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Management of tomato pests using effective macrooganism (EM) bio-pesticide as alternative pests control method in Bambili, Cameroon

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Abstract
Tuta absoluta, Bemisia tabaci and *Macrosiphum eurphorbiae* are important pests of tomato in Cameroon. The effect of EM a biopesticide containing *Bacillus thuringiensis* as active ingredient was evaluated on the suppression of these pests. A completely randomized design with 4 treatments [control (T_0); EM 10 ml/l (T_1); EM 20 ml/l (T_2); EM 30 ml/l (T_3)] and 3 replications were used. The biopesticide was sprayed weekly for seven weeks. The variation of insects' populations, *T. absoluta* impact and plants growth in different plots were compare using ANOVA test. Different concentrations of *B. thuringiensis* were effective in reducing pests populations and significant difference observed within treatments (DF =3, F=258.69, P=0.0000; DF=3, F=368.50, P=0.0000 and DF=3, F=44.21, P=0.0000) respectively. On the untreated control plots T_0, the populations of the three pests showed an exponential expansion within 7 weeks. No significant effect of *B. thuringiensis* was observed on the plant growth. This biological pesticide can save as alternative pests control for sustainable tomato production.

Keywords: Pests, EM biopesticide, *bacillus thuringiensis*, pests control, alternative

Introduction
Tomato (*Lycopersicon esculentum* Mill) is one of the important vegetable crops grown around the world for it nutritional, medicinal and economic values. In the Cameroon highlands, tomato is attacked by as many as 9 different insect pests (Heumou *et al*., 2015) [12]. Some of these tomato pests can show physical damage to the plant or parts (leaves, flowers, fruits, stems, roots) and others are agents of disease transmissions (Ntonifor *et al*., 2013) [15]. Among them, *Tuta absoluta, Bemisia tabaci* and *Macrosiphum eurphorbiae* are three phytophagous pests implicated in the attack of leaves, stems and flowers of the plant at all level of their development.

Tuta absoluta (Lepidoptera/Gelechiidae) commonly call Leaf miner moth attacks tomato at all developmental stages; from the seedling to mature fruits in both the fields and protected areas. The larva is the most dangerous stage of the life cycle it feeds between the leaf layers causing irregular mines and eventually tunnel into the stem and burrow into the roots (Deleva *et al.*, 2012) [7]. In tomatoes, leaf damage are caused through mine formation within the mesophyll by larvae, thus affecting the photosynthetic capacity of the plant and thereby lowering the yield (Desneux *et al.*, 2010; Biondi *et al.*, 2018) [8, 9]. In Bambili, one of the first invaded areas, *T. absoluta* was reported to damage up to 94% tomato leaves because of its high reproductive rate and within a short period of time (Heumou *et al*., 2022) [23].

Bemisia tabaci (Whitefly) is known as a serious polyphagus pest which has over 115 host plants in Sudan, e.g., cotton, tomato, eggplant, and cucurbits. However the first family host is Leguminosacae (Bellow and Powell, 1992) [25]. Whitefly has shown to deprive the host plants of growth and reduce the yield both quantitatively and qualitatively (Gameel, 1972) [11]. The insect also secretes sticky substance (honey-dew) which enhances the growth of black mould fungus, which cover the leaves and closing the stomata and affect photosynthesis. The whitefly is considered as a serious vector of plant viruses such as leaf curl disease. *Macrosiphum eurphorbiae* (Heminoptera: Aphididae) often called aphids or plant lice, are small, soft-bodied insects.

They attack tomatoes at any stage of development by sucking juices from plants, and can transmit many diseases to tomatoes (Djiéto-Lordon *et al.,* 2006) [9]. Aphids feed in colonies and remove sap from the plants with their piercing-sucking mouthparts. Tomato plants can tolerate large numbers of aphids without suffering yield loss. However, severe infestations can cause leaves to curl and leading to stunted plants. Decreased leaf area can increase sun scald to the fruit.

