



Research article

Assessment of ecotoxicological effects of agrochemicals on bees using the PRIMET model, in the Tiko plain (South-West Cameroon)

Daniel Brice Nkontcheu Kenko ^{a,b,*}, Norbert Tchamadeu Ngameni ^b^a Zoology Laboratory, Department of Animal Biology and Conservation, Faculty of Science, University of Buea, P.O. Box 63 Buea, South-West Region, Cameroon^b Biology and Applied Ecology Research Unit, Dschang School of Science and Technology, University of Dschang, P.O. Box 67 Dschang, West Region, Cameroon

ARTICLE INFO

Keywords:
 Agrochemical
 Ecotoxicological
 PRIMET
 Bees
 Risk assessment
 ETR

ABSTRACT

Pesticide utilization in agriculture has many harmful effects of non-target organisms. This study assessed pesticide risk to bees using PRIMET (Pesticide Risks in the Tropics to Man, Environment and Trade), a pesticide risk model. Data was collected on pesticide application scheme (active ingredient, crop, dose, number of applications, application interval) and ecotoxicological properties ($LD_{50\text{-Bee}}$). These two groups of variables were introduced one after the other in PRIMET 2.0 to obtain the Predicted Exposure Concentration (PEC_{bee}), No Effect Concentration (NEC_{bee}) and Exposure Toxicity Ratio ($ETR_{bee} = PEC_{bee}/NEC_{bee}$). Eight insecticides (out of 15 assessed) and 1 nematicide (out of 1) posed a *Definite Risk* to bees with imidacloprid ($PEC = 4412 \text{ g/ha}$; $ETR = 1.09E+07$) at the top position. Six insecticides (out of 16), and 1 nematicide (out of 1) posed a *Possible Risk* to bees. The insecticide oxamyl ($PEC = 2044 \text{ g/ha}$, $ETR = 87$) had the highest ETR in this category, followed by the nematicide ethoprophos ($PEC = 5.4E+04 \text{ g/ha}$; $ETR = 69$). The results of this study revealed that 27 compounds, including 1 insecticide (out of 15), 10 herbicides (out of 10) and 16 fungicides (out of 16) posed *No Risk* to bees. Herbicides and fungicides appeared “safer” for bees as compared to other pesticide families. The fungicides, mancozeb ($PEC = 1 \text{ g/ha}$, $ETR = 0.006$) and maneb ($PEC = 1 \text{ g/ha}$, $ETR = 0.006$) had the lowest ETR out of all the 43 compounds assessed in the study. Regulation on the importation, distribution and use should be reinforced for very hazardous compounds such as imidacloprid, carbofuran, thiamethoxam and metaldehyde. Substituting the most toxic pesticides with less toxic ones such as novaluron (insecticide), oxadiazon (herbicide), mancozeb (fungicide) and maneb (fungicide) may help to reduce pesticide pressure on the environment.

1. Introduction

The use of pesticides remains the most cost-effective means of controlling pests and weeds, allowing the maintenance of current yields and so, contributing to economic viability (Arias-Estevez et al., 2008). Unfortunately, a high percentage of pesticides applied affect non-target organisms with many acute lethal and chronic sublethal effects. Pesticide users often fail to follow safety measures and recommended doses, and suffer from post-application health disorders such as headache, impaired vision, irritation (Kenko & Kamta 2021; Kenko et al., 2017b; Tchamadeu et al., 2017). Pesticides have negative effects on male reproductive capacities (low sex hormone and sperm counts) as well as liver and kidney functions (Manfo et al. 2012, 2020). Pesticides are among the main chemicals involved in poisoning among patients referred to the Buea Regional Hospital, South-west Cameroon (Kenko Nkontcheu et al., 2020).

Pesticide effects on the environment and biota is routinely assessed via the use of bioindicators, biomarkers, bioassays and modelling. In this line of thought, many models have been used worldwide in EcoRA (Ecological Risk Assessment). In Thailand and Sri Lanka, a Preliminary Risk Assessment (PRA) was done as part of MAMAS (Managing Agrochemicals in Multi-Use Aquatic Systems) (Van den Brink et al., 2003). BEAST (Benthic Assessment of Sediment) has been used to evaluate and classify the level of environmental degradation (Moreno et al., 2009). AMRAP (Aquatic Macrophytes Risk Assessment for Pesticides) has been developed for macrophytes (Maltby et al., 2009). TOXSWA (TOxic Substances in Surface Waters) was developed for the fate of pesticides in fields (Adriaanse 1996). PEARL (Pesticide Emission Assessment of Regional and Local Scales) was made for local and regional evaluations of pesticide spray (Tiktak et al., 2000). PERPEST (Predicting the Ecological Risk of Pesticides) was developed to predict ecological risks related to pesticide (Van den Brink et al., 2002).

* Corresponding author.

E-mail address: kenko.daniel@ubuea.cm (D.B. Nkontcheu Kenko).

As toxicology studies are very expensive, toxicity data in Africa are often sourced from the northern hemisphere (Van den Brink 2008). Moreover, models used in EcoRA are mostly complex and intricate with a large number of required input parameters and data are not quite available. Models often focus on only certain risk aspects, making their applicability limited (Malherbe et al., 2013). These limitations are amplified in developing countries by lack of resources, thus restricting use of the models. The development of PRIMET (Pesticide Risks in the Tropics to Man, Environment and Trade) that require less data input, relevant to more chemical class and technical know-how was a necessity. PRIMET is a simple risk assessment model that requires few inputs and is suitable for use in developing countries (Peeters et al., 2008); it is easy to use even by people without specialist training (Malherbe et al., 2013). PRIMET has been used in South Africa (Malherbe et al., 2013), Cameroon (Fai et al., 2019; Kenko et al., 2017a), Vietnam (Stadlinger et al., 2018), Ghana (Onwona-Kwakye et al., 2020) and Ethiopia (Teklu et al., 2021).

