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Research Article

Toxicity to Honey Bees *Apis mellifera* Adansonii of Three Insecticides Used in Cotton Cultivation in Benin

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Abstract

Background and Objective: Protection and preservation of bees are a pledge in a sustainable and environment-friendly agriculture. The economic contribution of the insect's pollinators to world agriculture is estimated at billions of dollars. However, non-judicious choice of pesticides and absence of good plant protection practices, represent a real danger to bees. The objective of this study was to assess the toxicity of three insecticides commonly used in cotton protection on bees *Apis mellifera* adansonii in Benin. **Materials and Methods:** In this context, bees were taken from hives and transported to the laboratory. Active ingredients and different doses used were as followed: Emamectin benzoate, beta-cyfluthrin+imidacloprid and lamdex+chlorpyrifos at doses of: 2150 nanograms per bee, 125 nanograms per bee, 75, 50, 25, 12.5×10^3, 6, 2.5 and 1.25-0.96 ng per bee, depending on the active ingredient. Each treatment included three replications consisting of 25 bees. Bees were anaesthetized with ether before treatment. Each bee received by topical application on the pronotum, 1 µL of the formulation. **Results:** Observations were made after 2, 10, 24 and 48 h. Results indicated that, even the lowest doses of the pesticides: 0.96, 7.25 and 21.5 ng per b showed mortality higher than 90% to bees, 48 h after application. An insecticide with active ingredients including beta-cyfluthrin (45 g L^{-1}), which is a pyrethroid and Imidacloprid (100 g L^{-1}), a neonicotinoid actually forbidden in many European countries showed very high toxicity to honey bees, indicating values of the LD_{50} varying from 19.9 ng per bee for 10 h exposure to 1.1×10^{-2} ng per bee for 18 h and 5×10^{-4} ng per bee for 36 h. **Conclusion:** Results of the study indicated the urgent need of good plant protection practices in the frame of sustainable agriculture and bee's preservation and conservation. Promotion of strategies as part of the dissemination of good agricultural practices in plant protection is a guarantee which should ensure sustainable agriculture and environmental, human and biodiversity protection. These studies showed the urgency of the integrated plant protection in order to promote good agricultural practices for honey bee's protection.

Key words: *Apis mellifera* adansonii, pesticides, toxicity, lethal dose

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The economic contribution of bees to the world agriculture is estimated at US $117 billion. The impact of these pollinators is significant: At the global level, it represents about 10% of the turnover of the entire agriculture[1,2]. In the United States of America for 90 food plant pollinated by the foragers, the contribution of bees (pollination, fertilization, grains formation, harvest and selling) to the American GDP was estimated to be 15 billion dollars for 2000. In France, the increase in agricultural productions attributed to pollination by bees was estimated at 3 billion francs in 1982. Cotton plant, although an autogamic plant for the great majority, depends highly on bees and other pollinators[3] for its pollination. Pollination done by bees concerns also fruit trees. The pollen of fruit trees is big and cannot be moved only by the wind. Pollen of fruit trees is therefore, transported largely by honey bees[4-6]. Pollination of fruit trees is assured at more than 80% by bees and its success depends on the number of bees present in the plantation and climate conditions during flowering[2,4,7]. In 2007, in the United States for lack of pollination, cotton production recorded losses estimated at about 15 billion dollars[8]. A study highlighted the important role of bees in the pollination of the cotton plant[9]. In fact, the cotton pollen is heavy and cannot be transported by the wind and flowers of the cotton plant remain open just for one day[9]. Cotton production has recorded an increase in Benin, reaching 400,000 t of seed cotton in 2001/02 and in 2003/04 with a growth rate[10] of 2.5%. However, the cotton plant is very fragile and is sensitive to various pests and diseases[11]. During the campaign 2008-2009 only to produce 210,000 t of cotton, more than 1,000,000 L of chemical pesticides were used[12]. Benin economy is based on agriculture[13]. Agricultural production represents 40% of the GDP and occupies 70% of the total active population[12]. To meet their needs, farmers associate several activities including beekeeping. Although, having lot of potentialities because of its unique floristic biodiversity[14], beekeeping is still less developed and honey hunting represents more than 70% of the total production[15,16]. Moreover, for some years now, apiary weakening phenomena with a diminishing of bees activities, a sudden disappearance of the colonies and/or mass mortality of bees, leading to sudden grain yield losses and honey flow decrease were observed[6,16-21]. These phenomena provoke heavy losses in agricultural production. The absence of pollinators can reduce yield up to one quarter of the production[22]. Among the multiple reasons given responsible for bees' disappearance, the major one is the exposure of bees to different types of chemical pesticides used in crop protection etc.

