Research Article

Bioagents and Commercial Algae Products as Integrated Biocide Treatments for Controlling Root Rot Diseases of Some Vegetables under Protected Cultivation System

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1. Introduction

Vegetable crops are grown worldwide as a source of nutrients and fiber in the human diet. Fungal plant pathogens can cause devastation in these crops under appropriate environmental conditions. Root rot in vegetables strikes quickly and then ruins a whole crop. Several fungi may cause root rot in vegetable plants, transmitting the disease through the soil. Some common fungi include Fusarium, Rhizoctonia, Sclerotinia, and Pythium, each of which causes a root rot named for the specific fungi that cause it. While the presence of one of these fungi is the primary cause for disease, plants exposed to poor growing conditions, such as a soil that does not drain well, are most susceptible to root rot. The best way to avoid root rot is by eliminating these contributing causes and practicing sound cultivation techniques. It was reported that the soil-borne pathogens Rhizoctonia solani, Pythium ultimum, and Fusarium spp. can cause severe economic losses to field and greenhouse cucumber [1]. Also, Fusarium stem and root rot on greenhouse long English cucumber (Cucumis sativus L.) cvs. Bodega and Gardon was observed at four commercial greenhouses in Leamington, Ontario, Canada. Losses of 25 to 35%, representing 2.5 ha, were noted. Fusarium spp., Rhizoctonia spp., and Pythium spp. were isolated from tomato plants showing root and crown rot symptoms [2].

The pathogens, Alternaria solani, Fusarium solani, F. oxysporum, Rhizoctonia solani, Sclerotium rolfsii, Macrophomina phaseolina, and Pythium sp. were isolated from cucumber, cantaloupe, tomato and Pepper plants grown in plastic houses and showing root rot disease symptoms [3]. Different
approaches may be used to prevent, mitigate, or control plant diseases. Beyond good agronomic and horticultural practices, growers often rely heavily on chemical fertilizers and pesticides. Such inputs to agriculture have contributed significantly to the spectacular improvements in crop productivity and quality over the past 100 years. The current management strategy relies on the intensive use of fungicides. A promising strategy for the replacement of chemical pesticides has been the implementation of biological control. The recent development in the commercialization of biological control products has accelerated this approach [4]. The application of biological controls using antagonistic microorganisms has proved to be successful for controlling various plant diseases in many countries [5]. However, this is not an easy method, and it is costly to apply; however, it can serve as the best control measure under greenhouse conditions. *Trichoderma harzianum* introduced to the soil was able to reduce root rot incidence of faba bean plants significantly more than the fungicide Rizolex-T [1]. In recent years, several attempts have been made to overcome this obstacle by applying antagonistic microorganisms. *Trichoderma* spp. are well documented as effective biological control agents of plant diseases caused by soil-borne fungi [2, 3, 6]. Many investigators observed that the application of wheat bran colonized by *T. harzianum* to soil infested with *R. solani* and *S. rolfsii* reduced the incidence of root diseases caused by these pathogens [7–9]. Furthermore, soil drenched with *T. harzianum* was significantly able to reduce the incidence of bean root rot of bean and pepper wilt diseases [1, 10–12]. As for antagonistic bacteria, [13] it was found that seed treatment with *Bacillus* spp. was actively controlled by three fungal root diseases of wheat. Also, *Pseudomonas cepacia* or *Pseudomonas fluorescens* applied to pea seeds acted as biological control agent against *Pythium* damping-off and *Aphanomyces* root rot and was able to reduce diseases incidence [11]. In addition, recently algae are one of the chief biological agents that have been studied for the control of plant pathogenic fungi, particularly soil-borne diseases [14]. Cyanobacteria (blue-green algae) and eukaryotic algae had been reported to produce biologically active compounds, that is, antifungal activity [15, 16], antibiotic and toxic activity [17, 18], against plant pathogens. Moreover, *Anabaena* spp., *Scytomena* spp., and *Nostoc* spp. were reported to be efficient for controlling damping-off as well as the growth of soil fungus *Cunninghamella blakesleae* [19–22]. Also, [15] reported that culture filtrates or cell extracts from cyanobacteria and algae applied to seeds protect them from damping-off fungi such as *Fusarium* sp., *Pythium* sp. and *Rhizoctonia solani*. Furthermore, in previous work of the authors [23] it was found that commercial blue-green algae extracts, Weed-Max (blue-green algae extracts in powder phase), and Oligo-Mix (blue-green algae extracts in liquid phase) have potential for the suppression of soil-borne fungi and enhance the antagonistic ability of fungal, bacterial and yeast bioagents. These results led to the suggestion that another supporting factor is needed to raise the efficacy of the algae products. Therefore, application of the bioagents as a soil treatment in integration with algae products could be evaluated against root rot incidence of various plants.

