Biological control on plant pathogenic fungus, *Pythium* by using some plant extracts *in vivo* and *in vitro*

Biological control on plant pathogenic fungus, *Pythium* by using some plant extracts *in vivo* and *in vitro*

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Abstract. The inhibitory activity of 5 plant extracts, *Allium sativum, Opuntia ficus-indica, Malva parviflora, Mentha spicata* and *Plantago lanceolata* with concentration 50 % and 100 % were assessed against the mycelial growth of *Pythium* fungus *in vivo* and *in vitro*. Results showed that all the concentrations of plant extracts give significant inhibition in the mycelial growth of *Pythium* fungus; the highest concentration (100 %) prevented large amounts of mycelial growth followed by lower concentration (50 %) of plant extracts. *Opuntia* extract at 100 % concentration gave great effective in inhibition of *Pythium*`s mycelial production, which gave 100 per cent inhibition ratio while 50 per cent concentration gave 77.28 % inhibition ratio followed by other plant extracts, *Mentha*, *Allium sativum*, *Plantago*, then *Malva* respectively. *In vivo* experiments, tomato seed infused in five plant extracts separately and then sown with soil contaminated with *Pythium* fungus and watered after that with these five plant extracts. After 30 days, the length of the stems and roots were measured in centimetres. While the tomato seeds which were soaked with distilled water and then sown in polluted soil with fungus *Pythium*, and watered with distilled water (control) did not show any growth. .

Keywords. *Pythium*, biological control, plant extracts, *Mentha*, *Allium*, *Opuntia*, *Malva*, *Plantago*, *in vivo*, *in vitro*.

1. Introduction

Fungiform diseases cause a significant loss of many economic crops worldwide. Fungi have the greatest impact on the reduction of crop productivity or post-harvest losses, leading to a massive loss for the human race [1]. It is estimated that crop losses from fungal infection are around 14 percent [2]. World annual crop losses as a result of diseases were estimated at US$ 25,000 million, a major part of which is due to seed-borne fungal pathogens [3]. Several pathogens, including *Alternaria* species are degrading the fruit quality, reducing and making market values unsuitable for human consumption [4]. Plants are the sources of natural pesticides that lead to the development of new pesticides [5]. The use of plants or plant products as fungicides is very critical and requires further attention [6]. The extracts and oils of plants in particular formed the basis of many applications, including raw and processed food preservation, pharmaceutical, alternative medicinal products and natural therapies [7]. Extracts from many plants have recently become popular and have become scientifically interested in their

antibacterial and antifungal function [8]. With a view to creating an eco-friendly antifungal control strategy, in the present study, different concentrations of five plant extracts including *Allium sativum, Opuntia ficus-indica, Malva parviflora, Mentha spicata* and *Plantago lanceolata* were assessed for their antifungal activity.

2. Materials and Methods

2.1. Plant extracts preparation

Cold, aqueous extracts of five plants' leaves: *Allium sativum, Opuntia ficus- indica, Malva parviflora, Mentha spicata* and *Plantago lanceolata* were obtained to prepare of 50 % and 100 % of plant extracts by using sterilized distilled water; 10 g of leaves of each plant were washed, then mortar and pestle were used to grind leaves with 100 ml distilled water. The plants material was homogenized for 10 min by homogenizer then filtered with two layered of gauze then with filter paper. The filtrate was centrifuged at 3000 rpm for 15 minute, those solutions were considered traditional (100 per cent) solutions. The other 50 per cent concentration was prepared by adding equivalent amounts of distilled water to the normal solution.

2.2. The experiment (in vitro)

The plant extracts were combined with the medium potato dextrose agar (2 ml of extract for 18 ml of PDA), then Sprinkled on Petri dishes, Petri dishes were inoculated after media solidification by placing 4 mm mycelial disks of *Pythium* fungus (from the active colony) in the center of each plate. The pure fungus was obtained from laboratory fungi, Department of Biology, University of Al-Anbar. Three replicates for each concentration were packed, then the plates were incubated at 28º C and recorded the results after 7 days of incubation. Petri dishes without plant extracts considered as control.

