Toxic evaluation of Proclaim Fit ® on honey bee workers: lethal and sublethal effects

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Abstract

Impacts to honey bees due to exposure to agricultural pesticides is one of the most serious threats to the beekeeping industry. Our research evaluated the lethal and sublethal toxicity of Lufenuron+Emamectin benzoate (Proclaim Fit®) on the European honey bee *Apis mellifera* L. at field-realistic (worst-case) dose. Newly emerged (≤ 24-h old) and forager (unknown age) worker bees were treated with the field recommended dose of Proclaim Fit® using three routes of exposure including residual contact, oral, and spray within the laboratory. We also assessed the sublethal effects of Proclaim Fit® on the specific activity of some well-known detoxifying enzymes including α-esterase, β-esterase, and Glutathione S-transferase (GST) in the honey bees. In addition, toxicity of the insecticide was tested on 4th instar larvae within the hive. Based on estimated median survival times (MSTs), Proclaim Fit® was highly toxic to the bees, especially when applied as spray. According to hazard ratios, newly emerged bees were 1.47× and 2.78× more susceptible than foragers to Proclaim Fit® applied as residual contact and oral, respectively. Enzyme assays revealed the considerable involvement of the enzymes, especially GST and α-esterase, in detoxification of the Proclaim Fit®, but their activities were significantly influenced by route of exposure and age of bee. Notably, Proclaim Fit® was highly toxic to 4th instar honey bee larvae. Our results generally indicate a potent toxicity of Proclaim Fit® toward honey bees. Therefore, its application requires serious consideration and adherence to strict guidelines, especially during the flowering time of crops.

Introduction

Pesticides, including insecticides, acaricides, fungicides, herbicides, etc. are an integral part of modern agriculture and plant protection. Upon the report of Iran Plant Protection Organization (IPPO), approximately 35,000 tons of pesticides are used annually to control different species of insects, mites, fungi, weeds, etc.

Although pesticide manufacturers aim to produce effective compounds with low toxicity on ecologically-important animals such as the honey bee (*Apis mellifera* L.), commercial beekeepers report losses of colonies coinciding with pesticide applications on blooming crops that are visited by forager bees (Ricke et al. 2021). Although honey bees are faced with a myriad of biotic stressors (Boncristiani et al. 2021), abiotic ones like insecticides are often considered one of the main contributors to honey bee colony losses worldwide (Goulson et al. 2015; Sanchez-Bayo et al. 2016; Singla et al. 2021). This has made honey bee toxicology a top priority item for the researchers throughout the world.

When studying the effects of a pesticide towards honey bees, dose-mortality bioassays is one way to evaluate the toxicity. However, assessing their sublethal effects on the physiology, behavior, and longevity of the bees, as well as health of the whole colony provide a more comprehensive understanding (Desneux et al. 2007). Honey bees employ numerous detoxifying enzymes to rid themselves of xenobiotics (du Rand et al. 2015; Gong and Diao 2017). Esterases (ESTs) are the enzymes involved in Phase I xenobiotic detoxification reactions that degrade toxins via hydrolysis (Xu et al. 2013). These hydrolytic enzymes are

divided into alpha (α) and beta (β) esterases depending on their ability to hydrolyze the substrates alpha- and beta-naphthyl, respectively (Dahan-Moss and Koekemoer 2016). Glutathione *S*-transferases (GSTs), as the major conjugating enzymes, mainly contribute in Phase II detoxification by catalyzing the conjugation of oxidation/reduction products of Phase I with glutathione (GSH) for solubilization and transport (Xu et al. 2013; Berenbaum and Johnson 2015).

Proclaim Fit® is manufactured by Syngenta® Crop Protection Company and is a combination of two potent insecticides with contact and oral toxic effects. It contains 40% Lufenuron, which acts as a chitin synthesis inhibitor, and 10% Emamectin benzoate, which is classified as an avermectin, stimulating the release of GABA in the insect nervous system. This insecticide was registered on the list of authorized insecticides of IPPO in 2015 and is only recommended for the control of *Tuta absoluta* Meyrick on tomato fields. According to the safety data sheet provided by Syngenta®, Proclaim Fit® is highly toxic to the honey bees exposed to direct treatment or residue on blooming crops or weeds.

Recente policies of the IPPO call for elimination of high-risk pesticides to bees and the extension of using more eco-friendly compounds. This encouraged us to launch several projects assessing the lethal and sublethal effects of newly registered pesticides on honey bees. The present study was undertaken with the objective to (1) determine the residual contact, oral, and spray toxicity of the field-realistic dose of Proclaim Fit® on newly emerged and forager worker bees, (2) assess the sublethal effects of this insecticide on the specific activity of some important detoxifying enzymes, and (3) test the acute toxicity of the insecticide on 4th instar honey bee larvae within the hive.

Materials And Methods

Honey bees

Honey bees were obtained from healthy and queen-right colonies of *Apis mellifera* L. maintained in the apiary of Higher Education Complex of Shirvan, North Khorasan, Iran (N37°26'04"; E57°45'14"; 1067 m a.s.l.) during June to August, 2018. To obtain newly emerged worker bees (≤ 24-h old), frames of mature pupa of relatively homogeneous age were taken from different colonies and put in a wooden box with mesh walls and transferred to an incubator at controlled conditions (32 ± 2 °C; 60 ± 5% relative humidity; darkness). The newly emerged bees were collected every 24 h. To obtain forager bees, hive entrances were closed for 10 min so that the returning bees of unknown ages accumulated at the hive entrance. Then, they were gently brushed into a wooden hoarding cage and transported to the laboratory for testing.

