Original Article

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Avoidance Behavior of Honey bee, *Apis mellifera* from Commonly used Fungicides, Acaricides and Insecticides in Apple Orchards

Moonsu Kang[1] and Chuleui Jung[2,3]*

[1]Korea Plants Environmental Research Station, Suwon, Republic of Korea
[2]Agriculture Science and Technology Research Institute, Andong National University, Republic of Korea
[3]Department of Plant Medicine, Andong National University, Republic of Korea

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Abstract

Avoidance behavior is an important life history strategy to survive hazardous environment. The experiment was conducted to detect the avoidance tendency of the honeybee *Apis mellifera* against commonly used pesticides in apple production. Choice test given only 50% sucrose solution and pesticide-mixed sucrose solution as food estimated the avoidance in laboratory. Most of the acaricides and fungicides tested were shown avoided. Among insecticides, honeybee showed strong avoidance to cyhexatine, carbosulfan and fenpyroximate but low to diflubenzuron, tebufenpyrad, and acrinathrin. Avoidance behavior to neonicotinoid insecticides showed bifurcated; highly avoided from thiacloprid, acetamiprid while less avoided from imidacloprid, thiamethoxam and dinotefuran. From the field study, abamectin, fenthion, amitraz and acequinocyl showed highly avoided while fungicide of fenarimol, acaricides of acrinathrin and phosphamidon, IGR insecticide of diflubenzuron, neonicotinoid insecticide of imidacloprid, and carbamate insecticide of carbaryl showed less avoidance in the field. These results partly explained high bee poisoning from carbaryl in apple flowering period, and neonicotinoids during season.

Key words: *Apis mellifera* L., Avoidance tendency, Pesticide, Selection index

INTRODUCTION

Honey bees are vegetarians, usually consuming only nectar and pollen from plant blossoms or sweets like sugar syrup and honey dew (Atkin and Anderson. 1976 (1970)). These insects are highly social and a colony may contain as many as 10,000 to 100,000 individuals, depending on the time of year, prevailing weather conditions and availability of nectar and pollen sources (Robinson, 1979; Proctor *et al*., 1996). Honey bees provide not only the income sources for beekeepers but also pollination services for crop production (Degrandi-Hoffman, 1978; NRCS, 2005; Jung, 2008; Jung, 2014). Amongst beneficial insects, honey bees are considered as the most efficient and reliable pollinators of various agricultural crops (McGregor, 1976). Recently increased concerns on honey bee population decline have wide spread not only within Korea but over the world (Jung, 2014). There are several factors contributing to the decline of honey bees as well as the pollination services (Goulson *et al*., 2015), but considered as the combined stresses from parasites, pesticides and lack of foraging sources. Habitat destruction, including nesting and mating sites, and alternative forage, is the main issue in the decline of pollinators (Batra, 1995). Bee poisonings from diverse chemicals especially of insecticides are another important source of honeybee population decline

*Corresponding author. E-mail: cjung@andong.ac.kr

together with parasitic pests and diseases (Crane and Walker, 1983; Choi and Lee, 1986). Pesticides constitute an important component of modern agriculture without which the increase in agricultural production could never be achieved (Atkins *et al*., 1970). However, the large scale and indiscr-iminate use of pesticides has resulted in great conflict of interests because of simultaneously occurrences of harmful effects to biotic and abiotic agricultural environments (Kevan and Plowright, 1995). Many agrochemicals sprayed in the fields for crop protection against insect and disease pests are injurious to honeybees (Johansen, 1977). Impacts of these chemicals can occur directly as the mortality on contact or ingestion or indirectly elicit behavioral disorder or abnormal development of immatures. In many cases of acute toxicity, poisoned bees often become irritable (likely to sting), paralyzed or stupefied, appear to be 'chilled' or exhibit other abnormal behavior (Dharmawardena, 1994).

