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Compared effects of *Metarhizium anisopliae* ICIPE 69 and Chlorpyriphos-ethyl on the mango mealybug, *Rastrococcus invadens* Williams (Homoptera : Pseudococcidae) and its parasitoids in western Burkina Faso

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ABSTRACT

This study was carried out to evaluate the effects of the bio pesticide *Metarhizium anisopliae* ICIPE 69 and the chemical pesticide Chlorpyriphos-ethyl on *Rastrococcus invadens* and its parasitoids *Anagyrus mangicola* and *Gyranusoidea tebygi* in orchards. A Randomized Complete Block design consisting of 5 treatments was set up in Mahon and Niampédougou. There were the untreated control, the reference chemical Chlorpyriphos-ethyl (480 g/l) and 3 doses of the bio pesticide (10^8 conidia/ml, 10^9 conidia/ml and 2.10^9 conidia/ml). Live or dead mealybugs were counted on 20 leaves/mango trees before the first application of the chemicals and then successively each week for 3 to 4 weeks. Highest mortality rates of mealybugs were observed with Chlorpyriphos-ethyl (50-90%) followed by the higher dose of the bio pesticide (28-40%). The level of parasitoids expressed in log (N + 1) was low (0-0.86) on trees treated with Chlorpyriphos-ethyl compared to bio pesticide (0.15-1.86) and untreated control (0.33-1.53). Significant differences ($0.047 \leq P < 0.001$) were observed between treatments after the first application of the chemicals. Although the rate of mortality of mealybugs was twice as high with Chlorpyriphos-ethyl, *M. anisopliae* ICIPE 69 could be an alternative to synthetic insecticides for compatibility with parasitoids.

Keywords: Mango mealybug, biological control, entomopathogenic fungus, parasitoids, Burkina Faso.

INTRODUCTION

Mango is one of the fruits produced in most continents, particularly in Asia, Central America, South America and Africa. Its global production estimated at 40 million tons per year is concentrated in Asia and more specifically in India which alone produces on average 15 million tons per year (Mitra, 2016). African countries like Nigeria and Egypt are among the top 10 global producers

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of this fruit (Unctad, 2016). Mango is a food product with high nutritional and trade value. It contributes to improving the social welfare and health status of the populations (Passannet et al., 2017).

In Burkina Faso, mango represents the first fruit crop in terms of quantities produced and areas planted. About 400,000 tons of mangoes are produced each year, of which 30,000 to 50,000 tons are absorbed by the European market (Anonymous, 2016). Part of this production is processed by seventy-five units and processing industries. Dried mangoes account for 85% of these products (Kanté-Traoré et al., 2017). The mango sector is an important source of entry of foreign currencies through the export of fresh mangoes and derived products (dried mangoes, juices). In 2016, nearly 20,000,000 euros of revenue were generated in this sector in Burkina Faso. Most of the orchards (75%) and mangoes produced (68.5%) come from the western region (Mah, 2011). In this part of the country, the main production constraints of mango are due to diseases and insect pests. Diseases include bacterial, anthracnose and drying mango (Zombre, 2016). The main insect pests are fruit flies, mealybugs and termites (Dakouo et al., 2011). Three species of mealybugs are encountered on mango trees with a predominance of the invasive species *Rastrococcus invadens* (Nebié et al., 2016). The populations of the latter are naturally regulated by two hymenopteran parasitoids (*Anagyrus mangicola* Noyes and *Gyranusoidea tebygi* Noyes) which are under pressure from local hyper parasitoids *Marietta leopardina*, *Chartocerus hyalipennis* and *Pachyneuron* sp. (Hala et al., 2011; Nébié, 2017). Infestation focus may remain active for approximately 4 years. Faced with this phenomenon, the small growers of infested orchards use agro chemicals like Chlorpyriphos-ethyl, which is active in the short term while eliminating the two parasitoids associated with the pest (Nebié, 2011). In order to seek alternative control methods, we tested in laboratory and validated the pathogenicity of the fungus *Metarhizium anisopliae* ICIPE 69 on the developmental stages of *R. invadens* (Nébié, 2017). The high mortality rates (90-100%) of the pest recorded in the laboratory with this entomopathogen, *Metarhizium anisopliae* ICIPE 69 suggests that this bio pesticide could be an alternative to chemical insecticides used in the mango orchards. The objective of this study was to evaluate comparatively the efficacy of *M. anisopliae* ICIPE 69 and Chlorpyrifos-ethyl on *R. invadens* and the 2 parasitoids associated with it.

