Article

Effects of two herbicides on healthy and *Nosema* infected honey bee workers

Hossam F. Abou-Shaara, **Mohamed A. Abuzeid**

Department of Plant Protection, Faculty of Agriculture, Damanhour University, Damanhour, 22516, Egypt

E-mail: hossam.farag@agr.dmu.edu.eg

Received 7 March 2018; *Accepted 21 March 2018*; *Published 1 June 2018*

Abstract

Herbicides are commonly used by farmers to get rid of deleterious weeds. There is a common believe by beekeepers that herbicides have no harmful impacts on bee colonies. In this study, the effects of tow glyphosate herbicides (herbazed and glypho-up) on some parameters of honey bee workers were investigated. Three concentrations of each herbicide were tested. Concerning survival, the highest concentrations of herbazed and glypho-up showed significant impacts on caged bees than the control group (bees supplied with sugar syrup only) while the low concentrations showed the vice versa. The highest concentration of glypho-up caused the highest reduction in survival of caged bees. The tested herbicides showed no significant impacts on the navigation ability of bee workers than the control group. The proboscis extension reflex test was used to evaluate the learning ability of bee workers. The results showed insignificant impacts of the tested herbicides on the learning ability of bee workers than the control group. The survival was low when *Nosema* infected bees were exposed to the high concentration of glypho-up unlike *Nosema* infected bees and healthy ones. This study has a significant contribution towards understanding the potential impacts of herbicides on honey bees.

Keywords *Apis mellifera*; herbicides; survival; navigation; learning; *Nosema*.

Arthropods
ISSN 2224-4255
URL: http://www.iaees.org/publications/journals/arthropods/online-version.asp
RSS: http://www.iaees.org/publications/journals/arthropods/rss.xml
E-mail: arthropods@iaees.org
Editor-in-Chief: WenJun Zhang
Publisher: International Academy of Ecology and Environmental Sciences

1 Introduction

Honey bees (*Apis mellifera)* have many benefits to agriculture, economy, and rural sector in many countries. The high importance of honey bees as pollinator for many field and fruit crops is well known (Southwick and Southwick, 1992; Kamel et al., 2013; Klatt et al., 2014). Unfortunately, many hazards can impact honey bees passively and may cause the loss of bee colonies. In the last few years, a common phenomenon of losing honey bee colonies worldwide has been observed (Zhang, 2018). This phenomenon is known as colony collapse disorder (CCD). The main reason behind CCD is still unknown. However, several factors have been suggested as the potential causative reasons (Neumann and Carreck, 2010). Agricultural pesticides have been

accused with contributing to cause CCD (vanEngelsdorp et al., 2009; Zhang et al., 2011; Sanchez-Bayo and Goka, 2014). In fact, there are many environmental pollutants that could cause harmful impacts on honey bees (Johnson, 2015). Pesticides are considered the main pollutants for bee colonies (Zhang, 2018). For example, 121 different pesticides were detected within analyzed samples of wax, pollen, and bee (Mullin et al., 2010). At least one systemic pesticide was detected in about 60% of the wax and pollen samples while both acaricides fluvalinate and coumaphos, and fungicide chlorothalonil were found in over 47% of the samples. Understanding the effects of pesticides on honey bees are very important to help saving bee colonies.

Concerning herbicides, this specific group of pesticides is widely used in the agricultural lands to get rid of harmful weeds. Most beekeepers and farmers consider herbicides as safe chemicals to honey bees because the main target of herbicides is plants and not animals or insects. However, few studies have shown the adverse impacts of herbicides on honey bees including learning ability and navigation (Morton et al., 1972; Moffett and Morton, 1975; Herbert et al., 2014; Balbuena et al., 2015). These studies encourage performing additional investigations to understand the impacts of different herbicides on honey bees and potential interaction with bee diseases. Especially, very few investigations have been done on herbicides.

Pesticides can reach to honey bees by the contamination of nectar, pollen or water (Johnson, 2015). Residues of some pesticides have been detected in nectar and pollen of some plants (Barker et al., 1980; Dively and Kamel, 2012; Pohorecka et al., 2012). Herbicides could reach to honey bees directly during field application or indirectly during gathering contaminated water or water drained from a spray tank (Moffett and Morton, 1975). On the other side, many internal and external parasites can impact honey bees. *Nosema* spp. is considered as very dangerous internal parasite to bee health. *Nosema* spp. infected bees suffer from many problems including: less developed hypopharyngeal glands (Wang and Moeller, 1971), digestion problems (García-Palencia et al., 2010), and low flight ability (Kralj and Fuchs, 2010). Not only healthy bees but also infected ones could be impacted by herbicides. Especially, interactions between pesticides and *Nosema* spp. cause high negative impacts of on honey bees (Vidau et al., 2011; Doublet et al., 2015). The potential effects of herbicides on *Nosema* spp. infected bees are not well known.

