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Effectiveness of Weaver ants (*Oecophylla longinoda*), bait application (GF-120) and neem oil (*Azachdirachta indica*) combination in the control of fruit flies in mango orchards in Northern Côte d'Ivoire

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Abstract
Mango is Côte d'Ivoire's third most important export fruit after bananas and pineapples with a national production of 100,000 tons/year. Unfortunately, mango production, although flourishing in recent years, still faces various constraints, of which fruit flies are the most damaging. The objective of this work was to evaluate the effectiveness of Weaver ants (*Oecophylla longinoda),* biopesticide (Neem Oil) and bait food (GF-120) combination in the control of fruit flies in mango orchards. In each orchard, a 5 hectare plot was delimited into four sub-plots each 20 m apart with a treatment: Ants + Neem Oil, Ants + GF-120, Ants alone and a plot without ants. The treatments were repeated in three different locations (Korhogo, Ferké and Ouangolo). In each treatment, a sample of 100 mangoes was collected from ten trees. The harvested mangoes were weighed and incubated in the laboratory. Two weeks later, the mangoes were washed and the number of pupae was determined for each treatment. In all sites, the combination Ants + Nem protected mango orchards at 98.6%, followed by the combination Ants + GF-120 which is 98.43% and ants alone with a protection rate of 90.89%. The combination of control including Weaver ants effectively protects mango orchards than Weaver ants alone.

Keywords: Fruit flies, Weaver ants, biopesticides, combination, mango, Côte d'Ivoire.

1. Introduction

Mango (*Mangifera indica* L) is Côte d'Ivoire's third most important export fruit after banana and pineapple (Tchounand, 2017) [1]. Côte d'Ivoire is the third largest supplier of mangoes in the world on the European market with 32,600 t exported in 2017 compared to 10,179 t in 2011 after Brazil (100,000 t) and Peru (80,000 t) (Mieu, 2017) [2]. Mango production, although flourishing in recent years, continues to face various constraints, with fruit flies being the most damaging. These fruit flies damage mainly fruits that are close to maturity or in maturity (Quilici *et al*., 2005) [3]. They lay eggs in shallow fruits and larvae (worms or maggots), feed on the pulp of the fruit and dig tunnels. Secondary rots develop in the fruit and fall, causing many losses (Quilici *et al*., 2006) [4]. In Côte d'Ivoire, this damage is estimated at 17% at the beginning of the season; 69% in the middle of the season and 80% at the end of the season, and even 100% for some localities if there is no control (N'Dépo *et al*., 2010) [5].. To address these yield losses, research efforts are being intensified worldwide to develop alternative pest control strategies that are environmentally friendly. These strategies include the use of biopesticides and biological control agents such as *Oecophylla longinoda* (Adandonon *et al*., 2009) [6]. According to Vayssière *et al*. (2009) [7], the management and use of *Oecophylla* is a tool well adapted to the sustainable development of perennial cropping systems in Sub-Saharan Africa for their effectiveness in fruit fly management, improvement in fruit quality, and as a sustainable, economical and environmentally friendly fruit fly control method. It is with this mind that we conducted this study to show the effectiveness of *Oecophylla longinoda* in the control of fruit flies in mango orchards. Thus, in this work, we have combined weather ant with food bait (GF-120) and natural substance (Neem oil) to better protect mango orchards because one method cannot effectively control fruit flies.

