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Application of Fungicides Alternatives as Seed Treatment for Controlling Root Rot of Some Vegetables in Pot Experiments

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Abstract Different root rot fungi, *Alternaria solani Fusarium solani, F. oxysporum, Rhizoctonia solani, Sclerotium rolfsii, Macrophomina phaseolina* and *Pythium* sp. were isolated from various vegetables, *i.e*. Cucumber, Cantaloupe, Tomato and Pepper grown in plastic houses under protected cultivation system and showing root rot and or damping-off disease symptoms. The tested antagonistic fungi were *Trichoderma harzianum*, *T. Viride* and *T. hamatum, Bacillus subtilis, Pseudomonas fluorescens* and *Saccharomyces cerevisiae*. The efficacy of Calcium chloride, Thyme oil and /or bio-agents as seed dressing against disease incidence was evaluated in pot experiments under artificially infested with vegetables root rot causal organisms under greenhouse conditions. All applied treatments reduced significantly root rot incidence at both pre-, and post-emergence growth stages of Cucumber, Cantaloupe, Tomato and Pepper plants comparing with untreated check control. The obtained results showed that combination treatments of Calcium chloride, Thyme oil with bio-agents reduced significantly root rot incidence of all grown vegetables comparing with the application of each of them alone. Obtained results, in the present study, lead to suggest that integration between salt or essential oil and bio-agents is considered an applicable, safe and cost-effective method for controlling such soil-borne diseases.

Keywords Root Rot, Cucumber, Cantaloupe, Tomato, Pepper, Essential Oil, Calcium Chloride, Thyme Oil, Bio-Agents, Biological Control

1. Introduction

Root rot in vegetables strikes quickly and then ruin a whole crop. However the largest instruction course of actions is preventative measures, as therapy with fungicide does not normally work. Not all vegetables can arrangement root rot, but many standard vegetable crops are susceptible. Several fungi may cause root rot in vegetable plants, transmitting the disease through the soil. Some common fungi include *Fusarium, Rhizoctonia, Sclerotinia, Pythium* and others 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 soil that doesn't drain well, are most susceptible to root rot. The best way to avoid root rot is by eliminating these contributing causes, and practice sound cultivation techniques. The soil borne pathogens *Rhizoctonia solani, Pythium ultimum*, and *Fusarium* spp. can cause severe economic losses to field and greenhouse cucumber[1, 2]. 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[3]. 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[4].

Root rot disease occurs during the growing season of vegetable crops from seedling to flowering stages, and may cause pre-emergence 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 resistant survival structures.

The current management strategy relies on the intensive use of fungicides. In addition, chemical control does not give satisfactory control of the root disease. Application of biological control using antagonistic microorganisms has

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proved to be successful for controlling various plant diseases[5]. However, it is still not easy and costly in application. It can serve as the best control measure under greenhouse conditions. The concern of pesticides use with respect to human health and environment has brought increasing interest in alternatives use by avoiding negative effect on the environment. Essential oils are known for their natural components, such as mono terpenes, di terpenes, andhydro-carbons with various functional groups. Many other researchers have reported antifungal activities[6-8] of essential oils in food applications, pharmaceutical research and other areas as plant disease control.

The present research focuses on finding compounds that are safe to humans and the environment, *e.g.* Calcium chloride and Thyme oil as well as biocontrol agents which may provide an alternative control of many soil and seed-borne pathogens. The objective of the present work was to evaluate fungicide alternatives and /or bio-agents against root rot incidence when used as seed treatment under greenhouse

2. Materials and Methods

2.1. Plant Materials

Seeds of Cucumber (cv. Alpha), Cantaloupe (cv. Yatherb 7), Tomato (cv. Castel Rock) and Pepper (cv. California) were used in the present study.