In this study, both laboratory and field experiments were performed to investigate the effects of two herbicides on honey bees. Three concentrations of each herbicide were tested. In the laboratory, survival of bee workers, learning ability, and survival of *Nosema* spp. infected bees were studied while the effects of the tested herbicides on the navigation of forager bees were investigated in the field. The possible impacts of herbicides on bee workers were also presented in light of the obtained results.

2 Materials and Methods

Hybrids of Carniolan honey bee workers collected from an apiary at Damanhour city, Egypt were used in the following experiments. The first experiment was conducted in August 2016 while the other experiments were done during spring 2017.

2.1 Survival

Two herbicides, herbazed48% and glypho-up 41%, the active ingredient glyphosate isopropyl ammonium, were tested. Each herbicide was represented by three concentrations as; 0.5ml/50ml, 1ml/50ml, 1.5ml/50ml for herbazed; 0.7ml/50ml, 1.2 ml/50ml, 1.7 ml/50 ml for glypho-up. These concentrations are denoted as; herbazed1, herbazed2, and herbazed3, and glypho-up1, glypho-up2, and glypho-up3 for the three concentrations, respectively. Sugar syrup (2 sugar: 1 water, w/w) was used to prepare the treatments. A total of six treatments and one control group (sugar syrup only) were used. Bee workers from healthy colonies were collected from the lateral combs (forager bees). The bees were narcotized using low temperature (a refrigerator) for few minutes prior to place them in the cages. The cages were made using petri dishes (length 10 cm and

diameter 8.7 cm) following the method of Abou-Shaara et al. (2017). A small piece of beeswax (5X3 cm) was placed in each cage. Each treatment was represented by 3 cages, each having 30 bees, with a total of 90 bee workers. The bees were fed on sugar syrup only or the treatments over 12 days at room temperature (about 30°C). The daily number of dead bees in each cage was recorded.

2.2 Impacts on navigation

Honey bees have high precision in memorizing locations and avoiding locations depleted of food (Brown and Demas, 1994). It is practically known that forager bees can remember the location of their hives up to three days. Firstly, the opening of a beehive with healthy bees was closed by a small wooden piece. Then, forager bees were caught in front of this beehive and placed in plastic perforated jars (15 bees per jar per treatment, from which only 10 bees were included in the study after excluding dead or weak bees). The bees were marked prior the insertion into the jars using a marker pen. A small cotton piece saturated with one of the treatments (6 treatments, and sugar syrup as control) was placed in each jar prior the insertion of the bees. The jars were left in a dark place up to two days (48 hours), and then the bees were released from about one meter away from their beehive location at 7 am. This short distance was used to easily observe the bees during their return to the beehive, and to ensure that the bees did not face any problems (e.g. bee predators or other pesticides). The number of bees succeeded and unsucceeded in returning back to the beehive was recorded. Also, the time taken by forager bees to return back to the beehive was recorded and compared between treatments.

2.3 Impacts on learning ability

The highest concentrations of the two herbicides were tested in this experiment. Forager bees were collected from healthy colonies in perforated jars and were divided into three treatment groups (30 bees per group). Sugar syrup was presented to the first group (control group) while herbazad3 to the second group, and glypho-up3 to the third group over one day. In the next day the learning ability was assessed using Proboscis Extension Response Conditioning (PERC) for 10 bees per group, after excluding weak or dead bees. The PERC was performed following the method described by Abou-Shaara (2018). In brief, the bees were placed in modified Eppendorf tubes and were tightly fixed in the tubes without narcosis. Then, the training of the bees on odor was done using Eppendorf tubes with small piece of cotton saturated with 10 µl of rose oil (odor unit). The odor unit was used to ensure that the bees were exposed to the rose oil from the same distance. The bees were left for 12 hours before the learning sessions. The bees were trained by exposing them to the odor for 4 seconds followed by stimulating the antennae of the bees with droplets of sugar syrup during the last 1 second of the odor exposure period. These steps (learning session) were repeated with 25 seconds interval until the bees were learned that the odor is associated with syrup. The number of learning sessions needed until each bee was regarded as learned was recorded per each bee and compared between groups. The bee was regarded as learned when its proboscis was immediately extended after the onset of the exposure to the odor alone and vice versa (Fig.1).

