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Action of Black Soldier Fly Frass Fertilizer and Arbuscular Mycorrhizal Fungi on Growth Enhancement, Disease Expression Control, and Pest Attacks in *Lycopersicon esculentum* L. Plants

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Abstract The overall objective of this work was to apply beneficial fertilizers to improve growth in *L. esculentum* while evaluating their impact on *Fusarium oxysporum* and pest attacks. The work was conducted under two conditions (in greenhouses and outdoors). The Rio Grande and Kéro F1 varieties of *L. esculentum* were used. The experimental design was a complete block with four treatments applied, control, NPK, black soldier fly frass fertilizer (BSFFF), and arbuscular mycorrhizal fungi. Agronomic growth parameters, disease incidence and severity, and the various pests responsible for the attacks were evaluated. In the presence of the BSFFF treatment, an average height increase of *L. esculentum* plants of 30% was observed for the Kéro F1 variety in greenhouses and 19% for the Rio Grande variety (outdoors) compared to the controls. Similarly, the values of the parameters diameters at the collar, 48.44% for Rio Grande (greenhouse) and 17% for Kero F1 (outside greenhouse); leaf area, 45% for Rio Grande (greenhouse) and 60% for Rio Grande (outside greenhouse), are also influenced by BSFFF compared to the controls. In the greenhouse and outside the greenhouse, plants inoculated with AMF presented a colonization rate of 70% and 60% compared to the controls. The evaluation of the effect of BSFFF and AMF on plant health showed that the development of the disease occurs according to the treatments. There is a significant reduction in the severity of the diseases in plants having received AMF and BSFFF treatment compared to the controls, with respectively -15% and -7% (greenhouse) and -16% and -8% (outside greenhouse). This reduction appears more remarkable on *Fusarium* wilt caused by *F. oxysporum*. In view of these results, the use of BSFFF and AMF could be an avenue to explore in the context of soil fertilization and plant protection during the cultivation of *L. esculentum*.

Keywords: *Lycopersicon esculentum, black soldier fly frass fertilizer, arbuscular mycorrhizal fungi, Fusarium oxysporum, pest attack*

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1. Introduction

Lycopersicon esculentum (L.H. Karst.) is a vegetable grown worldwide for its edible fruits. According to FAO data [1], global production is estimated at 120 million tons, covering an area of approximately 5 million hectares [2]. Cameroon ranks 22nd with an estimated production of 1,090,212.34 tons, representing a 12.55% decrease from the previous year for an area of 86,553 hectares [2]. This widely consumed plant represents the world's second most important food resource after cereals. *L. esculentum* fruits are known to be a good source of phytochemicals and nutrients such as lycopene, potassium, iron, folate, and vitamin C [3,4]. Lycopene, present in the fruits, is a natural antioxidant that can help fight different types of cancer, including prostate, breast, lung, stomach, colorectal, oral, esophageal, pancreatic, bladder, cervical, and ovarian cancers [5,6]. However, despite its importance, the

cultivation of this vegetable crop not only has a high demand for fertilizers but also faces many constraints. These constraints include, among others, poor agricultural practices, declining soil fertility due to lack of fallow land, parasitic attacks of bacterial and fungal origin, and also attacks by insects, nematodes, and other crop enemies [7,8].

L. esculentum plants are almost entirely fertilized using chemical fertilizers. However, their low persistence over time not only contributes to environmental pollution but also presents risks to human and animal health. Today, the challenge is to propose solutions that tend to preserve the health of humans, animals, soils, and the environment. Plant fertilization methods using arbuscular mycorrhizal fungi and the droppings of *Hermitia illucens* larvae (black soldier flies or BSF), also called entomocompost or black soldier fly frass fertilizer (BSFFF), are increasingly being explored. It is also known that arbuscular mycorrhizal fungi are valued for several of their properties. They contribute to nutrition and improved growth in plants; influence soil enzymatic activities while promoting microbial activity; and are involved in defense in plants [9,10,11,12,13]. As for the use of *Hermitia illucens* lava droppings, authors mention that its application as an organic fertilizer contributes to improving plant growth thanks to its richness in black soldier fly frass fertilizer in mineral elements such as nitrogen, which is essential for plants [14,15,16]. However, what would be the influence of arbuscular mycorrhizal fungi and black soldier fly frass fertilizer on the growth of *L. esculentum* plants? Certainly, some reports also indicate that AMF protect plants against pathogens and, similarly, that the high chitin content of BSFFF contributes to improved plant health and disease resistance [16]. This work is therefore presented in this context, the objective of which was to apply beneficial fertilizers to improve growth in *L. esculentum* while evaluating their impact on *Fusarium oxysporum* expression and pest attacks.

2. Materials and Methods

2.1. Study Site and Materials Used

The work was conducted between August and October in the Centre region, Mfoundi Department, and more specifically on the campus of the Faculty of Sciences of the University of Yaoundé (03.8°59’56” North latitude and 11.5°00’39” East longitude). The Central region is covered by an equatorial climate of the Guinean type with bimodal rainfall, with an average annual temperature of around 25°C. The plant material used consisted of the seed grains of two varieties of *L. esculentum*: the hybrid variety Kéro F1, very adaptable, resistant to various diseases that can attack *L. esculentum*, and the variety Rio Grande, very adaptable, less resistant to various diseases that can attack *L. esculentum*. The substrate soil used had a clayey loam texture with 37% clay; 31.25% silt and 31.75% sand, an acid pH (pH-H_2O, 5.9 and pH-HCl, 4.9), a poor organic matter content of 2.76%, according to the standard proposed by [17] and an average CEC content of 12.5, a sum of exchangeable bases SBE is normal of 5.62 (1.4% Mg^+; 0.53% K^+; 0.02% Na^+; 3.68% Ca^+). This soil was rich in nitrogen, but had a very low available phosphorus content (Pass), which corresponds to the most biologically available phosphorus (labile organic and inorganic forms and microbial phosphorus) (21.18 ppm), according to the available phosphorus interpretation standards of [17].

