



Influence of the Foraging Activity of the Anthophilous Insects on *Talinum triangulare* (Waterleaf) fructification in Bafut (North West - Cameroon)

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ARTICLE INFO

ABSTRACT

Research Article

Received : 19/02/2021
Accepted : 04/10/2021

Keywords:

Talinum triangulare
Insect species
Flowers
Fruiting rate
Africa

Talinum triangulare is an herbaceous succulent plant eaten as a vegetable throughout the tropics including many countries in West and Central Africa and are an essential ingredient in traditional dishes. Experiments were made on the plant to examine the influence of foraging behavior of flowering insect on pollination and yields of this plant species in 2018 and 2019 at Bafut. Observations were made on 1615 to 4055 flowers per treatment. The treatments included unlimited floral access by visitors and bagged flowers to deny all visits. The study focused on the foraging behavior of flowering insects and their pollination activity (fruiting rate). The results show that 14 insect species visited waterleaf flowers and *Camponotus flavomarginatus* was the most frequent (33.20%). Insects foraged throughout the day light period. Their activity was highest between 10 am and 12 pm. Insect species foraged the flowers for pollen and nectar. The fructification rate of unrestricted floral access was significantly high than that of protected flowers to deny all visits. The maintenance of insect nest close to *T. triangulare* field is recommended to improve its fruits production.

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Introduction

Talinum triangulare is a non conventional vegetable crop which originates from tropical Africa and is widely grown in Asia, South America and West Africa (Schippers, 2000). It is an herbaceous succulent plant in the Portulacaceae family with common name as waterleaf, Ceylon spinach, fame flower (Siemonsna and piluck, 1993). Waterleaf is eaten as a vegetable throughout the tropics including many countries in West and Central Africa; it is cultivated in Nigeria and Cameroon; it is an essential ingredient in traditional dishes (Fontem and Schippers, 2004.). Waterleaf is a rich source of crude protein (22%), crude fibre (11.2%), ash (33.98%) (Enete and Okon, 2010) and content approximately 90.8g per 100g of water (Herrera, 1999). Waterleaf is a short-lived plant that grows up to 30cm to 100cm in height (Opabode,

2005).The flowers are visited by pollinators due to the rich nectar they have (Stevens, 1990). The fruit is dehiscent. (Schippers, 2000). Storage is a bit difficult thus should be used immediately when harvested (Okpala, 2016). The vegetable is used as a softener when cooking fibrous vegetables like *Gnetum africanum* (eru) (Ndaeyo et al., 2013), aid in digestion of food (Mensah et al., 2008, Okpala, 2016) and act as green forage for rabbit feed management (Aduku and Olukosi, 1990)

Since the increased popularity of eru in Cameroon and eastern Nigeria from around 1990 onwards, the demand for waterleaf has steadily risen, thus widening the domestic demand and supply gap of the product. It is now a common product on local markets, but no data on production and trade are available (Fontem and Schippers, 2004).

Waterleaf is a plant species for which information on insect pollination in Africa, particularly in Cameroon is still lacking. Much information exists for the species of waterleaf as food items (Nworgu et al., 2015; Ezekwe et al., 2013) and medicine (Aja et al., 2010; Liang et al., 2011; Ezekwe et al., 2013). There has been no previous research reported on the relationship between the plant and its anthophilous insects. This study was carried out to assess the effects of foraging behavior of flowering insect on pollination and yields of waterleaf. The information gained on the study could enable farmer's especially small scale farming household to develop management plans that could increase the overall quality and quantity of waterleaf yields in order to provide them a complete source of income.

