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THE EFFECT OF THE DIFFERENT CONCENTRATION AND PROPORTION OF MIXTURES OF ACTARA INSECTICIDE AND GINGER OIL IN THE MORTALITY RATE OF LARVAE OF GREATER WAX MOTH, *GALLERIA MELLONELLA* L.

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ABSTRACT:
During this study the toxic effect of Actara and ginger oil of *Zingiber officinale*, individually or in combination, on the 3rd and 5th instar larvae of greater wax moth, *Galleria mellonella* L. was investigated. The percentages of mortality of 3rd and 5th instars larval stage exposed for Actara, at concentrations of 0.1, 0.15 and 0.2 µl/ larva, after 24 hours exposure were 17.50, 25.00 and 32.50 %, and 10.00, 12.50 and 17.50 respectively. The percentages of mortality of 3rd and 5th instars larval stage exposed to mixture 1:1 of Actara insecticide and ginger oil at concentrations of 0.1, 0.15 and 0.2 µl/ larva were 26.60, 40.00 and 50.00% (for 3rd instar), and 20, 26.60 and 33.30% (for 5th instar) respectively, after exposure period of 24 hours. While the mortality of 3rd and 5th instars larval stage exposed to the mixing ratio 1:2 (Actara insecticide and ginger oil) of the same concentrations and same exposure period mentioned previously, were 36.60, 46.00 and 63.30 % (for 3rd instar), and 26.60, 36.60 and 46.60% (for 5th instar), respectively. Whereas in the mixing ratio 1:3(Actara insecticide and ginger oil) of same concentrations and same exposure period mentioned previously, the mortality (3rd and 5th instars larvae) were 53.30, 63.00 and 90.00 % (for 3rd instar), and 33.30, 46.60 and 63.00% (for 5th instar), respectively. The synergistic ratios for 3rd and 5th instars larval stage exposed to mixtures 1:1, 1:2 and 1:3 (different concentrations of Actara insecticide and ginger oil), were 1.25, 1.6 and 2.4 (for 3rd instar), and 1.88, 3.50 and 4.01 (for 5th instar), respectively, after 24 hours of exposure. This indicates that a combination of different concentrations of the Actara insecticide with ginger oil produced synergistic effect.

KEYWORDS: Ginger oil, Galleria mellonella L., Actara insecticide, Synergistic.

1. INTRODUCTION

The greater wax moth, *Galleria mellonella* is a useful insect, because its larvae is an excellent bait for fish. The wax moth is considered as one of the most serious pests for the honey bee colonies which feeds on comb wax and causes economic loss to the beekeeping industry (Coskun *et al.,* 2006). It deteriorates the honeycombs and creates nuisance to the honeybees. Damage is caused only by the caterpillars, which feed on combs, propolis, pollen larval skins and other protenaceous matters (ul-Haq *et al.,* 2004). The use of chemical insecticides such as sulphur, para dichloro benzene and calcium cyanide is harmful to bee population (Surendra *et al.,* 2010; Mohamed, 2014). In this context the use of plant products as insecticides is emerging as a major thrust area in controlling greater wax moth. Natural plant products possess insecticidal activity. The plants based insecticides being the natural plant products are safer and hence their use against pests has gained importance all over the world (Mekawa *et al.,* 2015; Gomes *et al.,* 2016). High toxic effects of botanicals products on the larvae of *G. mellonella* have been reported (Surendra *et al.,* 2010; Ahmad *et al.,* 2014). Very little information is available on the comparative efficacy of different plant products against the larval mortality of the greater wax moth, *Galleria mellonella* L. Therefore, this work is adopted to investigate the effect of combination of Actara and Ginger oil on larvae *G. mellonella* using spray method under laboratory condition.

