Effect of Four Plant Extracts (Vinca, Ak, Neem and Chinaberry) on The Level of Carbohydrate, Proteins and Lipids in The Body of The 2nd Instar Larvae of The Cabbage Worm, *Pieris rapae* L. (Lepidoptera, Pieridae)

Manal E.A. Elshaier[1], Hanaa I. Mahmoud[1], Horia A. Abd-El Wahab[2] and Ghada El S. A.[2]
[1]Faculty of Science – Girls Branch- Al Azhar Univ. Cairo, Egypt.
[2]Plant Protection Research Institute Dokki- Giza, Egypt

Received on: 3/12/2016 *Accepted: 30/8/2016*

ABSTRACT

Effect of four plant extracts (vinca Catharanthus roseus, ak Salix alaxensis, neem Azadirachta indica and chinaberry Melia azedarach) on the levels of carbohydrate, proteins and lipids were evaluated in the body of 2nd instar larvae of the cabbage worm, Pieris rapae L.(Lepidoptera, Pieridae). The results indicated that the total carbohydrate content in the body of the larvae decreased when treated with all tested concentrations of extracts (69.00, 151, 167 and 173.33 mg % for vinca, chinaberry, neem and ak extracts, respectively), compared with 174.43 mg % for control. Total protein content decreased in the body of larvae when treated with vinca, neem and chinaberry extracts at 118, 155.88 and 183.66 mg %, respectively, but increased in ak extract at 213 mg % compared with 187.66 mg % for control. Total lipid content decreased in the body of the larvae, when treated with all plant extracts (vinca, chinaberry, ak and neem extracts) at 31.66, 43.66, 68 and 69.33 mg %, respectively, compared with 81.66 mg % for control. These results indicated that the total percentages of these components were high in ak extract than in vinca extract, while the plant extracts of chinaberry and neem had moderate rate.

Key words: Pest control- Plant extracts- vinca- ak- neem– chinaberry-carbohydrate- proteins-lipids- cabbage worm, *Pieris rapae* L.

INTRODUCTION

The cabbage worm, *Pieris rapae* L.(Lepidoptera: Pieridae), is the most important foliar pest of Cole crop, occurring occasionally wherever crucifers are grown. The cabbage leaf worm develops on cabbage, cauliflower, broccoli, radish and turnip greens (Tuan *et al*., 2014). This economic importance is due to the larvae make the leaves riddled with large holes of irregular shape and size and also cause stunt head (Kamel, 1989).

In the past, the control strategies of plant pests were completely relied on synthetic insecticides (Sattar et al., 2014). In Egypt, many problems have been encountered as result of the extensive and random use of synthetic chemical insecticides including residual contamination of human foods, mammalian toxicity and pollution of the environment (Abd El- Wahab, 2003).

There is a growing interest in natural pesticides which extracted from plants(Elango *et al*., 2012). Many plant extracts were tested for their insecticide properties (Isman *et al*., 2006; Nisar *et al*., 2012).

Natural insecticides are one of the alternatives to chemical insecticides, which is regarded as safer, cheaper and useful control agents(Berebaum, 1989; Sarwar and Salman, 2015). Carbohydrate contribute to the structure and functions of all insect tissues (Chippendale, 1978). Proteins are essential to the process of cell division and control many reactions in the cellular metabolism (Agosin, 1978; Adel et *al*., 2014).

Lipids are fundamental structural components of the cell membrane and cuticle, they provide a rich source of metabolic energy for periods of sustained energy demand and they include important hormones and pheromones(Downer, 1978; Daylan *et al*., 2014). The present study was undertaken to investigate the effect of the tested plant extracts on the level of carbohydrates, proteins and lipids in the body of the 2nd larval instar of the cabbage worm, Pieris rapae L.

MATERIALS AND METHODS

Rearing of Pieris rapae L.

Larvae and pupae were collected from open field which is known as free of insecticides, reared in the laboratory then adults were maintained under semi-condition of greenhouse (big cage "200 × 250 × 300" cm). The source of food was naturally represented in balm plant (Ocimumbasilicum), geranium (Pelargonium gerveolens) and rose (Rosa gallica). Leaves of Brassicae oleracea Linn. plant with eggs were transferred to jars (250 cc) and covered with pieces of thin mesh fixed in place with a rubber band. The hatched larvae were provided with fresh cabbage leaves, in an incubator of 27± 2 ℃ and 60 ± 5 R.H.(Mona 1992).