Avoidance behavior is one of the behavioral survival mechanisms of organism together repair or tolerance of molecular or physiological damage from toxic chemicals (Bowler *et al*., 1992; Li-Byarlay *et al*., 2016), Avoidance behavior includes irritancy which is occurring after physical contact and repellency occurring without physical contact but often with olfactory means (Chareonviriyaphap *et al*., 1997). Here we assessed the avoidance tendencies of relatively low toxic pesticides such as acaricides and fungicides commonly used in apple production in Korea. Choice test given only 50% sucrose solution and pesticide-mixed sucrose solution as food estimated the avoidance in laboratory and field assessments were measured after spraying each selected chemical.

MATERIALS AND METHODS

Pesticide avoidance test in the laboratory

Pesticides: Sixty three pesticides (Table 1, 2, 3; 16 fungicides, 12 acaricides and 35 insecticides) were tested for the study. From the registry of pesticides on apple, selection was based on the chemicals that could be used in blooming season as the first priority and then mostly common use as the second priority.

Honeybee: Honey bees used in the experiments were from the experimental apiary of Andong National University. About 30 colonies of *Apis mellifera*, hybrids of *A. m. ligustica* were maintained with conventional methods but limited pesticide uses. For the avoidance assay, mixed ages of worker bees were collected from randomly chosen hives.

Cylindrical cages made of stainless wire (ø13.5 x H10.5cm) were used for this test. Five honey bees were kept in one cage considered as single replication and total 5 replications per pesticide were conducted. No food or water was provided for 2 hours before the experiments. Then, 50% sucrose solution and pesticide-mixed 50% sucrose solutions retaining recommended dose of pesticides were provided into two separate pots maintaining 9cm distance for detecting pesticide avoidance under 25±3°C, 50~80% RH and dark condition. Choice of each food source was checked for 5 minutes at the intervals of 1, 2 and 3 hours. When bee is on the food item and showed feeding behavior of touching food and retracting proboscis, it was considered as choice. The avoidance index was calculated by the formula:

$$AI = \frac{N_p}{N_c + N_p}$$

where AI = avoidance index, N_p = number of bees chosen pesticide-mixed sucrose solution, and N_c = number of bees chosen sucrose solution. The lower the avoidance index (AI) value, the higher the avoidance tendency. If the value is significantly lower than 0.5, it was considered as avoiding.

Pesticide avoidance test in the field

Field trial was conducted at the apple orchard in Geum-gok, Giran-myeon, Andong, Korea. Main cultivar is Fuji with conventional M-26 rootstock. 2-3m high apple trees were lined with 3m apart each other. The bee species *A. mellifera* cultured in Andong National University was used in this test. One fungicide (fenarimole), four acaricides (acequinocyl, amitraz,

Table 1. List of fungicides with chemical class, common name, amount of active ingredient (AI, %), formulation type and recommended concentration (g or ml of formulated product/20L) used in the laboratory test and in the field (**)

Class	Common name	AI%	Type	R.C.
Surfactant	Lime sulfur	22	Ls	200.0
Guanidine	Iminocatadine triacetate	3	Ec	13
Dicarboxmide	Iprodione	50	Wp	17
Benzimidazole	Benomyl	50	Wp	13
Anilinopyrimidine	Pyrimethanil	30	Wp	20
Dithiocarbamate	Mancozeb	75	Wp	40
Dithiocarbamate	Propineb	70	Wp	40
Carbamate	Carbendazim	60	Wp	20
Triazole	Diniconazole	5	Wp	10
Triazole	Difenoconazole	10	Wp	6.7
Triazole	Myclobutanil	6	Wp	13
Triazole	Bitertanol	25	Wp	20
Triazole	Imibenconazole	15	Wp	10
Triazole	Flusilazole	2.5	Wp	2.5
Pyrimidine	Fenarimol **	12	Wp	6.7
Antibiotic	Polyoxin b	10	Wp	20

Table 2. List of acaricides with chemical class, common name, amount of active ingredient (AI, %), formulation type and recommended concentration (g or ml of formulated product/20L) used in the laboratory test and in the field (**)