2. MATERIALS AND METHODS

2.1 Insect Rearing

Naturally-infested wax combs with greater wax moth were obtained from the apiary in Duhok and were taken to a rearing chamber in the Department of Biology/ Faculty of Science / Zahko University.
To ascertain pure culture, infested wax cubes (feeding medium) were cut and transferred to a clean 10 kg capacity glass jars. Emerged moths were taken to new jars fortified with uninfected waxes and left to copulate and lay eggs. Emerged larvae were monitored to obtain the desired instars for the following assay. Rearing and treatments were conducted at incubator conditions (LAB TECH. Korea) (at 28-30 ° C and 60-65 ±5% R.H). Larvae used in this study were 3rd and 5th instars.

2.2 Insecticides

Actara belongs to the subclass Thianicotinyl to the Neonicotinoides Group which is in the form of wettable Granules, containing 25% of active material. (Syngenta Group Company). (Syngenta. 2016).

2.3 Bioassays

For the implementation of the study, three different mixing ratios of 1:1, 1:2 and 1:3 (insecticide: Ginger oil), respectively, were used for each of the insecticide Actara concentrations 0.1, 0.15 and 0.2 Mg/L and Ginger oil (obtained from the local market) diluted with ethanol 1:1 (Ginger oil : ethanol) and then larvae treated were 3rd and 5th instar by spraying method and three replicates and each repeater includes 10 larvae for each concentration and the proportion of mixing and instar. The treated larvae were kept inside closed Petri dish to prevent escaping. The control group was treated with distilled water and ethanol according to mixing ratios for each experiment and kept in the incubator at a temperature of 28-30 °C and 60-65 ±5% R.H. The results were taken after 24 hours.

2.4 Estimating Synergistic Ratio (SR)

To calculate synergy ratios, the formula of Brattesten and Metcalf (1970) was used as follows

$$\text{Synergistic Ratio} = \frac{\text{LD50 or LC50 to pesticide only}}{\text{LD50 or LC50 to pesticide+synergistic}}$$

The synergistic ratio is equal to the number of times the increase in pesticide toxicity caused by the synergistic.

2.5 Data Analysis

The data were analyzed using the probit procedures with SAS program (SAS, 2002). For comparing the toxicity of different concentrations of Actara insecticide with different mixtures (Actara insecticide: Ginger oil).

3. RESULTS AND DISCUSSIONS

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3.1 The effect of Actara insecticide and proportion of mixtures between insecticide of Actara and ginger oil in the mortality of 3rd and 5th instar larvae after exposure period of 24 hours.

The percentages of mortality of 3rd instar Larvae of the greater wax moth, exposed to various concentrations of Actara insecticide are shown in table (1). The exposure of 3rd instar Larvae to concentrations of 0.1, 0.15 and 0.2 µl/ larvae produced mortality rates of 17.50, 25.00 and 32.50%, respectively; while exposure of the 5th instar larvae to the same concentrations the mortality rate produce of 10%, 12.50%, and 17.50%. The results of the present study agree with those of Osman (2010) who stated that Actara insecticide is toxic to larvae of Colorado potato beetle, *Leptinotarsa decemlineata* (Say). Since, the mortality rate increased with increasing the concentration. The 3rd instar larvae showed a higher sensitivity to the extract as compared with the 5th instar larvae

(Figure. 1). This is in accordance with the finding of Yong and Bruce (1994) who stated that, the newly hatched larvae were more sensitive to the insecticide of microbial origin (*Bacillus thuringiensis*) and their sensitivity decreased with the advancement of the larval age. Martin *et al*. (2000) and Abd El-Mageed and Shalaby (2011) also stated that, the sensitivity of the first or second larval instars to insecticides decreased with the increase in the larval age. The decrease in the sensitivity of the larval stage to the insecticides with the advancement of the larval stage can be attributed to increasing the level of metabolic enzymes of insecticides with the increase of larval instars. Ahmad and Brindley (1971) found that the level of enzymes in sixth instar larvae of greater wax moth was much higher than its level in the fifth instar larvae. While Gilbert and Wilkinson (1974) found that the level of effectiveness of these enzymes in the honey bee larvae was low, then increased with the advancement of the larval instar, and dropped too much before the pupal stage. In The fall armyworm larvae *Spodoptera frugiperda*, the level of the enzymes in the sixth instar larvae was much higher than its level in the second instar larvae (Yu and Hsu, 1993). Reed (1993) attributed the difference in the level of metabolic enzymes in the larvae of tobacco budworm *Heliothis virescens* against insecticide to the effectiveness of the metabolic enzymes in different instars.

