CATRINA (2022), 25(1):19-26

Efficacy of *Carum carvi* Essential oil Against the Parasitic Varroa Mite and its Impact on Honeybee *Apis mellifera* L.

Rehab M. El-Gendy[1], Rasha S. Sakla[2]
[1]Pest Physiology Research Department, Plant Protection Research Institute,
Agricultural Research Center, Dokki, Giza, Egypt.
[2]Bee Research Department, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

ABSTRACT

The Caraway, *Carum carvi* essential oil was assessed to behave as natural control of bee parasitic mite, *Varroa destructor* during fall season instead of chemicals methods. Caraway oil (100%) was used and compared to the synthetic acaricide; Apistan. The number of fallen mites and infestation percentages on both adult and brood were estimated regarding the effects of essential oil on honeybees *Apis mellifera.* The essential oil from *C. carvi* seeds were extracted by hydro distillation and characterized by GC/MS. Sixteen compounds were identified representing; 97.33% of the oil. The predominant components were Limonene (38.81%), Carvone (35.43 %), α-Myrcene (7.3%), Dihydrocarvone (5.58%) and Limonene oxide (5.13%). The infestation reduction percentages, recorded with Caraway oil, reached 84.42% and 70.65% on adult and brood workers after 21 days of treatments with no statistical difference with Apistan. The mean numbers of fallen Varroa mites were (67.67) and (74.65) in hives treated by essential oil and Apistan, respectively. The immune response of honeybee workers and pupa revealed significant elevation in phenol oxidase activity with Caraway oil however, remarkable inhibition in the enzyme activity observed with workers exposed to Apistan. The comet assay performed on worker honeybee as a biomarker of DNA damage; revealed statistically significant increase in DNA damage caused with Apistan (20.1%) and infested bee (21.64 %) compared to corresponding one treated with Caraway essential oil (12.4%) and control (9.6%). Caraway essential oil proved a safe way as natural controlling of *Varoaa destructive* with keep guard of honeybee social life and fit into IPM programs

Keywords: *Apis mellifera*; *Carum carvi*; essential oil; Genotoxicity Phenol oxidase; *Varroa destructor*.

INTRODUCTION

The bee parasitic mite (*Varroa destructor*) has become the most serious threat to bee colonies it causes great loss to honeybee (*Apis mellifera* Linnaeus) and beekeeping industry as well. Varroa mite is an ectoparasite of brood and adults, feeds on fat body of the bee (Ramsey, *et al*., 2019) it reduces the fitness of newly emerged individual bees and suppress their immune system (Yang *et al.,* 2005). Moreover, Varroa mites act as virus transmission that weaken colonies survival worldwide (Galindo-Cardona *et al.*, 2020), it can lead at the final stage through visible damaged signs in body and wings to the colony collapse (Genersch, 2010). Infested colonies require to be treated to avoid injury of brood and adults (Elzen *et al.,* 2000).

Miticides were successfully used inside bee colonies to suppress mite population. Despite of treated colonies with miticides showed more productivity than untreated ones, the continual use of synthetic miticides increase the chance of Varroa resistance development to these chemical products (Sammataro *et al*., 2005) furthermore, their harmful effects to human through hive products contamination (Bogdanov, 2006 and Alshafy, *et al*., 2021). On the other hand, miticides are also toxic to honeybee (Shoukry *et al*., 2013) and subsequently, activate the bee's detoxification mechanisms which considered an energy demanding and may affect their health. Thus, the use of naturally origin acaricides that are safe for non-target species as human and bees is highly being promoted. Many plant essential oils exhibit considerable acaricidal activity (Sabahi *et al.,* 2018) can be used as natural miticides and alternative to, or in rotation with synthetic miticides to give the colonies a break from chemical sublethal effects or combs contamination (Hýbl, *et al*., 2021). Caraway *(Carum carvi* L., family: Apiaceae) is a widespread plant grown everywhere in the world. Fruits of Caraway plant are used as beverages, foods flavoring agent besides various uses in ethnomedicine because of its own antimicrobial, spasmolytic, antioxidant, anti-inflammatory and immunomodulatory properties (Keshavarz, *et al.,* 2013). The current work aimed at evaluating the miticidal activity of Caraway essential oil compared to the synthetic miticide Apistan (tau-fluvalinate) which is frequently used inside bee colonies regarding their effect on honey bees, *A. mellifera.*

MATERIALS AND METHOD

This work was done in late Fall (2020) in the apiary of Honeybee Research Plant Protection Research Institute, Agric. Res. Center, El-Zagazig Branch, Sharqia Governerate. Adults and sealed broods of the honeybee colonies of Research department apiary were examined for mite infestation before selecting the experimental colonies. Nine honeybee *A. mellifera* colonies, in Langstroth beehives naturally infested by ectoparasitic *V. destructor* mites, were selected for this trial. Colonies were standardized for bee frame, brood and infestation levels and divided into three groups; each with three replicate colonies.

Essential oil extraction

The *Carum carvi* seeds were purchased at a local market in Sharkia province. Seeds of (500 g) were washed and then placed in a flask of hydro-distillation apparatus with 2 litres of distilled water and boiled for

three hours using a Clevenger-type apparatus. The essential oil was then collected and dried over anhydrous sodium sulphate.

