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# The Effectiveness of Chemical Inducers in Controlling Root Rot Disease in Maize and Compared with Fungicides

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**Abstract:** In the current study, abiotic agents (salicylic, oxalic, and benzoic acid) were used to examine the antifungal activity of these agents against maize root rot pathogens (*F. oxysporum, F. solani* or *R. solani) in vitro*. For these reasons, the experiments were done in the laboratory. Activity of antioxidants (salicylic, oxalic, and benzoic acid); pathogenic fungi there are significant differentiations among antioxidants and their concentrations. The linear growth of *F. oxysporum, F. solani,* and *R. solani* was decreased with increasing the concentration of antioxidants. in other meaning, there is an inverse relationship between increasing conc. of antioxidants and the growth of the examined pathogenic fungi. Generally, the minimum growth of three tested pathogenic fungi appeared under the high concentration of salicylic acid then a high oxalic level the high concentration of benzoic. Study of the tested fungicide, generally there is a negative relation between increasing the conc. of fungicide Tolex 500 wpm from 0 to 100 ppm and the linear growth of all studied pathogenic fungi. The high level (100 ppm) of fungicide suppressed the growth of all examined fungi. Also, there were variances in the sensitivity of the pathogens to fungicide concentrations; with *F. solani* being the most sensitive fungus.

**Keywords:**.Pathogens- Antioxidants- Fungicide Tolex 500 wpm

## INTRODUCTION

Maize are infected by many pathogenic fungal diseases worldwide, which greatly limit crop production. Fusarium spp. It has shown the most severe diseases affecting many commercially domesticated grassy crops. A chemical fungicide to control it seems important because seeds and soil that help the transmission of the disease. Potent antifungal formation of bioagents including antifungal microorganisms has been reported to be useful for the biocontrol of these fungi in pulses under field conditions. However, the effect of biological control affects microbial and plant interactions and the ecological fitness of the bioagents. Abdel-Razek et al., (2012) It was found that the symptoms of root rot are dwarfism. It was found that the symptoms are rot in the roots. change in the color of the plants, short stem size, and the plants become weak and easy to infect more than disease-resistant plants, and in many cases, the roots disappear. Delegation the cause of root rot disease was determined by the Rhizoctonia fungus. Root growth and development are weak. It includes obvious symptoms of root rot and its color to brown. Mackenzie and Williams, (2015) found that yield losses due to fungal diseases have been estimated depending on the environmental conditions surrounding the plant. Yield losses are due to the presence of different pathogens up to 60%. Da Silva, et.al (2017) found that maize (Zea mays L) is a crop of most significant cereals in the world. Both biotic and abiotic factors have an impact on the yield of this crop, as phytopathogenic fungi play a crucial role by lower yields levels and causing massive international economic damage. The phytopathogenic fungus Rhizoctonia solani is a significant maize pest that attacks all underground parts of the plant, including seeds and roots. In maize, R. solani is the causative agent of leaf in addition to sheath blight, although there are other symptoms such as leaf and sheath blight. Rashad and Moussa, (2020) found that pathogens cause huge losses in agricultural production and cause huge losses in many countries, and that necessary measures must be taken to maintain manufacturing to supply a growing population needs. The pathogens led to a significant decrease in the crop, therefore, the injury leads to many economic losses. Nohra et al., (2021) Legumes are infected by many pathogenic fungal diseases worldwide, which greatly limit crop production. Fusarium spp. It has shown the most severe diseases affecting many commercially domesticated legume crops. A chemical fungicide to control it seems important because seeds and soil that help the transmission of the disease. Potent antifungal formation of bioagents including antifungal microorganisms has been reported to be useful for the biocontrol of these fungi in pulses under field conditions. However, the effect of biological control affects microbial and plant interactions and the ecological fitness of the bioagents. Hence, the present evaluation aims to provide summary data on Fusarium pathogenesis, fungal-host pathogen interactions, and biological control strategies in relation to legume crops. Shahda, (2000) stated that the monitoring of root rot disease in various





