



Official publication of Pakistan Phytopathological Society

Pakistan Journal of Phytopathology

ISSN: 1019-763X (Print), 2305-0284 (Online)

https://pjp.pakps.com



RESEARCH ARTICLE

Suppressive Effect of Composts to Control Damping Off Disease Caused by *Fusarium Redolens* and *Pythium Ultimum* Var. *Ultimum* of Aleppo Pine Seedlings in Northwestern Algeria

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Article History:

Submitted: January 15, 2024; Revised: May 25, 2024; Accepted for Publication: November 12, 2024.

ABSTRACT

Damping off diseases are responsible for major production losses in agriculture and in the nursery forest in Algeria. The suppressive effects of four compost extracts were evaluated to suppress the damping off disease caused by *Fusarium redolens* and *Pythium ultimum* var. *ultimum* *in vitro* and *in vivo*. *In vitro* tests showed that the mediums prepared from the composts extracts C2 and C3 inhibited significantly the growth mycelia with (74.07%, 61.11% and 85.18%, 81.14%) respectively for the isolate of *F. redolens* and inhibited the two isolates of *P. ultimum* var. *ultimum* with (68.51%, 62.92% and 75.92%, 72.22%) for the isolate U3CR and (77.77%, 66.66% and 83.33%, 70.37%) for the isolate U7CR. These medias had high percentage of inhibition of the sporulation of *F. redolens* at (df=3; f=23.01; p=0.000) for the isolate U3CR at (df=3; f=0.37; p=0.7774) and for the isolate U7CR at (df=3; f=5.31; p=0.0118). the results showed no significant differences in inhibition germination caused by the four composts extract of the four composts compared with (P<0.05). The *in vivo* test demonstrated that these composts protect significantly the Aleppo pine seedlings from damping-off by reducing disease incidence. The findings indicate the potential of these composts to protect plants against damping-off diseases, with a remarkable reduction in disease incidence by up to 50%.

Keywords: Damping-off, compost, *Fusarium redolens*, *Pythium ultimum* var. *ultimum*, disease incidence.

INTRODUCTION

Damping-off disease of seedlings affect up to 80% of seedlings, leading to significant economic losses once established in nursery soils (Mangi *et al.*, 2021). Losses in crop production caused by damping-off pathogens such as *Pythium* sp. and *Fusarium* sp. are difficult to control (Raaijmakers *et al.*, 2009). In the recent years, alternative control methods are strongly desired for sustainable

agricultural, where organic amendments play an important role as environmentally friendly and sustainable alternative to protect plants against soil-borne pathogens. Composting is considered to be a sustainable, economical, and simple technology for disposing of organic waste (Song *et al.*, 2021). The use of organic agricultural wastes in this respect can be advantageous

both in soil fertility, recycling of agricultural residues and could provide a powerful tool for management of plant diseases (Khalil and El-Maghrabia, 2010).

Composting has become a preferable option to treat organic wastes to obtain a final stable sanitized product that can be used as an organic amendment (Sayara *et al.*, 2020). The process requires mostly aerobic conditions for the mineralization and humification of organic matter (OM) (Martínez-Blanco *et al.*, 2013). The type of compost was shown to be a critical factor in the efficacy (Haggag and Saber, 2007). Santhanarajan *et al.* (2021) concluded that a good establishment of the functional microbes within the composts would contribute to the biocontrol of the pathogens in the soil environment. Application of organic amendments, such as compost, has been reported as a management strategy against soil-borne pathogens, including *Fusarium*, *Pythium* and *Rhizoctonia* spp. Tewoldemedhin *et al.* (2015) reported that the suppressive effect of compost on damping-off disease was observed when a concentration of 30% tea compost filtrate inhibited 87% of *Rhizoctonia solani* population and it was completely inhibited at a concentration of 50% (Nofal *et al.*, 2021).

The suppressiveness of composts has been shown to depend on several parameters, including the composting process, the maturation stage and storage conditions. The main fungal and bacterial families occurring during different phases of composting processes have been identified by different methods such as high-throughput sequencing (Neher *et al.*, 2013).

The general objective of the present work was to corroborate the suppressive ability of composts towards damping off diseases caused by *Fusarium redolens* and *Pythium ultimum* var. *ultimum* the first aim was to study *in vitro* the inhibitory effect of the extract compost on mycelia growth, spore production and germination and the second aim was to study the suppressiveness of the compost of damping-off *in vivo*.

