

Effect of Humic Acid and Henna Extract *Lawsonia inermis* on Dubas Bug *Ommatissus binotatus* on Date Palm Trees in the South of Iraq

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Abstract

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Date palm is infected by many harmful insect pests, which lead to significant economic losses, among these pests is the dubas bug, *Ommatissus binotatus* (De Berg). This study was conducted to determine the effect of adding humic acid to NPK fertilizer and the use of henna extract *Lawsonia inermis* to reduce the effect of dubas bug on date palm trees. The spring insect infestation began at the beginning of March and reached its peak during April of 30.50% insect/leaf of untreated trees, 25% for trees treated with humic acid and henna extract, and 27.30% for trees treated with henna extract only. As for its autumn activity, it started at the beginning of October, and the peak infestation reached 25.60% for untreated trees, 18.70% for trees treated with humic acid and henna extract, and 20.60% for trees treated with henna extract only. The results also showed that trees treated with humic acid and henna extract were less infested with dubas bug, reaching 5.52%, with a significant difference from untreated trees, which amounted to 7.88%. The results also showed that treating infested leaves with dubas bug with humic acid increased the leaf content of total chlorophyll, wax and phenolic compounds compared with untreated leaves infested with dubas bug.

Keywords: Dubas bug, humic acid, *Lawsonia inermis*, date palm, phenolic substances.

Introduction

The palm trees *Phoenix dactylifera* L. are among the oldest fruit trees in the world. It is characterized by the exceptional importance that made it at the forefront of fruit trees because its fruits are highly nutritional (Al-Bakr, 1972; Al-Jubouri, 2002). The date palm is infested by many harmful insect pests, which lead to significant economic losses (ACSAD, 2001; Barbandi *et al.*, 2000). Among these pests is the dubas bug, *Ommatissus binotatus* var. *lybicus* (De Berg), which affects many date palm varieties. The economic damage of the insects comes from the feeding of both nymphs and adult insects on plant sap of leaves, stalks and fruits (Al-Rawi, 1998; Zarzis & Mohamed, 1992). In addition, the accumulation of dust due to the infestation leads to the fronds weakness and change in colour to yellowish green (Abdel-Hussein, 1985; Mokhtar & Nabhani, 2010). The severe and successive infestation, year after year, may decrease the growth and production of the trees leading in some cases to the death of the palm (Central Statistical Organization, 2000; Ibrahim & Khalif, 2003; Ibrahim, 2008). The availability of the nutrition elements in a balanced form is a significant factor that provides good growth for palm trees and enhances the plant ability to resist adverse environmental conditions, as well as pest infestations (Al-Amodi *et al.*, 2021). The method of adding fertilizers significantly affects nutrient absorption by the plant. Foliar spraying of nutrients ensures that the nutritional elements are absorbed directly into the plant tissues, which reduces energy consumption. The method can also be used by mixing nutrients with growth regulators, which saves a lot of effort and time (Focus, 2003).

Humic acid is the main component of soil organic matter, which has beneficial effects on the physical, chemical and microbiological properties of soil and can improve the growth of a various crops (Arancon *et al.*, 2006). Humic acid affects the cell membranes of plants, resulting in enhanced mineral transport, protein synthesis, plant hormone activity, photosynthesis, and modulating enzyme activities (Arancon *et al.*, 2003; Unay, 2011). In addition, it can increase the resistance of plants to insects and in some cases can positively affect natural enemies (Nasab *et al.*, 2019). The control with chemical pesticides is one of the most important methods that have been used to limit the spread of this insect and reduce the number of pathogens it transmits. However, the intensive use and wrong application led to toxicity of non-target organisms leading to an imbalance in the ecosystem.

The continued use of pesticides resulted into pesticide-resistance in pests, which is one of the major obstacles in agricultural production (Isman, 2006). Thus, attention has shifted to the use of natural products of plant origin, because pesticides of plant origin are effective on insects and have low toxicity to higher animals (Bowers & Jarvis, 1992). They also work as repellents, attractants and inhibit pest feeding and reproductive activity (Ibrahim, 2007; Raju *et al.* 1990; Schoonhoven, 1982). Al-Rubaie *et al.* (2000) indicated that the effectiveness of oil and water seed extracts of neem plant, *Azadiracta indica* and *Melia azedarach*, against the nymphs and adults of the dubas bug with high mortality of both stages.

