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Effects of *Apis mellifera* adansonii, L. 1758 (Apidae: Hymenoptera) pollination on yields of *Cucumeropsis mannii* (Naudin) in Kisangani, Democratic Republic of Congo

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ABSTRACT

The honeybees play an important role in the pollinations of many field crops. Here, we assessed the effect of the presence of honeybee colonies, *A. mellifera adansonii*, L. 1758 (Apidae: Hymenoptera) in the production of African melon crop, *C. mannii* (Naudin) (Cucurbitaceae) in Kisangani, Democratic Republic of Congo. The influences of distance to the apiary and flight orientation were also investigated. Generally, four fields of one hectare were cultivated in two study sites. At each site, both fields of one hectare were separated by more than 3 kilometers to avoid interference between treatments. Two honeybee colonies were installed in the middle of the first experimental field at each site (T_1) during flowering (when there were 10% of flowering plants). A second experimental field, without honeybee colonies, was the control (T_0). Each experimental field (with or without colonies) was divided into three concentric subplots around the apiary (P1: 0-10 meters to the apiary, P2: 10-30 meters, and P3: 30-50 meters to the apiary). The impact of pollination of honeybees on the yield of *C. mannii* was assessed on five components of yield (average number of seeds per fruit, average number of fruits per plant, average weight of seeds extracted per fruit, average length and average width of the seeds). The results of this study have shown that the pollination of introduced honeybees significantly improved the number of seeds per fruit by 83.78%, while the number of fruits per plant and the weight of seeds per fruit were improved by 422.89% and 185.61%, respectively. Indeed, the seed size was positively influenced by the presence of the apiary in the field. According to the findings in this study, the spatial distribution of plants (distance to apiary and orientation) did not influence the yield and the size of seeds in the field of *C. mannii*. The association "apiary - culture of *C. mannii*" could be integrated in the arsenal of strategies to enhance symbiotic interactions *Apis mellifera* with *C. mannii* in DRC.

Keywords: *Apis mellifera adansonnii*, L., pollination, *Cucumeropsis mannii* (Naudin), yield, Kisangani, RDC.

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INTRODUCTION

Entomophilous pollination is a key process in natural and agricultural ecosystems (Haubruge et al., 2006). In agricultural ecosystems, it has been displayed that 87 of the 124 major crops for human consumption in 200 countries around the world depend on different levels of pollination by insects (Holden, 2006; Klein et al., 2007). The abundance and biodiversity of pollinators are important factors for improving the performance of fruit crops in agricultural ecosystems. Hence, the needs for different current global partners reflect on the initiatives promoting the maintenance and mechanical protection of biodiversity of pollinators in the world (Azo'Oela et al., 2010).

The pollination of crops often depends on insects, mainly honeybees. Nevertheless, many researchers have indicated that there has been a decline in the numbers of insect pollinators in agroecosystems (Biesmeijer et al., 2006). For example, in 1990, American researchers published reports about the loss of pollinators' biodiversity in North America. A similar loss in the abundance of insect pollinators (particularly the honeybee species) was also observed in European countries. In 1980, researchers estimated a loss of 40% in Britain and 60% in the Netherlands. Several studies demonstrated that this decline in the numbers of pollinators may influence the entire planet and agriculture (Ghazoul, 2005; Steffan-Dewenter et al., 2005; Rasmont et al., 2006).

Also, many other factors including parasites, disease, climate change, agricultural intensification, urbanization growth, infrastructure installation, effects of pesticide and local and industrial pollution could be responsible for the decline of pollinators (Haubruge et al., 2006; Le Féon et al. 2010). Different agricultural practices may be taking into account to protect the biodiversity (Ricketts, 2000; Richards, 2001). Previous studies have shown that the introduction of honeybee colonies in crops helped improving the production of these crops, and good maintenance of pollinator biodiversity in these ecosystems (Azo'Oela et al., 2010). The latter authors observed that pollination of honeybees improved the production of fruits and seeds in the farming of rape and the African melon (*Citrullus lanatus*). Another crop, *Cucumeropsis mannii* (Cucurbitaceae) is considered as a nutritional source for the majority of African populations (Zoro Bi et al., 2003). It is cultivated in small fields in association with other major crops such as cassava, maize and banana. Now, this crop is a significant economic sector in Africa (Djè et al., 2006).