Preparation of plant samples and extraction:

Leaves of vinca, ak and chinaberry plants were left to dry at room temperature for one month then they were grinded into fine powder. Also, the seeds of neem were grind into fine powder in an electric mill. Powder of each plant was soaked in a mixture of hexane, acetone and ethanol solvents of equal proportion (1:1:1) in a flask for about one week.

Finally, the flasks were shaking in a shaker and their contents were filtered. The solvents were evaporated under reduced pressure; the crude extracts were weighted and kept in deep freezer until use.

Preparing the Stock Solution of the Tested Plant Extracts:

Convenient stock concentrations of each extract were prepared on basis of the tested plant weight and the volume of the distilled water(w/v) in the presence of tween 80(0.1%) as emulsifier. The stock concentrations were kept in glass stoppered bottles and stored under refrigeration. Four diluted concentrations for each plant extract were tested and obtained data were used to draw the LC-P Lines. Three replicates were used for each concentration.

Methods of application:

Under laboratory conditions, cabbage leaves were dipped in the tested concentration and left to dry. The 2nd instar larvae were allowed to feed on the treated leaves. Three replicates for each concentration were made. Mortality was recorded daily for 7 days after treatment and the living ones of the treatment were examined daily until final mortality, and percentage of mortality was calculated and corrected by Abbott´s formula (1925). Data were plotted on log dosage Probit Papers and statistically analyzed according to Finney (1952).

The same technique was used with water only and the emulsifier as a control.

Phytochemical Examination:

Previous studies explained that V. rosea contains vinblastine and vincristine which are alkaloid, C. procera contains calotropin which is glycoside, A. indica and M. azedarach contain azadirachtin, which is triterpenoid; so phytochemical examinations were made as a result of these theory as follows:

1- Test of Alkaloids and Nitrogenous Basis:

Alkaloids were tested according to the method of Linskens and Jacoson (1994).

2- Test of Glycosides:

Glycosides were tested according to the method of Stank *et al.* (1963).

3- Test of unsaturated sterols and/or Triterpenes:

-Liebermann- Burchard´s test(Liebermann and Burchard, 1890).

- Salkowski´s test: According to Wall *et al.*(1954).

Biochemical effects:

For this purpose, treated larvae of the highest concentrations were used to determine the total carbohydrate, protein and lipid content.

1-Determination of the total carbohydrate content:

Total carbohydrate content was determined as glycogen by the anthrone method that was described by Seifter *et. al.* (1950).

2-Determination of the total protein content:

Total protein in the whole insect homogenate was determined by the Biuret method (Wooten 1964).

3-Determination of the total lipid content:

Total lipid content was determined according to Knight et al. (1972).

Design of the experiments and statistical analysis of the obtained data were made according to Le Clerg et al.(1966). Duncan's new multiple range tests was used for testing the differences between treatments (Le Clerg et al., 1966).

RESULTS

1- Preliminary screening of phytochemical constituents of tested plants:

Data in Table(1) indicated that chinaberry leaves contained moderate amount of triterpens while neem seeds contained high amount of triterpenes (Jacobson, 1989; Koul, 1990 and Schmutterer 1990).

Also, data showed that vinca leaves contained highly amount of alkaloids(Rahman *et al.* 1994). Ak leaves contained highly amount of glycosides (Akinloye 2002; Ibrahim 2001).

In this respect, examination of the obtained results indicated that the importance role of plant species and parts as well as solvent of extraction in determining the phytochemical constituents of the tested plant extracts.

2- Effect of some plant extracts on the levels of carbohydrate, proteins and lipids in the body of the treated larvae of Pieris rapae L.:

2.1. Chemical analysis of the treated larvae of Pieris rapae L.

The effect of the plant extracts on total carbohydrate, total proteins and total lipids percentages was presented in Table (2).

2.2. Total carbohydrate:

The carbohydrate was not affected by all plant extracts for all treatments except that with vinca extract which decreased the carbohydrate content by about 69.00mg./dl compared with 174.43mg./dl for control.

2.3. Total proteins:

The proteins were not affected by the recording extracts 183.66mg./dl for chinaberry compared with 183.66mg/dl. for control. While the plant extracts, vinca and neem were decreased the proteins compared to control, 118, 155.88 and 187.66 mg./dl. respectively.

Table1: Preliminary phytochemical screening of the tested plant extracts.

Constituents \ Plant extract	Chinaberry (leaves)	Neem (seeds)	Vinca (leaves)	Ak (leaves)
1- Triterpenes	+ +	+ + +		
2- Alkaloids			+ + +	
3- Glycosides				+ + +

+ + + High amount + + moderate amount

Table 2: Effect of some plant extracts on components of the body of 2nd instar larvae of Pieris rapae L.

