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Effects of some insecticidal chemicals under laboratory condition on honeybees [*Apis mellifera* L. (Hymenoptera: Apidae)] that forage on onion flowers

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In Ethiopia, *Apis mellifera* L. are the most important honeybees to produce diverse bee products. In addition, bees also improve crop yields through their active and efficient pollination. However, they have been killed due to the misuse of various insecticides. For this reason this study was proposed to determine in the laboratory the level of toxicity of some insecticides used widely on honeybees foraging on onion flowers. Adult *A. mellifera* bandasii honeybees 21 to 24 days old were collected at the Adami Tullu Agricultural Research Center (ATARC) apiary. Easy to clean and well-ventilated 15 x 10 x 15 cm cages were used for feeding, contact and fumigation tests. Six insecticides: deltamethrin 2.5 EC, diazinon 60 EC, endosulfan 35 EC, lambda-cyhalothrin 5 EC, malathion 50 EC and profenofos 750 EC, were evaluated in the laboratory at the rates suggested for onion field spraying. Each treatment was replicated three times and arranged in a completely randomized design. All of the insecticides in the contact and feeding tests were toxic to honeybees and caused mortality within a few hours after exposure. In the fumigation test, profenofos did not cause honeybee mortality until the third hour, but the remaining insecticides caused varying mortality within 24 h.

Key words: Insecticides, toxicity, honeybee, bee products and crop yields.

INTRODUCTION

In Ethiopia, *Apis mellifera* races are the most economically important honeybees (Amssalu et al., 2004) which are used to produce diverse bee products, of which only honey and beeswax benefit small producers and the country as a whole. However, honeybees have been killed due to the misuse of various insecticides (MOWR, 2007). Reduction of honeybee colonies, which later result in reduction of bee products and crop yields are the major constraints of beekeeping in the country. This study was undertaken to determine the level of toxicity in the laboratory of some insecticides used widely, on honeybees that forage onion flowers.

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Table 1. Insecticides evaluated in laboratory toxicity tests on honeybees in the Adami Tullu District in 2012/2013.

Common name	Trade name	Rate (L/ha)	Spray volume (L)
Deltamethrin	Ethiodemethrin 2.5% EC	0.11	917
Diazinon	Ethiozinon 60% EC	0.50	1000
Endosulfan	Ethiosulfan 35% EC	0.20	1000
Lambda-cyhalothrin	Karate 5% EC	0.32	1000
Malathion	Ethiolathione 50% EC	0.20	1000
Profenofos	Selecron 750% EC	0.60	1200

MATERIALS AND METHODS

Adult *A. mellifera* bandasii bees 21 to 24 days old were collected in the evening from hives without brood at the Adami Tullu Agricultural Research Center (ATARC) apiary and set 5 min at 0°C in a refrigerator to reduce their mobility and aggressiveness. Wooden well-ventilated and easy to clean 15 x 10 x 15 cm cages were used to determine acute toxicity tests in feeding, fumigation and contact tests for deltamethrin, diazinon, endosulfan, lambda-cyhalothrin, malathion, and profenofos. Handling procedures, treatment and observations were conducted during day time at the commercial rates recommended for controlling thrips in onion plants (Table 1).

Feeding test

Twenty bees were placed in each of the test cages, which were placed randomly on a working table in the laboratory and after 2 h without food to homogenize their gut content they were exposed to the treatments and concentrations recommended. Only healthy bees were used. They were provided with 75 ml 50% honey solution in Petri dishes containing each of the aforementioned insecticide treatments. Insecticide toxicity was compared with that of 0.3 μg reference standard of the highly toxic dimethoate (Rogor) insecticide, and a separate control (bees fed on 50% honey solution only), with 3 replicates and a completely randomized design. Since bees did not survive 24 h after treatment, death losses were recorded at 0.5, 1, 2, 3, 4, 6, and 24 h after spraying (Amssalu, 2010).