MATERIALS AND METHODS

Composts: Four composts were purchased from the horticulture Company for Solid Waste Utilization. These composts were already tested for their ability to control pathogenic fungi and used as bio fertilizing. Four types of compost, prepared aerobically from six months to one year, were used in the present work as follows: Compost (C1): a mixture of different vegetables wastes with (70%), egg shell and cardboard boxes paper (30%). Compost

(C2): green wastes (80%) and sheep manure (20%). Compost (C3): poultry manure (20%), sheep manure (20%), and cereal straw (60%). Compost (C4): green waste (50%), straw, and dead leaves (50%). These types of composts were accumulated in inboxes of wood at a length varied between 0.50 m and 1.50 m, a width of 0.5 m, the overall weight is around of 50 kilos, the inboxes were covered with black plastic film.

During composting, the components of each compost are mixed carefully by adding little of water to provide humidity. The composts were aerated every two weeks. The turning is carried out mechanically. Then the temperature was measured every 14 days to allow the decomposition process and trigger the rise in temperatures (Znaïdi, 2002). To measure the temperature, we used a thermometer. The temperature of the mixture tells us the decomposition phase (mesophilic, thermophilic phase, cooling and maturation).

Fungi isolates: One isolate of *Fusarium redolens* (F5RS3) and two isolates of *Pythium ultimum* var. *ultimum* (U3CR and U7CR) were obtained during routine isolations from Aleppo pine seedlings exhibiting disease symptoms of damping-off. These isolates were identified on the basis of morphological and molecular characters and introduced in Genbank with accession numbers (F5RS3 with JX051323) (U3CR with JX191921 and U7CR with JX191922) these isolates were tested for their pathogenicity (Lazreg *et al.*, 2013a; Lazreg *et al.*, 2013b; Lazreg *et al.*, 2014; Lazreg *et al.*, 2016). In the present work we tested the suppressive effect of the compost to reduce the pathogenicity of these isolates towards the **Aleppo pine seedling: Inhibitory Effect of composts, *in vitro*:** The inhibitor effect of four tested composts as water extracts was examined against one isolate of *F. redolens* and two isolates of *P.ultimum* var. *ultimum in vitro* using the methods of pouring plate (El-Masry, 2002; Mouria *et al.*, 2014).

Media preparation: The culture media were prepared from the four extracts of the composts according to the method of Weltzien (1992). After filtration, the compost extracts are divided into two batches, each of two flasks containing the agar-agar for preparing the compost extract medium (ECM), the media were supplemented with glucose. The first batch is autoclaved at 120 °C and the second at 80 °C (Mouria *et al.*, 2014).

Evaluation of mycelial growth inhibition: A mycelia implant with 8 mm in diameter, taken on the edge of a

young colony from these three isolates, is deposited in the center of each petri dish containing one of these culture media with three repetitions for each isolate. Petri dishes were incubated at 25°C. For controls, *Fusarium* isolates were grown on PDA medium and *Pythium* was cultured on CMA medium. After 7 days of incubation, inhibition of mycelial growth was evaluated (Usman *et al.*, 2024; Yaqoob *et al.*, 2024).

Sporulation Assay: After 10 days of incubation the sporulation assay was performed as described by (Amadioha, 2003; Mouria *et al.*, 2014). Control tests were performed under the same conditions in the absence of treatments. The percentage of inhibition was calculated using the formula of Amadioha (2003): Inhibition (%) = $100 \times (C-T)/C$. Where, C and T represent the isolate sporulation in control and treated plates, respectively.

Spore Germination Assay: Spores germination assay was performed by using micro-culture on coverslip. Spore suspension of 0.1 mL (10^6 spores/mL) prepared in sterile distilled water (counting by a Malassez cell) was spread on slide containing media prepared from the composts. The slides were incubated at 25 °C for 24 h. For the control, the germination was evaluated with the same method without any treatment. The counting of spores germinated or un-germinated was determined under a microscope ($n = 100$ spores). A spore was considered germinated if the germ tube length was greater than its diameter (Hmouni *et al.*, 2003). The inhibition of the germination was calculated using the formula: $TIG = [(NSG0 - NSGt) / NSG0] \times 100$. Where, TIG = Inhibition of the germination (%), NSG0 = Number of germinated spores in control, and NSGt = Number of spores germinated in treatment.