2.2. Germination and Transfer of *L. esculentum* Seedlings under Different Treatments

To ensure the viability of the *L. esculentum* seeds used, the germination test was conducted both in the greenhouse and outside the greenhouse. For this purpose, twelve 10L basins, each containing 5kg of sterilized soil, were used for each condition. Six basins were used per variety, with three basins per growing condition. 300 seeds of each variety were sown at a rate of 50 seeds per basin, resulting in 600 seeds for both varieties. For each variety, the six basins were divided into two batches, three for the greenhouse condition and three for the outside-the-greenhouse condition. Whether in the greenhouse or outside the greenhouse, these basins were placed on a shelf 1 m above the ground. The number of germinated seeds and the germination rate were assessed every 5 days for 20 days. The germination rate, which is the ratio of the number of germinated seeds to the total number of seeds sown, was determined using the formula of [18]: T = (nG/N) x 100 (where T: germination rate, nG: germinated seeds, and N: total number of seeds germinated). After 20 days of growth, the germinated *L. esculentum* plants were transferred according to the treatments to be applied. There were 32 greenhouse trays and 32 outdoor trays. In each block unit, represented by the tray, 8 plants were transplanted in parallel. The distance between two rows was 10 cm, and 15 cm between plants. The treatments were applied 14 days after sowing.

2.3. Experimental Design and Fertilization

Two growing conditions were used. *L. esculentum* plants were placed in a greenhouse and outside the greenhouse. The experimental design used for each growing condition was a non-randomized complete block consisting of four treatments: control (T), fertilizer (NPK), arbuscular mycorrhizal fungi (AMF), and black soldier fly frass fertilizer (BSFFF). For each treatment, 15L of sterile soil substrate consisting of black soil and sand was introduced into trays at a ratio of 2:1 (10L soil: 5L sand). Each treatment consisted of 4 trays, in which one tray was replicated three times. The treatments were 0.5m apart, and the distance between the two varieties was 1.5m. 30g of each fertilizer was applied. The AMF used were a *Glomus* complex consisting of the strains *G. etunicatum, G. aggregatum, G. intraradices, G. mosseae, G. claroideum, and G. geosporum*, at a concentration of 20 spores/g of soil; the black soldier fly frass fertilizer obtained from the diet of BSF larvae and produced at the Laboratory of Animal Biology and Physiology at the University of Yaoundé I according to the protocol of [19], and NPK fertilizer (20.10.10).

2.4. Effect of the Treatments Applied on Agronomic Growth Parameters and Assessment of Mycorrhizal Status

The effect of the different fertilizers used was evaluated on the two varieties of *L. esculentum*. Morphological growth parameters were measured every 30 days for 90 days. These were height, average plant diameter, average collar diameter, average number of leaves, average leaf area, etc. [20,21]. Endomycorrhizal infection was detected by staining the fine roots of *L. esculentum* plants using the method of Phillips and Hayman (1970). The frequency and intensity of mycorrhization were assessed under an optical microscope at 400X magnification using the method of [22].

2.5. Influence of Fertilization on *Fusarium* Wilt Expression in *L. esculentum* Plants

During growth, phenotypically diseased plants were identified, counted, and labeled by unit and by variety. These *L. esculentum* plants were then counted by infection level, and disease quantification was performed by unit and over time. Disease incidence [23] and severity [24] were determined using the formulas: I (%) = Na /Nt × 100 (Where, I: is the disease incidence in %, Na: the number of affected plants and Nt: the total number of plants) and IS = 1x F1+2xF2+3xF3+4xF4+5x F5/N (IS: leaf symptom severity index; F: number of plants for each degree in the rating scale from 1 to 5; N: total number of plants used). The scale used for the severity of infection was that proposed by [24], with symptom classes varying according to the scale from 0 to 4, (0: Healthy plants, no symptoms; 1: Some leaves are infected; 2: Half of the leaves are infected; 3: More than half of the leaves are infected with or without wilting and 4: Dead plant). Furthermore, the susceptibility or resistance of *L. esculentum* plants was defined using the method of [25,26,27], showing that if $S < 1$, the cultivar is highly resistant; $1 < S \leq 2$, the cultivar is resistant; $S > 2$, the cultivar is susceptible; and $S \geq 3$, the cultivar is highly susceptible.

2.6. Isolation and Characterization of the *Fusarium* Strain from Diseased *L. esculentum* Leaves

Under both culture conditions, symptomatic *L. esculentum* leaves were washed with tap water and dried with blotting paper. These leaves were cut into fragments along the edges of the necrosis. The method of [28] modified, and [29] was used. Three fragments were placed per Petri dish on PDA medium. The sealed dishes were incubated in a growth chamber at 25°C. Mycelial colony development was observed from the leaf fragments. These colonies were purified by successive subcultures. The resulting pure fungal strains were used to reinfect healthy *L. esculentum* leaves according to Koch's postulate. Observations were made under a LEICA optical microscope at 400X magnification.

2.7. Identification of Insect Faunal Species Present on *L. esculentum* Plants

Insects were captured on *L. esculentum* plants grown outside the greenhouse in the morning and evening daily for 30 minutes. Depending on the insect group, different techniques were used, namely: sweep nets for flying insects, and vacuum cleaners and fine forceps for non-flying insects [30,31]. The captured insects were preserved in pill boxes containing 70% diluted ethanol. The identification of the specimens was done at the Zoology laboratory of the University of Yaoundé I, using an Olympus binocular microscope (X40). The specimens were classified by morphotypes, then identified up to the specific level for some orders and to the family level for others. The dichotomous keys of [32] were used for identification at the family level, while at the species level, those of [33] and [34] were used for Diptera, [35] and [36] for Hemiptera, and the African Insect Identification Guide (GBIF Secretariat, 2023) for other insect groups.

2.8. Statistical Analysis

The results were analyzed descriptively (mean ± standard deviation). The results are presented in graphs and tables (Microsoft Excel 2013 software). IBM SPSS Version 20.0 software was used to perform statistical analyses and compare means using analysis of variance (ANOVA) using the Student-Newman-Keuls test at the 5% threshold.

3. Results

3.1. Evaluation of Seed Germination Rates According to the Varieties

The results obtained showed that the seed germination rate of both *L. esculentum* varieties increased over time in both varieties under both conditions. The maximum germination rates were recorded on day 20, and were 92 and 100% for the Kéro F1 variety and 96 and 100% for the Rio Grande variety (Table 1). The low germination rate values were recorded on day 5 for both varieties. However, there was no significant difference between the average of the germination test, at $P<0.05$ using the Student Newman-Keuls test for both varieties under greenhouse and non-greenhouse growing conditions (Table 1).

3.2. Variation in Agronomic Growth Parameters According to the Treatments Applied

The average plant height of the two *L. esculentum* varieties used varied significantly using the Student Newman-Keuls test at 5%, depending on the treatments applied under both growing conditions (Table 2 and Table 3). Under greenhouse growing conditions, the results show a variation in the average plant height of L. esculentum between the different treatments over time.

