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Explorative socio-environmental survey for honey quality assessment in six target provinces of Burkina Faso

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Abstract: Honey bees and their products are optimal bio-indicators of environmental contamination. In this study, 12 honey and beeswax samples from 6 hives located in 6 different provinces of Burkina Faso, sited near agricultural crops, were analysed. The honey yield periods taken into account were 2: the main honey yield, occurring during the dry season, and the second, at the end of the rainy season. Physico-chemical parameters (water, total sugars, hydroxy-methyl-furfural content, conductivity, pH and acidity) and residual pesticide analyses were carried out to verify honey quality. In addition, melissopalynological analysis was conducted to establish the botanical origin of the honey samples. The samples identified as probably unifloral honeys were 5, with a predominance of *Lannea microcarpa* Engl. & K. Krause, *Vitellaria paradoxa* C. F. Gaertn., *Cassia mimosoides* L. and *Combretum* Loefl. genus, in relation to the predominant pollen. Pollen profiles were compared with plant biodiversity in the sampling area, which covered a surface area of 7 Km^2. A total of 90 beekeepers were interviewed about the impact of agricultural pesticide use on honey bee colonies. Considering the categories of agro-chemical contaminants analysed, no residues were found both in honey and beeswax. However, data showed that both honey quality and beekeeping techniques are in need of improvement. In general, further research is recommended in order to enhance the knowledge relating to the characteristics of honey from Burkina Faso. Moreover, additional research is required to verify the specific impact of pesticides on *Apis mellifera adansonii* Latreille life-cycle and products. Honey quality improvement could ensure a better-selling price and the possibility of opening new sale channels for burkinabé beekeepers and farmers. At the same time, it assists in guaranteeing pollination within the ecosystem and in biodiversity conservation.

Keywords: Honey, Environmental monitoring, Pesticide residues, Melissopalynology, Physico-chemical characterization, Burkina Faso

Introduction

In Africa, beekeeping is a centuries-old tradition and it plays an important social and economic role in rural economy (Nombré, 2003). In the western African country of Burkina Faso, beekeeping is a practice with an increasing tendency to qualify as a zootechnical practice, able to combat food instability.

The sale of bee products ensures an effective, additional source of income to rural families and allows small producers to diversify their activities, which have been traditionally focused on a small number of unprofitable crops (Bradbear, 2010; Olivier, 2010). In particular, in Burkina Faso, beekeeping yields a gross income of about 1.5 billion CFA francs (approximately 2 million euros), with a total number of beekeepers exceeding 20000 across the entire country, producing about 500 tons of honey per year.

Of the total, approximately 7000 beekeepers belong to the 7 main existing beekeeping centers (Ouedraogo, 2013). Despite the growth in beekeeping, several authors admit that in Burkina Faso, the current knowledge on *Apis mellifera adansonii* Latreille biology is still insufficient. In addition, a lack of information about melliferous flora, pollen morphology and physico-chemical characteristics of local honeys, persists (Méda *et al.*, 2005; Nombré *et al.*, 2002; Sawadogo and Guinko, 2001; Schweitzer *et al.*, 2014). Moreover, environmental monitoring techniques are not widespread. In burkinabé society, including the beekeepers themselves, beekeeping is merely viewed as a source of food production and the potential role of bees as environmental bio-indicators is not generally considered. Different papers reported that bees are excellent sentinels for monitoring environmental contamination (Bargańska *et al.*, 2015; Chiesa *et al.*, 2016; Di Marco *et al.*, 2012). In fact, old worker bees, which account for about ¼ of any given bee colony, and who take charge of the foraging activity (Porrini, 2009), can fly over a circumference of 7 Km^2 with a radius of 1.5 Km (Crane, 1984). In the flight area nearby the hive, bees can gather many environmental samples, and are able to accumulate air, water, soil contaminants in their products, thereby providing macroscopic and microscopic evidence of ecological alterations and pollutants. In particular, *Apis mellifera* responds to harmful substances with a significant mortality rate in the field and/or in the hive (direct indicator) (Bargańska *et al.*, 2015). If the compounds are not fatal, honey bees can act as an indirect indicator, providing information in the form of residues (Di Marco *et al.*, 2012; Johnson *et al.*, 2010). Therefore, plant protection products used in agriculture can not only cause mass poisoning of bees, but may also be transferred to bee products, especially honey affecting its quality, properties and posing a particular threat to human health (Bargańska *et al.*, 2014). Indeed, it is well-known that honey bees (and all the other pollinators) have a significant role in preserving plant biodiversity and the natural environmental *equilibrium* (Vaissière *et al.*, 2005). The disappearance of a plant species could cause drastic consequences to the environment, especially in a fragile system such as the Sahel (Contessi, 1994).

The aim of the present research was to examine the presence of contaminants in hive products and to provide a contribution to the knowledge of the physico-chemical parameters of honeys in Burkina Faso. Furthermore, the sources of nectars foraged by bees and local plant biodiversity were identified by palynological analysis. To achieve these objectives, 2 sampling experiments were carried out in 6 different target provinces that are involved in a rural development project implemented by the Italian Agency for Development Cooperation[1]. Some data were also gathered during specific surveys handed out to a group of beekeepers, focusing on their perception about agro-chemical product impact on bee colonies. The disorganized and massive use of such chemical products on the agricultural cropping area might cause significant harm to the environment and may compromise honey quality. As a consequence, this negative approach may preclude bee products into new trade channels. The global monitoring and protection of bees, not only on a local or national level, is necessary because of the ascertained ecological and economic value of bees as pollinators.

Materials and Methods

Study area

The study was carried out in 6 provinces of Burkina Faso, belonging to 3 different phytogeographical areas that differ in climate, flora and vegetation (Fig. 1). Rainfall pattern distribution follows a latitudinal gradient from the north (400 mm/year) to the south (1100 mm/year) (Desideri and Lucchese, 1995), associated with high spatio-temporal and inter-annual variability. The rainy season (the so-called *hivernage*) starts in May-June, while the dry season begins at the end of October and lasts about 8 months. The study area of the province of Gnagna was located in the Sub-Sahelian climatic zone where the precipitation is below 600 mm per year and is distributed within a 3-month rainy season.