2.3. Inhibition factor calculation (Percent)

The percent inhibition for Various concentrations were computed as follows:

Inhibition of growth (percent)= [(Dc-Dt)/Dc]* 100 (percent) [9].

Dc= Fungal colony average diameter in control

Dt= fungal colony average diameter in treatment.

2.4. The experiment (in vivo)

2.4.1. Seeds preparation

Tomato seeds *Lycopersicon esculentum* was soaked with plant extracts (100 %) in petri dishes for 24 h. In the first dish, tomato seeds was soaked in sterile distilled water (control); the second dish, tomato seeds was soaked with *Allium sativum* extract ; the third dish, tomato seeds was soaked with *Opuntia* extract; and soaked with *Plantago* extract in the fourth dish, and soaked with *Malva* and *Mentha* in the fifth and sixth dish.

2.4.2. Soil and peatmoss sterilization and planting tomato seeds

In our research, we performed a pot culture experiment to test the efficacy of five plant extracts against damping-off and root rot of tomato, *Lycopersicon esculentum*. Disinfected tomato seeds in 2

per cent sodium hypochlorite for 1 minute, rinsed in distilled sterile water and dried overnight [10]. Tomato seeds were grown at four seeds per pot. mixed soil and peat moss with ratio 1 : 2 and sterilized by autoclave for 30 min then left for 7 days, and putting in the pots. The soil polluted with *Pythium* fungus (three day-old actively growing mycelial discs) five discs, 4.5 mm for each pots then the seeds (soaking seeds) were planted in the pots and watered with the five extracts (each one alone) the control watered with distilled water every two days then left for growth (in greenhouse) until reach to 30 days in age. For each treatment three replicates were preserved . The shoot length and root length (cm) were recorded at 30 days after planting.

3. Results and Discussion

3.1. In vitro

The results of the study referred, That the inhibitory function of all plant extracts showed major variations against mycelial growth of the *pythium* fungus.

3.2. Impact of 5 plant extracts on in vitro growth of Pythium fungus.

The results showed (Table 1, Figure 1, 2, 3, 4, 5, 6) that five plant extracts triggered a significant inhibition of the growth of *Pythium* fungus. The 50 per cent concentration of *Opuntia* extract was the most effective against *Pythium* and caused the highest inhibition of mycelia production (77.28 %) followed by *Allium sativum* (60.60 %), *Mentha spicata* (53.30 %), *Plantago lanceolata* (26.65 %), *Malva parviflora* (15.06 %). The results also showed, *Opuntia* with concentration 100 % was the most effective against *Pythium* and gave great inhibition of mycelia growth (100 %) followed by *Mentha spicata* (69.85 %), *Allium sativum* (60.78 %), *Plantago lanceolata* (38.72 %) and *Malva parviflora* (38.35%). [11] Zena *et al*. (2013) mentioned that *Mentha* extract contain tannins, alkaloids, phenols, flavones, resins and saponins which have very important role in inhibition of many pathogenic organisms. Also [12] mentioned that the alkaloids compound has direct contact with plasma membrane of microorganisms which has proteins and lipids, or it interferes with a series of metabolic reactions of the microscopic organism which is very necessary for the growth and production of spores.

Table 1. Impact of 5 plant extracts on in vitro growth of *Pythium*.