The other reason for the difference in the sensitivity of various larval instars to the insecticides, is the nature of the cuticle and its thickness in various stages of the development as it increase with the advancement of larval instar, and this statement has been confirmed by Cercelius and Knowles (1976) while studying the effect of insecticide chlorodimeform on the larval instars of Cabbage looper *Trichoplusia ni* , they found the 3rd instar larvae were more sensitive due to increased permeability of their cuticle to insecticide as compared with 5th instar larvae.

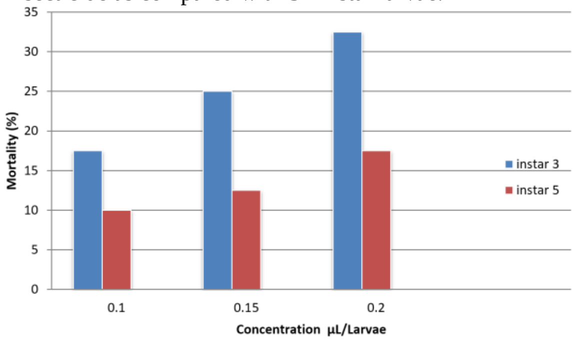

Figure 1. Effect of Actara insecticide in the mortality of 3rd and 5th instar larvae of the greater wax moth, *Galleria mellonella* L. after 24 hours of exposure.

The addition of ginger oil extract to the insecticide Actara caused a significant increase in the toxicity of the insecticide against the 3rd and 5th instar larvae (Table. 1). Since the percentages of the mortality was greatly increased as compared with the effect of the insecticide alone. This mean that ginger oil extract has a synergistic effect to the insecticide Actara.

The rate of mortality increased in 3rd instar larvae when they were exposed to a mixing ratio of 1:1 at concentrations of insecticide 0.1, 0.15, and 0.2 µL/larvae, after 24 hours treatment, becoming 26.60, 40.00 and 50.00%, respectively, this rate further increased when the mixing ratio increased to 1:2 the mortality rate becoming 36.60, 46.00 and 63.30% at the concentrations of the insecticide 0.1, 0.15 and 0.2 µL/larvae, after 24 hours treatment, respectively, and for the proportion of mixing 1:3 ratio and to the same concentrations the mortality percentage become 53.30%, 63.00%, 90.00%, after 24 hours treatment, respectively. (Figure 2).

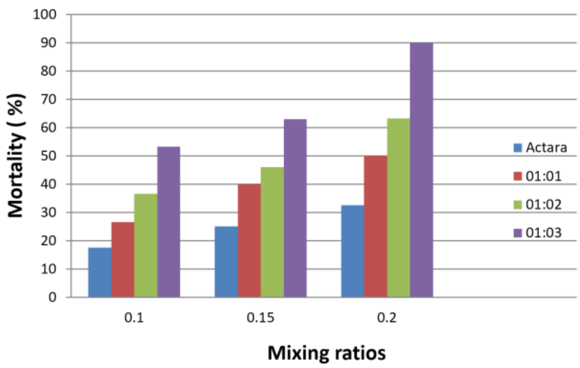

Figure 2. Effect of different mixing ratios of Actara insecticide on the mortality rate of 3rd instar larvae of the greater wax moth, *Galleria mellonella* L. after 24 hours of treatment.

Regarding the 5th instar larvae, when the mixing ratio was 1:1 the mortality percentage were 20.0, 26.60 and 33.30% at the concentrations of the insecticide 0.1, 0.15 and 0.2 µL / larvae, after 24 hours treatment, respectively, by increasing the mixing ratio to 1:2, and for the same concentrations the mortality percentage increased to 26.60, 36.60 and 46.60% after 24 hours treatment, respectively. The mortality rate increased more by increasing the mixing ratio to 1:3 to 33.30, 46.60 and 63.0% at the same concentrations of the insecticide (0.1, 0.15, 0.2 µL / larvae) after 24 hours treatment, respectively. (Figure 3).

