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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

THE INFLUENCE OF SOME INSECTICIDES ON THE ABUNDANCE AND FORAGING ACTIVITIES OF BROAD BEAN BEE POLLINATORS IN EGYPT: A CASE STUDY

Mısır'daki Bakla Arı Tozlayıcılarının Bolluğu ve Yiyecek Arama Faaliyetleri Üzerine Bazı İnsektisitlerin Etkisi: Bir Vaka Çalışması

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ABSTRACT

Insect pollinators provide many essential ecosystem services including pollination, and many others. However, pollinating insects are currently facing potential threats on an unprecedented scale with many species facing decline. Honeybee *Apis mellifera* comprise nearly 68% of those affected insect pollinators. Irrational Insecticides application, with special reference to neonicotinoides group is one of the main causes of this decline. The main objective of the current study is to investigate the impact of some insecticides application on the activity of broad bean flower-visiting bees. Tested inseticides were thiamethoxam, acetamiprid, thiacloprid (neonicotinoids), spinosyns A and D, beside some organophosphates. Changes in the daily activity of bees visiting broad bean flowers following insecticide application was recorded and compared to their activity before application, throughout until the end of blooming season.

Keywords: **Bee Decline, Colony Loss, Foraging, Neonicotinoides, Pollination**

ÖZ

Böcek tozlayıcıları, tozlaşma ve diğerleri dahil olmak üzere birçok temel ekosistem hizmeti sağlar. Bununla birlikte, tozlaşan böcekler şu anda birçok türün düşüşle karşı karşıya kalmasıyla eşi görülmemiş bir ölçekte potansiyel tehditlerle karşı karşıya. Bal arısı Apis mellifera, etkilenen böcek tozlaştırıcılarının yaklaşık %68'ini oluşturur. Neonicotinoides grubuna özel atıfta bulunulan irrasyonel İnsektisit uygulaması bu düşüşün ana nedenlerinden biridir. Mevcut çalışmanın temel amacı, bazı insektisit uygulamalarının bakla çiçeğini ziyaret eden arıların aktivitesi üzerindeki etkisini araştırmaktır. Test edilen insektisitler, bazı organofosfatların yanında tiyametoksam, asetamiprid, tiakloprid (neonikotinoidler), spinosinler A ve D. Bakla çiçeklerini insektisit uygulaması sonrasında ziyaret eden arıların günlük aktivitelerinde meydana gelen değişimler kayıt altına alınmış ve çiçeklenme döneminin sonuna kadar uygulama öncesindeki aktiviteleri ile karşılaştırılmıştır.

Anahtar Kelimeler: Arı Düşüşü, Koloni Kaybı, Toplayıcılık, Neonicotinoides, Tozlaşma

GENİŞLETİLMİŞ ÖZET

Amaç: Bu çalışmanın temel amacı, Mısır'ın İsmailia kentinde çiçek açma mevsimlerinde baklayı ziyaret eden bazı arı türlerinin (*Apis mellifera, Andrena ovatula, Colletes lacunatus, Xylocopa aestuans*) bazı insektisit uygulamalarının aktiviteleri üzerindeki etkisine ışık tutmaktır.

Giriş: Böcek tozlayıcıları, tozlaşma ve diğerleri dahil olmak üzere birçok temel ekosistem hizmeti sağlar. Bununla birlikte, tozlaşan böcekler şu anda birçok türün düşüşle karşı karşıya kalmasıyla eşi görülmemiş bir ölçekte potansiyel tehditlerle karşı karşıya. Bal arısı *Apis mellifera*, etkilenen böcek tozlaştırıcılarının yaklaşık %68'ini oluşturur. Neonikotinoid insektisitlerin, yani thiamethoxam ve thiacloprid'in öldürücü olmayan konsantrasyonlarının arıların yiyecek arama davranışı üzerindeki etkisi, laboratuvar koşullarında bazı çalışmalarda ele alındı, ancak saha çalışmaları oldukça yetersizdir.

Gereç ve yöntem: 2019 Çalışma, İsmailiye Valiliği'ndeki Süveyş Kanalı Üniversitesi'nin deney çiftliğinde gerçekleştirildi. Pestisit listesine neonikotinoidler Actara (25 WG - thiamethoxam), Clipper (%20 SL – asetamiprid (ve Calypso (%48 - thiacloprid) dahildir. Diğer insektisitler Radiant (%12 SC - spinosyns A ve D, *Saccharopolyspora spinosa*) ve malathion (%57 - organofosfat insektisit)

Bulgular ve tartışma: Sonuçlar, bakla çiçeklerini çeken farklı böcek türlerinin oluşumunun ve bolluğunun insektisit tedavisinden önce ve sonra önemli ölçüde değiştiğini göstermiştir. Ayrıca, düşüşün çiçeklenmenin sonuna kadar devam ettiği oldukça açıktı. Ortalama bal arısı sayısı tedaviden önce 8.57 arı/gün ile karşılaştırıldığında tedaviden önce 38.70 arı/gün olmuştur. insektisit tedavisinden sonra kaydedilen *C. lacunatus*, uygulamadan sonraki 10 günlük süre boyunca yalnızca bir kez kaydedilmiştir. Thiamethoxam, ana böcek tozlaştırıcısı olan bal arısı *A. mellifera* üzerinde en yüksek olumsuz etkiye neden oldu ve tedavi edilmeyen parsellerde 42,2 arı/gün ile karşılaştırıldığında, işlenmiş parsellerde ortalama 1,4 arı/gün olmuştur. Thiacloprid, faydalı böceklere karşı zararlı olması açısından Thiamethoxam'dan sonra ikinci sırada yer almakta, bunu organofosfatlar, spinosinler A ve D ve asetamiprid izlemektedir.