Plant extracts	Growth inhibition (%)	
	Mean. 50 conc.	Mean. 100 conc.
Allium sativum	60.60	60.78
Opuntia ficus-indica	77.28	100
Malva parviflora	15.06	38.35
Mentha spicata	53.30	69.85
Plantago lanceolata	26.65	38.72
Control (PDA without extract)	0	0

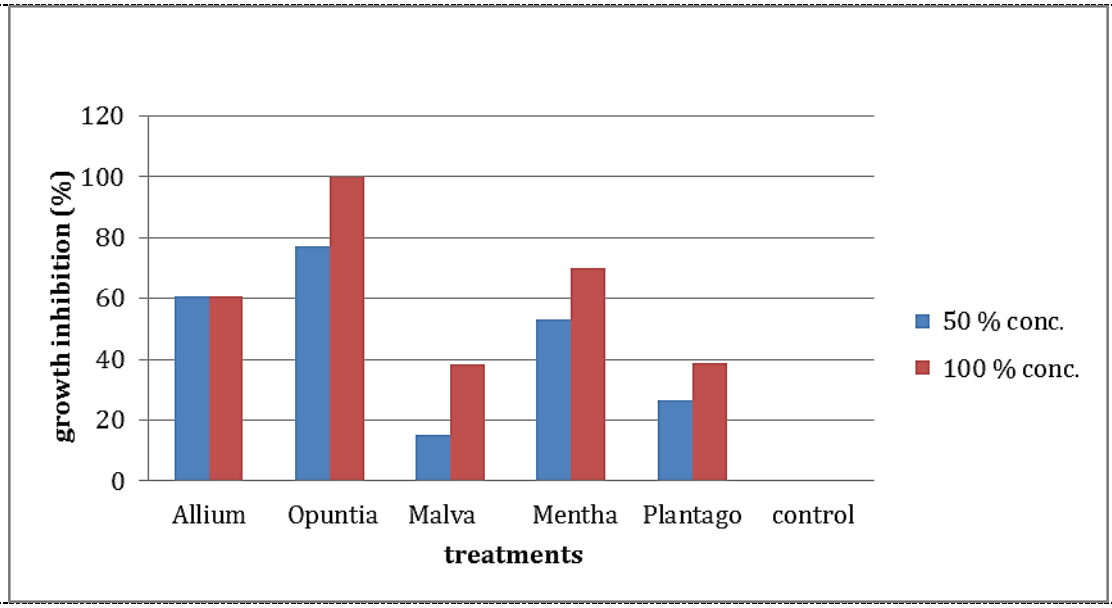


Figure 1. Impact of 5 plant extracts on in vitro growth of *Pythium*.