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crops can be made by different strategies, among which antioxidants in addition to biocontrol techniques are the most commonly recognized at the moment. Benzoic, salicylic, and ascorbic acid decreased the linear growth of Fusarium oxysporum, F. solani and Rhizoctonia solani then decreased the spore germination of Fusarium spp. The three antioxidants significantly reduced damping off. El-Moogy, (2002) stated that salicylic acid in addition to acetyl salicylic acid demonstrated antimicrobial effects on some fungal in addition to bacterial pathogens of plants then prevented their growth at certain concentrations in laboratory conditions. Application of salicylic acid (SA) in addition to acetylsalicylic acid (ASA) as a seed dressing or soil drench has decreased root rot infection of plants under greenhouse factors. El-Gendy et al., (2016) found that antioxidant-induced resistance is also a viable strategy for disease control caused by soil-borne pathogens. Systemic resistance induced by salicylic acid and oxalic acid in maize against root rot has been reported in environments under artificially controlled conditions. However, the use of bioremediation of seeds to inhibit root rot from soil-borne pathogens as an alternative to chemical fungicides is tolerable without causing any harm to humans, animals, or the environment. To mitigate the negative impact of root rot caused by Fusarium solani, Rhizo Bruisson, (2019) found that the focus is on developing strategies based on microbes that cause destructive plant diseases along with resistant cultivars. When applying anti-internal microorganisms is another important aspect of crop protection. Through a multitude of generally complex biochemical processes and signals, the mechanisms employed by endogenous cells include the production of plant growth regulators, like gibberellins, abscisic acid, cytokinins or auxins ctonia solani makes sense. Jarvis, (1988) stated that controlling pathogens with fungicides assured to be very difficult in addition to almost all fungicides are active only at phytotoxic levels. El-Mougy et al., (2013) Control of fungal diseases is achieved using fungicides that are dangerous or harmful to both humans and pets and result to environmental pollution.El-Gazzar et al., (2018) showed that significant environmental threats of fungicides were a main cause for promising biological or it is bio factors as well as suggesting it is utilization in the field to control plant fungal diseases. Trichoderma viride, mycorrhizae (Glomus mosseae) or Chitosan NPs, have been used individually or in combination to monitor maize late wilt caused by Fusarium oxysporum. Tested treatments offered an important reduce in disease incidence.Abdeljalil et al., (2021) stated that Fungicides are one of the most used methods to reduce the spread of pathogens. In most cases, agrochemicals are usually expensive or dangerous. Consistent application of fungicides may also lead to improve of fungicide resistance by the pathogen in many commercially domesticated legume crops. The chemical fungicide controlling it seems a task because seeds and soil are a means of transmitting the disease. Strong antifungal creation resulting from bio-agents including antagonistic microorganisms reported to be beneficial for bio control of this fungus in the pulses under field conditions. However, the biological controls impact is the effect on plant–microbial interactions and the ecological fitness of the bio-agents. Hence, the current assessment aims to offer brief data on Fusarium diseases, host–fungal pathogen interactions, and their bio controlling strategies in relation to the legume crop.

Aim of the study

This study aimed to avoid the use of fungicides and reduce infection by biological control and natural stimuli in maize plants and to fight pathogens using antioxidants

## MÉTHODOLOGY

**Isolation and identification of the pathogens:**

Samples of maize plants bearing clear signs of damping–off, root rot, and wilt were combined from dissimilar locations in Al Baha province, Saudi Arabia. The roots that were infected with diseases were prepared by washing them with tap water, then dividing them into small parts (about 1 cm). Thus, the diseased roots are prepared. Infected roots were divided into small pieces and then placed in a 0.5% sodium hypochlorite solution for two minutes, then put in sterile water for one minute and between two filter papers for drying. The prepared plant roots were placed in Petri dishes containing medium (PDA) with Add the antibiotic streptomycin 0.01% to prevent bacterial contamination. The dishes were positioned in the incubator at 25degrees Celsius and preserved at 4°C in a refrigerator for subsequent studies. After five days, each isolate



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was purified by isolating the tip of the mycelium after colonies were removed from the fungal mycelium Sinclair and Dhingra, (1985).

**Purification of pathogenic fungi:**

Fungi are purified by taking a part of the fungal mycelium and growing it on was a pure PDA environment. The resultant growth of the isolated fungi was identified depending on their morphological characteristics with light microscope description of Gilman (1957); Booth, (1985), and Barnett and Hunter, (1986). He made an estimate of each type of pathogenic fungi that was isolated and calculated the frequencies for each of them.