To address the problem of insect pests, famers generally use synthetic chemical pesticides as control method. There are used to reduce the pest population by poisoning them or repelling them from specified area. These pesticides are generally synthetic materials that directly kill or inactivate the pest. Insecticides are seldom used full strength, but are formulated in ways to dilute, extend, and make them easy to apply. The most common formulations are: dusts, granules, wet table powder, solutions, emulsifiable concentrations, aerosols and fumigants. The usage of chemical pesticide had simply double in all the regions of the world, Africa and South America being at the top (FAO, 1990). Nowadays, these chemical pesticides are known to have disadvantages such as persistence in the environment for long period of time, residues of chemicals in human food and livestock feed can adversely affect along the food chain, which cause serious diseases to human such as cancers, Alzheimer, Parkinson, Asthma, infertility, respiratory diseases. Akash *et al.* (2018) [1] reported that about 500,000 people in the world are annually poisoned by accidental exposure to insecticides with 1 to 5% as a fatality rate in developed countries. Moreover impact on non-targeted animals is very high. In many instances, increasing pest populations has been reported as a result of their natural enemies being destroyed by pesticides (Erdal et Clive, 1998) [10]. Loss of a large number of beneficial insects involved in pollinating crops, indirectly affect the yield of those crops. The used of some chemical pesticides has resulted in the development of resistance in the insects pests. The chemical pesticides can cause severe damage and residue problems on crops, livestock, and to the environment in general. This is why emphasis is being given to Integrated Pest Management (IPM) which focuses greatly on minimal use of chemicals and the integration with other environmental friendly control methods more. At the same time, research on safe insecticides to substitute the existing synthetic toxic insecticides has been intensified all over the world.

In this changing scenario, biological pesticides, such as the one that derived from bacteria, animals, or plants have many advantages (Arora *et al.,* 2012) [3]. Biological pesticides are usually inherently less harmful than conventional pesticides; they generally affect only the target pest and closely related organisms, in contrast to broad-spectrum conventional pesticides that may affect organisms as different as insects, birds, and mammals (USEPA, 2019) [22]. Biological pesticides often are effective in very small quantities and often decompose quickly, thereby resulting in lower exposures and largely avoiding the pollution problems caused by conventional pesticides. When used as a component of Integrated Pest Management (IPM) programs, biological pesticides can greatly decrease pest action than the use of conventional pesticides, while crop yields remain high. Within the range of bio-pesticides, over 90 species of naturally-occurring entomopathogenic bacteria have been isolated from insects, plants, and the soil, but only a few have been studied intensively (Olle *et al.,* 2013) [17]. Much attention has been given to *B. thuringiensis* a Gram positive bacterium that has been developed as a microbial insecticide. *B. thuringiensis* is the most widely used bacterium and as alternative to chemical insecticides for biological control of the forest and agricultural pests, vector of human, animal and plant diseases. During sporulation, different strains of this Gram-positive bacteria produced crystalline parasporal inclusion bodies composed of one or more toxic proteins known as endotoxin with high level of specificity against different species of Lepidopteran, Dipteran and Coleopteran, and some parasitic nematodes and Protozoan pathogens (Alejandra *et al*., 2007) [2].

Insecticides, Properties and mode of action of *Bacillus thuringiensis*

Unlike typical nerve-poison insecticides, *B. thuringiensis* acts by producing proteins (delta-endotoxin, the "toxic crystal") that reacts with the cells of the gut lining of susceptible insects. These *B. thuringiensis* proteins paralyze the digestive system, and the infected insect stops feeding within hours. *B. thuringiensis* affected insects generally die from starvation, which can take several days. Occasionally, the bacteria enter the insect's blood and reproduce within the insect. However, in most insects it is the reaction of the protein crystal that is lethal to the insect. Even dead bacteria containing the proteins are effective insecticides. After the spores and crystals of *B. thuringiensis* have been eaten by the larvae, the crystalline inclusions are soluble in highly alkaline mid gut lumen and converted to active toxin by trypsin-like proteases. The active toxin cross the peritrophic membrane and binds to specific receptors on the brush border apical membrane of mid gut columnar cells and insert into the membrane pore formation disrupts the ionic gradients and osmotic balance across the apical membrane and eventually causes the epithelial mid gut cells to lye, this leads to massive disruption of the epithelium and ultimately to the death of the larvae by starvation or septicemia (Alejandra *et al*., 2007, Bouslama *et al*., 2020) [2, 6].The most commonly used strain of *B. thuringiensis* (kurstaki strain) kills only leaf- and needle-feeding caterpillars. In the past decade, *B. thuringiensis* strains have been developed that control certain types of fly larvae (israelensis strain, or Bt). These are widely used against larvae of mosquitoes, black flies and fungus gnats. More recently, strains have been developed with activity against some leaf beetles, such as the Colorado potato beetle and elm leaf beetle (sandiego strain, tenebrionis strain). Among the various Bt. strains, insecticidal activity is specific. That is, *B. thuringiensis* strains developed for mosquito larvae do not affect caterpillars. Sareh *et al.* (2015) [18] demonstrated that Bt is active against on controlling other invertebrate of the phylum Nematoda. With the aims of searching of alternatives management strategies for sustainable protection of tomato cultivation, attempts were made to evaluate: (i) the efficacy of different concentrations of EM bio-pesticide on *T. absoluta, B. tabaci* and *M. eurphorbiae* populations on tomato; (ii) the effect of EM biological pesticide on the growth rate of tomato plant was also evaluated.

