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Effect of bio-pesticide on controlling pest, disease, and yield of green onion under greenhouse conditions

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Abstract

This study evaluated the effect of a spice bio-pesticide product derived from garlic, chili, ginger, onion and lemongrass on green onion pests and diseases, plant growth and yield, soil chemical properties and soil microbial populations under greenhouse conditions. A randomized design experiment with 13 treatments and two replications tested three different concentrations of the bioproduct at 8%, 6%, and 4% in combination with four different spray time intervals: every 3 days, every 7 days, every 10 days, and only at the appearance of pest/disease. The 6% bio-pesticide concentration sprayed every 10 days had the lowest anthracnose disease rate. The 4% bio-pesticide concentration sprayed every 7 days treatment compared to the control resulted in a 31.4% increase in yield of fresh green onions. The bio-pesticide increased soil pH in all treatments after two consecutive green onion crops compared to the control. Surveys of soil microorganisms after two consecutive crops showed the 4% bio-pesticide concentration significantly increased the number of soil bacteria such as beneficial nitrogen fixing bacteria, phosphate solubilizing bacteria and silicon solubilizing bacteria compared to the control. Bio-pesticide applications at any concentration reduced the soil fungal population; and had no effect on the actinomycetes population. While this bio-pesticide showed potential to biologically control green onion pests/disease, additional greenhouse and field experiments are needed to replicate the effects of this spice bio-pesticide application on yields and soil bacteria densities in different soil types and under different crop conditions.

Keywords: Biological control, Bio-pesticide, Garlic, Chili, Ginger, Green onion, Lemongrass, Soil microorganisms, Beneficial bacteria

1. Introduction

Green onion (*Allium fistulosum* L.) is one of the vegetable crops widely grown in the Mekong River Delta of Vietnam. Consumer demand for this vegetable is strong and green onion farmers can make 5-7 times higher profits compared to cultivating rice [1]. Vinh Long province is the largest onion growing region in the Mekong Delta with Binh Tan district having more than 1000 hectares of green onion under cultivation. The profitability of this crop leads farmers to intensively grow green onions crops continuously rather than rotating with other crops. This monocropping over many seasons causes an increase in pests and diseases especially anthracnose, rot and onion borer that reduce crop productivity. The primary strategy farmers use to control pest and disease pressure is to increase the amount and frequency of pesticides and synthetic fertilizers on their green onions.

These three serious green onion diseases, anthracnose, soft rot, and leaf tip wilt, threaten plant growth, capacity to reach maturity, yields, crop quality and farmer incomes. Soft rot disease is caused by *Erwinia carotovora* bacteria present in many green onion fields including this study site. About 95% of surveyed farmers in Binh Tan district had crops infected with this disease, leading to a big loss in green onion yields. A second common green onion disease is anthracnose, a fungal disease caused by *Colletotrichum* spp. Around 75% of surveyed farmers reported their crops had this fungal disease that caused their plants to wilt and shoots and leaf tissues to die. Leaf tip wilt disease is a third common disease appearing on green onions, however only 11.7% of surveyed farmers reported this in their green onion crop [2]. Farmers in this study reported they used a wide variety of pesticides to control pests and diseases on green onions and applied them at a higher dose and frequency than recommended. These pesticides had five types of active ingredients and farmers reported usage to manage diseases as follows: mancozeb (90%), difenoconazole (18.3%), hexaconazole (13.3%), and validamycin A (8.3%). Two other pesticides were used by 6.7% of surveyed farmers with active ingredients propiconazole and propineb; and some farmers reported fosetyl aluminum, trifloxystrobin, and azoxystrobin as active ingredients used for plant disease control. For pest control, pyridalyl was the most common active ingredient used by 35% farmers at this site, followed by emamectin benzoate (28.3%), and cyromazine (26.7%). Moreover, active ingredients such as chlorfluazuron, chlorantraniliprole, chlorfenapyr, spinetoram, permethrin, fipronil, cartap were also recorded and ranged from 1.7% to 10% of farmers using them. The volume of synthetic pesticides used by farmers was relatively high, averaging 908 g/mL/1000 m^2/crop. The amount of the fungicide used for controlling plant diseases was much higher, with applications averaging over 169 mL/1000 m^2/crop [2].

The indiscriminate and excessive use of pesticides increases production costs, degrades soil quality, and affects the quality and marketability of the green onion with pesticide residues on products a high concern of consumers. Farmers need alternative strategies to control pests and diseases on their green onion crops while achieving good yields with less harm to soil, water and other agroecosystem resources needed for them to successfully continue to farm. Biological approaches to managing crop disease and pests have been found to be environmentally friendly and there is a need for more scientific research to evaluate bio-pesticide disease and pest efficacy and environmental impacts [3]. Biological and non-chemical pest control methods are a cornerstone of organic farming and are increasingly used in sustainable integrated pest management plant protection strategies [4].

In recent years, a number of studies have applied extracts from popular spices such as onion, garlic, lemongrass, chili and ginger to protect and stimulate crop growth [5,6,7]. The research of Abd-El-Khair and Haggag [8] and Wei et al [9] found that garlic and ginger have the ability to protect many plants, give them resistance to some pathogens, and help to stimulate the growth of many crops [10,11]. Wang et al [12] suggested that garlic, ginger, lemongrass, and hot chili pepper could reduce the damage of pests and diseases on eggplant in a monoculture system, while Xiao et al [13] and Ahmad et al [14] reported on the efficacy of these extracts on cucumber and pepper. In Vietnam and world-wide there are few studies on the efficacy of bio-pesticide products from these spice extracts to control pests and diseases. The present study evaluates the efficacy of a bio-pesticide product containing onion, garlic, lemongrass, chili and ginger to control pest and disease and examines the effects on green onion growth and yields with specific attention to soil microbiological properties under greenhouse conditions.