2. Materials and Methods

2.1. Study sites

The study was conducted in mango orchards in northern Côte d'Ivoire. These mango orchards are located in the localities of Korhogo (09°23'800"N ; 005°43'429"W), Ouangolo (09°50'838"N ; 005°03.852"W) and Ferkéssedougou (09°44'803"N ; 05°15'668"W) (Figure 1). It is a vast mango production area characterized by a Sudanese type climate with two seasons, a dry season from November to April and a rainy season from May to October. The average annual rainfall is 1400 mm in a wet year and 1000 mm in a dry year. The natural vegetation consists of wooded savannah. Soils are ferralitic, moderately to highly desaturated (Djaha *et al.*, 2014) [8]. The temperature is characterized by a maximum (41°C) in March, and a minimum (16.5 °C) in January. A plot of 5 ha of Kent mango variety, mostly aged about 30 years and spaced 10 m × 10 m (about 100 trees per ha), was selected from each orchard. The selected plots were mainly colonized by Weaver ants. Each plot was subdivided into 04 sub-plots of 72 trees each with two rows of trees serving as borders. A subplot was used as a treatment site. The treatments were composed of: (i) Trees protected by Weaver ants + neem oil, (ii) Trees protected by Weaver ants + Bait application, (iii) Trees protected by Weaver ants and (iv) Trees not protected by Weaver ants.

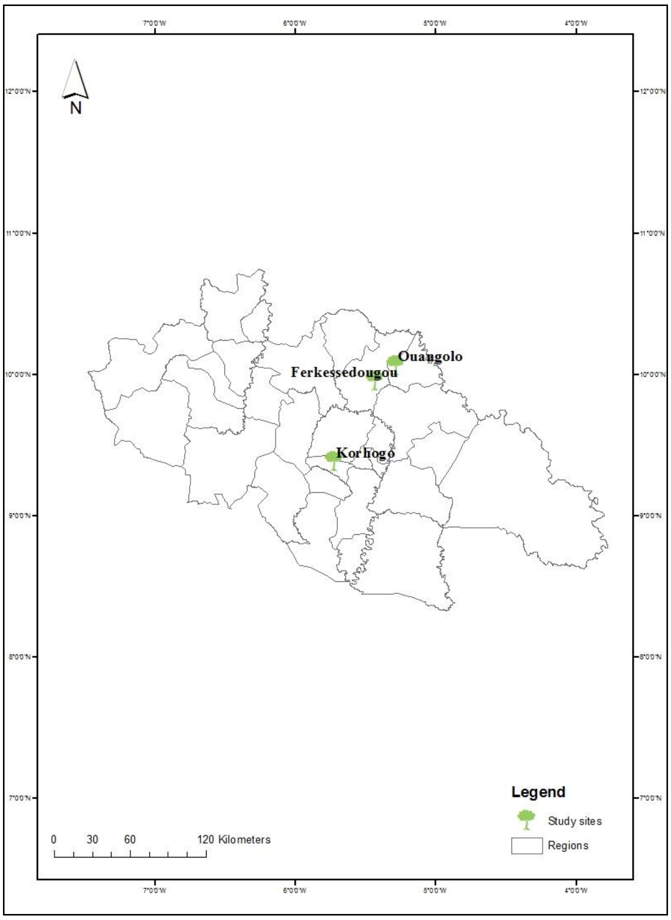


Fig 1: Study sites

2.2. Colonization of trees by *Oecophylla longinoda*

When transplanting Weaver ants into orchards, trees that were not colonized by Weaver ants were selected at the beginning of the experiment. Most of the trees sheltered the species *Pheidole megacephala*, a competitor of *Oecophylla longinoda* (Seguni *et al*., 2011) [9]. To protect the introduction of new colony into these trees, *P. megacephala* was controlled using chlorpyriphos ethyl 480g/l insecticide. Barriers were also established at the base of mango trees to prevent *Pheidole megacephala* and other ants from entering treated trees and plots. For trees containing Weaver ants, a mapping was carried out to determine the ants colonies present because weaver ants from different colonies fight, aggression tests were used to identify individual colonies (Peng *et al*., 2008). Ants of the same colonies were interconnected by twine and branches. Connecting trees between different colonies were pruned to avoid clashes between neighbouring ant colonies.

2.3. Distribution of Weather ant colonies

To determine the distribution of ant colonies, we used the cat food method described by Peng *et al*, (2008) as follow: One spoonful of cat food was placed on the main branch of each tree to attract and induce aggregation of weaver ants. After that one ant was picked with flexible forceps from one tree to an adjacent tree for ants' confrontation. If resident ants fight the introduced ants, then they belong to different colonies. Therefore, different color tapes were used to mark those two trees. In contrast if no fighting was observed on trees, then those ants belong to the same colony. In that case the same color tape was used to mark the two trees.

