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In vivo evaluation of liquid polyphenols obtained from OMWW as natural bio-chemicals against several fungal pathogens on tomato plants

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ABSTRACT

Olive mill wastewater (OMWW) is the result of the production of olive oil causing severe water pollution. In recent years, distinguished growing interest in isolation, testing, and utilization of agricultural waste or other sources of plant tissues rich in polyphenols has been arised. In this study, the evaluation of liquid polyphenols obtained from OMWW as natural bio-chemicals against several fungal pathogens on tomato plants was examined. Different concentrations of liquid formed polyphenol (LFP) at 5, 10, 20, and 30% were tested against 10 fungal pathogens causing plant diseases on tomato plants. After 40 d of application, tomato plants were harvested and plant height, number of flowers, plant fresh weight, root fresh weight, dry plant weight and dry root weight were calculated and LFP has presented encouraging results in protecting plants. Furthermore, the encapsulation of a polyphenol in encapsulating agent may help to slow down the degradation and protection from the frequent watering and runoff due to this action. Therefore, in order of major protection resulting from the use of polyphenol against major diseases classified the following phytopathogenic fungi: *Botrytis cinerea*, *Sclerotinia sclerotiorum*, and *Ascochyta lentis*.

Keywords: Polyphenols; Biocontrol; Plant diseases; OMWW

1. Introduction

Olive mill wastewater (OMWW) is responsible for the severe environmental impact of wastewater mills [1,2]. However, the total amount of the antioxidants in olive oil is only 1–2% and the rest of them are lost as waste (about 53%) [3]. Several phenolic compounds contained in OMWW are in particular interest since some of them have antioxidant effects preventing the degradation of fatty acids to glycerides [4,5]. Phenolic compounds are also being found in plant tissues [6] or in agricultural products such as olive oil.

Plants produce natural metabolites such as polyphenols, that linked phytotoxic and antimicrobial properties and inhibited microbial growth [2,7–15].

Nowadays, alternative, environmentally friendly methods of combating pests and diseases of plants are required [16] to reduce the impact of specialty chemicals to human health and the environment while several attempts to solve the environmental problem of disposal of OMWW is of great interest, especially in Mediterranean countries, such as Greece, Spain, and Italy [17].

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The purpose of this research work is the evaluation of liquid polyphenols obtained from OMWW as natural bio-chemicals against several fungal pathogens on tomato plants.

2. Materials and methods

Ascochyta lentis, *Verticillium dahliae*, *Sclerotinia sclerotiorum*, *Penicillium italicum*, *Aspergillus niger*, *Botrytis cinerea*, *Penicillium expansum*, *Rhizoctonia solani*, *Alternaria alternata*, *Fusarium oxysporum* f.sp. *lycopersici* obtained from Benaki Phytopathological Institute (BFI), were used for the evaluation of the antimicrobial activity of the examined polyphenolic compound. Different concentrations of liquid formed polyphenol (LFP) at 5, 10, 20, and 30% were applied in 40-d-old tomato plants, variety Majeo S1.

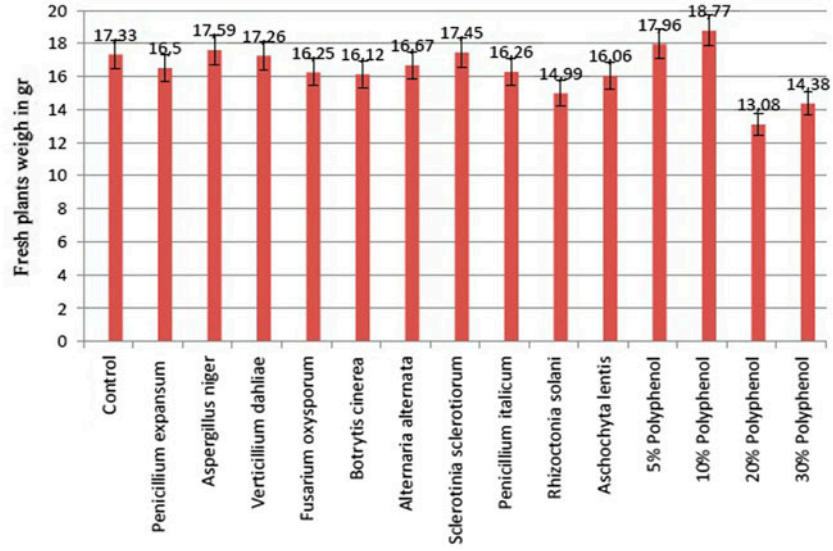

Fig. 1. Effect of 5% LFP on fresh plant weight of tomato plants infected with different fungal pathogens. Observation of phytotoxic effects of LFP. Fresh plant weight expressed in grams.

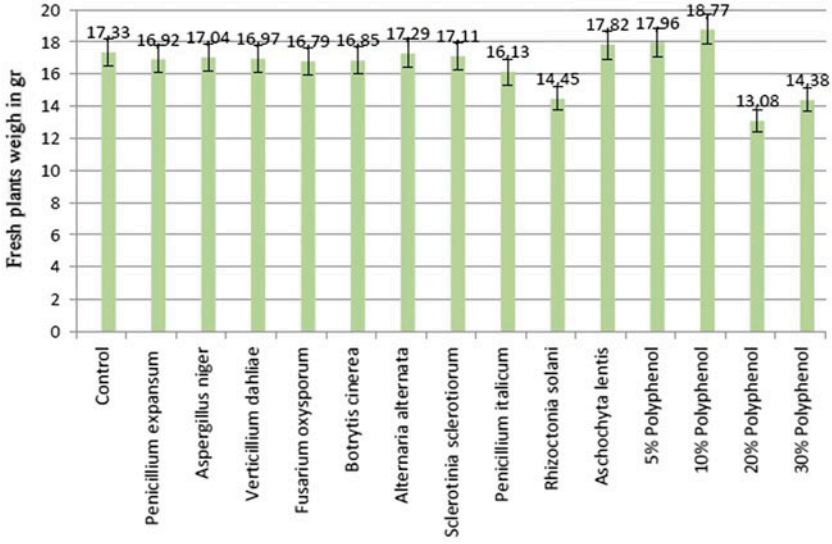

Fig. 2. Effect of 10% LFP on fresh plant weight of tomato plants infected with different fungal pathogens. Observation of phytotoxic effects of LFP. Fresh plant weight expressed in grams.