2.2. Fungicides Alternatives

The chemical salt, Calcium chloride ($CaCl_2$) was obtained from El-Nasr Company for chemical industry, Egypt. Meanwhile, pure-grade of Thyme oil was obtained from Cairo Company for oils and aromatic extractions CID, Egypt. The essential oil was stored in dark glass bottles at 4℃.

2.3. Pathogenic Fungi

The tested soilborne pathogenic fungi were *Alternaria solani Fusarium solani, F. oxysporum, Rhizoctonia solani, Sclerotium rolfsii, Macrophomina phaseolina* and *Pythium* sp. These fungi were isolated from various vegetables, *i.e*. Cucumber, Cantaloupe, Tomato and Pepper grown in plastic houses under protected cultivation system and showing root rot and or damping-off disease symptoms[4]. The isolated fungi proved their aggressive ability to induce root rot disease of those vegetables.

2.4. Bio-agents

The tested antagonistic fungi were *Trichoderma harzianum*, *T. Viride* and *T. hamatum, B. subtilis, P. fluorescens* and *S. cerevisiae*. These antagonists were isolated from cucumber, cantaloupe, tomato and pepper grown in plastic houses under protected cultivation systems and showing root rot disease symptoms[4]. The present bio-agents proved their antagonistic ability against the above mentioned pathogens.

2.5. Greenhouse Experiments

Evaluation of Calcium chloride, Thyme oil and /or bio-agents seed dressing under artificial infestation with vegetables root rot causal organisms against disease incidence was performed in pot experiments under greenhouse conditions of Plant Pathology Dept., National Research Centre, Egypt.

For root rot disease evaluation, experiments were carried out in a sandy loam soil artificially infested with root rot pathogens inocula. Inocula of pathogenic fungi, *Alternaria solani Fusarium solani, F. oxysporum, Rhizoctonia solani, Sclerotium rolfsii, Macrophomina phaseolina* and *Pythium* sp. were individually grown on autoclaved sand barlly medium (1:1,v:v + 40% water) for two weeks at 25±1℃[9]. The fungal inocula were then mixed together to obtain a mixture contains equal share of tested pathogens. Soil infestation was carried out through amended with a mixture of root rot pathogens inocula (5% w:w) after[10], then mixed thoroughly to ensure equal distribution of pathogenic fungal inocula. Infested soil was then filled in plastic pots (30-cm-diameter) and irrigated every second day for 1 week before sowing.

Evaluating the efficacy of Calcium chloride, Thyme oil, and/or bio-agents, *i.e. T. harzianum, T. viride, B. subtilis, P. fluorescens* and *S. cerevisiae* against root infection were applied as different treatments of seed dressing as follows:

- Calcium chloride 2% (20g/L).
- Calcium chloride (2%) + *T. harzianum* ($2x10^4$cfu/mL).
- Calcium chloride (2%) + *T. viride* ($2x10^4$cfu/mL).
- Calcium chloride (2%) + *B. subtilis* ($2x10^4$cfu/mL).
- Calcium chloride (2%) + *P. fluorescens* ($2x10^4$cfu/mL).
- Calcium chloride (2%) + *S. cerevisiae* ($2x10^4$cfu/mL).
- Thyme oil at the rate of 1% (10ml/L).
- Thyme oil (1%) + *T. harzianum* ($2x10^4$cfu/mL).
- Thyme oil (1%) + *T. viride* ($2x10^4$cfu/mL).
- Thyme oil (1%) + *B. subtilis* ($2x10^4$cfu/mL).
- Thyme oil (1%) + *P. fluorescens* ($2x10^4$cfu/mL).
- Thyme oil (1%) + *S. cerevisiae* ($2x10^4$cfu/mL).
- *T. harzianum* ($2x10^4$cfu/mL).
- *B. subtilis* ($2x10^4$cfu/mL).
- *T. viride* ($2x10^4$cfu/mL).
- *P. fluorescens* ($2x10^4$cfu/mL).
- *S. cerevisiae* ($2x10^4$cfu/mL).
- Untreated control