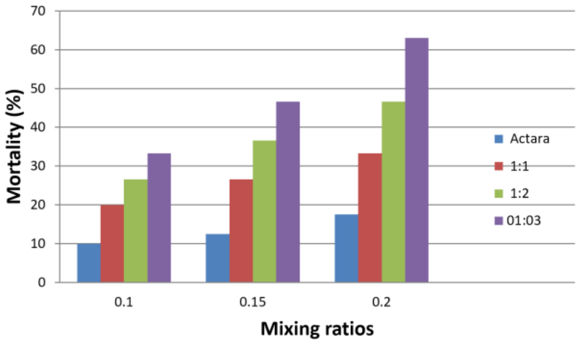

Figure 3. Effect of different mixing ratios of Actara insecticide on the mortality percentage of 5th instar larvae of the greater wax moth, *Galleria mellonella* L. after 24 hours of treatment.

The results indicated that the rate of the mortality increased, when using the mixing ratio of 1:1, as compared with insecticide alone. Ginger oil has a synergist effect which increased the effectiveness of the insecticide in increasing the mortality by using low doses of the extract in order to reduce the use of chemical insecticide to the lowest possible amounts and to reduce their harmful effects.

The synergist effect present in the ginger oil is due to the increasing the permeability of the insecticide through the cuticle, thereby, it reaches the targeted sites. Cassida (1970), and Sun, and Johnson (1972) confirmed that the fundamental mechanism to the synergists effect is the inhibition of oxidative enzymes in insects, and this is in the accordance of the present study. The variation in insect sensitivity to insecticides might be due to variation in the level of metabolic enzymes, and this is consistent with the findings of Frankling (1972) and Joffe *et al.* (2011) who stated that the percentage of inhibition depends on the concentration of synergist. Similarly. Al-Sinjari and Al-Attar, (2015). Reported the same findings.

Table (1) shows the effect of mixing ratios; regardless to the concentrations used as the highest mortality rates for the 3rd and 5th instar larvae were 51.57 and 35.72%, respectively.

Table 1. The effect of Actara insecticide and different proportions of mixtures between insecticide and ginger oil on the mortality of 3rd and 5th instar larvae of the greater wax moth, *Galleria mellonella* L after 24 hours of treatment

Larval insta	Insecticide	Mortality rate after 24 hours treatment				Effect of concentration
		Actara	Mixing ratio			
			1:1	1:2	1:3	
3rd instar	0.1	17.50	26.60	36.60	53.30	25.5 b
	0.15	25.00	40.00	46.00	63.00	32.33 b
	0.2	32.50	50.00	63.30	90.00	42.2 a
	Control	0	0	2.50	5.00	10.00 c
	Effect of Mixing ratios		29.1 c 5	36.47 b	51.57 a	
5th instar	0.1	10.00	20.0	26.60	33.30	16.98 c
	0.15	12.50	26.60	36.60	46.60	23.85 b
	0.2	17.50	33.30	46.60	63.00	31.53 a
	Control	0	0	0	0	0
	Effect of Mixing ratios		19.97 c	27.45 b	35.72 a	

3.2 Effect of combination of Actara insecticide and ginger oil on 3rd and 5th instar larvae.

The results of the synergistic effect of a combination of Actara insecticide and ginger oil revealed that the presence of ginger oil enhanced the toxicity of the insecticide Actara on 3rd and 5th instar larvae of the greater wax moth, as the effect increased with increasing the synergistic ratio.

3.2.1 The effects on 3rd instar larvae: As shown in table 2, the synergistic ratios were 1.25, 1.6 and 2.4 at a mixture ratios 1:1, 1:2 and 1:3, respectively. The synergistic ratios were increased with increasing the concentration of the ginger oil.

3.2.2 The effects on 5th instar larvae: As shown in table 2, the synergistic ratios were 1.88, 3.50 and 4.01 in the mixture ratios 1:1, 1:2 and 1:3, respectively. The synergistic ratios were increased with increasing the concentration of the ginger oil.