Insecticides

Formulated insecticides lufenuron+emamectin benzoate (Proclaim Fit®, WG 50%, Syngenta®/Switzerland) as our target insecticide and dimethoate (Rexion®, EC 40%, Hunan Haili/China)

as the toxic reference were purchased from a local agricultural chemical supplier in Iran. Water was used as the solvent as per application procedures of these formulations.

Toxicological bioassays

Toxicity of the field-realistic dose (worst-case scenario) of Proclaim Fit® (250 µg/ml) and Rexion® (dimethoate: 1000 µg/ml) towards newly emerged and forager bees were assessed using three routes of exposure as follows:

Residual contact toxicity

One ml of the insecticides' solutions at the field-realistic was placed on filter papers (Whatman No. 1, 13×11 cm) and left to dry for 30 min. The dried filter papers were attached to the bottom of the plastic disposable dishes (13×11×5 cm). Newly emerged or forager bees were randomly allocated to separate dishes in groups of 20 or 10 bees, respectively. Each round of testing included dishes containing 20 newly emerged bees or 10 forager bees treated with the solvent (water). Untreated newly emerged and forager bees were considered as our negative control. Sugar syrup (50% w/v) was provided *ad libitum* to all the bees during the exposure. Four replicates were considered for each treatment.

Oral toxicity

Twenty newly emerged bees and 10 forager bees were randomly allocated to separate dishes and starved for 1 h before the start of the tests. Then bees were allowed *ad libitum* access to 200 µl of sugar syrup (50% w/v) containing the test insecticides at the field-realistic dose for the subsequent 4 h. Afterwards, the insecticide-contaminated sugar syrup was removed from the dishes and the amount of syrup consumption was recorded. The bees were fed with insecticide-free sugar syrup for the rest of the experiment. Control bees received only sugar syrup as the solvent for whole of the treatment period. Each treatment was replicated four times.

Spray toxicity

In this experiment, a hand-calibrated sprayer was used to simulate direct exposure that could happen to honey bees in a field actively being treated with the insecticides. Twenty newly emerged bees and 10 foragers were randomly placed in separate dishes and kept in -20 °C for 1-3 min to cold anesthetize them (Human et al. 2013). Immediately, the bees in the dishes were sprayed with 500 µl of the insecticide solutions at the field-realistic dose. Bees sprayed with water were our solvent control bees and unsprayed ones were the negative control. All the bees were fed with sugar syrup during the tests. Tests were replicated four times.

All bioassays were conducted using a completely randomized design and carried out at 32 ± 2 °C and 65 ± 5% R.H. in darkness. Bee mortality was recorded at intervals of 2 hours and the dead bees were removed from the dishes. When no leg or antennal movements were observed upon prodding, the bees were considered dead. The tests lasted for 24 h.

Enzyme assays

Same experiment with three replications for each treatment and exposure route was concurrently conducted with the toxicity bioassays, in which upon reaching 50% mortality in newly emerged and forages bees exposed to the insecticides, the surviving individuals were taken from all treatment and control dishes and kept at -20 °C. The frozen bees were homogenized in 200 µl phosphate buffer (20 mM; pH 7.0) containing 0.1% Triton X-100. The homogenates were centrifuged (12,000×g for 15 min at 4 °C) and the supernatants were used for the enzyme assays. All tests were repeated three times.

For general esterases (ESTs), alpha-naphthyl acetate (α-NA) and beta-naphthyl acetate (β-NA) were used as substrates and the naphthol production was monitored by measuring absorbance at 450 and 540 nm, respectively, for α-NA and β-NA using the same microplate reader as a kinetic mode (Van Asperen 1962). The specific activity of glutathione *S*-transferase (GST) was determined based on the method of Habig et al. (1974) using CDNB (1-Chloro-2,4-dinitrobenzene) as substrate. Increases in absorbance were recorded at 360 nm using a microplate reader (Awareness Technology Stat Fax 3200®).

Larval toxicity

Acute toxicity of the insecticides to honey bee larvae were tested according to the method described by Gashout and Guzmán-Novoa (2009) with some modifications. One of the colonies used as the source of worker bees was selected for the tests with larvae. To obtain eggs with the same age, the queen of the colony was confined on a comb with empty cells for 24 h using a plastic excluder. Once the number of the eggs was sufficient, the queen was released and the comb containing eggs was left in the excluder for 6 further days until the emergence of 4^{th} instar larvae. The comb containing larvae was taken out of the hive and four cohorts of twenty 4^{th} larval instar was marked by pins of the same color for each treatment. Ten µl of the field-realistic dose of the insecticides prepared in sugar syrup (50% w/v) was applied at the bottom of each test cell, on which the larvae were laying. Two concurrent controls were used during the tests. Solvent control larvae were treated with 10 µl of insecticide-free sugar syrup and negative controls were left untreated. The comb was returned to the colony from which it had been taken. The cells were examined for larvae survival 3 days post treatment. The capped brood cells were considered alive, while the empty cells or those containing non-active or brown-colored larvae were considered dead. This experiment was conducted using a completely randomized design with four replications for each treatment.