In Benin, practices in the use of pesticides are more dangerous. The doses of pesticides applied per treatment are generally higher than those recommended, no matter the zone or the pesticide[23-25]. Faced with all these bad agricultural practices, what can be the consequences of such practices on bees? This study aims at evaluating the toxicity to bees of three pesticides used in cotton production in Benin.

MATERIALS AND METHODS

Study area: Bees were taken from an apiary in Gannou in the Commune of Parakou located in Borgou Department. Borgou has a surface area of 25,856 km^{-2}, a population of 724.171 inhabitants in 2002 and a density of 28 inhab km^{-2}. It has 8 communes including: Bembèrèkè, N'Dali, Nikki, Kalalè, Parakou, Pèrèrè, Sinendé and Tchaourou. Parakou is the departmental city and is located between 9°21 latitude North and 2°36 longitude East and stretches[26] on 441 km^2. The commune of Parakou is characterized by a humid tropical climate of Sudanese type with a big rainy season and a big dry season. The rainy season begins in April and lasts about 6-7 months. The dry season lasts about 5-6 months. The annual average temperature recorded is about 27.6°C with a maximum of 30.7°C in March and a minimum of 25.1°C in August. The relative moisture varies throughout the year between 26.5% (December) and 82% (August). The annual rainfall varies between 900 and 1.200 mm (ASECNA/station of Parakou-airport).

Materials: Laboratory works were carried out in the Laboratory of Plant Protection, Bees Pathology and Parasitology in Parakou.

The insecticides tested were the following: EMA 19.2 EC containing 19.2 g L^{-1} of emamectin benzoate; THUNDER 145 O-TEQ containing 45 g L^{-1} beta-cyfluthrin and 100 g L^{-1} of imidacloprid and the binary pesticide lamdex 30+chlorpyrifox 400.

For each of these insecticides, formulations of 5000, 3000, 2000, 1000, 500, 250, 100 and 50 ppm were prepared and corresponding to the doses per bee in a 1 μL:

- 96.0, 57.6, 38.4, 19.6, 9.6, 4.8, 1.92 and 0.96 ng μL^{-1} per bee for EMA 19.2 EC
- 725, 435, 290, 145, 72.5, 36.25, 14.5 and 7.25 ng μL^{-1} per bee for thunder 145 O-TEQ
- 2150, 1290, 860, 430, 215, 107.5, 43 and 21.5 ng μL^{-1} per bee for the insecticide containing lamdex 30+chlorpyrifox 400
- Control, where the bees were inoculated with water without insecticide

Each treatment was made of three replications. The bees were taken 2 h before the test and put in a cool box containing ice, at a temperature of 25°C, in the dark. The inoculation of the different treatments was done by applying 1 μL of each dose of the different treatments on the pronotum of the bee after having anesthetized the insects. Bees of the control treatment were inoculated with 1 μL of sterilized water. All the bees were afterward put in perforated boxes of polystyrene, recovered by a grill with fine mesh.

Analysis of statistical data: To determine the differences between the means of the treatments, the variance analysis was done after transformation of the raw data (percentage of the mortality of the bees), by the function (arsin(x)), before analysis on ANOVA. The variance analysis ANOVA was done using the software SPSS version 9.0 on the same data to compare treatments between themselves. In case of significant differences, the test of Student Newman-Keuls (SNK) was used to separate the means of the different treatments. All these parameters were analyzed to the significance threshold of 5%. Corrections relating to the mortality of the controls were made according to Abbott[27].

For the computation of lethal doses, the data obtained are treated using the software WinDL (CIRAD, Montpellier, version 1998), which allows modeling the effect of increasing concentrations of a molecule of pesticide on the mortality rate of insects groups (dose-effect relation). The software WinDL calculates an adjustment of the results to a straight line, after the logarithmic transformation of the concentrations and probit of cumulated frequencies of mortality (Henry straight line).

RESULTS

Evaluation of emamectin toxicity

Sensitivity to the different doses of emamectin: The mortality rates caused by the different doses of emamectin, 2 h after the test varied from 10.7±2.7% (0.96 ng per bee) to 100±0.0% (96 ng per bee) (Table 1).