The Komondjari and Tapoa sample sites were situated under a north Soudanian climate with an average annual rainfall ranging from 600 to 900 mm. The southern provinces utilized in the present research (Kénédougou, Léraba and Poni), occur under a south Soudanian climatic zone (900-1100 mm/year). Generally, in the northern Sahelian areas of the country, the *brousse*, that is the typical wooded savanna covering a large part of the national territory, becomes bushy and sparser, characterized by shrub-like, tree-layer, thorny and grassy steppes. Heading towards

[1] The Project "Développement de l'apiculture pour la sécurité alimentaire dans les provinces de Gnagna, Komondjari, Tapoa, Kénédougou, Léraba et Gaoua- Phase I", is funded by the Italian Agency for Development Cooperation (AICS) of the Italian Ministry of Foreign Affairs and International Cooperation (MAECI) and started in 2015.

the south, under a tropical-climatic regime, there is dense vegetation of *Forêt claire*, and savanna and gallery forests along rivers (Dembele, 2010; Dosio, 1996). According to Nombré (2003), in the more southern areas, the honey yield usually starts earlier (late January) and has a peak of production in March, before ending in early April. In the North-Soudanian zone, honey yield starts during the first weeks of February, reaching a maximum in April and ending in early May.

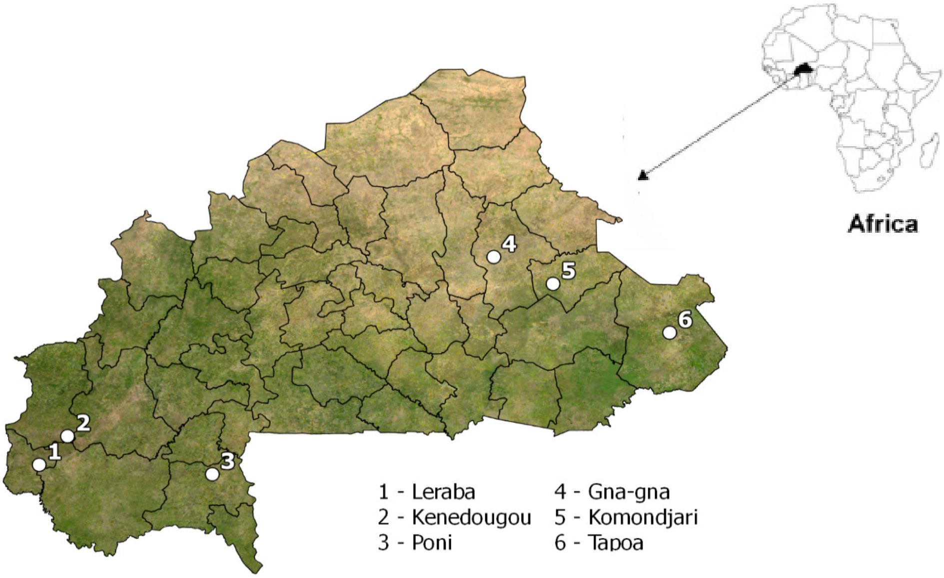

Figure 1 - Geographical location of the 6 study areas: 3 areas (Léraba, Kénédougou, Poni) are located in the western region, and the remaining 3 (Gnagna, Komondjari, Tapoa) in the eastern region of Burkina Faso, respectively.
Source: https://en.wikipedia.org/wiki/Burkina_Faso#/media/File:Burkina_sat.png (date of access: 08/02/2017; Scale 1:7.500.000, elaborated by the authors).

Data collection

A total of 12 samples of honey and 12 samples of beeswax were collected during the 2 main annual honey harvest seasons. These harvests seasons are characterized by a higher honey yield and a lower honey yield, respectively. The first sampling experiment was carried out in June 2016, at the end of the highest honey yield, when honey production reaches its maximum. The duration varies, ranging from February to May, reaching a peak around March-April. The second sampling experiment took place in September 2016, at the end of the rainy season, during the lower honey yield, which has a peak in September-October and, generally, a lower honey output (Nombré, 2003; Sawadogo and Guinko, 2001).

Both June and September have been considered as *mois de soudure* between the 2 seasons, and for this reason, conventionally, June has been included in the dry season. The samples collected during the second experiment were taken from the same 6 hives of the first one. Honey from Léraba was taken from traditional hives (called Canari)[2], while the remaining samples were taken from modern types of hives (Kenyan Top Bar)[3]. All samples from the above-mentioned hives were subjected to physico-chemical, melissopalynological and pesticide analyses. Physico-chemical and pesticide analyses were carried out at the Honey Research Centre of the University of Rome "Tor Vergata". Melissopalynological analyses were conducted at the Laboratoire de Biologie et Ecologie Végétales of Université de Ouagadougou, Burkina Faso. Finally, the vegetation within a 1.5 Km range around the sampling hives was examined in the field. Therefore, 6 field vegetation analyses were carried out and 15 plots (6000 m^2) were set up. A total of 90 beekeepers were interviewed about their perception of the impact of agro-chemical treatments on beekeeping activity.

Data analysis

Preliminary social survey

Information about pesticide impact on honey production and bee-family health status were collected within the framework of the Italian Agency for the Development Cooperation project. The survey was conducted from April to May 2016 and 90 informants, coming from the 6 different provinces, were interviewed. Data was gathered from semi-structured interviews with the aid of a smartphone application (KoboToolbox)[4] and then filled out on a Microsoft Excel spreadsheet. In order to understand the general perception of beekeepers about agro-chemical effects, the following topics were taken in consideration: a) capacity of observation and quantification of bee mortality; b) use and conditions of pesticide employment c) bee-family strength and health.

Physico-chemical, melissopalynological and pesticides analysis

All parameters relating to honey physico-chemical analysis were performed following the Italian Official Methods for the Analysis of Honey (Official Journal n. 185 of 11/08/2003). The approach of Bogdanov *et al.* (2004) was used as a reference.

