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# Antagonistic Fungi, Soil Amendment and Soil Solarization as an Integrated Tactics for Controlling Fusarium Root Rot of Lupine (*Lupinus termis*)

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**Abstract** The efficient control of soil-borne pathogens while avoiding environmental hazards and degradation of natural resources is the paramount challenge in crop protection sciences. *Lupinus termis* (lupine) is a fabaceous crop grown in Egypt for food, medical and industrial uses. The management of Fusarium root rot pathogens, which are responsible for serious losses on a number of economically important crops, including lupine, is being investigated. This study aimed to control Fusarium lupine root rot, under field conditions with no or low environmental impact; by using different control measures such as: antagonistic fungi, soil amendment and solarization singly or as an integrated disease management strategy. The integration of pest management methods is not only merely worthy, but also the more powerful practical solution for controlling soil-borne pests. Results showed that the solarized treated soil as well as the fungal antagonists, as single treatment methods in controlling Fusarium root rot revealed 80% and 81% healthy plants compared with their control that showed only 41%. Integrating soil solarization with mixed fungal inocula and soil organic amendment have been improving efficacy of controlling of lupine-fusarium-root rot by increasing the percentage number of healthy plants from 81 %, 79 % in treated unsolarized soil to 86 %, 82 % respectively in treated solarized soil. Using tactics such as solarization antagonistic fungi, and organic amendment as an integrated method, proved to be efficient for controlling the Fusarium root rot pathogen in *Lupinus termis.*

***Keywords:*** *Lupinus termis, Fusarium, root rot, solarization, soil amendment, biocontrol agent*

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## 1. Introduction

The white lupine (*Lupinus termis* Forsk) is economically important fabaceous crop which is grown in Egypt for food, medical and industrial purposes. The plant is cultivated for its seeds, which contains alkaloids, protein, oil, cholesterol, lecithin, salts (P & K) and carbohydrates [1]. The crop, however is suffering from various diseases, causing decrease in seed yield, including *Pythium* spp. *Rhizoctonia solani, Fusarium solani, and F. oxysporum*, causing decrease in seed yield. However, root rot pathogenic fungi *F. solani* is probably the most serious disease attacking lupine plant.

Development of sustainable agricultural methods by manipulating soil microbiota using soil and crop management practices is a basic tactic for improving crop production and management of plant diseases [2]. In this technique, biological methods of pest and disease control are confirmed. A range of specific soil microbes are playing an important role in the inhibition of plant infectious diseases and also in plant growth improvement [3]. Controlling plant diseases and pests require utilization of chemicals, which may cause environmental pollution, minimize soil microbial diversity and eventually increase crop diseases [4].

From several decades pesticides have been widely used to control soil-borne pathogens which cause severe losses of economically important crops, but due to the risks of fungicides on the human health and all life form in the environment, research interests, in the last few decades, focused on alternative control methods. [5]. The developed efficient methods to control soil-borne pathogens are usually need many process such as soil heating, biofumigation, biological soil disinfestation, use of organic improvement implementation, yield management, and use of biological control agents [6].

Many strategies to control Fusarium root rot disease in lupine have been investigated in the field. A promising tactic for the replacement of chemicals has been the implementation of biological control technology, used singly or as an integrated disease management component. The recent developments in the commercialization of biocontrol outputs have quickened this program. In order to reduce plant diseases in the field, with various degrees of success, biocontrol preparations of fungi, yeast and bacteria have been applied to seeds, seedlings, and implantation media in several ways [7].

A diverse group of beneficial micro-organisms, including bacteria, actinomycetes, yeasts and fungi, are being used as biocontrol agents. Generally, biocontrol agents are introduced singly against a pathogen or nematode, but recent studies clarify that mixtures of biocontrol agents may promote efficiency and precision of the biocontrol [8,9,10]. However, such mixtures which do not result in improved suppression of disease have been noticed by many researcher [11,12]. Such shortage is mainly because of inconsistent of the participant such as when two biocontrol agents inhibit each other. Consequently, compatibility of the co-inoculants is fundamental for the successful advancement of combining biocontrol agents [13].

