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# Study the Influence of Three Acaricides on Honeybee Workers *Apis mellifera* L. (Hymenoptera: Apidae) under Laboratory Conditions

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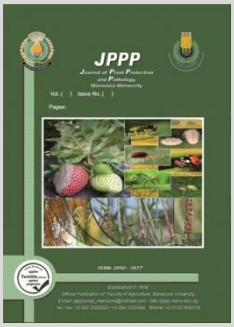


## ABSTRACT

Honey bees, *Apis mellifera* L. is a major global pollinator of crops and native plants. Insecticides have a negative and hazardous effect on *A. mellifera*, other pollinators, and beneficial organisms. This study was carried out to evaluate the influence of three acaricides; Ortus (fenpyroximate) 5% EC, Everken (abamectin)1.8% EC, and AgriFlex (abamectin+ thiamethoxam) 18.6% SC on the honeybee workers. These acaricides were chosen based on field observations, where those acaricides were used to control two-spotted spider mites on clover fields. A group of newly emerged honeybee workers was exposed to different dilutions (5, 7, 10, 13, and 15 ppm) of study candidate compounds. The control group was not treated with any acaricides. The mortality percentage was recorded after 24, 48, 72, and 96 hrs after acaricide applications, and $LC_{50}$ and $LC_{90}$ were calculated. Obtained results showed that everken and agriflex had significantly higher toxicity than ortus. Where $LC_{50}$ for everken and agriflex ranged between 2.75 ppm to 7.94 ppm after 24, 48, 72, and 96 hrs of acaricide applications, while ortus recorded 47.1 ppm after 24 hrs. Control treatment had the least honey bee mortality compared to all acaricide applications not exceeding (7%) followed by ortus (36%). Nevertheless, everken and agriflex reach 100% mortality after 96 hrs of the applications. Consequently, these results suggest that ortus can be included in the Integrated Pest Management (IPM) Program against harmful insects or honey bee pathogens and safe to sustainable, productive, and healthy honeybee stocks for the future.

**Keywords:***Apis mellifera*, Abamectin, Acaricides, Toxicity, Mortality, Fenpyroximate

## INTRODUCTION

Bee species and other beneficial insects have an essential role in plant pollination worldwide. The pollination process is the spirit of wild plant communities conservation all over the world (Naeem *et al*., 2020 and Loreau *et al*., 2001) and crop production (Costanza *et al*. 1997 and Kearns *et al*., 1998). Insect pollination value is determined to universal agriculture is $845 billion per year (Gallai *et al*., 2009). Despite the vast variation of plant pollinators, the honey bees, *Apis mellifera* L., is the most important global managed pollinator of agricultural crops and natural habitats (Calderone, 2012 and Hung *et al*., 2018). Additionally, approximately one-third of food consumption each day depends on pollination, fundamentally by honey bees (Holden, 2006). Therefore, honey bee's contribution to world food production is indispensable (Klein *et al*. 2007). Furthermore, honey bees provide honey, pollen, wax, propolis, and royal jelly to humans (Formato *et al*., 2011).

*Apis mellifera* is always exhibited to a vast range of biotic and abiotic stressors. Pathogens and pesticides are fundamental factors that affect honeybee survival. Interactions between stressors in honeybees could be one of the main reasons for the worldwide colony losses for more than ten years (Oldroyd, 2007; Potts *et al*., 2010; Van Engelsdorp and Meixner, 2010 and Van Engelsdorp *et al*., 2010). Despite insecticides having a harmful and hazardous effect on *Apis mellefera* (Nasr and Wallner, 2003; Pettis *et al*., 2004) and other pollinators, where they could contaminate nectar and pollen (Girolami *et al*., 2009 and Stoner and Eitzer, 2012). Beekeepers started to use pesticides inside bee colonies to control pests and pathogens (Johnson,2015). Consequently, insecticide accumulation in hive products because of chronic release to sublethal doses (Pilling *et al*., 2013), influences the colony case, messing with colony behavior and production (Sandrock *et al*., 2014).

