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Evaluating the Effects of Commonly Used Agro-Chemicals on the Health Status of *A. Mellifera Scutellata* in Southern Nations, Nationalities and Peoples' Region, Ethiopia

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Abstract: The study was conducted with the objective of to evaluate the potential effects of widely used agro-chemicals on the health status of Apis mellifera scutllata in Sidama zone, Southern Ethiopia. Seven different agro-chemicals (2, 4-D, Agrothoate 40% Malathion 50%, pyriban48%, Diazinon 60%, Macozeb 80% and Pallas 45 OD) were commonly applied on various crops in the study area. The acute toxicity of these agro-chemicals to honeybees was tested via feeding, contact and fumigation. The mortalities caused by individual agro-chemical were compared with positive control Agro-thoate40% (Diamethoate) and negative control (honey solution and water). Acute toxicity analysis in the laboratory indicated that all tested agro-chemicals were found significantly toxic to A.m. scuttlata compared to negative control via feeding. Agrothoate 40%, Malathion50% EC and Dianznon 60% were highly toxic with 100% of experimental bee mortality, less than an hour. All agro-chemicals were statically significant toxic to honeybee when compared ($P<0.01$) standard insecticides and control group through all exposure rout. Therefore, proper utilization agro-chemicals are important to minimize poisoning of honeybee.

Keywords: Agro-chemicals, Toxicity Effect, Sidama Zone

1. Introduction

The introduction of pesticide in Ethiopia to control agricultural pests' dates back to the 1960's [10, 11]. Although chemical pesticide use in Ethiopia was historically low and the volume fluctuates across the pesticide types, recent developments in increased food production and expansion in floriculture industry have resulted in the importation of about 3346.32 metric tons of agro-chemicals annually and higher consumption [12]. The most devastating phenomena that curtails the productivity of honeybee colonies, is poisoning of honeybees by agro-chemicals such as fungicides, pesticides, and herbicides. This daunting challenge not only affect the wellbeing of honeybees but also the wellbeing of human beings who utilize its products and also the ecology in which honeybees are main actors in pollination of plants to keep the ecology balances [3].

1.1. The Status and Cases of Agrochemical Effects on Honeybees

In many countries of the world, agro-chemicals have played major roles in increasing agricultural production dramatically but not without leaving their adverse effects on the environment. Population growth and land degradation contribute most to the increasing risk of food insecurity and famine in Ethiopia. On top of these obvious factors, the average crop loss due to pests is estimated to reach between 30 and 40% annually [23]. The need for agro-chemicals in modern agriculture is increasing and unsystematic use of these agro-chemicals has a subsequent effect on honeybees. The introduction of pesticide in Ethiopia dates back to 1960 and was mostly introduced for agricultural purposes. Although chemical pesticide use in Ethiopia was historically low, today Ethiopia is one of the African countries that use different kinds of Agro-chemicals for agricultural, industrial and health care purposes [11]. According to [7] report, Agro-chemicals applied on more than 3.2 million hectares of cultivated land in the year 2014/15 main production season. Every year on average 1262 tons of agro chemicals are imported and used. Yearly, about 541,467 liters of agro-chemicals are aerially sprayed on 514,923.6 hectares to control the migratory pests [2]. Use of agro-chemicals in Ethiopia is increasing from time to time. Illegal agro-chemicals were also seen marketed in open market and some shops and applied by farmers in the study areas (personal observation).

1.2. Random Use of Agro-Chemicals

Chemicals can poison pollinators or impair their reproduction, eliminate nectar and pollen sources and destroy larval host plants for moths and butterflies and deplete bees 'nesting materials [19]. To supply food for the increasing population of the world, controlling pests are essential weapons to fight these factors and produce high quality food. The need for these agro-chemicals is increasing in modern agriculture and without chemicals most of injurious insect populations cannot be held to low level necessary to obtain the high quality, damage free food and fibers that our modern society increasingly demands [17].