*Trichoderma* spp. which numerous in soil, root ecosystems and present as saprophytes, consider one of the most effective biocontrol agents. [14]. *Trichoderma* species are excellent competitors in the infection court, possess a capacity to modify the rhizosphere, are tolerant or resistant to soil pesticide, have the ability to grow and survives under unfavorable conditions, are efficient in utilizing soil nutrients, have powerful aggressiveness against phytopathogenic fungi, and also promote plant growth [15].

*Gliocladium* species are common soil saprophytes and several species have been reported to be parasites of many plant pests [16], as for example, *G. catenulatum* parasitizes *Sporidesmium sclerotiorum* and *Fusarium* spp. *G. catenulatum* has also been used as a wettable powder; this product can be applied to soils, roots, and foliage to reduce the severity of *Rhizoctonia solani* and *Pythium ultimum* damping-off disease in the greenhouse [17]. *G. virens* produces secondary metabolites such as gliotoxin which have anti-bacterial, anti-fungal, anti-viral and anti-tumor activities.

Many strains of *Chaetomium* species with potential to be biological control agents inhibit the growth of bacteria and fungi through competition (for substrate and nutrients), parasitism, anti-biosis, or diverse combinations of these [18,19]. *C. globosum* and *C. cupreum* in particular have been successfully used to control root rot disease of black pepper and strawberry and have been shown to reduce damping off disease of sugar beet [20].

As for organic amendments, they affect disease control by improving the nutrition of the host, thus increasing its resistance, or by reducing the inoculum potential of soil-borne pathogens in the soil. An increase in the ammonia concentration and in the soil pH factors that are involved in the suppression of Fusarium wilt as well as other root pathogens, has been reported in soil amended with organic amendments rich in nitrogen [21,22]. Investigations carried out under diversified environmental conditions have shown the capability of different members of the *Brassica* species, such as *B. juncea, B. campestris, B. nigra, B. napus, B. carinata,* and *B. oleracea,* to manage several soil-borne pathogens [23,24].

Still another important control measure technology, soil solarization is a non-chemical disinfestation practice that might utilize as a component of a prospective integrated pest management (IPM) program. The basic principle of soil solarization is to increase the temperature in a moist soil to a fatal level that directly influences the viability of harmful organisms. Soil solarization process also induces diverse ecological and biological changes in the soil that indirectly influence soil-borne pathogens as well as survival of useful organisms [25]. Actually, the most common practice of soil solarization is based on covering moistened soil with transparent polyethylene. The duration of soil mulching which is required for practical effect is usually five to seven weeks, depending on the pest, soil characteristics, climatic conditions and the transparent polyethylene properties [26,27]. Pest population and ecological status are uncontrolled variables, while soil moisture and transparent polyethylene properties could be modified as needed.

The use of combinations of different biocontrol agents can contribute to better control of pathogens over the use of single organisms [28]. Integration of microorganisms may enhance the degree and uniformity of control by providing multiple mechanisms of action, a more stable rhizosphere community, and efficacy over a broad range of environmental conditions. In particular, combinations of fungi and bacteria may provide prevention at different times or under different conditions, and occupy different or complementary niches; the combinations that may overcome disagreement in the performance of individual isolates [14]. Also, a positive synergistic interaction between bacterial antagonists, such as *Pseudomonas syringae* and strains of *Trichoderma* species has been reported for their combined enforcement in the control of plant pests [29]. Furthermore, a mixture of three different plant growth-promoting rhizobacteria (PGPR), utilized as a seed treatment, revealed intensive plant growth promotion and reduction of multiple cucumber diseases [8].