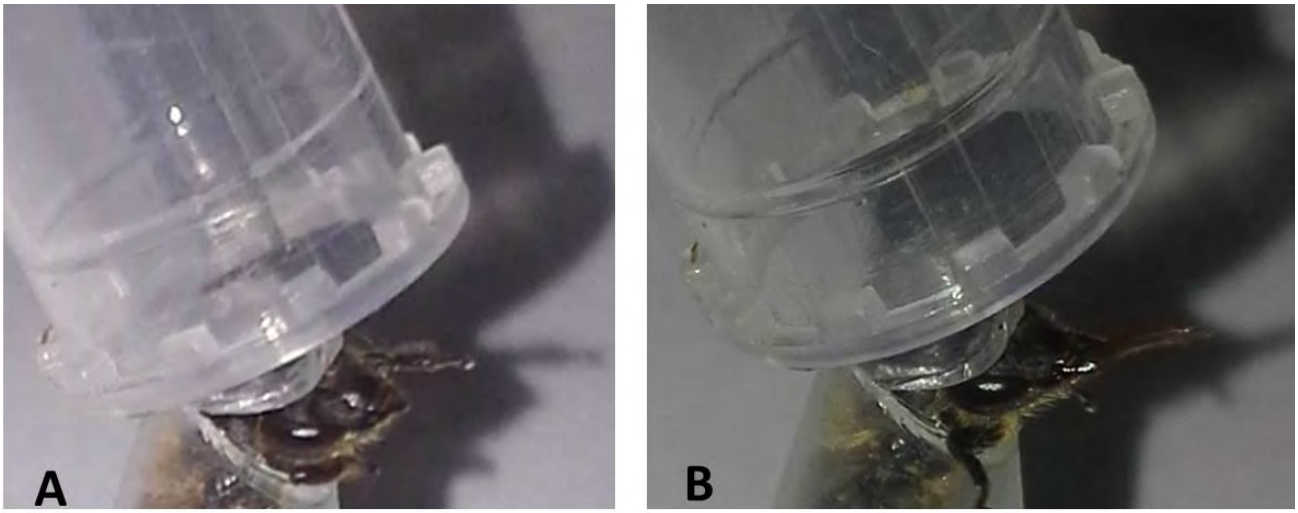

Fig. 1 Learning assessment of bee workers using Proboscis Extension Response Conditioning (PERC). A: the test bee shows no reaction to the odor only (unlearned bee), and B: the test bee shows reaction (proboscis extension) to the odor only (learned bee).

2.4 Survival of *Nosema* spp. infected bees

The herbicide with the highest impact on honey bees from the first experiment was tested to investigate whether the short term exposure to it could impact infected bees with *Nosema* spp. or not. The infection with *Nosema* spp. was done using fresh gut solution of diseased bees with *Nosema* spp. mixed with sugar syrup (2 syrup:1 solution, w/w) following some steps described in Fries et al. (2013). The gut solution was prepared as follows: 1) worker bees were collected from colonies with symptoms of *Nosema* spp. infection, 2) the guts were pulled out using a forceps and were placed separately in Eppendorf tubes filled with 0.5 ml of clean water, 3) the guts were mixed well with water, 4) a drop of each solution was examined under light microscope at 400x magnification to confirm the presence of *Nosema* spp. spores, 5) gut solutions of diseased bees were only mixed together and were filtered to remove any gut parts. This solution was used to infect the test bees.

For the test groups, the bees were collected from the lateral combs of healthy Carniolan bee colonies and were placed directly in the cages. Each group was replicated 4 times (4 cages, each having15 bees; the cage dimensions: 11.5 cm length and 6 cm diameter with porous covers). Three groups were tested: group 1) The bees were provided only with sugar syrup over the experimental period without any infection or exposure to the herbicide (control group), group 2) infected bees with *Nosema* spp. were provided with sugar syrup until the end of the experiment, and group 3) infected bees with *Nosema* spp. were provided over two days with a mixture of the herbicide and sugar syrup followed by sugar syrup only until the end of the experiment. The bees of group 2 and 3 were provided with 0.5 ml of the diluted gut solution of diseased bees to each cage over one day to ensure that the bees were infected. Then, the bees of group 3 were provided with a mixture of the herbicide and sugar syrup over two days (short term exposure). The sugar syrup (2 sugar: 1water, w/w) was presented to group 1 from the beginning of the experiment, to group 2 after the infection, and to group 3 after the infection and exposure to herbicide with a daily amount of 0.5 ml per cage. The number of dead bees was counted daily and for 10 days.

2.5 Statistical analysis

Herbicides (treatments) were considered as the independent factor while measured parameters as dependent factors in a completely randomized design (CRD). The values were presented as means ± standard errors (S.E.). For survival experiments, Kaplan-Meier test was used to compare survival curves using Log Rank (Mantel-Cox) test and to estimate survival means. For navigation and learning experiments, the groups were

compared using ANOVA followed by Post Hoc using Turkey's test to separate the means. The differences were considered significant when $P \leq 0.05$. The statistical analysis was performed using SPSS v. 16 and SAS v. 9.1.3.

3 Results

3.1 Survival

Concerning herbazed, the survival of bees decreased in the course of time for the three treatment groups more than the control group (Fig. 2). The means of estimated survival were 9.00± 0.36, 8.40±0.39, 7.75±0.39, and 6.85±0.41 days for control, herbazed1, herbazed2, and herbazed3, respectively. Survival of bees in control group was not significantly different than groups of herbazed1 and herbazed2 while in herbazed3 group was significantly less than control group (Table 1). Also, survival of bees in herbazed3 group was less significantly than herbazed1 (Mantel-Cox = 8.022 and P=0.005) and herbazed2 (Mantel-Cox = 5.848 and P=0.016).

