



# Efficiency of Plant Extracts and Fungicides in Controlling Root Rot Diseases in Peas

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## Abstract

Pea (*Pisum sativum L*) is an essential leguminous crop grown in many countries and Saudi Arabia. Peas are an important protein source for both humans and animals. Peas (*Pisum sativum L*) Pathogens attack the pea plant and cause severe damage to it, thus affecting its growth and production. In pathogenicity tests; twenty fastest-growing fungal isolates without regard to the isolation place including four isolates of *F. oxysporum*, *F. solani*, *R. solani* and *Alternaria solani* were examined for their pathogenicity on pea plants to select the most aggressive isolates to use in the further studies. In a study of the antifungal activity of plant extracts, i.e., Neem, Ginger, and *Artemisia* at concentrations of 0, 25, 50, 75, and 100%, all plant extracts at any concentration used occurred inhibition of the linear outgrowth of three examined pathogenic fungi. There is an opposite relation between increasing conc. of plant extracts from 0 to 100% and the linear outgrowth of three examined pathogenic fungi. In this respect, Neem and Ginger at 100% were the most efficacy. Neem extract at both 75 and 100% concentrations prevented the growth rate of *F. solani*.

Moreover, the high concentration (100%) also wholly suppressed the linear outgrowth of *F. oxysporum*. It caused more suppression in the linear outgrowth of *F. solani* in comparison with *F. oxysporum* in addition to *R. solani*. The reverse side, Ginger extract at high concentration wholly suppressed the linear outgrowth of *F. oxysporum* in addition to *F. solani*. Also, the concentration of 75% wholly suppressed the linear outgrowth of *F. solani*. In a study of chemical control, the Premium fungicide was more efficacious in lowering the linear spread of all examined fungi than the Tolex fungicide. Both high concentrations of Premium (75 and 100 ppm) prevented the outgrowth of *F. oxysporum* and *R. solani*. Tolex 500 wp fungicides at 100 and 75 ppm prevented the growth rate of *R. solani*. It is noteworthy that *F. oxysporum* was less effective with Tolex 500 while *R. solani* was more sensitive.

**Keywords:** Pathogenic fungi; chemical control; plant extracts; pea plants

## Introduction

Pea (*Pisum sativum L*) is a leguminous plant with many advantages as it fixes nitrogen through the atmosphere [1]. Pea is a crop grown in several countries, such as the K.S.A. Studies found that it contains a high nutritional value of protein, iron, phosphorus, and calcium, as well as a group of vitamins such as Shula, vitamins A and B [2]. In furthermore, many of these characteristics, it is a useful and important food for both humans and animals, as it reduces cholesterol [3,4]. In numerous regions of the world, peas are cultivated

and consumed as vegetables, as they are a significant source of protein and essential elements such as potassium and calcium. [5]. The pea plant is one of the most significant commercially plants due to its exposure to many diseases, especially root rot disease, and the harmful effect resulting from the use of fungicides, which are considered currently to be an effective treatment for the elimination of many diseases. However, scientists found that these pesticides threaten human and animal health, in addition to their ability to

eliminate soil vitality and fertility. [6,7]. Showed that plants infected with root rot showed significant genetic variation.

This difference is evident in the decrease in the number of plants, which causes a constant threat to the formation of peas, particularly in temperate zones, where peas are sown in spring in cool and wet conditions. Seedling death can be a significant factor limiting yield and causing severe economic loss [8] reviewed that *leguminous* crops are susceptible to many diseases that limit their productivity and reduce quality. It was found that some varieties of peas may be infected with nearly a hundred fungal causes around the globe. From among fungal diseases, *Fusarium* wilt is one of the worst diseases that affect legumes because it is a soil disease [9] found that root rot disease in peas is a complex disease caused by many fungi that are transmitted through the soil, and root rot disease is one of the significant aspects for determining crop yield. *Sclerotinia sclerotium*, *Rhizoctonia solani*, and *Fusarium oxysporum* were isolated from culture, recognized, and afterwards validated in a pathogenicity method as pathogens of root rot [10]. Peas are infected with many pathogens, whether fungal, bacterial, or viral, as well as nematodes, in addition to causing some physiological defects.

Fungal diseases, seedling death diseases, and root diseases are among the primary harmful conditions of the crop [11] found that *R. solani* is the highest significant destructive fungus caused a high % of both pre-emergence and post-emergence damping off 23.33% and 53.33% of viable plants. *F. oxysporum* showed (20, 26.66%) in pre- and post-damping off and 60% of survival plants. At the same time, *F. oxysporum* showed a high significant percentage in disease severity, followed by *R. solani*. *Pythium* spp. Gave 16.66%, 13.33% in both pre-damping and post-damping off, and 70 % of viable plants [12] showed that 14 isolates of *F. oxysporum* f. sp. pisi were evaluated in several areas of Manipur for their culture, morphogenetic and pathogenic activity. The color of the fungus varies from white to bright red, purple, and yellow. The development of pathogenic fungi from *Fusarium* isolates ranged from 5.4 cm to 8.9 cm at eight days after inoculation at twenty-six degree Celsius in 9 cm Petri plates. The isolates showed intermediate to abundant spores.

The size of the microconidia ranged from  $11.6 \times 3.1$  to  $25.2 \times 6.2$   $\mu\text{m}$  and the size of the microconidia varied from  $3.02 \times 2.1$   $\mu\text{m}$  to  $9.2 \times 5.6$   $\mu\text{m}$  [13] found that plant extracts give a natural and protective effect against pathogenic fungi such as *R. solani*. He discovered that developing antifungals would not only offer an effective tool for controlling pea root rot but would also pledge successful and superior multifunction alternatives to traditional fungicides for managing those certain plant diseases [14] tested 13 leaf extracts of various plants in the laboratory for their capacity to keep *Rhizoctonia solani* under control. Garlic, eucalyptus, lemon, joker or van Tulsi greatly hindered fungal outgrowth in addition to *sclerenchyma* formation, except for Tulsi, onion, aka, *jatropha*, and *bishram*, which led to a decrease in the spread of *Rhizoctonia solani* [15] discovered this through experiments on about thirteen plant extracts from leaf extracts of *Catharanthus roseus*, *Azadirachta indica*, *Lantana camara*, *Ocimum sanctum* *Ricinus communis*, *Saraca indica*, or *Thuja occidentalis* latex-producing plants *Calotropis procera*, *Nerium In-*

*dicum* and *Datura Ficus procera* or *Nerium Indicum* tested.

Bulbs of *Dinisoa*, *Allium cepa*, or *Allium siphon* for pathogen eradication *in vitro*. These extracts' antifungal activity was tested on fungi such as *Rhizoctonia solani*. It was discovered that when compared to the control, all-natural plant extracts reduced the pathogen [16] found that the methanol extract from (leaves, flowers, roots, fruit, and buds) of *Trachystemon Orientalis*, *Smilax excelsa*, *Rhododendron ponticum*, *Phytolacca americana*, or *Prunus laurocerasus*, these parts exhibited their activity against pathogenic fungi (*Alternaria solani*, *Botrytis cinerea*, and *Rhizoctonia solani*) [17] discovered that *clerodendrum* leaves (*Clerodendrum infortunatum* L), polyantha (*Polyalthia longifolia* Sonn), or ginger roots (*Zingiber officinale Roscoe*) were more efficacious towards *Colletotrichum muse* in addition to *Rhizoctonia solani* (Kühn) then resulted in growth suppression. Many fungi are pathogenic, whereas the highest potent plant extract is *clerodendrum* extract, which was found to be most potent towards the pathogen of rice sheath blight in either pot cultivation or field experiments [18] discovered extracts from *Moringa oleifera* Lam.

The plant parts (leaves, stem, fruit, and seeds) Strongly exhibited suppression in the spread of *Macrophomina phaseolina*, *Rhizoctonia solani* (Kühn), or *Fusarium oxysporum*, while the stem extract in addition to powder enhanced plant growth then demonstrated maximum suppression of root rot fungi on peas, so, this was clearly shown in bean crops under greenhouse conditions [19] that extracts of *Moringa* leaves and seeds contain antifungal properties that inhibit spread of *R. solani* in addition to *F. solani*. The antifungal effectiveness of the extracts was influenced by the concentrations of moringa extract [20] reported aqueous and ethanol-solvent extracts of four wild medicinal plants (*Moringa Olivera*, *Osmium Basilica*, *Cinnamomum cavort*, and *Lantana Camara*) were tested *in vitro* and under greenhouse for safeguarding of canola plants from root rot or wilt diseases. *In vitro*, all aqueous solvents in addition to ethanol extracts inhibited the linear growth of *F. solani*, *R. solani*, or *F. oxysporum* to various levels. *Moringa oleifera* was better than the fungal outgrowth of all examined fungi.

Additionally, all of the evaluated plants' aqueous extracts were less effective than the ethanol solvent [21] achieved the best production (66.55 q/ha) of peas when treating seeds with carbendazim and soil application of green manure + neem cake + antifungal. Piece [22] reported to identify the effect of indoor fungicide applications infected with root rot fungus of peas under greenhouse in addition to field conditions. Indoor fungicides generally lowered the severity of root rot, occasionally; the extent of seed treatment in the field is significantly exceeded. Even so, a difference between the level of control existed between hosts and pathogens in both greenhouses in addition to field experiments. *Prothioconazole* and flupyrim Penthiopyrad supplied the most regular outcomes across trials. According to the findings of these research, indoor application of fungicides offers growers a different method for controlling *Fusarium* root rot [23]. Found that fungicides of several classes like exogenous quinone inhibitors (QoIs), triazoles, Demethylation Inhibitors (DMIs) or Succinate Dehydrogenase Inhibitors (SDHIs) were employed for applications within limited locations.