Materials and Methods

Biological materials used in the experiment

Pests of tomato

The leaf moot *Tuta absoluta* (Fig 1A), a recent but much dangerous pests of tomato in Cameroon, it attack tomato at all the level of development and all the organs from leaves, stems flowers to fruits. The other pests are *Bemisia tabaci* Genn (Fig 1B) and *Macrosiphum euphorbiae* (Thomas 1878) (Fig 1C) are two Hemiptera of the family Aphididea they are among the oldest pests of tomato. Both of them are sap sucker of tomato and they higher impact on tomato is the fact that they are responsible of transmission of different viruses to the plant.

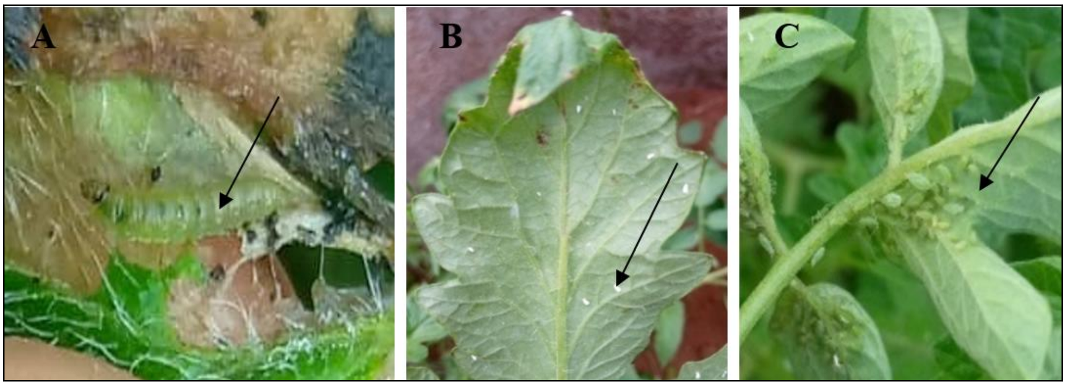

Fig 1: Pests of tomatoes in Bambili: larvae of *Tuta absoluta* in a leave (A), *Bemisia tabaci* on the underside leave of tomato (B) and a colony of *Macrosiphum euphorbiae* (C).

The EM bio-insecticide used for the trail

The Effective microorganism EM used was a commercial form bought in the marked. It is a mixture of live natural cultures of microorganisms isolated from fertile soils that are used to improve crop production (Olle *et al.*, 2013) [17]. The EM used in this trail was made up of a microorganism *Bacillus thuringiensis* which is a Gram positive bacterium. *B. thuringiensis* was isolated since 1901 by a Japanese bacteriologist Ishiwata it is classified as follows: Kingdom: Eubacteria; Phylum: Firmicutes Class: Bacilli; Order: Bacillales; Family: Bacillaceae; Genus: *Bacillus*; Species: *Bacillus thuringiensis.* The EM technology was developed over 40 years ago by Teruo Higa in Japan.