Sonuç: Bu çalışma, bal arısı ve diğer arı tozlayıcılarının Mısır'daki bazı neonikotinoidlere doğrudan maruz kalmasının etkisini ele alan ilk çalışmadır. Dolayısıyla, bu bulgu, umut verici bir şekilde, iyi tarım uygulamalarının tanıtılmasına ve uyarlanmasına ve tozlayıcıların çeşitliliğinin korunmasına ve bakla ve diğer mahsullerin iyi tozlaşma hizmetlerinin sağlanmasına yardımcı olacak entegre haşere yönetimi programlarının uygulanmasına yol açacaktır. Halkın ve karar vericilerin farkındalığını artırmak, arıların böcek öldürücüler gibi çevresel stres faktörlerini hafifletmesine yardımcı olabilir.

INTRODUCTION

Insect pollinators provide copies essential ecosystem service, including pollination and many others (Gill and Raine 2014). The economic contribution of insect pollinators to crop production globally is estimated to be €153 Billion, this equates to 9.5% of agricultural production (Gallai et al. 2009).

Bees, including honeybees and solitary bees, are generally considered the most important pollinators, accounting for about 80% of all flowering plants including most of the agricultural crops, confirming their major role in ecosystems (Goulson 2003).

Pollinating insects are facing potential threats on an unprecedented scale and are in decline, at worldwide level (Ollerton 2017). The decline of pollinating species, which has grown over the recent decades, may lead to a parallel decrease of plant species (Potts et al. 2010). Eventually this decline might result in great economic losses, human food crisis and loss of natural biodiversity (Allen-Wardell et al. 1998, Nellemann et al. 2009).

Broad bean is one of the strategic crops for Egyptian agriculture and cross-pollinations are crucial for good yield production (Bishnoi et al. 2012). The total cultivated area of broad bean in Egypt in 2019 was 40298 ha which produce about 139303 tones and the crop is bee-dependent (FAO 2019). The diversity of bee visiting broad bean in Egypt has been addressed in several studies (El Berry et al. 1974, Ibrahim 1979) but little was known about the current situation of its diversity since the decline of some bees was proved in terms of species richness and abundance (Shebl and Farag 2015).

Residues of the neonicotinoids insecticides were monitored in pollen and bees collected across the Nile Delta (Codling et al. 2018). Blacquiére et al. (20120) have performed some studies to evaluate the adverse effect of neonicotinoids insecticides on the foraging performance and larval development of some managed wild bees such as *Bombus impatiens*, *Osmia lignaria* and *Megachile rotudanta*. Nonetheless, there is no sufficient studies on the impact of neonicotinoids insecticides on honeybees in Egypt despite the extensive use of these insecticides for years. So the current study aims to evaluate the impact of some insecticides, with special reference to the neonicotinoids group on broad bean bee-pollinators in the Egyptian agroecosystem.

MATERIAL AND METHODS

Field Experiment:

Field study was conducted during the flowering seasons of broad bean, *Vicia faba*, variety Giza 843, from February to March 2019, at the experimental farm of Suez Canal University at Ismailia Governorate in North-Eastern Egypt (30°37'15"N 32°15'41"E). The location climate is characterized by mild winter with light rains and hot dry summer with some humidity. The soil texture of experimental site was sandy soil (94.5% sand, 2.5% silt and 3.0% clay) with pH of 7.8.

Insecticides used:

The insecticides used in the present study were Actara (25% WG - thiamethoxam), Clipper (20% SL - acetamiprid), Calypso (48% - thiacloprid), Radiant (12% SC - spinosyns A and D, *Saccharopolyspora spinosa*) and malathion (57%- organophosphate insecticide). All pesticides were purchased from the local market at Ismailia city.

These insecticides are widely used for controlling several pests on different crops such as aphids, whitefly and others. Solutions of all tested compounds were prepared in distilled water at the recommended field rate (FR) (Table 1). The tested concentrations of all tested insecticides in the present study were prepared one hour prior to conducting the experiments.

Table (1): List of commercial formulations, active ingredient and field rate of tested insecticides

Insecticides	Active ingredient	Recommended dose (FR)	Active ingredient (a.i.)
Actara 25 WG	Thiamethoxam	0.2 g/l	50 mg/l
Radiant 12% SC	Spinosyns A and D	0.5 ml/l	60 mg/l
Clipper 20% SL	Acetamiprid	0.25 ml/l	50 mg/l
Calypso 48% SL	Thiacloprid	0.3 ml/l	144 mg/l
Malathion 57% EC	Organophosphate	1.5 ml/l	855 mg/l

Field Bioassay:

Impact of insecticide application on the foraging activity of bee foragers:

Insecticides were applied using a backpack sprayer in a broadcast application on broad bean plants when the stage was at 70-100 days to flowering. The insecticides treatment was made of Five treatments with three replicate plots each (7 × 7 m). Each replicate was made of 10 rows of broad bean plants. Control treatment was also conducted with the same protocol, applying distilled water without any insecticide.

Population and foraging activity of different species of Hymenopterous bee pollinators visiting broad bean flowers were recorded. Regular counts of the insects above the flowers of broad bean plants were performed 15 days before insecticide application and again, 1, 3, 5, 7 and 10 days post treatment. Three counts a day were taken; *e.g.* morning at 10-12 am., early afternoon 12-2 pm, and 2-4 pm, with each count lasting about 5 minutes. During direct observation, the number of individuals for each species was counted.