2.4. Ants Treatment +Bait application (GF-120)

GF-120 is a combination of spinosad (neurotoxin) and protein baits (Salgado, 1998; Salgado *et al*., 1998) [10, 11] containing 0.02% spinosad (active ingredient) and 98.8% inert ingredients including water, sugars and attractants. It was applied at the recommended rate (1 L/ ha) as a foliar spray using an Apollo 16-AF hand-held sprayer (Berthoud Spayers Ltd, United Kingdom) with a conical nozzle (1 to 2 mm opening to deliver 2 to 6 mm droplets) (Vayssières *et al*., 2009) [7]. One square metre on each side of the tree according to the four cardinal points was sprayed in fruit-free areas. The product was applied every ten days from March to July.

2.5. Ants treatment + Neem Oil

The Neem insecticide, an organic product, was mixed with water at a concentration of 1%. The recommended rate (1 l / ha) was used. Prior to application, this rate was mixed with water in a 1:5 ratio (Dow Agrosciences, 2001) [12]. and applied as a foliar spray using an Apollo 16-AF hand sprayer (Berthoud Spayers Ltd, United Kingdom) with a conical nozzle (1 to 2 mm opening to deliver 2 to 6 mm droplets) (Vayssières *et al*., 2009) [7]. One square metre on each side of the tree according to the four cardinal points was sprayed at head height in fruit-free areas. The product was applied every ten days from March to July.

2.6. Control treatment (without ants)

In the control treatment, ants were kept out of control of the trees by applying an adhesive strip (Oecotak 5; Oecos Ltd, United Kingdom) around the trunk of the trees and ensuring that weeds did not cross this barrier.

2.7. Incubation of harvested fruit

The harvested mangoes were weighed and incubated in the laboratory in basins covered with sterile sand. Two weeks later, the mangoes were washed and the number of pupae from the mangoes in each treatment was determined.

2.8. Monitoring the abundance of Weaver ants in orchards

Monitoring of the abundance of Weaver ants was carried out using the method developed by Peng and Christian (2004) [13]. For each treatment, the trees were numbered from 1 to 10. Observations were made during the mango season from March to July on all the main branches, including those branched to the latter located at the level of the observer with the arms upright (~2.40m). Observations were made from 9:30 am onwards because the period of intense activity of Weaver ants is between 9:30 am and 4:30 pm (Vayssières *et al*., 2011) [14]. The Peng 2 method consisted in counting the number of ants present on the main branches according to a rating scale from 0 to 1, thus the score was given: 0 if there are no ants; 0.5 if the number of ants is between 1 and 10 and 1 if the number of ants is greater than 10.

Table 1: *Oecophylla longinoda* rating scale

Number of ants	**0**	**1 to 10**	**>10**
Scores	0	0.5	1

The percentage abundance of ants (% AF) per tree was then calculated by the following formula: (∑Scores / Me)*100 or% AF is the abundance percentage of ants and ∑Scores represents the sum of scores, Me is the total number of main branches on the tree. Then an average of the abundance of Weaver ants was determined for each treatment.

2.8. Data analysis

The data obtained were subjected to a one-factor analysis of variance (ANOVA 1) at the 5% threshold. The averages are ranked according to Fisher's LSD test using STATISTICA V.7.1 software to assess the abundance and the influence of Weaver ants. Furthemore, Attack rate (Ta), Infestation level (Ni) and Percentage of protection (Pp) was calculated by the following formula :

Ta = Mi/Me *100
Ni = Np/Pt
%Pp = (NT-T) /NT*100
Ta = Attack rate (%)
Mi = Number of infested mangoes
Me = Total number of mangoes in the sample
Ni = Infestation level (Pupes /Kg of fruit)
Np = Nomber of pupae collected
Pt = Weight of the mango sample
%Pp = Percentage of protection
NT = Number of pupae in the treated plot
T = Number of pupae in the control plot

