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Effectiveness of the insecticide BioELIT on *Bactrocera cucurbitae* (Diptera: Tephritidae) in cucumber *Cucumis sativus* (Linné, 1753)

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Abstract

The fruit fly *Bactrocera cucurbitae* (Diptera: Tephritidae) is an important pest of cucurbit fruits. It reduces the quality and yield of cucumber despite the use of chemical pesticides harmful to humans and the environment. Thus, this study was conducted in the Tonkpi region to compare the efficacy of the botanical extract BioELIT® to the chemical pesticides K-OPTIMAL 35 EC® and Cypercal 50 EC® commonly used by farmers. Foliar applications of these three insecticides were made from the 35th day after sowing. Only the control plots were not treated. For each treatment and the untreated, 5 replicates were made. Each elementary plot was two meters long and one meter wide. Sampling of *Bactrocera cucurbitae* adults were made weekly by using pitfall traps and direct observation, starting on the 35th day after planting just before the first foliar applications. From the 45th day after sowing, three visits with an interval of 4 days were made to count fruits attacked by *Bactrocera cucurbitae*. The results showed that the females of *Bactrocera cucurbitae* sting the young cucumber fruits, insert eggs and cause the loss of elaborated sap accumulated in the fruit, the malformation of the fruit, the browning then the necrosis of the fruit tissues. The botanical extract BioELIT® showed similar efficacy to the pesticides K-OPTIMAL 35 EC® and Cypercal 50 EC®. These treatments significantly reduced the number of *Bactrocera cucurbitae* on cucumber. The percentages of reduction of *Bactrocera cucurbitae* infested on cucumber varied between 12% and 70% during the trial. The number of fruits attacked ($F = 27.103$; $p < 0.0001$) and the fruit attack rates ($F = 73.352$; $p < 0.0001$) by *Bactrocera cucurbitae* were statistically identical in the plots treated with the botanical extract BioELIT® and the pesticides K-OPTIMAL 35 EC® and Cypercal 50 EC®. Thus, the botanical extract BioELIT® can replace chemical pesticides in the control of *Bactrocera cucurbitae*.

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Introduction

The fruit fly *Bactrocera cucurbitae* (Coquillet) (Diptera: Tephritidae) is an important pest of Cucurbitaceae and solanaceae fruits in the tropics and subtropics (McQuate and Teruya, 2015; Assi *et al.*, 2017; Shahzadi *et al.*, 2019). It reduced both fruits quality and the yield of cucumber. The insects *B. cucurbitae* cause abundant crop losses (Déclert, 1990; Koyama *et al.*, 2004) and crop losses vary between 30% and 100% despite chemical pesticides application (Dhillon *et al.*, 2005; Shahzadi *et al.*, 2019). In Côte d'Ivoire, farmers use pesticides to protect cucumber *Cucumis sativus* against *B. cucurbitae* (Doumbia and Kwadjo, 2009). However, in Côte d'Ivoire, several studies showed that, the recommended application rates were not respected and only 27% of pesticides used by farmers were registered (Doumbia and Kwadjo, 2009). Indeed, these pesticides used by farmers were persistent and accumulate in water, soil and air but also in food (Baglieri *et al.*, 2011; Horváth *et al.*, 2013). Furthermore, during foliar spraying, a proportion of pesticides always reach bacteria, earthworms, insects and soil fungi. Their toxic effects reduce the activity of the essential fauna for maintaining soil fertility.

In order to guarantee food safety for consumers and to preserve the environment, maximum residue limits for pesticides in food and water must be increasingly low. It is therefore important to provide an alternative solution to the use of pesticides by advocating the use of biopesticides. Thus, in Japan, the population of *B. cucurbitae* has been considerably reduced by the release of sterile insects (Koyama, 1994). In Thailand, parasitoids have been used to the control of *B. cucurbitae* (Ramadan and Messing, 2003). In Hawaii and Taiwan, the biopesticide Spinosad has been used against *B. cucurbitae* as an alternative to organophosphate pesticides that are harmful to humans and the environment (Hsu *et al.*, 2012).