Data analyses

Survival analysis was performed using the nonparametric method of Kaplan-Meier within GraphPad Prism ver. 8.0.2. Survival curves were compared using the Log-rank (Mantel-Cox) test. Enzyme assays data were analyzed by one-way ANOVA and the means were separated with Tukey's test ($P< 0.05$). Three-way ANOVA was used to assess the interaction effects of the treatments, the exposure routes, and age of the bees on the enzymes' activities. Statistical comparison between the enzymes' activity in newly emerged bees and forager bees was analyzed with independent-samples *t* test. Data were analyzed using

IBM SPSS ver. 24.0 software program (SPSS Science, Chicago, IL, USA). All data were expressed as mean ± S.E.

Results

Bioassays

Residual contact toxicity

The Log-rank test results showed that the treatments have significantly different residual contact toxicity to newly emerged bees (χ^2= 394.1; *df*= 3; *P*< 0.0001) (Fig. 1A) and foragers (χ^2= 249.6; *df*= 3; *P*< 0.0001) (Fig. 1B). The median survival time (MST) for newly emerged bees after contact with the residue of Proclaim Fit® and dimethoate occurred at 14 and 12 h, respectively, while the MST for the solvent and negative control bees was undefined (the curve never crossed the 50% survival probability line) (Fig. 1A). The MST for forager bees treated with the residue of Proclaim Fit® and dimethoate achieved after 14 and 6 h exposure, respectively (Fig. 1B).

Oral toxicity

The Log-rank test results showed that the treatments have significantly different oral toxicity to newly emerged bees (χ^2= 396.1; *df*= 3; *P*< 0.0001) (Fig. 2A) and foragers (χ^2= 208.8; *df*= 3; *P*< 0.0001) (Fig. 2B). The median survival time (MST) for newly emerged bees after oral exposure to Proclaim Fit® and dimethoate occurred at 12 and 16 h, respectively, while the MST for the solvent and negative control bees was undefined (Fig. 2A). The MST for forager bees orally exposed to Proclaim Fit® and dimethoate achieved after 17 and 4 h exposure, respectively (Fig. 2B).

Spray toxicity

The Log-rank test results showed that the treatments have significantly different spray toxicity to newly emerged bees (χ^2= 353.6; *df*= 3; *P*< 0.0001) (Fig. 3A) and foragers (χ^2= 170.5; *df*= 3; *P*< 0.0001) (Fig. 3B). The median survival time (MST) for newly emerged bees exposed to Proclaim Fit® and dimethoate as spray occurred at 4 h, while the MST for the solvent and negative control bees was undefined (Fig. 3A). The MST for forager bees exposed to Proclaim Fit® and dimethoate as spray achieved after 5 and 4 h exposure, respectively (Fig. 3B).

Susceptibility of newly emerged bees versus foragers to Proclaim Fit®

Based on estimated hazard ratio, newly emerged bees were 1.47 (95% CI= 1.02-2.12) and 2.78 (95% CI= 1.90-4.05) times more susceptible than foragers to Proclaim Fit® applied as residual contact (χ^2= 6.76; *df*= 1; *P*< 0.0093) and oral (χ^2= 35.86; *df*= 1; *P*< 0.0001), respectively. Spray toxicity of this insecticide for newly emerged bees was 1.08 (95% CI= 0.74-1.57) times of that for forages which was not significantly different (χ^2= 0.06; *df*= 1; *P*< 0.8023).

Changes in the detoxifying enzymes activities

α-esterase activity

The interaction effects of the treatments, the exposure routes, and age of the bees on α-esterase activity was significant (F= 41.42; $df_{t,e}$= 5,44; P 0.001). Contact exposure to the residue of Proclaim Fit® and dimethoate significantly decreased the activity of α-esterase in newly emerged bees rather than the solvent and negative controls (F= 20.75; $df_{t,e}$= 3,8; P< 0.001) (Fig. 4A). The activity of this enzyme in newly emerged bees orally exposed to Proclaim Fit® was not different from that to solvent control, but was significantly higher than that to dimethoate (F= 43.85; $df_{t,e}$= 2,6; P< 0.001) (Fig. 4B). Proclaim Fit® significantly increased α-esterase activity in newly emerged bees in comparison with the rest of the treatments when applied via spray (F= 56.69; $df_{t,e}$= 3,8; P< 0.001) (Fig. 4C). This enzyme showed highest activity when forager bees were exposed to Proclaim Fit® than the rest of the treatments via residual contact (F= 65.60; $df_{t,e}$= 3,8; P< 0.001) and oral (F= 5.63; $df_{t,e}$= 2,6; P< 0.042) (Fig. 4A and B). The activity of α-esterase in forager bees spray-exposed to Proclaim Fit® was not different from that to the solvent and negative controls, but was significantly lower than that to dimethoate (F= 13.05; $df_{t,e}$= 2,6; P< 0.002) (Fig. 4C). The comparison of α-esterase activity in newly emerged versus forager bees after being exposed to the test treatments via different routes of exposure is presented in Fig 4.