The effect of compost on the damping off of Aleppo pine seedling *in vivo*: The inoculums of the three isolates were produced using the modified Kirkpatrick *et al.*, 2006. 5mm cuttings of each isolate were placed in a sterilized 500ml flask containing 237.5g sand, 12.5g agar free CMA medium and 80ml sterile distilled H₂O. The flasks were stirred for 9 days to allow the colonization of the medium and the dispersion of the inoculum, then the contents of each flask were transferred to a 500 ml pots (Lazreg *et al.*, 2014). Seeds were stratified and wrapped in moistened, sterile cheese-cloth, enclosed in Petri dishes of 12 cm in diameter, and incubated at an ambient temperature of

about 25 °C for 3 weeks (Ocamb *et al.*, 2002). Inoculate of each isolate were then transferred to three 500 ml pots, and 10 Aleppo pine seeds were sown in each pot with three replications for each isolate. To test the effectiveness of the composts on disease suppression 30 seeds were sown in pots filled with contaminated soil mixed with compost at a concentration of 30%. For the control the seeds were grown in pots containing sterile soils. The pots are incubated at 25 °C and irrigated every 2 days.

Assessment of the degree of infection: After four weeks of incubation, the seedlings are carefully removed from soil and the roots were washed under running water for 2 min. The assessment of root rot was made according to the Okamb *et al.* (2002) and the disease index was calculated using the formula of Song *et al.* (2004). The inhibition of the disease incidence was calculated.

STATISTICAL ANALYSIS

Data was subjected to one-way ANOVA and significant differences between treatment means were compared by Tukey's HSD test. Statistix 8.0 software was use for data analysis.

RESULTS

Culture medias based on compost extracts sterilized at 80 °C and 120 °C induced inhibition on mycelia growth of *F. redolens* and the two isolates *P. ultimum* var. *ultimum* (U3CR and U7CR). According to results presented in Table 1, the media prepared from the composts 2 and 3 represents the greatest inhibitory effect, in particular these medias inhibited the mycelia growth of the isolate of *F. redolens* with (74.07%; 61.11% and 85.18%; 81.14%) respectively and inhibited the mycelia growth the two isolates *P. ultimum* var. *ultimum* U3CR with (68.51%; 62.92% and 75.92%; 72.22%) and U7CR with (77.77%; 66.66% and 83.33%; 70.37%) respectively Table1. The four extracts composts media sterilized at different temperature inhibited the growth of *F. redolens* with (df=3; f=1.89; p=0.1775) for the isolate U3CR was (df=3; f=0.66; p=0.5924) and for the isolate was (df=3; f=0.28; p=0.8401). The other media prepared from the composts 1 and 4 show $\geq 45\%$ inhibitory effect for all the damping off isolates. These composts had no significant differences in inhibitory effect on the mycelia growth on the three isolates ($P < 0.05$). The antagonistic power of this compost can be attributed to the diversity of its composition which can promote the growth of

antagonistic microorganisms and consequently their inhibitory effect on these isolates.

Table1. The percentage inhibition of the mycelia growth of two isolates of *Pultimum* var. *ultimum* (U3CR and U7CR) and one isolate of *F. redolens* (F5RS3) on different compost extract media sterilized at different temperature.

Isolates	Compost 1		Compost2		Compost3		Compost4	
	80 °C	120 °C	80 °C	120 °C	80 °C	120 °C	80 °C	120 °C
U3CR	44.44 bc	40.74 c	68.51a	62.96 ab	75.92 a	72.22 a	39.81c	44.44 bc
U7CR	59.25 bcd	53.33 cd	77.77 ab	66.66 abcd	83.33 a	70.37 abc	61.11 bcd	50.00 d
F5RS3	57.40 cd	51.85 cd	61.11 bcd	74.07 abc	85.18 a	81.48 ab	50.00 d	42.59 d
MSE	11.79	8.95	11.79	12.45	8.75	8.75	11.02	20.03

Means compared by one-way ANOVA, numbers followed by the same letters are not significantly different (P > 0.05).