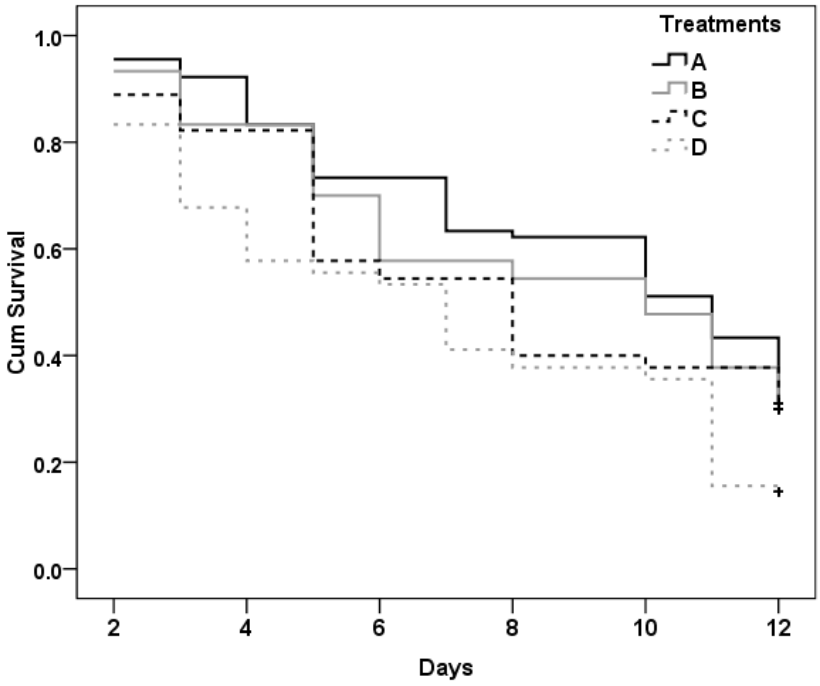

Fig. 2 Cumulative survival of bee workers over 12 days. A: control, B: herbazed1, C: herbazed2, and D: herbazed3 (3 cages per treatment and 30 bees per cage).

Increasing the concentrations of glypho-up caused high decrease in the survival of bee workers as shown in Fig. 3. The means of estimated survival were 9.00± 0.36, 7.84±0.43, 6.86±0.41, and 5.37±0.31days for control, glypho-up1, glypho-up2, and glypho-up3, respectively. The groups of glypho-up2 and glypho-up3 negatively impacted survival of caged bees more than control group with significant differences unlike glypho-up1. Also, survival of caged bees in glypho-up3 group was significantly less than glypho-up1 and glypho-up3 (Table 1).

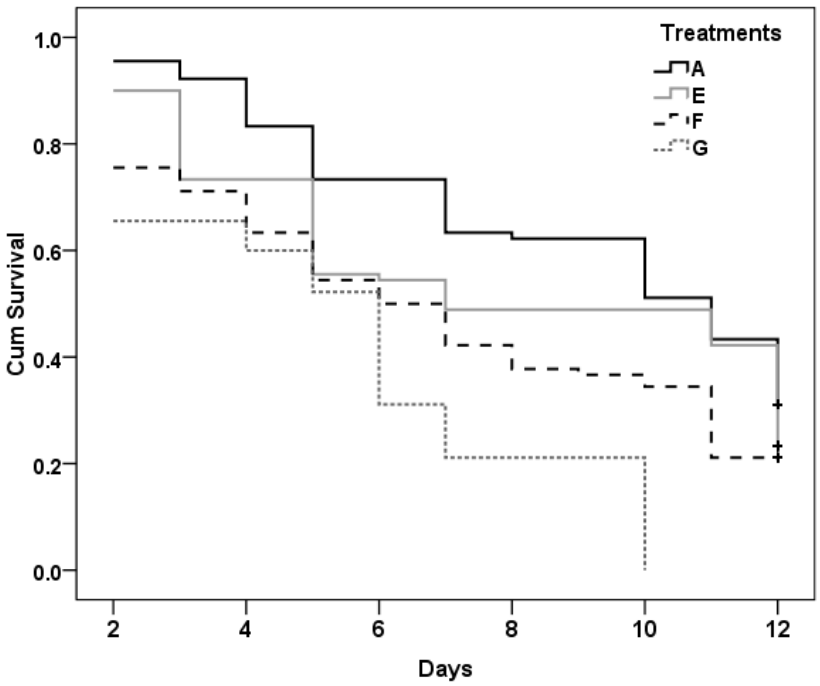

Fig. 3 Cumulative survival ofbee workers over 12 days. A: control, E: glypho-up1, F: glypho-up2, and G: glypho-up3 (3 cages per treatment and 30 bees per cage)

The treatment of glypho-up3 caused the highest reduction in survival of bees than the other treatments (Fig. 4). The survival of bees in glypho-up3 group was significantly less than all the other groups (Table 1). Also, this group had the lowest estimated survival mean. The treatments can be arranged in descending order according to the estimated survival means as; glypho-up3, herbazed3, glypho-up2, herbazed2, glypho-up1, and finally herbazed1.