Pesticide residues that remain in the food (honey or bee's products) may lead to potential health hazards to consumers. Thus, to confirm food safety and environmental protection, investigations on the proper use of pesticides in terms of authorization, registration, and compliance with maximum residue limits (MRL) should be considered. Also, the investigation of pesticide persistence in foodstuff and residues in agricultural fields needs more studies (Malhat *et al*. 2014).

Our current study focused on toxic assessment and calculation ($LC_{50}$) of the three acaricides; abamectin, fenpyroximate, and abamectin+ thiamethoxam on honeybee workers. Because pesticides are essential variables that could threaten honeybee survival, result in hive collapse and toxic residues in bee products.

## MATERIALS AND METHODS

### Honey Bee Hives

This study was carried out under laboratory conditions (Plant Protection Department, Faculty of Agriculture, Minia University, Egypt) in the summer of 2020. Wooden cages were used for breeding (15×14×6 cm), one side of the cage is covered with metal wire mesh,

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while the other side was made of glass. Each cage contained 100 newly emerged honeybee workers.

The cage was provisioned with 2 plastic droppers (20 ml) hung in the upper of the cage, one of these droppers filled with water, and the other one with sugar solution of (2 sugar: 1 water), (as a source of carbohydrates nutrition). The cage was also supplied with a paste placed in a small plastic cup at the base of the cage bottom (made of mixing pollen, sugar powder, and water) as a source of natural protein nutrition.

**Chemicals and Bioassay Methods**

Three different acaricides; everken (abamectin),1.8% EC, ortus 5% EC (fenpyroximate), and agriflex (3.3% abamectin+ 15.2% thiamethoxam as an active ingredients) 18.6% SC were assayed to evaluate their toxicity on honeybees. All tested acaricides were obtained from Syngenta Company to conduct the current experiment.

Bioassays were conducted using methods described by (Johnson et al. 2006). A range of acaricide concentrations (5, 7, 10, 13, and 15 ppm) were made through serial dilutions in acetone on the day of treatment for worker bioassays. Each concentrate from candidate acaricides was added to the sugar solution (2 sugar: 1 water) in a small dropper capacity of 20 ml. A group of hundred bees for each tested acaricide (20 bees/ concentration) were transferred from the rearing cage to another cage and allowed to feed on the dropper. Each treatment was replicated three times. Mortality for bee workers was recorded at 24, 48, 72, and 96 hrs after treatment. Probit analysis was established by plotting the probit units corresponding to 24,48,72 and 96 hrs mortality percentages versus concentration logarithms of tested chemicals. Bees fed with sugar solution only were used as a control.

Also, the Toxicity index (TI) assessment was estimated for each of the tested chemicals according to Sun (1950) as follows:

**Toxicity index = ($LC_{50}$ or 90 of the most effective compound/ $LC_{50}$ or $_{90}$ of the tested compound) *100**

**Data Processing and Statistical Analysis**

Data were inserted into Probit analysis (Finney 1971) to calculate $LC_{50}$ and $LC_{90}$ values of ortus, everken and agriflex assays values, slope and standard error intercept and its standard error, Pearson goodness of fit Chi-square ($X^2$), expected mortality and its residual, 95% confidence limits (CL) for the effective level of concentrations, and the heterogeneity factor in the calculation of the confidence limits using SAS (version 9.4) (SAS, 2008). Analysis of variance (ANOVA) was applied with the option of Fisher's LSD (least significant difference) method for mean separation at p = 0.05. additional t-tests were applied to reveal the statistical difference between the mortality of three chemical and control treatments.

