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Effects of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* on adults of *Coelaenomenodera lameensis* Berti and Mariau, 1999 (Coleoptera: Chrysomelidae) pest of oil palm (Daloa, Côte d'Ivoire)

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Abstract

Coelaenomenodera lameensis is the main pest of oil palm in West Africa, particularly in Côte d'Ivoire. This species is a leaf miner which, by proliferating, causes enormous damage to oil palm. The aim of this study was to evaluate the effect of the entomopathogenic fungi *Metarhizium anisopliae* (Met 358 and Met 359) and *Beauveria bassiana* (Bb 11) on *C. lameensis* adults. Trials were carried out under controlled infestation on an oil palm plot at the University Jean Lorougnon Guédé in Daloa. Male and female adults were captured and introduced into a muslin-covered cage containing leaflets. Each sex was divided into four batches: a 1st batch treated with Met 358, a 2nd batch treated with Met 359, a 3rd batch treated with Bb 11 and a 4th batch of controls. These adults were sprayed, 48 hours later, at the following concentrations 10^2; 10^4; 10^6; 10^8; 10^{10} and 10^{12} spores/ml for each fungal isolate (Met 358, Met 359 and Bb 11). Three replicates were carried out per treatment for each batch containing 40 adult males and 40 adult females. Concentrations of 10^{10} and 10^{12} spores/ml induced mortality rates of up to 100% in less than 7 days with the various fungi. These biopesticides could be an alternative to the abusive use of synthetic insecticides to reduce the damage caused by the pest *C. lameensis*.

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Introduction

Oil palm, grown for its oleaginous fruit in some twenty countries around the world, is the leading source of vegetable oil, accounting for 39% of vegetable oil production and 66% of marketed oils (Rival, 2020). In 2016, the oil palm sector recorded 65 million tons worldwide, 85% of which was supplied by Malaysia and Indonesia (Rival, 2020). In Côte d'Ivoire, the palm oil sector ranks 4th in the economy. It employs over a million people in the southern part of the forest zone and generates over 400 billion CFA francs in sales (D'Avignon, 2013), with production of 450,000 tons of crude palm oil a year (Cucumel, 2020). Côte d'Ivoire is the 2nd largest producer and 1st largest exporter in Africa. It also ranks 5th worldwide (Cucumel, 2020).

Unfortunately, this crop, at all stages of development, is exposed to numerous phytosanitary problems. These include attacks by several pests, the most important of which is *Coelaenomenodera lameensis* Berti et Mariau, 1999 (Anougba, 2022). During severe outbreaks, this insect causes extremely serious damage, leading to a drop in production of up to 30-50% over a period of 2-3 years (Mariau, 2001; Coffi *et al.,* 2012; Tano *et al.,* 2013). Controlling this pest is therefore a necessity.

There are many methods of combating this insect: chemical and biological. Unfortunately, these methods have not yet succeeded in completely eliminating this pest (Kouassi *et al.,* 2020). The massive use of synthetic insecticides creates numerous problems: environmental pollution and consequent human poisoning, the elimination of beneficial insects, the destruction of wildlife and the contamination of groundwater and rivers (Hénault-Ethier, 2015).

It would therefore be interesting to focus on other equally effective control methods that cause fewer ecotoxicological problems, including biological control of insect pests, which is a safe and environmentally friendly alternative to chemicals worldwide (Lacey *et al.,* 2015). This control involves the use of entomopathogenic fungi to control insect pest populations. They are responsible for infections in many insect species (Aby *et al.,* 2022). Among these entomopathogenic fungi, particularly those belonging to the *Metarhizium* and *Beauveria* genera show great promise against insect pests (Mnyone *et al.,* 2009; Lwetoijera *et al.,* 2010; Mnyone *et al.,* 2012).

The aim of this study was to evaluate the effects of the entomopathogenic fungi *Metarhizium anisopliae* (Met 358 and Met 359) and *Beauveria bassiana* (Bb 11) on *C. lameensis* adults.

Materials and methods

Study site

The study was carried out at the University Jean Lorougnon Guédé, located in the department of Daloa, in the Haut-Sassandra region. The University, located northeast of the town of Daloa, extends from latitude 6°54' north to longitude 6°26' west (Fig. 1), over an area of approximately 415 hectares. It is influenced by a humid tropical climate, with rainfall ranging from 1,200 to 1,600 millimeters per year (Coulibaly *et al.,* 2021). Temperature ranged from 25°C to 28°C, with an average of 26.62 ± 1.02°C. Relative humidity ranged from 73 to 84%, with an average of 79.83 ± 4.12%.