It is reasonable that plant losses from chronic herbicide use may be driving losses of pollinator species. Additionally, various broad-spectrum insecticides are not only applied on agricultural fields but also in residential gardens, recreational areas, forests as well as mosquito-ridden marshes and swamps. These chemicals can be equally toxic to beneficial insects as to the target species [15]. Chronic or sub-lethal exposure to agricultural or beekeeper applied agro-chemicals can weaken the honeybees immune system, and hamper the bees ability to fight infection.

1.3. Effects of Agro-Chemicals on Non-target Organisms (Pollinators)

Honeybees are vulnerable to many of the insecticides used to control damaging pest species by Fruit, vegetable, nut, and seed growers. The recent dramatic death of tens of thousands of honeybee colonies has left many beekeepers devastated and possibly many growers without the quantity and quality of bees needed to pollinate crops these [16]. Pesticide application also affects various activities of pollinators including foraging behavior, colony mortality and pollen collecting efficiency and eventually colony collapse occur due to Agro-chemicals application [12].

1.4. Agro-Chemicals Hazard to Honeybees

Since the use of agro-chemicals showed a steady growth over the past 50 years and currently with the development of the flower sector in Ethiopia, large quantities of agro-chemicals are imported annually to Ethiopia. In this regard, over 3000 tons of various types of agro-chemicals that are worth more than USD 20 million are imported annually [11].

1.5. Exposure of Honeybees to Agro-Chemicals

Honeybees can be exposed to agro-chemicals through either direct contact during foliar applications or contact with residues on the plant surface after foliar application or ingestion of residue in nectar and pollen or vapor drift. Honeybees of all ages and castes are susceptible to effects from pesticide exposure [12]. Adult bees may be exposed to agro-chemicals during flight and foraging. While nurse bees in hive may be exposed to agro-chemicals through contaminated pollen and nectar. Immature bees (brood) are exposed to agro-chemicals residues through contaminated comb cell walls or food sources. Queen bees may also be exposed to agro-chemicals by contact with contaminated bees, wax, and food. Egg laying and repeated contact of the abdomen to contaminated comb increases the risk of sub-lethal effects from pesticide residue exposure on queen bees [14].

Beekeepers and beekeeping experts of the SNNPR have always blame the indiscriminate use of agro-chemicals for the loss of honey bee colonies in the area. They repeatedly reported that honeybee colony population and swarms, honey production had declined in the area. According to their reports these are critical problems particularly during September-November when most agrochemicals are applied in cultivated field and dearth period honeybees are exposure to poisonous plants in the area. However, there were no substantial quantitative data on these cases. Therefore this study was initiated to evaluate the potential effects of widely used agro-chemicals on the health status of Apis *mellifera scutllata.*

2. Materials and Methods

2.1. Description of the Study Area

This particular study was conducted in Hawassa Agricultural research center soil laboratory. Hawassa has a variety of climate conditions; warm climate covers 54% of the area. Elevation of the area ranges from 1500 to 3500 m.a.s.l. and mean annual rainfall varies between 1200 mm to 1999 mm with 15°C to 19.9°C mean annual temperature.

2.2. Laboratory Analysis

Acute toxicity of seven agro-chemicals were selected during survey work and tested in the laboratory. Healthy adult worker bees were collected from strong and healthy colony. Bees were collected from the entrance of the hive at early morning and transported to laboratory using well-ventilated plastic container. Bees were anesthetized with CO_2, held in well-ventilated laboratory cages (5.5 x 8.5 x 10 cm), and placed at room temperature (25 ± 2°C) and humidity (60-70%) over study periods. The acute toxicity of these agro-chemicals to honeybees were tested via feeding, contact and fumigation. The mortalities caused by individual agro-chemical were compared with positive control Agro-thoate40% (Diamethoate) and negative control (honey solution and water).

Table 1. Recommended concentrations of frequently used agro-chemicals in the study area.

