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Agrochemical Application Practices and their Level of Toxicity on Honeybee

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Research Article

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ABSTRACT

In Ethiopia, the need for agrochemicals in agriculture is increasing but the unwise application has a subsequent effect on honeybees. Hence, the study was aimed to assess agrochemical application practices and identify the toxicity level of agrochemical on honeybee (*Apis mellifera bandasii*) at Lemo district, Southern Ethiopia. A multi-stage purposive sampling technique was used and one hundred five respondents were interviewed using a structured questionnaire. Toxicity of pesticides was identified using laboratory *via* feeding, contact, and vapor test. The survey data were analyzed by SPSS version 22·0 while the mortality of bees was analyzed using one-way analysis of variance employing Procedure of SAS. About 45% of the respondents applied agrochemical to their crops during bees' active foraging times. On sprayed fields, 53·6% of the beekeepers found dead bees, while, 28.6% faced the absconding bee colony. Among the respondents, 74·7% could not understand instructions and labels written on packages and bottles of agro-chemical. Malathion, Mancozeb, Pallas, Zura, Richway, and Ridomil used in different tests were significantly (P<0·05) toxic from the negative control.

The mean Lethal Dose (LD_{50}) of Malathion was <0.1 µl/bee, which indicates highly toxic. The mean LD_{50} of Pallas, Mancozeb, and Zura were 7-8 µl/bee, 7.5-8 µg/bee, and 6-7 µl/bee, respectively which indicate moderately toxic, whereas Richway and Ridomil were 11-12 µg/bee and 10-11 µg/bee respectively which indicate slightly toxic to the honeybee. The study concluded that agrochemical applications that occurred during optimal honeybee foraging time have a great chance to be exposed to Agrochemical; respondents had low awareness of safe handling and disposal of empty containers with low use of safety precautions. Hence, less persistent agrochemicals are used by

farmers and applied in the evening when bees were not flying. Besides, training of farmers on empty container and packages handling should be given.

INTRODUCTION

Agriculture is the backbone of Ethiopia's economy, accounting for 42 percent of the nation's Gross Domestic Product (GDP) and nearly 80 percent of employment World Bank [1]. The beekeeping sub-sector has been an integral part of agriculture in Ethiopia USAID and yields 1-3 percent of agricultural GDP [2,3]. However, the beekeepers in particular and the country, in general, are not benefiting from the subsector and share of beekeeping in the GDP and it has never been commensurate with the huge numbers of honeybee colonies and the country's potential for beekeeping. Productivity has always been low, leading to low utilization of hive products domestically and relatively low export earnings [4].

The decline of honeybee colonies is not only a global concern because of pollination services and food production reduction, but also due to a decline in honey production among other benefits. Multiple variables are responsible for the reduction of honeybee colonies which include pests, diseases, and loss of natural bee habitat. One of the probable causes for the population declines of pollinators, including honeybees, is the indiscriminate use of pesticides on agriculture particularly for crop production [5-10]. Bees can be exposed directly through bodily contact with pesticides or indirectly by consuming pesticide residue in the nectar and pollen of flowers [11].

The utilization of pesticides in developing countries is increasing [12]. Pesticides are agricultural technologies that enable farmers to control pests and weeds and constitute an important input when producing crops [13-15]. However, the majority of pesticides are not only targeting the pest but also affect non-target plants and animals during their application [16]. Declines in pollinator populations have affected global agricultural production and both food production and the economy [7].

The extensive and prolonged pesticide application reduces the bee population; this results in a reduction of flowering plants [17,18]. Also, it harms agricultural land, fauna, flora, and environmental sustainability [19]. Impacts on bees include a reduction in the yield of cross-pollinated crops, death of bees, impaired learning and orientation, reduce ability to collect food, and navigation back to their hive [11,20,21]. In Ethiopia, indoor and outdoor application of pesticides is a daily practice to increase productivity and to protect different food items from various pests before and after harvesting [22]. Misuse and overuse of pesticides are very common among farmers of developing countries and Ethiopia is not exceptional [23]. Despite these facts, in Ethiopia, there is a lack of stringent controlling mechanism on the importation of hazardous chemicals, absence of well-established institutions that provide farmers with the knowledge of pesticide application and about safety issues [24].

Farmers in the Lemo district practice a mixed farming system. Livestock production, cereal crop production, and horticultural crop are important components of the mixed farming system. Among cereals and horticultural crops grown wheat and potato are the major sources of food and cash income to the farmers in the area. However, in the past decade, the increasing population has forced farmers to intensify agricultural production and resulted in an increased prevalence of insect pests and disease problems. Hence, farmers of the Lemo district are countering the problems with the use of Agro-chemical to protect their crops from pests and diseases. Farmers who produce primarily crops use various types of Agrochemical without consideration of the damage on honeybee colonies. Owing to this, due to the expansion of agriculture packages to increase crop productivity, Agrochemical application has become popular among farmers of the Lemo district. The increase in the use of pesticides creates many problems for honey bee farmers of the area which include losses of honeybee colonies, a decline of honey yield, residual effect, and pollution of the environment due to lack of technical skills for proper and effective use of Agro-chemical.

However, there is scanty information, which shows the impact and damage of various Agro-chemicals on honey yield and honeybee population. Therefore, with the above background and justifications this research was initiated with the following objectives;

- To assess agrochemical application practices in the study area
- To identify toxicity level of agrochemical on honeybee (*Apis mellifera bandasii*) in the study area.