Class	Common name	AI%	Type	R.C.
Naphthoquinone	Acequinocyl **	15	Sc	20
Amidine	Amitraz **	20	Ec	20
Triflurea	Flufenoxuron	5	Sc	20
Sulfite ester	Propargite	30	Wp	27
Organochlorine	Tetradifon	8	Ec	25
Organophosphate	Fenthion **	50	Ec	20
Organophosphate	Phosphamidon **	50	Sl	20
Organotin	Azocyclotin	25	Wp	13
Organotin	Fenbutatin oxide	50	Wp	20
Carbazate	Bifenazate	23.5	Sc	10
Tetronic acid	Spirodiclofen	36	Wp	10
Machine oil	Machine oil	98	Ec	100

fenthion, phosphamidon), and 11 insecticides (diflubenzuron, acephate, dichlorvos, carbaryl, methomyl, imidacloprid, dinotefuran, acrinathrin, esfenvalerate, alpha cypermethrin, abamectin) were applied for this study (Table 1,2,3 with ** mark). Selection was made based on the toxicity data in the laboratory as well as the prevalent use in apple orchards (Kang, 2009).

Each pesticide was applied to branches of the tree. Pesticide was sprayed using the knapsack sprayer two times, 7 and 1 day prior to full blooming using the recommended dose with application rate of 400L per hectare. Then, three trees on which branches all 16 pesticides were sprayed were caged by using the nylon net (10x4x5m), after last spray. The one cage was considered as replicate. Then, one honeybee colony with queen right containing ap. 1500 workers was introduced into each cage in 1 day after final spray. Net was used to protect the honey bees from escaping. Numbers of honey bees visiting the flower blossoms were counted for 10 minutes for 2,3,4 DAT (day after treatment) and 8,9,10 DAT, respectively around noon.

Data analysis

Avoidance data from the laboratory study were analyzed by one tailed t-test with mean equal 0.5 using SAS program (SAS Institute, 2004). Avoidance index data from

Table 3. List of insecticides with chemical class, common name, amount of active ingredient (AI, %), formulation type and recommended concentration (g or ml of formulated product/20L) used in the laboratory test and in the field (**)

Class	Common name	AI%	Type	R.C.
Benzoylurea	Diflubenzuron **	25	Wp	8
Diacylhydrazine	Tebufenozide	8	Wp	20
Organophosphate	Trichlorfon	50	Sl	25
Organophosphate	Fenitrothion	50	Ec	25
Organophosphate	Monocrotophos	24	Sl	20
Organophosphate	Acephate **	50	Wp	20
Organophosphate	Azinphos methyl	25	Wp	40
Organophosphate	Chlorpyrifos	25	Wp	20
Organophosphate	Parathion	17	Ec	20
Organophosphate	Dichlorvos **	50	Ec	20
Organophosphate	Epn	45	Ec	20
Organotin	Cyhexatin	25	Wp	13
Carbamate	Carbaryl **	50	Wp	25
Carbamate	Methomyl **	45	Wp	13
Carbamate	Carbosulfan	20	Wp	20
Neonicotinoid	Acetamiprid	8	Wp	10
Neonicotinoid	Imidacloprid **	10	Wp	10
Neonicotinoid	Thiacloprid	10	Sc	10
Neonicotinoid	Thiamethoxam	10	Wp	10
Neonicotinoid	Clothianidin	8	Gr	10
Neonicotinoid	Dinotefuran **	10	Wp	20
Phenoxypyrazole	Fenpyroximate	5	Sc	10
Pyrazole	Tebufenpyrad	10	Ec	10
Pyridazinone	Pyridaben	20	Wc	20
Pyrethroid	Alpha cypermethrin	2	Ec	20
Pyrethroid	Deltamethrin	1	Ec	20
Pyrethroid	Bifenthrin	2	Wp	20
Pyrethroid	Acrinathrin **	3	Wp	6.7
Pyrethroid	Esfenvalerate **	1.5	Ec	20
Pyrethroid	Etofenprox	10	Wp	20
Pyrethroid	Fenpropathrin	5	Ec	20
Pyrethroid	Cypermethrin **	5	Ec	20
Pyrethroid	Lambda cyhalothrin	1	Wp	20
Antibiotic	Milbemectin	1	Ec	20
Antibiotic	Abamectin **	1.8	Ec	6.7

the field were compared by ANOVA, Turkey test. Honeybee visiting data to flower in the field between two time laps were compared with t-test.