The impact of honeybee pollination on the production of *C. mannii* crop has not yet been well documented in the Democratic Republic of Congo (DRC). The aim of the present study was first to evaluate the effect of the introduction of honeybee colonies on the production of *C. mannii* seeds and second to determine the effect of distance to the apiary and flight orientation on the yield and size of *C. mannii* seeds.

MATERIALS AND METHODS

Study site

This study was conducted in two outlying villages of Kisangani in DRC (Figure 1). Kisangani is located at 25°11'00" East longitude, 0°33'00" North latitude and at an altitude of 390-410 meters. Kisangani is dominated by a humid tropical climate belonging to the *Af* group according to the Köppen climate classification, which a monthly rainfall height of more than 60 mm in the driest month. The average rainfall recorded for Kisangani is around1700-1800 mm/year. Its average temperatures are around 24 °C and 25 °C.

The topography of Kisangani consists of large flat areas. The region is covered by abundant forest vegetation consisting of three types of formations: the primitive rainforest called 'the equatorial evergreen rainforest' along the banks of rivers, forming an edaphic grouping of lianas and epiphytes; the semi-deciduous forest land, and finally the swamp or periodically flooded forest. The soils are alluvial and aeolians, lateritic according to the French classification (Ngongo et al., 2009; Boyemba, 2011).

Biological material

The plant material used in this study consisted in seeds of *C. mannii* (Naudin) also called African melon, purchased locally from Kisangani's farmers. Its pollination is entomophilous and the bee remains a very effective pollinator (Zoro Bi et al., 2003). Pollinators introduced as part of our study consisted of four colonies of *A. mellifera adansonii, L.* that were captured by the trapping technique and domesticated in the Langstroth in Kisangani.

Experimental design

The observations were conducted at two different sites. Four fields of one hectare each were cultivated in two study sites. Two experimental fields were installed in each site and separated by more than 3 kilometers to avoid interference between treatments. In tropical conditions, the radius operating of wild bee colonies is estimated at 3 kilometers (Winston, 1993). At each site, two honeybee colonies were introduced into the middle of the treated fields (T1) during flowering (when there were 10% of flowering plants), while the second experimental field without an apiary was considered a control (T0). Generally, we introduced four honeybee colonies into two treated fields (plots containing apiaries). Each experimental field (with or without colonies) was divided into three concentric subplots around the apiary (P1: 0-10 meters to the apiary, P2: 10-30 meters and P3: 30-50 meters to the apiary). From the apiary, we charted four axes which were oriented to the four cardinal directions. Different distances used in the sampling and observations were also measured for each direction. These measurements were performed using topofil. For the study, the observations were performed at 5, 25 and 45 m from the apiary. The experimental design is schematically presented in the figure below (Figure 2).

Influence of honeybee pollination on *C. mannii* yield

Three months after flowering (corresponding to the harvest and maturity of seeds), the influence of honeybee pollination on the yield of *C. mannii* was evaluated using five yield parameters: (1) the average number of fruits per plant, (2) the average weight of seeds extracted per fruit, (3) the average number of seeds per fruit, (4) the average seed length and (5) the average seed width. The number of fruits per plant was determined by the framing method: data were collected from a sample of plants randomly selected in frames of 10 x 10 meters, which were placed 5, 25 and 45 meters from the apiary on each axis. The sub-sampling technique was used to measure parameters such as the average number of seeds per fruit, the average seed length, the average seed width and the average weight of seeds extracted per fruit. Measurements of these parameters were realized on sub-samples of 10 fruits or 10 seeds that were randomly selected in batches of fruits or seeds.