QoI fungicides behave at the quinol-binding site. Exogenous cytochrome bc1, which inhibits mitochondrial respiration then deactivates membrane formation by preventing demethylation, is categorized in the Fungicide Resistance Committee (FRAC) division 11. DMI fungicides deactivate membrane formation by suppressing the demethylation of sterol biosynthesis. The triazole fungicides are a subdivision of the DMI FRAC division 3. The SDHI fungicides, FRAC division 7, target the mitochondrial respiration chain; deactivate the tricarboxylate cycle in addition to the electron transport chain in mitochondria [24] found that the use of fungicides as an outcome of substantial lowering in disease severity contrasted with the untreated control. Some pesticides reduced infection by immersion; in addition to Fosetyl Aluminum suppressed pathogens effectively during seed treatment and spraying methods. Plant growth variables were also evaluated, and then a considerable enhancement was noticed in growth response of the treated plants contrasted with the untreated control. The study exhibited that fungicides can be used to effectively control peas. Wilt disease using the suitable application method. Thesis' objective, the purpose of this research was to investigate the following items.

- a) Groups of pea root rot inhibitors, fungi and their frequency from different fields in Beljurashi and Bani Kabir governorates,
- b) Isolation, purification, and identification of fungal root rot causes.
- c) Pathogenicity test for the fastest-growing pathogenic fungi.
- d) Investigating the effects of plant extracts (*Artemisia*, neem, and ginger) on growth of linear fungi.
- e) The impact of Tolex 500 wpm and Premium fungicides on the linear growth rate of fungi.
- f) Evaluation of seed soaking in the best biological control treatments, plant extracts, and fungicides against damping-off of pea seedlings and survival of plants under artificial soil with the most aggressive fungal pathogens in the greenhouse.

## Materials and Methods

### Source of Pea Seeds and Tested Materials

Pea seeds were obtained from the farm of Mr. Ahmed Ghoramallah in Beljurashi. While the used biological agents were isolated from the root of hygienic pea plants in the same sites in Beljurashi and Bani kabir provinces that were used to isolate fungal pathogens. Extracts from three plants have also been used as natural additives (neem, ginger, and *Artemisia*). Two fungicides, Tolex 500 and Premium, were used for comparison in this investigation.

### Fungal Pathogens and their Frequency

Samples of pea plants appearing damping-off or root-rot symptoms were gathered from various fields at Beljurashi or Bani kabir Provinces and transferred into laboratories of the College of Arts and Sciences, Beljurashi, Al-Baha University to prepare for isolation and identification of root rot fungal pathogens of pea plants. Fifty isolates of root rot pathogens were isolated from the roots of pea plants under natural infection from diverse locations in Beljurashi

and bani kabir Provinces, Saudi Arabia. As mentioned below, the frequency of isolated fungi species was recorded as a frequency percentage after isolated fungal genera.

### Isolation and Purification of the Pathogens

Samples of pea plants appearing apparent symptoms of damping-off and root rot diseases were gathered from similar areas to infected fields at both Beljurashi and Bani kabir provinces, Saudi Arabia. All diseased root samples were carefully washed in tap water to eliminate the adjacent soil particles, dried, and then chopped into small pieces (approximately 1 cm). The samples were surface sterilized by soaking them in 0.5% sodium hypochlorite solution for 2 min.; after this, it was carefully rinsed in sterilized water, followed by drying between two sterilized filter papers. The surface sterilized samples were transported onto plates of Petri dishes loaded with Potato Dextrose Agar (PDA) medium that has been provided with 0.01% Streptomycin antibiotic (100 mg/ml) to prevent bacterial contamination. Plate's Petri- dishes that contain infected root pieces were incubated at 25± degree Celsius for 5 days. The spreading of fungi was carefully moved to PDA slants and left at four degrees Celsius in a refrigerator for subsequent studies. The developing mycelium colons from pure cultures were got from each isolate were purified after five days by hyphal tip technique, in accordance with [25].

### Identification Studies

The developing fungi after five days from incubation were obtained randomly from the ends of the growing colonies and transported onto poured PDA medium plates. The isolated fungi that were growing were recognized based on their morphological characteristics using a light microscope [26-28]. Sections of the fungal growth were placed on clean slides using lacto phenol cotton blue stain to observe and identify the morphological structures [29]. The size, texture, and color of the fungal colonies were checked under the light microscope; the previously prepared slides were examined using the objectives of 40x for vegetative mycelia i.e. septation, diameters conidiophores or sporangiophores and also reproductive structures i.e. conidia and sporangiospores. The 10x objective of the light microscope was used to examine fungal colonies. The frequency and frequency percentage of the isolated fungal species, whether it is defined or undefined, were calculated.

### Pathogenicity Tests

The Pathogenicity tests were performed under greenhouse conditions of the College of Arts and Sciences, Beljurashi, at Al-Baha University. Twenty fastest-growing fungal isolates, including four isolates of *F. oxysporum*, six isolates of *F. solani*, five isolates of *R. solani*, and five isolates of *Alternaria solani* were examined for their pathogenicity on pea plants to select the most aggressive isolates to use in the further studies without regard to the isolation place.

### Preparation of fungal inoculum

Pots with a diameter of twenty-five cm were sterilized by soaking them in a five percent formalin solution for fifteen min., and afterward left to air-dry in the open air. The soil was sterilized by

extensively combining it with a five percent formalin solution. The treated soil was then lined with a plastic sheet for a week before removing it to allow the formalin to fully evaporate [30]. Fungi were cultured separately on sand-barley (SB) medium, which was made by combining twenty-five gram of clean sand, seventy-five gram of barley, and adequate water to lid the mix. Sterilized medium in flasks was inoculated with each individual fungus and incubated at a temperature of 25 degree Celsius for a period of two weeks. Each individual fungus was infested in the soil at four percent of the soil weight [31]. The soil in the pots was watered daily for a time of one week to promote the outgrowth of fungi. The soil in the control pots was combined with an equal quantity of sterilized Sand Barley (SB) medium that was free of fungus. Ten pea seeds were sterilized with a two percent sodium hypochlorite solution for 2 min, after that washed many times with sterilized water before being sown. For each treatment, three pots with a full of thirty seeds were utilized as replicates. The plants received a regular agricultural recommen-

ation for irrigation and fertilization. Pots were organized under the greenhouse.

### Sowing seeds

Healthy pea seeds. They were disinfected with one percent sodium hypochlorite solution for 5 min., after that washed in sterile water and sown at ten seeds per pot. For every treatment, three replicates were utilized. The plants received a regular agricultural recommendation for watering and fertilizing. The pots were set up in a totally randomized design under the conditions of a greenhouse.

### Disease assessment

The disease assessments were recorded as ratios of pre-emergence and post-emergence damping-off in addition to the hygienic surviving plants in every treatment at 15, 30, and 45 days after sowing, respectively, using the formula of [32] as follows:

$$\text{Pre-emergence damping-off \%} = \frac{\text{No. of non-germinated seeds after 15 days}}{\text{Total no. of the sown seeds}} \times 100$$

$$\text{Post-emergence damping-off \%} = \frac{\text{No. of dead seedlings after 30 days}}{\text{Total no. of the sown seeds}} \times 100$$

$$\text{Survived plants \%} = \frac{\text{No. of survival plants after 45 days}}{\text{Total no. of the sown seed}} \times 100$$

## In Vitro Studies

### Plant Extracts

#### Preparation of Plant Extracts

A quantity of three plant species (Neem, Ginger, and *Artemisia*), chopped leaves and seeds of each plant then washed thoroughly in sterile distilled water. Each sample's plant extract was made by mixing 20 grams of dried plant powder with 200 ml of distilled water in a glass flask (volume 500 ml). Large plankton is removed using two layers of gauze, and the filtrate is then separated from it using filter paper to provide a clear filtrate devoid of contaminants. Each plant sample's initial solution is this. The extracts were then put in a dark, airtight flask and kept in the refrigerator at 10 degrees Celsius until they were used [33]. The extracts were considered 100% concentration. The solvent extract was diluted with sterile distilled water to prepare other dilutions i.e., 25, 50, 75, and 100%.

#### Impact of Plant Extracts on Fungal Linear Outgrowth (mm)

The effects of plant extracts, i.e., Neem, Ginger, and *Artemisia* at concentrations of 0, 25, 50, 75, and 100% on fungal linear growth were tested in the laboratories of the College of Arts and Sciences, Beljurashi, Al-Baha University. All tested concentrations were combined separately with PDA medium prior solidification (1:9 v/v), after that emptied into sterilized Petri dishes. For each concentration, three plates were utilized as replicates. The plates were inoc-

ulated with fungal discs taken from the periphery of the 5-day-old culture of each tested fungi in the center of the plate. The check control was accomplished by growing one disc of the pathogen without any treatments. The plates were incubated at 27°C. The linear outgrowth of examined pathogenic fungi was estimated when the fungal cultivation covered the surface of any plate (each control). The most effective plant extracts were chosen for evaluation in a greenhouse experiment.

### Chemical Fungicides

#### Effect of Tolex 500 wpm and Premium via Fungal Linear Outgrowth

The effect of both Tolex 500wp (Tolclofos- methyl) and Premium fungicides on three pathogenic fungal spread were examined at the concentration of 0,10, 25,50, and 100 ppm according to the active ingredient. The prepared conc. of fungicide was added to the PDA medium just prior solidification and emptied into Petri-dishes. Three dishes were made for every treatment. The percentages of fungal outgrowth lowering were estimated by comparing the growth in various concentrations to the control treatment.

