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ORIGINAL RESEARCH ARTICLE

Field efficacy of *Metarhizium acridum* (Hypocreales: Clavicipitaceae) in the control of *Bactrocera dorsalis* (Diptera: Tephritidae) in citrus orchards in Senegal

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Abstract

The effectiveness of the “attract and kill” approach for the management of *Bactrocera dorsalis* in citrus orchards using autoinoculation strategy was evaluated in three locations in Senegal (Sindia, Sébikhotane and Ndoyene), between 2016 and 2018. Attractant Contaminant Traps (ACT) were treated with 0.3 g of *Metarhizium acridum*, and methyl eugenol was then deployed at densities of 25, 50 and 100 ACT to infect the flies. Recovery Traps (RT) containing methyl eugenol and a toxicant, Timaye were used to monitor the *B. dorsalis* population and the contamination rate. Results showed that the rate of contaminated flies increases with the number of ACT, at an average daily rate of contaminated flies of 68.1%, 85.44% and 99.67% at 25, 50, 100 traps, respectively. No contaminated flies were found in the control. The number of flies caught decreased from 21.7, 4.2 and 6.2 flies per day, respectively, for 25, 50, 100 ACTs and control in the first week, to 0.64, 0.71, 0.71 and 99.9 flies per day, respectively, for 25, 50, 100 ACTs and the control. All the flies caught at *M. acridum* treated sites were contaminated. No significant difference between the incidence of fruit damage in the three ACT densities and the control was found in the first week; however, there was a significant difference over time, from 90.0, 96.7 and 83.3% in the first week, to 30, 50 and 46.7% at the 14th week, respectively, for 25, 50 and 100 ACTs. No significant differences were found in the control. This present study demonstrated the efficacy of autoinoculative systems based-*M. acridum* for the management of *B. dorsalis* in citrus orchards in Senegal. This strategy is economical as it uses very little amounts of inoculum with locally made materials.

Keywords Autoinoculation · *Bactrocera dorsalis* · *Metarhizium acridum* · Attractive contaminant trap

Introduction

The horticultural sector is a growing industry in Senegal (FAO 2018), contributing significantly to the Agricultural Gross Domestic Product (GDP) (Konta et al. 2016). It also generates revenues in terms of foreign exchange earnings and employment creation through exports, not only for Senegal but also for the entire region (Binta and Barbier 2015). In Senegal, citrus fruits are mainly grown in the Niayes Zone and Casamance areas and comprise a wide range of fruit crops. Although very dynamic, the sector is permanently threatened by various emerging pests, among which are tephritid fruit flies (Ndiaye et al. 2012). The Tephritidae family comprises of 4000 species belonging to 500 genera, and constitutes one of the most economically significant insect groups (Ekesi et al. 2011). *Ceratitis cosyra* (Walker), *C. fasciventris*, *C. rosa*, *C. anonae*, *C. capitata* were the most prominent species before the arrival of the oriental or Asian invasive fruit fly, *Bactrocera dorsalis* (Hendel), on the continent (Ndiaye et al. 2008, 2012; Vayssières et al. 2012; Ekesi et al. 2016). Initially reported in 2003 in Kenya, *B. dorsalis* spread very rapidly in several countries (Lux et al. 2003; Schutze et al. 2017). In West Africa, losses due to *B. dorsalis* exceed 50% for

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cultivars of commercial interest, resulting in considerable income losses for growers (Vayssières et al. 2014). In addition to direct losses in orchards, fruit flies are responsible for the destruction of many mango export consignments, annually, from West Africa to Europe (De Meyer et al. 2009). In Senegal, crop losses attributable to *B dorsalis* are estimated at 40–60% in the Niayes region, and up to 70–80% in the South in Casamance (Vayssières et al. 2011; Ndiaye et al. 2012). Losses due to fruit flies seriously affect the livelihoods of producers and actors in the sector, including vendors, retailers, negotiators, sellers, exporters, and food processors (Grechi et al. 2013; Maertens 2009). Female fruit flies lay their eggs, in small groups, under the fruit epidermis, and emerging larvae develop in the pulp of the fruit.

Chemical control remains the main control strategy (Badji and Coly 2014). However, the recent introduction of strict quarantine measures and maximum residue limits of pesticides has motivated the search for alternatives to chemical control (Toledo et al. 2006; Deguine et al. 2016).

Fruit fly management includes several steps, ranging from monitoring, mass-trapping, control, to eradication. The Integrated Management Package includes the use of lures and baits, traps, orchard sanitation, an augmentorium, and mass release of parasitoids and biopesticides (Agunloye 1987; Warthen et al. 1998; McQuate et al. 2005; Ekesi 2016; Fombong et al. 2016). There is a growing interest in the use of the entomopathogenic fungi *Metarhizium* sp. in the management of tephritid fruit flies. However, huge quantities are required for blanket spraying, and the spore have limited viability due to UV. One innovative approach to minimize high volumes of inoculum and persistence is the “attract and kill concept”, which combines a male annihilation technique using the parapheromone Methyl eugenol (ME) and biopesticides (Ekesi and Billah 2007) in an autoinoculation approach. Methyl eugenol is a powerful male attractant of plant origin extensively used for trapping several species of fruit flies. The autoinoculation system allows male *B. dorsalis* to enter *Metarhizium* sp. treated traps where they can get infected with conidia of *M. anisopliae* before returning to the environment to disseminate it among mates or during copulation.

