

Botanicals, as a Sustainable Agroecological Alternative to Synthetic Pesticide for Controlling Leaf Miner (Pinworm) and Fusarium Wilt Disease of Tomato

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Abstract

Tomato (*Solanum lycopersicum* Mill) is a staple fruit vegetable widely grown worldwide for its rich nutrients including vitamins, minerals and proteins. In spite of its importance, yield and fruit quality in Buea, Cameroon is quite low compared to other countries. Pests and diseases are responsible for these limitations. Those of high importance are pinworm (*Tatu absoluta*) and Fusarium wilt respectively. Synthetic chemicals have been used to manage this pest/disease on tomato but this has not been very successful because of high cost and unavailability of these chemicals. Botanicals are known to have anti-microbial properties. 10% Leeks and marigold extracts were applied to tomato plants one week after planting. Five treatments were replicated four times in a randomized complete block. They include a control, a synthetic insecticide, leeks extract, marigold extract, and leeks/marigold extracts. Growth and yield parameters were evaluated and data obtained was analysed by descriptive statistics and ANOVA. Results showed that more tomato plant leaves were recorded in the leeks + marigold treatment (54) which differed significantly ($P = 0.05$) from the control (45). More fruits were harvested in the leeks + marigold treatment (32) which differed significantly ($P = 0.05$) from the control (18). Pinworm numbers were significantly higher in control (6) ($P = 0.05$) than the other treatments. Numerous leaf damage was observed in control (20) which differed significantly ($P = 0.05$) across treatments. More fruit damage was seen in control (14) and differed significantly ($P = 0.05$) from the other treatments. The least disease incidence was noted in Leeks +

Marigold treatment (27.5%), and the most in control (72.5%) which differed significantly ($P = 0.05$) across treatments. *Fusarium oxysporium* and *Fusarium semitectum* were identified. There was a significant difference in pathogen number in control compared to other treatments. Thus leeks and marigold extract increased yield of tomato by mitigating the effects of leaf miner and fusarium wilt.

Keywords

Leeks, Marigold, Disease Incidence, Fungal Pathogens

1. Introduction

Tomato is a Solanaceae that originates from the Andes, modern-day Chile, Bolivia, Ecuador, Colombia, and Peru [1]. China was the highest world tomato producer in 2017, and Cameroon ranked 20th with annual production of 1,068,495 [2]. This edible and nutritious fruit vegetable is a staple in Cameroon that is grown both on a small and commercial scale by local farmers. Tomato contains vitamins A, B, C and D; minerals including iron, calcium. It also has carbohydrate, proteins, water and cellulose

Nonetheless, the full production potential of this crop has been limited due to several factors, including insect pests like the fruit fly, fruit borer, whitefly and diseases like tomato blight, rot, leaf spot, wilt, and mildew [3] [4] [5] [6]. The most critical pest limiting production of tomato is the tomato leaf miner *Ta-tuabsoluta* which recently spread to Africa [5] [7] and a soil-borne systemic fungus (*Fusarium oxysporium*) [4]. In Cameroon, three regions (Northeast, West, and South) were reportedly affected by pinworm (*Tatu absulata*), with severe yield loss of 80% - 100% [8] [9] [10]. *Fusarium oxysporium*, on the other hand, causes wilting on tomato plants that result in loss of up to 80% in highly infested fields. The quest to increase tomato production has led to uncontrolled use of synthetic pesticides [11] [12].

Pesticides used by farmers in Cameroon for managing diseases are not limited to the production stage alone but extend to postharvest storage [13], as tomato is cultivated over intensive application of synthetic pesticides [11]. The incessant exploit of these non-natural pesticides helps curb the pest but has setbacks like ecological backlash of three “Rs” (Resistance, Resurgence and Replacement) [14] [15]. The use of synthetic pesticides inevitably leads to the presence of residues, which unfortunately deteriorates the health of farmers, consumers, and the quality of the environment [16] [17] [18]. Again, the high cost of imported synthetic pesticides makes it difficult for local farmers to purchase them. For instance, the annual cost of synthetic pesticides for controlling the most important tomato pests has been estimated at US\$3.25 billion. They are not always available and expensive [19].