MATERIALS AND METHODS

Study sites

The study was conducted in Niampedougou (11.04 ° N, 5.15 ° W) and Mahon (11.03 ° N, 5.22 ° W), two localities situated in the province of Kénédougou, western Burkina Faso. This area is characterized by a South-Sudanian tropical climate with a wet season from May to October and a dry season from November to April. The annual rainfall varies from 900 to 1,100 mm. Average temperatures range from 24.9 °C to 30.2 °C with relatively low thermal amplitude of 5.3 °C.

Pesticides

Bio pesticide

Metarhizium anisopliae was the bio pesticide used in this study. It is a ubiquitous, insect killing fungus from the soil, belonging to the order of Hypocreales and the Clavicipitaceae family. *Metarhizium anisopliae* strain ICIPE 69 was developed from the International Centre of Insect

Physiology and Ecology (icipe). It was used to control white flies, thrips, fruit flies and papaya mealybug *Paracoccus marginatus*. Upon coming into contact with the insect host, *M. anisopliae* spore will germinate and penetrate its host, where it will multiply in the haemocoel of the insect pest, killing it. Death will occur within 2 to 4 days, depending on the temperature, susceptibility and size of the insect pest.

Agrochemical

The agrochemical was Chlorpyriphos-ethyl (Pyrical® 480 EC), an insecticide from organophosphorus family formulated by Arysta LifeScience. Its formula is $C_9H_{11}C_{l3}NO_3PS$. On fruit trees, it is used to control mango mealybug *Rastrococcus invadens* and other insect pests like termites. It is also used to control mosquitoes, cockroaches, fleas and lice (Gapessie Ntiendjui et al., 2009). Chlorpyriphos-ethyl has a neurotoxic effect and shows a long persistence of action. It was listed by WHO as moderately hazardous pesticide though it is suspected to be endocrine disrupting chemical (Lema et al., 2014). In mango orchards, it is recommended to use one liter of product for 100 mango trees.

Insects: *Rastrococcus invadens* and their parasitoids

Mango mealybug, *R. invadens* is an ovoviviparous insect. Its life cycle is marked by three larval stages and an adult stage in both males and females. Both adult and immature stages are pestiferous causing serious damage to leaves, inflorescences and fruits. All stages of development were therefore targeted.

Two parasitoids species are associated with *R. invadens* in Burkina Faso. They include *Gyranusoidea tebegy* and *Anagyrus mangicola* which have some preference vis-a-vis the different stages of development of *R. invadens. Gyranusoidea tebygi* prefers larvae from first and second stages. On the other hand, *A. mangicola* prefers larvae from second and third stages. These two primary parasitoids are the hosts for other parasitoids like *Marietta leopardina*, *Chartocerus hyalipenis* and *Pachyneuron sp.* (Nébié, 2017).

Experimental design

A randomized complete block-type experimental design was set up and monitored between 20th July and 20th August 2016 at each site. The treatments consisted of the untreated control (UTC), the reference chemical (C) formulated with Chlorpyriphos-ethyl (480 g / l) and the 3 doses of the bio pesticide, *Metarhizium anisopliae* (low dose: $D1=10^8$ conidia/ml; medium dose: $D2=10^9$ conidia / ml and high dose: $D3=2.10^9$ conidia / ml). The concentration of 10^8 conidia / ml was obtained by diluting 1 ml of the commercial product with 9 ml of water. The concentration 10^9 conidia / ml corresponds to the nominal concentration. Concentration of 2.10^9conidia / ml was prepared by removing 2 ml of the crude commercial product. The mango tree was considered as an elementary unit in each replication. Thus, each mango tree was assigned to a treatment in a replication. In total, on each of the two sites, 15 mango trees were used for all 5 treatments, i.e. 3 mango trees per treatment.

Preparation and application of the solutions

The Chlorpyriphos-ethyl slurry was obtained by removing 12.5 ml of the commercial product which will be supplemented with 5 liters of water. As for the lower (10^8 conidia/ml) and medium (10^9 conidia / ml) concentrations of *M. anisopliae*, 15 ml of each were diluted with 5 liters of water. For the higher concentration (2.10^9 conidia / ml), 30 ml of the commercial product were diluted with 5 liters of water.

