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Role of Aqueous Plant Extracts, Humic Acid, and Salicylic Acid in Inhibition of *Rhizoctonia Solani* and Fusarium *Incarnatum* in Vitro

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Annotation: A laboratory experiment was carried out at a plant protection laboratory, Directorate of Diyala Agriculture, Iraq, during the season 2024 to evaluate the impact of aqueous plant extracts, viz., *Salvia officinalis*, *Silybum marianum*, and *Urtica dioica*, as well as salicylic acid and humic acid on the inhibition percentage of *Rhizoctonia solani* and *Fusarium incarnatum* in vitro. The findings revealed that the aqueous plant extracts such as *S. offisinalis* at concentrations of 30%, *U. dioica* at 20%, and *S. marianum* at 30% led to an increase in the inhibition percentage of *F. incarnatum*, which reached 59.25%, 69.99%, and 35.18%, respectively, while extracts of *S. offisinalis* at concentrations of 30%, *U. dioica* at 20%, and *S. marianum* at 10% resulted in an increase in the inhibition percentage of *R. solani*, which amounted to 6.47%, 54.62%, and 34.25%, respectively. whereas humic acid at a concentration of 5% was superior in the

inhibition percentage of *F. incarnatum* (48.14%) and *R. solani* (100.00%), and salicylic acid at a concentration of 0.5 ppm was outstanding in the inhibition percentage for each of *F. incarnatum* and *R. solani* (100.00 ppm).

Keywords: *Rhizoctonia solani*, *Fusarium incarnatum*, plant extracts, salicylic acid, and humic acid.

INTRODUCTION

Rhizoctonia and Fusarium are the most common soil-borne fungi that cause serious diseases in a variety of vegetable crops. *Rhizoctonia solani* is the most well-known and extensively studied fungus that lives in soil, is a non-obligate parasite, and causes damping off and necrosis on a variety of host plants, it is found in nature as vegetative hyphae and sclerotia due to the absence of conidia. One of the most efficacious and destructive soil-borne diseases is Fusarium wilt, which is brought on by Fusarium fungus and causes severe economic losses by infecting host roots and colonizing xylem vessels, which leads to plant wilting **(Salim et al., 2016).** Synthetic fungicides are widely used to suppress phytopathogenic fungi. The overuse of synthetic chemicals pollutes the ecosystem and causes damage to plants, so there is an important need to reduce chemical pesticides in order to minimize environmental pollution and improve plant growth, and as a result, eco-friendly methods must be adopted in order to boost crop health and yield, and botanical extracts are the most effective substitutes for these dangerous chemicals **(Salim et al., 2016; Salim et al., 2023).** Application of botanical extracts from medicinal plants to reduce pathogenic fungi is highly preferred due to their being less expensive and easily available. Extract of *Salvia officinalis* is the oil that possesses strong efficacy as fungicidal and bactericidal **(Bouaziz et al., 2009).** *Silybum marianum* has significant chemical components, such as flavonolignans, and has medicinal antioxidant characters **(Salehi et al., 2011).** The leaves of plants have antifungal activity against *Alternaria alternata*, as well as antibacterial and antioxidant properties (**Nematollahi and Sayidi 2017; Zarafshan, 2017).** Organic compounds such as humic acid and salicylic acid can also be used to reduce phytopathogenic fungi. Salicylic acid offers an interesting new option for the management of bacterial and fungal diseases **(Ellis et al., 2002; El-Khallal, 2007; Ali et al., 2009; and Yehia et al., 2011).** Humic acid stimulates plant growth and enhances natural resistance against numerous plant diseases **(Scheuerell and Mahaffee, 2004).** It stimulates the microorganisms in the soil, enhancing uptake of water and nutrients, increasing cell division **(Chen et al., 2004).** The objective of this trial was to assess the effect of aqueous plant extracts *S. officinalis, S. marianum,* and *U. dioica*, as well as humic acid and salicylic acid against *R. solani* and *F. incarnatum* in vitro.