In India, various seed and leaf extracts and plant bulbs significantly reduced *Bactrocera tau* (Walker) egg hatch (Thakur *et al.*, 2012). Thus, this study was conducted in the Tonkpi region to compare the efficacy of the biopesticide BioELIT® with the two chemical pesticides Cypercal 50EC® and K-OPTIMAL 35 EC® commonly used by farmers. The aim of this study is to compare the efficacy of the biopesticide BioELIT® and the two chemical insecticides on the level of infestation and the attack rate of cucumber fruits by *B. cucurbitae*.

Materials and methods

Presentation of the study environment

The cucumber *Cucumis sativus* TOKYO F1 (TECHNISEM, France) plots were established in the experimental plots of the University of Man (7°24'45''North and 7°33'13''West) located in the Tonkpi region (West of Côte d'Ivoire) (Fig. 1). The climate of this region is characterized by two seasons: a rainy season (April to October) and a dry season (November to March). The average annual rainfall is 1.632 mm and the average annual temperature varies around 25 °C (Saley, 2003; Ahoussi *et al.*, 2018).

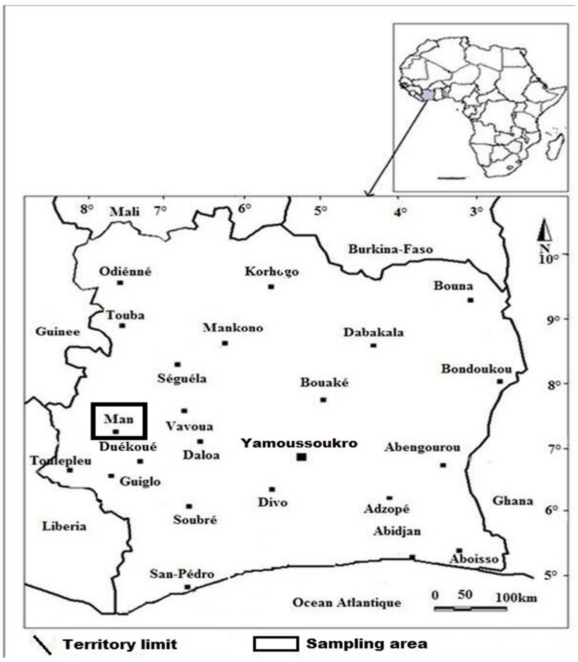


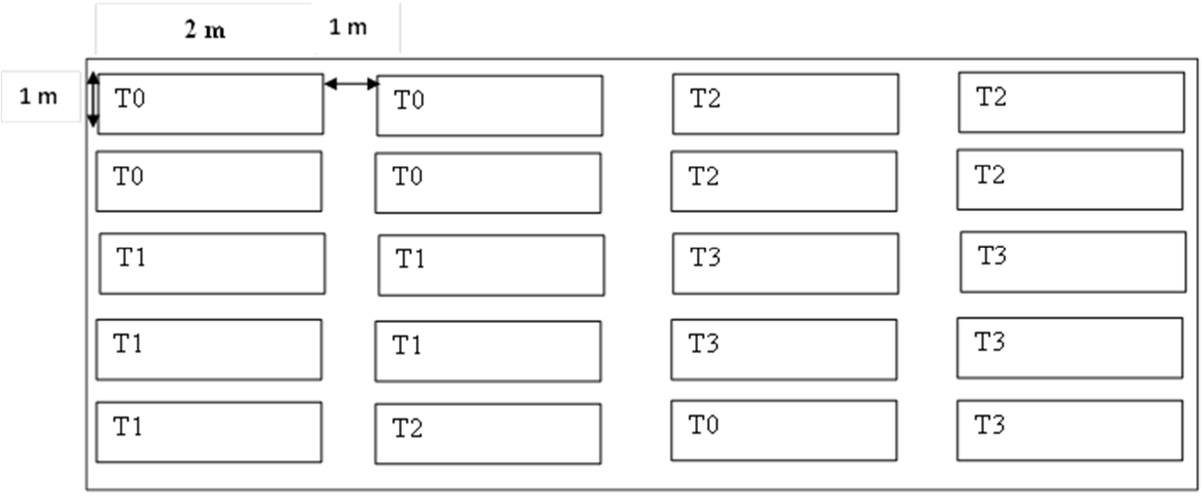
Fig. 1. Location of data collection site (Man, Côte d'Ivoire).

Experimental design

The grains of *C. sativus* were sown on 9th February 2020 on the beds. The experimental designs were five replications and four treatments including the control (untreated plots).

