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Impact of the entomopathogenic fungus *Metarhizium anisopliae* (Metchnikoff) Sorokin on *Spodoptera frugiperda* (J.E Smith), an invasive maize pest in Chad

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ABSTRACT

The invasion of agricultural plots by Spodoptera frugiperda (J.E Smith) constitutes a serious constraint to the sustainable maize production in Chad. Since then, farmers have unsuccessfully used available chemical insecticides to control this pest at that respect, the present study evaluated the bio-efficacy of two strains (Chinese and Senegalese) of Metarhizium anisopliae on the pest, both under laboratory and field conditions. Laboratory results showed that both strains were virulent against FAW resulting in a mortality of 90 ± 5.77% for the strain from Senegal and 75 ± 5.00% for that from China. In the field, significant differences were observed among the four treatments tested, namely the two M. anisopliae strains (2.5 kg/ha), the control and Emamectin benzoate (20g/ha) for both the infestation of maize plants and FAW densities ($P < 0.05$). Meanwhile, maize grain yields were significantly the highest in plots treated with Emamectin benzoate followed by plots treated with either of the two strains of the fungus whose yields were statistically similar ($df = 3$; $F = 27.13$; $P < 0.0001$). This biopesticide was more virulent under laboratory and field conditions and could be a promising biological control agent against S. frugiperda as an alternative to synthetic insecticides. However, additional studies that evaluate the implementation cost of the microbiological control are very necessary for more objective and robust conclusions.

Key words: Fall armyworm, microbiological control, *Zea mays*, Emamectin benzoate, biopesticides

RÉSUMÉ

Impact du champignon entomopathogène Metarhizium anisopliae (Metchnikoff) Sorokin sur Spodoptera frugiperda (J.E Smith), ravageur envahissant du maïs au Tchad

L'invasion des parcelles agricoles par le *Spodoptera frugiperda* (J.E Smith) constitue un défi à la production durable du maïs au Tchad. Depuis lors, les agriculteurs ont utilisé sans succès les insecticides chimiques disponibles pour lutter contre ce ravageur. La présente étude a évalué la bioefficacité de deux souches (chinoise et sénégalaise) de *Metarhizium anisopliae* sur le ravageur, à la fois en laboratoire et au champ. Les résultats de laboratoire ont montré que les deux souches étaient virulentes contre FAW, entraînant une mortalité de 90 ± 5,77 % pour la souche du Sénégal et de 75 ± 5,00 % pour celle de Chine. Au champ, des différences significatives ont été observées entre les quatre traitements testés, à savoir les deux souches de *M. anisopliae* (2,5 kg/ha), le témoin et l'Emamectine benzoate (20 g/ha) à la fois pour l'infestation des plants de maïs et les densités de FAW ($P < 0,05$). Parallèlement, les rendements en grains de maïs étaient significativement plus élevés dans les parcelles traitées avec l'Emamectin benzoate, suivies des parcelles traitées avec l'une des deux souches du champignon dont les rendements ($df = 3$; $F = 27,13$; $P < 0,0001$). Ce biopesticide s'est révélé plus virulent dans des conditions de laboratoire et de terrain et pourrait être un agent de lutte biologique prometteur contre *S. frugiperda* comme alternative aux insecticides synthétiques. Cependant, des études supplémentaires évaluant le coût de mise en œuvre de la lutte microbiologique sont très nécessaires pour tirer des conclusions plus objectives et plus solides.

Mots clés : Chenille légionnaire d'automne, lutte microbiologique, *Zea mays*, Emamectine benzoate, biopesticides.