RESULTS AND DISCUSSION

Pesticide avoidance test in the laboratory

In this study, honeybee showed significant avoidance to all tested fungicides (Fig. 1, $P<0.05$) in the laboratory test. This means that honeybee discriminate fungicide-mixed sucrose solution. Only to iminocatadine triacetate, the AI values was marginally significant ($P=0.07$). This chemical is used for apple bitter rot or valsa canker management. AI index of most fungicides used in apple orchards showed ranges from 0.12 to 0.37 with carbendazim lowest. Carbendazim is relatively non-toxic to honeybee in contact and feeding test (Kang, 2009).

Most of acaricides tested in the experiment showed lower than 0.2 AI values indicating significant avoidance tendency (Fig. 2). AI value of phosphamidon was not significant which means honeybee does not discriminate

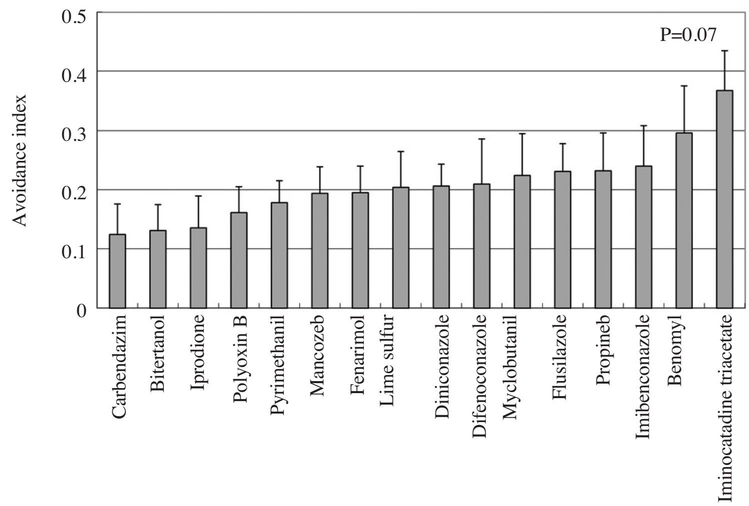

Fig. 1. Avoidance indices (Mean±SE) of honeybees to fungicides. Value significantly lower than 0.5 means high avoidance.

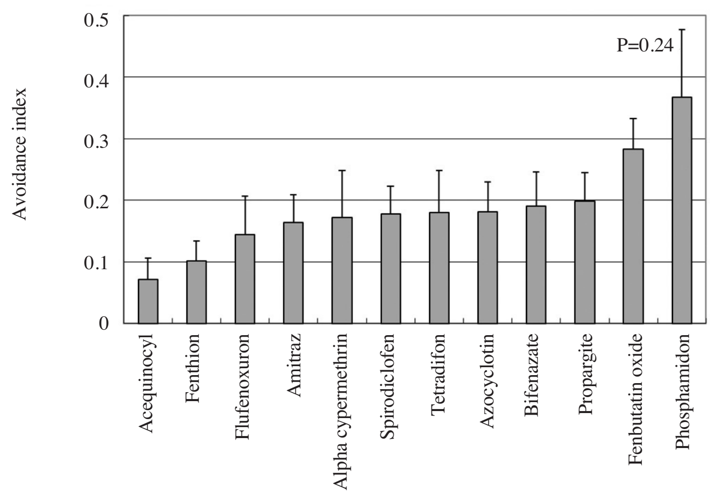

Fig. 2. Avoidance indices (Mean±SE) of honeybees to acaricides. Value significantly lower than 0.5 means high avoidance.