### Greenhouse Experiment

This experiment aimed to investigate the effectiveness of seed treatment in the best treatments of plant extracts, against the prevalence of damping off pea seedlings and survival plants under arti-

ficial soil with the most aggressive fungal pathogens. Pea seeds this study was done in sterilized pots (25cm), ten seeds for each one containing sterilized clay soil in the greenhouse. Both pots in addition to soil were sterilized with a 5% formalin solution. Plant extracts i.e., Neem and Ginger at the rate of 100%. as well as both fungicides (Tolex 500wp and Premium) at 100 ppm before sowing. A completely randomized design was used in this experiment. Soaked seeds were sown at rate of ten seeds per pot, six pots per replicate in each treatment. The inoculum of each of the three examined fungal pathogens was mixed with soil at a rate of 4% of soil weight. Disease incidences were recorded as percentages of pre-emergence and post-emergence damping-off in addition to the hygienic surviving plants in every treatment at 15, 30, and 45 days after sowing were assessed as mentioned before.

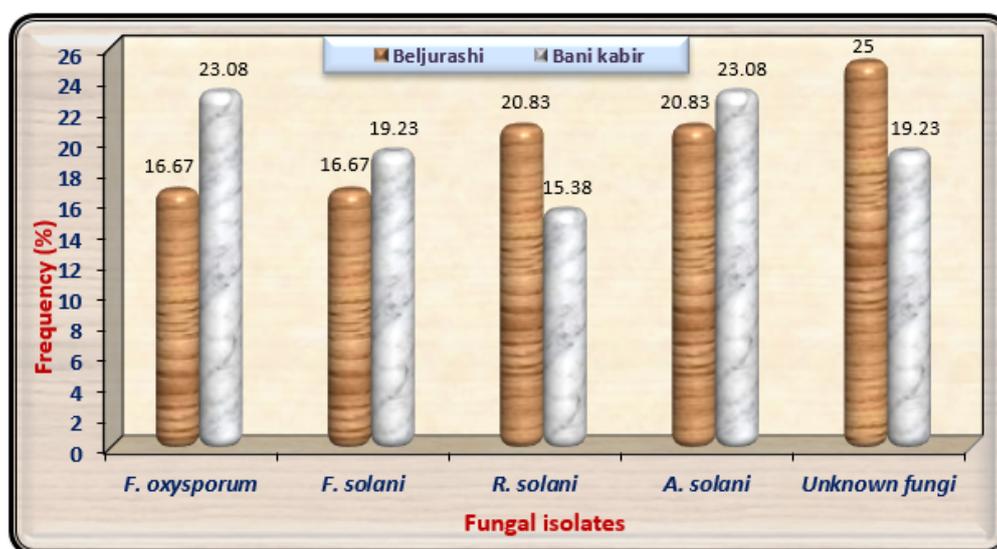
### Statistical analysis

All collected information was assessed or statistically analyzed using the software package [34] for comparing means at 5% in one way completely randomized.

**Table 1:** Isolated fungi and their frequency from pea plant roots appearing root rot disease symptoms at various fields in Beljurashi and Bani kabir Provinces.

Bani Kabir Root rot fungi	Beljurashi		Fungi isolated		Total
	*F	**F%	*F	**F%	
<i>Fusarium oxysporum</i>	4	16.67	6	23.08	10
<i>Fusarium solani</i>	4	16.67	5	19.23	9
<i>Rhizoctonia solani</i>	5	20.83	4	15.38	9
<i>Alternaria solani</i>	5	20.83	6	23.08	11
Unknown fungi	6	25.00	5	19.23	11
Total	24	100%	26	100%	50

\*F: Frequency, \*\*F%: frequency%

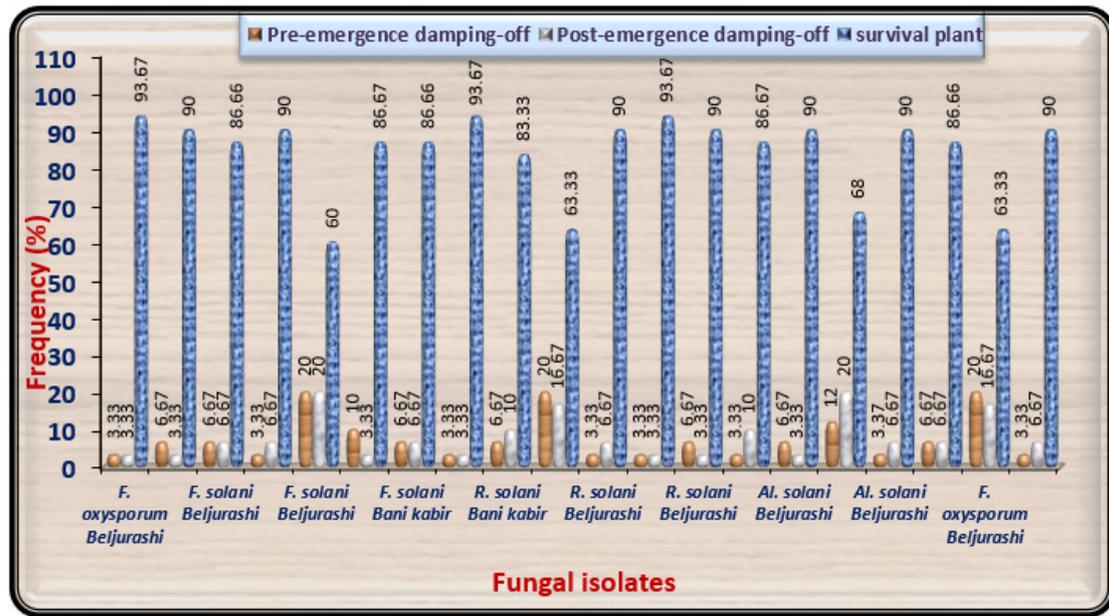


**Figure 1:** Isolated fungi from rotten roots of pea and their frequency (%) of Beljurashi and Bani Kabir governorates.

While *Fusarium oxysporum* and *Fusarium solani* came to an end with four isolates for both, which recorded 16.67% frequency. With respect to Bani kabir province, data in the same (Table) show that *Fusarium oxysporum* and *Alternaria solani* recorded the high isolates number “6 isolates for both” with high frequency “of 23.08%”. Both *Fusarium solani* and Unknown fungi came in the second order giving 19.23% frequency of both. However, *Rhizoctonia solani* came

lately by four isolates recorded 15.38% frequency. Totally, *Alternaria solani* and Unknown fungi recorded the highest fungal isolates giving eleven isolates for both in both provinces together. In this respect, *Fusarium oxysporum* came the second order recording ten isolates, followed by *Fusarium solani* and *Rhizoctonia solani*, which gave nine isolates for both.

**Pathogenicity Tests**



**Figure 2:** Pathogenicity test of 20 root-rot fungi isolated from pea roots collected from two governorates.

**Table 2:** Pathogenicity test of pre- and post-emergence damping-off in addition to survival of pea plants infected with isolated fungi (the fastest growing of twenty isolates).

Governorate	Isolated Fungi	Damping off %		Sur%
		Pre%	Post%	
Beljurashi	<i>F. oxysporum</i> (1)	3.33 c	3.33 d	93.67 a
Bani kabir	<i>F. oxysporum</i> (2)	6.67 bc	3.33 d	90.0 ab
Beljurashi	<i>F. solani</i> (1)	6.67 bc	6.67 d	86.66 bc
Beljurashi	<i>F. solani</i> (2)	3.33 c	6.67 d	90.0 ab
Beljurashi	<i>F. solani</i> (3)	20.00a	20.0 a	60.0 de
Beljurashi	<i>F. solani</i> (4)	10.0 b	3.33 d	86.67 bc
Bani kabir	<i>F. solani</i> (5)	6.67 bc	6.67 d	86.66 bc
Bani kabir	<i>F. solani</i> (6)	3.33 c	3.33 d	93.67 a
Bani kabir	<i>R. solani</i> (1)	6.67 bc	10.0 c	83.33 c
Bani kabir	<i>R. solani</i> (2)	20.00 a	16.67 b	63.33 d
Beljurashi	<i>R. solani</i> (3)	3.33 c	6.67 d	90.00 ab
Beljurashi	<i>R. solani</i> (4)	3.33 c	3.33 d	93.67 a
Beljurashi	<i>R. solani</i> (5)	6.67 bc	3.33 d	90.00 ab
Beljurashi	<i>AL. solani</i> (1)	3.33 c	10.0 c	86.67 bc
Beljurashi	<i>AL. solani</i> (2)	6.67 bc	3.33 d	90.00 ab
Beljurashi	<i>AL. solani</i> (3)	12.0b	20.0 a	68.00 e

Beljurashi	<i>AL. solani</i> (4)	3.33 c	6.67 d	90.00 ab
Bani kabir	<i>AL. solani</i> (5)	6.67 bc	6.67 d	86.66 bc
Beljurashi	<i>F. oxysporum</i> (3)	20.0 a	16.67 b	63.33 d
Beljurashi	<i>F. oxysporum</i> (4)	3.33 c	6.67 d	90.00 ab

Pathogenicity tests were performed under greenhouse conditions at Saeed El- Khazim farm in Beljurashi. Twenty fastest-growing fungal isolates without regard to the isolation place, including four isolates of *F. oxysporum*, six isolates of *F. solani*, five isolates of *R. solani*, and five isolates of *Alternaria solani* were examined for their pathogenicity on pea plants to select the most aggressive isolates to use in the further studies. Data in (Table 2) and illustrated in (Figures 2-2C) show that all examined fungi were pathogenic

and induced pre-emergence and post-emergence damping-off in pea seedlings. A high percentage of pre-emergence damping-off (20.0%) take placed under infected soil with *F. solani* No3 isolated from Beljurashi province and *R. solani* No2, which was isolated from Bani kabir province as well as *F. oxysporum* No three isolated from Beljurashi province. While *Alternaria solani* No three came in second order (Figure 2A).