In Cameroon, pesticide importation, distribution and use are done under conditions that are very far from ideal (Manfo et al., 2012). There are many studies on pesticide ecotoxicology in Cameroon. These include surveys on pesticide use patterns (Abang et al., 2013; Abdulai et al., 2018; Amuoh 2011; Dieudonné et al., 2015; Kenko & Kamta 2021; Kenko et al., 2017b; Matthews et al., 2003; Parrot et al., 2008; Tarla et al., 2013; Tchamadeu et al., 2017; Tetang and Foka 2008), laboratory bioassays (Kenko et al., 2017c; Manfo et al. 2012, 2020; Watching et al., 2020) and modelling (Fai et al., 2019; Kenko et al., 2017a). These studies gave evidence of human and environmental health implications of pesticide use. The Tiko plain has sandy alluvial and volcanic soil types with high agricultural potentials, making industrial agriculture one of the main activities of the municipality, among other activities such as trading, fishing and livestock (Tabi et al., 2018). The majority of the forest land (80%) of the Tiko municipality has been converted to oil palm, rubber and banana plantations by Cameroon Development Corporation (CDC) and only few patches of secondary forests exist. In addition to the CDC plantations, there are also small-scale farms producing cocoa and food crops (Neba et al., 2021). Because of pest attacks and in order to increase the yield, pesticide use in agriculture is inevitable. Therefore, many pesticides are used in the south-west region of Cameroon (Oyekale 2017; Tandi et al., 2014). However, pesticides have many harmful effects on non-target organisms (Ibekwe 2004; Sánchez-Bayo 2012; Stanley et al., 2016), including bees. Bees are among animal groups suffering from pesticide effects. Currently, there is a global concern about declining bee populations (Cresswell et al., 2012). Bees act as pollinators of many tropical crops (Hung et al., 2018); the western honeybee *Apis mellifera* is the most important crop pollinator species in the world (Gong & Diao 2017). Due to abundant agricultural activities, and the lack of environmental monitoring scheme by agro-industrial complexes of the municipality, this study aimed at assessing the risks posed by pesticide to bees using PRIMET, a pesticide risk model, in the Tiko plain, south-west Cameroon.

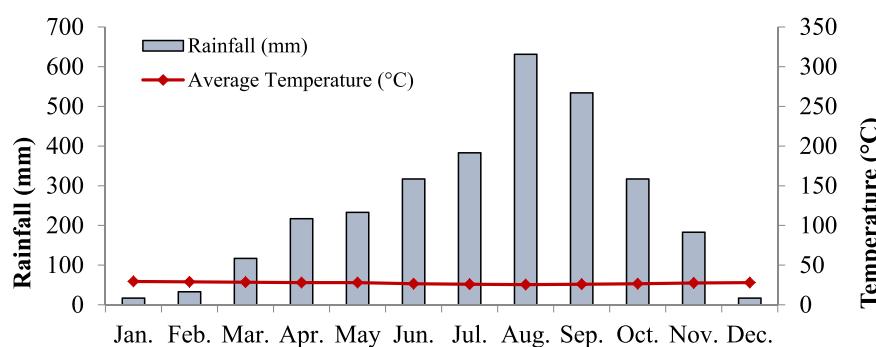


Figure 1. Ombothermic graph of the Tiko plain; Source: (CDC 2016).

2. Material and methods

2.1. Study area

The field work was carried out in the Tiko plain south-west region of Cameroon. Located between 4.08°N (Latitude) and 9.37°E (Longitude), the study site has an elevation of 52m and an annual rainfall of 3198mm (Tingem et al., 2008). The coldest and the雨iest month is August while the warmest month is January. The dry season runs from November to February (Figure 1) and the rainy season from March to October (CDC 2016). Tiko is located at the base of Mount Cameron, and it is close to the Atlantic coast of Cameroon, resulting in a humid climate. The main water courses in the Tiko municipality include the River Mungo, Ombe River, Ndongo and Benoe streams which empty into the Atlantic Ocean (Tabi et al., 2018).

2.2. Pesticide risk assessment

For pesticide risk assessment on bees, two sets of inputs parameters are required by the PRIMET model: pesticide application scheme in the study area and ecotoxicological properties of pesticides.

2.2.1. Survey on pesticide application scheme

Data on the pesticide application schemes were obtained from the survey using a structured questionnaire, and direct interviews of the CDC field assistants and local farmers. Informed consent was received from the participants in the questionnaire and interviews. Pesticide commercial name and active ingredients, applied dose (gram of active ingredient per hectare), number of applications per crop season, time between applications (days), crops on which pesticides are applied, were recorded (Tables 1, 2 and 3).

2.2.2. Ecotoxicological characteristics of pesticides used in the area

Pesticide ecotoxicological data (Table 4) for bees was obtained from the Pesticide Properties Data Base (<http://sitem.herts.ac.uk/aeru/ppdb/en/>) (Lewis et al., 2016).

2.3. Data processing and analysis

Parameters in Tables 1, 2, 3, and 4 were entered one at a time into the PRIMET Version 2.0 software. For each active ingredient, the PRIMET software calculated the Predicted Exposure Concentration (PEC_{bee}), the No Effect Concentration (NEC_{bee}) and the Exposure Toxicity Ratio (ETR_{bee}) (Peeters et al., 2008).

2.3.1. Predicted Exposure Concentration (PEC_{bee})

The exposure is established as the maximum single application rate expressed as gram active ingredient per hectare.

Table 1. Insecticides application schemes in the study area.

