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Effect of arbuscular mycorrhizal fungi on foliar feeding insect diversity and dynamic and on leaves damage incidence of *Solanum macrocarpum* L. in Togo

Effet des champignons mycorrhiziens arbusculaires sur la diversité et la dynamique des insectes phyllophages et l'incidence des dégâts sur les feuilles de *Solanum macrocarpum* L. au Togo

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Abstract

The present study has evaluated the potential effect of indigenous arbuscular mycorrhizal fungi (AMF) inoculation on the diversity and abundance of *Solanum macrocarpum* L. foliar feeding insects and their damage to leaves under field conditions. Seedlings inoculated with pure AMF isolates (*Funneliformis mosseae, Glomus Clarium* and *G. etunicatum*) or mixture indigenous AMF spores collected from *Solanum* sp. field, were transplanted six weeks after inoculation. The AMF inoculated seedling resulted in higher mycorrhizal colonisation of the plants. The most occurring insect species were *Bemisia tabaci* Gennadius (Hemiptera: Aleurodidae), *Empoasca* sp. Walsh (Hemiptera: Cicadellidae), *Syllepte derogata* Bsdv. (Lepidoptera: Noctuidae) and *Spodoptera littoralis* Fabricius (Lepidoptera: Crambidae) representing 97% of the total number of insects. Except *F. mosseae*, the AMF inoculation did not significantly affect the insect population diversity and dynamics. However, all the AMF strains significantly reduced pest damages on leaves, thus improving *S. macrocarpum* leaf quality. The results are discussed in term of using AMF strains as an alternative to chemical control for sustainable *S. macrocarpum* production.
***Keywords*:** Arbuscular mycorrhizal fungi, *Solanum macrocarpum*, insects pest control

Résumé

La présente étude a évalué l'effet potentiel de l'inoculation de champignons mycorhiziens à arbuscules (CMA) indigènes sur la diversité et l'abondance des insectes phyllophages et leurs incidences sur les feuilles de *Solanum macrocarpum* L. dans des conditions naturelles. Des plantules inoculées avec des isolats purs de CMA (*Funneliformis mosseae*, *Glomus Clarium* et *G. etunicatum*) ou un mélange de spores de CMA provenant des champs de *Solanum sp.*, ont été transplantées six semaines après l'inoculation. Le semis inoculé par l'AMF a entraîné une colonisation mycorhizienne plus élevée des plantes. Les espèces d'insectes les plus présentes étaient *Bemisia tabaci* Gennadius (Hemiptera: Aleurodidae), *Empoasca* sp. Walsh (Hemiptera: Cicadellidae), *Syllepte derogata* Bsdv. (Lepidoptera: Noctuidae) et *Spodoptera littoralis* Fabricius (Lepidoptera: Crambidae), représentant 97% du nombre total d'insectes. À l'exception de *F. mosseae*, l'inoculation de CMA n'a pas eu d'effet significatif sur la diversité et la dynamique des populations d'insectes. Cependant, toutes les souches de CMA ont réduit de manière significative les dommages causés par les ravageurs sur les feuilles, améliorant ainsi la qualité des feuilles de *S. macrocarpum*. Les résultats sont discutés en termes d'utilisation des souches de CMA comme alternative au contrôle chimique pour la production durable de *S. macrocarpum*.
Mots-clés: champignons mycorhiziens arbusculaires, *Solanum macrocarpum*, lutte contre les insectes

1. Introduction

In Sub-Saharan Africa, including Togo, urban agriculture has gained importance and an increase in cultivation area is observed during the last 20 years (Cofie et al. 2003). It is generating substantial revenue for smallholders and employment (Gockowski et al. 2003). The production of leafy vegetables represents an important part of urban agriculture and have increasingly become part of the daily diet of the population (Cofie et al. 2003). The consumption per capita and per year is estimated to 10kg with a daily consumption between 50 and 100g per capita (Rose and Richards 2004). Among these leafy vegetables, *S. macrocarpum* known as the African eggplant and called *gboma* in Togo, is highly valued and cultivated throughout the country for its nutritional importance (proteins and vitamins) (Uusiku et al. 2010).
Nevertheless, the leaves of *S. macrocarpum* are nowadays infested by several mites (Martin et al. 2010) and insects (Stephan et al. 2016) that affect the quality and the economic value of the crop, hence the consumer interest.
The management of *S. macrocarpum* foliar feeding insects is based mainly on the use of chemical products such as Organochlorines (DDT, Endrin), Organophosphates (Dimethoate, Profenofos, Malathion, Chlorpyrifos-ethyl, Acephate) and Pyrethroïds (Cypermethrin, Lambda-cyhalothrin, Deltamethrin) recorded by Agboyi et al. (2015) despite their negative effect on human health and environment (Aktar et al. 2009). The use of arbuscular mycorrhizal fungi (AMF) to control the foliar feeding pests has been suggested as a biological alternative to chemical control (Smith and Read 2008).
AMF species are reported to colonize the roots of over 80% of all plant crops (Bonfante-Fasolo 1987) and are considered as the most widespread symbionts in plants. The combination of plants and AMF generally increase plant resistance against its biotic and abiotic constraints (Vannette & Hunter 2009; Gianinazzi et al. 2010). Several studies reported that AMF induces resistance and increases tolerance to pest insects (Bennett and Bever 2007) and induces mineral uptake (Labidi et al. 2012) and plant development (Tchabi et al. 2016). In another study, the incidence of foliar feeding insect highly decreased when *Fragaria vesca* plants were inoculated with different strains of *Rhizophagus irregularis* (Roger et al. 2013). e.g. Barber et al. (2013) have reported 23% reduction using mixed spores inocula collected from conventional farms, organic farms, and a commercial AMF inculum (*Glomus intraradices*) at the inoculum spore density of 300 spores per potted plant. In addition, Gange and West (1994) in an interaction study between AMF and foliar-feeding insects in *Plantago lanceolate* L., have reported 70% of leaves damaged from control plants, compared with only 30% on inoculated potted plants. Nevertheless, there is a need for additional study on insect responses to inoculum of mycorrhizal fungi in a realistic field setting (Shaul et al. 1999).
The aim of the present study is to investigate the effect of different strains of AMF on the occurrence, diversity and dynamic of foliar feeding insects of *S. macrocarpum* and to evaluate the leaves damage impact. This is also a first report that describes the interaction between indigenous strains of AMF from Togo and the foliar feeding insects encountered of *S. macrocrpum*.

