Original Research Article

Effect of two biofertilisers based on endophytic fungi on fall armyworm infestations in maize production in the Kara region of Togo

ABSTRACT

Aims: The aim of the study was to assess the efficacy of two biofertilisers based on endophytic fungi on infestation rates, foliar damage and maize yield losses caused by the armyworm, *Spodoptera frugiperda*, a formidable maize pest.
Study design: Completely Randomized Design (CRD) with three types of treatments.
Place and Duration of Study: Agronomic Experimentation Station of Tchitchao, Kara (Togo), between October 2019 and March 2020.
Methodology:A complete randomized block design was used with three types of treatments: (i) maize seeds inoculated with *Glomus sp.* strain 142, (ii) maize seeds inoculated with Mycotri, (iii) maize seedlings fertilized with NPK and urea and finally (iv) a control consisting of seeds and/or seedlings of maize neither fertilized nor inoculated.
Results: The results show that the plots treated with endophytic fungi recorded significantly lower infestation rates compared with the fertiliser treatments and the control. The highest infestation rates were 54% at 38 days after sowing (DAS) and 45% at 31 DAS for treatments with 5.35 x 105 spores/ha *Glomus sp.* and 0.38 kg/ha Mycotri, respectively, compared with 78% at 31 DAS and 62% at 38 DAS for fertiliser treatments and the control. The highest level of leaf damage, 2.92, was observed at 33th days on the plots fertilised with fertiliser, compared with scores of 2.53 and 2.38 for the plots mycorrhised with *Glomus sp.* and Mycotri, respectively. Yields were significantly higher in the fertilised plots (3.87 t/ha), followed by the mycorrhised plots (2.62-3.01 t/ha) and the control (1.73 t/ha).
Conclusion: The endophytic fungi enabled the plants to tolerate fall armyworms attacks better than the controls, offering an alternative to chemical fertilisers and an effective option for managing soil fertility and controlling *Spodoptera frugiperda.*

Keywords: Maize, Spodoptera frugiperda, mycorrhizae, Glomus sp, biological control.

1. INTRODUCTION

The fall armyworm (CLA) *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae), is one of the most important pests in West Africa and Togo [1-3]. Native to tropical and subtropical regions of America [3,4], CLA feeds on more than 80 plant species [1,2,5], preferring maize and causing significant damage [6]. Studies by Prasanna et al. [7] show an increase in CLA infestations due to climate change, exacerbating maize yield losses. To better manage their crops, farmers are making massive spontaneous use of synthetic insecticides [8,9], which are known to have harmful effects on humans and the environment [10,11], natural enemies [10,13] and for the resistance phenomena they can induce in pests [14-17]. In a bid to solve these problems while ensuring sustainable management of CLA, FAO recommends integrated pest management, combining the most environmentally friendly strategies, including varietal control and the use of natural pesticides, biofertilisers and biopesticides in pest control [1]. In Togo, studies on the management of CLA have revealed that infestation rates on maize plants can exceed 68% [18] and several natural enemies of CLA are present in Togo [19]. In addition, Kpemoua et al. [20] have shown that some maize varieties grown in Togo seem to tolerate armyworm attacks as well as possible, while the study of Tcha-Moussa et al.[21] has shown that good plant fertilisation is a good option for managing CLA. The study of Kombieni et al.[22] showed that botanical pesticides can be an alternative to synthetic pesticides in the control strategy against CLA in Togo. Arbuscular mycorrhizal fungi (AMF) are considered a promising alternative for armyworm management because of their ability to improve plant health and resistance to biotic stresses, unlike biopesticides and botanical pesticides, which may be expensive or have limited effects [23,24]. For example, Gnamkoulamba et al. [25] showed that the symbiotic association of the fungi *Acaulospora spinosa*, *Glomus mosseae* and *G. hoï* with rice plants promotes better plant nutrition, increases their tolerance to pests and reduces the need for chemical products. Unlike chemical control, AMF offer a sustainable and environmentally friendly approach, avoiding resistance problems and negative impacts on CLA natural enemies [8,26]. This study, initiated as part of the national programme for the sustainable management of the Fall armyworm in Togo, aims to assess the bioprotective potential of endophytic fungi contained in two biofertilisers against the Fall armyworm under field conditions.