Metarhizium spp. has been widely studied because of its relatively narrow host range and environmental safety on non-target pests (Bischoff et al. 2009). It belongs to the class of Hyphomycetes. Some species of *Metarhizium* are effective biological control agents and are prescribed for various insect pests (Fernandes and Biteencourt 2008; Gao et al. 2011; Pattemore et al. 2014). *Metarhizium anisopliae* var. *acridium* IMI 330189 available as Green Muscle® has been shown to induce significantly high mortalities in *B. dorsalis* (between 93.7% to 94.8%) with a lethal time to 50% mortality (LT50) between 2.8 to 3.6 days (Ouna 2010).

The use of the autoinoculation approach was successfully proven by Zakari-Moussa et al. (2012), for the management of fruit flies *B. dorsalis* and *Ceratitis cosyra* (Walker) in the northern part of Guinea-Bissau. However, the effectiveness of such approach in Senegal is unknown.

Therefore, the aims of this study were (i) to demonstrate the field efficacy of various densities of autoinoculation devices-based *M. acridum* for the management of *B. dorsalis* populations in citrus orchards, and (ii) to determine the effect of such management approach on fruit damage.

Material and methods

Study site

The study was conducted in various parts of the country, such as Sebikhotane (14°44′29.3”N, 17°07′34.0”W and 14°44′27.3”N 17°08′24.5”W), Ndoyene (14°45′24.1”N, 17°07′27.0”W) and Sindia (14°33′54.5”N, 17°02′31.5”W) (Fig. 1). Sebikhotane has a savanna climate, with limited annual rainfall (300 mm–600 mm). It is classified as semi-arid, according to the Köppen-Geiger scale (BSh). The temperature average is 26.1 °C throughout the year and the precipitation average is 533 mm (Table 1). The vegetation is composed of citrus and mango trees (CLIMATE-DATA.ORG 2019). Sindia is also characterized by a savanna climate with limited rainfall. The classification of Köppen-Geiger is of semi-arid type (BSh). The temperature average is 25.9 °C throughout the year, and the precipitation average is 531 mm (Table 1). The vegetation is dominant composed of citrus (*Citrus sinensis*, *C. reticulata* et *C. paradisi*) and a few feet of mango trees (CLIMATE-DATA.ORG 2020).

Biopesticides

The entomopathogenic fungi *M. acridum* (IMI 330189) (Bischoff et al. 2009) was sourced from SenBiotech SA and stored at −30 °C. The viability of conidia was determined by spread-plating 0.1 ml of the conidial suspension (5×10^6 conidia mL^{-1}) on Sabouraud Dextrose Agar (SDA) plates. Sterile microscope cover slips were placed on each plate. The plates were incubated at a temperature of 25 °C and examined after 20 h for germination, using an optical microscope. Percentage germination was determined by counting approximately 100 spores for each Petri dish at 200 x magnification. The percentage germination was at 93.6% prior to the experiment.

Trap design and field experiments

Trap design

Two types of traps were used in the trials. Both traps were locally made, one using 0.5-l plastic bottles (24 cm in length), and the other, 1.5-l plastic bottles (32 cm in length).

The Attractant Contaminant Trap (ACT) was painted in yellow and the bottoms of the bottles were cut. The trap contained a

Fig. 1 Map of study orchards

dish made of cardboard rolled with velvet material, onto which 0.3 g of *M. acridum* (1.5×10^{10} conidia mL^{-1}) was applied. Methyl eugenol (4 ml) was applied on a cotton wick and placed at the middle of the tray to attract males of *B. dorsalis* (Fig. 2a). The inoculum was renewed every week, and the Methyl eugenol was renewed every 15 days.

The Recovery Traps (RT) comprised of a bottle with two opposite holes in the upper part. Ten (10) grams of Timaye (Methyl-eugenol + Deltamethrin), rolled in gauze fabric and tied with galvanized steel wire, are introduced into the RT (Fig. 2b). Methyl-eugenol attracts contaminated males *B. dorsalis* from ACTs to the RT, where they are killed by Deltamethrin. The Timaye is renewed every 30 days. Recovery traps are used to monitor fruit fly population and assess the contamination rate. Traps were hung on trees with galvanized steel wire, at a height of 1.5 m from the ground under the canopy.