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[2] Convex traditional hives made by baked clay.

[3] Modern wooden hives, easily constructed on site: they have a trapezoidal-shaped section, containing 32 cm width bars. The shape of this hive facilitate the extraction of honeycombs without causing damage to the bee-colony.

[4] Kobo-tool box is a free open-source tool for mobile data collection.

Pollen identification was performed following the method described by Louveaux *et al.* (1978) and Von der Ohe *et al.* (2004). Pollen types were classified by botanical genus or species names, only when they were reliably determined either at the genus or species level, respectively. In accordance with Von der Ohe *et al.* (2004), honey was considered as being derived predominantly from any given botanical origin (unifloral honey) if the relative frequency of the pollen of that *taxon* exceeded 45%. In Italy, honey is usually indicated as being unifloral if it is comprised of at least 45% of a normal-represented pollen derived from a single botanical species. Nevertheless, this criterion is not absolute since some unifloral honey are comprised of different percentages, according to pollen production, dispersion rate, and flower morphology of the species. For example, honey derived from *Castanea sativa* Miller and *Citrus* sp. plants, that are, respectively, characterized by a hyper-and hypo-pollen production, can be indicated as unifloral if they only contain 90 and 10% of the respective pollen (Persano Oddo *et al.,* 2000). In Burkina Faso, there are no reference regulations for melissopalynological analyses.

Therefore, we decided to indicate honey as being unifloral if the pollen composition derived from any given taxon was at least 45% of the total.

The 3 principal categories of agro-chemical pesticides, in accordance with the indications provided by local beekeepers, were analyzed in the honey samples. Pesticide residues were, respectively, extracted from honeys and waxes, according to the modified methods of Calatayud-Vernich *et al.* (2016) and Kamel and Al-Ghamdi (2006). For honey samples, 2 grams per specimen, was dissolved in 2 mL of hot distillated water (<80 °C) and sonicated for 10 minutes. The solution was purified through C18 Bakerbond SPE Columns, previously equilibrated with 3 mL of methanol and 3 mL of distilled water.

Moreover, 3 mL of distilled water were used to eliminate interfering compounds. Pesticides were finally eluted using 3 mL of methanol: dichloromethane (3:7). The extract was completely dried under nitrogen flow, re-suspended in 1 mL of methanol and analysed by Gas-Chromatography associated with Mass-Spectrometry (GC-MS). In the case of wax, 5 g of specimen samples were re-suspended in 10 mL of acetonitrile at 75 °C in agitation for 1 hour. The solution was frozen at -10 °C for 10 minutes, centrifuged 5 minutes at 5000 rpm and filtered. The filtered sample was then frozen again and subjected to the previous procedure. Finally, the wax specimen was concentrated by nitrogen flow, in order to attain a 1 mL volume, and subsequently analysed in GC-MS. Detection of the pesticides was then performed using a GC-MS-QP2010 system (Shimadzu, Kyoto, Japan) associated with a single quadrupole mass detector (electronic impact mode at 70 eV). The instrument was equipped with a diphenyl dimethyl polysiloxane SH-RTX-5MS column (length 30 m, diameter 0.25 mm, thickness 0.25 μm; Restek Corporation, Bellefonte, PA, USA), and 2 μL of supernatant was injected into the chromatograph. The GC oven was set as follows:

70 °C for 2 min; 200 °C (attained at a rate of 25 °C/min) for 5 min; 300 °C (attained at a rate of 3 °C/min) for 20 min. Helium was used as carrier gas at a constant flow of 1 mL/min.

Pesticides were identified and quantified by direct comparison with different concentrations of relative pure standard (Sigma-Aldrich), on the basis of retention time and mass fragmentation spectra. Results were expressed as μg of pesticide per kg of either honey or wax.

Vegetation analysis

For each target province, 1 hive was geo-referenced. This hive represented the center of a 7 Km^2 circle, which corresponded to the approximate mean area that honey bees are able to maintain under their control (Crane, 1984). With the aid of photo interpretation, different land surface covers, inside each circular area, were identified and classified according to the following codes: F = Forest; SF = *Forêt claire*[5]; RF = River Forest; DF = Degraded Forest, WET = Wetland. Regarding the cultivated areas, the following codes were adopted: AG (1, 2, 3) = different types of Agricultural area; TTC = Traditional Arboriculture; TTCA = Traditional Abandoned Arboriculture; ITC = Intensive Arboriculture; UG = Green zone within an Urban area. These cover classes do not correspond to any classical phytogeographical classification. They were created to be a functional measure of the existing vegetation.

Then, using this pattern, photos and field observations were carried out (Cencetti, 2016). Upon each land surface cover, squares of 20 x 20 meters (400 m^2) were installed, for a total of 15 plots (6000 m^2). For each cover plot, the following data were collected: a) plant species, b) plant species abundance/dominance and c) phenological phases (Cencetti, 2016). In the agricultural areas, besides the presence of annual rotations, annotations were also made of the main crops.

Results

Survey

The social survey results showed that 55% of the informants owned traditional hives, 12% Kenyan hives, while 33% used both. A total of 63% of beekeepers declared that they did not notice a reduction in the colonies caused by pesticides applied on cultivated areas, while 10% did not answer the question. Of the informants, 27% declared having noticed a reduction in the size of the bee families caused by chemical treatment. The informants stated that the dead bees were found both in front of

[5] We adopt forestry French classification for *Forêt claire* including forest covers where the herbaceous stratum replaces undergrowth bushes.

the hives and on the treated soils. It was, however, difficult to determine whether the cause of death was due to natural circumstances or a result of agro-chemicals applied by either the same beekeepers or neighboring farmers. The issue was even more difficult to understand, since beekeepers generally do not regularly control the hives and visit the apiaries. This practice is carried out even less when the hive is traditional. For example, the queen bee's presence is deduced only because the colony exists. Hence, a possible change in the bee's behavior, due to pesticide exposure, is hardly recognized by local beekeepers.