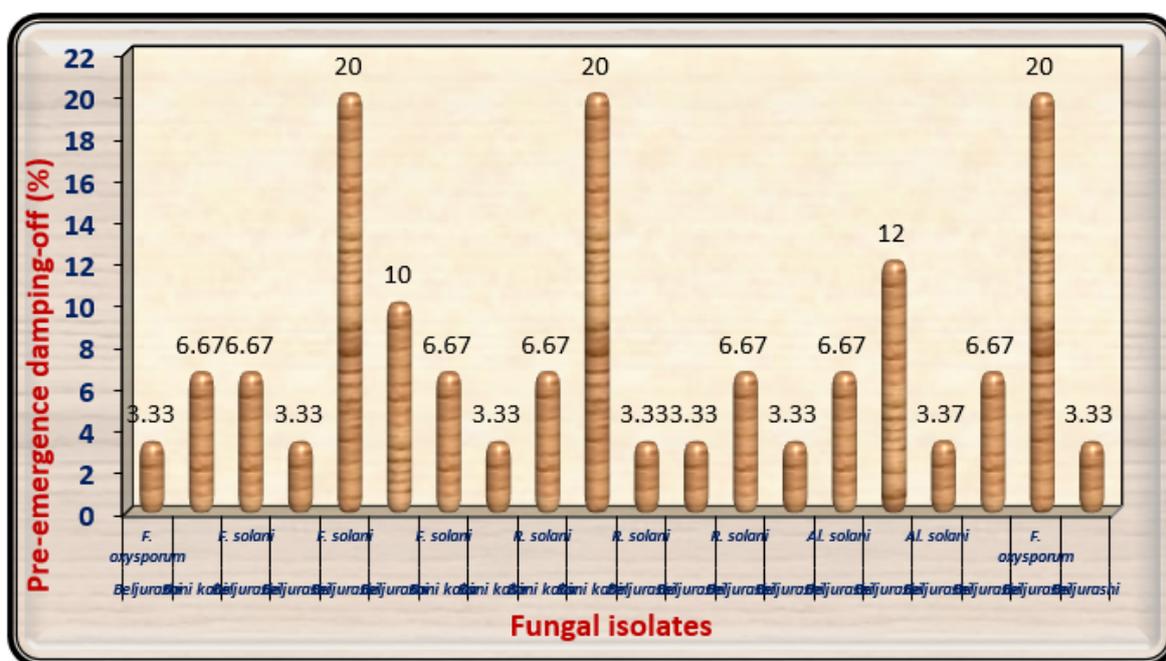


Figure 2A: Pathogenicity test of 20 root-rot fungi isolated from pea roots collected from two governorates.

On the other side, as a consequence of the pathogenicity tests on 30 days old pea seedlings as shown in (Figure 2B) indicated that both of *F. solani* No3 and *Alternaria solani* No 3 isolated from Beljurashi province were found to be the most hostile fungi on the base of post-emergence damping-off giving 20% infection. Moreover, *R. solani* No2 and *F. oxysporum* No3 came the second order in this respect giving 16.67% infection of post-emergence damping-off. Regarding healthy survival plants, it is clear from the same Table and illustrated in (Figure 2C) the lowest values of healthy survival pea plants after 45 days from sowing occurred under infected with

*F. solani* No3 (60%) accompanied by *R. solani* No2 equally with *F. oxysporum* No3 (63.33%). Moreover, *Alternaria solani* No3 came in the fourth order giving (68%). While other fungal isolates occurred a slight impact on this parameter. Based upon the seedling's mortality after 30 days and survival plants after 45 days from sowing, *F. oxysporum* No3, *F. solani* No3, and *R. solani* No2 were the most hostile and selected for further studies. Figures in the exact column that are accompanied by the same letter or letters do not differ significantly (p 0.05).

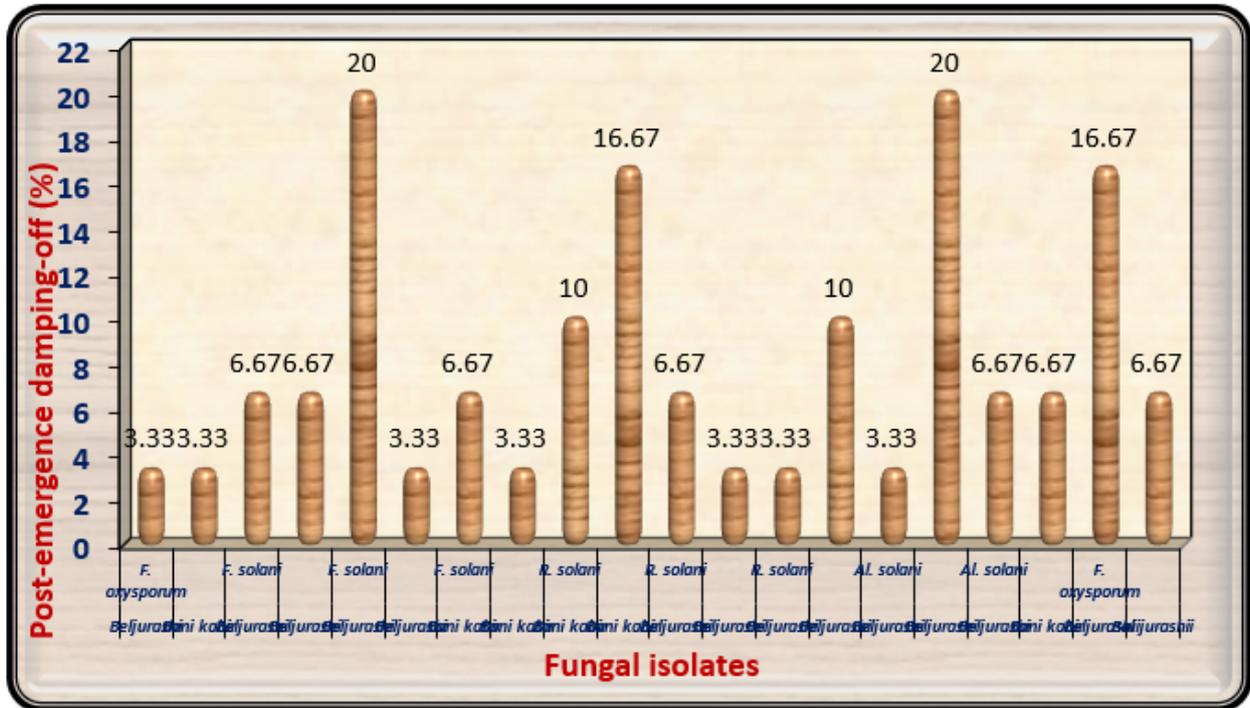


Figure 2B: Pathogenicity test of 20 root-rot fungi isolated from pea roots collected from two governorates.

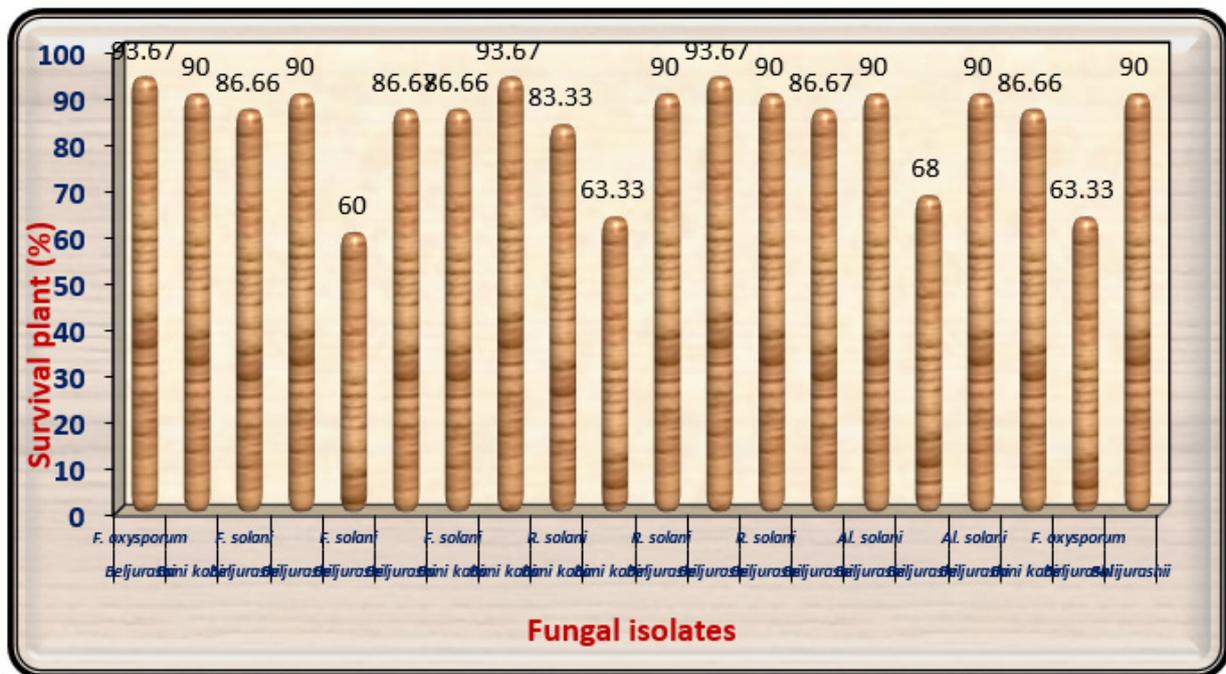


Figure 2C: Pathogenicity test of 20 root-rot fungi isolated from pea roots collected from two governorates.