The maximum plant heights were recorded on day 90 for Kero F1 plants treated with BSFFF and NPK (67.5 ± 6.28 and 67.19 ± 14.12 cm) and Rio Grande plants treated with BSFFF (65.16 ± 4.35 cm), respectively. However, outside of the greenhouse, on day 60, the NPK treatment for both *L. esculentum* varieties showed significantly higher values than the other treatments, with 43 cm and 46.5 cm, respectively. However, after 90 days, the maximum values were recorded with the BSFFF treatment for both varieties, with 60.16 ± 3.97 cm and 57.66 ± 4.71 cm, respectively. The smallest value was noted for the T treatment, at 17.59 cm.

As for the average stem collar diameter in the two *L. esculentum* varieties used, it did not vary significantly according to the 5% Student Newman and Keuls test, depending on the treatments applied under the two growing conditions (Table 2 and Table 3). Under greenhouse growing conditions, in both varieties, the maximum values for this diameter were recorded under the BSFFF treatment. It was very high in plants of the Rio Grande variety compared to the Kéro F1 variety. The maximum peak is 0.76 cm for the Rio Grande variety on day 90. In greenhouse cultivation, the average stem collar diameter of L. esculentum plants does not vary significantly between the different treatments over time. On day 30, the BSFFF treatment for both varieties shows the highest values, with 0.49±0.063 and 0.48±0.075 cm. Furthermore, on day 90, the AMF treatment for the Kéro F1 variety shows the maximum value, with 0.58±0.14 cm. It is also noted that the NPK and BSFFF treatments applied to the Kéro F1 variety show identical values, i.e., 0.56±0.017 and 0.56±0.016 cm, respectively (Table 2 and Table 3).

Table 1. Evolution of the germination rate in *Lycopersicon esculentum* varieties over time, depending on the cultivation conditions

	Rio grande		Hybride Kéro F1	
Time (Days)	Greenhouse	Outside greenhouse	Greenhouse	Outside greenhouse
5	17.33±00.57	20.00±01.73	15.00±00.00	15.33±002.30
10	49.00±07.81	38.33±02.08	45.00±05.29	30.33±01.52
15	89.33±01.15	85.00±05.56	75.00±03.60	66.66±04.72
20	100.00±00.00	96.33±01.52	100.00±00.00	92.00±00.00
Mean	63.91±2.38a	59.91±2.72a	58.75±2.66a	51.08±2.39a

Student-Newman-Keuls tests
The average values bearing the same letters are not significantly different at the 5% threshold of the SNK test.

Table 2. Evaluation of agronomic growth parameters in the two varieties of *L. esculentum* grown in greenhouses, according to the treatments applied. Cont: Control; NPK; chemical fertilizers; BSFFF: black soldier fly frass fertilizer and AMF: Arbuscular Mycorrhizal Fungi

Varieties	Time (Day)	Treatments	Average plant size (Cm)	Average collar diameter (Cm)	Average number of leaves	Average leaf area (Cm²)	Average number of flowers	Average number of fruits
Rio grande	30	**Cont**	19.00±02.74a	0.43±00.01a	10.00±00.01a	05.40±01.01a	00.00±00.00a	0.00±00.00a
		NPK	20.32±03.88a	0.40±00.01a	10.00±01.01a	10.31±03.55b	00.00±00.00a	0.00±00.00a
		BSFFF	19.17±07.56a	0.62±00.09ab	09.00±01.31a	10.67±02.57b	00.00±00.00a	0.00±00.00a
		AMF	23.67±04.12a	0.42±00.03a	09.00±02.01a	04.31±01.11a	00.00±00.00a	0.00±00.00a
	60	**Cont**	42.00±09.23b	0.48±00.00a	10.00±02.21a	03.45±01.41a	03.00±00.01ab	0.00±00.00a
		NPK	52.07±02.01cd	0.70±00.01abc	10.00±01.51a	09.41±02.32b	07.00±01.37bc	0.00±00.00a
		BSFFF	55.17±03.33cd	0.75±00.01abc	11.00±01.33a	09.84±02.11b	09.66±01.17c	0.00±00.00a
		AMF	48.00±02.41bc	0.58±00.00a	10.00±04.11a	04.23±01.01a	05.00±00.91abc	0.00±00.00a
	90	**Cont**	57.17±03.31cde	0.52±00.02a	08.00±01.71a	02.90±00.91a	04.00±01.01ab	2.00±00.01b
		NPK	60.52±01.64ef	0.63±00.01ab	08.00±01.89a	08.52±01.88b	13.21±04.76d	8.81±01.06d
		BSFFF	65.17±04.35f	0.77±00.01abc	09.00±02.01a	09.43±02.99b	15.33±03.01d	8.00±01.51c
		AMF	53.67±03.01cd	0.53±00.01a	08.00±01.01a	03.92±00.98a	05.33±01.36abc	2.00±00.01b
Hybride Kéro F1	30	**Cont**	20.50±01.45a	0.45±00.01a	09.00±02.61a	07.48±01.01b	00.00±00.00a	0.00±00.00a
		NPK	20.50±02.98a	0.28±00.01a	11.06±03.58a	10.65±03.77b	00.00±00.00a	0.00±00.00a
		BSFFF	25.00±05.77a	0.53±00.08a	10.66±01.13a	10.84±03.56b	00.00±00.00a	0.00±00.00a
		AMF	23.50±01.45a	0.42±00.04a	10.00±03.22a	07.36±01.11b	00.00±00.00a	0.00±00.00a
	60	**Cont**	52.00±00.63b	0.55±00.09a	09.00±01.05a	03.35±01.11a	05.00±00.96abc	0.00±00.00a
		NPK	55.18±02.01bc	0.60±00.00a	09.00±02.77a	09.79±01.11b	05.00±00.83abc	0.00±00.00a
		BSFFF	56.83±02.67bc	0.62±00.01ab	09.00±02.31a	09.84±02.72b	04.00±01.03ab	0.00±00.00a
		AMF	47.50±04.01b	0.47±00.03a	10.00±02.25a	04.63±02.01a	03.00±00.81ab	0.00±00.00a
	90	**Cont**	59.83±03.22cd	0.60±00.05a	08.00±02.01a	02.68±00.81a	03.00±00.43ab	2.00±00.04b
		NPK	67.20±06.01d	0.65±00.11ab	09.00±02.21a	07.97±03.01b	09.45±00.31c	6.01±01.11d
		BSFFF	67.50±06.28d	0.70±00.09abc	09.00±03.33a	10.15±02.37b	09.45±01.33c	6.00±01.09c
		AMF	56.67±03.61bc	0.58±00.01a	08.00±03.61a	04.63±01.71a	05.33±01.26abc	2.00±00.04b

Student-Newman-Keuls tests
The average values bearing the same letters in the same column are not significantly different at the 5% threshold of the SNK test.