The present research focuses on finding compounds that are safe to humans and the environment, for example, algae, as well as bioagents which may provide an alternative control against many soil- and seed-borne pathogens. The objective of the present work was to evaluate integrated bioagents, *Trichoderma harzianum*, *T. viride* or *Bacillus subtilis*, and *Pseudomonas fluorescens*, and commercial algae extract, Weed-Max or Oligo-X, against vegetables root rot incidence when used as soil drench under artificial infestation in pot experiment and natural infestation under commercial plastic house conditions.

2. Materials and Methods

Evaluation of integrated bioagents, *Trichoderma harzianum* or *Bacillus subtilis* and commercial algae extract, Weed-Max or Oligo-X, against Cucumber, Tomato, and Pepper root rot incidence, was carried out under greenhouse and plastic house conditions.

2.1. Tested Materials

2.1.1. Microorganisms. The bioagents fungal antagonists, that is, *Trichoderma harzianum* and *T. viride*, as well as bacterial antagonists, that is, *Bacillus subtilis* and *Pseudomonas fluorescens*, obtained from culture collection unit of Plant Pathology Department, National Research Centre, Egypt, were used in the present work. These bioagents proved their high antagonistic effect against wide spectrum of plant pathogens in many previous works at the same department. Also, highly aggressive isolates of *Fusarium solani* and *Rhizoctonia solani* were used in pot experiment.

2.1.2. Preparation of Microorganisms Inocula. The fungal inocula (pathogenic or bioagent) were grown individually for two weeks on sand-barley medium (1:1, w:w and 40% water) at 25 ± 2°C. Meanwhile bacterial bioagents were grown on nutrient broth medium for 48 hrs at 28 ± 2°C under shaking conditions.

2.1.3. Commercial Algae Extracts. Purchased commercial algae extracts, that is, Weed-Max (blue-green algae extracts in powder phase) produced by Inc. Trade S.A.E. Company, Naser City, Egypt, and Oligo-X algae (blue-green algae extracts in liquid phase) produced by Arabian Group for Agricultural Service, 114 King Fesal Street, Giza, Egypt were used in the present study. These commercial algae extracts were used in their commercial form without analyzing their composition.

2.1.4. Vegetables. Vegetables seeds or transplants of Cucumber (Hisham cv.), Cantaloupe (Yathreb cv.), Tomato (Agyaad cv.), and Pepper (Khyrratte cv.) were used for greenhouse and plastic house trails.