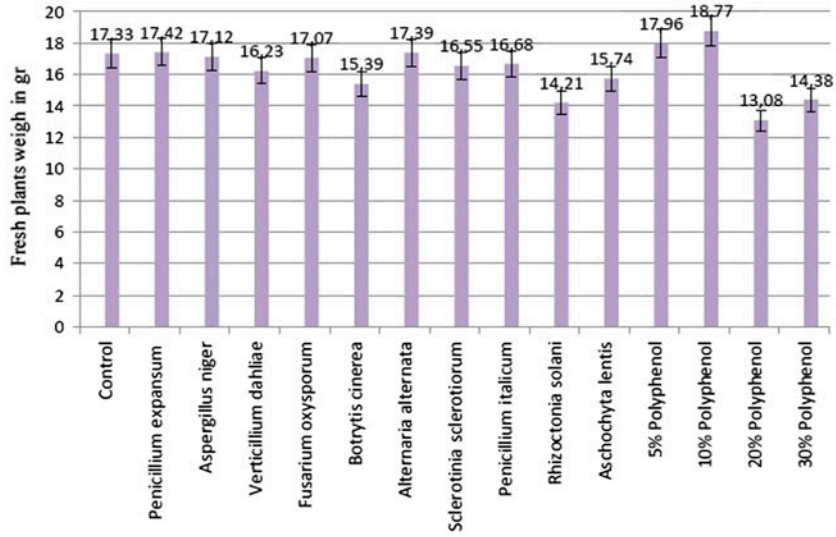

Fig. 3. Effect of 20% LFP on fresh plant weight of tomato plants infected with different fungal pathogens. Observation of phytotoxic effects of LFP. Fresh plant weight expressed in grams.

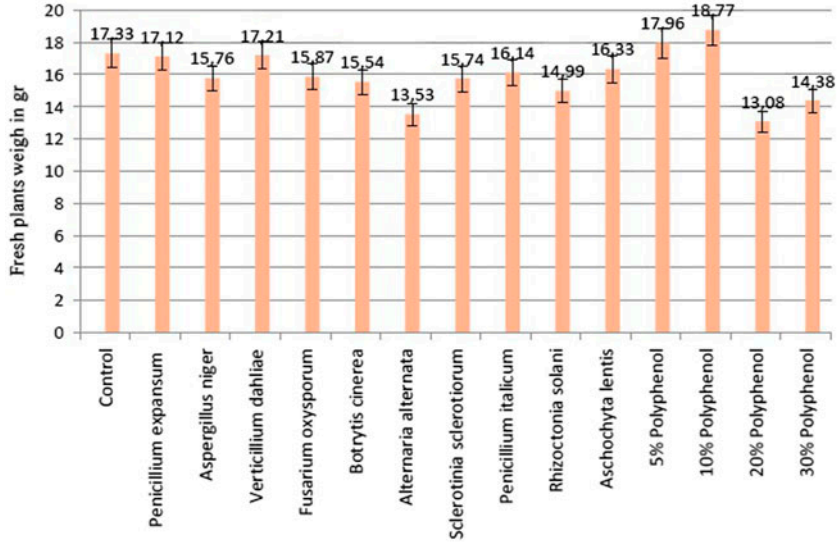

Fig. 4. Effect of 30% LFP on fresh plant weight of tomato plants infected with different fungal pathogens. Observation of phytotoxic effects of LFP. Fresh plant weight expressed in grams.

2.1. Preparation of the fungal pathogens

The microorganisms used in this scientific work were grown on medium PDA. The final spore suspension was counted using a haemocytometer and dilution of the spore suspension was applied where necessary. Before plant inoculation, the spore suspension was placed in the refrigerator at 3°C for about one week.

2.2. Preparation of plants-inoculums application

Majer S1 variety of 40-d-old tomato hybrids were used to ascertain the effectiveness of the antimicrobial activity of different concentrations of LFP against the examined fungal pathogens.

The plants were transplanted in disposable plastic pots which were filled with a mixture of high peat Potgrad P, suitable for the propagation of horticultural

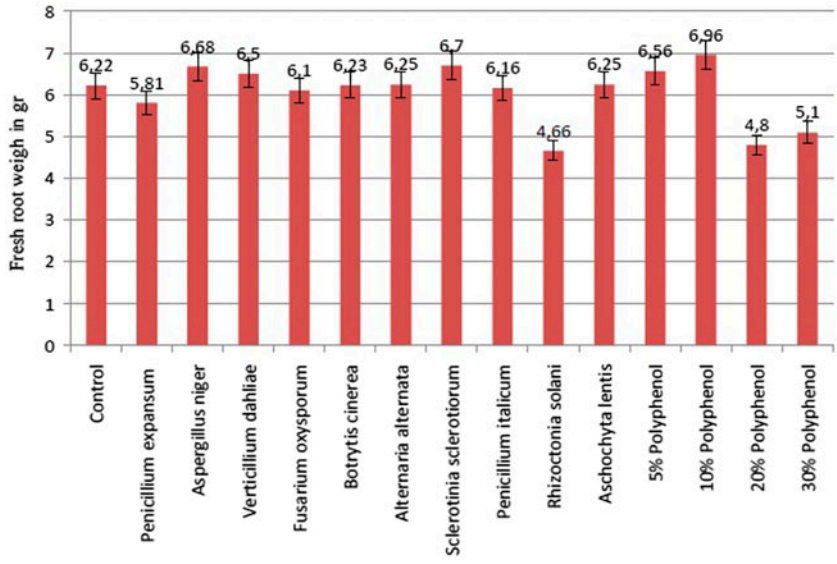

Fig. 5. Effect of 5% LFP on fresh root weight of tomato plants infected with different fungal pathogens. Observation of phytotoxic effects of LFP. Fresh root weight expressed in grams.

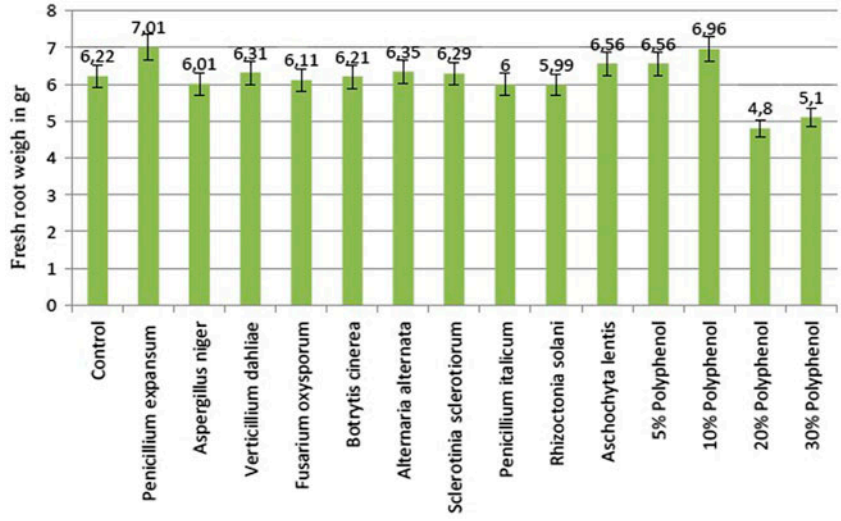

Fig. 6. Effect of 10% LFP on fresh root weight of tomato plants infected with different fungal pathogens. Observation of phytotoxic effects of LFP. Fresh root weight expressed in grams.

seedling obtained from Company Klassman-Dolmann GmbH Germany as imported in Greek market from AGROCHOUM SA. The pH of the soil was 5.5–6.5, the conductivity was 45 mS/m and the organic matter was 90–95%.