Field experiments

Four trials were conducted in the field during the rainy seasons of 2016, 2017 and 2018. The first three trials assessed the number of fruit flies contaminated at various densities (25,

Table 1 Annual weather data of Sebikhotane and Sindia

Sebikhotane	January	February	March	April	May	June	July	August	September	October	November	December
Mean T°C	22.4	22.4	23	23.4	24.5	27.1	27.8	27.5	27.9	28.2	26.7	23.8
Min T°C	16.6	16.7	17.3	18.2	19.9	23.1	24.5	24.3	24.4	24.1	21.4	18.4
Max T°C	28.3	28.2	28.8	28.7	29.1	31.1	31.1	30.8	31.4	32.3	31.9	29.3
Rainfall (mm)	0	1	0	0	0	17	78	213	160	36	2	1
Sindia												
Mean T°C	23	23.7	24.7	25.2	26	27.8	27.7	27.2	27.4	27.8	26.7	23.9
Min T°C	15.8	16.5	17.5	18.5	20.1	22.9	23.8	23.6	23.4	22.9	20.2	17.1
Max T°C	30.3	30.9	32	31.9	32	32.7	31.7	30.9	31.5	32.7	33.2	30.7
Rainfall (mm)	2	1	0	0	0	20	93	210	163	39	2	1

Sebikhotane and Ndoyene have similar climate profile

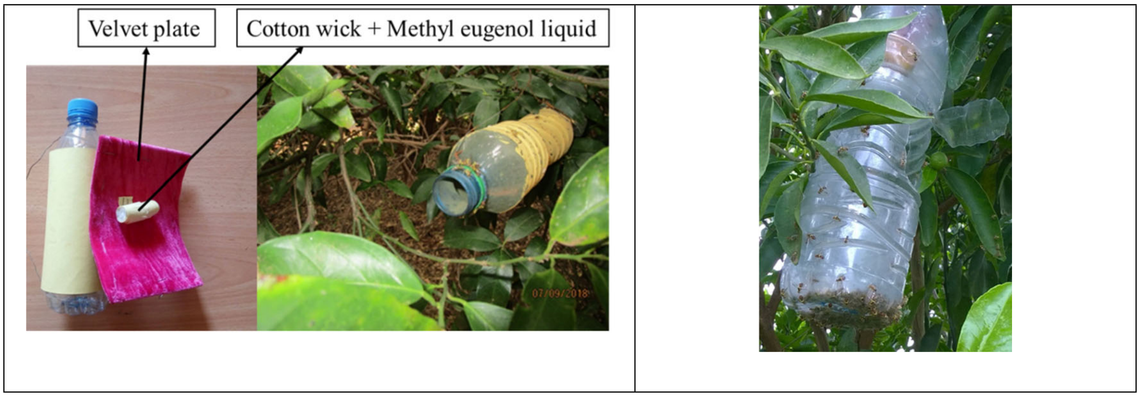


Fig. 2 Attractive contaminant trap (ACT) and Recovery trap (RT). a: Attractive contaminant trap (ACT) on citrus tree, **b**: Recovery traps (RT) of *B. dorsalis*

50 and 100) of the ACT over 12 days. The fourth trial evaluated the effect of ACT densities in the control of *B. dorsalis* population and their impact on fruit damage during three months between July and November 2018.

The first trial was conducted in 2016 (September 19 to October 01, 2016) on a 6-ha citrus orchard field in Sebikhotane. Four blocks of 1 ha each were identified and in each block, 25 ACTs and 10 RT were applied. In another hectare of citrus orchard located at Ndoyene, a control comprising 10 RTs was installed. Males flies of *B. dorsalis* were collected daily in each block. From the flies recovered in each treated block, three batches of 100 flies were randomly selected to determine the rate of contamination. Flies contaminated with *M. acridum* were then counted in each batch, and the average number was used as the contamination rate in the block. The contamination rate was estimated as the proportion of trapped flies that had at least 3 of the 4 following characters that indicated their contamination: a pink-colored cuticle, modified eyes, flat and curved abdomen, and translucent femur (Ekesi et al. 2007; Faye et al. 2017; Toledo et al. 2017). Two other trials were conducted at Sindia in 2017. The first one (August 18 to August 30, 2017) was conducted in a 12.5 ha-field containing citrus orchards with 220 trees per hectare. Four blocks, receiving 50 ACT and 10 RTs per hectare, were identified in the first field. In a 2.5-ha of citrus orchard at Sebikhotane, one hectare was delimited and considered as a control, containing 10 RTs. Flies were collected daily. The second one was conducted in the same field (September 18 to September 30, 2017) with 100 ACT and 10 RTs per hectare for the field test, and 10 RTs in the control. For all three tests, RTs were installed one day after ACTs and monitored daily for 12 days to find out the average daily rate of contaminated flies for each density.

Field trials on the efficacy of three ACT densities

The last experiment was conducted in 2018 (July 27 to November 3) in a citrus orchard in Sindia, which had an area of 12.5 ha, with 220 trees per hectare. A citrus orchard with an area of 2.5 ha, composed mainly of citrus and some mango trees served as a control in Sebikhotane. Seven hundred (700) ACT and 52 RTs were deployed and monitored weekly for three months. The treatments were as follows:

- 25 ACT and 4 RT per hectare;
- 50 ACT and 4 RT per hectare;
- 100 ACT and 4 RT per hectare.