Previous studies indicated that treatment with the aqueous henna extract affects the biochemical activity of the cotton leaf worm *S. littoralis*, with changes in all tested

enzymes (phenoloxidase, chitinase, protease, alkaline and acid phosphatase) and disturbances in the protein levels at all concentrations (Abdel Razzik *et al.*, 2018). This study investigated the effect of humic acid added to NPK fertilizer and henna extract on reducing the number of dubas bug on date palm trees.

Materials and Methods

Infestation rate

This study was conducted during the 2020 and 2021 seasons in an orchard in Abu Al Khasib area in Basrah governorate, where liquid humic acid at 1% concentration added to NPK fertilizer was used monthly to irrigate four meters high date palm trees of the Halawi variety infested with dubas bug. Samples were collected from date palm trees infested with dubas bug. Three trees homogeneous in age and vegetative growth were selected for each treatment, in addition to the control trees without treatment. Infestation rate with dubas bug was assessed. A nine leaflets sample was taken per treatment (3 trees x 3 leaflets) and the number of bugs were counted. Assessment was made monthly from November 5, 2019 to October 25, 2021. The infested samples were placed inside nylon bags and transferred to the laboratory for examination.

Henna plant sample collection and alcoholic extract preparation

Samples of the henna plant *Lawsonia inermis* were collected and the leaves were washed with tap water and then placed on filter papers in a suitable air stream with continuous stirring to dry them and prevent them from rotting. After that, the leaves were ground and the dry powder was kept in the refrigerator until use. Using ethanol as a solvent, 20 gm of plant dry powder was placed in Thambles paper containers, then placed in the Soxhlet extractor using 200 ml of solvent at a temperature of 40°C for 24 hours, and the extract was dried with a rotary evaporator (Almansoori *et al.*, 2021).

Effect of the plant extract on the adult and different nymph stages of dubas bug in vitro

The base solution (stock solution) was prepared from the henna plant *Lawsonia inermis* by dissolving 1 gm of dry residue in 5 ml of alcoholic solvent and the volume was completed to 10 ml with distilled water so that the original solution was at a concentration of 10%, and concentrations of 25, 50, 75 and 100% were prepared. For each concentration, 1 ml of liquid paraffin was added as an adhesive and two drops of Tween 80 as a diffuser for every 100 ml of concentration, 80 ml of distilled water, liquid paraffin and Tween 80 (Harborne, 1984). In 9 cm plastic Petri dishes, palm fronds pieces were placed on filter papers moistened with distilled water. The different concentrations were evaluated separately, with three replications for each. The nymph mortality rate was recorded 24, 48 and 72 hours after treatment and corrected according to Schenider and Orell's equation (Shaban & Al-Mallah, 1993).

Then the values were shifted angularly, and the data were analyzed as a factorial experiment with C.R.D design at P= 0.01 (Al-Rawi & Khalaf–Allah, 1980).

$$\text{Corrected mortality (\%)} = \frac{\text{Mortality rate in treated plot} - \text{mortality rate in control plot}}{100 - \text{Mortality rate in control plot}} \times 100$$

Effect of alcoholic extract on nymphs and adults of field Dubas bug

The henna alcoholic extracts were sprayed on 4 years old date palm trees in an orchard in the Abu Al-Khasib area in Basrah governorate, at the rate of 500 ml/tree and at 0.0, 25, 50, 75 and 100% concentration, with three replicates for each treatment. The readings were taken 3, 5, 7 and 10 days after treatment. The mortality rate was calculated according to Schenider and Orell's equation (Shaban & Al-Mallah, 1993).

Total chlorophyll content of leaves

The total chlorophyll in the leaves of palm trees (Al-Halawi) was estimated according to Mackinney (1941). One hundred mg of leaves was cut into small pieces by scissors and ground in a ceramic mortar by adding 6 ml of acetone 80% until the color of the precipitate became free of green dye. The extract was centrifuged, and the supernatant was collected in volumetric tubes covered with opaque paper to prevent the dye's photo-oxidation. The filtrate volume was completed by adding acetone, and then the optical density was measured at 645 and 663 nm wavelength in a Shimadzu UV-visible (Type 1700) spectrophotometer.

Leaf wax content

Wax was measured according to a method published earlier (AOAC, 1975), by taking 100 gm of dried palm leaves in an electric oven at 40°C for 72 hours and then placed in a Soxhlet continuous thermal extraction device using a hexane solvent for seven cycles at 60°C for each category.