To avoid dryness, the transplanted plants were watered thoroughly and after two days a total amount of 1 ml of the fungal spore suspension and the different concentrations of LFP were applied by making a hole near the root system. Five plants were used in each treatment.

Then, the plants were placed in the greenhouse and were irrigated daily with automatic flushing for 30 min. To reduce photoperiod matter, special artificial lighting lamps were used extending the daytime for 4 h.

After 40 d of application, plants were harvested and the following measurements were taken: plant

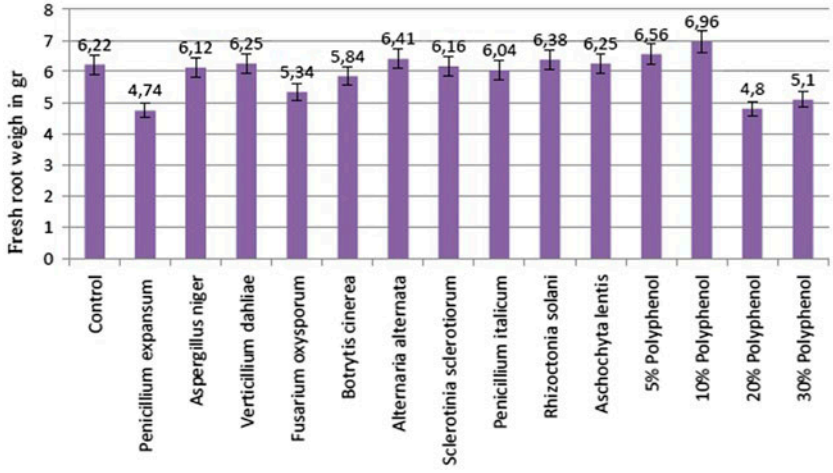

Fig. 7. Effect of 20% LFP on fresh root weight of tomato plants infected with different fungal pathogens. Observation of phytotoxic effects of LFP. Fresh root weight expressed in grams.

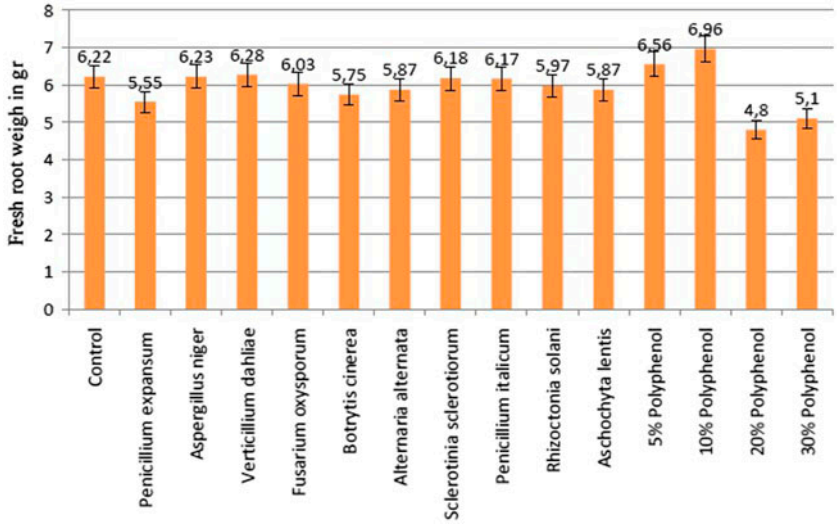

Fig. 8. Effect of 30% LFP on fresh root weight of tomato plants infected with different fungal pathogens. Observation of phytotoxic effects of LFP. Fresh root weight expressed in grams.

height, number of flowers, plant fresh weight, root fresh weight, dry plant weight, and dry root weight. For the determination of dry plant weight and dry root weight, the examined plants were placed in paper bags and dried in a drying oven at 60°C for 24 h.

3. Results

3.1. Effect of LFP on fresh root weight

From Figs. 1–8, it was observed that the use of LFP at 5, 10, 20, and 30% concentration prevented the appearance of the symptoms of infection in most of the infected plants. However, there was statistically important difference between the treatment of the fungus *R. solani* and all the other treatments including control while LFP at 5–10% concentration was not sufficient to control infection of *R. solani*. Moreover, in graph three, it was observed that LFP at a concentration of 20% was also not sufficient to control *B. cinerea* and *R. solani* infection. Also, in graph four it was observed that LFP at a concentration of 30% was not sufficient to control *A. alternata* and *R. solani* infection. Furthermore, it was observed in graph six that on treatment with 10% of LFP + *P. expansum*, the fresh root weight was higher even than those of the control.

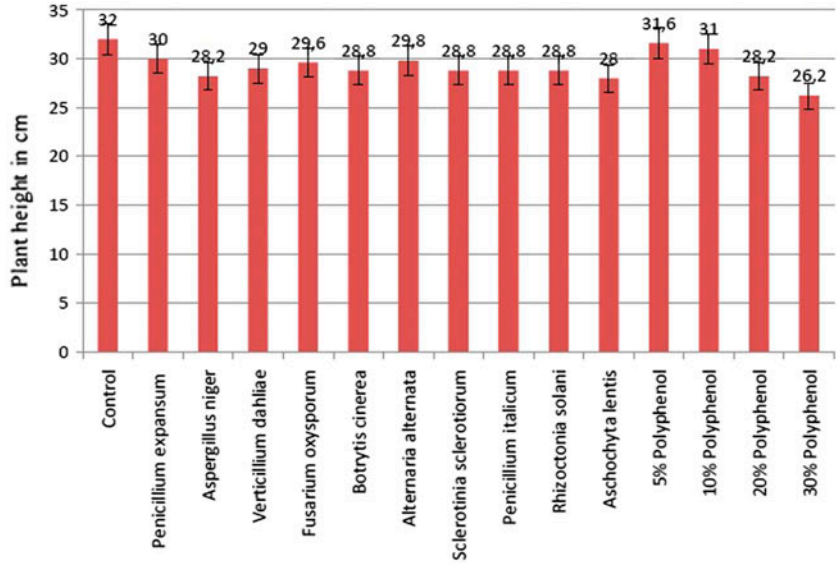

Fig. 9. Effect of 5% LFP on height of tomato plants infected with different fungal pathogens. Observation of phytotoxic effects of LFP. Height expressed in cm.

