Evaluation the effect of ectomycorrhizal fungi on Prunus cerasifera x salicina (Rosaceae) growth compared with chemical and organic fertilizer

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Biofertilizer is considered as an alternative to chemical fertilizer to increase soil fertility and crop production in sustainable farming. The use of biofertilizer is steadily increased in agriculture and offers an attractive way to replace chemical fertilizers, pesticides, and supplements. The main objective of this study was to evaluate the effect of local ectomycorrhizal fungi isolated from the roots of some plants, on growth of Prunus cerasifera x salicina under greenhouse conditions. The impact of symbiotic fungus on the plant growth parameters was measured by comparing the inoculated plants, with control plants and plants treated with chemical fertilizer and compost. The fungus isolated from P. cerasifera (myrobalan) roots in PDA media, and pure culture was obtained. 50 plants were grown 10 for each parameter as follow: “10” seedlings planted in sterile soil without fungus and fertilizer (control); “10” seedlings planted in sterile soil was fertilized with the isolated fungus; “10” seedlings planted in sterile soil was fertilized with chemical fertilizers without fungus; “10” seedlings planted in sterile soil 50% and 50% compost; ”10” seedlings planted in 100% compost. Our results showed a positive influence of the ectomycorrhizal fungi on the growth parameters of P. cerasifera x salicina seedling compared with control, chemical fertilizer and compost, in all growth parameters. The different growth parameters were measured after incubation of plant seedlings in the green house for four months. We concluded that the use of ectomycorrhizal fungi gave positive influence on the growth of plant. According to these results, we strongly recommend the use of symbiotic fungi as total or partial substitute of other fertilizer.

Key words: Biofertilizer, ectomycorrhizal fungi, symbiotic fungi, Prunus cerasifera, myrobalan.

INTRODUCTION

Mycorrhization is a mutualistic association (non-pathogenic association) between soil borne fungi and the root of the higher plants. The term mycorrhiza (Gr. Mykes=Fungus or Mushroom; Rhiza=Root) i.e. “Fungal Root” was coined by the German Pathologist (Frank, 1885) to describe the union of two different beings to form a single morphological organ in which the plant nourishes the fungus and fungus does the same for the plant. Mycorrhiza confers many attributes to plants such as growth stimulation due to increased nutrient uptake, tolerance of plants to adverse conditions and bio-control of root disease (Molina et al., 1992). Between the seven types of mycorrhizae described (arbuscular, ecto,

ectendo-, arbutoid, monotropoid, ericoid and orchidaceous mycorrhizae), arbuscular mycorrhizae and ectomycorrhizae are the most abundant and widespread in forest communities (Rossi et al., 2007; Allen et al., 2003). Ectomycorrhizal (ECM) fungi are a more recently evolved association (approximately 125 million years ago) and despite their widespread distribution, associate with only 3% of vascular plant families (Rossi et al., 2007). Almost all ECM fungi belong to the Ascomycota and Basidiomycota phyla and the ECM mutualism is thought to have been derived several times independently from saprophytic lineages (Hibbett et al., 2000). The mycorrhizal symbiosis between plant roots and fungus is essential for the survival of both the partners (Harley and Smith, 1983). It has been suggested

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that this symbiosis was a prerequisite for the successful colonization of the terrestrial environment by plants some 400 million years ago (Pyronzinski and Mallock, 1975). The term mycorrhiza describes a range of symbiotic structures formed between the fine root and different fungi. An established ECM symbiosis is characterized by three structural components: the hyphal sheath or mantle, the Hartig net or intraradical mycelium, and the extraradical mycelium. The hyphal sheath or mantle encloses the root completely. The structural composition of the mantle is very diverse and can range from relatively thin, loosely arranged assemblages of hyphae to very thick, multilayered and pseudoparenchymatous mantles. The surface of the mantle can be compact and smooth or rough with numerous emerging hyphae and hyphal strands or rhizomorphs. The fungal sheath is involved in nutrient storage and controls the nutrient transfer to the host. The fungal mantle can represent a significant apoplastic barrier (Bücking et al., 2002). The Hartig net plays the key role in the nutrient transfer between both partners. The Hartig net is formed by hyphae that penetrate into the root cortex intercellularly. The penetration depth of the Hartig net differs between angiosperms and gymnosperms. Most angiosperms develop an epidermal Hartig net and confine the penetration of the Hartig net to the outer epidermis, which is often radially elongated. By contrast, the Hartig net in gymnosperms normally encloses several layers of cortical cells and sometimes extends up to the endodermis (Smith and Read, 2008).