Treatment	Total carbohydrate%	Total protein%	Total lipids%
Vinca	69.00[c]	118.00[b]	31.66[c]
Chinaberry	151.00[b]	183.66[a]	43.66[c]
Neem	167.00[a]	155.66[a]	69.33[b]
Ak	173.13[a]	213.00[a]	68.00[b]
Control	174.43[a]	187.66[a]	81.66[a]

Also, the plant extract ak was increased the proteins compared to control with 213 and 187.66 mg./dl., respectively.

2.4. Total lipids:

Data in Table (2) cleared that the total lipids decreased by all treatments, to 31.66, 43.66, 68 and 69.33 mg./dl. With the plant extracts vinca; chinaberry, ak and neem, respectively, compared with control 81.66 mg./dl. The simple correlation analysis showed that there was a positive correlation between the total carbohydrate, total proteins and total lipids contents in the body of larvae of *Pieris rapae* L. and mortality percentages of the four plant extracts; vinca, chinaberry, ak and neem.

On the other hand, the total carbohydrate was significant with vinca and chinaberry, and high significant with neem and ak extracts. While total proteins were high significant with vinca and neem extracts. The total proteins were significant with ak and non-significant with chinaberry extracts. The total lipids were high significant with vinca and neem extracts but significant with chinaberry and ak extracts.

The correlation between phytochemical components of the body of larvae of Pieris rapae L. treated with some plant extracts and mortality:

Data represented in Table (3) and Fig. (1) showed the total carbohydrate, total proteins, total lipids and mortality percentages of 2nd instar larvae of P. rapae L.

The simple correlation analysis showed that there was a positive correlation between the total carbohydrate, total proteins and total lipids contents in the body of larvae of *Pieris rapae* L. and mortality percentages of the four plant extracts; vinca, chinaberry, ak and neem.

DISCUSSION

1-Preliminary screening of phytochemical constituents of the tested plants:

Results indicated that chinaberry and neem contained achievely highly amounts of triterpenes which identified as azadirachtin by Jacobson 1989, Koul 1990 and Schmutter 1990.

Obtained results that Vinca contained mainly highly amount of alkaloids were agreement of Rahman et al.(1994), who proved that these alkaloids were vinblastine and vincristine.

Present results indicated also that, ak contained mainly highly amount of glycosides which recorded by Akinloye *et al.* (2002) as cardiac glycoside.

Table 3: The correlation between phytochemical components of the body of 2nd instar larvae of Pieris rapae L. treated with some plant extracts and mortality.

Components (mg.)	General mortality mean %					L.S.D.0.05
	Vinca	Chinaberry	Neem	Ak	Control	
	65%	60%	60%	77.55%	---------	
Total carbohydrate	69.00	151	167	173.13	174.43	---------
R.	0.965**	0.953**	0.977***	0.989***		
Total protein	118	183.66	155.88	213	187.66	---------
R.	0.998***	0.528*	0.977***	0.714**		
Total lipids	31.66	43.66	69.33	68	81.66	---------
R.	0.982***	0.844***	0.99***	0.822***		
	2.25± 0.25	2.75± 0.25	2.25±0.5	2.5±0.5	2.05±0.05	0.862

