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Residual toxicity of selected insecticides on *Aphis gossypii* and their safety limits on honeybees

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RESEARCH ARTICLE

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ABSTRACT

Evaluation studies were carried out to simulate realistic field exposures of sulfoxaflor and flonicamid against *Aphis gossypii* at foraging time of *Apis mellifera*. Semi-field trials of field rates of sulfoxaflor and flonicamid against *A. gossypii* laboratory strain at 48 h of exposure had equipollent overall mean of mortality of 62.50 and 63.50%, respectively in season of 2020, likewise 60.50 and 62.50%, respectively in season of 2021. Lethal time values (LT_1) had ranges of 51.33–32.46 days for sulfoxaflor and 49.00–39.55 days for flonicamid. Laboratory trials on foraging honeybees (~21 days old) at 5 h of exposure showed an excellence for sulfoxaflor (5.00%) in overall mean of mortality compared to flonicamid (2.75%) in season of 2020. Likewise, sulfoxaflor (4.75%) surpassed flonicamid (2.75%) in season of 2021. The highest LT_1s on honeybees for sulfoxaflor and flonicamid reached 27.45 and 10.94 days, respectively. International Organization for Biological Control classified both insecticides to be harmless on honeybees. Survival foraging bees exposed to LD_{50}s of the tested insecticides had malformed digestive tracts gradually vanished along week of exposure. Suggestions for foliar spray stoppages prior to flowering period were mentioned for both insecticides.

KEYWORDS

cotton aphids, piercing-sucking insects, *Apis mellifera*, pollinators, long-term toxicity

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INTRODUCTION

The cotton aphid, *Aphis gossypii* (Homoptera: Aphididae) is a worldwide polyphagous species which evolves multiple biotypes that realizes reasonable variances in host adaptation. This target pest causes a high level of economic and ecological problems associated with the occurrence of sooty mold and lethal virus diseases to plants. It also possesses a rapid reproduction and high insecticide resistance (Quan et al., 2019; Zhao et al., 2021; Morando et al., 2021).

Foraging activity of non-target pollinators occasionally might coincide with invasive cotton aphids during flowering period on crops. One of the most valuable and predominant pollinators is honeybee, *Apis mellifera* (Hymenoptera: Apidae), which plays a key role in pollinating crops and wild plants accompanied by significant increases to the yield production. Tragic failure of honeybee colonies to do their pollination missions in some regions may be due to some negative environmental impacts (Hora et al., 2018; Li et al., 2018). Various environmental stresses, including the extensive use of insecticides, could cause negative impact on non-target pollinators and subsequently crop production. Moreover, honeybees are not repelled by these insecticides and may be exposed to the treated plants in the field during their foraging time (Gomes et al., 2020).

In the recent year, new generations of pesticides have been adopted in controlling piercing-sucking insects in the agricultural field. Sulfoxaflor is one of the most novel insecticides, which belongs to chemical class of sulfoximines, which is widely used in controlling crop pests. However risk assessments of sulfoxaflor generally reported high toxic effect to pollinators, particular moderate toxicity was exhibited on adult honeybees (Tamburini et al., 2021). Nevertheless, relevant concentrations of sulfoxaflor could cause significant death and growth failure to larvae and newly emerged honeybees (Li et al., 2021). According to a few European union (EU) member states, sulfoxaflor spray application a week ago prior to bloom period might be safe on honeybee's life whom exposed to the treated areas (Li et al., 2021; Tamburini et al., 2021).

On the other hand, flonicamid is considered as a novel selective feeding inhibitor and potential alternatives in Integrated Pest Management (IPM) programms. It belongs to a new chemical class of pyridine-carboxamides. It owns a systemic action and long-term toxicity against various sucking insects such as aphid species, *Frankliniella occidentalis* (Pergande) and *Bemisia tabaci* (Gennadius). Nevertheless, flonicamid possessed a selective toxicity on bumble bee *Bombus terrestris* and met with safe limits for pollination activity of the apiaries. It also had neglectful impacts on beneficial natural enemies of *Orius laevigatus* (Fieber) and *Amblyseius swirskii* (Athias-Henriot) (Fanigliulo et al., 2009; Colomer et al., 2011).

In this respect, our objectives were directed to evaluate the residual toxic effects of sulfoxaflor and flonicamid on cotton aphids, *A. gossypii.* This study tried to simulate a realistic field exposure to these selected insecticides against cotton aphids in coincide with foraging time of honeybee, *A. mellifera* during flowering period of summer squash plant. Moreover, we tried to establish safety limits for these insecticides on foraging honeybees prior to flowering period. Finally, this study was supported with a particular physiological dissection studies on digestive tracts of survival foraging honeybees exposed to sub-lethal doses of these selected insecticides to evaluate their probable impacts.