Therefore, plant disease/pest management alternatives that are environmentally friendly, without side effects on humans, and economically affordable to farmers are needed to improve yield and fruit quality [20] [21] [22]. Plant bioactive extracts are known to have anti-microbial and anti-insecticidal properties which are ecologically friendly, available, durable, affordable and effective for pest/disease management. This is because, plants synthesize hundreds of chemical compounds for various functions including defense and protection against insects, fungi, diseases and herbivorous mammals [23] [24]. Therefore, this work aims at evaluating extracts of botanicals (10% Leek extract, 10% Marigold extract, and 10% Marigold/Leek extract) in controlling tomato leaf miner and fusarium wilt as a cost-effective and eco-friendly alternative to synthetic pesticides.

2. Materials and Methods

2.1. Description of Experimental Site

This research was carried out at the University of Buea, Faculty of Agriculture and Veterinary Medicine Teaching and Research Farm. The location of the site is at the foot of Mount Cameroon, Southwest Region, situated between latitudes 3°27' and 4°27'N and longitude 8°58' and 9°25'E and at an elevation of about 500 to 1000 m above sea level. Buea has a mono-modal rainfall regime with 86% relative humidity and sunshine between 900 to 12,000 hours per annum. The dry season starts from October to March, and the rainy season from March to September, with a mean annual rainfall of 2800 mm. The mean monthly air temperature ranges from 19°C - 30°C, and soil temperature at 10 cm depth decreases from 25°C to 15°C. The soil is derived from weathered volcanic rocks dominated by silt and clay [25] [26] [27].

2.2. Botanical Extracts Preparation and Management of Leaf Miner

Botanical extracts were prepared from fresh leaves of pot marigold and Leek. Pot marigold leaves were harvested from the surroundings, and the mint leaves were weighed by using an electronic balance (S METTLER) to obtain 200 g. Water extract of botanicals was prepared as follows; 200 g of the fresh pot marigold leaves were blended in a kitchen blender and mixed with 2 L of distilled water. 25 g detergent (SABA power detergent manufactured by PACIFIC INTER-LINK Malaysia) was added to the mixture and kept for 24 hours at room temperature. The mixture was then filtered through a 250-micron sieve to obtain liquid extracts from the plant. Fresh leeks (wild leeks) were bought from the local Muea market. The same procedure for preparing marigold extract was used to prepare leek extract. The third treatment was a 50:50 combination of leek and pot marigold. The extracts were sprayed directly after preparation without diluting with the aid of a knapsack sprayer. Synthetic insecticide, Emamectin benzoate 50 g/kg at a rate of 7.5 g/15l and diluted in water. All applications were done weekly from the first week after planting until flowering.

Establishment of tomato nursery

A ridge measuring 2 m by 1 m was raised to 20 cm high with a hoe. 15 kg of poultry manure was mixed on the mound. The ridge was disinfected by spreading 5 g of Terbufos mixed with 80 g of Mancozeb in a 15 L of water knapsack sprayer. 10 g of Cobra F1 26 hybrid seeds were then broadcasted on the raised bed. Watering was done before and after broadcasting. The ridge was covered with plantain leaves to initiate a microclimate for rapid germination and removed three days after germination.

2.3. Experimental Design

Meter tape was used to measure 210 m² of the experimental field. The field was manually cleared and sprayed with systemic herbicide. Five treatments were replicated four times in a randomized complete block experimental design (**Figure 1**). Twenty plots measuring 2 m × 2 m were demarcated and raised 25 cm high with hoe. Replicates plots were segregated by 0.5 m and a replicate to another by 1 m. Spacing of 50 cm × 75 cm were used to marked the planting spots and pegs were placed at the spots giving 3 inter and 4 intra rows with 12 stands per plot.

2.3.1. Transplanting of Tomato Seedlings

Transplanting was done in the evening periods, between 4 - 6 pm by hand three weeks after seeding on the nursery and when seedlings had at least four true leaves. The nursery beds were watered for 2 hours before transplanting into the experimental plots according to the planting distance and treatments (**Table 1**). Two seedlings were planted per stand, giving 24 plants per experimental plot.