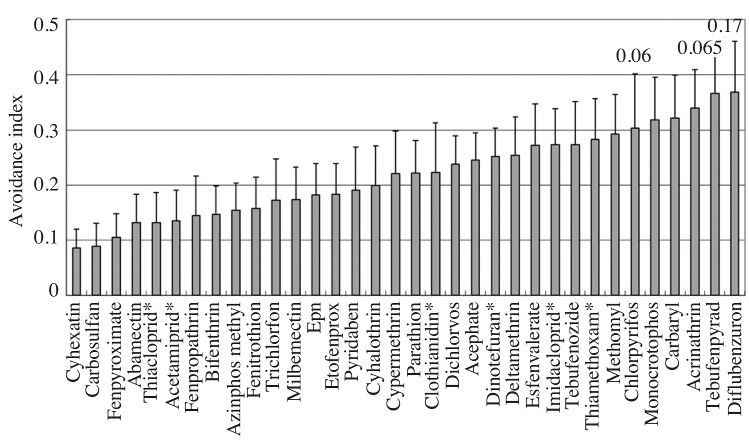

Fig. 3. Avoidance indices (Mean±SE) of honeybees to insecticides. Neonicotinoid insecticides were marked with *. Value significantly lower than 0.5 means high avoidance.

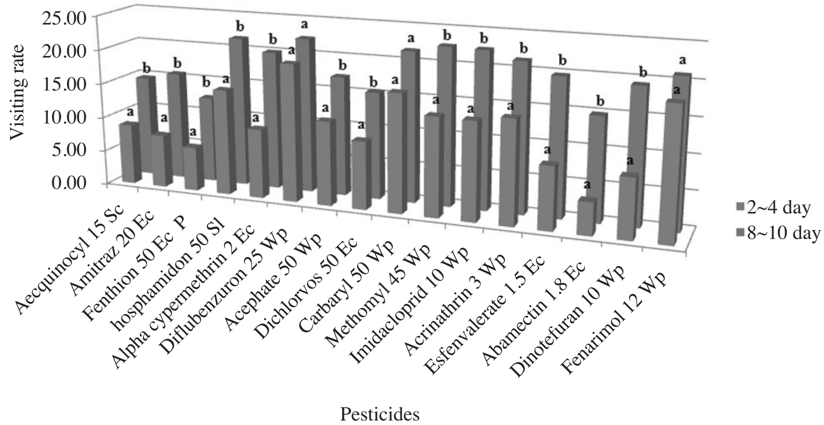

Fig. 4. The comparison of visiting rate of honeybees to the apple flowers at 2 to 4 and 8 to 10 days after spray. Sixteen pesticides were sprayed and flower visiting rates (No. bees/blossoms/10 min) were estimated. On the same chemical, different letters on the bar indicate significant difference of visiting rates in two-time frames.

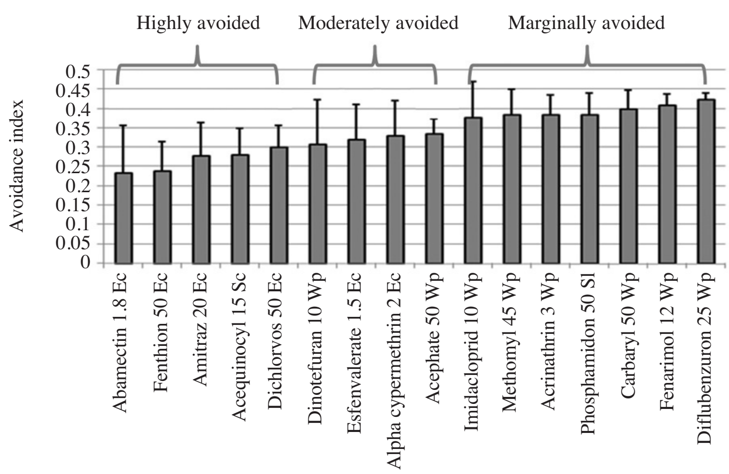

Fig. 5. Avoidance tendency of honeybees from the flower blossoms where 16 pesticides were sprayed in apple orchards.