β-esterase activity

The activity of β-esterase was significantly influenced by the interaction effects of the treatments, the exposure routes, and age of the bees (F= 3.54; $df_{t,e}$= 5,44; P 0.009). The activity of this enzyme in newly emerged bees exposed to the residue of Proclaim Fit® did not differ from that of controls, but was significantly higher than that of dimethoate (F= 9.43; $df_{t,e}$= 3,8; P< 0.005) (Fig. 5A). This enzyme showed similar activities when newly emerged bees were exposed to the test treatments orally (F= 0.14; $df_{t,e}$= 2,6; P< 0.870) and via spray (F= 2.00; $df\ t,e$= 3,8; P< 0.193) (Fig. 5B and C). In foragers, β-esterase was more active when the bees were exposed to the residue of Proclaim Fit® than other treatments (F= 4.90; $df_{t,e}$= 3,8; P< 0.032) (Fig. 5A). The activity of this enzyme in the forager bees orally exposed to Proclaim Fit® was statistically equal to that to the dimethoate, but was significantly higher than that to the solvent (F= 9.50; $df_{t,e}$= 2,6; P< 0.014) (Fig. 5B). The activity of β-esterase in forager bees spray-exposed to Proclaim Fit® was not different from that to the rest of the treatments (F= 2.83; $df_{t,e}$= 3,8; P< 0.106) (Fig. 5C). The comparison of β-esterase activity in newly emerged versus forager bees after exposure to the test treatments via different routes of exposure is presented in Fig 5.

GST activity

The activity of GST was significantly affected by the interaction effects of the treatments, the exposure routes, and age of the bees (F= 49.77; $df_{t,e}$= 5,44; P 0.001). Proclaim Fit® significantly induced GST activity in newly emerged bees in comparison with the rest of the treatments when applied as residual

contact (F= 72.49; $df_{t,e}$= 3,8; P< 0.001) and spray (F= 35.08; $df_{t,e}$= 3,8; P< 0.001) (Fig. 6A and C). This enzyme showed the lowest activity when newly emerged bees were orally exposed to Proclaim Fit® than dimethoate and solvent control (F= 35.15; $df_{t,e}$= 2,6; P< 0.001) (Fig. 6B). In case of foragers, the activity of GST in Proclaim Fit®-treated bees was significantly higher than that in control bees when exposed as residual contact (F= 45.40; $df_{t,e}$= 3,8; P< 0.001) and oral (F= 43.55; $df_{t,e}$= 2,6; P< 0.001) (Fig. 6A and B). The activity of this enzyme in the forager bees exposed to Proclaim Fit® via spray did not differ from that to dimethoate, but was significantly lower than that in control bees (F= 12.53; $df_{t,e}$= 3,8; P< 0.002) (Fig. 6C). Fig 6. also shows the comparison of GST activity between newly emerged and forager bees after exposure to the test treatments via different routes of exposure.

Since the interactions of exposure route, bee age, and type of enzyme were significant (F= 39.12; $df_{t,e}$= 4, 36; P 0.001), determining which enzyme is more involved in detoxification of Proclaim Fit® depends on how and at what age the bees are exposed to this insecticide. Nonetheless, it seems that GST and α-esterase are more likely to be induced than β-esterase when Proclaim Fit® molecules entered into the bees' body.

Toxicity to worker bee larvae

The acute toxicity of the field-realistic dose of the treatments on honey bee larvae results clearly indicate that Proclaim Fit® (86.25% mortality) and dimethoate (93.75% mortality) are highly toxic to the honey bee larvae compared to the controls (F= 130.00, $df_{t,e}$= 3,15, P< 0.001) (Fig. 7).

Discussion

Forager bees are on the frontlines of exposure to pesticides. If they survive a severe toxic exposure, they can take pesticide-contaminated pollen or nectar back to the colony and potentially affect the bees and brood inside the hive. Herein, we assessed the toxicity of different routes of exposure to the field-realistic dose of Proclaim Fit® for newly emerged and forager worker bees under laboratory conditions. In agreement with the manufacturing company report, we provided significant evidence that formulated Proclaim Fit® is highly toxic to the bees. It is also noteworthy that the information presented in the data safety sheet of Proclaim Fit® is exclusively for its active ingredients, whereas in the present study we worked on its commercial formulation. It has been reported that the toxicity of the formulations to honey bees is often greater than that of the corresponding active ingredients, because the initial toxic property of the active ingredients are enhanced by adding inert materials during the formulation process (Zhu et al. 2014; Badawy et al. 2015; Mullin et al. 2016; Han et al. 2018). In the present study, we did not compare the toxicity of formulated Proclaim Fit® against the technical-grade lufenuron+amamectine benzoate on the honey bee, but we hypothesize that the toxicity of formulated Proclaim Fit® may be beyond that which is reported by the manufacturer. This is demonstrated by our bioassay results that showed the toxicity of Proclaim Fit® is comparable with that of dimethoate, which is one of the most toxic compounds to honey bees (Dai et al. 2019).

Relatively little is known about how the susceptibility of honey bees to xenobiotics change with age. Rinkevich et al. (2015) showed that as honey bees aged, the susceptibility to phenothrin significantly decreased, but the sensitivity to naled significantly increased. In another study, it was found that younger bees (7-day old) exhibited lower mortality than older bees (42-day old) in response to five individual insecticides belonging to five different insecticide classes (Zhu et al. 2020). In the present research, we hypothesized that forager bees would be more tolerant than newly emerged bees to Proclaim Fit®, but our findings indicated that sensitivity of the honey bees to Proclaim Fit® was dependent on both the route of exposure and the age of the bees. Newly emerged bees were as susceptible as foragers to Proclaim Fit® applied as spray, whereas this insecticide was more toxic to newly emerged bees than foragers when administrated as contact and oral.

