Biocontrol of *Spodoptera frugiperda* using indigenous entomophagous fungi from Côte d'Ivoire

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Introduction

The fall armyworm (*Spodoptera frugiperda*; *Lepidoptera*: *Noctuidae*), a pest native to the Americas, was first reported in Côte d'Ivoire in 2017 and is now infesting 50 African countries. It feeds on more than 353 plant species, including many food crops, but especially maize, causing losses of up to 100% (Fig. 1). Chemical control has proven ineffective against this pest. In addition, its frequent use leads to problems such as the development of resistance within pest populations and contamination of the environment and food supplies. Finding effective alternative control methods is an absolute necessity. The objectives of this work were to verify the virulence on *S. frugiperda* in semi–*in vitro* conditions and the safety on *Apis mellifera* i*n in vitro* conditions of Ivorian entomopathogenic strains already selected during a previous work on their virulence against development stages of *S. frugiperda* in *in vitro* conditions.

Material and methods

Semi-*in vitro* assay on *S. frugiperda*

An experimental plot with an area of 332.5 m^2 (35m x 9.5 m) was set up at the University of Nangui-Abrogoua. The experimental plot was divided using insect netting into 21 elementary sub-plots of 7.5 m^2. Each plot contained 15 maize plants. Five adult males and five adult females of *S. frugiperda*, were inoculated, when maize plants reached one month old. Daily monitoring was then carried out to observe the damage evolution. When the pest threshold of 20% of infested whorls was reached, the plants were treated by spraying with spore solutions at the concentration of 10^6 spores/mL (Fig.2). To this end, the strains of *Beauveria bassiana* (A211, A214a and A214b), *Metarhizium sp*. (T34) and *M. anisopliae* (T331) which showed the highest pathogenicity *in vitro* on larvae were applied, as well as negative and positive controls (with or without *S. frugiperda* and treatment). The ANOVA one-way statistical procedures was carried out using the Minitab® 22 program.

In vitro assays on *A. mellifera larvae*

The modified protocol for in vitro rearing of *A. mellifera* larval stages developed by Schmehl et al., 2016 was used for tested the virulence against pollinator of this five selected strains. The larvae were collected from a healthy hive in HEPIA apiary, using a methodology of caging the queen for 24 hours on a Cupularve Nicot® (Fig.3). An experimental unit was made up of 12 individuals and was repeated 4 times. The application of the strains was carried out orally by mixing different spore concentrations from $2*10^4$ to $2*10^7$ spores/mL with food. Mortality readings were carried out daily, noting the absence of respiratory movement on the *larvae* or black and white necrotic spots on the nymphs. The cumulative mortality in % was calculated, as well as the LC50 and LT50 with log-probit procedures.

In vitro assays on *A. mellifera* adult

The five selected strains were applied to the adults of *A. mellifera*. Living bees were collected using a mouth aspirator and kept alive using the methodology of Evans and al., 2009 (Fig.4). The bees were then anesthetized for 6.5 min at -20°C, and brought into contact with concentrated spore solutions between $5*10^3$ and $5*10^7$ spores/mL (Fig.5). Two methods of spore inoculation were tested: a topical spraying to observe acute intoxication, and a trophic application in food to observe chronic intoxication. An experimental unit consisted of 10 individuals and was repeated 4 times. The cumulative mortality in % was calculated, as well as the LC50 and LT50 with log-probit procedures and the Kruskal-Wallis statistical procedures were carried out using the Minitab® 22 program.

Optimal temperatures

The optimal temperatures of the selected strains were verified by the modified protocol developed by Fargues et al. (1992), by testing their growth at 7 temperatures (6.5°C, 12.5°C, 18.5°C, 25°C, 30°C, 35°C and 40°C). Each experimental unit was repeated six times and radial growth was measured every two days. Growth rates were calculated and the statistical procedures of Kruskal-Wallis were performed using the Minitab® 22 program.