2. Materials and Methods

2.1. Experiment site description

The field experiment was conducted at the Research Centre of the Faculty of Agronomy, University of Lomé, Togo (6°10.563N and 1°12.782E). The site is characterized by Guinean climate with two rainy seasons, April to July and September to November with two dry seasons in between. The soil of the experimental site is classified as a ferralsol soil. The following surface (0-15cm) soil properties were found out by Laboratory of Soil and Chimical of Agronomy Faculty of University of Lomé after soil analysis: organic matter (OM) 1.87%; total N 0.15%; pH 6.50; available Phosphorus (P2O5) 0.5 mg/kg; Potassium (K2O) 0.46mg/kg and Magnesium (MgO) 0.01mg/kg.

2.2. Arbuscular Mycorrhizal Fungi (AMF) inocula and seeds inoculation

A total of eight inocula were used from which three (*F. mosseae*, *G. Clarium* and *G. etunicatum)* were isolated and identified as single spore culture at University of Basel, Botanical Institut while five were mixed spores inocula collected from the *Solanum* sp. field in different areas in Togo: Dapaong, Kara, Sokodé, Kpalimé and Baguida. The spores' morphotype were mainly of *Glomus* sp. and *Acaulospora* sp. and the inoculum spore densities were 256 spores/25g (Dapaong); 201 spores/25g (Kara); 301 spores/25g (Sokodé); 269 spores/25g (Kpalimé); 354 spores/25g (Baguida). The inocula were maintained in pot culture at International Institute of Tropical Agriculture (IITA), Benin Station for six months before being used as inoculum. The spore densities of three pure inocula culture, 479 spores/25g (*Funneliformis mosseae*), 300spores/25g (*Glomus clarium*) and 544 spores/25g (*G. etunicatum*) were used. Seeds inoculation was done during nursery period in plastic tanks (50×40×20cm) in the greenhouse. The substrate used for nursery consisted of soil from the arable land from the Experimental site and beach sand (w/w, 2:1). The soil was collected from a depth of 0-25cm and passed through a 1mm aperture sieve to remove roots and debris. The marine sand was

thoroughly washed with tap water to remove salt. The substrate mixture was oven sterilized at 80°C for 72h.
The tank filled with sterilized soil was watered and three stripes were made in the length direction of the plastic tank about 1cm deep as a seedbed. Thereafter, 50g of corresponding strain inoculum was spread in each stripe before putting the seeds and closed it with the sterilized marine sand. The control plastic tank had not received any AMF strain inoculum, but sterilized substrate used for inocula production. One plastic tank was used for each inoculum making in total, nine plastic tanks.

2.3. Experimental design at the field

The treatments were arranged in a completely randomized block design with nine treatments. Four repetitions were tested for each treatment. Each replicate consisted of one plot of 3×6m (18m^2). Six weeks old plants from the nursery were transplanted at 25×25cm in each plot. The number of plants per plot was 220. Every plot was separated by one-meter space as an edge. The plots were regularly watered and weeded until harvest. AMF roots colonisation was assessed two months after transplanting. The insect pest diversity, population dynamic, and insect damage on the leaves were assessed.

2.4. Assessment of AMF root colonisation and spore density

AMF root colonisation and spore density were assessed two months after transplanting at the field. The roots of five plants per treatment of each plot were randomly sampled, cleaned with tap water and conserved in the vials (capacity 48mL) containing tap water for the analysis. The AMF spores were isolated by wet sieving and sucrose density gradient centrifugation (Oehl et al., 2003). AMF root colonisation was determined according to Brundrett et al. (1996). A 1.0g subsample of the roots was excised from the five plants, to assess the percentage of AMF colonisation. At 90~ on a hot plate, the root samples were cleared in KOH (100g/l) for 1 h and stained with trypan blue (0.5g/l) in lactoglycerol (Kormanik and McGraw 1996) at 90~ for 30 min. Percentage colonisation of host plant roots was estimated by visual observations of stained root segments mounted in lactoglycerol by the grid-line intercept method (Giovannetti and Mosse 1980) by subtracting the initial weight of the filter paper from the weight of mycelia and filter paper.