The RTs were situated away from the experimental site and considered as a control treatment. Each trap density was repeated 4 times, and the twelve treated blocks were placed in a completely randomized design (Fig. 3a and b). The 4 RTs in each block carrying ACT were placed centrally, as shown in Fig. 2, to monitor the *B. dorsalis* population. RT handles were oiled to prevent ants from eating caught flies. The flies caught were counted weekly and the contamination rate assessed.

Dynamics of damage on fruits

The infestation rate was assessed at the start, and at the 5th, 9th and 14th weeks of the trial in the plots, with the three separate trap densities. At each site, ten trees with ripening fruits were randomly selected and on each tree, 10 fruits were randomly chosen from the accessible fruits of the tree and inspected for signs of female oviposition. The symptoms observed on fruits included signs of decay, sap flow and sting scars. Pressure was applied with the thumb to release the sap from the already stung fruit. Inspected trees were tagged in red to avoid double counting.

Data analysis

The figure for Daily trapped flies (DTF) was evaluated by using the following formula:

$$DTF = \frac{Weekly\ trapped\ flies}{Trap\ number\ x\ number\ of\ trapping\ days}$$

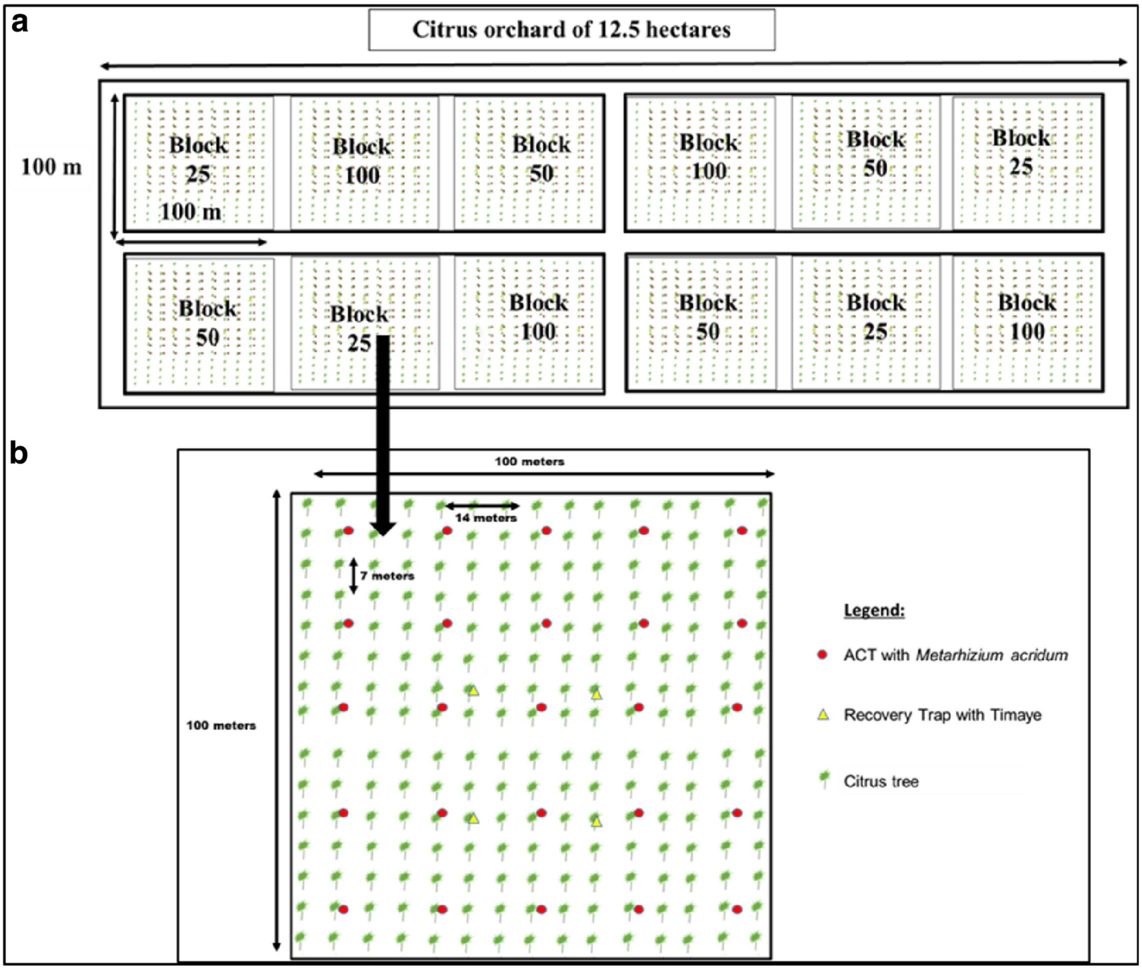


Fig. 3 Experimental design of three densities trial (**a**) and details of one block of 25 ACT density (**b**)