**Pathogenicity tests**:

Pathogenicity tests were performed *in vivo* AI-Baha – Shatiba 2022 Four isolates of *F.oxysporum* , three isolates of *F.solani*, and five isolates of *R.solani*. These isolates were tested to select fungi that have a high ability to cause infection. These seedlings (25 cm) were disinfected with 5% formalin for 15 minutes before being utilized in culture and then left in the open air until dryness. The inocula of the isolated fungi were prepared on sand-barley (SB)medium (25g clean sand,75g barley, and sufficient water to encompass the mixture) the additive mixed and bottled then autoclaved for 20 minutes at 1.5 air pressure, flasks containing sterilized medium were inoculated with agar discs acquired from the periphery of each isolated colony of 5-day-old fungi It was incubated at 25 °C for two weeks. By thoroughly mixing the soil with a 5% formalin solution, the soil was sterilized. Then the treated soil was coated plastic sheet for a week and then the plastic sheet was taken away to permit for this complete evaporation of formalin Whitenhead, (1957) soil infestation with each individual fungal was carried out at the rate of 3% of soil weight Metwelly,(2004) soil mixed thoroughly with fungal inoculum then watered daily for one a week to boost fungal growth, the soil of monitoring pots were blended with the same amount of sterilized fungus-free sand- barley (SB)medium, Apparently healthy of maize seeds were surface sterilized using 2% sodium hypochlorite for 2 min, washed varied times with sterilized water than sow at an average of 10 seeds per pot, Three replicate pots with a total of 30 seeds were used for each treatment disease assessment:

The deadening percentages per and post emergence above the soil surface were determined as well as the percentage of surviving plants 15, 30, and 45 days after sowing, respectively, by the equation based on El-Helaly *et al*., (1970).

Per-emergence (%) =Number of non-germinated seeds "at15days" / a Total number of sown seeds ×100.

Post-emergence (%) = Number of dead seedlings "at 30 days" / a Total number of sown seeds ×100.

Survived plant (%) =Number of survived plants "at 45 days" / a Total number of sown seeds ×100.

***In-vitro* evaluation of antioxidants on the linear growth of pathogenic fungi:**

The impact of the examined antioxidants i.e., oxalic acid, salicylic acid, and benzoic acid at various concentrations. were examined to know their impact on the mycelial linear growth of pathogenic fungi *in vitro.* Three concentrations i.e,0,5,10 Mm were ready to use then added separately to conical flasks containing sterilized PDA medium to achieve the suggested concentrations, then mixed gently and dispensed in sterilized petri plates. Plates were individually inoculated at the center with equal disks (5mm) and 25°c. for 7days.The average of the fungal linear growth was then enumerated Saber M.M.*et al.*, (2009).

**Effect of the fungicide on Tolex 500 wpm on the linear growth of filamentous pathogenic fungi:**

The impact of Tolex 500 wpm (Tolclofos- methyl) on the pathogenic fungal growth was examined at the concentration of 0,10,25,50 and 100 ppm relied on the active additive. The prepared concentration of fungicide was added to the PDA medium just before solidification and poured in Petri- dishes. In each treatment 3 replicates were used. Three Petri-dishes were used as replicates for each treatment. The percentages of reduction in fungal growth were counted as the growth in various concentrations versus to that in check treatment.

## RESULTS:

**Frequency of fungal pathogens:**

Thirty fungal isolates were isolated from maize root showing typical symptoms of root rot grown under natural infection in AL-Baha Province, Saudi Arabia. It is clear from Table (1) that the fungal isolated which were identified included three fungal genera (*Rhizoctonia*, *Fusarium*, and *Alternaria*) while two fungal isolates did not identified *Fusarium* genus came the highest frequency (18 isolates) and included four species viz., *oxysporum* “5 isolates”, *solani* “4 isolates”,