INTRODUCTION

Maize, Zea mays L. (Poaceae) constitutes the staple food in many African countries, including Chad (Cairns et al., 2013; Badu-Apraku and Fakorede, 2017). Unfortunately, its production is hampered by the fall armyworm *Spodoptera frugiperda* J. E Smith (Lepidoptera: Noctuidae) which invaded the African continent in 2016 (Goergen et al., 2016), and constitutes a real threat to security food for hundreds of millions of people around the world (Chhetri and Acharya, 2019; Devi, 2018). It is therefore urgent to develop effective and sustainable strategies for the control of this invasive pest (Tepa-Yotto et al., 2021). In Africa, chemical control based on the use of synthetic chemical pesticides remains the method most commonly used by producers. However, the massive application of conventional insecticides as a single method of combating this pest can promote the development of resistant populations on the continent and could, in the long term, be ineffective (Day et al., 2017). Additionally, multiple applications or high doses of insecticides are known to be lethal to many beneficial insects, including the natural enemies of the fall armyworm, and for their ability to pollute the environment and incur risks serious health risks for both farmers and consumers (Gutiérrez-Moreno et al., 2019; Kebede and Shimales, 2021; Siegwart 2021). Thus, taking into account the cropping systems, the income level of farmers as well as the small size of farmers in Africa, several authors recommend against this pest, an integrated fight based on a reasonable combination of different control methods (Day et al., 2017). Hence, the need to develop and introduce biological alternatives for sustainable control of FAW in its area of spread in Africa. Thus, in the Chadian context, several control methods are being evaluated against this pest. Recently, Mbaidiro and his team assessed, in 2021, the effect of the length of the development cycle of certain maize varieties on their susceptibility to FAW, while Mbaidiro & Onzo (2022) reported that neem oil could be a biological insecticide effective against this pest. Tests on sowing dates were also carried out by Mbaidiro et al. (2023) as part of cultural and agronomic control. Another alternative to chemical control is biological control, although its implementation requires time and a lot of investment (Graffin, 1982; Lachemi, 2020). However, its microbiological component based on the use of entomopathogenic fungi has currently been identified as an effective alternative management strategy against many harmful insects, not only as autonomous control agents (Douro-Kpindou et al., 2012; Vanden Bussche and Tech, 2021), but also as a major component in integrated pest management (Opisa et al., 2018; Abdelaziz and Senoussi, 2019). In a recent study, Day et al. (2017) reported that several microorganisms including entomopathogenic fungi, entomopathogenic nematodes, viruses "and bacteria" were associated with FAW in the United States of America; and recommended that the entomopathogens associated with this pest in Africa be inventoried, and that their impact on the pest be assessed. Indeed, the performance of entomopathogenic fungi is generally largely affected by climatic factors, notably temperature and relative humidity; which may result in marked variability in their effectiveness as a control agent for target pests, in this case in novel FAW habitats (Visalakshi et al., 2020). However, when optimal conditions are met, particularly high relative humidity, external sporulation of the fungus contributes to its spread and can cause epizootics in FAW populations (Khan, 2015), as observed for other pests (Onzo et al., 2013; Latiff et al., 2022). Several species of entomopathogens are recognized as potential biological control agents to counter FAW. These include: *Beauveria bassiana* (Akutse et al., 2019; Cruz-Avalos et al., 2019), *Metarhizium rileyi* (Varma and Upendhar, 2020), *M. anisopliae* (Cruz-Avalos et al., 2019) , *M. robertsii* (Hernandez-Trejo et al., 2019) and *Fusarium solani* (Hernandez-Trejo et al., 2019). Among the entomopathogenic fungi most used in biological control, *M. anisopliae*, strains of which have proven virulent against different species of insect pests and on different crops (Douro-Kpindou et al., 2008; Hala et al., 2018). Recently, Akutse et al. (2019), reported that some isolates of *M. anisopliae* caused mortality rates greater than 90% in early immature stages of *S. frugiperda*. Ramanujam et al. (2020) reported mortalities greater than 67% in populations of *S. frugiperda* treated with the ICAR-NBAIR Ma-35 isolate of *M. anisopliae* and a subsequent increase in yield of up to 44%. In laboratory tests, Romero Arenas et al. (2014) reported a mortality rate of 72.5% at 72 h after treatment of third instar FAW larvae with *M. anisopliae* strain CP-MA1, while other authors reported mortality rates of larvae or eggs ranging from 87% to 100% following the application of various strains of *M. anisopliae* (Gutiérrez-Cárdenas et al., 2019).

In Chad, *M. anisopliae* var acridium was used as part of the biological control of the desert locust (Brader et al., 2003). Given the recognized importance of this entomopathogenic fungus against numerous crop insects around the world and its potential against FAW, the present study evaluated the pathogenicity of two strains of *M. anisopliae* on the larvae of *S. frugiperda* under laboratory growing conditions, and their ability to reduce *S. frugiperda* infestation in the real environment.

MATERIAL AND METHODS

Study site

The study was conducted at the Agronomic Research Station of Bébédjia (8°40'34'' N; 16°33'58'' E; 397 m altitude). This station is located in the Sudanian zone of Chad, 524 km south of N'Djamena (the country's capital). It benefited a tropical Sudanian climate characterized by alternation of a rainy season, which extends from April to October (4 to 5 months), and a dry season, which extends from November to March (7 to 8 months). Annual rainfall is between 600 mm and 1200 mm (DMN, 2019; Mbaidiro et al., 2021; Mbaidiro and Onzo, 2022) . Rainfall data as well as temperature and relative humidity collected at the Bébédjia meteorological station, at 3 km from the experimental site, were used to characterize the test. These include, in particular, the average daily precipitation as well as the number of rainy days recorded during the test. Overall, the average monthly rainfall collected at the experimental site during the study was 272.95 mm while the average temperature varied between 22°C and 33°C, with the minimum in August and the maximum in October. The relative humidity during the study period varied between 84.66% and 96.23% with an average of 90 ± 0.43%.