Each elementary plot was a bed measuring 2m on 1m (Fig. 2). Each elementary plot has two lines of *C. sativus* plants spaced to 0.9 m and the distance between the *C. sativus* plants on the line was 0.5m.

After emergence, a disbudding was performed to obtain two plants of *C. sativus* per cluster, i.e. 8 plants per square meter. Rice compost and small quantities of fertilizer (N-P-K) were used.



T0: untreated, T1: BioELIT®, T2: K-OPTIMAL 35 EC®, T3: Cypercal 50 EC®

Fig. 2. Experimental setup.

Evaluation of the efficacy of different treatments on Bactrocera cucurbitae in cucumber plots

Application of the treatments

Foliar applications C. sativus with the biopesticide BioELIT® (Fig. 3) and with the chemical pesticides Cypercal 50 EC® (50 g/L cypermethrin) and K-OPTIMAL 35 EC® (Lambda-cyhalotrin 25 g/L and Acetamiprid 20 g/L) (Fig. 3) were made with the insecticides sprayer 16 L every week from 6 to 8 hours. The first foliar applications of these insecticides were made 35th days after *C. sativus* seeds planting.

Fig. 3. Insecticides BioELIT®, K-OPTIMAL 35 EC® and Cypercal 50 EC®.

The biopesticide BioELIT® is an insecticide based on *Azadirachta indica* and were manufactured by Kabod group located in Côte d'Ivoire. The active ingredients of this biopesticide are azadirachtin, salanin, nimbine and melandriel. It is applied at the rate of 250 mL/16 L of water for spraying 500 m². This biopesticide is a growth inhibitor and has antifeedant properties. The recommended dose of Cypercal 50 EC® was 40 mL in 15 L of water to spray 400 m². However, the recommended dose of K-OPTIMAL 35 EC® was 40 mL of the product diluted in 15 liters of water to spray 400 m². These insecticides BioELIT® and K-OPTIMAL 35 EC® used in this study were acting by contact and by ingestion and Cypercal 50 EC® is acting by contact. Only the control plots were not treated.

Sampling of Bactrocera cucurbitae adults in cucumber plots

Setting of traps or barber traps in cucumber plots

The enumeration of *B. cucurbitae* adults on cucumber plants started from the 35th day after sowing, just before the first treatment. The sampling of these insects was done by setting pitfall traps or Barber traps.

The pitfall traps were set 4 times, 3 days after the treatments at one-week intervals (from day 32[th] to day 53[th] after seeds planting). Three days after the traps were set, the trapped insects were collected. In each elementary plot, two yellow tray traps were set in the soil between the rows of cucumber plants, which constitutes 10 traps per treatment. Each trap is filled with water to two thirds. To this water, soapy liquid is added (breaks the surface tension of the water and thus causes drowning) as well as salt and vinegar at 6° for insects' preservation).

Conservation and identification of insects

The insects collected were grouped by similarity and preserved in 70% alcohol. The identification of the insects was done using a binocular magnifying glass which reveals the distinctive characters used in the dichotomous keys of determination to the orders and families of insects.

Percentage reduction of Bactrocera cucurbitae in cucumber Cucumis sativus plots

The effectiveness of the different treatments were evaluated from the surveys of *B. cucurbitae* adults collected in *C. sativus* plots. From these surveys, reduction rate was calculated using the following formula:

$$\text{Reduction rate}(\%) = \frac{(ICBT-ICAT)*100}{\text{ICBT}}, (1)$$

with:

ICBT= Insects collected before treatment

ICAT= Insects collected after treatment.

Attack rate of Bactrocera cucurbitae on cucumber Cucumis sativus fruits

From the 45th day after sowing, three visits with an interval of 4 days were made to count fruits attacked by *B. cucurbitae* in *C. sativus* plots. In fact, Cucumber *C. sativus* fruits were collected from 1 m² of each elementary plot of each treatment and from the untreated plots. The number of cucumber *C. sativus* fruits attacked by *B. cucurbitae* was counted. The attack rate of cucumber *C. sativus* fruits attacked by *B. cucurbitae* was then calculated by using the following formula:

$$\text{Ar} = \frac{Nfat*100}{\text{Nt}} (2),$$

with:

Ar = Attack rate (%),

Nfat = Number of fruits attacked by *Bactrocera cucurbitae* per treatment,

Nt = Total number of fruits harvested per treatment.