2.5. Assessment of insect population diversity

Population diversity was determined by weekly collecting samples of insect specimens encountered with hoover, net-sweeping and beating sheet in each treatment plot throughout the cropping season.

The insect diversities were performed by categorizing the insects collected in orders, families and species. To achieve this classification, sampled adult insects were conserved in the vials (48 ml containing 70% ethanol) and larvae on fresh leaves in Petri dishes (150×20mm^2) and kept in the entomology and nematology laboratory at "Ecole Supérieure d'Agronomie, Université de Lomé". The larvae were reared until adult stage. The adult species were sent to International Centre of Insect Physiology and Ecology (ICIPE), Kenya for species identification by the taxonomists. Shannon–Weaver diversity index (H) was computed to consider both abundance and evenness of species present in the community in each treatment plot as follow (Magurran 1988):

$$H = \sum_{i=1}^{n} (Pi \times \ln(Pi)) \text{ , where } \quad Pi = \frac{Ni}{N}$$

H: Shannon diversity index, i: insect species; n: number of insect species; N: total number of all insect species collected in a particular treatment plot; N_i: total number of individual insect species in the same treatment plot; ln: natural logarithm.

2.6. Assessment of insect population development

Thirty days (considered as day 0 for insects counting) after transplanting, the plants were carefully inspected for the occurrence of the insect pests. Insect counting was focused on the species which were mainly represented. The assessment was done each week from the thirty days until the last harvest of the leaves. Therefore, ten plants were randomly selected per plot each week and checked thoroughly for the occurrence of insects counted according to the species. In addition, each species was recorded after 5 to 6 sampling periods so that the data collected on each treatment plot could be representative of the community and may be used in species abundance and diversity studies.
Since the density of insect populations in treated plots depends not only on treatments but also on natural occurring changes, we calculated corrected percentage efficiency of each AMF strain on insect density reduction according to modified Henderson and Tilton's formula:

$$\%Efficacy = \left[1 - \left(\frac{T_a}{T_b}\right) \times \left(\frac{C_b}{C_a}\right)\right] \times 100$$

With $T_b = C_b$;

$$\%Efficacy = \left[1 - \left(\frac{T_a}{C_a}\right)\right] \times 100$$

where T_b and C_b represent densities before inoculation in treated and control plots and T_a and C_a represent densities after inoculation in treated and control plots respectively (Henderson and Tilton 1955).

2.7. Assessment of insect damages incidence on leaves

The insect damages were evaluated through the percentage of the number of plants injured (Incidence at plot level) and number of leaves damaged per plant (Incidence at plant level) in each treatment plot. For the evaluation, ten plants were biweekly and randomly sampled throughout the plots before every harvest of leaves and damaged leaves were considered as leaves perforated or rolled by insects. Leaf chlorosis and leaf withering were also considered for piercing and sucking insects damages. The parameters were calculated as follow:

$$I_1(\%) = \frac{PI}{NP} \times 100 \quad \text{and} \quad I_2(\%) = \frac{LD}{NL} \times 100$$

I1 = Incidence at plot level, *PI* = number of plants injured, *NP* = total number of plants sampled, *I2* = Incidence at plant level, *LD* = number of leaves damaged, *NL* = total number of leaves per plant.

2.8. Statistical analysis of the data

The data collected were analyzed with the Statistical Package for Social Science (SPSS version 20.0, GLM procedure) by analysis of variance (ANOVA) in which significant differences were observed between treatments whenever the 95% confidence limits failed to overlap, and the means were discriminated using the Student-Newman-Keuls test. Densities data and percentages were respectively transformed into x'=log10(x+1) and Arcsinus √ (percent x/100) before analysis for data standardization, x is the number of insects (Gomez and Gomez 1984). A t-test was used to compare the diversity index between the treatments through R statistic software, version 3.3.2 and all figures were created using SIGMAPLOT version 8.0 for Windows.

3. Results

3.1. Arbuscular mycorhizal fungi root colonisation and spore density

Roots mycorhization was recorded in all treatments including control (Table 1) with significantly higher AMF colonisation rate (F = 29.36; df = 8; $P \leq 0.0001$) from inoculated plants compared to the control plants. The highest rates were obtained with *F. mosseae* and Kara strain (45.53%; 46.04% respectively) and the lowest mycorrhization was obtained with Kpalimé strain. For spore production, AMF spore density was significantly higher (F = 5.20; df = 8; P = 0.01) in the AMF inoculated plants compared to the control. Within AMF inoculated treatments, the spore density was significantly higher with plants inoculated with *G. Clarium* compared to other inoculated plants (Table 1).