Gas Chromatography analysis of *Carum carvi* oil

GC/MS assay of Caraway oil was performed at National Research Center (NRC), Egypt to screened its components using a Thermo Scientific, Trace GC Ultra/ISQ Single Quadrupole MS, TG-5MS instrument with 30m, 0.251mm, 0.1 mm film thickness as silica capillary column. Electron ionization energy (70 eV) and helium gas as the carrier were used at 1mL of oil /min at 280 °C in GC/MS detection. The volatile oil exposed to initial temperature 40 °C (hold 3 min) to 280 °C as a final temperature at an increasing rate of 5 °C/min (hold 5 min) in programmed oven. The quantity of the identified components was exhibited by using relative peak area as percentages. The unknown compounds were identified based on the NIST, WILLY library data of the GC/MS system in comparison with their mass spectra.

Experimental design and treatments application

Three groups were designed to evaluate the efficiency of Caraway essential oil compared to Apistan, commen and simplest defence against the Varroa mite, and control group. First group, two ml/colony of Caraway essential oil, at 100% concentration, was applied using two stripes of carton (2 x 10 cm) held between the brood nests. The treatment was performed three times, each with a seven-day interval. For second group, Apistan (10% fluvalinate) was administrated as one Apistan strip/colony hanged between two brood combs in treated colonies. The third control group, three untreated colonies were left as control. Colonies were received sugar syrup solution (50%) weekly throughout the experiment time.

Efficiency of treatment applied

The effectiveness of the treatment was verified by a reduction in the percentages of Varroa mite infestation. The Varroa infestation percentages in sealed brood cells and on worker bees were recorded before and after each treatment.

For sealed brood

Samples of 50 sealed cells were selected randomly from the brood frames in each tested colony. The cells were scratched, prepupae or pupae were removed, carefully examined and the infested cells were counted. The infestation percentage was calculated as follow:

$$Infestation\ \% = \frac{Number\ of\ infested\ cells}{Number\ of\ examined\ cells} X\ 100$$

For adult workers

Bees were taken from the hive combs where, about 100 workers bee sample were collected randomly from both the brood chamber and the honey super of each colony using washing technique. The bees were immersed in water with detergent, shaking well and then removed using a wire net. The fallen mites and the collected bees were counted (Ritter, 1981).

The percent of infestation was calculated as:

$$Infestation\ \% = \frac{Total\ number\ of\ mites}{Total\ number\ of\ bee}\ X100$$

During treatment period the infestation percentages in both worker brood and adult bee were estimated weekly post each treatment. The reduction percentages in mite infestation were calculated according to the equation of Henderson and Tilton (1955).

$$Reduction\ of\ infestation\ \% = 1 - [\frac{TaXCb}{TbXCa}]\ X100$$

Where: *Ta*, % infestation of treated colonies after treatment; *Cb*, % infestation of untreated colonies before treatment; *Tb*, *%* infestation of treated colonies before treatment and *Ca*, % infestation of untreated colonies after the time period of treatment.

Mites fall and dead worker bees

The fallen mites were counted using plastic sheet coated with vaseline and covered with wire mesh placed on the hive bottom board. The sheet was replaced with new clean one at the end of each treatment and dropped mites were recorded. Dead worker bees were also checked post each treatment.

Biochemical assay

Phenol oxidase

Samples of honeybee workers and pupae were collected after 14 days post treatment with essential oil and Apistan then weighed and frozen. The frozen samples were homogenized in 1ml of distilled water using chilled glass Teflon homogenizer (MPW-309 Mechanic-Preczyina, Poland) and then centrifuged (Hettich, Germany) at 6.000g for 10 min at 5°C. The supernatant of each group was used.

The activity of phenol oxidase was determined according the method of Ishaaya (1971) in which using catechol as the substrate. The activity was measured at 405 nm after mixing 0.5 mL phosphate buffer (0.1 M, pH 7) with 200 µL enzyme solution and 200 µL catechol solution (2 %).

Genotoxicity (DNA damage marker)

DNA integrity using comet assay

The simple evaluation of cellular DNA damage in honeybee worker after treatment with Caraway oil and Apistan after 14 days was obtained by comet assay method (The single cell gel electrophoresis assay, SCGE) under alkaline conditions as maintained by Singh *et al.*, 1988**.**

The individual cell of a honeybee worker was first mixed with low melting agarose before being applied on the Oxi Select Comet Slide, and then embedded cells were treated with a lysis buffer and alkaline solution to denaturate and relax the DNA. the slide samples were then electrophoresed in a horizontal chamber to separate intact DNA from damaged pieces. Following alkaline electrophoresis, the samples were dried, stained with ethidium bromide dye (12 ug/ml), and examined using epifluorescence microscopy. Under these conditions, damaged DNA (containing cleavage and strand breaks) will move further than int-

act DNA, resulting in a "comet tail" shape which can be calculated as follow:

Tail moment (arbitrary unit) = Length of DNA migration (um) × percentage (%) of migrated DNA.

Statistical Analysis

All the data were expressed in means ±S.E and percentages. The results were statistically evaluated using one-way (ANOVA). Comparison of treatments to determine means that are significantly different Tukey's HSD was applied. Costat (Costat, 2005) statistical software package was used. The significance differences determined at $p \leq 0.05$. level.

