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How far pesticide contamination from agro-systems can affect adjacent protected forests? Case study in Benin, West Africa

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Abstract

Due to pest pressure, nowadays, conventional cotton farming necessitates the use of more and more pesticides. This study measured how far agrochemicals used in farming areas can pollute adjacent protected habitats. Experimental beehives were installed in farming areas, in buffer zone and in a forest (protected habitat) to monitor pollutants presence. Hives with bee colony were monthly weighted for 20 consecutive months to monitor beekeeping success year round. In parallel the food/flower resources estimated at each site, the dead bees continuously trapped per site to explain eventual fluctuation in beehive weight progress. Honey and dead bee were sampled for pesticide analysis to check if dead bees contain higher pesticide traces and thereby their numbers trend per site. Results show contamination of honey and bee from all sites. The food/flower resources availability followed the same trend as the beehives' weight progression. We concluded of general contamination of bees and honey from farming areas to protected habitats (forest). However, these findings don't have a clear impact on beekeeping and subsequent pollination services. But, the food resources availability appeared to most affect the beekeeping success.

Key words : Apiculture, honeybee, pollination, agrochemicals, Kétou

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Quelle est la portée de contamination des pesticides utilisés dans les agrosystèmes vers les forêts protégées adjacentes? Étude de cas au Bénin, Afrique de l'Ouest

Résumé

En raison de la pression des ravageurs, le système conventionnel de production du coton, de nos jours, nécessite l'utilisation de plus en plus de pesticides. Cette étude a mesuré dans quelle mesure les pesticides utilisés dans les zones agricoles peuvent polluer les habitats protégés adjacents. Des ruches expérimentales ont été installées dans des zones agricoles, en zone tampon et dans une forêt (habitat protégé) pour surveiller la présence de polluants. Les ruches colonisées ont été pesées mensuellement pendant 20 mois consécutifs afin de surveiller le succès de l'apiculture tout au long de l'année. Parallèlement, la disponibilité des ressources en fleurs/nourriture a été estimée sur chaque site, les abeilles mortes piégées en continu par site pour expliquer une éventuelle fluctuation de la progression du poids des ruches suivies. Le miel et les abeilles mortes ont été échantillonnés pour la recherche des pesticides afin de vérifier si les abeilles mortes contiennent des traces de pesticides plus élevées et, par conséquent, expliquer la tendance de leur nombre par site. Les résultats montrent une contamination du miel et des abeilles de tous les sites. La disponibilité des ressources en fleurs / nourriture a suivi la même tendance que la progression du poids des ruches. Nous avons conclu à une contamination générale des abeilles et du miel des zones agricoles aux habitats protégés (forêt). Cependant, ces résultats ne montrent pas clairement un impact de la contamination par les pesticides sur le succès de l'apiculture et les services de pollinisation subséquents. Mais, la disponibilité des ressources alimentaires semblait affecter le plus le succès de l'apiculture, sur la base de nos résultats.

Keywords : Apiculture, abeille, pollinisation, pesticide, Kétou

INTRODUCTION

The rapid human population growth in developing countries would require additional effort to feed the growing population. Conventional agriculture would necessitate intensive use of production inputs, including pesticide to meet the food demand. Pesticides actions and mechanism on pests is well documented their impacts on useful organisms are poorly known (Köhler and Triebskorn, 2013). These useful organisms are, for example, pollinators, seed dispersers, pest control agents, etc. that are useful in agro-systems and for crop production (Fischer et al., 2006). Different evidence of population decline of ground insects as well as flying ones was reported in pesticide application contexts (Mone et al., 2014). There is then a confirmation that useful organisms are also destroyed when pesticides are applied in farming areas to fight pests. There is evidence stating that the loss of insect diversity and abundance is expected to create subsequent effects on food webs and to threaten ecosystem services (Hallmann et al., 2017).

Benin as many other developing African countries, is promoting agricultural intensification to secure food for the growing population, but successive governments are also promoting cash crops - cotton and cashew crops to improve the GDP and create new economic opportunities. In this context, agrochemicals are increasingly used for pest control in targeted crops. As part of West Africa, the main cotton-producing region on the continent, it claims together with other sub-region countries up to 55% of the pesticides market in Africa (Ferrigno et al., 2017).

Similar to previous studies that stated important impact of agricultural intensification and pesticide use on useful organisms and ecosystem services (Tscharntke et al., 2012), we expect comparable impacts on the local habitats and their mutualistic organisms mainly in cotton farming systems in Benin. In such context we wonder how the subsequent pollution is affecting natural habitats like protected areas that are not allocated to agricultural activities. Parks or protected areas were reported to be by far much efficient to conserve biodiversity than any other habitat in front of man-made disturbances (Bruner et al., 2001). Agrochemical pollution from adjacent agro-systems to protected forests will then cause insidious negative effect on hosted biodiversity. Since protected forests are known to be the last refuges to biodiversity, these threats should be considered when comes to the conservation of ecosystem services to guarantee food security for the constantly growing human population. We monitored different parameters on targeted mutualistic animal, honey bee, from farming areas through buffer zone till inside protected forest to document how far agrochemical contaminations are affecting living organisms and their possible effects on ecosystem services such as pollination services provided by bees.