Common name	Types	Recommended concentration
2,4-D	Herbicide	0.5ml/80mlH_2O
Agro-thoate40%	Insecticide	0.125ml/37.5mlH_2O
Malathion50%	Insecticide	0.5ml/50mlH_2O
Pyriban48%	Insecticide	0.5ml/50mlH_2O
Mancozeb80%	Fungicide	1gm/500mlH_2O
Diazinon60%	Insecticide	0.5ml/50mlH_2O
Pallas 45 OD	Herbicide	1 ml/100ml H_2O

2.2.1. Feeding Test

Thirty pre-determined healthy worker bees were placed in laboratory cages and starved for up to two hours before the beginning of the test. Bees provided with 50% honey solution containing the recommended concentration of 300µg (10µg/bee) of each test agro-chemicals to determine the toxicity [21]. The recommended concentration of each test agro-chemicals indicated on the table 2, each treatment was replicated 3 times. mortality of Honeybees and injured honeybees were recorded in 15, 30, 45 min, 1, 2, 4, 6, 12, 24, 48, and compared with 50% honey solution (nontoxic) and 0.3 µg of reference standard toxic chemicals, Agro-thoate40% (Dimethoate). Honey solution was replenished for all experimental bees in all test categories when required (when they finished the supplied resource [13].

2.2.2. Vapor or Fumigation Test

Thirty healthy bees were held in laboratory cage and placed over the Petridis filled with recommended concentration of each pesticide in three replications. The mortality of bees and injured honeybees were recorded in an hour interval for maximum of two days. The death rate was compared with concentration of the standard toxic chemicals (Dimethoate) and non-toxic control (petridish filled with water). All bees in the cages fed 50% honey solution during the experiment [13].

2.2.3. Contact Test

Finally, in all laboratory tests percent of mortality caused by each agro-chemical in each test were calculated using Abbott method [1] indicated below.

$$\%\,of\,mortality = \frac{\%\,mortality\,treatment\,-\,\%\,mortality\,control}{100\,-\,Mortality\,control} \times 100$$

2.3. Data Management and Statistical Analysis

The data collected from the survey were coded and stored into computer loaded SPSS software programs version 20, and cleaned for consistency and accurateness. The statistical analysis used in the study varied depending on the type of variable and information obtained. Summarized data were presented in the form of tables and figures. Chi-square was used to test the significance difference between or among values whenever necessary and the constraints of the apiculture in the study areas were prioritized using rank index.

The variances of laboratory data analyzed using GLM and Tukey's honest significant difference (HSD) at 1% level of significance was used for mean separation whenever significant results encountered.

Model: $Y_i = \mu + Ai + \varepsilon i$ Where;

Y_i=an observation in honeybee mortality,

μ=the overall mean

A_i=the effect of various agrochemicals on honeybee mortality (i=7)

ε_i=random error

3. Results and Discussion

3.1. Toxicity Test Results in the Laboratory Condition

Generally, oral exposure through contaminated food is considered to be typical means for the exposure of honeybees to agro-chemicals in the field. Accordingly, based on the study conducted to test toxicity of each of the agro-chemicals (2,4-D, Agrothoate 40%, Malathion 50%, Pyrabine 40%, Macozeb 80%, Diazinon 60% and Pallas 45 OD) used by respondent farmers, in the laboratory condition using direct feeding, contact and vapor methods [20, 21], results have been summarized as follows.

3.2. Toxicity Test Using a Feeding Technique

In this test, Agrothoate40% as positive and 50% honey solution as negative control have been used as standards. Accordingly, all the agro-chemicals tested indicated a laboratory acute toxicity results with highly significant acute toxicity differences between the positive and negative controls and among tested agro-chemicals at ($P< 0.01$) confirming that all tested agro-chemicals were very significantly toxic to local honeybees in the study area compared to negative control through direct feeding (Table 2). More specifically, though LD_{50} of 2, 4-D has been determined to be between 6-8 μg/bee and [5, 6] has recommended the use of this herbicide in the vicinity of bees if dosage, timing and method of application are in accordance of instructions, we found that 2, 4-D has killed 63.3% of experimental sample honeybees in the laboratory (Figure 1). Of course, authors have clarified that the chemical should not be applied directly on bees. Conversely, even if [4] has indicated that 2, 4-D was not toxic to central high land bees, *A. m. bandasii*, we found that the 2,4-D was significantly toxic to southern Ethipia bees, *A. m. scutellata*. However, these toxicity differences might be differences in environmental condition, temperature and honeybee race variations. Agro chemicals are fast ingredients acting at worm and humid air condition than cool climate conation.