Statistical analysis

All statistical analyses were performed using R version 2, 14. 1. 2011. Three ways analysis of variance (ANOVA) were used to compare the differences and similarities of yield components between the three different factors: pollination (with or without apiary); distances to the apiary (P1, P2, P3) and orientation (N, S, E, W). Data were transformed ($\sqrt{x+0.5}$ and arcsine) before parametric tests in order to normalize the data. All probabilities were appreciated at 5%.

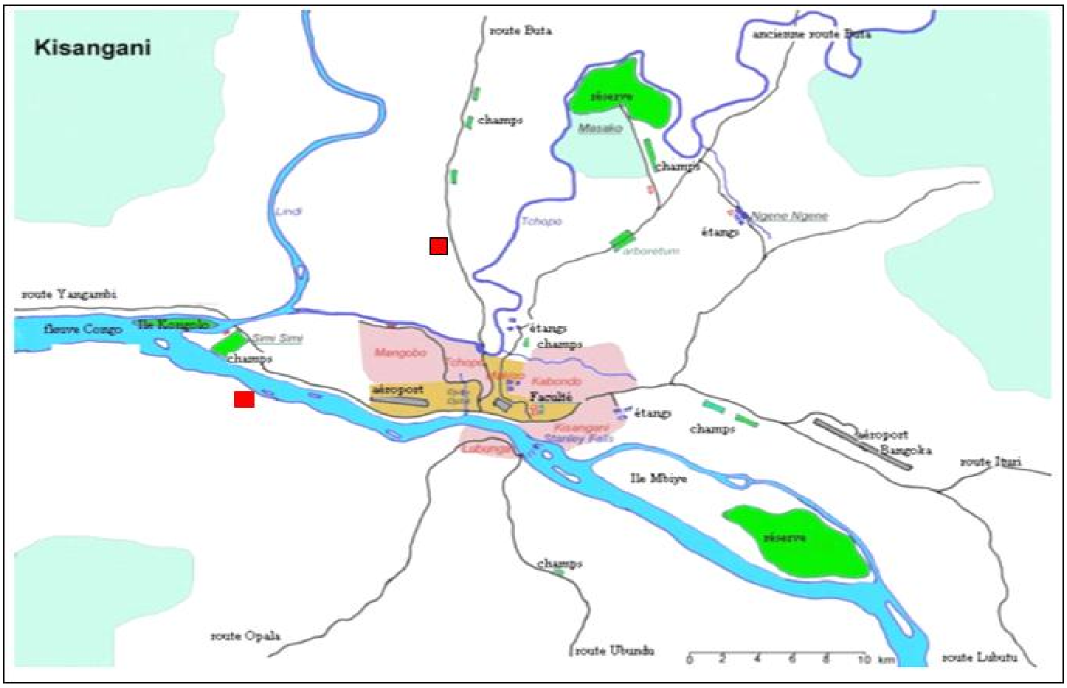
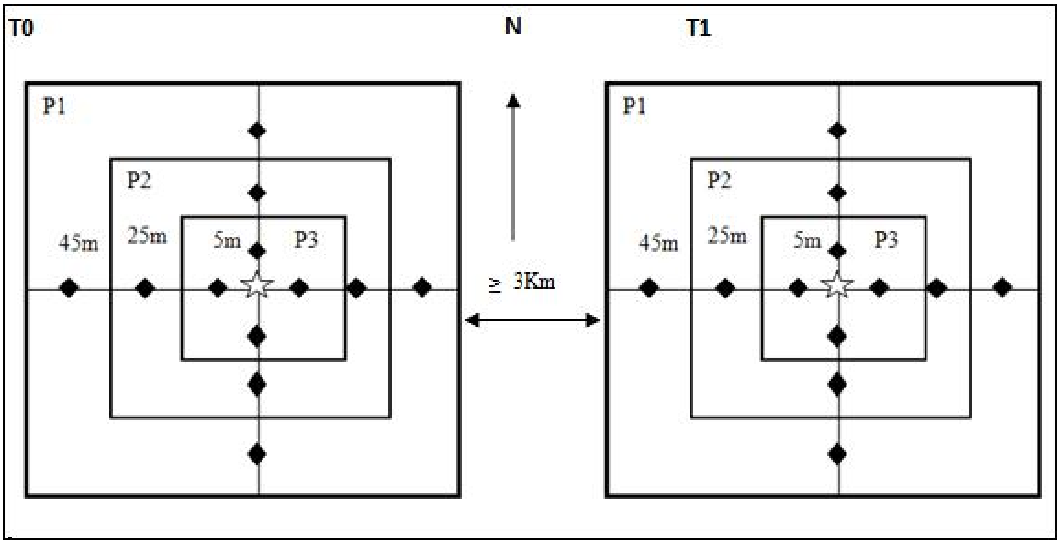