2. Materials and methods

2.1 Materials

2.1.1 Bio-pesticide product

The ingredients for the formulation of the bio-pesticide tested in this study were obtained from local sources. These materials were onion (*Allium cepa*), garlic (*Allium sativum*), chili pepper (*Capsicum frutescens* L.), ginger (*Zingiber officinale*) and lemongrass (*Cymbopogon sp*.). The bio-pesticide product was prepared according to Otunola et al. [15]. Briefly, 1 kg onion, 1 kg garlic, 1 kg chili pepper, 1 kg ginger and 1 kg of lemongrass were finely ground, then mixed with 5 L of white wine and poured into a 10 L glass jar to ferment under standard laboratory conditions for 5 days. Then more wine was added to the jar to reach 75% volume of the jar and allowed to continue to ferment for another 5 days. After 10 days the mixture was filtered using a strainer and the fluid collected. This fluid was the base formulation for the bio-pesticide used in this experiment.

2.1.2 Soil samples

The soil for the greenhouse pot experiment was collected from a green onion farm in Tan Binh commune, Binh Tan district, Vinh Long province at a depth of 0-20 cm from several points in the field, and then thoroughly mixed into a large sample. A portion of the soil sample was used for analysis of physical, chemical and biological properties of soil at the Laboratory of Soil Physics, Soil Chemistry and Soil Biology, the Department of Soil Science, Faculty of Agriculture, Can Tho University, Vietnam.

The soil pH was measured with a 744 pH Meter-Metrohm meter made in Switzerland and electrical conductivity (EC) was measured with an EC Schott meter model 960 made in Germany. The pH and EC values were measured using an electrode in an extract with a soil:water ratio of 1:2.5. The soil was shaken for about 2 h on a Gerhardt shaker made from Germany at 150 rpm for 2 h, then centrifuged at 2,000 rpm for 3 min on a Tomy RC 130 centrifuge manufactured from Virtue.

Total N, total P and available K content were determined based on the soil sample inorganic method and then quantified by absorption spectroscopy at wavelengths 650, 880 and 766.5 nm respectively according to the method of Keeney et al. [16], Fatharani et al. [17], respectively. The baseline physical, chemical, and biological properties of the soil sampled for the experiment are presented in Table 1.

Table 1 The characteristics of the soil sample used in the greenhouse pot experiment collected from green onion farm in Binh Tan district, Vinh Long province, Vietnam.

Characteristics	Unit	Value
pHH_2O	-	5.23
EC	mS/cm	0.25
Organic matter	%	1.90
Total N	%	0.09
Total P	%	0.08
Available K	meq/100 g	0.25
NH_4^+-N	mg/kg	9.19
Available P	mgP/kg	11.0
Bacteria	log_{10} (CFU/g)	5.51
Fungi	log_{10} (CFU/g)	2.49
Soil texture		Silty clay

2.1.3 Green onion seedlings

The green onion Hanh Huong variety with red roots (*Allium fistulosum* L.) was used in the experiment. This is the local variety commonly grown in Binh Tan district, Vinh Long province.

2.2 Efficacy of bio-pesticide product from garlic, chili, ginger, onion and lemongrass on pest and disease controlling, growth and yield of green onions and selected soil properties under greenhouse conditions

2.2.1 Experimental layout

The experiment was conducted in the greenhouse of the Department of Soil Science, Faculty of Agriculture, Can Tho University for two consecutive green onions crops grown in soil pots from April 2021 to July 2021. The experiment was arranged in a randomized design with 13 treatments containing three different concentrations of 8%, 6%, and 4% bio-product in a combination with four different spray intervals: every 3 days, every 7 days, every 10 days spray, and the appearance of pests/disease. The treatment number, name and descriptions for each of the 13 treatments are listed in Table 2. Each treatment corresponded to 1 soil pot and each soil pot was planted with 3 onions plants.

Green onion cultivation techniques were applied by the method of Ba and Thuy [1] and all treatments used the same formula chemical fertilizer (100N-85 P_2O_5-40 K_2O) for green onion. The base formulation of the bio-pesticide was diluted to reach the target concentrations (4%, 6%, and 8%) for the experimental treatments. The bio-pesticide was sprayed after planting according to treatment until harvest time. An aliquot of 10 mL of bio-pesticide was sprayed at 5:00 pm evenly on stems, leaves of green onions and the soil in the pot. The green onion plants in each pot were watered everyday with tap water. Weeds in each pot were controlled manually.

Table 2 Details of treatments under greenhouse conditions.

Number	Treatment	Description of treatments
1	Control	Control without bio-pesticide spray
2	BP8%-3D	10 mL bio-pesticide 8% every 3 days
3	BP8%-7D	10 mL bio-pesticide 8% every 7 days
4	BP8%-10D	10 mL bio-pesticide 8% every 10 days
5	BP8%-A	10 mL bio-pesticide 8% when pests /disease appear
6	BP6%-3D	10 mL bio-pesticide 6% every 3 days
7	BP6%-7D	10 mL bio-pesticide 6% every 7 days
8	BP6%-10D	10 mL bio-pesticide 6% every 10 days
9	BP6%-A	10 mL bio-pesticide 6% when pests /disease appear
10	BP4%-3D	10 mL bio-pesticide 4% every 3 days
11	BP4%-7D	10 mL bio-pesticide 4% every 7 days
12	BP4%-10D	10 mL bio-pesticide 4% every 7 days
13	BP4%-A	10 mL bio-pesticide 4% when pests /disease appear

2.2.2 Collected parameters

Disease parameters were determined according to the formula of disease rate (%) = number of diseased leaves/total number of leaves in each plant. Leaves were identified as diseased when there were lesions on the leaves and agronomic parameters were taken at 15, 30, and 45 days after sowing (DAS). Plant diameter and canopy diameter were collected at harvest time. The yield of green onions was determined at the harvest stage in each crop by weighing the fresh weight and the dry weight of green onions in each pot.