Seeds of Cucumber, Cantaloupe, Tomato and Pepper were disinfested by emerging in sodium hypochlorite solution (3%) for 2 min, then rinsed and washed in sterilized water and air dried. Disinfected seeds were soaked for 3 h individually in the tested treatment solutions, and then left to air dry before sowing. Stock solutions (500 ml) of each of the tested treatment, Calcium chloride, Thyme oil and /or bio-agents were prepared in certain concentrations by dissolving in sterilized distilled water in form of sticky suspensions (1 mL of Arabic gum/100mL suspension). Two mL of Tween 80 (0.2% of water volume) were added to the essential oil solutions to obtain an aqueous emulsion. Tween 80 also known as polysorbate 80 is a nonionic surfactant and emulsifier derived from sorbitol obtained from various types of fruit. Polysorbate 80 is a water-soluble somewhat yellowish amber liquid that is used as a dispersing agent to mix oil and water and to solubilize fragrances and essential oils[11]. All stock solutions were prepared in black glass bottles kept in refrigerator until used. Coated seeds were sown as three seeds per pot, five pots per replicates in each treatment. Another set of soil amended only with a mixture of root rot pathogens (5% w:w) and sown with surface sterilized seeds was kept as control check treatment. The average percentage of root rot incidence at the pre- and post-emergence of growth stages was recorded up to 15 and 45 days of sowing date, respectively.

Statistical analysis

All experiments were set up in a complete randomized design. One-way ANOVA was used to analyze differences between inhibitor effect and linear growth of pathogenic fungi *in vitro*. A general linear model option of the analysis system SAS[12] was used to perform the ANOVA. Duncan's multiple range test at $P < 0.05$ level was used for means separation[13].

3. Results and Discussion

The obtained results in Table (1) and Fig (1) showed the efficacy of applied Calcium chloride, Thyme oil and /or bio-agents as seed treatment against tested vegetables root rot diseases incidence. Presented data revealed that all applied treatments reduced significantly root rot incidence at both pre-, and post-emergence growth stages of Cucumber, Cantaloupe, Tomato and Pepper plants comparing with untreated check control. Data also showed that combination treatments of Calcium chloride, Thyme oil with bio-agents reduced significantly root rot incidence of all grown vegetables comparing with the application of each of them alone. At pre-emergence growth stage the treatments of the bio-agents, *T. harzianum* and *B. subtilis* recorded higher significant reduction in root rot incidence when combined with calcium chloride (3.3-7.0%), Thyme oil (3.3-8.4%) and/or applied alone (17.3-25.5% & 17.0-25.8%) comparing with the other bio-agents *P. fluorescens* (33.6-38.1% & 27.3-30.3% & 31.8-38.5%) and *S. cerevisiae* (29.6-47.0% & 36.2-47.7% & 28.8-32.9%), in respective order. Meanwhile, the root rot incidence for Cucumber, Cantaloupe, Tomato and Pepper were 42.9, 44.7, 47.3 and 42.5%, respectively in untreated seeds check treatment. Data in Table (1) and Fig. (1) revealed that treatments of *T. harzianum* and *B. subtilis* combined with either calcium chloride or Thyme oil seem to have the superior treatments for controlling root rot disease incidence of tested vegetables.

Also, data in Table (1) showed another feature concerning *S. cerevisiae* and *P. fluorescens* that application of *S. cerevisiae* treatment alone was significantly reduced root rot incidence comparing with combination of either Calcium chloride or Thyme oil treatments, while when *P. fluorescens* combined with Thyme oil resulted in significant reduction in root rot incidence comparing with either its application alone or when combined with Calcium chloride, respectively. Regarding *T. viride* treatments, recorded results in Table (1) showed fluctuated response against root rot incidence for individual host plant. No significant differences were observed between *T. viride* treatments in cucumber, while *T. viride* combined with calcium chloride and Thyme oil were significantly differed than *T. viride* alone for reducing disease incidence of Cantaloupe and Tomato.