Study Material

Spodoptera frugiperda is the only animal species that was the subject of this study (Figure 1). As for the plant material, it was made up of the TZEEW maize variety, recognized as sensitive to FAW (Mbaidiro et al., 2021).

The spores of the entomopathogenic fungus *M. anisopliae* used in this study were obtained from a company in China (Henan Au Loddy Commerce Trade Co., Ltd.) for the Chinese strain; and from the Agricultural Zoology Laboratory of the Directorate of Plant Protection of Senegal for the Senegalese strain. As for the chemical insecticide, Emamectin benzoate 50g/kg (Emacot 050WG), it was acquired locally from an approved distributor of agricultural inputs.

Figure 1. Larvae of *Spodoptera frugiperda*; Georg Goergen, IITA, (2016)

Methods

Fungal spore suspension

The fungal suspension was prepared by making a mixture of 1 g of the talc formulation of each strain in 9 ml of sterile distilled water containing 0.01% Tween 80 (Ramanujam *et al*., 2020). Before bioassays, conidial suspensions were adjusted to a concentration of 1 × 10^8 spores/ml by dilution using the Neubauer Improved Hemocytometer (Akutse et al., 2013, 2019).

Laboratory evaluation of the virulence of *M. anisopliae* on FAW

The experimental design was a complete randomized block with 3 objects and 4 replicates and was as follows:

- object 1:Ten 3rd instar larvae of FAW sprayed with sterile water;
- object 2: Ten 3rd instar larvae of FAW sprayed with a suspension of *M. anisopliae* (China strain): 1 × 10^8 spores/ml;
- object 3:Ten 3rd instar larvae of FAW sprayed with a suspension of *M. anisopliae* (Senegal strain): 1 × 10^8 spores/ml;

The larvae were transferred into transparent plastic boxes (16-x-16-cm), and fed at 24 h intervals with freshly detached maize leaves. Plastic boxes with *S. frugiperda* larvae were incubated at 25 ± 2 °C and 90 ± 5% RH for 10 days in a Memmert UNE-600 incubator (manufactured in Belgium).

Field assessment of the impact of *M. anisopliae* on FAW

The experimental design was a complete randomized blocks comprising four blocks and four objects. The treatments represented by the two biopesticides, a chemical insecticide and the control and were as followed:

- object 1: Untreated maize (control);
- object 2: maize sprayed with *M. anisopliae* (China) at a rate of 2.5kg/ha (at the manufacturer's recommended field rate)
- object 3: maize sprayed with *M. anisopliae* (Senegal) at a rate of 2.5kg/ha ;
- object 4: maize sprayed with Emamectin benzoate 50 g/kg WG at a rate of 20g/ha

A single block contained with four elementary plots receiving each of the four treatments. The plots were 5 m long and 3.6 m wide and had six sowing rows. 1.5 m path separated elementary plots from each other by and blocks separated by 2 m path. Maize ("TZEEW" variety) was sown at a rate of three seeds per pocket and at a spacing of 0.60 m x 0.40 m, after a useful rain of at least 20 mm of water from the rain gauge. Regarding the maintenance of the plots, a first weeding was done on the 14th day after emergence, followed by a second weeding 21 days after the first. Cereal fertilizer N, P, K (20-10-10) was applied as background fertilizer, at a quantity equivalent to 150 kg/ha. It was buried in furrows drawn 10 cm from the sowing line. Urea was added as a cover fertilizer in two fractions, respectively, at the 10-leaf stage and at male flowering, in quantities equivalent to 25 kg/ha (Mbaidiro et al., 2021).

The fungal formulations were prepared in the evening of the day of application. For this purpose, 5g (1x10^8 cfu/g) (Ramanujam et al., 2020) of spore powder of each strain of *M. anisopliae* was previously mixed with a liter of water to which 5 ml of liquid soap was added to allow the fungi spores to adhere to corn plants. Regarding the chemical insecticide treatment, its solution was made by mixing 0.14 g of Emamectin benzoate with one liter of water. These products were applied using two sprayers model (XFB III) (manufactured in China by Zhejiang Dingfeng Plastic Co., Ltd.) with 5 liter capacity. The first sprayer exclusively used for fungal formulations while the second was reserved for the application of chemical insecticide in order to avoid contamination. The effectiveness of entomopathogenic fungi is generally affected by factors like ultraviolet rays from the sun, high heat, etc. (Rajamani and Negi, 2021). Consequently, for the fungi as for the chemical insecticide, applications took place in the evening (between 4:30 p.m. and 5:30 p.m.) on the 20th, 30th, 40th and 50th days after sowing (i.e. at an interval of 10 days (Ramanujam et al ., 2020).