The improvement of mycorrhizal plants' nutrition by enhancing N, P and potassium (K) uptake, among other nutrients, has been known for decades (Smith and Read, 2008). Mycorrhizal fungi have a competitive advantage in harvesting nutrients, since they do not need to compete as much for primary carbon reserves (Haselwandter et al., 2004). Some ECM fungi have the ability to access organic N and P pools by nutrient mobilization from natural organic substrate (Bending and Read, 1995, 1997; Pérez- Moreno and Read, 2000). ECM fungi can also obtain P by inducing dissolution of phosphate rock (Finlay and Rosling, 2006; Liu et al., 2005; Rosling, 2009). The often-extensive belowground ECM extramatrical mycelium networks can connect trees of the same or different species (Newman, 1988; He et al., 2006). ECM associations are important in plant water uptake, especially in areas with low water availability (Morte et al., 2001; Marjanovic et al., 2005; Smith and Read, 2008). Similarly, ECM associations can confer host plant tolerance to heavy metals and salt in areas exposed to salinization (Smith and Read, 2008). Mycorrhizas can protect host plants roots against pathogens (Fitter and Garbaye, 1994; Sen, 2001) and insect herbivory (Gehring and Whitham, 2002). The aim of this study was to evaluate the effect of ECM on Prunus cerasifera trees growth under greenhouse conditions.

MATERIALS AND METHODS

Fungi

The fungus used in this study was isolated from roots of Prunus cerasifera trees grown in private gardens.

Plants

One type of plants from the family (Rosaceae) was selected, P. cerasifera x salicina. This plant is widely cultivated in Gaza Strip.

Isolation of fungus

The fungi used in this study were isolated from roots of the genus Prunus found in private area in Jabalia city–Gaza strip, these areas are relatively distant from agricultural area where the chemical fertilizer is frequently used. Roots were taken out and prepared for the isolation of the fungus as follow: Roots were washed with running water; disinfected by different concentrations of sodium hypochlorite ranging from 2 to 10% for 1 to 5 min; washed with distilled water.

Culturing and multiplication of fungus

The short roots obtained from plants were subjected to the following treatments: Cutting to short pieces (2 - 3 cm); washed again with sterile water; the roots then cultured on MMN (modified melin-norkrans) media for 7 days; the fungus mycelia were sub-cultured for multiplication. All these steps took place in an axenic condition.

Identification of fungus

Our research aim was not to identify type of the fungus. However, to evaluate its influence on plant growth, the identification of the fungus was carried out by type's symbiosis ectomycorrhizal of fungi with the genus Prunus and compared it with our mycelia obtained from root (Tataranni, 2012).

Preparation of inoculum

The inoculum preparation was carried out according to Brundrett et al. (1999) as follow: Flasks containing fungal cultures grown in partially solidified nutrient solution (0.3% agar). These static cultures were periodically shaken to break up the mycelium into smaller segments. Flasks containing ECM fungi in semi-liquid culture (as in 1) were incubated with continuous agitation by a rotary shaker. Note that fungal mycelium has formed into large or small balls, which can be directly used as inoculum. Homogenizing mycelia from a culture with sterile water to
produce mycelial slurry inocula. Mycelial slurries, ready to inoculate Prunus seedlings in the greenhouse or nursery.

**Preparation of Prunus cerasifera x salicina seedling groups**

The seedlings of *Prunus cerasifera x salicina* obtained from Modern Agriculture Arboretum planted on sterilized normal soil inside pots (d=20 cm, h = 30 cm).