2.2. Pot Experiment. Pot experiment was carried out in the greenhouse of Plant Pathology Department, National
Research Centre, Egypt, for evaluating the efficacy of bioagents, *Trichoderma harzianum* or *Bacillus subtilis*, and commercial algae extract, Weed-Max or Oligo-X, introduced to the soil, as soil treatments, against root rot disease incidence. Cucumber and tomato representing cucurbits and solanaceous crops were used in this experiment as a model of widespread vegetable crops mainly grown under protected cultivation system in Egypt and showed susceptibility to attack with root rot pathogens [3]. The pathogenic isolates of *Fusarium solani* and *Rhizoctonia solani* were used. Loamy soil was artificially infested individually (at the rate of 5% w:w) with inoculum of each pathogenic fungus, through mixing thoroughly with the soil and then filled in plastic pots (20 cm in diameter). The infested soils were then mixed thoroughly with inocula of bioagents either fungi (*Trichoderma harzianum, T. viride*) or bacteria (*Bacillus subtilis, Pseudomonas fluorescens*) relevant to the specific treatment. The fungal inocula were added to the soil at the ratio of 5% (w:w) of soil weight, while bacterial inocula as liquid culture (3×10^6 cfu/mL) at the ratio of 100 mL/cubic foot of soil. Algae extracts Oligo-X (liquid phase) and Weed-Max (solid phase) were added at different concentrations, that is, 0.5, 1.0, and 20.0 (mL or g/L), were added individually or combined with bioagents at the rate of 250 mL/cubic foot of soil.

Another set of varied infested soils only with pathogens was used for comparison check treatment. The applied treatments in infested soil with either *Fusarium solani* or *Rhizoctonia solani* were as follows:

1. Oligo-X
2. a mixture of Oligo-X + *T. harzianum*
3. a mixture of Oligo-X + *T. viride*
4. a mixture of Oligo-X + *B. subtilis*
5. a mixture of Oligo-X + *P. fluorescens*
6. weed-Max
7. a mixture of Weed-Max + *T. harzianum*
8. a mixture of Weed-Max + *T. viride*
9. a mixture of Weed-Max + *B. subtilis*
10. a mixture of Weed-Max + *P. fluorescens*
11. *T. harzianum*
12. *T. viride*
13. *B. subtilis*
14. *P. fluorescens*
15. infested Untreated control
16. uninfested untreated control.

Five seeds of Cucumber or Tomato were sown in prepared artificially infested soils in each pot, and five pots were used as replicates for each particular treatment. Percentages of pre- and postemergence root rot incidence throughout the experimental period were recorded. Percentage of root rot incidence at the preemergence stage was calculated as the number of absent seedlings relative to the number of sown seeds. Meanwhile, the percentage of root rot incidence at the postemergence stage was calculated as the number of diseased plants relative to the number of emerged seedlings. Percentage of root rot incidence at preemergence growth stage was recorded after two weeks from sowing date. Meanwhile, percentage of root rot incidence at postemergence stage was calculated 45 days after emergence. This experiment was repeated twice in order to confirm the obtained results. At the end of the experiment, survived plants were carefully pulled out from pots after being flooded with water in order to leave the root system undamaged. Plant roots showing rot lesions in addition to the visual root rot symptoms on the shoot system were considered diseased plants. Isolation from infected germinated seeds at the preemergence stage and infected plants at the postemergence stage was carried out. Undeveloped, germinated seeds which were picked up from the soil and the diseased plants were washed and sterilized with 3% sodium hypochlorite and then subjected to reisolation in order to identify the causal pathogens.