*non-Significant ** Significant *** highly Significant

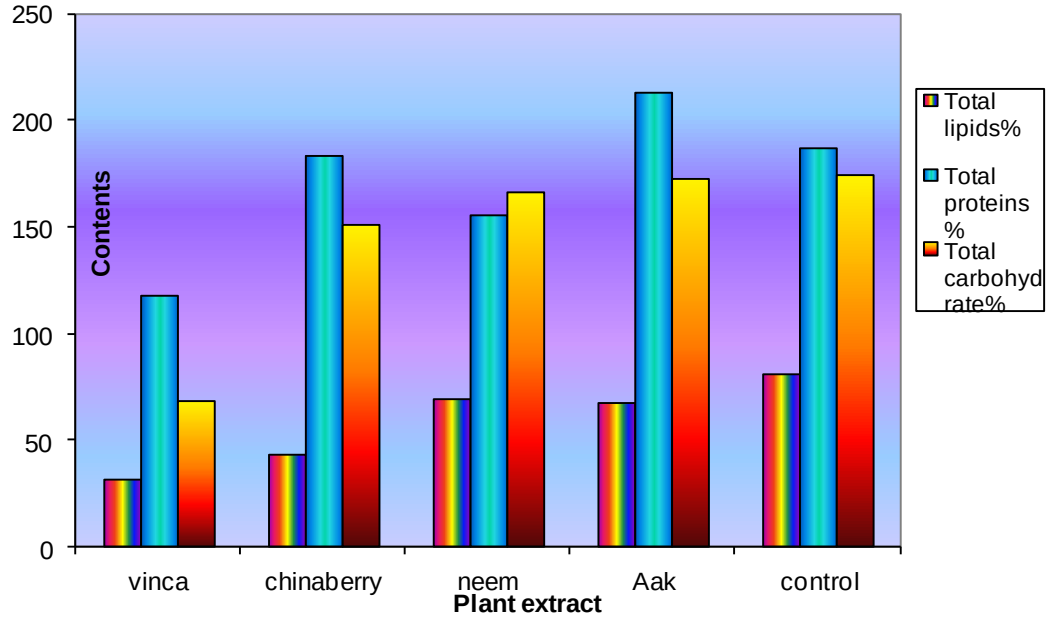

Fig. 1: Effect of some plant extracts on the levels of carbohydrates, proteins and lipids in the body of the treated larvae of Pieris rapae L.

2- Effect of some plant extracts on the levels of carbohydrate, proteins and lipids in the body of the 2nd instar larvae of Pieris rapae L.

The results indicated that total carbohydrate, proteins and total lipids suffered considerable reduction in the treated 2nd instar larvae of *Pieris rapae* L. due to the treatment with various plant extracts (vinca, ak, chinaberry and neem) but protein content increased when larvae treated with ak extract, compared with control.

In this connection, our results could be supported by the work of Taha et al (1989) which recorded a highly significant reduction in the glycogen content of the 4th nymphal instar of Spodoptera littoralis previously treated with V. rosea acetone extract. Moreover, they observed a significant decrease of the nymphal total lipid and protein contents.

Our results agreed with Zhang and Chiu(1992) who found that the activities of proteinases in the larval midgut of Pieris rapae L., decreased as the result of treatment of larvae with toosendanin(botanical material from the bark of chinaberry).

Also, obtained results agreed with Abo El-Ghar et al. (1995) who showed that the treatment of 6th instar larvae of Agrotis ipsilon with extracts of Melia azedarach and Vinca rosea resulted in considerable reduction in the total protein, lipids and carbohydrates. Schmidt et al. (1998) proved that protein content in the hemolymph of Sposdoptera littoralis and A. ipsilon was decreased significantly due to larval treatment with M. azedarach extract.

In conclusion, the results obtained in these investigations may encourage further research of practical nature for cabbage leaf worm control in the future. Ak extract was recommended for control of this pest. Similar conclusion was previously reported by Chaudhry (1992) and Ibrahim (2001).

REFERENCES

Abbott,W.S.(**1925**): A method for computing the effectiveness of an insecticide. J. Econ. Entomol., **18**: 265-267.

Abo EL-Ghar G.E.S.;. Khalil, M.E. and Eid, T.M.(**1995**): Some biochemical effects of plant extracts in the black cutworm, Agrotis ipsilon (Hufnagel). Bull. Entomol. Soc. Egypt, Econ. Ser., **22**, 85.

Abdel-Wahhab, M.A. and Aly, S.E.(**2003**): Antioxidants and radical scavenging properties of vegetable extracts in rats fed aflatoxin-contaminated diet. J Agric Food Chem. **2003** Apr **9; 51(8):**2409-14.

Adel, B.; Jalal, J.S.; Arash, Z. and Roya, K.(**2014**): Effect of heavy metals (Cd, Cu, and Zn) on feeding indices and energy reserves of the cotton boll worm Helicoverpa armigera Hübner(Lepidoptera: Noctuidae) Journal of Plant Protection Research vol. **54**, No. **4**

Agosin, M.(**1978**): Functional Role of Protein. In "Biochemistry of Insects" (Ed. by Rockstein, M.). Academic Press. New York, San Francisco, London. 94- 143 pp.

Akinloye, A.K.; Abatan, M.O.A. and Oke , B.O. (**2002**): Histo-morphometric and histopathological studies on the effect of Calotropis procera (Giant milkweed) on the male reproductive organs of wistar rates. African J. Biomedical Res. **5**:1-2, 57-61.

Berebaum, M.R.b(**1989**). North American ethnobotanicals as sources of novel plant – based insecticides, 11-24 pp.