Table 3 Method describing for collecting agronomic parameter of green onions.

Parameters	Methods
Plant height (cm)	Measured from the ground to the top of the highest leaf
Plant diameter (cm)	Measured the maximal diameter of the stem
Number of shoots/plants	Counted all the shoots which were completely separated
Number of leaves/plants (pcs)	The number of leaves per each plant
Stem length (cm)	The length of the stem from the root collar to the leaf collar at harvest

Soil chemical and biological characteristics were obtained at harvest after the second crop for all treatments. The soil was analyzed for pH, EC (mS/cm), fungi (log_{10} colony forming unit ((CFU)/g), actinomyces (log_{10} CFU/g), bacteria (log_{10} CFU/g), and nitrogen fixing bacteria, phosphorus solubilizing bacteria, and silicate mineral solubilizing bacteria. Tryptone Soya Agar (TSA), Potato Dextrose Agar (PDA), Starch Agar, Burk Agar, National Botanical Research Institute's phosphate growth medium (NBRIP) Agar, and Soil Extract Agar (SEA) were used to determine the numbers of bacteria, fungi, actinomycetes, nitrogen-fixing bacteria, phosphate solubilizing bacteria and silicate mineral solubilizing bacteria in the soil [18-21]. Number of bacteria, fungi, actinomycetes, nitrogen fixing bacteria, phosphate solubilizing bacteria and silicon soluble bacteria were determined according to the method of Gerba and Pepper [18]. Samples were extracted with buffer phosphate solution at a ratio of 1:10 (soil: buffer) for 1 h at 150 rpm. Aspirate 50 µL of suspension at dilution concentrations and spread on TSA, PDA, Starch Agar, Burk Agar, NBRIP Agar, SEA, respectively for determination of populations of bacteria, fungi, actinomycetes, nitrogen-fixing bacteria, phosphate solubilizing bacteria and Si-soluble bacteria in the soil [19-21]. Samples were placed in an incubator at 30°C to allow microorganisms to grow and determine the number of bacterial colonies growing on different types of media.

2.3 Data analysis

The data collected were subjected to analysis of variance (ANOVA) to ascertain the significance of treatments. The means of significant effects were compared using the Tukey test at 5% level of significance with MINITAB software 16.2 version.

3. Results and discussion

3.1. The efficacy of bio-pesticide product on pathogen disease control under greenhouse conditions

During two consecutive crop experiments (Crop 1 and Crop 2), anthracnose was the only plant disease recorded in Crop 2 at almost harvest time. The efficacy of the bio-pesticide made from onions, peppers, ginger, garlic, and lemongrass on anthracnose disease is presented in Figure 1. In general, the treatments were effective in different ways in controlling anthracnose disease. The percentage of infected leaves ranged between 10% and 38.4%.

The bio-pesticide treatment of 6% concentration sprayed every 10 days was the most effective in reducing the rate of anthracnose infested leaves of green onion to 10%. This treatment had a significantly lower infestation rate than all other treatments as well the control treatment without bio-pesticide application (16.2%) ($p<0.05$). One explanation could be that bioactive compounds from onions, peppers, ginger, garlic, and lemongrass have anti-fungal properties enabling this bio-pesticide to control the anthracnose disease fairly well. Previous research by Corzo et al. [22] similarly demonstrated that garlic had active ingredients such as polyphenols, sulfur-based substances (allicin, diallyl disulphide, S-ethyl cysteine, and N-acetyl cysteine) and other biologically active substances capable of supporting human immune system and serving as a biological control against plant diseases. In addition, Jiménez-Reyes et al. [23] showed that garlic contains a significant number of sulfurous compounds, which exhibit potent antifungal properties [24,25]. These compounds include: allicin (diallyl-dithiosulfinate); ajoene [(E,Z)-4,5,9-trithiadodeca-1,6,11-triene-9-oxide]; DAS, diallyl disulphide (DADS) and allyl methyl disulfide, allivin. Similarly, the study of Dania et al. [26] showed that the use of garlic in combination with *Trichoderma harzianum* fungus inhibited *Pythium aphanidermatum* causing seedling death on tomato; with an inhibitory effect up to 91.2% under laboratory conditions and significantly reduced disease in tomato plants under greenhouse conditions. In addition, the alpha-zingiberene compound in ginger has been used against fungal pathogens such as *Botryodiplodia theobromae*, *Colletotrichum camelliae*, *Curvularia eragrostidis*, *Fusarium udum*, *Pestalotiopsis theae*, and *Sclerotinia sclerotiorum* because of its zingiberene content [27]. Wilson and Demmig-Adams [28] showed that the organosulphur compounds in garlic and onions spices inhibit harmful bacterial growth via interaction with sulphur-containing enzymes. Moreover, lemongrass oil (*Cymbopogon citratus*) at 500 mg/L, completely inhibits sporulation and germinal tube generation of *Colletotrichum coccodes*, *B. cinerea*, *Rhizopus stolonifer* and *Cladosporium herbarum* fungus, which can cause diseases in tomato plantations. This suggests that lemongrass oil could work as a natural fungicide for the product when stored and limit the pathogen spread by reducing spores in the atmosphere and storage surfaces [29]. Another compound is the oleoresin capsaicin (8-methyl-vanillyl-6- nonenamide), a spicy component of red pepper species, which works as antifungal against *B. cinerea* [30]. Consequently, integrating these compounds into a biological control for plant diseases could be effective. The *A. melegueta* ethanolic extracts, the garlic (*A. sativum*) methanolic extracts, as well as the ginger (*Zingiber officinale*), can also inhibit *A. niger* fungus growth [25]. The results are similar in the studies of many other authors on the effectiveness of the solution with water or ethanolic of garlic, ginger, onion, and lemongrass in the prevention and treatment of plant diseases. [24,31-35].