MATERIALS AND METHODS

Materials collection

The plants under study were collected as shown in Table (1), while humic acid from Baqubah Nursery and salicylic acid from the Plant Pathology Lab, Directorate of Diyala Agriculture. *R. solani* and *F. incarnatum* that were molecularly diagnosed after isolated from the infected tomato were obtained from the Plant Pathology Lab, Directorate of Diyala Agriculture under recording codes *F. incarnatum* (OQ357846-OQ357847), and *R. solani* (OQ357844-OQ357845).

Pathogenicity test on tomato seeds

Ten ml of sterile distilled water was added to each petri dish of fungi, *R. solani* and *F. incarnatum*, then the spores and mycelium were harvested by scraping them with a glass slide and filtering through three layers of muslin cloth. Then, 1 ml of each fungus suspension was put

in 9 ml of sterile distilled water to prepare a dilution of 0.1, then sprayed on the ten tomato seeds placed on the blotting paper, while the control seeds were sprayed with distilled water and incubated at room temperature. After that, the germination percentage was assessed (Table 2 and Figure 1). The number of *F. incarnatum* spores was calculated by a hemocytometer, which reached $5x10^6$ spores/ml.

Aqueous extracts preparation

The leaves of *S. marianum* and *U. dioica* were weighted at a rate of 50 g and washed with tap water, then added to 200 ml of distilled water and mixed by an electric blender. Also, 50 g of *S. officinalis* powder was added to 500 ml of distilled water and mixed by blender, and each of them was left for two days in the refrigerator, then filtered through three layers of muslin cloth on the third day.

Poisoned food technique

The concentrations of treatments were prepared with PDA medium (Potato Dextrose Agar), which included 10, 20, and 30% for each plant extract, 1, 3, and 5% for humic acid, and 0.1, 0.3, and 0.5 ppm for salicylic acid, then poured separately into petri dishes (9 cm) with three replications for each concentration, and the control treatment included only PDA medium. After the medium became solid, agar discs (6 mm) of *R. solani* and *F. incarnatum* were transferred to the center of the petri dishes, then incubated at 25±2 °C for five days. The orthogonal diameters mean of fungi growth was measured, and the inhibition percentage was calculated according to the following formula: % inhibition = (C-T)/C×100

C = diameter of the fungal colony in the control

T = diameter of the fungal colony in the treatment

Analytical statistics

ANOVA, or one-way analysis of variance, was used to assess the data **(Fisher and Yates, 1968).**

Table 1. Plants used in preparing aqueous plant extracts

Common name	Scientific Name	Family	Used parts	Site of collection
Milk thistle	*Silybum marianum*	Asteraceae	Leaves	Plant Pathology Lab, Directorate of Diyala Agriculture
Sage	*Salvia officinalis*	Lamiaceae	Leaves	Local market
Stinging nettle	*Urtica dioica*	Urticaceae	Leaves	Plant Pathology Lab, Directorate of Diyala Agriculture

Table 2. Germination percentage of tomato seeds

Days	Germination%			
	F. incarnatum	**Control**	***R. solani***	**Control**
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	20	10	20
5	10	40	20	40
6	20	70	30	70
7	30	70	50	70
8	30	70	60	70
9	30	70	60	80

10	40	80	60	80
11	40	80	60	80
12	40	80	60	80
13	40	80	60	80
14	40	80	60	80
15	40	80	60	80

