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Evaluation of Kz Mineral Oil for Molluscicidal and Biochemical Activities against White Garden Snail *Theba pisana* (Müller) as a Safe Alternative to Pesticides and Honey Bee *Apis mellifera* L. Friendly Compound

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ABSTRACT



This work was conducted to evaluate the molluscicidal activity of Kz mineral oil compared to methomyl insecticide against one of the most serious land snails; white garden snail *Theba pisana* under laboratory conditions. The toxicity changes in juveniles and adults of *T. pisana* snail treated with three concentrations (1.25, 2.5 and 5%) for Kz oil and (0.031, 0.063 and 0.125%) for methomyl were evaluated using dipping technique of lettuce and cabbage leaves. Results indicated that the tested concentrations of Kz oil had toxic effect on both juveniles and adults increasing with time passage and concentration. The highest mortality percentage obtained with Kz (5%) recorded (53.33%) and (46.67%) for juveniles & adults with lettuce leaves, respectively however the same concentration gave (40%) and (26.67%) mortality for juveniles & adults mortality effect increased also by time passage and concentration where the highest mortality in both juveniles & adults recorded (66.67% & 46.67%), respectively when lettuce leaves are dipped in 0.125% methomyl. Treatment with all concentrations of Kz oil and methomyl exhibited remarkable reduction in amylase and invertase activities compared to control. Furthermore, Kz oil showed very low toxic effect to adults of *Apis mellifera* by feeding whereas, toxicity was almost non- exist when bees were in contact with treated leaves.

Keywords: Theba pisana - Mineral oil - Apis mellifera L. - Vital enzymes.

INTRODUCTION

The white garden snail Theba pisana (Muller) is considered an extremely injurious pest to ornamental flowers, shrubs beside wide varieties of vegetables, fruits, and citrus in most areas of its distribution Baker (1986). Terrestrial gastropods T. pisana snail consider the most significant threats to sustainable agriculture around the world Barker (2002) where, damage mainly caused due to feeding and contamination with their bodies, faces or slime leading to deterioration of the product quality and financial loss Lglesias et al. (2003). Theba pisana snail was also recorded to be harmful snails in many districts of Egypt attacking various plants Eshra (2013). Unfortunately, about 25 million agricultural workers in developing countries are poisoned yearly by pesticides Jeyaratnam (1990). In addition, applied pesticides to a variety of agricultural crops adversely impact non-target beneficial insects as honey bees (Apis mellifera L.) when come to contact with various pesticides during their foraging activity which considered a major cause of bees decline in cultivated areas. Certainly, honey bees are most important pollinators worldwide affecting about 35 % of the world food crops; that play essential role for the maintenance of agricultural productivity Potts et al. (2010). Therefore, it is more crucial to study the effect of investigated pesticides on non-target benefit insects as honey bee while controlling a wide variety of agricultural pests through pesticides Desneux et al. (2007). Attention is increasingly being paid to the use of

mineral oils where they considered one of the safest methods in controlling pests possess no health hazards when compared with synthetic pesticides furthermore, no resistant populations were developed to them Aly *et al.* (1984). Also, they play a fundamental role in integrated pest management programs IPM for many pests Helmy *et al.* (2012).

This study aimed to evaluate the impact of Kz mineral oil for controlling the white garden snail *Theba pisana* (Müller) using dipping technique of cabbage and lettuce leaves in comparable with recommended methomyl pesticide with regarding to its effect on two digestive enzymes; amylase and invertase. Kz oil is also tested for its toxicity to adult *Apis mellifera* workers as nontarget beneficial insects.

MATERIALS AND METHODS

Theba pisana (Muller) snail: The white garden snail *T. pisana* juveniles and adults were collected from orchard cultivated with navel orange, *Citrus sinensis* L. at Menia El-Kamh district, Sharkia Governorate, Egypt. The collected snails were transferred immediately to the laboratory using white cloth bags. Healthy and nearly identical individuals were selected and kept in terrarium glass filled with moist clay soil adjusted at 75 % of water field capacity. Snails were fed daily with lettuce or cabbage leaves for two weeks before treatment for acclimation.