## RESULTS AND DISCUSSION

**Toxicities of candidate acaricides to honey bee**

The results showed the effect of acaricides on honeybee Apis mellifera L. when they fed on a sugary solution laced with insecticide under laboratory conditions. The bees were provisioned with the sugar solution with different concentrations for each acaricide. The control bees were fed on sugar solution only without any chemicals. Our research results of lethal concentration (LC50) that has been studied to evaluate those three insecticides used ortus, agriflex, and everken (Table 1 and Figure 1). Firstly, everken and agriflex are required (LC50 = 6.01 ppm and (LC50 = 7.94 ppm), respectively as a lethal concentration for 50% of honeybees after 24 hrs of treatment, while ortus is needed (LC50 = 47.14 ppm). This means that everken and agriflex had significantly higher toxicity than ortus. Results after 48 hrs and 72 hrs had a similar manner, where ortus showed the lowest toxicity against honeybees (LC50s = 37.15ppm and 31.62 ppm, respectively) compared to everken and agriflex which ranged between (LC50 = 2.75-4.47 ppm). Total mortality was observed on honeybees after 96 hrs of agriflex application. Followed by the high toxicity of everken on honeybees. Otherwise, the least toxicity with the highest LC50 was recorded for ortus after 96 hrs too (LC50 = 25.12 ppm).

In addition, the safety factor for each pesticide was estimated after 24, 48, 72, and 96 hrs on honeybees. Nevertheless, the highest safety factor was noted on everken after24, 48, and 72 hrs (100, 100, and 67.57 ppm). Followed by agriflex, which recorded a range of 74.25-100 safety factors. In contrast, ortus showed a lower safety factor after 24, 48, 72, and 96 hrs ranging between 12.03-14.45.

**Table 1. Probit data (LC-P line data) established from plotting the probit units corresponding to 24,48,72 and 96h mortality percentages versus concentration logarithms of tested chemicals.**

| Treatments | Line Equation | Slope ± SE | df | $LC_{50}$ as ppm(95%CL) | $LC_{90}$ as ppm(95%CL) | TI-( $LC_{50}$) | TI-( $LC_{90}$) |
|---|---|---|---|---|---|---|---|
| | | | | 24 hrs after exposure | | | |
| Everken | y = 3.091x + 2.590 | 3.091 | 4 | 6.01a | 15.49a | 100 | 100 |
| Ortus | y = 2.018x + 1.623 | 2.018 | 3 | 47.14b | 204.17b | 12.75 | 7.59 |
| AgriFlex | y = 2.087x + 3.116 | 2.087 | 4 | 7.94a | 32.36c | 75.69 | 47.87 |
| | | | | 48 hrs after exposure | | | |
| Everken | y = 3.845x + 2.502 | 3.845 | 4 | 4.47a | 9.55a | 100 | 100 |
| Ortus | y = 1.882x + 2.038 | 1.320 | 4 | 37.15b | 177.83b | 12.03 | 5.03 |
| AgriFlex | y = 2.576x + 2.991 | 2.576 | 4 | 6.02a | 19.05c | 74.25 | 50.13 |
| | | | | 72 hrs after exposure | | | |
| Everken | y = 3.856x + 2.649 | 3.856 | 3 | 4.07a | 8.71a | 67.57 | 95.39 |
| Ortus | y = 1.797x + 2.278 | 1.797 | 4 | 31.62b | 158.49b | 8.69 | 4.57 |
| AgriFlex | y = 2.609x + 3.848 | 2.609 | 3 | 2.75a | 8.51a | 100 | 85.08 |
| | | | | 96 hrs after exposure | | | |
| Everken | y = 4.014x + 2.744 | 4.014 | 2 | 3.63a | 7.59a | - | - |
| Ortus | y = 1.858x + 2.367 | 1.858 | | 25.12b | 125.89b | 14.45 | 6.029 |
| AgriFlex | 100% mortality | - | - | - | - | - | - |

***$LC_{50}$ and $LC_{90}$ values having different letters within column for each time after exposure separately are significantly different. (-) means no living honeybee after 96 hrs after AgriFlex application**