Specimens of the bee pollinators were collected and preserved in the laboratory for identification based on reference collection in the Department of Plant Protection, Faculty of Agriculture, Suez Canal University.

Statistical analysis

All obtained data were statistically analyzed using ANOVA. When F-test was significant, means were separated using Tukey's test at the 0.05 level of significance. SPSS and CoStat software were used.

RESULTS

A total of 5 bee species; three long tongued bees and two short tongued bees were recorded on flowers of untreated broad bean plants. The long tongued bee species were *Apis mellifera* L. and *Xylocopa aestuans* Spinola of Apidae and *Chalicodoma siculum.* Meanwhile, Rossi of Megachilidae was recorded only in the early season. The short tongued bee species recorded were *Andrena ovatula* Kirby of Andrenidae and *Colletes lacunatus* Dours Colletidae.

Abundance of main bee pollinators on broad bean flowers before and post insecticide application

Occurrence and abundance of different bee species attracted to broad bean flowers significantly varied before and after insecticides treatment. Mean number of honeybees was 38.70 bees/day before insecticide treatment compared to 8.57 bees/day post treatment. Likewise, *A. ovatula* count was 8.53 bees/day before treatment compared to 2.52 bees/day post treatment. Meanwhile *X. aestuans* count was 27.67 bees/day before treatment, and totally disappeared post insecticide treatment (Fig. 1).

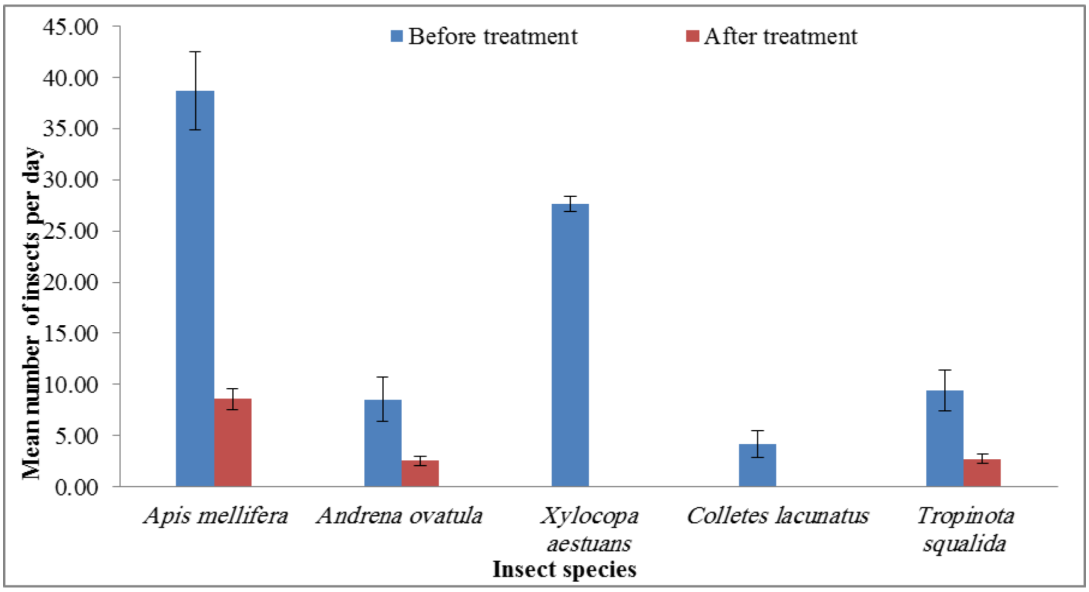


Fig. (1): Impact of insecticide applications on the activity of bee pollinators and *Tropinota squalida* on broad bean flowers

Seasonal activity of main bee pollinators on broad bean flowers before and post insecticide application:

Significant decrease in the activity of all bees visiting broad bean flowers was recorded post insecticide application compared to their activity before application, and that decline continued until the end of the blooming season. The decrease was more pronounced in the case of honeybees *A. mellifera*, which was significantly more active prior to insecticide treatment compared to post treatment. The activity of *A. ovatula* decreased gradually after insecticide application, while *C. lacunatus* was recorded only once over the period of 10 days post application. It is worth noting that no individual of *X. aestuans* was recorded on broad bean flowers after insecticide treatment (Fig. 2). Counts of *Tropinota squalida*, a non pollinator nor a flower visitor was performed for comparison purposes only.