The various slurries were applied to the mango foliage on the same day using an atomizer (CIFARELLI®). Duration of 7 days was observed between two successive applications at each site. In total, 4 applications of each product were made in Mahon against 3 applications in Niampédougou.

Evaluation of the effects of the treatments on the populations of *Rastrococcus invadens*

The density of the mealybug was evaluated in order to know the effects of the insecticides after their application on the populations of the insect. For this purpose, a series of weekly observations were made at each site before and after application of the slurries. In total, 5 series of observations were carried out in Mahon against 4 series in Niampédougou. These observations were made on the leaves and concerned the number of live or dead mealybugs, and their associated parasitoids. In each series of observations, 4 leafy branches were taken using a perch on the foliage of each tree along the 4 cardinal points (East, West, North and South). Twenty leaves, or five leaves/cardinal point, were then taken at random and carefully observed under a hand-held magnifying glass to count the number of live or dead mealybugs.

Evaluation of the effects of the treatments on the associated parasitoids of *Rastrococcus invadens*

This evaluation consisted first of all in collecting the full mummies (pupae of the parasitoids) on the leaves sampled during the different series of observations. Solid mummies are hard to touch and brown in color. They were incubated in plastic vials to allow the parasitoids to emerge. The flasks were labeled to bear the number of the trees on which the insects were taken, the date of observation and the name of the locality. In contrast, the leaves were incubated in Kraft A4 size paper envelopes to allow newly parasitized mealybugs to complete their developmental cycle. A period of 15 days was observed before stripping the incubated leaves. The parasitoids emerged from the samples of each tree were harvested and stored in vials containing 70 ° C alcohol.

Identification of parasitoids associated with *Rastrococcus invadens*

The parasitoids were identified under a binocular microscope using identification manuals developed by Annecke and Insley (1972), Goergen and Neuenschwander (1994) and Noyes and Hayat (1994). Available specimens were also used during that identification. The number of each species of parasitoids was determined and recorded by date of observation and treatment.

Statistical analysis of collected data

Statistical analysis was carried out on the mortality rate of mealybugs and the emergence of parasitoids observed according to treatments and dates of observation. To do this, the data were first entered and then transformed into log (N + 1) base 10 with the Microsoft Office Excel 2010 software to have a normal distribution. The transformed values were used to calculate the mortality rate of mealybugs according to the following formula: $\frac{\log(N1+1)}{[\log(N1+1)+\log(N2+1)]} x100$ where N1 is the number of dead mealybugs / leaf and N2 the number of live mealybugs / leaf. The parasitoid emergence values were expressed as log (N + 1) per batch of 20 incubated leaves. All the processed data were subjected to an analysis of variance with the Genstat Discovery Edition 4 software. The Student-Newman-Keuls test was used to separate the means when significant differences between them were highlighted at the threshold of probability 5%.

RESULTS

Comparative effects of M. anisopliae ICIPE 69 and Chlorpyriphos-ethyl on populations of *Rastrococcus invadens*

Site of Mahon

The mean mortality rate of mealybugs varied from 8.15 to 89.43% depending on the treatments and the dates of observation (Table 1). Trees treated with Chlorpyrifos-ethyl (P) showed the highest average mortality rate (50.74 to 89.43% dead mealybugs / leaf). This value was about 2-3 times higher than the average mortality rate observed on trees treated with the higher dose (D3) of *M. anisopliae*.

The analysis of variance did not reveal any significant difference (P = 0.070) between the treatments during the first observation carried out before the first application of the insecticides. A significant to highly significant difference ($0.021 \leq P < 0.001$) was observed between the treatments after the 1st, 2nd, 3rd and 4th applications of the solutions. Three homogeneous groups were identified or classified by the Student-Newman-Keuls test. The first two groups included the untreated control (UTC) and the 3 doses (D1, D2 and D3) of *M. anisopliae*, while the 3rd group was represented by the single dose (P) of Chlorpyrifos-ethyl.

Site of Niampédougou

Observations carried out before the 1st application of the insecticides showed an average rate of mortality of mealybugs between 1.6 and 4% depending of the treatments (Table 2). The analysis of variance did not reveal any significant difference (P = 0.073) between treatments at that date. On the other hand, the average mortality rate increased on treated and control trees after the application of the insecticides. The mango trees treated with *M. anisopliae* showed mortality rates ranging from 25.61 to 39.97%. The highest dose (D3) of *M. anisopliae* had the highest mortality rate (28.62 to 39.97% dead mealybugs / leaf) compared with the other two doses (D2 and D1). These mortality rates were 2 to 3 times lower than those observed on trees treated with Chlorpyriphos-ethyl (56.19 to 86.59% dead mealybugs / leaf). Control trees showed the lowest values, between 7% and 9% dead mealybugs / leaf.