Honey bee workers: Honey bee foragers workers *Apis mellifera* L. (Hymenoptera: Apidae) were used in the experiments. Workers obtained from the apiary of Plant

Protection Research Institute, Agriculture Research Center according to Iwasa *et al.* (2004). The bees were collected from a single strong colony (to minimize the genetic variations as possible) by placing clean plastic container over opening in the entrance block. The container was transferred to the laboratory and bees were anesthetized by cooling at (4 °C for 1 min) before placing inside the experimental cages. Bees kept in plastic cylindrical cages (7 cm diameter× 14 cm height) at 25±2 °C and 65±5% RH and fed with 50% (w/v) sucrose solution using Eppendorf.

Tested materials

KZ mineral oil (95% EC): A petroleum-derived oil, produced by Kafr El-Zayat Company for Pesticides & Chemicals, Egypt was used in this investigation.

Methomyl (Lannate 90 % SP): carbamate insecticide was also procured from Kafr El-Zayat Company.

Toxicity assay for tested snail: This study was carried out at the laboratory of Plant Protection Research Institute. For the toxicity assay, three concentrations of 1.25, 2.5 and 5 % for Kz mineral oil and three concentrations 0.031, 0.063 and 0.125 % for methomyl pesticide were prepared. Similar leaf discs (5 cm- diameter) of fresh leaves of lettuce and cabbage were immersed for one minute in the three concentrations of Kz oil and methomyl pesticide suspensions, then left in air to dry before feeding application. Five juvenile and five adult individuals of T. pisana snail were introduced into each plastic box, then covered with muslin cloth and secured with rubber band to prevent snail from escaping. Each concentration was replicated three times. After 48 hours of exposure period, the treated leaves were replenished daily with non-treated leaves for 21 successive days. For control replicates, lettuce and cabbage fresh leaves were dipped in water free from pesticide or oil. Dead individuals were counted using stainless steel needle according to El-Okda (1980). Mortality percentages were calculated after 1, 3, 7, 14 and 21 days and corrected by Abbott's formula (1925).

Toxicity assay for honey bee: Two ways of exposure were used to evaluate the acute toxicity of Kz oil on foraging workers of honey bees:

- **Oral application** by feeding bees with tested Kz oil prepared in 50% (w/v) sucrose solution while bees fed with pure sucrose solution as control.
- Acute contact toxicity with oil residues on treated fresh leaves (using dipping technique) and bees fed with 50% (w/v) pure sucrose solution through experiment period. Treatments applied for 24 hours for the two methods. The experiment was carried out using the three concentrations of the tested oil (1.25, 2.5 and 5%) for oral and contact bee toxicity test. Each concentration for each way of exposure comprised 3 replications with 15 adult bees for a replicate. The mortality was assessed at 24, 48 and 72h post treatment where bees that did not respond to mechanical stimuli were scored as dead.

Biochemical studies:

Preparation of snails for biochemical assay: The mollusca shells of adults of *T. pisana* snails were removed and the soft tissues were weighed, pooled and homogenized as 1:10 (w/v) in distilled water. The homogenates were centrifuged at 5000 r.p.m for 20 minutes at 5 °C according to Abd El-Haleim *et al.* (2006). The supernatants were immediately assayed to determine the activities of amylase and invertase enzymes by the method of Ishaaya and

Swiriski (1976). The enzymatic activity was expressed as (μ g glucose /g. b. wt. /min) and relative activity percentage (RA%) calculated as follow: [(Treatment – Control) / Control] × 100.

Statistical analysis: The statistical analysis was determined by using one way test, (ANOVA), Cohort Software (2005).

RESULTS AND DISCUSSION

Kz oil was evaluated for molluscicidal activity on white garden snail under laboratory conditions compared to methomyl the most tradition known pesticide against terrestrial snails. Three low concentrations for methomyl were used to enable toxicological and biochemical comparison with investigated oil. The insecticides were applied to the fresh leaves surface before feeding application using dipping technique to simulate a field spraying.

Toxicity assay:

Impact of Kz mineral oil on juveniles of T. pisana snail compared to methomyl pesticide: Data obtained in Table (1) illustrate the molluscicidal effect of three different concentrations of the tested Kz oil on juveniles of T. pisana snail using dipping technique of lettuce and cabbage fresh leaves at different time intervals. Results indicated that all tested concentrations of tested oil (1.25, 2.5 and 5%) have significant toxic effect on juveniles of tested snail compared to the control. Juveniles mortality increased by the passage of time where, the highest mortality percentages were obtained after three weeks post feeding treatment recorded (13.33, 33.33, 53.33 %) for lettuce leaves and (6.67, 20, 40.%) for cabbage leaves with 1.25, 2.5 and 5 % of Kz oil, respectively. Whereas, the tested concentrations of methomyl (0.031, 0.063 and 0.125 %) gave mortality percentage (26.67, 40, 66.67%) for lettuce leaves and (20.0, 33.33, 53.33%) for cabbage leaves, respectively.