Table 3. Evaluation of agronomic growth parameters in the two varieties of *L. esculentum* grown outside the greenhouse, according to the treatments applied. Cont: Control; NPK; chemical fertilizers; BSFFF: black soldier fly frass fertilizer and AMF: Arbuscular Mycorrhizal Fungi

Varieties	Time (Day)	Treatments	Average plant size (Cm)	Average collar diameter (Cm)	Average number of leaves	Average leaf area (Cm²)	Average number of flowers	Average number of fruits
Rio grande	30	**Cont**	18.08±01.21a	0.42±00.01a	09.17±02.77a	02,35±00.99a	00.00±00.00a	0.00±00.00a
		NPK	23.67±03.34a	0.38±00.02a	09.83±02.01a	10,11±01.59b	00.00±00.00a	0.00±00.00a
		BSFFF	19.50±01.64a	0.48±00.06a	06.67±01.01a	07,54±01.23b	00.00±00.00a	0.00±00.00a
		AMF	18.33±02.51a	0.40±00.09a	09.69±03.01a	03,16±01.55a	00.00±00.00a	0.00±00.00a
	60	**Cont**	37.80±01.66b	0.42±00.00a	09.38±01.01a	01,59±00.89a	03.00±00.44ab	0.00±00.00a
		NPK	43.00±00.78bc	0.50±00.01a	09.00±02.01a	09,55±02.67b	03.00±01.12ab	0.00±00.00a
		BSFFF	37.70±02.70b	0.53±00.01a	09.38±03.01a	05,11±01.33a	04.00±01.01ab	0.00±00.00a
		AMF	39.98±01.01b	0.45±00.04a	08.85±01.01a	02,31±00.32a	04.00±01.11ab	0.00±00.00a
	90	**Cont**	46.32±01.01bc	0.50±00.03a	08.82±02.11a	02,04±01.15a	03.00±00.01ab	02.00±00.78b
		NPK	53.83±00.99cd	0.52±00.01a	08.67±02.01a	06,55±02.11ab	05.00±01.01abc	05.00±01.47c
		BSFFF	60.17±03.97d	0.55±00.01a	10.33±01.50a	05,60±01.01a	13.00±01.61d	04.00±00.98bc
		AMF	47.83±02.66bc	0.53±00.01a	09.66±01.51a	02,37±00.33a	03.00±01.01ab	02.00±00.71b
Hybride Kéro F1	30	**Cont**	17.59±020.0a	0.39±00.03a	09.11±01.33a	05,58±01.67a	00.00±00.01a	00.00±00.00a
		NPK	20.17±01.01a	0.33±00.01a	09.33±01.56a	10,42±03.34b	00.00±00.00a	00.00±00.00a
		BSFFF	22.00±01.55a	0,50±00.07a	09.33±01.67a	06,10±01.45ba	00.00±00.00a	00.00±00.00a
		AMF	19.50±01.51a	0,42±00.02a	08.75±03.22a	04,19±01.67a	00.00±00.00a	00.00±00.00a
	60	**Cont**	40.67±02.21bc	0.53±00.00a	08.64±01.11a	01,83±00.34a	05.00±01.66abc	00.00±00.00a
		NPK	46.50±02.67bc	0.55±00.01a	09.00±01.88a	08,65±02.15b	03.00±00.99ab	00.00±00.00a
		BSFFF	41.17±01.11bc	0.53±00.01a	09.00±02.01a	04,98±01.77a	03.00±00.56ab	00.00±00.00a
		AMF	37.78±03.01b	0.42±00.04a	09.90±03.15a	02,56±00.48a	03.00±01.01ab	00.00±00.00a
	90	**Cont**	45.92±03.03bc	0.52±00.02a	07.90±02.41a	02,21±00.67a	04.00±01.01ab	02.00±00.01b
		NPK	55.83±02.07d	0.57±00.03a	09.67±01.66a	06,69±01.28ab	09.00±00.01c	08.84±01.39d
		BSFFF	57.67±04.71d	0.57±00.09a	10.00±01.51a	04,91±01.87a	09.33±01.58c	08.39±02.22d
		AMF	52.02±02.61cd	0.58±00.14a	10.66±01.03a	02,43±00.57a	05.00±01.87abc	02.00±00.01b

Student-Newman-Keuls tests
The average values bearing the same letters in the same column are not significantly different at the 5% threshold of the SNK test.

3.3. Average Leaf Number in the Two *L. esculentum* Varieties According to the Treatments Applied

The average leaf number in the two *L. esculentum* varieties used does not vary according to the 5% Student Newman and Keuls test, according to the treatments applied under the two growing conditions (Table 2 and Table 3). Under greenhouse conditions, the highest values are obtained in the NPK and BSFFF treatments for the Kéro F1 variety on day 30 with 11.06 ± 3.58 leaves and 10.66 ± 1.63 leaves, respectively. This average leaf number decreases over time. The low values are generally recorded on day 90 for all treatments. In non-greenhouse cultivation, the results show that on day 30, the highest value is 11.16 ± 2.58 leaves for plants of the Rio Grande variety. On day 60, the AMF treatment for Kéro F1 and BSFFF for Rio Grande gave significantly higher values, i.e., 9.9±3.15 leaves and 9.66±1.5 leaves. On day 90, average values of 10.66±1.03 and 10.33±1.5 leaves were recorded, respectively, in the plants of the AMF treatment for Kéro F1 and the BSFFF treatment for Rio Grande (Table 2 and Table 3).