Breeding of C. lameensis

Adults (males and females) of *C. lameensis* were used as animal material.

Two types of cylindrical sleeves, made of 0.50 mm white muslin, were made for the tests on these adults. One large (300 cm × 80 cm) for rearing (Fig. 2A) and the other small (100 cm × 80 cm) for treatment (Fig. 2B) of *C. lameensis*. The sleeves were fitted with an opening bordered by adhesive strips to prevent the emergence of insects placed on the leaflets. These insects were captured in the town of Divo and then transferred to the study site in breeding cages for multiplication. Careful and safe control of the breeding was carried out to prevent the insects from escaping.

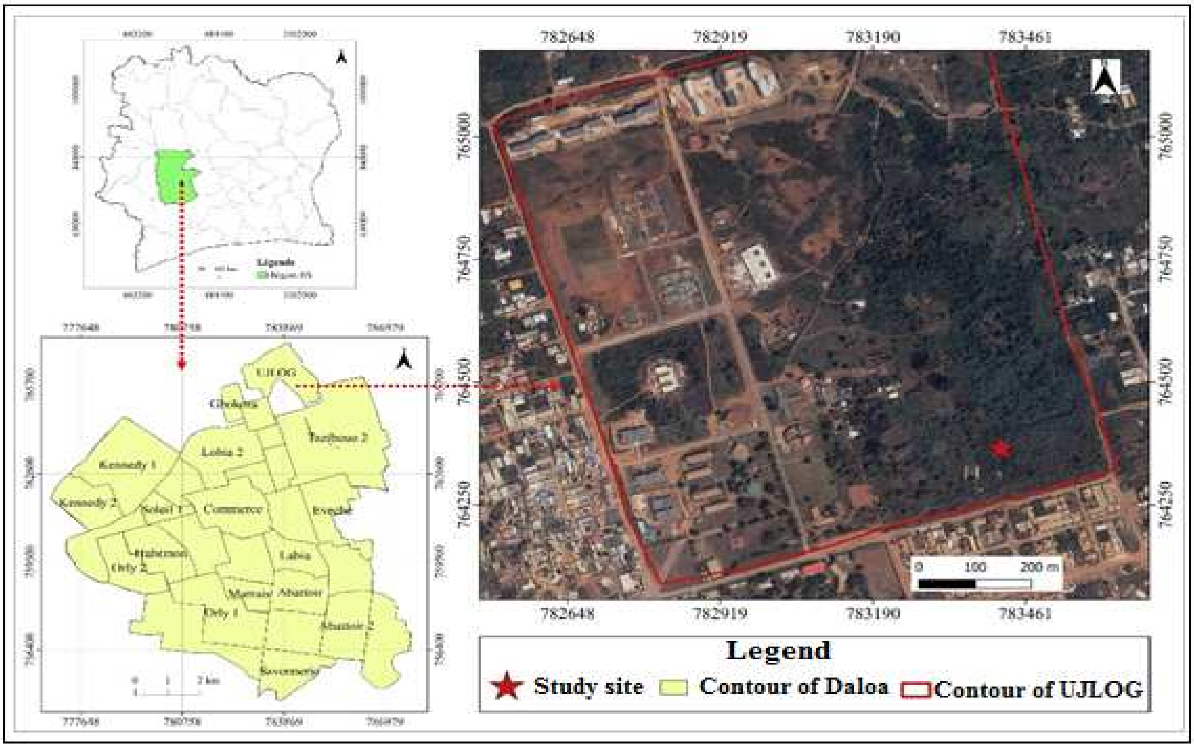

Fig. 1. Map showing the location of the study site.

Using 8 cm-diameter, 10 cm-high cylindrical boxes with lids, adult pairs of *C. lameensis*, including egg-laying females, were placed on leaflets covered with muslin sleeves. The pairs were monitored for 120 days, during which time new individuals were obtained for testing.

Production of entomopathogenic fungi

Two isolates of *M. anisopliae* (Met 358 and Met 359) and one isolate of *B. bassiana* (Bb 11) from the bank of the Institut International d'Agriculture Tropicale du Bénin (IITA-Benin) were used as pathological material.

A quantity of 39 g of Potato Dextrose Agar (PDA) powder was dissolved in 1 l of distilled water in a beaker.