3. Results

3.1. Average abundance of *Oecophylla longinoda* in mango orchards

The Peng 2 index showed that *Oecophylla longinoda* is abundant in mango orchards at more than 50%. However, the orchard of Korhogo (63.88 ± 2.51%) was abundant in Weaver ants, followed by Ferké (62.78 ± 3.04%) and Ouangolo (55.31 ± 2.66%) (Figure 2).
Anova 1, at the 5% threshold, the averages assigned to the same letters are not statistically significant

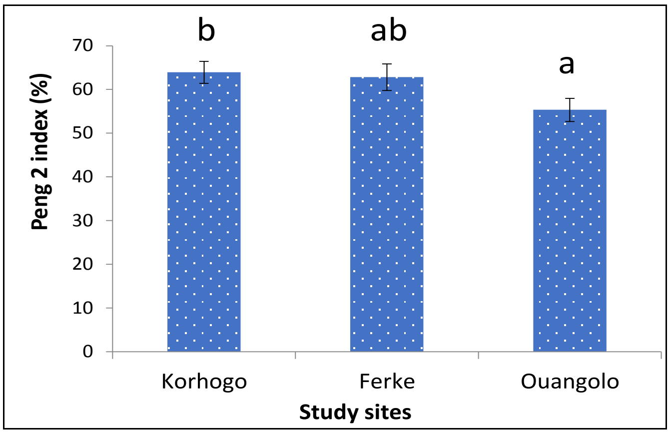
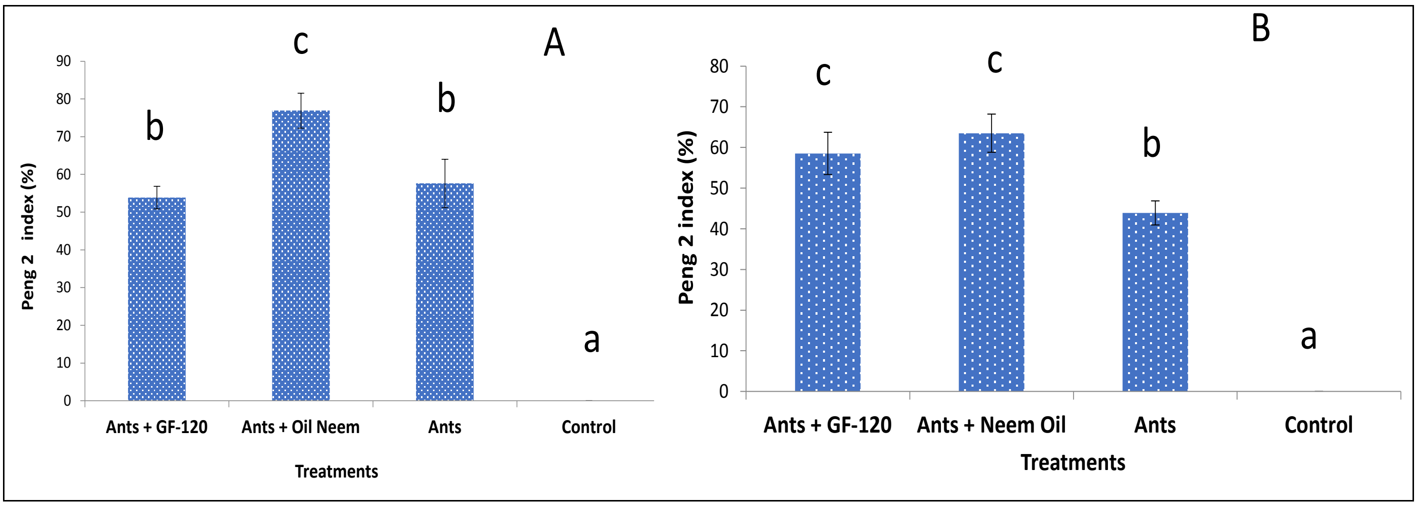
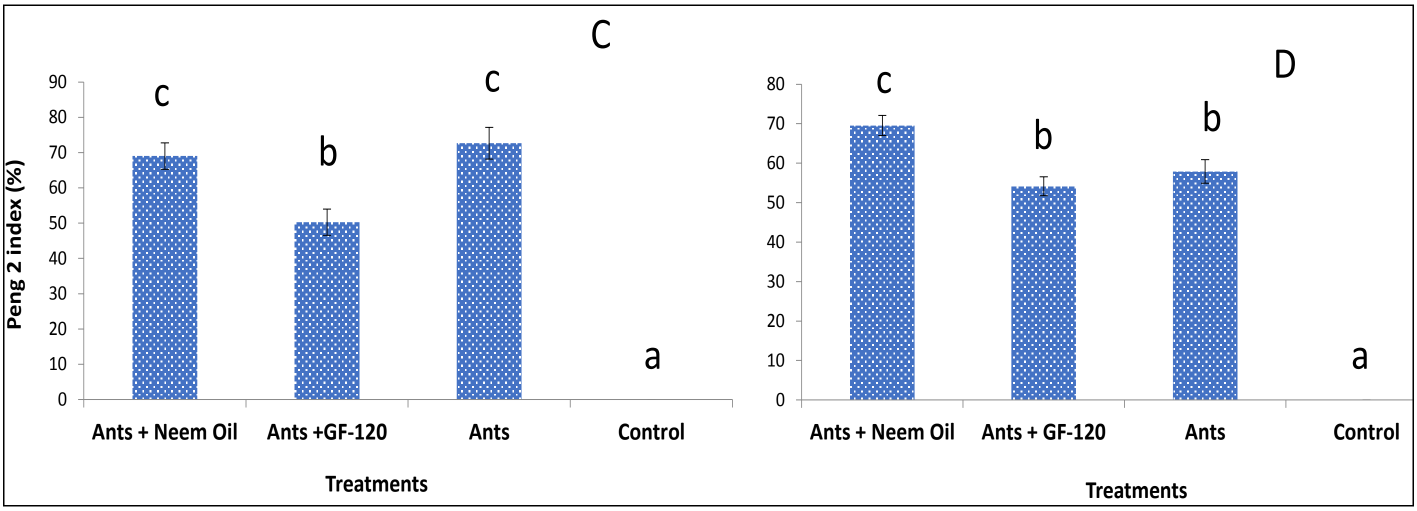