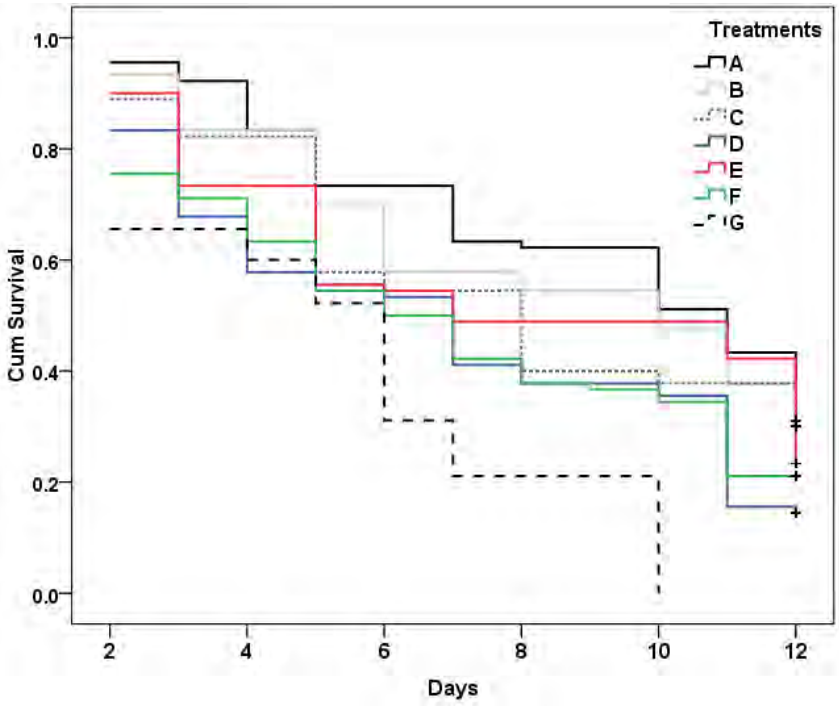

Fig. 4 Cumulative survival of bee workers over 12 days. A: control, B: herbazed1, C: herbazed2, D: herbazed3, E: glypho-up1, F: glypho-up2, and G: glypho-up3 (3 cages per treatment and 30 bees per cage).

Table 1 Comparisons between test groups (control group vs. treatment groups and glypho-up3 group vs. other groups). A: control, B: herbazed1, C: herbazed2, D: herbazed3, E: glypho-up1, F: glypho-up2, and G: glypho-up3. The impacts are significantly different when $P \leq 0.05$.

	Comparisons					
	A*B	**A*C**	**A*D**	**A*E**	**A*F**	**A*G**
Mantel-Cox	0.317	0.864	12.901	1.863	7.830	59.506
P value	0.573	0.353	0.000	0.172	0.005	0.000
	G*B	**G*C**	**G*D**	**G*E**	**G*F**	
Mantel-Cox	44.744	28.408	19.240	35.020	17.130	
P value	0.000	0.000	0.000	0.000	0.000	

3.2 Impacts on navigation

Bees from all the groups either the treatments or the control group were able to navigate and to locate correctly their beehive. Also, no significant differences were recorded between groups in the spent time to return to the beehive (DF= 6, F=0.59, *P*=0.74). The spent time varied from 14 to 26 seconds (Fig. 5) with mean of 18.40±0.97, 19.30±1.03, 19.40±0.99, 18.60±0.73, 19.60±0.80, 20.20±0.98, and 20.40±1.19 seconds for control, herbazed1, herbazed2, herbazed3, glypho-up1, glypho-up2, and glypho-up 3, respectively. Thus, the treatments did not impair the navigation ability of honey bees.

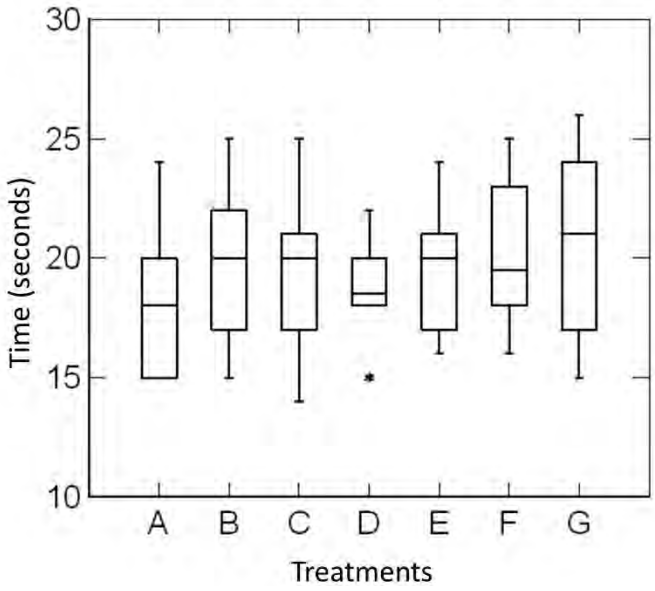

Fig. 5 Variations (range and median) in the spent time by forager bees to return to their beehive. A: control, B: herbazed1, C: herbazed2, D: herbazed3, E: glypho-up1, F: glypho-up2, and G: glypho-up3 (10 bees per group).