As for Pepper, *T. viride* combined with Thyme oil reduced significantly root rot incidence followed by *T. viride* combined with calcium chloride or applied alone. At post-emergence of tested vegetables growth stage (Table, 2 & Fig. 2), treatments of the bio-agents, *T. harzianum* and *B. subtilis* combined with calcium chloride, Thyme oil and/or applied alone were significantly caused higher protection against root rot disease incidence.

Also, treatment of *S. cerevisiae* alone was significantly reduced disease incidence of Cucumber, Cantaloupe and Pepper followed by its combination with Calcium chloride and Thyme oil, while no significant differences were observed between these treatments with Tomato plants, although they differed significantly with untreated check control treatment. The other bio-agents, *T. viride* and *P. fluorescens* combined with either Calcium chloride or Thyme oil had superior effect for reducing root rot incidence comparing with their application alone. In general data in Table (1 and 2) and fig (1 and 2) revealed that application of *T. harzianum* and *B. subtilis* either alone or combined with Calcium chloride and Thyme oil proved to be the most significant seed treatments for controlling root rot incidence of Cucumber, Cantaloupe, Tomato and Pepper in pot experiment. Similar results were also recorded by many investigators. Within the large reservoir of natural fungicides that exist in plants examples exist that would serve as safe and effective alternatives to synthetic fungicides. Such compounds (volatile components, essential oils), if properly formulated and applied, could be used directly or could serve as templates for synthetic analogs.

In the present study application of Thyme oil as seed coating revealed its efficacy against seed or plant invasion under *in vivo* conditions which resulted in a significant reduction in root rot incidence of grown vegetables under greenhouse conditions. The suppression of root rot development under greenhouse condition seems to corresponds with the ability of this essential oil to reduce disease incidence. The major components of the used Thyme oil are carvacrol and thymol. These results could be correlated with the stability and concentration as well as volatility of the active component in the essential oil used. Essential oils from oregano and thyme for 24 h as fumigants were applied against the mycelia and spores of *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus ochraceus*, as well as against natural microflora of wheat grains[14]. Also, they recorded that their findings emphasize the toxicity of oregano and thyme essential oils as fumigants against fungi attacking stored grain and strengthen the possibility of using them as an alternative to chemicals for preserving stored grains.

Moreover, it was recorded that that in poison food medium thyme oil had greatest antifungal activity against *Penicillium digitatum* and *Rhizopus stolonifer* at 1,000 and ≥600 µL/L, respectively[15]. In vapour phase, thyme oil at ≥5 µL completely inhibited the mycelial growth of pathogens.

Thyme essential oil was tested against the fungi, *Aspergillus, Penicillium, Alternaria*, *Ulocladium*, *Absidia, Mucor, Cladosporium, Trichoderma,Rhizopus* and *Chaetomium* [16]. They found that the vaporous phase of the thyme essential oil (82 µg l^{-1}) in glass chambers strongly suppressed the sporulation of moulds during 60 days of exposure. Moreover, [17] reported that Thyme and Egyptian geranium oil inhibited growth of all test fungi, *Aspergillus niger*, *Trichoderma viride*, and *Penicillium chrysogenum* on southern yellow pine for 20 weeks. Similar results were also reported by many researchers indicating the efficacy of essential oils as antifungal inhibitors for a large number of soilborne pathogens[18-21].

The mode by which microorganisms are inhibited by essential oils and their chemical compounds seem to involve different mechanisms. It has been hypothesized that the inhibition involves phenolic compounds, because these compounds sensitize the phospholipid bilayer of the microbial cytoplasmic membrane causing increased permeability and unavailability of vital intracellular constituents[22].