Contact test (residual exposure method)

The experiment and normal feeding (that is, 50% honey) were set as that for the feeding test above. 9 cm diameter filter papers were immersed in 100 ml of solutions containing the recommended concentrations of the insecticides and later they were set to dry, and then they were attached to a wall of the cages, where 20 worker honeybees were introduced for exposure. Contact actions of each insecticide treatment were compared with the standard (0.15 μg dimethoate) and the control (paper immersed in distilled water only). Mortality losses of honeybees were recorded separately at 0.5, 1, 2, 3, 4, 6, and 24 h after exposure (Amssalu, 2010).

Fumigation test

The experiment set-up and normal feeding to bees (that is, 50% honey solution) were the same as for the feeding and contact tests. Glass tubes were filled with 10 ml of each treatment and were tapped with cotton wicks for slow diffusion, and placed into the test cages. The death rate was compared with 0.0009% of the standard toxic chemical dimethoate (Rogor) and the control (the tube filled with water). Fumigation action of each treatment was recorded through honeybee mortality at 0.5, 1, 2, 3, 4, 6, and 24 h after exposure, as in the other tests (Amssalu, 2010).

Data collection

Mortality (%)

Mortality of bees at each observation time was recorded for each treatment and compared with the standard toxic chemical and the control, and they were corrected by Abbott (1925):

$$\text{-\% of mortality: Correct mortality} = \frac{\text{\% mortality in treatment - \% mortality in control}}{\text{100- Mortality in control}} \times 100$$

Average insecticide mixed food consumption per g in the feeding test was recorded at the end.

Data analysis

Results were analyzed using SAS software version 9.1. The insecticide treatments were compared with the controls and the standard, and the 5% LSD test was used for mean separation whenever significant differences occurred. Before data analysis, data were revised for normality and when they were not normally distributed, they were transformed by $\sqrt{(x + 0.5)}$.

RESULTS AND DISCUSSION

Feeding test

Insecticides differed significantly ($p \leq 0.001$) in causing mortality to honeybees in the feeding test (Table 2). All of them caused significant bee mortality 0.5 h after exposure when compared with the control, that is, water. On the other hand, all the insecticides, except lambda-cyhalothrin, were not statistically different in bee mortality from the toxic standard dimethoate at 0.3 μg. Maximum honeybee mortality was recorded from bees fed on lambda-cyhalothrin (14.71%), followed by diazinon (11.54%). Also, all insecticides were toxic to honeybees 1 h after feeding exposure, although endosulfan (6.46%) was relatively less toxic.

Toxicity levels of all insecticides were not significantly different from the standard 2, 3, 4, and 6 h after feeding exposure, but they were significantly different from the

Table 2. Toxicity of insecticides to honeybees in a laboratory feeding test in 2012/2013.

Treatments	Corrected mortality of honeybees in percent (%)						
	0.5 h	1 h	2 h	3 h	4 h	6 h	24 h
Deltamethrin	8.14 (2.94)^{b}	9.55 (3.17)^{c}d	21.59 (4.70)^{a}	21.59 (4.70)^{ab}	19.75 (4.50)^{a}	15.02 (3.94)^{bc}	0.00 (0.71)d
Diazinon	11.54 (3.47)^{ab}	18.25 (4.33)^{a}	21.59 (4.70)^{a}	23.22 (4.87)^{a}	11.54 (3.47)^{bc}	11.54 (3.47)^{c}	0.00 (0.71)d
Dimethoate	5.70 (2.49)^{b}	13.26 (3.71)^{abc}	21.59 (4.70)^{a}	21.59 (4.70)^{ab}	16.56 (4.13)^{ab}	15.02 (3.94)^{bc}	0.00 (0.71)d
Endosulfan	5.70 (2.49)^{b}	6.47 (2.64)d	16.56 (4.13)^{b}	16.15 (4.08)^{ab}	14.71 (3.90)^{abc}	15.02 (3.94)^{bc}	13.86 (3.79)^{a}
Lambda-cyhalothrin	14.71 (3.90)^{a}	16.56 (4.13)^{ab}	20.02 (4.53)^{a}	14.71 (3.90)^{b}	9.99 (3.24)^{c}	18.25 (4.33)^{ab}	0.00 (0.71)d
Malathion	8.14 (2.94)^{b}	8.14 (2.94)^{c}d	20.02 (4.53)^{a}	19.93 (4.52)^{ab}	15.02 (3.94)^{ab}	19.75 (4.50)^{a}	5.26 (2.40)^{c}
Profenofos	8.14 (2.94)^{b}	11.53 (3.47)^{bc}	20.02 (4.53)^{a}	18.25 (4.33)^{ab}	11.54 (3.47)^{bc}	15.02 (3.94)^{bc}	12.17 (3.56)^{ab}
Control	0.00 (0.71)^{c}	0.00(0.71)e	0.00 (0.71)^{c}	0.00 (0.71)^{c}	0.00 (0.71)d	0.00 (0.71)d	7.79 (2.88)^{bc}
LSD (5%)	**0.88**	**0.81**	**0.37**	**0.82**	**0.69**	**0.54**	**0.71**
CV (%)	**17.6**	**14.7**	**5.4**	**11.7**	**11.5**	**8.6**	**21.1**

