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EFFICACY OF ISOLATES OF AN ENTOMOPATHOGENIC FUNGUS ON COFFEE BERRY BORER

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ABSTRACT

The coffee berry borer (*Hypothenemus hampei* F.; Coleoptera: Scolytidae) is an important pest of coffee in sub-Saharan Africa (SSA), which causes enormous damage to the crop and yet is difficult to control. The objective of this study was to evaluate the efficacy of *Beauveria bassiana,* an entomopathogenic fungi, on *H. hampei* F. pest under laboratory conditions. Isolates of *B. bassiana* were isolated using the suspension-dilution method on Beauveria Selective Medium (MSB), from Daloa (Cote d'Ivoire) soil sample. Five isolates of *B. bassiana,* namely TE3.211, TE3.222, TE7.311, G.233 and G.232, were tested on coffee berry borer, through two inoculation methods (active and passive). The isolation results showed that 65.51% mortality rates caused by *B. bassiana* varied from 48.48 to 99.66%. Isolate TE7.311 caused a mortality rate of 96.66% for active inoculation of bark beetles and 70% for passive inoculation of cherries. Isolate TE3.211 by active inoculation of bark beetles and isolate G232 by active inoculation of cherries generated mortality rates of 98.33 and 99.66%, respectively. The pathogenicity tests carried out on the bark beetle presented LT50s, which varied from day 2 to day 9 for the passive method. In contrast, as for the active method, TL50 were observed from day 1 to day 2. The results of this study confirm the efficacy of *B. bassiana* as biological control agents against coffee berry borer.

Key Words: Bark beettle, pathogenisty test, TL 50

RESUME

Le scolyte des fruits du caféier (*Hypothenemus hampei* F. ; Coleoptera: Scolytidae) est un ravageur important du caféier en Afrique subsaharienne (SSA), qui cause d'énormes dégâts aux cultures et est difficile à contrôler. L'objectif de cette étude était d'évaluer l'efficacité de *Beauveria bassiana*, un champignon entomopathogène, sur *H. hampei* F. ravageur, dans des conditions de laboratoire. Des isolats de *B. bassiana* ont été isolés par la méthode suspension-dilution sur milieu Sélectif Beauveria

(MSB), à partir d'un échantillon de sol de Daloa (Côte d'Ivoire). Cinq isolats de *B. bassiana,* à savoir TE3.211, TE3.222, TE7.311, G.233 et G.232, ont été testés sur le scolyte des fruits du caféier, par deux méthodes d'inoculation (active et passive). Les résultats de l'isolement ont montré que les taux de mortalité de 65,51 % causés par *B. bassiana* variaient de 48,48 à 99,66 %. L'isolat TE7.311 a provoqué un taux de mortalité de 96,66 % pour l'inoculation active des scolytes et de 70 % pour l'inoculation passive des cerises. L'isolat TE3.211 par inoculation active de scolytes et l'isolat G232 par inoculation active de cerises ont généré des taux de mortalité de 98,33 et 99,66 %, respectivement. Les tests de pathogénicité réalisés sur le scolyte ont présenté des LT50 qui variaient du jour 2 au jour 9 pour la méthode passive. En revanche, comme pour la méthode active, des TL50 ont été observées du jour 1 au jour 2. Les résultats de cette étude confirment l'efficacité de *B. bassiana* comme agents de lutte biologique contre le scolyte des fruits de caféier en Côte d'Ivoire.

Mots Clés : Scolyte, test de pathogénicité, TL 50

INTRODUCTION

Coffee production in sub-Saharan Africa (SSA), is invariably exposed to a milliard of pests, the most devastating of which is currently the coffee berry borer (*H. hampei*). It causes damage at all phases of coffee fruiting (Ohoueu *et al*., 2021), starting with falling of young infested crops; which is sometimes significant and can affect up to 30% of the crop (Ohoueu *et al*., 2021).

The bark beetle's entry galleries in cherries encourage infestation by bacteria (*Erwinia* sp.) and fungi (*Aspergillus* sp., *Fusarium* sp. and *Penicillium* sp.), which can be harmful to human health. The presence of mycotoxins in particular is frequently observed (Ohoueu *et al*., 2022). *Hypothenemus hampei* larvae continue to evolve in cherries from harvesting, during machining, as long as living conditions are favourable for them.

A coffee crop which contains borer is fragile, leading to a significant loss of yield during hulling; with production losses varying from 20 to 80% (Vega *et al*., 2015; Ohoueu *et al*., 2022). Several insecticides routinely used to control the pest have various limitations within the growers' environment. Moreover, use of chemical insecticides is increasingly discouraged to avoid their harmful effects on the entomofauna of natural enemies of *H. hampei*; as well as to safeguard the health of the consumers of finished products (Ohoueu *et al.*, 2022). Therefore, it is imperative to orientate the pest fight towards methods more specific to environments and the health of consumers.

Biological control methods are among the most preferred options for controlling this pest. Entomopathogenic fungi, such as *Beauveria bassiana* and *Metarhizium* sp. are commonly used for this purpose (Ohoueu *et al*., 2022). *Beauveria bassiana* has already shown its effectiveness in combating bark beetles in Hawaii (Kawabata *et al*., 2017) and Puerto Rico (Mariño *et al*., 2017). This is also the case for *Metarhizium anisopliae*; which is also known as an entomopathogenic fungus against several other pests. The objective of this study was to evaluate the efficacy of *Beauveria bassiana,* an entomopathogenic fungus against the coffee berry borer.