Table 1: AMF colonisation and spore density of *S. macrocarpum* roots

Mycorrhizal strains	AMF root colonisation	AMF spores density
Dapaong strain	32.06 ± 1.34 **bc**	9.33 ± 2.18 **a**
Kara strain	46.04 ± 25.35 **a**	20.00 ± 2.64 **a**
Sokodé strain	38.77 ± 5.30 **ab**	14.67 ± 3.71 **a**
Kpalimé strain	25.35 ± 2.59 **c**	10.33 ± 5.89 **a**
Baguida strain	31.98 ± 2.03 **bc**	8.67 ± 2.96 **a**
G. etunicatum	31.04 ± 2.28 **ab**	6.67 ± 2.73 **a**
G. Clarium	40.87 ± 1.91 a**b**	45.00 ± 19.73 **b**
F. mosseae	45.53 ± 0.35 **a**	14.00 ± 4.62 a
Control	5.39 ±1.72 **d**	1.33 ± 0.88 **c**
P	≤ 0.0001	0.002
F	29.364	5.201
df	8	8

Means in the same column followed by the same letters did not differ significantly (Student-Newman-Keuls, $P < 0.05$).

3.2. Insects population diversity

Overall, 4,471 insects, belonging to 12 species, 12 families and 5 orders were collected on *S. macrocarpum* throughout the cropping season, (Table 2). The most important species were *Bemisia tabaci* Gennadius [Hemiptera: Aleurodidae] (47.43%); *Empoasca* sp. Walsh [Hemiptera: Cicadellidae] (41.71%); *Spodoptera littoralis* Bsdv. [Lepidoptera: Noctuidae] (6.37%) *and Syllepte derogata* Fabricius [Lepidoptera: Crambidae] (1.81%). The occurrence of all other species was about 2.68%. The highest percentage of insects was collected from the control plot (12.82%) while the lowest percentage of insects was collected from plants inoculated with *F. mosseae* (8.45%) (Table 2). Furthermore, the Shannon diversity index (H) showed that plants inoculated with *F. mosseae* presented a significant lower diversity of insect species compared to control ($t_{9.77}$ = 2.76; P = 0.02) (Table 3).

Table 2: Species present (as number and percentage of total insects collected from the nine treatments plots on *S. macrocarpum*) during the cropping season at the agricultural research station of Lomé, southern Togo

Order	Family	Species	Treatments									Total
			Dapaong strain	Kara strain	Sokodé strain	Kpalimé strain	Baguida strain	*G. etunicatum*	*G. Clarium*	*F. mosseae*	Control	
Hemiptera	Aleurodidae	*Bemisia tabaci*	246 (50.40)	248 (43.89)	253 (53.94)	187 (41.28)	220 (44.00)	229 (44.37)	275 (51.98)	181 (47.63)	282 (49.21)	2121 (47.43)
	Pseudococcidae	*Pseudococcus* sp.	4 (0.81)	2 (0.35)	1 (0.21)	3 (0.66)	1 (0.20)	0 (0.00)	2 (0.37)	0 (0.00)	6 (1.04)	19 (0.42)
	Cicadelidae	*Empoasca* sp.	174 (35.65)	245 (43.36)	158 (33.69)	228 (50.33)	244 (48.80)	225 (43.16)	204 (38.56)	181 (47.63)	206 (35.95)	1865 (41.71)
	Pyrrhoridae	*Dysdercus* sp.	3 (0.61)	4 (0.70)	1 (0.21)	1 (0.22)	5 (1.00)	3 (0.58)	0 (0.00)	1 (0.26)	3 (0.52)	21 (0.47)
	Aphididae	*Aphis craccivora*	0 (0.00)	0 (0.00)	10 (2.13)	0 (0.00)	0 (0.00)	3 (0.58)	0 (0.00)	5 (0.13)	0 (0.00)	18 (0.40)
Lepidoptera	Crambidae	*Syllepte derogata*	5 (1.02)	12 (2.12)	11 (2.34)	10 (2.20)	5 (1.00)	8 (1.55)	8 (1.51)	7 (0.18)	15 (2.61)	81 (1.81)
	Noctuidae	*Spodoptera littoralis*	48 (9.83)	47 (8.31)	24 (5.11)	18 (3.97)	24 (4.80)	41 (7.94)	33 (6.28)	0 (0.00)	50 (8.72)	285 (6.37)
Orthoptera	Pyrgomorphidae	*Zonocerus variegatus*	3 (0.61)	0 (0.00)	4 (0.85)	2 (0.44)	0 (0.00)	1 (0.19)	2 (0.37)	0 (0.00)	3 (0.52)	15 (0.34)
Diptera	Diopsidae	*Diopsis* sp.	2 (0.40)	1 (0.17)	3 (0.64)	1 (0.22)	0 (0.00)	3 (0.58)	0 (0.00)	1 (0.26)	2 (0.34)	13 (0.29)
Coleoptera	Meloidae	*Meloe* sp.	0 (0.00)	3 (0.53)	1 (0.21)	0 (0.00)	0 (0.00)	2 (0.38)	0 (0.00)	1 (0.26)	1 (0.17)	8 (0.18)
	Chrysomelidae	*Podagrica* sp.	1 (0.20)	3 (0.53)	2 (0.42)	1 (0.22)	1 (0.20)	0 (0.00)	3 (0.56)	0 (0.00)	4 (0.69)	15 (0.34)
	Coccinellidae	*Chelomenes* sp.	2 (0.40)	0 (0.00)	1 (0.21)	2 (0.44)	0 (0.00)	1 (0.19)	2 (0.37)	1 (0.26)	1 (0.17)	10 (0.22)