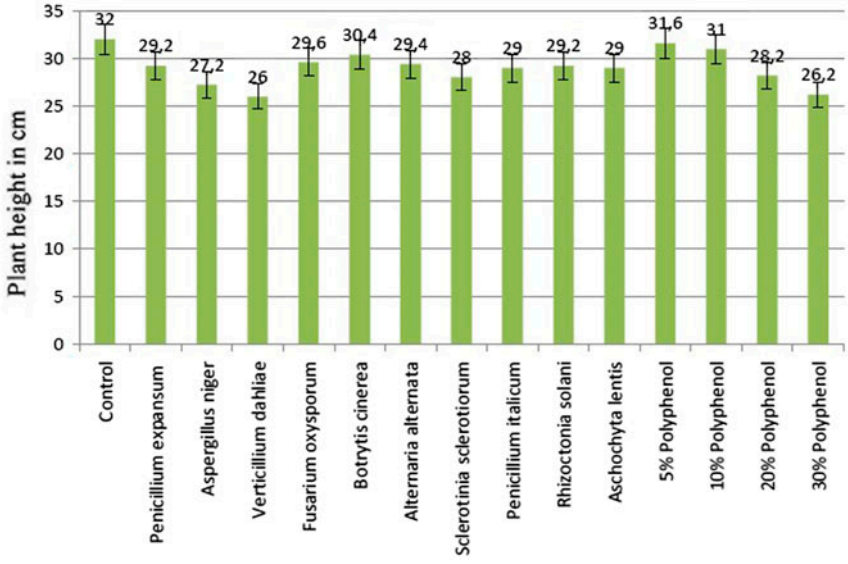

Fig. 10. Effect of 10% LFP on height of tomato plants infected with different fungal pathogens. Observation of phytotoxic effects of LFP. Height expressed in cm.

From the results presented in graph seven, it was observed that the 20% concentration of LFP was not sufficient to control infections by *P. expansum* and *F. oxysporum* while in graph 8, 30% of LFP did not control *P. expansum* infection. Also, it was observed that when LFP was applied at 20–30% concentration, potential phytotoxical symptoms appeared. Furthermore, the use of LFP at 5–10% concentration favored the growth of plants at levels even higher than those of the control, while the use of LFP at 10% concentration favored the growth of roots.

3.2. Effect of LFP on plant height

From Fig. 9, it was observed that the use of LFP at 5% concentration did not prevent the appearance of the symptoms of infection in some of the treatments such as *P. expansum*, *F. oxysporum*, and *A. alternata*.

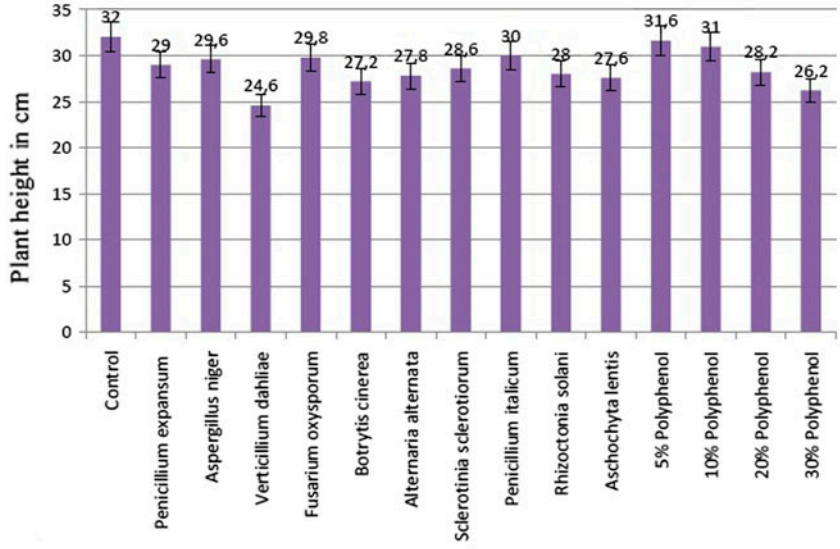

Fig. 11. Effect of 20% LFP on height of tomato plants infected with different fungal pathogens. Observation of phytotoxic effects of LFP. Height expressed in cm.

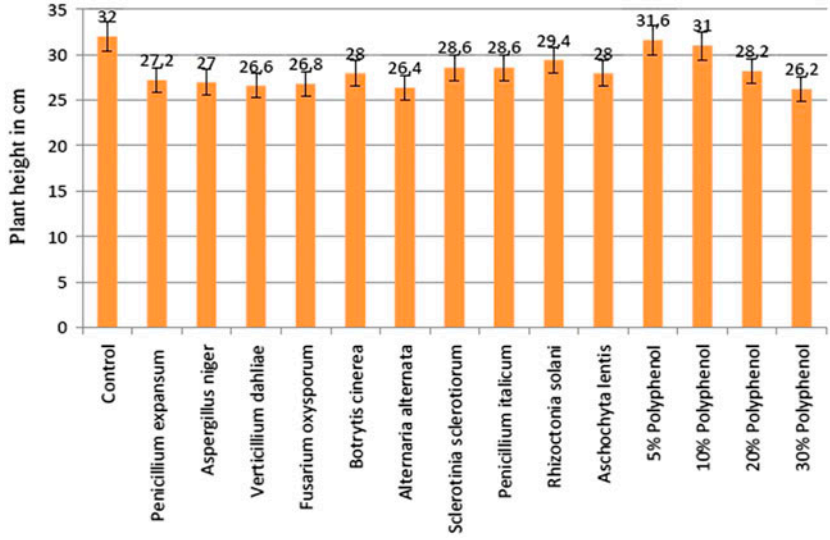

Fig. 12. Effect of 30% LFP on height of tomato plants infected with different fungal pathogens. Observation of phytotoxic effects of LFP. Height expressed in cm.

However, there was statistically important difference between control and treatments infected with *A. niger*, *V. dahliae*, *B. cinerea*, *S. sclerotiorum*, *P. italicum*, *R. solani*, and *A. lentis*.

Furthermore, LFP at 10% concentration (Fig. 10) did not prevent the appearance of the symptoms from pathogens such as *P. expansum*, *F. oxysporum*, *B. cinerea*, *A. alternata*, and *R. solani*. However, there was statistically important difference between control and treatments infected with *A. niger*, *V. dahliae*, *S. sclerotiorum*, *P. italicum*, and *A. lentis*.

Moreover, LFP at 20% concentration (Fig. 11) did not prevent the appearance of the symptoms from pathogens such as *P. expansum*, *A. niger*, *F. oxysporum*, and *P. italicum*. However, there was statistically important difference between control and treatments infected with *V. dahliae*, *B. cinerea*, *A. alternata*, *S. sclerotiorum*, *R. solani*, and *A. lentis*.