MATERIAL S AND METHODS

Study site. The study was carried out in the entomology laboratory of the National Agronomic Research Center (CNRA) of Anguededou in Côte d'Ivoire. This station is located at 2 kilometers on northern highway, between 4° and 5° west meridian and 5° and 6° parallel precisely on the northwest outskirts. The temperature in laboratory ranged from 24 to 28 °C and relative humidity from 60 to 70%; conditions similar to the ambient conditions of the study area.

Biological material. The biological material included soil samples, symptomatic coffee cherries, healthy coffee cherries, coffee berry borer, and the entomo-pathogenic fungus *Beauveria bassiana*.

Coffee berry borer. The coffee berry used for this study were collected from the field of coffee trees and then transferred into sterile jars to avoid fungal contamination, before transporting them to the laboratory for storage at room temperature (about 24°C). All adult coffee berry borers used during this study were bred in Divo laboratory.

Isolation of *Beauveria bassiana.* Fungi *B. bassiana* were isolated from soil samples collected from three coffee plantations, namely Gboli, Tcheglologuhe and Gbolitapia, all in Daloa, Cote d'Ivoire. The choice of localities was based on the intensity of coffee berry borer populations.

The samples were taken separately from the first five centimeters of the top soil. A sufficient quantity of soil was taken from the 10 to 15 cm horizon depth; then deposited using a spatula on an aluminum sterile sheet, after removing stones and plant debris. Each sample was collected in a sterile bag, then transported for refrigeration at 4 °C.

Isolation of *Beauveria bassiana* from soil. Soil suspensions were prepared using 10 g of each sample, well sieved and diluted in 100 ml of sterile distilled water; and finally homogenised for ten minutes using a mechanical shaker. This suspension after filtering, represented the stock solution from which further dilutions were prepared up to 10^{-4}. Then 0.1 ml of each solution of a sample was spread on the surface of the Beauveria Selective Mediums, and labelled with a code designating its origin and its degree of dilution on the container boxes. The boxes were incubated at 28 °C, in the dark and fungi were examined daily for 3 days.

Purification and conservation of isolated strains. The fungi were media transplanted on PDA (Potato Dextrose Agar) comprising of the following: 5 g of Agar, 5 g of D-glucose, 5 g of mashed potatoes, 0.6 g of chloramphenicol, and 02 g of 'citric acid. The medium was autoclaved for 30 minutes and then shaken with a stirrer before being distributed into the Petri dishes. These dishes were incubated at 28 °C in the dark and observed daily by carrying out successive and repetitions until pure strains were obtained, after confirmation using microscopic examination.

Identification of isolated strains. Identification of the fungal strains was essentially using cultural characterisation (macroscopic identification) and morphological (microscopic) identification. Growth and development were evaluated after 4, 8, 12 and 16 days of incubation at 28 °C; using unaided eyes.

Macroscopic examination. Macroscopic observation was done using unaided eyes to determine the colour of the colony during development. This observation reveals the identity of the strains and the colour of aerial mycelium and its variation over time, and the colour of dish coating (Boukhalfa, 2018)

Microscopic identification. The observation of the morphology of the spore chains and the mycelium was done by lightly pressing a piece of adhesive tape on the strain. The manipulation was carried out under a flow hood to avoid pure strain contamination. The tape containing the strain was glued to a sterile slide and examined on an optical microscope using X10 and X40 magnification.

Coffee berry borer breeding and preparation of spore suspensions. The coffee berry borer populations used in this study were bred at Divo breeding centre in

Côte d'Ivoire, using the Ohoueu *et al*. (2022) method.

Mycelial fragments of *Beauveria* spp. from 15 days old on PDA medium were introduced separately into 10 ml of sterile distilled water. The set up was then adjusted to the concentration of 10^7, to which 2 drops of Tween 80 reagent were added to allow maximum release of spores; while maintaining the concentration of the entomopathogenic solution at 10^7 conidia per millilitre. The solution was then evaluated using a hematometric cell (Malassez cell) (Boukhalfa, 2018).

Study design and treatments. The treatment factors included *Beauveria bassiana* isolate (Absolute control, TE3.211, TE3.211, TE7.311, G.233 and G.232). The petri dish containing 10 healthy ripe cherries and 10 adult bark beetles represented the experimental unit.

Inoculation procedure. Two inoculation methods were used, namely passive and active inoculation method. The passive method was carried out on fungal colonies in petri dishes; while the active inoculation, was done with a sporal solution. Thirty coffee berry borers were used per test, and these were replicated thrice per isolate.

Passive inoculation. Passive inoculation consisted initially of infested coffee berry borer on fungal colonies contained in the petri dish for 5 minutes, then transferring them to a petri dish containing healthy cherries. These coffee berry borers were previously soaked in bleach for 3 minutes and rinsed twice with sterile distilled water. Thereafter, the bark beetles were placed in petri dishes containing healthy cherries. The same process was repeated with infested cherries and healthy coffee berry borers. The experimental unit, and the petri dish, contained 10 ripe cherries and 10 coffee berry borers.