Five groups of seedlings were prepared: Control (C) 10 seedlings in sterilized normal soil without fungus; ectomycorrhizae fungi (SECM) 10 seedlings in sterilized normal soil treated by the suspension of the isolated ECM fungi; chemical fertilizer (CHF), 10 seedlings in sterilized normal soil treated by chemical fertilizer (14-14-14) 10 ml/L every two weeks through the experimental study period; 50% compost (COM 50), 10 seedlings in 50% sterilized normal soil mixed by 50% compost; 100% compost (COM 100), 10 seedlings in 100% compost.

**Inoculation with ECM**

The seedlings in second group (SECM) were inoculated with ectomycorrhizae fungi suspension one-time only.

All seedlings were incubated in the green house for 3 months (from November 2013 to March 2014).

**Statistical analysis**

Data were collected and computed by using version 18 of Statistical Package for Social Science (SPSS). One way ANOVA was the main statistical test used in our study.

**RESULTS**

We have confirmed that the isolated fungi from plant's root was *Hebeloma hiemale* that was used in our study. We had identified it according to morphological features of the *Prunus* tree symbiotic fungi, as external hyphae, mantle and Hartig net.

As shown in Figure 1 the growth of mycorrhized plants is better than those of control plants in all measures, and with clear statistical significance at P < 0.05.

As shown in Figure 2 the growth parameters of (CHF) plants are better than those of (C) plants in all measures except (RL), and with clear statistical significance.

As shown in Figure 3 the growth of (C) plants is significantly better than plants grown in (COM 50) in all measures except (SDW, RDW).

Figure 4 showed that the growth parameters of (C) plants were significantly better than those of (COM 100) plants in all measures except (SDW).

As shown in Figure 5 the growth parameters of (SECM) plants were better compared with the (CHF) treated plants.

As shown in Figure 6 the growth parameters of chemical fertilizer (CHF) treated plants were significantly better than those of 50% compost (COM 50) plants in all measures.

The growth parameters of (CHF) plants were better than those of (COM 100) plants in all measures with clear statistical significance (Figure 7).

The growth parameters of (SECM) plants were better than those of (COM 50) and (COM 100) plants in all measures, with clear statistical significance (Figures 8 and 9).

As shown in Figure 10, the compost used is not beneficial for plant growth, it showed poisonous effects on plants which led to death.

**Discussion**

Soil fungi are playing an essential role in equilibrium of ecosystem either by parasitic, symbiotic, saprophytic. Despite its negative role in causing a number of plant diseases, fungal positive effects are particularly important. Its symbiotic effect is considered as a main
Figure 2. Comparison between control (C) and chemical fertilizer (CHF) plants. Leaf number (LN); branch number (BN); stem length (SL); root length (RL); stem wet weight (SWW); root wet weight (RWW); stem dry weight (SDW); root dry weight (RDW).

Figure 3. Comparison between control (C) and 50% compost (COM 50) plants. Leaf number (LN); branch number (BN); stem length (SL); root length (RL); stem wet weight (SWW); root wet weight (RWW); stem dry weight (SDW); root dry weight (RDW).

Figure 4. Comparison between control (C) and 100% compost (COM 100) plants. Leaf number (LN); branch number (BN); stem length (SL); root length (RL); stem wet weight (SWW); root wet weight (RWW); stem dry weight (SDW); root dry weight (RDW).
source of mineral nutrients for a number of plants and trees. It is noteworthy to mention that symbiotic plants represent more than 95% of all plants (Rossi et al., 2007). Moreover, limited agricultural areas with intensive agriculture are particularly in need of such symbiotic organisms in order to limit the use of chemical fertilizers and reduce the ground water pollution. Gaza strip is a good example for such areas with agriculture representing a backbone for population life. In this regard, this study focused on using fungi isolated from the environment as a partial or complete alternative for chemical fertilizers. It may aid in reducing the consumption of these fertilizers and thus minimize the environmental and health burden on human life. The main aim of our study was to show the influence of locally isolated fungi from soil in Gaza Strip on the growth of local plants Prunus cerasifera x salicina by measuring different growth parameter such as (LN, BN, SL, RL, SWW, RWW and specially RDW, SDW). The second objective of our study was to compare the mycorrhizae on plant growth and the role of compost and chemical fertilizer by measuring the growth parameters of the plant. Biofertilizers will be the best solution to replace chemical fertilizers to overcome the harmful effects of chemical fertilizers and to maintain soil fertility and clean groundwater. Therefore, we carried out this research on an important plant, Prunus cerasifera x salicina.