MATERIAL AND METHODS

Tested insecticides

Sulfoxaflor (Closer (24% SC); applied field rate 15 mL/100 L) was obtained from Dow Agro Science, Canada and flonicamid (Teppeki (50% WG); applied field rate 20 gm/100 L) was obtained from Sumitomo Corporation, Egypt. The applied field rates of the tested insecticides followed the recommendations performed by Agriculture Pesticides Committee of Egyptian Agriculture ministry.

Target insect pest

Insect sampling and rearing. Collected samples of target cotton aphid, *A. gossypii* from broad weeds in late winter seasons of 2020 and 2021 were reared on summer squash seedling plants *Cucurbita pepo* in plastic pots (diameter, 10 cm x height, 8 cm), covered with muslin cloth. These rearing pots were incubated into a purposed-built incubator chamber equipped with digital thermostat, hygrometer and automatic shut-off 24 h timer. This incubator chamber provided approximate rearing conditions of 25 ± 2 °C, 60% RH and 16 h of daylight. This procedure simulated the rearing method performed by Gaimari and Turner (1996). Damaged plants by reared aphids were replaced whenever needed with appropriate quantities of new healthy ones to avoid excessive crowding of aphids. A new *A. gossypii* laboratory strain (LS) was obtained after approximate 5 successive generations.

Semi-field trial design on cotton aphid. Field experiments were achieved during seasons of 2020 and 2021 on summer squash at Ezbit Zaki Afandi, Abohomous, Al-Behera, Egypt. All plantation practices tracked the guidance of optimal production processes of crop. All treatments were assigned to 40 m^2 micro-plots in a randomized complete block design with four replicates. The treatments of the tested insecticides were sprayed using Knapsack sprayer

Table 1. Overall mean of mortality percentages of sulfoxaflor and flonicamid at their field rates against adults of *Aphis gossypii* (LS) at 48 h of exposure, seasons of 2020 and 2021

Season	Treatments	Overall mean of mortality %[1] ±SD[2]		LT_1[3] (days)
2020	Sulfoxaflor	62.50[a]	±0.67	51.33 (29.39–89.65)
	Flonicamid	63.50[a]	±0.58	49.00 (29.38–81.73)
2021	Sulfoxaflor	60.50[a]	±1.03	32.46 (21.87–48.18)
	Flonicamid	62.50[a]	±1.01	39.55 (25.71–60.85)

[1]Calculated within intervals of 0, 2, 4, 7, 9 days.
[2]Standard deviation.
[3]It is the time required to kill 1% of cotton aphids at 48 h of exposure to treated leaves by the tested insecticides, which had been sampled from the field at each interval of 2, 4, 7 and 9 DAT. It refers to the estimated time where the residual efficacy is mostly dissipated.
Means of overall mortality for each growing season aside with the same letter are not significantly different according to the $LSD_{0.05}$, where the values $LSD_{0.05}$ for season 2020 = 8.894 and season 2021 = 8.107.

equipment (CP3) at their recommended field rates (FR) (Table 1). The total spray volume was 4 L water per each micro-plot. Control treatment was sprayed by water only.

Newly emerged leaves of treated and untreated summer squash plants were collected from each micro-plot in perforated bags and transfer to laboratory at intervals of 0, 2, 4, 7 and 9 days after treatment (DAT). Lab test was accomplished according to susceptibility test method of Insecticide Resistance Action Committee (IRAC) (2016). Two leaf disks were placed on a hardened surface of agar gel 1% poured into the base of a glass container (7 cm in diameter) with 1 cm gap from its rim. Each glass container was covered with micro holes gauze for ventilation and fitted with lids. Ten of adult cotton aphids (LS) were transferred to each container. Each treatment was replicated four times. The treatments were incubated under 21 °C and 60% RH. Mortality percentages of treated and untreated aphids were recorded at 48 h of exposure and corrected according to formula of Abbott (1925). Lethal time required to kill 1% (LT_1) of adult aphids was estimated by probit analysis (Abd El Rheem, 2005; Patil, 2015).

Non-target pollinator

Insect collecting. Honeybee, *A. mellifera* was selected as a common non-target insect pollinator. The age of 21 days of adult honeybees refer to the active age where a maximum foraging activity could be accomplished (Vance et al., 2009). Uniformed ages of bees at 21 days old in laboratory were obtained by simulating the standard methods of Williams et al. (2013). Strong, massive and healthy colonies of honeybees were selected, according to the measurements established by Delaplane et al. (2013), for the sampling of honeybees in the apiary of Agriculture Research Center, Al-Sabhia, Alexandria, Egypt. Frames contained majority of uniformed fertilized eggs within 24 h were marked by date until capped brood almost about to be full emerged. The marked frames were picked up and all adult honeybees were removed gently by shaking the frame over the colony. Each selected frame was place in appropriate frame cage. The caged frames of broods were transferred to a laboratory incubator maintained at 35 °C and 50–70% RH. The frame was checked before full emergency of newly adults every 12–24 h to obtain age homogeneity using a forceps. Each 5 newly emerged adults were inserted in glass containers (100 cm^3) covered with plastic caps ventilated with 6 holes (3 mm, diameter) and fitted with disposable pipette plastic dropper to provide fresh daily prepared artificial feeding with 50% sucrose solution. The container of newly emerged adults were reared in laboratory incubator at 30 °C and 50–70% RH preluding for tests of acute oral toxicity and simulated trials for field exposure on aged adults of 21 days.