Pesticide active ingredients	Crop	Application Interval (Days)	Applied Dose (g.a.i./ha)	Number of Applications Per Crop Cycle
Acetamiprid	Cocoa	30	1 000	4
Bifenthrin	Tomato	21	147	2
Cadusafos	Banana	180	5 600	2
Carbofuran	Banana	180	5 600	2
Chlorpyrifos	Corn	30	73.5	2
Cypermethrin	Tomato	7	441	7
Deltamethrin	Corn	60	73.5	6
Dimethoate	Tomato	15	14.7	8
Fipronil	Cocoa	60	88	6
Imidacloprid	Cocoa	56	4 412	3
λ -Cyhalothrin	Cocoa	30	1 000	4
Lindane	Cocoa	180	735.3	2
Malathion	Beans	184	441	2
Novaluron	Tomato	21	147	2
Oxamyl	Banana	180	2 044	2
Thiamethoxam	Cocoa	7	2 500	9

Table 2. Fungicides application schemes in the study area.

Pesticide active ingredients	Crops	Application Interval (days)	Applied Dose (g/ha)	Number of Applications Per Crop Cycle
Azoxystrobin	Banana	180	100	2
Bitertanol	Banana	180	300	2
Carbendazim	Rubber	36	40	10
Chlorothalonil	Banana	180	1 000	2
Cu(OH) ₂	Cocoa	3	50	40
Difenoconazole	Banana	180	100	2
Epoxiconazole	Banana	180	100	2
Fenpropimorph	Banana	180	616	2
Imazalil	Banana	180	1	2
Mancozeb	Banana	180	2 000	2
Maneb	Tomato	2	100	31
Metalaxyl	Cocoa	20	50	15
Propiconazole	Banana	180	100	2
Pyraclostrobin	Rubber	180	100	2
Tebuconazole	Cocoa	30	59	4
Thiabendazole	Banana	180	500	2

2.3.2. No effect concentration (NEC_{bee})

For the effect assessment, a “safe” concentration was calculated from the toxicity values and an assessment correction factor (to convert from $\mu\text{g}/\text{bee}$ to g/ha) (Eq. (1)).

$$\text{NEC}_{\text{bee}} = \text{EF}_{\text{bee}} \times \text{LD50}_{\text{bee}} \quad (1)$$

where,

NEC_{bee} = No effect concentration for bees (g/ha)

LD50_{bee} = concentration (oral or contact) that kills 50% of bees ($\mu\text{g}/\text{bee}$), the most sensitive endpoint of oral LD50 and contact LD50.

EF_{bee} = extrapolation correction factor for effect assessment of bees, to convert from $\mu\text{g}/\text{bee}$ to g/ha (default value = 50).

2.3.3. Risk assessment for bees

The risk, expressed in Exposure Toxicity Ratio (ETR) as a result of application is computed according to Eq. (2):

Table 3. Herbicides, nematicides and molluscicides application schemes in the study area.

Pesticide active ingredients	Crop	Application Interval (Days)	Applied Dose (g.a.i./ha)	Number of Applications Per Crop Cycle
Herbicide				
2,4-D amine	Weeds	60	221	6
Clethodim		120	147	1
Diuron		365	295	1
Glufosinate-NH ₃		365	735	1
Glyphosate		180	588	2
Glyphotrimesium		365	588	1
Nicosulfuron		30	147	3
Oxadiazon		365	29.5	1
Paraquat		90	442	3
Triclopyr		21	551	3
Molluscicide				
Metaldehyde	Banana	365	12 000	1
Nematicide				
Ethoprophos	Banana	120	54 000	3

$$\text{ETR}_{\text{bee}} = \frac{\text{PEC}(\text{bee})}{\text{NEC}(\text{bee})} \quad (2)$$

where,

ETR_{bee} = Exposure Toxicity Ratio due to application

PEC_{bee} = Exposure concentration = individual dose applied (g/ha)

NEC_{bee} = No Effect Concentration for bees (g/ha)

- $\text{ETR} < 1$, there is No Risk
- $1 \leq \text{ETR} \leq 100$, there is a Possible Risk
- $\text{ETR} > 100$, there is a Definite Risk

ETR values were interpreted as seen in Table 5 following (Peeters et al., 2008):

2.3.4. Distribution of ETRs

The Kruskal-Wallis's test (non-parametric) was used to check the distribution of ETRs and compare medians according to pesticides families. The spearman method was used to check the statistical correlation between LD50_{bee} and ETR_{bee}.

3. Results

3.1. Insecticides effects on bees

The present study revealed that almost all the insecticides (75%) used in the area posed a possible and a definite risk to bees. The insecticide imidacloprid ($\text{PEC} = 4 412 \mu\text{g}/\text{bee}$; $\text{ETR} = 1.09E+07$) posed the highest risk followed by carbofuran ($\text{PEC} = 5 600 \mu\text{g}/\text{bee}$; $\text{ETR} = 3 111$). Nova-luron ($\text{PEC} = 147 \mu\text{g}/\text{bee}$, $\text{ETR} = 0.03$) is the only insecticide posing “No Risk” to bees (Table 6).

3.2. Effects of herbicides, molluscicides and nematicides on bees

All the herbicides evaluated in the study area posed “No Risk” ($\text{ETR} < 1$). Metaldehyde (molluscicide) posed a definite risk ($\text{ETR} = 2 124$) to bees while ethoprophos (nematicide) posed a possible risk ($\text{ETR} = 69$) to bees (Table 7).

Table 4. Ecotoxicological characteristics of pesticides.

Insecticides	Fungicides	Herbicides	
Active Ingredient	LD ₅₀ (µg/bee)	Active Ingredient	LD ₅₀ (µg/bee)
Acetamiprid	1.72	Azoxystrobin	200
Bifenthrin	0.02	Bitertanol	200
Carbofuran	0.036	Carbendazim	50
Chlorpyrifos	0.059	Chlorothalonil	40
Cypermethrin	0.023	Cu(OH) ₂	44.46
Deltamethrin	0.0015	Difenoconazole	100
Dimethoate	0.1	Epoxiconazole	100
Fipronil	0.0059	Fenpropimorph	100
Imidacloprid	0.081	Imazalil	39
λ-Cyhalothrin	0.038	Mancozeb	85.3
Lindane	0.23	Maneb	100
Malathion	0.16	Metalaxyl	200
Novaluron	100	Propiconazole	100
Oxamyl	0.47	Pyraclostrobin	100
Thiamethoxam	0.024	Tebuconazole	200
-	-	Thiabendazole	34

Table 5. ETR range, risk categories and corresponding colours.