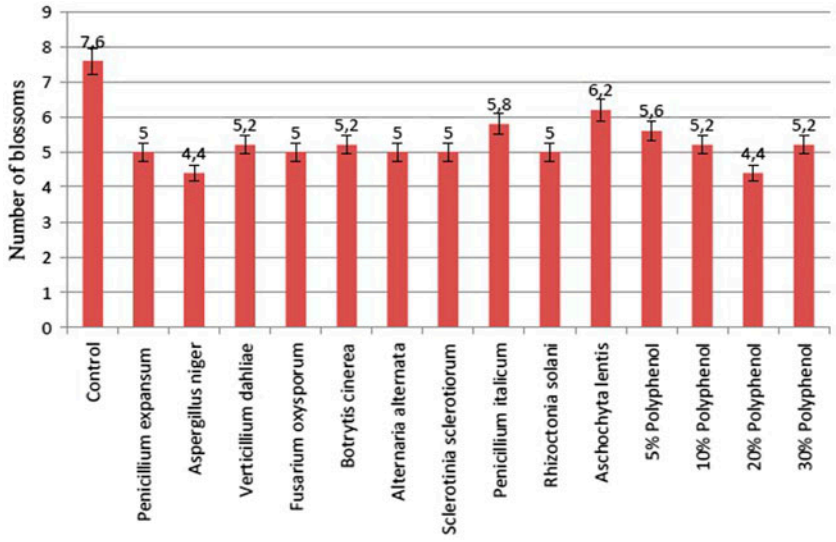

Fig. 13. Effect of 5% LFP on number of blossoms of tomato plants infected with different fungal pathogens. Observation of phytotoxic effects of LFP.

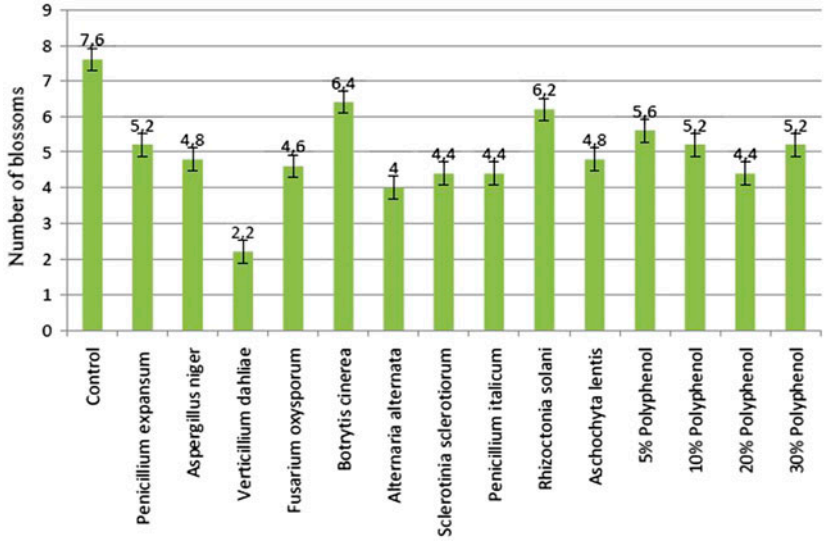

Fig. 14. Effect of 10% LFP on number of blossoms of tomato plants infected with different fungal pathogens. Observation of phytotoxic effects of LFP.

Also, LFP at 30% concentration (Fig. 12) did not prevent the appearance of the symptoms from pathogens such as *P. expansum*, *A. niger*, *V. dahliae*, *F. oxysporum*, *B. cinerea*, *A. alternata*, *S. sclerotiorum*, *A. lentis*, and *P. italicum*. However, there was statistically important difference between control and treatment infected with *R. solani*.

Finally, it was observed that when LFP was applied at 20–30% concentration, potential phytotoxical symptoms appeared.

3.3. Effect of LFP on number of blossoms

From Fig. 13, it was observed that the use of LFP at 5, 10, and 20% concentration did not prevent the appearance of the symptoms of infection showing that control treatment differs statistically from all other treatments. In 5% concentration of LFP, the lower number of blossoms was observed in treatment inoculated with *A. niger* and in 20% LFP showing potential phytotoxical effects. However, the use of 5% LFP in

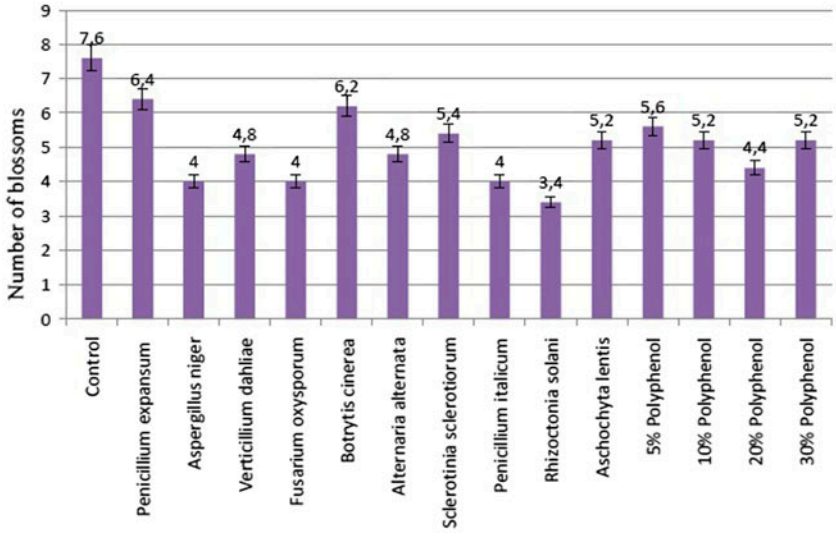

Fig. 15. Effect of 20% LFP on number of blossoms of tomato plants infected with different fungal pathogens. Observation of phytotoxic effects of LFP.

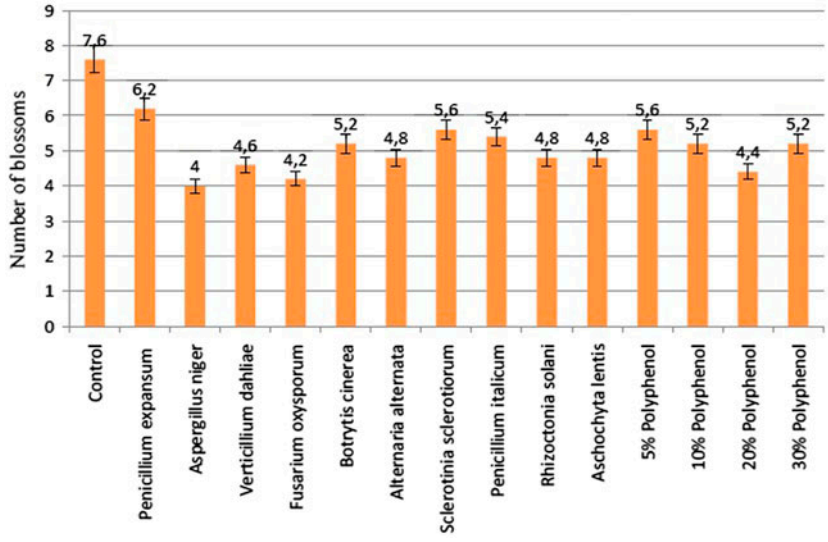

Fig. 16. Effect of 30% LFP on number of blossoms of tomato plants infected with different fungal pathogens. Observation of phytotoxic effects of LFP.

treatments infected with *A. lentis* and *P. italicum* suppresses damage from fungi as expressed in number of blossoms.

