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The Effect of UV-C Rays and An Insect Growth Regulator on the Growth and Development of Potato Tuber Moth *Phthorimaea operculella* .

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Abstract

The study aimed to control the potato tuber moth *Phthorimaea operculella* (Zeller) by using Insect Growth Regulator (Match) and UV-C with a wavelength of 254 nm on adults and incomplete stages (eggs and larvae). The results indicated that (1-2) days old eggs treated by UV-C rays with different exposure times (4,8,12 and 16 min.), from a distance of 50 cm and insect growth regulator had almost the same effect on the eggs' hatching rates. The highest non-hatching rates of eggs that sprayed by insect growth regulator was 59.30% at a concentration of 2 ml /L. While the highest non-hatched rates of eggs that exposed to UV-C rays was 62.20% at a 16-minute exposure period. The results also showed that of (1-2) day old eggs treated with ultraviolet rays or treated with the insect growth regulator (Match) were more vulnerable than eggs at (5-6) days old. As the proportionality was direct between the rates of eggs non-hatching and the duration of exposure to radiation or the concentration of the insect growth regulator. The results showed that the first instar larvae were more affected than the fourth instar larvae by both insect growth regulator and UV-C treatments, at different concentrations and exposure times. The highest mortality rates for first-stage larvae under the influence of growth regulator were 42.20% at a concentration of 2 ml/L and 52.20% under the influence of UV-C rays, exposure time of 16 min., compared with 5.33 and 8.33% in the control treatment, respectively. As for the effect of ultraviolet radiation on the adults of the potato tuber moth, the difference in fertility results (number of laid eggs / female) decreased dramatically, as the number of eggs

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laid in the control treatment was 113.00 eggs / female. The lowest number of eggs laid was 23.55 eggs / female when insects were exposed to UV rays for 16 min., while the percentages of hatching, pupation and emergence were 76.33, 68.30 and 60.30% in the control treatment, and decreased to 22.30, 18.10 and 8.00% respectively, when insect exposed to UV-C rays for 16 minutes. The results indicate the possibility of using UV-C rays and the insect growth regulator (Match) of chemical pesticides to control potato tuber moth.

Keywords: Economic insect, potato tuber moth, ultraviolet (UV) radiation, insect growth regulation.

تأثير الأشعة فوق البنفسجية UV-Cومنظم النمو الحشري في نمو وتطور عثة درنات البطاطا *Phthorimaea operculella* (Zeller)

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الخلاصة

هدفت الدراسة الحالية دراسة تأثير كلاً من منظم النمو الحشري Match والأشعة فوق البنفسجية UV-C بطول موجيnm 254 على الأدوار الكاملة وغير الكاملة (بيض ويرقات) لعثة درنات البطاطا (Zeller) *Phthorimaea operculella*. أشارت النتائج ان البيض بعمر (1-2) يوم المعامل بالأشعة فوق البنفسجية بأوقات تعرض مختلفة (12،8،4 و16 دقيقة) ، من مسافة 50 cm ومنظم النمو الحشري لهما نفس التأثير تقريباً في نسب عدم فقس البيض ، إذ كانت أعلى نسب عدم الفقس للبيض بتأثير منظم النمو هي 59.30 % عند التركيز 2مل /لتر، أما أعلى نسب عدم الفقس للبيض بتأثير أشعة UV-C هي 62.20 % عند مدة تعرض 16 دقيقة . بينت النتائج أيضاً أن البيض بعمر (1-2) يوم المعامل بالأشعة فوق البنفسجية أو المعامل بمنظم النمو الحشري Match كان أكثر نسبة في عدم الفقس من البيض بعمر (5-6) يوم ، إذ كان التناسب طردياً بين نسب عدم فقس البيض ومدة التعرض للأشعة أو تركيز منظم النمو الحشري . أظهرت النتائج ان يرقات الطور الأول كانت أكثر تأثراً من يرقات الطور الرابع بالنسبة لمعاملات منظم النمو الحشري Match وكذلك بالنسبة لمعاملات أشعة UV-C ، وذلك في جميع التراكيز والمدد الزمنية ،إذ كانت أعلى نسب هلاك ليرقات الطور الأول بتأثير منظم النمو هي 42.20 %عند التركيز 2مل/لتر و 52.20% بتأثير أشعة UV-C مدة تعرض 16 دقيقة بالمقارنة مع 5.33 و 8.33% في معاملة السيطرة على التوالي . أما تأثير الأشعة فوق البنفسجية في بالغات عثة درنات البطاطا ، تبين اختلاف في نتائج الخصوبة (عدد البيض الملقى / انثى) اختلافاً كبيراً ، إذ كان عدد البيض الملقى في معاملة السيطرة 113.00 بيضة/ انثى