Solarization can also be integrated with a wide range of organic amendments, such as composts, crop residues, green manures and animal manures and inorganic fertilizers to increase the pesticidal effect of the combined treatments [30,31,32,33]. Incorporation of these organic amended may act to reduce the number of soil-borne pests in soil by altering the composition of the inhabitant microbiota or of the soil physical environment. Using these materials along with solarization can sometimes extremely increase the microbicidal activity of the amendments. However, this appears to be an inconsistent phenomenon, and such outcomes should not be generalized before performing confirmatory research. The concentrations of volatile compounds (from the soil organic materials) have been shown to be significantly higher by applying solarization [32].

The addition of biocontrol agents to the soil prior, through or following the solarization process in order to gain enhanced and constant pesticidal efficacy has long been surveyed by many researchers. There have been considerable prospect of adding specific antagonistic and/or plant growth- encouragement microorganisms to the solarized soil in order to reassure a long-term disease-suppressive effect to thereafter planted crops [34].

The present investigation aimed to control Fusarium lupine root rot, under field conditions with no or low environmental impact; by using different control measures viz.: beneficial fungi, soil amendment and solarization singly or as an integrated disease management strategy.

# 2. Materials and Methods

## 2.1. Experimental Design

Trials were carried out over two years on three consecutive crops of lupine (local cultivar) at the Botanical garden of the Faculty of Science, University of Suez Canal at Ismailia city in Egypt, in a field naturally infested with the *F. solani*.

Infested field was divided into two plots ($12m^2$ each) in which both plots were further divided into six sections, each measuring 1 × 2.20 m (six solarized and six unsolarized). For both solarized and unsolarized plots, four were amended with dried powder of cabbage residue at a rate of $1kg^-$ / plot (Figure 1) and four were treated with antagonistic fungi (*Chaetomium, Gliocladium* & *Trichoderma*) at a rate of 750g sorghum sand inocula/plots were also considered out of the twelve plots.

### 2.1.1. Preparation of Antagonistic Fungi

To prepare inocula of antagonistic fungal candidates (*Chaetomium, Gliocladium* & *Trichoderma*), 500 g sorghum grains, 50 g sand and 90 ml tap water (media) were poured into conical flasks (1000 ml) mixed thoroughly and stopped by cotton plug. The flasks were then sterilized in autoclave for 30 min. at 121°C, on 2 consecutive days. Twenty mycelial agar discs (5 mm diameter) cut from a five-day-old PDA culture plate of antagonistic fungal candidates transferred into conical flasks and mixed with sorghum grains sand medium then incubated at 28°C for three weeks. After incubation the fungi colonized sorghum grains sand medium were air dried and stored in refrigerator at 4 C until use. Inocula of both dried powder of cabbage residue and air dried antagonistic fungal candidates were manually mixed thoroughly with the soil to a depth of 8 - 10 cm at a rate of $1kg^-$ / plot and 750g / plots respectively.

### 2.1.2. Soil Solarization and Soil Temperature

Out of twelve plots only six were solarized, mulching was carried out on soil moistened by irrigation to increase the thermal sensitivity of resting structures, and to improve heat conduction. Soil was tilled before mulching and covered with transparent polyethylene sheets (200 µm thick) in the summer months. The polyethylene remained intact for 28 days. Soil temperatures have been recorded daily with a soil thermometer in solarized and unsolarized (amended & unamended) plots.

### 2.1.3. Planting

Lupine (local cultivar) was sown then planted. The crop was seeded at 10 seeds per row, irrigated routinely and amended with inorganic fertilizer. The plots were planted at 20 cm apart from each other. After a growth period for 9 weeks under field conditions, diseased plants were sorted out and the incidence percentage of root rot was calculated.

**Disease Incidence percentage:**

$$\text{Disease Incidence } (\%) = \frac{\text{No. Infected plants}}{\text{Total No. plants}} \times 100.$$

## 2.2. Soil Analyses

To study the effects of soil solarization and cabbage residue amendments on the chemical characteristics of the soil, various analyses pertaining: pH, organic matter, total nitrogen, extractable phosphorous, calcium, potassium, magnesium and chloride were done by using standard methods according to. The pH value of fresh soil was determined potentiometrically in water (1: 2.5, soil: water, w/v) by using electronic pH meter, model HI 8014 Hanna Ins. Italy) [35].