Table 1. Effect of applying Calcium chloride, Thyme oil and /or bio-agents as seed treatment against vegetables pre-emergence root diseases caused by soil-borne pathogenic fungi under open greenhouse conditions

Treatment	Root diseases incidence (%) pre-emergence stage			
	Cucumber	Cantaloupe	Tomato	Pepper
Ca Cl_2	32.1 b	41.8 a	45.1 a	41.8 a
Ca Cl_2 + *T. harzianum*	5.1 e	3.3 e	3.6 e	5.1 e
Ca Cl_2 + *T. viride*	26.2 c	28.1 c	28.8 c	31.4 b
Ca Cl_2 + *B. subtilis*	7.0 e	4.4 e	4.0 e	4.0 e
Ca Cl_2+ *P. fluorescens*	37.7 b	36.6 b	38.1 b	33.6 b
Ca Cl_2 + *S. cerevisiae*	29.6 c	32.9 b	47.0 a	40.0 a
Thyme oil	27.3 c	43.3 a	31.8 b	25.9 c
Thyme oil + *T. harzianum*	3.3 e	4.7 e	4.4 e	5.9 e
Thyme oil + *T. viride*	29.6 c	25.9 c	29.9 c	25.5 c
Thyme oil + *B. subtilis*	4.8 e	4.7 e	5.5 e	8.4 e
Thyme oil + *P. fluorescens*	27.7 c	30.3 b	29.2 c	27.3 c
Thyme oil + *S. cerevisiae*	41.0 a	47.7 a	36.2 b	41.4 a
T. harzianum	25.5 c	24.4 c	17.3 d	19.2 d
T. viride	28.8 c	31.0 b	31.8 b	31.8 b
B. subtilis	25.8 c	27.7 c	17.0 d	17.3 d
P. fluorescens	29.9 c	38.5 b	32.2 b	31.8 b
S. cerevisiae	28.8 c	32.9 b	32.2 b	29.9 c
Control	42.9 a	44.7 a	47.3 a	42.5 a

Mean values within columns followed by the same letter are not significantly different (P ≤ 0.05)