After homogenization in a water bath for 5-10 minutes, the resulting mixture was autoclaved for 15 minutes at a temperature of 120˚C and a pressure of 15 PSI for sterilization. Next, the medium was poured into sterile Petri dishes (diameter= 9cm, height= 1.5 cm) under a laminar chamber. After cooling, a small quantity of conidia from the fungal isolates was removed using a sterilized bacteriological needle and spread over the entire surface of the solidified medium (PDA). Petri dishes were covered with parafilm. Each dish was marked with the isolate name and the date of subculturing.

These Petri dishes were incubated in a photo period of 12 h of light and 12 h of darkness for 21 days.

Inoculum preparation

After 21 days' incubation of the fungi, a few culture fragments were removed and introduced into 9 ml sterile distilled water. After 10 min of agitation, the concentration of the suspension was determined using a Neubauer hematimetric cell. Dilutions were made in distilled water until six concentrations were obtained (10^2, 10^4, 10^6, 10^8, 10^{10} and 10^{12} spores/ml).

Application of entomopathogenic fungi on C. lameensis adults

A 2430 m^2 (54 m × 45 m) plot containing 30 oil palms was used for this trial. Cages containing adult males and females of *C. lameensis* were placed on the palms. Each sex was divided into four batches: a 1st batch treated with Met 358, a 2nd batch treated with

Met 359, a 3rd batch treated with Bb 11 and a 4th batch of controls. Three replicates were carried out per treatment for each batch containing 40 adult males and 40 adult females. Controls were not treated.

For each treatment, mean mortality rates were calculated and corrected using the Abbott (1925) formula.

Mortality was corrected according to Abott's (1925) formula:

$$M = \frac{\text{Number of dead insects}}{\text{Total number of insects}} \times 100$$

$$Mc = \frac{Mo - Mt}{} \times 100$$

$$100 - Mt$$

With MC: corrected mortality; Mo: observed treatment mortality rate and Mt: control mortality rate.

The lethal dose (LD) was determined by the regression model using log probit.

Statistical analysis

Data processing was carried out using the software Statistica version 7.1. An analysis of variance (ANOVA) was used to identify significant differences between the data. The Student-Newman-Keuls test at the 5% threshold was used to classify means into homogeneous groups.

LD_{50} and LD_{90} were determined using Rstudio software version 4.3.2. The regression model used to determine lethal doses is log probit, which allows values to be predicted.

Results

Effect of entomopathogenic fungi on adult males and females of C. lameensis

Effect of M. anisopliae (Met 358) on C. lameensis

Mortality rates ranged from 23.89 ± 0.37% to 100 ± 0% for males and 39.70 ± 7.75% to 100 ± 0% for females during the 15-day control period. In males, the lowest concentration (10^2 spores/ml) failed to cause the death of 50% of the insects. Concentrations of 10^4, 10^6 and 10^8 spores/ml resulted in mortality rates of over 50% (51.35 ± 3.52; 66.38 ± 5.36 and 82.36 ± 6.47%) after 15 days. The highest mortality rates (100 ± 0%) were obtained with concentrations of 10^{10} and 10^{12} spores/ml on day 6 and day 4 (Fig. 3A). The pathogenicity of Met 358 on females after 15 days at concentration 10^2 spores/ml caused a mortality of 39.70 ± 7.75%. Mortality rates increased with the following concentrations: 10^4, 10^6 and 10^8 spores/ml to give 59.38 ± 10.48; 79.31 ± 7.70 and 86.19 ± 7.88% respectively.

Table 1. LD_{50} and LD_{90} values on day 4 after treatment.

					Lower		Upper		X^2	
B.	Isolates	LD	Males	Females	Males	Females	Males	Females	Males	Females
bassiana	Bb 11	LD50	2.46×10^{8}	2.07×10^{8}	1.91×10^{7}	8.81×10^{7}	5.30×10^{9}	5.10×10^{8}		
		LD90	1.96×10^{13}	4.58×10^{12}	3.21×10^{11}	10^{12}	6.74×10^{16}	3.05×10^{13}	14,7	5,11
	Met 358	LD50	7.35×10^{7}	1.31×10^{7}	1.29×10^{6}	9.37×10^{4}	8.25×10^{9}	2.07×10^{9}		
M.		LD90	1.34×10^{12}	3.06×10^{11}	1.09×10^{10}	1.97×10^{9}	1.31×10^{18}	3.08×10^{18}	37,6	45
anisopliae	Met 359	LD50	2.25×10^{7}	10^{7}	1.94×10^{7}	4.57×10^{5}	1.80×10^{5}	5.55×10^{8}		
		LD90	1.73×10^{11}	9.02×10^{10}	2.79×10^{11}	2.41×10^{9}	1.27×10^{9}	6.13×10^{15}	37,6	37,3

The highest mortality rate (100 ± 0%) was obtained with concentrations of 10^{10} and 10^{12} spores/ml on days 6 and 4 (Fig. 3B). Statistical analysis revealed significant differences between mortality rates for the different concentrations (adult males: F= 32; ddl= 6; p=0.000; adult females: F= 16.04; ddl= 6; p=0.000).