MATERIAL AND METHOD

Study site

The field experiment has been conducted in South Benin, precisely in Kétou, Dogo and Adakplamè village territories (District of Plateau, eastern of Bénin) one of the favourable honey beekeeping regions of the country (Yédomonhan, 2009). These territories are connected to two joint protected forest reserves (Forêts classées de Dogo-Kétou, 7°29'N, 2°25'E) (Figure 1), and form a well-known agricultural region where farmers plant different crops including cotton that requires massive use of pesticides.

The local climate is a tropical bimodal rainfall-type characterized by two rainy seasons (March to July and from September to October) alternating with two dry seasons (in August and from November to February). The total covered area of forest is 42,850 ha (Houndagba et al., 2007). The Dogo-Kétou forest is bounded at the Northern side by the Issanhoun river, on the Western side by the Ouémé river, that receive water from all water catchments in the vicinity, in the Eastern and Southern side by the notched edges of the plateau of Kétou.

The relief is a plateau of low altitude (between 100 and 200 m), characterized in some places by depressions more or less pronounced (Figure 1). Although this forest benefited from a management plan under the PGFTR project (CIRAD-TERA, 1998), it is mainly occupied by farming activities carried by different ethnic groups nowadays. In addition, heavy wood extraction and charcoal-making activities are fragmenting and destroying the forest. All these show a noticeable unsustainable use of these natural resources.

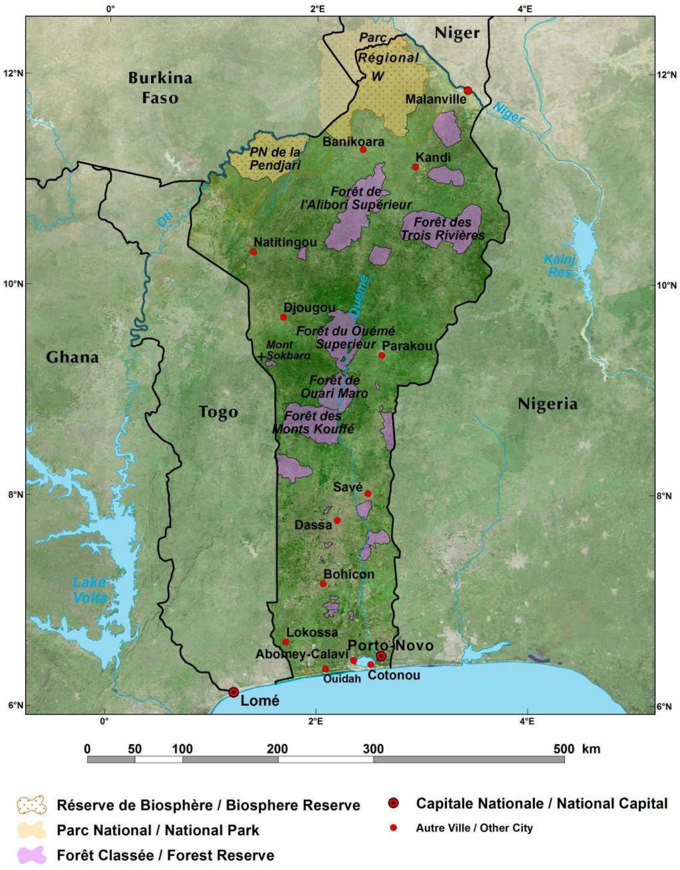

Figure 1: Map of Benin showing the study sites

Data collection and analysis

Experimental design

Bee activities were measured through an experimental beekeeping system. 10 experimental beehives were installed in the farming areas, 10 in the buffer zone of the forest and 10 in the core zone of the forest far from cropping activities. This density is based on Betayene (2008) who recommended a maximum of 10 beehives per 50 m² located at about 4 to 7 km between two apiaries.

Data collection

Hives that received bee colonies were monthly weighted during 20 consecutive months to monitor honey production year-round. Beehives with colonies were weighted with scale of capacity 50 kg, precision 10 g. Colonies were weighted between nightfall and midnight to avoid heavy disturbances that would have been caused on colonies if they were weighted during day time.