Figure 1 reveals a distinct pattern of an increase in plant disease rate associated with higher concentrations of the bio-pesticide product applied within shorter time intervals. Frequent applications of high concentrations of the bio-pesticide may be damaging the leaf tissues of the green onion. The mechanical wounds of leaf tissue may open the opportunity for pathogens to attack the plant and cause the disease. This hypothesis needs to be tested in future experiments. In addition, the experiment results showed that spraying the bio-pesticide product only when disease appeared had no effect on preventing anthracnose disease on green onion. Based on these findings we would recommend that bio-pesticide products are most effective when used to prevent the disease rather than to treat when the diseases appear. This result is consistent with the work of Iamba et al. [36]. They investigated concentration effects of chili and ginger extracts against diamondback moth (*Plutella xylostella* L.) on round cabbage. The results showed that chili extract was effective at lower concentrations than at higher dose because high concentrations and frequent applications of chili and ginger extract damaged leaves and defoliated cabbage.

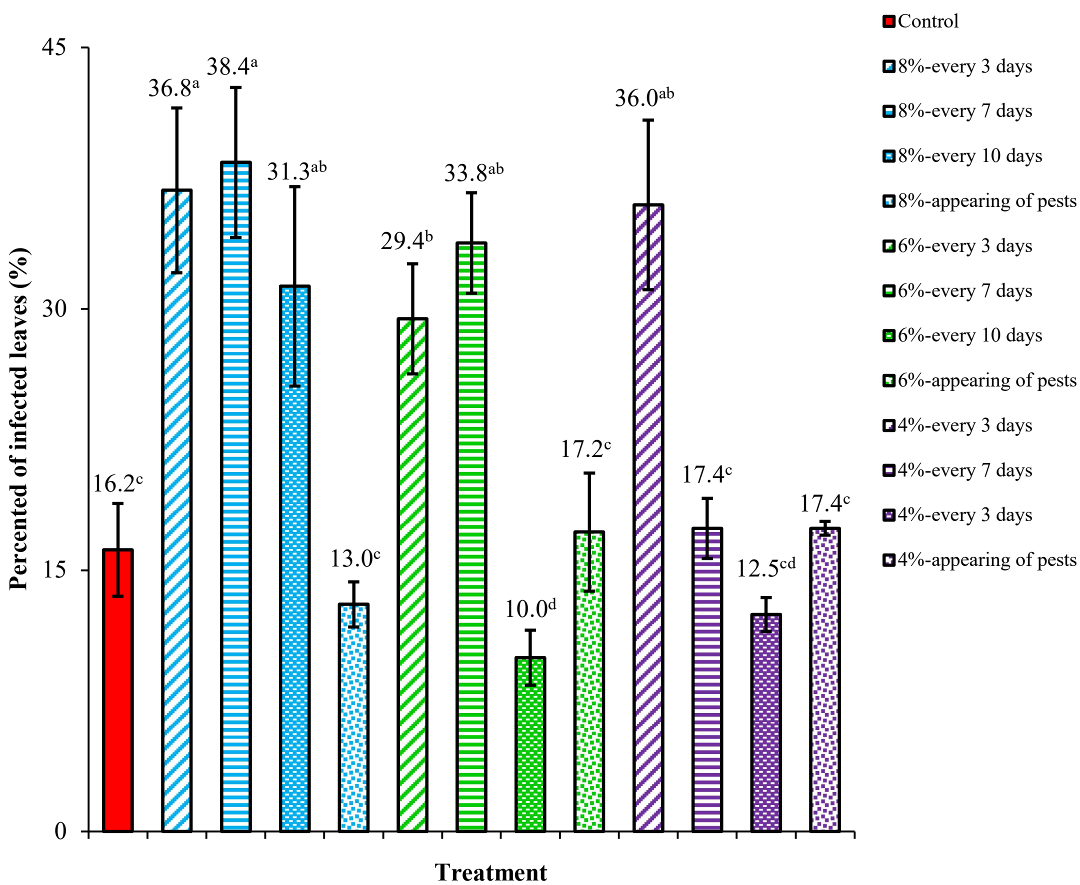

Figure 1 Rate of onion leaves infected with anthracnose disease in Crop 2 under the greenhouse condition.

3.2 Effects on growth and yield of green onion under greenhouse conditions

3.2.1 Plant height

Plant height did not seem to be affected by the bio-pesticide application. There was no significant difference in plant height among the green onion plants receiving different bio-pesticide treatments or the control treatment without bio-pesticide applications (Table 4).

Table 4 Agronomic parameters of green onion in replicated experiments, Crop 1 and Crop 2 under greenhouse conditions.