Effect of M. anisopliae (Met 359) on C. lameensis

During the 15-day control period, mortality rates ranged from 27.41 ± 2.67% to 100 ± 0% for males and 43.97 ± 2.65% to 100 ± 0% for females. In males, the lowest concentrations (10^2 and 10^4 spores/ml) failed to cause the death of 50% of the insects. Concentrations of 10^6 and 10^8 spores/ml resulted in mortality rates of over 50% for 15 days (64.56 ± 7.92 and 77.36 ± 4.29%, respectively). The highest mortality rates (100 ± 0%) were observed with concentrations of 10^{10} and 10^{12} spores/ml on days 7 and 4 (Fig. 4A). Concerning the pathogenicity of Met 359 on females after 15 days at the concentration of 10^2 spores/ml, a mortality of 43.97 ± 2.65% was observed. Mortality rates increased with the following concentrations: 10^4; 10^6 and 10^8 spores/ml, reaching 50.83 ± 3.26; 69.86 ± 3.55 and 80.97 ± 12.04% respectively. The highest mortality rates (100 ± 0%) were recorded on day 6 at 10^{10} spores/ml and day 4 at 10^{12} spores/ml (Fig. 4B). Statistical analysis revealed significant differences between the mortality rates of the different concentrations (adult males: F= 20,17; ddl= 6 ; p =0,0001; adult females: F= 13,29; ddl= 6 ; p=0,0001).

Fig. 2. Breeding (A) and processing (B) sleeves.

Effect of B. bassiana (Bb 11) on C. lameensis

Over the 15-day control period, mortality rates ranged from 31.84 ± 2.25% to 100 ± 0% for males, and from 31.87 ± 3.60% to 100 ± 0% for females. It's important to note that the lowest concentrations (10^2 and 10^4 spores/ml) in males failed to kill 50% of the insects. In contrast, concentrations of 10^6 and 10^8 spores/ml caused mortality rates in excess of 50% for 15 days, recording 50.45 ± 2.74 and 68.09 ± 5.77% respectively. The highest concentrations (10^{10} and 10^{12} spores/ml) induced mortality rates of 100 ± 0% on days 7 and 5 respectively (Fig. 5A). With regard to the pathogenicity of Bb 11 on females after 15 days, the concentrations that induced mortality rates below 50% were 10^2, 10^4 and 10^6 spores/ml with 31.87 ± 3.60; 39.63 ± 3.52 and 49.98 ± 3.42% respectively. As for the 10^8 spores/ml concentration, it induced a mortality rate of over 50%. The highest concentrations (10^{10} and 10^{12} spores/ml) induced a maximum mortality rate of 100 ± 0% on days 6 and 5 (Fig. 5B). Statistical analyses revealed significant differences between mortality rates for the different concentrations (adult males: F = 18,46; ddl = 6; p = 0,0001; adult females: F = 26,9; ddl = 6; p = 0,0001).

Lethal doses (LD_{50} and LD_{90}) on day 4 after application of Met 358, Met 359 and Bb 11 to adult males and females of C. lameensis

The corresponding LD_{50} and LD_{90} on day 4 after treatment were determined by transforming the corrected mortality percentages into Probits and the doses of entomopathogenic fungi used into the natural logarithm. It took 2.46×10^8 and 2.07×10^8 spores/ml of isolate Bb 11 to kill 50% of male and female *C. lameensis* adults respectively.

In contrast, 7.35×10^7 and 1.31×10^7 spores/ml of isolate Met 358 and 2.25×10^7 and 10^7 spores/ml of isolate Met 359 were required to kill 50% of male and female *C. lameensis* adults respectively (Table 1).

To kill 90% of *C. lameensis* males and females, 1.96×10^{13} and 4.58×10^{12} spores/ml of isolate Bb 11 were required. In contrast, 1.34×10^{12} and 3.06×10^{11} spores/ml of isolate Met 358 and 1.73×10^{11} and 9.02×10^{10} spores/ml of isolate Met 359 were required to kill 90% of adult males and females (Table 1).