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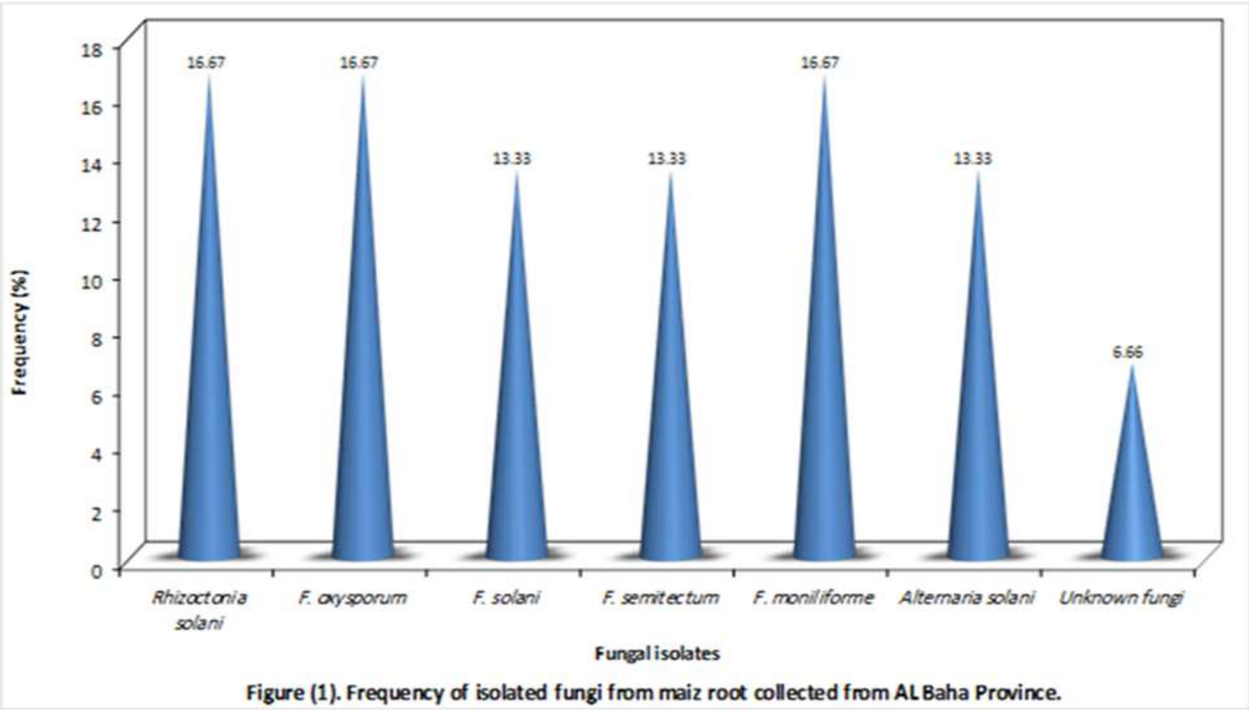
*semitectum* “4 isolates” and *moniliforme* “5 isolates”. Generally, *Rhizoctonia solani*, *F.oxysporum*, and *F.moniliforme* recorded the superiority in frequency percentage (16.67%).

**Table (1):** Frequency of isolated fungi from maize root collected from AL Baha Province

| **Fungal isolates** | **AL Baha** | |
|---|---|---|
| **Root–rot Fungi** | ***F** | **F%**** |
| ***Rhizoctonia solani*** | 5 | 16.67% |
| ***F.oxysporum*** | 5 | 16.67% |
| ***F.solani*** | 4 | 13.33% |
| ***F.semitectum*** | 4 | 13.33% |
| ***F.moniliforme*** | 5 | 16.67% |
| ***Alternaria solani*** | 4 | 13.33% |
| **Unknown fungi** | 2 | 6.66% |
| **Total** | 30 | |

***F: Frequency**
****F%: Frequency%**




**Figure(1).** Frequencyofisolated fungi from maiz .



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**Pathogenicity tests:**

Testing for pathogenicity *F. oxysporum* isolates four, *F. solani* isolates three, and *R. isolates* five. For their ability to be the pathogenic agents causing pre- and post-emergence damping-off in maize plants. Information in Table (2) showed that the most examined isolated fungi were pathogenic then causes typical signs of both pre and post-emergence damping-off with different degrees. *R. solani* No1 was the most aggressive isolate on the base of seedlings mortality recorded at 60% survival plants. *F.oxyporum*(3) came next followed by *F.solani*(3) giving 63.33% and 70.0% survival plants, respectively. It is worthy to mention that *F.oxyporum*(1) had no effect on pre-emergence damping-off. Moreover, the same trend occurred under *F.oxyporum*(4) and *F.solani*(2) in post-emergence damping-off. Hence, the highest percentages of survival plants (96.67%) was recorded under both infection of *F.oxyporum*(1) and *F.oxyporum*(4) then *F.solani*(2) which gives (93.33%). Based on these results *R. solani*(1), *F.oxyporum*(3), *and F.solani*(3) were selected for further investigations.

**Table (2):** Pathogenicity test of the fungi isolates fromrot root maize (*Zea mays* L)

| | **Damping off %** | | |
|---|---|---|---|
| **Isolated fungi** | ***pre%** | ****post%** | *****sur%** |
| *F.oxyporum(1)* | 0.00 d | 3.33 cd | 96.67 a |
| *F.oxyporum (2)* | 3.33 cd | 3.33 cd | 93.34 ab |
| *F.oxyporum(3)* | 20.00 a | 16.67 ab | 63.33 de |
| *F.oxyporum(4)* | 3.33 cd | 0.00 d | 96.67 a |
| *F.solani(1)* | 6.67 c | 3.33 cd | 90.00 abc |
| *F.solani(2)* | 6.67 c | 0.00 d | 93.33 ab |
| *F.solani(3)* | 16.67 a | 13.33 b | 70.00 d |
| *R. solani (1)* | 20.00 a | 20.0 a | 60.00 e |
| *R. solani (2)* | 6.67 c | 6.67 c | 86.66 bc |
| *R. solani (3)* | 3.33 cd | 6.67 c | 90.00 abc |
| *R. solani (4)* | 10.00 b | 6.67 c | 83.33 c |
| *R.solani (5)* | 3.33 cd | 3.33 cd | 93.34 ab |