Data collection

Larval mortality of *S. frugiperda* and sporulation of mummies in the laboratory

Larval mortality due to *M. anisopliae* infection was confirmed by the presence of green conidia on the surface of the corpse after examination under a light microscope (Onsongo et al., 2019; Munywoki et al., 2022). The number of dead larvae observed was carefully recorded at 24 h, 48 h, 72 h and 96 h after application of the fungal suspensions. The larval mortality rates for each object were calculated using the Abbott formula (Abbott, 1925) as follows:

Corrected mortality (%) = (1- (nTa/nCa)) x 100

Where nTa is the number of surviving individuals in the batch of treated insects after treatment and nCa is the number of surviving individuals in the control batch after treatment. Larval sporulation rates were calculated using the Abbott formula (Abbott, 1925) based on dead individuals (larvae) in each object as follows:

RS= (1- (nLS/TndLM)) x100,

where, nSL = number of sporulated larvae; TndL = total number of dead larvae.

Field testing

Estimation of *S. frugiperda* attacks on maize plant

The estimation of the number of maize plants infested by *S. frugiperda* was carried out on a sample of 25 plants chosen randomly from four central lines of each elementary plot: i.e. a total of 100 plants per objet. Observations started just before the application of the treatments, and continued every 5 days preceding each pesticide application. Thus, the first observations were made on the 20^{th} day after sowing when the incidence of attacks is high while the last ones took place on the 60th day after sowing, corresponding to the stage of physiological maturity of the plants and where borer attacks become negligible (Pannuti et al., 2016). The infestation rate of the corn plots was then determined by making the ratio between the number of infested plants (i.e., those harboring the pest) and the total number of plants sampled.

Determination of relationship between meteorological parameters and Faw infestation rate on maize plants

Data on meteorological parameters, namely temperature, relative humidity and precipitation, were recorded during the trial period and the influence of these parameters on the infestation rate of maize plants by the CLA has been determined.

Evaluation of larval mortality in the field and sporulation of FAW cadavers after incubation in the laboratory

A visual count of dead S. frugiperda larvae was carried out before each application of the object and 5 days afterwards. Thus, mortality of FAW larvae was determined in each object by collecting, mummified larvae per treatment and per plot and the collection were placed in a Petri dish (110 × 60 mm). These Petri dishes were then covered with damp filter paper, then sealed with parafilm paper and incubated in the laboratory at 25 ± 2 °C and 90 ± 5% RH to ensure sporulation of the fungus. Sporulation rates were calculated and a comparison between the two strains of the fungus was made.

Estimation of damage on maize ears

Damage to the ears was assessed on a sample of 25 ears selected randomly from the four central rows of each elementary plot. The extent of pest damage on each ear was determined using a scale of 1 to 9, as described by (Mbaidiro and Alexis, 2022; Kamweru et al., 2022).

Evaluation of maize yield in the different objects

To evaluate corn dry grain yield, all corn plants from the four central rows of each elementary plot were harvested. The ears were detached, despatched, dried and then shelled manually. The grains were then dried at 12% of residual humidity level and weighed with a Steinberg brand electronic balance (300 kg; internal precision 50 g). The production thus determined is that of the four central lines of each elementary plot (i.e. 12 m^2). This yield was then reported per hectare for greater clarity and to facilitate comparisons with other research results.

Statistical analysis of data

Pest density, infestation rate, FAW fungal mortality rate, sporulation rate and damage score underwent the Shapiro–Wilk normality test (Shapiro and Wilk, 1965), before being submitted to analyzes of variance (ANOVA). In case the data were not normally distributed, they were transformed by Arcsine√(X/100) (for proportions) and by log (x+1) (for count data) before being analyzed. . When the analysis of variance revealed significant differences between treatments, the means of the different treatments were separated using the Student-Newman-Keuls (SNK) multiple separation test at the 5% threshold. Linear regression analysis was carried out to determine the relationship between rainfall and meteorological data and the infestation rate of the plots. All statistical analyzes were performed using XLSTAT Version 2016.02.27444 software.