**Plant Extracts**

The antifungal activity of plant extracts, i.e., Neem, Ginger; and *Artemisia* at concentrations of 0, 25, 50, 75, and 100% on fungal linear outgrowth of the examined pathogenic fungi are displayed in (Tables 3-3C) as well as illustrated in (Figures 3-3C). Data in (Ta-

ble 3A) and (Figure 3A) showed an opposite correlation between raising the concentration of neem extract from 0 to 100% and the linear outgrowth rate of three examined pathogenic fungi. By other means, by raising the concentration of neem extract, the linear growth rate of examined pathogenic fungi decreased. Neem extract

at both 75 and 100% concentrations prevented the development of *F. solani*. Moreover, the high concentration (100%) also wholly suppressed the linear growth of *F. oxysporum*. It induced more sup-

pression in the linear outgrowth rate of *F. solani* in comparison with *F. oxysporum* in addition to *R. solani*.

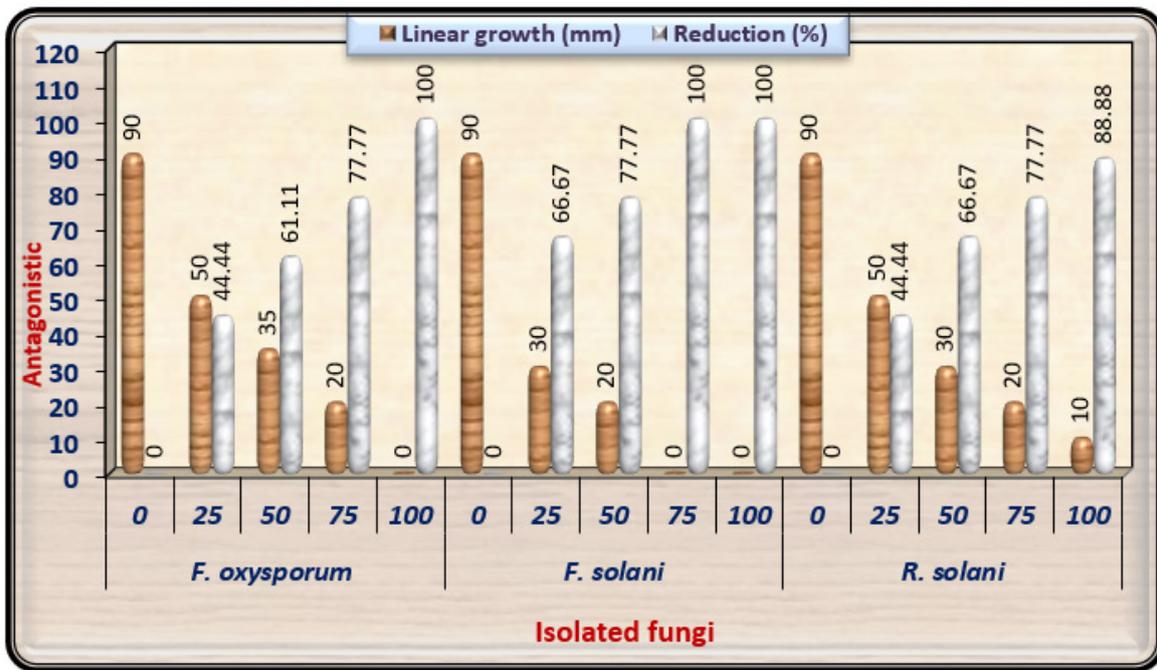


Figure 3A: Impact of plant extract (neam) at various conc. on linear outgrowth root-rot pathogens.

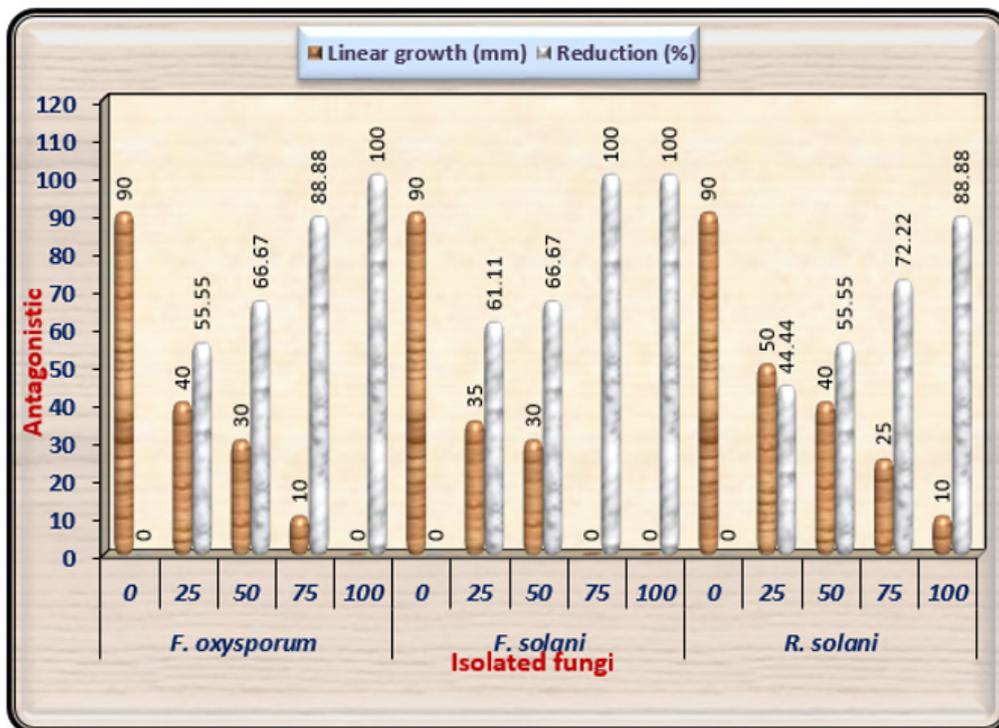


Figure 3B: Impact of ginger plant extract at diverse conc. on linear outgrowth root-rot pathogens.

The results exhibit that the genus *Rhizoctonia* demonstrated greater tolerance for neem extract than the genus *Fusarium*. The impacts of various concentrations of Ginger plant extract on fungal linear outgrowth of the examined causal pathogens of pea root rot disease are displayed in (Table 3B) and illustrated in (Figure 3B). Results indicate that a gradual increase in the concentration of Ginger extract from 25% to 100% led to lowering in mycelium linear outgrowth of three examined pathogenic fungi. The minimum growth of three tested pathogenic fungi appeared under the high concentration of Ginger extract (100%) followed by the concentration of 75%. Ginger extract at high concentrations wholly suppressed the linear outgrowth of *F. oxysporum* in addition to *F. solani*. Furthermore, the 72-5% concentration wholly suppressed the linear outgrowth of *F. solani*. And it is worth noting that *R. solani* was more tolerant to Ginger plant extract than both species of *Fusarium*.

The antifungal activity of *Artemisia* plant extract at various conc. on linear outgrowth of pea root rot pathogens is presented in (Table 3C) and illustrated in (Figure 3C). There is a negative correlation between raising the concentration of this extract as well as the development of the examined pathogenic fungi. Also, there is a clear difference among pathogenic fungi in their impact on different concentrations of *Artemisia* plant extract. *Artemisia* extract at high concentration showed an increased effect as complete inhibition of mycelium linear growth of *R. solani* only. At the same time, the same concentration gives 88.88% and 77.77% reduction of *F. solani* and *F. oxysporum* linear growth, respectively. The results of plant extracts found that *Artemisia* extract was the least efficacious on the linear outgrowth of the examined pathogenic fungi, so it is neglected in the greenhouse experiment.

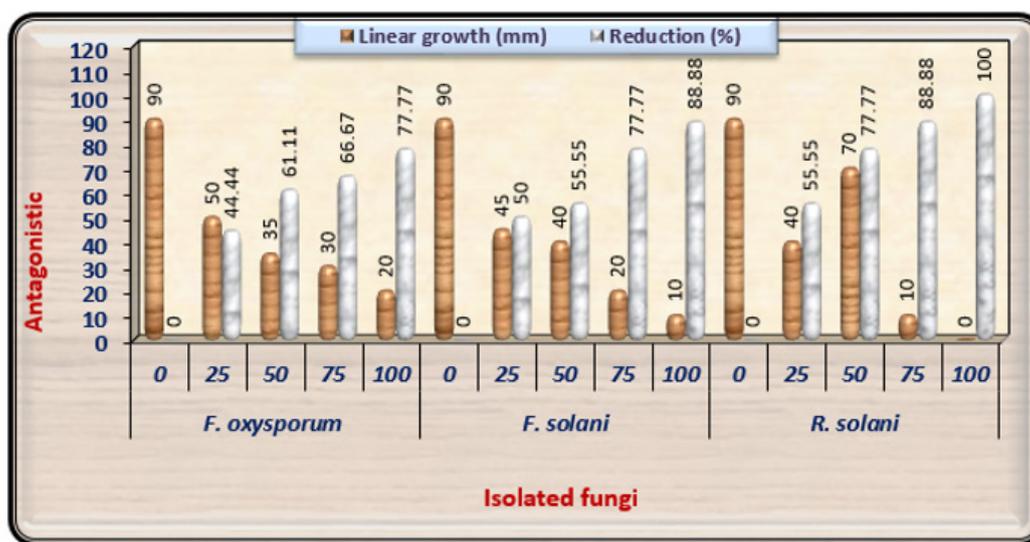


Figure 3C: Impact of artemisia plant extract at diverse conc. on linear outgrowth root-rot pathogens.