The prepared media from the four composts extract had an inhibitory effect on sporulation of these isolates. These Medias affected significantly the spore production whereas the media sterilized at 80°C completely inhibited sporangia production of isolate U7CR with 92.36%. The medias prepared from the compost extract C2 and C3 had high percentage of inhibition with (86.33%; 79.74% and 81.19%; 89.80%) for the isolate *F. redolens* F5RS3 at temperature 80°C and 120°C respectively. The inhibitory effects on sporange production of *P. ultimum* var *ultimum* U3CR and U7CR were (77.01%; 62.52% and 85.18%; 80.53%); (84.05%; 72.64% and 92.36%; 88.76%) respectively (Table 2). These medias inhibited the sporulation of *F. redolens* at (df=3; f=23.01; p=0.0000) for the isolate U3CR at (df=3; f=0.37; p=0.7744) and for the isolate U7CR at (df=3; f=5.31; p=0.0118).

The compost extracts from C2 and C3 qqQJQ exhibited the highest inhibitory effect on spore germination of the tested isolates. The germination significantly inhibited for the both genus of fungi Tables 3. Moreover, the composts 2 and 3 showed very important inhibitory

effect on spore's germination of these isolates. The germination of the sporangia of *P. ultimum* was significantly reduced by this compost. The percentage inhibition of the spore germination of the isolate of *F. redolens* F5RS3 was (77.16%; 85.16% and 82.50%; 88%) respectively on the media prepared from the extracts composts 2 and 3 respectively whereas the sporangia germination inhibition of the two isolates of *P. ultimum* var *ultimum* U3CR and U7CR with (58.33%; 57.50% and 84.33%; 76.66%) and (63.66%; 62.50% and 87.33%; 86.33%) respectively. The germination was inhibited by the media prepared from the compost extracts C1 and C4 was (70.33%; 69.33%; 55%; 47.50 and 62.50%; 62.50%) respectively for the isolates F5RS3, U3CR and U7CR. The germination was of the isolate F5RS3 was inhibited at (df=3; f=2.46; p=0.1058) for the isolate U3CR at (df=3; f=1.83; p=0.1881) and for the isolate U7CR at (df=3; f=1.62; p=0.2305). The results showed no significant differences in the inhibition of the germination caused by the extracts of the four composts compared with (P<0.05).

Table 2. The percentage inhibition of spore production of two isolates of *Pultimum* var. *ultimum* (U3CR and U7CR) and one isolate of *F. redolens* (F5RS3) on different compost extract media sterilized at different temperature.

120°C	Compost4		Compost3		Compost2		Compost 1		Isolates
	80°C	120°C	80°C	120°C	80°C	120°C	80°C	80°C	
50.35 a	65.57 a	80.53 a	85.18 a	62.52 a	77.01 a	56.33 a	58.90 a	U3CR	
43.52 d	68.55c	88.76 a	92.36 a	72.64 bc	84.05 ab	65.88 c	69.57bc	U7CR	
64.56 c	79.74 b	89.80a	81.19 b	86.33 ab	79.74 b	65.18c	69.64 c	F5RS3	
5.03	6.04	10.00	6.07	6.37	5.07	10.22	5.44	MSE	

Means compared by one-way ANOVA, numbers followed by the same letters are not significantly different (P > 0.05).

Table 3. The percentage inhibition of the spore germination of two isolates of *P. ultimum* var. *ultimum* (U3CR and U7CR) and one isolate of *F. redolens* (F5RS3) on different compost extract media sterilized at different temperature.

Isolates	Compost 1		Compost2		Compost3		Compost4	
	80 °C	120 °C	80 °C	120 °C	80 °C	120 °C	80 °C	120 °C
U3CR	55.00 bc	42.33c	58.33 b	57.50 b	84.33 a	76.66 a	47.50 bc	45.83 bc
U7CR	62.50 b	59.16 b	59.00 b	63.66 b	87.33 a	86.66 a	62.50 b	57.16 b
F5RS3	70.33 b	67.66 b	77.16 ab	85.16 a	82.50 a	88.00 a	69.33 b	69.16b
MSE	10.48	7.53	7.83	5.85	7.34	4.06	7.3	6.97

Means compared by one-way ANOVA, numbers followed by the same letters are not significantly different ($P > 0.05$). Data presented in *table 4* showed that the damping off disease incidence of pine seedling was significantly decreased by composts application at rate of 30%. Compost (C2 and C3) had a greater suppressive effect against the three pathogenic fungi, whereas the disease reduction reached to 47% and 42.33% for *F. redolens*, 44.66 and 57.66% for U3CR, 45.66% and 44% for U7CR. Results also revealed that the other composts (C1 and C4) had the moderate suppressive effect against these isolates caused damping off Allepo pine seedling. The compost (C1 and C4) reduce the disease incidence with (41.33%; 39.33%; 42.33%; 41%; 41% and 37.33%) respectively for the isolates (F5RS3, U3CR and U7CR). These tested compost reduce significantly the disease incidence for the isolate F5RS3 was reduced with ($df=3$; $f=9.05$; $p=0.0060$) and for the isolate U3CR with ($df=3$; $f=67.2$; $p=0.0000$) and for the isolate U7CR with ($df=3$; $f=2.00$; $p=0.1933$).