Means in parenthesis are data transformed by square root; those in the column followed by the same letter are not significantly different at the 5% probability level.

water-treated control. However, at 2 and 4 h after exposure, deltamethrin caused maximum mortality, while at the 3 and 6 h time period diazinon (23.22%) and malathion (19.75%) were markedly toxic to honeybees. In the water-treated control no mortality occurred throughout the test. However, after 24 h feeding exposure, higher mortality was recorded in endosulfan (13.86%), profenofos (12.17%), malathion (7.79%) and water fed (5.26%) honeybees. In the other treatments, the bees died before 24 h.

All the tested insecticides were toxic when fed to honeybees. Also, they exhibited some unusual behaviors, such as lack of coordination, trembling and tumbling. Our results agree with those of Atkins et al. (1981), who also found that ingestion of deltamethrin resulted in mortality and impaired learning ability in honeybees. Similarly, diazinon mixed in the food of the bees was extremely toxic when ingested. Gary and Mussen (1984) also reported that malathion caused significant mortality of honeybees in the laboratory. Kidd and James (1991) found that endosulfan is toxic to honeybees and profenofos remained highly toxic to them when tested orally. According to the USEPA (1988), lambda-cyhalothrin is highly toxic to bees when they are fed orally.

Exposure of honeybees to insecticides may result in acute mortality and sublethal effects. Data in Table 3 indicate that all insecticides mixed with honey were not readily accepted by honeybees. When they ingested insecticide poisoned food, they exhibited unusual behavior, like paralysis, and abnormal jerky and spinning movements. In contrast, honeybees fed normal food consumed more than those provided with insecticide tainted food. Bees fed with diazinon laced food consumed the lowest.

Contact test (residual exposure method)

All the insecticide treatments killed significantly ($p \leq 0.01$) more honeybees than the control (Table 4). Moreover, 0.5 h after exposure, all insecticides were not statistically different from the highly toxic standard dimethoate at 0.15 μg. The honeybees died at the same level (9.99%) when exposed to profenofos, endosulfan, lambda-cyhalothrin, and diazinon, whereas low mortality occurred equally (6.47%) in bees exposed to malathion and deltamethrin. However, 1, 2, 3 and 4 h after exposure, there were no significant differences ($p \geq 0.05$) between the toxic standard and all the insecticides tested.

An hour after exposure, minimum and maximum mortality occurred with profenofos (8.14%) and diazinon (13.26%), respectively. However, 6 h later, profenofos, endosulfan, diazinon, malathion, and deltamethrin caused significantly more honeybee deaths than the toxic standard dimethoate. At the 24 h exposure there were more dead bees with the water control than in those exposed to the insecticides. Bee mortality in the water control might be attributed to their exhaustion when they were trying to leave the cage. There were also some dead bees among those exposed to lambda-cyhalothrin, malathion, and deltamethrin. In the contact test all insecticides

Table 3. Effect of insecticide treatments on honeybee food consumption in the Adami Tullu Jido Kombolcha District in 2012/2013.