Simulated laboratory trials for insecticide-field exposure on honeybees

Laboratory trials were achieved against uniform foraging *A. mellifera* (~21 days old) in coincidence with the time of semi-field trials accomplished against *A. gossypii* on summer squash plant during seasons of 2020 and 2021. The experiments were initiated during flowering stage of summer squash plant in pots (diameter, 30 cm x height, 25 cm). Each pot was covered with wire net cage (hole size, 0.45 x 0.3 mm) with dimensions of (diameter, 28 cm x height, 60 cm). The treatments of the selected insecticides were sprayed using hand sprayer equipment at their field recommended dosages (Table 1). The total volume of spray solution was 50 mL water per each pot. Control treatment was sprayed by water only. Each treatment was replicated four times.

Twenty of honeybees were inserted in each cage at intervals of 0, 2, 4, 7 and 9 DAT. Mortality percentages of honeybees were recorded at 5 h of exposure (approximate total journeys duration of foraging bees per day) and corrected according to formula of Abbott (1925). LT_1 values on treated honeybees were estimated by probit analysis (Abd El Rheem, 2005; Patil, 2015).

Safety limits assessment of the selected insecticides on honeybees

Adverse effects of the tested insecticides on honeybees were classified according to a classification system performed by International Organization for Biological Control (IOBC) (Hassan, 1992). This classification system included class 1 that meant by harmless at mortality percentages of <30%, class 2 meant by slightly harmful at mortality percentages of 30–79%, class 3 meant by moderately harmful at mortality percentages of 80–99% and class 4 meant by harmful at mortality percentages with > 99%.

Effects of selected insecticides on the digestive tracts of survival foraging honeybees

Oral toxicity tests on honeybees in laboratory. Evaluations of dietary exposure were achieved on *A. mellifera* (~21 days old) exposed for 24 h to values of LD_{50} of sulfoxaflor (0.44 mg L^{-1}) and flonicamid (102.10 mg L^{-1}). According to empirical studies of Williams et al. (2013), Nicolson et al. (2013), Knopper et al. (2016), Harano and Nakamura (2016) and Rodney and Purdy (2020), the average consumed amount of nectar by one honeybee for each foraging journey (~30 min total residing time on flower) is 40 mg. Then, 10 journeys (~5 h) per day x 40 mg nectar x 5 bees = 2,000 mg total weight of nectar per day. Nectar contains about 30% sugar (mainly sucrose). Simulated daily artificial diet contains fresh-prepared insecticide concentration in 0.6 gm (30% sucrose) dissolved in 2,000 mg distilled water (DW). The dietary portion was introduced via disposable pipette plastic dropper into glass container (100 cm^3). Each container contained 5 honeybees. Seven concentrations were prepared for each insecticide. Each concentration was replicated four times. Control treatments contained 2,000 mg of 30% sucrose solution in DW. All the treatments were incubated at 21 °C, 60% RH in Shel-lab incubator (model 15450, Sheldon manufacturing, Inc.). Mortality percentages of honeybees were observed after 24 h of treatment, corrected by formula of Abbott (1925) and submitted to probit analysis (Finney, 1971).

Digestive tracts dissection

This experiment underwent the aforementioned design of the simulated laboratory trial on honeybees (~21 days old). Five honeybees were inserted for 24 h in each cage during flowering period treated with values of LD_{50} of sulfoxaflor and flonicamid at 0, 2, 4 and 7 DAT. Cages of control treatment were sprayed with water. The digestive tracts of the survived honeybees were dissected under a binocular microscope (6 × magnification) in Ringer's solution (0.42 gm KCl, 0.2 gm $NaHCO_3$, 9.0 gm NaCl, 0.48 gm $CaCl_2$ in 1,000 mL DW) according to the method of Junqueira and Carneiro (1980).

Statistical analysis

All the obtained data were subjected to analysis of variance (ANOVA). Software of Statistical Analysis System Institute (SAS), (2002) was used to determine the mean values using LSD test for significance at 0.05.