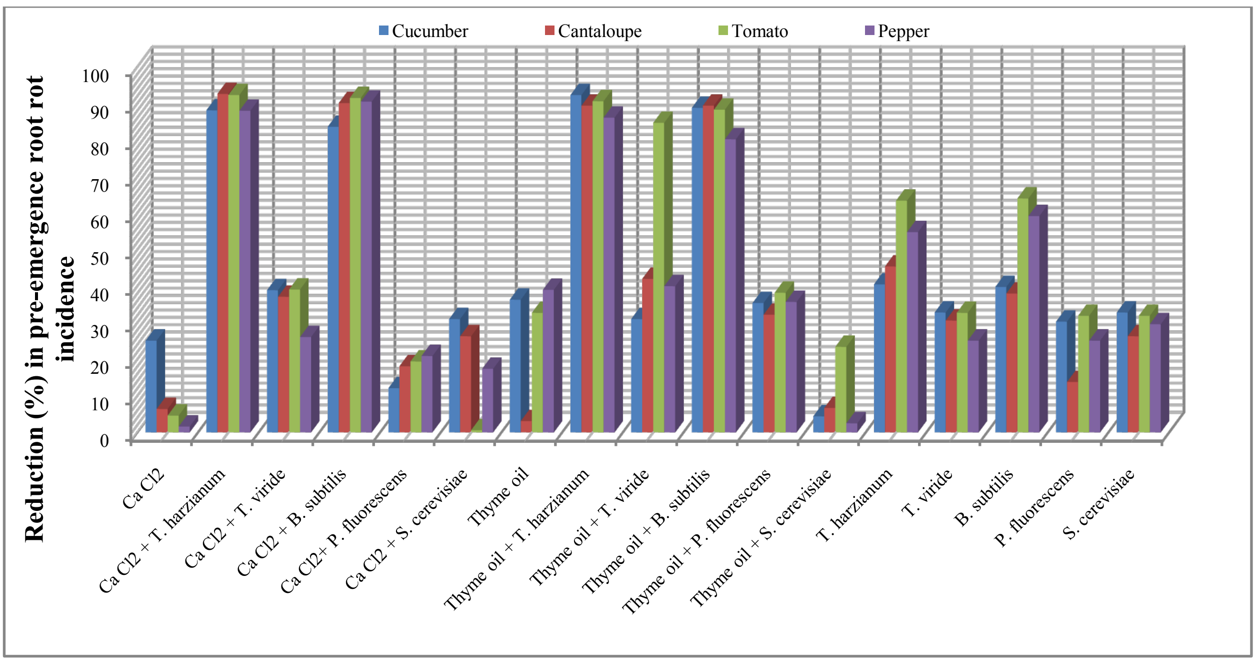

Figure 1. Reduction in vegetables root diseases (Pre -emergence) caused by soil-borne pathogenic fungi in response to applying Calcium chloride, Thyme oil and /or bio-agents as seed treatment under open greenhouse conditions

Table 2. Effect of applying Calcium chloride, Thyme oil and /or bio-agents as seed treatment against vegetables post-emergence root diseases caused by soil-borne pathogenic fungi under open greenhouse conditions

Treatment	Root diseases incidence (%)			
	post-emergence stage			
	Cucumber	Cantaloupe	Tomato	Pepper
Ca Cl_2	42.6 d	61.2 c	69.1 c	63.4 c
Ca Cl_2 + *T. harzianum*	4.6 g	5.9 g	5.3 g	6.2 g
Ca Cl_2 + *T. viride*	37.5 e	32.3 e	36.4 e	37.5 e
Ca Cl_2 + *B. subtilis*	5.1 g	6.5 g	6.5 g	6.8 g
Ca Cl_2+ *P. fluorescens*	44.5 d	35.8 e	49.3 d	45.2 d
Ca Cl_2 + *S. cerevisiae*	32.4 e	38.1 e	70.7 b	65.0 c
Thyme oil	41.9 d	76.0 b	46.0 d	40.7 d
Thyme oil + *T. harzianum*	7.1 g	5.7 g	8.4 g	5.4 g
Thyme oil + *T. viride*	38.5 e	40.0 d	41.7 d	39.2 e
Thyme oil + *B. subtilis*	5.3 e	4.0 g	6.9 g	5.2 g
Thyme oil + *P. fluorescens*	48.8 d	42.3 d	42.4 d	40.3 d
Thyme oil + *S. cerevisiae*	72.1 b	67.9 c	48.8 d	51.9 cd
T. harzianum	32.5 e	30.5 e	30.4 e	30.9 e
T. viride	40.6 d	39.7 e	48.3 d	45.6 d
B. subtilis	24.6 f	21.7 f	30.1 e	30.3 e
P. fluorescens	39.9 e	46.3 d	49.1 d	48.3 d
S. cerevisiae	41.8 d	44.2 d	49.6 d	50.1 cd
Control	81.5 a	89.1 a	86.4 a	85.7 a

Mean values within columns followed by the same letter are not significantly different ($P \leq 0.05$)