A randomised complete block design with three repetitions was set up for infested cherries and for infested coffee berry borers. Coffee berry borer mortality was determined daily for 15 days after the start of the experiment. Mortality was recorded using the Aby *et al.* (2010) procedure.

Active inoculation. As for active inoculation, 10 ml of the sporal solution with a concentration of 10^7 conidia per ml, was sprayed on coffee berry borers in petri dishes containing healthy cherries. These coffee berry borers were previously soaked in bleach for 3 minutes and rinsed twice with sterile distilled water. Thereafter, the coffee berry borers were placed in petri dishes containing healthy cherries. The process was repeated with infected cherries and healthy coffee berry borers.

The petri dish which represented the experimental unit, contained 10 ripe cherries and 10 adult coffee berry borers. A randomised complete block design, with three repetitions, was set up for infested cherries and for infested coffee berry borers. Coffee berry borer mortality was determined daily for 15 days after the start of the experiment.

Data collection

Coffee berry borer mortality rate. The mortality rate was estimated according to the Abbott (1925) formula as recommended by the FAO and WHO insecticide tests.

$$Mc = \frac{Mo - Me}{100 - Me} \times 100 \quad \text{..... Equation 1}$$

Where:

Mc = Corrected mortality (%);
Mo = Mortality observed in treated lots (%) ; and
M = Mean of mortalities from distilled water controls.

Lethal time for 50 (LT50) and 90% (TL90). Projections of TL50 and TL90 were made on

the mortality rates curves developed according to the days of observation graphically against the LT50 and 90.

Sporulations on insects. Dead coffee berry borer populations were removed from Petri dishes and disinfected individually with sodium hypochlorite (1%), for 30 seconds. Then, they were put in Petri dishes containing moistened filter paper and then incubated in the dark. The mycelial down rate was calculated and compared to the mortality rate; and the fungus taken from the insect was identified under an optical microscope.

Data analysis. The data collected were analysed using R.2.0 software. The Kruskal Wallis test was allowed to compare the mortality rate of bark beetles according to the isolates of *B. bassiana* fungi. In the event of a significant difference, the U-Man Whitney test was used to classify the homogeneous groups.

The ANOVA test was used to compare the inoculation methods according to the mortality rates of the fungi tested. In the cases of significant differences, the Fischer LSD test was used to classify the homogeneous groups.

RESULTS

Isolation. In total, 58 mushroom isolates were obtained in the three sites studied, including 24 from Tchebloguhé, 33 from Gboli and one from Gbolitopia (Fig. 1). These were distributed in 38 of the genus *Beauveria* spp. and 20 from the genus Penicillium. The isolation rate of *B. bassiana* was 65.51%; while that of *Penicillium* spp. was 34.48%.

Effect of *Beauveria bassiana* on the coffee berry borer. All strains were pathogenic against the coffee berry borer, with a mortality rate ranging from 41 to 99% (Table 1). Mode of inoculation caused a significant difference between mortality rates. Also, there was a significant difference between the mortality rate due to control and due to the five isolates for passive and active inoculations. At the level of passive inoculation, the TE7.311 strain was the most pathogenic with a mortality rate of 96.66%; while in terms of active inoculation of bark beetle populations, the TE3.211 strain was the highest mortality rate (98.33%). For the active inoculation of cherries, G232 strain had the highest mortality rate (99.66%) and

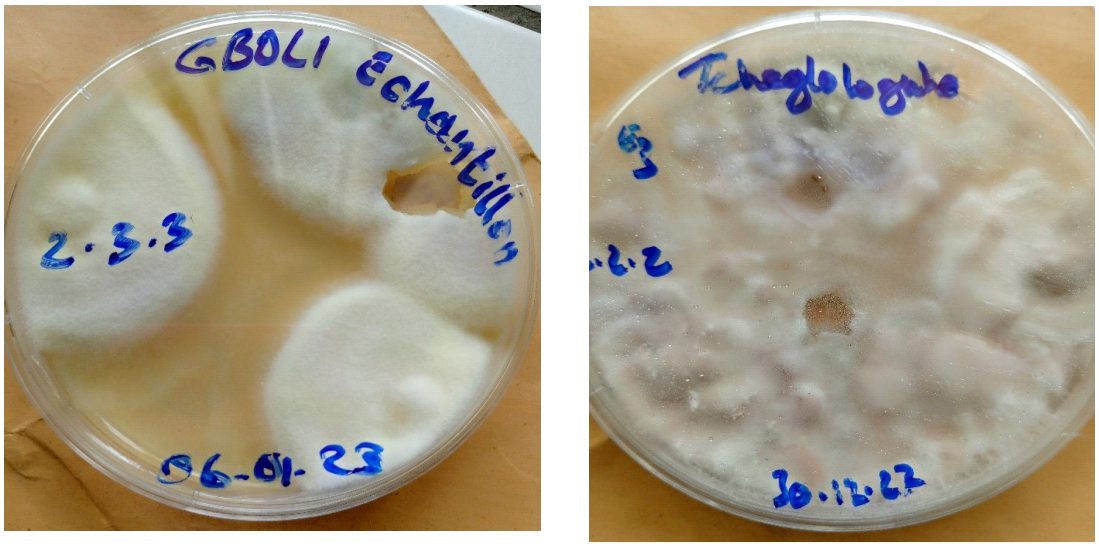

Figure 1. Fungus *Beauveria bassiana.*

TABLE 1. Mortality rate of beetles bark caused by the fungi *Beauveria bassiana* depending on the inoculation method