Discussion

Moralities induced by entomopathogenic fungi on male and female adults of C. lameensis

Sustainable pest management strategies aim to minimize the economic loss caused by insect pests. In the present study, the entomopathogenic potential of two biological control agents (*Metarhizium anisopliae* and *Beauveria bassiana*) was evaluated against *Coelaenomenadera lameensis* adults. Biological tests showed that the entomopathogenic fungi used can infect and induce the death of *C. lameensis* individuals by contact with. These results confirm those of Nébié *et al.* (2022) and Mureed *et al.* (2023), who showed the efficacy of *M. anisopliae* and *B. bassiana* isolates on the mango mealybug *Rastrococcus invadens* and the red palm weevil *Rhynchophorus ferrugineus*. These results are also in line with those of Hala *et al.* (2018) and Aby *et al.* (2022), who have also shown in their studies the efficacy of the entomopathogenic fungus *M. anisopliae* on *Prosoestus* spp, oil palm pests, and on the banana weevil *Cosmopolites sordidus*. Other authors have also shown the susceptibility of *Helicoverpa armigera* larvae, the okra flea beetle *Podagrica* spp, *Spodoptera frugiperda* larvae and the Senegalese locust *Oedaleus senegalensi* to isolates of *M. anisopliae* and *B. bassiana* (Douro Kpindou *et al.*, 2012; Tounou *et al.*, 2018; Ganha, 2020; Bechiri and Hanachi 2020).

