Effect of biofumigation by *Calligonum polygonoides*, dry olive leaves, and ash of olive leaves on chilli pepper growth and recovery of *Rhizoctonia solani*

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ABSTRACT

Rhizoctonia solani J.G. Kühn is a serious soilborne pathogen in chilli fields worldwide. This study examined the effect of biofumigation using arta (Calligonum polygonoides L.) and olive (Olea europaea L.) plant material on chilli growth and recovery of R. solani from chilli plants. The experiment was conducted under greenhouse conditions in potted soil amended with no plant material (control) or with plant material from Calligonum and Olea (olive leaves, or ash of olive leaves). Chilli was planted in the amended soils and inoculated with R. solani. Plant height, number of fruits, and frequency of recovery of R. solani from chilli were recorded. Soil amendment with Calligonum plant material resulted in the lowest frequency of recovery of R. solani. Moreover, Calligonum treatment increased dry mass and height of chilli plants compared to other treatments. Also, treatment with olive plant parts inhibited R. solani growth and enhanced growth of chilli compared to the control treatment. Thus, C. polygonoides and O. europaea are potential biofumigant plants to control R. solani.

Key words: chilli pepper; *Capsicum annuum*; ; biofumigation; *Calligonum polygonoides*; olive; *Rhizoctonia solani*; inoculation

IZVLEČEK

UČINKI BIOFUMIGACIJE Z VRSTO Calligonum polygonoides, SUHIMI LISTI OLJKE IN PEPELOM IZ OLJČNIH LISTOV NA RAST ČILIJEV IN REGENERACIJO GLIVE Rhizoctonia solani

Gliva Rhizoctonia solani J.G. Kühn je pomembne talni pathogen na poljih čilijev širom po svetu. V raziskavi je bil preučen učinek biofumigacije z vrsto dresnovk (Calligonum polygonoides L.) in pripravki iz oljke (Olea europaea L.) na rast čilijev in njihovega uspevanja po inokulaciji z glivo R. solani . Poskus je bil izveden v rastlinjaku kot lončni poskus, v katerem prsti niso dodali ničesar (kontrola) ali z dodatkom rastlinskih delov vrst Calligonum in Olea (oljčni listi ali pepel iz njih). Čiliji so bili posajeni v prst z dodatki in inokulirani z glivo R. solani. Zabeležena je bila višina rastlin, število plodov in frekvenca okrevanih rastlin po inokulaciji z glivo R. solani. Dodatki delov vrste Calligonum k prsti so rezultirali k najmanjši obnovi glive R. solani. Še več, dodatki delov te vrste so povečali višino in suho maso čilijev v primerjavi z drugimi obravnavanji. Tudi obravnavanja s pripravki iz oljke so zavrla rast glive R. solani in pospešila rast čilijev v primerjavi s kontrolo. Pripravki iz vrste C. polygonoides in oljke so lahko biofumigacijska sredstva za nadzor talne glive R. solani.

Ključne besede: čili; Capsicum annuum; biofumigacija; Calligonum polygonoides; oljka; Rhizoctonia solani; inokulacija

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1 INTRODUCTION

Chilli pepper (Capsicum annuum L.) hereafter referred to as 'chilli', is an important vegetable crop worldwide (Castillo et al., 2009; Scholberg et al., 2009). It is grown for several purposes such as fresh market, processing, extraction of food coloring, pharmaceuticals, and other products (Goldberg, 2001). Chilli yield has declined clearly during the last decade for a variety of reasons (Sanogo and Carpenter, 2006). Soilborne pathogens belonging to genera Phytophthora, Verticillium, Fusarium, and Rhizoctonia significantly reduced yield and quality of chilli (Sultana et al., 2008; Tariq et al., 2009). Plant pathogens such as R. solani J.G. Kühn are of great importance since they can attack plants throughout the cropping cycle, causing death of young seedlings early in the season and root rot in mature plants in the mid to late season (Woodhall et al., 2007).

Complete control of *R. solani* is not possible, but the activities of the pathogen can be minimized (Abeysinghe, 2009). Application of certain chemical fungicides could be the best solution to reduce the problems caused by *R. solani* and other soilborne plant pathogens (Bubici et al., 2006; Cimen et al., 2004; Jarvis, 1993). However, fungicides are not the most effective way to manage this pathogen. Moreover, fungicides has negative impact on the environment (Lazarovits et al., 2008; Powelson and Rowe, 1993). Therefore, environmental friendly control methods, such as biofumigation, are highly recommended and increasingly being used.