RESULTS

Effect of compared on larval mortality of *S. frugiperda* and sporulation of corpses: laboratory test

Table 1 below presents the average number of dead larvae, corrected mortality rate and sporulation rate of *S. frugiperda* caterpillar corpses. Overall, the average number of dead larvae on the ten FAW 3^{rd} instar larvae used varied from 0.25 ± 0.25 (Control treatment) to 8.75 ± 0.48 (*M. anisopliae* Senegal strain). The analysis of variance showed a significant impact of the treatments on the average number of dead larvae (df = 2; F = 155.17; P < 0.0001), the Metarhizium strain from Senegal having recorded the greatest number of dead larvae. Analysis of variance also revealed a significant effect of the two strains of Metarhizium on larval mortality of *S. frugiperda* (df = 2; F = 198.20; P < 0.0001) with a mortality rate of 65 ± 2 .88% recorded with the strain from China and that of 87.50 ± 4.79% recorded with the strain from Senegal. Similarly, analysis of variance revealed a significant difference between the two strains of the fungus with regard to the sporulation rate of FAW mummies (df = 1; F = 8.00; P < 0.030). Thus, the Senegal strain recorded the highest sporulation rate compared to the Chinese strain (Table 1).

Table 1. Larval mortality and sporulation of corpses caused by *M. anisopliae*

Traitements	**Average number of dead larvae**	**Adjusted mortality rate (%)**	**Sporulation rate (%)**
Control	0.25 ± 0.25 c	0	-
Metarhizium strain Senegal	8.75 ± 0.48 a	87.50 ± 4.79 b	90 ± 5.77 a
Metarhizium strain China	6.50 ± 0.29 b	65.00 ± 2.88 c	75 ± 5.00 b
ddl	2	2	1
F	155.17	198.20	8.00
Pr > F	0.0001	0.0001	0.030

In the same column, the means (± SE) followed by the same letter are not significantly different according to the Student-Newman-Keuls Test.

Effect of compared objects on infestation of maize plants by *S. frugiperda* in the field environment

The evaluation of the infestation of maize plants by FAW on the different treatments (Table 2) showed that the infestation rate varied from 16 ± 3.88% to 71.50 ± 2.35%. The analysis of variance revealed a significant impact of the treatments on the infestation rate (df = 3; F = 39.03; P < 0.0001), with Emamectin benzoate (19g/ha) having recorded the lowest infestation rate, followed by Metarhizium from Senegal, then the strain from China.

Table 2. Effect of treatments on field infestation of corn plants by *S. frugiperda*

Treatments	**Pre-treatment** Average infestation rate (%) per 100 plants	**Post treatment** Average infestation rate (%) per 100 plants
Control	83.75 ± 6.25 a	71.50 ± 2.35 a
Metarhizium strain Senegal	71.25 ± 6.57 a	31.25 ± 3.12 c
Metarhizium strain China	75.00 ± 6.45 a	50.25 ± 2.52 b
Emacot	68.75 ± 6.57 a	16 ± 3.88 d
ddl	3	3
F	1.02	62.81
Pr > F	0.410	< 0.0001

The numbers in parentheses represent the average number of infested plants out of the 100 plants sampled per plot. In a column, the means (± SE) followed by the same letter are not significantly different according to the Student-Newman-Keuls test at the 5% threshold.

Relationship between *S. frugiperda* infestation of corn plants, average temperature, relative humidity and cumulative number of rainy days

Linear regression analysis reveals a significantly negative relationship between the number of rainy days and the FAW infestation rate (Figure 2), with a coefficient of determination $R^2 = 0.171$ (Slope = -1.510; $P < 0.0001$). However, although the relationships between infestation rate and, respectively, average temperature and relative humidity are positive ($R^2 = 0.0106$ and $R^2 = 0.0004$ respectively), they are not significant ($P > 0.05$).