Materials and Methods

Study site, experimental plot and biological material

The experiment was carried twice, April to June 2018 and 2019 at Bafut (06°50'N and longitude 10°06' E and 1200m above sea level), Mezam, North West Region of Cameroon (Figure 1) and covers an area of roughly 340 km² (Anonymous, 2005). Bafut is characterized by a humid sub-Equatorial climate with a rainy season from March to November which recharges groundwater and a dry season from December to February. It has a mean annual precipitation of 2657.2 mm, humidity of 80% and temperatures ranging from 22.3°C to 26.7°C (Etia, 1980). Soils in Bafut are conditioned chiefly by topography and climate including alluvial soils, ferruginous soils and hydromorphic soils common in swampy valleys (Gavaud and Muller, 1980). The natural vegetation of Bafut is the grassland savannah, marked by grasses mixed with deciduous shrubs and stunted trees here and there, meanwhile the swampy valleys are dominated by raffia bushes and palm trees (Letouzey, 1980). This natural vegetation is strongly modified by human activities mainly farming and demographic pressure imposed by a rapidly increasing population (Acho-Chi, 1998). The experimental plot was selected in the South of Bafut at Mambu (Figure 1) and was 10 m long and 10 m wide (100 m²).

The biological material was represented by all the insects naturally present in the environment of study site. The Stem of *T. triangulare* (Figure 2) were provided from an already mature waterleaf farm. The vegetation near *T. triangulare* field had various spontaneous and cultivated species.

Sowing and weeding

On May 9, 2018 and 2019, the experimental plot having been previously plowed, the transplanting of the waterleaf's stem was carried out on 8 subplots of 4 m in length, 2 m in width and 50 cm in height each. The stems were harvested of height 5 to 7 cm. The distance between the subplots was 50 cm. The planting was done in line, at the rate of four lines per subplots with a spacing of 50 cm between the lines and 30 cm on the lines. For the plants to easily grow without the majority dying, it was watered till the rain was constant and plant which did not do well were replaced. Weeding was performed manually as necessary to maintain plot weeds-free.

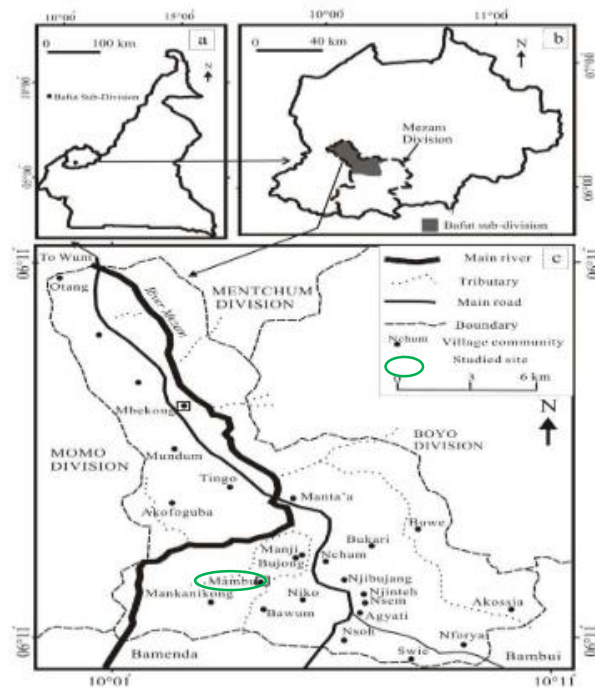


Figure 1. Study area location.
(a) Cameroon map showing position of the Bafut sub-division; (b) Map of North West Region of Cameroon locating the Bafut Sub-division; (c) Map of Bafut Sub-division (Anonymous, 2005) showing the studied site (modified by Otiobo, 2019)

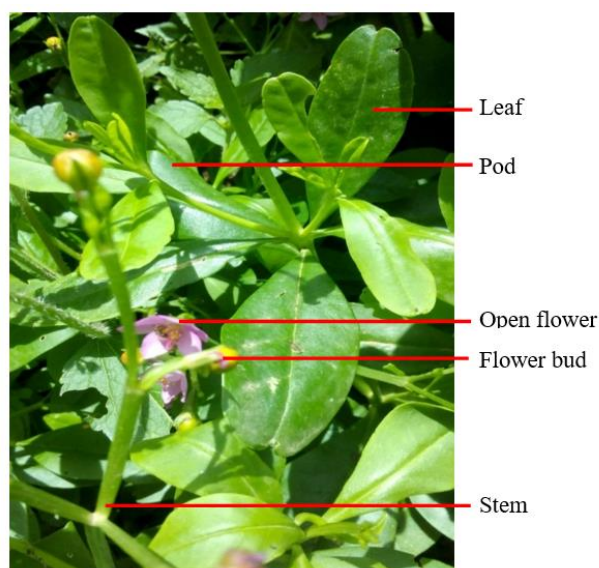


Figure 2. *Talinum triangulare* Plant (Otiobo, 2019)