Flower blooming as food resources available at each site was estimated as it is known to affect honey production. The phenology of the plants on which honeybees forage were then monitored monthly all over the same beehive weighting period. The selection of these plants has been done using a list obtained from previous study (Yédomonhan et al., 2009) and direct observations of bees foraging activities. Due to absence of method we referred to Szigeti (2016) on the quadrat method to estimate fruit resources. On each selected plant, the food potential was estimated through the selection of three quadrats of 1 m² each chosen from three different sides of the plant canopy. All flowers were counted and a mean value calculated for 1 m². This value was used to extrapolate the food potential multiplying the mean value per m² with the tree crown surface calculated with the measured radius. All individuals of each plant species were sampled the same way and summed during a given monitoring period. Every selected flowering plant was treated similarly. Then, per period, the food potential was estimated to explain possible beehive weight fluctuation that cannot be linked with agrochemical pollutions.

The dead bees were trapped per site on three beehives selected within those hosting large bee colonies. This information helps explaining possible fluctuation of beehive weight progress. Locally made small netted cage that fit the bee fly hole was installed on selected beehives and monitored weekly to count dead bees to follow the progress in the goal to explain possible beehive weight fluctuation imputable to be colony diminution. Three netted cages were fixed to three different beehives hosting large colonies per site resulting in a total of 9 netted cages. A total of 195 counts were done (84 in farming areas, 60 in buffer zone and 51 in forest).

Pesticides analysis

Pesticide traces search in honey and bee tissue samples was conducted from 15 g for honey and dead bees. In total 12 samples of honey and 18 dead bee samples were collected only from Dogo (Kétou). Samples were collected during (June-August) and after (February-April) the pesticide application periods. At each sampling period, samples were collected as followed: 3 honey samples and 2 dead bee samples respectively from farmed lands (Agrosystem), Buffer zone and Forest. For comparison, only honey samples were collected from heavy cotton cultivation regions in the North Benin situated about 400 – 500 km far from Kétou District (Banikoara : 8 honey samples (4 during (June-August) and 4 after (February-April) pesticide application), Tanguiéta: 4 honey samples (2 during (June-August) and 2 after (February-April) pesticide application). These samples were used for pesticide traces search in the laboratory first to compare doses found in Dogo (Kétou) samples and link findings with dead bee number or honey production patterns. Samples from heavy cotton cultivation regions were to check whether the intensity of exposure will result in accumulation of active substances. All collection sites were adjacent to protected habitats: Tanguiéta site to Pendjari Park, Banikoara site to W National Park and Dogo-Kétou to the Protected Forest of the same name.

The Table 1 bellow presents the list of pesticides used in cotton cultivation in Benin during the study period.

Table 1 Pesticides used in Benin for cotton cultivation during the study period

Commercial names	Active compounds	Year
Cutter 112 EC	Chlorpyrifos	2012-2013
Profenofos 500 EC	Profenofos	2012-2013
Cobra 120 EC	Acetamipride Spinetorame	2012-2013
EMA Super 56 DC	Emamectine benzoate Acetamipride	2012-2013
Lambdacal P 645 EC	Lambdacyhalothrine Profenofos	2012-2013
Cotonnix	Deltamethrine, Chorpyriphos-ethyl Acetamipride	2012-2013
Thunder	Betacyflutrine Imidacioprid	2012-2013
Aceta star	Acetamipride Bifenthrin	2012-2013
Alphacal P 218 E	Alphacypermetrine Profenofos	2011-2012
Calfos 375EC	Profenofos	2011-2012
Laser 480 EC	Spinosad	2011-2012
Chango 122 SE	Indoxacarbe Cypermethrine	2011-2012
Ato IBI 01	Betacypermethrine Chorpyriphos-ethyl	2011-2012
Thian 175 O-TEQ	Flubendiamide Spirotetramate	2011-2012
Decis T 258,75	Deltamethrine	2011-2012
Nurelle D 36/200	Cypermethrine Chorpyriphos-ethyl	2011-2012

Depending on the pest pressure in each region, the combination of pesticides varied from one region to another. But everywhere, six consecutive treatments were done. These six treatments were divided into 3 different windows. Sprays started short before flower sets and were separated by two weeks periods.

Gas Chromatography-Mass Spectrometry used to analyse pesticide samples

Gas Chromatography (GC) analysis was carried out on Agilent 6890N GC-Coupled MSD 5972 with chem-station-software-based data acquisition. The injector temperature was maintained at 220 °C, and the detector one was 280°C. Sample was injected in the split less mode, and the split less was opened after 60 s. 1µl sample injection volume was utilized. A fused silica capillary column measuring 30mx0.25mm with a film thickness of 0.25mm composed of 95% Dimethylpolysiloxane (30 m 0.25 mm I.D., 0.25 ím) with chemically bonded phases DB-5 was used. The carrier gas used was Helium at a flow rate of 0.5ml/min. The oven temperature was programmed as follows: initial temperature of 150°C, held for 1 min, increased to 230 °C at 3 °C min-1, held for 5 min, and then increased to 250°C at 3°C min-1 and held for 15 min. The MS ionization potential was 7 eV from 500000, and the temperatures were as follows: ion source 250 °C, transfer line 200 °C, and analyzer 230 °C. GCMS was analysed using electron impact ionization at 70eV and data was evaluated using Total Ion Count (TIC) for compound identification and quantification. The spectrums of components were compared with the database of spectrum of known components stored in the GCMS library. Analysis was performed in SIM mode monitoring specific ions of each analyte as it is shown in. The most intense ion was used for quantification and the second and third ion for confirmation. Identification criteria were based on (a) MSD chem station library and (b) the relative peak heights of the three characteristic masses in the sample peak that must be within 20% of the relative intensity of these masses in the mass spectrum of the standard analysed in the GC/MS system.