The synergistic effect of ginger oil on the toxic effect of Actara may be attribute to increasing the permeability of the insecticide through the cuticle and facilitate its entrance to reach the target site. This finding agree with that of Shufeng et al. (2005) who stated that the synergistic effect may be due to facilitating the entry of the insecticide through cuticle.

On the other hand, Michalets (1998) and Mckinnon et al. (2008) suggested another mechanism for the synergistic effect by inhibition of the function of oxidative enzymes which are responsible for the degradation (metabolized) to insecticide.

As mentioned previously that the variation of insects sensitivity to insecticides may be attributed primarily to the contrast enzymes levels that metabolize the insecticide, similarly Franklin (1972) stated that the percentage of inhibition ratio depends on the synergistic concentration. Furthermore, Khalequzzaman and Rumu (2010) studied the effect of mixing cardamom essential oil with prirmiphos-methyl pesticide (1/20) and they observed a synergistic effect by increasing the percentage of mortality against adult cowpea weevil.

Table 2. The synergistic effect of ginger extract oil and Actara on the mortality rate of 3rd and 5th instars larvae of the greater wax moth, after 24 hours exposure.

Larval instars	Insecticide		exposure periods (24 Hours)		
			synergistic ratios		
			mixture ratios		
	Insecticide Conc. L/Larvaµ	Mortality rate	1:1	1:2	1:3
3rd instar larvae	0.1	17.50	1.25	1.6	2.4
	0.15	25.00			
	0.2	32.50			
5thinstar larvae	0.1	10.00	1.88	3.50	4.01

4. CONCLUSSION

The increase of the mixing proportion of ginger oil extract with insecticide Actara increases the mortality rate of *G. mellonella*, and the toxic effect varied with the concentrations.

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كورتيا لێكولينێ:

ئەڤ ڤەكولينە يا هاتيە ئەنجام دان لە سەر كارتيكرنا ژهراوى يا (Actara) و (Zingiber officinale) ب تينێ يان پيكەڤە, لە سەر ژيت سێ و پينج كرمێت بچووك يێ (Galleria mellonella L.). و كو رێژا گوشتنا كرميت بچووك ژيێ وانا سێ و پينج (دوده الشمع) بكارئينانا دەرمانى كيمياو (Actara) هندەبوو 17.50, 25.00 و 32.50%, 10.00, 12.50 و 17.50 كو تركيزا وى هندە بوو 0.1, 0.15 و 0.2 ميكرولتر / يرقه, پشتێ بورينا 24 سەعتا لە ديف ئيك. رێژا گوشتنا كرميت بجووك لە ژيێ سێ و پينج يت (دوده الشمع) ژير كارتيكرنا ريژا تيكەل 1:1 (زيت الزنجبيل: مبيد الاكتارا) كو تركيز زى ئەڤ بوون 0.1 و 0.15 و 0.2 ميكرولتر / يرقه, كو ريژا مرنێ هند بوو 26.60, 40.00 و 50.00 % (ژيێ سێ) , و 20, 26.60 و 33.30 % (ژيێ پينج) لە ديف ئيك, پشتێ بورينا 24 سەعتا. بەلێ رێژا گوشتنا كرميت بجووك لە ژيێ سێ و پينج يت (دوده الشمع) ژير كارتيكرنا ريژا تيكەل 1:2 (زيت الزنجبيل: مبيد الاكتارا) كو تركيز زى ئەڤ بوون 0.1 و 0.15 و 0.2 ميكرولتر / يرقه, كو ريژا مرنێ هند بوو 36.60, 46.00 و 63.30 % (ژيێ سێ) , و 26.60, 36.60 و 46.60 % (ژيێ پينج) لە ديف ئيك, پشتێ بورينا 24 سەعتا. هەروەسا رێژا گوشتنا كرميت بجووك لە ژيێ سێ و پينج يت (دوده الشمع) ژير كارتيكرنا ريژا تيكەل 1:3 (زيت الزنجبيل: مبيد الاكتارا) كو تركيز زى ئەڤ بوون 0.1 و 0.15 و 0.2 ميكرولتر / يرقه, كو ريژا مرنێ هند بوو 53.30, 63.00 و 90.00 % (ژيێ سێ) , و 33.30, 46.60 و 63.00 % (ژيێ پينج) لە ديف ئيك, پشتێ بورينا 24 سەعتا. رێژا (تازر زيت الزنجبيل لمبيد الاكتارا) كو كارئينانە لە سەر كرميت (دوده الشمع) ژيێ وان سێ و پينج بين ريژا تيكەل1:1 و 1:2 و 1:3 (زيت الزنجبيل: مبيد الاكتارا) كو 1.25, 1.6و 2.4 (ژيێ سێ) , و 1.88, 3.50و 4.01 (ژيێ پينج), لە ديف ئيك, پشتێ بورينا 24 سەعتا. ئەڤ ديار دكەت كو تيكەلێ زيت الزنجبيل لەگەل تركيزێ ژيك جودا يت مبيد الاكتارا بو مە درئيخت كارتيكرنا تازرى.