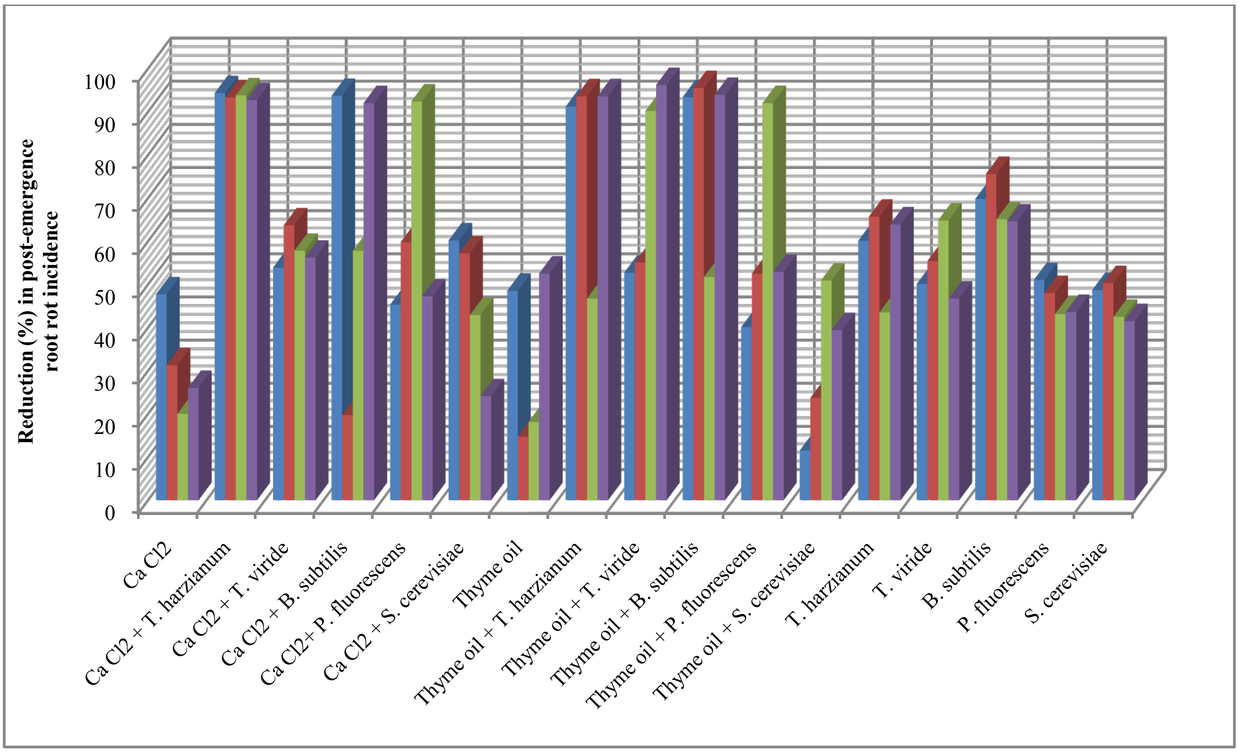

Figure 2. Reduction in vegetables root diseases (Post-emergence) caused by soil-borne pathogenic fungi in response to applying Calcium chloride, Thyme oil and /or bio-agents as seed treatment under open greenhouse conditions

Many authors emphasized that the antimicrobial effect of essential oil constituents has been dependent on their hydrophobicity and partition in the microbial plasmatic membrane. Effect of specific ions due to their addition in/on plasmatic membrane had great a effect on the protons motive force, intracellular ATP content and overall activity of microbial cells, including turgor pressure control, solutes transport and metabolism regulation[23]. The used thyme oil is reported to contain many volatile compounds[14].

So, it seems that the antifungal effects are the result of compounds acting synergistically. This means that the individual components by themselves are not sufficiently effective. This result confirmed elsewhere[24] who reported that carnation, caraway, thyme, peppermint and geranium essential oils used to coat seeds at a concentration of 4% sown in soil treated with the bio-agent *Trihoderma harzianum,* gave pronounced protection to emerged bean seeds against the invasion of root rot pathogenic fungi, *Fusarium solani, Rhizoctonia solani, Sclerotium rolfsii* and *Macrophomina phaseolina* under greenhouse and field conditions. Also, [25] reported that Under storage conditions, application of carnauba wax formula containing either *S. cerevisiae* or *S. cerevisiae* (CBY) combined with peppermint oil (1%) had more superior effect for reducing gray mould, soft rot and black rot incidence caused by *Botrytis cinerea*, *Rhizopus stolonifer* and *Alternaria alternate* the causal agents of tomato fruit decay, reaching up to 100% under artificial inoculation of decay pathogenic fungi. They also conclude that, the application of carnauba wax containing 1% peppermint oil combined with *S. cerevisiae* or *S. cerevisiae* (CBY), could control several post-harvest diseases of tomato fruit without affecting tomato fruit quality under storage conditions[25].