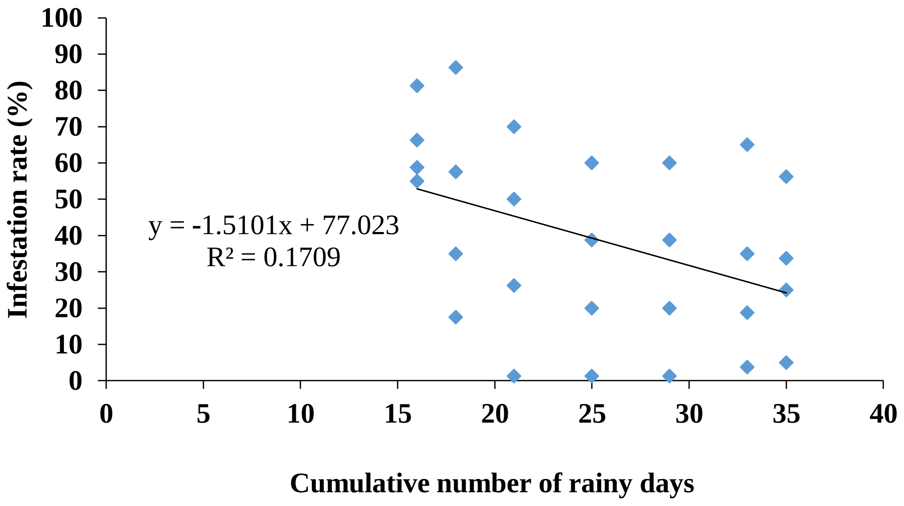

Figure 2. Relationship between FAW infestation rate and cumulative number of rainy days

Effect of compared objects on larval density of *S. frugiperda* in the field

Temporal variation of *S. frugiperda* larval densities on 25 maize plants (Figure 3) showed high densities of FAW larvae at the 25th day after sowing (25th DAS) across all treatments. These densities gradually decreased from the 30th DAS to reach their lowest levels on the 55th DAS, corresponding to the last day of sampling. Mean population densities of *S. frugiperda* larvae varied from 0.71 ± 0.18 (Emacot) to 5.29 ± 0.65 caterpillars (control treatment) on 25 plants. Analysis of variance revealed a significant influence of treatments on the population density of S. frugiperda larvae (df = 3; F = 27.40; P = 0.0001); the untreated control recorded the highest density while the chemical insecticide (Emacot) and the two strains of *M. anisopliae* experienced the lowest densities which are statistically similar.

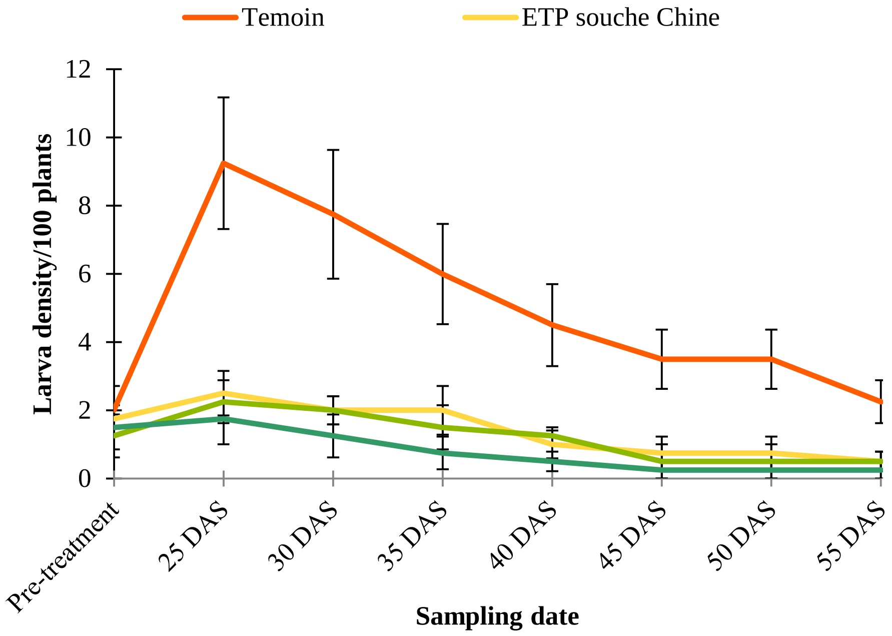

Figure 3. Evolution of the average density of FAW larvae per 100 plants (JAS: Day after sowing). The bars represent the standard errors of the mean.

Larval mortality in the field and sporulation rate of corpses after incubation in the laboratory

A total numbers of dead *S. frugiperda* larvae collected were 50 and 35 larvae with sporulation rates which were 72% and 62.86%, respectively for the Senegalese strain of *M. anisopliae* and that of China. It follows that the Senegalese strain recorded the greatest number of dead larvae, and also experienced the highest rate of sporulation of corpses after incubation of the mummies in the laboratory.

Effect of objects on *S. frugiperda* damage on ears

Between treatments, the average number of maize ears attacked on 25 maize plants per elementary plot varied from 9.50 ± 0.50 to 13.25 ± 2.72, (Table 3). For this parameter, the analysis of variance revealed no significant difference between the treatments (df = 3; F = 1.15; P < 0.368). As for the damage index on the cob, it varied from 1.00 ± 0.00 (Emacot) to 5.00 ± 0.58 (Control); with a significant difference revealed by the analysis of variance (ddf = 3; F = 21.15; P < 0.0001). Emacot treatments and the Metarhizium strain from Senegal recorded the lowest damage indices, followed by the Metarhizium strain from China.