RESULTS

Reduction percentage of Varroa infestation on adult workers

Data in table (1) revealed the mean reduction percentages of Varroa mites on adult honeybees treated by Caraway oil and Apistan. Post the first treatment, significant difference was observed in the reduction percentages of Varroa infestation between the tested essential oil and Apistan ($p \leq 0.005$) recorded 27.65 and 41.20 for Caraway oil and Apistan, respectively. Post the third treatment data indicated a clear reduction in the infestation percentage in both Caraway oil (84.42%) and Apistan (88.31%) with no significance difference between (p=0.2594).

Reduction percentages of Varroa infestation on worker brood

The mean reduction percentages of Varroa mite infestation on worker brood after treating the honeybee colonies with Caraway essential oil and Apistan strips were illustrated in table (2). Data revealed gradual reduction in Varroa mite infestation percentages on sealed brood from the first to the third week post treatments reached (70.65%) and (75.58%) with essential oil and Apistan, respectively. The reduction percentages with Apistan group was significantly higher after the first treatment $p= 0.0052$ whereas, the statistical test (one way ANOVA, Tukey's HSD) performed no significant difference ($p >0.005$ between the two treatments after the second and third treatments.

Mites fall and dead worker bees

Results obtained (Table 3) indicated that the highest number of fallen mites/treatment was recorded after the first and second treatment with both tested essential oil and Apistan compared to control colonies. Apistan treatment recorded higher total number of fallen Varroa (74.65) followed by Caraway oil (67.67) and control (44.99). Data presented in (Table 3) indicated that the experimented Caraway essential oil and Apistan didn't cause mortality of worker bee throughout the experimental period.

Identification of Caraway oil

Carum carvi oil was analyzed using the GC/MS analysis. A total of 16 compounds were identified in the essential oil, with 97.33% of the total oil as shown in (Fig. 1). Data in table (4) showed that Caraway oil contain Limonene and carvone as a major compound with 38.81% and 35.43 % (Fig. 2) followed by α-Myrcene (7.3%), Dihydrocarvone (5.58%), Limonene

Table (1): The mean values of *V. destructor* infestation and reduction (%) of honeybee workers after different of exposures intervals to Caraway oil and Apistan strips.

Treatments	Varroa infestation and reduction rate (%)						
	Before treatment	Exposure intervals					
		First		Second		Third	
		infestation	reduction	infestation	reduction	infestation	Reduction
Control	21.48±0.74	22.5±1.47	-	26.21±0.99	-	27.92±1.22	-
Caraway oil	29.00±1.73	24.00±0.56	27.65 ±1.63	11.53±0.96	67.28±1.87	5.88±0.32	84.42±1.67
Apistan	27.30±0.98	18.22±0.97	41.20 ±0.96	9.11±1.00	72.31±1.62	4.21±0.31	88.31±2.44
LSD	-	-	5.2864	-	6.8861	-	8.2328
p	-	-	0.0021 **	-	0.1128 ns	-	0.2594 ns

Data expressed as in means ±S.E. Different letters means per column are significant effect based on Tukey's HSD test, at $p \leq 0.05$. **, is highly significant and ns, non-significant. LSD, means low significance differences at probability value ($p \leq 0.05$).

Table (2): The mean values of *V. destructor* infestation and reduction (%) of honeybee workers brood after different of exposures intervals to Caraway oil and Apistan strips.

Treatments	Varroa infestation and reduction rate (%)						
	Before treatment	Exposure intervals					
		First		Second		Third	
		infestation	reduction	infestation	reduction	infestation	reduction
Control	18.30±1.19	19.66±0.42	-	21.66±1.02	-	24.00±1.73	-
Caraway oil	23.63±0.85	19.70±1.38	22.45 ±1.09	12.90±0.52	53.83±1.47^{b}	9.10±0.81	70.65±1.63
Apistan	26.00±1.53	19.20±0.67	31.40 ±1.18	12.66±0.29	58.86±1.45^{b}	8.30±0.46	75.58±1.66
LSD	-	-	4.4797	-	5.7587	-	6.4903
p	-	-	0.0052 **	-	0.0724 ns	-	0.1028 ns

Data expressed as in means ±S.E. Different letters means per column are significant effect based on Tukey's HSD test, at $p \leq 0.05$. **, is highly significant and ns, non-significant. LSD, means low significance differences at probability value ($p \leq 0.05$)

oxide (5.13%). On the other hand, the minor compounds are α-Bourbonene (0.07%), (+) Spathulenol (0.09%), Perillaldhyde (0.11%), transCaryophyllene (0.12%), α-elemene (0.13%) and α-ocimene (0.41%), respectively.

Phenol oxidase activity

The observed data in figure (3) cleared significance inhibition ($p \leq 0.005$) of the activity of phenol oxidase in honeybee worker with Apistan (34.19 U/g.b.wt) whereas, remarkable increase in enzyme activity were detected with Caraway volatile oil (92.26 U/g.b.wt) in comparison to control (83.48 U/g.b.wt). Data showed Caraway oil caused moderate significance elevation in phenol oxidase activity of honeybee pupa with essential oil compared to control and Apistan treatment **(***P*=0.0015).