Table 3: Shannon diversity index (H) comparison between insect species type within AMF strains and control plots

Treatments	Dapaong strain		Kara strain		Sokodé strain		Kpalimé strain		Baguida strain		*G. etunicatum*		*G. Clarium*		*F. mosseae*		H
	df	t	*df*	t	*df*	t	*df*	t	*df*	t	*df*	t	*df*	t	*df*	t	
Control	8.12	0.326^{ns}	9.25	0.803^{ns}	8.10	0.855^{ns}	9.85	1.390^{ns}	9.91	-1.884^{ns}	8.89	0.647^{ns}	8.56	-1.272^{ns}	9.77	2.761*	1.04
Dapaong strain			6.88	0.658^{ns}	6.20	0.725^{ns}	7.55	1.347^{ns}	7.68	-1.938^{ns}	6.63	0.484^{ns}	8.12	0.326^{ns}	8.89	3.126*	1.00
Kara strain					9.49	0.156^{ns}	9.72	0.431^{ns}	9.63	-0.840^{ns}	9.94	0.104^{ns}	9.80	-0.487^{ns}	8.50	-1.442^{ns}	0.89
Sokodé strain							8.71	-0.205	8.57	-0.553^{ns}	9.75	0.244^{ns}	9.91	-0.287^{ns}	7.40	-1.029^{ns}	0.85
Kpalimé strain									9.99	-0.443^{ns}	9.45	0.524^{ns}	9.16	-0.125^{ns}	9.31	-1.082^{ns}	0.80
Baguida strain											9.33	-0.915^{ns}	9.03	-0.242^{ns}	9.44	0.601^{ns}	0.72
Glomus etunicatum													9.95	-0.569^{ns}	8.13	-1.487^{ns}	0.91
G. Clarium															7.81	-0.733^{ns}	0.77
F. mosseae																	0.62
Total																	0.84

*H : Shannon index. Signif. Codes: 0.001'***' ; 0.01'**' ; 0.05'*' ; no significant "ns"*

3.3. Monitoring of insects' population development

Population development was focused on *B. tabaci*, *Empoasca* sp., *S. littoralis* and *S. derogata* species which were mainly represented in the population structure (Fig. 1, 2, 3 and 4). Overall, the number of *B. tabaci* recorded on *S. macrocarpum* plants during the observation period did not vary significantly among treatments (F = 1.42; df = 8; P = 0.18). However, considering the date of

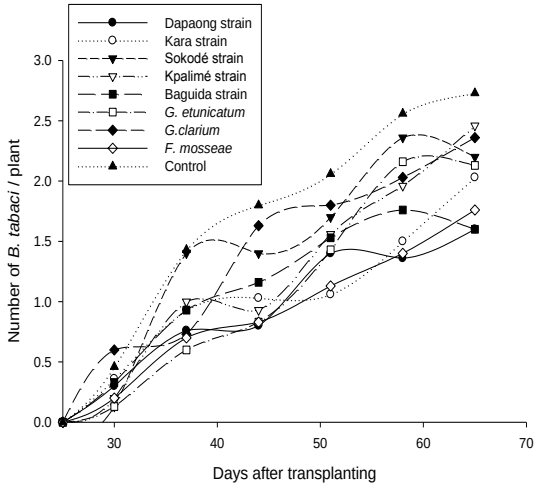

Figure 1: Mean count of *Bemisia tabaci* per plant over a period of 65 days in untreated plots, plots treated with different strains of arbuscular mycorrhizal fungi.

assessment, the numbers of *B. tabaci* recorded at 58 days after transplanting (DAT) was significantly higher on plants inoculated with *F. mosseae*, *G. etunicatum* and Dapaong strain compared to uninoculated control plants (F = 2.24; df = 8; P = 0.02).

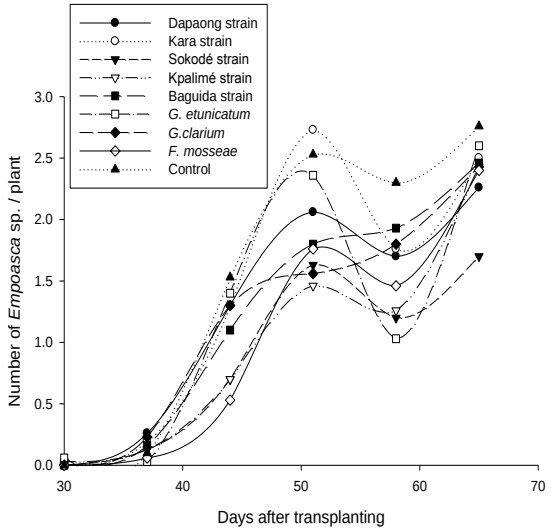

Figure 2: Mean count of *Empoasca* sp. per plant over a period of 65 days in untreated plots, plots treated with different strains of arbuscular mycorrhizal fungi.