3.4. Average Leaf Area of the Two *L. esculentum* Varieties According to the Treatments Applied

The results show that the average leaf area values of the two *L. esculentum* varieties used vary significantly according to the 5% Student Newman and Keuls test, according to the treatments applied under the two growing conditions (Table 2 and Table 3). Under greenhouse growing conditions, the maximum values are obtained with NPK and BSFFF treatments. On day 30, the plants treated with BSFFF for both varieties show the highest values with 10.84 ± 3.56 cm² and 10.67 ± 2.57 cm², respectively. However, on day 60, the same trend is observed with BSFFF treatment, which shows the same value for both varieties with 9.84 ± 3.37 cm². Similarly, on day 90, the highest value with the BSFFF treatment for Kero F1 was 10.15 ± 2.37 cm². In greenhouse cultivation, NPK treatments had the greatest influence on the average leaf area parameter in both varieties. The highest values were 10.42 ± 1.45 and 10.11 ± 1.59 cm², respectively, on day 30. These values decreased between days 60 and 90 (Table 2 and Table 3).

3.5. Mean Number of Flowers and Fruits in the Two Varieties of *L. esculentum* Depending on the Treatments Applied

The mean number of flowers observed in the two varieties of *L. esculentum* varied significantly using the 5% Student Newman and Keuls test, depending on the treatments applied under both growing conditions (Table 2 and Table 3). Flower emergence was observed between days 60 and 90. Under greenhouse conditions, the average

number of flowers varied over time. Plants treated with BSFFF produced the most flowers in the Rio Grande variety compared to other treatments. NPK and BSFFF treatments significantly influenced flower production in both varieties (Table 2 and Table 3). Maximums of 13.21±04.75 and 15.33±03.01 were noted for the NPK and BSFFF treatments in the Rio Grande variety in the greenhouse. At D_{90}, plants treated with BSFFF and AMF presented values of 5.33±1.36 and 5.33±1.26 flowers, respectively, for the Rio Grande and Kéro F1 varieties. In non-greenhouse cultivation, the analysis of the results shows a variation in the average number of flowers of L. esculentum plants according to the different treatments over time, with maxima for the NPK and BSFFF treatments (Table 3). The influence of the applied treatments, compared to the control plants, was recorded for the Rio Grande variety compared to the Kero F1 variety. The high average flower count was noted on day 90, with 13±7.61 and 9.33±5.68 flowers, respectively, in *L. esculentum* plants treated with BSFFF for the Rio Grande and Kero F1 variety (Table 2). The average fruit count in the two *L. esculentum* varieties used varied significantly according to the 5% Student Newman and Keuls test, depending on the treatments applied in the two growing conditions (Table 3 and Figure 1). Whether under growing conditions, in the greenhouse, or outside the greenhouse, the maximum fruit counts were obtained in the NPK treatments applied. We noted 8.81±1.06 and 8.84±1.39 fruits, respectively, in Rio Grande and Kero F1, in the greenhouse and outside the greenhouse (Table 2 and Table 3).

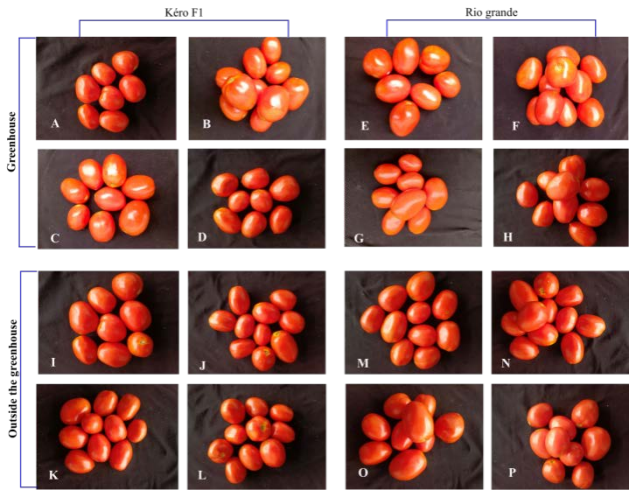

Figure 1. Appearance of *L. esculentum* fruits after 90 days of cultivation under both conditions. Greenhouse cultivation conditions (from A to H) and outside greenhouse (from I to P). Treatments applied: in the Kéro F1 variety; Controls (A and I), NPK (B and J), BSFFF (C and K) and CMA (D and L) and in the Rio Grande variety; Controls (E and M), NPK (F and N), BSFFF (G and O) and CMA (H and P)

3.6. Mycorrhizal Status Assessment

Analysis of the mycorrhization parameters assessed at D_{90} shows that when *L. esculentum* plants are grown in shaded conditions, the frequency and intensity of mycorrhization are more significant compared to those grown outside greenhouses (Figure 2). Under greenhouse conditions, the maximum frequency is 70% for plants of the Rio Grande variety. It is noted that the Kéro F1 variety has the lowest frequency (65%). Similarly, the mycorrhization intensity value is very low for the Kéro F1 variety (6.44%). However, under greenhouse conditions, the Rio Grande variety has the lowest mycorrhization frequency (52.5%) compared to the Kéro F1 variety (60%). Mycorrhization intensity values are lower outside the greenhouse compared to greenhouse conditions. We note 2.79 and 5.23% respectively for the Rio Grande and Kéro F1 varieties.

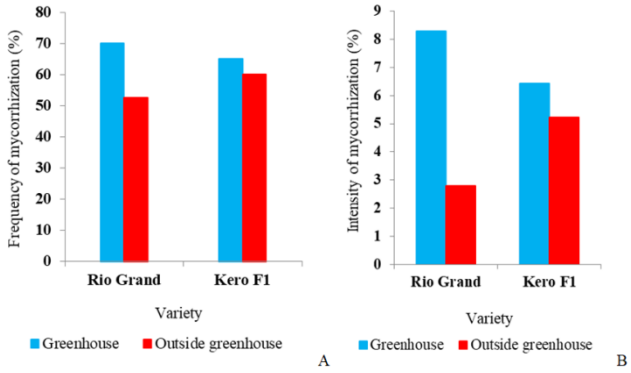

Figure 2. Mycorrhizal status of roots in the two varieties of *L. esculentum* under the two growing conditions. Mycorrhization frequency (A) and mycorrhization intensity (B)

3.7. Influence of Applied Fertilization on the Expression of *Fusarium* wilt in *L. esculentum*

Macroscopic study of *Fusarium oxysporum* strains isolated and cultivated on PDA medium revealed three morphotypes: cottony (with a dense colony, with a diffuse border and a very abundant cottony aerial mycelium) (Figure 3A), senescent Ras (extremely sparse, low-viscosity aerial mycelium with a very slow growth rate) (Figure 3B), and fluffy (with a sparse colony and often a clear outline, thus presenting a poorly developed and fairly short aerial mycelium) (Figure 3C). Microscopic observations of these three morphological aspects of *Fusarium oxysporum* strains revealed the abundant presence of microconidia, macroconidia of varying size, chlamydospores, and septate mycelial hyphae (Figure 4).