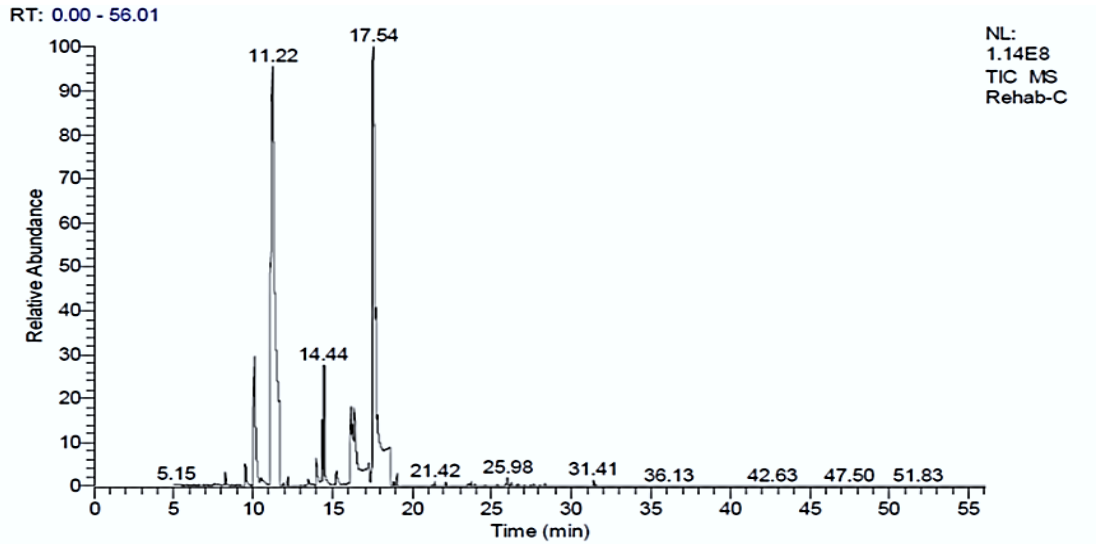

Figure (1): GC-MS chromatogram of the volatile Caraway essential oil.

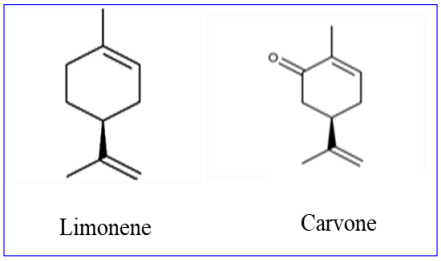

Figure (2): The main components of *Carum carvi* L. oil identified by GC-MS analysis.

Gentoxicity

Comet DNA assay

Genetic damage in treated and infested workers obtained through the comet assay was presented in figure (4) and table (5). The highest values of genetic damage were observed in the infested adult and Apistan treatment where, DNA damage percentages were (21.63% &20.1%) and tail length (8.39 &10.84), respectively. Low damage in comet percent observed with Caraway treatment recorded a close value (12.4) to that of control (9.6) with significant lower values in tail length (7.29 and 7.87) than Apistan treatment. There were non-significance differences in tail moment and olive tail moment when compared with control group at (P= 0.2397 and 0.7801).

DISCUSSION

The current work was carried out during Fall to evaluate the efficacy of *Carum carvi* essential oil against Varroa mites compared to Apistan a widely used synthetic miticide inside bee colonies. The percentages of Varroa mite infestation were gradually reduced after each treatment on both the workers brood and adults with essential oil and Apistan. Nevertheless, Apistan significantly reduced Varroa infestation after the first week compared to Caraway oil, insignificant difference was observed after the second and third weeks of treatment. The toxicity of the tested essential oil to Varroa mite increased by increasing the exposure time, the same trend was observed by Damiani *et al.* (2009) with essential oils they tested. Moreover, the decrease of brood during late fall and early winter makes Varroa very susceptible to control by essential oils where most Varroa mites will exist on worker bees (Noel and Amrine, 1996).

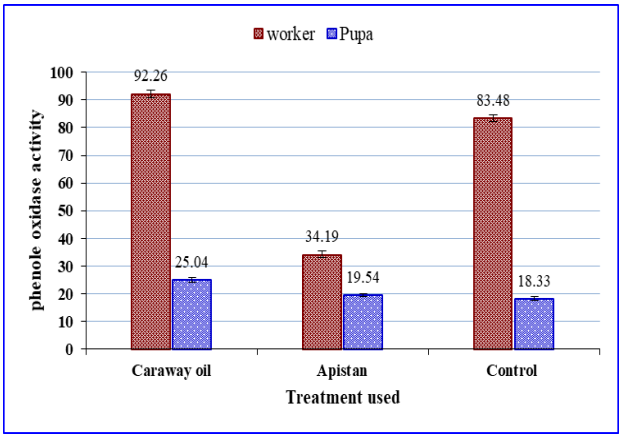

Figure (3): Effect of Caraway volatile oil and Apistan on phenol oxidase activity in worker and pupa *Apis mellifera*.