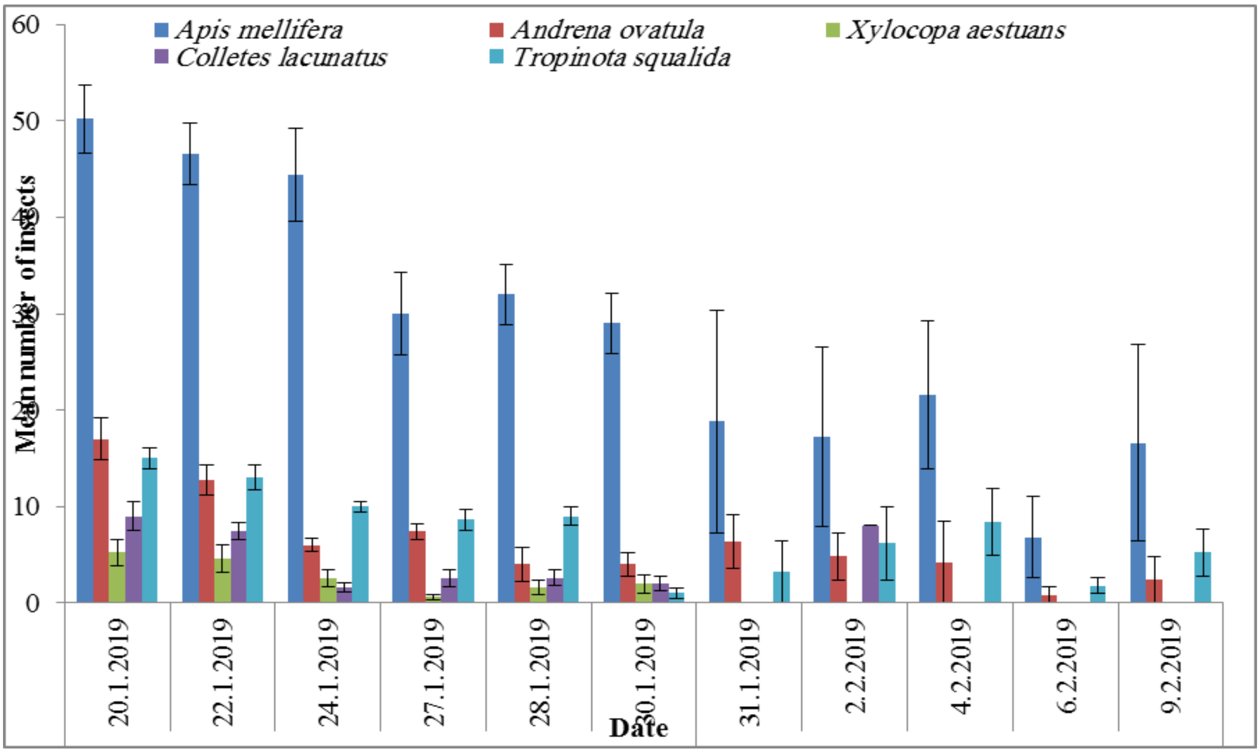


Fig (2): Activity of main insect visitors on broad bean flowers, before and post insecticide application, Ismailia 2019

Daily activity of main bee pollinators and *Tropinota squalida* on broad bean flowers before and post insecticide application:

Daily activity of insects visiting broad bean flowers varied greatly, showing significant decrease after insecticides application compared to their activity before application (Fig. 3). Highest activity of all observed insects, especially honeybee *A. mellifera* was recorded at 12:00-2:00 p.m. before insecticides treatment, meanwhile their activity has significantly decreased after the treatment. The mean averages of *A. mellifera*, *A. ovatula* and *Tropinota squalida* were 14.8, 3.07 and 3.13 individual/interval before treatment compared to 6, 1.13 and 1.77, respectively post treatment.

Impact of tested insecticides on the number and activity of broad bean insect visitor in Ismalia g**overnorate:**

Figure (4) indicated that all tested insecticides clearly led to significant decline in the number of insects visiting broad bean flowers. Thiamethoxam caused the highest negative impact on the main insect pollinator, *Apis mellifera*, with an average of 1.4 bees/day in treated plots compared to 42.2 bees/day in untreated plots. Thiacloprid came second in terms of its harmful effect against the beneficial insects, since it caused a cut down of attracted honey bees to broad bean flowers, though this reduction was less than that caused by Thiamethoxam, accounting for 2.6 bees/day. Meanwhile, the decline in the number of insects visits were even less for organophosphate, spinosyns A and D and acetamiprid with averages of 3.6, 35.8 and 37.8 bees/day, respectively. Similar trend of visits decline was also recorded for the other insect pollinators as well as on *Tropinota squalida*.

As shown in Figure (5) the deleterious impacts of the tested insecticides on the activity of the main insect visitors of broad bean flowers were observed throughout the three various time intervals per day, with no significant differences between the different intervals.

Data presented in Figure (6) indicates that the harmful effects of all tested insecticides on the activity patterns of the main insect visitors of broad bean flowers were recorded shortly one day after application. These effects had increased gradually with the time till the 7th day post application, then decreased again in the 10th day of application except in case of thiacloprid and thiamethoxam, where the impact continued without significant decrease.

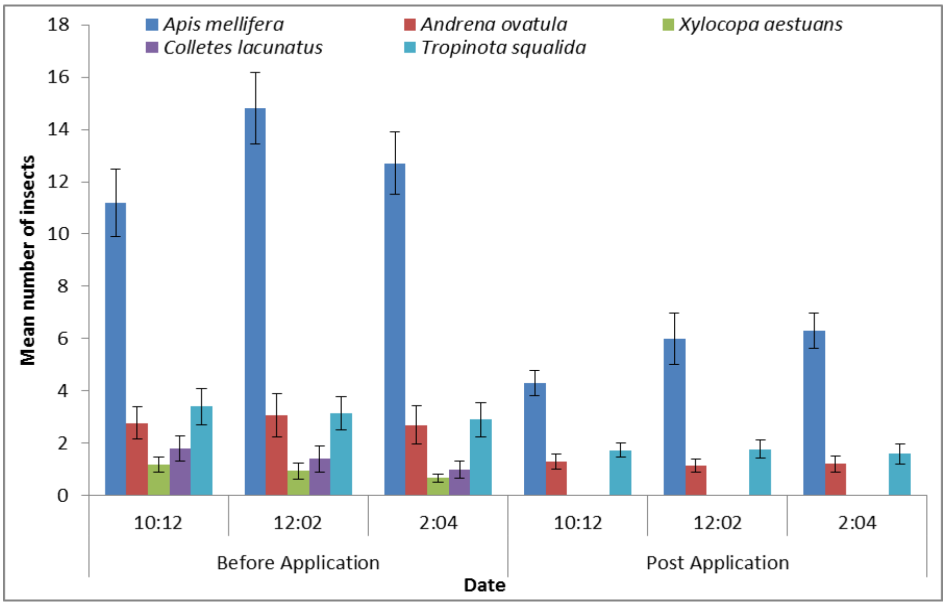


Fig. (3): Daily activity at three collected times of bee pollinators on broad bean before and after insecticide application, Ismailia 2019