Preparation and application of EM bio-insecticide

Different quantities of the EM were mesured and diluted in a chlorine free water to make acquious solution of four concentrations: T0, control; T1, EM 10 ml/L; T2, EM 20 ml/L and T3, EM 30 ml/L. These EM concentrations constituted treatments that were applied on tomato plants using pre-pressurized knapsack sprayer (2L capacity). The treatments were applied ones a week for seven weeks. By spraying the plants with the EM mixture, cells and spores of *B. thuringiensis* were deposited on the leaves and other tomato organs. Insects surely ingested cells and spores of the pathogen while feeding on the treated tomato plants. A completely randomized design with 4 treatments in 3 replications was used.

Evaluation of pests populations in different plots

Every week and for seven weeks from the first EM application, a sample of 10 plants was taken from each treated plots and the parameters such as; abundance of insects, severity of *T. absoluta* infestation of leaves were measured. This was done in the morning before weekly EM application to avoid warming up of over-nighting insects and their mobility. Leaf miner, whitefly and aphids were counted on the different plots and the average numbers of insects were noted. For *T. absoluta*, the impact on the leaves was evaluated. The percentage of leaves infestation (IL) was calculated using the formula:

$$\mathrm{IL}(\%) = 100 \times \frac{\text{Number of leaves Infested by the pest on 10 plants}}{\text{Total number of leaves on 10 plants}}$$

Where: (IL=Infested leaves)

Effect of the treatments on the growth rate of plants

Groups of 10 plants were randomly selected in each treated group and the effect of biopesticide on the plants growth was evaluated. Data on plant height was measured using a tape.

Statistical analysis

Data were introduced in Statgraphic software and analysed using the statistical test of ANOVA, means were separated with Tukey test and figures drawn with Excel. All statistical analysis was done at the significant level of 5%.

Results

Effects of *Bacillus thuringiensis* on leaf miner moth, whitefly and aphids

The results revealed that *B. thuringiensis* showed insecticidal activity on the populations of *T. absoluta*, *B. tabaci* and *M. euphorbiae* on tomato plants in the treated plots as compared to the control plot.

Effects of *Bacillus thuringiensis* treatments on the population of *Tuta absoluta* Leaf miner moth

The EM biological pesticide had a significant effect on reducing the population of leaf miner moth, the ANOVA tests showed a significant difference between treatments (df=3, F=258.69, P=0.0000). Two weeks after transplantation the flies were found on all the plants and their population remains stable till the 3th week before reducing to 0 for all the treated plants (T1, T2 and T3) within four to five weeks after treatments while the population of *T. absoluta* increased in the control experiment (Figure 2).

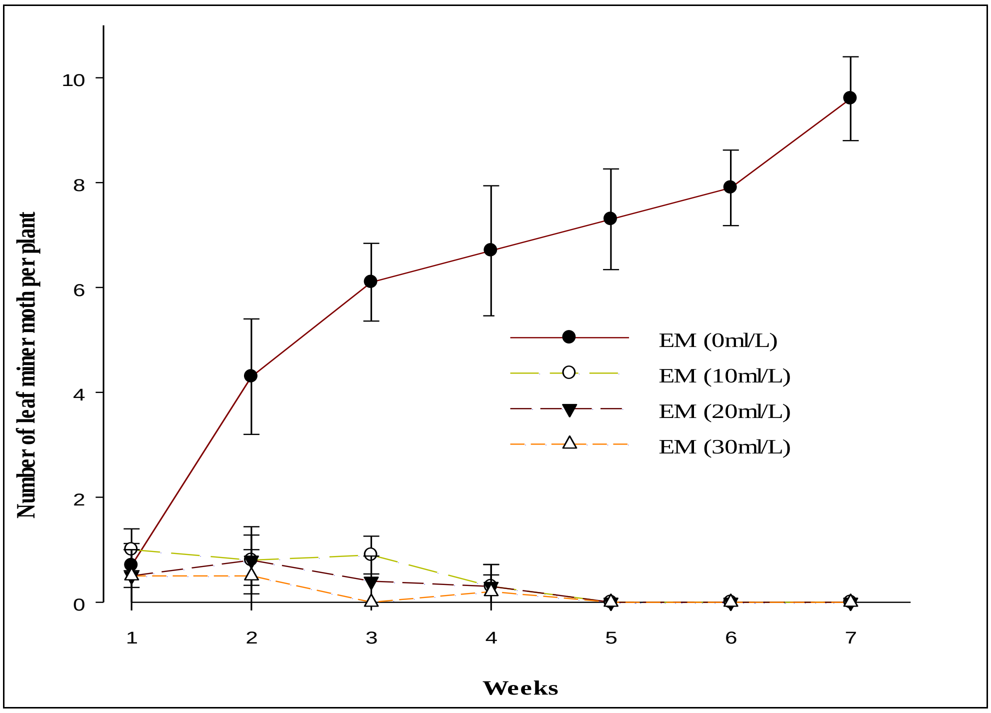

Fig 2: Evolution of leave miner *Tuta absoluta*'s population according to different treated plots with time.