Regarding the dropped mites, the highest total numbber of Varroa mites on the sheet was recorded after the first and second treatments with the essential oil and Apistan in comparison to control. Increasing of fallen Varroa mites in honeybee colonies exposed to Caraway essential oil may attribute to the activation of the defence behaviour mechanisms of worker bees against Varroa mite by plant essential oil (Noor Islam *et al*., 2016). In the same context, Salem *et al*., (1998) suggested that some changes occurred in the haemolymph of honeybee workers may be due to feeding neem extract, resulted in an increased of Varroa mite fallen on the sheet.

The GC-MS analysis of *C. carvi* volatile oil revealed that the major components are limonene and carvone with 38.81% and 35.43 %, respectively. Many previous literatures mentioned carvone and limonene as the main constituent's portion of Caraway oil with different percentages (Laribi *et al*., 2010; Rivera *et al*., 2010; Baananou *et al*., 2013; Abou El-Soud *et al*., 2014; Chizzola, 2014 and Garaya *et al*., 2016). The ratio of these two major compounds is responsible for expressing the quality of Caraway oil. The ratio of limonene to carvone may impairment by increasing the storage time as maintained by (Sedlakova, 2001; Mahboubi, 2019 and Gajić *et al*., 2020). On the other hand, *C. carvi* oil contains small amount of carvone on the authority of Hajlaoui *et al*. (2021).

It is proposed that toxicity may be due to the combination of *C. carvi* oil constitutes. The major concern nowadays is the use of essential oils for Varroa control instead of chemical control as Apistan while very few data regarding their effects on honeybee are known particularly their intervention with immune response and genotoxicity. Therefore, the current work evaluates the impact of Caraway oil and Apistan on the activity of phenol oxidase of honeybee adults and pupa beside their acaricidal action.

Table (3): The effect of tested Caraway essential oils and synthetic Apistan on the number of fallen Varroa mites and bee mortality.

Treatments	No. of fallen mite before treatments	No. of fallen mite after treatments			Total Number	Bee mortality
		Exposure intervals				
		First	Second	Third		
Control	12.33	13.67	14	17.33	44.99	0.00
Caraway oil	17.33	23.33	28.33	16.00	67.67	0.00
Apistan	18.66	31.67	24.33	18.66	74.65	0.00

Table (4): The identified GC-MS phytochemical compounds of *Carum carvi* L. essential oil

No.	Rt	Compound name	Area%
1	8.23	α-ocimene	0.41
2	9.46	α-Pinene	1.17
3	10.06	α-Myrcene	7.3
4	11.22	dl-Limonene	38.81
5	12.16	α-Phellandrene	0.28
6	13.41	Linalool	1.45
7	14.44	Limonene oxide	5.13
8	16.36	Dihydrocarvone	5.58
9	17.27	5Caranol, (1S,3R,5S,6R)	0.99
10	17.53	Carvone	35.43
11	18.84	Perillaldhyde	0.11
12	21.25	α- Bourbonene	0.07
13	21.42	α- elemene	0.13
14	22.1	transCaryophyllene	0.12
15	25.9	(+) spathulenol	0.09
16	25.98	(-) Caryophyllene Oxide	0.26
Total			97.33

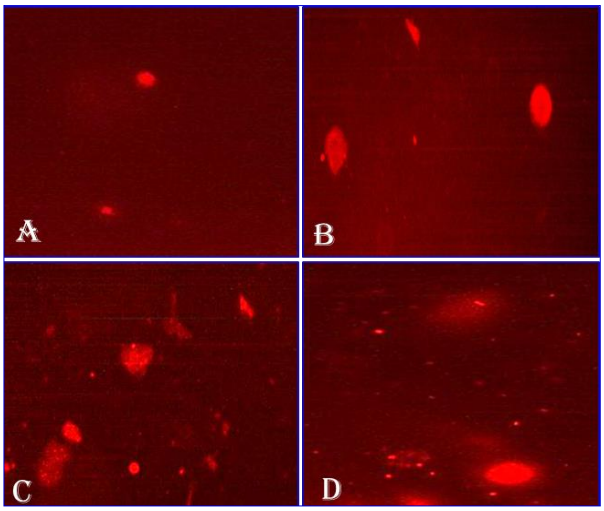

Figure (4): Fluorescent microscopic images of worker bee cells injury in different treatment groups Comet assay showing DNA damage degree. A, control group; B, Caraway oil-treated group; C, Apistan-treated group and D, infested-untreated group.

Regarding the pupal stage, Caraway oil cause significant increase in the activity of PO compared with control and Apstain treatment. During this stage, it increases cuticle pigmentation and sclerotization to build the adult bee's exoskeleton. Therefore, PO is

considered a key enzyme for honeybee development. In the opinion of Zufelato *et al*. (2004), the exo skeleton differentiation in the adults of honeybee is due to increase melanization of pupal cuticle related to increase of enzyme activity. In the same context, the inhibition of the PO activity is the reason of delaying cuticle hardness in honeybee pupae and insequence its development (Zufelato *et al*., 2000).

It is noted from the current data that Apistan treatment significantly reduced PO activity (34.19 U/g.bwt) than control (83.48 U/g.b.wt), this reduction may cause weakness in the immune response of adults when faced different stressors. Fortunately, Caraway essential oil caused increases in the enzyme activity (92.26 U/g.b.wt) in comparison to control.