Impact of Kz mineral oil on adults of T. pisana snail compared to methomyl pesticide: Data in Table (2) summarized the molluscicidal effect of the three concentrations of Kz oil on adults of T. pisana snail using dipping technique of lettuce and cabbage leaves at different time intervals. The mortality percentages of Kz oil increased by time, where the highest values (6.67, 20, 46.67%) for lettuce leaves and (6.67, 13.33, 26.67%) for cabbage leaves were observed after 3 weeks of treatment for the 1.25, 2.5 and 5 % of Kz oil, respectively. Regarding the methomyl action after three weeks from treatment, mortality percentages were (13.33, 26.67, 46.67%) for lettuce leaves and (6.67, 20, 40 %) of cabbage leaves for the three concentrations 0.031, 0.063 and 0.125 %, respectively. Generally, statistical analysis revealed significant difference in mortality percentages between the three concentrations of tested oil and methomyl pesticide by time compared to control for both lettuce and cabbage leaves. However, no statistical difference was found in mortality percentages of juveniles using cabbage leaves and adults using lettuce leaves compared to methomyl after three weeks post treatments (Table 1,2). In addition, results indicated more notable molluscicidal potency of tested materials with lettuce leaves compared to cabbage leaves. Many authors recorded the mortality of pests as a result of treatment with mineral oils as, Helmy et al. (1982) used various qualities of mineral oils as curatives to down insects and mites. Aly et *al.* (1984) recorded 99.2% reduction of soft scale insect *Pulvinaria psidii* Maskell (Hemiptera: Coccidae) infesting guava trees after 60 days from treatment of mineral oil star oil. Beside, oil was found to be efficient against scale insects, mites and most respondents little toxicity Johnson and Caldwell (1987). Few studies have examined effects of mineral oils on land snails however, in our study using Kz oil for controlling white garden snail *Theba pisana* (Müller) considered novel method. The toxic action of oils is more physical than chemical which might be due to contact that affecting respiration process, function or structure of cell membrane. They also may kill insect by disrupting their

feeding on oil- covered surfaces and acting as poisons that interact with the fatty acids of the insect and disturbing normal metabolism Helmy *et al* (2012). They also added that oil may preventing habitation of the newly hatched individuals on plant parts covered with oil layer. Our study also revealed a superiority in mortality effect of treated lettuce leaves which could be a result of difference in heavy metals concentration in plant leaves. In connection, Boamponsem *et al.* (2012) detected higher concentration of Cu, Mn, Pb, Fe, Cd and Zn in lettuce leaves compared to cabbage leaves.

Pesticides	% Conc.	Mortality percentages					Mortality percentages					
		lettuce leaves					cabbage leaves					
		One day	Three days	One week	Two weeks	Three weeks	One day	Three days	One week	Two weeks	Three weeks	
Kz mineral Oil	1.25	0.00^{b}	0.00 ^c	0.00 ^c	6.67 ^c	13.33 ^{de}	0.00^{b}	0.00^{b}	0.00^{b}	6.67 ^{cd}	6.67 ^{cd}	
	2.5	0.00^{b}	6.67 ^{bc}	13.33 ^{bc}	20.00^{bc}	33.33 ^{cd}	0.00^{b}	0.00^{b}	6.67 ^b	13.33 ^c	20.00 ^{bc}	
	5	6.67 ^b	20.00 ^{ab}	26.67 ^b	46.67 ^a	53.33 ^{ab}	6.67 ^{ab}	13.33 ^{ab}	20.00 ^{ab}	26.67 ^b	40.00 ^{ab}	
Methomyl	0.031	0.00 ^b	6.67 ^{bc}	13.33 ^{bc}	20.00 ^{bc}	26.67 ^{cd}	0.00 ^b	6.67 ^{ab}	6.67 ^b	13.33 ^c	20.00 ^{bc}	
	0.063	6.67 ^b	20.00 ^{ab}	26.67 ^b	33.33 ^{ab}	40.00 ^{bc}	6.67 ^{ab}	13.33 ^{ab}	20.00 ^{ab}	26.67 ^b	33.33 ^{ab}	
	0.125	20.00 ^a	33.33 ^a	46.67 ^a	53.33 ^a	66.67 ^a	13.33 ^a	20.00^{a}	33.33 ^a	46.67 ^a	53.33 ^a	
Control		0.00 ^b	0.00 ^c	0.00 ^c	0.00 ^c	0.00 ^e	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^d	0.00 ^d	
LSD0.05		10.81*	15.91**	14.64***	22.06**	24.96 ***	7.64*	17.09 ^{ns}	20.22*	13.24***	33.31*	