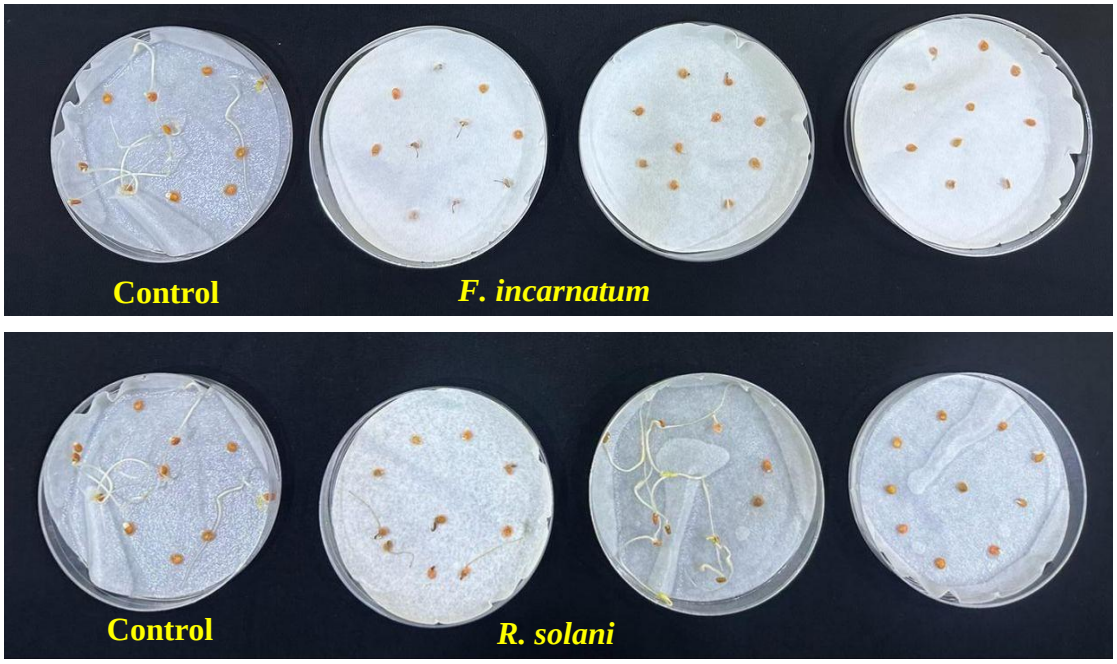


Figure 1. Germination percentage of tomato seeds

RESULTS

The findings in Fig. 2 showed that *S. offisinalis* concentrations led to a significant increase in the inhibition percentage of *F. incarnatum*, where a concentration of 30% was significantly superior in the inhibition percentage of mycelial growth, which reached 59.25%, followed by concentrations of 20% (50.92%) and 10% (45.55%), while there were no significant differences between the concentrations against *R. solani*.

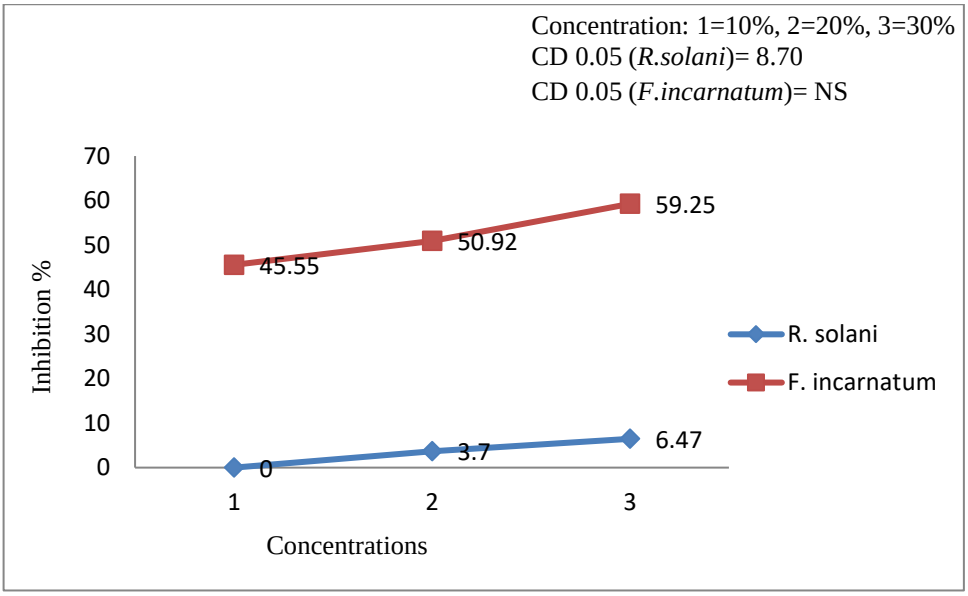


Figure 2. Effect of various concentrations of *S. officinalis* extract on growth inhibition of *F. incarnatum* and *R. solani* in vitro

Fig. 3 shows that a concentration of 20% of *U. dioica* resulted in a significant increase in the inhibition percentage of mycelial growth of *F. incarnatum* (69.99%) and *R. solani* (54.62%), followed by concentrations of 10% (64.81 and 41.66%) compared to concentrations of 30% (48.14 and 0.0%), respectively.