The interactions between the treatments and DAT were significant at 51 DAT (F = 1.98; df = 40; P = 0.04) only for mixture inoculum from Dapaong.

Overall number of *Empoasca* sp. on *S. macrocarpum* was significantly lower in plant inoculated with mixture inoculum from Sokodé compared to mixture inoculum from Kara and Baguida and the control plants (F = 2.69; df = 8; P = 0.01). However, the numbers of *Empoasca* sp.

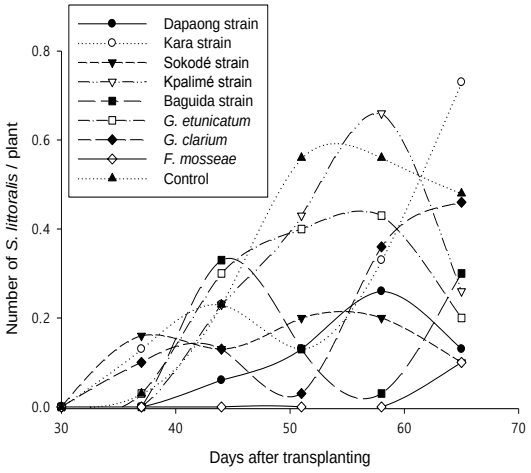

Figure 3: Mean count of *Spodoptera littoralis* per plant over a period of 65 days in untreated plots, plots treated with different strains of arbuscular mycorrhizal fungi.

recorded at each day after transplanting (DAT), was increased significantly from 37 to 65 DAT (F = 104.85; df = 5; P < 0.0001). The highest number of *Empoasca* sp. was recorded at 65 DAT. The interactions between treatments and DAT were significant (F = 0.77; df = 40; P = 0.84).

The number of *S. derogata* and *S. littoralis* populations was low in all the treatments. No significant differences were observed in the number of *S. derogata* larvae recorded in control plants compared to the inoculated plants (F = 1.85; df = 8; P = 0.06). Furthermore, there was no significant differences observed between DAT (F = 2.44; df = 3; P = 0.06) and their interactions with the treatments (F = 0.49; df = 24; P = 0.98). Similar results were observed for *S. littoralis.*

No significant difference was found between treatments (F = 1.41; df = 8; P = 0.18), DAT (F = 2.27; df = 4; P = 0.05) and their interactions with the treatments (F = 0.66; df = 32; P = 0.92).

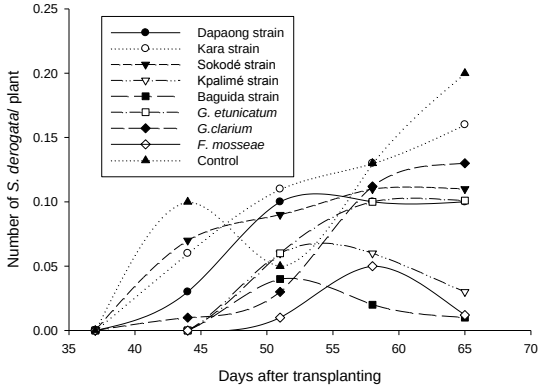

Figure 4: Mean count of *Sylepte derogata* per plant over a period of 65 days in untreated plots, plots treated with different strains of arbuscular mycorrhizal fungi.

Table 4: Corrected densities of *B. tabaci*, *Empoasca sp.*, *S. littoralis* and *S. derogata* based on counts on 30 days and subsequently at 7-day intervals until the 65th day using a modified Henderson and Tilton's formula (1955)

Mycorrhizal strains	Main insect species in the population structure			
	B. tabaci	*Empoasca sp.*	*S. littoralis*	*S. derogata*
Dapaong strain	42.91 ± 3.54 **ab**	15.56 ± 4.29 **a**	75.43± 7.38 **ab**	35.76 ± 15.31 **a**
Kara strain	35.84 ± 4.25 **ab**	9.58 ± 4.50 **a**	23.57± 15.49 **a**	15.00 ± 9.57 **a**
Sokodé strain	23.46 ± 10.14 **ab**	35.21 ± 9.41 **a**	50.24 ± 13.78 **ab**	22.59 ± 9.65 **a**
Kpalimé strain	32.08 ± 7.05 **ab**	30.74 ± 10.47 **a**	33.80 ± 18.60 **a**	59.71 ± 22.10 **a**
Baguida strain	32.85 ± 2.30 **ab**	16.78 ± 5.43 **a**	61.78 ± 18.93 **ab**	74.90 ± 18.57 **a**
G. etunicatum	41.97 ± 9.14 **ab**	29.24 ± 13.82 **a**	22.02± 10.79 **a**	50.83 ± 20.43 **a**
G. clarium	17.54 ± 6.85 a	17.41 ± 6.30 a	35.60 ± 17.03 a	47.01 ± 14.76 a
F. mosseae	47.90 ± 3.09 b	37.07± 8.45 a	95.83 ± 4.16 b	83.88 ± 8.54 a
F	2.869	1.185	3.326	2.029
P	0.015	0.338	0.008	0.092
df	8	8	8	8

Percentage efficiency of AMF strain inoculation are summarized in Table 4. Treatment efficacy differed significantly on *B. tabaci* and *S. littoralis* (F = 2.86; df = 7; P = 0.01 / F = 3.32; df = 7; P = 0.01), with *F. mosseae* inducing the greatest population reduction followed by Dapaong strain.