Table 3A: Impact of neem plant extract at various conc. on linear outgrowth of pea root- rot pathogens.

Plant extract	Fungi	Concentrations %	Linear growth	Reduction%
Neem	<i>F. oxysporum</i>	0.0	90.0a	0.0g
		25	50.0b	44.44f
		50	35.0c	61.11e
		75	20.0de	77.77c
		100	0.0f	100.0a
	<i>F. solani</i>	0.0	90.0a	0.0g
		25	30.0cd	66.67d
		50	20.0de	77.77c
		75	0.0f	100.0a
		100	0.0f	100.0a
	<i>R. solani</i>	0.0	90.0a	0.0g
		25	50.0b	44.44f
		50	30.0cd	66.67d
		75	20.0de	77.77c
		100	10.0ef	88.88b

**Table 3B:** Impact of ginger plant extract at various concentrations on linear outgrowth of pea root- rot pathogens.

Plant extract	Fungi	Concentrations %	Linear growth	Reduction %
Ginger	<i>F. oxysporum</i>	0.0	90.0a	0.0h
		25	40.0c	55.55f
		50	30.0de	66.67d
		75	10.0f	88.88b
		100	0.0g	100.0a
	<i>F. solani</i>	0.0	90.0a	0.0h
		25	35.0cd	61.11e
		50	30.0de	66.67d
		75	0.0g	100.0a
		100	0.0g	100.0a
	<i>R. solani</i>	0.0	90.0a	0.0h
		25	50.0b	44.44g
		50	40.0c	55.55f
		75	25.0e	72.22c
		100	10.0f	88.88b

**Table 3C:** Impact of Artemisia plant extract diverse concentrations on linear outgrowth of pea root- rot pathogens.

Plant extract	Fungi	Concentrations %	Linear growth	Reduction %
Artemisia	<i>F. oxysporum</i>	0.0	90.0a	0.0i
		25	50.0b	44.44h
		50	35.0de	61.11e
		75	30.0e	66.67d
		100	20.0f	77.77c
	<i>F. solani</i>	0.0	90.0a	0.0i
		25	45.0bc	50.0g
		50	40.0cd	55.55f
		75	20.0f	77.77c
		100	10.0g	88.88b
	<i>R. solani</i>	0.0	90.0a	0.0i
		25	40.0cd	55.55f
		50	20.0f	77.77c
		75	10.0g	88.88b
		100	0.0h	100.0a

## Chemical Control

### Effects of Tolex 500 wpm and Premium on the Linear Outgrowth of Pea Root Rot Pathogenic Fungi

Results are displayed in (Table 4) and illustrated in (Figure 4) shows the effects of various concentrations of both chemical fungicides used on the linear outgrowth of the examined pathogenic fungi. It was observed that the impact of both chemical fungicides on the linear outgrowth rate of tested pathogenic fungi differed according to the type of fungicide, concentrations, and treated fungi. The reduction percentage rose as the fungicide concentration rises irrespective of fungal species. Generally, Premium fungicide was

more efficacious in lowering the linear growth rate of all examined fungi than Tolex fungicide. Both high concentrations of Premium (75 and 100 ppm) prevented the outgrowth of *F. oxysporum* in addition to *R. solani*. While *F. solani* was less effective with the treatment of this fungicide, the high concentration only led to complete suppression of the linear growth rate of this fungus. On the other side, Tolex 500 wp fungicides at 100 and 75 ppm prevented the outgrowth of *R. solani*. And it is worth noting that *F. oxysporum* was less effective with Tolex 500 while *R. solani* was more sensitive. Based on these results, Tolex 500 and Premium at 100 ppm of both were used to evaluate under greenhouse conditions.

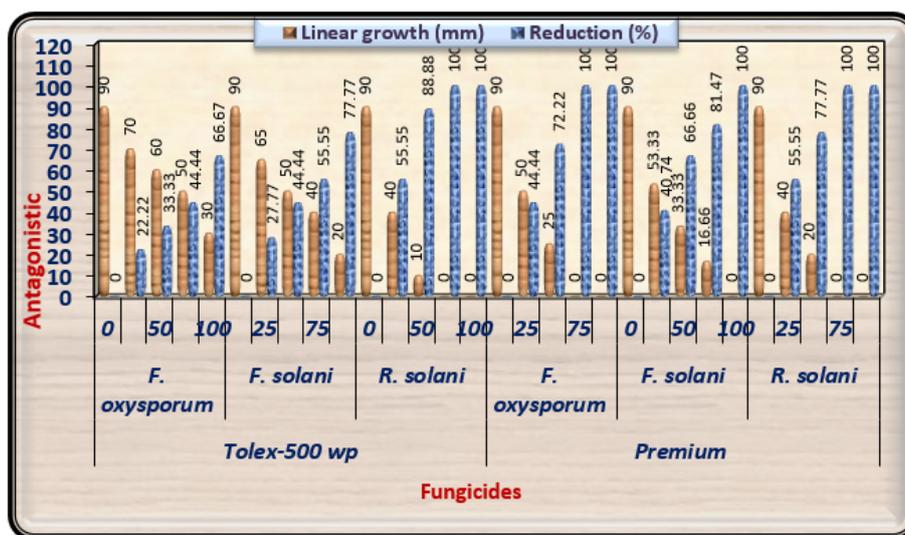


Figure 4: Effect of different fungicide Tolex-500 wp and premium on root-rot pathogenic in pea.

Table 4: Effect of fungicides (Tolex 500 wpm and premium) on linear outgrowth of pea root- rot pathogens.

Plant extract	Fungi	Concentrations %	Linear growth	Reduction %
Tolex 500	<i>F. oxysporum</i>	0.0	90.0a	0.0m
		25	70.0b	22.22i
		50	60.0c	33.33j
		75	50.0d	44.44h
		100	30.0fg	66.67f
	<i>F. solani</i>	0.0	90.0a	0.0m
		25	65.0bc	27.77k
		50	50.0d	44.44h
		75	40.0e	55.55g
		100	20.0fg	77.77d
	<i>R. solani</i>	0.0	90.0a	0.0m
		25	40.0e	55.55g
		50	10.0j	88.88b
		75	0.0k	100.0a
		100	0.0k	100.0a
Premium	<i>F. oxysporum</i>	0.0	90.0a	0.0m
		25	50.0d	44.44h
		50	25.0gh	72.22e
		75	0.0k	100.0a
		100	0.0k	100.0a
	<i>F. solani</i>	0.0	90.0a	0.00m
		25	53.33d	40.74i
		50	33.33f	66.66f
		75	16.66i	81.47c
		100	0.0k	100.0a
	<i>R. solani</i>	0.0	90.0a	0.0m
		25	40.0e	55.55g
		50	20.0hi	77.77d
		75	0.0k	100.0a
		100	0.0k	100.0a

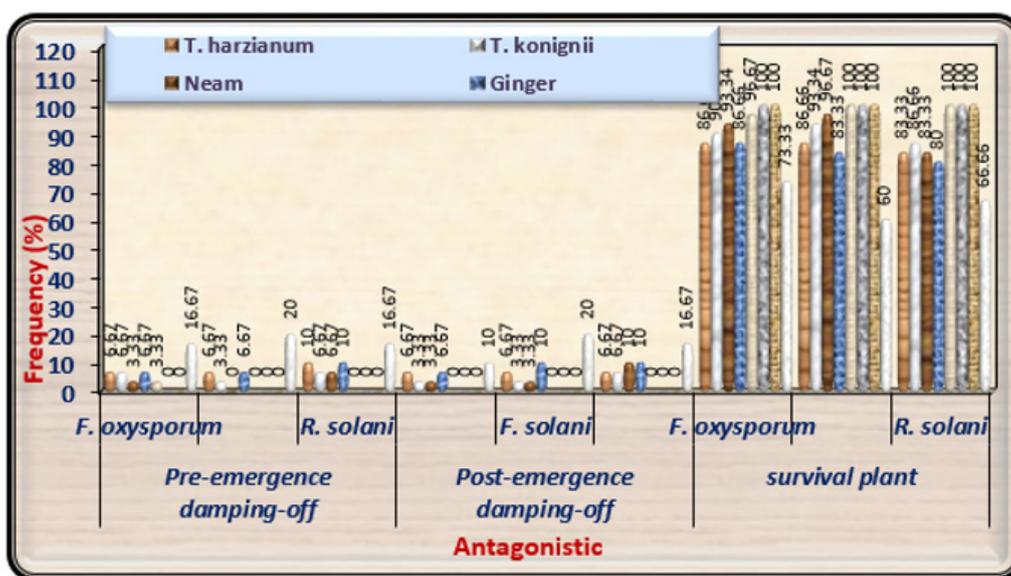
### Greenhouse Experiment

The purpose of this experiment was to examine the efficacy of seed the best treatments of plant extracts (Neem and Ginger at 100%), and fungicides (Tolex and Premium at 100 ppm) against the incidence of damping off on pea seedlings in addition to survival plants. The evaluation of both plant extracts, and chemical fungicide on disease prevalence (pre-emergence and post-emergence damping-off) of pea plants under infested with *F. oxysporum*, *F.*

*solani*, and *R. solani* were presented in (Table 5) and illustrated in (Figures 5-5C). All tested treatments considerably lowered damping-off disease and significantly increased healthy survival plants compared with infected control under three tested pathogenic fungi. Generally, premium fungicide was more efficient in this regard, accompanied by Tolex 500 fungicide, then Neem extract, Ginger extract came at least in order without significant differences between them. Concerning healthy control (uninfected) grown in sterilized soil, it was observed that no recorded disease incidence.