Chaudhry, M. I.(**1992**): Efficacy of botanical pesticides against Plecoptera reflexa Guen. (Noctuidae, Lepidoptera), shisham defoliator.Pakistan J. Forestry,**42**: 4,199-202.

Chippendale,G.M.(**1978**): The Functions of Carbohydrates in Insect Life Process. In "Biochemistry of Insects" (Ed. by Rockstein, M.), Academic Press, New York, San Francisco, London. 1-55 pp.

Daylan A.; Tzompa, S.1.; Yi, L.; Hein, J.F. and Valenberg, I.(**2014**): Insect lipid profile: aqueous versus organic solvent-based extraction methods; Food Research International 62(**2014**) 1087–1094

Downer, R.G.H.(**1978**): Functional Role of Lipids in Insects. In "Biochemistry of Insects" (Ed. by Rockstein, M.), Academic Press, New York, San Francisco, London. 58-91 pp.

Elango G, Rahuman AA, Kamaraj C, Bagavan A, Zahir AA, Santhoshkumar T, Rajakumar G.(**2012**): Efficacy of medicinal plant extracts against Formosan subterranean termite, Coptotermes formosanus. Industrial Crops and Products **36(1)**, 524-530.

Finney, D. J.(**1952**): Probit analysis (Second Edition). Cambridge Univ. Press, London, 1-661. pp.

Ibrahim,K.I.(**2001**): The effect of some plant extracts as rodenticides on certain rodent species. Ph. D. Thesis, Fac. of Agric., Al- Azhar Univ., Cairo, Egypt.145 pp.

Isman, M. B.(**2006**): Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annual Review of Entomology **51**, 45-66.

Jacobson, M., ed.(**1988- 1989**): Focus on phytochemical pesticides, Vol.1: The Neem Tree. CRC Press, Boca Raton, FL.178pp.

Kamel,M.H.M.(**1989**). Ecological and Biological studies on the cabbage worm, Pieris rapae L., M. Sc. Thesis, Fac. Agric. Cairo Univ., 99pp.

Knight, J.A.; Anderson, S. and James, M. R.(**1972**): Chemical basis of the sulfo- phosphovanillian reaction for estimation of total serum lipids. Clinical chemistry. Vol.**18**,No.**3**:199-202.

Koul,O.; Isman, M.B. and Ketter, C.M.(**1990**): Properties and uses of Neem, Azadirachta indica, Canadian J. Bot. 68:1-11.

Le Clerg, E.L.; Leonard, W.H. and Anderow, G.C.(**1966**): Field plot technique. 2nd Ed., Burgess publ. Co., Minn eapolis, Minnesota, U.S.A. 373 pp.

Liebermann,C. and Burchard, H.(**1890**): Chem. Intr. Quoted by Halaweish. F.T. 61p.

Linskens,H.F. and Jackson ,J.F,(**1994**): Isolation of alkaloids(Extraction) Modern Methods of Plant Analysis(Alkaloids), Vol.**15**, Germany.2-3 pp.

Mona, B.R.(**1992**): Studies on the biological effects of Bacillus thuringiensis Berliner and Gamma Radiation on Pieris rapae L., M.Sc.Thesis, Fac. Sci. Cairo. 146pp.

Nisar, M.S.; Ahmed, S.; Ashfaq, M. and Sahi, ST.(**2012**): Effect of Leaf and Seed Extracts of Jatropha curcas Linn. On Mortality and Tunneling of Subterranean Termites, Odontotermes obesus (Ramb.) Termitidae.

Rahman, A.U.; Iqbal, Z. and Nasir, H.(**1994**): Synthetic approaches to vinblastine and vincristine- anticancer alkaloids of Catharanthus roseus. Studies in natural products chemistry, Vol.14, H.E.J. Res. Institute of chem., Univ. Karachi, Pakistan 805-806 pp.

Sarwar, M. and Salman, M.(**2015**): Success Stories of Eco-friendly Organically Acceptable Insecticides as Natural Products; Discovery International Journal of Materials Chemistry and Physics; Vol. 1, No**. 3, 2015**, pp. 388-394

Sattar, A.; Naeem M, Ehsan-ul-Haq.(**2014**): Efficacy of Plant Extracts Against Subterranean Termites i.e. Microtermes obesi and Odontotermes lokanandi (Blattodea:Termitidae). Journal of Biodiversity Bioprospecting and Development 1,122.