Nonetheless, in the study area, 25% of all interviewees claimed to pulverize pesticides and/or herbicides as foliar sprays on crops, which are often situated in the vicinity of the apiary. Such distribution occurs primarily in conjunction with the rainy season. The herbicide Glyphosate and pyrethroid based pesticides, such as Deltamethrin, were the most utilized by beekeepers who practice agriculture in the area. The most pulverized crops were respectively, green beans (48%), followed by cashew (17%), cotton (13%), sesame (13%) and maize (13%).

Physico-chemical analysis

The results, including moisture, total sugar content, HMF, pH, acidity and conductivity, are summarized in Tables 1 and 2. In those tables, the detected levels were compared to the limit values set by Italian law (legislative decree n. 179/2004 implementing directive 2001/110/CE on the production and marketing of honey in the EU). According to the law, HMF limits for tropical honey should not exceed 80.00 mg/kg. Of the honey samples in the present study, 5 out of 12, had optimal values, while 7 exceeded the limit. Water content and free acidity seemed to be the least respected parameters.

Table 1 - Physico-chemical results of the honey analysis. Samples were collected during the dry season.

Target Province	Sampling Period: Dry season							
	Moisture g/100g	Sugars g/100g	HMF mg/kg	pH	Acidity meq/kg			Conductivity mS/cm
					Free	Combined	Total	
Tapoa	19.00	79.30	20.36	3.93	37.50	15.00	52.50	0.679
Komondjari	20.60	77.50	3.74	4.58	25.00	5.00	30.00	1.044
Gnagna	17.60	80.75	2.24	4.80	17.50	5.00	22.50	1.141
Kénédougou	21.20	77.20	11.23	4.18	32.50	12.50	45.00	0.827
Léraba	22.60	75.80	8.20	3.97	72.50	15.00	87.50	1.014
Poni	20.80	77.50	19.46	3.65	55.00	15.00	70.00	0.609
Limit	20.00	> 60.00	80.00		50.00			

Table 2 - Physico-chemical results of the honey analysis. Samples were collected during the rainy season.

Target Province	Sampling Period: Rainy season							
	Moisture g/100g	Sugars g/100g	HMF mg/kg	pH	Acidity meq/kg			Conductivity mS/cm
					Free	Combined	Total	
Tapoa	19.10	79.50	2.24	3.60	45.00	17.50	62.50	0.463
Komondjari	16.80	81.80	2.00	4.20	22.50	5.00	27.50	0.562
Gnagna	19.50	79.20	0.75	3.92	80.00	17.50	97.50	0.817
Kénédougou	20.90	77.75	3.74	3.65	75.00	25.00	100.00	0.778
Léraba	14.30	84.50	23.20	3.49	67.50	15.00	82.50	0.739
Poni	14.00	88.00	8.50	4.20	25.00	15.00	40.00	0.845
Limit	20.00	> 60.00	80.00		50.00			

Vegetation analysis

The study of vegetation type allows the identification of where bees are foraging predominantly, thereby providing useful information about the botanical species involved in honey production (Nombré, 2003). In Table 3, for each target province, different types of vegetation cover are reported (Agriculture/Agriculture type 1,2,3 – Forest - Traditional/Intensive Arboriculture – Wetland - River/Degraded forest - *Forêt claire* - Traditional Abandoned Arboriculture - Green zone within an Urban area) (Cencetti, 2016).

As a whole, 3850 hectares of vegetation cover were classified using GIS contouring. Table 4 shows both the extension of contoured areas (hectares) and the number of plant species for each target province. The total number of species (137) does not take in consideration species redundancy.

Gnagna province represented the poorest area in plant species, while the richest was the Kénédougou province.

The maximum distance (differential) expressed as percentage of the number of plant species was 70%. Figure 2 shows the variation in percentage of the number of plant species along the target province.

In the first 3 eastern provinces, there was a certain quantitative uniformity of species, while in the latter western 3 provinces, a positive variation takes place (increase).

Table 3 - Vegetation cover categories (code and legend) and surface area cover in hectares (areas), for the 6 target provinces.

Target Province	Code	Legend	Area (hectares)
Gnagna	AG	Agriculture	13.41
	F	Forest	4.75
	SF	*Forêt claire*	532.60
	TTC	Traditional Arboriculture	0.63
	WET	Wetland	28.35
Kénédougou	AG	Agriculture	210.48
	F	Forest	18.84
	ITC	Intensive Arboriculture	78.45
	RF	River Forest	22.69
	SF	*Forêt claire*	230.75
	TTC	Traditional Arboriculture	113.20
Komondjari	AG	Agriculture	360.17
	DF	Degraded forest	143.98
	F	Forest	98.96
	WF	Wetland	36.02
Léraba	AG1	Agriculture type 1	4.04
	AG2	Agriculture type 2	37.43
	AG3	Agriculture type 3	222.45
	F	Forest	3.41
	SF	*Forêt claire*	328.33
	TTC	Traditional Arboriculture	0.70
	TTCA	Traditional Abandoned Arboriculture	6.86
	WET	Wetland	72.66
Poni	AG	Agriculture	252.66
	SF	*Forêt claire*	366.88
	WET	Wetland	47.29
Tapoa	AG1	Agriculture type 1	530.16
	AG2	Agriculture type 2	58.93
	UG	Green zone within an Urban area	5.76
	WET	Wetland	19.28

Table 4 - Extension in hectares of vegetation cover in each target provinces and the number of plant species.