ETR range	Risk category	Colour
ETR < 1	No Risk	Green
1 ≤ ETR ≤ 100	Possible Risk	Orange
ETR > 100	Definite Risk	Red

Table 6. Risks posed by insecticides on Bees.

Pesticide active ingredients	PEC _{bee} (g/ha)	NEC _{bee} (g/ha)	ETR _{bee}
Acetamiprid	1 000	86	12
Bifenthrin	147	1	147
Carbofuran	5 600	1.8	3 111
Chlorpyrifos	73.5	2.95	25
Cypermethrin	441	1.15	384
Deltamethrin	73.5	0.08	980
Dimethoate	14.7	5	3
Fipronil	88	0.3	298
Imidacloprid	4 412	0.4	1.09E+07
λ-Cyhalothrin	1 000	1.9	526
Lindane	735.3	11.5	64
Malathion	441	8	55
Novaluron	147	5 000	0.03
Oxamyl	2 044	23.5	87
Thiamethoxam	2 500	1.2	2 083

3.3. Effect of fungicides on bees

Analyses indicated that all the assessed 16 fungicides posed "No Risk" to bees with ETR below 1. This suggests that fungicides are less toxic to bees in the study area (Table 8).

3.4. ETRs according to pesticides families

The Kruskal-Wallis's test revealed that the distribution of ETRs was significantly ($p < 0.05$) higher for insecticides, as compared to herbicides and fungicides (Figure 2).

Table 7. Risks posed by herbicides, molluscicides and nematicides on Bees.

SN	Pesticide active ingredients	PEC _{bee} (g/ha)	NEC _{bee} (g/ha)	ETR _{bee}
1	2,4-D	221	5 000	0.04
2	Clethodim	147	2 550	0.06
3	Diuron	295	5 385	0.05
4	Glufosinate-NH ₃	735	1.73E+07	0.04
5	Glyphosate	588	5 000	0.12
6	Glyphotriimesium	588	20 000	0.03
7	Nicosulfuron	147	3 800	0.04
8	Oxadiazon	29.5	5 000	0.006
9	Paraquat	442	463	0.95
10	Triclopyr	551	5 000	0.11
11	Metaldehyde	1.2E+04	5 650	2 124
12	Ethoprophos	5.4E+04	780	69

1-10: Herbicides; 11: Molluscicide; 12: Nematicide.

4. Discussion

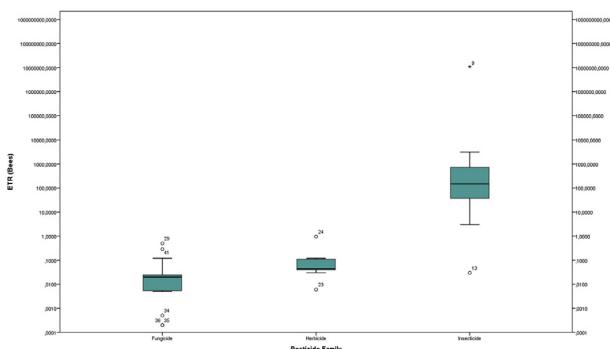
4.1. Pesticides with no risk effects to bees

In the insecticide family, only novaluron (out of 15 insecticides) posed "No risk" to bees with ETR of 0.03. Novaluron (chitin synthesis inhibitor) is an insect growth regulator that is generally less toxic to bee ($LD_{50,bee} = 100\mu\text{g}/\text{bee}$) (Lewis et al., 2016) as compared to other insecticides, hence its ability to pose "No Risk"; moreover, this compound was used at relatively low dosage (147 g/ha) by tomato farmers in the study area. In fact, a pesticide with relatively high $LD_{50,bee}$ is expected to have a low ETR. The spearman correlation revealed that $LD_{50,bee}$ had a very strong positive and significant ($r^2 = 0.997$; $p < 0.0001$) correlation with NEC_{bee} , and a strong negative and very significant ($r^2 = -0.70$; $p < 0.0001$) correlation with the ETR_{bee} . A previous study revealed that novaluron had not sublethal effects among bumblebees, *Bombus terrestris* (Malone et al., 2007). Nevertheless, novaluron, even at full field rate (147 g/ha) is very harmful to immature alfalfa leaf-cutting bees, *Megachile rotundata* (Hymenoptera: Megachilidae) (Hodgson et al., 2011).

Regardless of the dose, all the herbicides and fungicides in this study posed "No Risk" to bees. Bees have the ability to develop tolerance to some insecticides, acaricides and fungicides using P450 genes that produce detoxification enzymes (Gong & Diao 2017), but this capacity is

Table 8. Risks posed by fungicides to Bees.

Pesticide active ingredients	PEC _{bee} (g/ha)	NEC _{bee} (g/ha)	ETR _{bee}
Azoxystrobin	100	10 000	0.01
Bitertanol	300	10 000	0.03
Carbendazim	40	2 500	0.02
Chlorothalonil	1000	2 000	0.5
Cu(OH) ₂	50	2 223	0.02
Difenconazole	100	5 000	0.02
Epoxiconazole	100	5 000	0.02
Fenpropimorph	616	5 000	0.12
Imazalil	1	1 950	0.0005
Mancozeb	1	4 265	0.0002
Maneb	1	5 000	0.0002
Metalaxyl	50	10 000	0.005
Propiconazole	100	5 000	0.02
Pyraclostrobin	100	5 000	0.02
Tebuconazole	59	10 000	0.006
Thiabendazole	500	1 700	0.29

**Figure 2.** Distribution of ETRs in pesticide families.

often lowered when pesticides are combined. Joint toxicity of pesticides mixture may be more toxic than individual chemical compounds (Almasri et al., 2020).