2. MATERIAL AND METHODS

2.1 Experimental site

The study was conducted at the Agronomic Experimentation Station of Tchitchao, Kara (Togo), with geographical coordinates of 9°37'2.968'' north latitude and 1°6'51.645'' east longitude. The study area has a tropical Sudanian climate, with a single main rainy season from April to October and a dry season from November to March. The soils are ferralitic types relatively poor.

2.2Mycorrhiza inocula and maize variety

Four pure strains of 472 (*Glomus sp*) at a rate of 6000 spores/kg of substrate were used. The strainsisavailable at "Laboratoire des Sciences Agronomiques et Biologiques Appliquées", (LaSABA-University of Kara).

2.3Experimental design and seeds inoculation

A completely randomised block design of six treatments with replications was used, with a distance of one meter between the different blocks and 0.5 m between experimental units within the same block. The experimental unit consisted of a 5.25 m x 5 m maize plot. Sowing was carried out in two-seed stacks with a spacing of 0.25 m between successive stacks on the same row and 0.80 m between successive rows, i.e. 7 rows of 20 plants (140 plants) per plot unit. The plants were removed two (2) weeks after emergence, leaving only one plant per cluster for the trial. For Mycorrhiza 142 (*Glomus sp*), the maize grains were sown in pits containing the planned dose of spores in order to promote early mycorrhization. Two doses - 8.02 x 105 spores/ha and 5.35 x 105 spores/ha - of mycorrhiza were used for sowing, i.e. 1.5 g/packet and 1 g/packet respectively. For MYCOTRI, the 1.5 g and 1 g powder was mixed with 10 g of maize seed for application rates of 0.38 kg/ha and 0.25 kg/ha respectively, the latter being the rate recommended by the developer. The absolute control consisted of maize varieties neither fertilised nor inoculated with the fungus strain. The positive control consisted of maize fertilised with NPK 15-15-15 and urea, which were applied at 15th days after sowing (DAS) and 45th DAS respectively.

2.4AMF root colonization

The AMF root colonization and spore density were assessed after two months of inoculation by harvesting (taking root out from the soil) 5 plants per treatment. Roots were extracted by wet sieving and decantation [27]. AMF root colonization was determined according to Brundrett et al. [28], using trypan blue to stain mycorrhizal structures. The gridline-intersect technique [29] was applied to analyze AMF colonization using magnifying glass, at up to 90-fold magnification.

2.5Effect of inoculation on the infestation rate of armyworms

The infestation rate was assessed on a sample of 50 plants from each plot unit following the W approach described by the FAO [2]. A plant was considered infested when it showed at least one egg mass, live larva or fresh droppings. These data were collected from 10 DAS and at 7 days frequency until the appearance of the male inflorescence on half of the plants on the experimental plot. The proportion of the leaf surface attacked was assessed using the pre-established scale of Davis et al. [30]. To do this, 10 plants were sampled per plot every fortnight from the 15th day of the month until the appearance of the male flowers in order to assess the level of damage by visual observation of the symptoms.

2.6Effect of inoculation on armyworm damage levels

Damage levels were assessed using the method described by Kombieni et al. [22]. It consisted in noting, by visual observation, the proportion of the leaf surface consumed or damaged by armyworm using the pre-established scale of Davis et al. [30], which ranges from 1 (no leaf damage) to 9 (severe leaf damage). This assessment was carried out 3 times on 10 randomly selected plants, at two-week intervals, starting on 15 DAS.