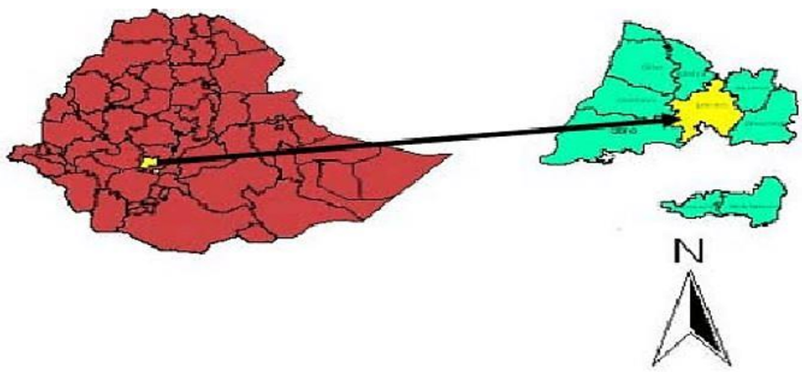
MATERIALS AND METHODS

Description of the study area

The study was conducted in the Lemo district of Hadiya Zone, Southern Ethiopia. The district is found around the capital of Hadiya Zone, Hosanna town, which is located 232 km south of Addis Ababa, the capital of Ethiopia. The district is located between 7°.22”-7.45” N latitude and 37°.40-38°.00” E longitudinal line at an altitude of 1900–2700 meters above sea level with annual rainfall received ranges from 250 -1200 mm, which covers an area of 38,140 hectares. Annual precipitation means minimum and maximum temperatures are 13°C and 23°C, respectively. The district was densely populated and characterized by two agroecological zones with 91% of mid-altitude, 9% of high-altitude areas. The district has 33 kebeles and is bordered on the South by the Kembata Tembaro Zone, on the Southwest by Duna and Soro districts, on the West by Gomibora district, on the Northwest by Misha district, on the Northeast by Ana Lemo district, and on the Southeast by Shashogo district [25].

The existing land use system consists of 88.5% cultivated land, 2.5% grazing land, 9% forest, shrub, and bushlands. The major farming activity was the mixed farming system (rearing of livestock, beekeeping, and crop production). There were two cropping seasons in the area the short rainy season (Belg) from March to April and a long rainy season (Meher) from June to September. The estimated livestock population in the area is about 91,853 cattle; 43,439 sheep; 31,788 goats; 14,924 equines; 103,559 chickens and 102,176 bee hives (Figure 1).[25]

Figure 1. Map of Study Area.



Sampling technique and sample size determination

For this study, Multi-stage purposive sampling procedures were used to select representative sample respondents. In the first stage of sampling, the Lemo district from Hadiya Zone was selected purposively. In the second stage, three kebeles were selected from the crop potential area, which was based on the vegetation cover, their suitability, and potential for crop production were selected purposively from 33 kebeles of the district. Accordingly, Gana, Lareba, and Leisana were selected. Finally, from a stratum of the selected kebeles, thirty-five (35) respondents were selected from each kebeles purposively based on the potentials of crop production. Thus, the total household selected for the study was 105 respondents.

Data collection method

The data were collected using a semi-structured questionnaire with the researcher and development agent in each Kebeles. The questionnaire was prepared in English, translated into the local language (Hadiyessa). During the study, Focus Group Discussion, Key informant interviews, field observations, and document analysis were applied. Both primary and secondary data have been relied on. Qualitative and quantitative data were generated using the conventional survey method.

Laboratory analysis

The experimental part of the study was performed at the Holeta bee research center with the honeybee *Apis mellifera bandasii*. Acute toxicity of commonly used Agro-chemical identified during the survey was tested in the laboratory. Healthy adult worker bees, collected from the frame without brood were anesthetized with CO_2 and held in well-ventilated laboratory cages (5.5 x 8.5 x 10 cm) and placed in 25 ± 2°C temperature and 60-70% of humidity over the study periods. The acute toxicity of the agrochemical to honeybees was tested through feeding, contact, and fumigation. The concentration of each test pesticide causing 50% death of experimental bees and degree of toxicity hazard was determined. The mortalities of bees amongst Agro-chemical were also compared with standard toxic chemicals, Dimethoate (positive), and 50% honey solution (negative) controls.

Feeding test: Thirty healthy worker bees were placed in laboratory cages and starved for up to two hours before the commencement of the test. A bee was being provided with a 50% honey solution containing the recommended concentration of 300 μg (10 μg /bee) of each test Agro-chemical to determine the toxicity (OEPP ⁄ EPPO, 2010). Each treatment was replicated three times and arranged in a completely randomized design. Data on mortality and any injuries were collected every 0.25, 0.5 1, 6, 12, 24, and 72 hrs and compared with 50% non-toxic honey solution and 0.3 μg of reference standard toxic chemicals (Dimethoate). Food consumption in each test was also recorded and replenished every 24 hrs [26].

Vapor test: Similar to the feeding test, thirty healthy adult worker bees were held in a laboratory cage and placed over the Petri dish filled with a recommended concentration of each pesticide in three replications. The deaths of bees and injured honeybees were recorded in 0.25, 0.5, 1, 6, 12, 24 hours and for a maximum of three days. The death rates of bees were compared with the concentration of the standard toxic chemicals (Dimethoate) and non-toxic control (Petri dish filled with water). All bees in the cages were fed 50% honey solution until the end of the study period [26].

Contact test: Filter papers were immersed in a recommended concentration of each test Agrochemical and allowed to dry at room temperature. These papers were enclosed separately in Lab cage containing 15 worker honeybees. Toxicity effects of each concentration of test materials were compared with standard chemicals (Dimethoate) and control (paper immersed in pure tape water). Each treatment was replicated three times [26]. The deaths of bees and injured honeybees were recorded in 0.25, 0.5, 1, 6, 12, 24 hrs and for a maximum of three days. Finally, in all laboratory tests, the percent of mortality caused by each agrochemical in each test was calculated using the as indicated below [27].

$$\text{Correct mortality(Abbot)} = \frac{\%\ \text{mortality treatment} - \%\ \text{mortlity control}}{100} * 100$$

Determination of LD_{50}: The mortality data for each pesticide should be analyzed using appropriate statistical methods to determine LD_{50} for each pesticide. The lethal dose at which 50 percent of experimental bees have died for each commonly used agrochemical was determined using different concentration levels. The LD_{50} is assessed for the toxicity level of Agrochemical to bees. The number of doses and replicates tested meet the statistical requirements for determination of LD_{50} at 5% confidence limits (OECD, 1998) and (EPPO, 2010) [28,29].