4.2. Pesticides with possible risk effects to bees

Six insecticides (acetamiprid, dimethoate, lindane, chlorpyrifos, malathion and oxamyl) out of 15 (40%) and the only nematicide (ethoprophos), posed a possible risk to bees with oxamyl (PEC = 2044 µg/bee, ETR = 86.98) indicating the highest risk. These findings may be related to the fact that oxamyl (AChE inhibitor), a soil-applied insecticide (Lewis et al., 2016), was used at relatively high dosage (2044 g/ha). Additionally, compounds such as acetamiprid ($LD_{50} = 1.72\mu\text{g}/\text{bee}$), dimethoate ($LD_{50} = 0.1\mu\text{g}/\text{bee}$), lindane ($LD_{50} = 0.23\mu\text{g}/\text{bee}$), chlorpyrifos ($LD_{50} = 0.059\mu\text{g}/\text{bee}$), malathion ($LD_{50} = 0.16\mu\text{g}/\text{bee}$), and oxamyl ($LD_{50} = 0.47\mu\text{g}/\text{bee}$) are very toxic to bees because their $LD_{50} < 2 \mu\text{g}/\text{bee}$ (Vázquez et al., 2015). This work gave evidence of negative correlation between pesticides LD_{50} and ETR. Acetamiprid and dimethoate seem to be less toxic in the aquatic milieu as a previous study reported them to pose minor aquatic risk; oxamyl was predicted by PRIMET to pose a possible risk to the aquatic milieu while lindane, chlorpyrifos, malathion posed a definite aquatic risk (Kenko et al., 2017a). Lindane, chlorpyrifos, malathion seem to elicit higher toxicity in water than on land. However, they pose risk both for terrestrial and aquatic ecosystems. Lindane and

dimethoate which posed a possible risk to bees are banned in Cameroon (MINADER 2013a, b, c). This is an indication that some agrochemicals may still enter the country through unorthodox routes as earlier reported (Manfo et al., 2012). This stresses the necessity to follow up and reinforce legislation on the importation, distribution and utilization of agrochemicals in Cameroon.

Ethoprophos (PEC = 5.4E+04, ETR = 69.23) has a moderate toxicity to bee ($LD_{50} = 15.6\mu\text{g}/\text{bee}$) but it posed a possible risk probably because of its use at high dosage (54 000 g/ha), every 4 months by farmers. This broad spectrum nematicide has been predicted by PRIMET to pose a definite aquatic risk to the Benoe River, South-West Cameroon (Kenko et al., 2017a). As it posed a definitive risk to bees, ethoprophos (nematicide) is risky both for aquatic and terrestrial ecosystems.

4.3. Pesticides with definite risk effects to bees

Eight insecticides (bifenthrin, carbofuran, cypermethrin, deltamethrin, fipronil, imidacloprid, λ -cyhalothrin and thiamethoxam) out of 15 (53%) posed a definite risk to bees. Imidacloprid (neonicotinoid) indicated the highest ETR. The sensitivity of bees to neonicotinoids such as imidacloprid and thiamethoxam is determined by cytochrome P450s of the CYP9Q subfamily (Manjon et al., 2018). In fact, neonicotinoids, organophosphates, triazoles, carbamates, dicarboximides and dinitroanilines pesticides have a huge bioaccumulation potential in honeybee bodies with concentrations ranging from 0.3 to 81.5 ng/g (Kasiotis et al., 2014). Additionally, some pesticides strongly inhibit honey bee cytochromes CYP9Q2 and CYP9Q3 (Haas & Nauen 2021) which are involved in xenobiotic detoxification in bees (Berenbaum & Johnson 2015).

Bifenthrin, a sodium channel modulator, posed a definite risk to bees because of their high toxicity ($LD_{50} = 0.02\mu\text{g}/\text{bee}$) even though it was used at relatively low dosage (147 g/ha) twice a season on tomatoes. Bifenthrin is a serious aquatic contaminant (Ensminger et al., 2013) which has previously been predicted to pose a possible aquatic risk. Carbofuran's capacity to pose risk may be related to its use at relative high dosage (5 600 g/ha). This insecticide is also risky to the aquatic ecosystem; it has been banned for use in Cameroon (MINADER 2013a), so its use in the study area is completely illegal. Cypermethrin is used by many farmers in the area; it is very toxic to bee ($LD_{50} = 0.023\mu\text{g}/\text{bee}$) indicating its capacity to be risky even at low dosage (444 g/ha). Deltamethrin, a fast-acting pyrethroid insecticide, posed a definite risk. This may be because of its repeated application (6 times/season). In the same line of thought, deltamethrin posed a possible risk when used on maize (Ansara-Ross et al., 2008), and a definite risk when used on corn and cotton (Ansara-Ross et al., 2008; Kenko et al., 2017a).

Previously reported to pose minor aquatic risk (Kenko et al., 2017a), fipronil (broad spectrum insecticide) posed a definite risk to bees probably because it was applied six times a crop season on cocoa. Thiamethoxam, an insecticide with broad spectrum systemic action, was used at a relative higher dosage (2 500 g/ha) on cocoa, hence its ability to pose risk to bees. Nevertheless, thiamethoxam, has low aquatic toxicity because it posed no risk to the Benoe stream (Kenko et al., 2017a). Moreover, the potential acute risk of thiamethoxam to freshwater organisms was found to be minimal (Finnegan et al., 2017). λ -Cyhalothrin (pyrethroid insecticide) was used 4 times per crop season, monthly at 1 000 g/ha on cocoa; it is very toxic to bees ($LD_{50} = 0.038\mu\text{g}/\text{bee}$). These may be the reason for its ability to pose a definite risk.