**There is no significantly difference between figures in the same column that begin with the same letter(s). ($p \leq 0.05$).**

***pre% = pre-emergence damping off **post% =post-emergence damping off and ***sur% =survival plant.**

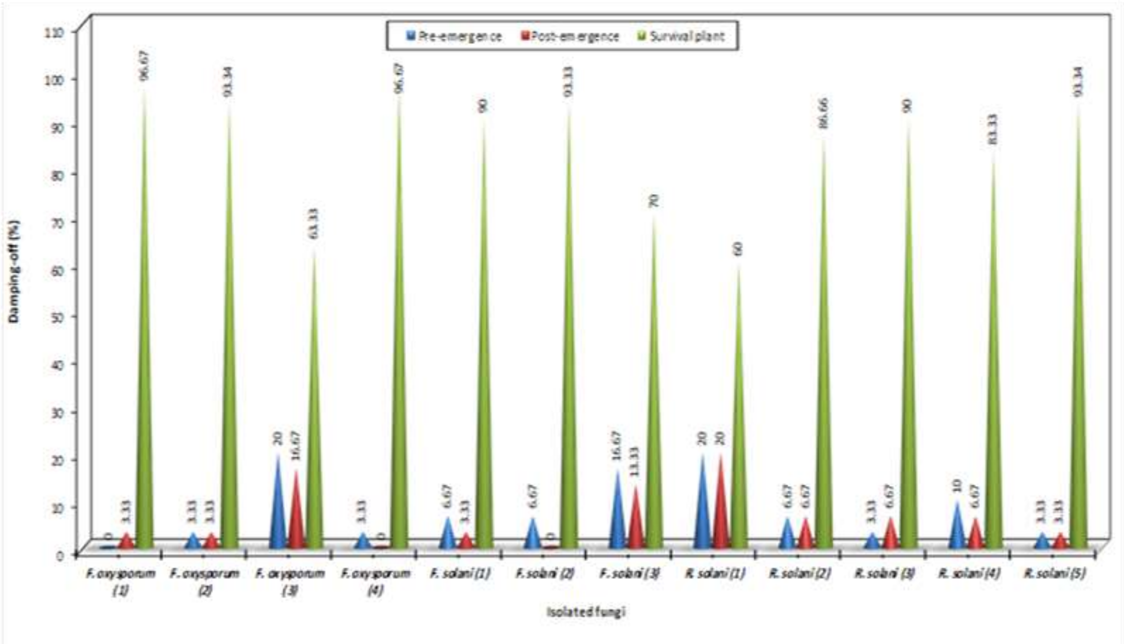



**Figure (2). Pathogenicity test of the fungi isolates from rot root maize (*Zea mays* L).**

**Figure(2).**pathogenicity test of the fungi isolates fromrot reet maize (Zea maysL)

**Antifungal activity of the tested antioxidants:**

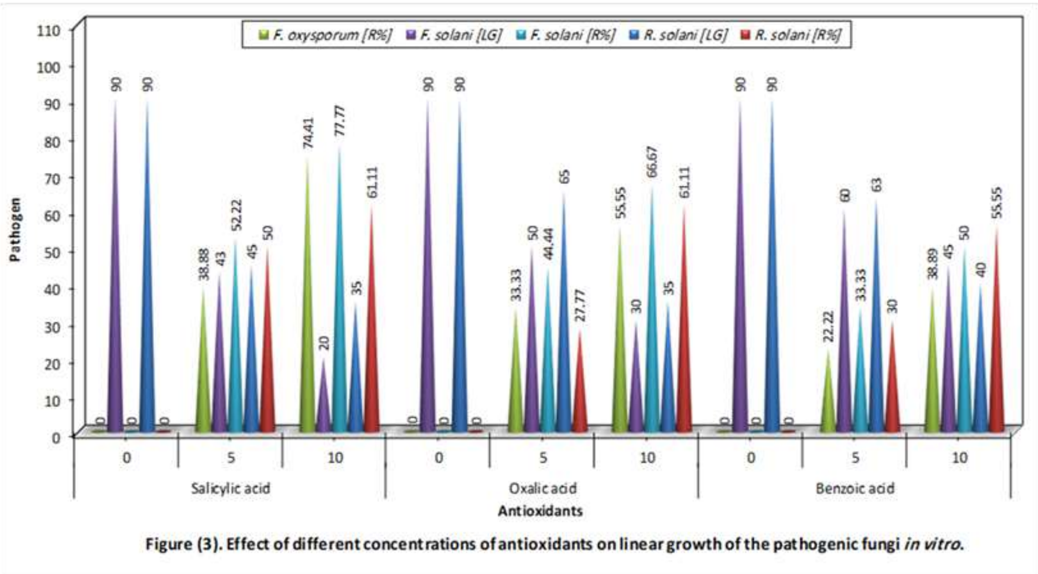
The impact of three tested antioxidants (salicylic, oxalic, and benzoic acid) at both concentrations (5 and 10 Mm) on the linear growth of the three examined pathogenic fungi (*F. oxysporum*, *F. solani*, and *R. solani*) as compared with non-treated control (media free antioxidants) are present in Table (3) The are a significant differentiation among antioxidants and their concentrations. The negative relation between increasing the concentration of antioxidants and the growth of the examined pathogenic fungi is easily discernible. Generally, the lower limit growth of three tested pathogenic fungi occurred under the high concentrate of salicylic acid then a high oxalic level the high concentrate of benzoic. The highest lowering percentage was recorded under the application of salicylic acid at 10 Mm giving 74.41%, 77.77%, and 61.11% reduction for *F. oxysporum*, *F. solani*, and *R. solani*, respectively. In this regard, oxalic acid at 10 Mm came next followed by benzoic acid at the same level.