Impact of different treatments on *Tuta absoluta*'s damages on tomato leaves

The impact of the treatments was also directly evaluated by counting the number of leaves damaged by the pest in the plots treated with different concentration of EM. They was a significant difference on leave infestation among the treatments (df=3, F=32.11, P=0.0000). The number of infected leaves increased gradually in the control treatment (T0), from tree weeks 95% the leaves of the plants showed sign of infestation. On the contrary the number of new leaves infected decreased with time in the three treated plots (T1, T2, and T3) and these infestations totally disappeared within after five weeks of treatment (figure 3).

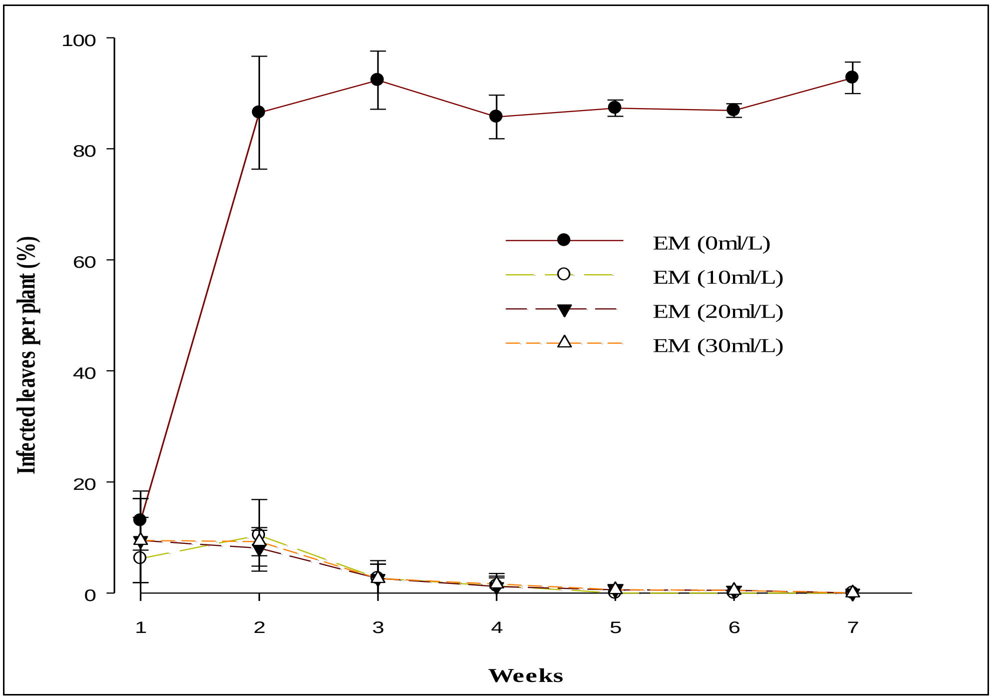

Fig 3: Evolution of the percentage of leaves infestation by *Tuta absoluta* according to treatments.

Effects of *Bacillus thuringiensis* treatments on the population of *Bemisia tabaci* (White fly)

The effect of *B. thuringiensis* the bio-insecticide on the whitefly's population was highly positive. There was a significant difference among the treatments (df=3, F=368.50, P=0.0000). In the negative control plot (T0) the population of whitefly *B. tabaci* increased from the first week after transplantation to attain the level of 30 individuals per plant within two weeks and remain more than 20/plant all over the sampling period. At the same time, in treated plots, the abundance of this pest decreased gradually from the first week to reach the level of 0 within 4 weeks (for concentrations T3 and T2) and five weeks for T1. However, there was no significant variation of *B. tabaci* abundance within (T1, T2 and T3) concentrations of the *B. thuringiencis* in the treated plots (Figure 4).