2.7Effect of inoculation on maize agronomic performances

At harvest, 20 maize plants per plot were selected. The length and circumference of each cob were measured individually using a tape measure. After drying in the sun, the cobs were dehulled and the weight of each batch, corresponding to each plot, was determined. The weight obtained per plot sample was then extrapolated to represent a density of 50,000 plants per hectare, in accordance with the cropping pattern adopted

2.8Data analysis

Data obtained were analysed using SAS software version 9.2 (SAS Institute 2005). In order to stabilise variances for statistical analysis, infestation and mycorrhization rates were transformed by the function $x' = \arcsin(\sqrt{p})$, with $p = x/100$; damage levels, plant heights, maize cob circumferences and lengths, and yields were transformed by the function $x' = \log(x + 1)$. Data on damage levels and plant infestation were compared using ANOVA (PROC GLM), and means were separated using the Student-Newman-Keuls (SNK) test at the 5% threshold.

3. RESULTS AND DISCUSSION

The results showed that over the whole trial, the plots fertilised with NPK and urea recorded the highest infestation rates. Plots treated with MYCOTRI -0.25kg/ha and 0.38kg/ha -

recorded the lowest armyworm infestation rates. The highest infestation rates, significantly ranging from 78% to 75% and 44% to 62%, were recorded from 24 to 38 DAS by the NPK + urea treatment and the absolute control respectively. On the other hand, infestation rates of 36% to 56% for MYCOTRI treatments and 44% to 54% for *Glomus sp.* treatments were obtained at the same dates after sowing (Fig. 1).

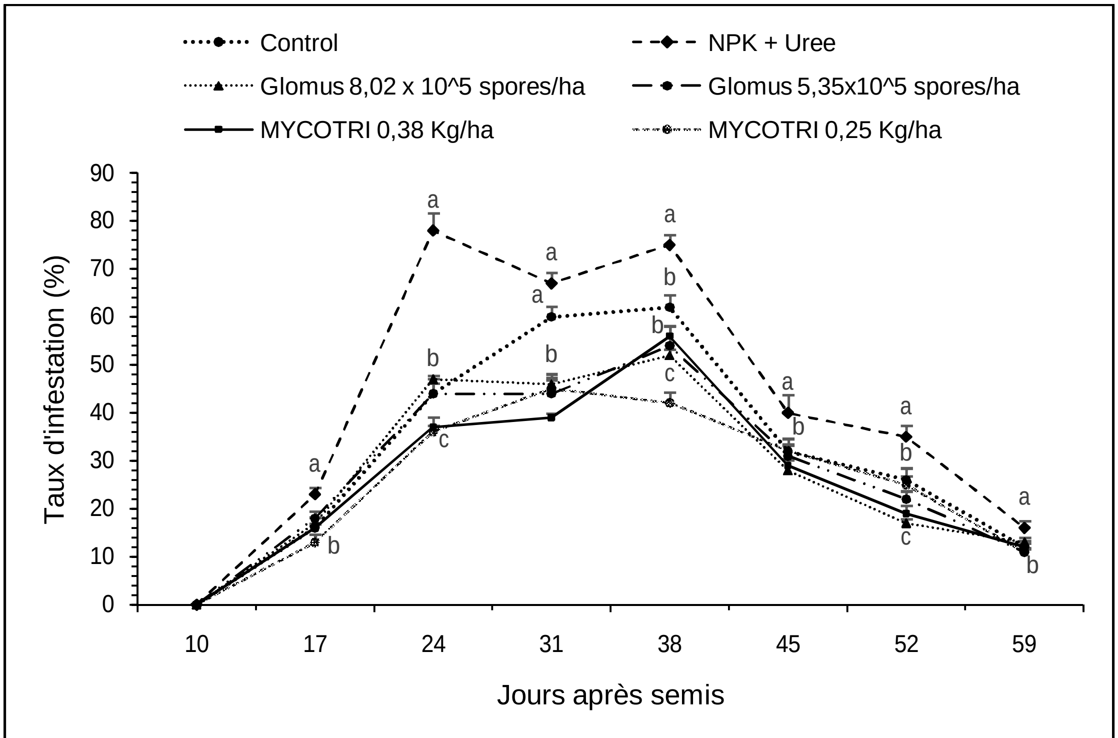

Fig. 1. Mean plant infestation rates by armyworm. Means of treatments with error bars affected by different letters are statistically different at the 5% threshold following the same day after sowing.