Biofumigation depends on the breakdown of plant metabolites in soil to produce volatile compounds that can suppress plant pathogens, nematodes, and weeds (Larkin et al., 2011). Many crops have been proven to work as natural biofumigants against several soilborne pests. Those plants can release glucosinolates and isothiocyanates through soil and inhibit soilborne pathogens (Aires et al., 2009; Kirkegaard et al., 2000; Laegdsmand et al., 2007; Matthiessen and Kirkegaard, 2006). Examples of biofumigant plants are *Brassica carinata* A. Braun (Porras et al., 2009; Snapp et al., 2007), *B. oleracea* L. (Fan et al., 2008), and *B. napus* L. (Laegdsmand et al., 2007). Those plants were intensively studied as biofumigants worldwide.

Other plants might have the potential to act as biofumigants and are still undiscovered and require intensive research work. Examples include *Calligonum* spp. *and O. europaea* L... The *Calligonum* genus descended from the Polygonaceae plant family and it is known as "arta" (Abrahim et al., 2014). Around 80 species across the Mediterranean region, Asia and North

America belong to the Calligonum genus (Halis, 2007). Most Calligonum plants are shrubs with diffuse and irregular woody branches. These plants have scale-like or filiform simple leaves with perfect flowers having 10-18 stamens with filaments connate at the base. Among Calligonum species is C. polygonoides L., which is a small shrub, usually 10 cm to 15 cm high, but occasionally may reach 25 cm in height with a girth of 2.5 to 5 cm (Kaul, 1963). Calligonum contains several chemicals which encouraged researchers to investigate the potential of these plants for medical uses (Kamil et al., 2000). Examples of chemicals found in Calligonum plants include anthraquinones, flanovonids, and dehydrodicatechin (Farid et al., 2007; Ghazanfar 1994; Kamil et al., 2000). Anthraquinones found in Calligonum showed high antimicrobial potential (Alkhalifah, 2013; Zaki et al. 1984). Similarly, these plants might have some components that have potential to control certain oilborne plant pathogens. Also, it was found that *Calligonum* plant material inhibit growth of two plant fungal pathogens, Alternaria spp. and Rhizopus spp. (Abrahim et al., 2014).

Another promising biofumigant plant is O. europea. Olive leaves were studied widely for their extracts and for their medicinal properties and uses (Benavente-Garcia et al., 2000). Extracts from olive-mill wastes, for example, include organic matter. potassium, carbohydrates, fats, polyphenols, and polyalcohols (Romero et al., 2005). Our interest in olive leaves is based on bioassays that showed antifungal effect of olive leaves against some human pathogens at laboratory level (Pinelli et al., 2000). Moreover, production of olive oil becomes a very common industry (Nogales et al., 2006), and a huge number of olive trees are growing worldwide. Consequently, huge amounts of olive leaves are lost during pruning and harvesting of olive fruit as waste. Traditionally, many farmers used to burn the pruned twigs with leaves and the resulting ash is incorporated with soil. Also, olive waste ash was used as soil amendment (Nogales et al., 2006). However, little research, if any, had focused on the effect of fresh olive leaves or leaf ash on soilborne plant pathogens. Therefore, our long-term goals are to encourage farmers and agricultural practioners to use the widely available plants as biofumigants to inhibit soilborne pathogens and consequently minimize the use of chemicals in the agroecosystem. Our objectives were to study the effect of incorporating C. polygonoides above-ground plant parts, olive leaves or olive ash (leaves and twigs ash) into soil on the recovery of R. solani from roots of chilli plants and on dry mass, number of fruits, and plant height of chilli pepper.

2 MATERIALS AND METHODS

2.1 Inoculum preparation

The *R. solani* isolate used was recovered from infected tomato plants displaying typical symptoms of *Rhizoctonia* root rot. The isolate was grown on potato dextrose agar in petri plates.

2.2 Soils

A large composite sample of silty clay soil (calcareous), was collected from the top 15 cm of a field at the Hashemite University, Zarqa, Jordan. The soil was thoroughly mixed, and stored until use. Round plastic pots (10 cm upper radius, 7.5 cm lower radius, and depth of 18 cm) were used. Three openings at the bottom of each pot were made for drainage. The pots were filled with soil (4 kg) mixed with each type of biofumigant plant materials. The pots were irrigated with tap water as needed throughout the study period.

2.3 Biofumigant plant materials

The aboveground *Calligonum* parts were collected from fields near the Hashemite University during summer 2014. Plant parts were spread over sheets of cheesecloth and allowed to air dry at room temperature (20-25 °C). Then, dry plant materials were milled and homogenized with a plant grinder (MF 10 basic-Thomas No. 1203C62), and collected and stored in plastic bags until use. By the time of olive fruit harvesting (October 2014), olive twigs were collected from local olive orchards at the Hashemite University. Leaf blades and peduncles were removed from the collected twigs, dried at room temperature for two weeks, and grinded. Olive twigs and leaves were collected at the time of pruning. Then, they were burned and their ash was collected and stored in plastic bags until use.