Data management and statistical analysis

The data were organized and analyzed using one-way Analysis of Variance (ANOVA) *via* Statistical Package for Social Sciences (SPSS version 24.0, 2012). Descriptive statistics such as frequency, means, percentages, and standard error of the means was employed to have a summary description of the data collected from 105 households' response. Some of the study parameters were prioritized using the rank index.

$$\text{Index} = \frac{\sum[(\text{n} \times \text{no of p 1st}) + (\text{n} - 1 \times \text{no of p 2nd}) + \cdots + (1 \times \text{no of p last})]}{\sum[(\text{n} \times \text{no of p 1st}) + (\text{n} - 1 \times \text{no of p 2nd}) + \cdots + (1 \times \text{no of p last})] \text{ for all factors}}$$

RESULTS AND DISCUSSION

Socioeconomic characteristics of smallholder farmers

The socioeconomic characteristics of smallholder farmer's respondents are described in Table 1. Out of the total interviewed respondents, about 93.3% of interviewed respondents were male. The respondents' age status indicated that 34.3% of interviewed respondents fall within the range of 40-60 years. While 32.9% of interviewed respondents are between 20 and 40 years. Moreover, 6.4% of interviewed respondents are above 60 years and

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1.4% of the respondent's strata are below 20 years. Regarding the marital status of interviewed smallholder farmer's respondents in the study area, higher percentages (93.4%) were married. The educational achievement of interviewed smallholder farmer's respondents indicated that larger percentages (38.1%) were uneducated followed by those who attended junior grades (5-8) (18.1%) (Table 1).

Table 1. Socioeconomic characteristics of smallholder farmers in the study area (n=105).

Parameters	Selected kebeles			Respondents (n=105)
	Gana	Lareba	Leisana	n(%)
Sex				
Female	2	1	4	7(6.7%)
Male	27	33	38	98(93.3%)
Age in years				
<20		1	1	2 (1.4)
20-40	15	14	17	46 (32.9)
40-60	18	15	15	48 (34.3)
Above 60	5	2	2	9 (6.4)
Educational level				
Illiterate	15	10	15	40 (38.1)
Read and write	8	2	2	12 (11.4)
Primary grade (1-4)	5	8	3	16 (15.2)
Junior grades (5-8)	5	6	8	19 (18.1)
Secondary school (9-12)	3	4	7	14 (13.3)
Higher education	2	1	1	4 (3.8)
Marital status				
Married	34	33	32	98 (93.4)
Single	1	1	1	3 (2.9)
Divorced	1		1	2 (1.9)
Widowed		1	1	2 (1.8)

Utilization and application practices of agrochemical in Lemo district

Commonly used agro-chemical in Lemo district: Commonly used Agro-chemical in the study area is listed in Table 2. These Agro-chemical were similar to Agrochemical reported by Gebremichael, in Ejere District, West Shoa, Zewdie in Chilga District, North Gondar Zone, Amhara Region [30,31] (Ridomil, Selectron, Mancozeb, Ethiotate, Cruze, Profit, Karate and Malathion in irrigated vegetable growing farmers' fields). Most of the Agro-chemical in use in the Lemo district was herbicides followed by fungicides which fall in slightly hazardous to highly toxic according to WHO classification (Table 2).

Table 2. Commonly used Agro-chemical in Lemo district in order of their rank.

Agro-chemical used by farmers	Classification of Pesticide by target organism	WHO's toxic class	1st	2nd	3rd	4th	5th	Index	Rank
Pallas	Herbicide	II	32	8	6	3	2	0.277	1st
Zura	Herbicide	III	12	28	21	10	6	0.273	2nd
Mancozeb 80% WP	Fungicide	II	14	13	20	11	6	0.269	3rd
Richway	Herbicide	III	14	12	4	18	10	0.129	4th
Malathion 50% EC	Insecticide	II	5	8	6	5	10	0.064	5th
Ridomil Gold68 WG	Fungicide	III	4	2	1	3	1	0.020	6th

Note: II=Moderately Hazardous; III=Slightly Hazardous; WP=Wettable Powder; EC=Emulsifiable Concentrates; WG=Wettable Granular

Agro-chemical application month and time

The study revealed that the majority of the respondents (45%) applied Agro-chemical during the morning, 20% during mid-day, 15% during late morning whereas 20% of farmers applied during any time of the day as shown in Table 3. Application during mid-day and late morning is not recommended because honeybee is on active foraging time. On the other hand, the majority of the respondents spray agrochemical from July to September. This shows that the Agrochemical application time and months correspond with the active foraging time and with months of honeybee's floral calendar in the study area. This means that pesticide application happens during the peak honey bee foraging activities and peak flowering period for many honey plants. Hence the honeybees have a great chance to come in contact with the chemical. In other words, honeybees are exposed to Agro-chemical [32-35].

The type of application for Agrochemical being used in the study area was liquid, dust, granule, and wettable powder. From the type of application in the study area liquid spraying (93%) is practiced, which is in line with the study of Alemu, Hiluf, Ayalew and Melisie. Similarly, Desalegn reported that 85.03% of farmers apply the liquid (emulsified), 8.84% powder, and 4.6% both liquid and powder forms in western Amhara [33,35,36-38]. Applying agrochemical, having a residual hazard to bees in the late evening, after the bees have stopped foraging and mid-night are the best times to protect honeybees against the effect of agrochemical [39]. Farmers must know the features of pesticide formulations can choose the appropriate sprayer and timing of their spraying operations (Table 3).

Table 3. Agro-chemical application time and formulations of pesticide.

	Response	Frequency	Percentage
Time of application	Morning	47	45
	Late morning	21	20
	Midday	16	15
	Afternoon	21	20
Method of pesticide		98	93
	Liquid spraying		
	Granules	2	2
	Dust spray	2	2
	Powder form	3	3

Exchange of information among smallholders on agrochemical utilization

According to the study result, about 98% of the respondent beekeeper does not announce the Agrochemical application time and their plan before application. This implies that farmers have a poor exchange of information among smallholders. While only a few (2.1%) of the respondent beekeeper inform the neighbor beekeepers about pesticide application time and their plan before Agro-chemical application. Similarly, 98.1% of the respondents of non-beekeeper pesticide users do not announce pesticide application time and their plan, and only a few (1.9%) users inform beekeepers in the study area when they apply and their plan before application. This result result is in agreement with Marta who reported that none of the pesticide users announce before they apply the chemical in the Mecha district, reported that about 93.3% of the beekeepers in South Wollo and Waghimra zones do not announce the beekeeper before application of Agro-chemical. Similarly, Marta reported that none of the agrochemical users in the Mecha District of West Gojjam Zone make any attempt to announce their intention to spray before they apply the agrochemical [32,33,40].