A significant to highly significant difference ($0.017 \leq P < 0.001$) was observed between treatments after the first, second and third applications of the insecticides at the 5% probability threshold. The Student-Newman-Keuls test was used to separate treatment averages and classify them into 3 homogeneous groups during the 2nd and 4th observations, which occurred 7 days after the first and the third application of the insecticides respectively. The first group comprised the un-treated control (UTC) and the second group the 3 doses (D1, D2 and D3) of *M. anisopliae*. The third group was represented by the single dose (P) of Chlorpyriphos-ethyl. Four homogeneous groups were detected during the 3rd series of observations carried out 7 days after the 3rd application of the insecticides. This was the un-treated control for the 1st group, the low and medium doses of *M. anisopliae* for the 2nd group. The higher dose of *M. anisopliae* and that of Chlorpyriphos-ethyl represented respectively the 3rd and 4th groups.

Comparative effects of *Metarhizium anisopliae* ICIPE 69 and Chlorpyriphos-ethyl on parasitoids of *Rastrococcus invadens*

The population of emerged parasitoids was dominated by *G. tebygi* on all the study sites. Considering all the treatments used, the proportion of *G. tebygi* varied from 74.71 to 91.34% and from 82.01 to 89.33% respectively at the Mahon and Niampédougou sites. The number of *Anagyrus mangicola* represented 8.66 to 25.29% of emerged parasitoids.

Cases of Anagyrus mangicola

The average number of individuals in the emerged parasitoids (log (N + 1) varied from 0 to 1.01 and from 0 to 1.16 on the Mahon and Niampédougou sites respectively (Tables 3 and 4). This average number was lower (0 to 0.56) on trees treated with Chlorpyriphos-ethyl (P) than on the un-treated control (UTC) trees and those treated with the D1, D2, D3 doses of *M. anisopliae*.

Analysis of variance revealed a significant difference (P = 0.03) between treatments before the first application of the insecticides at the Mahon site (Table 3). A non-significant difference (P>0.05) was observed between the treatments during the 2nd and 3rd series of observations carried out 7 days after the 1st and 2nd applications of the insecticides. Also, a significant difference (0.006≤P≤0.047) was observed between treatments in the 4th and 5th series of observations. Two homogeneous groups were constituted: D1, D2, D3 and UTC for the first group and P for the second group.

No significant difference (P>0.05) was observed between treatments before the first application of the insecticides at the Niampédougou site (Table 4). The same result was obtained in the second observation followed by a significant difference (0.001< P<0.002) in the 3rd and 4th series of observations. The separation of the averages revealed two homogeneous groups consisting of the doses D1, D2, D3 and UTC on the one hand and P on the other hand.

Case of Gyranusoidea tebygi

The mean number (log (N + 1)) of emerged *G. tebygi* individuals varied from 0 to 1.42 and from 0.20 to 1.87 respectively at the Mahon and Niampédougou sites (Tables 5 and 6). The lowest values were obtained on trees treated with Chlorpyriphos-ethyl.

No significant difference (P>0.05) was observed between treatments in the first series of observations performed before the first application of the insecticides in Mahon (Table 5). The same result was obtained in the 2nd and 3rd series of observations. On the other hand, very significant differences (P<0.001) were observed between treatments at the 4th and 5th series of observations. The Student-Newman-Keuls test allowed to classify the treatments into 2 homogeneous groups: the untreated control (UTC) and the 3 doses (D1, D2 and D3) of *M. anisopliae* for the first group, and Chlorpyriphos- ethyl (P) for the 2nd group.

For the Niampédougou site, the analysis of variance did not reveal any significant difference (P = 0.058) between treatments in the first series of observations (Table 6). Significant to very highly significant differences (0.011≤P<0.001) were observed between treatments in the 2nd, 3rd and 4th series of observations. Two homogeneous groups were also observed. These were the untreated control (UTC) and the 3 doses (D1, D2 and D3) of *M. anisopliae* for the 1st group and Chlorpyriphos-ethyl (P) for the 2nd group.