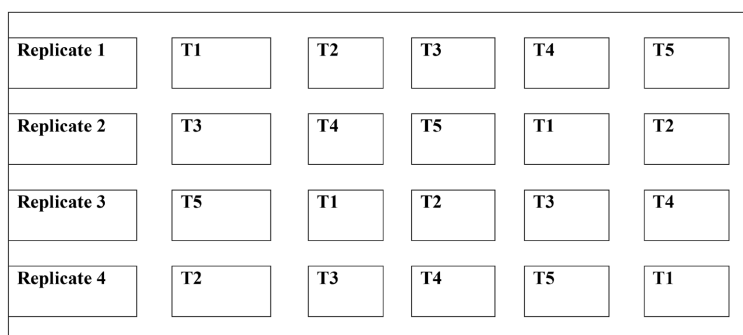


Figure 1. Randomize complete block design with five treatments and four replicates.

Table 1. Treatments.

Codes	Treatments
T1	Control
T2	Synthetic pesticide
T3	Leeks extract
T4	Marigold extract
T5	Leek + Marigold extract

2.3.2. Maintenance of Tomato Crop

Nutrients Provided for the Growth/Yield of Tomatoes

Immediately after transplanting, watering was done, and the pattern was maintained every evening at 5 pm when it did not rain or if there was a decrease in soil moisture due to increased sunshine. 40 g of poultry manure was applied at the planting spots two weeks before transplanting and 3 weeks after transplanting. Mineral amendment (N:P:K—20:10:10) was used in a ring form at a rate of 10 g per stand.

2.4. Data Collection

2.4.1. Vegetative, Pinworm Damage, and Yield Data

Data collection started three weeks after transplanting (WAT) and continued weekly. Data was collected from five tagged plants per experimental plot. Data collected were; leaf number, damaged number of leaves by pinworms, number of fruits harvested, and number of fruits damaged by pinworms.

2.4.2. Disease Data Collection

1) Inspection of Field and Samples Collection for Identification

The experimental field was visited weekly, three weeks after planting, to observe wilt symptoms on plants. Infected materials were collected from each plot, labeled based on treatment, plot number and symptom type. Samples collected were put in zip-locked bags before being taken to the University of Buea Faculty of Science Laboratory for Fusarium identification studies. Sample collection and transportation were done following plant quarantine guidelines. The samples were stored in a cold room and fusarium identification was done using diagnostic keys of Rayner [28] and Kirk *et al.* [29].

2) Methods; Isolation and Identification of Fusarium

The preparation of samples was as described by Summerell *et al.* [30]. Glassware were washed using detergent, while dipping scalpels, cork borer, inoculation needles into 70% ethanol before applying red hot flame from the burner for sterilization. Beakers, agar plates and pipettes were sterilized at 120°C in an oven for one hour and allowed to cool. Before samples were plated on semi solid Potato Dextrose Agar (PDA), powering of the laminar flow was done for 2 hours. 1 L conical flask with distilled water was used to suspend PDA in a water bath and heated at 50°C for 20 minutes, subsequently autoclaved for 20 minutes at 121°C for sterilization and stored at 4°C in the refrigerator after cooling.

As directed by Summerell *et al.* [30], about 2 mm² of plant tissue was cut using sterile scissors. It was washed in tap water and sterilized for 5 minutes in a solution of 1% Sodium hypochlorite. A 9 cm diameter Petri-dish was used to rinsed the plant tissue with distilled water four times at a one-minute interval. Separated plating of four pieces of the planting material was done at the edges of Petri-dish containing Potato Dextrose Agar (PDA) and the other on a selective medium for isolating Fusarium, Peptone-Pentachloronitrobenzene (PPA). Mi-

croscopic examination and colony characteristics procedure were as described by Agbor *et al.*, 2022 while description of micro conidia, and colony were done according to Kirk *et al.* [29], Rayner's morphological chart [28] and Hawsksworth *et al.* [31]

$$\text{Incidence of disease (\%)} = \frac{\text{number of infected plants}}{\text{Total number of sampled plants}} \times 100 \quad [32].$$

Abundance = Number of isolated individual pathogens per total number of samples collected per treatment.