Statistical analysis

The number of *B. cucurbitae* adults collected, the number of cucumber fruits attacked and the attack rate were subjected to an analysis of variance (ANOVA main effect) at the 5% threshold using the IBM SPSS Statistic version 20 software. The obtained means were discriminated with Fisher's test (LSD) at the 5% threshold using XLSTAT 2016 software.

Results

Effectiveness of treatments on the number of Bactrocera cucurbitae adults in cucumber plots

Before the treatments of the insecticides Cypercal 50 EC®, K-OPTIMAL 35 EC® and BioELIT®, the number of *B. cucurbitae* adults were statistically identical at the level of the different plots including the control plots.

This number of *B. cucurbitae* adults varies between 6 and 9 adults of *B. cucurbitae* in the plots (ddl=19; F=1,508; p=0,251). Three days after the first treatment (3DAT1), the insecticides Cypercal 50 EC®, K-OPTIMAL 35 EC® and BioELIT® tested have significantly reduced the number of *B. cucurbitae* adults in cucumber plots compared to the untreated (Control). The botanical extract BioElit® have a similar effectiveness as the pesticides Cypercal 50 EC® and K-OPTIMAL 35 EC® (ddl=19; F=19.707; p <0.0001).

All of these insecticides significantly reduced also the number of *B. cucurbitae* in cucumber plots compared to the untreated (control), three days after the second treatment (3DAT2) (ddl=19; F=25.778; p <0.0001) and three days after the third treatment (3DAT3) (ddl=19; F=10.495; p <0.0001) respectively. There are not significant differences between the pesticides Cypercal 50 EC® and K-OPTIMAL 35 EC® and the botanical extract BioELIT® tested against *B. cucurbitae* adults (Table 1).

Table 1. Mean of *Bactrocera cucurbitae* number on cucumber plots

Treatment	Number of *Bactrocera cucurbitae* ± SE			
	1DBT	3DAT1	3DAT2	3DAT3
Untreated (T0)	6.40 ± 0.894	8.00 b ± 0.707	6.40 b ± 1.342	3.20 b ± 1.483
BioELIT® (T1)	6.60 ± 1.140	3.4 a ± 0.894	2.60 a ± 0.548	1.40 a ± 0.548
K-OPTIMAL® 35 EC (T2)	6.80 ± 2.287	2.60 a ± 1.517	1.67 a ± 1.140	0.60 a ± 0.548
Cypercal® 50 EC (T3)	8.40 ±1.817	2.60 a ± 1.817	1.60 a ± 0.894	0.40 a ± 0.548
ddl	19	19	19	19
F	1.508	19.507	25.778	10.495
P	0.251	<0.0001	<0.0001	<0.0001

SE= Standard error, 3DAT1= Three days after the first treatment, 3DAT2= Three days after the second treatment, 3DAT3= Three days after the third treatment. The means assigned to the same letter within the same column are not significantly different (Fisher test (LSD), P < 5%).

Percentage reduction of Bactrocera cucurbitae adults by the different treatments

The insecticides BioELIT®, K-OPTIMAL 35 EC® and Cypercal 50 EC® have significantly reduced the number of *B. cucurbitae* adults on cucumber plots after the first treatment. The reduction rate of Batrocera cucurbitae adults by the insecticides BioELIT®, K-OPTIMAL 35 EC® and Cypercal 50 EC® on cucumber were 46.143%, 59.722% and 67.045%, respectively. After the second treatment, the percentage reduction of *B. cucurbitae* adults on cucumber was low and ranged from 12 to 26%.

After the third treatment, the reduction rate of *B. cucurbitae* adults by the insecticides used on cucumber foliar applications was higher. The highest reduction rate was made by the pesticides Cypercal 50 EC® and was 70%. This reduction rate was followed by those of the botanical extract BioELIT® (46.667%) which was higher compared to those obtained with the pesticide K-OPTIMAL 35 EC® (43.33%) (Fig. 4).

Effectiveness of the treatments on cucumber fruits attacked by Bactrocera cucurbitae

The treatments have increased the number of cucumber fruits harvested per m² in cucumber plots than the untreated plots (Control) (8.600 fruits). The number of cucumber fruits harvested in *C. cucumis* plots treated with the pesticides K-OPTIMAL 35 EC® (18,800 fruits) and Cypercal 50 EC® (19.400 fruits) gave highest number of cucumber fruits per m² than those of the biopesticides BioELIT® (16.600 fruits) (ddl=19; F=69.887; p <0.0001) (Tab. 2).