Furthermore, at 10% concentration of LFP (Fig. 14), the lower number of blossoms was observed in treatment inoculated with *V. dahliae* and *A. alternata*. However, the use of 10% LFP in treatments infected with *B. cinerea* and *R. solani* suppresses damage from fungi as expressed in number of blossoms.

Moreover, at 20% concentration of LFP (Fig. 15), the lower number of blossoms was observed in treatment inoculated with *R. solani*, *A. niger*, *F. oxysporum*, and *P. italicum*. However, the use of 20% LFP in treatments infected with *B. cinerea* and *P. expansum* suppresses damage from fungi as expressed in number of blossoms.

Finally, at 30% concentration of LFP (Fig. 16) a lower number of blossoms was observed in treatment

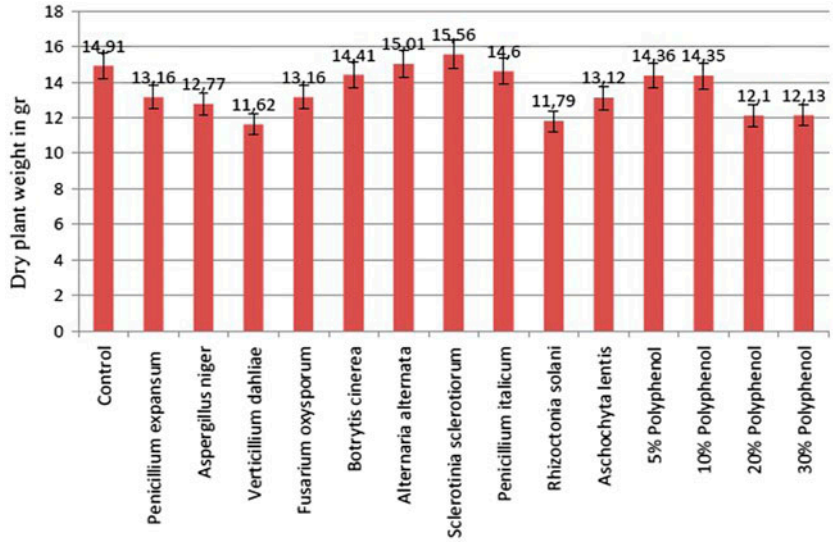

Fig. 17. Effect of 5% LFP on number of dry plant weight of tomato plants infected with different fungal pathogens. Observation of phytotoxic effects of LFP.

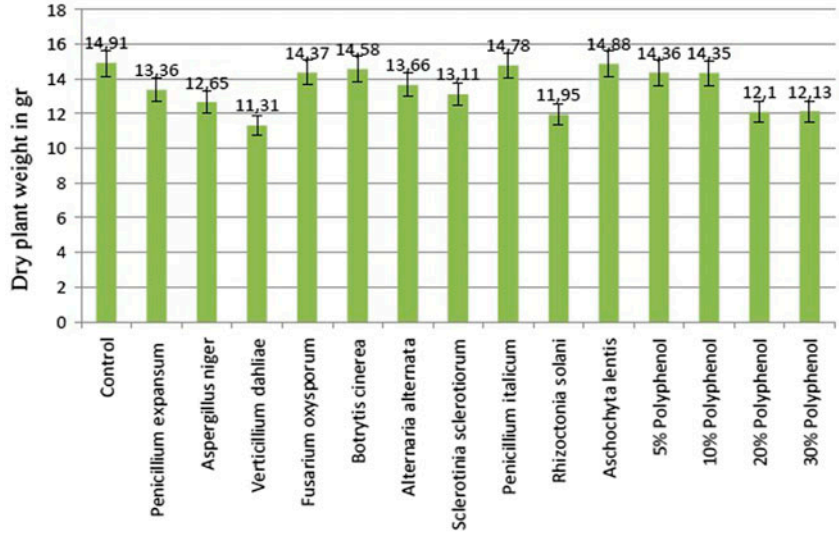

Fig. 18. Effect of 10% LFP on number of dry plant weight of tomato plants infected with different fungal pathogens. Observation of phytotoxic effects of LFP.

inoculated with *A. niger*, *F. oxysporum*, and *V. dahliae*. However, the use of 30% LFP in treatment infected with *P. expansum*, suppresses damage from fungus as expressed in number of blossoms.

3.4. Effect of LFP on dry plant weight

From Fig. 17, it was observed that the use of LFP at 5% concentration prevented the appearance of the symptoms of infection in some of the treatments such as *B. cinerea*, *A. alternata*, *S. sclerotiorum*, and *P. italicum*. However, there was statistically important difference between control and treated plants infected with *P. expansum*, *A. niger*, *V. dahliae*, *F. oxysporum*, *R. solani*, and *A. lentis*.

Furthermore, in Fig. 18 it was observed that the use of LFP at 10% concentration did not prevent the appearance of the symptoms of infection in some of

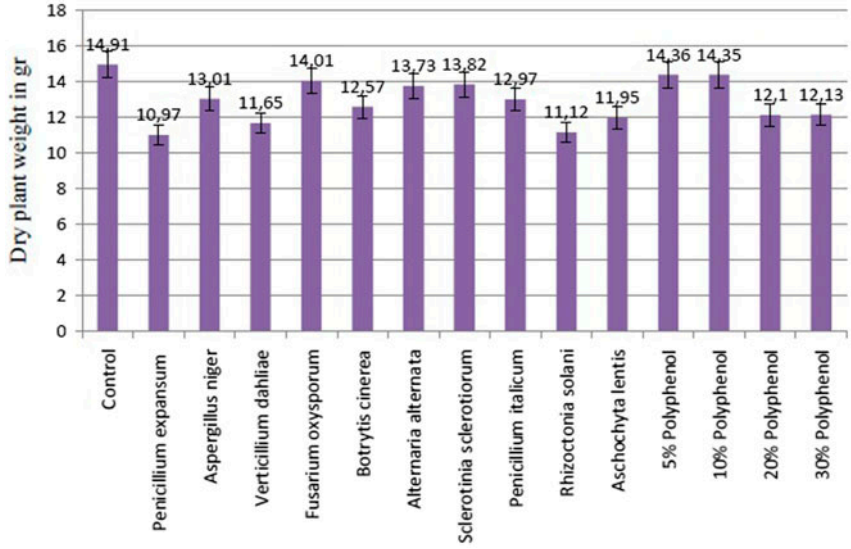

Fig. 19. Effect of 20% LFP on number of dry plant weight of tomato plants infected with different fungal pathogens. Observation of phytotoxic effects of LFP.