Figure 1: Map of Kisangani and the location of the experimental site symbolized by ■, source: Nshimba, 2008.



☆ : Location of apiary or center; ◆ : sampling location;

T_0 : Plot without apiary; T_1 : Plot with apiary; P_1, P_2, P_3: Subplots;

N : Geographic North.

Figure 2: Schema of the experimental design.

RESULTS

Influence of honeybee pollination on the yield of *C. mannii*

The data on influence of honeybee pollination on the yield of *C. mannii* are presented Table 1. The data indicate that the presence of honeybee colonies has statistically improved the three quantitative components of the yield of *C. mannii* (P <0.001). The average number of seeds per fruit was 250.73 ± 126.96 in plots provided with apiaries compared with 136.43 ± 97.16 for control plots (without apiaries), which is an improvement in the production of seeds per fruit of 83.78% more than the control (P <0.001).

The average number of fruits per plant was 4.34 ± 2.79 in plots containing apiaries against 0.83 ± 0.94 for the control plots, representing a growth of 422.89% (P <0.001). The average weight of seeds extracted per fruit was 44.67 ± 31.79 g in plots pollinated by honeybees compared with 15.64 ± 14.22 g for control plots, which represents an increase of 185.61% of yield (P <0.001).

The presence of honeybee colonies positively influenced seed size (Table 1) : the average seed length was 1.95 ± 0.13 cm in plots equipped with apiaries against 1.87 ± 0.14 cm for control plots (without apiaries), which was an improvement in the production of seeds per fruit of 4.28% more than the control (P <0.001). The average seed width was 1.00 ± 0.09 cm in plots equipped with apiaries against 0.97 ± 0.09 cm for control plots (without apiaries), indicating an improvement in the production of seeds per fruit of 3.09% more than the control (P <0.001).

Influence of distance to the apiary and flight orientation on the performance of *C. mannii*

The data on influence of distance to the apiary and flight orientation on the performance of *C. mannii are* presented in Tables 2 and 3. The three components of the yield were statistically similar between the three levels of distance to apiaries (P > 0.05). Thus, the distance to the apiary does not influence the performance during our trials. Seed length and width were statistically different between the three levels of distance to the apiary (P <0.05). However, the trends of seed length and the seed width were not consistent and varied between size parameters (Table 2).

All performance parameters were statistically different between the four directions (North, South, East and West) of experimental fields, except for the weight of seeds per fruit (P <0.05). However, the observed trends for all performance parameters between the four orientations were not consistent and varied between components themselves (Table 3).