The highest dose 96 ng per bee has shown higher mortality than all other doses 24 h after inoculation with almost 100% mortality of the bees. All treatments have shown mortality higher than 85%, 48 h after the application.

No significant difference was observed between the different doses used for this period. Twenty four hours later, the highest dose used (96 ng per bee) caused the highest level of mortality (98.7±1.3), followed in decreasing order by the other doses (F = 60.56, p<0.0001). For each of the doses used, the mortality rates increased with the exposure time of bees. No matter the exposure time, the controls recorded mortality rates lower than 10%.

Evaluation of the lethal doses at 50 and 90% of the emamectin pesticide: The values of the Lethal Doses (LDs) LD_{50} and LD_{90} (Table 2) were obtained by analyzing the effect of the increasing doses of the active ingredients on the mortality rate of the groups of bees. The results have showed that $3.49\times10^{34}\pm1.11\times10^{3}$ g L^{-1} are needed to kill 90% of the bees in 2 h, $2.41\times10^{14}\pm2.01\times10^{2}$ g L^{-1} to kill 50% of the bees in 2 h and $1.66\times10^{-6}\pm7.1\times10^{2}$ g L^{-1} to kill 10% of the bees in 2 h.

The lower and higher limits of each LD varied from 1×10^{-38} to 1×10^{38} g L^{-1} for all values of the lethal doses. The correlation coefficient 0.678 obtained after the regression between the mortality rate and the applied dose has shown that the proportion of dead individuals has a close relationship with the applied dose (χ^2 = 3.991, ddl = 6, p = 0.678).

The results showed a better adjustment to the regression model and the reliability of the LD_{50}. This shows that it has a dose-response effect. In other words, the mortality increases when the dose increases. There is therefore, a good adjustment of the model. The same results were obtained 24 h after the test.

Table 1: Effect of increasing doses of active ingredients of the emamectin insecticide on the mortality of the bees

		Mortality (%)			
Treatments	Doses (ng μL^{-1} per bee)	2 h	10 h	24 h	48 h
Control	None	0.0 ± 0.0^{b}	4.0 ± 0.0^{d}	5.3 ± 1.3^{d}	9.3 ± 1.3^{d}
50 ppm	0.96	10.7 ± 2.7^{a}	36.0 ± 0.0^{ab}	74.7 ± 1.3^{ab}	90.7 ± 1.3^{bc}
100 ppm	1.92	14.7 ± 2.7^{a}	48.0 ± 8.3^{ab}	78.7 ± 4.8^{bc}	93.3 ± 2.7^{ab}
250 ppm	4.8	12.0 ± 0.0^{a}	52.0 ± 16.2^{ab}	72.0 ± 4.6^{c}	88.0 ± 6.1^{c}
500 ppm	9.6	21.3 ± 1.3^{a}	70.7 ± 6.7^{b}	90.7 ± 2.7^{c}	100.0 ± 0.0^{a}
1000 ppm	19.6	17.3 ± 11.4^{a}	74.7 ± 9.6^{ab}	89.3 ± 2.7^{ab}	97.7 ± 1.3^{ab}
2000 ppm	38.4	16.0 ± 2.3^{a}	78.7 ± 5.8^{a}	90.7 ± 1.3^{ab}	100.0 ± 0.0^{a}
3000 ppm	57.6	13.3 ± 3.5^{a}	64.0 ± 9.2^{a}	94.7 ± 3.5^{a}	98.7 ± 1.3^{ab}
5000 ppm	96.0	14.7 ± 5.8^{a}	68.0 ± 4.6^{b}	98.7 ± 1.3^{a}	100.0 ± 0.0^{a}
$F_{8,18}$		4.21	9.71	60.56	85.17
p-value		0.0054	<0.0001	<0.0001	<0.0001

NB: There is no significant difference between the means having the same letter on the columns

Table 2: Lethal doses of emamectin and their lower and higher limits 2, 24 and 48 h after the test of toxicity