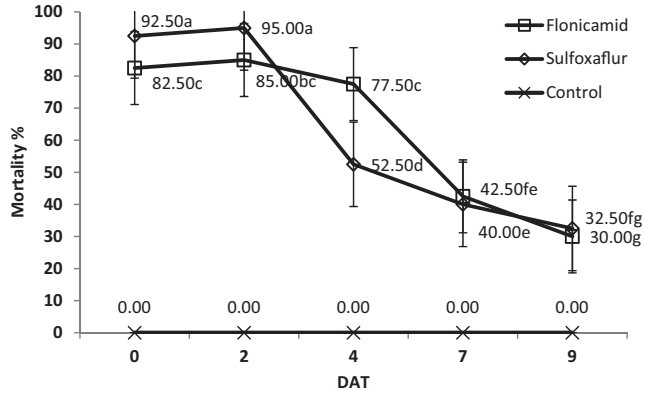
RESULTS

Residual toxicity of sulfoxaflor and flonicamid against *Aphis gossypii* (LS)

Residual toxicity (Figs 1 and 2) and overall mean of mortality percentages (Table 1) against adults of *A. gossypii* (LS) at 48 h of exposure to the applied FRs of sulfoxaflor and flonicamid were evaluated during flowering periods of *Cucurbita pepo* plants in seasons of 2020 and 2021.

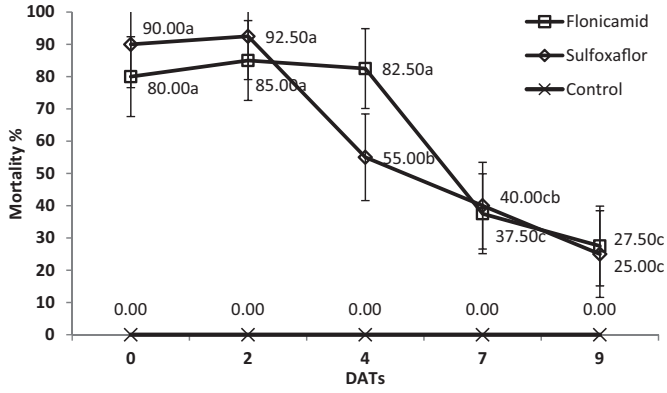
Data of season 2020 demonstrated that sulfoxaflor and flonicamid achieved strongest toxic effects against *A. gossypii* (LS) over the first 2 DAT. The toxic effects at 0 and 2 for sulfoxaflor (92.50 and 95.00%, respectively) were significantly higher than flonicamid (82.50 and 85.00%, respectively). Contrariwise, the period at 7 and 9 DAT showed significant decreases in the toxic effects with non-significant difference between sulfoxaflor (40.00 and 32.50%, respectively) and flonicamid (42.50 and 40.00%, respectively) at (Fig. 1). In addition, the results of overall mean of mortality percentages at 48 h of exposure along the 9 DAT had no significant differences between sulfoxaflor (62.50%) and flonicamid (63.50%). Meantime, the estimated data of LT_1 on *A. gossypii* (LS) were 51.33 and 49.00 days for sulfoxaflor and flonicamid, respectively (Table 1).

Data of season 2021 was identical to the previous season. Strongest toxic effects with non-significant difference between sulfoxaflor (90.00 and 92.50%, respectively) and flonicamid (80.00 and 85.00%, respectively) exhibited at 0 and 2 DAT against *A. gossypii* (LS). In contrary, the period from 7 to 9 DAT showed significant declines in the toxic effects of sulfoxaflor (40.00 and 25%, respectively) and flonicamid (37.50 and 27.50%, respectively) (Fig. 2). Moreover, the results of overall mean of mortality percentages at 48 h of exposure along the 9 DAT had no significant differences between sulfoxaflor (60.50%) and flonicamid (62.50%). Meanwhile, the data of estimated LT_1on *A. gossypii* (LS) reached 32.46 and 39.55 days for sulfoxaflor and flonicamid, respectively (Table 1).



- Mortality percentages values were supported with standard error.
- Mortality percentages with the same letter are not significantly different according to the $LSD_{0.05}$ for the interactions between both treatments.

Fig. 1. Residual toxicity of sulfoxaflor and flonicamid at their field rates against adults of *Aphis gossypii* (LS) at 48 h of exposure, season 2020

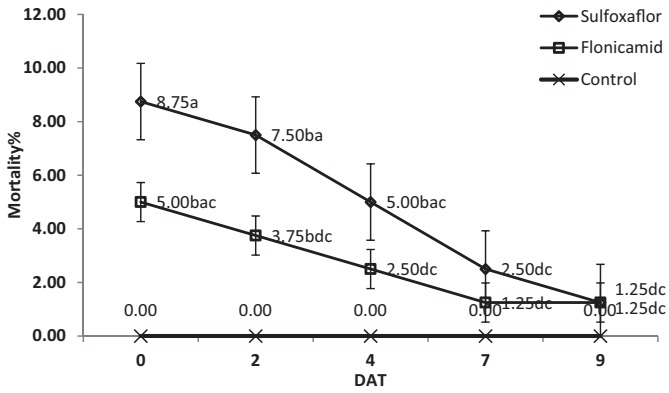


- Mortality percentages values were supported with standard error.
- Mortality percentages with the same letter are not significantly different according to the $LSD_{0.05}$ for the interactions between both treatments.