Insecticide treatments	Food ingested by honeybees (g)
Deltamethrin	1.48[c]
Diazinon	1.32[d]
Dimethoate	1.58[c]
Endosulfan	1.73[b]
Lambda-cyhalothrin	1.84[b]
Malathion	1.56[c]
Profenofos	1.74[b]
Control	5.33[a]
LSD (5%)	**0.15**
CV (%)	**4**

Means in the column with same letter are not significantly different at the 5% probability level.

Table 4. Residual effects of insecticides on honeybees in the laboratory in the Adami Tullu Jido Kombolcha District in 2012/2013.

Treatments	Corrected mortality of honeybees (%)						
	0.5 h	**1 h**	**2 h**	**3 h**	**4 h**	**6 h**	**24 h**
Deltamethrin	6.47 (2.64)[b]	8.14 (2.94)[b]	13.41(3.73)[a]	15.02 (3.94)[ab]	11.40 (3.45)[a]	18.51 (4.36)[ab]	7.40 (2.81)[b]
Diazinon	9.99 (3.24)[a]	13.26 (3.71)[a]	14.94 (3.93)[a]	15.02 (3.94)[ab]	9.80 (3.21)[a]	18.51 (4.36)[ab]	0.00 (0.71)[b]
Dimethoate	9.99 (3.24)[a]	8.14 (2.94)[b]	13.41 (3.73)[a]	16.56 (4.13)[a]	12.75 (3.64)[a]	13.41 (3.73)[c]	0.00 (0.71)[b]
Endosulfan	9.99 (3.24)[a]	8.14 (2.94)[b]	13.41 (3.73)[a]	15.02 (3.94)[ab]	9.80 (3.21)[a]	20.38 (4.57)[a]	0.00 (0.71)[b]
Lambda-cyhalothrin	9.99 (3.24)[a]	11.54 (3.47)[ab]	15.26 (3.97)[a]	13.26 (3.71)[b]	11.40 (3.45)[a]	13.41 (3.73)[c]	5.8 (2.51)[b]
Malathion	6.47 (2.64)[b]	8.14 (2.94)[b]	11.75 (3.50)[a]	15.02 (3.94)[ab]	12.75 (3.64)[a]	16.89 (4.17)[b]	8.14 (2.94)[b]
Profenofos 750	9.99 (3.24)[a]	8.14 (2.94)[b]	13.41 (3.73)[a]	15.02 (3.94)[ab]	12.75 (3.64)[a]	16.89 (4.17)[b]	0.00 (0.71)[b]
Control	0.00 (0.71)[c]	0.00 (0.71)[c]	1 .06 (1.25)[b]	0.00 (0.71)[c]	0.00 (0.71)[b]	0.00 (0.71)d	16.56 (4.13)[a]
LSD (5%)	**0.47**	**0.76**	**0.88**	**0.31**	**0.76**	**0.37**	**1.54**
CV (%)	**9.6**	**15.3**	**14.6**	**5.1**	**13.9**	**5.7**	**17.0**

Means in parenthesis are data transformed by square root; those in the column followed by the same letter are not significantly different at the 5% probability level.

were toxic to honeybees throughout the test hour. This result was the same in other studies (Hagler et al., 1989; Pilling, 1992), where diazinon and lambda-cyhalothrin were highly toxic to honeybees within 24 h on an acute contact basis. Endosulfan causes also acute contact toxicity to bees (WHO, 1998).

Tomlin (2006) found also malathion, deltamethrin, and profenofos highly toxic to honeybees in contact toxicity tests.

Fumigation test

The fumigation toxicity test yielded also highly significant ($p \leq 0.001$) mortality on honeybees (Table 5). Mortality due to deltamethrin (11.13%),

Table 5. Fumigation effects of insecticides on honeybee in the laboratory in the Adami Tullu Jido Kombolcha District in 2012/2013.