**Table 5:** Influence of bio control agents, plant extracts and fungicides on pea seedling under greenhouse conditions.

Treatments	Pre- emergence damping of %			Post- emergence damping of %			Survival plant %		
	<i>F. oxysporum</i>	<i>F. solani</i>	<i>R. solani</i>	<i>F. oxysporum</i>	<i>F. solani</i>	<i>R. solani</i>	<i>F. oxysporum</i>	<i>F. solani</i>	<i>R. solani</i>
Neem	3.33c	0.00 c	6.67c	3.33c	3.33d	10.00 b	93.34 abc	96.67 ab	83.33 bc
Ginger	6.67b	6.67b	10.00 b	6.67b	10.00 b	10.00 b	86.66c	83.33 c	80.00 c
Tolex 500	3.33c	0.00 c	0.00 d	0.00 d	0.00 d	0.00 d	96.67ab	100.00 a	100.00a
Premium	0.0d	0.00 c	0.00 d	0.00 d	0.00 d	0.00 d	100.00 a	100.00 a	100.00 a
Control (uninfected)	0.0d	0.00 c	0.00 d	0.00 d	0.00 d	0.00 d	100.00 a	100.00 a	100.00 a
Control (infected)	16.67a	20.0a	16.67a	10.00 a	20.0a	16.67a	73.33d	60.00 d	66.66d



**Figure 5:** Influence of biocontrol agents, plant extracts and fungicides on pea seedling under greenhouse conditions.

About survival plants, Premium fungicide nullified the effects of all pathogenic fungi on healthy survival plants leading to 100% healthy plants under-infested with the three pathogenic fungi. Moreover, Tolex 500 fungicide overcomes the harmful effects of *F. solani* and *R. solani* giving 100% healthy plants, but it is providing 96.67% healthy plants under infested with *F. oxysporum*. As shown in (Table 5) and (Figure 5A), pre-emergence damping-off of pea seedlings after fifteen days from sowing was decreased significantly due to the application of all treatments used at any dose under all infested soil by three tested pathogenic fungi as compared

with infected control. Premium fungicide was more effective in this respect, where it completely inactivates the fungal pathogens, followed by Tolex 500. In relation to the effects of bio and natural additives on infected pea seedlings by pre-emergence damping-off, Neem extract was the most efficacious hence nullifying the injurious effect of *F. solani*. In contrast, both bio additions and Ginger extract come in the second order without significant differences. On the other side, both fungicides lead to the complete nullification of the tested pathogenic fungi in occurrence post-emergence damping-off, as shown in (Figure 5B).

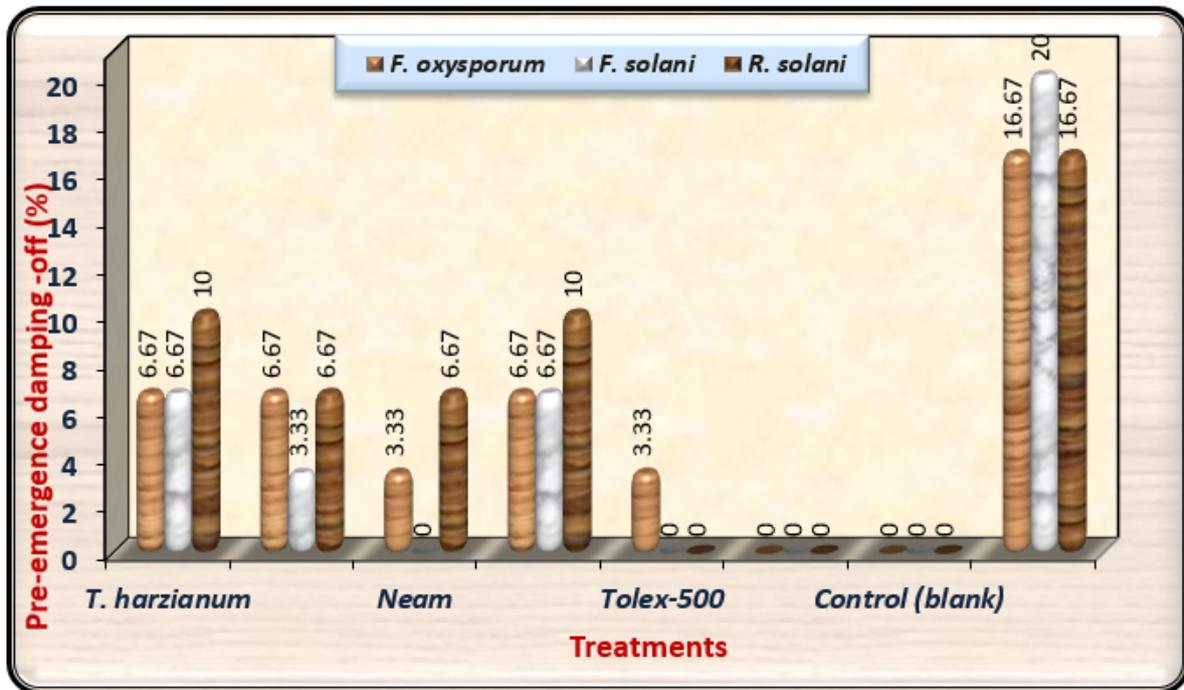


Figure 5A: Influence of biocontrol agents, plant extracts and fungicides on pea seedling under greenhouse conditions.

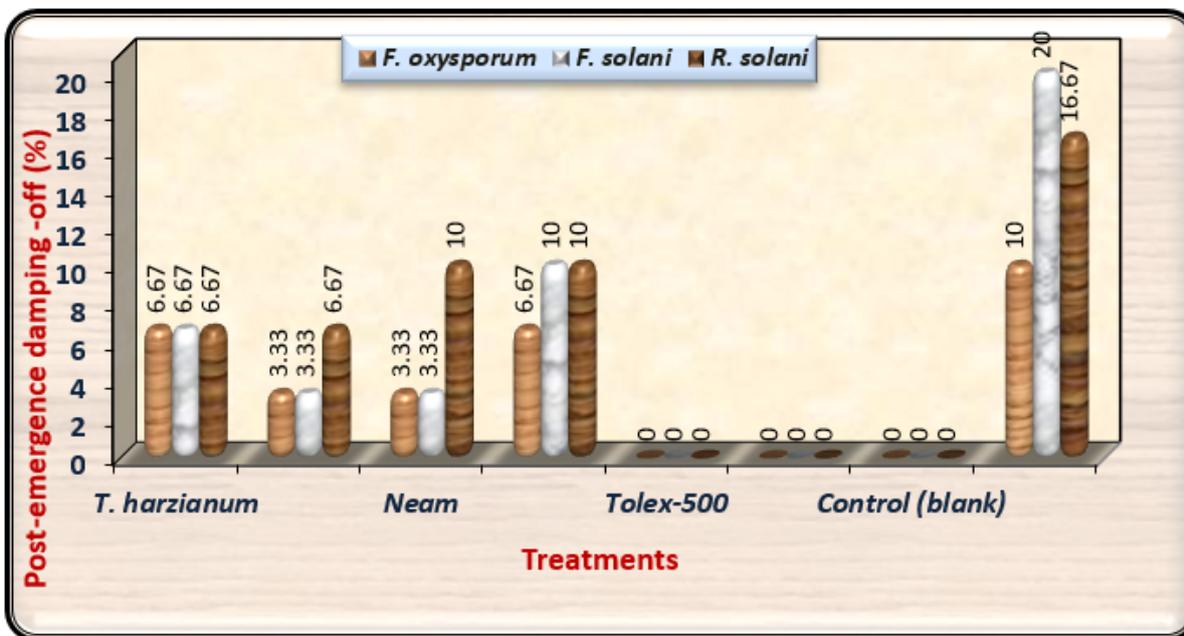
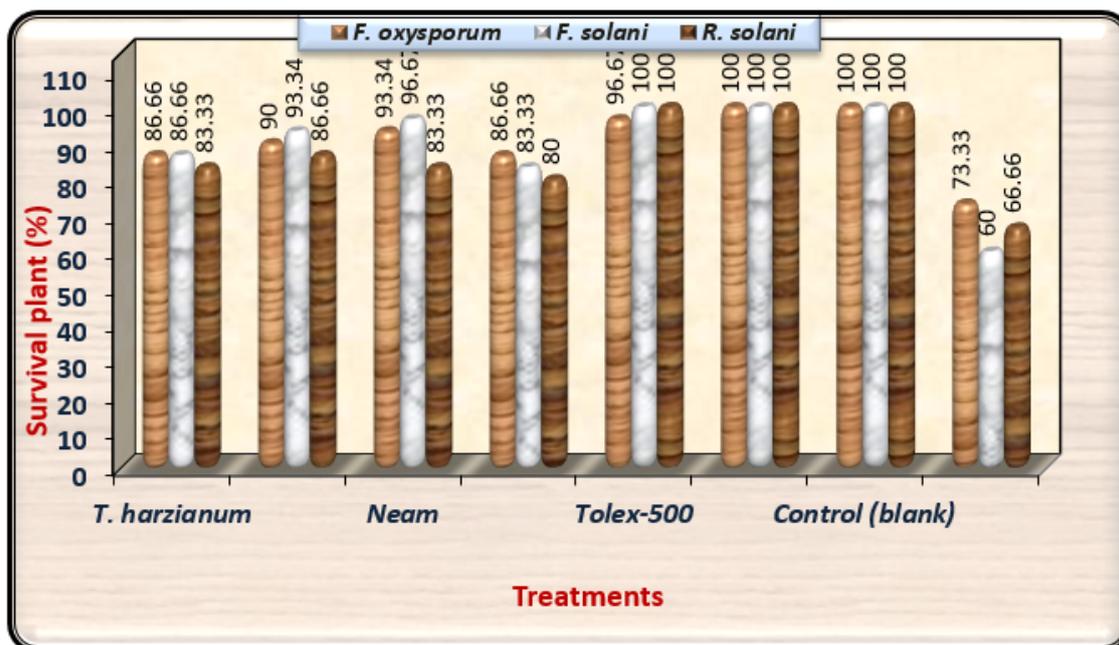


Figure 5B: Influence of biocontrol agents, plant extracts and fungicides on pea seedling under greenhouse conditions.