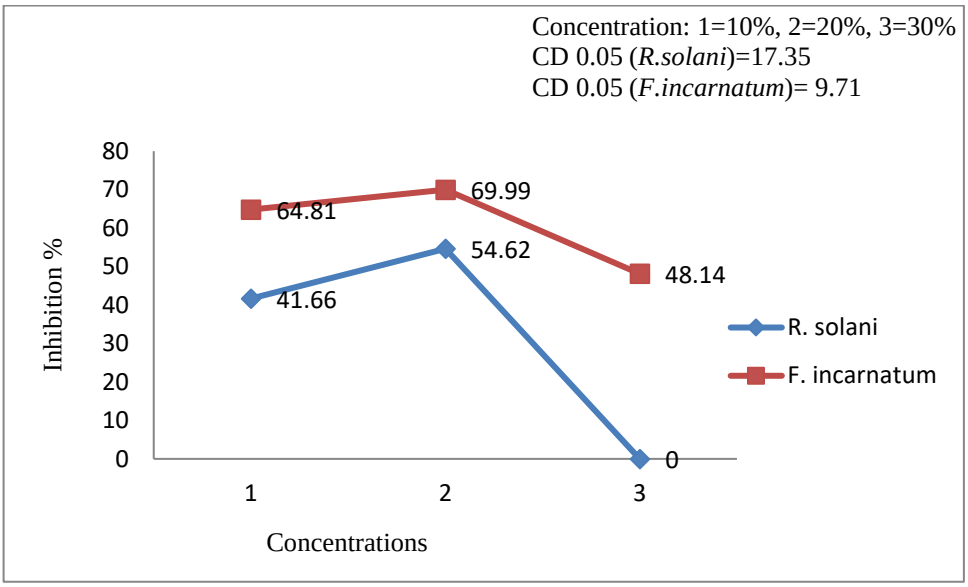


Figure 3. Effect of various concentrations of *U. dioica* extract on growth inhibition of *F. incarnatum* and *R. solani* in vitro

From Fig. 4, there were no significant differences between the concentrations of *S. marianum* in the inhibition percentage of *F. incarnatum*; in contrast, a concentration of 10% was significantly superior in the inhibition percentage of *R. solani* (34.25%), followed by concentrations of 20% (15.73%) and 30% (0.0%).

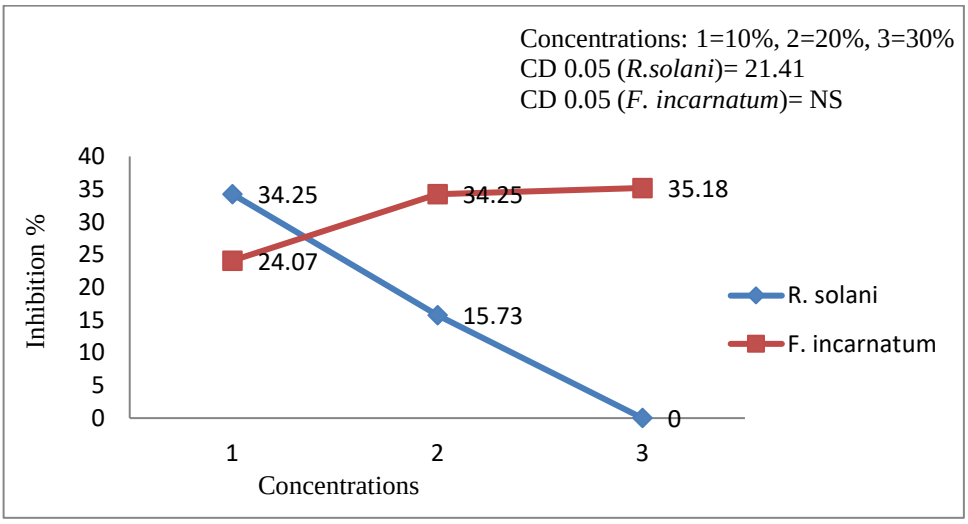


Figure 4. Effect of various concentrations of *S. marianum* extract on growth inhibition of *F. incarnatum* and *R. solani* in vitro