Lethal doses	Lethal doses at 50% (g L^{-1})	Lower limit (g L^{-1})	Higher limit (g L^{-1})
LD_{90} (2 h)	$3.49\times10^{34}\pm1.11\times10^{3}$	1×10^{-38}	1×10^{38}
LD_{50} (2 h)	$2.41\times10^{14}\pm2.01\times10^{2}$	1×10^{-38}	1×10^{38}
LD_{10} (2 h)	$1.66\times10^{-6}\pm7.1\times10^{2}$	1×10^{-38}	1×10^{38}
LD_{90} (24 h)	$2.05\times10^{-2}\pm1.83\times10^{-1}$	9.26×10^{-3}	5.47×10^{-2}
LD_{50} (24 h)	$1.34\times10^{-4}\pm3.82\times10^{-1}$	1.04×10^{-5}	5.07×10^{-4}
LD_{10} (24 h)	$8.70\times10^{-7}\pm7.5\times10^{-1}$	5.06×10^{-9}	1.1×10^{-5}
LD_{90} (48 h)	$1.46\times10^{-3}\pm3.53\times10^{-1}$	2.82×10^{-19}	7.94×10^{-3}
LD_{50} (48 h)	$1.64\times10^{-5}\pm1.01\times10^{0}$	1×10^{-38}	3.4×10^{-4}
LD_{10} (48 h)	$1.84\times10^{-7}\pm1.77\times10^{0}$	1×10^{-38}	3.13×10^{-5}

Table 3: Effect of increasing doses of active ingredients of thunder insecticide on bee's mortality

		Mortality (%)			
Treatments	Doses (ng μL^{-1} per bee)	2 h	10 h	24 h	48 h
Control	None	0.0 ± 0.0^{f}	4.0 ± 0.0^{e}	5.3 ± 1.3^{b}	9.6 ± 1.3^{b}
50 ppm	7.25	17.3 ± 1.3^{de}	65.0 ± 5.8^{d}	94.0 ± 2.6^{a}	98.6 ± 1.3^{a}
100 ppm	14.5	10.6 ± 2.6^{e}	70.0 ± 1.3^{cd}	96.0 ± 2.3^{a}	96.0 ± 2.3^{a}
250 ppm	36.25	28.0 ± 4.0^{d}	85.0 ± 1.3^{bc}	94.6 ± 2.6^{a}	98.6 ± 1.3^{a}
500 ppm	72.5	12.0 ± 2.3^{e}	78.6 ± 5.8^{cd}	93.3 ± 4.8^{a}	96.0 ± 2.3^{a}
1000 ppm	145	42.6 ± 8.7^{c}	74.7 ± 9.6^{bcd}	97.3 ± 1.3^{a}	98.6 ± 1.3^{a}
2000 ppm	290	52.0 ± 6.1^{bc}	81.3 ± 2.6^{ba}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
3000 ppm	435	64.0 ± 2.3^{ba}	92.0 ± 4.0^{ba}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
50000 ppm	725	69.3 ± 1.3^{a}	92.0 ± 2.3^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
$F_{8,18}$		51.25	60.37	66.80	131.36
p-value		<0.0001	<0.0001	<0.0001	<0.0001

N.B.: There is no significant difference between the means having the same letter on the columns

These data have shown that $1.46\times10^{-3}\pm3.53\times10^{-1}$ g L^{-1} are needed to kill 90% of the bees in 48 h, $1.64\times10^{-5}\pm1.01\times10^{0}$ g L^{-1} to kill 50% of the bees in 48 h and $1.84\times10^{-7}\pm1.77\times10^{0}$ g L^{-1} to kill 10% of the bees in 48 h, (Table 2). The estimated percentage of natural mortality in the trial: 9.39%. The adjustment test of the model to the data (chi square test) gave the following results: χ^2 calculated = 15.358, ddl = 6, exceeding p of χ^2 = 0.018%, indicating that there is no response-dose effect. In other words, the mortality rate does not increase necessarily when the dose increases.

Evaluation of the toxicity of thunder

Sensitivity of the bees to the different doses of thunder applied: The results of bees sensitivity to the insecticide are given in Table 3. The results given in Table 3 have shown that, 10 h after the application of thunder, even the lowest doses of this formulation have caused mortality rates higher at 65%. After 24 h of exposure, all the doses have shown mortality higher than 90%. The highest doses (725, 435 and 290 ng per bee) have caused during the same period 100% mortality. Bees of the control treatments have shown a mortality rate lower than 10%.