Cooperation between beekeepers and non-beekeepers concerning wise utilization of Agrochemical was very weak and certainly did not consider mutual benefits and environmental protection in the study area. In this regard, Desalegn pointed out that the effects of Agrochemical due to none beekeepers' indiscriminate uses and actions are showing, absences of governing policy that put in place forcing measures to criminals that can be penalized [38]. About 95.6% of respondents indicated that they took no measure to protect honeybee colonies from agrochemical poisoning while the rest of the respondents close the entrance of the hive with coarse cloths to prevent honeybee exposure to sprayed pesticides in the study area. It may be better to apply the agro-chemical at the appropriate time of the day means during low foraging (evening) of the bee (Table 4).

Table 4. Information exchange among smallholders on Agrochemical application and measures taken to protect bee colonies from poisoning.

Variable	Category	F	%
Would Agro-chemical (beekeeper) users inform beekeepers before application time?	Yes	2	2%
	No	103	98%
	Total	105	100%
Do Agro-chemical (non-beekeeper) users inform neighbor beekeepers before application time?	Yes	2	1.9%
	No	103	98.1%
	Total	105	100%
Are farmers willing to use cultural weed and pest controlafter awareness?	Yes	-	-
	No	105	100%
	Total	105	100%
Measures are taken to protect bee colonies from Agro-chemical poisoning	No option	99	95.6
	Closing the hive entrance by coarse cloth	6	4.4
	Total	105	100%

Purpose of agrochemical utilization

Purposes of Agrochemical utilization in the study area are indicated in Table 5. Herbicides are the most widely used Agro-chemical in the study area to control weeds in crops (wheat, pea, bean, maize, and teff). This may be due to different reasons: to enhance crop productivity, save time and weeding requires huge labor to control. These reasons were supported by key informants, focus group discussion, and field observation. About 15.9% of respondents indicated that they use Agro-chemical for controlling fungi and, 6.5% of them use them for insect pest control. Moreover, 3.7 and 1.7% of respondents use Agro-chemical for rodent control, and veterinary uses, respectively. This finding is in line with Hiluf and Ayalew report that 93.2% for weed control, 89.93% use insect pest control, 37.5% use fungi control, 13.54% for rodent control, 24.3% for veterinary uses, and 1.74% for other purposes in North Shoa Zone of Amhara Region (Table 5) [35].

Table 5. Purpose of agrochemical utilization in Lemo district.

Purpose of Agrochemical utilization	Frequency	Percentage
Weed control	76	72.2
Insect pest control	7	6.5
Fungi control	17	15.9
Rodent control	3	3.7
Veterinary purpose	2	1.7

Farmer's awareness on handling and application of agro-chemical

The study revealed that about 62% of the respondents in the study area obtained information regarding Agro-chemical use and application from development agents, 23.7% from neighbor framers/friends, and 3.3% from traders, while the rest 6.1% from reading labels and 4.9% from farmer's association as presented in Table 6. The finding was in agreement with Desalegn, Fikre and Zewdie [35,38,41]. Almost all (97.1%) of respondents are not trained on the safe use and handling of Agro-chemical. However, a few of them (2.9%) received training about the safe use of agrochemicals and applications.

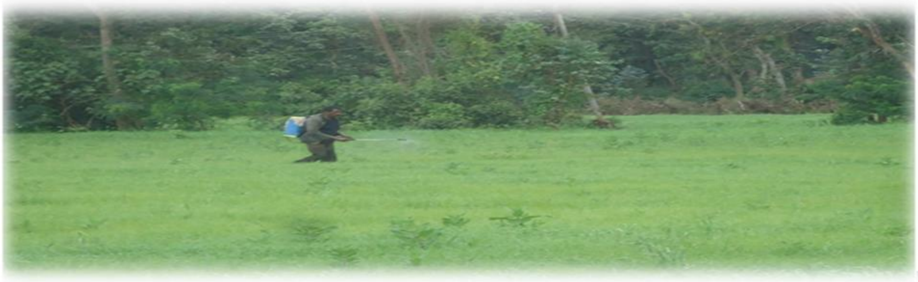
The majority (74.7%) of respondents could not understand instruction and labels written on packages and 25.3% of can understand instruction and labels written on packages as shown in Table 6. Most of the respondents could not read or understand instructions on Agrochemical packages and bottles. This is due to the educational attainment of respondent sprayers, this result tallied with Negatu and Ligani Fikre [41-43], who reported that only 27, 26 and 30% of the respondents could understand and follow instructions indicated labels on pesticide containers in a different part of the country. Similarly, Mekonnen and Agonafir also reported that written information on pesticide packaging was not read by the sprayers in general while Melisie [37] stated that even literate farmers, who can read, do not follow instructions on labels.

Almost all (97.6%) of respondents could not use Personal Protective Equipment (PPE) while spraying agrochemicals that they use just normal clothes, which implies that farmers have low awareness about using PPE, and 2.4% use personal protective equipment while spraying agrochemicals. This is consistent with the research results of Hiluf similarly Ligani, reported that 92.48% were any ordinary suit during spraying and formulation [42,44]. The main reason for not using PPE in the study area may be the low level of knowledge about safety measures and shortage of money to buy protective clothes. Regarding checking the expiry date on the containers and bottles of the agrochemicals used, 58% of farmers did not check the expiry date of the Agro-chemical they purchased. this is maybe due to farmers' belief in their pesticide providers and lack of knowledge on the importance of the expiry date while the rest check an expiration date, which corresponds with a finding which corresponds with a finding of Mengistie and Zewdie (Table 6) (Figure 2).[35,44]

Table 6. Farmer's knowledge on Agrochemical utilization and application.

Question and Responses	n (%) response
Where do you get Information regarding Agrochemical use and application?	
From reading from label	6(6.1)
From development agents	65(62)
From traders/suppliers	3(3.3)
From neighbor farmers	26(23.7)
From farmer's association in the area	5(4.9)
Have you had training on the safe use and handling of Agrochemical?	
Yes	3(2.9)
No	102(97.1)
Can you understand instructions and follow instructions written on Pesticide packages and bottles?	
Yes	27(25.3)
No	78(74.7)
Do you use personal protective equipment while spraying agrochemical?	
Yes	3(2.4)
No	102(97.6)
Would you check the expiry date on the containers of the Agrochemical pesticide that you use?	
Yes	44(42)
No	61(58)
NB: Numbers in parenthesis are percentages while others indicate the frequency	

Figure 2. Farmer applying Agro-chemical without PPE.