Table 4. The reduction of the incidence diseases by different composts

Isolates	Compost 1	Compost2	Compost3	Compost4
U3CR	41.00 a	44.66 bc	41.00 c	37.33 b
U7CR	42.33 a	44.00 a	45.66 a	41.00 a
F5RS3	41.33 a	42.33 ab	47.00 b	39.33 b
MSE	9.92	4.73	3.85	2.65

Means compared by one-way ANOVA, numbers followed by the same letters are not significantly different ($P > 0.05$).

In this study we have presented evidence for the role of compost in suppression of damping-off in *Pythium* suppressive compost. A majority of the compost samples significantly ($P < 0.05$) suppressed damping-off caused by *P. ultimum* var. *ultimum* and *F. redolens*. Identifying suppressive compost samples using the dual criteria of having significantly healthier seedlings than the infested control, and having at least 80% healthy seedlings compared with the non-infested control. The compost C2 and C3 were usually the most efficient in increasing growth as compared to the other compost. This is presumably due to its equilibrate initial composition. Positive effect of compost extracts on growth may be also due to the presence of nutrients and beneficial microorganisms that were known to also promote root growth.

DISCUSSION

The media prepared from the compost extracts inhibited the mycelia growth, sporulation and germination of the tested pathogens (*F. redolens* and *Pythium ultimum* var. *ultimum*). Our results were similar to that found by (Hibar *et al.*, 2006). When culture media and compost teas were compared in terms of their impact on *Fusarium oxysporum* f. sp. *radicis-lycopersici*, it was observed that compost teas had a pronounced inhibitory effect on the pathogen's mycelial growth. Our results are in accordance with that found by and Barghouth *et al.* (2023).

The composts (C2 and C3) highly inhibited the mycelia growth whereas the composts (C1, and C4) gave

moderate inhibition zone against the tested isolates of *F. redolens* and *P. ultimum* var. *ultimum*. Same results were observed by Attia (2019) when treated with compost tea. These results are agreement with Estrella *et al.* (2012) of significant inhibition of mycelium development of *Fusarium oxysporum* f. sp. *melonis* by using compost of agriculture product in agar plates in laboratory condition. The reduction of mycelium growth in the 40% concentration of sterilized compost extract may be due to some chemical compounds elaborated during composting process and remained after the sterilization step or some thermo stable extracellular metabolites (El kinany, 2017).

Extract of (CBECTR) has a significant inhibition of mycelium development of *F. solani* isolates in agar plates through decreasing growth 33-55% (Ali *et al.*, 2020). Ebrahimi *et al.* (2018) found household-waste compost (HC); spent mushroom compost (SMC); and vermicompost (VC) against *Pythium ultimum* on pea composts had a suppressive effect on the pathogen, and HC had the most limiting effect on the mycelium. HC suppressed the mycelium disk up to 34 mm, while SMC, VC and control showed 46, 64 and 106 mm mycelium inhibition, respectively. This experiment showed that a higher percentage of composts have significantly more liming effect on *P. ultimum*.

The inhibition of sporulation of *F. redolens* and *P. ultimum* var. *ultimum* on media prepared from the four composts extracts had no significant differences compared with ($P < 0.05$).

Mouria *et al.* (2013) found that the mycelia growth and sporulation of five strains selected were inhibited by the extracts with 95.24% and 100%.

In vitro and *in vivo*, Znaidi (2002) and Hibar *et al.* (2006) also found that compost extracts can reduce the development of *Fusarium* spp. infecting potato and tomato. Previous studies of *Pythium* suppressive soils and composts have provided some key observations about the role of resident microbial communities in affecting pathogen responses to plants and subsequent disease development in these systems.