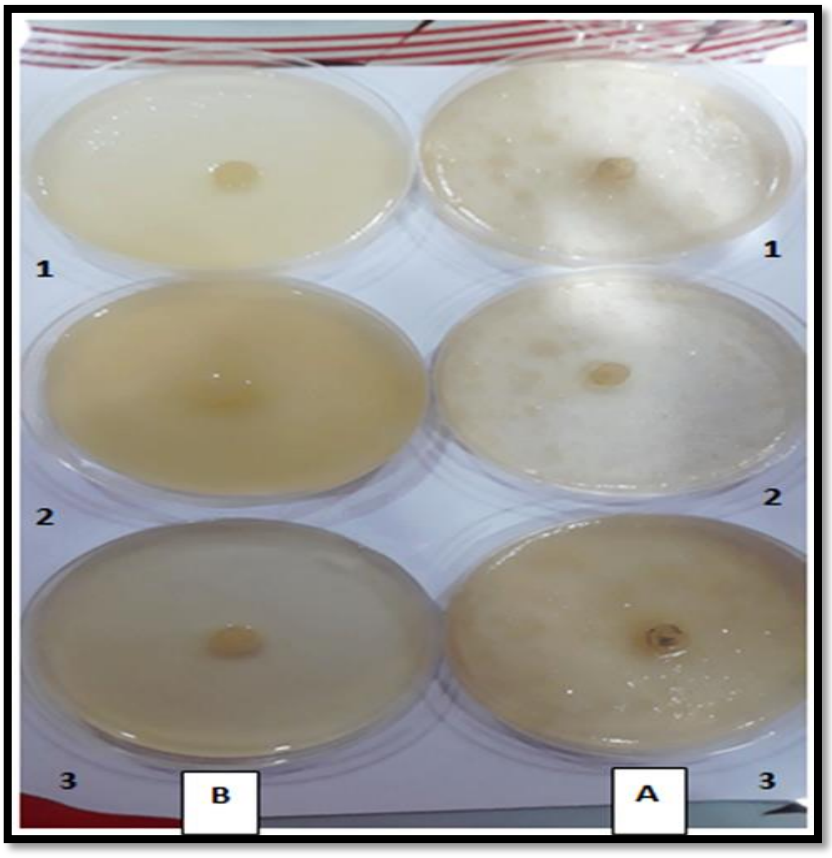


Figure 2. A (replicate; 1,2,3) PDA with 50% Opuntia extract and cultured with Pythium fungus.
B (replicate; 1,2,3) PDA with 100% Opuntia extract and cultured with Pythium fungus.

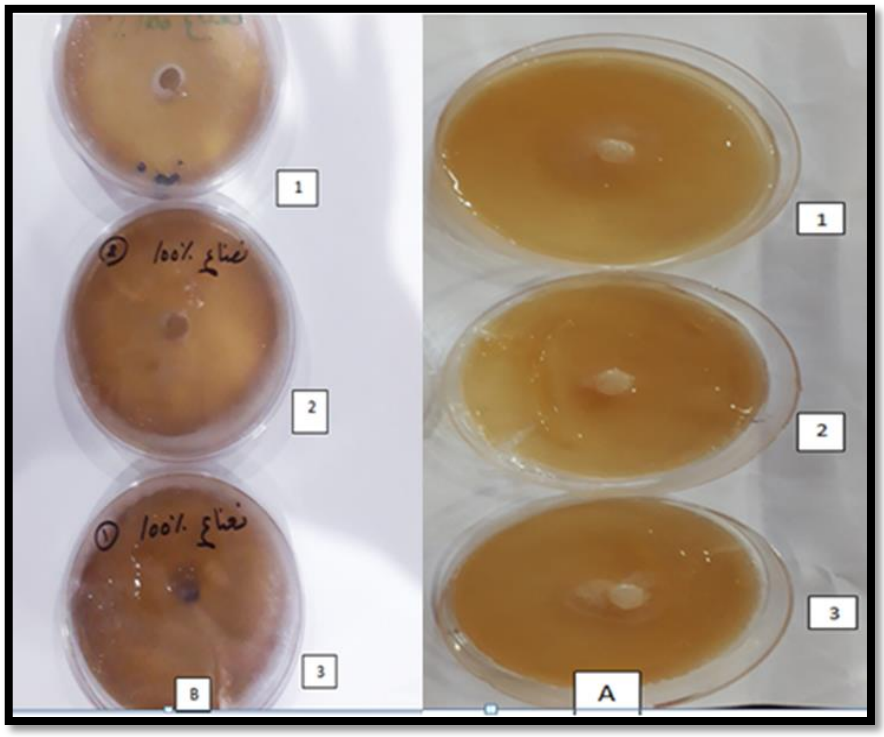


Figure 3. A (replicate; 1, 2, 3) PDA with 50% Mentha extract and cultured with *Pythium* fungus
B (replicate; 1,2,3) PDA with 100% *Mentha* extract and cultured with *Pythium* fungus.