The infestation rate was evaluated at the beginning of the trial, and at 5, 9 and 14 weeks later, from 100 fruits (10 trees sampled and 10 fruits per tree per hectare), for the 25, 50 and 100 ACT densities, as follows:

$$I = \frac{Number\ of\ bitten\ fruits}{Number\ of\ observed\ fruits} x100$$

A One-Way ANOVA was used to compare the daily contamination rate for each density against the control plot. For the field trials on efficacy of 3 ACT densities, the weekly data on captured oriental fruit flies were transformed to number of flies per trap per day. Count data were transformed with $\sqrt{X+1}$ to normalize data and for the homogeneity of variances. The Student-Newman-Keuls (SNK) test, at $\alpha = 5\%$, was used for mean separation. GenStat 9^{éme} Edition software was used for carrying out after the ANOVA (GenStat 2006). A Pearson's correlation test was performed with InfoStat software (2018 version) to determine the influence of ACT density, contamination rate, and time on the dynamics of fruit fly populations (Di Rienzo et al. 2018).

Results

Effect of ACT density of fruit fly contamination

The results of preliminary trials conducted in 2016 and 2017 are given in Table 2. There was a significant variation in the average rate of contaminated flies for each of the three ACT densities and the control (DF = 3; F = 1065.56; $p < 0.001$). The rate of contaminated flies increases with the number of ACT (Fig. 4a and b). The Pearson correlation test, in Table 3, showed a positive correlation (r = 0.92; p < 0,0001) between trap densities and the contamination rate. With 25 ACT, the average daily rate of contaminated flies was 68.1 ± 1.87%, while at 50 ACT, the rate was at 85.44. At 100 ACT, the rate was 99.67 ± 0.180%. No contaminated fly was found in the control during the two years (Table 2).

Daily rates of contaminated flies at 25 ACT varied from 83.5% on the second day to 55.25% on the sixth day. The 0.3 g dose of the fungus was renewed on the sixth day, with the result of an increase in the rate of infected flies to 73% on

Table 2 Number of flies caught and the contamination rates at different ACT densities at 25 (Sebikhotane) and 50 and 100 ACT (Sindia) over 12 days

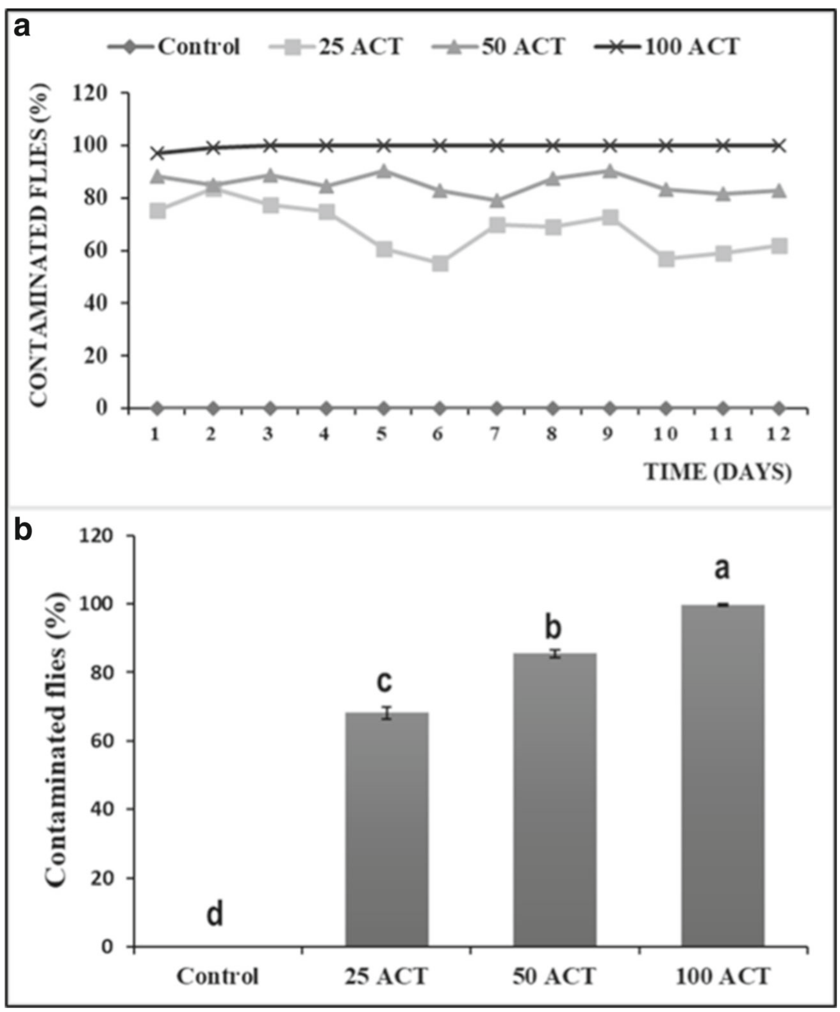
Density of ACT	Year	Caught flies	Contaminated flies	% of contamination	Standard Error
25	2016	23,360	15,908	68.10	1.870
Control 1	2016	9686	0	0	–
50	2017	34,088	29,125	85.44	1.057
Control 2	2017	16,594	0	0	–
100	2017	16,960	16,904	99.67	0.180
Control 3	2017	6365	0	0	–