Plants treated with chemical fertilisers showed significantly greater damage, with the highest damage level score of 2.85 at 45 DAS. Damage scores on plots treated with biofertilisers varied between 1.23 ± 0.07 and 2.53 ± 0.04 from 15 to 45 DAS, statistically identical to those of the absolute control (1.16 ± 0.08 and 2.58 ± 0.11) (Fig. 2).

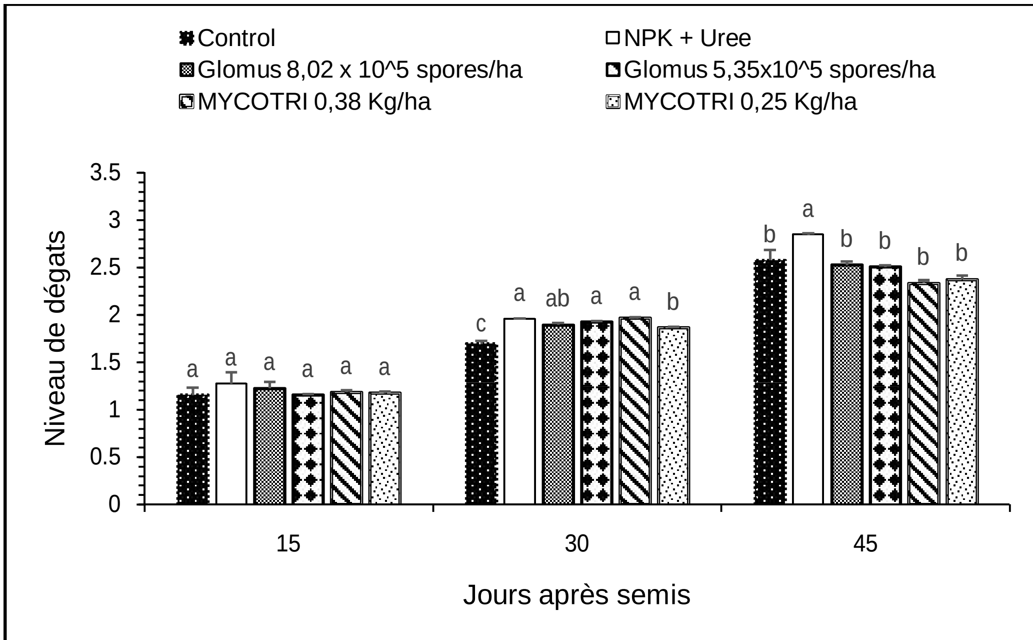

Fig. 2. Level of leaf damage in plants infested by armyworm. Means of treatments with error bars affected by different letters are statistically different at the 5% threshold following the same day after sowing.

The results illustrated in Fig. 3 showed that the difference in plant growth was significant between treatments, indicating that plants fertilised with NPK+Urea grew faster (P< 0.05), followed by plants mycorrhised with *Glomus sp.* Plots treated with MYCOTRI showed similar or even lower plant growth than the absolute control at certain dates after sowing. Mycorrhization of the roots resulted in the presence of vesicles and a network of arbuscules in the soil (Fig. 4).