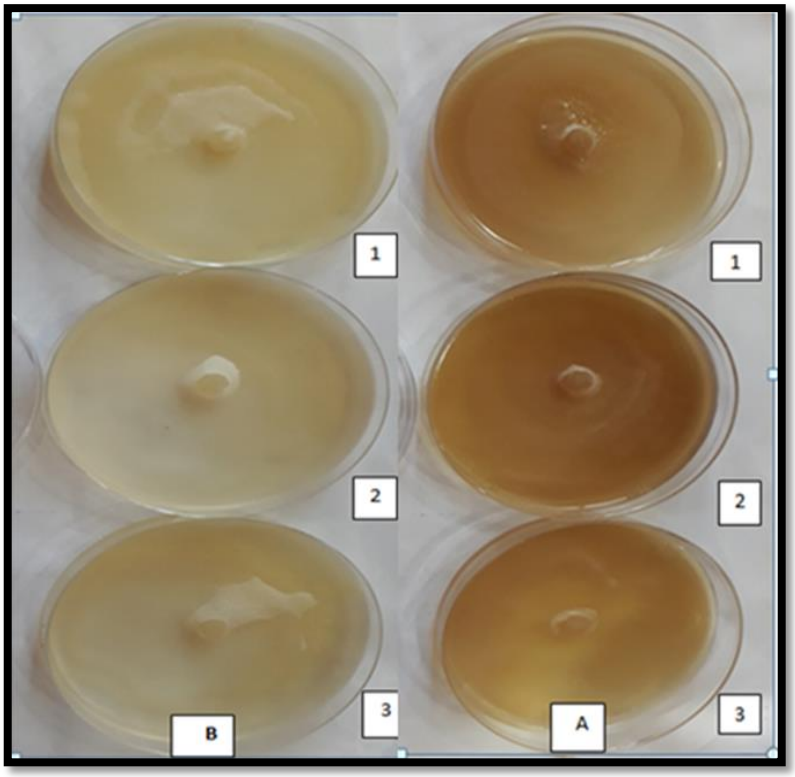


Figure 4. A (replicate; 1, 2, 3) PDA with 50% *Plantago* extract and cultured with *Pythium* fungus.
B (replicate; 1, 2, 3) PDA with 100% *Plantago* extract and cultured with *Pythium* fungus.

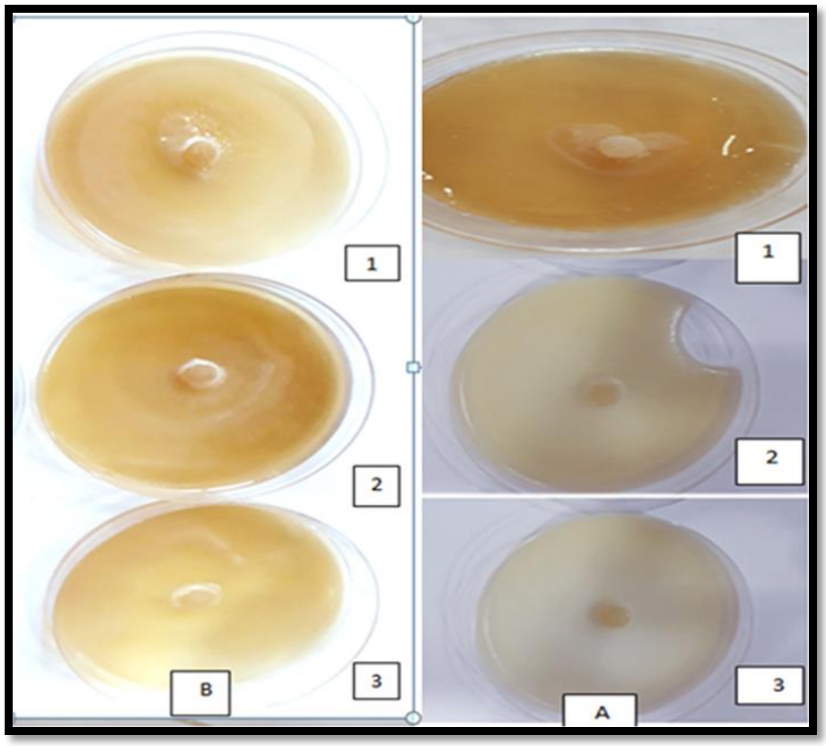


Figure 5. A (replicate; 1, 2, 3) PDA with 100% *Allium sativum* extract and cultured with *Pythium* fungus.
B (replicate; 1, 2, 3) PDA with 50% *Allium sativum* extract and cultured with *Pythium* fungus.