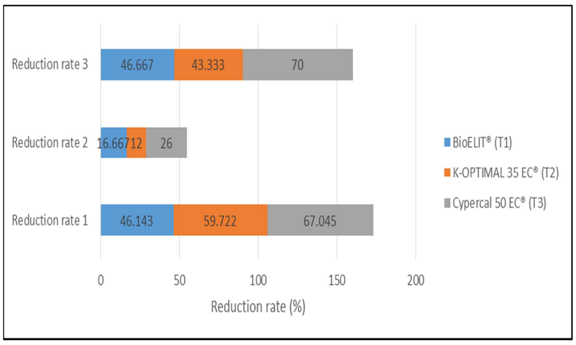


Fig. 4. Reduction rate of *Bactrocera cucurbitae* adults by the treatments in cucumber plots.

The insecticides BioELIT®, K-OPTIMAL 35 EC® and Cypercal 50 EC® have significantly reduced the number of cucumber fruits attacked by *B. cucurbitae* (ddl=19; F= 27.103; p <0.0001) and they were reduced cucumber fruits attack rate (ddl=19; F= 73.352; p <0.0001) compared to the control. Indeed, the insecticides BioELIT®, K-OPTIMAL 35 EC® and Cypercal 50 EC® have similar efficiencies in the number of cucumber fruits attacked by *B. cucurbitae* and have a similar attack rate. However, the number of fruits attacked and the fruit attack rate were moderately higher in the plots treated with the botanical extract BioELIT® (2 fruits; Ar =12.168%) than those treated with the pesticides K-OPTIMAL 35 EC® (1.6 fruits; Ar = 8.310%) and Cypercal 50 EC® (1.4 fruits; Ar =7.174%). The number of cucumber fruits attacked by *B. cucurbitae* and the fruits attack rate wer higher in the control plots and were 5.600 fruits per m² and 65.373%, respectively (Tab. 2).

Bactrocera cucurbitae adult attack on cucumber fruit

Bites of *B. cucurbitae* on young cucumber fruits cause the loss of elaborated sap accumulated in the fruit (Fig. 5A). During the stings, the females of *B. cucurbitae* lay their eggs in the fruits. These bites have also caused cucumber fruits malformation (Fig. 5B).

Moreover, the puncture zones were browning and then the fruit tissues were necrosis (Fig. 5C & D). Thus, *B. cucurbitae* have reduced both the quality and the yield of cucumber fruits.

Table 2. Mean of cucumber fruits harvested, fruits attacked and fruits attack rate by *Bactrocera cucurbitae*

Treatment	Number of fruits harvested/m² ± SE	Number of fruits attacked/m² ± SE	Attack rate (%) ± SE
Untreated (T0)	8.600 c ± 1.140	5.600 b ± 1.140	65.373 b ± 13.079
BioELIT® (T1)	16.600 b ± 1.140	2.000 a ± 0.707	12.168 a ± 4.622
K-OPTIMAL® 35 EC (T2)	18.800 a ± 1.789	1.600 a ± 0.894	8.310 a ± 4.101
Cypercal® 50 EC (T3)	19.400 a ± 1.140	1.400 a ± 0.548	7.174 a ± 2.634
ddl	19	19	19
F	69.887	27.103	73.352
p	<0.0001	<0.0001	<0.0001

SE= Standard error

The means assigned to the same letter within the same column are not significantly different (Fisher test (LSD), $P < 5\%$).