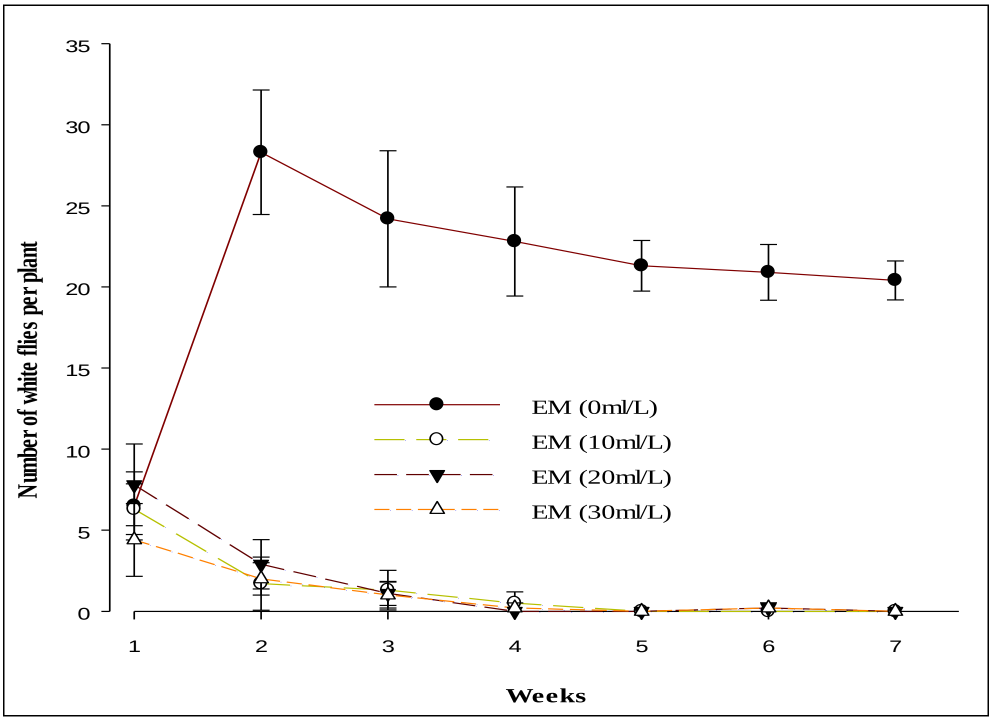

Figure 4: Evolution of *Bemisia tabaci* population with time for the four treatments.

Effects of *Bacillus thuringiensis* treatments on the population of *Macrosiphum euphorbiae* (Aphids)

The bio-insecticide treatments reduced population of aphids. There was a significant difference among the four treatments (df=3, F=44.21, P=0.0000). In the untreated control plot, the number of aphids increased gradually to attend a maximum of more than 2500 individuals within 7 weeks. On T1, T2 and T_3 all the plant that were treated by *B. thuringiensis* were free from the aphids all over the experimental period (Figure 5).