2.5. Data Analysis

Data collected were subjected to descriptive statistics and they were keyed into Microsoft Excel spreadsheet 2016, after which they were transferred into compatible software, IBM SPSS Statistics for Windows (SPSS v26). Variables were exposed to univariate analysis of variance (ANOVA, $P < 0.05$) to test the effect of treatments ($n = 5$) as categorical predictors. Significant means were separated by Duncan Multiple Range Test (DMRT) $P = 0.05$.

3. Results

3.1. Effect of Treatments on the Number of Leaves and Fruits of Tomato

The number of leaves of the tomato plant ranged from 45 to 54 leaves per plant (Figure 2) and differed significantly ($F_{4,11} = 4.674$, $P = 0.012$) athwart treatments, likewise more number of leaves in leeks + marigold treatment (54) and fewest in control (45) (Figure 2). The number of fruits of tomato ranged from 18 to 32 per plant (Figure 3) and differed significantly ($F_{4,11} = 15.466$, $P = 0.000$) athwart treatments, with the highest in synthetic insecticide treatment (32) and lowest in control (18) (Figure 3).

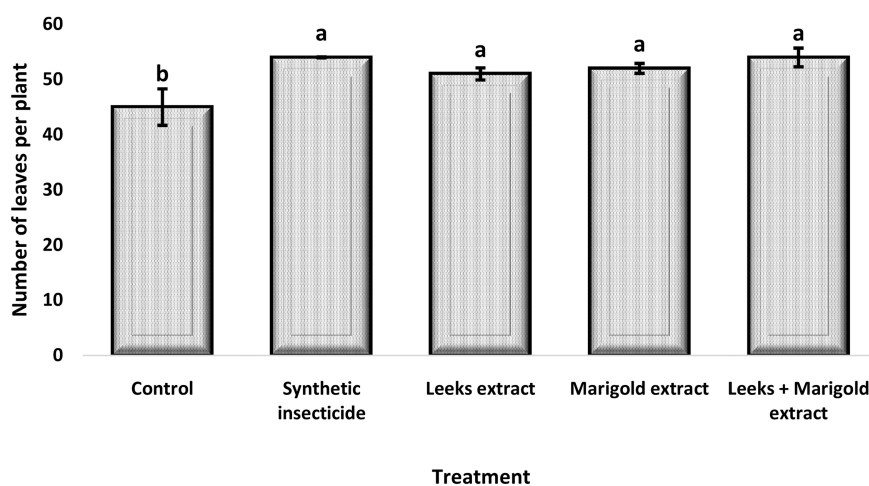


Figure 2. Effect of treatments on the number of leaves of tomato. Different letter columns are significantly different ($P < 0.05$), Duncan Multiple Range Test.

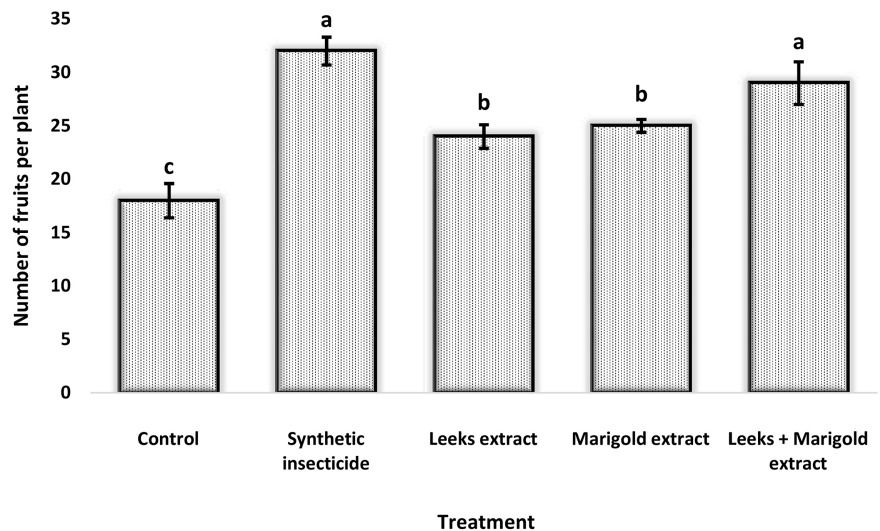


Figure 3. Effect of treatments on the number of fruits of tomato. Different letter columns are significantly different ($P < 0.05$), Duncan Multiple Range Test.