The genetic biomarker as comet assay (single cell gel electrophoresis) is now frequently used in genetic toxicology since it is simple to measure DNA damage on an individual cell level (Azqueta and Collins, 2013, Villar and Ojeda, 2019). The low damage in comet % observed with Caraway treatment recorded a value that was close to that of the control (12.4 and 9.6 %, respectively). The high damage observed with infested workers followed by Apistan treatment (21.63 and 20.1%, respectively) demonstrates the damage that Apistan can induce to honey bees. Meanwhile, increasing the amount of DNA damage and comet tail length may cause cell apoptosis (El-Gendy, 2021).

Caraway essential oil, in general, proven a safe app roach as a natural control of Varoaa damaging with the

Table (5): Genotoxicity of Caraway oil and Apistan verses untreated worker honeybee cell integrity using a comet assay.

Treatments	Quantified DNA damage			
	Damage%	Tail length	Tail moment	Olive tail moment
Control	9.60^{c}	7.87^{ab}	0.43	0.63
Caraway	12.40^{b}	7.29^{b}	0.80	0.92
Apistan	20.10^{a}	10.84^{a}	0.96	0.70
Infested worker	21.63^{a}	8.39^{ab}	0.69	0.85
LSD	1.22	2.37	0.55	0.72
p	0.0000^{***}	0.0376 *	0.2397^{ns}	0.7801^{ns}

Data expressed in Means±S.E; Same letters per column mean non-significant effect; different letters mean significant effect at $p \leq 0.05$ according to Tukey's HSD test; ***, highly significant; *, significant; ns, non-significant. LSD means low significance differences at probability value ($p \leq 0.05$)

protection of honeybee community interaction due to its beneficial components. Caraway oil includes Carvone, which, when combined with Limonene and Carvacrol, has anti-inflammatory, antioxidant, and antibacterial properties. Also, monoterpenes as Carvone and limonene possess an immunomodulatory activity of Caraway essential oil. These observations were supported by the findings of Keshavarz, *et al*. (2013) and Tarek *et al*. (2014).

CONCLUSION

Recently, the use of natural materials for Varroa mite management has supported the need to overcome resistance to currently used acaricides and provide residue-free hive products. It may be concluded that the studied essential oil *Carum carvi* is efficient against Varroa mite infection and can be used safely inside bee colonies as an alternative to, or in rotation with, synthetic miticide (like Apistan) and may fit well into an IPM programme for Varroa mite control. On the other hand, it is critical to investigate the impact of in-hive miticides on the vital functions of honeybees in order to protect their social lives from injury.

REFERENCES

ABOU EL-SOUD, N.H., N.A. EL-LITHY, G. EL-SAEED, . S.WAHBY, M. YKHALIL, F. MORSY, AND N. SHAFFIE. 2014. Renoprotective effects of Caraway (*Carum carvi* L.) essential oil in streptozotocin induced diabetic rats. J of Applied Pharmaceutical Science 4(2): 27

ALSHAFY, A.G., E.W. ZIDAN, R., M. REZK AND W.S. MESHRIF. 2021. Effect of some antimicrobial drugs on the fitness of honeybee *Apis mellifera* L. (Hymenoptera: Apidae). Catrina: The International Journal of Environmental Sciences, 25-33. doi: 10.21608/cat.2022.100375.1109

AZQUETA, A., AND A. R. COLLINS. 2013. The essential comet assay: a comprehensive guide to measuring DNA damage and repair. Archives of toxicology 87(6): 949-968.

BAANANOU, S., E. BAGDONAITE, B. MARONGIU, A. Piras, S. Porcedda, D. Falconieri, AND N. BOUGHATTAS. 2013. Extraction of the vol-atile oil from *Carum carvi* of Tunisia and Lithuania by supercritical carbon dioxide: chemical composition and antiulcerogenic activity. Natural prod-uct research 27(22): 2132-2136

BOGDANOV, S. 2006. Contaminants of bee products. Apidologie 37(1):1-18.

CHIZZOLA, R. 2014. Composition of the essential oil of wild grown Caraway in meadows of the Vienna region (Austria). Natural product communications 9(4):581-582.

COSTAT STATISTICAL SOFTWARE, 2005. Microcomputer program analysis version, 6.311. Co Hort Software, Monterey, California, USA.

DAMIANI, N., L.B. GENDE, P. BAILAC, J.A. MAR-

CANGELI, AND M.J. EGUARAS. 2009. Acaricidal and insecticidal activity of essential oils on Varroa destructor (Acari: Varroidae) and Apis mellifera (Hymenoptera: Apidae). Parasitology Research 106:145-152.

EL-GENDY, R.M. 2021. Toxicological, histological and biochemical effects of Lepidium sativum seeds extract on Galleria mellonella L. (Lepidoptera: Pyralidae) larvae. Catrina: The International Journal of Environmental Sciences, 23(1): 1-10. doi: 10.21608/cat.2021.65612.1074

ELZEN, P.J., J.R. BAXTER, G.W. ELZEN, R. RIVERA, AND W.T. WILSON, 2000. Evaluation of grapefruit essential oils for controlling Varroa jacobsoni and Acarapis woodi. American Bee Journal 140(8): 666-668.