Figure 1 : Damage from *S. frugiperda* attacks observed on corn leaves and ears

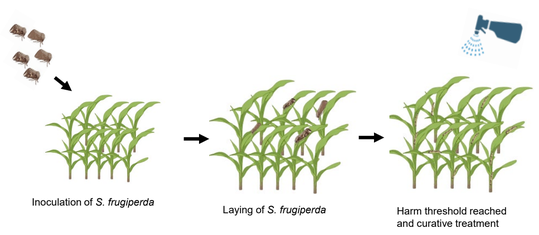

Figure 2 : Scheme of plant inoculation by *S. frugiperda* and application of spore solutions for curative purposes

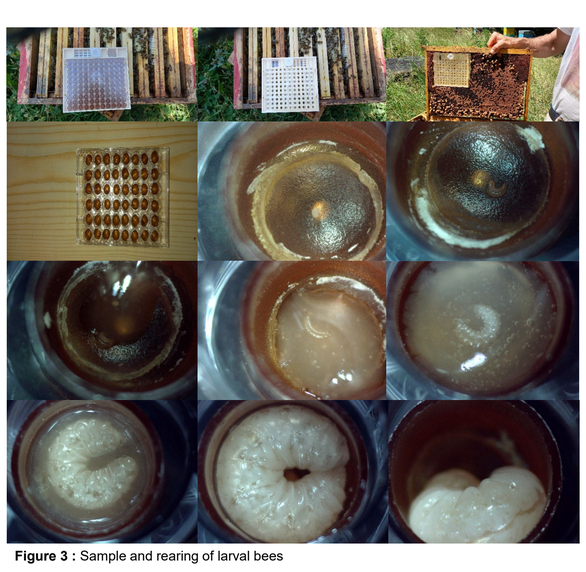

Figure 3 : Sample and rearing of larval bees

Figure 4 : Sample of adult bees

Results

Semi-*in vitro* assay on *S. frugiperda*

The severity and incidence of all treatments differed statistically from those of the negative control and the positive control (Fig. 5 and Fig. 6). Moreover, they did not differ from each other. In addition, strains A211, A214a, A214b and T331 showed bio stimulant capacity with an additional yield ranging from 1.33 and 3.33 t/ha (Fig. 7).

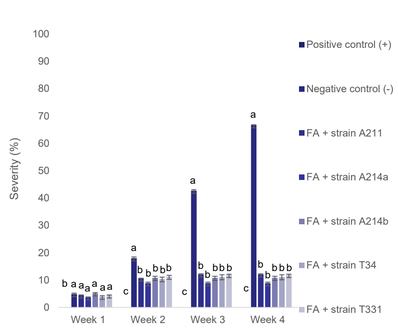

Figure 5 : Severity of *S. frugiperda* on maize

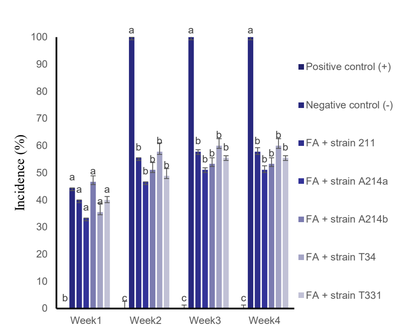

Figure 6 : Incidence of *S. frugiperda* on maize

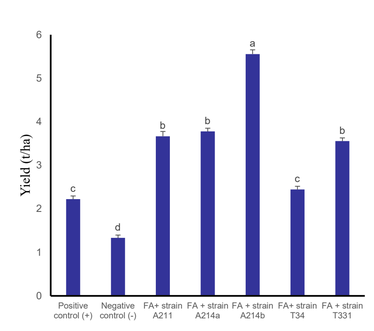

Figure 7 : Yield of maize depending of treatments

In vitro assays on *A. mellifera larvae*

No treatments differed from the negative control at 7th day. In addition, the negative control demonstrated 40% mortality, so no statistical procedures could be performed (Fig.8). However, safety trends are evident, especially at the CL50 and LT50 levels (Table.1).

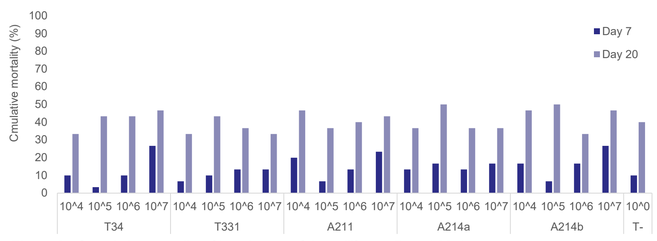

Figure 8 : Cumulative mortality of larval stage of *A. mellifera* at 7- and 20-days post treatments

Table 1: CL50 and LT50 of larval stage of *A. mellifera* depending of treatments

Strains	CL50 (spores/mL)	LT50 (days)
T34	$10^{12109000}$	9,87
T331	$10^{51463333}$	11,49
A211	$10^{23586000}$	29,89
A214a	$10^{4945769}$	13,89
A214b	$10^{2007285}$	45,38