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بينما كان أقل عدد بيض ملقى 23.55 بيضة / انثى عند معاملة الحشرات بالأشعة فوق البنفسجية مدة 16 دقيقة ، أما النسب المئوية للفقس والتعذر والبزوغ كانت 76.33 ، 68.30 و 60.30 % في معاملة السيطرة، وانخفضت الى 22.30 ، 18.10و 8.00% على التوالي عند المعاملة بالأشعة فوق البنفسجية مدة 16 دقيقة، تشير النتائج الى امكانية استخدام وسائل بديلة للمبيدات الكيميائية للسيطرة على عثة درنات البطاطا.

الكلمات المفتاحية: حشرة اقتصادية، عثة درنات البطاطا، الأشعة فوق البنفسجية، منظم النمو الحشري.

Introduction

The potato crop, *Solanum tuberosum* L., which belongs to the family Solanaceae, is one of the important vegetable crops because it constitutes a main food in many countries of the world due to its abundant yields and cheap production costs [1]. It is also considered one of the most important strategic vegetable crops in the Arab world, this crop is exposed to many insect pests, especially the potato tuber moth, *P. operculella* (Zeller), which is one of the main pests that attack potatoes in the field and storage [2]. Insect pest control operations are one of the most important pillars of agricultural and animal. Production given the total dependence on pesticides during the past decades in pest control, and the resulting imbalance in the natural balance and the destruction of environmental components. The modern strategies in pest control depends on reducing the use of those pesticides, and replacing them with alternative safe methods, to preserve the environment and return the natural balance to its previous era [3] . One of the most important elements of this strategy was the use of insect growth regulators(IGRs) and UV-C as trends of integrated pest control to protect the crops and increase yield.

The newly emerged larvae of potato tuber moth (PTM) burrows into the leaves and stems, causing them to dry out. They dig tunnels into the tubers to complete their life cycle in the storeroom, the adults come out to mate and the females lay their eggs in the eyes of the tubers and buds to return to infection [4]. Due to its continued reproduction, the pest reached 100% within a month in the event that no, field treatments were conducted to control the insect [5].

In order to reduce the problems caused by these pesticides, researchers resorted to finding more effective and selective pesticides that are safe for the environment, such as growth regulators.

They are highly specialized in their impact on the target species, as these substances can cause a physiological defect that affects transformation and morphology, thus growth regulators have been developed to mimic, prevent or interact with the hormonal system of insects [6].

Insect growth regulators are also manufactured chemicals that are used in pest control, and there is an accelerating global trend towards the use of alternatives to pesticides, especially those environmentally friendly biological compounds. In recent years, the world has turned the other modern techniques that used in pest control, is the use of Ultra-Violet Rays, which have been used at different wavelengths, as they reduce the damage caused by pests to minimum without sacrificing the components of the environment and beneficial organisms. One of the physical methods that have been widely used during the last decades in the control of storehouse insects is the use of electromagnetic energy such as infrared and UV rays [7]. The current study aimed to demonstrate the effect of different concentrations of the insect growth regulator (Match) and UV rays at wavelength 254nm in the eggs and larvae of potato tuber moth.

Materials and methods

Breeding potato tuber moth *Phthorimaea operculella* (Zeller) under laboratory conditions

Potato tubers of the diamond variety were placed in cages measuring (30 x 40 x 60 cm) under laboratory conditions temperature was 25±2 C^{o}, relative humidity was 60±5 % and light period was (12L : 12D) hours. The experiments lasted from 3/10/2020 to 5 /11/2021 in laboratory of the general directorate of Karbala education. Five newly emerged pairs (5 males + 5 females) were released to establish the colony also potato tuber moth placed potato tubers with three pairs (3 males + 3 females) in 3L plastic pots fitted with a cotton swab moistened every day with a 10% sugar solution in order to feed the butterflies and covered with a cloth (soft chiffon) and these pots were placed in the same previous conditions. The newly emerged insects were collected and kept in 1L plastic containers with wide openings covered with a black cloth to see insect eggs clearly, and provided with a cotton swab saturated with 10% sugar solution to feed the butterflies. A 10 cm diameter petri dish was placed on top of the cloth cover, containing a

black filter paper that was moistened with water daily, to form a support base for the ovipositor of females. The eggs laid by females on this paper were collected daily using a soft, damp brush for testing.