In the present study the coating vegetables seeds with only the tested bioagents, *T. harzianum*, *T. Viride* and *T. hamatum, B. subtilis, P. fluorescens* and *S. cerevisiae* could reduce root rot incidence comparing with control check treatment. This reduction was increased when these bioagents combined with calcium chloride or Thyme oil. Similar results were reported[9] who stated that *T. harzianum* introduced to the soil was able to reduce root rot incidence of faba bean plants significantly. Moreover, the application of biological controls using antagonistic microorganisms, has proved to be successful for controlling various plant diseases in many countries[5,26,29]. Furthermore, the ability of biocontrol agents, fluorescent *Pseudomonas*, non-pathogen *Fusarium* strain and *Trichoderma harzianum* T-22 when applied in combination and alone, to control of *Fusarium oxysporum* f. sp. *Lycopersici* the causal of tomato wilt in the greenhouse was studied[30]. They found that combination of *T. harzianum* T-22 + fluorescent *Pseudomonas* isolate gave the best control (70.2%)[30].

On the other hand**,** Calcium chloride as $CaCl_2$ was reported to suppress growth of the citrus mould pathogen *Penicillium digitatum*[31]. Calcium chloride effectively reduced silver scurf lesions on potato tubers, but not sporulation of *Helminthosporium solani.* It is known that addition of calcium chloride can also improve the activity of biocontrol agents[31,32]. Certain strategies, such as pre- or postharvest application of calcium salts, hydrogen peroxide and chitosan against fruit decay are proposed[33,34]. Pre- and postharvest calcium applications have been used to delay ageing or ripening to reduce postharvest decay and control of many diseases in fruits and vegetable[35]. Postharvest calcium treatment of apples was reported to provide broad spectrum protection against the postharvest pathogens of *Penicillium expansum* and *Botrytis cinerea*[36]. Furthermore, [37] reported that the commercial backing yeast *S. cerevisiae* (CBY) enhanced the inhibitory effect of tested sodium bicarbonate or calcium chloride salts reflected in increasing *Alternaria solani,* the causal agent of early blight disease of potato, mycelial fungal growth reduction when combined at the rate of 1:1 at each concentration tested. In pot experiment, under artificial infestation with pathogenic fungus, application of sodium bicarbonate or calcium chloride significantly reduced the early blight incidence and severity by increasing their concentrations. Superior effect of sodium bicarbonate or calcium chloride in disease reduction was observed when they combined with *S. cerevisiae*. The mechanisms by which calcium salts inhibit mycelia fungal growth are not well known. One hypothesis is that high external concentrations of Ca^{2+} may lead to increased concentration of Ca^{2+} in the cytosol[31]. Since maintenance of low basal concentrations of internal Ca^{2+} is essential for normal cell functions, organisms with the inability to regulate intracellular Ca^{2+} may exhibit compromised growth and development. Calcium ions may reduce the incidence of fungal infection by directly inhibiting fungal growth and by inhibiting cell wall-degrading enzymes produced by the pathogens[31-40]. Also, [41] earlier recorded that Calcium application reduces bean root rot caused by *Rhizoctonia solani* probably by altering pectin metabolism of the host.

The present study demonstrated that the salt, Calcium chloride or the thyme, essential oil application as a seed coating combined with fungal or bacterial bioagents resulted in a significant reduction in root rot incidence of vegetables, Cucumber, Cantaloupe, Tomato and Pepper under greenhouse conditions. It may be concluded that integration between salt, essential oil and bioagents is considered an applicable, safe and cost-effective method for controlling such soilborne diseases.

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