Leaf content of total phenolics

The total phenols in palm fronds were determined using the Ciocalteau-Folin method, where 2.5 ml of 10% Folin reagent and 2 ml of 2% Na₂NO₃ were added to 1 ml of the plant sample extract, then the mixture was incubated for 15 minutes at room temperature, and then the sample absorbance was measured using a spectrophotometer at a wavelength of 765 nm using galic acid as a standard compound (Aiyegoro & Okoh, 2010).

Statistical analysis

Values obtained from laboratory experiments were analyzed according to a C.R.D. design at a probability level of 0.01. Values obtained from field factorial experiments were analyzed according to R.C.B.D. design at a probability level of 0.05, and compared based on the least significant difference (LSD) (Al-Rawi & Khalaf–Allah, 1980). Statistical analysis was performed using the software SPSS Statistics for Windows Version 21 (IBM, 2012).

Results and Discussion

Dubas bug infestation rate

The results obtained (Figure 1) showed that the dubas bug started their spring activity at the beginning of March, where

the infestation rate was 7.91% for untreated trees, 4.97% for trees treated with humic acid and henna extract, and 5.30% for trees treated with henna extract alone. Peak infestation reached 30.50% during April for untreated trees, 25% for trees treated with humic acid and henna extract, and 27.30% for trees treated with henna extract alone. The number of the insects then started to decrease and disappeared during July and August, whereas its autumn activity started during the beginning of October and reached the peak of infestation of 25.60% for untreated trees, 18.70% for trees treated with humic acid and henna extract and 20.60% for trees treated with henna extract alone. The insect population then began to decrease and disappeared during December, January and February. Results also showed that trees treated with humic acid and henna extract had the lowest infestation with dubas bug of 5.52%, with a significant difference from the control treatment (7.88%).

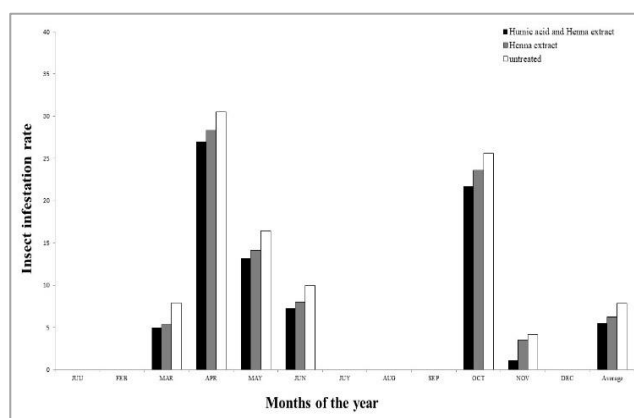


Figure 1. Infestation rate of date palm with dubas bug during the months of the year.

The results obtained in this study were in agreement with previous studies (Yali & Nassab, 2020) in relation to the efficiency of natural enemies such as the seven-point ladybird *Coccinella septempunctata* L in controlling the cabbage aphid *Brevicoryne brassicae* when treated with humic and salicylic acid. Another study Sharif (2011) showed significant superiority of spraying with humic acid at a concentration of 2.5% before or at the beginning of pollen blooming in increasing total yield compared to other treatments. Furthermore, Kock & Mengel (1977) indicated that potassium has an important role in the process of stimulating the transfer and storage of photosynthesis products, which could slightly increase the resistance of trees to pests. The effect of humic acid on the average weight of the fruit may be attributed to the high water content of the fruits. Balanced nutrition is necessary to promote good growth of palm trees with a better ability to resist adverse environmental conditions and pest infestation (Al-Amodi *et al.*, 2021).

The effect of plant extracts on the dubas bug

The results obtained (Table 1) show the effect of different concentrations of the alcoholic henna extract in vitro on the egg, nymph and adult instars of dubas bug. All henna extract concentrations used did not have an effect on the egg stage