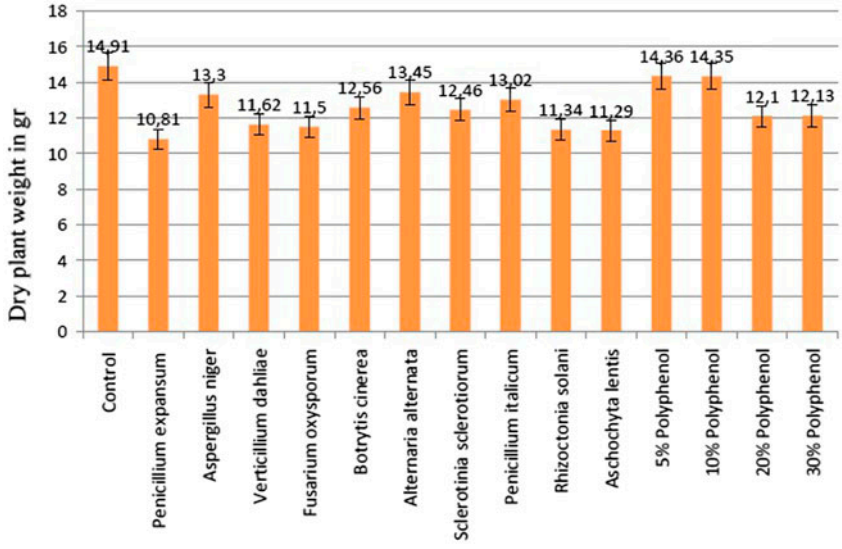

Fig. 20. Effect of 30% LFP on number of dry plant weight of tomato plants infected with different fungal pathogens. Observation of phytotoxic effects of LFP.

the treatments such as *P. expansum*, *A. niger*, *V. dahliae*, *S. sclerotiorum*, and *R. solani.* However, there was no statistically important difference between control and treatments infected with *F. oxysporum*, *B. cinerea*, *A. alternata*, *P. italicum,* and *A. lentis.*

Moreover, in Fig. 19 it was observed that the use of LFP at 20% concentration did not prevent the appearance of the symptoms of infection in some of the treatments such as *P. expansum*, *A. niger*, *V. dahliae*, *B. cinerea*, *P. italicum*, *R. solani,* and *Ascocyta lentis.* However, there was no statistically important difference between control and treatments infected with *F. oxysporum*, *A. alternata,* and *S. sclerotiorum.*

Also, in Fig. 20 it was observed that the use of LFP at 30% concentration did not prevent the appearance of the symptoms of infection in all the treatments because of the possible phytotoxic effect.

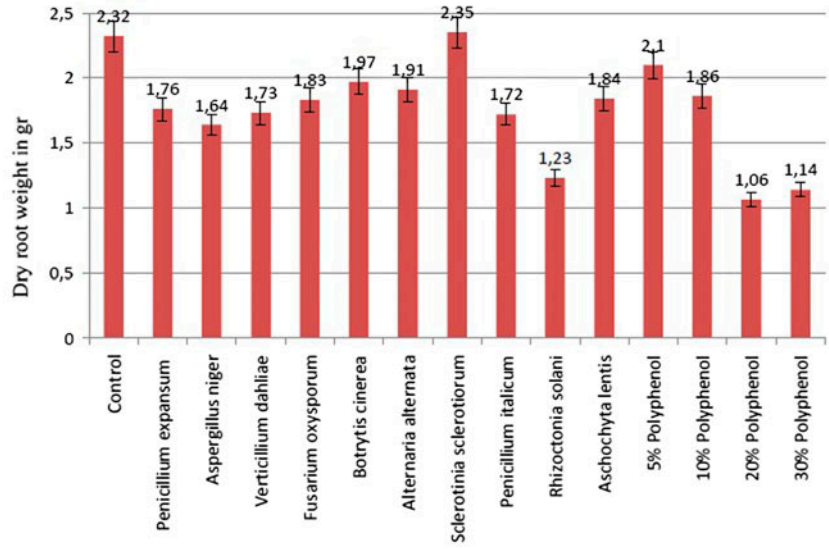

Fig. 21. Effect of 5% LFP on number of dry root weight of tomato plants infected with different fungal pathogens. Observation of phytotoxic effects of LFP.

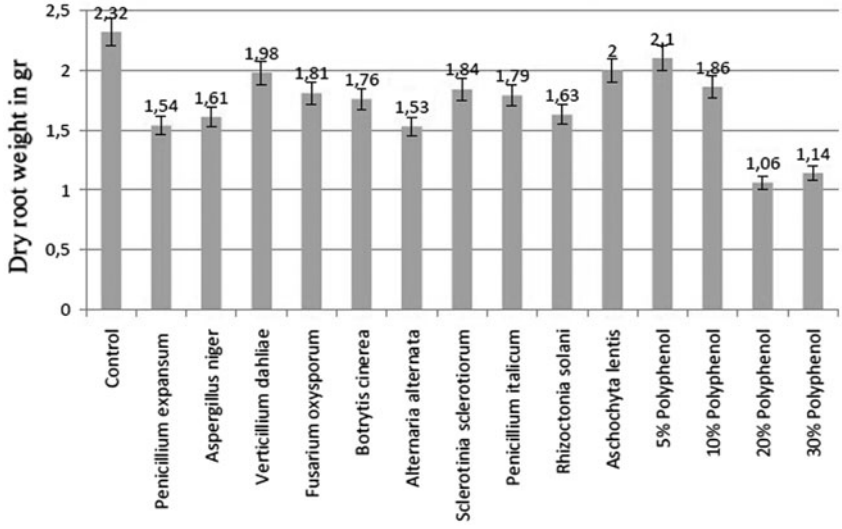

Fig. 22. Effect of 10% LFP on number of dry root weight of tomato plants infected with different fungal pathogens. Observation of phytotoxic effects of LFP.

Finally, it was observed that when LFP was applied at 20–30% concentration, potential phytotoxical symptoms appeared while concentrations of 5–10% LFP did not differ statistically from the control.

3.5. Effect of LFP on dry root weight

From Fig. 21, it was observed that the use of LFP at 5% concentration prevented the appearance of the symptoms of infection from *S. sclerotiorum*. However, there was statistically important difference between control and treated plants infected with *B. cinerea*, *A. alternata*, *P. italicum*, *P. expansum*, *A. niger*, *V. dahliae*, *F. oxysporum*, *R. solani*, and *A. lentis*. The lower dry root weight was observed in the treatment inoculated with the fungus *R. solani*.