Treatment	Plant height (cm)		Leaf/plant (leaves)		Shoots/plant (shoot)		Stem diameter (cm)	
	Crop 1	Crop 2	Crop 1	Crop 2	Crop 1	Crop 2	Crop 1	Crop 2
Control	50.1	47.6	16.3^{bc}	23.7^{bcd}	4.25	5.5^{ab}	0.63^{bc}	0.80
BP8%-3D	46.1	42.0	18.1^{abc}	11.1^{f}	4.29	3.38^{b}	0.65^{abc}	0.64
BP8%-7D	50.5	40.0	17.2^{abc}	17.4^{e}	3.21	4.0^{ab}	0.78^{ab}	0.65
BP8%-10D	46.1	43.8	15.1^{cd}	18.0^{e}	4.17	4.83^{ab}	0.74^{abc}	0.71
BP8%-A	45.8	47.8	20.3^{a}	18.9^{de}	4.61	4.11^{ab}	0.64^{abc}	0.79
BP6%-3D	45.2	46.3	11.6^{e}	21.2^{cde}	3.20	4.96^{ab}	0.54^{c}	0.76
BP6%-7D	47.1	43.1	18.9^{ab}	20.1^{de}	4.58	6.38^{a}	0.60^{bc}	0.62
BP6%-10D	52.2	43.4	17.3^{abc}	30.8^{a}	3.75	5.75^{ab}	0.78^{ab}	0.81
BP6%-A	46.8	47.0	12.2^{de}	20.7^{cde}	2.83	4.67^{ab}	0,73^{abc}	0.85
BP4%-3D	44.7	47.1	11.1^{e}	24.3^{bcd}	2.46	6.00^{ab}	0.58^{bc}	0.68
BP4%-7D	50.0	47.1	15.5^{c}	27.2^{ab}	2.92	5.33^{ab}	0.88^{a}	0.86
BP4%-10D	51.5	42.9	19.7^{a}	25.7^{abc}	3.75	5.45^{ab}	0.74^{abc}	0.79
BP4%-A	46.7	44.1	11.6^{e}	21.5^{cde}	3.42	4.75^{ab}	0.65^{abc}	0.78
F	ns	ns	*	*	ns	*	*	ns
CV (%)	10.3	9.30	21.2	24.0	32.5	25.01	18.7	15.9

Note: Values in the same column with different letters are significant difference at 5% level by Tukey's test.

3.2.2 Number of leaves/plants

The results of the number of leaves/plants in Crop 1 and Crop 2 grown under greenhouse conditions are presented in Table 4. Overall, the number of green onion leaves/plant in the treatments and the control group in Crop 1 was lower than that of Crop 2. Although grown in similar greenhouse conditions from April to July, differences in plant growth may reflect changes in the pot soil from Crop 1 to Crop 2 since these crops were grown sequentially in the same soil. This study evaluates soil pH and bacteria at the end of Crop 2 harvest. Future research that analyzes the soil at the end of each crop season may shed light on plant growth differences among sequential crops grown in the same location. An examination of the number of leaves per plant within each crop season for each treatment show the use of the bio-pesticide at different concentrations and time intervals resulted in statistically different number of leaves/plants among treatments ($p<0.05$). The number of leaves/plant in Crop 1 ranged from 11.1 to 20.3 leaf/plant, with the number of leaves/plant in the 8% bio-pesticide concentration when pests first appeared (Treatment 5) having the highest number of leaves at harvest time (20.3 leaves/plant). Treatment 8, the application of the 6% bio-pesticide at 10 day intervals had the highest number of leaves in Crop 2 (30.8 leaves/plant). This compares to the control treatment in Crop 1 (16.3 leaves/plant) and in Crop 2 (23.7 leaves/plant) with lower leaf/plant counts than these two treatments. However several treatments in Crop 2 had a lower number of the leaves/plant as compared to the control treatment such as the 8% concentration treatments at 3 days, 7 days, and 10 days (Treatments 2, 3, 4); the 6% concentration applied at 3 day intervals (Treatment 6) and when the pest/disease appeared (Treatment 9); and the 4% concentration when the pest first appeared (Treatment 13). In Crop 1, Treatments 4, 6, 9, 10, and 13 had fewer leaves/plant than the control. Crop 2 shows a distinct pattern of fewer leaves/plant in the use of the 8% concentration bio-pesticide regardless of spray frequencies, suggesting 8% may be too high of a concentration. And even the 6% concentration used at frequent intervals (3 days and 7 days) may be an over application as leaf numbers seem to be reduced. The results from Crop 1 are a little more mixed and more replications would provide a clearer pattern.

In summary, the use of bio-product ethanoic extracts from onion, garlic, chili pepper, ginger, and lemongrass impacted the number of leaves/plant with the bio-pesticide concentration and frequency of applications affecting the number of leaves that develop. In this study, applying bio-pesticide products with concentration of 8% when disease symptoms just appear or with 6% at every 10 days seem to increase the number of leaves/plant of the green onion under greenhouse conditions.

3.2.3 Number of shoot/plants

The results of the number of shoots/plant of green onions in Table 4 showed that in Crop 1 no significant difference in the number of shoot/plant was found among treatments. In Crop 2, numbers of shoots/plant were highest in the bio-pesticide treatment 7, a 6% concentration applied at 7 day intervals which had the highest number of shoots/plant (6.38 shoots). However, this was not significantly different from the control treatment (5.5 shoots) (p>0.05).

3.2.4 Stem diameter

The stem diameters of green onion in different treatments were significantly different in the first season, Crop 1 but not significantly different in the second season, Crop 2 (Table 4). Stem diameter of all treatments ranged between 0.54 and 0.88 cm in Crop 1, where Treatment 11, the 4% concentration of bio-pesticide product applied at 7 day intervals had the highest diameter (0.88 cm). This was significantly different from the control treatment without bio-pesticide spraying (0.63 cm). In Crop 2, the stem diameter of green onion fluctuated from 0.64 to 0.86 cm with no significant difference.

The results of this green onion experiment show that the use of a bio-pesticide formulated from onions, garlic, chili peppers, lemongrass and ginger can affect plant growth as measured by number of shoots, number of leaves, and stem diameter. Specifically, applications of the bio-pesticide at different concentrations and time intervals will have differing effects on the crop growth. This is consistent with previous studies. A study by Abdel-Moneim al et. [37] concluded that applying garlic extract increased the diameter of the green onion stem diameter. Research by Awais et al. [38] demonstrated that several compounds from *Allium* species act as growth promoters for plants. Similarly, Esmat et al [39] showed that applications of ginger extract increased rose plant height, number of branches, and the number of flowers but reduced the number of non-flowering branches. Spray applications of ginger extract also increased essential oil content, sugar, anthocyanins and total carotenoids in roses.