2.3. Plastic House Experiments. The tested treatments in previous pot experiment which revealed promising results against root rot incidence were applied in plastic houses experiments under natural infestation conditions. This experiment was carried out on growing vegetables under protected cultivation system in commercial plastic houses of Ministry of Agriculture and Soil Reclamation, Egypt, at Dokki, Nubaria, and Haram locations. The applied integrated treatments were a mixture of Oligo-X (2 mL/L) + *T. harzianum* and a mixture of Weed-Max (2 g/L) + *B. subtilis*. The recommended fungicide Rizolex-T 50% as transplanting dipping at the rate of 2 g/L was used as comparison treatment. The experimental plastic house consists of 6 rows each contains two cultivated row sites. Each cultivated row site (0.9 × 60 m, width × length) was divided into 2 parts 30 m long each, and every part was considered as one replicate. Three replicates were used for each particular treatment in complete randomized design. The proposed treatments were prepared in laboratory of Plant Pathology Department, NRC, and sent to certain locations for soil application. The soil drenched at the cultivated row site with bioagents inocula of *T. harzianum* was grown previously on sand-barley medium at the ratio of 120 g/m^3^ [10] as well as inocula of *B. subtilis* were grown previously on nutrient broth medium at the rate of 500 mL/row (3 × 10^6 cfu) after [13]. Bioagents inocula were incorporated into the top of 20 cm of the soil surface at planting row sites considering relevant treatments. The prepared solution of algae mixture was also incorporated into the same cultivated row site at the rate of 30 L/row (distributed for the three replicates) as combined treatment. These treatments were applied 5 days before vegetables transplanting. The transplanted vegetables were Cucumber (at Dokki plastic house location); Cantaloupe (at Nubaria location); Tomato and Pepper (at Haram plastic house location). The cultivated vegetables received traditional agriculture practices, that is, irrigation, fertilizers, and so forth. At all locations, the growing vegetables in the experimental plastic houses received traditional programs recommended by Committee of Protected Cultivation Administration Office, Ministry
of Agriculture and Soil Reclamation, for controlling foliar diseases and harmful insects, that is, Aphids, Thrips, Whitefly, and so forth.

Monitoring and scouting of root rot incidence were recorded up to 60 days from transplanting date. Percentage of root rot disease incidence was recorded as the number of diseased plants relative to the number of planted seedlings and then the average of disease incidence in each treatment was calculated. At the end of growing season the obtained accumulated yield of cultivated vegetables for each relevant treatment was calculated.

2.3.1. Statistical Analysis. All experiments were set up in completely randomized design (CRD). The data collected from greenhouse experiment were analyzed by MSTAT-C program [24]. The means differences were compared by least significant difference test (LSD) at 5% level of significance. In plastic house experiments, one-way ANOVA was used to analyze differences between applied treatments. A general linear model option of the analysis system SAS [25] was used to perform the ANOVA. Duncan's multiple range test at $P \leq 0.05$ level was used for means separation [26].

3. Results

3.1. Pot Experiment. Evaluating the efficacy of bioagents and/or algae extracts as soil drench against root rot disease incidence was carried out in the greenhouse. Data presented in Tables 1 and 2 showed that all applied treatments as soil drench reduced significantly root rot incidence at both pre- and postemergence growth stages of Cucumber and Tomato plants compared with untreated check control. Data also revealed that applied treatments of the bioagent in combination with algae extracts resulted in higher significant reduction in root rot incidence than each of them alone. The presented data showed that treatments of $T. harzianum$ or $B. subtilis$ either alone or combined with algae extracts were significantly superior for reducing root rot disease for two tested vegetable plants compared with the other tested treatments as well as control. It is also observed that (Tables 1 and 2) rising concentrations of either Oligo-X or Weed-Max were reflected in more disease reduction. Presented data also showed that the recorded root rot incidence in $T. harzianum$ and $B. subtilis$ as single treatments at preemergence growth stages was 20% for Cucumber and Tomato in infested soil with either $F. solani$ or $R. solani$. Meanwhile, lower significant disease incidence were recorded when these bioagents combined with algae extracts. In this regard, root rot incidence of cucumber at preemergence stage (Table 1) was recorded as 16.0, 12.0, and 8.0% and 20.0, 16.0, and 12.0% at combined treatment of $T. harzianum$ and Oligo-X and Weed-Max at concentration of 0.5, 1.0, and 2 mL/L in infested soil with $F. solani$ and $R. solani$, respectively. Meanwhile root rot incidence of Tomato at preemergence stage (Table 2) was recorded as 20.0, 16.0, and 12.0% and 20.0, 20.0, and 12.0% at the same previous treatments in respective order. On the other hand, root rot incidence at preemergence growth stage was recorded as 32.0, 36.0% and 32.0, 32.0% for Cucumber and Tomato in untreated infested soil with $F. solani$ or $R. solani$, respectively.