Evaluation of the lethal doses at 10, 50 and 90% of the thunder-based insecticide: The values LD_{10}, LD_{50} and LD_{90} (Table 4) were obtained by analyzing the effect of the increasing doses of the active ingredients on the mortality rate of the bees. Table 4 presents the values of the different LDs, 2, 10, 24 and 48 h after the test.

The results have shown that $7.67\times10^{0}\pm5.91\times10^{-1}$ g L^{-1} are needed to kill 90% of the bees in 2 h against $2.49\times10^{-1}\pm4.08\times10^{-1}$ g L^{-1} for 50% of the bees to die in the same period of time. The values of the lower and higher limits of these lethal doses have varied between 2.76×10^{-1} and 2.52×10^{-2} g L^{-1} for the lower limits at 2.12×10^{2} and 2.48×10^{0} g L^{-1} for the higher limits. Forty eight hours after, the values of the lethal doses at 90 and 50% were $5.41\times10^{-4}\pm1.01\times10^{0}$ and $4.97\times10^{-7}\pm2.5\times10^{0}$ g L^{-1}, respectively.

The lethal doses at 50% provoked at the different steps of the observation have shown the following values for 2, 24 and 48 h: $8.13\times10^{-3}\pm1.22\times10^{0}$, $1.1\times10^{-5}\pm1.22\times10^{0}$ and $4.97\times10^{-7}\pm2.5\times10^{0}$ g L^{-1}, respectively (Table 4).

The data analysis at 24 and 48 h after the application has shown that there is a response-dose effect which meant that, mortality increases when the dose increases with, respectively (χ^2 calculated = 9.618, ddl = 6, p = 0.142%) for 24 h and (χ^2 calculated = 7.21, ddl = 6 and p = 0.302%) for 48 h.

Table 4: Lethal doses of thunder and their lower and higher limits 2, 24 and 48 h after the test of toxicity

Lethal doses	Lethal doses at 50% (g L^{-1})	Lower limit (g L^{-1})	Higher limit (g L^{-1})
LD_{90} (2 h)	$7.67\times10^{0}\pm5.91\times10^{-1}$	2.76×10^{-1}	2.12×10^{2}
LD_{50} (2 h)	$2.49\times10^{-1}\pm4.08\times10^{-1}$	2.52×10^{-2}	2.48×10^{0}
LD_{10} (2 h)	$8.13\times10^{-3}\pm1.22\times10^{0}$	8.38×10^{-6}	7.8×10^{0}
LD_{90} (10 h)	$2.64\times10^{-1}\pm1.49\times10^{-1}$	1.5×10^{-1}	6.5×10^{-1}
LD_{50} (10 h)	$1.99\times10^{-3}\pm2.57\times10^{-1}$	3.82×10^{-4}	4.93×10^{-3}
LD_{10} (10 h)	$1.49\times10^{-5}\pm5.85\times10^{-1}$	3.24×10^{-7}	1.13×10^{-4}
LD_{90} (24 h)	$3.93\times10^{-3}\pm4.19\times10^{-1}$	2.96×10^{-5}	1.43×10^{-2}
LD_{50} (24 h)	$1.10\times10^{-5}\pm1.22\times10^{0}$	1.33×10^{-12}	3.22×10^{-4}
LD_{10} (24 h)	$3.10\times10^{-8}\pm2.07\times10^{0}$	4.92×10^{-20}	8.91×10^{-6}
LD_{90} (48 h)	$5.41\times10^{-4}\pm1.01\times10^{0}$	1×10^{-38}	6.17×10^{-3}
LD_{50} (48 h)	$4.97\times10^{-7}\pm2.5\times10^{0}$	1×10^{-38}	1.53×10^{-4}
LD_{10} (48 h)	$4.56\times10^{-10}\pm4.02\times10^{0}$	1×10^{-38}	4.39×10^{-6}

Table 5: Effect of the increasing doses of active ingredients of the insecticide on bee's mortality