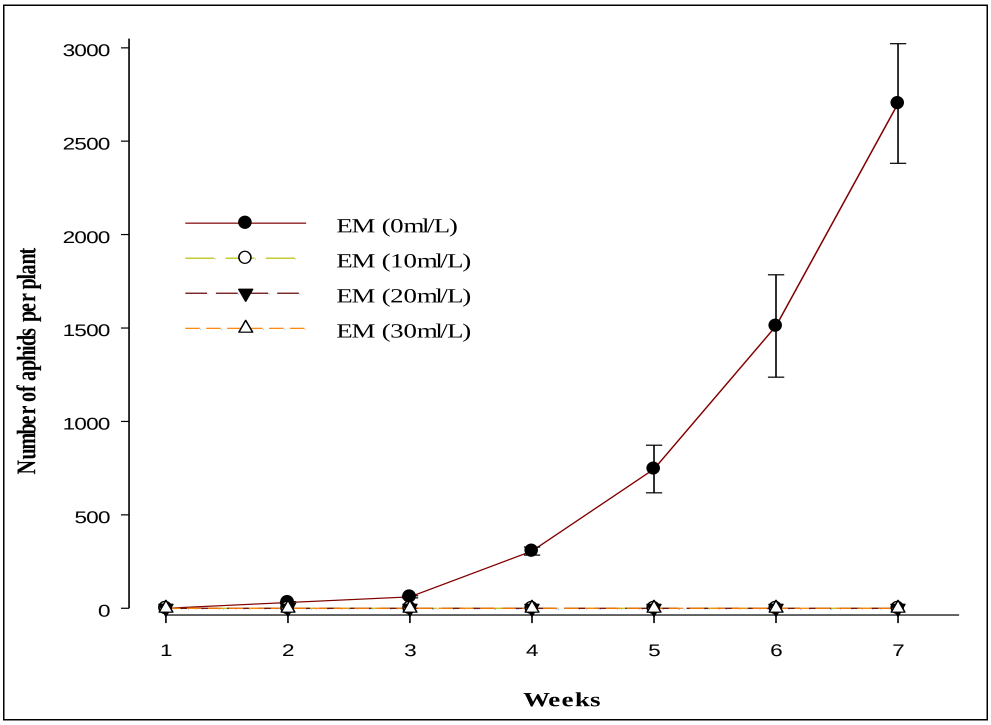

Fig 5: Population dynamic of *Macrosiphum euphorbiae* with time for the 4 treatments.

Effect of EM biological pesticide on the growth rate of tomato plant

At the end of the experiment, we observed that the growth rate of tomato for the control treatment (T0) where no EM was applied, was similar to the growth rate of tomato in the other three treated plots (T1,T2 and T3). The biological pesticide has no significant effect on the growth parameters of the plants (df=3, F=44.21, P=0.882) (Figure 6).

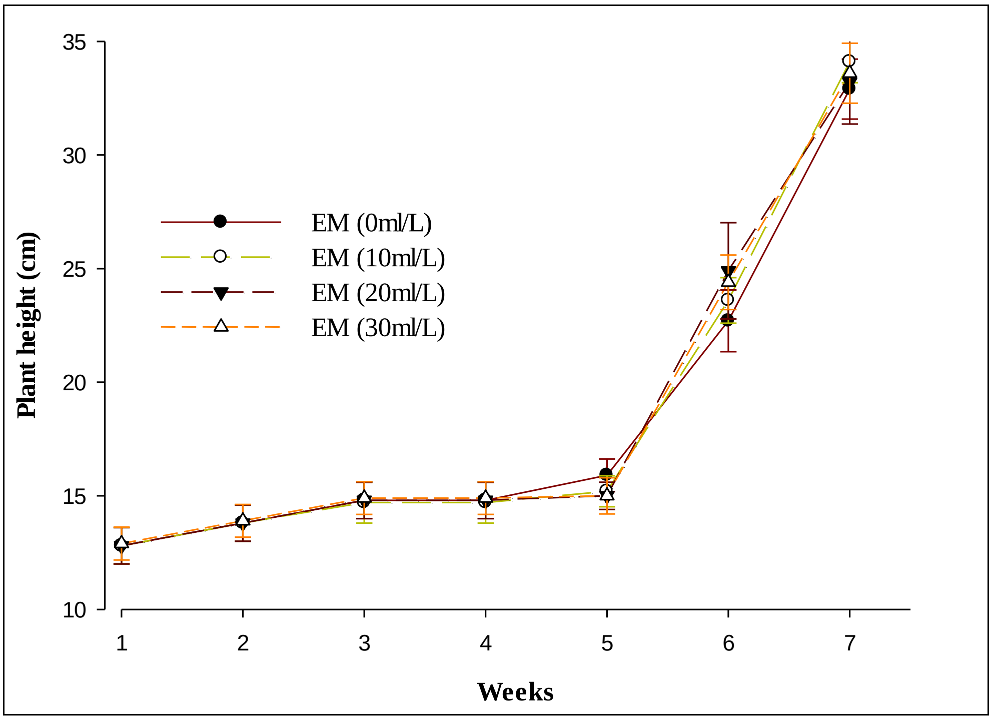

Fig 6: Evolution of growth rate of tomato plants treated by 4 different concentrations of EM.