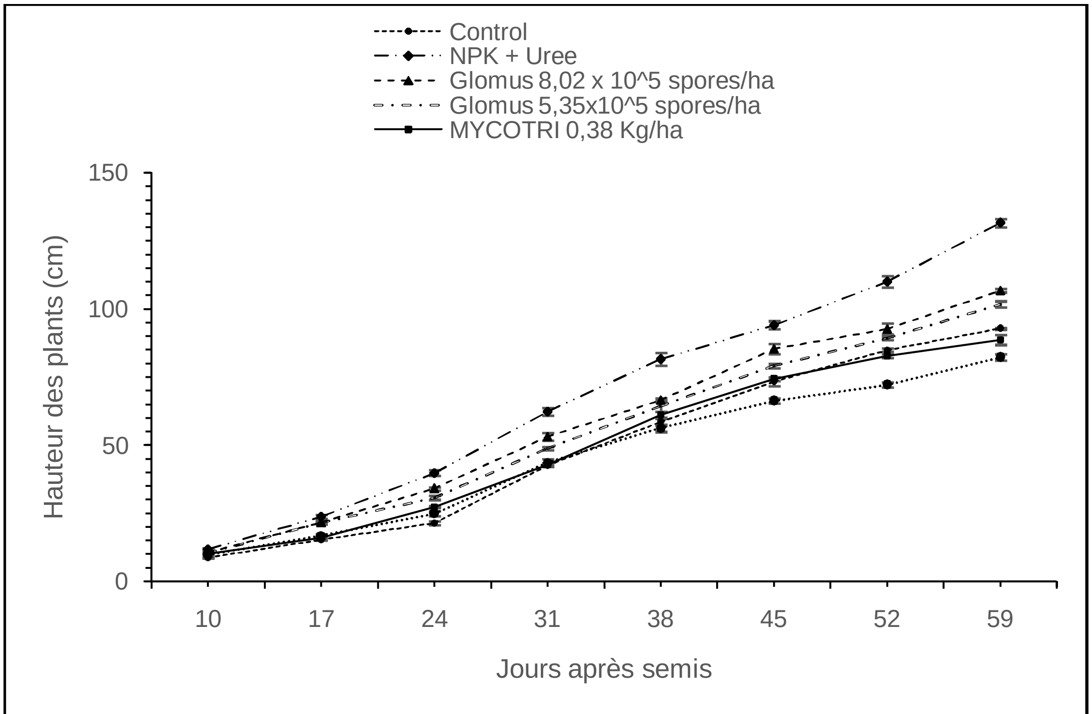

Fig. 3. Evolution of plant heights of treatments according to days after sowing.