Target Province	Area (hectares)	Plants Species
Tapoa	614	13
Komondjari	639	14
Gnagna	580	12
Kénédougou	674	40
Léraba	676	31
Poni	667	27
Total	3850	137

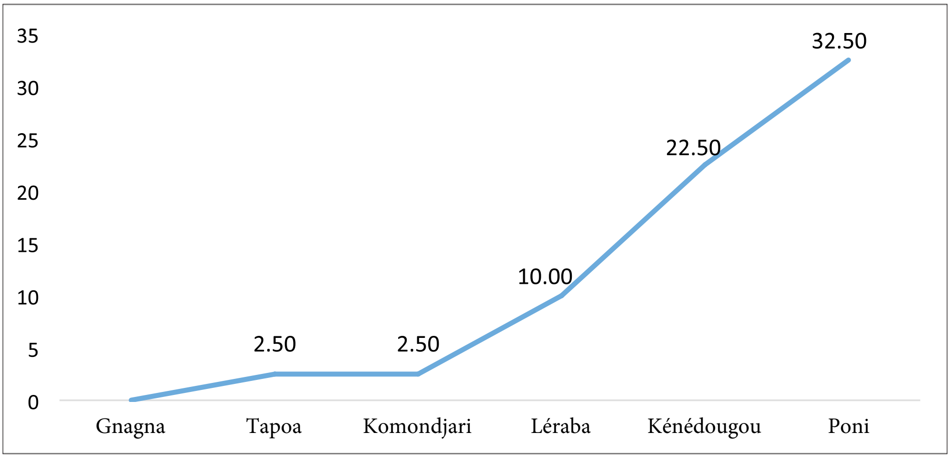

Figure 2 - Variation in percentage of the number of plant species along the 6 target provinces.

Because the same plant species may exist repeatedly along the study areas in target provinces, Figure 3 shows the distribution of redundancy, expressed as increasing frequency values. The absolute number of plant species found in all areas was 64.

In particular, there were 101 redundant forest tree species, which were distributed in the various areas of target provinces, as reported in Table 5.

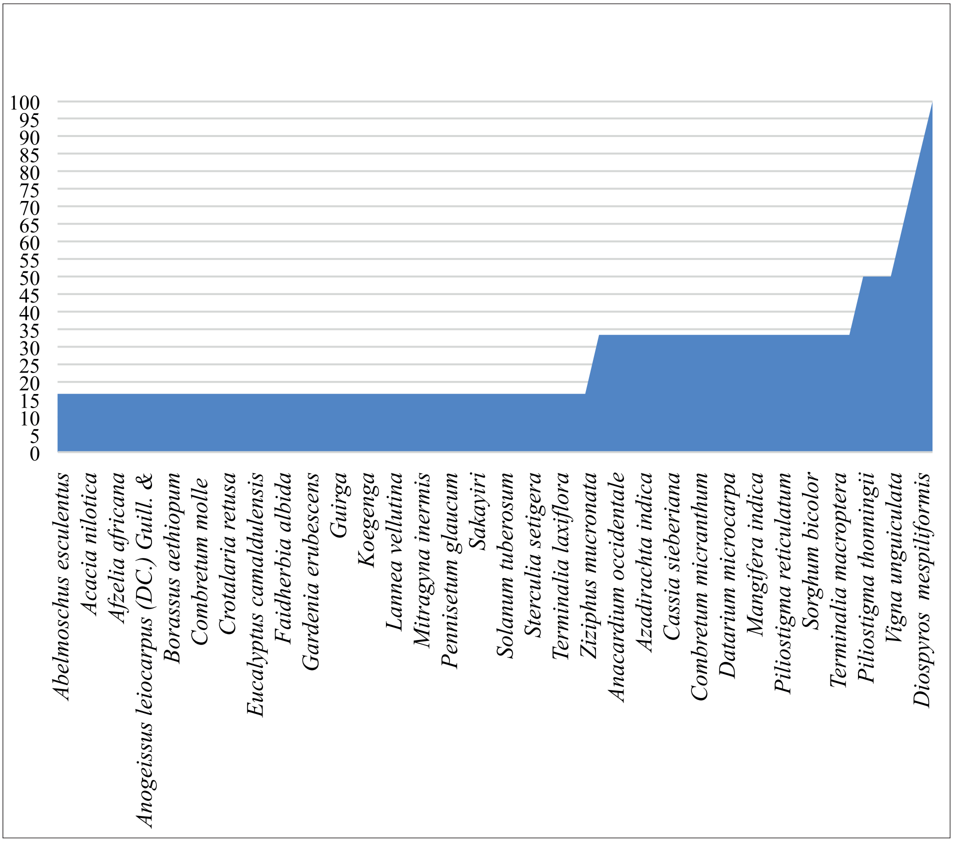

Figure 3 - Distribution of plant species redundancy in the areas of target provinces.

Table 5 - Forest cover extension in each target province, number of individuals and redundant forest tree species.

Target Province	Forest cover (hectares)	Number of Individuals	Forest tree species
Tapoa	19.30	38	4
Komondjari	279.00	38	12
Gnagna	537.40	23	4
Kénédougou	272.30	160	33
Léraba	331.70	68	24
Poni	414.20	67	24

On the basis of the number of individuals and species, the Margalef Index was calculated (S-1/Ln N), as reported in the Table 6. In general, the Margalef Index assumes a linear relationship between the number of species and the number of individuals for each species. This relationship is similar to that occurring between the number of species and extension (hectares) in a sampling area. There was a positive correlation (+ 0.17) between forest tree species richness (Margalef Index) and forest cover extension, considering all forest categories: Forest, *Forêt claire*, River Forest, Degraded forest and Forests growing on wetland.

These forested areas represented about 48% of all sampled areas, and about 74% of all identified plant species, respectively.

Table 6 - Variation of forest tree species richness (Margalef Index) and forest cover extension for each of the target provinces.

Target Province	Margalef Index	Forest cover (hectares)
Tapoa	0.80	19.30
Gnagna	1.00	537.40
Komondjari	3.00	279.00
Léraba	5.50	331.70
Poni	5.50	414.20
Kénédougou	6.30	272.30

Melissopalynological analysis

The melissopalynological analysis is based in experimental unifloral honeys analysed by Demianowicz (1964) and in subsequent researches. In Figure 4 the main pollen species found in the honey samples are listed. Thus, there were probably 5 unifloral (42%) and 7 multifloral (58%) honey samples, respectively (Table 7).