Fig. 2. Residual toxicity of sulfoxaflor and flonicamid at their field rates against adults of *Aphis gossypii* (LS) at 48 h of exposure, season 2021

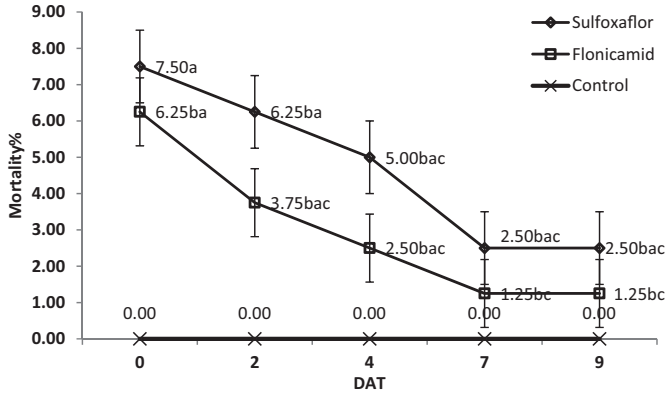
Residual toxicity of sulfoxaflor and flonicamid against honeybees

Laboratory trials evaluated residual toxicity (Figs 3 and 4) and overall mean of mortality percentages (Table 2) of the tested insecticides against *A. mellifera* (~21 days old) at exposure time of 5 h (total journeys duration of foraging bees per day). This laboratory trial was accomplished



- Mortality percentages values were supported with standard error.
- Mortality percentages with the same letter are not significantly different according to the $LSD_{0.05}$ for the interactions between both treatments.

Fig. 3. Residuals toxicity of sulfoxaflor and flonicamid at their field rates against *Apis mellifera* (~21 days old) at 5 h of exposure to treated *Cucurbita pepo* at flowering stage in lab. trial of 2020



- Mortality percentages values were supported with standard error.
- Mortality percentages with the same letter are not significantly different according to the $LSD_{0.05}$ for the interactions between treatments.

Fig. 4. Residuals toxicity of sulfoxaflor and flonicamid at their field rates against *Apis mellifera* (~21 days old) at 5 h of exposure to treated *Cucurbita pepo* at flowering stage in lab. trial of 2021

Table 2. Overall mean of mortality percentages of sulfoxaflor and flonicamid at their field rates against *Apis mellifera* (~21 days old) at 5 h of exposure in lab. trials of 2020 and 2021

Season	Treatments	Overall mean of mortality %[1]	±SD[2]	LT_1[3]	IOBC Classification[4]
2020	Sulfoxaflor	5.00[a]	±3.19	20.42 (1.75–237.82)	Harmless
	Flonicamid	2.75[b]	±1.63	10.94 (1.88–63.57)	Harmless
2021	Sulfoxaflor	4.75[a]	±2.24	27.45 (0.84–897.55)	Harmless
	Flonicamid	2.75[b]	±2.24	10.94 (1.88–63.57)	Harmless

[1]Calculated within intervals of 0, 2, 4, 7, 9 days.
[2]Standard deviation.
[3]It is the time required to kill 1% foraging honeybee at 5 h of exposure to the treated plant by the tested insecticides in each interval of 0, 2, 4, 7 and 9 DAT. It refers to the estimated time where the residual efficacy is mostly dissipated.
[4]Based on field studies included limits of mortality % of (< 30%) meant by harmless, (30–79%) meant by slightly harmful, (80–99%) meant by moderately harmful and (> 99%) meant by harmful (Hassan 1992). Means of overall mortality for each growing season aside with the same letter are not significantly different according to the $LSD_{0.05}$, where the values $LSD_{0.05}$ for season 2020 = 2.253 and season 2021 = 5.592.

in coincidence with the time of semi-field trials against *A. gossypii* on flowering stage of *C. pepo* in seasons of 2020 and 2021.

Regarding to data of mortality percentage against *A. mellifera* in laboratory trial of 2020, the strongest toxic effects of sulfoxaflor (8.75 and 7.50%) were revealed at 0 and 2 DAT, respectively. On the 7th and 9th DAT, toxic effects for sulfoxaflor showed significant declines reached 2.50 and 1.25%, respectively (Fig. 3). No significant differences between mortality percentages of

flonicamid along the 9 DAT. Moreover, mortality percentages between sulfoxaflor and flonicamid had no significant differences along the 9 DAT. In addition, the results of overall mean of mortality percentages at 5 h of exposure for sulfoxaflor (5.00%) were significantly higher than flonicamid (2.75%) along the 9 DAT. Meantime, the data of LT_1 values of sulfoxaflor and flonicamid on *A. mellifera* reached 20.42 and 10.94 days, respectively (Table 2).

Data of laboratory trial of 2021 showed that there were no significant differences between mortality percentages of each of flonicamid and sulfoxaflor along the 9 DAT against *A. mellifera*. Moreover, mortality percentages between sulfoxaflor and flonicamid had no significant differences along the 9 DAT. Furthermore, the result of overall mean of mortality percentages at 5 h of exposure along the 9 DAT for sulfoxaflor was 4.75% and followed by 2.75% for flonicamid. Meanwhile, the data of LT_1 values on *A. mellifera* reached 27.45 and 10.94 days when exposed to sulfoxaflor and flonicamid, respectively (Table 2).