Data Analysis

All data have been computed, and Excel was used to draw curves with dead bee mean numbers and the mean beehive weights; histograms drew with values of different pesticides concentrations in samples and polar charts with the mean values of estimated food resources. SigmaStat 3.5 was used to run ANOVA on Ranks to compare series of active substances detected in samples from three sites and Mann-Whitney Rank Sum Test to compare series while comparing two sites. As all data series didn't fall in normal distribution, we were forced to run these non-parametric analyses.

RESULTS

Pesticides accumulation in honey and bees

Active substances detected in homey and bee tissues samples collected in different regions of Benin, different land use practices and different seasons (during and out of pesticide application period) are showed as followed (Figure 2).

The honey was contaminated everywhere the pesticides have been used in cotton cultivation and the detected concentrations seem not to depend clearly on the intensity of the cotton cultivation. Usually, bees forage in radius of 3 km in normal conditions. Therefore, Banikoara (fig. 2a & b), extreme North-western Benin, that is the major cotton cultivation region; Tanguiéta (fig. 2a & b), extreme North-western Benin, that is another cotton cultivation region showed comparable pesticide traces concentrations with Kétou (fig. 2a & b) where cotton cultivation also exists but much less than the two first localities about 400 – 500 km from Kétou. Moreover, samples collected from the same localities during and out the pesticides application didn't show any statistical significant difference in active substances detection and concentrations (Dokossouan February-April vs. Dokossouan June-August; Mann-Whitney Rank Sum Test; T = 353, n (small) = 18, n (big) = 18, p = 0,537; Batia February-April vs. BatiaJune-August; Mann-Whitney Rank Sum Test; T = 302, n (small) = 18, n (big) = 18, p = 0,334; Dogo February-April vs. Dogo June-August; Mann-Whitney Rank Sum Test; T = 286, n (small) = 18, n (big) = 18, p = 0,141). Finally, difference in land use also didn't reveal any statistical significant difference (fig. 2c) (n = 18; ANOVA on Ranks; H = 0,146; d.f = 2; p = 0,929).

When we consider the pesticides accumulation in honey bees tissues, results are showed as followed (figure 3).