On the other hand, LD_{50} of Malathion50% EC and Diazinon 60% was determined to be less than 0.1 μg/bee [15, 18], we found that these agro-chemicals have killed 100% of the experimental sample honeybees in short period of time (6 hrs) (Table 2). These agro-chemicals were comparable to highly toxic standard pesticide Agro-thoate 40% EC. These toxicity findings were found to be partially consistent with result of [2]. According to their toxicity actions to honeybees, we categorized that Malathion 50% EC, Pyrabin48% and Diazinon 60% as fast acting and highly significantly toxic agro-chemicals to *A. m. scutellata* honeybees. Whereas, Mancozab 80% and Pallas 45 OD, which have killed 43.3% and 80% of the experimental honeybees in 12hrs time respectively (Figure 1), could be classified as significantly toxic agro-chemicals to the local honeybees at (P < 0.01). Hence, all these data could explain enough that local honeybee decline has been aggravated by misuse of toxic agro-chemicals in the local conditions without appropriate considerations.

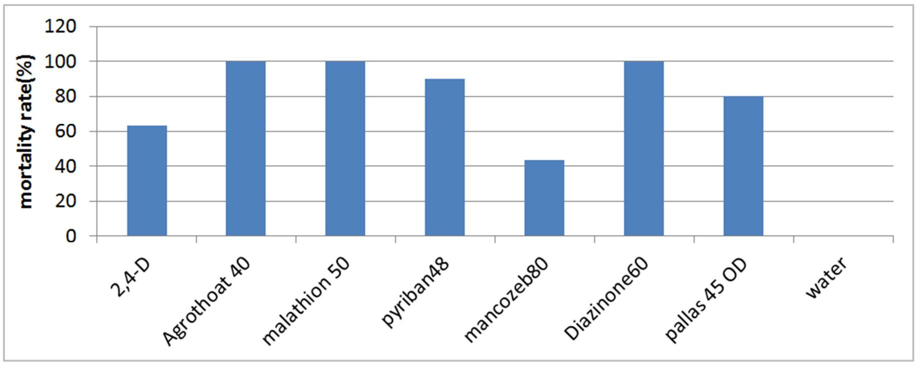

Figure 1. *Toxicity effects of various agro-chemicals to A.m. scutellata via feeding.*

Table 2. *Extent of toxicity of agro-chemicals vs time intervals to A.m. scutellatavia.*

Type of agro-chemicals	Number of dead honeybees in different time									LD50	Toxicity classification
	15 min	30 min	45 min	1 hr	2 hr	4 hr	6 hr	12 hr	48hr		
2,4-D	0	0	1	5	8	3	2	1	0	5-7 μg /bee	Moderately toxic
Agrothoat40%	5	20	5	0	0	0	0	0	0	< 0.1 μg /bee	Highly toxic
Malathion50%	4	7	19	0	0	8	2	0	0	< 0.1 μg /bee	Highly toxic
Pyriban48%	3	4	10	0	0	8	2	0	0	5-8 μg /bee	Moderately toxic
Mancozeb80%	2	4	3	0	0	0	3	1	0	33-44 μg /bee	Slightly toxic
Diazinon60%	12	15	3	0	0	0	0	0	0	< 0.1 μg /bee	Highly toxic
Pallas 45 OD	0	1	4	10	5	3	1	0	0	8-9 μg /bee	Moderately toxic
Honey solution	0	0	0	0	0	0	0	0	0		

Table 3. *Multiple Comparisons of Feeding test by using Tukey HSD at (p < 0. 01).*