GALINDO-CARDONA, A., A.C. SCANNAPIECO, R. RUSSO, K. ESCALANTE, M. GERIA, N. LEPORI, M.M. AYUP, I. MUNTAABSKI, M.C. LIENDO, L. LANDI, T. GIRAY, A.C. MONMANY-GARZIA. 2020. Varroa destructor parasitism and genetic variability at honeybee (Apis mellifera) drone congregation areas and their associations with environmental variables in Argentina. Frontiers in Ecology and Evolution 8: 394.

GAJIĆ, I., L. STANOJEVIĆ, A. DINIĆ, J. STANOJEVIĆ, L. NIKOLIĆ, V. NIKOLIĆ, AND V. SAVIĆ. 2020. The chemical composition of the essential oil and volatile compounds from Caraway fruit (Carum carvi L.) extracted by headspace-solid phase microextraction and the antioxidant activity. Advanced Technologies 9(1): 37-43.

GARAYA, A., W. DHIFI, M. NEHIRI, A. ECHCHELH, M. EBNTOUHAMI, A. CHAOUCH, W. MNIF, AND R. B. E. N. CHAOUACHA-CHEKIR. 2016. Chemical composition and anti-corrosive activity of Carum carvi seed essential oil. Journal of New Science 30(3): 1719-1724

GENERSCH, E. (2010). Honeybee pathology: current threats to honey bees and beekeeping. Applied microbiology and biotechnology, 87(1), 87-97.

HAJLAOUI, H., S. ARRAOUADI, E. NOUMI, K. AOUADI, M. ADNAN, M.A. KHAN, A. KADRI, AND M. SNOUSSI. 2021. Antimicrobial, antioxidant, anti-acetylcholinesterase, antidiabetic, and pharmacokinetic properties of Carum carvi L. and Coriandrum sativum L. essential oils alone and in combination. Molecules 26(12): 3625.

HENDERSON, C.F., E.W. TILTON. 1955. Test with acaricides against the brown wheat mite. J Econ. Entomol 48(2):157-161.

HÝBL, M., A. BOHATÁ, I. RÁDSETOULALOVÁ, M. KOPECKÝ, I. HOŠTIČKOVÁ, A.VANÍČKOVÁ, AND P. MRÁZ. 2021. Evaluating the Efficacy of 30 different essential oils against Varroa destructor and Honeybee workers (Apis mellifera). Insects 12(11): 1045.

ISHAAYA, I. 1971. Observation on the phenoloxidase system in the armored scales Aonidiella auranti and chrysomphalus aonidum. Comp. Biochem. physiol. 39:935 – 943.

KESHAVARZ, A., M. MINAIYAN, A. GHANNADI, AND P. MAHZOUNI. 2013. Effects of Carum carvi L. (Caraway) extract and essential oil on TNBS-induced colitis in rats. Research in pharmaceutical sciences 8(1): 1.

LARIBI, B., K. KOUKI, A. MOUGOU, AND B. MARZOUK. 2010. Fatty acid and essential oil composition of three Tunisian Caraway (Carum carvi L.) seed ecotypes. Journal of the Science of Food and Agriculture, 90(3): 391-396

MAHBOUBI, M. 2019. Caraway as important medicinal plants in management of diseases. Natural products and bioprospecting, 9(1): 1-11.

NOEL B., J. AMRINE. 1996. More on essential oils for mite control. Amer. Bee J.136(12):858-859.

NOOR ISLAM, M. A., E.S. EHSAN-UL-HAQ, AND F. NAZ. 2016. Management of Varroa destructor by essential oils and formic acid in Apis mellifera Linn. Colonies.

RAMSEY, S.D., R. OCHOA, G. BAUCHAN, C. GULBRONSON, J.D. MOWERY, A. COHEN, D. LIM, J. JOKLIK, J. M. CICERO, J. D. ELLIS, D. HAWTHORNE, AND D. ENGELSDORP. 2019. Varroa destructor feeds primarily on honey bee fat body tissue and not hemolymph. Proceedings of the National Academy of Sciences 116(5): 1792-1801.

RITTER, W. 1981. Varroa disease of the honeybee Apis mellifera. Bee world 62(4): 141-153.

RIVERA, L.L., G. VILAREM, R.S. GÓMEZ, M.J. ESTRADA, AND J.V. FEIJOO. 2010. Water soluble fractions of Caraway (Carum carvi L.) essential oil. Latin American and Caribbean Bulletin of Medicinal and Aromatic Plants. 9 (6): 495-500.

SABAHI, Q., M.M. HAMIDUZZAMAN, J.S. BARAJAS-PÉREZ, J.M. TAPIA-GONZALEZ, AND E. GUZMAN-NOVOA. 2018. Toxicity of anethole and the essential oils of lemongrass and sweet marigold to the parasitic mite Varroa destructor and their selectivity for honeybee (Apis mellifera) workers and larvae. Hindawi Psyche Volume 2018, Article ID 6196289, 8 pages, doi.org/10.1155/-2018/6196289 .

SALEM, M., M. NOUR, S. EL MAASARAWY, AND M. ZAKARIA 1998. Testing medical plants extracts compounds on haemocytes and varoatosis in honey bees. J. Agric. Sci. Mansoura Univ 23(1): 447-460.

SAMMATARO, D., P. UNTALAN, F. GUERRERO, AND J. FINLEY. 2005. The resistance of Varroa mites (Acari: Varroidae) to acaricides and the presence of esterase. International Journal of Acarology, 31(1), 67-74.