Fig 2: Average abundance of *Oecophylla longinoda* by the Peng2 method in mango orchards

3.2. Influence of treatments on the abundance of *Oecophylla longinoda*

In mango orchards, there is greater tree colonization by Weaver ants in plots where neem has been applied in all study sites. Indeed, Weaver ants are more abundant in the Neem Oil treatment (76.89 ± 4.61% ; 63.51 ± 4.69% ; 69.63 ± 3.18% ; 69.56 ± 2, 54) than the GF-120 treatment (53.86 ± 2.98% ; 58.52 ± 5.16% ; 50.29 ± 3.71% ; 54.15 ± 2.41%) respectively in Ferké, Ouangolo and Korhogo and in all the sites. Statistical treatments reveal a significant difference ($p<0.05$) between treatments and the abundance of Weaver ants in mango orchards (Figures 3A, 3B, 3C and 3D).





Anova 1, at the 5% threshold, the averages assigned to the same letters are not statistically significant

Fig 3: Influence of treatments on the abundance of *Oecophylla longinoda* by the Peng 2 method, Feké Orchard (A); Ouangolo Orchard (B); Korhogo Orchard (C); All Orchard (D).

3.3. Effect of combinations of control methods on fruit fly infestations.

In all sites, the protection parameters (Attack rate, Infestation level and protection percentage) showed that Ants + Oil Neem treatment and the Ants + GF-120 treatment protected mango orchards more than the Ants treatment alone.