**Table (3).** Impact of various concentrations of antioxidants on linear growth of the pathogenic fungi *in vitro*

| Antioxidants | Concentration Mm | *F. oxysporum* | | *F. solani* | | *R. solani* | |
|---|---|---|---|---|---|---|---|
| | | LG | R% | LG | R% | LG | R% |
| **Salicylic acid** | 0 | 90.00 a | ---- | 90.00 a | ---- | 90.00 a | ---- |
| | 5 | 55.00b | 38.88 | 43.00 b | 52.22 | 45.00 b | 50.00 |
| | 10 | 23.00 c | 74.41 | 20.00 c | 77.77 | 35.00 c | 61.11 |
| Mean | | 61.11 | ---- | 51 | ---- | 56.67 | ---- |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Oxalic acid** | 0 | 90.00 a | ---- | 90.00 a | ---- | 90.00 a | ---- |
| | 5 | 60.00b | 33.33 | 50.00 b | 44.44 | 65.00 b | 27.77 |
| | 10 | 40.00 c | 55.55 | 30.00 c | 66.67 | 35.00 c | 61.11 |
| Mean | | 63.33 | ---- | 56.66 | ---- | 63.11 | ---- |
| **Benzoic acid** | 0 | 90.00 a | ---- | 90.00 a | ---- | 90.00 a | ---- |
| | 5 | 70.00b | 22.22 | 60.00 b | 33.33 | 63.00 b | 30.00 |
| | 10 | 55.00c | 38.89 | 45.00 c | 50.00 | 40.00 c | 55.55 |
| Mean | | 71.67 | ---- | 65.00 | ---- | 64.33 | ---- |

**Means within different letter(s) under the same treatment on each pathogen significantly differ (p ≤ 0.05) LG = Linear Growth, R% = Reduction Percentage, A = Antioxidants, B = Concentration and AxB = interactions.**