Similar trend was also observed concerning root rot incidence at postemergence growth stage. The highest reduction in disease incidence was recorded at treatments of bioagents combined with algae extracts at concentration of 2 mL/L and 2 g/L. At postemergence stage, the recorded root rot incidence of Cucumber (Table 1) at $T. harzianum$ + Oligo-X and $T. harzianum$ + Weed-Max treatments was 8.6, 9.0% and 8.3, 8.6% in infested soil with $F. solani$ or $R. solani$, respectively. Also, in Table 2 the root rot incidence of Tomato in infested soil with $F. solani$ or $R. solani$ was recorded as 13.6% at $T. harzianum$ combined treatment with either Oligo-X or Weed-Max. Announced reduction in root rot incidence at postemergence growth stage of Cucumber and Tomato was observed at $B. subtilis$ combined with Weed-Max treatments. At concentration of 2 g/L of Weed-Max combined with $B. subtilis$ the root rot incidence recorded as 4.1, 4.1 and 9.0, 8.6% for Cucumber and Tomato plants in soil infested with $F. solani$ or $R. solani$, respectively (Tables 1 and 2).

The obtained results concerning applied treatments of bioagents alone or combined with commercial algae extracts against root rot incidence of some vegetables grown in artificially infested soil with root rot pathogens under greenhouse conditions revealed that the most promising treatments for controlling disease incidence are combined treatments between bioagents, $T. harzianum$, $B. subtilis$, and algae products at the highest concentration tested. Therefore, these treatments were evaluated under natural infections in plastic house conditions.

3.2. Plastic House Experiments. Different treatments were evaluated against root rot incidence under plastic houses conditions at different locations. Combined soil drench treatments, Oligo-X (2 mL/L) + $T. harzianum$ and (Weed-Max (2 g/L) + $B. subtilis$) were applied compared with the fungicide Rizolex-T (2 g/L) as transplants dipping. These treatments were carried out before Cucumber, Cantaloupe, Tomato, and pepper being transplanted. Presented data in Table 3 revealed that the applied combined treatments significantly reduced root rot incidence compared with fungicide and check control treatments.

At all locations it was observed that Oligo-X (2 mL/L) + $T. harzianum$ and Weed-Max (2 g/L) + $B. subtilis$ reduced disease incidence of grown vegetables compared with fungicide treatment and control as well. In this concern, data in Table 3 showed that no significance was observed between the two applied combined treatments. Also, data showed that the recorded disease incidence was 5.6%, 4.8% and 6.3%, 5.0% compared with 8.6%, 6.8% of Tomato, and Pepper plant treatments, Oligo-X (2 mL/L) + $T. harzianum$, Weed-Max (2 g/L) + $B. subtilis$, and the fungicide Rizolex T, in respective order. Meanwhile, root rot incidences as 17.6% and 18.7% were recorded in untreated control.

Reduction in disease incidence of Cucumber grown at Dokki location (Table 3) was recorded as 86.5% and 85.8% at Oligo-X (2 mL/L) + $T. harzianum$ and Weed-Max (2 g/L) + $B. subtilis$ compared with 65.3% at fungicide treatments. As for Cantaloupe plants grown at Nubaria location (Table 3) the reduction in root rot incidence was recorded as 73.0%, 74.6%,
and 53.9% at treatments, Oligo-X (2 mL/L) + T. harzianum, Weed-Max (2 g/L) + B. subtilis and fungicide, respectively. At Haram location similar trend was observed for the reduction in root rot incidence of Tomato and Pepper plants.