Determination of the reproduction mode of *Talinum triangulare*

On 28th May 2018, eight subplots carrying 326 plants with 3819 flowers at the bud stage were labeled. Four subplots carrying 172 plants with 2204 flowers were left un-attended (treatment 1) and four subplots carrying 154 plants with 1615 flowers were bagged to prevent visitors

(treatment 2). On 30th May 2019, eight subplots carrying 388 plants with 7741 flowers at the bud stage were labeled. four subplots carrying 200 plants with 4055 flowers were left un-attended (treatment 3) and four subplots carrying 188 plants with 3686 flowers were bagged to prevent visitors (treatment 4). Twenty days after shedding of the last flower, the number of fruits was assessed in each treatment. The fruiting index (Fi) was then calculated as described by Tchuenguem et al. (2001):

$$Fi = F1/F2$$

where F2 is the number of fruits formed and F1 the number of viable flowers initially set. The allogamy rate (Alr) from which derives the autogamy rate (Atr) was expressed as the difference in fruiting indexes between treatment X (unprotected flowers) and treatment Y (bagged flowers) as follows (Dermaly, 1977):

$$Alr = [(Fi_X - Fi_Y) / Fi_X] \times 100$$

where Fi_X and Fi_Y are respectively the fruiting average indexes of treatments X and Y.

$$Atr = 100 - Alr.$$

Study of the foraging activity of insects on *Talinum triangulare* flowers

The methodology used was modified from that of Tchuenguem et al. (2004). The frequency of insects in the waterleaf was determined based on observations of flowers every two days, during each of the following daily time frame, from 2th to 28th of June 2018 and from 2th June to 4th July 2019: 7 - 9 am, 10 am - 12 pm, 1 - 3 pm and 4 - 6 pm. In a slow walk along all plants, the identity of all insects that visited the waterleaf was recorded. Specimens of all insect taxa were caught with an insect net and for each species, 3 to 5 insect specimens were captured. These insects were conserved in 70% ethanol for subsequent taxonomy determination except for Lepidoptera which were conserved in wrapper following Borror and White (1991) recommendations. All insects encountered on flowers were registered and the cumulated results expressed in number of visits to determine their relative frequency in the anthophilous entomofauna of waterleaf. In addition to the determination of the floral insects' frequency, direct observations of the foraging activity on flowers were made on insect pollinator fauna in the experimental field. The floral products (nectar or pollen) harvested by the insect species during each floral visit were registered based on their foraging behavior. Nectar foragers were seen extending their proboscis to the base of the corolla while pollen gatherers scratched anthers with the mandibles or the legs. During the same time that the insects encountered on flowers were registered, the types of floral products collected by those insect species were noted. In the morning of each sampling day, the number of opened flowers carried by each subplot was counted and was registered. During each daily period of observations, the temperature and relative humidity of the station were registered using a mobile thermo-hygrometer, every one hour.

Evaluation of the impact of insects on *Talinum triangulare* fruit yields

For each year, this evaluation was based on the impact of insect visiting flowers on pollination, the impact of pollination on fructification of waterleaf, and the comparison of fruiting rate of treatment X (unprotected flowers) and treatment Y (bagged flowers). At maturity, fruits grown from each treatment were harvested and were individually tagged. Comparison was done on the fruit set rate between treatments. The fruit set rate [(number of fruits formed/number of flowers formed) × 100] was determined based on the number of flowers and the number of actual fruits formed. The percentage of the fruit set rate due to the influence of the foraging insects was calculated based on the relative difference in fruit set between bagged (treatment 2 & 4) and open-pollinated flowers (treatment 1 and 3). (Tchuenguem et al., 2004).

Data analysis

Data were analyzed using descriptive statistics, student's t-test for the comparison of means of two samples, correlation coefficient (r) for the study of the association between two variables, chi-square (χ^2) test for the comparison of two percentages using SPSS statistical software (version 19.0; SPSS, Inc., Chicago, Illinois, USA) and Microsoft Excel 2003.