In vitro assays on *A. mellifera* adult

Mortality rates on adult honeybees varied depending on the treatments. Indeed, strains T34 and T331 caused statistically significant mortalities at the threshold of 1 ‰ (*) compared to the negative controls and this from the concentration of $5*10^6$ spores/mL, both for acute and chronic toxicities. The A211 strain caused a significant incidence at a concentration of $5*10^7$ spores/mL, acutely and chronically respectively from the 9th and the 12th day (Fig.9). **Strains A214a and A214b did not cause any significant mortality,** trends are evident, especially at the CL50 and LT50 levels (Table.2).

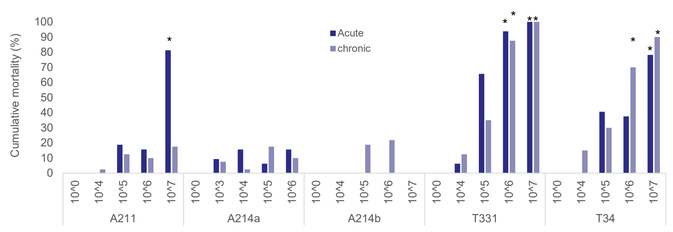

Figure 9 : Cumulative mortality of *A. mellifera* with acute and chronical toxicity depending o treatments

Tableau 2: CL50 and LT50 of adult stage of *A. mellifera* depending of treatments and their applications

Strains	CL50 acute (spores/mL)	CL50 chronic (spores/mL)	LT50 acute (days)	LT50 chronic (days)
T331	4,63E+04	6,44E+04	5,78	7,15
T34	1,56E+06	2,88E+05	7,89	7,58
A211	2,26E+06	6,03E+07	9,56	10,59
A214a	6,49E+07	8,39E+12	11,73	12,68
A214b	-	6,91E+06	-	18,26

Optimal temperatures

Optimal temperatures demonstrated the ability of *Metarhizium* strains to grow at higher temperatures than *B. bassiana* isolates. Indeed, optimal temperatures were 21.5°C, 22.5°C, 24°C, 26.5°C, and 27.5°C for strains A211, A214a, A214b, T34, and T331, respectively. Moreover, in the Kruskal-Wallis procedure, all growth rates above 60% of the optimum showed statistically significant differences compared to the minima at the 5% (*) and 1‰ (**) thresholds (Fig.10).

Discussion

All strains provided similar and satisfying protection for corn. In addition, strains A211, A214a, A214b, and T331 appear to have a biostimulant potential. A214a and A214b did not show virulence toward *A. mellifera*, unlike T331, T34, and A211. However, bees maintain high temperatures within the colony. The ability of *Metarhizium* strains to withstand these temperatures confers virulence toward *A. mellifera*. Conversely, these extreme temperatures appear to be responsible for the low virulence toward bees of strain A211.

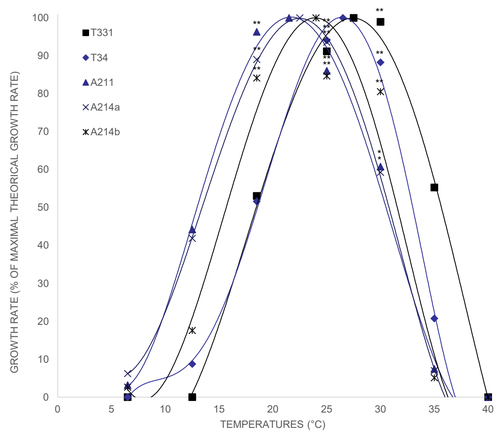

Figure 10 : Optimal temperatures curve of entomopathogenic fungi strains

Conclusion

The semi-*in vitro* test validated the pathogenicity against *S. frugiperda* of the five strains previously selected based on their in vitro virulence.
It also identified an unexpected biostimulant potential of four out of these 5 strains. Finally, the application of these strains to adults and larvae of *A. mellifera*, coupled with a cardinal temperature profile, made it possible to identify and eliminate strains virulent against eusocial pollinators, such as *A. mellifera*, and to explain this interaction. In conclusion, this work identified strains A214a and A214b as the best candidates: they protect maize crops against *S. frugiperda,* they concomitantly promote plant growth, and they are not lethal to eusocial pollinators, such as *A. mellifera*.

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