The effectiveness of the bioproduct was not consistent over the two experimental seasons (Crop 1 and Crop 2). More replications are needed to better understand bio-pesticide plant responses, fluctuations in plant growth and to determine sources of plant grow variability over multiple seasons. Potential reasons for variability may come from the biopesticide itself, the shelf life of the volatile compounds as formulated may be too short; and storage conditions may affect its potency. Another factor in plant growth is the soil that plants are grown in. It may also be that the soil characteristics, pH and bacteria present and densities in the soil change over multiple seasons of bio-pesticide applications and influence plant growth patterns and responses to the bio-pesticide.

3.2.5 Fresh yield and dry biomass

The efficacy of the bio-pesticide treatments on fresh and dry biomass of green onion under greenhouse conditions is presented in Table 5. In general, the results of bio-pesticide treatments on fresh yield and dry biomass of green onion plants were inconsistent between Crop 1 and Crop 2. Crop 1 highest fresh yield of green onion was 123 g/pot using Treatment 11, the 4% bio-pesticide applied at 7 day intervals which showed a 31.4% increase of fresh yield compared to the control treatment of 91.7 g/pot (significant difference at p<0.05). However, in Crop 2, the application of the bio-pesticide did not significantly increase the yield of green onion compared to the control treatment where no bioproduct was applied. The fresh yield of green onion in the sprayed treatments varied from 38.3 to 121.3 g/pot with the control treatment yield at 114.3 g/pot. There seems to be an emerging pattern of low yields where the bio-pesticide was applied at high concentration or at short intervals between spray applications. For example, in Crop 2 the 8% concentration at 3 day or 7 day intervals resulted in a reduction in fresh yield of green onion (38.3 g/pot and 52.1 g/pot, respectively). In Crop 1, three day interval applications of the bio-pesticide substantively reduced fresh yields at 6% and 4% concentrations, 35.4 g/pot and 38.3 g/pot respectively. More replications of this experiment are needed to clarify the bio-pesticide spray frequency effect on yields.

Similar to the fresh biomass, in Crop 1 Treatment 11, the 4% bio-pesticide concentration applied every 7 days had the highest dry weight (9.8 g/pot) which was significantly higher than the control treatment (7.3 g/pot) (p<0.05). Although Treatment 11 in Crop 2 yielded the highest dry weight (11 g/pot) of all the bio-pesticide treatments, it was not significantly different from the control dry biomass at 11.5 g/pot. A pattern similar to reduced fresh yields, shows dry biomass reductions when the bio-pesticide was applied at 3 day intervals. For example, biomass yields in Treatment 2, 8% concentration applied at 3 day intervals in Crop 2 were 3.6 g/pot; and in Crop 1 were 6.6 g/pot. Crop 1 treatments applied at 3 day intervals, even at lower concentrations (Treatment 6, 6% concentration and Treatment 10, 4% concentration) resulted in very low dry biomass weight, 2.8 g/pot and 3.1 g/pot respectively.

Findings from Crop 1 and Crop 2 experiments gave mixed results. Additional replications of the green house experiment as well as field experiments are needed in order to draw sufficient conclusions to make recommendations about use of this spice bio-pesticide from garlic, chili, ginger, onion and lemongrass. Our findings show that the bio-pesticide concentration and the frequency of applications effect green onion yields and show potential to increase yields when applied at appropriate concentrations and frequencies. For example, the 4% concentration at 7 day intervals and the 6% concentration applied at 10 day intervals increased the fresh yield and dry biomass of green onion, while treatments with high concentration of the bio-pesticide at 8% sprayed at 3 day intervals showed a strong reduction in the green onion yield and dry biomass suggesting possible bio-pesticide overdose. This may be due to some of the chemical compounds in the formulation-such as capsaicin in chili peppers, essential oils, oleoresin and gingerol in ginger, etc., which could cause damage to the roots, stems and leaves of green onion when sprayed at high concentration on the soil and leaves. Gong et al. [40] studied the effect of low concentration at 2% raw garlic straw extract against root-knot nematodes (*Meloidogyne incognita*) in tomato and found this concentration inhibited the nematodes and increased the tomato yield. Jess et al. [41] investigated the potential of garlic oil to control *Megaselia halterata* in commercial mushroom production and found that garlic solutions at low level concentrations (0.1–20%) successfully repelled adult female *M. halterata*. Other research shows that bio-pesticides made with onions are not recommended for use on peas and small beans because they inhibit their growth [42].

Table 5 Fresh yield and dry biomass of green onion under greenhouse conditions.

Treatment	Fresh biomass (g)		Dry biomass (g)	
	Crop 1	Crop 2	Crop 1	Crop 2
Control	91.7^{cd}	114.3^{ab}	7.3^{cd}	11.5^{a}
BP8%-3D	82.5^{d}	38.3^{d}	6.6^{d}	3.6^{h}
BP8%-7D	95.9^{cd}	52.1^{d}	7.7^{cd}	6.0^{fg}
BP8%-10D	84.7^{d}	107.7^{abc}	6.8^{d}	9.1^{cd}
BP8%-A	94.7^{cd}	108.4^{abc}	7.6^{cd}	10.5^{abc}
BP6%-3D	35.4^{f}	90.8^{c}	2.8^{f}	9.3^{bcd}
BP6%-7D	85.4^{cd}	53.1^{d}	6.8^{cd}	4.8^{gh}
BP6%-10D	113.6^{ab}	121.3^{a}	9.1^{ab}	10.7^{a}
BP6%-A	55.8^{e}	100.7^{abc}	4.5^{e}	9.1^{bcd}
BP4%-3D	38.3^{f}	95.3^{bc}	3.1^{f}	8.7^{de}
BP4%-7D	123.0^{a}	119.5^{a}	9.8^{a}	11.0^{a}
BP4%-10D	102.0^{bc}	107.0^{abc}	8.2^{bc}	9.3^{bcd}
BP4%-A	52.0^{ef}	92.6^{c}	4.2^{ef}	7.4^{ef}
F	*	*	*	*
CV (%)	33.9	33.9	29.7	28.5

Note: Values in the same column with different letters are significant difference at 5% level by Tukey's test.