No differences were observed between treatments efficacy on *Empoasca sp.* and *S. derogata* (Table 4).

Means in the same column followed by the same letters did not differ significantly (Student-Newman-Keuls, $P < 0.05$).

3.4. Effect of AMF inoculation on leaves damage incidence

The data of leaves damage incidence of insect attacks are presented in tables 5 and 6. The percentage of plants attacked was significantly higher in uninoculated control plants compared to inoculated plants while the lowest was recorded on *G. etunicatum* plot (Table 5). Considering the DAT, the highest incidence on plants were recorded at 75 DAT compared to the other assessment days (F = 24.44; df = 4; P < 0.0001).

The percentage of leaves attacked was significantly lower in inoculated plots compared to uninoculated control (Table 6). No significant difference was recorded considering the DAT. The interactions between the treatments and DAT were also not significant (F = 0.23; df = 24; P = 0.90).

Table 5: Impact of AMF on incidence at plot level of foliar feeding insect damages on *S. macrocarpum* during cropping season

Mycorrhizal strains	Days After Transplanting (DAT)					Mean
	30	45	60	75	90	
Dapaong strain	16.67 ± 3.83 **ab**	53.33 ± 6.67 **a**	53.33 ± 6.67 **a**	60.00 ± 3.33 **a**	56.67 ± 8.81 **a**	48.00 ± 3.05 **a**
Kara strain	40.00 ± 5.77 **bc**	56.67 ± 3.33 **a**	60.00 ± 11.54 **a**	60.00 ± 5.77 **a**	56.67 ± 8.81 **a**	54.67 ± 6.36 **a**
Sokodé strain	43.33 ± 8.81 **c**	56.67 ± 8.82 **a**	53.33 ± 3.33 **a**	53.33 ± 6.67 **a**	66.67 ± 3.33 **a**	54.67 ± 4.67 **a**
Kpalimé strain	20.00 ± 5.77 **ab**	46.67 ± 3.33 **a**	46.67 ± 3.33 **a**	46.67 ± 3.33 **a**	60.67 ± 5.77 **a**	44.00 ± 2.05 **a**
Baguida strain	13.33 ± 3.33 **ab**	40.00 ± 5.77 **a**	40.00 ± 5.77 **a**	40.00 ± 5.77 **a**	53.33 ± 6.67 **a**	37.33 ± 4.37 **a**
G. etunicatum	15.00 ± 5.00 **ab**	33.33 ± 8.81 **a**	40.00 ± 10.00 **a**	33.33 ± 16.67 **a**	50.00 ± 5.77 **a**	34.33 ± 6.69 **a**
G. clarium	36.67 ± 8.81 **bc**	53.33 ± 12.01 **a**	40.00 ± 5.77 **a**	43.33 ± 8.81 **a**	60.00 ± 5.77 **a**	46.67 ± 4.80 **a**
F. mosseae	11.67 ± 1.67 **a**	36.00 ± 8.81 **a**	40.00 ± 5.77 **a**	43.67 ± 8.81 **a**	53.33 ± 6.67 **a**	37.00 ± 4.72 **a**
Control	71.67 ± 7.26 **d**	88.33 ± 4.40 **b**	91.67 ± 4.40 **b**	90.00 ± 5.77 **b**	88.33 ± 6.01 **b**	86.00 ± 3.60 **b**
F	10.98	4.80	6.02	4.28	3.03	12.39
P	< 0.0001	0.003	0.001	0.005	0.024	< 0.0001
df	8	8	8	8	8	8

Means in the same column followed by the same letters did not differ significantly (Student-Newman-Keuls, $P < 0.05$)

Table 6: Impact of AMF on incidence at plant level of foliar feeding insect damages on *S. macrocarpum* during cropping season