Mortality rate (%)	Souches	Inoculation method			
		Passive		Active	
Genres		Bettle bark	Cherry	Bettle bark	Cherry
Beauveria	TE3.222	64,81 ± 10,58 b	41,48 ± 1,48 c	89,62 ± 5,78 ab	93,33 ± 3, 33 a
	TE3.211	86,66 ± 3,33 a	54,44 ± 10,94 b	98,33 ± 1,66 a	85 ± 3 ab
	TE7.311	96,66 ± 6,66 a	70 ± 15,27 a	96,66 ± 3,33 a	73,33 ± 8,81 b
	G233	63,33 ± 8,81 b	41,48 ± 5,96 c	96,67 ± 3,33 a	85,55 ± 9,87 a
	G232	60 ± 10 b	60 ± 15,28 ab	76,66 ± 6,66 b	99,66 ± 0,33 a
Absolute control	AC	10,34 ± 3,99 c	12± 3,94 d	10,18 ± 2,57c	10,03 ± 1,32c
	H	10,25	10,21	11,90	11,25
	p	0,042	0, 039	0,036	0,047
		P>0,05	P<0,05	P<0,05	P<0,05

In each column, the values assigned the same letter are statistically identical at the 5% threshold

presented a significant difference with the mortality rate of the TE3.211 strain (73.33%). Mortality rate of bark beetles based on the method of inoculation

There was a significant difference between the two inoculation methods (Fig. 2). Active inoculation had the highest mortality rates; 87.19% for active bark beetle inoculation, and 81.20% for active cherry inoculation. The lowest mortality rate was obtained with the passive cherry inoculation method (53.17%) (Fig. 2).

Lethal times for 50 and 90% by passive inoculation. Passive inoculation of bark beetles made it possible to observe TL50 on five isolates (G232, G232, TE3.211, TE3.222 and TE7.311). Isolate TE7.311 reached its LT50 at 2.5 days, followed by isolates TE3.211, TE7.311, TE3.222 and G233 (Fig. 3). On the other hand, TL90 was not reached during this inoculation.

TL50 of mushrooms by passive inoculation of cherries. Data for TL50 for the three isolates (G232, TE3.211 and TE7.311) under passive inoculation of cherries are presented in Figure 4. Isolate G232 reached LT50 at 4 days; followed by isolates TE7.311 and TE3.211, which reached LT50 at 7 and 9 days, respectively. In contrast, TL90 was not reached by this inoculation (Fig. 4).

TL50 and90 of fungi by active inoculation of bark beetles. Data for TL50 on the five isolates on the 1st day under active inoculation of cherries are shown in Figure 5. A total of four isolates (G233, TE3.211, TE3.222 and TE7.311) reached TL90. Isolate G233 recorded a TL90 at 1.8 days, followed by isolates TE7.311 and TE3.211, which reached TL90 on the 2nd day and isolate TE3.222 reached its TL90 on the 3rd day (Fig. 5).

TL50 and TL90 of mushrooms by active inoculation of cherries. Active inoculation of cherries reached TL50 on the five isolates on the Day 1 (Fig. 6). Three isolates (G232, TE3.211 and TE3.222) attained TL90. Isolate G232 was the first to record TL90 at 2 days after; followed by isolates TE3.211 and TE3.222, which respectively reached TL90 on the 4th and 5th day after (Fig. 6).

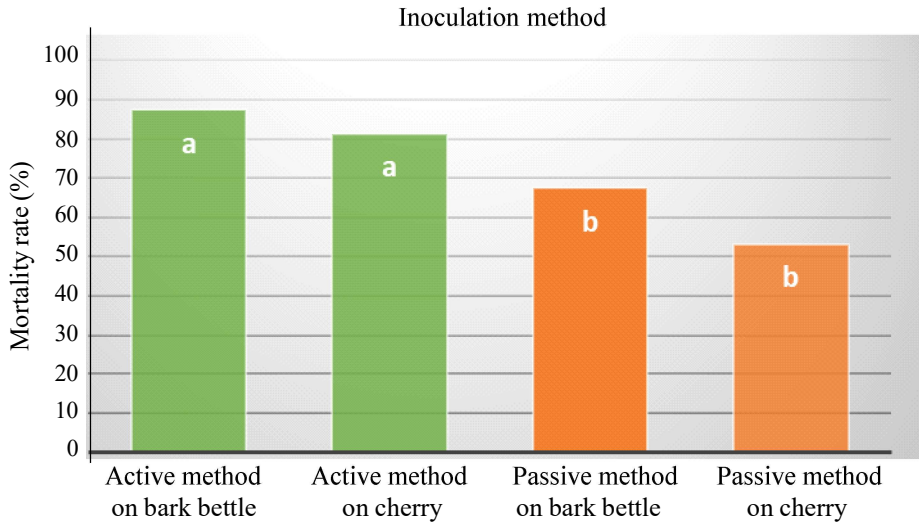

Figure 2. Bark beetle mortality rate depending on inoculation methods. a and b: represents homogeneous groups.