3.2. Treatment Effect on Pinworms Number and Their Damage on Leaves and Fruits

The number of pinworms ranged from 2 to 6 per plant (Figure 4) and differed significantly ($F_{4,11} = 17.909$, $P = 0.000$) among treatments, with the least in synthetic insecticide treatment (2) and the most in control (6) (Figure 4). The number of leaves damaged ranged from 12 to 20 leaves per plant (Figure 5) and differed significantly ($F_{4,11} = 5.671$, $P = 0.006$) among treatments, with the least in leeks + marigold treatment (12) and the most in control (20) (Figure 5). The number of damaged fruits of tomato ranged from 7 to 14 per plant (Figure 6) and differed significantly ($F_{4,11} = 6.137$, $P = 0.004$) among treatments, with the least in synthetic insecticide treatment (7) and the most in control (14) (Figure 6).

3.3. Symptoms of Fusarium Wilt on Tomato

Plant leaves lost turgidity and shriveled fallen downwards. The stem and branches were weak and could not support the plant upright. The plant eventually withers and dies. Lesions and browning were noticed on infected roots and there was a general decrease in root hairs. Roots finally got rotted as the plant dies (Figure 7).

Fungal Pathogens Morphological Features after Isolation and Culture

Banana-shaped *Fusarium oxysporium* (FO) isolates had microconidia with three main septa and were foot-shaped at the basal cell. Their microconidia were elongated, uniformed and two-celled (Figure 8). *Fusarium semitectum* had a pale violet colony coloration on PDA. The microconidia had variable septations with 3 - 5 septa, single septate with 2-celled microconidium, polyphialides were paired, and the chlamydospores in chains (Figure 8 and Figure 9).

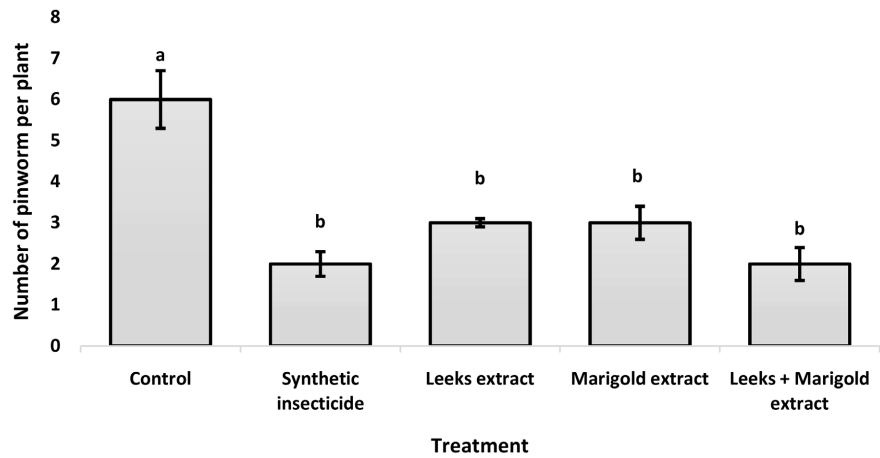


Figure 4. Effect of treatments on the number of pinworms on tomato. Different letter columns are significantly different ($P < 0.05$), Duncan Multiple Range Test.