the chemical (P=0.24). Phosphamidon is an organophosphate insecticide developed in 1960s. It acts as a choline esterase inhibitor, and showed high toxicity to honeybee in the laboratory experiments (Kang, 2009), while the lowest avoidance index was observed from acequinocyl (0.07), which showed very low toxicity to honeybee in the contact and feeding test (Kang, 2009).

Honeybees showed significant avoidance to most of the insecticide tested (Fig. 3, P<0.05), except chlorpyrifos (P=0.06), tebufenpyrad (P=0.07) and diflubenzuron (P=0.17). The avoidance index was found lowest (0.09) in cyhexatin and highest in diflubenzuron (0.37).

Interestingly, avoidance behavior to neonicotinoid insecticides showed bifurcated patterns; highly avoided (AI<1.2) to thiacloprid, acetamiprid while less avoided (AI>2.5) to thiacloprid, imidacloprid, thiamethoxam and dinotefuran. Imidacloprid and thiaclopriod are much more toxic to honeybee than acetamiprid or thiacloprid in the laboratory contact or feeding trials or field, leading EU ban of those chemicals (Cressey, 2013; Lee *et al*., 2016). Less discrimination of these chemicals by honeybee could result in synergized toxicity in the field to honeybee (Woodcock *et al*., 2016; Alburaki *et al*., 2017) or bumblebee (Arce *et al*., 2017). If bees are exposed to guttation of the plants where neonicotinoids were applied into the soil such as seed coating, the negative impact could exacerbated by the increased concentration of the systemic pesticide (Girolami *et al*., 2009).

Pesticides avoidance test in the field

From the field trials evaluating avoidance tendency of honeybee from pesticide-treated flower blossoms, flower visiting rates were variable on treatments. Fenarimol, diflubenzuron and carbary showed no difference on the flower visiting rates of honey bees measured 2-4 DAT and 8-10 DAT (Fig. 4). This implies that those chemicals would not elucidate the behavioral changes of honeybee foraging possibly via contact or olfactory cures. Flower visiting rates were relatively lower in acequinocyl, amitraz, fenthion, acephate, diclorovos and abamectin. All treatments showed avoidance. AI values were ranged from 0.23 to 0.42 (Fig. 4). The responses were categorized into three groups; highly, moderately and marginally avoided. Abamectin, fenthion, amitraz and acequinocyl showed highly avoided which showed high avoidance in the laboratory test, too. While fungicide of fenarimol, acaricides of acrinathrin and phosphamidon, IGR insecticide of diflubenzuron and neonicotinoid insecticide, imidacloprid showed less avoidance in the field test. Interestingly, fewer numbers of honeybees were foraging abamectin and fenthion sprayed flowers, but high numbers of honeybees were found forging on flowers where difulbenzuron and carbaryl were sprayed (Fig. 4). Recently more apple growers spay carbaryl for flower and fruit thining, higher toxic effects on foraging honeybee as well as broods are expected. This result could partly explain the massive bee poisoning during apple flowering season. Kim *et al*. (2014) reported that carbaryl is the most responsible chemical imposing greater risk to honeybee during apple flowering season.

Avoidance is an adaptive behavior through which an organism could escape the hazard for better survival. Through the series of laboratory and field experiments, we found that honeybee showed significant avoidance tendencies from the toxic pesticides. Interestingly, avoidance tendencies were much strong to fungicides and acaricides than to insecticides. Lower physiological susceptibility to fungicides or acaricides together with strong avoidance would benefit to survival of honeybees. But weaker avoidance to some insecticides including neonicotinoids of imidacloprid or thiamethoxam could lead to danger of bee poisoning.

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