The concentration of 5% of humic acid resulted in a significant increase in the inhibition percentage of *F. incarnatum* (48.14%) and *R. solani* (100.00%), followed by concentrations of 3% (24.99 and 38.88%) and concentrations of 1% (18.51 and 0.0%), respectively (Fig. 5).

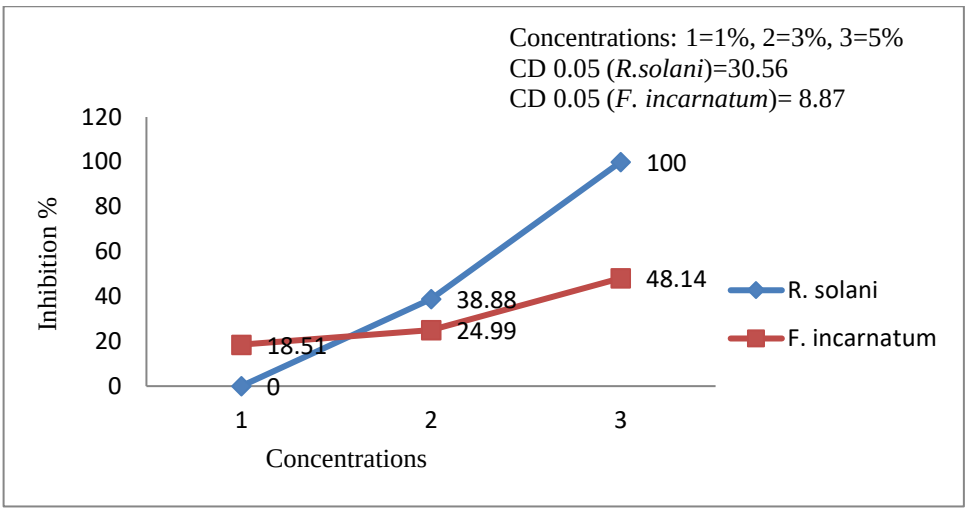


Figure 5. Effect of various concentrations of Humic acid on growth inhibition of *F. incarnatum* and *R. solani* in vitro

The concentration of 0.5 ppm of salicylic acid led to a significant increase in the inhibition percentage of *F. incarnatum* (100.00 ppm), followed by concentrations of 0.3 ppm (94.443 ppm) compared to concentrations of 0.1 ppm (73.70 ppm), whereas there were no significant differences between the concentrations against *R. solani* (Fig. 6).

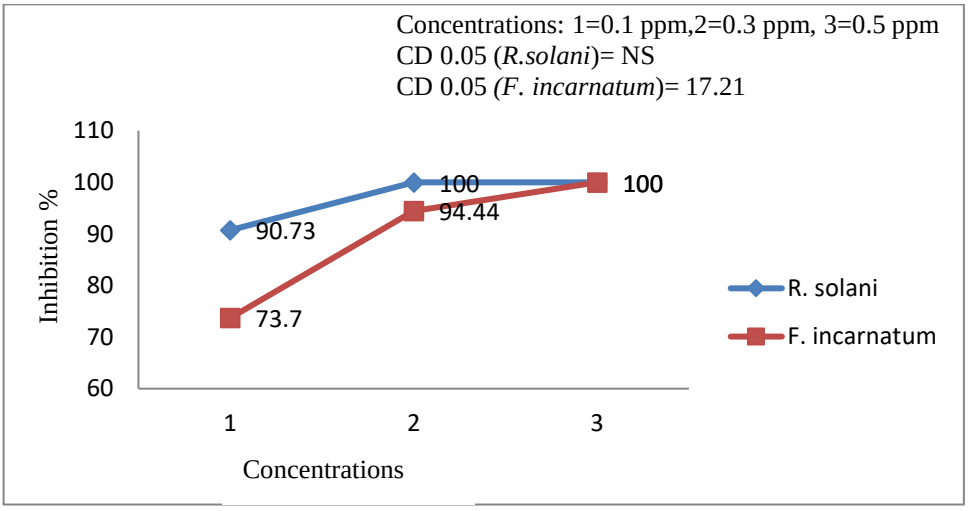


Figure 6. Effect of various concentrations of salicylic acid on growth inhibition of *F. incarnatum* and *R. solani* in vitro