3.3 Impacts on learning ability

The mean number of the learning sessions needed until the bees were considered as learned was 7.90±1.03, 8.90±1.14 and 9.10±1.08 sessions for control, herbazad3 and glypho-up3, respectively. The number of sessions ranged from 3 to 13, 4 to 15, and 5 to 15 for the three groups, respectively (Fig. 6). No significant differences were found between the control group and the two treatment groups (DF=2, F= 0.35, *P*= 0.70).

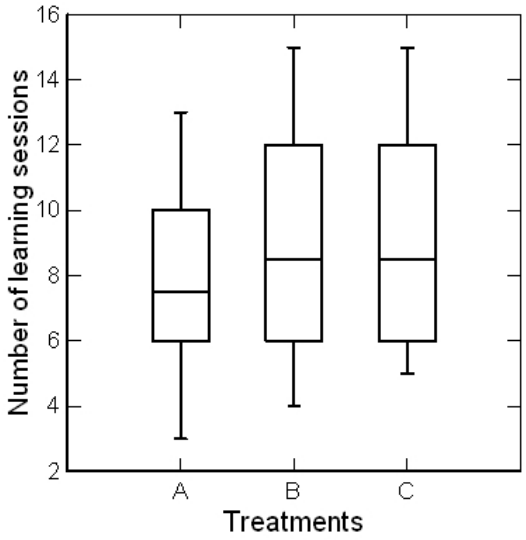

Fig. 6 Variations (range and median) in the required numbers of the learning session until the bees were considered as learned. A: control group, B: herbazad3, and C: glypho-up3 (10 bees per group).

3.4 Survival of *Nosema* spp. infected bees

The ability of test bees to survive declined over days (Fig.7). The estimated survival means were 8.56± 0.27, 7.43±0.33, and 7.05±0.28 days for bees fed on sugar syrup only (group A), *Nosema* spp. infected bees (group B) and *Nosema* spp. infected bees exposed to glypho-up 3 (group C), respectively. The survival of bees in group B (Mantel-Cox =9.154 and *P*=0.002) and group C (Mantel-Cox =26.866 and *P*=0.000) was significantly less than group A. Also, survival of bees in group C was significantly less than group B (Mantel-Cox=4.356and *P*=0.037).

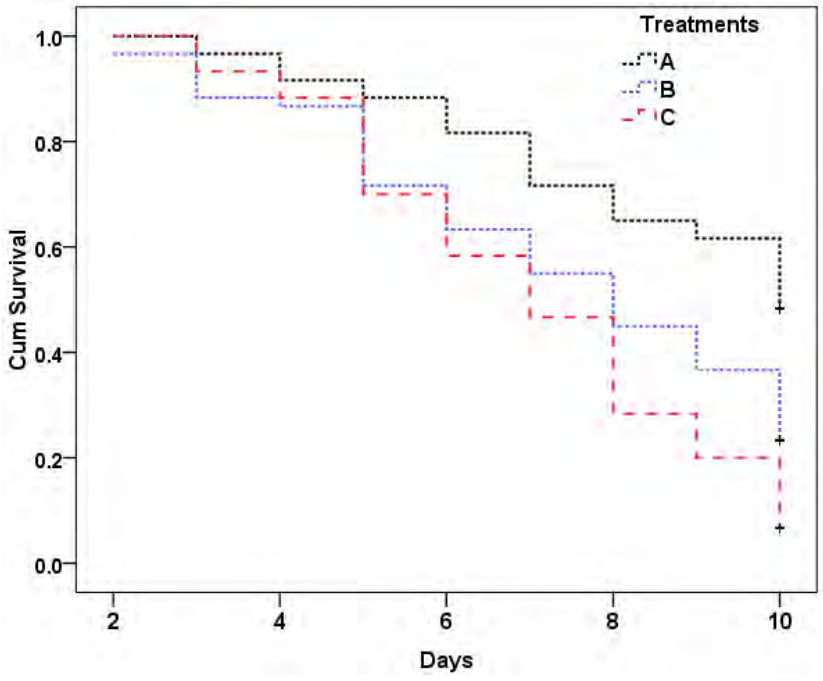

Fig. 7 Cumulative survival of bee workers over 10 days. A: control, B: Nosema spp. infected bees, and C: bees infected withNosema spp. and exposed to glypho-up 3 (4 cages per treatment and 15 bees per cage).

4 Discussion

4.1 Survival

The survival rates of caged bees decreased with increasing the concentration of herbazed and glypho-up. This result indicates the potential toxicity of tested herbicides to honey bees. The highest concentration of glypho-up caused the highest passive effects on caged bees than the other treatments with the lowest estimated survival mean. The differences between the two herbicides could be attributed to their formulation. In a similar way, Morton et al. (1972) found variations among tested herbicides with different formulations in their impacts on bee workers. The herbicide itself may not be toxic to honey bees unlike the carrier materials. The oil carrier has been found to be toxic to bee workers and not the herbicide (Moffett and Morton, 1975). Apart from the toxic part of the herbicide, exposing bee workers to high concentrations of these herbicides should be avoided.