the ninth day, which decreased to 62% on the twelfth day. The rate of infected flies at 50 ACT varied from 88.6% on the second day to 79% on the sixth day. The rate increased after the renewal of the fungus on the sixth day to 90.4% on the eighth day, and then decreased to 83% on the twelfth day. The rate of infected flies at 100 ACT varied from 99% on the second day to 100% on the sixth day. It did not change after the fungus was renewed on the sixth day, and it was 100% by the twelfth day (Fig. 4b).

Field efficacy for three ACT densities

Evaluation of daily trapped flies

The population dynamics of the *B. dorsalis* in the fungus-treated plot (Sindia) and in the control (Sebikhotane), during August to November 2018, are shown in Fig. 5. The flies trapped per day decreased with the various treatments of ACT, whereas in the control, the DTF increased over a period



(DF = 3; F = 1065.56; p < 0.001)

Fig. 4 Daily rate (**a**) and means (**b**) of contaminated adults of B. dorsalis per density over 12 days at 2016 (Sebikhotane) and 2017 (Sindia) ($DF = 3$; $F = 63.45$; $p < 0.001$)

Table 3 Matrix of correlation coefficients of Pearson and *P* values between population dynamics and the three study factors

Variable 1	Variable 2	n	Pearson	p value
DTF	DTF	48	1	< 0,0001
DTF	Contamination (%)	48	−0,62	< 0,0001
DTF	Density of traps	48	−0,61	< 0,0001
DTF	Time	48	−0,38	0,0082
Contamination (%)	DTF	48	−0,62	< 0,0001
Contamination (%)	Contamination (%)	48	1	< 0,0001
Contamination (%)	Density of traps	48	0,92	< 0,0001
Contamination (%)	Time	48	−0,04	0,7716
Density of traps	DTF	48	-0,61	< 0,0001
Density of traps	Contamination (%)	48	0,92	< 0,0001
Density of traps	Density of traps	48	1	< 0,0001
Density of traps	Time	48	0	> 0,9999
Time	DTF	48	-0,38	0,0082
Time	Contamination (%)	48	-0,04	0,7716
Time	Density of traps	48	0	> 0,9999
Time	Time	48	1	< 0,0001

of 5 weeks, with an DTF of 126.8 ± 4.8 flies. There was a significant positive correlation between density of trap and DTF (r = 0.65; $p < 0.0001$). An ANOVA test showed significant differences in number of flies caught between the control block and the three densities of ACT treated blocks (DF = 3; F = 63.45; $p < 0.001$) (Fig. 5). The first week surveys indicated an average fly daily trapped of 21.71 for the density of 25 ACT, 4.21 ± 4.82 for the density of 50 ACT, 6.21 ± 4.82 for the density of 100 ACTs, and 69.11 ± 4.82 flies for the control. At the sixth week, the DTF for the density of 25 ACTs, was 0.64 ± 4.82 flies, against 0.71 ± 4.82 for the density of 50 ACTs, and 0.71 ± 4.82 flies for the density than 100 ACTs. At the 14th week, the DTF was 1.14 ± 4.82 flies for the density of 25 ACTs, 0.24 ± 4.82 flies for the density of 50 ACTs, and 0.56 ± 4.82 flies for the density than 100 ACTs. All the flies caught on the treated sites were contaminated.

There is no significant difference between treated orchards from week 4 until the end of the trial. In contrast, throughout the experiment, the DTF was significantly higher in the control orchard than in the treated ones. All the flies caught at the treated sites were contaminated. The Pearson correlation test showed no significant correlation (r = −0.12; $p = 0.3602$) between trap catches and the rate of contaminated flies. The same test showed no significant correlation (r = 0.59; $p < 0{,}0001$) between the sites and the number of contaminated flies.

Dynamics of fruits damage

Analysis of variance showed that there was no significant difference between the incidence of fruit damage in the three ACT densities and the control (DF = 3; F = 1.75; $p < 0.174$), with 64.17% for 25 ACTs, 74.17% for 50 ACTs, 69.17% for 100 ACTs, and 92.5% for the control.

However, significant difference of the incidence of fruit fly damage were found over time (DF = 3; F = 24.24; $p < 0.001$). Damage decreased significantly from 90.0%, 96.7%, and 83.3% in the first week, to 30%, 50%, and 46.7% in the 14th week, respectively, for 25, 50 and 100 ACT in the treated orchard (Fig. 6). For the control, there was no significant reduction in the incidence of damage, over time.