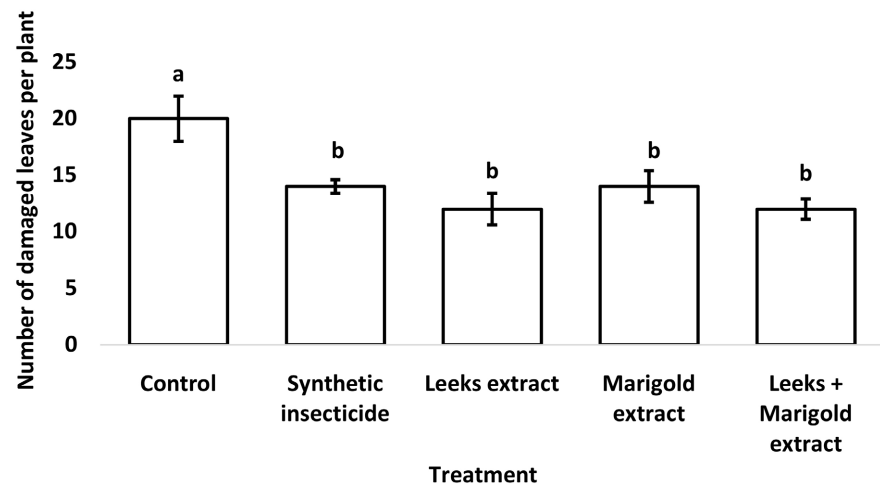


Figure 5. Effect of treatments on the number of damaged leaves of tomato. Different letter columns are significantly different ($P < 0.05$), Duncan Multiple Range Test.

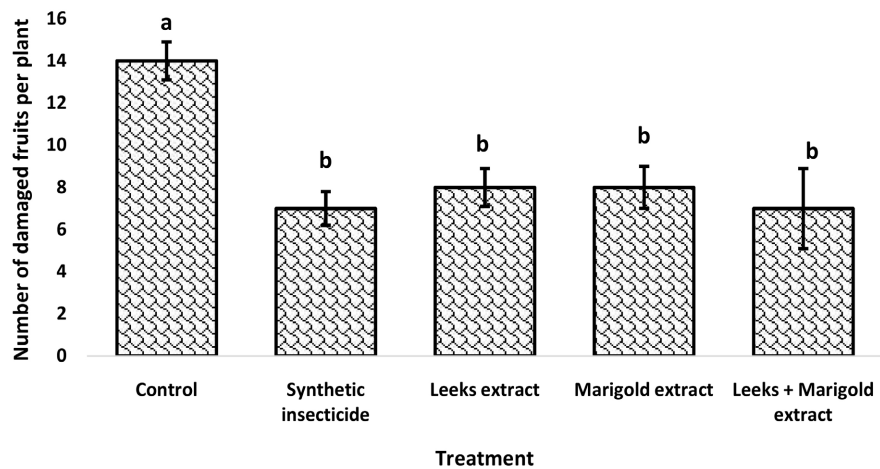
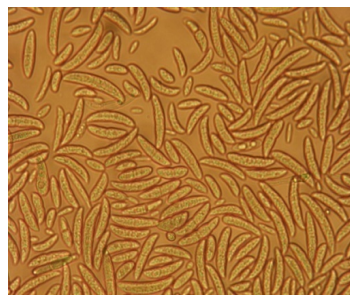


Figure 6. Effect of treatments on the number of damaged fruits of tomato. Different letter columns are significantly different ($P < 0.05$), Duncan Multiple Range Test.



Figure 7. Wilt symptom on tomato plant.

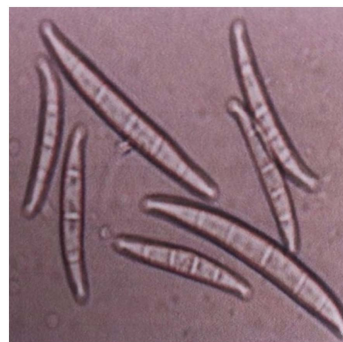


(a)



(b)

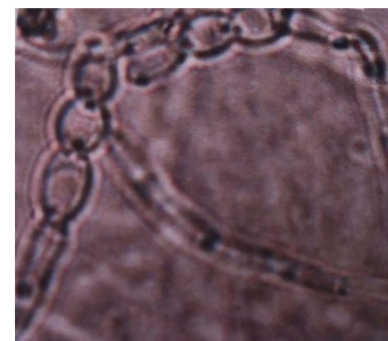
Figure 8. *Fusarium oxysporium*. (a) Microconidia which is foot-shaped at the basal end; (b) Elongated uniform two-celled microconidium.



