect pest management systems. In: Plant Resistance to Insects, ed., Wiley, New York, **1989a**, p 286.
[11] Holland, D., Hatip, K. and Bar-Yaakov, I. Pomegranate: Botany, horticulture and breeding. In: Janick, J. Horticultural reviews, ed., John Wiley & Sons Inc. **2009**, pp: 127-191.
[12] Mars, M. Pomegranate plant material: Genetic resources and breeding, a review. In: Melgarejo, P., Martínez-Nicolás, J.J. and Martínez-Tomé, J. Production, Processing and Marketing of Pomegranate in the Mediterranean Region. Ed., Adv. Res. Technol., Zaragoza: CIHEAM. 2000, pp: 55-62.
[13] J.A. Khan, S. Hanee, *Int J Appl Biol Pharmaceutical Tech*, **2011,** 2, (3), 23–27.
[14] R.P. Singh, K.N. Chidambara Murthy, G.K. Jayaprakasha, *J Agric Food Chem*, **2002,** 50, (1), 81–86.
[15] P.S. Negi, G.K. Jayaprakasha, B.S. Jena, *Food Chem*, **2003,** 80, (3), 393-397.
[16] L.C. Vasconcelos, F.C. Sampaio, M.C. Sampaio, S. Pereira Mdo, J.S. Higino, M.H. Peixoto, *Braz Dent J*, **2006,** 17, (3), 223-227.
[17] E.P. Lansky, R.A. Newman, *J Ethnopharmacol*, **2007**, 109, (2), 177-206.
[18] M. Reddy, S. Gupta, M. Jacob, S. Khan, D. Ferreir, *Planta Med*, **2007**, 73, 461-467.
[19] J.S. Jurenka, *Altern Med Rev*, **2008**, 13, (2), 128-144.
[20] A.A. Tayel, A.F. El-Baz, M.F. Salem, M.H. El-Hadary, *J Plant Dis Protec*, **2009**, 116, (6), 252-256.
[21] C. Augusta, F. Bonzanini, G. Palla, M. Cirlini, R. Bruni, *Plant Foods Hum Nutr*, **2010**, 65, 277-283.
[22] S. Abdollahzadeh, R.Y. Mashouf, H. Mortazavi, M.H. Moghaddam, N. Roozbahani, M. Vahedi, *J Dent (Tehran)*, **2011**, 8, (1), 6.
[23] M.A. Dkhil, *Parasitol Res*, **2013**, 12,(7), 2639-2646.
[24] J.G. Eldiasty, M.M. Hassan, O.M.H.M. Kamal, *Egypt Acad J Biol Sci*, **2014**, 6, (1), 1-16.
[25] V.I. Hukkeri, G.A. Kalyani, B.C. Hatapaki, F.V. Manvi, *Fitoterapia*, **1993**, 64, 69-70.
[26] Tripathi, S.M. and D.K. Singh, *Braz J Med Biol Res*, **2000**, 33,(11), 1351-1355.
[27] S.M. Tripathi, V.K. Singh, S. Singh, D.K. Singh, *Phytotherapy Res*, **2004**, 18,(7), 501-506.
[28] Osman, G.Y., Mohamed, A.H., Salem, T.A. and Elmalawany, A.M. Immunoparasitological effect of *Punica granatum* in *Schistosoma mansoni* infected mice. 10th International Conference on Future Horizon of Environmental Sustainable Development in Arab Countries and Facing the Challenges Sharm El-Sheikh, Egypt. 21-24/12/2013.
[29] A.N. Sharma, M. Rajguru, *Soybean Res*, **2009**, 7, 102-105.
[30] S.M. Mahmood, *Tikrit J Pure Sci*, **2010**, 15, (2), 174-180.
[31] S.A. Mansour, R.F. Bakr, L.S. Hamouda, R.I. Mohamed, *Biopestic Int*, **2010**, 6, (2), 129-145.
[32] S.A. Mansour, R.F.A. Bakr, L.S. Hamouda, R.I. Mohamed, *Egypt Acad J Biolog Sci*, **2012**, 5, (1), 151-167.
[33] N. Ghandi, S. Pillai, *Int J Agric Biol*, **2011**, 13, 535-540.
[34] H.H. Mohammad, *J Agric Sci Technol*, **2012**, A, 2, 1175-1181.
[35] A. Ben Hamouda, A. Mechi, K. Zarred, I. Chaieb, A. Laarif, *Tun J Plant Prot*, **2014**, 9, (1), 91-100.
[35] J.G. Eldiasty, M.M. Hassan, O.M.H.M. Kamal, *Egypt Acad J Biol Sci*, **2014**, 6, (1), 1-16.
[36] K. Ghoneim, A. Al-Daly, M. Amer, A. Mohammad, F. Khadrawy, M.A. Mahmoud, *Entomol Applied Sci Lett*, **2014a**, 1, (1), 1-10.
[37] K. Ghoneim, M. Amer, A. Al-Daly, A. Mohammad, F. Khadrawy, M.A. Mahmoud, *Int J Current Res Acad Rev*, **2014b** , 2, (6),18-34.
[38] K. Ghoneim, M. Amer, A. Al-Daly, A. Mohammad, F. Khadrawy, M.A. Mahmoud, *Entomol Appl Sci Lett*, **2014c**, 1,(2), 9-19. Ava
[39] M.S.J. Simmonds, W.M. Blaney, S.V. Ley, G. Savona, M. Bruno, B. Rodríguez, *Phytochem*, **1989**, 28, 1069-1071.
[40] Z.L. Liu, S.H. Goh, S.H. Ho, *J Stored Prod Res*, **2007**, 43, 290-296.
[41] G.P. Waldbauer, The consumption and utilization of food by insects. *Adv Insect Physiol*, **1968**, 5, 229–288.
[42] R.R. Farrar, J.D. Barbour, G.G. Kennedy, *Ann Entomol Soc Am*, **1989**, 82, 593-598.
[43] T. Koide, M. Nose, M. Inoue, Y. Ogihara, Y. Yabu, N. Ohta, 1998. *Planta Med*, **1998**, 64, 27-30.
[44] C.L. Cespedes, S.C. Molina, E. Muñoz, C. Lamilla, J. Alarcon, S.M. Palacios, M.C. Carpinella, J.G. Avila, *Ind Crop Prod*, **2013**, 42, 78-86.
[45] N. Gandhi, S. Pillai, P. Patel, *Int J Agric Biol*, **2010**, 12, 616-620.