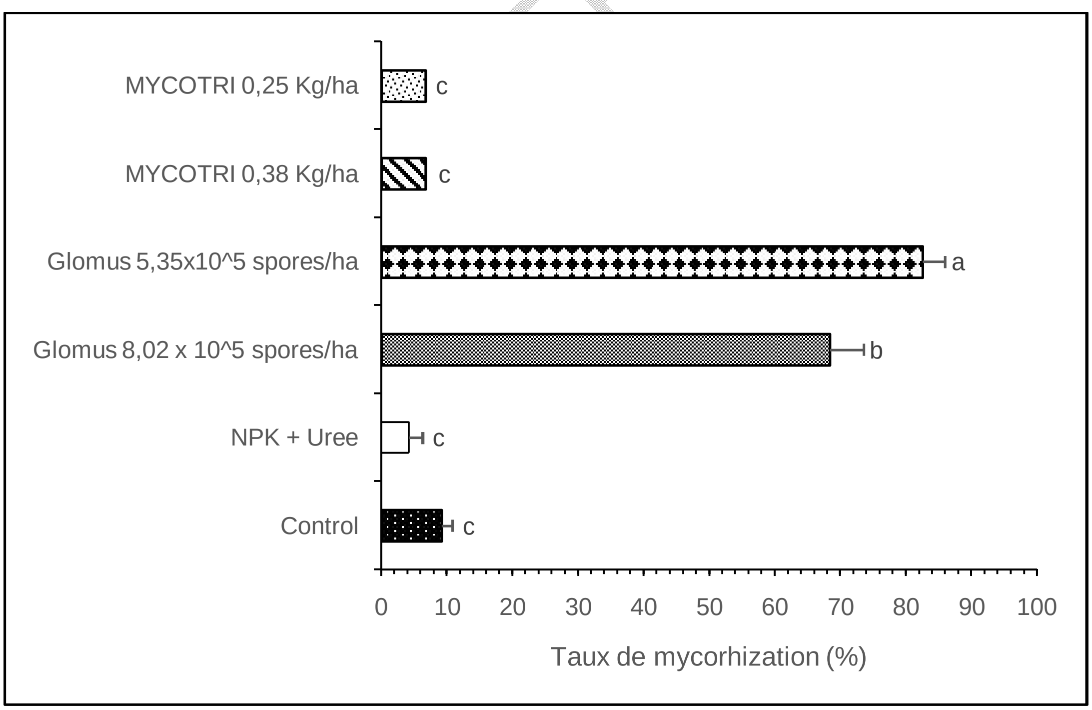

Fig. 4. Average mycorrhization rate of plants. Means of treatments with error bars affected by different letters are statistically different at the 5% threshold following the same day after sowing.

The best root mycorrhization rates of 82.5% and 68.33% were obtained with *Glomus sp* at 8.02 x 105 spores/ha and 5.35 x 105 spores/ha respectively (F=168.81; *P*< 0.0001) (Fig. 5).

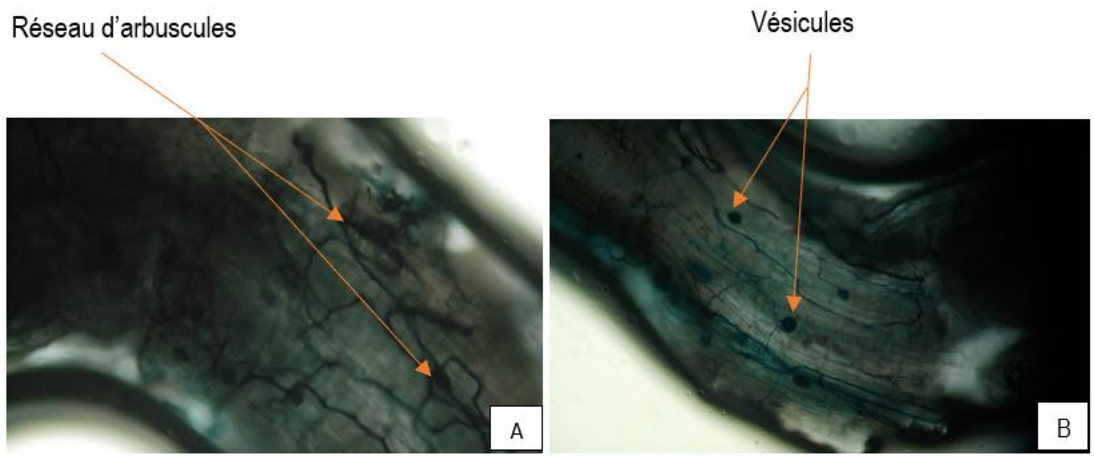

Fig. 5. Mycorrhizal status of maize

At harvest, spikes from fertilised plants were the longest (F=19.82; *P*<0.0001) and spike size was statistically identical between treatments (F=2.574; *P* = 0.0632) (Table 1). The average yields of the different treatments show that the best yield of 3.86 ± 0.12 t/ha was obtained with mineral fertilisation, followed by plots inoculated with *Glomus sp.* (3.00 ± 0.05 and 2.62 ± 0.15 t/ha), plots treated with MYCOTRI (2.44 ± 0.15 and 1.90 ± 0.14 t/ha) and the control (1.73 ± 0.19 t/ha).

Table 1. Effect of treatments on maize productivity parameters

Treatments	Ear size (cm)	Earcircumference (cm)	Yield (t/ha)
Control	9,72±0,90 b	11,22±0,64 a	1,73±0,19 d
NPK + Urée	14,34±0,87 a	13,11±0,82 a	3,86±0,12 a
*Glomus*_8,02 x 10^5 spores/ha	11,21±0,90 b	12,40±1,47 a	3,00±0,05 b
*Glomus*_5,35 x 10^5 spores/ha	11,33±0,66 b	11,78±0,52 a	2,62±0,15 cb
MYCOTRI 0,38 kg/ha	9,68±0,55 b	12,02±0,85 a	2,44±0,15 c
MYCOTRI 0,25 kg/ha	10,14±0,75 b	11,23±0,79 a	1,90±0,14 d
F	19,82	2,574	28,95
P	<0,0001	0,0632	<0,0001

Means followed by the same lower case letters in the same column are not significantly different at the 5% threshold.