An obvious yield increase in all treatments was significantly higher than that in the control. Also, the harvested yield in applied combined treatments at all locations was significantly higher than that in the fungicide and control treatments (Table 4). The treatments of Oligo-X (2 mL/L) + T. harzianum showed higher yield production than those treatments using Weed-Max (2 g/L) + B. subtilis, and Rizolex-T. At Dokki location (Table 4) the effective treatment which helped increase Cucumber yield was Oligo-X (2 mL/L) + T. harzianum (47.4%), followed by Weed-Max (2 g/L) + B. subtilis (37.8%) and Rizolex-T (32.9%), respectively. At Nubaria location (Table 4) grown Cantaloupe in soil drenched with Oligo-X (2 mL/L) + T. harzianum resulted in the highest increase in produced yield estimated as 28.6% followed by...
Table 2: Effect of integrated soil treatment with algae compound and bio-agents against root rot incidence of tomato under greenhouse conditions.

<table>
<thead>
<tr>
<th>Soil infestation</th>
<th>Soil treatment</th>
<th>Pre-emergence</th>
<th>Post-emergence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Fusarium solani</strong></td>
<td>Oligo-X</td>
<td>28.0</td>
<td>24.0</td>
</tr>
<tr>
<td></td>
<td>Oligo-X + <em>T. harzianum</em></td>
<td>20.0</td>
<td>16.0</td>
</tr>
<tr>
<td></td>
<td>Oligo-X + <em>T. viride</em></td>
<td>24.0</td>
<td>24.0</td>
</tr>
<tr>
<td></td>
<td>Oligo-X + <em>B. subtilis</em></td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>Oligo-X + <em>P. fluorescens</em></td>
<td>24.0</td>
<td>24.0</td>
</tr>
<tr>
<td>Weed-Max</td>
<td></td>
<td>28.0</td>
<td>24.0</td>
</tr>
<tr>
<td><strong>Rhizoctonia solani</strong></td>
<td>Oligo-X</td>
<td>28.0</td>
<td>24.0</td>
</tr>
<tr>
<td></td>
<td>Oligo-X + <em>T. harzianum</em></td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>Oligo-X + <em>T. viride</em></td>
<td>24.0</td>
<td>24.0</td>
</tr>
<tr>
<td></td>
<td>Oligo-X + <em>B. subtilis</em></td>
<td>16.0</td>
<td>16.0</td>
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<tr>
<td></td>
<td>Oligo-X + <em>P. fluorescens</em></td>
<td>24.0</td>
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<tr>
<td>Weed-Max</td>
<td></td>
<td>20.0</td>
<td>20.0</td>
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<tr>
<td>Weed-Max + <em>T. harzianum</em></td>
<td></td>
<td>20.0</td>
<td>16.0</td>
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<tr>
<td>Weed-Max + <em>T. viride</em></td>
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<td>20.0</td>
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<tr>
<td>Weed-Max + <em>B. subtilis</em></td>
<td></td>
<td>16.0</td>
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<tr>
<td>Weed-Max + <em>P. fluorescens</em></td>
<td></td>
<td>24.0</td>
<td>24.0</td>
</tr>
<tr>
<td>Weed-Max + <em>T. harzianum</em></td>
<td></td>
<td>20.0</td>
<td>16.0</td>
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<tr>
<td>Weed-Max + <em>T. viride</em></td>
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<td>Weed-Max + <em>B. subtilis</em></td>
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<td>16.0</td>
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<tr>
<td>Weed-Max + <em>P. fluorescens</em></td>
<td></td>
<td>24.0</td>
<td>24.0</td>
</tr>
<tr>
<td>Untreated</td>
<td></td>
<td>32.0</td>
<td>24.0</td>
</tr>
</tbody>
</table>

LSD at 5%  
Infestation (I): ns  
Treatment (T): 2.0  
Concentration (C): 4.0  
Between (I × T × C): 4.0