Table 2. Impact of Kz mineral oil on adults of <i>T. pisana</i> snail compared to methomyl pesticide	Table 2. Impac	t of Kz mineral o	il on adults of <i>T</i> .	nisana snail com	pared to methomyl pesticide.
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Pesticides	% - Cerro	Mortality percentages					Mortality percentages					
		lettuce leaves					cabbage leaves					
	Conc.	One day	Three days	One week	Two weeks	Three weeks	One day	Three days	One week	Two weeks	Three weeks	
Kz mineral oil	1.25	0.00 ^c	0.00^{b}	0.00 ^b	0.00 ^c	6.67 ^{cd}	0.00 ^b	0.00^{b}	0.00 ^c	0.00 ^b	6.67 ^{bc}	
	2.5	0.00°	0.00^{b}	6.67 ^b	6.67 ^{bc}	20.00 ^{bc}	0.00^{b}	0.00^{b}	0.00 ^c	6.67 ^b	13.33 ^{bc}	
	5	0.00°	6.67 ^{ab}	20.00 ^{ab}	26.67 ^{ab}	46.67 ^a	0.00^{b}	6.67 ^{ab}	13.33 ^b	20.00 ^{ab}	26.67 ^{ab}	
Methomyl	0.031	0.00 ^c	6.67 ^{ab}	6.67 ^b	6.67 ^{bc}	13.33 ^{bc}	0.00 ^b	0.00^{b}	0.00 ^c	6.67 ^b	6.67 ^{bc}	
	0.063	6.67 ^b	13.33 ^{ab}	20.00 ^{ab}	20.00 ^{ab}	26.67 ^b	0.00^{b}	6.67 ^{ab}	13.33 ^b	20.00 ^{ab}	20.00 ^b	
	0.125	13.33 ^a	20.00^{a}	33.33 ^a	40.00 ^a	46.67 ^a	6.67 ^a	13.33 ^a	26.67 ^a	.33ª33	40.00^{a}	
Control		0.00 ^c	0.00^{b}	0.00 ^b	0.00 ^c	0.00 ^d	0.00 ^b	0.00 ^b	0.00 ^c	0.00 ^b	0.00 ^c	
LSD0.05		6.24**	17.09 ^{ns}	20.22*	20.21**	16.51 ***	4.41*	7.64*	13.24**	20.22*	22.06*	

Impact of Kz mineral oil on adults of honey bee workers *Apis mellifera* L. using contact and oral applications: The results of toxicity bioassays of Kz oil to honey bees (*A. mellifera* L.) are summarized in Table (3). In direct contact assay no obvious mortality was observed where, bees kept moving and feeding normally for 48 h after treatment and mortality only detected at 2.2% for Kz (5%) after 72h post treatment whereas, no bee mortality was recorded with both concentrations 1.25% and 2.5% of Kz oil after 72h of application. Regarding oral toxicity test of Kz oil, the mortality percentage does not exceed 9.09% with Kz (5%) and 4.55% with Kz (2.5%) and confirming the result of

contact toxicity test Kz oil (1.25%) was found to be the safest recording (0.0%) mortality after 72h of application. Our results showed that the three concentration of Kz oil tested for controlling white garden snail had very low toxic effect to honey bees by feeding application and it is almost non- existent in direct contact assays. Otherwise, methomyl was mentioned previously as a highly toxic pesticide to honey bees on contact (LD $_{50}$ <0.5 m g/bee US EPA (1998). Methomyl was present with other pesticides in honey bee brood combs beside, its residues was detected in pooled dead bees ranging from 0.04 to 3.4 mg/L Anderson and Wojtas (1986).