		Mortality (%)			
Treatments	Doses (ng µL^{-1} per bee)	2 h	10 h	24 h	48 h
Control	None	0.0 ± 0.0^{f}	4.0 ± 0.0^{d}	5.3 ± 1.3^{b}	9.3 ± 1.3^{b}
50 ppm	21.54	17.3 ± 3.5^{e}	70.6 ± 3.5^{c}	88.0 ± 4.0^{a}	96.4 ± 4.0^{a}
100 ppm	43.0	22.6 ± 1.3^{ed}	82.6 ± 4.8^{c}	96.0 ± 4.0^{a}	100.0 ± 0.0^{a}
250 ppm	107.5	29.3 ± 1.3^{ed}	85.3 ± 2.6^{bc}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
500 ppm	215.0	33.3 ± 3.5^{d}	78.6 ± 3.5^{bc}	93.3 ± 4.8^{a}	98.6 ± 1.3^{a}
1000ppm	430	50.6 ± 1.3^{c}	69.3 ± 4.8^{bc}	89.3 ± 2.7^{a}	100.0 ± 0.0^{a}
2000 ppm	860	73.3 ± 7.4^{b}	78.6 ± 4.8^{bc}	93.3 ± 2.6^{a}	100.0 ± 0.0^{a}
3000 ppm	1290	74.6 ± 4.8^{b}	90.6 ± 3.5^{ba}	96.0 ± 4.0^{a}	100.0 ± 0.0^{a}
5000 ppm	2150	88.0 ± 2.3^{a}	96.0 ± 0.0^{b}	98.6 ± 1.3^{a}	100.0 ± 0.0^{a}
$F_{8,18}$		75.05	52.22	40.45	150.6
p-value		<0.0001	<0.0001	<0.0001	<0.0001

NB: There is no significant difference between the means having the same letter on the columns

Table 6: Lethal doses of lamdex+chlorpyrifox and their lower and higher limits 2, 10 and 24 h after

Lethal doses	Lethal doses at 50% (g L^{-1})	Lower limit (g L^{-1})	Higher limit (g L^{-1})
LD_{90} (2 h)	$5.01654\times10^{0}\pm1.20013\times10^{-1}$	3.12993×10^{0}	9.51526×10^{0}
LD_{50} (2 h)	$2.89271\times10^{-1}\pm5.37743\times10^{-2}$	2.26222×10^{-1}	3.70173×10^{-1}
LD_{10} (2 h)	$1.66803\times10^{-2}\pm1.19149\times10^{-1}$	8.83624×10^{-3}	2.66474×10^{-2}
LD_{90} (10 h)	$7.68461\times10^{0}\pm1.11089\times10^{0}$	1.49643×10^{-2}	3.94628×10^{3}
LD_{50} (10 h)	$1.47169\times10^{-4}\pm2.13368\times10^{0}$	9.15438×10^{-10}	2.36593×10^{1}
LD_{10} (10 h)	$2.81843\times10^{-9}\pm5.21124\times10^{0}$	5.42715×10^{-22}	1.46367×10^{4}
LD_{90} (24 h)	$5.57\times10^{-3}\pm2.82\times10^{0}$	7.33×10^{-10}	4.23×10^{4}
LD_{50} (24 h)	$1.94\times10^{-11}\pm1.57\times10^{1}$	1×10^{-38}	4.7×10^{27}

Evaluation of the toxicity of the pesticide lamdex 30+chlorpyrifos 100

Sensitivity to the different doses of the applied insecticide: Table 5 shows the sensitivity of the bees to the different doses of the lamdex insecticide, after 2, 10, 24 and 48 h. The data have shown that the lowest dose (21.5 ng per bee) induced mortality lower at 20% against mortality higher at 85% for the highest dose (2.15×10^{3} ng per bee).

After 24 h all the doses used induced mortality rates higher at 88%. There is no significant difference between the different mortality rates observed at this period of observation. The bees of the control treatment have shown mortality lower at 10%. There is a highly significant difference between the mortality rates of the bees of the control test and the treatments at the different periods of observation.

Forty eight hours after all the doses used have induced mortality nearly equivalent to 100%, whereas, the control treatment showed 9.3 ± 1.3% mortality. There is a highly significant difference between the mortality rates of the control treatment and all the doses used ($p<0.0001$).

Evaluation of the lethal doses at 10, 50 and 90% of the lamdex 30 and chlorpyrifos 100 based insecticide: The study of the different lethal doses induced by the insecticide lamdex+chlorpyrifos after their application is summarized in Table 6. The results have shown that $5.01654\pm1.20013\times10^{-1}$ g L^{-1} are needed to kill 90% of the bees in 2 h, against $7.68461\pm1.11089\times10^{0}$ g L^{-1} in 10 h and $5.57\times10^{-3}\pm2.82$ g L^{-1} in 24 h.