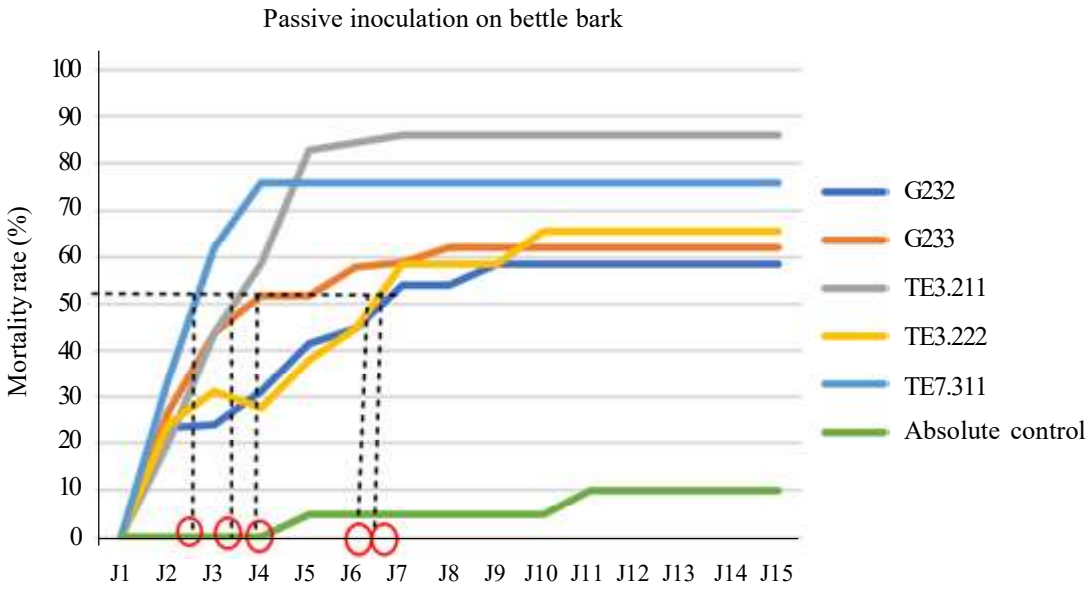

Figure 3. Bark beetle mortality rate after passive inoculation on bark beetle.

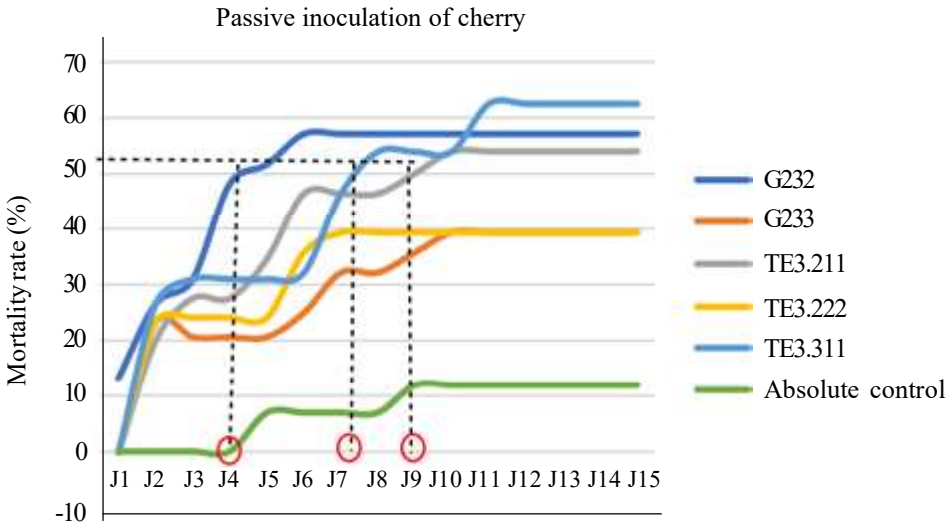

Figure 4. Bark beetle mortality rate after passive inoculation of cherries.