Metaldehyde, a systemic molluscicide for controlling terrestrial slugs and snails (Joyce et al., 2020) posed a definite risk to bees (PEC = 1.2E+04; ETR = 2124). Metaldehyde is practically non-toxic to the adult honey bee on both an acute oral and contact exposure (Bieri 2003; Joyce et al., 2020) but its application at high doses may explain why it posed risks to bees. The negative impact of pesticides on bees may affect crop yield and lower seed vigour as bees are the main agents of crop pollination (Gong & Diao 2017).

4.4. Toxicity according to pesticide families

Unlike insecticides with significantly higher ETRs, fungicides and herbicides had low ETRs. These findings give evidence of a very high risk associated with insecticides as compared to other pesticide families. Insecticides ingested from nectars and pollens of flowers of threatened crops have been identified as one potential threat to bees (Cresswell et al., 2012). This is a warning signal for other insects, arthropods, organisms, and the ecosystem as a whole as honey bees are not more sensitive to pesticides than other insect species (Hardstone & Scott 2010).

5. Conclusion

From the results of the present study, there are indications that the present level of application of pesticides in the Tiko municipality, southwest Cameroon render bees vulnerable to pesticides. The regulation on the importation, distribution and utilization of pesticides should be reinforced in Cameroon, especially for chemicals whose high toxicity on non-target organisms has been proven in the study. Substituting the most toxic pesticides with less toxic ones may help to lower reduce pesticide pressure on the environment. Further studies should be done using PRIMET in other agroecological regions of the country and the world. Assessing the bioaccumulation capacity of agrochemicals will also give valuable information of their ecotoxicology.

Declarations

Author contribution statement

Daniel Brice Nkongtcheu Kenko: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Norbert Tchamadeu Ngameni: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