It was observed that the enzymes activities varied significantly with the type of the treatments and the age of bees. ESTs are less involved in the detoxification of insecticides in honey bees (Gong and Diao 2017). In our study, the activity of α-esterase in foragers exposed to Proclaim Fit® as residual contact and oral was significantly higher than that to dimethoate and controls. In newly emerged bees, only spray application of Proclaim Fit® caused significant increase in α-esterase activity rather than other treatments. β-esterase was not as much as α-esterase induced by Proclaim Fit®. Han et al. (2019) reported that acetamiprid and propiconazole, alone or combination, significantly increased the GST activity in honey bee foragers after one day oral exposure. They found no differences between the GST activities of the treated newly emerged bees and the controls. In a recent study, Zhu et al. (2020) observed that the correlation between the GST activity and honey bee age was not significant. We found that this enzyme was 1.4× and 4.8× more active in foragers than newly emerged bees when Proclaim Fit® administrated as residual contact and oral, respectively. Thus, the greater tolerance of foragers than newly emerged bees to oral exposure to Proclaim Fit® could be linked to more activation of the detoxifying enzymes, especially GST. It could be concluded that the variations in the enzyme activities is likely due to the differences in the physiology of newly emerged and forager bees.

It is important to note that in the present study honey bees were kept in the laboratory and exposed to the field recommended dose of the treatments. While Proclaim Fit® showed outstanding toxicity to the honey bee adults and larvae, this is certainly unrealistic of normal field exposure, as honey bees, especially those inside the hive, are less likely to directly encounter the drops of pesticides as long as the products are applied according to the label.

As previously mentioned, the usage of Proclaim Fit® in Iran is restricted to the control of *T. absoluta* in tomato fields. Tomatoes are not commercially pollinated by honey bees, as the pollen is enclosed inside the anthers and the flowers do not provide much nectar reward; thus, providing very little incentive for honey bee visits (Bispo dos Santos et al. 2009). Therefore, it is less likely that honey bees would be directly exposed to pesticides in tomato fields. Nevertheless, the possibility of spraying on any honey bee-friendly plants, especially weeds, that are close to tomato fields should be considered. Since other bees are used to pollinate tomato crops (Cooley and Vallejo-Marín 2021), given the high toxicity of Proclaim

Fit® to honey bees, future testing should include that of other bees found pollinating tomato crops. We hope our findings provide a comprehensive and robust support for the IPPO, Iran's Department of Environment, and other decision makers to take better strategies to protect honey bee colonies from harmful pesticides.

Declarations

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Authors Contribution The authors contributed equally to this work.

Conflict of interest The authors declare that they have no conflict of interest.