of dubas bug. However, the 100% concentration, 72 hours after treatment, led to 61.42% and 57.37% mortality of nymphs and adults, respectively, with a significant difference compared to the control treatment, which amounted to 1.49% and 1.56%, respectively. The period following treatment (24, 48 and 72 hours) had an effect on mortality rate. The mortality rate of nymphs and adults reached 35.07%, 72 hours after treatment, was significantly different from the mortality rate 24 hours after treatment (24.89%), whereas 100% extract concentration 72 hours after treatment gave a higher significant difference from the rest of the treatments for both nymph and the adult stages, with a mortality rate of 68.94% and 63.02%, respectively. On the other hand, the lowest mortality rate was obtained by the 25% extract concentration 24 hours after treatment which amounted to 25.57% and 22.39%, respectively. The results obtained in this study were similar to previous reports (Khalif & Abd, 2017) which indicated the lack of henna extract effect on the eggs of the house fly *Musca domestica*, as the incubation period of the egg reached 1 day with a concentration of 1250 mg/liter. The extract also affected the average weight of the pupa, as the lowest average weight of the pupa was 0.036 gm compared with the control treatment 0.092 g. The deadly effect of these extracts was attributed to the fact that they act as a food inhibitor or hinder the growth and development of the insect. The cause of deaths in the nymph treated with plant extracts may be due to the ability of the extracted materials to inhibit the feeding of the insect through its effect on the enzyme protease and the membrane of the middle alimentary canal midgut, as well as reducing the levels of sugar and the total protein hemoglobin (Chun, 1994).

Table 1. Effect of henna extract on corrected mortality ratio of dubas bug instars in the laboratory.

Extract	Insect stage	Mortality rate (%) after			
		24h	48h	72h	Average
25%	Egg	0.00	0.00	0.00	0.00
	Nymph	25.57	29.89	36.13	30.53
	Adult	22.39	26.65	32.46	27.16
50%	Egg	0.00	0.00	0.00	0.00
	Nymph	30.02	41.56	54.07	41.88
	Adult	31.83	39.71	50.44	40.66
75%	Egg	0.00	0.00	0.00	0.00
	Nymph	41.78	50.96	59.88	50.87
	Adult	40.23	48.11	55.92	48.08
100%	Egg	0.00	0.00	0.00	0.00
	Nymph	55.23	60.11	68.94	61.42
	Adult	51.63	57.47	63.02	57.37
Average		24.89	29.53	35.07	
Control treatment		0.00	1.49	1.56	

LSD for interference between the nymph and the concentration of the extract =9.724; LSD for interference between the adult and the concentration of the extract =8.910; LSD for interference between the period after treatment and the concentration of the extract =4.963.

Effect of henna extract on nymphs and adults of dubas bug in the field

Results obtained (Table 2) showed that the henna extract did not affect the egg stage in the field for all concentrations used. It also showed the superiority of the henna plant extract at 100% concentration 10 days after treatment which led to 54.58% mortality, with a significant difference from the rest of the concentrations used in the study. Highest mortality rate of 54.81% and 54.36% was reached for the nymphs and adults of dubas bug, respectively, 10 days after treatment with 100% henna extract. whereas the lowest mortality rate was reached for nymphs and adults was 20.46% and 20.19%, respectively, three days after treatment with 25% henna extract, which is consistent with a previous study (Khalif & Abd, 2017).

Table 2. Effect of henna extract on corrected mortality rate of dubas bug stages in the field.

Concentration	Period after treatment	Mortality rate (%)		
		Nymphs	Adults	Average
25%	3 days	20.46	20.19	20.32
	5 days	23.10	22.44	22.77
	7 days	34.07	31.75	32.91
	10 days	35.41	33.56	34.48
50%	3 days	28.04	24.66	26.35
	5 days	28.76	25.21	26.98
	7 days	37.12	32.09	34.60
	10 days	36.45	34.80	35.62
75%	3 days	32.68	30.93	31.80
	5 days	37.73	35.27	36.50
	7 days	42.89	41.46	42.17
	10 days	45.97	43.33	44.65
100%	3 days	35.42	33.99	34.70
	5 days	40.04	40.01	40.02
	7 days	50.73	50.52	50.62
	10 days	54.81	54.36	54.58
Average		36.48	34.66	

LSD for interference between the insect stage and the concentration of the extract =1.937; LSD for interference between the insect stage and the period after treatment=4.016; LSD for interference between the period after treatment and the concentration of the extract =3.736.

A previous study Metspalu *et al.* (2001) showed that some toxic compounds in the plant may not lead to rapid killing of larvae, but rather lead to weak growth, as some toxic compounds affect the movement of the digestive canal as well as digestion and absorption activities that occur inside the insect, thus reducing the efficiency of converting food to tissues in favor of the insect. Furthermore, another study Al-Rubaie *et al.* (2000) reported the use of oil and aqueous seed extracts of the neem *Azadiracta indica* and *Melia azedarach* plants against the nymphs and adults of the dubas bug and attributed its effect to the presence of azadirachtin, which has a killing effect on insects.