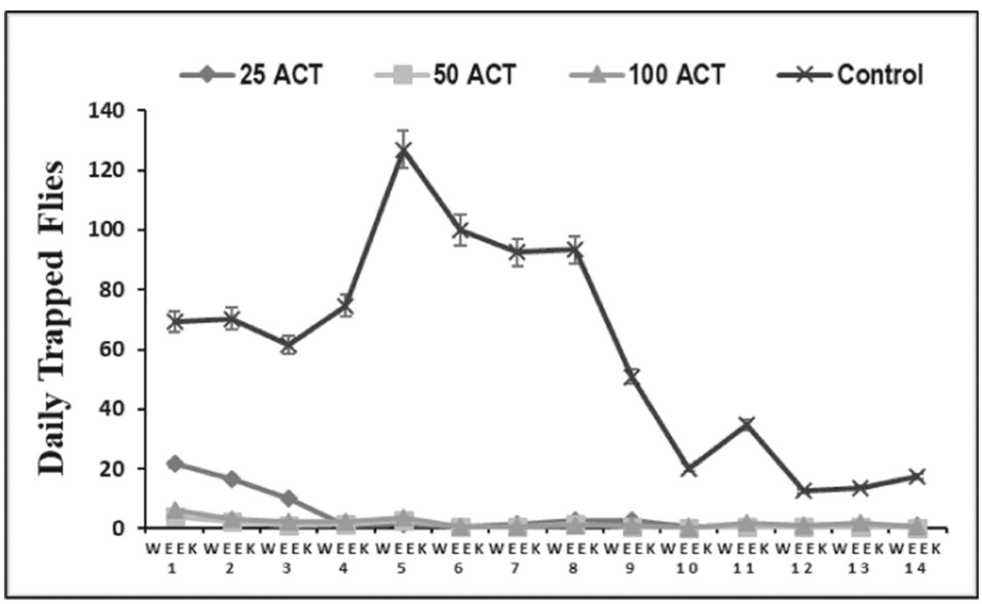


Fig. 5 Population dynamic of *B. dorsalis* according to number of ACT per hectare in 2018 at Sindia (treated orchard) and Sebikhotane (control)

(DF = 3; F = 63.45; p < 0.001)

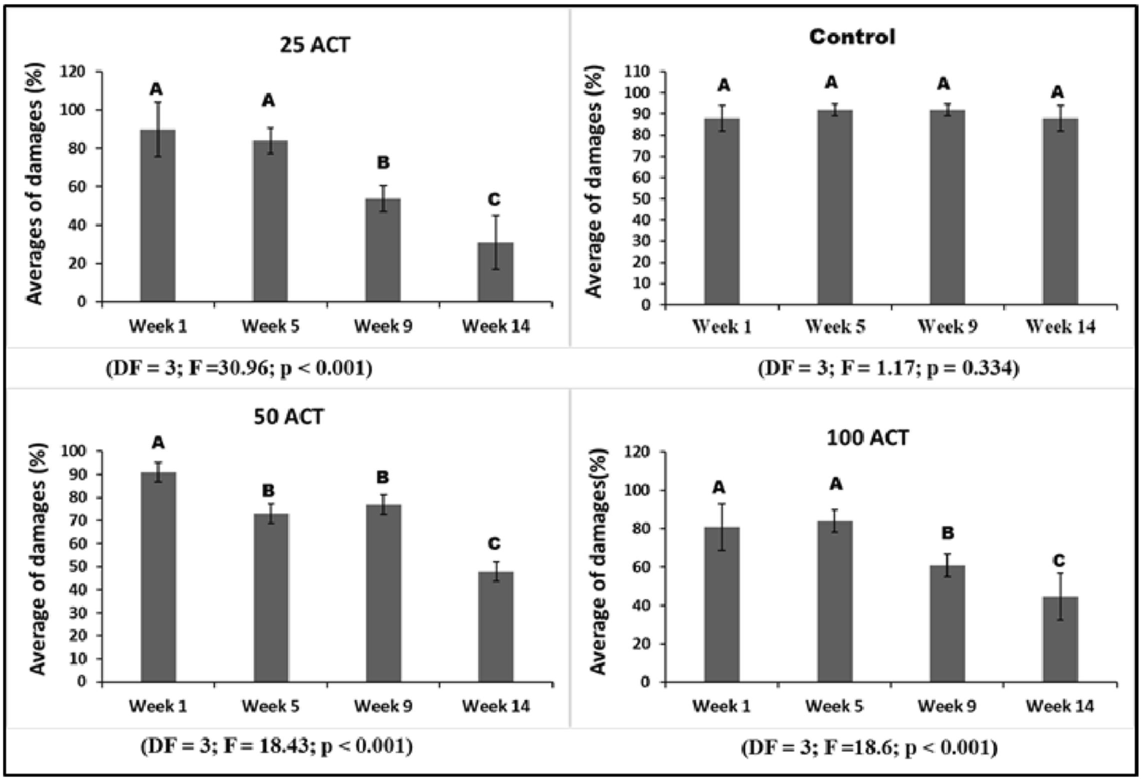


Fig. 6 Evolution of infested fruits rate (±ES) of treated orchards (Sindia) and the control (Sebikhotane) over time in 2018