Comparative effects of *Metarhizium anisopliae* ICIPE 69 and Chlorpyriphos-ethyl on hyperparasitoids of *Rastrococcus invadens*

Three species of hyperparasitoids of *R. invadens* were recorded in this study. There were *Chartocerus hyalipennis*, *Marietta leopardina* et *Pachyneuron sp.*. The number of these hyperparasitoids, transformed into log (N + 1), varied on average from 0 to 1.6 and from 0.1 to 1.7 respectively in Mahon and Niampédougou according to treatments and frequencies of observations (Tables 7 and 8). The lowest values were observed on trees treated with Chlorpyrifos-ethyl (P).

Analysis of variance did not reveal any significant difference (P>0.05) between treatments prior to the first application of the insecticides in Mahon (Table 7). The same result was observed after the 1st and 2nd applications. On the other hand, very significant differences (P <0.001) were detected after the 3rd and 4th applications of insecticides. The Student-Newman-Keuls test made it possible to separate the treatments into 2 or 4 homogeneous groups. In both cases, the control and the different doses of *M. anisopliae* belonged to the same group.

The results of the analysis of variance did not show a significant difference between the treatments before the first application of the insecticides in Niampédougou (Table 8). It was after this first application that significant differences (0.023≤P <0.001) were observed between treatments. Two to three homogeneous groups were formed from the Student-Newman-Keuls test. The untreated control (UTC) and the 3 doses of *M. anisopliae* belonged to the same homogeneous group.

Table 1: Mortality rate (%) of *R. invadens* according to insecticide treatments and dates of observation in Mahon.

Dates of observation		AvA1	7JA1	7JA2	7JA3	7JA4
Insecticide applications		**A1**	**A2**	**A3**	**A4**	-
	UTC	14,53 a	13,48 a	15,04 a	15,42 a	13,99 a
	D1	16,75 a	24,93 b	26,72 b	30,06 b	25,18 b
Treatments	**D2**	19,31 a	30,68 b	28,79 b	31,70 b	27,74 b
	D3	8,15 a	35,35 b	33,32 b	33,26 b	28,56 b
	P	15,66 a	58,54 c	80,17 c	89,43 c	50,74 c
Probability		0,075	0,021	<0,001	<0,001	0,041
Signification		NS	S	VHS	VHS	S

In the same column, the means followed by the same letter are not significantly different from each other according to the Student-Newman-Keuls test at the 5% probability threshold.
NS: No Significant; S: Significant; VHS: Very Highly Significant.
D1, D2, D3: Lower, medium and higher doses of the bio pesticide; P: Reference product; UTC: Un-Treated Control.
A1, 2, 3, 4: Applications 1, 2, 3, 4; AvA1: Prior to 1st Application; 7JA1, 2, 3, 4: 7 Days after the 1st, 2nd, 3rd, 4th Application.

Table 2: Mortality rate (%) of *R. invadens* according to insecticide treatments and dates of observation in Niampédougou.

Dates of observation		AvA1	7JA1	7JA2	7JA3
Insecticide applications		**A1**	**A2**	**A3**	**-**
	UTC	1,626 a	8,19 a	7,16 a	7,21 a
	D1	1,745 a	26,71b	25,61 ab	31,82 b
Treatments	**D2**	1,817 a	27,81 b	26,85 ab	33,91b
	D3	1,845a	28,62b	38,53b	39,97b
	P	3,893 a	56,19c	81,64c	86,59 c
Probability		0,326	0,017	<0,001	<0,001
Signification		NS	S	VHS	VHS

In the same column, the means followed by the same letter are not significantly different from each other according to the Student-Newman-Keuls test at the 5% probability threshold.
NS: No Significant; S: Significant; VHS: Very Highly Significant.
D1, D2, D3: Lower, medium and higher doses of the bio pesticide; P: Reference product; UTC: Un-Treated Control.
A1, 2, 3: Applications 1, 2, 3; AvA1: Prior to 1st Application; 7JA1, 2, 3: 7 Days after the 1st, 2nd, 3rd Application.

Table 3: Mean number ((log (N+1)) of *A. mangicola* individuals emerged according to insecticide treatments and dates of observation in Mahon.