Results

Reproduction mode of *Talinum triangulare*

The fruiting index of *T. triangulare* was 0.86, 0.77, 0.88 and 0.79, respectively for treatments 1, 2, 3 and 4. Thus, in 2018 allogamy rate was 10.60% and autogamy rate was 89.40%. In 2019, the corresponding figures were 10.74% and 89.26%. For the two years combined, allogamy rate was 10.67% and autogamy rate was 89.33%. It appears that *T. triangulare* used in our experiments has a mixed reproduction mode with the predominance of autogamy over allogamy.

Diversity and abundance of flowering insects

In total, 14 insect's species belonging to 13 Families under six Orders visiting the waterleaf plant were recorded during the two studied periods (table 1). Out of these, five species belonged to Hymenoptera (54.34%), two to Coleoptera (27.53%), Diptera (11.20%), Hemiptera (12.38%) and Lepidoptera (4.97%) respectively. The Orthoptera order ends with one species (2.52%).

The hymenopterans, dipterans and lepidopterans were mostly found on the flowers. Among the hymenopteran species *Camponotus flavomarginatus* was the most frequent (33.20%) insect visitor followed by *ceratina* sp (10.80%) and *Lasioglossum* sp. (7.49%), the others species (*Apis mellifera* and *Vespa diabolica*) have a percentage less than 2%. The dipteran species were represented by *Musca domestica* (6.47%) and *Drosophila* sp. (4.73%). For lepidopterans, the two species (*Acraea acerata* and *Papillio demodocus*) were poorly represented (< 5%). *Latridius* sp (8.52%) and *Scirtes* sp. (6.07%) were the two species of the order Coleoptera. Hemiptera represented by *Acanthicephala thomasi* (4.89%) and *Lygaeus* sp. (7.49%) were mostly found sapping the plant. Order Orthoptera was poorly represented by *Dissosteira Carolina* species (5.37% of the visits).

Products harvested

On a waterleaf plant, insects collect either nectar and pollen (Apidae, Halictidae, Muscidae and Vespidae) or only nectar (Acraeidae, Drosophilidae, Formicidae, Papilionidae and Scirtidae). A part from the floral product, we have insect that suck sap from the plant (Latritidae, Lygaeidae and Coreidae). Globally, pollen and nectar collectors were numerically predominant, representing up to 76.58% of the number of visits of individuals collected on waterleaf plant.

Rhythm of visits according to the flowering stages

In general, visits were most numerous when the number of open flowers was highest (Figure 3). Furthermore, we found a positive and significant correlation between the number of open flowers and the number of insect visits in 2018 ($r_{2018} = 0.86$ [$df = 26$; $P < 0.05$]) and in 2019 ($r_{2019} = 0.89$; [$df = 32$; $P < 0.05$]).

Table 1. Insect species recorded on *Talinum triangulare* flowers in 2018 and 2019, number and percentage of visits of different insects.

Order	Insects		2018		2019		Total (2018+2019)	
	Family	Genus, species	n ₁	p ₁ (%)	n ₂	p ₂ (%)	n _T	p _T (%)
Coleoptera	Latrididae	<i>Latridius</i> sp ^a	-	-	108	16.07	108	8.52
	Scirtidae	<i>Scirtes</i> sp [*]	-	-	77	11.46	77	6.07
	Total Coleoptera		-	-	185	27.53	185	14.59
Diptera	Drosophilidae	<i>Drosophila</i> sp. [*]	33	5,54	27	4.02	60	4.73
	Muscidae	<i>Musca domestica</i> **	82	13,76	-	-	82	6.47
	Total Diptera		115	19.30	27	4.02	142	11.20
Hemiptera	Lygaeidae	<i>Lygaeus</i> sp ^a	-	-	95	14.14	95	7.49
	Coreidae	<i>Acanthicephala thomasi</i> ^μ	62	10,40	-	-	62	4.89
	Total Hemiptera		62	10.40	95	14.14	157	12.38
Hymenoptera	Formicidae	<i>Camponotus flavomarginatus</i> *	201	33,72	220	32.74	421	33.20
	Apidae	<i>Apis mellifera</i> **	11	1.85	10	1.49	21	1.66
		<i>ceratina</i> sp**	71	11.91	66	9.82	137	10.80
	Halictidae	<i>Lasioglossum</i> sp.**	52	8.72	43	6.40	95	7.49
	Vespidae	<i>Vespa diabolica</i> **	15	2,52	-	-	15	1.18
	Total Hymenoptera		350	58.72	339	50.45	689	54.34
Lepidoptera	Acraeidae	<i>Acraea acerata</i> *	26	4.36	26	3.87	52	4.10
	Papilionidae	<i>Papillio demodocus</i> *	11	1,85	-	-	11	0.87
	Total Lepidoptera		37	6.21	26	3.87	63	4.97
Orthoptera	Acridae	<i>Dissosteira Carolina</i> ^μ	32	5,37	-	-	32	2.52
	Total Orthoptera		32	5.37	-	-	32	2.52
Total			596	100	672	100	1268	100
			11 species		9 species		14 species	