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Figures

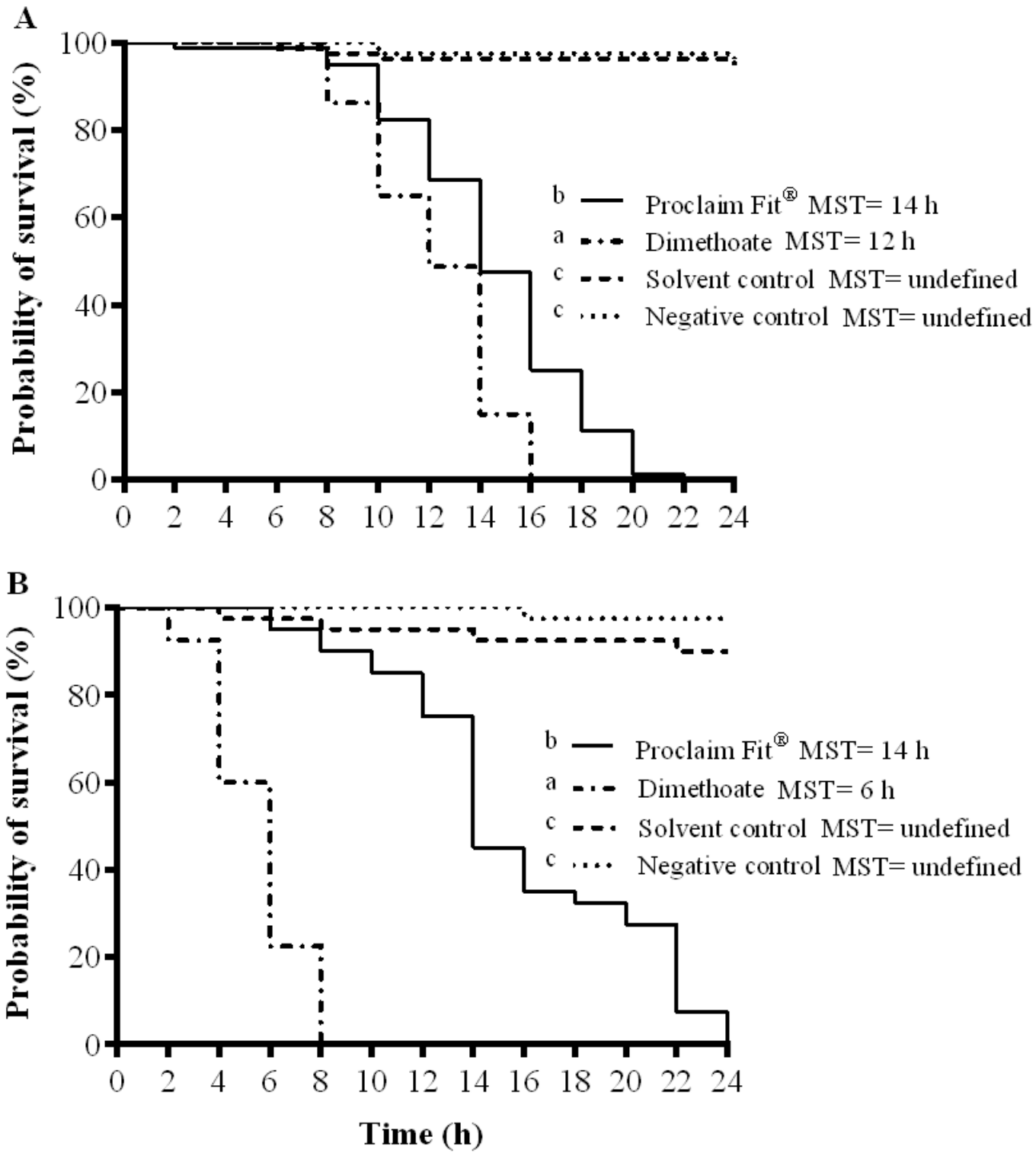

Figure 1

Survival curves from h0 to h24 and the median survival time (MST) of newly emerged (A) and forager (B) bees exposed to Proclaim Fit®, dimethoate, solvent control, and negative control applied as residual contact. Legends with different letters indicate significant differences at $P \leq 0.05$, Log-rank (Mantel-Cox) test.

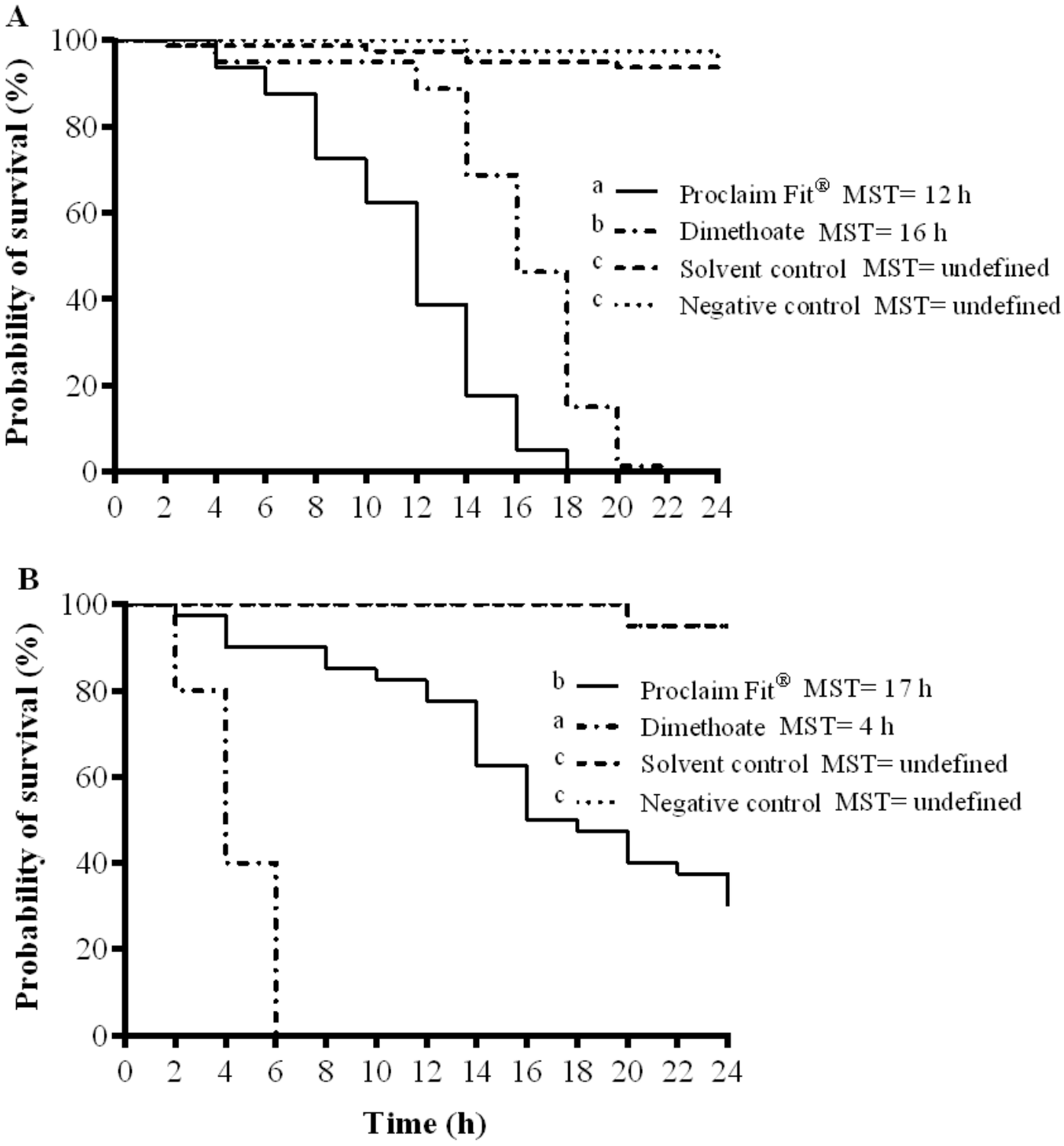

Figure 2

Survival curves from h0 to h24 and the median survival time (MST) of newly emerged (A) and forager (B) bees exposed to Proclaim Fit®, dimethoate, solvent control, and negative control applied as oral. Legends with different letters indicate significant differences at $P \leq 0.05$, Log-rank (Mantel-Cox) test.