By comparing the different variables of plant growth, the statistical analysis of the results has shown a real positive role of ECM on prunus plant growth compared to CHF, C, and COM 50 and COM 100 plants. These results agree with the results obtained by different researches (Pyasi et

Figure 5. Comparison between ectomycorrhizae fungi (SECM) and chemical fertilizer (CHF) treated plants. Leaf number (LN); branch number (BN); stem length (SL); root length (RL); stem wet weight (SWW); root wet weight (RWW); stem dry weight (SDW); root dry weight (RDW).

Figure 6. Comparison between chemical fertilizer (CHF) and 50% compost (COM 50) treated plant. Leaf number (LN); branch number (BN); stem length (SL); root length (RL); stem wet weight (SWW); root wet weight (RWW); stem dry weight (SDW); root dry weight (RDW).
Figure 7. Comparison between chemical fertilizer (CHF) and 100% compost (COM 100) treated plant. Leaf number (LN); branch number (BN); stem length (SL); root length (RL); stem wet weight (SWW); root wet weight (RWW); stem dry weight (SDW); root dry weight (RDW).

Figure 8. Comparison between ectomycorrhizal (SECM) and 50% compost (COM 50) treated plant. Leaf number (LN); branch number (BN); stem length (SL); root length (RL); stem wet weight (SWW); root wet weight (RWW); stem dry weight (SDW); root dry weight (RDW).
This can explain the role of these fungi in the plants nutrition by increasing the uptake of N, P, K from soil, and other benefits of ECM is the increase water absorption and other hand protection against pathogens. These results supported our main objective in this study.
which motivates us to encourage the farmers and the agricultural officials to begin using the biological fertilizer instead of the chemical fertilizer. These results are in concordance with most similar previous studies (Niemi et al., 2004).

The results have shown that the use of commercial compost were so bad on the plant growth and even lethal. No doubt, we have used high rate of compost (50 - 100%), which may be the reason of plant death, but this does not prevent the chemical analysis for compost in order to know its accurate composition.

The influence of chemical fertilizer in all parameters was better than control, except root length. This can be explained as the chemical fertilizer may contain nutrient elements that limited the root growth or because the nutrient availability around the plants root. These results are in concordance with most similar previous studies (Lyr and Hoffman, 1967).

The growth of control plants was higher than of (50 & 100%) compost plants with statistical clear significance, except RDW, SDW in 50% compost and RDW in 100% compost, because the compost keeps a lot of water than soil. This allows the plant to absorb a lot quantity of water. These results are in good agreement with different previous studies (El-Kichaoui et al., 1995; Niemi et al., 2004; Pyasi et al., 2013).

In general, we can say that using the biological fertilizer (fungi) was the better way for obtaining a good growth. Therefore, we can confirm that the use of ECM as fertilizer will be very useful for plant growth and environment health.

Conclusion

The present study investigated the influence of ECM fungus isolated from local soil on the growth of Prunus cerasifera x salicina plants in Gaza Strip. We have adopted to determine the effect of fungus on plant growth by comparing plants inoculated with C, CHF, COM 50 and COM 100 plants. The information that can be concluded from this study are:

i.) A net increasing of growth of Prunus cerasifera x salicina in the presence of ECM suspension when compared to CHF, C, COM 50 and COM 100 plants.

ii.) Our statistical analysis illustrated the clear difference in Prunus cerasifera x salicina growth parameters in the presence of chemical fertilizer or SECM than control plants.

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REFERENCES