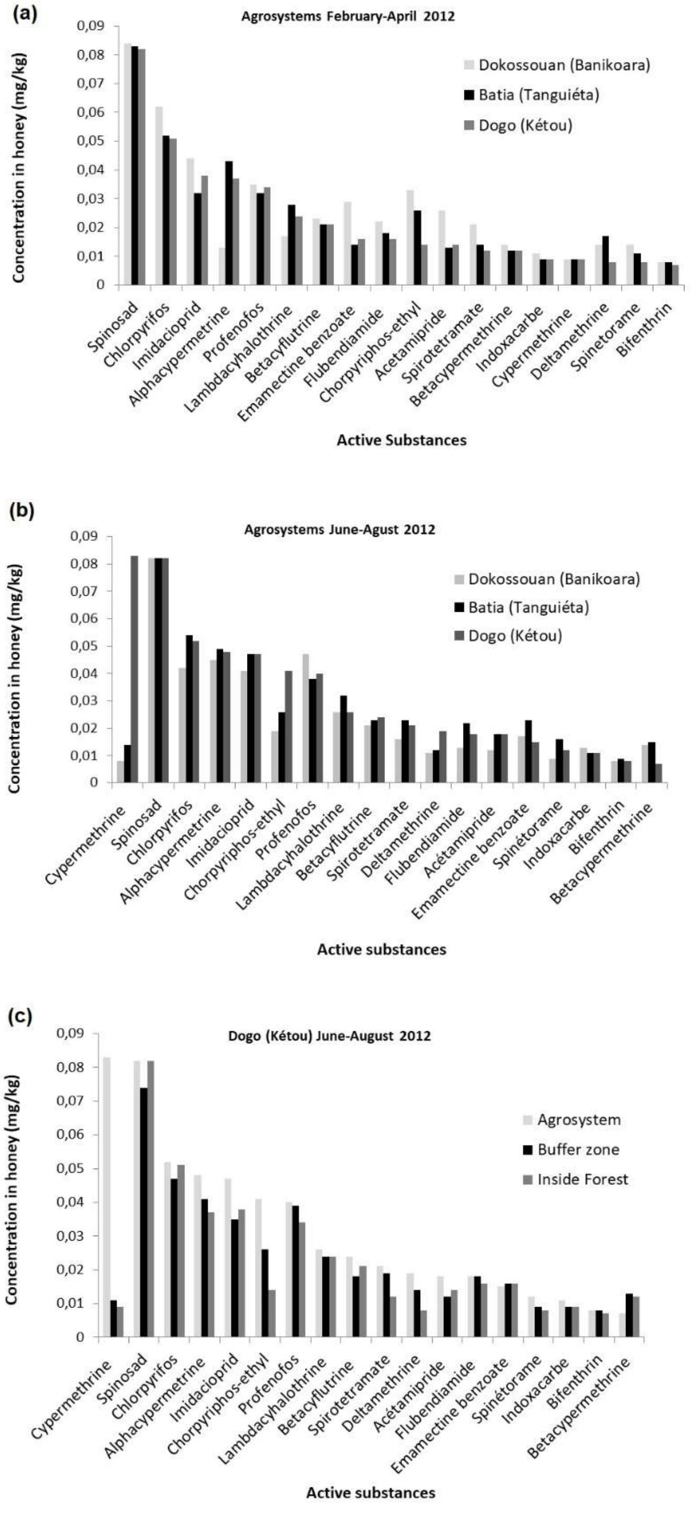

Figure 2: Pesticide active compounds detected in honey samples from the different localities(a) and different land use types (agrosystems = farmed lands; buffer zone = edge of forest; inside forest) during (June-August) and out (February-April) pesticides application periods (24 honey samples, one of the result listing all active compounds is presented here) (a: n=18; ANOVA on Ranks; H = 1,050; d.f = 2; p = 0,592; b: n= 18; ANOVA on Ranks; H=1,705;d.f = 2; p = 0,426; c: n=18; ANOVA on Ranks; H = 2,567;d.f = 2; p = 0.277)

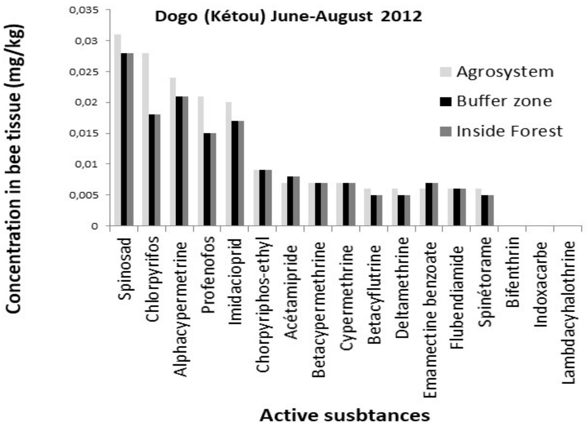

Figure 3: Pesticide traces detected in bee tissue samples from different land use types during pesticides application period (June-August 2012) at Dogo (agrosystem = farmed lands; bufferzone = edge of forest; forest = inside forest) (18 bee samples, one of the result listing all active compounds is presented here)

The pesticides traces were detected overall similarly from farmed lands to forest (n = 18; ANOVA on Ranks; H = 0,146; d.f = 2; p = 0,929) but not all active substances detected in honey were present in bee and concentrations were roughly more than two times lower in bee than in honey. As reference, the pesticides officially used to fight pests in cotton cultivation in Benin at the period the research was carried out are listed in the table 1. It is noticeable that all active substances mentioned in pesticide commercial names were detected in bee and thereby in honey.

Dead bee abundances

Trapped dead bee number at experimental beehives within and out of pesticide application periods is showed as followed (Figure 4). Increase of dead bee's abundances showed some peaks that match sometime cotton cultivation periods (June-August) but not always. The difference in the dead bee number from one site to another maybe due to the colony size since the number of dead individuals is known to be correlated with the total number of individuals present in the colony/beehive.

Food resources availability

Food potential for bees monitored monthly in the different sites on plants on which bee forage is presented as followed (figure 5). We noticed from the graph that food resource availability was high on all sites from June to August. Moreover, the food resource diversity increased from village to forest.