Previous studies concluded that applications of extracts from ginger, garlic and lemongrass at the right concentration and frequency helped to increase plant growth [5,6,7], especially the study of Esmat et al. [39] who reported that using ginger root extract increased rose yield up to 19.9% as compared to the control. Additionally, a study by Nadia et al. [43] demonstrated that the yield of dill significantly increased when sprayed with a 400 mg/L concentration of garlic. The study by Ali et al. [44] showed that spraying garlic extract increased eggplant yield by 23.6% as compared to the control treatment. Similarly, Begna et al. [45] showed that cabbages sprayed with chili extract at appropriate concentrations were able to produce good yields. These results are explained by Corzo et al. [22] findings that bioproducts have a number of organic compounds with plant growth stimulating functions, typically polyphenols, sulfur-based compounds such as allicin, diallyl, disulphide and other biologically active substances. Thus, different bio-pesticide spray concentrations and frequencies can stimulate the growth of plants in different ways.

3.3 Effect on selected soil properties after two consecutive green onion crops under greenhouse conditions

3.3.1 Soil chemical properties

The results of selected soil chemical properties after two consecutive green onion crops grown in the same soil pot and measured after Crop 2 harvest are presented in Table 6. There was significant difference among treatments in soil chemical properties pH and EC. Specifically, the soil pH value of the experimental treatments ranged from 5.09 to 6.29, in which all the bio-pesticide applied treatments had significantly higher soil pH, ranging from 6.17 to 6.29 as compared to the control treatment (5.09). However, there was no statistical difference among the sprayed treatments ($p>0.05$). The bio-pesticide product formulated of onions, peppers, ginger, garlic and lemongrass used for all treatment concentration levels and at various time intervals throughout the growing season increased the pH after two consecutive green onion crops in the same soil as compared to the control treatment (no bio-pesticide) under greenhouse conditions.

Soil EC values ranged from 157 to 183 µS/cm after two consecutive green onions crops in the same soil pot under greenhouse conditions. Only one treatment had a significantly different soil EC value from the control ($p<0.05$). Specifically, Treatment 13, the 4% concentration bio-pesticide applied when the disease appeared gave the highest EC value (183 µS/cm) which was significantly different from the control treatment (161 µS/cm). The soil EC values of the other bio-pesticide sprayed treatments were not significantly different from the control treatment.

Table 6 Effect of bio-pesticide product on selected soil chemical and biological properties after two consecutive green onion crops grown in the same soil pots under greenhouse conditions.

Treatment	Density of microorganisms (Log_{10}(CFU/g)						
	pH	EC (µS/cm)	Bacteria	NFB	PSB	SSB	Fungi	ACT
Control	5.09^{c}	161^{cde}	5.43^{d}	4.95^{h}	4.65^{e}	3.82^{b}	3.48^{a}	4.14
BP8%-3D	6.21^{ab}	157^{e}	5.55^{d}	4.92^{gh}	4.81^{abc}	3.97^{ab}	2.87^{f}	4.18
BP8%-7D	6.20^{ab}	159^{e}	5.55^{d}	5.04^{fg}	4.88^{ab}	3.92^{ab}	3.02^{ef}	4.16
BP8%-10D	6.20^{ab}	$177^{a\text{-}d}$	5.76^{c}	5.18^{de}	4.91^{a}	3.96^{ab}	3.16^{cde}	4.17
BP8%-A	6.15^{bc}	160^{de}	6.47^{b}	5.24^{cde}	$4.75^{a\text{-}d}$	3.98^{a}	3.40^{abc}	4.19
BP6%-3D	6.17^{ab}	161^{cde}	6.48^{b}	5.14^{ef}	4.85^{ab}	3.97^{ab}	3.01^{ef}	4.19
BP6%-7D	6.22^{ab}	$178^{a\text{-}d}$	6.76^{a}	5.28^{cd}	4.98^{ab}	3.95^{ab}	3.08^{def}	4.20
BP6%-10D	6.27^{a}	$183^{a\text{-}d}$	6.59^{ab}	5.43^{ab}	4.83^{abc}	4.05^{a}	$3.22^{b\text{-}e}$	4.23
BP6%-A	6.29^{a}	$181^{a\text{-}d}$	6.63^{ab}	5.39^{ab}	$4.82^{a\text{-}d}$	4.01^{ab}	$3.35^{a\text{-}d}$	4.18
BP4%-3D	6.26^{ab}	$162^{b\text{-}e}$	6.60^{ab}	5.35^{bc}	4.71^{de}	4.00^{ab}	3.55^{a}	4.19
BP4%-7D	6.19^{ab}	$180^{a\text{-}d}$	6.71^{a}	5.50^{a}	$4.76^{b\text{-}e}$	4.06^{a}	$3.32^{a\text{-}d}$	4.20
BP4%-10D	6.22^{ab}	$169^{a\text{-}e}$	6.68^{a}	5.43^{ab}	4.63^{cde}	4.03^{ab}	3.58^{a}	4.16
BP4%-A	6.21^{ab}	183^{a}	6.71^{a}	5.49^{a}	4.87^{a}	3.95^{ab}	$3.35^{a\text{-}d}$	4.17
F	*	*	*	*	*	*	*	ns
CV (%)	2.26	7.03	8.01	3.46	1.97	1.65	7.00	2.15

Note: Values in the same column with different letters are significant difference at 5% level by Tukey's test; NFB: nitrogen fixing bacteria; PSB: phosphate solubilizing bacteria; SSB silicate mineral solubilizing bacteria.