3.8. Disease Incidence and Severity

Disease incidence and severity, assessed in the two *L. esculentum* varieties used, varied significantly using the Student's Newman and Keuls test at 5%, depending on the treatments applied under the two growing conditions (Table 4). The results show a variation in disease incidence in *L. esculentum* plants between the different treatments. This incidence appears very low in all mycorrhizal plants under both conditions, in both *L. esculentum* varieties, compared to the other treatments (Table 4). It is noted that this disease expression appears higher in greenhouse plants compared to plants grown outside the greenhouse. Outside the greenhouse, we note that on Day 60, values of 22.22% and 28% were recorded in the BSFFF treatment for the two varieties, respectively, in Kéro F1 and Rio Grande. Furthermore, the highest incidence values were obtained in the control treatment for

both varieties, with 45% and 44%, respectively. On day 90, the same trend was observed, with the BSFFF treatment showing 81.48% for the Kéro variety and 82.12% for the Rio Grande variety. However, in the greenhouse on day 90, the incidence rate in plants of both *L. esculentum* varieties was 100% for all treatments except the AMF treatment (Table 4). Furthermore, there was a variation in disease severity in *L. esculentum* plants depending on the different treatments applied. This severity remained very high in plants of both varieties under greenhouse conditions compared to the outdoor conditions, but appeared low in mycorrhizal plants (Table 4).

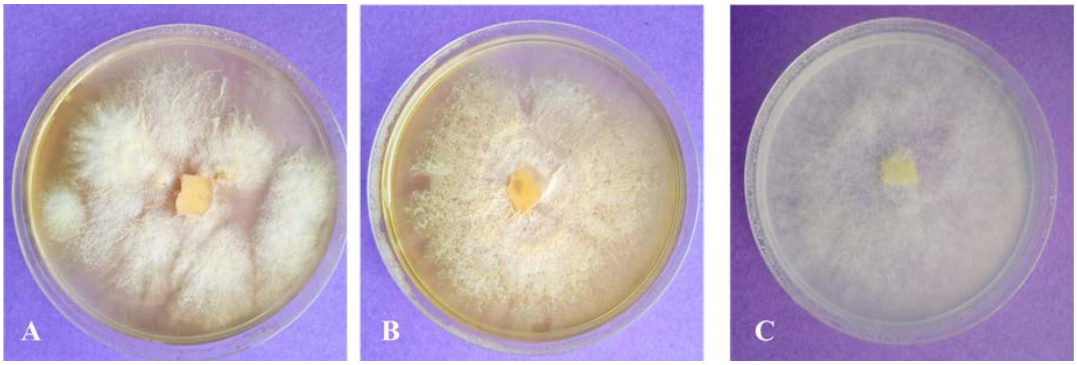

Figure 3. Appearance of pure culture of *Fusarium oxysporum* isolates isolated from leaves. Cottony (A), and senescent (B), downy (C)

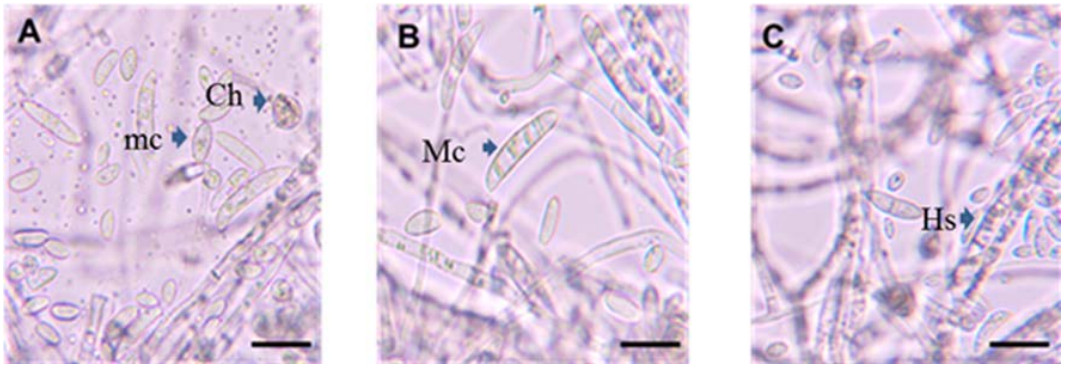

Figure 4. Structures of conidia and hyphae of *Fusarium oxysporum* observed under a 400X light microscope; with mc: microconidia; Mc: macroconidia; Ch: chlamydospores; Hs: septate hypha

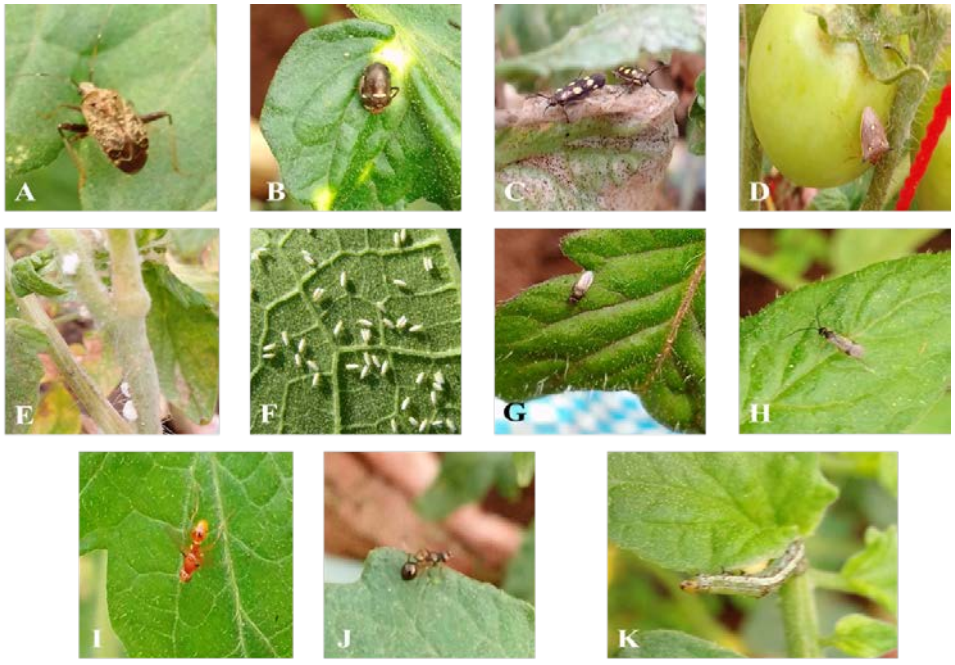

Figure 5. Entomofauna species observed on *L. esculentum* plants under cultivation conditions outside of a greenhouse. A: Proscisdocoris; B: chrysomelidae sp.1; C: Miridae sp.2; D: aspavia armigera; E: coccidae sp.3; F: aleurodidae sp.4; G: scenopinidae sp.5; H: stratiomidae sp.6; I and J: tephritidae sp.7 and sp.9; K: noctuidae sp.8