Schmidt, G.H.; H.; Rembold, A.; Ahmed, A.I. and Breuer, M.(**1998**): Effect of Melia azedarach fruit extract on juvenile hormone titer and protein content in the haemolymph of two species of noctuid lepidopteran larvae(Insecta: Lepidoptera: Noctuidae). Phytoparasitica, **26**:4, 283- 292.

Schmutterer, H.(**1985**): Trials of biological and integrated pest control in tropics. Giessene Beitrage zur Entwicklungsforschung 1 symposien, **12**, 143-150.

Seifter, S.; Dayton, S.; Novic ,B. and Muntwyler, E.(**1950**): Estimation of glycogen with the anthrone reagent, Arch. Biochem. **25**:191- 200.

Stank, J.; Cerny, M.; Kocourek, J. and Pacak, J.(**1963**): The Monosaccharides; Prague. Advanced in Carbohydrate Chemistry 1. 329-344.

Taha, M.A.; Ibrahim, F.I.; Nabil, A.S.; and Salem, S.A.T.(**1989**): Effect of selected plant extracts on feeding activity and some biological parameters of the desert locust Schistocerca gregaria(Forsk.). J. Fac. Edu. Egypt, No. 14, 237- 253 pp. Biochemistry. Churchill, London, 4th Ed. Basal Karge, 264-269 pp.

Taha, N. M.; Anh, B. L. and Bui-Anh, N. H.(**2014**): Efficacy of Garlic and Chili Combination Solution on Cabbage Insect Pests and Crop Growth in Vietnam. International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering Vol:**8**, No:**10**, 2014

الملخص العربي

تأثير اربع مستخلصات نباتية (الونكا، الزنزلخت، العشار والنيم) علي المحتوي الكلي للبروتين والكربوهيدرات والدهون للعمر اليرقي الثاني لفراشة ابو دقيق الكرنب(حرشفية الاجنحة: بيريدي)

منال السيد عبد العزيز الشاعر[١]، هناء إبراهيم محمود[١]، حورية عبد الوهاب[٢]، غادة السيد[٢]

[١]كلية العلوم– فرع البنات– جامعة الازهر القاهرة

[٢]معهد وقاية النباتات بالدقي– جيزة

هدفت الدراسة الحالية الي تقييم تأثير اربع مستخلصات نباتية (الونكا Vinca rosea، العشار Calotropis procera، الزنزلخت Melia azedarach والنيم Azadirachta indica) علي مستوي الكربوهيدرات والبروتين والدهون ليرقات الجيل الثاني لديدان فراشة ابو دقيق الكرنب حيث اظهرت النتائج ان:

المحتوي الكلي للكربوهيدرات في جسم اليرقات انخفض(مع جميع المستخلصات المستخدمة إلى الآتي: ٦٩,٤٤، ١٥١، ١٦٧ و١٧٣,٣٣ مجم /دسل. عند المعاملة بكل من الونكا، الزنزلخت، النيم والعشار، على التوالى، مقارنة باليرقات الغير معاملة ١٧٤,٤٣ مجم /دسل. تقريبا.

انخفض المحتوى البروتينى لليرقات المعاملة بالونكا، النيم والزنزلخت إلى ١١٨، ١٥٥,٨٨، ٦٦, ١٨٣ مجم /دسل. على التوالى، بينما زاد بالنسبة لليرقات المعاملة بمستخلص العشار إلى ٢١٣ مجم/دسل. مقارنة باليرقات غير المعاملة ١٨٧,٦٦ مجم/دسل. تقريبا.

أيضا، انخفض المحتوى الدهنى فى اليرقات المعاملة بكل من(الونكا، الزنزلخت، العشار والنيم) إلى ٣١,٦٦، ٤٣,٦٦، ٦٨ و٦٩,٣٣ مجم/دسل.، على التوالى، مقارنة باليرقات الغير معاملة ٨١,٦٦ مجم/دسل. تقريبا.

وقد أوضحت النتائج أن النسبة الكلية للمكونات السابقة كانت عالية فى اليرقات المعاملة بمستخلص العشار أكثر من الونكا، بينما كانت متوسطة فى اليرقات المعاملة بكل من مستخلصى الزنزلخت والنيم.

وتوصي الدراسة بتوسيع الدراسات المعملية المستقبلية علي هذه المستخلصات في مكافحة افة ابو دقيق الكرنب وأن مستخلص نبات العشار يوصي به لمكافحة تلك الافة.

الكلمات الدليلية: مكافحة– مستخلصات نباتية– الونكا– العشا– الزنزلخت– والنيم– بروتين– الكربوهيدرات– الدهون– فراشة ابو دقيق الكرنب.