*Visitor collected nectar; ** Visitor collected nectar and pollen; α: Visitor sapping the plant; μ: Predator. n₁: number of visits on 2204 flowers in 14 days; n₂: number of visits on 4055 flowers in 17 days; P₁ and p₂: percentages of visits; p₁ = (n₁ / 596) × 100; p₂ = (n₂ / 672) × 100; p_T = (n_T / 1268) × 100.

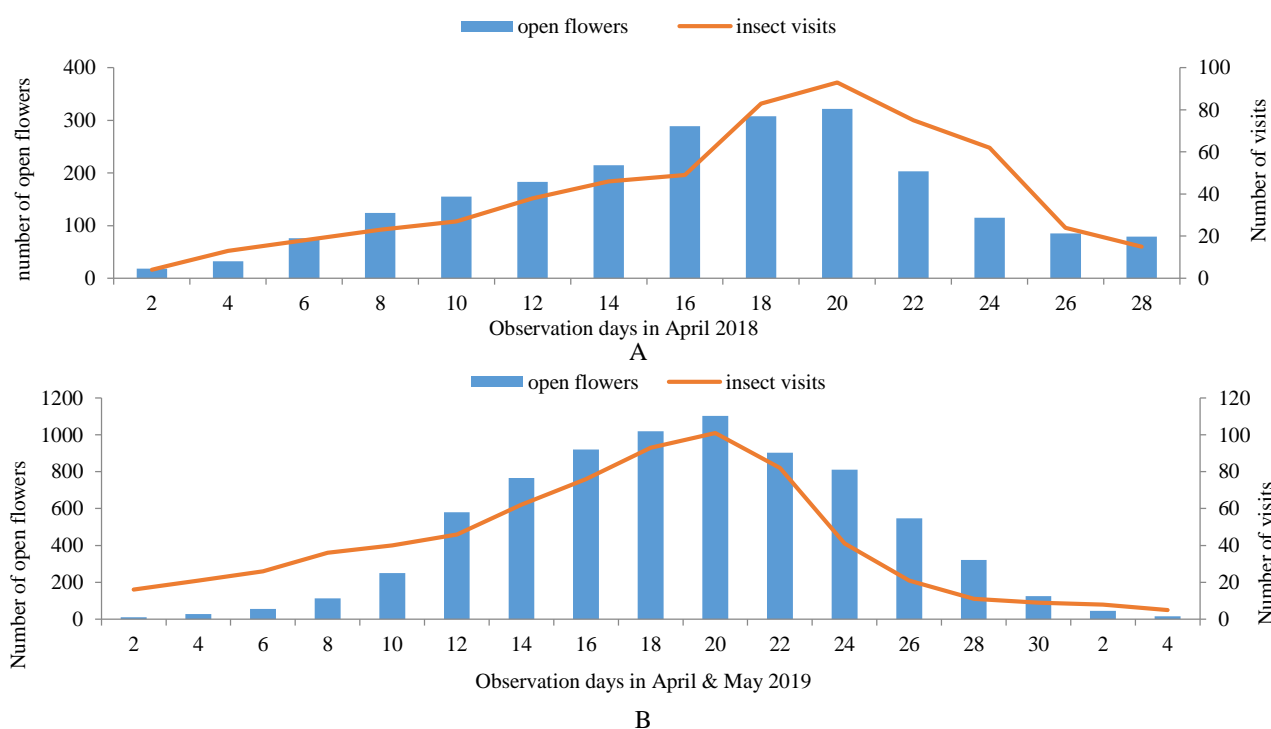


Figure 3. Daily variation in the number of visits of insects observed on the flowers of *Talinum triangulare* according to the number of blooming flowers in 2018 (A) and 2019 (B) in Bafut; Av: April, Ma: May.

Table 2. Daily distribution of insects visits on 2204 over 14 days in 2018 and 4055 flowers over 17 days in 2019, mean temperature and mean humidity of the study site.

Year	Parameters	Daily period			
		7 - 9 am	10 am-12 pm	1 - 3 pm	4- 6 pm
2018	Number of visits	158	265*	137	36
	Temperature (°C)	19.77	25.07*	23.47	20.65
	Hygrometry (%)	59.57	45.43*	42.50	48.07
2019	Number of visits	169	369*	92	42
	Temperature (°C)	19.90	24.57*	23.14	20.71
	Hygrometry (%)	67	60.53*	55.76	56.59

Table 3. Fruiting rate according to the treatments of *Talinum triangulare*

Year	Treatments	Number of flowers studied	Number of fruits formed	Fructification Rate (%)
2018	1 (Unprotected plants)	2204	1887	85.16
	2 (Protected plants)	1615	1093	67.68
2019	3 (Unprotected plants)	4055	2733	67.40
	4 (Protected plants)	3686	2134	57.89

Comparison of fruiting rates: $\chi^2_{(1/2)} = 174.97$ (df= 1, P<0.005, VHS). $\chi^2_{(3/4)} = 303.36$ (df= 1, P<0.05, VHS).

Daily rhythm of visits