4.2 Impacts on navigation

The results showed the absence of any passive impacts on the navigation ability of bee workers. The bees exposed to glypho-up or herbazed also showed no significant differences than the control group in regard to the spent time by bees until returning to their beehive. Accordingly, Herbert et al. (2014) did not record negative effects of glyphosate on the foraging behavior of honey bees, suggesting that the forager bees can easily transport this herbicide to nest-mates. This finding supports the current study because the treated bees returned successfully to their beehive. In another study, no impacts of glyphosate on the returning ability of treated bees to their beehives were found (Balbuena et al., 2015). Their study supports the current study in regard to the successful arriving of the treated bees to their beehives apart from the spent time in flying.

4.3 Impacts on learning ability

Bees exposed to the tested herbicides required more number of the learning sessions than the control bees. However, worker bees from herbazad3 and glypho-up3 groups did not differ significantly in their learning ability than those from the control group. Thus, herbazad3 and glypho-up3 showed no impact on the learning ability of bees. This finding is supported by the navigation experiment as these herbicides did not impact the navigation ability. On the contrary, bees chronically exposed to glyphosate showed reduction in learning performance (Herbert et al., 2014). Also, negative impacts on the olfactory learning of honey bees were found to sublethal doses of the insecticide fipronil (El Hassani et al., 2005). It is clear that the tested herbicides in this study did not impact the learning of honey bees especially at short term of exposure.

4.4 Survival of *Nosema* spp. infected bees

The results showed that survival of *Nosema* spp. infected bees was less than the control group. This can be explained by the role of *Nosema* spp. spores in causing digestion problems to the bees leading to the high mortality. Also, infected bees with *Nosema* spp. and exposed to glypho-up for short period showed less survival than bees infected only with *Nosema* spp. and the control group. This can be explained by the passive effects of glypho-up and *Nosema* spp. spores on bee health. The first experiment supports the passive role of glypho-up on survival of bee workers. It is expected that the interaction between these two stressors are high. The current findings are supported by the previous investigations using different pesticides. Mortality rates were significantly increased when *Nosema* infected bees were exposed to sublethal doses of insecticides (Vidau et al., 2011). It was found that bees exposed to high pesticide residue from brood combs had more susceptibility to *Nosema* spores at younger age than bees exposed to low pesticide residue (Wu et al., 2012). The interaction between *Nosema* infection and neonicotinoid (imidacloprid) increased death rates of bees (Alaux et al., 2010). Also, the infection with *Nosema* was significantly higher in bees exposed to imidacloprid than the control colonies (Pettis et al., 2012). The death rates increased when bees were exposed to *Nosema* infection and neonicotinoid (thiacloprid) (Doublet et al., 2015).

5 Conclusion

High concentrations of tested glyphosate herbicides caused significantly less survival of bee workers than the control group especially glypho-up. The tested herbicides had no unfavorable effects on the navigation or learning abilities of bee workers. Thus, the present study presented additional confirmation on the ability of forager bees to transport herbicides from the field to their colonies. The exposure of *Nosema* spp. infected bees to the high concentration of glypho-up for a short time can reduce the survival. The present findings highlight that reducing the survival rate of bee workers is the main noticeable impact caused by the tested herbicides. The toxic components of the tested herbicides along with the mode of action inside bees require further investigations.

References

Abou-Shaara HF. 2018. An inexpensive method to assess the associative learning in honey bees using the proboscis extension reflex test. Bee World, 95(1): 6-8

Abou-Shaara HF, Staron M, Cermáková T. 2017. Impacts of oxalic acid, thymol, and potassium citrate as *Varroa* control materials on some parameters of honey bees. Turkish Journal of Veterinary and Animal Sciences, 41(2): 238-247

Alaux C, Brunet J-L, Dussaubat C, Mondet F, Tchamitchan S, Cousin M, Brillard J, Baldy A, Belzunces LP, Le Conte Y. 2010. Interactions between *Nosema* microspores and a neonicotinoid weaken honeybees (*Apis mellifera*). Environmental Microbiology, 12: 774-782

Balbuena MS, Tison L, Hahn M-L, Greggers U, Menzel R, Farina WM. 2015. Effects of sublethal doses of glyphosate on honeybee navigation.Journal of Experimental Biology, 218: 2799-2805

Barker R, Lehner Y, Kunzmann MR. 1980. Pesticides and honey bees: Nectar and pollen contamination in alfalfa treated with dimethoate. Archives of Environmental Contamination and Toxicology, 9: 125-133

Brown MF, Demas GE. 1994. Evidence for spatial memory in honeybees (*Apis mellifera*). Journal of Comparative Psychology, 108: 344-352

Dively GP, Kamel A. 2012.Insecticide residues in pollen and nectar of a cucurbit crop and their potential exposure to pollinators. Journal of Agricultural and Food Chemistry, 60: 4449-4456