Type of agro-chemicals	2,4 D	Agrothoat40% EC	Malathion 50% EC	Pyriban 48%	Mancozeb 80% WP	Diazinone 60% EC	Pallas 45 OD	Control (Honey solution)
2,4 D		11.0000* P=0.000	-11.0000* P=0.000	-8.0000* P=0.000	6.0000* P=0.000	11.0000* P=0.000	-5.0000* P=0.001	19.0000* P=0.000
Agrothoat40% EC	11.0000* P=0.000		0.0000 P=1.000	3.0000* P=0.035	17.0000* P=0.000	0.0000 P=1.000	6.0000* P=0.000	30.0000* P=0.000
Malathion 50% EC	11.0000* P=0.000	11.0000* P=0.000		3.0000* P=0.035	17.0000* P=0.000	0.0000 P=1.000	6.0000* P=0.000	30.0000* P=0.000
Pyriban 48%	8.0000* P=0.000	-3.0000* P=0.035	-3.0000* P=0.035		14.0000* P=0.000	-3.0000* P=0.035	3.0000* P=0.035	27.0000* P=0.035
Mancozeb80% WP	-6.0000* P=0.000	-17.0000* P=0.000	-17.0000* P=0.000	-14.0000* P=0.000		-17.0000* P=0.000	-11.0000* P=0.000	13.0000* P=0.000
Diazinone 60% EC	11.0000* P=0.000	0.0000 P=1.000	0.0000 P=1.000	3.0000* P=0.035	17.0000* P=0.000		6.0000* P=0.000	30.0000* P=0.000
Pallas 45 OD	5.0000* P=0.001	-6.0000* P=0.000	-6.0000* P=0.000	-3.0000* P=0.035	11.0000* P=0.000	-6.0000* P=0.000		24.0000* P=0.000
Control (Honey solution)	-19.0000* P=0.000	-30.0000* P=0.000	-30.0000* P=0.00	-27.0000* P=0.000	-13.0000* P=0.000	-30.0000* P=0.000	-24.0000* P=0.000	

*. The mean difference is significant at p< 0.01 level.

3.3. Toxicity Results from Contact Exposure Tests

Toxicity results obtained from a direct contact treatment revealed that there was a highly significant toxicity difference between the negative control and agro-chemicals and among each of the agro-chemicals tested at (P< 0.01). Specifically, 2,4-D, Malathion 50% EC and Diazinon 60% EC have caused a 10%, 100% and 99% honeybee mortality when contacted with these agro-chemicals while Pyriban 48%, Mancozeb80% and Pallas 45 OD killed 70%, 13% and 20% of sample honeybees respectively (Figure 2). Except 2,4-D and Mancozab80%, tested agro-chemicals were high significantly toxic to honeybees during contact compared to the negative control groups at (P<0.01). Whereas, all tested agro-chemicals showed comparable toxicity to sample honeybees compared to the standard chemical Agrothoate40% at P<0.01 (Table 4).

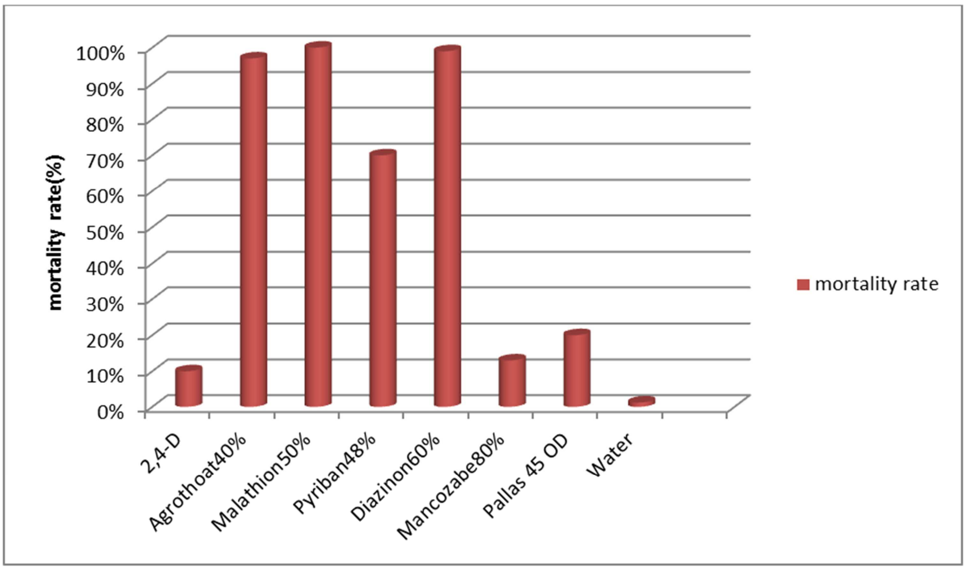

Figure 2. *Toxicity effects of different agro-chemicals to A.m. scutellata via contact.*