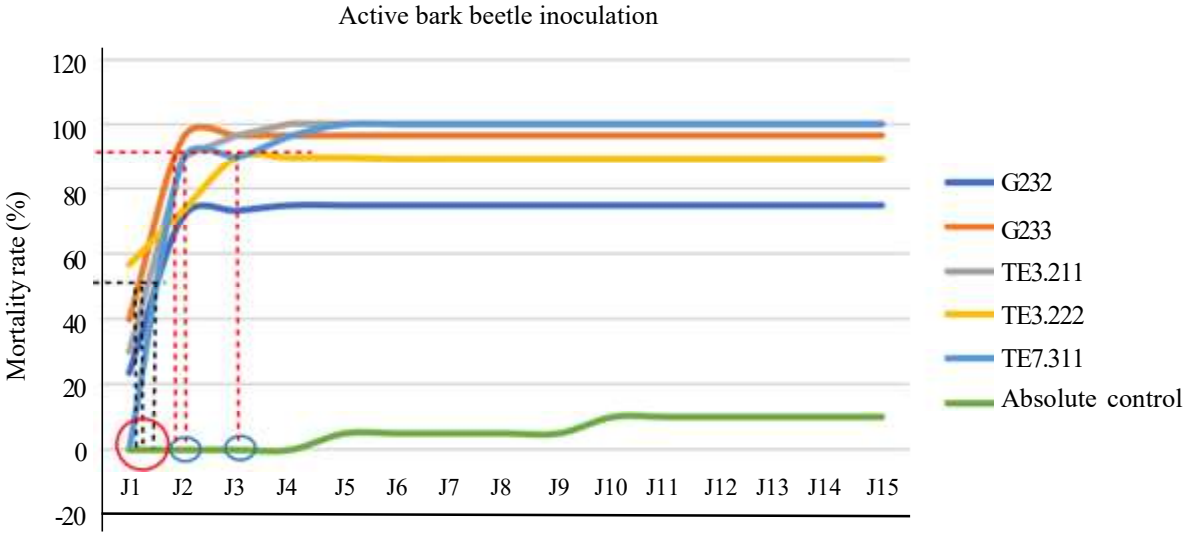

Figure 5. Bark beetle mortality rate after active bark beetle inoculation.

Sporulation of dead bark beetles. The rates of dead bark beetles showing sporulations (fluff) of the fungi tested were all lower than the mortality rates previously obtained (Fig. 7). Populations of dead bark beetles in the presence of distilled water (absolute control) did not show any sporulation (fluff) of *B. bassiana*.

DISCUSSION

Isolation. The presence of a total of 58 mushroom isolates in the samples of the three sites (Table 1), implies that the isolation process was successful. These isolates were distributed in 38 of the genus *Beauveria* spp. and 20 from the genus Penicillium. The isolation rate of *B.*

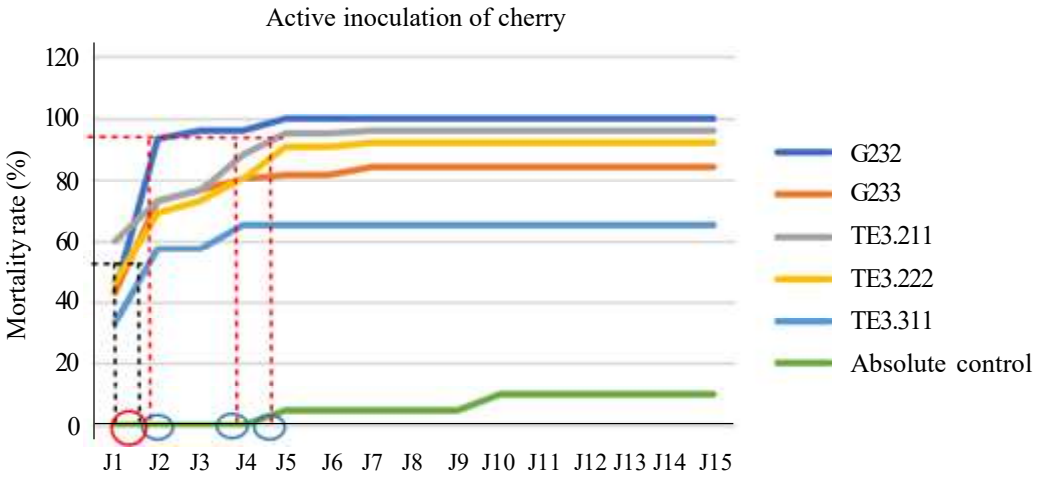

Figure 6. Mortality rate of bark beetles after active inoculation of cherries.

bassiana was 65.51%; while that of *Penicillium* spp. was 34.48% (Fig. 1). These results are consistent with those of Boubacar *et al*. (2009), who carried out isolations from soils under cocoa trees and showed that these soils constitute preferred sites for indigenous microorganisms.

The high isolation rate of *B. bassiana* confirms the selectivity carried out at the medium level (MSB). This selectivity could be explained by the possible presence of nutrients (vitamins, antibiotics) favourable to *B. bassiana*'s development.

This is the first study on *B. bassiana* isolation in this locality (Daloa). It is imperative that actual validation of the present laboratory results is done in the fields under natural conditions and on a larger scale.

Efficacy of *Beauveria bassiana*. The pathogenicity of all five fungal strains against the coffee berry borer, with a mortality rate ranging from 41 to 99% (Table 1), could be explained by the mode of infection of the fungus, which is by direct contact with the insect when conditions are favourable for their development. This infection continues through a mechanism of recognition and compatibility of the spores with the cells of the insect's integument, followed by the production of certain enzymatic compounds such as lipases, chitinases and proteases and also pressures; mechanisms, which favour the penetration of fungus (Aby *et al*., 2010).

All of these factors play a major role in host penetration. Onofre *et al*. (1999) stated that secondary metabolites are produced by many fungi, for example *B. bassiana*, which produces a range of diverse chemical secondary metabolites such as beauvericin, bassianolides, bassianin, tenellin and cyclosperin. These metabolites promote infection of the hosts.