Preparation of chitin synthesis inhibitor (Match)

The chitin synthesis inhibitor (Match) was obtained from Syngenta at a concentration of 5% as a concentrated emulsion. Different concentrations of it were prepared on the basis of the active substance Lufenuron by dissolving 2 ml in a liter of distilled water to obtain a stock solution. From the stock solution the different concentrations (0.5, 1, 1.5 and 2 ml/L) were prepared using the dilution law: V1 X C1 = V2 X C2

V1= first volume, C1= first concentration, V2= second volume, C2= second concentration

Effect of chitin synthesis inhibitor (Match) on Potato Tuber Moth Eggs

The eggs on the filter papers were collected and divided into two age groups (1-2) (Light white in color) and (5-6) days old (dark white color). The process of collecting eggs lasted for a week .Then, it was placed in petri dishes a diameter of 9 cm). 20 eggs were placed in each dish, with three replications for treatment group(a total of 15 dishes) . The eggs were sprayed from a distance of 20 cm with chitin synthesis inhibitor (Match) at a concentration of (0.5, 1, 1.5 and 2 ml / L), while the control treatment, it was sprayed with distilled water only. The treated eggs were transferred to volumetric flasks with a capacity of 500 ml containing potato tubers of the diamond variety at a rate of 100 g for each beaker to continue their development. All petri dishes were placed in an incubator under laboratory conditions of 25±2 C^o and relative humidity of 60±5 % and light period was (12L : 12D) hours. The percentage of eggs non-hatching after five days was calculated from the following equation:

$$\text{Death rates} = \frac{\text{The number of non-hatched eggs}}{\text{Total number of eggs}} \text{ X } 100 \%$$

Effect of UV-C radiation on potato tuber moth eggs

The eggs on the filter papers were collected and divided into two age groups (1-2) and (5-6) days old. Then they were placed in plates with a diameter of 9 cm and in each plate 20 eggs, with three replications for each age group(a total of 15 dishes) and for each period of time. The plates were placed at a distance 50cm from a UV exposure source at a wavelength of 254nm and exposed for four periods (4,8,12 and 16 min) . For the control treatment, it was not exposed to radiation, the eggs were transferred to volumetric flasks with a capacity of 500 ml containing potato tubers at a rate of 100 g for each beaker to follow their development. All flasks were incubated under the same previous conditions.

Effect of chitin synthesis inhibitor (Match) on Potato Tuber Moth Larvae

Larvae were collected from infected potato tubers in breeding cages and the larvae were divided into two groups (first and fourth instar larvae), the color of the larva in the first instar is whitish-gray and smaller in size, while the larvae of the fourth instar are larger and reddish-gray in color , then placed in 9 cm diameter dishes and in each dish 20 larvae with three replications for each age group and for each concentration, the larvae were sprayed from a distance of 20 cm with a match chitin synthesis inhibitor at concentrations (0.5, 1, 1.5 and 2 ml/L). As for the control treatment, it was sprayed with distilled water only, the larvae were transferred to volumetric flasks with a capacity of 500 ml containing potato tubers at a rate of 100 g per beaker to follow their development. All flasks were incubated under the same previous conditions. The dishes were checked daily and the dead larvae were removed and their number was recorded.

Effect of UV-C radiation on potato tuber moth larvae

Larvae were collected from infected potato tubers in breeding cages, and the larvae were divided into two groups (first and fourth instar larvae), then placed in 9 cm diameter dishes and in each dish 20 larvae with three replicates for each age group. The dishes were placed at a distance of 50 cm from the source of exposure to ultraviolet radiation. Violet with a wavelength

of 254nm and was exposed to four periods of time (4,8,12 and 16 min.). As for the control treatment, it was not exposed to radiation. The larvae were transferred to volumetric flasks with a capacity of 500ml containing potato tubers at a rate of 100g for each beaker to follow their development. All dishes were incubated under the same previous conditions.

Effect of UV-C radiation on potato tuber moth adults

Three pairs (3 males + 3 females) of newly emerged unmarried adults were placed in a 9 cm diameter petri dish with three replicates for each treatment in addition to the control treatment. The plates were placed at a distance of 50 cm from the UV exposure source (LPHO Genesis Technologies، Inc. company) at a wavelength of 254nm and exposed for four durations (4,8,12 and 16 min.), As for the control treatment that was not exposed to radiation, these adults were transferred to 3L plastic pots for mating, containing 100g of potatoes for laying eggs and feeding the larvae on them. It was also provided with a cotton swab moistened every day with a 10% sugar solution in order to feed the butterflies and covered with a cloth (soft chiffon), and the pots were placed in the same conditions as before. Then the percentages of the number of eggs laid/female, hatchability, pupation and emergence percentages were recorded for each treatment.

Statistical analysis.

The data was analyzing using SPSS (version 26.0) statistical software. Significant differences were evaluated using t- test analysis at the 5% probability level [8].