Insect activity begins in the morning with the opening of the flowers and decreases sharply around 2 p.m. The daily period of optimal insect activity is between 10 a.m. and 12 p.m. in both 2018 and 2019 (Table 2). Climatic conditions seem to have an influence on the foraging activity of insects on waterleaf flowers. The correlation was significant between the number of insect visits and the temperature in 2018 ($r = 0.68$ [df= 6; P<0.05]) and in 2019 ($r = 0.63$; [df= 6; P<0.05]), then not significant between the number of insect visits and the relative humidity in 2018 ($r = -0.06$; [df= 6; P>0.05]) and in 2019 ($r = 0.39$; [df= 6; P>0.05]).

Impact of insect activity on pollination and yields of *Talinum triangulare*

During nectar and pollen harvest from *T. triangulare*, foraging insects always shook flowers and are regularly made contact with the anthers and stigma, increasing the possibility of *T. triangulare* pollination. The comparison of the fruiting rate (Table 3) revealed that the differences observed were highly significant between treatment 1 and treatment 2 ($\chi^2 = 174.97$ [df= 1; P<0.05]) and between treatment 3 and treatment 4 ($\chi^2 = 303.36$ [df= 1; P<0.01]). In all the visits, Nectar and pollen collectors made contact with the anthers and stigma and carried pollen. With this pollen, they flew frequently from flower to flower of the same species. The fruit formation was higher in flowers left unprotected for unlimited visits (where high diversity of insects was observed) than in the bagged flowers. This suggests a good pollination in the crop, indicating the need for anthophilous insect's management to increase fruiting.