In summary, all the experimental treatments using the bio-pesticide product increased the soil pH values after two green onion consecutive cropping seasons, but the soil EC after two consecutive crops showed little significant change. The increasing soil pH may be due to the pH contributions of the combined materials used to formulate this bio-pesticide. For example, white wine pH is 7 [45], ginger extract pH is 5.6-5.9 [46], chili extract pH is 5.5-7.5 [47], and garlic extract pH is 5-6 [48]. Thus, soil applications of this bio-pesticide product caused an increase of soil pH values. A study by Nghia et al. [49] using microbial preparations non-pharmaceutical measures (NPISi) showed increasing soil pH and EC values and positive correlations with increased growth and increased onion yield. These results are also similar to the findings of Lee [50] and Desire et al. [51].

3.3.2 Population of soil microorganisms

Soil survey results after Crop 2 harvest on the number of soil microorganisms following two consecutive green onion crops in the same soil pot under greenhouse conditions are presented in Table 6. There was a statistically significant difference ($p>0.05$) among all treatments regarding the number of soil microorganisms including bacteria, fungi, nitrogen fixing bacteria, phosphate solubilizing bacteria and silicate mineral solubilizing bacteria, with the exception of actinomycetes.

The density of bacteria in the soil varied between 5.43-6.71 log_{10} (CFU/g). The treatments (10, 11, 12, 13) sprayed with a 4% bio-pesticide concentration had a significant increase in the number of soil bacteria as compared to the control treatment. The increase in soil bacteria was accompanied by the population increase of beneficial bacteria such as nitrogen fixing bacteria, phosphate solubilizing bacteria and silicon solubilizing bacteria (Table 6). This finding was consistent with the study of Ray [52]. The interaction of tree root hairs with organic manures increased the activity of the nitrogen-fixing bacteria in the soil; and organic manures also acted as a carrier medium for the development of several beneficial microorganisms such as *azospirillum*, *azotobacter*, *rhizobium* and *phosphobacteria.*

The soil fungi survey showed that most of the bio-pesticide treatments had a lower number of fungi compared to the control treatment. However, for actinomycetes, the application of the bio-pesticide did not change the density of actinomycetes in soil. In summary, the application of the bio-pesticide product at a 4% concentration increased the densities of bacteria and beneficial bacteria in the soil. However, spraying the bio-pesticide product at any concentration reduced the soil fungal population, but had no effect on actinomycetes numbers in the soil under greenhouse conditions after two consecutive green onion crops grown in the same soil pots.

These findings compare to previous studies that showed that the use of extracts from garlic, chili, ginger, and lemongrass had antimicrobial effects. For example, Farzad et al. [53] found that allicin from garlic extract had antifungal activity and inhibited the growth of mycelium of *T. rubrum*. Kim [54] also found the use of onion extract inhibited *Streptococcus mutans, Streptococcus sobrinus, Prevotella intermedia* and *Porphyromonas gingivalis*. Shaista et al. [55] showed that the use of garlic and ginger extracts gave good resistant capacity to crops against pathogenic bacteria strains such as *E. coli, Staphylococcus aureus, Salmonella typhi*. However, there has been no research indicating that the use of plant extracts such as garlic, ginger, chili, onion and lemongrass increases the number of bacteria in the soil. The soil microbial findings of this bio-pesticide experiment uniquely reveal that a bio-pesticide formulated from extracts of garlic, ginger, peppers, lemongrass, and onions have a significant effect on the microbial population in the soil. This is a promising area for future research and additional soil and crop experiments will build on these findings.

There is a need for additional research on the effectiveness of botanical pesticides to manage agricultural pests that damage crop economic value and to prevent degradation of soil, water, and other environmental resources. Bio-pesticides offer an alternative to current synthetic pesticide use and can be effective in pest and disease management in both conventional and organic agricultural systems. A growing body of research is finding bio-pesticides can be cost-effective, easy to use and are increasingly available and accessible [56]. A number of studies compare organic pesticides with the use of inorganic pesticides to evaluate potential beneficial effects of bio-pesticides on soil microorganisms. Current findings show one of the strongest benefits of using natural pesticides and insecticides are their environmental friendliness and promotion of microbial diversity. Because pesticides derived from plants volatilize quickly and have short persistence times and low vertebrate toxicity, they present less risk to the environment, microorganisms, insects and animals [57-59].

4. Conclusion

This experimental study on a bio-pesticide extracted from onions, garlic, chili peppers, ginger and lemongrass showed that a 6% bio-pesticide concentration applied at 10 days intervals until harvest was the most effective treatment in prevention of anthracnose disease in green onions grown in greenhouse conditions. Further, spraying with a 4% bio-pesticide concentration at 7 days intervals until harvest increased the fresh yield of green onions by 31.4% as compared to the control treatment. Lastly, the application of the bio-pesticide product at all concentrations increased soil pH and the number of soil bacteria and beneficial bacteria such as nitrogen fixing bacteria, phosphorus solubilizing bacteria and silicate mineral solubilizing bacteria. The results of this study suggest that a bio-pesticide extracted from onions, garlic, chili peppers, ginger and lemongrass have great potential in biological control of green onion pathogens and can be used in green onion protection as an alternative to synthetic pesticides while improving soil health.

There is a need for additional greenhouse replications and field trials to evaluate the efficacy of this bio-pesticide formulated from onion, garlic, chili pepper, ginger and lemongrass on the growth, yield of green onions as well as on other vegetable crops and different types of soil. Future research is needed to better understand the effectiveness of botanical pesticides, crop and soil impacts of formulations, active ingredients, application rates, storage stability, and volatility under ultraviolet light so as to enhance the significant commercialization of botanical pesticides.

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