The attack rate (Ta) in all sites is (2.25 ± 1.29% ; 2.95 ± 1.41% ; 8.41 ± 3.44% and 23.04 ± 8.28%) respectively in the Ants + Oil neem ; Ants + GF-120 ; Ants and the control treatments. The highest attack rate was experienced in Korhogo (6.75 ± 2.19%; 7.52 ± 2.47%; 19.23 ± 6.45% and 45.12 ± 15.95%) while the lowest was experience in Feké (0 ± 0%; 0 ± 0%; 0 ± 0%; 3.33 ± 0.8% and 20 ± 11.54%) and Ouangolo (0 ± 0%; 1.33 ± 0.33%; 2.66 ± 1.33% and 4.00 ± 2.30%) respectively in the Ants + Oil Neem treatments; Ants + GF-120 treatments; Ants treatments and the control. (Table 2).

The infestation level (Ni) in all sites is (1.04 ± 0.53 ; 1.48 ± 0.69 ; 4.24 ± 1.78 and 47.97± 22.91) respectively in the Ants + Oil Neem ; Ants + GF-120 ; Ants and control treatments. The highest infestation rate was experienced in Korhogo (3.13 ± 0.37 ; 4, 10 ± 0.74 ; 11.27 ± 0.43 and 123.04 ± 44.57) while the lowest was experience in Ferké (0 ± 0; 0, 33 ± 0.08; 1.24 ± 0.77 and 6.14 ± 3.10) and Ouangolo (0 ± 0; 0, 33 ± 0.08; 1.24 ± 0.77 and 6.14 ± 3.10) respectively in the Ants + Oil neem treatments; Ants + GF-120 ; Ants and the control (Table 3).

The percentage of protection (%PP) in all sites is (95.89 ± 1.25 ; 95.47 ± 1.83 ; 84.89 ± 8.29 and 0.00 ± 0.00, 00) respectively in the Ants + Oil Neem ; Ants + GF-120 ; Ants and control treatments. The highest percentage of protection was experienced in Ferké (100 ± 0.0 ; 100 ± 0.0 ; 99.17 ± 0.83 and 0 + 0) while the lowest was experience in Ounagolo (100 ± 0.00 ; 97.22 ± 2.78 ; 88.61 ± 6.28 and 0.00 ± 0.00, 00) and Korhogo ((95.89 ± 1.25 ; 95.47 ± 1.83 ; 84.89 ± 8.29 and 0.00 ± 0.00) respectively in the Ants + Oil Neem treatments; Ants + GF-120 ; Ants and control (Table 4).

Table 2: Attack rate (TA) of orchards in study sites

Treatments	Ferké	Ouangolo	Korhogo	All the sites
Ants	3.33 ± 0.8 a	2.66 ± 1.33 a	19.23 ± 6.45 ab	8.41 ± 3.44 a
Ants + Oil Neem	0 ± 0 a	0 ± 0 a	6.75 ± 2.19 a	2.25 ± 1.29 a
Ants + GF-120	0 ± 0 a	1.33 ± 0.33 a	7.52 ± 2.47 a	2.95 ± 1.41 a
Control	20 ± 11,54 a	4.00 ± 2.30 a	45.12 ± 15.95 b	23.04 ± 8.28 b
F	2.53	1.33	4.18	4.43
P	0.12	0.32	0.04	0.01

Averages on the same lines with the same letters are not significantly different (Newman Kauls test: α =0.05)

Table 3: Infestation level (Ni) of orchards in study sites

Treatments	Ferké	Ouangolo	Korhogo	All the sites
Ants	0.22 ± 0.05 a	1.24 ± 0.77 a	11.27 ± 0.43 a	4.24 ± 1.78 a
Ants +Oil Neem	0 ± 0 a	0 ± 0 a	3.13 ± 0.37 a	1.04 ± 0.53 a
Oécophylles + GF-120	0 ± 0 a	0. 33 ± 0.08 a	4.10 ± 0.74 a	1.48 ± 0.69 a
Control	14.73 ±7.67 a	6.14 ± 3.10 a	123.04 ± 44.57 b	47.97± 22.91 b
F	3.64	3.15	6.89	3.96
P	0.06	0.08	0.01	0.01