The chi square test done at 2, 10 and 24 h after the test has shown a dose-response effect at 2 h. In other words, the mortality increases when the dose increases (χ^2 = 10.969, ddl: 6, excess probability of χ^2 = 0.089%).

DISCUSSION

The results of this study have shown the impact on bees of three insecticides commonly used in agriculture in Benin. Used to control pests, these insecticides have shown that their noxiousness to the major pollinators, bees *Apis mellifera* adansonii is quite worrying. To the best of our knowledge, very few studies on the toxicity of pesticides with regard to bees have been carried out in sub-Saharan Africa in particular. These studies are all the more important that already in 1967, it was reported that carbaryl, a cotton insecticide in the US destroyed 70 000 colonies of bees of which 33,000 colonies in the state of Washington where it is used to treat maize[28]. The number of colonies has also decreased[29] from 4.3 million in 1985 to 2.7 million in 1995. In Europe, the number of bee hives has reduced to about 16% between 1985 and 1991 and the number of beekeepers about[30] 8%. In France, the number of beekeepers and bee hives has reduced, respectively[31] from 20 and 30% in 1996 and 2001. In this context, several studies have been conducted on the toxicity of pesticides to bees, especially insecticides. These studies led to different results, depending on the active ingredients and laboratory conditions. Emamectin is one of the molecules newly introduced in the protection of cotton production in Benin. It is a biological insecticide, containing abamectin benzoate, a lactone produced from the fermentation of *Streptomyces avermitilis*. The application of the increasing doses of 9.6, 1.92 at 96 ng per bee gave values of LD_{50} varying from 0.134 ng per bee after 24 h exposure to 1.64. 10^{-2} ng per bee after about 48 h exposure of the bees. Let us point out that the value of LD_{50} after 2 h exposure was $2.41\times10^{14}\pm2.01\times10^{2}$ value showing the nature of the product and its harmlessness since the first exposure.

The present studies also addressed the issue of toxicity of thunder, an insecticide with active ingredients including beta-cyfluthrin (45 g L^{-1}), a synthesis pyrethroid and Imidacloprid (100 g L^{-1}) which is an insecticide of the family of the neonicotinoid actually forbidden in many European countries. These values of the LD_{50} varied from 19.9 ng per bee for 10 h exposure at 1.1×10^{-2} ng per bee for 18 h and 5×10^{-4} ng per bee for 36 h. Results tally with those of Suchail *et al.*[32] who obtained for the LD_{50} at 24 and 48 h, 24 ng per bee per topical application of imidacloprid on *Apis mellifera* mellifera. The same researchers show the values of 14 µg per bee for *Apis mellifera* caucasica per topical application. A LD_{50} varying from 49 at 102 ng per bee at 48 h per topical application is obtained[33].

In France, Gaucho, an imidacloprid insecticide is suspected to provoke a decrease in the population of bees and honey production. A study by Araki *et al.*[34] has proven extremely high toxicity of imidacloprid (LD_{50} equals 600 mg kg^{-1} bees) and two of its metabolites: 5-hydroxy-imidacloprid (LD_{50} = 2600 mg kg^{-1} bees) and olefin (LD_{50} = 300 mg kg^{-1} bees)[35]. Beta-cyfluthrin is an active ingredient, that shows an insecticide effect and that belongs to the chemical family of synthesis pyrethroids. In thunder, the efficiency of the two active ingredients is made possible thank to an O-TEQ formulation. This synergism allows higher efficiency, which explains these results very lower than those obtained by various authors with imidacloprid alone[32,33,22].

The studies carried out with the insecticide containing lamdex 30 and chlorpyrifos 400 have shown the values of the LD_{50} which have varied from 0.15 ng per bee after 10 h of exposure to 1.94×10^{-8} ng per bee after 18 h exposure. The results tally with those of Worthing[36] and Stevenson[37] which show that the LD_{50} of chlorpyrifos ethyl at 24 and 48 h on the bee *Apis mellifera* mellifera is 59 ng per bee per application. A value of 250 ng per bee is obtained after topical application[38]. On the contrary[22], obtains 34.55 ng per bee per topical application. The values obtained during these studies were lower than all these values of LD_{50} mentioned by the different authors. Chlorpyriphos ethyl is generally classified as toxic for bees. Sublethal effects of chlorpyriphos ethyl on bees are not known. These results can be explained partly by the presence of two active ingredients that are lamdex 30 and chlorpyrifos 100 in the insecticide. The possible synergism of these two active ingredients would explain the low values of LD_{50} observed, therefore its high toxicity to bees.