In addition, several authors have shown the stimulant and suppressive effect of different compost applications in many cropping systems against several pathogens such as *Fusarium oxysporum*, *Verticillium dahliae*, and *Rhizoctonia solani* (Markakis *et al.*, 2016).

indeed, as confirmed by other research, compost extract might have contained antagonistic mycoparasites, such as *Trichoderma* sp., *Penicillium* sp, *Petriella* sp or plant growth promoting bacteria (PGPB) like genera of *Azotobacter*, *Pseudomonas*, *Stenotrophomonas*, *Bacillus*, *Flavobacterium*, *Streptomyces* or *Actinomyces* group found in the chestnut compost which has colonized mycelia and inhibited sclerotia germination of *Sclerotium rolfsii* (Danon *et al.*, 2007; Zmora-Nahum *et al.*, 2008)

Our results indicate that these composts have strong suppressive to *Fusarium* and *Pythium* causing damping-off of Aleppo pine seedling in pots experiments. In these studies, suppression of pathogens by the application of compost was observed to be between 37.33 % and 57%. Results showed also that addition of compost extracts to growing media had improved Aleppo pine seedling growth despite to the presence of the pathogen EL-saiid *et al.* (2018) recorded that the mixed treatment of agricultural compost tea and olive pomace was significantly superior over the individual treatments and showed 63.52% and 44.07% percent inhibition against *R. solani* and *F. oxysporum* respectively. Zhou *et al.* (2023) found in their results that the reduction in *A. solani* by 48% and *F. oxysporum* by 54% in the post-harvested soil with compost treatment (5%) against the control demonstrated the anti-phytopathogenic efficiency of compost.

The findings of the present study corroborate with the findings of Islam (2018), Lamprecht and Tewoldemedhin (2017) who used two commercial composts (compost A and compost B) from independent suppliers were

evaluated for disease suppression under glasshouse conditions. Both composts significantly suppressed damping-off caused by *Fusarium* spp., but in a few instances, compost B was more effective than compost A in disease suppression of individual isolates of both species.

Compost can stimulate the rate of vegetation establishment, enhances root development (Donn *et al.*, 2014), and allows the establishment of beneficial microorganisms and the suppression of soil pathogens (Siedt *et al.*, 2021).

Cayueta *et al.* (2008) found that application of composts to soil has been proposed to control different diseases. However, not all types of compost have been shown to exert beneficial effects on plant growth and health. The compost extracts used in the present study were found to be effective when used as fertilizers. EL-Mohamdy (2004) showed that the biocompost application as soil amendment were able to suppress diseases caused by *R. solani* and *Fusarium* spp. on a number of economic crops. The suppressive effect of three green waste composts in peat based potting mixtures at an inclusion rate of 20% (v/v) was evaluated and the results showed that the suppression of the disease was consistent and suggested that inclusion of compost in peat as a potting mixture component to replace 20% of the peat can be used to reduce this disease in commercial production sites (Van der Gaag *et al.*, 2007).

Pugliese *et al.* (2014) reported that composting process and the type and nature of wastes and raw materials influence the maturity, quality and suppressiveness of composts.

Pesticides are extensively used in agriculture to control plant disease and are known to cause major environmental problems in the world with high risk for pollution and residual effect. The utilization of organic agricultural wastes in this respect is considered as a safe non-chemical control method.

CONCLUSION AND RECOMMENDATION

The present study has demonstrated that use of the composts clearly improves their efficacy to suppress the pathogenicity of soil-borne fungi without any risk. The biological test *in vitro* and *vivo* confirm that our compost inhibited and reduced the mycelia growth, sporulation, and spore germination of the two pathogenic fungi. These results provided evidence that the compost extract has the potential to become a good

method for biological control against soil borne fungi. The biological control using compost has become a reliable and simple method for improving the quality and quantity of our yield. Equally important is the attainability of healthy and 100% organic product. Our compost is promoted as an alternative to biological control to protect crops and can be applied as a soil fertilizer in farming. Further studies are required to isolate and select effective microorganisms inhabiting this compost. Also, it is necessary to evaluate the

antifungal activity of this compost in vivo and in naturally infected soils disease.

AKNOWLEDGEMENT

The authors are grateful to Belabid Lakhdar a Dean of Faculty of Life Science and Nature of the University of Mascara Algeria for the generous help and assistance to realize this work and manuscript. In this presented work we have treated damping off disease caused by *Fusarium redolens* and *Pythium ultimum var ultimum* by using composts as biological tool.

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