Averages on the same lines with the same letters are not significantly different (Newman Kauls test: α =0.05)

Table 4: Percentage of protection (%Pp) of orchards in study sites

Treatments	Ferké	Ouangolo	Korhogo	All the sites
Ants	99.17 ± 0.83 b	88.61 ± 6.28 bc	84.89 ± 8.29 a	90.89 ± 3.69 b
Ants + Oil Neem	100 ± 0.0 b	100 ± 0.00 c	95.89 ± 1.25 a	98.63± 0.77 c
Ants + GF-120	100 ± 0.0 b	97.22 ± 2.78 b	95.47 ± 1.83 a	98.49 ± 0.92 c
Control	0 + 0 a	0.00 ± 0. 00 a	0.00 ± 0. 00 b	0.00 ± 0. 00 a
F	14672	194.44	194.44	194.44
P	0.0000001	0.000001	0.000001	0.000001

Averages on the same lines with the same letters are not significantly different (Newman Kauls test: α =0.05)

4. Discussion

The current study demonstrates the potential use of a combination of Weather ant, Bait application and Neem oil in the control of fruit flies in mango orchards in northern Côte d'Ivoire.

The average abundance of Weaver ants in mango orchards has shown that Weaver ants are more than 50% abundant in mango orchards in northern Côte d'Ivoire. This abundance could provide good protection for mango orchards. According to Peng *et al*, (2008) [15], the abundance of Weaver ants per tree must exceed 50% per tree to have effective orchard protection. The high abundance of Weaver ants in these localities could be due to favourable climatic factors, the high availability of host plants and the presence of high prey in these orchards. According to Lokker, (1990) [16], climatic factors (temperature, rainfall and relative humidity) can affect the abundance of Weaver ants. In addition, the mango tree is a preferred host plant for Weaver ants thanks to its young and light leaves for nest building but also because of the predation that ants lead in the mango tree. According to (Lokker, 1990) [16], the appearance of young flexible leaves and nectar at certain development stages of host plants, for example, cashew nuts, may temporarily influence the abundance of Weaver ants. The secretion of aphids on the respective shoots of hosts is considered to promote the stability and increase of Weather Ant colonies (Van Mele and Cuc, 2007) [17]. Indeed, aphids produce sweet honeydew and protein as food for Weaver ants, which in turn protects them (Van Mele and Cuc, 2007) [17].

Treatments of natural substance (Neem Oil) and Food Bait (GF-120) have played a positive role on the population of Weaver ants in mango orchards. The treatments maintained the balance of ants on the tree by increasing their populations. According to Peng and Christian, (2005) [18], the combination of Weaver ants and low-dose chemicals based on liquid Potassium and white oil has no effect on Weaver ants but are harmful to insect pests. Indeed, the 1L/ha rate at the ten (10) day treatment frequency played a positive role in the abundance of *Oecophylla longinoda*. After each treatment, the Weaver ants approached and sucked away the fine droplets of food bait and natural substance. However, they were more concentrated on the droplets of the natural substance than on the food bait. In addition, Weaver ants were more abundant in the Neem treatment than food bait treatment. This could be explained by the fact that *Azadirachta indica* is a natural plant that grows wild in forests, in and around mango orchards. So Weaver ants would be in contact with this plant species before migrating to the mango tree. In addition, azachdiradine is a natural insecticide of the terpenoid family that gives it intrinsic properties (Faye, 2010) [19]. As for the food bait, it was less prefered by Weaver ants than Neem Oil. Indeed, this could explain why food bait is not a natural substance such as Neem Oil. In fact, food bait consists of food attractants to attract fruit flies and a low-level insecticide (spinosad) that kills fruit flies slowly once after eating the food (Dow Agrosciences, 2001) [12]. According to Peng and Christian, (2006) [20]. the combination of Weaver ants and non-toxic chemicals reduces the number of fruit fly larvae in mango orchards and in this way significantly reduces the level of fruit rejection.