(a)



(b)



(c)

Figure 9. *Fusarium semitectum*. (a) Microconidia with variable septations; (b) Paired polyphialides; (c) Chlamydospores in chains.

3.4. Impact of Treatments on Wilt Disease Incidence of Fusarium and Abundance of Pathogen in Tomato

Tomato disease incidence differed significantly ($F_{4,15} = 12.132$, $P = 0.000$) among treatments with the least in Leeks + Marigold treatment (27.5%) and the most in the control 72.5% (Figure 10).

In Table 2, incidence of *Fusarium oxysporium* differed significantly ($F_{4,15} = 9.441$, $P = 0.001$) among treatments with the most in control (8) and the least in Leeks + Marigold treatment (4). *Fusarium semitectum* (FS) differed significantly ($F_{4,15} = 12.132$, $P = 0.000$) among treatments with the most in control (7) and least in Leeks + Marigold treatment (3). Overall, more of the fusarium pathogens were found in control (15), which differed significantly ($F_{4,15} = 11.238$, $P = 0.000$) among treatments and less pathogens seen in Leeks + Marigold treatment (7) (Table 2).

4. Discussion

4.1. Effect of Treatments on Tomato Number of Leaves and Fruit Yield

The high number of leaves and the number of fruits produced in the botanical

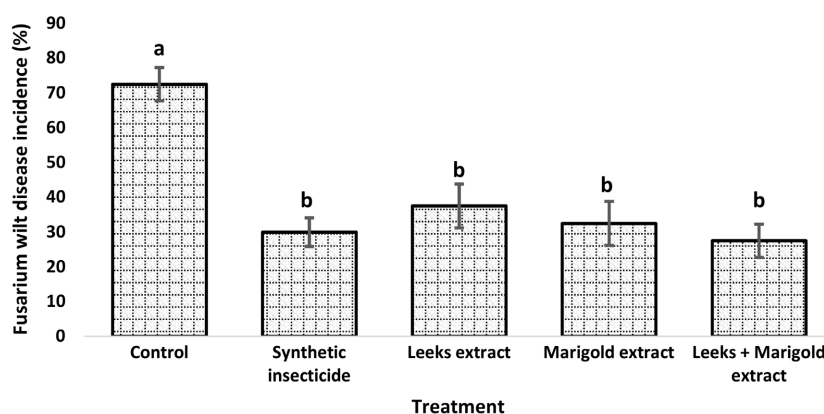


Figure 10. Effect of treatments on tomato disease incidence. Different letter columns are significantly different ($P < 0.05$), Duncan Multiple Range Test.

Table 2. Effect of treatments on the abundance of tomato fungi pathogens.

Treatment	<i>Fusarium oxysporium</i> (FO)	<i>Fusarium semitectum</i> (FS)	Total pathogens
Control	8 ± 0.5 ^a	7 ± 0.5 ^a	15 ± 1.0 ^a
Synthetic insecticide	4 ± 0.5 ^b	3 ± 0.5 ^b	7 ± 1.0 ^b
Leeks extract	5 ± 0.5 ^b	4 ± 0.5 ^b	9 ± 1.0 ^b
Marigold extract	4 ± 0.5 ^b	3 ± 0.5 ^b	8 ± 1.0 ^b
Leeks + Marigold extract	4 ± 0.5 ^b	3 ± 0.5 ^b	7 ± 1.0 ^b

Values within the column with the same letters are not significantly different according to Duncan Multiple Range Test, $P < 0.05$.

treatments illustrate their ability to control pinworm and fusarium wilt disease of tomato plants. This allows the plants to produce more leaves, increasing the photosynthesis surface, translating to more fruits [33]. Coincidentally, other narratives have demonstrated compelling results of botanical extracts eloquently increasing crop productivity compared to none treated crops [34]. The fewer leaves and fewer fruits in control showcase the absence of a defense mechanism against pinworm and fusarium wilt disease, thus giving them a leeway to devour the crop, drastically reducing their performance [24]. It can also be noticed that the botanical extract, especially the Leeks + marigold extract treatments, has very comparative effects on the performance of tomato plants to that of synthetic insecticide, as also seen in other works [35] [36]. Pinworm alone has been shown to reduce up to 80% - 100% of tomato fruit yield, meaning its control in the applied treatment plants massively boosted their performance [10]. While fusarium wilt damage can amount to 80 % of crop loss, it is evident in this study that the treatments successfully mitigated the disease effect, which conforms with other views [6].