Table 3. Impact of Kz mineral oil on Apis mellifera L. workers using contact and oral applications

Tested		Mortality percentages							
oil	Concentrations		Oral		Contact				
011	-	24h	48h	72h	24h	48h	72h		
W7 -:1	1.25%	0.00	0.00	0.00	0.00	0.00	0.00		
KZ oil	2.5%	2.27	4.55	4.55	0.00	0.00	0.00		
	5%	4.55	6.82	9.09	0.00	0.00	2.22		
Control		0.00	0.00	0.00	0.00	0.00	0.00		

Moreover, methomyl is classify as a highly toxic pesticide to humans through oral exposures so, great careful should be taken when applying products containing methomyl for agricultural to protect nontarget species from toxicity as honey bees. Claudianos *et al.* (2006) attributed

the sensitivity of bees to pesticides to a marked deficient in the number of genes encoding detoxification enzymes.

Biochemical assay for tested snail: The change in the activities of amylase and invertase enzymes (μ g glucose /g. b. wt. /min) were estimated to clarify the physiological response of *T. pisana* snail to the tested compounds.The

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supernatant obtained from adult snail homogenates were estimated after one day, three days and one -week post treatments with 1.25, 2.5 and 5 % of Kz oil and 0.031, 0.063 and 0.125 % of methomyl using dipping technique of lettuce and cabbage leaves. Generally, all the tested concentrations of Kz oil and methomyl caused remarkable inhibition in the activities of both invertase and amylase enzymes which associated with time elapsing and concentrations of the compounds as compared to control.

Invertase and amylase activity using dipping technique of lettuce leaves: Data in Fig. (1) showed that the decreasing percentages in the amylase activity reached its maximum level after one week of treatment recording (-5.79, -15.20 & -36.34) for Kz oil at (1.25, 2.5 and 5%) and (-20.58, -34.22 & -47.96) for methomyl at (0.031, 0.063 and 0.125 %). Regarding invertase activity Fig. (2) the decreasing percentages recorded (-6.91, -11.58 & -26.86) and (-13.27, -22.19 & -37.09) for Kz oil and methomyl, respectively as compared to control.

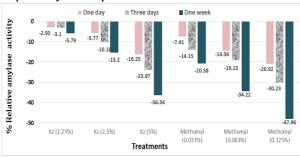


Figure 1. Relative activity percentages of amylase enzyme in *T. pisana* snail adults treated by Kz oil and methomyl using dipping technique of lettuce leaves.

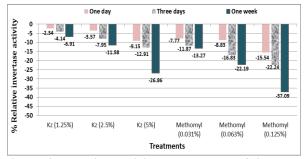
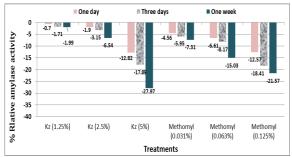
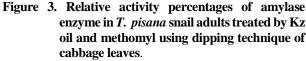


Figure 2. Relative activity percentages of invertase enzyme in *T. pisana* snail adults treated by Kz oil and methomyl using dipping technique of lettuce leaves.

Invertase and amylase activity using dipping technique of cabbage leaves: It is obvious from Fig. (3) that the level of amylase activity in the treated adult of *T. pisana* snail was inhibited by time. The maximum reduction was (-1.99, -6.54 & - 27.97) for Kz oil and (-7.31, -15.03 & - 21.57) for methomyl after one week of treatment. Concerning invertase relative activity to control the highest reduction percentages observed after one-week post treatment gave (-4.17, -5.42 & -10.13) and (-3.99, -8.93 & -24.66) for Kz oil (1.25, 2.5 and 5%) and methomyl (0.031, 0.063 and 0.125 %) , respectively Fig.(4). Invertase and amylase are two important digestive enzymes involved in carbohydrates metabolism and play an important role in the digestion and utilization of carbohydrates for energy production Naveed *et al.* (2009). Our results showing an inhibitory action of both Kz oil and methomyl action on amylase and invertase activities. The reduction effect could be attributed to the direct toxic effect of Kz oil and methomyl caused irregularity of enzymatic activity Sobhi *et al.* (2020). These results are in agreement with those reported by Radwan, *et al.* (2008) who revealed that treatment of *Eobania vermiculata* snail with methomyl and methiocarb caused decrease in carbohydrate, lipid and protein contents. In accordance, Gaaboub *et al.* (2012) recorded decrease in the activity of amylase and invertase enzymes in cotton leafworm *Spodoptera littoralis* (Boisd.) surpass in toxicity effect with lettuce leaves over cabbage leaves treated by methomyl.