Eventually, all mortality values and overall mean of mortalities of sulfoxaflor and flonicamid on *A. mellifera* along the 9 DAT during 2020 and 2021 did not exceed the limits of harmless (< 30%) indicated by IOBC classification.

Toxicity of the selected insecticides against honeybees in laboratory

Laboratory studies were carried out to determine the acute oral LD_{50} of the selected insecticides on *A. mellifera* (~21 days old) at 24 h of exposure (Table 3). LD_{50} of sulfoxaflor (0.44 mg L^{-1}) was significantly higher than the toxic value of flonicamid (102.10 mg L^{-1}).

Malformation effects of sulfoxaflor and flonicamid on honeybees

The digestive tracts of survival foraging honeybees (~21 days old) exposed for 24 h to acute oral LD_{50} of sulfoxaflor and flonicamid along the 7 DAT were tested to check the honey stomach, ventriculus, rectum and all parts of alimentary canal in general. The present results of the two insecticides illustrated the malformed digestive tracts shaped by steep dwarfing signs at 0 DAT (Figs 5E and 6E) as well as a particularly damage in honey stomach, ventriculus and rectum of digestive parts at 2 DAT (Figs 5D and 6D). Malformations began to subside at the 4th DAT (Figs 5C and 6C) and seemed to be neglectful at the 7th DAT (Figs 5B and 6B) in compare to the control treatment (Figs 5A and 6A).

DISCUSSION

Potential risks could be arising due to the irrational usages of insecticides in controlling invasive insect pests that occasionally coinciding alongside with foraging activity of non-targeting pollinators during the flowering period. Meanwhile, current debates have been spreading out on the

Table 3. Toxicity of the selected insecticides at their oral LD_{50}s on *Apis mellifera* (~21 days old) at 24 h of exposure

Tested insecticides	LD_{50} (mg L^{-1})	Confidence limits (mg L^{-1})	Slope	x^2	*df*
Flonicamid	102.10	27.11–384.45	1.960	0.102	5
Sulfoxaflor	0.44	0.34–0.54	1.046	1.892	5

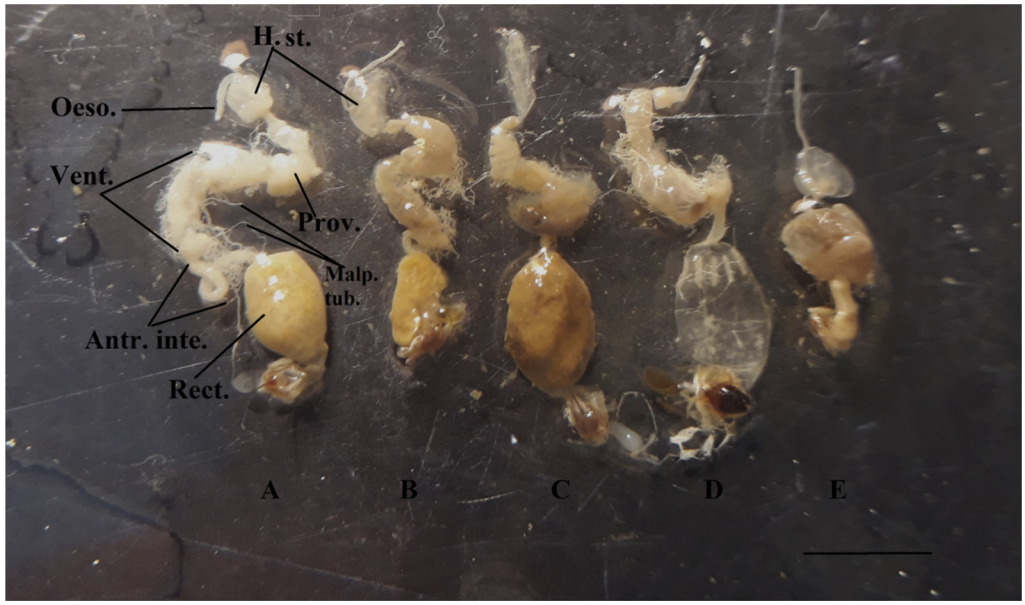


Fig. 5. A, Normal digestive tract and B, C, D, E, Malformed digestive tracts in adults of honeybees exposed to sulfoxaflor at 7, 4, 2 and 0 DAT, respectively. Oeso.: Oesophagus, H.st.: Honey stomach, Prov: Proventriculus, Vent.: Ventriculus, Malp. tub.: Malpighan tubules, Antr. Inte: Antrerior intestine, Rect.: Rectum. (Bar = 50 mm)

phenomena of bee's deaths, which often return to the agricultural practices associated with the applications of highly toxic insecticides. Based on the foregoing cause, our study tried to evaluate more selective and ecofriendly insecticides that should be adopted to reduce the impacts on pollinators and retain their pollination activities (Fanigliulo et al., 2009; Gomes et al., 2020).