Storage of agro-chemicals and empty container handling practice

This study revealed that 41.7% of respondents store agrochemicals anywhere in the house, 23.8% of respondents hang in the ceiling/wall, 17.9% of respondents store in a locked box, 12.5% store Agro-chemical in separate stores with other agricultural equipment while few 6.3% reported that they store in the kitchen along with other utensils, a practice which might expose children and adults to hazardous risks as shown in Table 7.Thus, unsafe storage of Agrochemical was common among respondents in the study area. This corresponds with the studies conducted by Lekei, Hiluf, Ayalew and Mengistie [45].

The study revealed that 42.9% of respondents dumped anywhere the empty containers of Agrochemical, 26.1% of respondents use for domestic purposes [44]. This is maybe due to the shape and size of empty containers and bottles are conducive for holding milk, kerosene, table salt, and edible oil. About 20% of respondents used for storage of other Agrochemicals, 4.1, 2.9, and 4.1% of respondents collected and sold, buried, and kept with other waste materials, respectively as shown in Table 7. This tallied with the finding 77.2% of respondents used empty pesticide containers for various household purposes (example for food and water storage) [35,44]. However, quite a considerable number of respondents dispose of the empty pesticide containers within the farm as well as nearby (14.2%), by incineration (5.2%), and burying (3.5%) Butajira district in the Gurage zone, Southern Ethiopia (Table 7).

Table 7. Storage of Agro-chemicals and empty container handling practice in Lemo district.

The fate of empty Agro-chemical container	n (%)
Collect and sold	4(4.1)
Dumped anywhere	46(42.9)
Kept with other waste materials	4(4.1)
Used for domestic purpose	27(26.1)
Buried	3(2.9)
Used for storage of other Agro-chemicals	21(20)
What did you do with the remaining pesticides after application or	
Used to next year or season	84 (79.6)
Discarded it	5(4.5)
Sell it.	16(15.9)
Storage of Agro-chemicals	
Kitchen	7 (6.3)
Separate agricultural equipment store	13(12.5)
Locked box	18(17.9)
Anywhere in the house	43(41.7)
Hanging in the ceiling/wall.	24(23.8)

Note: Numbers in parenthesis are percentages while others indicate the frequency.

Impact of agro-chemicals on honeybee

About 92% of respondent beekeepers were aware of the effects of Agrochemicals on honeybees. These respondents got this concept from extension agents (72.2%), lessons from friends (16.4%) and from their observation (11.4%) as shown in Table 8. These findings are coherent with the findings of Marta, Alemu, Asaminew and Maria and Zewdie. Similarly, Fetene also reported that 96.9% of respondents in Tigray, 97.8% in Amhara, and 95.6% in Oromia revealed that they will understand and recognize the undesirable effects of Agro-chemicals on the livelihoods of their bees. Besides, that 69% of the beekeepers in selected districts of the Amhara Region have got an extension service and are already aware of when and how to properly use Agrochemical without producing effects on the environment and honeybees [32,33,35,38,45,46].

Accordingly, 53.6, 28.6, 9.3, and 8.6% of the respondents of the study areas did see dead bees on the sprayed field, absconding of bee colonies, dwindled honeybee colonies, and dead bees on hive entrance, respectively.

Furthermore, the study indicates that there was a high loss of honeybee population and colony due to the unwise application of Agrochemicals on agricultural farms in the study area. This result is coherent with the findings of Melisie and Fetene. Similarly, Tesfaye reported several bee colonies either die or abscond from their hives due to the extensive and unsafe use of agro-chemicals (Table 8). [38,46,47]

Table 8. Perception of farmers and their observation on effect of Agro-chemicals on honeybee.

Are you aware of pesticides' effects on honey bees?	**n (%)**
Yes	97(92.1)
No	8(7.9)
Who and how do you get the concept?	
Personal observation	12(11.4)
Lesson from friends	17(16.4)
Awareness from extension workers	76(72.2)
What type of effect on honeybee you can observe after the application of Agrochemicals?	
Dead bees on sprayed field	56(53.6
Absconding of bee colony	30(28.6
Dwindled honeybee colony	10(9.3)
Dead bees on the hive entrance	9(8.6)

Note: Numbers in parenthesis are percentage whil e others indicate frequency, n=number of sampled respondents.

Laboratory test results on commonly used agro-chemicals.

The toxicity level of commonly used Agrochemicals in the study area (Zura, Pallas, Richway, Ridomil, Malathion 50% EC, and Mancozeb 80% WP) was determined using the standard laboratory toxicity test procedures (OECD/OCDE, 1998; EPPO, 2010) [28,29]. The Agrochemicals have been tested on the honeybee race of the study area, *Apis mellifera bandasii via* feeding, contact, and vapor test. The control treatment for the feeding test experiment was the honey solution (negative control) and highly standard toxic Agrochemicals (Dimethoate) as positive control while water was used as a negative control for contact and vapor test and Dimethoate as a positive control.

Toxicity level of agro-chemicals on honeybees *via* feeding

The Agrochemicals were tested under laboratory conditions *via* feeding of honeybees and as compared to with positive control treatment group (Dimethoate) and negative control treatment group (honey solution). Laboratory feeding test indicated that Malathion 50% EC was highly significantly ($P<0.01$) different in toxicity as compared to Richway, Ridomil, and negative control treatment group (Honey solution) as shown in Table 9 and had 100% killing effects on honeybees as shown in Table 9 and Figure 3.

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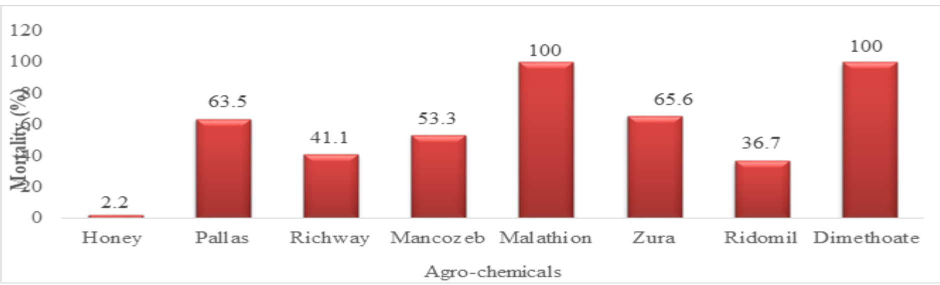
Table 9. Feeding test multiple comparisons using Tukey HSD.