All multifloral honey samples were collected during the rainy season.

Pesticide analysis

In Tables 8 and 9 residual pesticides in honey samples were compared with the content limits (μg/kg) set by the National Action Plan for Residues 2016, as developed by the Italian Ministry of Health, taking into consideration the EU regulations (directive 96/23/CE). When considering the categories of agro-chemical contaminants analyzed, no residues were detected in both honey and wax.

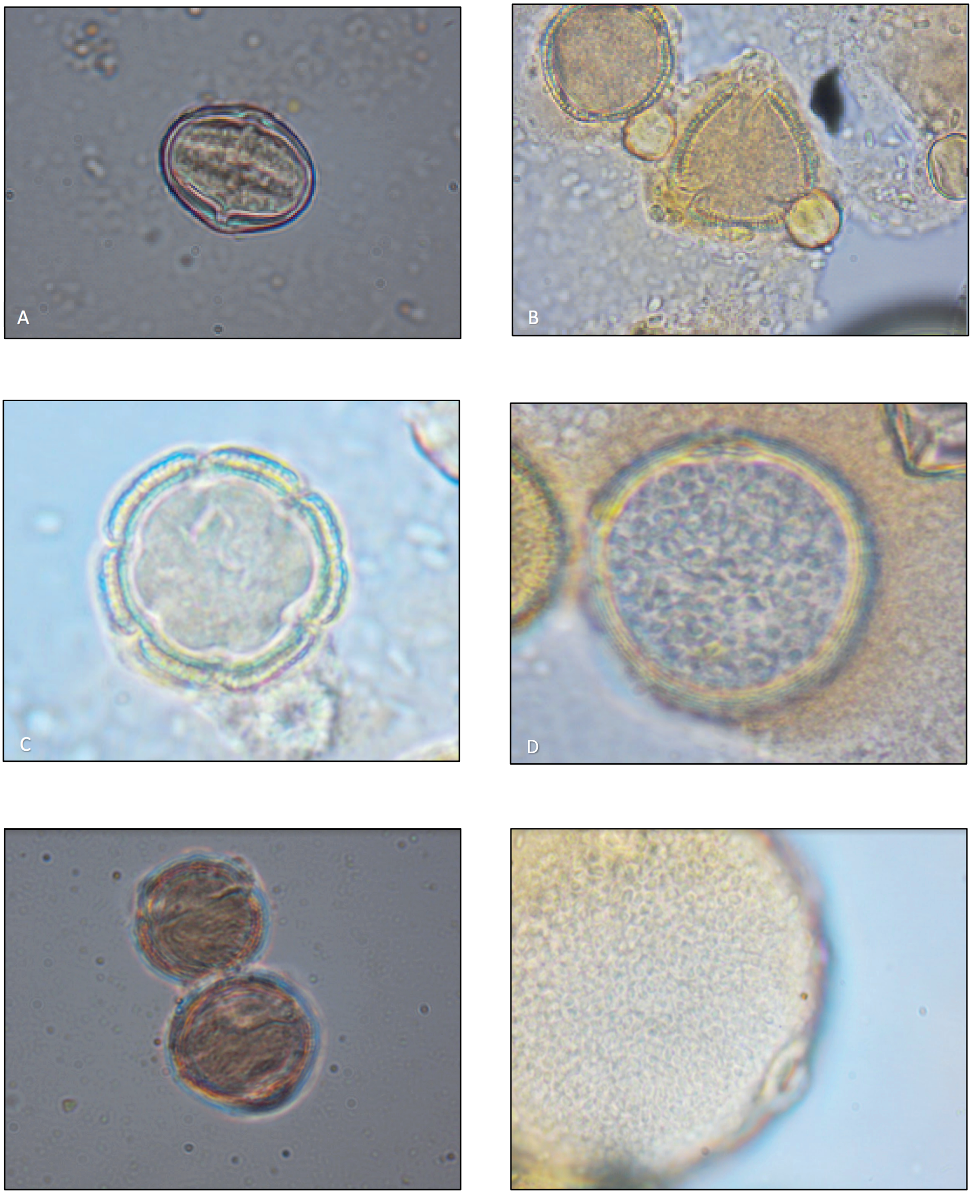

Figure 4 - Pollen grains. (a) Vitellaria paradoxa; (b) Mytragina inermis; (c) Mitracarpus scaber; (d) Bombax costatum; (e) Mangifera indica; (f) Gramineae type.

Table 7 - Typologies of pollen recovered in honey samples collected at the end of the dry season and during the rainy season of 2016.

Tapoa	*Lannea microcarpa* (55%), *Lonchocarpus laxiflorus* (21%), *Vitellaria paradoxa* (7%), *Cissus quadrangularis* (5%), *Combretum* type (4%), *Balanites aegyptiaca* (3%), *Ziziphus mauritiana* (2%), *Parkia biglobosa* (1%), *Berlinia grandiflora* (1%), undetermined (1%)	*Piliostigma thonni*ngii (32%), *Diospyros mespiliformis* (14%), *Cochlospermum planchonii* (8%), *Ceratotheca sesamoides* (12%), *Cissus quadrangularis* (12%), *Gramineae* type (7%), *Cleome viscosa* (7%), *Boerhavia* type (3%), *Mitracarpus scaber* (2%), *Acacia* type (1%), *Hibiscus* type (1%), undetermined (1%)
Komondjari	*Vitellaria paradoxa* (49%), *Combretum* type (15%), *Lannea microcarpa* (11%), *Terminalia avicennioides* (10%), *Ziziphus mauritiana* (9%), *Acacia* type (5%), undetermined (1%)	*Piliostigma thonningii* (36%), *Vigna unguiculata* (12%), *Gramineae* type (11%), *Acacia nilotica* var. *adansonii* (7%), *Vernonia pauciflora* (7%), *Sesamum indicum* (7%), *Diospyros mespiliformis* (6%), *Waltheria indica* (5%), *Sida acuta* (5%), *Commelina benghalensis* (4%), undetermined (1%)
Gnagna	*Combretum* type (47%), *Vitellaria paradoxa* (27%), *Terminalia avicennioides* (11%), *Mitragyna inermis* (6%), *Grewia bicolor* (4%), *Xeroderris stuhulmannii* (3%), *Triumfetta rhomboidea* (1%), undetermined (1%)	*Grewia bicolor* (37%), *Piliostigma thonningii* (11%), *Acacia nilotica* var. *adansonii* (11%), *Gramineae* type (11%), *Boerhavia diffusa* (8%), *Ipomoea eriocarpa* (6%), *Sida acuta* (5%), *Borreria stachydea* (4%), *Vernonia pauciflora* (3%), *Mitracarpus scaber* (3%), undetermined (1%)
Kénédougou	*Combretum* type (47%), *Bombax costatum* (21%), *Borassus aethiopum* (21%), *Anacardium occidentale* (4%), *Parkia biglobosa* (3%), *Tapinanthus dodoneifolius* (2%), undetermined (2%)	*Piliostigma thonningii* (21%), *Detarium microcarpum* (16%), *Citrus aurantifolia* (14%), *Cochlospermum planchonii* (12%), *Cucumis melon* (12%), *Malvaceae* type (8%), *Boerhavia* type (6%), *Borreria* type (6%), *Bridelia ferruginea* (2%), *Gramineae* type (2%), undetermined (1%)