Table 4. Variation in the incidence and severity of the disease in the two varieties of *L. esculentum* grown outside the greenhouse and in the greenhouse, according to the treatments applied. Cont: Control; NPK; chemical fertilizers; BSFFF: black soldier fly frass fertilizer and AMF: Arbuscular Mycorrhizal Fungi

Varieties	Time (Day)	Treatments	Incidence (%)		Severity (%)	
			Greenhouse	Outside greenhouse	Greenhouse	Outside greenhouse
Rio grande	30	**Cont**	00.00±00.00a	00.00±00.00 a	00.00±00.00a	00.00±00.00a
		NPK	00.00±00.00a	00.00±00.00 a	00.00±00.00a	00.00±00.00a
		BSFFF	00.00±00.00a	00.00±00.00 a	00.00±00.00a	00.00±00.00a
		AMF	00.00±00.00a	00.00±00.00 a	00.00±00.00a	00.00±00.00a
	60	**Cont**	60.22±00.01c	45.00±00.01c	2,34±00.01a	1,45±00.01a
		NPK	64.62±00.01c	34,62±00.01b	2,62±00.01a	1,35±00.01a
		BSFFF	38.00±00.01b	28.00±00.01b	1,98±00.01a	1,28±00.01a
		AMF	27.33±00.01b	18,33±00.01b	1,16±00.01a	1,05±00.01a
	90	**Cont**	100.00±00.01d	95.00±00.01d	2,69±00.01a	2,45±00.01a
		NPK	100.00±00.01d	88,43±00.01d	2,33±00.01a	2,38±00.01a
		BSFFF	100.00±00.01d	72.00±00.01d	2,37±00.01a	1,71±00.01a
		AMF	60.00±00.01c	45,71±00.01c	1,37±00.01a	1,00±00.01a
Hybride Kéro F1	30	**Cont**	00.00±00.00a	00.00±00.00a	00.00±00.00a	00.00±00.00a
		NPK	00.00±00.00a	00.00±00.00a	00.00±00.00a	00.00±00.00a
		BSFFF	00.00±00.00a	00.00±00.00a	00.00±00.00a	00.00±00.00a
		AMF	00.00±00.00a	00.00±00.00a	00.00±00.00a	00.00±00.00a
	60	**Cont**	60.00±00.01c	44.00±00.01c	2,22±00.01a	1,44±00.01a
		NPK	62.37±00.01c	37,04±00.01b	2,90±00.01a	1,37±00.01a
		BSFFF	35.22±00.01b	22,22±00.01b	1,86±00.01a	1,22±00.01a
		AMF	32.28±00.01b	18,57±00.01b	1,32±00.01a	1,03±00.01a
	90	**Cont**	100.00±00.01d	92.00±00.01d	2,56±00.01a	2,24±00.01a
		NPK	100.00±00.01d	92,59±00.01d	2,75±00.01a	2,37±00.01a
		BSFFF	100.00±00.01d	81,48±00.01d	2,50±00.01a	2,04±00.01a
		AMF	73,00±00.01c	73,33±00.01c	1,50±00.01a	1,29±00.01a

Student-Newman-Keuls tests
The average values bearing the same letters in the same column are not significantly different at the 5% threshold of the SNK test.

Table 5. Characterization of the entomofauna species of *L. esculentum* (var. Rio grande and Kéro F1)

Orders	Families	Genera	Trophic groups	
Coleoptera	chrysomelidae	chrysomelidae gen.sp.1	Plant pest	Phytophages
Diptera	Scenopinidae	Scenopinidae gen.sp.5		
	Tephritidae	Tephritidae gen.sp.7	Plant pest	Sap-sucking insect
	Tephritidae	Tephritidae gen.sp.6	Plant pest	Sap-sucking insect
	Stratiomidae	Stratiomidae gen.sp.9		
Hemiptera	Aleyrodidae	*Bemisia* gen.	Plant pest	Sap-sucking insect
	Miridae	Miridae gen. sp.2	Plant pest	Sap-sucking insect
	Miridae	*Proscisdocoris* gen.	Plant pest	Sap-sucking insect
	Coccidae	Coccidae gen. sp.3	Plant pest	Sap-sucking insect
	Pentatomidae	*aspavia armigera*	Plant pest	Sap-sucking insect
Lepidoptera	Noctuidae	Noctuidae gen. sp.8	Plant pest	Phytophages

3.9. Identification of the Insect Species Present on *L. esculentum*

The results show that the insects collected from the experimental site are diverse. These insects, which inhabit the *L. esculentum* plant during the study period, belong to four insect orders, 11 families, and 11 genera (3 identified) (Table 5). The Diptera and Hemiptera orders were the most representative, with four and five families each. The Coleoptera and Lepidoptera orders were the least represented, with one family each. Considering the lifestyle and mouthparts of the species encountered, they can be categorized into five groups: piercing-sucking insects, leaf-eating insects, saprophagous insects, and predators.

4. Discussion

The overall objective of this work was to apply beneficial fertilizers to improve growth in *L. esculentum* while evaluating their impact on *Fusarium oxysporum* and pest attacks. The results obtained varied significantly over time, depending on the fertilizers applied under greenhouse and outdoor growing conditions. The morphological growth parameters evaluated varied significantly using the Student and Keuls t-test at $P<0.05$. The nursery growth phase for both varieties showed 92 and 96% recovery, reflecting the good health of the L. esculentum seeds used. In greenhouses and outdoors,