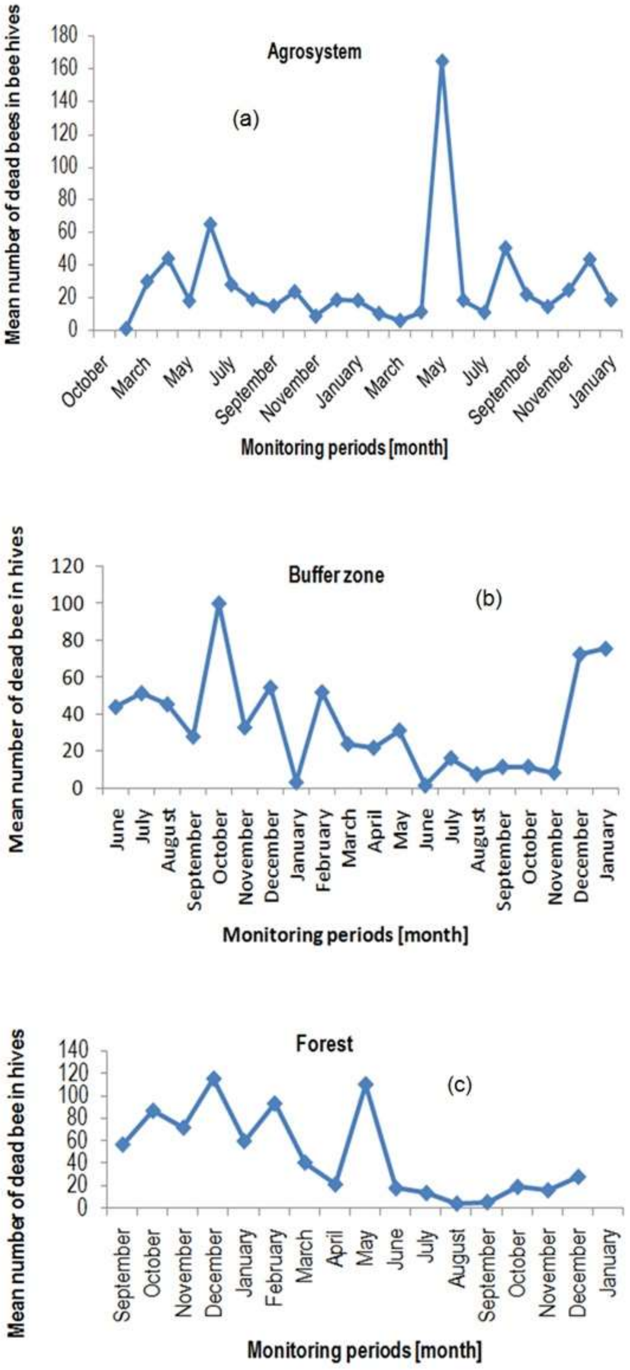

Figure 4: Numbers of dead bees trapped at beehives in different land use types from September 2011 to January 2013 (a: agrosystem = farmed lands (84 samples); b: buffer zone= edge of forest (60 samples); c: forest = inside forest (51 samples)