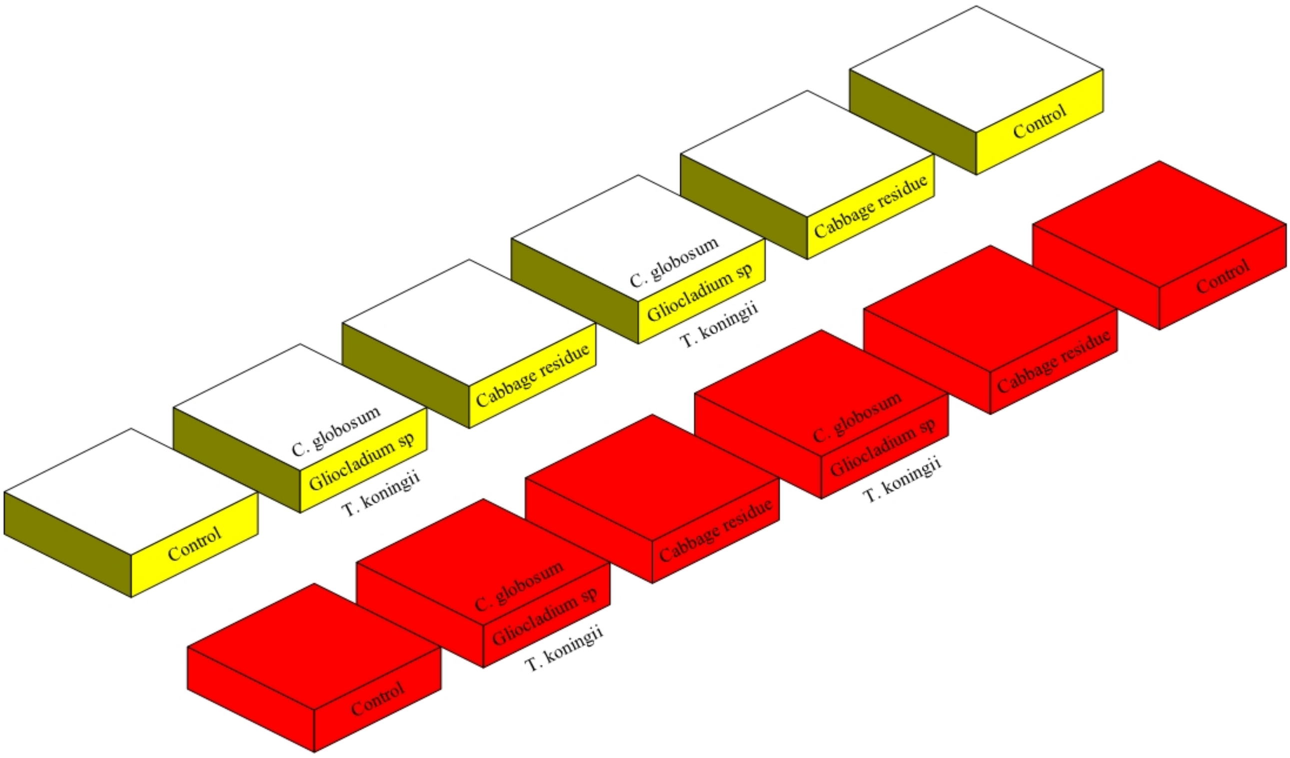


**Figure 1.** Experimental design showing different treatments

### 2.3. Data analysis

The data were subjected to Mann-Whitney (Nonparametric Tests) and statistically analyzed using two independent random samples employed to denote significant differences between treatments. Least significant differences were calculated at $P < 0.05$.