Doublet V, Labarussias M, de Miranda JR, Moritz RFA, Paxton RJ. 2015. Bees under stress: sublethal doses of a neonicotinoid pesticide and pathogens interact to elevate honey bee mortality across the life cycle. Environmental microbiology, 17: 969-983

El Hassani AK, Dacher M, Gauthier M, Armengaud C. 2005. Effects of sublethal doses of fipronil on the behavior of the honeybee (*Apis mellifera*).Pharmacology Biochemistry and Behavior, 82: 30-39

Fries I, Chauzat M-P, Chen Y-P, Doublet V, Genersch E, Gisder S, Higes M, McMahon DP, Martín-Hernández R, Natsopoulou M, Paxton RJ, Tanner G, Webster TC, Williams GR. 2013. Standard methods for *Nosema* research. Journal of Apicultural Research, 52(1)

García-Palencia P, Martín-Hernández R, González-Porto A-V, Marin P, Meana A, Higes M. 2010. Natural infection by *Nosema ceranae* causes similar lesions as in experimentally infected caged-worker honey bees (*Apis mellifera*). Journal of Apicultural Research, 49: 278-283

Herbert LT, Vázquez DE, Arenas A, Farina WM. 2014. Effects of field-realistic doses of glyphosate on honeybee appetitive behavior. Journal of Experimental Biology, 217: 3457-3464

Johnson RM. 2015. Honey bee toxicology. Annual Review of Entomology, 60: 415-434

Kamel SM, Blal AH, Mahfouz HM, et al. 2013. The most common insect pollinator species on sesame crop (*Sesamum indicum* L.) in Ismailia Governorate, Egypt. Arthropods, 2(2): 66-74

Klatt BK, Holzschuh A, Westphal C, Clough Y, Smit I, Pawelzik E, Tscharntke T. 2014. Bee pollination improves crop quality, shelf life and commercial value. Proceedings of the Royal Society B, 281: 20132440

Kralj J, Fuchs S. 2010. *Nosema* spp. influences flight behavior of infected honey bee (*Apis mellifera*) foragers. Apidologie, 41: 21-28

Moffett JO, Morton HL. 1975. How herbicides affect honey bees. American Bee Journal, 115: 178, 179, and 200

Morton HL, Moffett JO, Macdonald RH. 1972. Toxicity of herbicides to newly emerged honey bees. Environmental Entomology, 1: 102-104

Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, vanEngelsdorp D, Pettis JS. 2010. High levels of miticides and agrochemicals in north American apiaries: Implications for honey bee health. PLoS ONE, 5: e9754

Neumann P, Carreck NL. 2010. Honey bee colony losses. Journal of Apiculture Research, 49: 1-6

Pettis JS, vanEngelsdorp D, Johnson J, Dively G. 2012. Pesticide exposure in honey bees results in increased levels of the gut pathogen *Nosema*. Naturwissenschaften, 99: 153-158

Pohorecka K, Skubida P, Miszczak A, Semkiw P, Sikorsk P, Zagibajlo K, Teper D, Koltowski Z, Skubida M, Zdalska D, Bober A. 2012. Residues of neonicotinoid insecticides in bee collected plant materials from oilseed rape crops and their effect on bee colonies. Journal Apicultural Science, 56: 115-134

Sanchez-Bayo F, Goka K. 2014. Pesticide residues and bees- A risk assessment. PLoS ONE, 9: e94482

Southwick EE, Southwick L. 1992. Estimating the economic value of honey bees (Hymenoptera: Apidae) as agricultural pollinators in the United States. Journal of Economic Entomology, 85: 621-633

vanEngelsdorp D, Evans JD, Saegerman C, Mullin C, Haubruge E, Nguyen BK, Frazier M, Frazier J, Cox-Foster D, Chen Y, Underwood R, Tarpy DR, Pettis JS. 2009. Colony collapse disorder: A descriptive study. PLoS ONE, 4: e6481

Vidau C, Diogon M, Aufauvre J, Fontbonne R, Vigues B, Brunet JL, Texier C, Biron DG, Blot N, El Alaoui H, Belzunces LP, Delbac F. 2011. Exposure to sublethal doses of fipronil and thiacloprid highly increases mortality of honeybees previously infected by *Nosema ceranae*. PLoS ONE, 6: e21550

Wang DI, Moeller FE. 1971. Ultrastructural changes in the hypopharyngeal glands of worker honey bees infected by *Nosema apis*. Journal of Invertebrate Pathology, 17: 308-320

Wu JY, Smart MD, Anelli CM, Sheppard WS. 2012. Honey bees (*Apis mellifera*) reared in brood combs containing high levels of pesticide residues exhibit increased susceptibility to *Nosema* (Microsporidia) infection. Journal of Invertebrate Pathology, 109: 326-329

Zhang WJ. 2018. Global pesticide use: Profile, trend, cost / benefit and more. Proceedings of the International Academy of Ecology and Environmental Sciences, 8(1): 1-27

Zhang WJ, Jiang FB, Ou JF. 2011. Global pesticide consumption and pollution: with China as a focus. Proceedings of the International Academy of Ecology and Environmental Sciences, 1(2): 125-144