Relationship between insect infestation and chlorophyll, wax and phenolic content

Severity of palm leaves dubas bug infestation varied (Figure 2), and the total chlorophyll content of the leaves differed significantly between the studied treatments. The chlorophyll content in palm fronds treated with humic acid and henna extract was significantly higher than in fronds of infested and untreated trees. It reached 5.771 mg/100 mg in the palm tree leaves treated with humic acid and henna extract, but trees treated with humic acid or henna extract only contained 5.438 and 5.116 mg/100 mg leaves, respectively. The results were similar to what has been reported earlier Al-Dosari (2009), who demonstrated a negative correlation between the severity of the scale insect infestation and the chlorophyll content of palm leaves. In this study, the high chlorophyll content of date palm leaves was associated with low infestation rate of dubas bug, which is in agreement with earlier findings that insects are generally attracted to the long waves of the yellow color, whereas the green color is insect repellent (Almansoori *et al.* 2021).

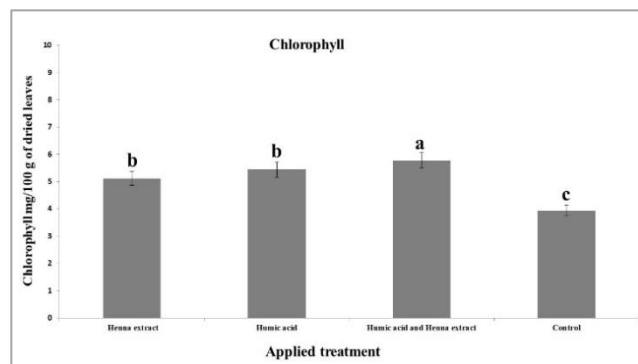


Figure 2. The total chlorophyll content of date palm leaves infested with dubas bug following different treatments.

The results obtained also showed (Figure 3) that the wax content of palm fronds treated with humic acid and henna extract was significantly different from the untreated trees, and it was 1.09 mg/100 g of dried leaves. This shows that the thickness of the waxy layer plays an important role as it impedes the insect's access to the vascular bundles to absorb the plant sap. A discrepancy was found between the severity of infestation and the leaves wax content, and this discrepancy may be attributed to the fact that insects cannot digest fats (wax) externally, because the enzyme responsible for digesting fats, lipids, is found within the tissues of the middle alimentary canal (Al-Haj Ismail, 2009). Thus, the insect cannot digest the waxy layer surrounding the leaf, and thus the resistance of the palm leaves to insect attack increases with the increase in the leaves wax content (Ibrahim & Khalif, 2003).

There was also an inverse relationship between the severity of insect infestation and the leaves content of phenolic compounds after different treatments (Figure 4). Phenolic compounds content of fronds treated with humic acid + henna extract and humic acid alone was significantly different from that of untreated fronds, and it reached 0.191 and 0.177 mg/1 ml leaf extract, respectively.

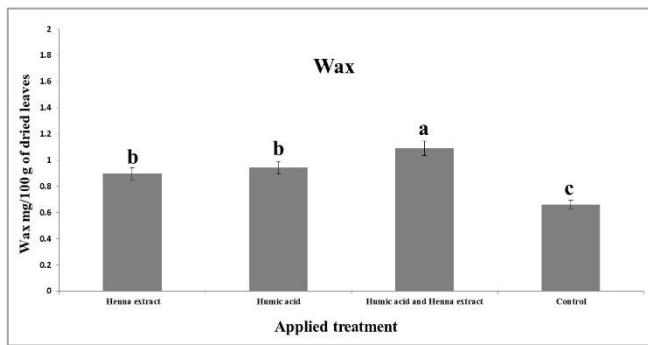


Figure 3. The wax content of palm leaves resulting from the infestation of dubas bug following different treatments.