4. DISCUSSION

The use of arbuscular mycorrhizal fungi in agriculture is an innovative method for coping with abiotic and biotic stress in crops [31-33]. The results show that CMAs (*Glomus sp*.) were able to reduce *S. frugiperda* infestations, thus providing an opportunity to integrate mycorrhizal inoculation into integrated armyworm management. Similar work by Gnamkoulamba et al. [25] and Sokame et al. [34] highlighted the effect of CMAs on the population dynamics and damage of insect pests. Indeed, Gnamkoulamba et al. [25] showed that biofertilisers did not prevent infestation but mycorrhised rice plants were less attacked by the armyworm. Similarly Sokame et al.[34] found that MACs significantly reduced pest damage to *S. macrocarpum* leaves and ensured better crop quality. Schenck and Kellan [35] showed that MACs do not directly affect the pest, but rather cause changes in host tissues, in particular the development of cell lignification, which can interfere with the normal consumption of leaves by caterpillars, which prefer tender leaves. Other authors such as Wardle et al. [36] and Bezemer and van Dam [37] have shown that microorganisms such as MACs contribute to the development of plant resistance and tolerance to insect attack. CMAs colonise plant roots, promoting the exchange of hexose sugars, phosphorus, nitrogen and water resources that are essential for plant resistance and tolerance to insect attack [38-41].Our study showed that plants fertilised with chemical fertiliser were consumed more by CLA. The CLA preference for these plants can be explained by the high concentrations of nutrients in the tissues of plants fertilised with chemical fertilisers[42,43]. Mineral fertilisation provides the plant with a greater quantity of rapidly accessible nutrients. Our results also show that mycorrhised plants have low levels of damage compared with chemical treatments. MCAs promote the induction of systemic defence responses in host plants, notably through the activation of hormonal signalling pathways (such as those for jasmonic acid and ethylene) involved in insect resistance [24]. Studies of Cameron et al. [44] show that mycorrhizal inoculation can also improve plant tolerance to biotic and abiotic stresses by increasing nutrient uptake and modifying the composition of root exudates, which influences root colonisation by insects. CMAs also influence interactions between plants and herbivorous insects by modifying characteristic plant traits [45-47]. Furthermore, Gange and West [48] concluded in their study that a reduction in mycorrhizal colonisation of plantain led to an increase in insect damage.

Maize grain yield is higher in mycorrhised plots than in absolute controls, which corroborates the results found by Fagbola et al. [49], Ananthakrishnan et al. [50], Tchabi et al. [51], Ibiremo et al. [52], Saïdou et al. [53], Proborini et al. [54], Johnson et al. [55], Aguegue et al. [56], and Haro et al. [57]. The supply of nutrients to the plant therefore enables it to express itself better in terms of productivity, as concluded by Ndonda[58] on cassava and Gnamkoulamba et al. [59] on rice. As MYCOTRI main function is to protect plants against telluric attacks, its fertiliser function seemed less important depending on the agronomic parameters. Nevertheless, its ability to accelerate the decomposition of organic matter and strengthen root development (according to the CRPCC/CHAMPIMIX designer, AGIDE-TOGO), would have resulted in better yields than the absolute controls.

5. CONCLUSION

This study demonstrated the bioprotective potential of *Glomus sp.* and MYCOTRI against the armyworm, the main maize pest in Togo. The results of this study showed that CMAs and MYCOTRI significantly reduced attacks by armyworms on maize plants. In view of the results, CMAs and MYCOTRI appear to be a reliable alternative to synthetic chemical pesticides for sustainable, environmentally-friendly production. Further work is needed, however, to assess the effect of combining CMAs and chemical fertilisers, and to investigate the presence of indigenous CMAs in the context of sustainable management of the armyworm in different agro-ecological zones of Togo.

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