The obtained data of the selected insecticides throughout the semi-field trials on *A. gossypii* as well as laboratory trials on honeybees were normal and the variances were homogeneous according to analysis of variance by ANOVA. Semi-field trials in this study on seasons of 2020 and 2021 showed that flonicamid and sulfoxaflor had potent toxic effects against *A. gossypii* (LS) during the first 4 DAT and followed by significant declinations in their toxic effects up to 9 DAT. Both of the insecticides showed non-significant differences in their overall mean of mortality percentages along the 9 DAT. These findings came in accordance to the other studies demonstrated the common used of flonicamid against cotton piercing sucking pests such as *Bimisia tabaci*, *Oxycarenus hyalinipennis* and *A. gossypii* in Pakistan (Gore et al., 2013; Koo et al., 2014; Ullah et al., 2021). Furthermore, sulfoxaflor exhibited potent and quick initial action against *B. tabaci* and *A. gossypii* during summer plantation of cucumber in Nubarya district, El-Beheira Governorate, Egypt (Barrania et al., 2019). Nevertheless, sulfoxaflor and flonicamid might be alternatively used in regions that characterized with relatively high resistance insect pests to other conventional insecticides. Thus, the rotational use of sulfoxaflor and flonicamid were quiet effective for controlling populations of jassid, Amrasca devastans collected from different locations on okra and cotton in Southern Punjab, Pakistan (Abbas et al., 2018).

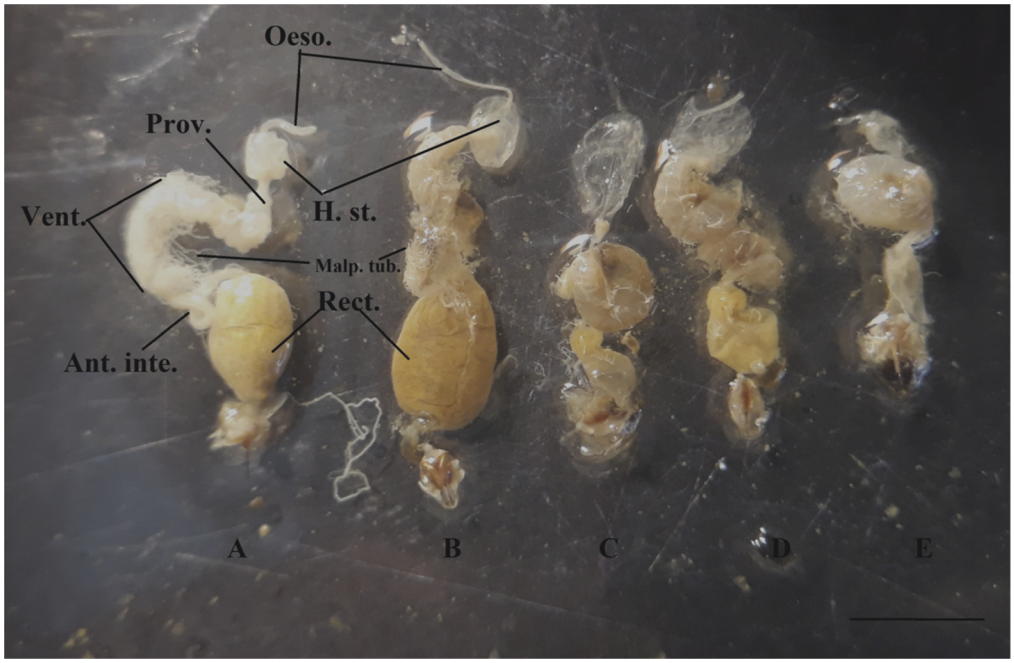


Fig. 6. A, Normal digestive tract and B, C, D, E, Malformed digestive tracts in adults of honeybees exposed to LC_{50} of flonicamid at 7, 4, 2 and 0 DAT, respectively. Oeso.: Oesophagus, H.st.: Honey stomach, Prov: Proventriculus, Vent.: Ventriculus, Malp. tub.: Malpighan tubules, Antr. Inte: Antrerior intestine, Rect.: Rectum. (Bar = 50 mm)

Meantime, the data of our semi-field trials in both seasons concerning the LT1s of the tested insecticides, could express the full withhold protection times against *A. gossypii* (LS). It refers to the estimated time where the residual efficacy of the tested insecticides is mostly dissipated. Median LT1s of sulfoxaflor approximately served in our study 4–7 weeks while Ghafoor et al. (2019) estimated the median LT50 of sulfoxaflor to be 31.67 days against mealybug Drosicha mangiferae at 2nd instar nymphs. In addition, our study showed that median LT1 of flonicamid served 5–7 weeks which apparently controversial to the short-termed time of medium LT50 at range of 2.33–3.83 days on brown planthopper, Nilaparvata lugens (Mizhu, 2018).