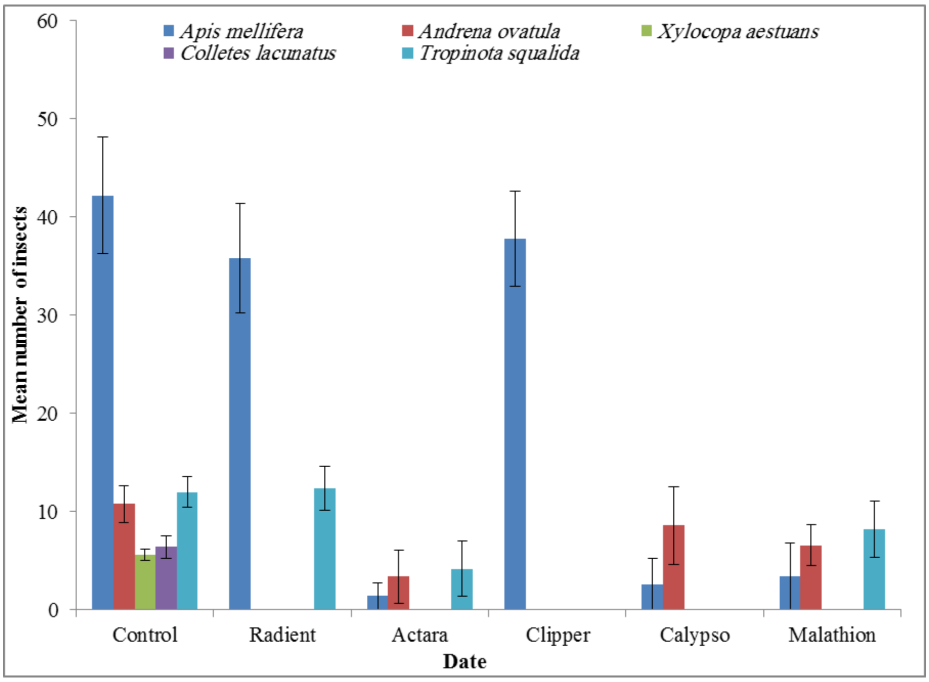


Fig. (4): Effect of insecticide applications on the activity of bee pollinators on broad bean flowers, Ismailia 2019