Discussion

In our experiments, *T. triangulare* has a mixed reproduction mode with the predominance of autogamy over allogamy. This can be explained by the fact that *T. triangulare* is a self-pollinated crop but there is the tendency of the population to be heterogenous due to their floral propensity for insect pollinations (Gill, 1988). In habitats where pollinators are available, the species is likely to show mixed mating system involving both cross-

pollination as a result of floral visitors and autogamy by perianth closing (Shivanna, 2019). Such a mechanism gives the species resilience under pollinator constraint as a means of reproductive assurance (Kalisz and Vogler, 2003; Eckert et al., 2006, 2010; Shivanna, 2014, 2015)

Flowers of *T. triangulare* are actinomorphic and of open type; any floral visitor is able to harvest the reward (Pellmyr, 2002). On a *T. triangulare* flowers, insects harvested nectar (Scirtidae, Drosophilidae, Formicidae, Acraeidae, Papilionidae, Muscidae, Apidae, Halictidae) this results is in accordance with some authors results whom found that Pollinators like bees, ants, grasshopper and flies visit waterleaf flowers due to the rich nectar they have (Stevens, 1990). In addition to the nectar, some insects also collected pollen (Muscidae, Apidae, Halictidae) while others sucks the sap (Latrididae, Lygaeidae, Coreidae) and one group is predator (Acridae). These insects' predators' help to control the populations of other insect's species in the farm (Ewusie and Quay, 1977). These different diets are based on their feeding strategies. This diversity of insect herbivores reflects diversity in diet and feeding behavior (Bernays, 1998). Our studies revealed that waterleaf plant attracted broad range of insects. Among those insect species, *Camponotus flavomarginatus* (ant) was the most frequent insect on the *T. triangulare* plants where they moved up and down linearly on the different parts of the plant, this was due to the fact that they are extremely abundant in most ecosystems. Several authors have acknowledged the fact that ants could aid in pollination in different ways (Oliveira et al., 1999; Gomez, 2000; Ghazoul, 2001; Philpott et al., 2006).

the presence of insects on the experimental plot were high in the morning from 10 am to 12 pm and is correlated with the availability of nectar and/or pollen in *T. triangulare* flowers; similarly, this is the period during which the stigma of waterleaf flowers has optimal receptivity for pollen or the weather conditions. In fact, Bramel et al. (2004) reported that the weather during bloom affects abundance and foraging of insect pollinators. Insects as poikilothermic animals change their activity visibly depending on the temperature of the surrounding environment (Bale et al., 2002; Menéndez et al., 2007).

The positive and significant correlation between the number of open waterleaf flowers and the number of insect visits highlights the high attractiveness of the nectar and / or pollen of this Portulacaceae to insects. Insects carry pollen from flower to flower and can thus play a positive role in geitonogamy (Rosa et al., 2011), by placing the pollen of one flower on the stigma of another flower of the same plant. Insects that pass from flower to flower on different plants carry pollen from one plant to another. They can thus promote xenogamy (Rosa et al., 2011), by putting the pollen of one plant on the stigma of a flower of another plant. The latter form of pollination is all the more likely as allogamy exists in *T. triangulare* as also reported by Rachman (2008). The insects shake the flowers during their visits. These movements would facilitate the optimal release of pollen for the occupation of the stigma. The optimal pollen load on the stigma would be favorable for the formation of fruits and seeds

The positive and significant contribution of insects in the fruit yields of waterleaf is justified by their actions on pollination. The fruit yields recorded in all the treatment can be attributed to the predominance of autogamy of this specie and the important role of the pollinating insects. The flowers that were exposed to pollinators provided more fruits than protected plants; this result is in agreement to previous results reported on *T. triangulare* crop in Indonesia (Rachman, 2002). According to McGregor (1976), Fruiting is mainly dependent on the intensity of pollination.

Conclusion

Talinum triangulare is a plant species that benefits from the foraging activity of a broad range of insects species. 14 species of insects distributed into six Orders visited the flowers of *T. triangulare* to harvest nectar and/or pollen. These insects foraged the flowers of this plant species from 7 am to 6 pm, with a peak activity located between 10 am and 12 pm. *T. triangulare* is plant that benefits from pollination by insects despite its capacity of self-pollination. The significant increase in yields of *T. triangulare* in the presence of anthophilous insects is the consequence of the foraging activity of these insect species on the pollination of the flowers of the plant studied. Installation and preservation of insect nests near the population of *T. triangulare* should be recommended in order to benefit from the ecosystem service of pollinating insects thus increasing in fruit yield of Waterleaf

Acknowledgements

The authors thank all those who facilitated this work, especially Youmbi Chirelle Audrey and Shu Constance Bi for their help in data collection.

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