Agro-chemicals.	Hon.	Pal.	Ric.	Man.	Mal.	Zur.	Rid.	Dim.	Mean±SEM
Hon.		-55.6**	-32.9	-40.4*	-	-	-27.8	-99.9**	-0.05^{a} ± 4.49
Pal.	55.6**		22.8	15.3^{a}	-44.3*	-5.1	27.8	-44.3*	55.69^{b} ± 14.26
Ric.	32.9	-22.8		-7.6	-67.1**	-27.8	5.0	-67.1**	32.91^{bc} ± 10.36
Man.	40.4*	-15.2	7.6		-59.5*	-20.3	12.6	-59.5*	40.51^{b} ± 5.52
Mal.	99.9**	44.3*	67.1**	59.5*		39.3*	72.1**	0.000	100.00^{d} ± 0.00
Zur.	60.7*	5.1	27.8	20.3	-39.3*		32.9	-39.3*	60.76^{b} ± 6.33
Rid.	27.8	-27.8	-5.0	-12.6	-72.1**	-32.9		-72.1**	27.85^{bc} ± 7.91
Dim.	99.9**	44.3*	67.1**	59.5*	0.000	39.3*	72.1**		100.00^{d} ± 0.00

Note: Hon=Honey; Pal=Pallas; Ric=Richway; Man=Mancozeb; Mal=Malathion; Zur=Zura; Rid=Ridomil; Dim=Dimethoate; ^{a,b,bc,b,d,b,bc,d}: Means in the column with the same letter not significantly different at 5%; P=Probability; *=Significant at P<0.05; **= Highly Significant at P<0.01; SEM=Standard Error of Mean

Figure 3. Toxicity effects of Agro-chemicals on honeybees through feedings.



Furthermore, there was no significant toxicity difference between this Agrochemical and the toxic standard, Dimethoate. This implies that the toxicity of Malathion 50% EC was comparable to the standard toxic Agrochemical and it causes rapid and severe mortality of honeybees because its effect might be acute or lethal. Thus, Malathion pesticide was highly toxic to honeybees (*Apis mellifera bandasii*). This result was supported by the LD_{50} recorded during the laboratory test <0.1 μl, indicating that the value falls in the highly toxic category. This result also indicated that Malathion 50% EC has caused significantly high mortality of honeybees within half an hour after this chemical application compared to other tasted chemicals and negative control. The study findings tallied with results of Elisie report whose toxicity level of Malathion 50% EC was not significantly differing from standard toxic chemical and Zewdie report on Malathion50% EC that was highly toxic and killed 100% of experimental bees in a shorter time (less than an hour). The LD_{50} of agro-chemicals is in the standard range given for highly toxic substances (<2 μl/bee) [15,37].

Pallas was highly significantly (P<0.01) toxic to honeybees as compared to negative control treatment group and significantly toxicity (P<0.05) difference as compared to positive control treatment group (Dimethoate) as shown in Table 9 and had 63.5% killing effects on honeybees (*Apis mellifera bandasii*) as shown in Figure 3. Thus Pallas was moderately toxic to honeybees (*Apis mellifera bandasii*). The mean LD_{50} recorded during the laboratory test is 7-8 μl, indicating that the value falls in the moderately toxic category as shown in Table 12 and Figure 6.

Zura was highly significant (P<0.01) toxicity difference as compared to negative control treatment group (Honey solution) and significantly (P<0.05) toxicity difference as compared to positive control treatment group (Dimethoate) as shown in Table 9 and has 65.6% killing effects on honeybees (*Apis mellifera bandasii*) as shown on Figure 3. The mean LD_{50} recorded during the laboratory test was 6-7 μl, indicating that the value falls in the moderately toxic category as shown in Table 12 and Figure 6. Mancozeb was found significantly (P<0.05) toxic to honeybee compared to positive control treatment (Dimethoate) and Malathion 50% EC as shown in Table 9 and had 53.3% killing effects on honeybees as shown in Figure 3. The mean LD_{50} recorded during the laboratory test is 7.5-8 μg, indicating that the value falls in the moderately toxic category as shown in Table 12 and Figure 6.

Richway and Ridomil had 41.1 and 36.7% killing effects on honeybees, respectively as shown in Figure 3. Thus, Richway and Ridomil Agro-chemicals were relatively less toxic to the honeybee (*Apis mellifera bandasii*) as compared to the negative control. However, Richway and Ridomil may cause severe honeybee losses when they are applied on crops in bloom and foragers are active. This result was supported by the LD_{50} recorded during the laboratory test and their mean LD_{50} of Richway and Ridomil chemical were 11-12 μg and 10-11 μg, respectively which indicates that the value falls in the slightly toxic category as shown in Table 12 and Figure 6.

Toxicity level of agro-chemicals on honeybees *via* contact

Laboratory contact tests for commonly used Agrochemicals in the study area (Zura, Pallas, Richway, Ridomil, Malathion 50% EC, and Mancozeb 80% WP) were conducted and compared with highly standard toxic chemical

(Dimethoate) and negative control treatment group (water). Laboratory contact toxicity results revealed that there was a highly significant (P<0.01) toxicity difference on tested Agrochemicals and negative control treatment (Water). Malathion 50% EC was found highly significant (P<0.01) in toxicity difference as compared to other tested Agrochemicals (Zura, Pallas, Richway, Ridomil, and Mancozeb 80% WP). Malathion 50% EC had 100% killing effects on honeybees as shown in Figure 4. Furthermore, there was no significant toxicity difference between this Agrochemical and the toxic standard, Dimethoate. This implies that the toxicity of Malathion 50% EC was comparable to the standard toxic agrochemical to the honeybee (*Apis mellifera bandasii*). Thus, Malathion pesticide was highly toxic to honeybees (*Apis mellifera bandasii*) *via* contact. However, except Malathion, all pesticides were less toxic compared to standard toxic (Dimethoate). This was in line reported that Malathion is a highly toxic Agrochemical to honeybee in contact toxicity tests [35,37,48].