Dates of observation		AvA1	7JA1	7JA2	7JA3	7JA4
Insecticide applications		**A1**	**A2**	**A3**	**A4**	-
	UTC	1,0111 b	0,3333 a	0,5017 a	0,6737 b	0,7611 b
	D1	0,4184 a	0,1590 a	0,3333 a	0,6344 b	0,4824 b
Treatments	**D2**	0,8812 ab	0,2817 a	0,8421 a	0,6191 b	0,4601 b
	D3	0,8421 ab	0,4601 a	0,6108 a	0,7656 b	0,6414 b
	P	0,6608 ab	0,2594 a	0,2007 a	0,1003 a	0,0000 a
Probability		0,030	0,837	0,229	0,047	0,006
Signification		S	NS	NS	S	S

In the same column, the means followed by the same letter are not significantly different from each other according to the Student-Newman-Keuls test at the 5% probability threshold.
NS: No Significant; S: Significant.
D1, D2, D3: Lower, medium and higher doses of the bio pesticide; P: Reference product; UTC: Un-Treated Control.
A1, 2, 3, 4: Applications 1, 2, 3, 4; AvA1: Prior to 1st Application; 7JA1, 2, 3, 4: 7 Days after the 1st, 2nd, 3rd, 4th Application.

Table 4: Mean number ((log (N+1)) of *A. mangicola* individuals emerged according to insecticide treatments and dates of observation in Niampédougou.

Dates of observation		AvA1	7JA1	7JA2	7JA3
Insecticide applications		**A1**	**A2**	**A3**	**-**
	UTC	0,6414 a	0,7934 a	0,5511 b	0,7195 b
	D1	0,7741 a	0,6191 a	0,6778 b	0,6344 b
Treatments	**D2**	0,9201 a	0,9454 a	0,8863 b	0,7224 b
	D3	0,8105 a	0,7670 a	0,7834 b	1,0908 c
	P	1,1655 a	0,5663 a	0,0000 a	0,2007 a
Probability		0,523	0,550	0,002	0,001
Signification		NS	NS	S	S

In the same column, the means followed by the same letter are not significantly different from each other according to the Student-Newman-Keuls test at the 5% probability threshold.
NS: No Significant; S: Significant.
D1, D2, D3: Lower, medium and higher doses of the bio pesticide; P: Reference product; UTC: Un-Treated Control.
A1, 2, 3: Applications 1, 2, 3; AvA1: Prior to 1st Application; 7JA1, 2, 3: 7 Days after the 1st, 2nd, 3rd Application.

Table 5: Mean number ((log (N+1)) of *G. tebygi* individuals emerged according to insecticide treatments and dates of observation in Mahon.

Dates of observation		AvA1	7JA1	7JA2	7JA3	7JA4
Insecticide applications		**A1**	**A2**	**A3**	**A4**	-
	UTC	1,211 a	1,044 a	1,278 a	1,423 b	1,1742 b
	D1	0,802 a	1,329 a	1,123 a	1,342 b	1,3389 b
Treatments	**D2**	1,418 a	1,273 a	1,089 a	1,400 b	1,1854 b
	D3	1,278 a	1,258 a	1,302 a	1,376 b	1,0974 b
	P	1,140 a	0,674 a	0,534 a	0,100 a	0,0000 a
Probability		0,203	0,204	0,168	<0,001	<0,001
Signification		NS	NS	NS	VHS	VHS

In the same column, the means followed by the same letter are not significantly different from each other according to the Student-Newman-Keuls test at the 5% probability threshold.
NS: No Significant; VHS: Very Highly Significant
D1, D2, D3: Lower, medium and higher doses of the bio pesticide; P: Reference product; UTC: Un-Treated Control.
A1, 2, 3, 4: Applications 1, 2, 3, 4; AvA1: Prior to 1st Application; 7JA1, 2, 3, 4: 7 Days after the 1st, 2nd, 3rd, 4th Application.

Table 6: Mean number ((log (N+1)) of *G. tebygi* individuals emerged according to insecticide treatments and dates of observation in Niampédougou.

Dates of observation		**AvA1**	**7JA1**	**7JA2**	**7JA3**
Insecticide applications		**A1**	**A2**	**A3**	**-**
	UTC	1,405 ab	1,530 b	1,505 b	1,325 b
	D1	1,324 a	1,683 b	1,453 b	1,474 b
Treatments	**D2**	1,862 b	1,612 b	1,702 b	1,447 b
	D3	1,541 ab	1,866 b	1,758 b	1,571 b
	P	1,471 ab	0,860 a	0,460 a	0,201 a
Probability		0,058	0,011	<0,001	<0,001
Signification		NS	S	VHS	VHS

In the same column, the means followed by the same letter are not significantly different from each other according to the Student-Newman-Keuls test at the 5% probability threshold.
NS: No Significant; S: Significant; VHS: Very Highly Significant.
D1, D2, D3: Lower, medium and higher doses of the bio pesticide; P: Reference product; UTC: Un-Treated Control.
A1, 2, 3: Applications 1, 2, 3; AvA1: Prior to 1st Application; 7JA1, 2, 3: 7 Days after the 1st, 2nd, 3rd Application.