On the other hand, data of our laboratory trials, which simulate the field exposure on foraging honeybees (~21 days old) to sulfoxaflor and flonicamd during the same flowering time of summer squash plants in seasons 2020 and 2021. Sulfoxaflor had higher toxic effects than flonicamid at the first 5 h. Both insecticides had gradual declinations in their toxic effects and showed steep declines by the 9th DAT. Furthermore, the data of overall mean of mortality percentages along the 9 DAT for sulfoxaflor were significantly higher than those in flonicamid. These data were confirmed by the similar studies showed severe toxicity on honeybees when exposed to sulfoxaflor at the first 6 h (Chakrabarti et al., 2020). Flonicamid showed a slight toxic effect and selectivity on adults of honeybees (Thomazoni et al., 2009). Meantime, our estimated of LT1s during the two seasons on *A. mellifera* might express the safe pre-flowering intervals

that help in setting the right decision for foliar spray stoppage intervals of the tested insecticides. In addition, the overall mean of mortality percentages of both treatments were classified by their insignificant effects and met the safety limits of IOBC/West Palaearctic Regional Section (WPRS). Therefore, LT1s of sulfoxaflor on honeybees might suggest a median banned period of 3 or 4 weeks prior to flower stage. These data were confirmed by Jiang et al. (2020) who proved that a contact and dietary exposure to treated cotton plant with sulfoxaflor before or during flowering exhibited higher risk to honeybees. Therefore, new recommendation for sulfoxaflor application via drip irrigation on cotton plant before and during flowering realized significant eliminations in sulfoxaflor residues on pollen and nectar. Moreover, the safety period of sulfoxaflor was prescribed in a few EU member states within 5–6 days prior to bloom period (Li et al., 2021; Tamburini et al., 2021). Furthermore, our study estimated LT1 of flonicamid on honeybees that might suggest a median banned period of 10.94 days prior to follower stage, which was relatively shorter than sulfoxaflor. Similar findings were manifested by the tunnel tests and field studies on honeybees exposed to flonicamid that exhibited short-termed toxic effect on foraging bees and minimal effect on non-foraging bees (Australian Pesticides and Veterinary Medicines Authority (APVMA), 2014).

Finally, our research accomplished dissection studies on digestive tracts of survival foraging honeybees (~21 days old) exposed to sulfoxaflor and flonicamid at their values of LD50 for 24 h. Both insecticides showed steep dwarfing signs appeared in the digestive tract at the first 5 h of exposure. Distinct damages in honey stomach, ventriculus and rectum of digestive parts could be realized at 2 DAT. Malformations significantly subside at 4 DAT and seem to be neglected at the 7th DAT compared to control treatment. These malformations may be justified by the fact that flonicamid have anti-feeding activity inhibits insect stylet to attach to the plant for feeding and subsequently suffer from starvation then died within few days (Morita et al., 2007; Cho et al., 2011). On the other hand, the malformed digestive tracts that caused by sulfoxaflor could emulate role of neonicotinoids. This assumption based on the fact that sulfoxaflor likewise neonicotinoids could target nicotinic acetylcholine receptor which could be expressed by epithelial tissue of the mid-gut of insects. For instance, imidacloprid could interfere with the differentiation of regenerative cells beside reduction in numbers of digestive and endocrine cells in mid-gut epithelium of the 4th instar larvae of the mosquito *Stegomyia aegypti* (Insecticide Resistance Action Committee (IRAC) International MoA Working Group, 2020; Fernandes et al., 2015).

CONCLUSION

According to the given data of semi-field trials in both seasons, we could recommend sulfoxaflor and flonicamid as novel insecticides own potent toxic effects against cotton aphid, *A. gossypii* (LS). In general, the two tested insecticides had equipollent overall toxic effects on cotton aphid. Both tested insecticides had the strongest toxicity during the first 2 DAT and followed by significant declines from 7 up to 9 DAT. The LT1 values against *A. gossypii* (LS) for sulfoxaflor could refer to median withhold periods ranged between 4 and 7 weeks as well as flonicamid ranged between 5 and 7 weeks. On the other hand, toxicity tests in laboratory on *A. mellifera* showed that the survived foraging honeybees exposed to oral LD50s of the tested insecticides had the most critical impacts associated with malformed digestive tracts, which soon vanished

by the end of 7th DAT. Moreover, simulated trials in laboratory for field exposures of both tested insecticides against foraging *A. mellifera* along the 9 DAT could realize insignificant effects according to the safety limits of IOBC/WPRS. Based on LT1s on foraging honeybees, we might suggest permissive pre-flowering intervals for foliar treatments stoppages that might confirm more safety limits alongside with IOBC classification. Thus, banned period prior to follower stage might be 3 or 4 weeks for sulfoxaflor and 10.94 days for flonicamid. Therefore, flonicamid might consider being safer and more applicable than sulfoxaflor during bee foraging time.

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