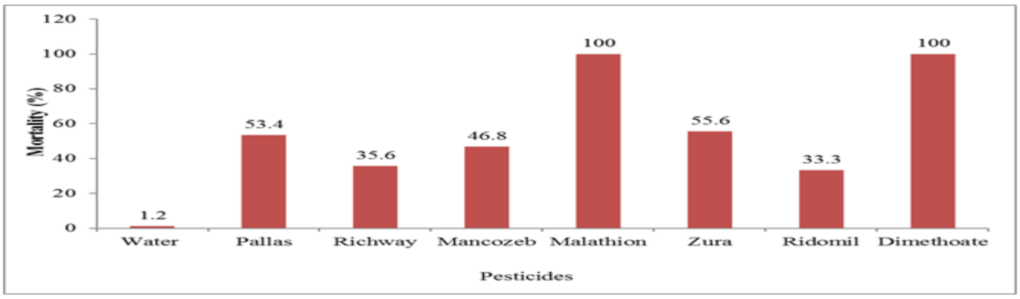
Pallas was significantly toxic (P<0.05) to honeybee as compared to Richway and Ridomil *via* contact test. Furthermore, there was no significant toxicity (P>0.05) difference between this pesticide and Mancozeb 80% WP and Zura as shown in Table 10. Pallas had 53.4% killing effects on honeybees as shown in Figure 4. Furthermore, there was no significant toxicity (P>0.05) difference between this pesticide and Pallas, Zura, and Richway as shown in Table 10. Mancozeb 80% WP had 46.8% killing effects on honeybee (*Apis mellifera bandasii*). Zura had 55.6% killing effects on honeybees (*Apis mellifera bandasii*) as shown in Figure 4 [49-51]. Richway and Ridomil had 35.6 and 33.3% killing effects on the honeybee, respectively as shown in Figure 4. From this finding, apart from toxicity, it might be having sub-lethal effects on flight, navigation, and learning in bees (Table 10) (Figure 4).

Table 10. Contact tests multiple comparisons using Tukey HSD.

Agro-ch	Water	Pal.	Ric.	Man.	Mal.	Zur.	Rid.	Dim.	Mean±SEM
Wat.		-	-	-	-100.0**	-	-30.3**	-	-2.03^a ± 2.35
Pal.	51.2**		18.6*	6.9	-48.8**	-2.3	20.9*	-48.8**	51.2^d ± 4.03
Ric.	32.6**	-18.6*		-11.7	-67.5**	-20.9*	2.3	-67.3**	32.5^{bc} ± 2.33
Man.	44.2**	-6.9	11.7		-55.8**	-9.3	13.9*	-55.8**	44.2^{cd} ± 4.03
Mal.	100.0**	48.8**	67.5**	55.8**		46.5**	69.8**	0.00	100.0^e ± 0.00
Zur.	53.5**	2.3	20.9*	9.3	-46.5**		23.3*	-46.5**	53.5^d ± 2.33
Rid.	30.3**	-20.9*	-2.30	-13.9*	-69.8**	-23.3*		-69.8**	30.2^b ± 4.03
Dim	100.0 **	48.8**	67.5**	55.8**	0.000*	46.5**	69.8**		100.0^e ± 0.00

Note: Agro-ch=Agro-chemicals; Hon=Honey; Pal=Pallas; Ric=Richway; Man=Mancozeb; Mal=Malathion; Zur=Zura; Rid=Ridomil; Dim=Dimethoate; ^{a,d,bc,cd,e,d,b,e}: Means in the column with the same letter not significantly differently at 5% P=Probability; *=Significant at P<0.05; **= Highly Significant at P<0.01; SEM=Standard Error of Mean

Figure 4. Toxicity effects of Agro-chemicals on honeybees *via* contact.



Toxicity level of agro-chemicals on honeybees *via* vapor under laboratory condition

Fumigation toxicity test for the Agrochemicals was estimated by comparing with standard toxic chemical (Dimethoate) and non-toxic chemical (water). Laboratory vapor test results revealed that there is a highly significant toxicity difference ($P<0.01$) in the tested Agrochemicals (Zura, Pallas, Richway, Ridomil, Malathion 50% EC and Mancozeb 80% WP) as compared to negative control treatment (Water) *via* fumigation test as shown on Table 11. Malathion 50% EC was found highly significant ($P<0.01$) toxicity difference as compared to other tested Agrochemicals (Zura, Pallas, Richway, Ridomil, and Mancozeb 80% WP) as shown in Table 11. Malathion 50% EC had 100% killing effects on honeybees as shown in Figure 5. Furthermore, there was no significant toxicity difference between this pesticide and the toxic standard, Dimethoate. This implies that the toxicity of Malathion 50% EC was comparable to the standard toxic pesticide *via* vapor test. Thus Malathion 50% EC was highly toxic to honeybees (*Apis mellifera bandasii*) (Table 11 and Figure 5).

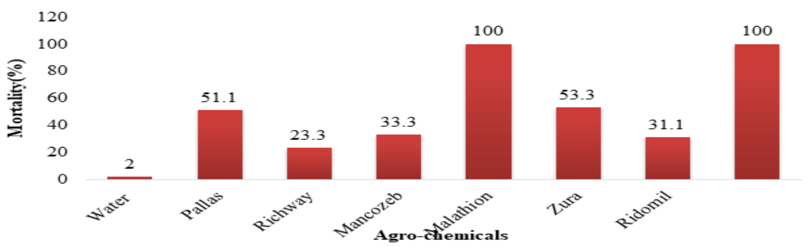
Pallas was found highly significant ($P<0.01$) in toxicity compared to negative control treatment and Mancozeb 80% WP, Richway, and Ridomil. Furthermore, there is no significant toxicity difference between this pesticide and Zura as shown in Table 11. Pallas had 51.1% killing effects on honeybees (*Apis mellifera bandasii*) as shown in Figure 5. Mancozeb 80% WP was found highly significant toxicity difference ($P<0.01$) as compared to Pallas and Zura while significantly toxic ($P<0.05$) compared with Richway. Furthermore, there was no significant ($P>0.05$) toxicity difference between this pesticide and Ridomil as shown in Table 11 [52-54]. Mancozeb 80% WP had 46.8% killing effects on honeybee (*Apis mellifera bandasii*). Furthermore, there was no significant ($P>0.05$) toxicity difference between this Zura and Ridomil. Zura had 53.3% killing effects on honeybees (*Apis mellifera bandasii*) (Figure 5). Richway and Ridomil had 35.6% and 33.3% killing effects on the honeybee, respectively as shown in Table 11 and Figure 5.

Table 11. Vapor test multiple comparisons using Tukey HSD.