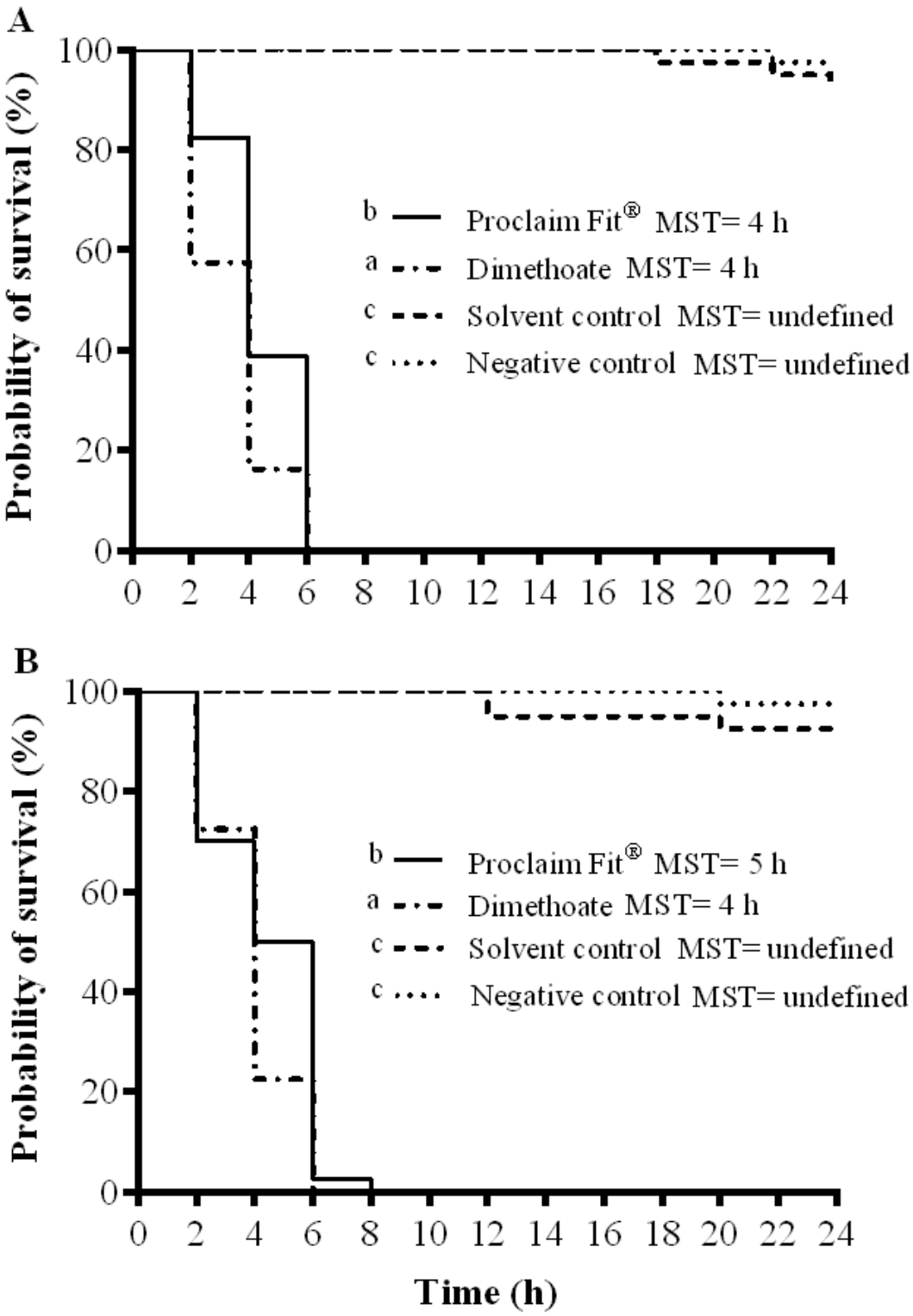

Figure 3

Survival curves from h0 to h24 and the median survival time (MST) of newly emerged (A) and forager (B) bees exposed to Proclaim Fit®, dimethoate, solvent control, and negative control applied as spray. Legends with different letters indicate significant differences at $P \leq 0.05$, Log-rank (Mantel-Cox) test.

Figure 4

Effect of Proclaim Fit®, dimethoate, solvent control, and negative control applied as residual contact (A), oral (B), and spray (C) on the α-esterase specific activity (mean±S.E.) in newly emerged and forager bees. Means followed by different letters (large letters for newly emerged bees and small ones for forager bees) indicate significant differences at $P \leq 0.05$, Tukey's test. Means of the enzyme activity in newly emerged and forager bees were compared pairwise by Student's *t*-test and their statistically differences are denoted as * ($P < 0.05$), ** ($P < 0.01$), and *ns* (no significant difference).