Results and discussion

The results of Table (1) showed that (1-2) days old eggs were more affected by the match insect growth regulator and UV-C than (5-6) days old eggs, in all concentrations and time periods. Perhaps the reason for the increase in the non-hatching rates for (1-2) days old eggs more than eggs (5-6) days of age is due to the negative effect that rays or the insect growth regulator have on the egg shell, as it is thinner and more flexible in eggs at the age (1-2) days, which leads to an increase in the lethal effect of growth regulators or UV-C rays, and perhaps the reason for

this is because the younger eggs are more affected by insect growth regulators less the older eggs, because the layers that cover the older eggs are thicker than the youngest eggs, as the eggs have the highest non-hatching rates. The effect of the insect growth regulator, was the highest at a concentration of 2 ml / L, which reached 59.30 and 33.90% for (1-2) and (5-6) days old eggs, respectively, compared to 6.33 and 2.00% in the control treatment. Anwar [9] pointed out the ability of chitin-synthesis inhibitors to penetrate through the egg shell, which leads to inhibition of embryonic development and thus loss of its ability to hatch. Abd [10] mentioned a significant decrease in the rate of hatching of eggs of *Trogoderma granarium* at different concentrations of the insect analogue Insegar. Faruki [11] mentioned the effect of lufenuron on female house flies carrying eggs at different concentrations led to a decrease in hatching rates, as the percentage of non-hatching eggs was up to 93% at the concentration of 3ppm. The results of Table (1) showed that the non-hatching percentages for eggs exposed to UV-C rays were 62.20 and 58.00% at 16 min., for (1-2) and (5-6) days old eggs, respectively, compared to 8.33 and 4.20% in the control treatment.

Table 1: Non-hatching rates of the potato tuber moth (1-2) and (5-6) days old eggs exposed to the insect growth regulator (Match) and UV-C rays

% of non-hatching eggs that exposure to UV-C rays			% of non-hatching eggs of that sprayed with the growth regulator (Match)		
Age (5-6) days	Age (1-2) days	time/minute	Age (5-6) days	Age (1-2) days	Concentration ml /L
4.20	8.33	the control	2.00	6.33	the control
32.33	39.22	4	28.20	35.33	0.5
38.20	41.30	8	31.22	42.10	1
42.30	43.21	12	34.30	45.20	1.5
58.00	69.20	16	42.10	59.30	2
39.20	48.23	the average	33.90	45.40	the average
6.8			6.2		Arithmetic
2.02			2.01		T tabular P<0.05

The results of Table (1) shows that the non-hatching rates of (1-2) and (5-6) days old eggs increased steadily with the increase in the concentrations of the growth regulator, as well as with the increase in the duration of exposure to UV-C rays. Ghamen [12] mentioned that exposing *T. granarium* eggs to ultraviolet rays damaged the egg's placenta, leading to the

leakage of the internal contents of the egg. The early embryonic stages of the potato tuber moth eggs are more sensitive to UV-C rays than the late embryonic stages, as a proportion of the (5-6) days old eggs that were exposed to radiation with a wavelength of 254 nm hatched and developed to reach the full stage. Brower [13] indicated during his study of the radio sensitivity of eggs of the Indian flour moth *P. interpunctella* (Hub.) that the sensitivity of eggs to radiation increased in the stage of cleavage, blastula stage and gastrula stage compared to the advanced embryonic stages when organogenesis is formed. The results of Table (2) showed that first instar larvae were more affected by match insect growth regulator treatments and UV-C than fourth instar larvae in all concentrations and time periods. The highest mortality rates of first and fourth instars larvae were 42.20 and 36.10% at the concentration of 2 ml/L compared to 5.33 and 2.00% in the control treatment, respectively, it is clear from the results that the larvae of the first instar were very sensitive to the growth regulator, and this depends on the age, behavior and physiological of the larva. It is also noted that the lower killing efficiency in the last instar compared to the first instar, at least partly due to the cellular defense mechanisms in the first larval age, in addition to the fact that the larvae of the instar stage the last start to reduce food intake. Tariq [14] indicated that the use of chitin formation inhibitors in the treatment of the last larval stage of the corn stem borer insect *Sesamia cretica* (Led.) led to a decrease in the average lifespan of males and females, and this decrease is inversely proportional to the increase of the used concentrations.

Table 2: Mortality rates of first and fourth instar larvae of potato tuber moth that exposure to insect growth regulator (Match) and UV-C rays.