SEDLAKOVA, J., B. L. A. N. K. A. KOCOURKOVA, AND V. KUBAN. 2001. Determination of essential oils content and composition in Caraway (*Carum carvi* L.). Czech Journal of Food Sciences 19(1): 31-36.

SINGH, N.P., M.T. MCCOY, R.R. TICE AND E.L. SCHNEIDER. 1988. A simple technique for quanti-

tation of low levels of DNA damage in individual cells. Experimental cell research 175(1):184-191.
SHOUKRY, R.S., A.M., KHATTABY, A.A., EL-SHEAKH, A.H., ABO-GHALIA, AND S.M. ELBANNA. 2013. Effect of some materials for controlling Varroa mite on the honeybee drones (Apis mellifera L.). Egyptian Journal of Agricultural Research, 91(3), 825-834.
TAREK, N., H.M. HASSAN, S.M. ABDELGHANI, I.A. RADWAN, O. HAMMOUDA, AND EL-GENDY, A. O. (2014). Comparative chemical and antimicrobial study of nine essential oils obtained from medicinal plants growing in Egypt. Beni-Suef University Journal of Basic and Applied Sciences 3(2): 149-156.
VILLAR, S., AND P. OJEDA. 2019. Measurement of genetic damage in Apis mellifera caused by agrochemicals using comet assay. Current Topics in Toxicology, 15:133-139
YANG, X., AND D.L. COX-FOSTER. 2005. Impact of an ectoparasite on the immunity and pathology of an invertebrate: evidence for host immunosuppression and viral amplification. Proceedings of the National Academy of Sciences 102(21): 7470-7475.
ZUFELATO, M.S., M.M.G. BITONDI, Z.L.P. SIMO˜ES, K. HARTFELDER. 2000. The juvenile hormone analog pyriproxyfen affects ecdysteroid-dependent cuticle melanization and shifts the pupal ecdysteroid peak in the honeybee (Apis mellifera). Arthropod Struct. Dev. 29:111–119.
ZUFELATO, M. S., A. P. LOURENÇO, Z. L. SIMÕES, J. A. JORGE, AND M.M. BITONDI. 2004. Phenoloxidase activity in Apis mellifera honey bee pupae, and ecdysteroid-dependent expr-ession of the prophenoloxidase mRNA. Insect biochemistry and molecular biology 34(12): 1257-1268.

فاعلية زيت الكراويا العطرى *Carum carvi* فى مكافحة طفيل الفاروا وتاثيره على نحل العسل *Apis mellifera* L.

ريحاب محمود الجندي[1] و رشا شوكت شكرى سكلا[2]

[1] قسم بحوث فسيولوجيا الافات معهد بحوث وقاية النباتات، مركز البحوث الزراعية، دقى، جيزة، مصر.
[2] قسم بحوث النحل معهد بحوث وقاية النباتات، مركز البحوث الزراعية، دقى، جيزة، مصر.

الملخص العربـــــي

هدف البحث الى تقييم فاعلية زيت الكراويا العطرى *Carum carvi* كمركب طبيعى فى مكافحة طفيل الفاروا الذي يصيب مستعمرا ت نحل العسل *Apis mellifera* وذلك خلال فصل الخريف. و لقد تم اختبار الزيت العطرى بتركيز 100٪ ومقارنته بمبيد الفاروا الأبستان. تم تحديد نسبة الإصابة بالطفيل على الشغالات و فى الحضنة بالاضافة الى عدد الفاروا المتساقط ، قبل وبعد كل المعاملة. تم استخلاص الزيت العطري من بذور *C. carvi* بالتقطير المائي واجراء التحليل الكميائى بـ GC / MS. حيث تم تحديد ستة عشر مركبا تمثل 97.33٪ من الزيت. وكانت المكونات السائدة الليمونين (38.81٪) ، كارفون (35.43٪) ، ألفا ميرسين (7.3٪) ، دي هيدرو كارفون (5.58٪) وأكسيد الليمونين (5.13٪). أوضحت النتائج أن الزيت العطري المختبَر أظهر تاثير فعال على طفيل الفاروا حيث سجلت نسبة الخفض فى الإصابة بعد نهاية المعاملة 84.42٪ و 70.65٪ على كل من الشغالات والحضنة دون وجود فرق معنوى مقارنة بالأبستان كما كان العدد الإجمالي للفاروا المتساقط (67.67) و (74.65) في خلايا النحل المعاملة بالزيت العطري والأبستان. أظهرت الاستجابة المناعية لشغالات نحل العسل والعذارى ارتفاع معنوي في نشاط الفينول أوكسيديز مع زيت الكراوية فى حين أظهر الابيستان تثبيطا ملحوظا في نشاط الإنزيم . كما أظهرت نتائج تحليل (Comet assay) الذي تم إجراؤه على شغالات نحل العسل كمؤشر حيوي لتلف الحمض النووي ؛ زيادة معنوية في تلف الحمض النووي فى الشغالات المعاملة بالابيستان(% (20.1) و المصابة بالفاروا (21.64٪) مقارنة بالشغالات المعاملة بالزيت العطري (12.4٪) و غير المعاملة (9.6٪).