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 Agr-o-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
scutellata.

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
CO₂, 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/80mlH₂O             |
| Agro-thoate40% | Insecticide | 0.125ml/37.5mlH₂O         |
| Malathion50% | Insecticide | 0.5ml/50mlH₂O             |
| Pyriban48%  | Insecticide | 0.5ml/50mlH₂O             |
| Mancozeb80% | Fungicide   | 1gm/500mlH₂O              |
| Diazinon60% | Insecticide | 0.5ml/50mlH₂O             |
| Pallas 45 OD| Herbicide   | 1 ml/100ml H₂O            |

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, Agrothoate40%, 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 LD₅₀ 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 short period of time (6 hrs) (Table 2). These agro-chemicals were comparable to 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: \( Y_i = \mu + A_i + \epsilon_i \) Where;
\( Y_i = \) an observation in honeybee mortality,
\( \mu = \) the overall mean
\( A_i = \) the effect of various agrochemicals on honeybee mortality (i=7)
\( \epsilon_i = \) random error
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, Pyribin48% and Diazinon 60% as fast acting and highly significantly toxic agro-chemicals to *A. m. scutellata* honeybees. Whereas, Mancozeb 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.](image)

### 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 Pyribin 48%, Mancozeb80% and Pallas 45 OD killed 70%, 13% and 20% of sample honeybees respectively (Figure 2). Except 2,4-D and Mancozeb80%, tested agro-chemicals were highly significantly toxic to honeybees during contact compared to the negative control groups at (P<0.01). Whereas, all tested

| Type of agro-chemicals | Number of dead honeybees in different time intervals (LD50) | Toxicity classification |
|------------------------|-------------------------------------------------------------|------------------------|
|                        | 15 min | 30 min | 45 min | 1 hr | 2 hr | 4 hr | 6 hr | 12 hr | 48 hr |                      |
| **2,4-D**              | 5-7 µg/bee | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| **Agrothoat40%**       | <0.1 µg/bee | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| **Malathion50%**       | 33-44 µg/bee | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| **Pyribin48%**         | <0.1 µg/bee | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| **Mancozeb80%**        | 4-7 µg/bee | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| **Pallas 45 OD**       | <0.1 µg/bee | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| **Honey solution**     | 8-9 µg/bee | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

*The mean difference is significant at p< 0.01 level.*
agro-chemicals showed comparable toxicity to sample
honeybees compared to the standard chemical Agrothoate40%
at P<0.01 (Table 4).

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%, Mancozeb 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 Diazino 60% caused high mortality
of central honeybees, A, m. bandasii through vapor.
Different types of agrochemicals

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 Agrochemicals | 2,4 D | Agrothoat40% | Malathion 50% | Pyriban 48% | Mancozeb80% | Diazinone 60% | Pallas 45 OD | Control (water) |
|-----------------------|-------|--------------|--------------|-------------|-------------|--------------|--------------|----------------|
| 2,4 D                 | P=0.000 | P=0.000     | P=0.000     | P=0.000    | P=0.000    | P=0.000     | P=0.000     | P=0.000       |
| Agrothoat40%          | 15.00000* | 10.44000*   | 16.50000*   | 14.90000*  | 17.91000*  | 7.74000*    | 10.50000*   | P=0.000       |
| EC                    | P=0.000 | P=0.000     | P=0.000     | P=0.000    | P=0.000    | P=0.000     | P=0.000     | P=0.000       |
| Malathion 50%         | -10.44000* | 8.88000*    | -14.90000*  | -17.91000* | -9.240000  | -7.74000*   | -18.24000*  | P=0.000       |
| EC                    | P=0.000 | P=0.000     | P=0.000     | P=0.000    | P=0.000    | P=0.000     | P=0.000     | P=0.000       |
| Pyriban 48%           | -1.50000* | 1.41000     | 2.970000    | 17.910000  | 8.670000   | 26.910000  | 18.240000   | P=0.000       |
| WP                    | P=0.116 | P=0.000     | P=0.000    | P=0.000    | P=0.000    | P=0.000    | P=0.000     | P=0.000       |
| Diazinone 60%         | 16.410000 | 11.85000    | 9.240000    | 9.00000    | 26.910000  | 25.500000  | 23.940000   | P=0.000       |
| EC                    | P=0.000 | P=0.157     | P=0.001     | P=0.000    | P=0.000    | P=0.000    | P=0.000     | P=0.000       |
| Pallas 45 OD          | 7.740000 | 3.180000    | -5.70000    | 9.240000   | -8.670000  | -18.240000 | 18.240000   | P=0.003       |
| Control (water)       | P=0.000 | P=0.000     | P=0.000     | P=0.000    | P=0.000    | P=0.000    | P=0.000     | 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, Agrothoate40%, Malathion80%, Pyriban48%, Diazinone60%, Mancozeb80% 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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