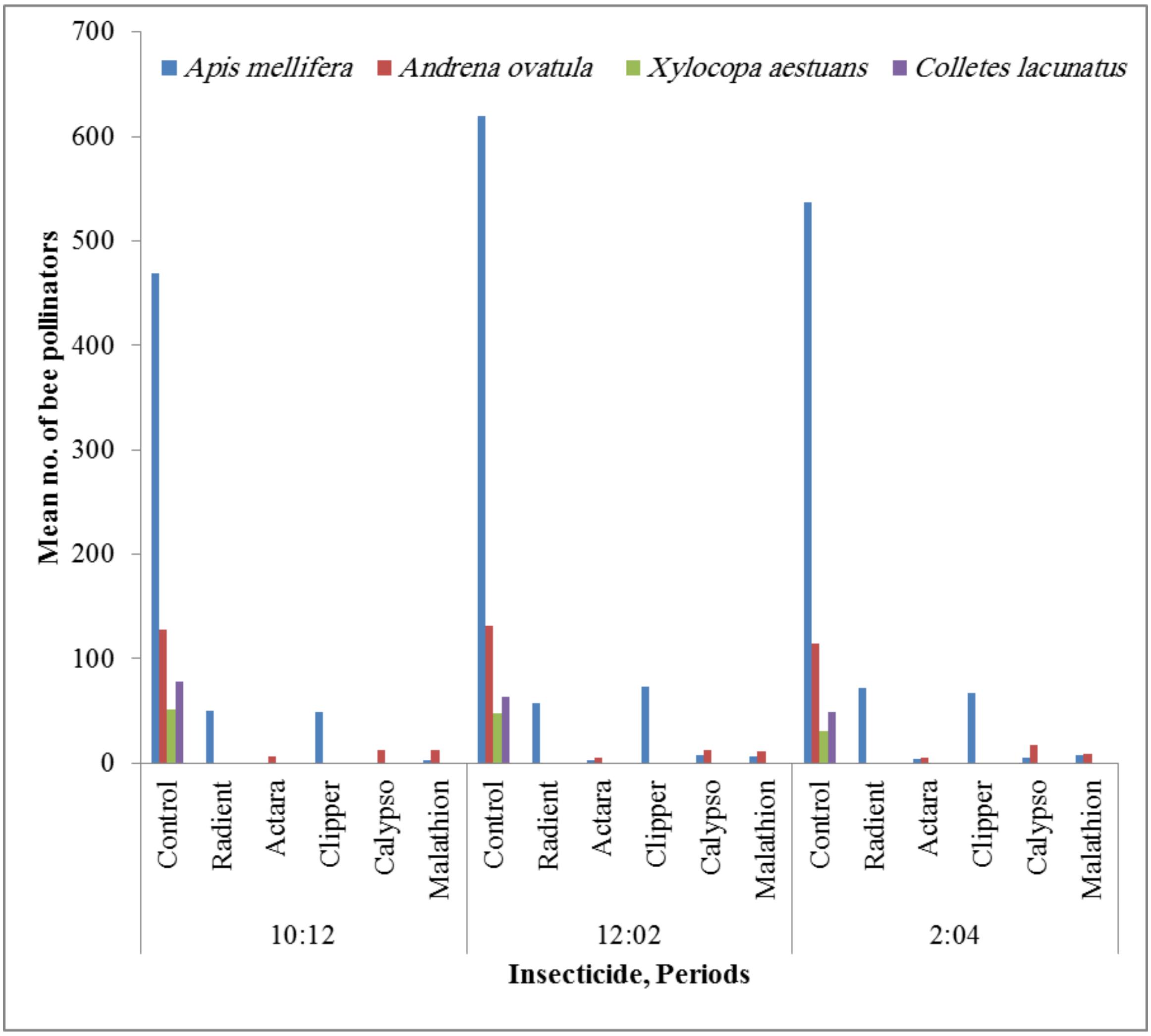
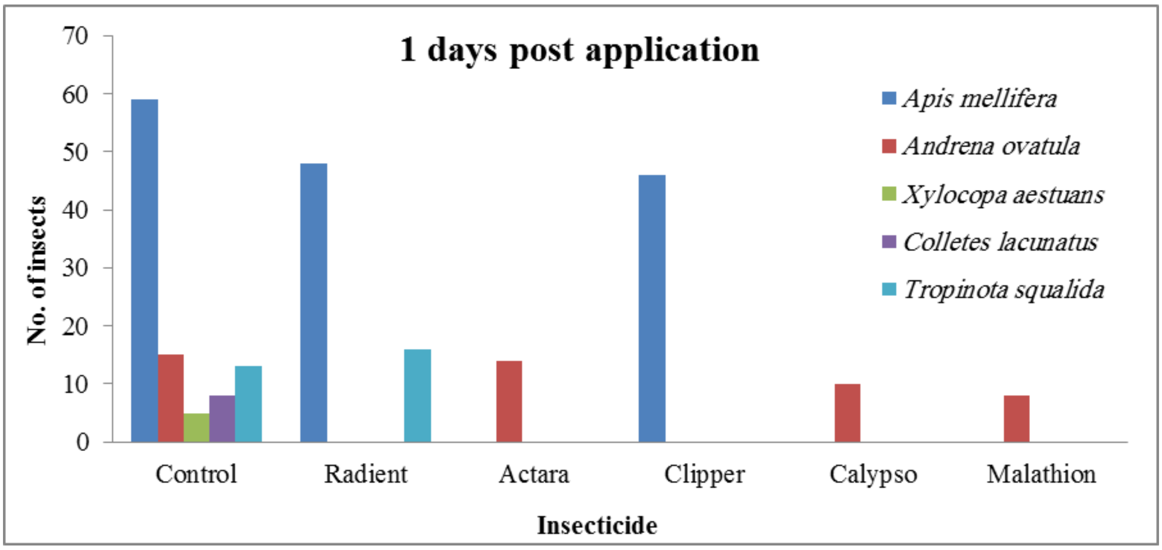
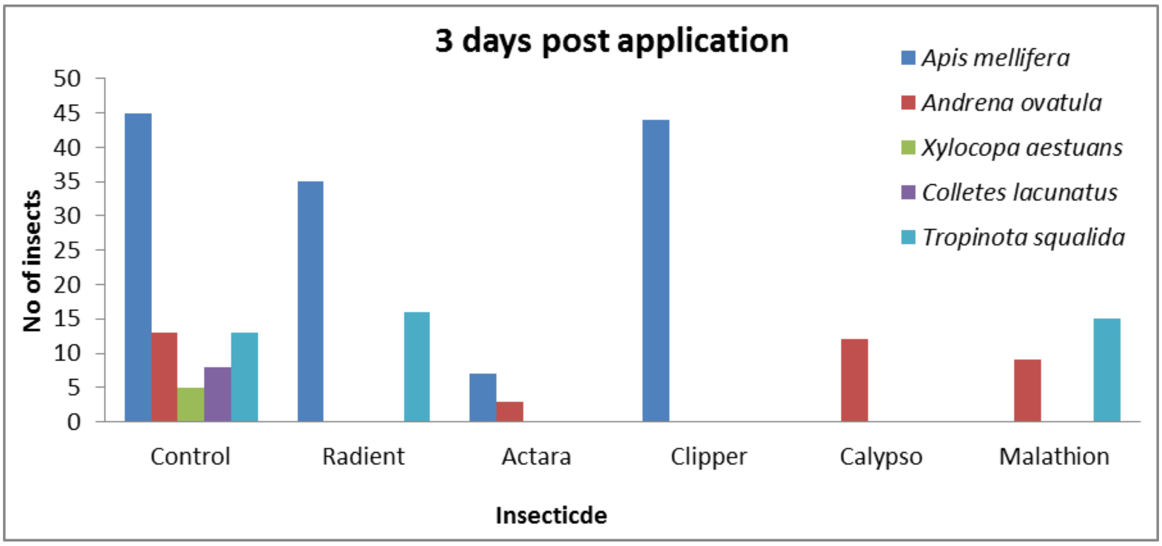


Fig. (5): The effect of insecticide applications on the daily activity of bee pollinators on broad bean flowers, Ismailia 2019


1 days post application
No. of insects
70
60
50
40
30
20
10
0
Apis mellifera
Andrena ovatula
Xylocopa aestuans
Colletes lacunatus
Tropinota squalida
Control
Radient
Actara
Clipper
Calypso
Malathion
Insecticide