Table 7 - continued

Léraba	*Alternanthera* type (37%), *Mangifera indica* (23%), *Anacardium occidentale* (11%), *Borassus aethiopum* (11%), *Parkia biglobosa* (8%), *Acacia* type (4%), *Mitracarpus scaber* (3%), *Gramineae* type (1%), *Waltheria indica* (1%)	*Detarium microcarpum* (23%), *Cochlospermum planchonii* (17%), *Nauclea latifolia* (13%), *Piliostigma thonningii* (12%), *Diospyros mespiliformis* (11%), *Alternanthera* type (7%), *Mimosa pigra* (4%), *Acacia* type (4%), *Crotalaria goreensis* (3%), *Mitracarpus scaber* (3%), *Gramineae* type (1%), *Waltheria indica* (1%), undetermined (1%)
Poni	*Cassia mimosoides* (54%), *Baissea multiflora* (26%), *Bombax costatum* (7%), *Combretum* type (6%), *Acacia* type (3%), *Anacardiaceae* type (2%), undetermined (2%)	*Detarium microcarpum* (32%), *Vigna unguiculata* (12%), *Cochlospermum planchonii* (12%), *Hibiscus* type (10 %), *Gramineae* type (7%), *Grewia bicolor* (7%), *Annona senegalensis* (6%), *Nymphaea lotus* (5%), *Sarcocephalus latifolius* (4%), *Gardenia erubescens* (2%), *Hyptis* sp. (1%), undetermined (2%)

Table 8 - List of analysed pesticides on honey samples.

HONEY		
DRY AND RAINY SEASON		
Pesticide	Result	Limit (μg/kg)
Deltamethrin	Absent	-
Glyphosate	Absent	-
Atrazine	Absent	-

Table 9 - List of analysed pesticides on bees wax samples.

BEESWAX		
DRY AND RAINY SEASON		
Pesticide	Result	Limit (μg/kg)
Organophosphates*	Absents	0
Organochlorine**	Absents	0
Amidine***	Absents	200
Pyrethroids****	Absents	0
Glyphosate	Absent	0
Atrazine	Absent	0

* Malathion, Chlorfenvinphos, Coumaphos (for the Coumaphos the limit of action is 100 μg/kg)
** DTT, DDD, DDE, Lindane, a b c BHC, Heptaclor, Aldrin R, I, II sulphate Endosulfan
*** Amitraz
**** Fluvalinate, Cyfluthrin, Cypermethrin, Flumethrin, Deltamethrin

Discussion and conclusion

Pollen analysis and physico-chemical properties provided elements for an appropriate characterization of honey samples, derived from the 6 provinces of Burkina Faso. In particular, quality parameters, pesticide content and botanical origin were analysed.

Physico-chemical results showed that 5 out of 12 honey samples had values, in terms of moisture, total sugar contents, HMF, pH, acidity and conductivity, that were below the limits set by EU regulations.

Of the 5 samples, 2 were derived from the Tapoa and Gnagna hives, respectively, and both samples had been collected in June. The remaining 3 honey samples were collected in September, and were derived from the Tapoa, Komondjari and Poni hives, respectively. Despite the low values obtained by the physico-chemical results, honey from the Gnagna province (collected in June) contained spherical bacteria, evidence of contamination probably due to incorrect management. In addition, honey collected from Léraba in June, produced in traditional hives was shown to be inedible.

As a result, the water content in 5 honey samples exceeded the limits, suggesting possible problems relating to conservation. As the honey itself is a concentrated solution of simple sugars (primarily glucose and fructose), only few microorganisms can potentially survive and eventually multiply.

It is generally acknowledged that a low water percentage does not favor microbial metabolism (Pressi, 2015). In contrast, a higher quantity of water promotes the multiplication of yeasts, favoring a possible fermentation process, which can irreversibly alter honey quality, rendering it unsuitable for consumption.

Regarding the above-mentioned 5 honey samples, 4 were collected during the period of high honey yield (dry season) and the remaining sample during low honey yield (rainy season). In particular, all samples collected from the highest rainfall, western provinces (Kénédougou, Léraba, Poni), during high honey yield, had higher humidity contents, thereby favoring the growth of yeasts in honey samples from Poni and Léraba. In turn, this resulted in an acidity content, exceeding legal limits (respectively, 72.50 meq/kg and 55.00 meq/kg). High humidity, acidity and yeast presence were also detected in the honey from Kénédougou, collected in September. Therefore, samples gathered during low honey yield, except that of Kénédougou, showed a lower water content. Frequently, because of the scarcity of basic professional equipment and specific training, beekeepers remove immature honey, resulting in an alteration in the quality and capacity for conservation.