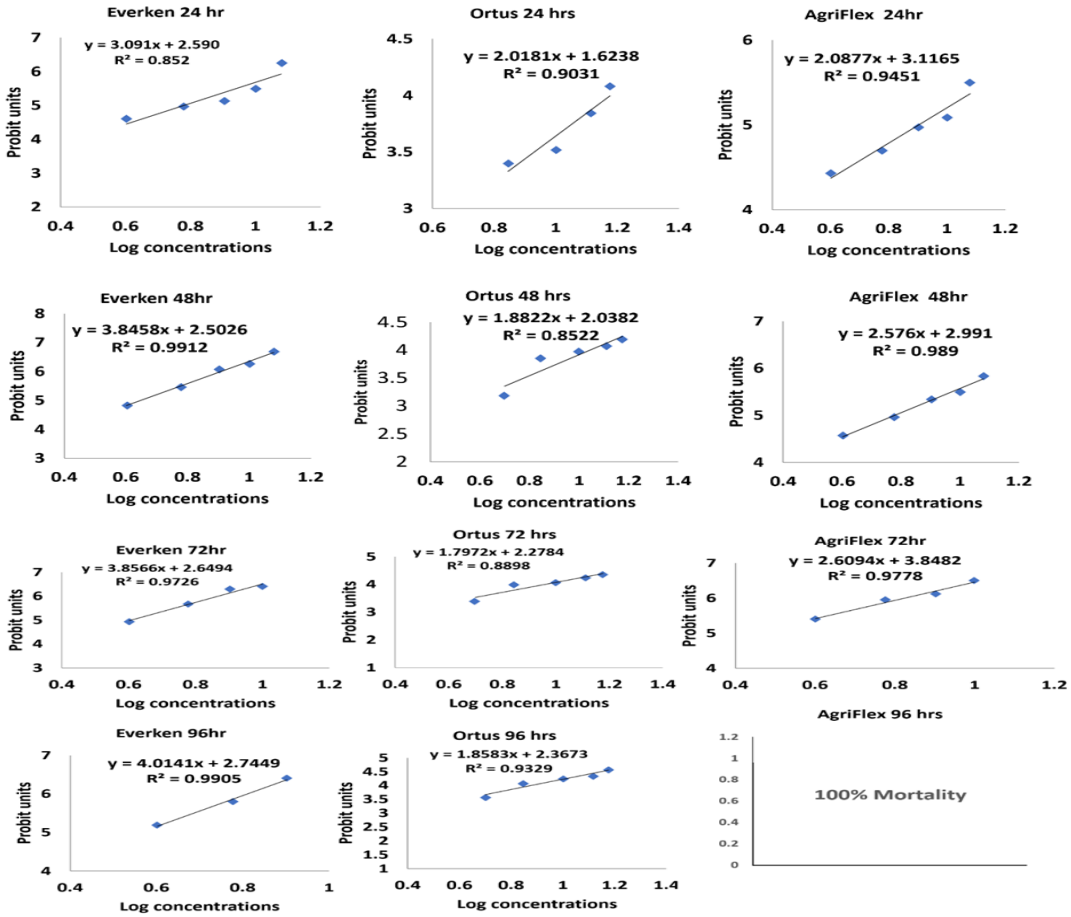


**Figure 1. Probit data (LC-P line data) established from plotting the probit units corresponding to 24,48,72 and 96h mortality percentages of …. versus concentration logarithms of tested pesticides**

**Honey bees mortality**

Honey bee mortality was recorded daily for four days after (24, 48, 72, and 96 hrs) of acaricide applications. Each acaricide was assayed in five concentrations (5, 7, 10, 13, and 15 ppm) and each concentration was replicated five times. Also, control treatment was applied, but without any acaricide applications. Observed data for honey bee mortality was illustrated in (Figure 2). Generally, honey bee mortality is increasing with the increase in acaricide concentration. Data showed that less mortality was detected in control and ortus after 24, 48, 72, and 96 hrs at concentration 5 ranging between 3.3-10.67%. But in other concentrations (conc. 7, 10, 13, and 15), control had the least mortality after all acaricide application times. Whilst everken recorded the highest honey bee mortality after 24 and 48 hrs (95.67%) for all tested concentrations except conc. 5 (30-40%) and conc. 10 (50-60%) after 24 hrs. Where similar high mortality was observed for everken and agriflex. After 72 hrs of acaricide applications, similar mortality percentages were observed at all concentrations (ranging between 76.33-100%) except conc. 5, where agriflex showed the highest mortality compared to all acaricides. Otherwise, 100% mortality occurred on and agriflex at all concentration, followed by everken (ranged between 60.67-100%). While, honey bees mortality percentage did not exceed 40% after 96 hrs of application.