- Abang, A., Kouame, C., Abang, M., Hannah, R., Fotso, A., 2013. Vegetable growers perception of pesticide use practices, cost, and health effects in the tropical region of Cameroon. *Int. J. Agron. Plant Prod.* 4, 873–883.
- Abdulai, A.N., Konje, C.N., Achiri, T.D., Tarla, D.N., Nsobinenyui, D., 2018. Pesticide use practices by market gardeners in the santa area of the north west region of Cameroon. *Int. J. Phys. Soc. Sci.* 26, 1–11.
- Adriaanse, P., 1996. Fate of Pesticides in Field Ditches: the TOXSWA Simulation Model. SC-DLO.
- Almasri, H., Tavares, D.A., Pioz, M., Sené, D., Tchamitchian, S., Cousin, M., Brunet, J.-L., Belzunges, L.P., 2020. Mixtures of an insecticide, a fungicide and a herbicide induce high toxicities and systemic physiological disturbances in winter *Apis mellifera* honey bees. *Ecotoxicol. Environ. Saf.* 203, 111013.
- Amuoh, C.N., 2011. A case study of health risk estimate for pesticide-users of fruits and vegetable farmers in Cameroon. Master Biosci. Eng. Ghent Univ. Belg. 58.
- Ansara-Ross, T., Wepener, V., Van den Brink, P., Ross, M., 2008. Probabilistic risk assessment of the environmental impacts of pesticides in the crocodile (west) Marico catchment, North-west province. *WaterSA* 34, 637–646.
- Arias-Estévez, M., López-Periago, E., Martínez-Carballo, E., Simal-Gándara, J., Mejuto, J.-C., García-Río, L., 2008. The mobility and degradation of pesticides in soils and the pollution of groundwater resources. *Agric. Ecosyst. Environ.* 123, 247–260.
- Berenbaum, M.R., Johnson, R.M., 2015. Xenobiotic detoxification pathways in honey bees. *Curr. Opin. Insect Sci.* 10, 51–58.
- Bieri, M., 2003. The environmental profile of metaldehyde. In: BCPC Symposium Proceedings. British Crop Protection Council, pp. 255–262.
- CDC, 2016. Rainfall, temperature, daily sunshine hours of the Tiko plain. In: Plain CoTT (Hrsg.). Research Office, Group Banana Manager, Cameroonian Development Corporation, Tiko.
- Cresswell, J.E., Page, C.J., Uygur, M.B., Holmbergh, M., Li, Y., Wheeler, J.G., Laycock, I., Pook, C.J., de Ibarra, N.H., Smirnoff, N., 2012. Differential sensitivity of honey bees and bumble bees to a dietary insecticide (imidacloprid). *Zoology* 115, 365–371.
- Dieudonné, N., Ngwa, N.E., Olivier, D.S., Bertrand, F.L., Barbara, A.T., 2015. Environmental and health impact associated with the dissemination of persistent organic pollutants (POPs) in yaoundé. *J. Health Environ. Sci.* 2, 1–5.
- Ensminger, M.P., Budd, R., Kelley, K.C., Goh, K.S., 2013. Pesticide occurrence and aquatic benchmark exceedances in urban surface waters and sediments in three urban areas of California, USA, 2008–2011. *Environ. Monit. Assess.* 185, 3697–3710.
- Fai, P.B.A., Ncheuveu, N.T., Tchamba, M.N., Ngealekeleoh, F., 2019. Ecological risk assessment of agricultural pesticides in the highly productive Ndop flood plain in Cameroon using the PRIMET model. *Environ. Sci. Pollut. Contr. Ser.* 26, 24885–24899.
- Finnegan, M.C., Baxter, I.R., Maul, J.D., Hanson, M.L., Hoekstra, P.F., 2017. Comprehensive characterization of the acute and chronic toxicity of the neonicotinoid insecticide thiamethoxam to a suite of aquatic primary producers, invertebrates, and fish. *Environ. Toxicol. Chem.* 36, 2838–2848.
- Gong, Y., Diao, Q., 2017. Current knowledge of detoxification mechanisms of xenobiotic in honey bees. *Ecotoxicology* 26, 1–12.
- Haas, J., Nauen, R., 2021. Pesticide risk assessment at the molecular level using honey bee cytochrome P450 enzymes: a complementary approach. *Environ. Int.* 147, 106372.
- Hardstone, M.C., Scott, J.G., 2010. Is *Apis mellifera* more sensitive to insecticides than other insects? *Pest Manag. Sci.* 66, 1171–1180.
- Hodgson, E.W., Pitts-Singer, T.L., Barbour, J.D., 2011. Effects of the insect growth regulator, novaluron on immature alfalfa leafcutting bees, *Megachile rotundata*. *J. Insect Sci.* 11.
- Hung, K.-L.J., Kingston, J.M., Albrecht, M., Holway, D.A., Kohn, J.R., 2018. The worldwide importance of honey bees as pollinators in natural habitats. *Proc. Biol. Sci.* 285, 20172140.
- Ibekwe, A.M., 2004. Effects of fumigants on non-target organisms in soils. *Adv. Agron.* 83, 2–37.
- Joyce, J.L., Donovan, E., Spatz, D., Chief, B., Branch III, E.R., 2020. Draft Ecological Risk Assessment for the Registration Review of Metaldehyde.
- Kasiotis, K.M., Anagnostopoulos, C., Anastasiadou, P., Machera, K., 2014. Pesticide residues in honeybees, honey and bee pollen by LC-MS/MS screening: reported death incidents in honeybees. *Sci. Total Environ.* 485, 633–642.
- Kenko, D.B.N., Kamta, P.N., 2021. Human and environmental health implications of pesticide utilization by market gardeners in the western highlands of Cameroon. *Asian J. Environ. Ecol.* 14, 44–56.
- Kenko, N.D.B., Fai, P.B.A., Taboue, C., Tchamadeu, N.N., Ngealekeleoh, F., Mbida, M., 2017a. Assessment of chemical pollution with routine pesticides using PRIMET, a pesticide risk model in the Benoe stream in the south-west region of Cameroon. *Eur. Sci. J.* 13, 153–172.
- Kenko, N.D.B., Patricia, B.A.F., Ngameni, T.N., Mpoame, M., 2017b. Environmental and human health assessment in relation to pesticide use by local farmers and the Cameroonian development corporation (CDC), fako division, south-west Cameroon. *Eur. Sci. J.* 13, 454–473.
- Kenko, N.D.B., Tchamadeu, N.N., Ngealekeleoh, F., Nchase, S., 2017c. Ecotoxicological effects of imidacloprid and lambda-cyhalothrin (insecticide) on tadpoles of the African common toad, *Amietophryne regularis* (Reuss, 1833)(Amphibia: bufonidae). *Emer. Sci. J.* 1, 49–53.
- Kenko Nkongtcheu, D.B., Ngwe-Bell, M.-U., Ngameni Tchamadeu, N., 2020. Five year (2013–2017) trends in poisoning among patients of the Buea regional hospital, south-west region (Cameroon). *Braz. J. Biol. Sci.* 7, 209–216.
- Lewis, K.A., Tzilivakis, J., Warner, D.J., Green, A., 2016. An international database for pesticide risk assessments and management. *Hum. Ecol. Risk Assess.* 22, 1050–1064.
- Malherbe, W., Van Vuren, J., Wepener, V., 2013. Preliminary risk assessment of common-use pesticides using PRIMET and PERPET pesticide risk models in a semi-arid subtropical region. *Water Sa* 39, 599–610.
- Malone, L., Scott-Dupree, C., Todd, J., Ramankutty, P., 2007. No Sub-lethal Toxicity to Bumblebees, *Bombus Terrestris*, Exposed to Bt-corn Pollen, Captan and Novaluron. *Maltby, L., Arnold, D., Arts, G., Davies, J., Heimbach, F., Pickl, C., Poulsen, V., 2009. Aquatic Macrophyte Risk Assessment for Pesticides. CRC Press.*
- Manfo, F.P.T., Moundipa, P.F., Déchaud, H., Tchana, A.N., Nantia, E.A., Zabot, M.T., Pugeat, M., 2012. Effect of agropesticides use on male reproductive function: a study on farmers in Djutitsa (Cameroon). *Environ. Toxicol.* 27, 423–432.
- Manfo, F.P.T., Mboe, S.A., Nantia, E.A., Ngoula, F., Telefo, P.B., Moundipa, P.F., Chongwa, F., 2020. Evaluation of the effects of agro pesticides use on liver and kidney function in farmers from Buea, Cameroon. *J. Toxicol.* 2020.
- Manjon, C., Trocza, B.J., Zaworra, M., Beadle, K., Randall, E., Hertlein, G., Singh, K.S., Zimmer, C.T., Homem, R.A., Lueke, B., 2018. Unravelling the molecular determinants of bee sensitivity to neonicotinoid insecticides. *Curr. Biol.* 28, 1137–1143.e5.
- Matthews, G., Wiles, T., Baleguel, P., 2003. A survey of pesticide application in Cameroon. *Crop Protect.* 22, 707–714.