Table 1: Impact of honeybee pollination on the yield and seed size of *C. mannii.*

Sites	Parameters	N	Bee pollination		Pr (< F)
			With apiary	Without apiary	
Yangambi	Weight of seeds (g) per fruit	90	69.02 ± 28.93 a	25.22 ± 19.60 b	<0.001***
	Number of seeds per fruit	90	44.67 ± 31.79	44.67 ± 31.79	<0.001***
	Number of fruit per plant	90	4.31 ± 2.26 a	0.53 ± 0.55 b	<0.001***
	Seed length	90	1.95 ± 0.13 a	1.82 ± 0.13 b	<0.001***
	Seed width	90	1.04 ± 0.10 a	0.92 ± 0.07 b	<0.001***
Bamanga	Weight of seeds (g) per fruit	90	20.67 ± 10.48 a	12.71 ± 10.18 b	<0.001***
	Number of seeds per fruit	90	153.55 ± 70.37 a	107.04 ± 75.76 b	<0.001***
	Number of fruit per plant	90	5.47 ± 3.23 a	1.33 ± 1.19 b	<0.001***
	Seed length	90	1.96 ± 0.11 a	1.96 ± 0.12 a	0,9
	Seed width	90	0.97 ± 0.08 a	1.02 ± 0.09 b	<0.001***
Overall mean	Weight of seeds (g) per fruit	90	44.67 ± 31.79 a	15.64 ± 14.22 b	<0.001***
	Number of seeds per fruit	90	250.73 ± 126.96 a	136.43±97.16 b	<0.001***
	Number of fruit per plant	90	4.34 ± 2.79 a	0.83 ± 0.94 b	<0.001***
	Seed length	90	1.95 ± 0.13 a	1.87 ± 0.14 b	<0.001***
	Seed width	90	1.00 ± 0.09 a	0.97 ± 0.09 b	<0.001***

Significative codes : 0 '***' 0.001 '**' 0.01 '*' 0.05 'ns' 0.1 ' ' 1
***: very highly significant difference; **: very significant difference, *: significant difference and ns: no significant difference.

Table 2: Impact of distance to the apiary on the yield and seed size of *C. mannii*.

Sites	Distance to the apiary				Pr (<F)
	Parameters	0 to 10m	10 to 30m	30 to 50m	
Yangambi	N	20	80	80	
	Weight of seeds (g) per fruit	44.92 ± 34.88 a	53.87 ± 31.58 a	56.66 ± 34.88 a	0.466
	Number of seeds per fruit	243.9 ± 120.8 a	299.6 ± 124.0 a	313.7 ± 135.0 a	0.164
	Number of fruit per plant	1.10 ± 0.74 a	2.60 ± 2.78 a	2.86 ± 2.48 a	0.150
	Seed length	1.85 ± 0.18 a	1.90 ± 0.15 a	1.89 ± 0.13 a	0.365
	Seed width	0.98 ± 0.07 a	0.99 ± 0.13 a	0.97± 0.08 a	0.322
	N	20	80	80	
Bamanga	Weight of seeds (g) per fruit	16.94 ± 10.69 a	16.30 ± 11.92 a	17.08 ± 10.22 a	0.909
	Number of seeds per fruit	154.67±98.63 a	122.75 ± 73.42 a	133.10 ± 73.21a	0.266
	Number of fruit per plant	2.10 ± 2.42 a	2.00 ± 0.11 b	3.38 ± 3.08 a	0.346
	Seed length	1.90 ± 0.12 a	1.01 ± 0.09 a	1.92 ± 0.11 a	<0.001***
	Seed width	0.99 ± 0.09 a	1.01 ± 0.09 a	0.99 ± 0.09 a	0.186
Overall mean	N	20	80	80	
	Weight of seeds (g) per fruit	30.11 ± 28.51 a	32.72 ± 29.34 a	34.26 ± 31.15 a	0.756
	Number of seeds per fruit	196.70±117.0 a	200.1 ± 132.0 a	210.7 ± 137.3 a	0.761
	Number of fruit per plant	2.70 ± 2.00 a	5.53 ± 2.81 a	4.90 ± 2.73 a	0.6699
	Seed length	1.87 ± 0.15 a	1.95 ± 0.14 b	1.91 ± 0.12 b	<0.001***
	Seed width	0.98 ± 0.08 a	1.00 ± 0.11a	0.98 ± 0.09 a	0.072