% of larvae killed as a result of the effect of UV-C rays			% of larvae killed as a result of the effect of the growth regulator Match		
Fourth instar Larvae	first instar larvae	time/minute	Fourth instar Larvae	first instar larvae	Concentrations ml /L
4.20	8.33	Control	2.00	5.33	Control
34.33	39.22	4	28.20	15.22	0.5
35.20	41.30	8	31.22	18.10	1
39.30	43.21	12	30.10	28.00	1.5
48.00	52.20	16	36.10	42.20	2
39.2	43.9	the average	33.9	45.4	the average
7.7			8.2		Arithmetic
2.02			2.13		t tabular P<0.05

Miyamoto [15] mentioned that the inhibitors of chitin formation mainly target insect larvae, as the treated larva continues to grow until it reaches the moulting stage, but it fails because it could not form chitin. The highest mortality rates of first and fourth instar larvae under UV-C rays were 52.20 and 48.00% at 16 min. of exposure, compared with 8.33 and 4.20% in the control treatment, respectively (Table 2). This could be explained by the UV-C rays may damage neurons as a result of their high sensitivity to radiation, which leads to inhibition of the secretion of the moulting hormone, and then the death of the larvae[15]. In addition, the reason may be due to other functional factors such as the reduced ability of the larvae to digest food as a result of the influence of radiation in the process of absorbing digested food. Foster [16] mentioned that radiation has negative effects on the secretion of hormones to control the processes of morphogenesis. Table(2) also shows the mortality rates of first and fourth instar larvae increased steadily with the increase in the concentration of growth regulator, as well as with the increase in the duration of exposure to UV-C rays. Al-Zubaidi [17] mentioned that the first larval age of the cotton leaf worm *Spodoptera littoralis* is more sensitive than the advanced ages.

Saour and Makee [18] mentioned that the radiation doses, especially the high ones, play a major role in reducing the rate of the number of developed larvae, as they cause changes in the treated organisms ranging from sterility to rapid death. Table (3) shows the fertility results (number of eggs laid/female) decreased dramatically, as the number of eggs laid in the control treatment was 113.00 eggs/female, while the lowest number of eggs laid was 23.55 eggs/female when insects were treated with ultraviolet radiation for 16 min.. As for the percentages of hatching and pupation, they were 76.33 and 68.30% in the control treatment, and decreased to 22.30 and 18.10%, respectively, when treated with UV rays for 16 min., as for the percentages of emergence, they decreased and reached 18.30 and 8.00% when treated with ultraviolet rays for 12 and 16 min., respectively, compared with 60.30% in the control treatment, and the results of Table (3) showed a gradual decrease in the number of eggs laid/ females and percentages of hatching, pupation and emergence with increasing duration of exposure to UV-C rays.

Table 3: The effect of different exposure durations of UV-C rays on different aspects of potato tuber moth adults.

%emergence rate	%pupation rate	% hatchability	(Fertility) The average number of eggs laid/female	exposure time/min
60.30	68.30	6.337	113.00	The control
39.33	42.64	55.30	71.12	4
24.50	32.20	41.00	58.30	8
18.30	26.52	32.66	42.16	12
8.00	18.10	22.30	23.55	16
22.53	29.84	37.81	48.78	the average
4.2	5.1	6.2	6.8	Arithmetic
2.02	2.35	2.01	2.13	t tabular P<0.05

The decrease in the number of eggs laid by the females treated by UV-C rays can be attributed to the abnormalities that may occur in the fatty bodies and the ovaries, thus impeding the formation of eggs and decreasing their fertility. A study showed that the rusty red flour beetle *Tribolium castaneum* was exposed to ultraviolet rays with a wavelength of 254nm for 16 min., the number of eggs laid by the female decreased to 23.50 eggs / female, and the hatchability rate was 29.30%, the pupation rate was 24.90% and the emergence rate was 4.10% [19]. Through the results shown in Table (3), it is clear that UV-C rays affected the physiological processes in insects that reached the adult stage of the treated adults, through the delay of the moulting process in the larval stage and the emergence of distorted, short-lived adults with coiled wings, although the cuticle layer was very thin, as well as the loss of some legs and parts of antennae compared to the untreated insects.

Conclusion

The results indicated that ultraviolet rays and insect growth regulator (Match) have almost the same effect on(1-2) days old eggs treated. The results also showed that (1-2) day old eggs treated with ultraviolet rays or treated with an insect growth regulator (Match) have higher mortality than (5-6) day old eggs. As the first instar larvae were more affected than the fourth instar larvae when treated by the insect growth regulator treatments, as well as UV-C. As for the effect of ultraviolet radiation on the adults, the difference in fertility results (number of eggs

laid / female) decreased dramatically, as the percentages of hatching, pupation and emergence are decreased.

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