**Figure(3).**Effect of different concentrations of antioxidantd on linear growth of the pathogenic fungi *in vitro*



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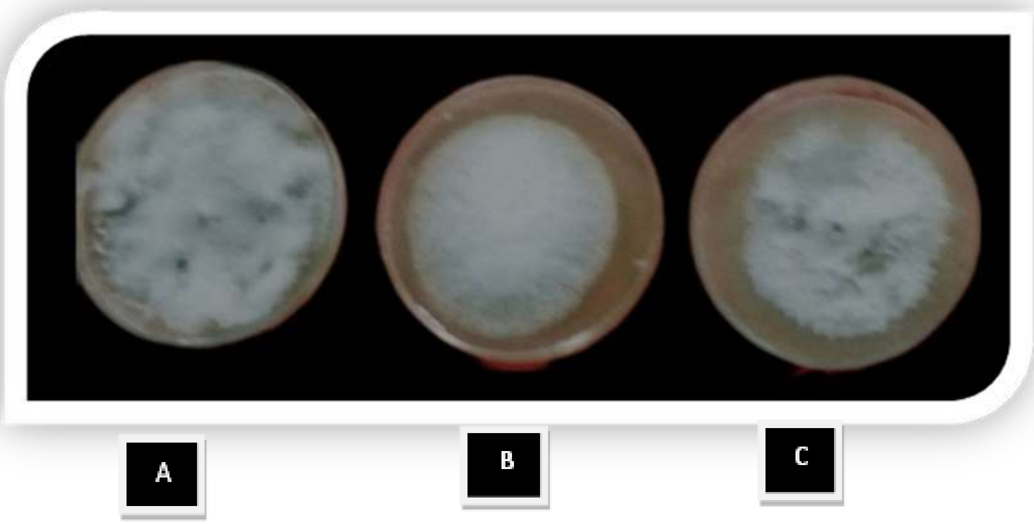
A:concentrations(0)
B:concentration (5)
C:concentration (10)

**Figure (4).** Effect Salicylic acid on fungi F.solani




A:concentrations(0)
B:concentration (5)
C:concentration (10)

**Figure (5).** Effect oxalic acid on fungi *R.solani.*

**Effect of the fungicide on Tolex 500 wpm on the linear growth of filamentous pathogenic fungi:**

Chemical fungicides are considered an important treatment for seeds due to plant seeds need them to guarantee an enough plant position in the field and its expected degradation when introduced into the soil. Table (4) show that there is negative correlation between increasing the concentration of fungicide Tolex 500 wpm from 0 to 100 ppm and the linear growth of all tested pathogenic fungi. High level (100 ppm) of fungicide suppressed the growth of all tested fungi. Data also indicate that there were differences in the sensitivity of the pathogens to fungicide concentrations; F. solani





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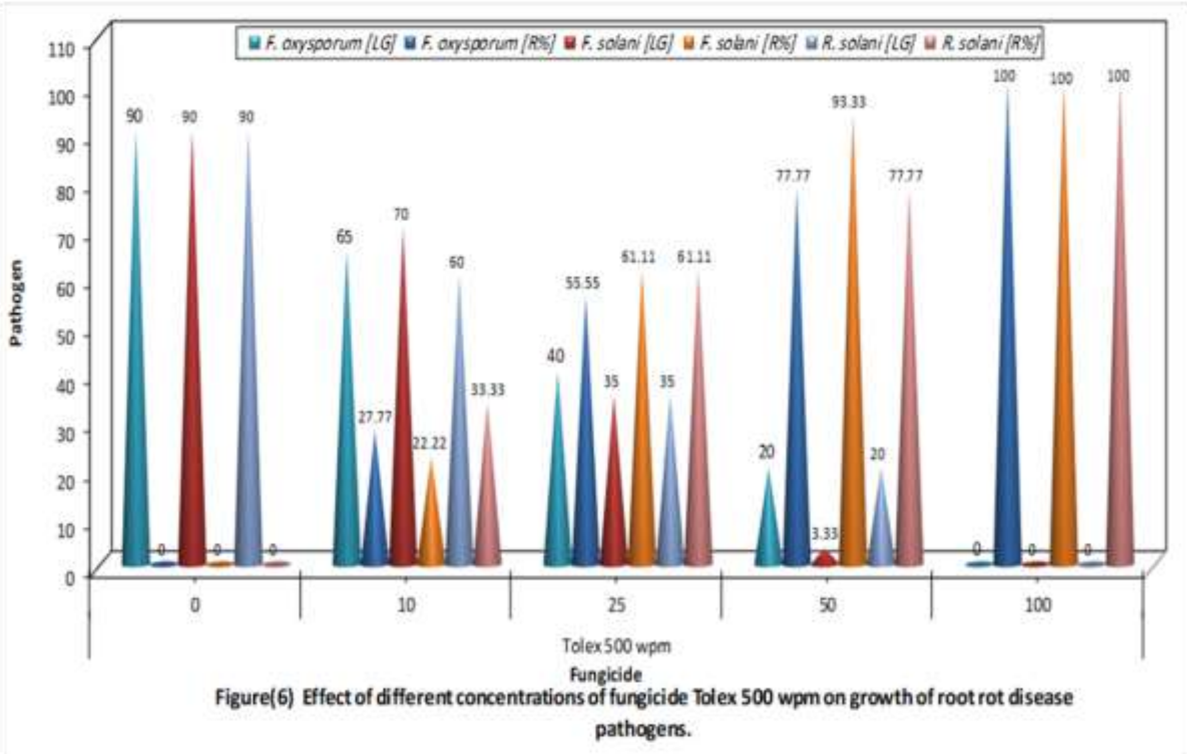
being the most sensitive fungus recorded a 93.30% reduction % under 50 ppm.