Table 3. Influence of treatments on *S. frugiperda* damage to corn cobs

1	**Treatments**	**2**	**Average number of ears attacked**	**3**	**Average damage index on ears**
4	Control	5	13.25 ± 2.72 a	6	5.00 ± 0.58 a
7	*Metarhizium* souche from China	8	11.50 ± 1.32 a	9	3.25 ± 0.25 b
10	*Metarhizium* souche from Senegal	11	10.00 ± 0.71 a	12	2.00 ± 0.41 c
13	Emacot	14	9.50 ± 0.50 a	15	1.00 ±0.00 c
16	ddl	17	3	18	3
19	F	20	1.15	21	21.15
22	Pr > F	23	0.368	24	0.0001

In a column, the means (± SE) followed by the same letter are not significantly different according to the Student-Newman-Keuls Test at the 5% threshold.

Effect of different objects on maize production

Over all objects, the average yield (± standard error) estimated in dry maize grains per hectare varied from 734.99 ± 42.93 kg/ha to 1694.52 ± 36.80 kg/ha (Figure 4). The analysis of variance revealed a significant impact of treatments on performance (df = 3; F = 27.13; P < 0.0001). Thus, the Emacot-based object recorded the highest yield followed by *M. anisopliae* strains from Senegal and China which recorded statistically similar grain yields. The lowest yield was obtained with the control treatment.

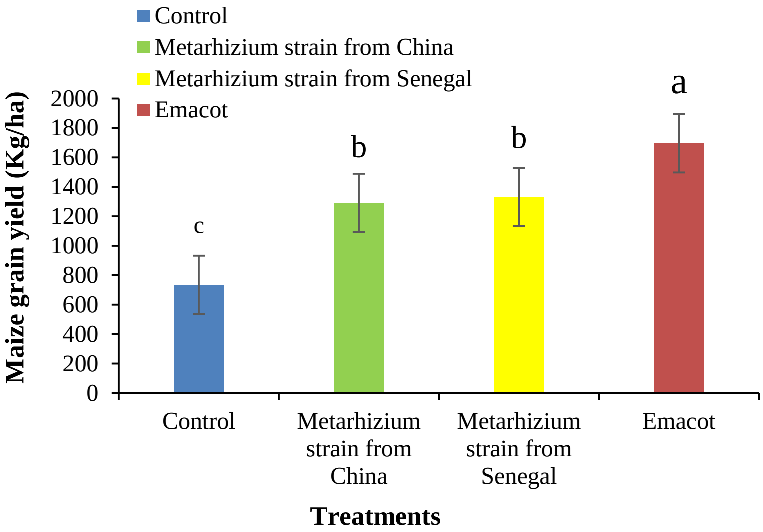

Figure 4. Maize grain yield on the different treatments. The means (± SE) followed by the same letter are not statistically different (SNK test at the 5% threshold)