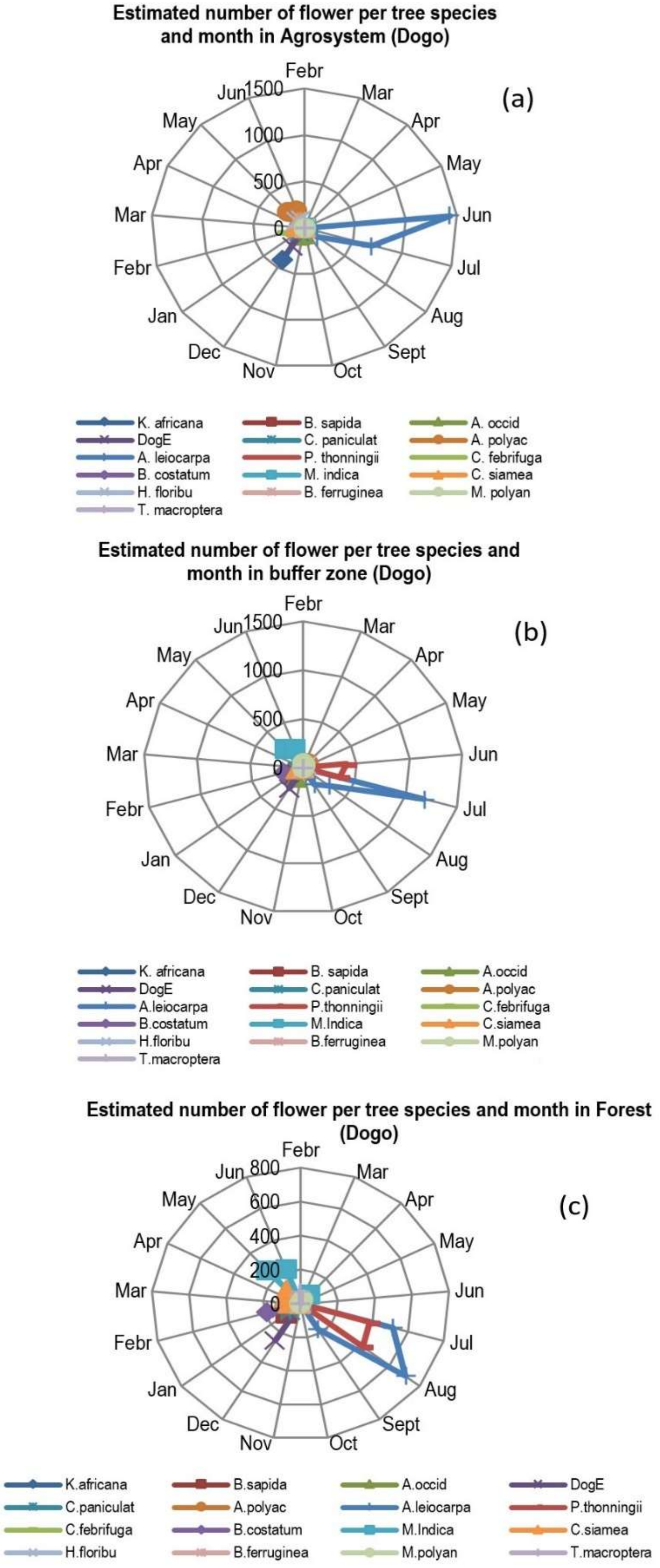

Figure 5: Food resources availability patterns in different land use types (a: agrosystem =village area = farmed lands; b: buffer zone = edge of forest; c: forest = inside forest)

Beehives weight progress

Results of beehives weighted monthly to follow the progress in honey production are showed as followed (Figure 6). From the curves, actives substances traces in honey and in dead bees didn't yield clearly in a reduction of honey production as the pesticides application periods and short after the beehive weight progress didn't show any inflexion. In contrast, the patterns seemed to fit the rainy season (March-July) in the southern, key study region and the rainy season (September-October) in the northern, the heavy cotton cultivation region where the majority of plants set flowers and fruits used by bees.

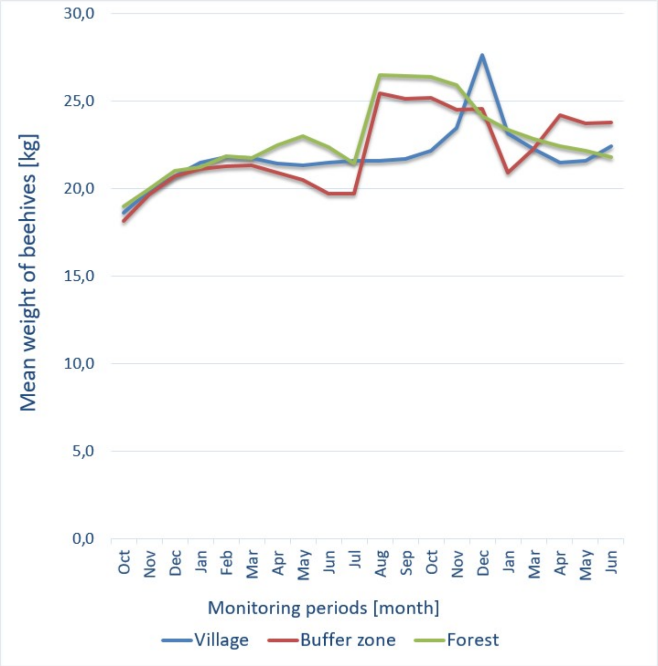

Figure 6: Beehive weight progress in different land use types (village area; buffer zone = edge of forest; inside forest)

DISCUSSION

Our results reported a general presence of actives substances in bees and honey from agro-system to adjacent protected forest situated about 10 km. This demonstrates possible transfer of agrochemicals pollution from agrosystems quite far from sources to key ecosystems such as protected forests known. Bueno and da Cunha (2020) reported pesticide drifts but not on so long distances. Pollutants transport from farmed land to protected habitat is likely to cause negative effect on living organisms. Nowadays, it is widely recognized that the most important condition to maintain life on the earth is biodiversity (Pavlov and Bukvareva, 2007). Thus, well-documented services on which all living organisms, including humans, are constantly provided by biodiversity elements of fauna, flora, fungi, etc. These are unique characteristics of the earth on which humans are living (Bradley et al., 2012). When we consider provisioning ecosystem services that include food and wood production, pollination services delivered by pollinator are major driving factors (Djossa et al., 2015). Although a large number of mutualistic organisms are responsible of pollination services, insects are by far the most important in term of individual numbers (Gianessi, 2010). Many other insects are also providing biological control services on their hosts that are pests both in natural habitats and in farmed lands. Negative impact of pesticides used in farmed lands on these useful organisms is becoming widely recognized. Hallmann et al. (2017), reported massive decline of insect populations for no clear reasons with a long-term population assessment in protected habitats of Germany using standardized methods. The same study also suspected cascading consequences of this important decline on food and ecosystem services production.