**Table (4).** Effect of different concentrations of fungicide Tolex 500 wpm on the growth of root rot disease pathogens.

| Fungicide | Concentration ppm | ***F. oxysporum*** | | ***F. solani*** | | ***R. solani*** | |
|---|---|---|---|---|---|---|---|
| | | LG | R% | LG | R% | LG | R% |
| **Tolex 500 wpm** | 0 | 90.00 a | 0.00 | 90.00 a | 0.00 | 90.00 a | 0.00 |
| | 10 | 65.00 b | 27.77 | 70.00 b | 22.22 | 60.00 b | 33.33 |
| | 25 | 40.00 c | 55.55 | 35.00 c | 61,11 | 35.00 c | 61.11 |
| | 50 | 20.00 d | 77.77 | 3.33 d | 93.30 | 20.00 d | 77.77 |
| | 100 | 0.00 e | 100.00 | 0.00 e | 100.00 | 0.00 e | 100.00 |

**There are no significant variance between the figures when the same letter(s) are in the same column ($p \leq 0.05$).**
**LG = Linear Growth and R% = Reduction Percentage.**




**Figure(6).**Effect of different concentration Tolex500wpm on growthof root rot disease pathogens.



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## DISCUSSION

Data in this investigation indicated that all tested antioxidants have direct antifungal activity relying on the fungal structure in addition to chemical concentrations. A high concentration of salicylic acid followed by a high oxalic level, the high concentration of benzoic was more effective in leading to a decrease in the growth of the pathogenic fungi on which the study is being conducted. Abiotic agents are considered one of the alternative methods to fungicides in controlling plant pathogens. Antioxidants can reduce the negative oxygen radicals released during fungal Salicylic acid induces infections Shahda, (2002). the expression of pathogens-related proteins Metwally *et al.*, (2003). It decreased the linear growth of many fungi including *F. solani*, *F. oxysporum*, and *R. solani* El-Mougy, (2002). The same other added that Salicylic acid (SA) was highly toxic to fungal mycelial linear growth and has a direct antifungal activity. The mode of action of antioxidants may be explained in one or more of these mechanisms; a) Scavenger activity of free radicals, b) Quencher activity of singlet excited states, c) Chelation of transition metals and d) In activator activity of enzymes Shahda, (2002). Moreover, Nesci *et al.*, (2003**)** explained the antimicrobial impact of antioxidants results from one or more of the subsequent factors: a) The oxidised compounds prevent several enzymes from functioning properly, b) soluble in membrane lipids and intervene with membrane functions, c) intervene with the formation of protein, RNA or DNA and, d) act on specific hydroxyl groups on the phenol compounds which raise poisoning to microorganisms.The application of chemical fungicides as a seed treatment before sowing is a common practice to prevent damping off, root rot, and other fungal pathogens in seed and soil-borne. Chemical fungicides are considered an important treatment for seeds results from plant seeds needs them to make an sure the field has a sufficient plant stand and it is expected degradation when introduced into the soil. In a laboratory experiment, four concentrations of fungicide (Tolex 500 wpm) were utilized to evaluate their impact on the linear growth of the examined pathogenic fungi as compared with a check (media-free fungicide). The tested concentrations of fungicide proved to be effective against pathogenic fungi. There is an opposite correlation between increasing the concentration of fungicide Tolex 500 wpm from 0 to 100 (ppm) and the linear growth of all studied pathogenic fungi. The high level of 100 (ppm) of fungicide inhibited the growth of all examined fungi. *F. solani* being the most sensitive fungus. The differences in the reaction may be due to the selective reaction between fungicide and fungus. These outcomes somewhat concur with those disclosed by Ismail, (1994) The use of fungicide as seed treatment decreases root rot incidence results from the predicted decay of this fungicide when put into the soil and subjected to the environmental factors Abd El- Hai and Ali, (2017).In this investigation, the harmful effects of fungicide Tolex 500 wpm may be due to the active substance in fungicide composition which decomposes in fungus media and causes toxicity.

## RECOMMENDATIONS

The research recommends paying attention to safe alternatives and avoiding the use of fungicides under any circumstances to preserve the environment and biological organisms, as well as to preserve human health.

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