DISCUSSION

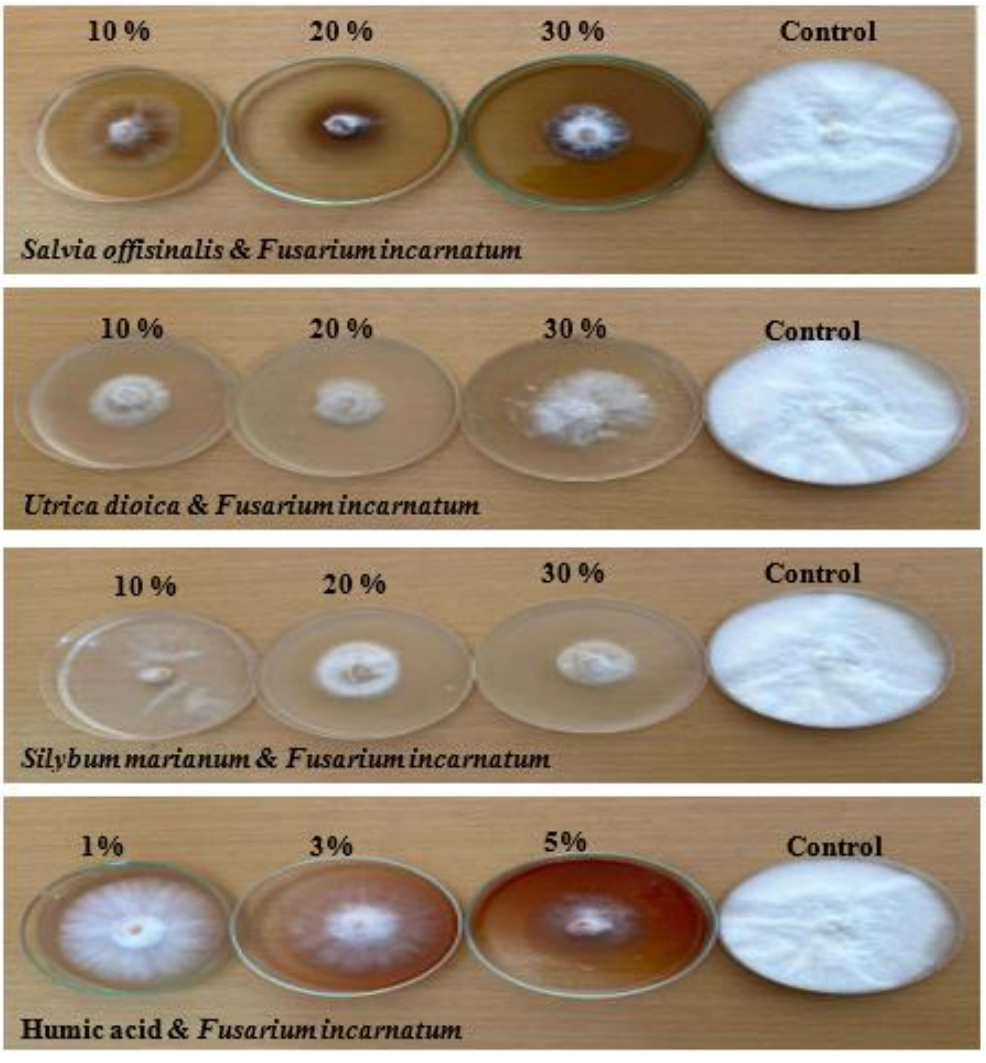
The previous findings revealed that aqueous plant extracts, viz. *S. offisinalis*, *U. dioica* and *S. marianum*, as well as humic acid and salicylic acid led to an increase in the inhibition percentage of *F. incarnatum* and *R. solani* (Figs. 7 and 8).