خلاصة البحث:

تم خلال هذا البحث دراسة التأثير السام لمبيد الاكتارا Actara وزيت الزنجبيل *Zingiber officinale*، منفردا أو مجتمعا، على العمرين الثالث والخامس ليرقات دودة الشمع الكبرى *Galleria mellonella* L.. وقد كانت نسب الوفيات ليرقات العمرين الثالث والخامس لهذه اليرقات عند المعاملة بالمبيد الكيميائي الاكتارا هي 17.50, 25.00 و 32.50٪، و10.00، 12.50 و 17.50 عند التراكيز 0.1, 0.15 و 0.2 ميكرولتر / يرقة، بعد 24 ساعة من التعرض على التوالي. بلغت نسب الوفيات ليرقات العمرين الثالث والخامس لدودة الشمع المعاملة بنسبة خلط 1:1 من (زيت الزنجبيل و مبيد الاكتارا) عند التراكيز 0.1 و 0.15 و 0.2 ميكرولتر / يرقة, 26.60، 40.00 و 50.00٪ (للعمر الثالث)، و 20، 26.60 و33.30٪ (للعمر الخامس) على التوالي، بعد فترة التعرض 24 ساعة. بينما بلغت نسب الوفيات ليرقات العمرين الثالث والخامس لدودة الشمع المعاملة بنسبة خلط 1:2 من (زيت الزنجبيل و مبيد الاكتارا) عند تراكيز المبيد 0.1 و 0.15 و 0.2 ميكرولتر / يرقة, 36.60، 46.00 و 63.30٪ (للعمر الثالث)، و 26.60، 36.60 و46.60٪ (للعمر الخامس) على التوالي، بعد فترة التعرض 24 ساعة. في حين كانت نسب الوفيات ليرقات العمرين الثالث والخامس لدودة الشمع المعاملة بنسبة خلط 1:3 (زيت الزنجبيل و مبيد الاكتارا) عند تراكيز المبيد 0.1 و 0.15 و 0.2 ميكرولتر / يرقة, 53.30، 63.00 و 90.00٪ (للعمر الثالث)، و 33.30، 46.60 و 63.00٪ (للعمر الخامس) على التوالي، بعد فترة التعرض 24 ساعة. بلغت نسب تأزر زيت الزنجبيل لمبيد الاكتارا عند تعريض العمرين الثالث والخامس ليرقات دودة الشمع الكبرى بنسب الخلط 1:1, 1:2 و1:3 (زيت الزنجبيل و تراكيز مختلفة لمبيد الاكتارا) 1.25, 1.6 و 2.4 (للعمر الثالث) و1.88, 3.50 و4.01 (للعمر الخامس), على التوالي. بعد فترة تعريض 24 ساعة. تشير هذه النتائج ان خلط زيت الزنجبيل مع تراكيز مختلفة من مبيد الاكتارا ينتج عنه تاثير تأزري.