Phenols are one of the natural compounds found in the palm fronds, and these substances work with proteins to inhibit active enzymes and reduce ready-made proteins in the insect (Sahayaraj *et al.*, 2008), since the insect depends in its production of eggs on the content of its body of proteins (Al-Haj Ismail, 2009), consequently, the lack of proteins in the insect's body reduces the number of eggs laid, and this in turn, leads to reduction in the insect's numbers. In a previous study (Al-Dosari, 2005) it was shown that the fruits of date palm varieties with high phenolics content are less affected by dust mites compared with fruits of varieties with low phenolics content. The variation in infestation severity may be attributed to the different environmental conditions and

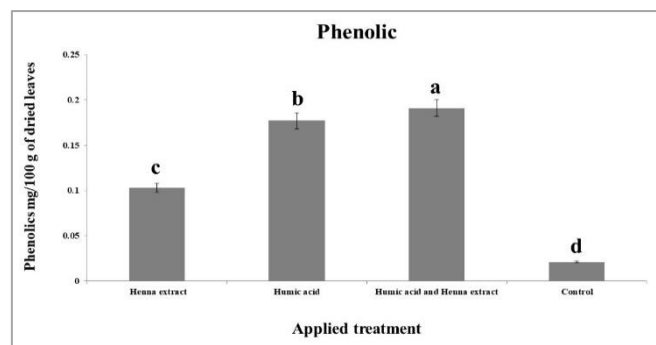


Figure 4. The phenolic compounds content of palm leaves infested with dubas bug following different treatments.

practices in each region. The removal of old and infested fronds, reduces infestation with the dubas bug, and the contact of palm fronds with each other is the most important factor (Hilal & Abbas, 2004).

In conclusion, results obtained in this study indicated that the use of locally available plant extracts of the henna plant *Lawsonia inermis* can be used as a safe and effective component for inclusion in integrated pest management (IPM) programs against *Ommatissus binotatus*. Furthermore, more studies are still needed to improve the efficiency of dubas bug control by combining the use of plant extracts together with other management measures such as predator release, pheromone traps and insecticides to achieve desirable results.

الملخص

الساعدي، غزوان فيصل. 2023. تأثير حمض الهيوميك ومستخلص الحناء *Lawsonia inermis* في حشرة الدوباس *Ommatissus binotatus* التي تصيب أشجار النخيل في جنوب العراق. مجلة وقاية النبات العربية، 41(1): 58-64. <https://doi.org/10.22268/AJPP-41.1.058064>

يصاب نخيل التمر بالعديد من الآفات الحشرية الضارة، والتي تؤدي إلى خسائر اقتصادية كبيرة، ومن بين هذه الآفات حشرة الدوباس (*Ommatissus binotatus*) أجريت هذه الدراسة لمعرفة تأثير إضافة حمض الهيوميك إلى سماد NPK، واستخدام مستخلص الحناء *Lawsonia inermis* لتقليل تأثير حشرة الدوباس على أشجار النخيل. بدأت الإصابة بالحشرات خلال الربيع في بداية شهر آذار/مارس وبلغت ذروتها خلال شهر نيسان/أبريل، حيث بلغت 30.50% حشرة/ورقة للأشجار غير المعالجة، و 25% للأشجار المعالجة بحمض الهيوميك وخلص الحناء، و 27.30% للأشجار المعالجة بمستخلص الحناء فقط. أما عن نشاطها في الخريف، فقد بدأ في بداية شهر تشرين الأول/أكتوبر، وبلغت ذروة الإصابة 25.60% للأشجار غير المعالجة، و 18.70% للأشجار المعالجة بحمض الهيوميك وخلص الحناء، و 20.60% للأشجار المعالجة بمستخلص الحناء فقط. كما أظهرت النتائج أن الأشجار المعالجة بحمض الهيوميك وخلص الحناء كانت أقل إصابة بحشرة الدوباس، حيث بلغت 5.52%، مع وجود فرق معنوي عن الأشجار غير المعالجة والتي بلغت 7.88%. كما أظهرت النتائج أن معالجة الأوراق المصابة بحشرة الدوباس بحمض الهيوميك أدى إلى زيادة محتوى الأوراق من الكلوروفيل الكلي والشمع والمركبات الفينولية مقارنةً بأوراق الشاهد المصاب غير المعامل.

كلمات مفتاحية: حشرة الدوباس، حمض الهيوميك، *Lawsonia inermis*، نخيل البلح، مواد فينولية.

عناوين الباحثين: غزوان فيصل الساعدي*، مركز بحوث نخيل البلح، جامعة البصرة، البصرة، العراق. *البريد الإلكتروني للباحث المرسل: ghazwan.khalaf@uobasrah.edu.iq

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