DISCUSSION

The results of the present study aimed at evaluating the effectiveness, in laboratory conditions and in a real environment, of two strains (Chinese and Senegalese) of the entomopathogenic fungus *M. anisopliae* against *S. frugiperda*, showed a significant impact of this entomopathogen on third instar larvae (L3) of *S. frugiperda*. In a first part, the evaluation under laboratory conditions showed high mortality of 3rd instar larvae of *S. frugiperda* following the application of the two strains of the fungus. In addition, the presence of growths of green mycelium and conidia on the mummified corpses of *S. frugiperda* larvae constituted proof that the mortality of the larvae was indeed due to the activity of the fungus. Indeed, as demonstrated by several authors, it is the fungal conidia, which are responsible for the infection of the host and its death (Gabarty et al., 2014; Lacey et al., 2015). Thus, several research results have demonstrated that *M. anisopliae* exhibits insecticidal activity against different groups of plant pest insects including, among others, *Spodoptera exigua* (Hübner, 1808) and *S. litura* (Fabricius, 1775) (Javar and Sajap, 2020), *Plutella xylostella* (L.) (Shehzad et al., 2021), *Aphis gossypii* Glover (Latiff et al., 2022). The effectiveness of *M. anisopliae* has also been reported by several authors against FAW with high mortality in laboratory conditions (Akutse et al., 2019; Gutiérrez-Cárdenas et al., 2019; Ullah et al., 2022). However, in the present study, the pathogenicity of this entomopathogen under laboratory conditions revealed greater virulence of the Senegal strain with a greater number of sporulating caterpillars compared to the China strain. This difference between strains of the fungus could be linked to intraspecific variations between fungal strains as documented by several authors on other insect species (Tiago et al., 2014; Vega and Kalkum, 2011). In the second part of this study, the evaluation in real environment revealed after the four applications of the fungus, a lower rate of larval infestation of maize plants by *S. frugiperda* in the plots treated with *M. anisopliae* than in those untreated. These low infestation rates recorded in plots treated with the fungus nevertheless remain higher than those obtained with the chemical insecticide Emacot. The same applies to the densities of the pest, which, although being significantly lower on the plots treated with the fungus than in the controls, they remain higher than those recorded on the plots treated with Emacot. The application of the entomopathogenic fungus effectively induced remarkable mortality among FAW larvae, making it possible to significantly reduce the infestation rate and damage of *S. frugiperda* on corn in the field. Similar to the results obtained in laboratory conditions, the strain from Senegal was significantly more virulent than that from China. This difference in virulence could be explained by the fact that the Senegal strain coming from a country whose climatic and environmental conditions are closer to those prevailing in Chad, and would be better adapted compared to the Chinese strain (Ferron et al., 1991; Hunt et al., 1994). Thus, there followed a subsequent improvement in yield compared to the productivity of untreated control plots. Here too, plots treated with the chemical insecticide Emacot recorded the best corn grain yield. These results support those reported by several authors on the effectiveness of *M. anisopliae* in reducing the FAW infestation rate (Mallapur et al., 2018;

Ramanujam et al., 2020), as well as on other pests such as *Tuta absoluta* Meyrick on tomatoes in the field (Aynalem et al., 2022). However, plots treated with the chemical insecticide Emacot recorded the best corn grain yield. Epizootics linked to entomopathogenic fungi generally depend on climatic factors such as wind, rain or the frequency of contacts between insects (Abdelaziz and Senoussi, 2019). In addition, like other insect pests, the population dynamics of the FAW is known to be affected by the weather conditions characterizing each season; which explains its variation in Chad depending on sowing dates (Mbaidiro et al., 2023). Thus, it appears from our results that precipitation and in particular the number of rainy days can influence FAW infestation rate. It follows that the low infestation rates observed in the real environment by the pest would be due to the rain frequency received by the crops during the first stages of vegetative growth of the maize plants; this would have contributed to the leaching of the first and second instar larvae, preventing them from settling on plants (Anandhi et al., 2020; Kumar et al., 2020; Pragya et al., 2022). At the same time, high ambient relative humidity is a factor favoring the germination of fungal spores and therefore its negative impact on the density of FAW larvae (Ahmad and Ibrahim, 2021; Reddy et al., 2020). It therefore turns out that the frequency of precipitation synergistically affects FAW density when the entomopathogenic fungus is present in the environment. This significant impact of entomopathogens on the abundance of the pest would explain why grain yield was significantly higher in plots treated with chemical insecticide or by the two strains of *M. anisopliae* than in untreated control plots (Aynalem et al., 2022; Munywoki et al., 2022). Although the impact of the insecticide remains significantly higher than that of entomopathogens, this relative effectiveness of chemical treatment could well be counterbalanced by the sustainable and non-toxic nature of the application of the entomopathogenic fungus. Indeed, the yield gain with Emacot compared to *M. anisopliae* strain from Senegal is only 28% while *M. anisopliae* strain from Senegal improved corn yield by 80% compared to the control. However, additional studies, particularly on the cost of treatments, are necessary for a more robust conclusion.

CONCLUSION

The present study demonstrated sensitivity of third instar larvae of *S. frugiperda* to the two strains of *M. anisopliae* in laboratory conditions and FAW larvae in the field. The third instar larvae were more susceptible to *M. anisopliae* strain from Senegal compared to the strain of china under laboratory. The application in the field result in a high reduction of infestation rate with the strain from Senegal compared to the china strain. In general, *M. anisopliae* conidia was more virulent under laboratory and field conditions by reducing larval infestations, decreasing damage and subsequently increasing the production yield of maize in plots treated with entomopathogen fungi compared to the untreated plots. This entomopathogen fungus could be a promising biological control agent against *S. frugiperda* as an alternative to synthetic insecticides in maize cropping systems. However, the prospecting, collection and field evaluations of *M. anisopliae* strains isolated locally against FAW and its natural enemies constitute prospects for future research work on the sustainable control of this pest.

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