Discussion

The use of autoinoculation systems is seen as an innovative approach for pest management control especially when combined with semiochemicals and biopesticides. Methyl eugenol is a natural chemical compoundfound in many plants. When ingested by *Bactrocera* males, it acts as a booster to the male sex pheromonal components, thereby increasing their mating success (Ekesi et al. 2016). One of the advantages of entomopathogenic fungi is that they infect their host through direct contact and do not need to be ingested. Results of the current study showed variations in contamination rates with *M. acridum* as a function of the density of ACTs. This implies that male *B. dorsalis* picked infection from ACTs which have caused mortality with minimal dose of 0.3 g of *M. acridum* spores.

The maximum amount of fungus inoculum used in this study was 30 g of spores per hectare for the highest density of ACT. Ekesi and Billah (2007) suggested an application of 80 to 100 g of spores per week of *M. anisopliae* for 100 self-inoculating devices for a mango orchard with 100 trees per hectare. It can be concluded that if appropriate number of traps are used, less inoculum will be required thereby confirming the cost-effectiveness of the lure and kill approach.

There was a significant reduction in *B. dorsalis* population in Sindia, as compared with the control orchard in Sebikhotane. According to the International Atomic Energy Agency (IAEA 2003), an orchard in which DTF <1 is considered to have been the object of a fruit fly suppression/removal. All the three trap densities were effective in the management of *B. dorsalis* in the orchard treated after six weeks at an DTF less than 1 for all densities. This low DTF, could be explained by possible horizontal transmission of *M. acridum* from infected flies to healthy flies. Fungal disease is transmitted by simple contact, or during mating, or by ingestion in ticks that devour their dead infected congeners (Sanjaya et al. 2013). Studies on the horizontal transmission of a fungal infection from infected insects to healthy insects have already been reported in termites, butterflies and houseflies (Daniel and Wyss 2008; Carcamo et al. 2015; Opisa et al. 2019). In the laboratory, Dimbi et al. (2013) and Sookar et al. (2014) showed that contaminated flies, before dying, were capable of transmitting fungal disease to healthy flies, causing mortality between 71 and 100%, at 10 to 15 days after inoculation. Hedström and Monge-Nájera (1998) reported that infected females or males that copulate more than once increase the chances of fungal disease being transferred to healthy populations of fruit flies. In addition to the horizontal transmission of the fungus from an infected male to the healthy female, an infected male can spread the fungal disease through contact with another healthy male during leks (Banelli et al. 2014; Toledo et al. 2017). Additionally, Shelly et al. (2016) demonstrated that males of *Bactrocera* produce and diffuse sex pheromones that invite the receptive females over long distances. Furthermore, in Hawaii, Shelly and Kaneshiro

(1991) observed groupings of males, or leks, in a citrus orchard, which attracted females for mating in the canopy.

Males of *B. dorsalis* that visited the ACTs spent much time there around the cotton wick soaked in methyl eugenol. Haq et al. (2016) demonstrated that a methyl eugenol diet of males of the *B. dorsalis* complex improves their mating competitiveness, thereby increasing their ability to transmit fungal infection to healthy females. San Andrés et al. (2014) demonstrated in the laboratory the effectiveness of the use of ACTs based on *M. anisopliae* against *C. capitata*. Navarro-Llopis et al. (2015) showed that a density of 24 ACTs with *M. anisopliae* was effective against *C. capitata* in a citrus orchard in Valencia, Spain.

There was a density relatedness between ACT traps and population reduction and infection with *M. acridum* resulted in reduced fruit damage. Blocks with the density of 25 ACT have the lowest fruit damage rate, which dropped from 90% to 30%. Fungal infection has been shown to have an effect in infected females by reducing fertility (Dimbi et al. 2013). Orchard sanitation was not applied during our study and the fallen fruits served as breeding grounds for the surviving population of *B. dorsalis*. Re-infestation of the orchard by flies was noted from these fallen citrus fruits. Vargas et al. (2010), Deguine et al. (2013) and Ekesi et al. (2016) showed that sanitation is the foundation of integrated fruit fly control, without which any other control method is effective (Vargas et al. 2010; Deguine et al. 2013; Ekesi et al. 2016).

This study demonstrated the effectiveness of the combination of *M. acridum* treated traps and parapheromone for the dissemination of fungal spores by male of *B. dorsalis*. This technique significantly reduced the fruit fly population, uses minimal amount of fungus inoculum and is made of locally available and affordable materials. The study suggests a more in-depth study on the cost-benefit analysis and the development of strategies for the scaling of this technique as a first-line management strategy during early seasons, in conjunction with other IPM techniques such as orchard sanitation.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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