2.4 Pot preparation and plant production

On March 2015 and 2016 in experiments 1 and 2, soil of each pot was mixed thoroughly with 150 ml of the grinded plant materials according to each treatment (*Calligonum*, olive leaves, and olive ash). No plant materials were added to pots in the control treatment. One chilli seedling ('AZ20') at the six to eight leaf growth stage was transplanted into each pot at a depth of 10 cm. The chilli seedlings were grown in germination trays containing a mixture of peat moss and perlite (1:1volume basis). The pots were irrigated immediately after transplanting and maintained in the greenhouse with a minimum air temperature of 20 °C and maximum air temperature of 32 °C (average greenhouse air temperature was 25 °C).

2.5 Inoculation

Shortly before inoculation, two holes were made to a depth of 2.5 cm and approximately 1 cm away from the seedling. On April 2015 and 2016, pots were inoculated simultaneously for each experiment. Seedlings in both experiments were at the 6-8 leaf growth stage. All pots were inoculated by placing one mycelium plug, 1 cm in diameter, of *R. solani* colony grown on potato dextrose agar into each hole. All holes were filled with soil after inoculation and the pots were irriagated with tap water.

2.6 Rhizoctonia solani assessment

Three and a half months after inoculation tap roots were collected and stored in a refrigerator until plating. Each tap root was cut into top, middle and bottom parts. The middle part of the tap root was then clipped into eight homogeneous segments under aseptic conditions, and four segments were plated on a 9 cm diameter petri plate containing potato dextrose agar. Two petri dishes were assigned to each treatment. All plates were then incubated at room temperature (22 to 25 °C). Two weeks after incubation, colonies of *R. solani* were counted to estimate the *R. solani* frequency based on 8 tap root segments.

2.7 Experimental design and data analysis

Each experiment was conducted in a randomized completly block design with four replicates per each of the four treatments: control, *Calligonum*, olive leaves, and olive ash. The measured variables were: (i) *R. solani* frequency of recovery from tap root segments, (ii) dry mass of the above-ground plant parts, (iii) plant height, (iv) and number of fruits. All data were subjected to an analysis of variance (ANOVA) using the Proc GLM procedure in SAS version 9.2 (SAS institute, Cary, NC). Proc GLM was used to calculate F statistics for the overall treatment effect. Least significant difference (LSD) was used to carry out pairwise comparisons to separate treatment means. All statistical tests were assessed at 5 % significance level.

3 RESULTS AND DISCUSSION

3.1 Rhizoctonia solani assessment

The overall F test for both experiments (P < 0.0001 in experiment 1 and P = 0.002 in experiment 2) implies that the frequency of *R*. *solani* recovered from chilli tap

100 a Frequency of recovery of *Rhizoctonia solani* (%) 90 80 70 60 50 40 h 30 20 10 0 Control Calligonum **Olive leaves** Olive ash Treatments

Figure 1: Frequency of recovery of *Rhizoctonia solani* from tap root segments of chilli in experiment 1. Each number is the mean of four individual plants, comprised of four replicates per treatment. Bars with the same letter are not significantly different based on the LSD at 5 % level of significance.

In experiment 1, the frequency of *R. solani* from root segments was significantly the lowest in the *Calligonum* treatment compared to all other treatments (Fig. 1). While in experiment 2 (Fig. 2), olive ash treatment

resulted in the lowest frequency of *R. solani* compared to other treatments. Thus, mixing soil with *Calligonum*, olive leaves, and olive leaves ash reduced *R. solani* recovery from tap roots significantly.



Figure 2: Frequency of recovery of *Rhizoctonia solani* from tap root segments of chilli in experiment 2. Each number is the mean of four individual plants, comprised of four replicates per treatment. Bars with the same letter are not significantly different based on the LSD at 5 % level of significance.

root segments was significantly lower when soil was mixed with plant materials than that in control treatment (Fig. 1 & 2).

3.2 Plant growth

In experiment 1 and 2 (P = 0.002), dry mass was significantly higher for plants grown in soil mixed with

20 18 Dry mass of above ground parts (g) 16 a 14 12 b 10 b 8 b 6 4 2 0 Control Calligonum Olive leaves Olive ash Treatments

Figure 3: Dry mass of aboveground parts of chile in experiment 1. Each number is the mean of four individual plants, comprised of four replicates per treatment. Same letters on bars of the same parameter are not significantly different based on the LSD at 5 % level of significance



Figure 4: Dry mass of aboveground parts of chilli in experiment 2. Each number is the mean of four individual plants, comprised of four replicates per treatment. Bars with the same letter are not significantly different based on the LSD at 5 % level of significance.

Calligonum than plants grown in soil under all other treatments (Fig. 3 & 4).