Treatments	Corrected mortality of honeybees in percent						
	0.5 h	1 h	2 h	3 h	4 h	6h	24h
Deltamethrin	11.13 (3.41)[ab]	8.62 (3.02)[ab]	17.45 (4.24)[a]	17.65 (4.26)[a]	17.65 (4.260)[a]	25.00 (5.05)[ab]	0.00 (0.71)[c]
Diazinon	8.92 (3.07)[b]	8.92 (3.07)[ab]	17.65 (4.26)[a]	19.93 (4.52)[a]	19.93 (4.52)[a]	25.00 (5.05)[ab]	0.00 (0.71)[c]
Dimethoate	5.34 (3.98)[a]	11.13 (3.41)[a]	19.93 (4.52)[a]	17.65 (4.26)[a]	15.34 (3.98)[ab]	22.44 (4.79)[b]	0.00 (0.71)[c]
Endosulfan	0.00 (0.71)d	5.26 (2.40)[b]	15.34 (3.98)[ab]	19.93 (4.52)[a]	15.34 (3.98)[ab]	26.44 (5.19)[ab]	0.00 (0.71)[c]
Lambda-cyhalothrin	7.00 (2.74)[b]	5.26 (2.40)[b]	15.34 (3.98)[ab]	15.34 (3.98)[a]	19.75 (4.50)[a]	25.00 (5.05)[ab]	4.65 (2.27)[b]
Malathion	2.89 (1.84)[c]	6.90 (2.72)[ab]	10.79 (3.36)[b]	15.34 (3.98)[a]	17.65 (4.26)[a]	27.69 (5.31)[a]	6.26 (2.60)[a]
Profenofos	0.00 (0.71)d	0.00 (0.71)[c]	0.00 (0.71)[c]	5.26 (2.40)[b]	11.13 (3.41)[b]	13.34 (3.98)[c]	5.26 (2.40)[ab]
Control	0.00 (0.71)d	0.00 (0.71)[c]	0.00 (0.71)[c]	0.00 (0.71)[c]	0.00 (0.71)[c]	0.00 (0.71)d	23.40 (4.89)[a]
LSD (5%)	**0.88**	**0.86**	**0.85**	**0.59**	**0.85**	**0.46**	**0.33**
CV (%)	**23.6**	**21.4**	**15.1**	**9.5**	**13.1**	**6.0**	**10.1**

Means in parenthesis are data transformed by square root; those in the column followed by the same letter are not significantly different at the 5% probability leve**l.**

diazinon (8.92%), and lambda-cyhalothrin (7.00%) 0.5 h after fumigation were significantly greater than the toxic standard, which suggests that these insecticides were applied under the 0.0009% concentration level. Similarly, 1 h after fumigation diazinon (8.92%), deltamethrin (8.62%), and malathion were not significantly different from the toxic standard. However, 2 and 3 h after fumigation, all the insecticides except profenofos, caused similar degrees of mortality as the toxic standard. However, profenofos did not cause mortality until 3 h after the beginning of the

test. Similarly, at the 4 and 6 h fumigation all the insecticides caused significant mortality, although profenofos caused relatively less mortality than the toxic standard. After 24 h fumigation, there were more dead honeybees in the water control than in the insecticide fumigated bees. As in the honeybee consumption test, bee mortality in the water control might be attributed to their suffocation when they were trying to leave the cage.

The insecticides evaluated did not knock down honeybees but nearly all of them caused significantly more mortality than the control. This could be due to their fume properties. According to U.S. Department of Health and Human Services (2006), diazinon volatilizes in the air at room temperature and is transformed to diazoxon (an even more potent ChE inhibitor than diazinon), where it

remains active. This property of diazinon makes it more toxic to honeybees. The same Agency (2009) also reported that deltamethrin has a higher potential to volatilize from water compared with other pyrethroids. The USEPA (1988) also indicates that lambda-cyhalothrin and endosulfan are moderately toxic to invertebrate animals, while malathion is very low in toxicity to animals when inhaled (USEPA, 2006).

Conclusion

All of the insecticides evaluated were toxic to honeybees and caused mortality within a few hours after exposure to laced food. In the fumigation test, profenofos did not cause honeybee mortality until the third hour, but the other insecticides caused varying mortality within 24 h. The government ought to exclude products that are toxic to honeybees and the environment in general as criteria for registration and marketing of imported as well locally formulated insecticides. Further research is needed on these insecticides to determine their LD_{50} on Ethiopian bee races.

Conflict of Interest

The authors have not declared any conflict of interest.

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