Agro-ch	Water	Pal.	Ric.	Man.	Mal.	Zur.	Rid.	Dim.	Mean±SEM
Wat.		-	-20.7**	-31.1**	-100**	-51.7*	-28.7*	-100**	0.00^{a} ± 0.00
Pal.	49.4**		28.8**	18.4**	-50.6**	-2.3	20.7**	-50.6**	49.4^{d} ± 2.19
Ric.	20.7**	-28.7**		-10.37*	-79.3**	-31.1**	-8.1	-79.3**	20. 7^{b} ± 1.93
Man	31.1**	-18.4**	10.4*		-68.9**	-20.7**	2.3	-68.9**	31.1^{c} ± 3.33
Mal.	100.0**	50.6**	79.3**	68.9**		48.3**	71.3**	0.00	100.0^{e} ± 0.00
Zur.	51.7**	2.3	31.1**	20.7**	-48.3**		23.0**	-48.7**	51.7^{d} ± 1.93
Rid.	28.7**	-20.7**	8.1	-2.3	-71.3**	-23.0**		-71.3**	28.7^{bc} ± 1.11
Dim	100.0**	50.6**	79.3**	68.9**	0.00	48.3**	71.3**		100.0^{e} ± 0.00

Note: Agro-ch=Agro-chemicals; Hon=Honey; Pal=Pallas; Ric=Richway; Man=Mancozeb; Mal=Malathion; Zur=Zura; Rid=Ridomil; Dim=Dimethoate; $^{a, d, b, c, e, bc}$: Means in the column with the same letter not significantly differently at 5% P=Probability; *=Significant at P<0.05; **=Highly Significant at P<0.01; SEM= Standard Error of Mean.

Figure 5. Toxicity effect of Agro-chemicals on honeybees through fumigation test.



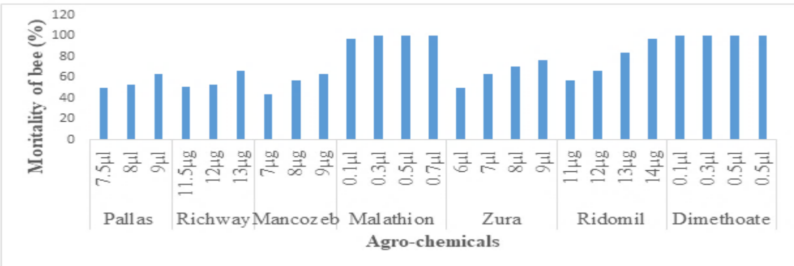
The LD_{50} of agrochemicals was in the standard range given for highly toxic substances (<2 µl/bee), moderately toxic (acute LD_{50}, 2-10.99 µg/bee), slightly toxic (acute LD_{50}, 11-100 µg /bee) and non-toxic (acute LD_{50}>100 µg/bee) to adult bees [15].

The LD_{50} based on mortality data of bee corrected for control mortality using Abbott's formula and LD_{50} value was expressed in µg or µl of test agrochemicals per test honeybee. As shown in Table 12 and Figure 6. The study finding revealed that the LD_{50} of Malathion 50% EC and the standard toxic Dimethoate were less than 0.1 µl. This was in line with Zewdie. LD_{50} of Pallas was7-8 µl/bee, Mancozeb was 7.5-8 µg/bee, Richway was 11-12 µg/bee, Zura was 6-7 µl/bee and Ridomil was 10-11 µg/bee as shown in Table 12 and Figure 6.

Table 12. LD_{50} of agrochemicals and their toxicity level.

Agro-chemicals	LD_{50}	Toxicity level
Malathion 50% EC	<0.1 µl	Highly toxic
Pallas	7-8 µl	Moderately toxic
Mancozeb	7.5-8 µg	Moderately toxic
Richway	11-12 µg	Slightly toxic
Zura	6-7 µl	Moderately toxic
Ridomil	10-11 µg	Slightly toxic
Dimethoate	<0.1 µl	Highly toxic

Figure 6. LD_{50} of Agrochemicals at 24 hours.



CONCLUSION

Zura, Pallas, Richway, Ridomil, Malathion 50% EC, and Mancozeb 80% WP were the common Agrochemicals used for various purposes in the study areas. Farmers utilize herbicides predominately that were accessible in trader's shop including open market, Hosanna farmer service, and agricultural office. Based on the result of this study, respondents have not been trained on pesticide handling, utilization, empty container, package handling practice, and IPM. In addition to this, most of the respondents could not understand instructions and labels written on packages and bottles. About 97.6% of respondents could not use PPE while spraying and formulation of agrochemicals and 58% of farmers did not check the expiry date of the Agrochemicals they purchased. About 92% of respondent farmers were aware of the effects of pesticides on honeybees. The most common method of Agrochemical application used was liquid in the form of spray-applied during the morning of July-September.

The Agro-chemicals (Malathion, Mancozeb, Pallas, Zura, Richway, and Ridomil) which were identified during the study period were tested *via* different tests (feeding, contact, and fumigation) were significantly ($P<0.05$) toxicity difference from the negative control treatment (Water for contact test and Honey solution for feeding and fumigation tests). The mean Fifty Percent Lethal Dose (LD_{50}) of Malathion 50% EC was less than 0.1 µl/bee, which indicates that in a highly toxic category. The mean LD_{50} of Pallas, Mancozeb 80% WP and Zura were 7-8 µl/bee, 7.5-8 µg/bee, and 6-7 µl/bee, respectively which indicate in moderately toxic, whereas Richway and Ridomil were 11-12 µg/bee and 10-11 µg/bee respectively and indicate slightly toxic to a honeybee.

The finding of this study concluded that agrochemicals application occurs during the peak honey bee foraging activities and flowering period for many honey plants hence the honeybees were exposed to the Agrochemicals. Laboratory investigation indicated that all test Agrochemicals were toxic to the honey bee (*Apis mellifera bandasii*) with different toxicity levels. From tested Agrochemicals, Malathion 50% EC was the most toxic Agro-chemical.

Based on the findings of the study, the following recommendations are suggested to be considered in future intervention strategies:

- Less persistent Agrochemicals like Richway and Ridomil were used by farmers and applied in the evening when bees were not flying.
- Further research was needed on Agrochemicals to determine their LD_{50} and Hazard Quotient (HQ) on Apis mellifera bandasii at the field level.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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