## 3. Results

### 3.1. Effect of Solarization and Amendment on Soil Temperature

The maximum soil temperatures were always higher in the solarized soil than unsolarized plots. In solarized soil, the maximum temperatures increased to 50°C, while in unsolarized plots it did not exceed 39°C. Mulching of moist soils accordingly raised the soil temperature and the highest temperature was in the upper layer at 5 cm depth (Figure 2). The differences in the maximum temperatures between solarized amended & unamended and non-solarized amended & unamended soils after four weeks were 11°C & 13°C and 12°C & 13°C at the 5 and 10 cm depth, respectively. Although temperature in amended soils (solarized & unsolarized) revealed temperature higher than that of corresponding unamended, three to four degrees, the difference was not significant.

### 3.2. Effect of Solarization on Root Rots Severity

The efficacy of solarization towards the lupine root rot severity was evaluated. While unsolarized soil (control) revealed only 41 % healthy plant, solarized soil showed 80 % healthy plants (Figure 3).

### 3.3. Effects of Antagonistic Fungi and Soil Amendment on Root Rot

In field soil, antagonistic fungi, and soil amendment with dried powder cabbage residues have been reduced the severity of lupine root rot when they added to unsolarized soil. The addition of conidial fungi, and cabbage residues without solarization resulted in comparable reduction in root rot severity (Figure 3). Where co-inoculum (fungal antagonists) showed 81 %, soil amendment with dried powder cabbage residues revealed 79 % in comparison with untreated soil (control) which revealed only 41%.