Significative codes : 0 '***' 0.001 '**' 0.01 '*' 0.05 'ns' 0.1 ' ' 1
***: very highly significant difference; **: very significant difference,. *: significant difference and ; ns: no significant difference

Table 3: Influence of flight orientation on the yield and seed size of *C. mannii.*

Sites	**Parameters**	**Orientation**			
		North	**South**	**East**	**W**
	N	**160**	**160**	**160**	**16**
Yangambi	Number of seeds per fruit	258.8 ± 119.0 a	310.2 ± 128.6 a	313.3 ± 122.7 a	343.7 ±
	Number of fruit per plant	2.05 ± 1.82 a	2.10 ± 1.94 a	3.80 ± 2.86 a	3.20 ±
	Weight of seeds (g) per fruit	51.29 ± 34.88 a	53.38 ± 32.52 a	54.87 ± 31.76 a	61.56 ±
	Seed length (cm)	1.86 ± 0.13 a	1.88 ± 0.17 ab	1.90 ± 0.12 ab	1.95 ±
	Seed width (cm)	0.97 ± 0.06 ab	0.96 ± 0.14 a	1.03 ± 0.11b	0.98 ±
Bamanga	N	160	160	160	16
	Number of seeds per fruit	103.3 ± 80.9 a	121.9 ± 56.0 a	150.7 ± 61.3 a	135.3 ±
	Number of fruit per plant	4.55 ± 3.66 ab	2.10 ± 1.33 a	4.80 ± 4.09 b	2.80 ±
	Weight of seeds (g) per fruit	13.10 ± 10.05 a	13.38 ± 8.90 a	20.87 ± 10.81 b	19.25 ±
	Seed length (cm)	1.95 ± 0.12 a	1.94 ± 0.14 a	1.95 ± 0.10 a	2.01 ±
	Seed width (cm)	1.00 ± 0.08 ab	0.99 ± 0.10 ab	0.97 ± 0.08 a	1.04 ±
Overall mean	N	160	160	160	16
	Number of seeds per fruit	168.3 ± 124.6 a	216.1 ± 136.7 a	211.0 ± 118.5 a	226.5 ±
	Number of fruit per plant	3.30 ± 3.12 ab	2.10 ± 1.65 a	4.37 ± 3.60 b	3.00 ±
	Weight of seeds (g) per fruit	29.30 ± 30.09 a	33.10 ± 30.97 a	33.48 ± 26.66 a	38.12 ±
	Seed length (cm)	1.90 ± 0.13a	1.91 ± 0.16 a	1.93 ± 0.11ab	1.98 ±
	Seed width (cm)	0.99 ± 0.07 a	0.98 ± 0.12 a	0.99 ± 0.10 a	1.01 ±

Significative codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 'ns' 0.1 ' ' 1 ,***: very highly significant difference; **: very significant difference,*: significant difference; and ns: no significant difference.

DISCUSSION

Influence of honeybee pollination on the yield of *C. mannii*

The attempt was to assess the effect of honeybee pollination on the yield of *C. mannii*. The data show positive effects of the introduction of honeybee pollination on the yield of *C. mannii*. The analysis of variance revealed that the presence of honeybee colonies statistically improved the average number of seeds per fruit, the average weight of seeds extracted per fruit and the average number of fruits per plant. The increase in these three components was considered by Quiévy (2008) as a quantitative improvement of the yield of fruit crops (e.g *C. mannii)*. These results confirmed the findings of Mesquida and Renard (1981) and Azo'Oela et al. (2010). These authors observed that the pollination of honeybees improved the production of fruits and seeds in the farming of rape and the Cucurbitaceae (particularly *C. lanatus*).This study has shown that the pollination of the introduced honeybees positively influenced the seed size. These results differ from the findings of Mesquida and Renard (1981), who reported on the farming of rape. These authors observed a decrease in the size of seeds when honeybees were provided during the farming of rape. The variation in experimental conditions could explain these differences (e.g. cultivated species, climate, soil).