Figure 5

Effect of Proclaim Fit®, dimethoate, solvent control, and negative control applied as residual contact (A), oral (B), and spray (C) on the β-esterase specific activity (mean±S.E.) in newly emerged and forager bees. Means followed by different letters (large letters for newly emerged bees and small ones for forager bees) indicate significant differences at $P \leq 0.05$, Tukey's test. Means of the enzyme activity in newly emerged and forager bees were compared pairwise by Student's *t*-test and their statistically differences are denoted as * ($P < 0.05$), ** ($P < 0.01$), and *ns* (no significant difference).

Figure 6

Effect of Proclaim Fit®, dimethoate, solvent control, and negative control applied as residual contact (A), oral (B), and spray (C) on the glutathione S-transferase (GST) specific activity (mean±S.E.) in newly emerged and forager bees. Means followed by different letters (large letters for newly emerged bees and small ones for forager bees) indicate significant differences at $P \leq 0.05$, Tukey's test. Means of the enzyme activity in newly emerged and forager bees were compared pairwise by Student's *t*-test and their statistically differences are denoted as * ($P < 0.05$), ** ($P < 0.01$), and *ns* (no significant difference).

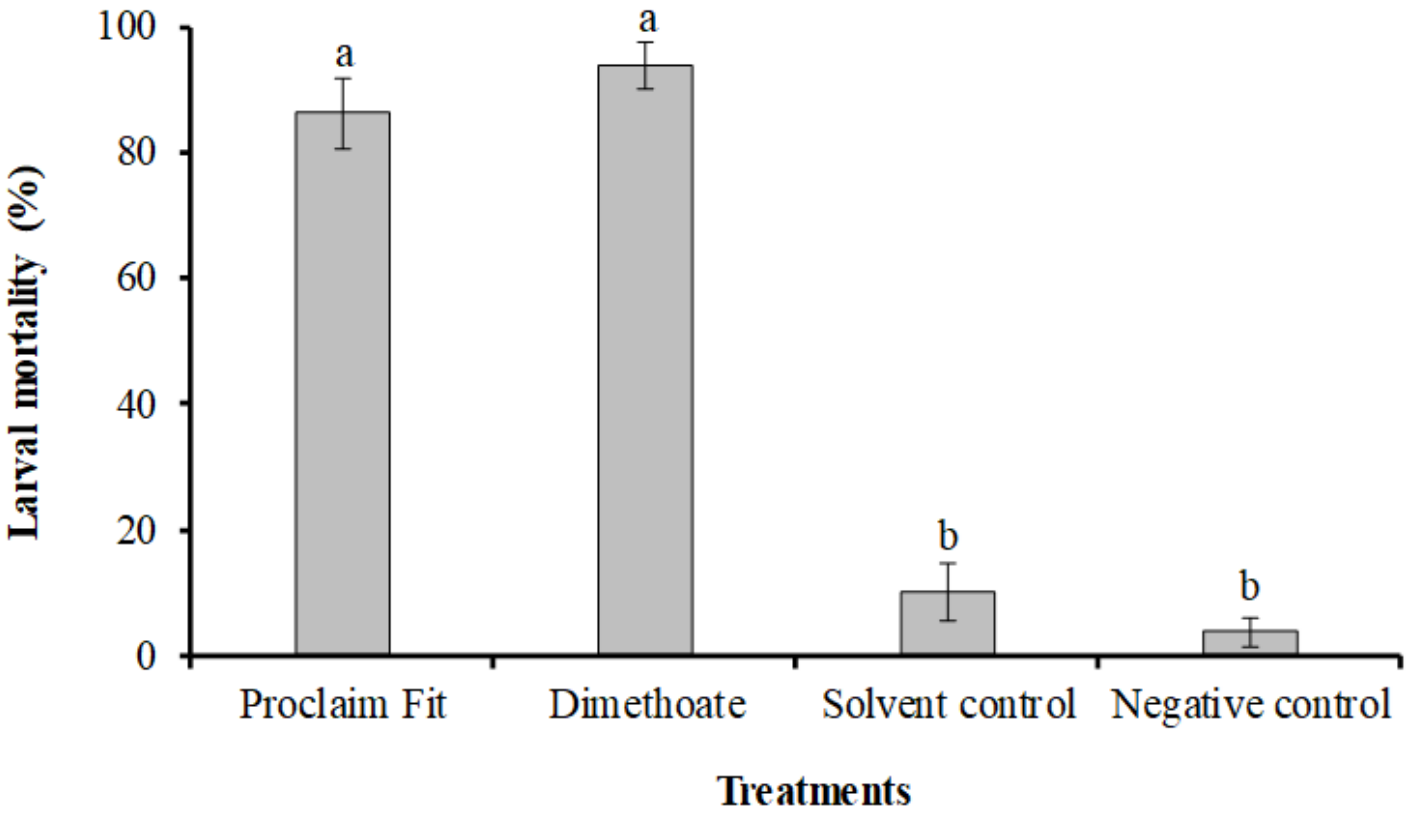

Figure 7

Percentage of mortality (mean±S.E.) of 4th instar honey bee larvae after exposure to Proclaim Fit®, dimethoate, solvent control, and negative control. Means followed by different letters indicate significant differences at *P* 0.05, Tukey's test.