Table 4. *Multiple comparison of contact test by using Tukey HSD.*

Type of Agro-chemicals	2,4 D	Agrothoat40% EC	Malathion 50% EC	Pyriban 48%	Mancozeb80% WP	Diazinone 60% EC	Pallas 45 OD	Control (water)
2,4 D		-26.067* P=0.000	-27.000* P=0.000	-18.0000* P=0.000	-0.90 P=0.767	-24.700* P=0.000	-3.000* P=0.002	-1.000 P=0.669
Agrothoat40% EC	26.067* P=0.000		-0.933 P=0.735	8.067* P=0.000	25.167* P=0.000	1.367 P=0.09	23.067* P=0.000	25.067* P=0.000
Malathion 50% EC	27.000* P=0.000	0.933 P=0.735		9.000* P=0.000	26.100* P=0.000	2.300* P=0.019	24.000* P=0.000	26.000* P=0.000
Pyriban 48%	18.000* P=0.000	--8.067* P=0.000	-9.000* P=0.000		17.100* P=0.000	-6.700* P=0.000	15.000* P=0.000	17.000* P=0.000
Mancozeb80% WP	0.900 P=0.139	-25.167* P=0.000	-26.100* P=0.000	-17.100 P=0.000		-23.800* P=0.000	-2.100* P=0.002	-0.100 P=1
Diazinone 60% EC	24.700* P=0.000	-1.367 P=0.0319	-2.300* P=0.019	6.700* P=0.000	23.800* P=0.000		21.700* P=0.000	23.700* P=0.000
Pallas 45 OD	3.000* P=0.002	-23.067* P=0.000	-24.000* P=0.000	-15.000* P=0	2.100* P=0.036	-21.700* P=0.000		2.000* P=0.050
Control (water)	1.000 P=0.103	-25.067* P=0.000	-26.000* P=0.00	-17.000* P=0.000	0.100 P=0.865	-23.700* P=0.000	-2.000* P=0.050	

*. The mean difference is significant at the 0.01 level.

3.4. Fumigation Test Results

Toxicity due to honeybee fumigation tests by agro-chemicals were evaluated against the standard highly toxic pesticide Agrothoate40% (diamethoate) and the negative control (water). Accordingly, all tested agro-chemicals have been found to cause a highly significant mortality to sample honeybees at (P< 0.01). Honeybee death due to 2, 4-D, Malathion 50%, Mancozab 80% and Pallas 45 OD was calculated to be 35%, 50.2%, 30% and 47.7% respectively. Statically, these chemicals were significantly less toxic than that of the standard used. Therefore, they exhibited a moderately vapor toxicity levels while Pyriban 48% which killed 79.2% of sample honeybees were significantly greater than all other agro-chemicals tested. But Diazinone 60% EC (87.7%), were significantly greater than all agro-chemicals tested and were comparable to toxic standards Agrothoate 40%EC (Table 5 and Figure 3). At this point, these results showed that tested agro-chemicals caused significant honeybee mortality through vapor in which differences might be attributed to the differences in nature of their active ingredients. This result partiality in agreement with work of [8,9] who indicated that some chemicals including Diazinone 60% EC have potential to volatize even at room temperature and [3] who showed that Diazono 60% caused high mortality of central honeybees, *A, m. bandasii* through vapor.