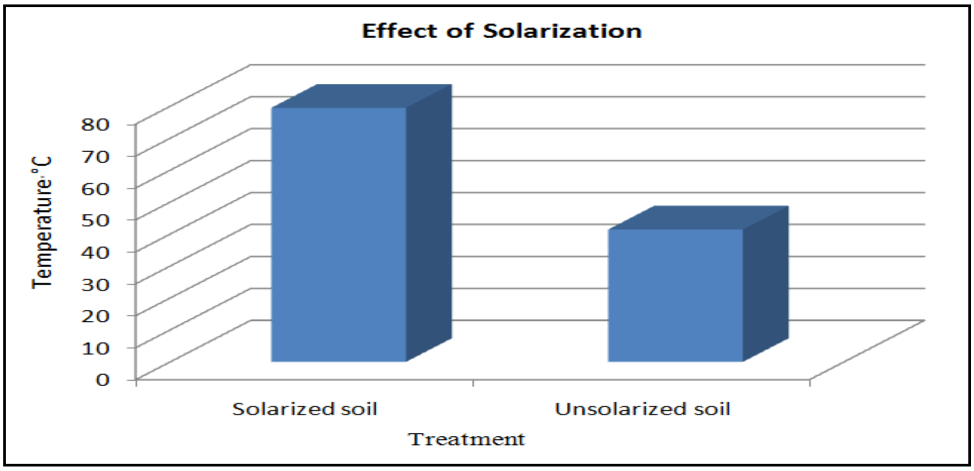


**Figure 2.** Soil temperature, in solarized and unsolarized (amended & unamended) plots in 5cm and 10cm depth

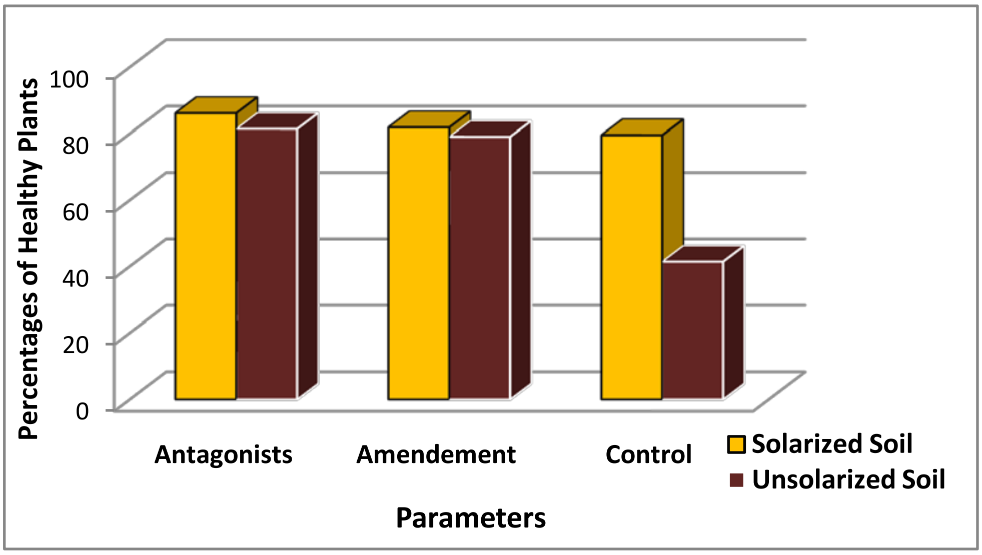


**Figure 3.** Effects of soil solarization, antagonistic fungi, and soil amendment on lupine root rot severity

**Table 1. Some chemical properties of solarized and unsolarized sandy loam field soil amended with organic materials**

| Parameter / Plot | pH | Ca (meq/l) | Mg (meq/l) | Na (meq/l) | Cl (meq/l) | (ppm) | P (ppm) | N (µg/kg) | OM% |
|---|---|---|---|---|---|---|---|---|---|
| Unsolarized | 7.95 | 2.3 | 0.8 | 11.3 | 15 | 2.1 | 480 | 390 | 1.36 |
| Unsolarized amended | 7.45 | 3.80 | 1.9 | 10.3 | 11.3 | 2.39 | 685 | 470 | 1.55 |
| Solarized | 7.80 | 2.40 | 1.2 | 10.85 | 10 | 2.35 | 595 | 437 | 1.75 |
| Solarized amended | 7.60 | 3.25 | 1.4 | 13.9 | 11.5 | 2.9 | 790 | 675 | 1.3 |

### 3.4. Efficacy of Integrated Soil Solarization with Antagonistic Fungi and Amendment on Root Rot Incidence

Soil solarization with or without the addition of fungal antagonists, and amendment with dried powder cabbage residues reduced the disease incidence significantly, whereas they showed infected plants only 14, 19 and 21 % respectively, as compared with 59 % for the unsolarized control (Figure 3). However integrating soil solarization with mixed fungal inocula, and soil organic amendment, have been improving efficacy of controlling of lupine root rot by increasing the percentage number of healthy plants from 81 %, 79 %, in treated unsolarized soil to 86 %, 82 %, respectively in treated solarized soil (Figure 3).

### 3.5. Effects of Solarization and Amendment on Chemical Properties of Field Soil

Organic materials (OM) contents variable but generally higher levels of plant nutrients, organic carbon, and salts than did field soil (Table 1). OM % was highest for the dried powder of cabbage residues amended soil in both solarized and unsolarized soils. Cabbage residues amended soil generally contained higher concentration of NPK and Mg than that of untreated soils in both solarized and unsolarized soil.

## 4. Discussion

The management of Fusarium root rot, which responsible for serious losses on a number of economically important crops including lupine termis (*L. termis*), is indeed being investigated, with special attention to the use of control measures with no hazard or low environmental impact [21,22,36,37,38,39,40]. The use of organic amendments (compost, animal manure, vegetable residues, and organic waste) on several soil-borne pathogens has received intensive research interest for long time [41,42]. Finding the most efficient strategy (single or integrated tactics) to control soil borne disease (more specifically, the Fusarium root rot) with no hazard or low environmental impact is the main goal of this research.