The significant difference between the mortality rate due to the control and those of the five isolates for passive and active inoculations (Fig. 2), is a clear confirmation of the efficacy of the isolates for control of the pest. The superiority of the TE7.311 strain with a mortality rate of 96.66%, at the level of passive inoculation signifies use these isolates to control coffee berry borer in field (Aby, 2010). Similarly, in terms of active inoculation of bark beetle populations, the superiority of TE3.211 strain attaining the highest mortality rate (98.33%), also implies that active inoculation of *B. bassiana* is efficacious on coffee berry borer and could be used in field.

For the active inoculation of cherries, G232 strain had the highest mortality rate (99.66%)

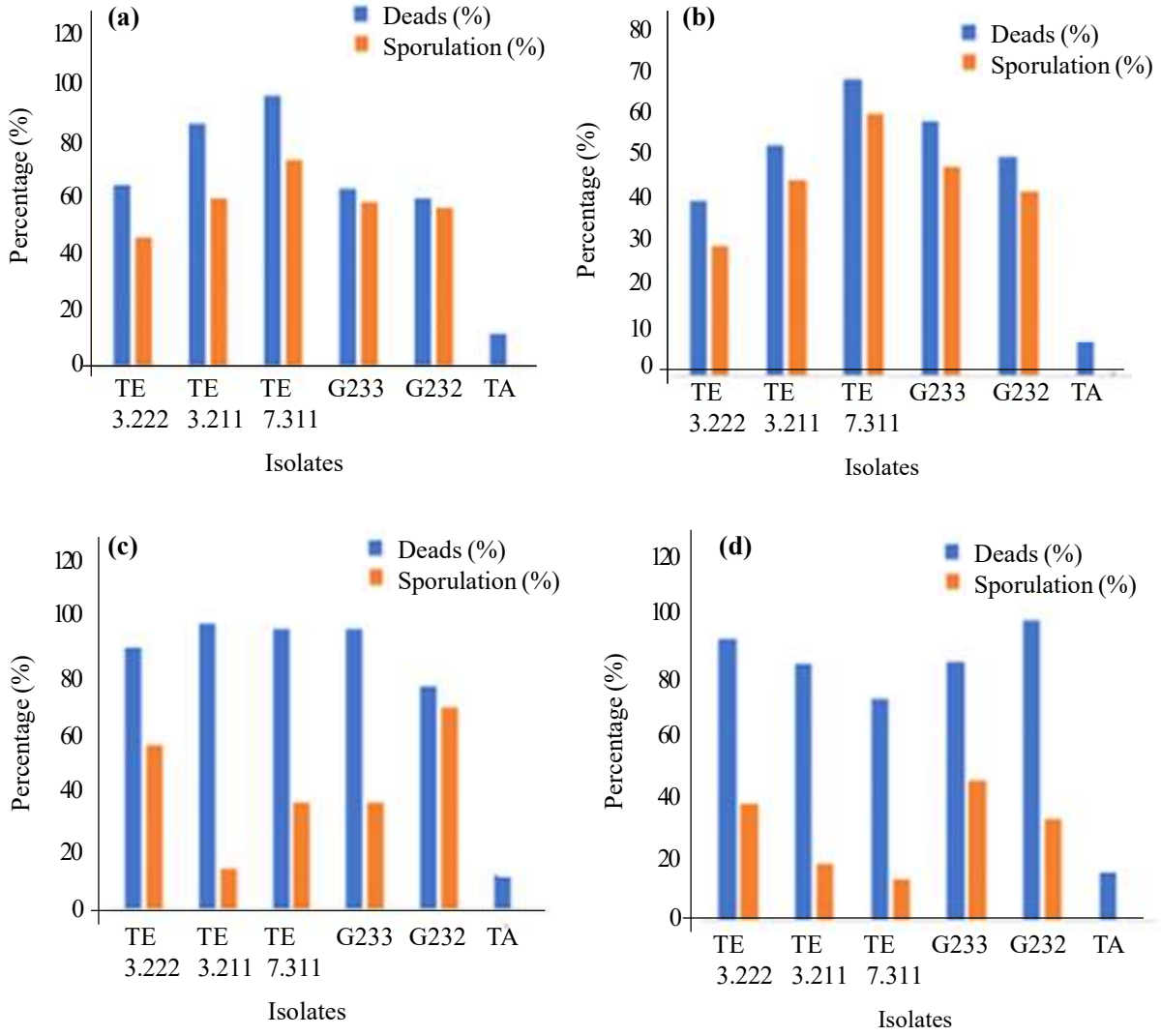

Figure 7. Mortality and sporulation rates depending on *B. bassiana* strains.

and presented a significant difference with the mortality rate of the TE3.211 strain (73.33%). From these results, it can be concluded that passive and active inoculation of *B. bassiana* are efficacious; although the latter inoculation was the best. This conclusion is provisional pending field verification to obtain the actual picture under natural conditions.

The control of the survival of adult coffee berry borer populations by *B. bassiana*, irrespective of active or passive inoculation (Fig. 2), is not entirely new. However, the active method (sporal solution) caused a greater mortality rate than its passive counterpart (working on fungal colonies). The superiority of effectiveness was attributed to the time taken by the spores to adhere to the cuticle of the insect and degrade it (Aby *et al.*, 2010).