**Figure 5C:** Influence of biocontrol agents, plant extracts and fungicides on pea seedling under greenhouse conditions.

## Discussion

Pea (*Pisum sativum* L) is a crop high in protein and fixes atmospheric nitrogen. It is considered one of the plants that many countries cultivate, in addition to containing a high percentage of protein and carbohydrates and some vitamins. The pea plant is very important for its nutritional value for humans in addition to animals then plays a critical role in reducing cholesterol in the blood. Most countries in the world grow it for consumption in the form of vegetables because this plant is a significant source of protein and some fundamental elements. Through previous studies, it was found that several factors contribute to a decrease in the yield of the pea plant, like wilt diseases and root rot, which is considered the most effective disease because it is transmitted through the soil, which increases the damage resulting from many pathogens. Thus, these causes affect the growth of the crop and decrease its production. The pea plant is considered one of the plants that are severely affected by root rot disease, which is transmitted through the soil [35].

Wilting and seedling death are diseases induced by many fungi present in the soil that cause diseases, such as *Sclerotinia*, *Fusarium*, *Thielaviopsis*, *Aphanomyces*, *Rhizoctonia*, *Pythium* and *Phoma* [36]. In this research, fifty isolates of root rot pathogens were isolated from the roots of pea plants exposed to natural infection from different sites in the Beljurashi and Bani Kabir governorates in the Kingdom of Saudi Arabia. 24 fungal isolates were isolated from Beljurashi in addition to 26 from the yard. It was observed that fungal species were identified from four species of three genera (*Fusarium*, *Rhizoctonia*, and *Alternaria*). In all, *Alternaria solani* recorded the highest number of fungal isolates, which gave 11 isolates in

both counties together, while *Fusarium oxysporum* came in first place. The second order recorded ten isolates, accompanied by *Fusarium solani* then *Rhizoctonia solani*, which gave nine isolates for both. These outcomes matched those that were noted by [37,38].

The difference in the number, frequency, and species of fungal isolates according to the isolated province may outcome from the variation in soil texture, moisture, and composition of the soil, as well as the varying capability of fungal genera to thrive and adjust to diverse conditions. The present results of the pathogenicity tests study suggested that the twenty examined fungal isolates were pathogenic in addition to inducing pre- and post-emergence damping-off in pea seedlings. The largest proportion of pre-emergence damping-off (20.0%) happened under infected soil with *F. solani* No3 isolated from Beljurashi province and *R. solani* No2 isolated from Bani kabir Province as well as *F. oxysporum* No3 isolated from Beljurashi province. While *Alternaria solani* No3 came in second order. On the contrary, findings obtained from the pathogenicity experiments conducted on thirty-day-old pea seedlings revealed that both *F. solani* No3 and *Alternaria solani* No3 isolated from Beljurashi province were found to be the most aggressive fungi because of post-emergence damping-off accompanied by *R. solani* No 2 then *F. oxysporum* No 3.

The lowest values of healthy survival pea plants after 45 days from sowing occurred under infected with *F. solani* No 3 accompanied by *R. solani* No2 equally with *F. oxysporum* No3. Generally, *F. oxysporum* No 3, *F. solani* No3, and *R. solani* No2 were the most aggressive and selected for further studies. These results may be due to genetic variance by many fungal isolates. These outcomes are consistent with those achieved by [39-42]. In addition, the vari-

ation in pathogenic ability by many fungal isolates could be due to genetic variations of them. The negative consequences of the tested pathogenic fungi are caused by seed rot and damage, in addition to root system death, which reduces the absorption surface or consumption of water in addition to essential nutrients [43]. This is agreed with the present study where the three examined pathogenic fungi induce the destruction of pea root structure, in turn, causes damping-off. Moreover, [44] resulted that the negative consequences of fungal pathogens may be caused by enzyme formation, which results in rotten lesions on seed cotyledons, seed rot, and plumule soft rot, which causes damping off.

As for plant extracts, the outcomes revealed that all extracts that were used had the ability to inhibit pathogens at any concentration, where there is a negative relationship between plant extracts and pathogens from zero to 100% f. In this regard, 100% neem and ginger were the most efficient, so it was used for the greenhouse experiment. Neem extract at a concentration of 75 and 100% suppressed the outgrowth of *F. solani*. Moreover, the high concentration (100%) suppressed the linear outgrowth of *F. oxysporum*. It caused more significant inhibition of the linear outgrowth of *F. solani* compared to *F. oxysporum* and *R. solani*. So ginger extract has been shown to increase the inhibition rate of pathogens of *F. oxysporum* in addition to *F. solani* completely. Also, the 5-72% concentration suppressed the linear outgrowth of *F. solani*. Under greenhouse conditions, neem extract was the most effective and thus had the most substantial effect on *F. solani*. It is also most effective in reducing damping off after pea emergence 30 days after planting under soil infested with both types of *Fusarium*.

These results are like those of [45] they found that garlic oil has antimicrobial in addition to antiviral activities [46] tested some oils for inhibition of the growth of many pathogens and found that garlic juice suppressed linear growth rate of *F. moniliforme*, *Helminthosporium Oryza*, *Sclerotium bataticola*, *Penicillium italicum*, *Alternaria citri*, *Aspergillus niger* as well as Clove juice, suppressed the formation of *Macrophomina phaseolina* sclerotia. Plant extracts can produce secondary metabolites, and some of them, in addition to their derivatives, have antimicrobial properties, like phenolic compounds, which may sensitise phospholipids, preventing fungi movement [47-50] used thyme, cumin, clove, and rosemary essential oils which prevented the spread of *Aspergillus parasiticus* and aflatoxin formation. The effectiveness of different plant extracts towards fungal pathogens differs due to their solubility in water and/or the existence of inhibitors to fungi toxic principle in the composition of plant extract.

In this connection, [51] stated that garlic oil possesses antifungal as well as antioxidant properties due to includes sulfur, phenolic compounds, terpenoids, allicin, and saponins [52] stated that there is an antifungal activity of many plant extracts i.e., Garlic, *Artemisia*, *Capsicum*, *Caraway*, *Fleabane*, *Cumin*, *Camphor*, *Anise*, *Black piper*, and *Thyme* against *Aspergillus niger* and *F. oxysporum* the causal pathogens of onion rot. Some plant oils i.e., *caraway*, *cumin*, *coriander*, and *fennel* completely suppressed the linear outgrowth of *Rhizoctonia solani*, *Fusarium oxysporum*, and *Sclerotium rolfsii* [53]. Also, seed treatment with garlic and neem extracts were the

most efficacious treatment for dominating the damping-off disease of bean due to activated soil microbes, decreasing the population of *R. solani* [54,55]. When fungicides are used to study their effect on pathogens according to the type of fungicide and its concentration lowered the growth of pathogens. The growth rate of pathogens lowered with a rise in the concentration of the pesticide. Overall, the premium fungicide was more effective in lowering the linear spread of all fungi examined than the Tolex fungicide.

Both high concentrations of Premium (75 and 100 ppm) suppressed the spread of *F. oxysporum* in addition to *R. solani*. Tolex 500 wp fungicides at 100 and 75 ppm inhibited the spread of *R. solani*. It is noteworthy that *F. oxysporum* was less effective with Tolex 500 treatment while *R. solani* was more sensitive. Also, under the greenhouse, this premium fungicide was most efficacious in reducing damping-off disease, thus significantly increasing the healthy survival of the plants. The effects of the differences in chemical fungicides may be due to a selective reaction between the fungicide and the fungus. These outcomes are rather like those presented by [56-58] and suggested that the utilization of a fungicide as a seed treatment reduces the incidence of root rot due to the anticipated decomposition of this fungicide when introduced. soil and its exposure to environmental conditions. The decrease in harmful effects of fungal pathogens with the use of both fungicides may be due to the active ingredient in the fungicide formulation degrading in the fungal medium and causing toxicity.

## Conclusions and Recommendation

From the foregoing results and discussions, it is possible to conclude that the use of neem extract from plant extractions could be utilized as substitute methods and antifungal agents towards fungal pathogens (*F. oxysporum*, *F. solani* or *R. solani*) which were discovered to be associated to symptoms of pea damping off and root rot diseases.

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