Different reasons are given to explain this variability. The toxicity of the active ingredients varies depending on the mode of application (topical, tarsal contact, collective ingestion or individual ingestion), the experimental conditions (temperature, relative moisture), experimental parameters (number of bees in each group, number of replications, sugar concentration in the syrup, quantity of acetone to dilute the active ingredient)[22,39-41]. Some studies have shown that the toxicity of the pyrethroids varies with the temperature[42,43].

Several studies have been carried out on the effects of deltamethrin on bees *Apis mellifera* mellifera[37,36] find a LD_{50} at 24 and 48 h of 51 ng per bee per topical application. The same value as the two previous per topical application is indicated[44]. The value of 0.067 µg per bee topical application is found by Atkins and Anderson[45]. Other studies show a value of the LD_{50} of 109.72 ng per bee per topical application and 239.50 ng per bee for the collective ingestion[22]. A value of 0.027 µg per bee is obtained for the topical application[22,44]. These results show that the values of LD can vary depending on several factors. In fact, the LD_{50} can vary depending on the conditions the experiment is carried out. The studies conducted on the toxicity per topical application of deltamethrin for the bees show that, when the experiment is carried out at 32°C and at 50% HR, an LD_{50} varying from 0.025-0.037 µg per bee is obtained[46]. On the contrary when the temperature conditions are 20°C and the relative moisture at 30%, it is obtained a value of the LD_{50} between[22] 0.006 and 0.011 µg per bee.

In the face of all the aforementioned, reasoned control (rational utilization of chemical pesticides) of crop pests is one of the factors that condition the success of plant protection and the conservation of our environment. The different impacts of the toxicity of the pesticides to bees tackled only direct impacts, which are visible due to bad utilization of an active ingredients. However, there are many other consequences of this bad agricultural practice in crop protection. A few of them are: Change in behavioral attitudes and aptitudes of the bees, toxicity of beehive products and increased sensitivity of the bees to the different attacks of parasites and diseases, all this leading to desertion of the beehives and the disappearance of several hundreds of swarms of bees, a phenomenon already observed by beekeepers in Benin.

Among behavioral aptitudes, learning and memorizing performances allow the bees to adapt themselves to the variations of the signal learnt initially[47,48]. A sublethal dose of permethrin[49] or the sub-chronic administration of imidacloprid and endosulfan[41] or the applications of doses of endosulfan and cyfluthrin in the farm reduce the olfactory learning capacity[50].

CONCLUSION

The use of insecticides as part of the integrated control is most often indispensable to ensure abundant, regular and quality production. Insecticides are developed to kill pests and not crop auxiliaries. However, their mode of utilization is often not compatible with bee's activities. These studies have shown the urgency of the integrated protection aspect of bees in all protection strategies of our crops in order to associate the promotion of good agricultural practices in crop protection. The pesticides tested in the frame of this study are among the most used in cotton, market gardening and arboriculture protection. These crops depend on at least 90% or more bee's pollination and other pollinators for their yield. Non-judicious choice, as well as bad practices in plant protection is a real handicap to achieving the objectives of modern agriculture which aim at increasing yields and food security. A codification of the use of most insecticides studied should safeguard crop auxiliaries by increasing considerably yields. Promotion of strategies as part of the dissemination of good agricultural practices in plant protection is a guarantee which should ensure sustainable agriculture and environmental, human and biodiversity protection.

SIGNIFICANCE STATEMENTS

Pollination of fruit trees is assured at more than 80% by honey bees and its success depends on the number of the pollinators in the plantation and climate conditions during flowering. The absence of pollinators can reduce yield up to one quarter of the production. For some years now, apiary weakening phenomena with a diminishing of honey bees activities, a sudden disappearance of the colonies and/or mass mortality of bees, leading to sudden grain yield losses and honey flow decrease were observed in Benin. Among the multiple reasons the major one is the exposure of honey bees to pesticides. In Benin, doses of applied pesticides are generally higher than those recommended for cotton protection. What can be the consequences of such practices on honey bees? Results indicated that, even lowest doses of the pesticides showed mortality higher than 90% to honey bees, 48 h after application. Urgent needs of the integrated plant protection to promote good agricultural practices for honey bee's protection is stressed.

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