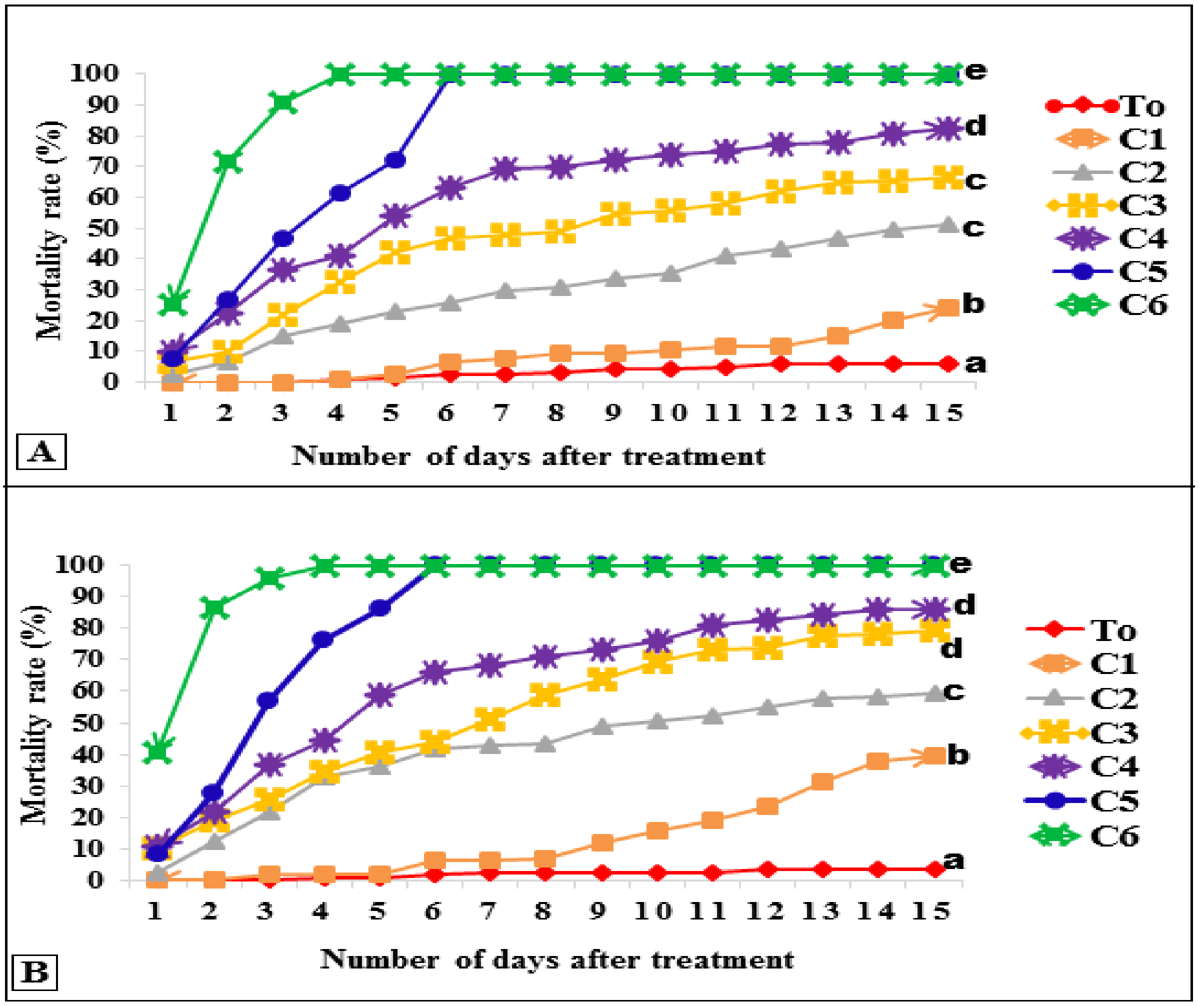

Fig. 3. Variation in mortality of *C. lameensis* males (A) and females (B) after treatment with *M. anisopliae* (Met 358).

For the different entomopathogenic fungi used, mortality rates increased with concentration. This is in line with the work carried out on *Maruca vitrata* larvae by Toffa *et al.* (2014), which states that mortality rates are a function of the concentration applied. Demirci *et al.* (2011) and Mahot *et al.* (2019) have also shown in their work on *Planococcus citri* and *Sahlbergella singularis* that mortality rates increase with inoculum concentration, which is an important factor in the pathogenicity of entomopathogenic fungi.

The quantity of conidia must be high enough to cause insect death (Inglis *et al.,* 2001). Our results also showed that treatment of the different sexes with entomopathogenic fungi had no significant effect.