Obtained results of our ongoing research showed that ortus (fenpyroximate) had the least honey bee mortality (less than 30%). Bahreini *et al*., (2022) mentioned that fenpyroximate (pyrazole class) had lower bee mortality after 24 hrs of treatment. Additionally, both compounds could provide effective Varroa control and alternative options for managing Varroa resistance to be included in current IPM practices, and enable sustainable, productive, and healthy honeybee stocks for the future. Also, he mentioned that these compounds with>80% efficacy and safe for honeybees are good candidates for future registration in Canada.

Furthermore, everken (abamectin) had significantly the greatest mortality after 24 and 48 hrs of acaricide application up to 95% mortality. Abamectin acts on insects by interfering with neural and neuromuscular transmission. It acts on a specific type of synapse located only within the brain and is protected by the blood-brain barrier (Hayes and Laws 1990). Also, Sun *et al*. (2013) reported that abamectin had high toxicity ($LC_{50}$ =1.690 µg/ml) against aphids in many studies. Aljedani (2016) declared that abamectin has a negative impact on honeybees, which is a notable influence on the lethal time (LT50), where abamectin was faster than deltamethrin in the honeybee workers' death. Additionally, he mentioned that abamectin impact on cytotoxic midgut cells results in midgut digestive disorders, consequently, the formation of epithelial tissue after digestive cells die during morphological alterations.

After 72 and 96 hrs of chemical applications, agriflex (abamectin+ thiamethoxam) showed higher mortality compared to everken at some tested concentrations and reached 100% mortality after 96 hrs of treatment at all tested concentrations. Kakmand *et al*., (2008) observed that there is damage to the midgut epithelium of honeybees as a consequence of acute exposure to the insecticides malathion, deltamethrin, and thiamethoxam. Also, FAO, (2000) described thiamethoxam as non-toxic to fish, daphnia, and algae, mildly toxic for birds, highly toxic to midges, and acutely toxic for bees. Thiamethoxam is one of the neonicotinoids compounds known to affect honeybees (Iwasa *et al*., 2004; Friol *et al*., 2017; Tavares *et al*., 2015 and 2019). At high levels, neonicotinoids lead to paralysis and death of target and non-target insects by binding to nicotinic acetylcholine receptors (nAChRs) which are expressed in the insect nervous system (Matsuda *et al*. 2001; Goulson, 2013 and Tsvetkov *et al*., 2017) As well, Carvalho *et al*., (2009) found also independent of the form of contamination, thiamethoxam was extremely toxic to bees, causing the death of more than 80% of the specimens after 3 days. Our results suggest using ortus in IPM programs to control harmful pests and bee's pathogens with least effect on *A. mellifera*.