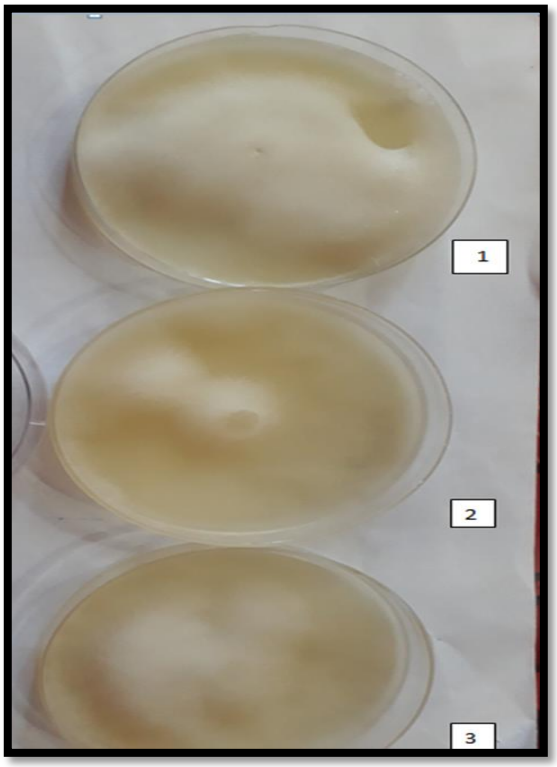
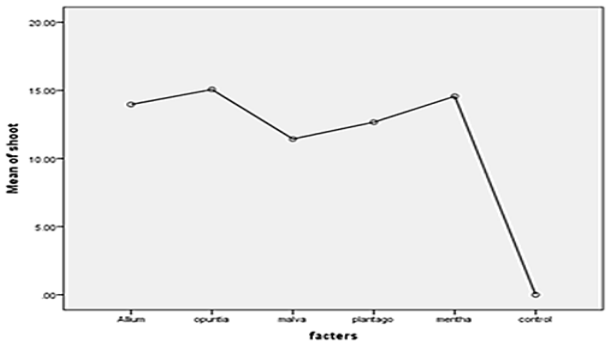


Figure 6. Control (Replicate; 1, 2, 3) PDA without plant extract and cultured with *pythium* fungus.

Table 2. Effect of five plant water extracts on shoot length.

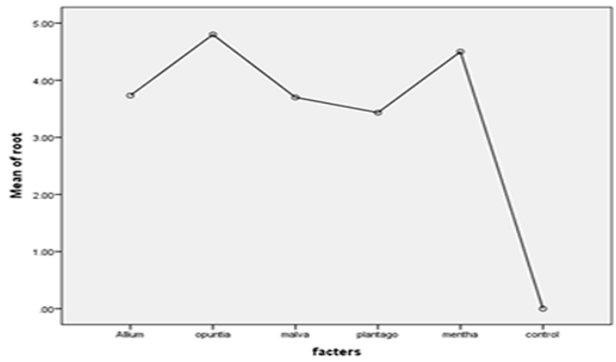
Shoot length(cm)			
Plant extracts	mean		Std. Deviation
Allium	13.966	cd	1.436
Opuntia	15.066	e	1.563
Malva	11.433	b	0.611
Plantago	12.666	bc	0.568
Mentha	14.566	de	0.650
control	0.000	a	0.000



Numbers with similar letters are not significantly different from each other.

Table 3. Effect of five plant water extracts on root length.

Root length (cm)			
Plant extract	mean		Std. Deviation
Allium	3.733	bc	0.568
Opuntia	4.800	d	0.458
Malva	3.700	b	0.435
Plantago	3.433	b	0.503
Mentha	4.500	cd	0.871
control	0.00	a	0.00



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