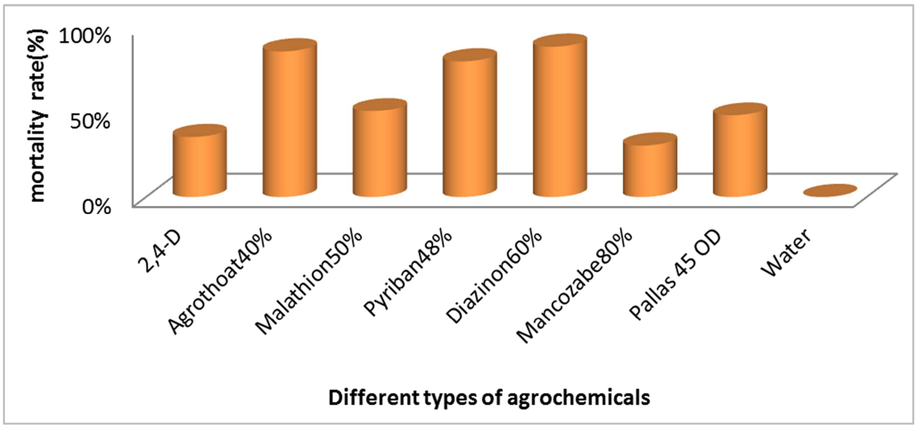

Figure 3. *Toxicity of agro-chemicals to A.m.scuttlata tested through fumigation technique.*

Table 5. *Multiple comparisons of Fumigation test using Tukey HSD.*

Type of Agro-chemicals	2,4 D	Agrothoat40% EC	Malathion 50% EC	Pyriban 48%	Mancozeb80% WP	Diazinone 60% EC	Pallas 45 OD	Control (water)
2,4 D		-15.0000* P=0.000	-4.5600* P=0.000	-13.4400* P=0.000	1.5000 P=0.116	-16.4100* P=0.157	-7.7400* P=0.000	10.5000* P=0.000
Agrothoat40% EC	15.0000* P=0.000		10.4400* P=0.000	1.5600 P=0.094	16.5000* P=0.000	-1.4100 P=0.031	7.2600* P=0.000	25.5000** P=0.000
Malathion 50% EC	4.5600* P=0.000	-10.4400* P=0.000		-8.8800* P=0.000	6.0600* P=0.000	-11.8500* P=0.000	-3.1800* P=0.000	15.0600* P=0.000
Pyriban 48%	13.4400* P=0.000	-1.5600* P=0.094	8.8800* P=0.000		14.9400* P=0.000	-2.9700* P=0.000	5.7000* P=0.000	23.9400* P=0.000
Mancozeb80% WP	-1.5000 P=0.116	-16.5000* P=0.000	-6.0600* P=0.000	-14.9400* P=0.000		-17.9100* P=0.000	-9.2400* P=0.000	9.0000* P=0.000
Diazinone 60% EC	16.4100* P=0.000	1.4100 P=0.157	11.8500* P=0.001	2.9700* P=0.000	17.9100* P=0.000		8.6700* P=0.000	26.9100* P=0.000
Pallas 45 OD	7.7400* P=0.000	-7.2600* P=0.000	3.1800* P=0.000	-5.7000* P=0.000	9.2400* P=0.000	-8.6700* P=0.000		18.2400* P=0.003
Control (water)	-10.5000* P=0.000	-25.5000* P=0.000	-15.0600* P=0.000	-23.9400* P=0.000	-9.0000* P=0.000	-26.9100* P=0.000	-18.2400 P=0.000	

* The mean difference is significant at the 0.01 level.

4. Conclusion and Recommendation

In conclusion, the results of the present study show that agro-chemicals are considered as a powerful weapon or magic bullets in the study area in order to enhance the agriculture productivity. The utilization of agro-chemicals is increasing from time to time and mainly used to control weeds, pests, and diseases of crops and animals. Common agro-chemicals use in sidama zone was 2,4-D, Agrotheote40%, Malathion80%, Pyriban48%, Diazinone60%, Macozeb80% and Pallas 45 OD. Laboratory toxicity test indicated that all agro-chemicals which were applied on various crops were toxic to *A. m. scuttlata* honeybee with different toxicity level. Malathion50% EC, pyriban48% and Diazinon 60% were fast acting and highly toxic to honeybees and the rest were of moderate and slightly toxicity categories.

According to the result of this study some of the suggested issues that require consideration by beekeepers and any development organizations are high lightened on the integrated efforts are very important to educate farmers on proper agro-chemical handling, management, utilization, appropriate safety precautions, effects of pesticide on honeybee health and Integrated Pest Management (IPM).

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