Mycorrhizal strains	Days After Transplanting (DAT)				Mean
	30	45	60	75	
Dapaong strain	24.61 ± 0.36 **a**	25.33 ± 0.65 **a**	24.30 ± 1.30 **a**	24.87 ± 1.41 **a**	24.78 ± 1.14 **a**
Kara strain	24.96 ± 1.56 **a**	25.23 ± 1.50 **a**	27.43 ± 1.93 **a**	29.85 ± 1.89 **a**	26.87 ± 1.93 **a**
Sokodé strain	25.05 ± 1.12 **a**	24.85 ± 1.71 **a**	25.38 ± 0.06 **a**	27.11 ± 1.12 **a**	25.59 ± 1.64 **a**
Kpalimé strain	25.22 ± 2.21 **a**	25.42 ± 1.78 **a**	25.42 ± 2.46 **a**	25.52 ± 1.46 **a**	25.39 ± 1.70 **a**
Baguida strain	23.36 ± 1.17 **a**	22.92 ± 1.22 **a**	23.75 ± 0.82 **a**	24.96 ± 1.72 **a**	24.75 ± 1.22 **a**
G. etunicatum	25.32 ± 2.13 **a**	24.39 ± 3.16 **a**	25.35 ± 0.65 **a**	23.76 ± 2,54 **a**	24.71 ± 1.87 **a**
G. clarium	23.47 ± 3.46 **a**	27.60 ± 2.69 **a**	26.25 ± 3.42 **a**	27.17 ± 1.42 **a**	26.12 ± 1.97 **a**
F. mosseae	23.71 ± 1.10 **a**	24.79 ± 1.15 **a**	24.79 ± 1.27 **a**	26.37 ± 0.48 **a**	24.92 ± 1.00 **a**
Control	40.62 ± 1.62 **b**	44.05 ± 2.05 **b**	42.14 ± 1.97 **b**	43.81 ± 2.03 **b**	42.65 ± 1.52 **b**
F	9.80	12.02	10.35	14.67	18.83
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
df	8	8	8	8	8

4. Discussion

The Arbuscular Mycorrhizal Fungi (AMF) colonisation of *S. macrocapum* root was observed in all plot treatments including control plot. Plant species from the family of Solanacea (e.g., *Solanum* sp.) are generally susceptible to AMF (Harikumar et al. 2014). The AMF colonisation observed in control plot may have explained the agricultural soils supporting of an active indigenous AMF community. Bissadou et al. (2012) have reported similar results in tomato (*Lycopercicon esculentum* Mill.) planted in the same field conditions showing AMF root colonisation in the non-inoculated plants.

In the present study, the insect community structure recorded in all of the treatments was very close. However, their abundance distribution differed significantly between species. Other field studies have also showed that the biological control of herbivore insects resulted on differences on insect species abundance (Altieri 1999; Tounou et al. 2008). For instance, numerical domination of *B. tabaci*, *Empoasca* sp., *S. littoralis* and *S. derogata* populations were more obvious regardless the treatments. This variation in distribution amongst communities with close species composition might be a consequence of certain factors such as the availability of food resources, constraints of natural enemies, interspecific competitions, ecology habitat and evolution of competitive interactions (Altieri 1999).

Concerning the insect population development, our study showed that there is no significant difference between the DAT and their interactions with treatments. The present result reveals a lower effectiveness of the repellent effect of AMF at higher leaf feeding pest densities. Other field studies indicated that plant roots colonization by arbuscular mycorrhizal (AM) fungi has minor (Roger et al. 2013) or no insecticides effect on herbivory of foliar-feeding insects (Gange & West 1994). Pacovsky et al. (1985) showed that the AMF colonisation of sorghum has no effect on reproductive behaviour by the aphid *Schizaphis graminum* Rondani (Hemiptera: Aphididae).

Although the AMF strains used have no significant reducing effects on leaf-feeding insect population diversity and abundance, they reduced significantly the levels of incidence and severity attacks of these insects. The present findings can be explained by an increase in tolerance or resistance of plants to insect attack due to AMF root colonisation (Bennett et al. 2007). Cely et al. (2016) demonstrated that AMF in symbiosis with plant alters the quality of food for the next higher trophic level. This type of indirect effect, where one organism modifies the relationship between two other organisms, is referred to as "interaction modification" (Wootton 1994). Other studies on the effect of AMF on plant-herbivore relations showed that nature of interaction modification depends on the environment and more organisms engaged in the interaction (Gange et al. 1994; Gange and West 1994). Lin and Kogan (1990) found that performance of Mexican bean beetle larvae *Epilachna varivestis* Mulsant (Coleoptera: Coccinellidae) is more sensitive to induce resistance in soybean than is the performance of soybean looper caterpillars *Pseudoplusia includens* Walker (Lepidoptera: Noctuidae). Compared to leaf-chewing insects, Mexican bean beetles ingest relatively little structural tissue as they scrape the leaf surface to release juices. Insects that feed primarily on plant juices may be more influenced by factors that alter concentrations of soluble nutrients and toxins than are insects that process a great deal of structural tissue.

AMF fungi may also exert other types of indirect effects. For example, fungus and herbivore both consume

photosynthates and so may engage in exploitative competition. If photosynthates are limiting, increased attacks would reduce the amount of photosynthates available to the AMF. Reduced AMF colonisation with grazing is not uncommon (Gehring and Whitham 1994); consistent with the hypothesis that AMF and herbivores compete. At the same time, colonisation by AMF may increase primary productivity and thus benefit herbivores by increasing the amount of food available. Indirect effects of AMF colonisation on herbivore performance may be complex. If there is a general trend in the effects of AMF colonisation on plant-herbivore relations, it will not be apparent until more fungus-plant-herbivore systems are examined over a range of environmental conditions.

5. Conclusion

This study has shown that the inoculation of *S. macrocarpum*, a leafy vegetable crop, with indigenous AMF should be considered as an alternative solution to chemical control to improve leaves quality as each strain of AMF used increased plants tolerance to insect pests related to leaves attacks.

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Conflict

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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