As for the passive method, the spores probably needed more time to germinate and release the enzymes necessary for the degradation of the insect's homocoel. Zouleika (2021) carried out similar work with the spore solution of *B. bassiana,* but obtained lower

results (46.33%) with aphids during pathogenicity tests, compared to the present work on the coffee berry borer.

From these results, therefore, it can be concluded that our *B. bassiana* isolates are more virulent. However, this conclusion pends further field validation under natural conditions; otherwise, the active inoculation method is potentially more efficacious for the control of the pest under laboratory conditions.

Lethal times for 50 and 90%. The TL50 and TL90 results showed virulence of *B. bassiana* isolates on treated coffee berry borer. This could be due to the rapidity of these isolates in causing death of the insect when conditions are favourable for their development. TL is the best for assessment of the efficacy of *B. bassiana* isolates on coffee berry borers. These results show lethal times higher than those of Aby *et al*. (2010), who showed LT50, 6 to 10 days and LT90, 17 to 22 days after *Metarhizium anisopliae* infection on banana weevils under similar laboratory conditions. In light of these results, it could provisionally be concluded that *B. bassiana* isolates can kill quickly 90% of coffee berry borer when it is used in normal condition.

Sporulation of dead bark beetles. The occurrence of sporulations (fluff) of the *B. bassiana* fungi after 15 days of conditioning the dead coffee berry borer (Fig. 6), confirmed that the mortalities observed were linked to the action of this fungus. However, the sporulation rates recorded were all lower than the mortality rates. Thus, the death of coffee berry borer could be due to other actions of these fungi or to the temperature and humidity which may not favour the development of the spores ingested or present on the cuticle of coffee berry borer.

The results of the present study conformed with those of Ouorou *et al*. (2010), who compared mortality rates and sporulation rates of *Helicoverpa amigera* corpses. They obtained significantly higher sporulation rates at a dose of 10^7 conidia per insect; and attributed their results to the weight, age and dose applied to insect. For field tests we can vary the concentration of conidia to increase coffea berry borer mortality and sporulation rate.

CONCLUSION

The search for entomopathogenic fungi effective against the coffee beetle made it possible to isolate 38 strains of the fungus of *B. bassiana*, 5 of which were tested during this study. The tests showed that the fungi tested were all pathogenic on adult coffee berry borers *in vitro*, regardless of inoculation method. IsolateTE7.311 was the most effective by the passive method, while isolate TE3.211 was more pathogenic by active method on cherry; and isolate G232 by active method on coffee berry borer. Isolates of *B. bassiana* from Côte d'Ivoire were proven to be effective in this study. Their TL50 and TL90 showed aggressive insecticide potential against the coffee berry borer. However, inoculation by the active method remains the most effective. A field inoculation test would confirm these results obtained in the laboratory.

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REFERENCES

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Enomologyt* 18:265-267.

Aby, N., Kobenan, k., Kehe, M., gnonhouri, P., kone, D. and Zouzou, M. 2010. *Metarhizium anisopliae*: Parasite du charançon noir du bananier cosmopolites

sordidus dans les bananeraies ivoiriennes. *Journal of Animal et Plant Sciences* 7(1): 729-741.

Kawabata, A.M., Nakamoto, S.T., Curtiss, R.T., Shriner, S. and Aristiza´bal, L.F. 2017. Recommandations for CBB Integrated Pest Management in Hawaii. UH-CTAHR. 41pp.

Mariño, Y. A., Vega, V.J., Garcýa, J.M., Verle Rodrigues, J.C., Garcý´a, N.M. and Bayman, P. 2017. The coffee berry borer (Coleoptera: Curculionidae) in Puerto Rico. Distribution infestation, and population per fruit. *Journal of Insect Science* 17:1-8.

Ohoueu, E.J.B., Diabaté, D., Adja, N.A., Aïdara, S., Amoa, A.J., Légnaté, H., Keli, J. and Bouet, A. 2021. Variation saisonnière des populations du scolyte des fruits de caféiers (*Hypothenemus hampei)* dans les zones de production d'Abengourou en Côte d'Ivoire. *Journal of Applied Biosciences* 161:16632-16641.

Ohoueu, E.J.B., Bouet A., Amoa A.J., Beugré, D.I., Sery, D.J-M., Legnate, H. and Wandan, E.N. 2022. Effect of aqueous extracts of *Azadirachta indica* a. juss, *Jatropha curcas* l. and *Moringa oleifera* lam. on coffee berry borer (*Hypothenemus hampei* f.; coleoptera: scolytidae) in laboratory. *International Journal of Biological and Chemical Sciences* 16:2289-2301.

Vega, F.E., Infante, F. and Johnson, A.J. 2015. The genus hypotenemus, with emphases on *H. hampei,* the coffee berry borer. Biology and ecology of native species. London, UK: Elsevier/Academic Press. pp. 427-494.

Zouleikha, H. 2021. Les champignons entomopathogènes et leur utilisation en lutte biologique. Mycologie et Biotechnologie Fongique Master, Mycologie et biotechnologie fongique, Université des Frères Mentouri Constantine Faculté des Sciences de la Nature et de la Vie, Constantine, Algérie. pp. 28-32.