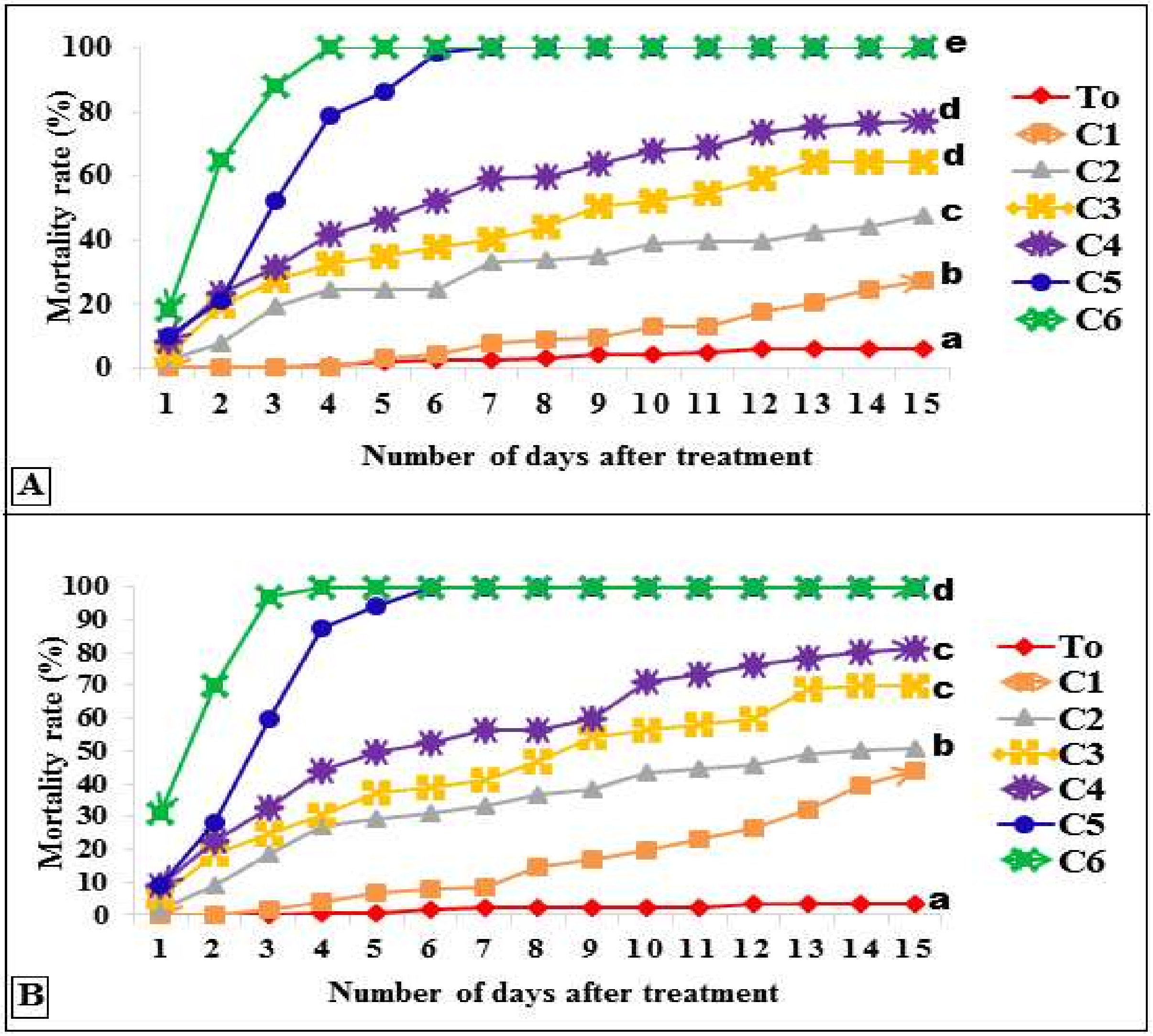

Fig. 4. Variation in mortality of *C. lameensis* males (A) and females (B) after treatment with *M. anisopliae* (Met 359).

Of the six concentrations of each fungus tested, two concentrations, 10^{10} and 10^{12} spores/ml, each achieved 100% mortality. These high mortality rates (100%) testify to the high virulence of these pathogens. These results are in line with those of Valda *et al.* (2003), Benserradj (2014) and Toffa *et al.* (2014). These authors obtained 100% mortality of *Plutella xylostella*, *Culex pipiens* and *Maruca vitrata* larvae with different concentrations of *B. bassiana* and *M. anisopliae* isolates. Similarly, Simarani and Umaru (2020), in their work on *Elasmolomus pallens* peanut seed bugs, showed 100% mortality on day 7 after treatment with *M. anisopliae*. Furthermore, François *et al.* (2016) also reported in their studies that *Metarhizium* sp. and *B. bassiana* induced significant virulence and entomopathogenic potential (100% mortalities) against the aphid *Myzus persicae* after 6 days of treatment. The high and rapid virulence of these fungi is explained by their compatibility with certain compounds found in insects. These compounds are the fatty acids, amino acids and glucosamines found in the insect epicuticle (Shahid *et al.,* 2012). Studies have shown that the elimination of insects by entomopathogenic fungi involves a series of successive steps that can lead to the death of the host depending on its ontogenic or immune response stage.

These stages involve: adhesion of fungal conidia to the insect integument; germination of conidia; degradation of the cuticle to allow penetration and conversion of hyphae into blastospores. The blastospores use nutrients from the host hemocoel and release toxins within the hemolymph.