3 days post application
No of insects
50
45
40
35
30
25
20
15
10
5
0
Apis mellifera
Andrena ovatula
Xylocopa aestuans
Colletes lacunatus
Tropinota squalida
Control
Radient
Actara
Clipper
Calypso
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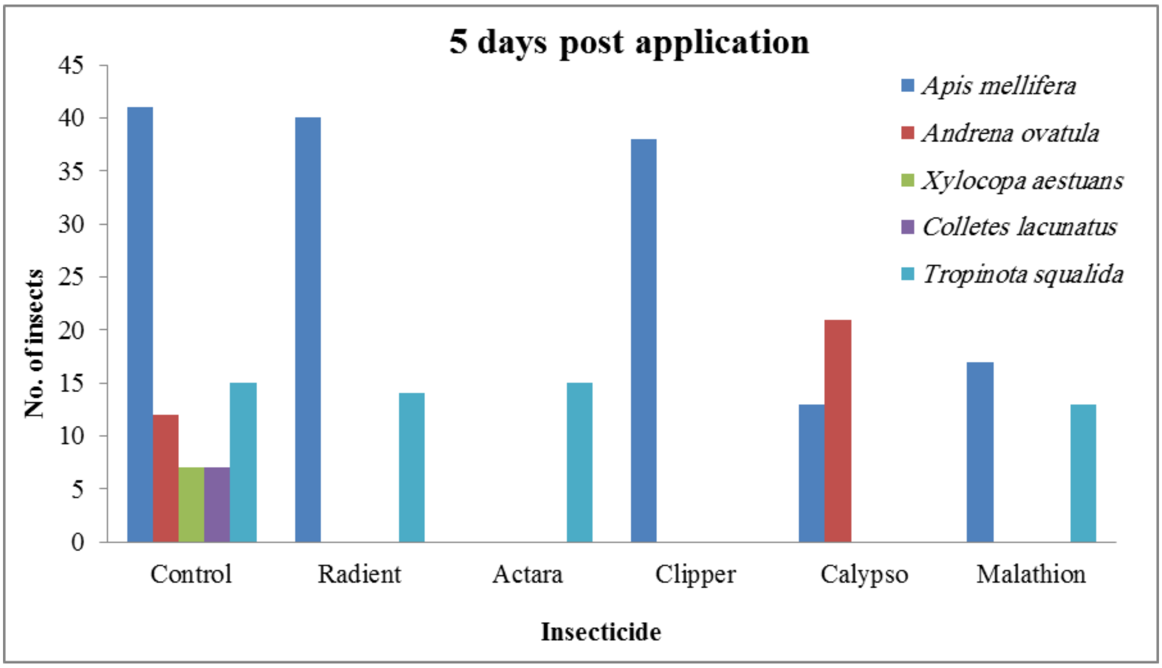
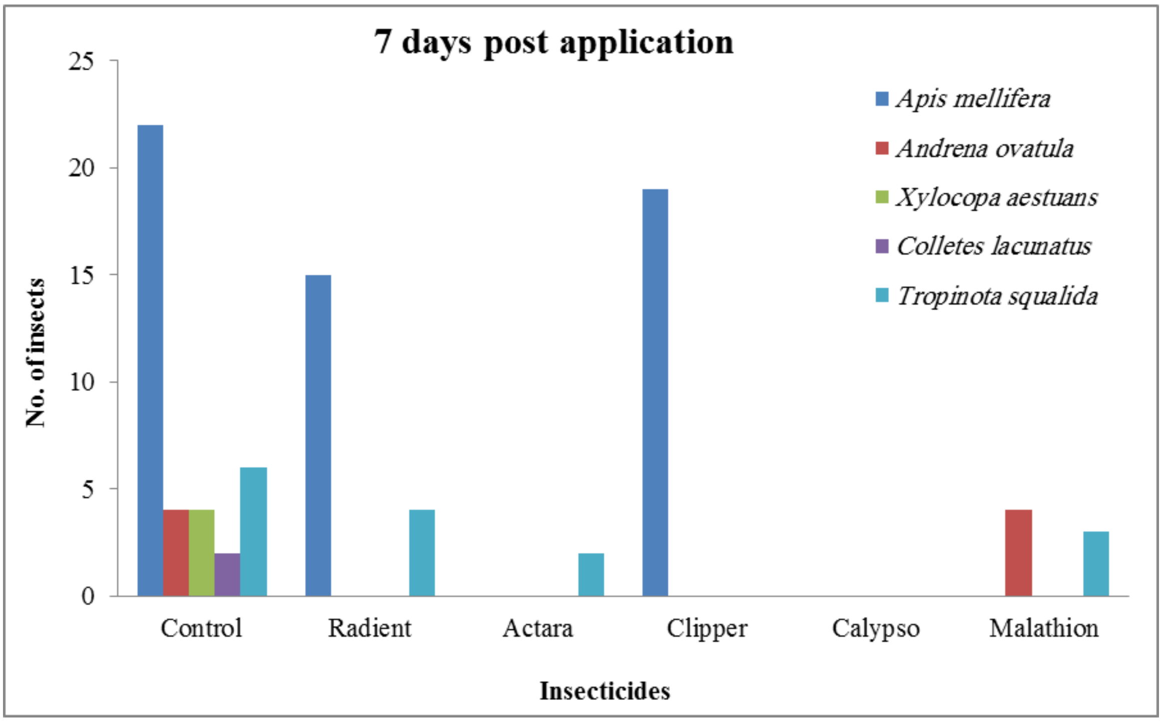

5 days post application
No. of insects
45
40
35
30
25
20
15
10
5
0
Apis mellifera
Andrena ovatula
Xylocopa aestuans
Colletes lacunatus
Tropinota squalida
Control
Radient
Actara
Clipper
Calypso
Malathion
Insecticide