Influence of distance to apiary on the yield of *C. mannii.*

The aim was to assess the effect of distance to apiary on the yield of C. mannii. The data demonstrated that all of the observed components of the yield were statistically similar between the three levels of distance to apiary. In this study, distance to the apiary did not affect the yield of *C. mannii*. These results differ from those of Manning and Wallis (2005), who showed that the seed yield of rape was relatively heterogeneous between plants located in remote bands of 30 metres. The lack of pollination gradient observed in our study may be due to the relatively small size of our experimental plots (one hectare) compared with the operational areas of wild bee colonies in tropical conditions (radius operating estimated at 3 km) (Winston, 1993). Our results showed that seed size was statistically different between the three levels of distance to the apiary. However, the trends of seed length and seed width were not consistent and varied between components themselves. Therefore, distance to apiary did not influence seed size in our study. These results did not differ from those obtained by Quiévy (2008) on the farming of rape.

Influence of flight orientation on the yield and seed size of *C. mannii*

The attempt was to assess the effect of flight orientation of honeybee on the yield of *C. mannii*. The results demonstrated that flight orientation of honeybees did not affect yield and seed size of *C. mannii* because the trends of all performance parameters were not consistent and varied between sites and components themselves. These results correspond to the ecological conditions of Kisangani where the average wind speed varies from 0 to 10 km/hour (Boyemba, 2011). The choice of direction is necessary in ecosystems where the wind speed is higher. Beyond 45km/hour, wind negatively influences the mobility and the activity of honeybees (Pesson and Louveaux, 1984).

Conclusion

Honeybee pollination is an important factor for improving the yield of fruit crops in agricultural ecosystems. The seed yield of Cucurbitaceae grown in Africa is directly or indirectly dependent on the pollination insect activity, especially that of honeybees. The purpose of this work was to investigate the impact of pollination of introduced honeybees on the yield of *C. mannii* in Kisangani. Three months after flowering (corresponding maturity of seeds), the impact of honeybee pollination on the yield of *C. mannii* was significant. This study showed that the pollination of introduced honeybees positively influenced the yield and the seed size of *C. mannii*. Moreover, the distance to the apiary and the flight orientation did not affect the yield and seed size of *C. mannii*. The lack of pollination gradient observed in our study may be due to the relatively small size of our experimental plots (one hectare) compared with the operational areas of wild bee colonies in tropical conditions. Low wind speed in Kisangani significantly reduced the effects of the flight orientation of bee on yield of *C. mannii*. The association "apiary–*Cucumeropsis mannii* crop" could be successfully used to improve the pollination efficiency and increase *C. mannii* yield in ecosystems where a loss of species diversity in the community of pollinators has been reported.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

BNP is the main actor of this research. He participated in the development of the protocol, the execution of the protocol, the analysis and interpretation of the results and the writing of the manuscript; EH, the promoter of this research, contributed to the development of the initial protocol, the execution of the protocol of this work, the analysis and interpretation of the results; BKN, Co-Proponent of this work, contributed to the development of the initial protocol, the execution of the protocol of this work, the analysis and interpretation of the results; FF, Head of Functional and Evolutionary Entomology at the University of Liège-Gembloux Agro-BioTech, is the person who oversaw all the research work from the development of the research protocol to the analysis and interpretation of the results; The analysis and interpretation of the results were carried out with the advice and assistance of YB, Researcher at the Statistics and Informatics Unit of the University of Liège-Gembloux Agro-BioTech and GLG, Researcher at the Biodiversity Unit of the Catholic University of Louvain-la-Neuve; PM of the University of Kinshasa and M-LS, researcher at the Royal Belgian Institute of Natural Sciences, agreed to devote their time and energy to improve the present manuscript.

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