Table 7: Mean number ((log (N+1)) of hyperparasitoids individuals emerged according to insecticide treatments and dates of observation in Mahon.

Dates of observation		**AvA1**	**7JA1**	**7JA2**	**7JA3**	**7JA4**
Insecticide applications		**A1**	**A2**	**A3**	**A4**	-
	UTC	1.396 a	1.276 a	1.537 a	1.375 bc	0.999 b
	D1	1.281 a	1.343 a	1.376 a	1.214 b	1.427 b
Treatments	**D2**	1.295 a	1.303 a	1.501 a	1.395 bc	1.149 b
	D3	1.333 a	1.419 a	1.377 a	1.573 c	1.215 b
	P	1.404 a	1.244 a	0.926 a	0.000 a	0.000 a
Probability		0.867	0.463	0.091	<0.001	<0.001
Signification		NS	NS	NS	VHS	VHS

In the same column, the means followed by the same letter are not significantly different from each other according to the Student-Newman-Keuls test at the 5% probability threshold.
NS: No Significant; VHS: Very Highly Significant.
D1, D2, D3: Lower, medium and higher doses of the bio pesticide; P: Reference product; UTC: Un-Treated Control.
A1, 2, 3, 4: Applications 1, 2, 3, 4; AvA1: Prior to 1st Application; 7JA1, 2, 3, 4: 7 Days after the 1st, 2nd, 3rd, 4th Application.

Table 8: Mean number ((log (N+1)) of hyperparasitoids individuals emerged according to insecticide treatments and dates of observation in Niampédougou.

Dates of observation		**AvA1**	**7JA1**	**7JA2**	**7JA3**
Insecticide applications		**A1**	**A2**	**A3**	**-**
Treatments	**UTC**	1.589a	1.349b	1.157b	1.091b
	D1	1.555a	1.359b	1.200b	1.120b
	D2	1.675a	1.449b	1.386b	1.392b
	D3	1.687a	1.700b	1.629b	1.711c
	P	1.359a	0.667a	0.159a	0.100a
Probability		0.367	0.023	<0.001	<0.001
Signification		NS	S	VHS	VHS

In the same column, the means followed by the same letter are not significantly different from each other according to the Student-Newman-Keuls test at the 5% probability threshold.
NS: No Significant; S: Significant; VHS: Very Highly Significant.
D1, D2, D3: Lower, medium and higher doses of the bio pesticide; P: Reference product; UTC: Un-Treated Control.
A1, 2, 3: Applications 1, 2, 3; AvA1: Prior to 1st Application; 7JA1, 2, 3: 7 Days after the 1st, 2nd, 3rd Application.

DISCUSSION

Comparative effects of *Metarhizium anisopliae* ICIPE 69 and Chlorpyriphos-ethyl on mango mealybug *Rastrococcus invadens*

The results obtained in the Mahon and Niampédougou sites did not reveal significant differences between treatments before the first application of the insecticides. After each of the 3 to 4 weekly applications, the rate of mortality of mealybugs increased both on trees treated with *M. anisopliae* and those treated with Chlorpyriphos-ethyl. The effects of 3 doses of *M. anisopliae* was similar on pest populations but with a higher mortality rate of trees treated with the highest dose (2.10^9 conidia / ml). Chlorpyrifos-ethyl caused an average mortality rate of the pest 2-3 times higher than that obtained with each of the 3 doses of *M. anisopliae*. Under laboratory conditions, the highest dose (2.10^9conidia / ml) of *M. anisopliae* resulted in a mortality rate (90-100%) similar to that obtained with Chlorpyrifos-ethyl (Nébié, 2017). The inferiority of the efficacy of *M. anisopliae* as compared with that of Chlorpyriphos-ethyl in the orchard would probably be related to the adverse effects of certain abiotic factors. According to Braga et al. (2001), the exposure to sunlight even for a few hours can completely inactivate the conidia of *M. anisopliae*. Similarly, changes in temperature and relative humidity affect the rate of infection of insects by inhibition of spore germination, which in turn affects the formation of the germinating tube and penetrates through the cuticle of the insect (Fargues and Luz, 2000, Soza-Gomez and Alves, 2000). Kuboka (2013) reported that temperatures below 15 ° C and above 35 ° C are not favorable to fungus activity. Moreover, the mode of action of each insecticide partly explains its effectiveness on the insect. Chlorpyrifos-ethyl acts by contact and ingestion with an immediate effect on insects. *M. anisopliae* also acts by contact and ingestion. The time of death of the insect sensitive to this fungus is 3 to 10 days under normal conditions (Benserradj, 2014). The mode of infection of the fungus is divided into