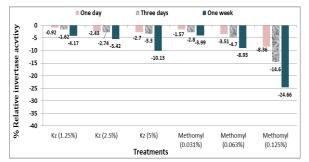


Figure 4. Relative activity percentages of invertase enzyme in *T. pisana* snail adults treated by Kz oil and methomyl using dipping technique of cabbage leaves.

CONCLUSION

The current study suggested that Kz mineral oil has potential for using in the control of white garden snail *Theba pisana* (Müller) as safe alternative to synthetic methomyl pesticide. Kz oil found to be possess marked toxic and biochemical effects against juveniles and adults of white garden snail and was safe to the most important pollinators honey bee (*Apis mellifera* L.). Further studies are required to evaluate the surpass in toxicity effect with lettuce leaves over cabbage leaves.

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تقييم الزيت المعدنى Kz كمبيد للقواقع وتاثيرة على النشاط البيوكيميائى لقوقع الحدائق الابيض Theba pisana تقييم الزيت المعدنى Kz كمبيد القواقع وتاثيرة على النشاط البيوكيميائى لقوقع الحدائق الابيض Muiller) كبديل امن للمبيدات ومركب صديق لنحل العسل . رشا شوكت شكرى سكلا ، محمد فرج نور الدين غازى فرج و أسماء محمد عبدالمجيد السيد معهد بحوث وقاية النباتات مركز البحوث الزراعية – الدقى – الجيزة – مصر

لقد تم تقييم الزيت المعدنى Kz كمبيد ضد الأفراد الصغيرة والبالغة لقوقع الحدانق الأبيض Theba pisana مقارنة بمبيد الميثوميل تحت الظروف المعملية عند تركيزات (١,٢٥ و ٢,٥٠ و ٥٪) لزيت Kz و(٢٠,٠ و ٢٢٠,٠ و ٢٢٠) لمبيد الميثوميل باستخدام تقنية غمر أوراق الخس والكرنب. أشارت النتائج الى زيادة نسبة موت القواقع المعاملة بزيادة التركيز وبمرور وقت التجربة حيث كانت أعلى نسبة موت عند معاملة أوراق الخس بزيت Kz ٥٪ هى (٣٣,٣٣ و ٢٦،٢٤ ٪) للأفراد الصغيرة و البالغة على الترتيب ، بينما كانت أعلى نسبة موت عند معاملة أوراق الكرنب بنس بزيت Kz ٥٪ هى (٣٣،٣٣ و 7،٣٣ و ٢،٣٤ ٪) للأفراد الصغيرة و البالغة على الترتيب ، بينما كانت أعلى نسبة موت عند معاملة أوراق الكرنب بنفس التركيز هى (٤٠ و ٢٠,٣٢ ٪) على التوالى وذلك عند الأسبوع الثالث من المعاملة. أما بالنسبة لمبيد الميثوميل كانت أعلى نسبة موت عند معاملة أوراق الكرنب بنفس التركيز هى (٤٠ و ٢٠,٣٢ ٪) على التوالى وذلك عند الأسبوع الثالث من المعاملة. أما بالنسبة لمبيد الميثوميل كانت أعلى نسبة موت الذر الصغيرة و البالغة القوقع هى (٢٦,٣٣ و ٤٦,٣٣ ٪) على التوالى وذلك عند موال الحسوم المعاملة. أما بالنسبة لمبيد الميثوميل كانت أعلى نسبة موت اللأفراد الصغيرة و البالغة القوقع هى (٢٠ و ٤٦,٣٠) على التوالى وذلك عند معاملة أوراق الخس بالمورات (٢٠)، و ٢٠,٠٠ ٪ والم وذلك عند الأسبوع الثالث من المعاملة. أما بالنسبة لمبيد الميثوميل كانت أعلى نسبة موت الموراد الصغيرة و البالغة القوقع هى (٢٦,٣٠ و ٤٦,٣٠) على التوالى وذلك عند معاملة أوراق الخس بالمبيد بتركيز ٥٠,٠٠ ٪ وأظهرت النتائج أيضا المعنى من المعاملة راد الصغيرة و الانفرتيز للأفراد البالغة للقوقع المعامل بالزيت على منيالات نحل العسل. المعدني Kz أمن على نحل العسل .