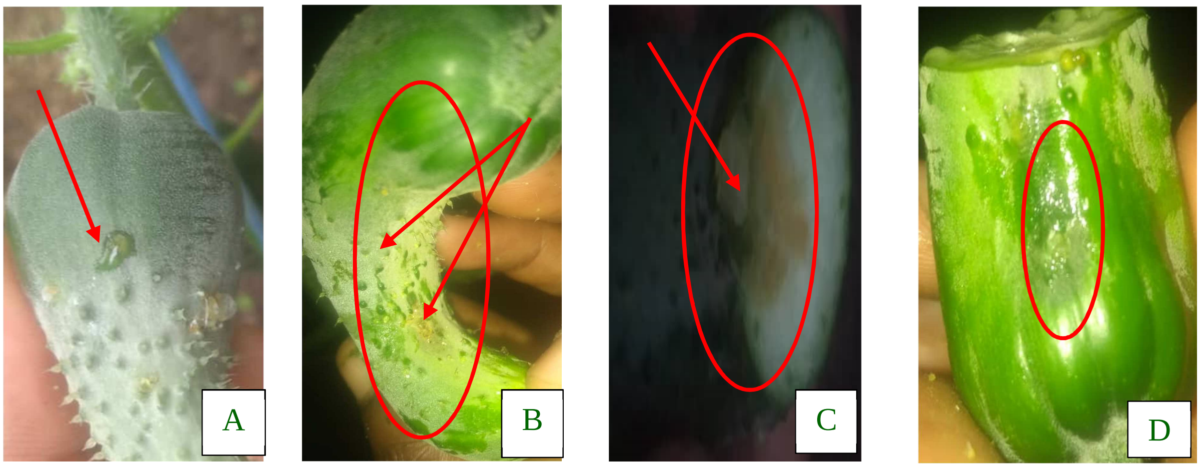


Fig. 5. Damage of *Bactrocera cucurbitae* bites on cucumber fruits (A: loss of elaborated sap accumulated in the fruit, B: malformation of the fruit, C and D: browning and the necrosis of the fruit tissues).

Discussion

Bactrocera cucurbitae female's bite on cucumber young fruits and lays their eggs. At the bite zone of *B. cucurbitae*, there is the loss of the elaborated sap accumulated in the cucumber fruits attacked. The larvae from the eggs consume the tissues of the cucumber fruits and cause the damage of the fruit, the browning then the necrosis of the fruit tissues. This infestation of cucumber fruits by *B. cucurbitae* reduced both the quality and the yield of cucumber fruits. Foliar applications of the insecticide BioELIT® on cucumber plants reduced significantly the number of *B. cucurbitae* on cucumber plants, the number of cucumber fruits attacked and the damage rate of fruit by *B. cucurbitae*. This efficacy of the insecticide BioElit is thought to be related to the fact that this biopesticide inhibits the hatching of *B. cucurbitae* eggs. Indeed, Thakur and Gupta (2012) showed that the insecticide based on *A. indica* prevent the hatching of eggs of insects of the genus *Bactrocera*. This efficacy of the insecticides BioELIT® can be explained by the fact that this insecticide based on *A. indica* contains triterpenoids such as azadirachtin, azadirone, nimbine, and salanine (Islam *et al.*, 2007). These toxic substances have repellent and antifeedant effects that reduce the attack of cucumber fruits by *B. cucurbitae*. According to Diabaté *et al.* (2014a), the extracts based on *A. indica* seeds and their leaves reduced the number of insect pests and their damage on plants (Diabaté *et al.*, 2004 a). These extracts cause mortality of insect larvae and their adults by contact and ingestion (Diabaté *et al.*, 2004 b). According to Aerts and Mordue (1997), most insect groups treated with *A. indica* extracts die during molting and the few survivors show wing and thorax deformities as adults.

According to Islam *et al.* (2007), triterpenoids such as azadirachtin, azadirone, nimbine and salanin in neem *A. indica* extracts give it a high rate of antifeedant. These metabolites of A. indica extracts are thought to act on the chemoreceptors of insects and prevent food intake. According to Mordue and Blackwell (1993), azadirachtin contained in aqueous extracts of *A. indica* acts on chemoreceptors and inhibits food intake.

Conclusion

B. cucurbitae stings on young cucumber fruits cause the loss of elaborated sap accumulated in the fruit, malformation of the fruit, browning and then necrosis of the fruit tissues. The biopesticide BioELIT® and the chemical insecticides K-OPTIMAL 35 EC® and Cypercal 50 EC®.have significantly reduced the number of *B. cucurbitae* in cucumber plots.

The reduction rate of *B. cucurbitae* in cucumber plots were ranged between 46% and 70%. The number of fruits attacked and the fruit attack rates by *B. cucurbitae* were statistically identical in the plots treated with the biopesticide BioELIT® and with those treated with the pesticides K-OPTIMAL 35 EC® and Cypercal 50 EC®. Thus, the biopesticide BioElit can substitute chemical pesticides in the control of *B. cucurbitae*.

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