4 distinct stages: adhesion, germination, penetration and dissemination. Each of these stages depends on the host and the environmental factors.

Comparative effects of *Metarhizium anisopliae* and Chlorpyriphos-ethyl on the 2 parasitoids associated with *Ratrococcus invadens*

Regarding the emergence of *R. invadens'* parasitoids, no significant differences were observed between treatments prior to the first application of the insecticides. This led to a decrease in the emergence and even the total disappearance of parasitoids on trees treated with Chlorpyrifos-ethyl. These results are similar to those reported by Nébié (2011) in the same area and Hala et al. (2013) in Côte d'Ivoire. The toxic effect of Chlorpyriphos-ethyl would probably be the cause of the disappearance of parasitoids on trees treated with this product. Indeed, the parasitoids can be directly reached during the application of the product; which helps to reduce the level of parasitism on the mealybug populations. In addition, newly parasitized mealybugs absorb the product and die prematurely; which reduces the emergence of parasitoids. The level of emergence of parasitoids remained high on the control trees and those treated with the different doses of *M. anisopliae*. In general, entomopathogens are known for their effect compatible with other biological control agents such as parasitoids. Stolz et al. (2002), using different strains of *M. Anisopliae* in the laboratory, reported that this entomopathogen did not have a significant negative impact on *Apoanagyrus lopezi* and *Phanerotoma* sp parasitoids. Similar results were reported with various strains of this fungus on the parasitoid *Prorops nasuta* (Rosa et al., 2000). Some authors have reported that melanization reactions induced by the parasitism process can inhibit entomopathogenic fungus penetration in the insect cuticle (Avery et al., 2008). The parasitized insects before the application of the entomopathogen can thus complete their developmental cycle. Other authors have found that fungi can be inhibited by fungal metabolites produced by the parasitoid on the parasitized host (Blackburn et al., 2002). The effects produced by Chlorpyriphos-ethyl and *M. Anisopliae* on the two parasitoids were similar to those observed on hyperparasitoids. This is the 4^{th} link in the mango-mealybug-parasitoid-hyperparasitoid food chain. Their survival depends on that of the parasitoids within which they develop.

Conclusion

This study made it possible to determine the relative effectiveness of the biopesticide *M. anisopliae* ICIPE 69 on mango mealybug and its impact on its parasitoids in orchards. Three doses of this product were tested and compared to the single dose of Chlorpyriphos-ethyl (480 g / l). Significant to very highly significant differences were recorded between treatments with respect to the mortality rate of *R. invadens* after application of the insecticides. Chlorpyriphos-ethyl was more toxic than the 3 doses of *M. anisopliae*. Indeed, the mortality rate of mealybugs was 2 times higher on trees treated with Chlorpyriphos-ethyl than those treated with *M. anisopliae*. The level of emergence of parasitoids decreased considerably until they disappeared completely on mango trees treated with Chlorpyrifos-ethyl. *M. anisopliae* may constitute an alternative to chemicals in view of its compatibility with the action of parasitoids associated with *R. invadens*. However, in a context of small family farms, as is the case with the majority of mango orchards in Burkina Faso, the cost of this bio pesticide will ultimately be the most decisive factor in its adoption by smallholder farmers.

COMPETING INTERESTS

The protocol was drafted by Karim Nébié. The experimentation was implemented by Karim Nébié who also drafted this manuscript. Souleymane Nacro reviewed carefully the manuscript and translated from French to English. He also selected the journal and coordinated all the submission process. Rémi Dabiré and Lenli Claude Otoibiga reviewed the manuscript.

AUTHORS' CONTRIBUTIONS

All authors contributed to the work and to the preparation of the manuscript.

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