Root rot disease occurs during the growing season of vegetable crops from seedling to flowering stages and may cause preemergence infection. So far, apart from scientific and practical difficulties, there is no economic way to control the crop diseases. The management of soil-borne plant pathogens is particularly complex because these organisms live in or near the dynamic environment of the rhizosphere and can frequently survive a long period in soil through the formation of rhizosphere.
Table 3: Application of algae compounds and bioagent formula against root rot disease of cucumber grown in plastic houses under protected cultivation system at different locations.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dokki Cucumber</th>
<th>Nubaria Cantaloupe</th>
<th>Haram Tomato</th>
<th>Haram Pepper</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DI&lt;sup&gt;1&lt;/sup&gt;</td>
<td>R&lt;sup&gt;2&lt;/sup&gt;</td>
<td>DI</td>
<td>R</td>
</tr>
<tr>
<td>Oligo-X + <em>T. harzianum</em></td>
<td>2.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>86.5</td>
<td>3.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>73.0</td>
</tr>
<tr>
<td>Weed-Max + <em>B. subtilis</em></td>
<td>2.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>85.8</td>
<td>3.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>74.6</td>
</tr>
<tr>
<td>Rizolex-T (2 g/L)</td>
<td>5.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>65.3</td>
<td>5.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>53.9</td>
</tr>
<tr>
<td>Untreated control</td>
<td>15.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
<td>12.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
</tr>
</tbody>
</table>

Mean values within each column followed by the same letter are not significantly different (*P* ≤ 0.05).

<sup>1</sup>Disease incidence calculated as the number of diseased plants relative to the whole number of examined plants in percentage.

<sup>2</sup>Reduction in disease incidence calculated as disease incidence in treatment relative to disease incidence in control in percentage.

Table 4: Effect of applied algae compounds and bio-agent formula on produced yield of some vegetables grown in plastic houses under protected cultivation system at different locations.

<table>
<thead>
<tr>
<th>Treatment</th>
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<th>Nubaria Cantaloupe</th>
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<th>Haram Pepper</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Y&lt;sup&gt;1&lt;/sup&gt;</td>
<td>I&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Y&lt;sup&gt;1&lt;/sup&gt;</td>
<td>I&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oligo-X + <em>T. harzianum</em></td>
<td>299.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>47.4</td>
<td>183.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>28.6</td>
</tr>
<tr>
<td>Weed-Max + <em>B. subtilis</em></td>
<td>280.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>37.8</td>
<td>175.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.0</td>
</tr>
<tr>
<td>Rizolex-T (2 g/L)</td>
<td>270.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.9</td>
<td>161.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.2</td>
</tr>
<tr>
<td>Untreated control</td>
<td>203.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
<td>142.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
</tr>
</tbody>
</table>

Mean values within each column followed by the same letter are not significantly different (*P* ≤ 0.05).

<sup>1</sup>The obtained accumulated yield of cultivated vegetables for each relevant treatment was calculated as Kg/row.

<sup>2</sup>Percentage of the increase in obtained accumulated yield of cultivated vegetables for each relevant treatment was calculated relative to yield in control treatment.

Resistant survival structures. Several commercially available products have shown significant disease reduction through various mechanisms to reduce pathogen development and disease. Plant diseases need to be controlled to maintain the quality and abundance of food, feed, and fiber produced by growers around the world. However, the environmental pollution caused by excessive use and misuse of agrochemicals, as well as fearmongering by some opponents of pesticides, has led to considerable changes in people's attitudes towards the use of pesticides in agriculture. Many strategies to control this fungal pathogen have been investigated in the field [27, 28]. Currently, the most effective method in preventing soil-borne diseases is to mix the seed with chemical fungicides. The application of chemical fungicide induces other problems, such as the harm to other living organisms and the reduction of useful soil microorganisms [29, 30]. Therefore, public concern is focused on alternative methods of pest control, which can play a role in integrated pest management systems to reduce our dependence on chemical pesticides [31].