The use of LFP at 10% concentration (Fig. 22) did not prevent the appearance of the symptoms of infection in treated plants while control differs statistically from all the other treatments having the heavier dry

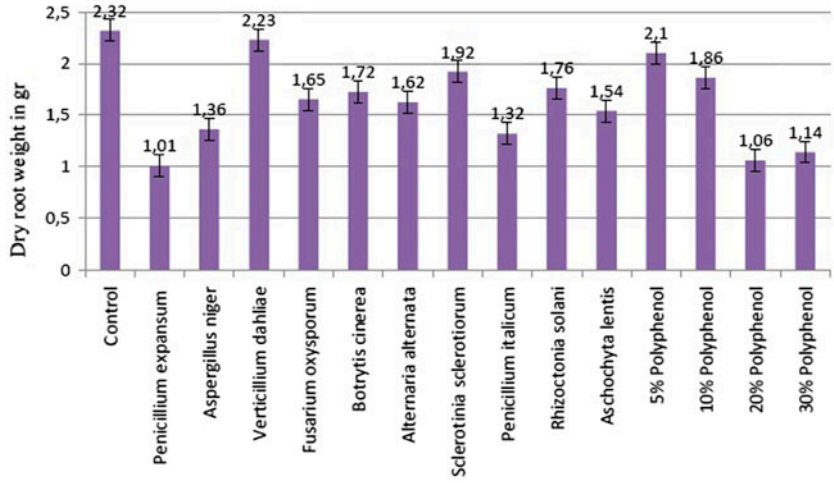

Fig. 23. Effect of 20% LFP on number of dry root weight of tomato plants infected with different fungal pathogens. Observation of phytotoxic effects of LFP.

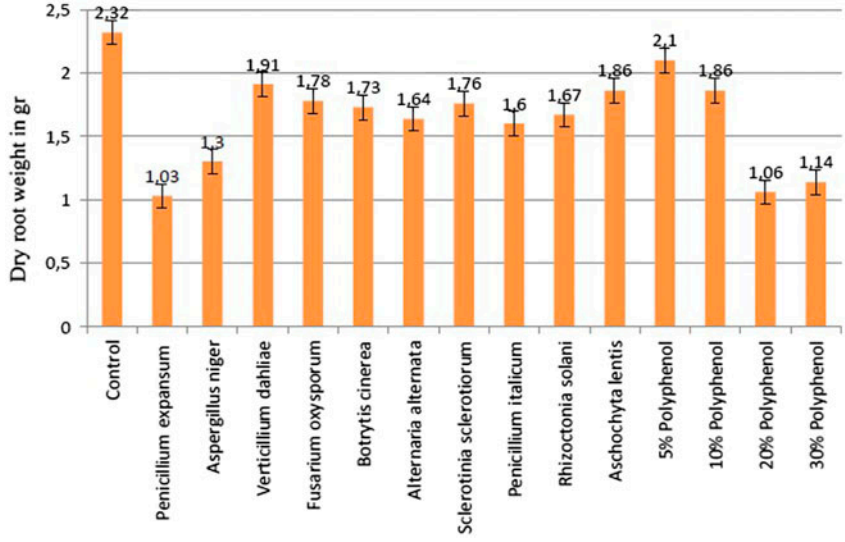

Fig. 24. Effect of 30% LFP on number of dry root weight of tomato plants infected with different fungal pathogens. Observation of phytotoxic effects of LFP.

root weight. The lower dry root weight was observed in the treatments inoculated with the fungi *P. expansum* and *A. alternata*.

Furthermore, the use of LFP at 20% concentration (Fig. 23) did not prevent the appearance of the symptoms of infection in treated plants while control differs statistically from all the others besides the treatment inoculated with *V. dahliae*. The lower dry root weight was observed in the treatments inoculated with the fungi *P. expansum*, *P. italicum*, and *A. niger*.

Also, the use of LFP at 30% concentration (Fig. 24) did not prevent the appearance of the symptoms of infection in treated plants while control differs statistically from all the other treatments. The lower dry root weight was observed in the treatments inoculated with the fungi *P. expansum* and *A. niger*.

Finally, it was observed that when LFP applied at 20–30% concentration, potential phytotoxical symptoms appeared in plant growth.

4. Discussion and conclusions

In recent years, distinguished growing interest in isolation, testing and utilization of agricultural waste or other sources of plant tissues rich in polyphenols, such as tissues of species *Olea europaea*, *Prunus amygdalus*, *Stevia rebaudiana*, etc. and their waste, such as OMWW has been arised [18–24]. Therefore, the use of these by-products which are ideal as wastes may offer new products with high added value, also reducing environmental risks and pollution levels if they were discharged into the environment.

The use of low concentration of LFP at 5–10% could control, in some cases, fungal pathogens. However, with a higher concentration of LFP (20–30%) appeared possible phytotoxic effects. The number of blossoms is a very important factor since productivity is depending of them. However, poor root and stem growth are also important factors since fruit development is depending also on water and nutrient absorption from the soil. Therefore, all measured parts of the plant are important. It was observed that LFP controlled the growth of fungi such as *B. cinerea* and *S. sclerotiorum*. However, fungi such as *R. solani*, *P. expansum*, and *A. niger* showed affected plant growth mainly due to their rapid mycelium growth, the large number of produced spores and the rapid dissemination into the tissues of the host.

Additionally, in many measurements, the development of plant tissues was favored when the soil contained small amount of LFP at 5–10%. This action could show a possible use of LFP as natural component affecting plant growth in a similar way as fertilizer. However, the effectiveness of LFP against plant and fungi was not clear in many cases likely due to external factors such as air temperature and photoperiod affecting plant growth.

Plant pathogenic fungi such as *F. oxysporum* and *V. dahliae* requires longer period of symptoms appearance from first infection due to their development within the plant vascular tissues. For this reason, it is proposed at a later stage of examined LFP that the *in vivo* experiments are to be extended more than 40 d.

From the above, it is understood that the LFP has presented encouraging results, but the effectiveness should be studied further. The encapsulation of a polyphenol as an encapsulating agent may help to slow the degradation and protection from the frequent watering and runoff due to this action.

Therefore, in order of major protection resulting from the use of polyphenol against major diseases classified the following phytopathogenic fungi: *B. cinerea*, *S. sclerotiorum* and *A. lentis*.

4.1. Suggestions–Future Actions

(1) Evaluation of LFP against post-harvest diseases in actual storage conditions.
(2) Use of test panel to evaluate the flavor and other characteristics in products that they have applied LFP.
(3) Combined use of LFP with copper compounds to control plant diseases.
(4) Evaluation of the effect of time and storage conditions on post-harvest quality products.
(5) Attempts to encapsulate LFP in other substances than protein and maltodextrin.

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