In conventional agriculture in general and conventional cotton cultivation system in particular, pesticide is commonly used because of the pressure of pest population. Pesticides in agriculture are well known in their actions and mechanisms to control target pests on interest crops but negative effects on non-targeted organisms in the same area are not usually well documented (Köhler and Triebskorn, 2013) and considered for biodiversity conservation needs. Different evidence of population decline of ground insects as well as flying ones was reported in pesticide application contexts (Mone et al., 2014). Since our study reported active substances deposition at all sites in honey bee tissues and the honey they produced, this demonstrated the long-distance spread-out of agrochemical pollutant far from sources (agro-systems) beyond what was reported by Bueno and Cunha (2020). The detection of these harmful matters in protected forest honey bee community, depending on how sensitive living organisms are over there, would have affected insects and other living organisms as well. Moreover, pollutants would have accumulated on vegetal materials for indirect poisoning later.

Considering the different active substances present at each site, almost the 5 first ones were everywhere present in honey but during and out the application periods concentrations varied. It was no major differences between northern (intense cotton cultivation zones, Banikoara & Tanguiéta) and the central regions (less intense cotton cultivation zone, Kétou) in term of number and concentration of active substances (February-April/no pesticide use period: n=18; ANOVA on Ranks; H = 1,050; d.f = 2; p = 0,592; June-August/pesticide use period: n= 18; ANOVA on Ranks; H=1,705;d.f = 2; p = 0,426; c: n=18; ANOVA on Ranks; H = 2,567;d.f = 2; p = 0.277). One could infer on the persistence of these harmful matters. However, there was a surprisingly higher concentration of Cypermethrine compound at Kétou (Dogo) compared with the northern region (0.084 mg/kg (Dogo) vs. 0.014mg/kg (Batia) and 0.008 mg/kg (Dokossouan)). The difference was also reported between Kétou (Dogo) agrosystem (0.083 mg/kg) and forest areas (0.009 mg/kg) that concentrated much less. The later was reported to be persistent till about 5 days after spray and can contaminate honey bee (Pashte and Patil, 2017). One can then imagine that pesticide application contaminated food resources available in agro-systems both in the northern and the central regions and bee foraging activities occurred soon after they were deposited. Since honey bees are always active working and collecting materials to hives for honey production, they didn't keep long time collected materials and fortunately concentrated about half of active pesticide substances in their tissues compared with what was detected in honey. In another hand one could explain higher concentration in honey from the drying out process of nectar collected by bees to obtain honey that is likely to concentrate more active substances contained.

When talking about insect pollinators, honey bees are worldwide recognized to be the best in this ecosystem service on which many wild and cultivated plant rely for their production (Toni et al., 2018). Thus, any damage on honey bee population is expected to create a disaster in food web in ecosystems and thereby for human.

Non-target organisms are, for example, other pollinators, seed dispersers, pest control agents, etc. that are also useful in agro-systems and for crop production (Fischer et al., 2006). There was a clear report that the loss of insect diversity and abundance is expected to create subsequent effects on food webs and to threaten ecosystem services (Hallmann et al., 2017).

With the promotion of cotton cultivation to support Benin's economy, the heavy use of pesticide similar to West African countries (up to 55% of the pesticides market in Africa (Ferrigno et al., 2017)), there is a need of deep screening of ecosystem services to be potentially affected. Based on findings on possible impact of these pesticide uses proposition could be formulated in fine for good practices and application techniques or timing to mitigate negative effects on useful organisms and ecosystems. It is crucial to really address this question because it is nowadays difficult to keep agricultural goods production in conventional system without pesticide application.

Similar to previous studies that stated important impact of agricultural intensification and pesticide use on useful organisms and ecosystem services (Tscharntke et al., 2012), we expect comparable impacts on local habitats and their mutualistic organisms like honey bees and other insects (Sgolastra et al., 2016) mainly in cotton cultivation context in Benin.

Our study reported that dead bee numbers fluctuation as well as bee hive weight progress didn't show any clear link with pesticide application nor with the concentration of active compound in bee tissues and honey. Our findings can then be explained by the report of honey bees less sensitivity to pesticides compared with other insects (Hardstone and Scott, 2010); what would be a good situation in term of ecosystem service they deliver's conservation but the quit high concentration we detected in honey may result in human health problem with long term consumption of honey in this context.

Coming back to our study, what came out as major production factor in bee keeping success both in forest and in agrosystems was food resources availability and distribution in time as reported but not the pesticide substances presence, at least with actual study.

From the present study, based on current findings, we can conclude of not heavy pesticide direct impact on bee colonies and thereby beekeeping activities. However, long term effect on colony via brood could be an insidious and then not easy to detect with method that target mature bee individuals. Deep and more accurate studies are then necessary to conclude on such sensitive debate.

CONCLUSION

The present study reported on the impact of pesticide uses in agriculture and cotton cultivation on beekeeping success and bee pollination services. Findings cannot support to conclude a clear negative impact. In contrast, food resources availability and distribution in time showed more visible influence on beekeeping success. We concluded in a need of more detailed and large screening of exosystems exposed to the use of pesticides to drow solide conclusion.

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