### 4.1. Single Control Measure

#### 4.1.1. Soil Solarization

By applying soil solarization as a single control approach our data revealed that while unsolarized soil showed only 41 % healthy plant, solarized soil accommodates 80 % healthy plants. This difference, in number of healthy plants, is highly significance implying that solarization highly effective in controlling faba bean Fusarium root rot. The efficacy of pesticidal activity of solarization is found to consequent from an integration of physical, chemical and biological effects, as described in several universal publications [25,43,44,45,46]. Our result is in constant with the outcomes obtained in Egypt as well as in other countries all over the world [27,46-53].

#### 4.1.2. Soil Organic Amendment

Organic amendments are often used to improve soil quality as they can change the general suppressiveness of soil, because of the enriched microbial activity**.** Application of organic matter might directly or indirectly change the progression of disease caused by soil-borne mould pathogens. The impact of organic amendments in the suppression of soil-borne diseases comprise stimulating specific microbial, biological and chemical changes, has been investigated in several pathosystems [21,22]. Organic amendments directly suppress pathogenic fungi like *Rhizoctonia*, *Sclerotinia*, and *Fusarium* [54], and impact the vulnerability of crops to phytopathogenic fungi [55]. Organic fertilizers indirectly decrease fungal disease by altering soil biological balance and enhance suppressive microorganisms such as mycorrhizal fungi, thermotolerant biocontrol agents such as *Trichoderma* spp., *Talaromyces flavus* and *Pseudomonas* spp. [26,56]. It is suggested that changes in the microbial community structure within the soil is related to the incidence of root rot [57].

Data of the field experiment revealed that the efficacy soil amendment by dried cabbage residues in controlling of lupine root rot by showing only 21 % diseased plant, while infested soil showing 59 % diseased plant. Regarding the beneficial effect organic amendment (i.e. dried cabbage residues in infested soil), result of several investigation came in agreement with our outcome. According to [58] in the light of the obtained results, it could be suggested that introducing composts to the soil is considered as potential strategy for biological control of cucumber root-rot pathogen and enhance the plant growth as well. [59] reported that rapeseed rotation caused shifts in the soil microbiota toward communities dominated by diversified bacterial groups hence promote control soilborne pathogens. [60] stated that the increase of organic matter in agriculture soils may impact both biological and chemical processes which may in turn support pathogen suppression. Results produced revealed that *Brassica* spp., in decomposition, release isothiocyanates (ITCs) which significantly suppressed the growth of fungal potato pathogens. [40] conclude that, the potential use of soil amendments in the management of *Fusarium* wilt of monoculture lettuce grown in short rotations: *B. carinata* (pellets and flour) and compost represent a good opportunity to integrate pest management programmes, with a positive impact on reducing the inoculum potential of the pathogen after two consecutive crop cycles. [61] demonstrated that, in controlled situation, undecomposed olive mill dry residue influenced the crop species growth (phytotoxic effect) and the phytopathogenic fungi (substrate effect).

Furthermore, studies carried out in different countries under different environmental conditions have shown the capability of different members of the *Brassica* species, such as *B. juncea*, *B. napus*, *B. nigra*, *B. oleracea*, *B. carinata*, *B. campestris* and *B. oleracea*, to control several soilborne pathogens [23,24,62,63]. The use of Brassicaceae in crop rotations or as green manure in biofumigation treatments has been found to reduce the incidence of some soilborne pathogens (including Fusarium root rot) through their release of isothiocyanates [64,65].

It's of worthy to mention that, the use of organic agricultural wastes in this regard can be an advantageous both in improving soil fertility, recycling of agricultural remains and could supply a constant tool for management of plant diseases.

## 4.2. Integrated Control

Practically it's possible to combine various biocontrol agents, either with each other, or with other control methods (e.g. agronomical, chemical, or physical) for the aim of obtaining a synergistic effect, rather than additive or antagonistic.