Despite the high tropical temperatures, all HMF values were below the legal limits. The pH values of honey samples ranged from around 3.49 (Léraba) up to 4.80 (Gnagna). It is well known, that pH value provides a quick indication of the acidity strength in honey. Generally, the pH is acid falling within a range between 3.5 and 4.5. This acidity is in part caused by the presence of numerous organic acids, already contained in the nectar or honeydew, and in part from the bees. Honey acidity usually increases with age and fermentation.

Through the vegetation analysis, conducted within the foraging areas surrounding the hives, different agricultural sites were identified. These sites were cultivated with sesame, green beans, sorghum and maize. As reported by the interviews with farmers/beekeepers, cash crops such as green beans and cotton, for which the revenue can exceed the cost of purchasing chemical products, rely on pesticide usage, especially during the rainy season. The pesticides detected represent those effectively used by peasants and available on the local market. Of the screened pesticides, no contaminant residues were detected in honey and beeswax. Many active pesticide ingredients are lipophilic and easily dissolve into a wax matrix. Due to its chemical inertia and conservation capacity, the wax tends to accumulate large quantities of these substances, thereby decreasing the degradation of the pesticide residues. The absence of pesticides in beeswax samples is quite significant, especially for the sample collected during the rainy season. Therefore, it is suggested to take into consideration all the major pesticide category effects on colony health (multiresidue analysis) and also other apiarian matrices need to be studied (Wiest *et al.,* 2011). According to Amulen *et al.* (2017), to ensure a high level of product quality control, routine analytical pesticide monitoring is always recommended. The absence of the examined categories of contaminants in the honey, would suggest that bees exposed to agro-chemical products at least in part die directly on the treated ground before being able to carry the contaminants back to the hive.

Alternatively, it is also possible that, especially during wet season, the pesticides could be washed away by rainwater before entering into contact with bees.

Considering these hypotheses, it may be useful to estimate the mortality of honey bees in the field. This estimation is, however, difficult to set, for 2 main reasons: a) weather conditions, which favor a rapid decomposition of dead bees and b) the proliferation of many predators (ie. soil microfauna), which can quickly eat the dead bees on the ground.

As reported in Table 7, the study of the pollen collected during the dry season shows that 5 out of 6 honey samples are probably unifloral, and in particular, there are 2 samples of *Combretum* Loefl. type, and 1 of *Vitellaria paradoxa* C. F. Gaertn, *Lannea microcarpa* Engl. & K.Krause and *Cassia mimosoides* L. Honey from Léraba was instead made up by a mix of pollens, in which the genus *Alternanthera* Forssk. (37%) and the species *Mangifera indica* L. (23%) were the most represented botanical taxa, respectively.

These palynological results have been in part confirmed by the vegetation analysis carried out in Komondjari (eastern provinces), where the vegetation cover has been classified as forest and humid forest, and the dominant species is shown to be *Vitellaria paradoxa*, comprised of 10 individuals. This tree species blooms between January and March, during the dry season, representing a good source of nectar and pollen (Nombré *et al.*, 2009). In Gnagna (eastern provinces), the same species were both found in the *Forêt claire* during the vegetation analysis, and later detected in the corresponding honey sample. *Lannea microcarpa* is a tree species blooming approximately between February and April; it occurred 2 times, respectively in Komondjari and Léraba. In Kénédougou (western provinces), and especially in the areas of forest classified as fluvial, *Combretum molle* G.Don (fam. Combretaceae) plants were detected.

Pollens belonging to this botanical family were prevalent in the honey collected in June (47%). In honey from Léraba (western provinces) there were pollens of *Anacardium occidentale* L. (fam. Anacardiaceae) (11%). This tree grows in an area of traditional agriculture. Pollens of *Bombax costatum* Pellegr. & Vuillet (fam. Bombacaceae), *Combretum* and *Acacia* type, were detected in honey samples from Poni (western provinces). All honey samples collected in the rainy season were multifloral and characterized by a mix of pollens, where the 45% threshold of specific pollen content was never surpassed. All samples contained a variable percentage of pollens from plants belonging to the Gramineae family. In the rainy season, the bee flora is dominated by the flowering of herbaceous polliniferous plants, such as *Sorghum bicolor* L., *Zea mays* L., *Pennisetum glaucum* (L.) R. Br. On the contrary, the dry season is dominated by the flowering of woody species, such as *Vitellaria paradoxa* and *Lannea microcarpa*, producing mainly nectar (Nombré *et al.*, 2009; Sawadogo and Guinko, 2001).

In some cases, in the melissopalynological analysis, there was no evidence of the pollen of plants encountered in the field during vegetation analysis. This could be an indication of the possibility that honey bees flew over a sampling area wider than 7 Km^2, in search of plants with the highest sugar content.

In fact, the foraging area is linked to the quality of the nectar. According to Nombré *et al.* (2009), bees can reach a distance exceeding 3 Km from the hive to attain plant species with more than 15% sugar content.

Currently, about 70% of burkinabè honey production is sold on the domestic market, while the remaining 30% is exported to the sub-regional market, in particular to that in Nigeria (Carboni, 2015).

Improving honey quality could ensure an improved selling price, as well as the possible opening of new sales channels that could increase the cash income of burkinabé beekeeping farmers. Lack of adequate training and the persistent use of unsuitable techniques are among the main factors that restrict this burgeoning sector. Even if the majority of beekeepers interviewed provided evidence of not having full knowledge and control of the bee colonies, the general transition trend towards to a modern beekeeping system is, however, observed. Furthermore, the absence of targeted pesticide categories in hive products, is favorable suggesting the possibility of expanding the honey market but it is necessary to lay the groundwork for a large-scale impact assessment of pesticides. However, it would be recommendable to improve both physico-chemical qualities of honey and beekeeping techniques. In conclusion, this research may contribute towards enriching the knowledge of Burkina Faso honey where only a few studies, especially on agro-chemical contaminations levels, have been performed to date (Schweitzer *et al.,* 2014).

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