7 days post application
No. of insects
25
20
15
10
5
0
Apis mellifera
Andrena ovatula
Xylocopa aestuans
Colletes lacunatus
Tropinota squalida
Control
Radient
Actara
Clipper
Calypso
Malathion
Insecticides

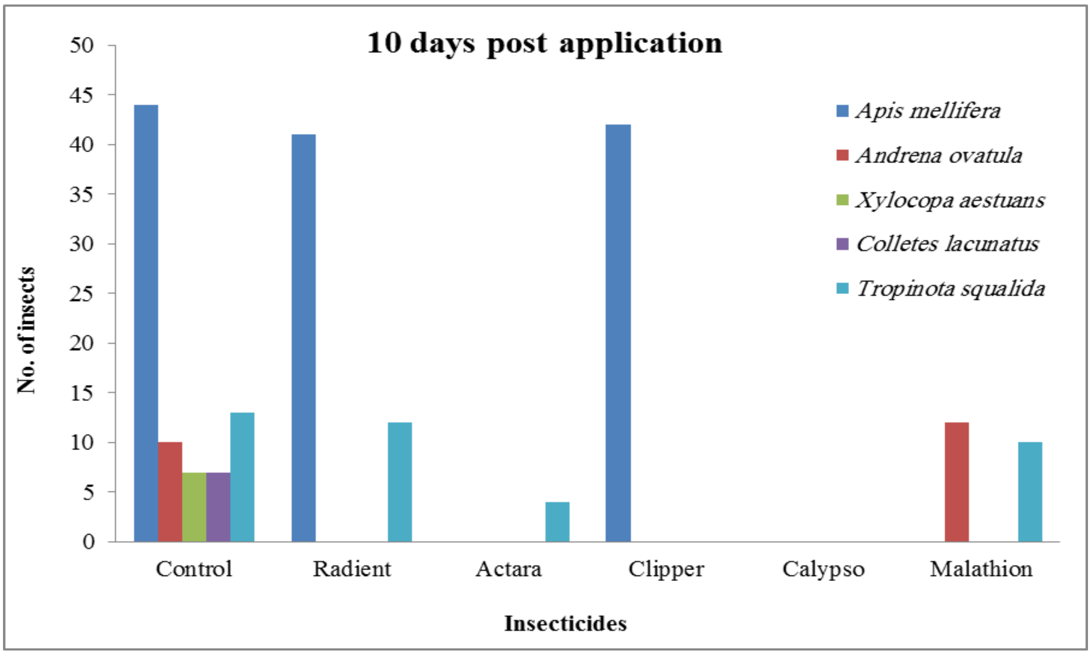


Fig (6): Number of bee pollinators visited broad bean flowers 1, 3, 5, 7 and 10 days post insecticide application, Ismailia 2019.

DISCUSSION

In Egypt, several studies have been addressing levels of pesticides residues in several honeybees colonies products such as honey and pollens (Al Naggar et al. 2015, Abdallah et al. 2017, Codling et al. 2018, El-Hady et al. 2019, El-Nahhal 2020). However, the impact of direct exposure of forager bees to insecticides was not fully studied. The present study is an attempt to fill this gap, trying to figure out the impact inflicted on social and solitary bees forager bees after exposure to neonicotinoids insecticides under field conditions.

The present findings revealed a sharp decline in the abundance of four tested bee species before and after direct exposure to pesticides. Both groups of short and long tongued bees visiting broad bean were affected after direct exposure, and the number of foragers were much less in *A. ovatula*, *A. mellifera*, *C. lacunatus* and *X. aestuans*.

The present findings shed some light on the importance of direct exposure of non-*Apis* species to insecticides, specially thiacloprid and thiamethoxam that negatively affected the pollination services provided by these species. (Shebl and Farag 2015) have also recorded some significant decline affecting some potential bee pollinators, with special reference to species of genu *Anthophora* due to insecticides use and some intensive agricultural practices.

Strobl et al. (2021) suggested that the mortality is not necessarily the only impacts since colony individual survival and fertility might also be affected by exposure to insecticides. Low doses of thiamethoxam and thiacloprid reduced colony size, queen production and bumblebees worker survival which could have a negative influence on their foraging behavior (Mommaerts et al. 2010, Giri et al. 2018).

Similarly, Giri et al., 2018 reported that thiamethoxam had a negative impact on foraging activity and mortality of worker honeybees 3-4 days post spraying in field conditions. Williamson et al., (2014) have also reported that the bee exposure to thiamethoxam was combined with more time grooming and the exposure to sublethal for 24h has a subtle influence on bee behavior. However, some further work should be carried out to fully ascertain the impact of neonicotinoids on biological and physiological parameters of non *Apis* bees, especially managed species such as leafcutting bees and mason bees.

Conclusion
The major emphases of the present study have focused on how neonicotinoids and some other insecticides would influence pollinators decline, that lead to weaken the role they play in pollination services, agriculture production, biodiversity and other major ecosystem services. Raising awareness of the public and decision makers is one major goal of the present study as the first step to enforce special measures to promote rational use of pesticides in Egypt, and to limit and / or ban the use of neonicotinoids insecticides one of the most serious environmental stressors on bees.

Competing interests: The authors declare that they have no competing interests

Authors' contributions: DE, MS, MT and MO planed and designed the work. DE and MS did field work and collected data. MO and MT analyzed the data and writing the manuscript. All authors read and approved the final manuscript

Source of Finance: Not applicable because there is no funding source for this study

Ethical issue: Not applicable because this study on bees, animals or humans.

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