### 4.2.1. Combinations of Biocontrol Agents

The concept of using mixed inocula has been suggested by many investigators [14,28,29,66-72]. They recommended the application of two or more biocontrol agents that (i) are able to intensify antagonistic effect of each other, (ii) possess different antagonistic mechanisms, (iii) have different substrate preference, and (iv) are insensitive to secondary metabolites produced by pathogen. The idea behind this is based on the assumption that pairs of inocula will have an impact upon each other as well as the target pathogen. This impact can be positive (beneficial) or negative (harmful) i.e. the reaction between two inocula may be synergistic or antagonistic. Practically, mixtures of biocontrol agents with different plant colonization patterns may be advantageous for the biocontrol of diverse plant pathogens through different mechanisms of disease suppression. Furthermore, combinations of diversified organisms may promote the level and consistency of pest management by providing diverse mechanisms of action, a more stable rhizosphere community, and efficiency over a wider range of environmental conditions.

The efficacy of using mixed fungal inocula was tested in a field trial to assess its efficiency to control lupine-fusarium-root rot. The data of this experiment showed that the effectiveness of fungal antagonists in controlling *Fusarium* root rot by revealing 81 % healthy plant by comparing with control which accommodate only 41% this difference is highly significance. Such result is consistent with many investigations in Egypt and other countries [69,70] tested the potentiality of pair inocula of certain fungal taxa in controlling of tomato fusarium wilt, under field conditions, results showed three distinct types of pairs; synergistic pairs, in which the potentiality of the pairs is higher than that of the single inocula forming the pairs; antagonistic pairs, in which the potentiality of the pairs is less than that of the single inocula forming the pairs; neutralistic pairs, in which the potentiality of the pairs is equal to that of single inocula. [6,68] demonstrated that a synergistic impact can be gained in controlling *F.oxysporum* f.sp. *radicis lycopersici* by combining *Pseudomonas* spp. with avirulent *F. oxysporum.* According to a long-term field study was accomplished to test the impact of crop management on the abundance of actinomycetes, fungal antagonists *Trichoderma* spp., and *Gliocladium* spp., as prospective biocontrol agents (PBAs), and their relation with the incidence of peanut root rot caused by *F. solani* [73]. Data obtained suggest a likely role of PBAs in the management of *F. solani.* Also our result agreement with an array of finding all over the world [59,71,72,74,75,76,77,78].

### 4.2.2. Improving Disease Control Measure by Solarization

In particular, combinations of different antagonistic fungal agents, mycorrhizal fungi, and soil organic amendment with solarization may provide protection at different times or under varied conditions, and establish various or complementary niches. Such combinations may overcome disagreement in the efficacy of individual isolates [14]. By combining solarization with biofumigation and organic fertilizers, the reintroduction of biocontrol candidates like *Trichoderma* spp. and *Bacillus* spp. may be more effective than every processing alone in controlling soil-borne pests. Populations of these two microbial antagonists relatively abundant more than other microorganisms in solarized soil. Integrating organic amendments with soil solarization has been an effective method for management of phytopathogenic moulds, weeds and other pests [45,42,36].

Data of field experiment revealed that integrating soil solarization with mixed fungal inocula, and soil organic amendment, has been improving efficacy of controlling of lupine-fusarium-root rot by increasing the percentage number of healthy plants from 81 %, 79 % in treated unsolarized soil to 86 %, 82 % respectively in treated solarized soil. However, these differences statistically are not significant. Our result is in agreement with many outcomes [21,22,33,36,41,42,79,80,81].

In conclusion, the efficiently control soil-borne pests while avoiding environmental hazards and degradation of natural resources is the paramount challenge in crop protection sciences. Biocontrol agents, soil amendment and soil solarization are an additional tool for accomplishing this mission, when it is used in convenient conditions. The integration of pest management methods instead of depending on one potent control agent, is not only merely worthy but also the only practical solution for participate for controlling soil-borne pests. There are many challenges needed for the further development of integration of pest management methods: improvements in implementation technology and control effectiveness, and a better understanding of control mechanisms which can lead to more effective disturbance of pathogens' life cycles.

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