The presence of Weaver ants in mango orchards has significantly reduced fruit fly damage. The level of infestation in the Ants treatment was low, protecting the mango orchard by more than 90%. According to Peng and Christian, (2005) [18], Weaver ants increased producers' incomes by 70% compared to conventional treatment programs in mango orchards in northern Australia. In Côte d'Ivoire, similar studies on coconut palms have shown that *Oécophylla longinoda* has reduced chinch bug damage (*Pseudotheraptus devastans* DISTANT) (Allou *et al*., 2006) [21].

However, integrated pest management using Weaver ants has considerably reduced fruit fly damage compared to *Oecophylla longinoda* alone. This is due to the fact that in addition to the actions of ants on fruit flies, the combination with biopesticides and food baits has contributed to the reduction of fruit flies in mango orchards. In our study, six applications were made at ten-day intervals. According to Vayssière *et al*, (2009) [7], the reduction in infestation is due to the number of applications and the date of the first treatment. Insecticide Neem showed insecticidal activity on adults of fruit flies. These insecticidal activities of Neem Oil extracts are attributed to the presence in different parts of the plant of several compounds of the terpenoid family (azadirachtin, nimbine, salaninne, deacetylnimbine, deacetylsalaninne) (Faye, 2010) [19]. Indeed, according to Schmutterer, (1995) [22], the ability of Neem Oil to repel insects was first reported in the scientific literature in 1928 and 1929 by two Indian researchers, who used 0.001% of an aqueous suspension of neem seeds to repel locust soil. The toxic and repellent effects of Neem could depend on their chemical composition and the level of insect sensitivity. According to Mouffok *et al*, (2008) [23], azadirachtin has been shown to be effective on the white pine weevil, which is a Beetle. As for food bait, it is a low dose insecticide-based on food bait (spinosad) that kills flies after ingestion. This food bait is widely used to control fruit flies in mango orchards. In Côte d'Ivoire, studies by N'Dépo, (2010) [24] have shown that bait success has reduced fruit fly infestation in mango orchards. (Vayssière *et al*, (2009) [7] showed that the level of fruit fly infestation in mango orchards in Benin was 81% in 2006 after 7 weeks of application and 89% in 2007 after 10 weeks of application. The combination of ants, natural substances and food bait effectively strengthens the fight against crop predators. For example, Peng and Christian, (2005a) [25] reported that weaver ants used in an integrated management program (IPM) with non-toxic chemicals (white oil and liquid potassium) may be more effective than using Weaver ants alone because they alone were unable to reduce the entire population of pests. According to Anato *et al*, (2015) [26], the Ants + GF-120 treatment obtained the highest yield of cashew nuts than the other treatments compared to controls.

The combination of Weaver ants and Neem Oil (98.63 ± 0.77%) protected the mango orchard than the combination of Weaver ants and GF-120 (98.49± 0.92%) than the *Oecophylla longinoda* treatment alone (90.89± 3.69%) because the Neem promote the abundance of Weaver ants in mango orchards than food bait.

5. Conclusion

The potential use of a combination of Weather ant, Bait application and neem oil in the control of fruit flies showed that combination has significantly reduced fruit fly damage compared to treatment with *Oecophylla longinoda*. Weaver ants are abundant in mango orchards in northern Côte d'Ivoire and can provide good protection against fruit flies if they are protected. However, they are more abundant in the locality of Korhogo than Ferké and Ouangolodougou. In addition, ants were compatible with food bait and biopesticide. In the search for alternative pest control strategies that respect the environment, it would be interesting to integrate the combination of Weaver ants and food bait (GF-120) or Weaver ants and biopesticides (Neem Oil) into mango orchards to more effectively control these insect pests because one method is not efficient to fight against pest.

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