Discussion

Results revealed that different concentrations of the bio-insecticide containing *B. thuringiensis* reduced the population of leaf miner moth, whitefly and aphids. The Highest number of these pests was recorded in the untreated plot. The toxicity of *B. thuringiensis* on tomato pests has been reported by many authors amongst which Thameur *et al.* (2020) [21], in the control of *H. armigera* a pests of tomato in India and also by Shahini *et al.* (2021) [19] in the control of *T. absoluta* in Albania, Mediterranean basin. Shaukat *et al.* (2010) [20] confirmed the efficacy of *B. thuringiensis* on many other crop pests. But Olle *et al.* (2013) [17] in his investigation argued that, among 22 reports on the effects of EM on the yields of vegetables 85% were positive, 4% were negative and 11% showed no significant influence. The microorganism *B. thuringiensis* acts by producing proteins (delta-endotoxin, the "toxic crystal") that reacts with the cells of the gut lining of susceptible insect pests. These *B. thuringiensis* proteins paralyze the digestive system, and the infected insect stops feeding within hours. *B. thuringiensis* affected insects generally die from starvation, which can take several days. Occasionally, the bacterium enters the insect's blood and reproduces within the insect (Shaukat *et al*., 2010) [20]. Therefore, the level of infestation of the pests reduced with time as *B. thuringiensis* bio-insecticide was being applied to the tomato plants. In the control plot, where *B. thuringiensis* was not applied, the population of the pests increased significantly with time (especially the aphids). This high population of aphids and other sap sucking pests of tomato plants caused the leaf to shrink and assume a needle-like shape. The consequence of this is retarded growth and poor yield since the photosynthetic capacity of the leaf is reduced (Okolle *et al*., 2005) [16]. However, we have not observed effect on the length of tomato plants.

The severity of attack was examined on the leaves particularly for *T. absoluta*. On the leaves, larvae penetrate and feed on the mesophyll parts. This results in irregular mines on the leaf surface, subsequently, decreasing the photosynthetic capacity of the plant. The galleries and mines in the leaves alter the general development of the plant and can cause necrosis (Biondi *et al.*, 2018) [5]. Under severe attacks, more than (95%) of the leaves were attacked and the plants in the garden generally present burnt appearance. Moreover, we suggest that they be included in the protocols to control *T. absoluta*, the application of IPM strategies that include the association of parasitoids mirids (Heumou *et al.*, 2022) [23] and usage of pheromones for mass trapping (Shahini *et al.*, 2021) [19] can constitutes an effective basis for the control of *T. absoluta* using biological methods.

In this study, *B. thuringiensis* at different concentration affected the rate of infestation of the pests differently on the treated plots. The least concentration of the bio-insecticide (10ml/L) took a longer time to reduce the pest population to zero while the 30ml/l took the shortest time. On the three different pests, 4 to 5 *B. thuringiensis* weekly applications repetitions were sufficient to reduce the pest populations to zero. Amongst the three pests studied, *B. thuringiensis* showed the highest efficacy on *M. eurphorbiae* since no individual was found on the treated plots. In a comparison study between the bio and synthetic chemical pesticide on the 2nd instars larvae of *T. absoluta*, Mohamed *et al.*, (2020) [24] showed under laboratory conditions that *B. thuringiensis* had significantly higher toxicity than Spinosad a synthetic chemical pesticide.

B. thuringiensis didn't show any effect on the tomato plants growth parameters such as plant height. This could be explained by the fact that EM bio-pesticide lack bio-fertilizing components. Moreover, high level of infestation of these pests was attained when the plants were mature and the impact couldn't be appreciated by then. This is contrary to the work of Okolle *et al*., (2005) [16] who showed that high level of infestation of these pests could limit the growth of the plant since the sap is being sucked and rate of photosynthesis reduced.

Conclusion

The study revealed that EM bio-pesticide containing *B. thuringiensis* have potential in controlling phytophagous insects that affect plants. In particular the effectiveness of *B. thuringiensis* in reducing the populations of insect pests like *T. absoluta*, *B. tabaci* and *M. eurphorbiae* is promising. No bio-fertilizing effects were observed on the plant growth. However bio-insecticide of *B. thuringiensis* base formulation can be introduced in Integrated Pests Management program of tomato agrosystems to bring insect pests below economic injury level.

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