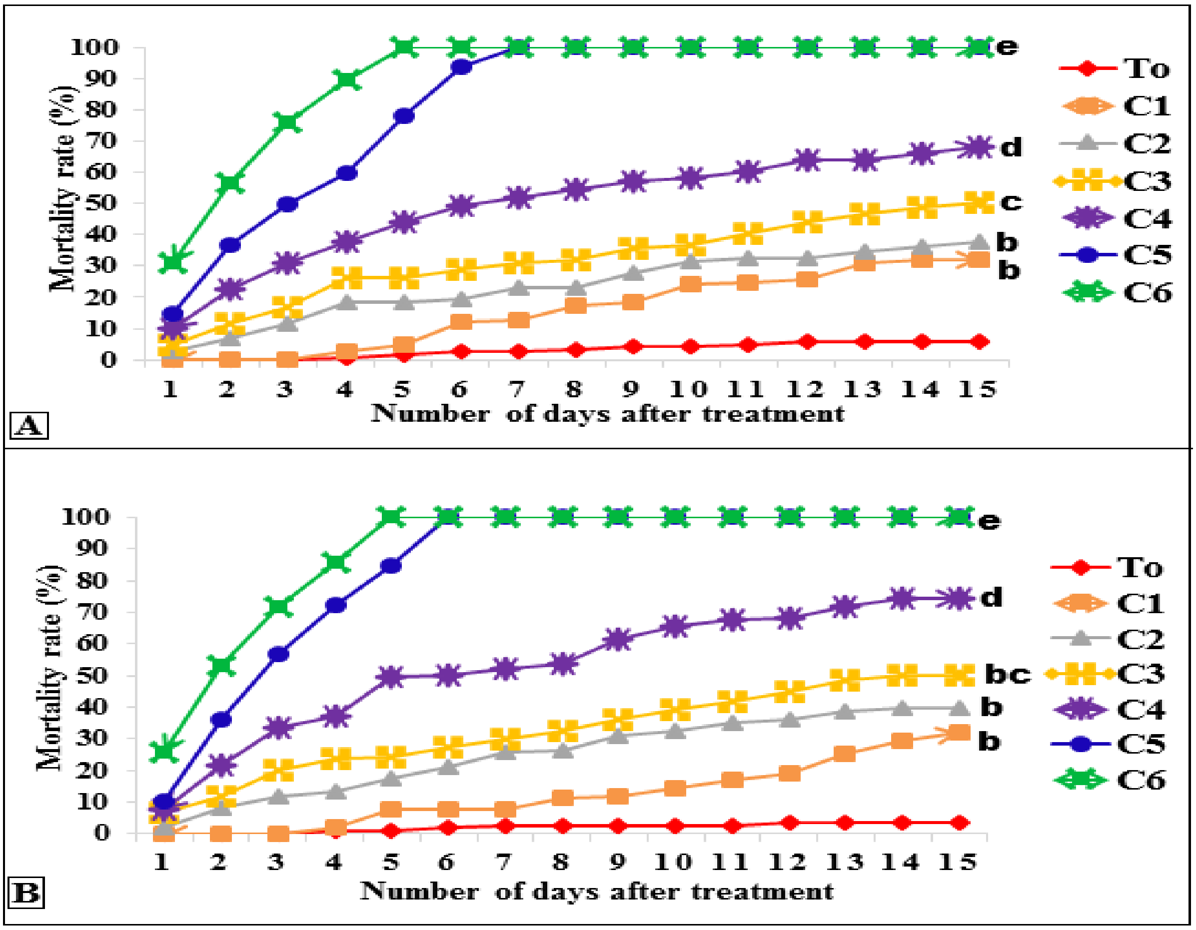

Fig. 5. Variation in mortality of *C. lameensis* males (A) and females (B) after treatment with *Beauveria bassiana* (Bb 11).

The fungus exits the host through openings in the cuticle to produce spores on the surface of the cadaver (Khan *et al.,* 2016).

Lethal doses (LD_{50} and LD_{90}) induced by entomopathogenic fungi on adult males and females of C. lameensis

The ability of a pathogen to cause insect death is essential in the selection of strains of entomopathogenic fungi. A very important parameter in this selection is its virulence (Inglis *et al.,* 2001). The LD50 could be used as a parameter for assessing the virulence of a biological control agent. Sabbahi (2008) also reports that LD50 is used to reveal the insecticidal potential of entomopathogenic fungi. Our work revealed that the lowest LDs (2.25×10^7 and 10^7 spores/ml) causing the death of 50% of adult males and females respectively, were obtained with the Met

359 isolate. Next come the LDs (7.35×10^7 and 1.31×10^7 spores/ml) of isolate Met 358. The highest LDs (2.46.108 and 2.07.108 spores/ml) inducing 50% male and female mortality respectively were obtained with isolate Bb 11. Our work is similar to that of Benserradj and Mihoubi (2014), who showed in their studies that the *M. anisopliae* LK9953010 (M3) fungus strain has an LD50 = 2.3×10^7 spores/ml on day 4 after treatment. Similarly, Narin (2017) revealed in his work on *Dendroctonus simplex* that the dose causing 50% mortality in these insects after 6 days was estimated at 7.4×10^8 spores/ml with *B. bassiana* isolate INRS-242. Ahmed and Freed (2021) also reported that the virulence of *B. bassiana* on *Rhynchophorus ferrugineus* larvae collected in four provinces of Pakistan gave the following LD50s: 1.3×10^7; 1.5×10^7; 5.3×10^7 and 1.02×10^8 spores/ml. With regard to LD90, our results on adult males and females of *C. lameensis* gave respectively 1.96×10^{13} and 4.58×10^{12} spores/ml for isolate Bb 11; 1.34×10^{12} and 3.06×10^{11} spores/ml for isolate Met 358 and 1.73×10^{11} and 9.02×10^{10} spores/ml for isolate Met 359.

These results are not very close to those of Narin (2017), who stated that for a dose of 2.6×10^{10} spores/ml, the fungus can cause the death of 90% of the insect population 6 days after treatment.

Conclusion

Our results showed that the time/concentration response was significant for the different concentrations of entomopathogenic fungi used on male and female *Coelaenomenadera lameensis* adults. Of the 6 concentrations used (10^2; 10^4; 10^6; 10^8; 10^{10} and 10^{12} spores/ml) for each fungus, the 10^{10} and 10^{12} spores/ml concentrations were the most virulent, with mortality rates of 100% on day 4 for 10^{12} spores/ml and 100% on day 6 for 10^{10} spores/ml. The 10^{10} spores/ml concentration could be tested in the field for *C. lameensis* management.

Declaration of interests

The authors declare that they have no conflicts of interest in relation to this article.

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