The revolutionized therapy of infectious diseases by the use of antimicrobial drugs has certain limitations due to the changing patterns of resistance in pathogens and they side effects they produced. These limitations demand for improved pharmacokinetic properties, which necessitate continued research for new antimicrobial compounds for the development of drugs [32]. So accordingly, pharmaceutical industries are giving importance to the compounds derived from traditional sources (soil and plants) and less traditional sources like marine organisms [33]. Hence, the interest in marine organisms as a potential and promising source of pharmaceutical agents has increased during recent years [34]. Marine algae or seaweeds are a rich and varied source of bioactive natural products, so they have been studied as potential biocidal and pharmaceutical agents [35]. Hence, the interest in marine organisms as a potential and promising source of pharmaceutical agents has increased during recent years [34]. Marine algae or seaweeds are a rich and varied source of bioactive natural products, so they have been studied as potential biocidal and pharmaceutical agents [35]. Hence, the interest in marine organisms as a potential and promising source of pharmaceutical agents has increased during recent years [34].
nitrosulphuric-heterocyclic, sterols, dibutanoids, proteins, peptides, and sulphated polysaccharides [37]. The antibacterial activity of seaweeds is generally assayed using extracts in various organic solvents, for example, acetone, methanol, toluene, ether, and chloroform-methanol [40]. Using of organic solvents always provides a higher efficiency in extracting compounds for antimicrobial activity [41]. Several extractable compounds, such as cyclic polysulfides and halogenated compounds, are toxic to microorganisms, and therefore, responsible for the antibiotic activity of some seaweeds [42]. Also [36] it was revealed that the extraction of antimicrobials from the different species of seaweeds was solvent dependent; methanol was a good solvent for extraction of antimicrobials from brown seaweeds, whereas acetone was better for red and green species.

Furthermore, application of seaweeds as organic soil amendment has increased in recent years due to raising awareness about the adverse effect of chemical pesticides [43–47]. Also, in previous study the suppression of root rotting fungi and root knot nematode of chili by a red alga *Soritaria robusta* has been reported [44].

The present investigation has demonstrated the antifungal activity of all treatments tested. This work proves that some algae products have potential and could be useful when integrated with bioagents against root rot fungal pathogens. From the earlier reports [32, 36] it is evident that some of the marine algae products have antifungal compounds which do have the capacity to inhibit the fungal pathogens. Therefore, there is increasing interest in obtaining alternative antimicrobial agents for use in plant disease control systems. One of the main procedures used in the research of biologically active substances is a systematic screening for the interaction between microorganisms and plant products. This procedure has been a source of useful agents to control the microbial survival [48]. In the present study the introduction of the bioagent *T. harzianum* or *B. subtilis* to the soil increased the efficacy of both algae products against root rot incidence under greenhouse and plastic houses conditions. Similar results were also reported [10]. It was stated that *T. harzianum* introduced to the soil was able to reduce root rot incidence of faba bean plants significantly more than the fungicide Rizolex-T. Moreover, the application of biological controls using antagonistic microorganisms has proved to be successful for controlling various plant diseases in many countries [6, 49–52]. Combination between Topsisin-M and drench with either *B. subtilis* or *B. megaterium* induced more significant reduction in disease incidence and yield increase than seed coating with bioagents and/or each treatment alone. In addition, *Bacillus cereus* has proven to have beneficial effects on crop health including enhancement of soybean yield, suppression of damping-off of tomato [53], and *alfalfa* [54].

5. Conclusion

The present work demonstrated that application of commercial algae extracts, Oligo-X and Weed-Max, combined with bioagents, *T. harzianum* and *B. subtilis*, as integrated soil treatment under greenhouse and plastic houses trials, resulted in plant health and improved the obtained yields in numerous tested vegetable crops. These treatments caused a significant reduction in root rot incidence of Cucumber, Cantaloupe, Tomato, and Pepper. Furthermore, an obvious yield increase in all applied combined treatments at all locations was significantly higher than that in the fungicide and control treatments. It may be concluded that application of commercial algae products combined with active bioagents is considered an applicable, safe, and cost-effective method for controlling such soil-borne diseases.

Conflict of Interests

The authors declare that they do not have a direct financial relation with the two mentioned companies “El-Nile Company, Egypt, and Chemical Industrial Development Company (CID), Egypt,” that might lead to a conflict of interests.

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