plants receiving the BSFFF treatment were found to have a higher average height, collar diameter, leaf area, and flower count than control plants. This translates to the fact that the enrichment of the substrate by BSFFF would have led to an improvement in the organic matter content, the major consequence of which would also be an increase in microbial activity, thus contributing to the availability of nutrients beneficial to cultivated *L. esculentum* plants, because the excrement is rich in nitrogen, phosphorus, potassium, and micronutrients. The applied BSFFF would have revitalized the soil substrate used, which was acidic, poor in organic matter, and nutrients such as phosphorus. [37], emphasize that black soldier fly (BSF) excrement has significant potential to revitalize marginalized soils. Rosmiati et al. [38] also highlight good growth performance in lettuce plants fertilized with BSFF produced from Coffee Husk. Moreover, the work of [39] showed that the applications of BSF treatments in watermelon plants not only significantly influenced the growth parameters of watermelons compared to the control but also contributed to the availability of P, while improving the soil pH compared to other amended treatments. However, the good growth observed at D_{90} in *L. esculentum* plants that received the NPK treatment is justified by the fact that NPK immediately makes available to plants mineral elements such as nitrogen, phosphorus, and potassium, directly assimilable by plants. Although urea provides sufficient nitrogen to the plant compared to BSFFF, BSFFF also provides other nutrients such as phosphorus, potassium, calcium, and magnesium, essential for plant growth. On the other hand, the results obtained from the growth parameters show that the plants in the AMF treatment do not present any significant difference compared to the control plants. This would indicate that the symbiosis is, on the one hand, costly for the *L. esculentum* plants concerning the applied mycorrhizal complex. This established symbiosis appears to be conditioned not only by the clayey loam nature (which shows that it was a little compact), the volume of the substrate used (which would partly influence the quantity of available nutrients), but also by the quantity of non-assimilable phosphorus to be assimilated by the mycorrhizal strains of the complex used. This also explains the low yield observed in all the mycorrhizal plants. The ineffectiveness of the mycorrhizal inoculation on the height growth and the diameter at the collar of the *L. esculentum* plants obtained could also indicate that the action of the applied AMF would have intervened rather for the protection of plants against *Fusarium oxysporum*.

It is also noted that the results on mycorrhization show that the roots of *L. esculentum* were mycorrhized by the mycorrhizal isolates used, and the absence of mycorrhizal infection on the roots of the controls shows that the treatments are free from any mycorrhizal contamination. Previous work on the colonization of *L. esculentum* roots by AMF has shown a frequency and intensity of mycorrhization greater than 90% and 30% **[21]**. However, the results of the present study show a frequency of mycorrhization not exceeding 70% and an intensity of less than 10% in both culture conditions. Similar rates were obtained by [41], who observed low mycorrhization rates in *L. esculentum* plants in the nursery and further emphasized that post-transplanting soil cultivation may also be responsible for the low mycorrhization rate observed in the field.

Disease incidence varied significantly at day 90 for all treatments under both growing conditions, suggesting that under these conditions, despite the fertilization applied, all *L. esculentum* varieties were susceptible to attack. The authors of [40] report that not all organic fertilizers have the potential to protect plants against infection. Some can be sources of contaminants if improperly treated. However, the low severity obtained with BSFFF treatments, and much lower in the presence of AMF, in the greenhouse, could be explained by their ability to induce resistance. The applied BSFFF would have made the plants more vigorous. But it should be noted that both varieties used are susceptible to *F. oxysporum*. So, these two treatments would have allowed the plants to tolerate attacks compared to the Control and NPK treatments applied. [41] points out that insect excrement functions as a nematicide and fungicide, and at the same time promotes plant growth. Similarly, the work of [16] states that insect excrement is rich in chitin from the shed exoskeleton, which is known to have several beneficial effects on plant growth and health. In addition to this protection against pathogens, some authors like [42] have shown that good quality compost can also stimulate the defenses of the entire plant. [43] showed that the pretreatments of the *S. miltiorrhiza* plant with AMF reduce the progression of *Fusarium* wilt, thereby preventing a loss of biomass and photosynthesis. The morphological study of *F. oxysporum* isolates reveals three different morphotypes: the cottony appearance, the senescent Ras appearance, and the downy appearance. The analysis of the structure of the conidia of the different fungal isolates confirms the identification of *F. oxysporum* with the presence of the three types of conidia, which are macroconidia, microconidia, chlamydospores, and septate hyphae, by the characteristics defined by [44].

The results obtained on the identification of pests show that market garden crops in general and those of *L. esculentum* in particular harbor enough enemies. The results of the present study show that the insects associated with the cultivation of *L. esculentum* belonged to 4 orders, 11 families, and 12 genera (3 identified). The low diversity of the insects listed could be explained by the environmental factors, the method, and the period of the collections, which would have influenced the antomaufone species. However, previous work on the Characterization of the entomofauna of L. esculentum (*Lycopersicon esculentum* Mill) in the field in Cameroon showed that the insects associated with the cultivation of L. esculentum belonged to 8 orders, 21 families, 22 genera, and species by [45]. The authors of [46] on the evaluation of harmful insects of *Capsicum annuum* in cultivation in Yaoundé identified 7 orders and 28 species of insects. In the group of piercing-sucking insects, the order of Hemiptera constitutes the greatest pest of *L. esculentum*. These observations are contrary to those of [47], who showed that the order of Lepidoptera was the major pest of *L. esculentum* in the field in Senegal. It should be noted, however, that the majority of insects inventoried were present at the vegetative stage of the plant, particularly on the leaves and stems. Lepidoptera larvae were more remarkable and frequent on the fruits of the plant. These observations are supported by the work of [48] on the

population dynamics of *Tuta absoluta* in Algeria. These species attack the vegetative organs of the *L. esculentum* plant much more and prevent their development. These different pests are also believed to have contributed to the spread of the disease in plants.

5. Conclusion

The nursery recovery showed germination percentages, highlighting the good health of the seed grains used. The treatments applied influenced growth differently depending on the variety and growing conditions. The BSFFF treatment was generally the most effective in greenhouses, while NPK showed good potential at the beginning of outdoor cultivation. Evaluation of the effect of BSFFF and AMF on plant health showed that disease development occurred according to the treatments and time. Over time, BSFFF and AMF plants were found to be the least affected by the disease than the control treatments, and this was confirmed by the disease index obtained. The identified insect pests caused several types of damage to different parts of the *L. esculentum* plant. Leaf and fruit perforations, as well as flower drop and rotting of infested fruits, were observed. These various pests would also have contributed to the spread of disease in plants such as leaf curl disease in *L. esculentum*.

Author Contribution

All authors contributed to the conception, implementation, analysis and interpretation of the results obtained and also to the writing of the article. Furthermore, their approval for publication of the final version was subject to its critical revision for important intellectual and scientific content.

Conflict and Interest

The authors declare no conflicts of interest regarding the publication of this paper.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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