- MINADER, 2013a. Arrêté N° 00699 A-MINADER-SG-CNHPCAT du 23 juillet 2013 Portant Interdiction D'utilisation Des Produits Phytosanitaires Contenant Le Carbofurane. In: MINADER (Hrsg.). Ministry of Agriculture and Rural Development, Yaoundé, Cameroon.
- MINADER, 2013b. Arrêté No-00829 A-MINADER-SG-CNHPCAT du 30 Juillet 2013 Portant Interdiction D'utilisation Des Produits Phytosanitaires Contenant Le Dimethoate. In: MINADER (Hrsg.). MINADER, Yaoundé, Cameroon.
- MINADER, 2013c. Liste des pesticides homologués Au cameroun Au 31 juillet 2013. Liste réservée Au grand public. In: MINADER (Hrsg.). National Registration Commission of Phytosanitary Products and Certification of Sprayers, Yaoundé, Cameroon, p. 40.
- Moreno, P., França, J., Ferreira, W., Paz, A., Monteiro, I., Callisto, M., 2009. Use of the BEAST model for biomonitoring water quality in a neotropical basin. *Hydrobiologia* 630, 231–242.
- Neba, G.A., Anyinkeng, N., Mumbang, C., Fonge, A.B., 2021. Benthic algal community in relationship to perturbation in the Tiko mangrove estuary Cameroon. *Open J. Ecol.* 11, 540–564.
- Onwona-Kwakye, M., Hogarh, J.N., Van den Brink, P.J., 2020. Environmental risk assessment of pesticides currently applied in Ghana. *Chemosphere* 254, 126845.
- Oyekale, A., 2017. Cocoa farmers' safety perception and compliance with precautions in the use of pesticides in centre and western Cameroon. *Appl. Ecol. Environ. Res.* 15, 205–219.
- Parrot, L., Dongmo, C., Ndoumbé, M., Poublon, C., 2008. Horticulture, livelihoods, and urban transition in Africa: evidence from South-West Cameroon. *Agric. Econ.* 39, 245–256.
- Peeters, F.M., van den Brink, P.J., Vlaming, J., Groenwold, J.G., Beltman, W.H., Boesten, J.J., 2008. PRIMET Version 2.0, Technical Description and Manual: a Decision Support System for Assessing Pesticide Risks in the Tropics to Man, Environment and Trade. 1566-7197. Alterra.
- Sánchez-Bayo, F., 2012. Insecticides mode of action in relation to their toxicity to non-target organisms. *J. Environ. Anal. Toxicol.* 4, S4–2.
- Stadlinger, N., Berg, H., Van den Brink, P.J., Tam, N.T., Gunnarsson, J.S., 2018. Comparison of predicted aquatic risks of pesticides used under different rice-farming strategies in the Mekong Delta, Vietnam. *Environ. Sci. Pollut. Contr. Ser.* 25, 13322–13334.
- Stanley, J., Preetha, G., Stanley, 2016. Pesticide Toxicity to Non-target Organisms. Springer.
- Tabi, E.S.B., Eyong, E.M., Akum, E.A., Löve, J., Cumber, S.N., 2018. Soil-transmitted Helminth infection in the Tiko Health District, South West Region of Cameroon: a post-intervention survey on prevalence and intensity of infection among primary school children. *Pan Afr. Med. J.* 30.
- Tandi, T.E., Wook, C.J., Shendeh, T.T., Eko, E.A., Afoh, C.O., 2014. Small-scale tomato cultivators' perception on pesticides usage and practices in Buea Cameroon. *Health* 6, 2945.
- Tarla, D., Meutchieye, F., Assako, V., Fontem, D., Kome, J., 2013. Exposure of market gardeners during pesticide application in the western highlands of Cameroon. *Sch. J. Agric. Sci.* 3, 172–177.
- Tchamadeu, N.N., Nkонтчeu, D., Nana, E.D., 2017. Evaluation des facteurs de risques environnementaux liés à la mauvaise utilisation des pesticides par les maraîchers au Cameroun: le cas de Balessing à l'Ouest Cameroun. *Afrique Sci.* 13, 91–100.
- Teklu, B.M., Haileslassie, A., Mekuria, W., 2021. Pesticides as water pollutants and level of risks to environment and people: an example from Central Rift Valley of Ethiopia. *Environ. Dev. Sustain.* 1–20.
- Tetang, T., Foka, G., 2008. Utilisation des pesticides dans la zone agricole du Moungou—évaluation de l'impact sur l'environnement, la santé des populations et solutions envisageables: cas de la localité de Njombe dans l'arrondissement de Njombé-Penja. *Afr. Front Prot. Nat. Man.*
- Tiktak, A., Van den Berg, F., Boesten, J., Leistra, M., Van der Linden, A., Van Kraalingen, D., 2000. Pesticide emission assessment at regional and local scales: user manual of pearl version 1.1. RIVM report 711401008 142.
- Tingem, M., Rivington, M., Bellocchi, G., Azam-Ali, S., Colls, J., 2008. Effects of climate change on crop production in Cameroon. *Clim. Res.* 36, 65–77.
- Van den Brink, P.J., Roelsma, J., Van Nes, E.H., Scheffer, M., Brock, T.C., 2002. Perpest model, a case-based reasoning approach to predict ecological risks of pesticides. *Environ. Toxicol. Chem.: Int.* 21, 2500–2506.
- Van den Brink, P.J., Sureshkumar, N., Daam, M., Domingues, I., Milwain, G., Beltman, W., Perera, M., Satapornvanit, K., 2003. Environmental and Human Risks of Pesticide Use in Thailand and Sri Lanka; Results of a Preliminary Risk Assessment. Alterra.
- Van den Brink, P.J., 2008. Ecological Risk Assessment: from Book-Keeping to Chemical Stress Ecology. ACS Publications.
- Vázquez, P.P., Lozano, A., Uclés, S., Ramos, M.G., Fernández-Alba, A., 2015. A sensitive and efficient method for routine pesticide multiresidue analysis in bee pollen samples using gas and liquid chromatography coupled to tandem mass spectrometry. *J. Chromatogr. A* 1426, 161–173.
- Watching, D., Tamgno, B., Kamdem, I.N., Zilbinkaye, M., Ngassoum, L.N.T.M., 2020. Volatile organic compounds as markers for detection of *Callosobruchus maculatus* (Fabricius) BRIWELL 1929 (Coleoptera: chrysomelyidae), major pest of Bambara Groundnut [*Vigna subterranea* (Linneaus), Fabaceae] by the hymenopteran parasitoids. *J. Entomol. Zool. Stud.* 2020 (8), 1441–1447.