4.2. Effect of Treatments on Tomato Number of Pinworm, Leaves, and Fruits Damaged

The reality that the applied strategies managed the malicious pinworm infestations more than the control treatments means that they effectively limit its damage. Particularly with the botanicals, this efficacy in abating pinworm results from secondary metabolites they harbor [23] [37]. Leeks contain the compound saponins and allicin, while Marigold subsumes geraniol [24] [38]. These compounds could have worked as neurotoxins, inhibiting acetylcholinesterase and octopamine, inflicting insect dysfunction and dead [39] [40]. This led to reduced pinworm infestation in botanical extract treatments. Botanical extracts of leeks and marigold have been evinced to exhibit pest-control potential [35]. The reduced number of pinworms has translated to few leaves and fruits damaged in the administered treatments, while control treatments suffer the consequence of no form of applied control measure [41]. Looking at these results, the botanical extracts had overall comparative performance in suppressing tomato pinworm and limiting damage to leaves and fruits with synthetic insecticide; however, they are substantially unique from the control, which is in line with preceding articles [42] [43]. Botanical extracts derived from leeks and marigold have shown evidence for pest control, and their effectiveness against insects has been reported [35] [36]. The dominant force of synthetic insecticide over leeks and marigold extracts is affiliated to their broad spectrum of activity [44].

4.3. Impact of Treatments on Wilt Disease Incidence

Fusarium wilt disease on tomato plants caused by *Fusarium oxysporium* and fusarium crown and root diseases caused by *Fusarium semitectum* (FS) is the most

devastating soil-borne diseases of tomato [4]. They occur worldwide in the field and lead to significant losses in tomato production. From this study, the identification of the symptoms of lesions, browning, loss of turgidity, and wilting of leaves, as well as the isolation of the fungal pathogens, enabled a clear view of the compelling actions of the botanical treatments in combating the disease which is consistent with Agbor *et al.* [6]. Further laboratory investigations of the fungal isolates gathered, including cultural attributes observed on PDA such as the microconidia with mainly three septa and foot-shaped at the basal cell and a pale violet colony coloration confirmed the pathogens *Fusarium oxysporium* and *Fusarium semitectum*. The high pace of disease incidence in control depicts the absence of a diseases regulation system, in this way reassuring diseases to multiply openly, while the opposite was found in different therapies applied with minimal frequency in the leeks and marigold extracts confirming their pesticidal properties [4] [33] [45]. Swarmed leaves investigation uncovers two parasitic fungal pathogens devouring tomato plants' coherent development, resulting in infections that decrease yield [6]. It can be seen that leeks and marigold extract limit the mischief brought about by these fungal pathogens altogether [46] [47]. This outcome is in line with the speculation of this study.

5. Conclusion

This study reveals the biocontrol efficacy of leeks and marigold botanical extracts in limiting pinworm and fusarium wilt on tomato plants. The botanicals massively reduced the disease incidence, the number of pinworms, leaves, and fruits damaged, as well as increased the number of leaves and fruits of the tomato plants, affirming their pesticidal potential under field conditions. These results allow resource-poor farmers to use leeks and marigold botanical extracts as a source of effective and affordable biocontrol eco-friendly alternatives to synthetic insecticide in managing pests and diseases of tomato.

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Authors' Contributions

This work was carried out in collaboration with all authors. Author DTA processed data, performed statistics, and literature searches, and wrote the first manuscript draft. Author GTM, DBA, EETB and EAE designed and established field trials, collected data, and performed literature searches. Author TTO did laboratory analysis, coordinated field site, manuscript preparation, and conducted literature searches.

The final manuscript has been read by all authors.

Conflicts of Interest

The authors say they have no conflicting interests regarding this paper.

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