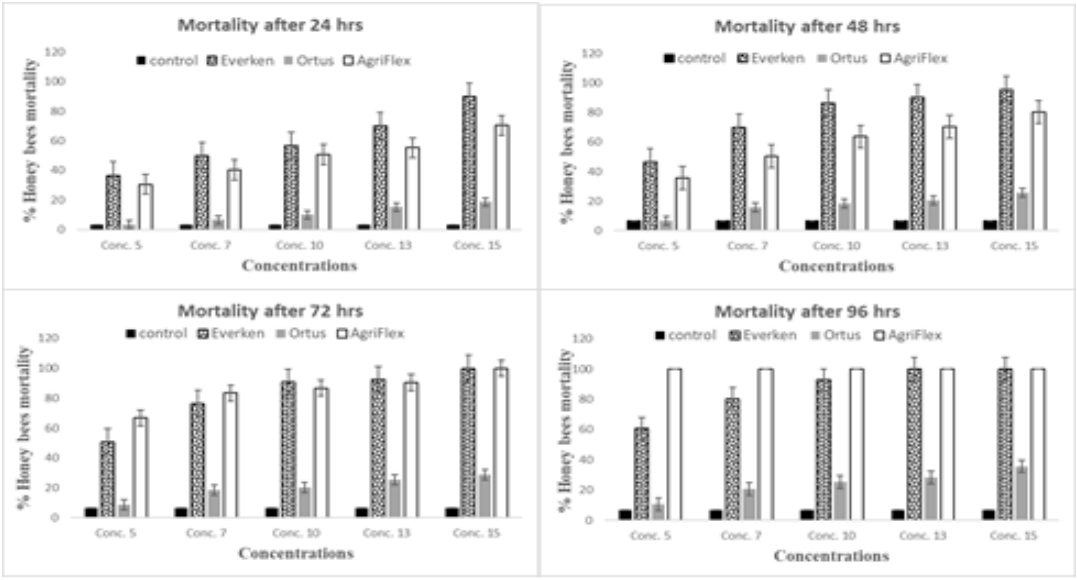


**Figure 2. Honey bee mortality percentages after 24, 48, 72 and 96 hrs of control and acaricide applications at five different concentrations (conc.)**

## CONCLUSION

Through the current results, we can conclude that abamctin and agriflex had high toxicity on honeybees colony health and vitality, especially honeybee workers. On the other hand, ortus could be included in IPM practices, and enable sustainable, productive, and healthy honeybee stocks for the future.

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## دراسة تأثير ثلاثة مبيدات اكاروسية مختلفة علي شغالات نحل العسل تحت الظروف المعملية

**محمود جمعة درويش، علي مصطفي علي و مروة فاروق كامل علي**

**قسم وقاية النبات ـ كلية الزراعة ـ جامعة المنيا**

### الملخص

يعتبر نحل العسل من اكبر ملقحات المحاصيل والنباتات المحلية عالميا. للمبيدات تأثير سلبي وخطير علي نحل العسل ،الملقحات الاخري والكائنات النافعة. نفذت هذه الدراسة لتقييم تأثير ثلاثة مبيدات اكاروسية Ortus 5%SC (fenpyroximate), Everken 1.8% EC (abamectin) and AgriFlex 18.6% SC (abamectin+ thiamethoxam)علي شغالات نحل العسل. تم اختيار هذه المبيدات الاكاروسية بناءا علي الملاحظات الحقلية، حيث استخدمت هذه المبيدات في مكافحة اكاروس العنكبوت الاحمر في حقول البرسيم . تم تعريض مائة من الحشرات الكاملة الحديثة لشغالات نحل العسل لتخفيفات مختلفة من المركبات المرشحة للدراسة (٥، ٧، ١٠، ١٣ و ١٥ جزء في المليون). تم تزويد النحل بالماء ،الغذاء، البروتين الطبيعي ومحلول سكر مع المبيدات الاكاروسية. اما الكنترول لم يتم معاملته بأي مبيدات اكاروسية. تم تسجيل النسبة المئوية للموت بعد ٢٤، ٤٨: ٧٢ و ٩٦ ساعة من المعاملة بالمبيدات الاكاروسية وتم حساب التركيز القاتل ل ٥٠% و ٩٠%. أظهرت النتائج المتحصل عليها ان AgriFlex and everkenعلي سمية معنويا مقارنة ب ortus. حيث كان التركيز القاتل ل ٥٠% لمبيد AgriFlex and everken يتراوح بين ٢,٧٥ -٧,٩٤ جزء في المليون بعد ٢٤، ٤٨، ٧٢ و ٩٦ ساعة من المعاملة بالمبيدات. بينما سجل ortus ٤٧,١ جزء في المليون بعد ٢٤ ساعة. اما معاملة الكنترول كانت لها اقل نسبة موت لنحل العسل مقارنة بمعاملات المبيدات الاكاروسية (٧%) يتبعها ortus (٣٦%). وصلت نسبة الموت ل AgriFlex and everken الي ١٠٠ % بعد ٩٦ من المعاملات. وبالتالي فهذه النتائج تقترح ان ortus يمكن ان يكون ضمن برنامج المكافحة المتكاملة ضد الحشرات الضارة وممرضات نحل العسل وبشكل امن علي الانتاجية المستدامة لمخزون نحل العسل الصحي في المستقبل