In experiment 2, the dry mass of the above-ground parts of the chilli plants grown in soil mixed with olive leaves was significantly (P = 0.0035) higher than that of plants

grown in soil mixed with olive ash. On the other hand, plant height was significantly different among treatments in experiment 1 (Fig. 5).



Figure 5: Plant height (cm) in experiment 1. Each number is the mean of four individual plants, comprised of four replicates per treatment. Bars with the same letters are not significantly different based on the LSD at 5 % level of significance

Plants grown in soil mixed with *Calligonum* or olive ash were significantly (P = 0.025) taller than plants grown in soil mixed with olive leaves or control soil. However, there were no significant differences among plants height under all treatments of experiment 2 (data not shown). Similarly, all comparisons indicated no significant effect of biofumigant plant materials on the number of chile fruits in both experiments.

Under field conditions, chilli plants are exposed to numerous soilborne pathogens such as R. solani (Skaggs et al., 2000; Sanogo, 2003). Rhizoctonia solani is well known as a potent pathogen that causes huge losses in yield of many crops (Siddiqui and Shaukat, 2005). Controlling soilborne fungal pathogens was achieved by several methods including the use of chemicals. On the other hand, alternative methods including biofumigation have been used. In this experiment, locally available plant residues were used. Calligonum and olive plant parts showed significant reduction in the frequency of R. solani recovered from tap roots of chile plants. Chilli was selected as an indicator crop since it is widely grown worldwide and has a great value .Calligonum plant material inhibited R. solani in both experiments significantly (Fig. 1 & 2). Incorporation of grinded Calligonum aboveground plant parts into soil was effective in reducing the recovery of R. solani from chilli tap roots. This result provides an evidence that

Calligonum contains substances that are able to inhibit R. solani. In agreement with this evidence, Abrahim et al. (2014) concluded that Calligonum species have antifungal compounds effective in the treatment of plant diseases. Different factors might have affected the efficiency of R. solani recovery and chilli responses. These factors include the inoculum level of R. solani which was two mycelium plugs, diameter of 1 cm , of the colony grown on potato dextrose agar, and the amount, and timing and method of application of Calligonum. On the other hand, Calligonum plant material gave the highest dry mass of chilli aboveground parts (Fig. 3 & 4). It is possible that Calligonum plant material enhanced chilli crop to uptake water and nutrients since it inhibited growth of R. solani. Additionally, the use of Calligonum encouraged chilli roots growth which possibly had lead to better performance of chilli plants. Calligonum treatments produced the tallest chilli plants (Fig. 5). This could be attributed to the inhibition of R. solani, thus allowing chilli plant to grow well.

Similarly, olive leaves used as dry material or ash showed significant reduction in the frequency of *R*. *solani* from chilli tap roots. Plants grown in soil amended with olive plant material showed comparable increase in dry mass and plant height. The grinded, burned, or decomposed plant parts used in these experiments might have also contributed to the growth and development rate of chilli not only as inhibitors of *R. solani*, but also as a source of nutrients. In agreement with our results, Solomon et al. (2015) concluded that olive leaves incorporated into soil increased soil fertility and increased plant growth. Similarly, it was found that olive waste ash increased dry mass of chilli stems and leaves significantly (Nogales et al., 2006). Also, it was found that adding dry olive-mill wastes to the soil increases phophorus and potassium contents of chilli leaves (Benitez et al., 2000). Furthermore, olive leaves contain many substances reported to have inhibitory effects on several fungi. It is assumed that *R. solani* was inhibited in our experiments because of the effects of such substances.

The primary active compounds in the raw olive leaves include oleuropein and hydroxytyrosol, as well as several other polyphenols and flavonoids such as oleocanthal and elenolic acid (Vivioli et al., 1998). It is also expected that plant materials added to the soil in our experiments encouraged microbial activity in the rhizosphere of chilli. Consequently, beneficial soil microorganisms could compete with R. solani and reduce the activities of this pathogen. Benitez et al. (2000) suggested that olive plant material increased microbial number and activity in chilli rhizosphere. Our results showed promising results of using olive leaves, dry or ash, to control R. solani. Additional research is needed in order to explore more aspects of using biofumigant plants to inhibit soilborne pathogen in major crops. Our current study may be the first research to investigate the use of Calligonum, olive leaves, and ash of olive leaves as biofumigants to control R. solani on chilli crop.

4 CONCLUSION

We showed that incorporating *C. polygonoides*, olive leaves, and ash of olive leaves in soil infested by *R. solani* is effective in reducing the frequency of recovery of the pathogen from chilli, and in increasing dry mass of chilli aboveground plant parts. The incorportated plant materials can also serve as a source of nutrients to

support plant health and plant growth. Future work should be conducted with focus on the relationship between plant growth and plant health and the amount, and timing and method of application of the biofumigants, and inoculum levels of *R. solani*.

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