The results of the current study are in agreement with those of **Geuenich et al. (2008) and Edris et al. (2007),** who reported that the aqueous extract of *Salvia officinalis* has been shown to have antibacterial, antioxidant, antiviral, and fungicidal properties. The inhibition ability of the Salvia species can be attributed to the flavonoid and phenolic compounds, which have antimicrobial activity **(Hu and Kitts, 2003; Xavier et al., 2009; Badiee et al., 2012; Oliveira et al., 2013; Matejczyk et al., 2018).** Theaqueous extract of *Salvia officinalis* was effective against the growth of Botrytis cinerea **(Rabilu et al., 2021).** The great efficacy of *Utrica dioica* extract against *R. solani* and *A. alternata* indicates that it might be applied as a chemical supplement to control pathogen fungi in plants **(Hadizadeh et al., 2009).** The various concentrations of *Urtica dioica* exhibited antifungal potential against *Alternaria alternata* **(Sayidi and Nematollahi,**

2017). According to **Salim et al. (2017)**, the ethanolic extract of *Silybum marianum* demonstrated a strong inhibitory effect on *R. solani* mycelial in vitro. Humic acid increases the natural resistance against diseases and pests **(Scheuerell and Mahaffee, 2004). Yigit and Dikilitas (2008), Abdel-Monaim et al. (2011), and Abdel-Kader et al. (2012)** reported that using humic acid can reduce a number of plant diseases. **Pascual et al. (2000) and Loffredo et al. (2007)** reported that humic acid may be able to inhibit the mycelial growth of a variety of plant-pathogenic fungi, including *Fusarium oxysporum*, *Alternaria alternata*, *Fusarium culmorum,* and *Phytium ultimum*. Humic acid may be inhibitory to the fungi *Fusarium culmorum* and *Alternaria alternata* in PDA medium **(Moliszewska and Pisarek, 1996).** According to **Van Loon et al. (1998)**, the induction of disease resistance could be dependent on the accumulation of salicylic acid or jasmonic acid. Application of *Pseudomonas fluorescens* with a variety of salicylic acid compounds reduced the root rot caused by *Rhizoctonia solani* in cucumber and beans **(Kataria et al. 1997).** Using salicylic acid at a concentration of 4.0 g/L completely prevented the mycelial growth of *Sclerotinia sclerotiorum* **(Hilal et al. 2006). Abdel-Monaim (2012)** found that when 200 ppm of salicylic acid was added to liquid and solid media, it had the highest inhibitory effect on *F. oxysorum*. f. sp. *lycopersici* **.**

Conclusion

Under in vitro conditions, *F. incarnatum* and *R. solani* were well controlled by aqueous extracts of *S. officinalis, S. marianum,* and *U. dioica,* as well as humic acid and salicylic acid.



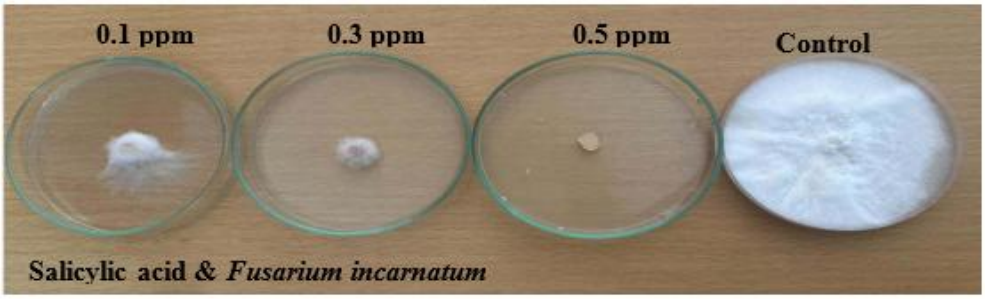


Figure 7. Effect of various concentrations of *S. offisinalis, U. dioica, S. marianum* extracts, Humic acid and Salicylic acid on growth inhibition of *F. incarnatum in vitro*

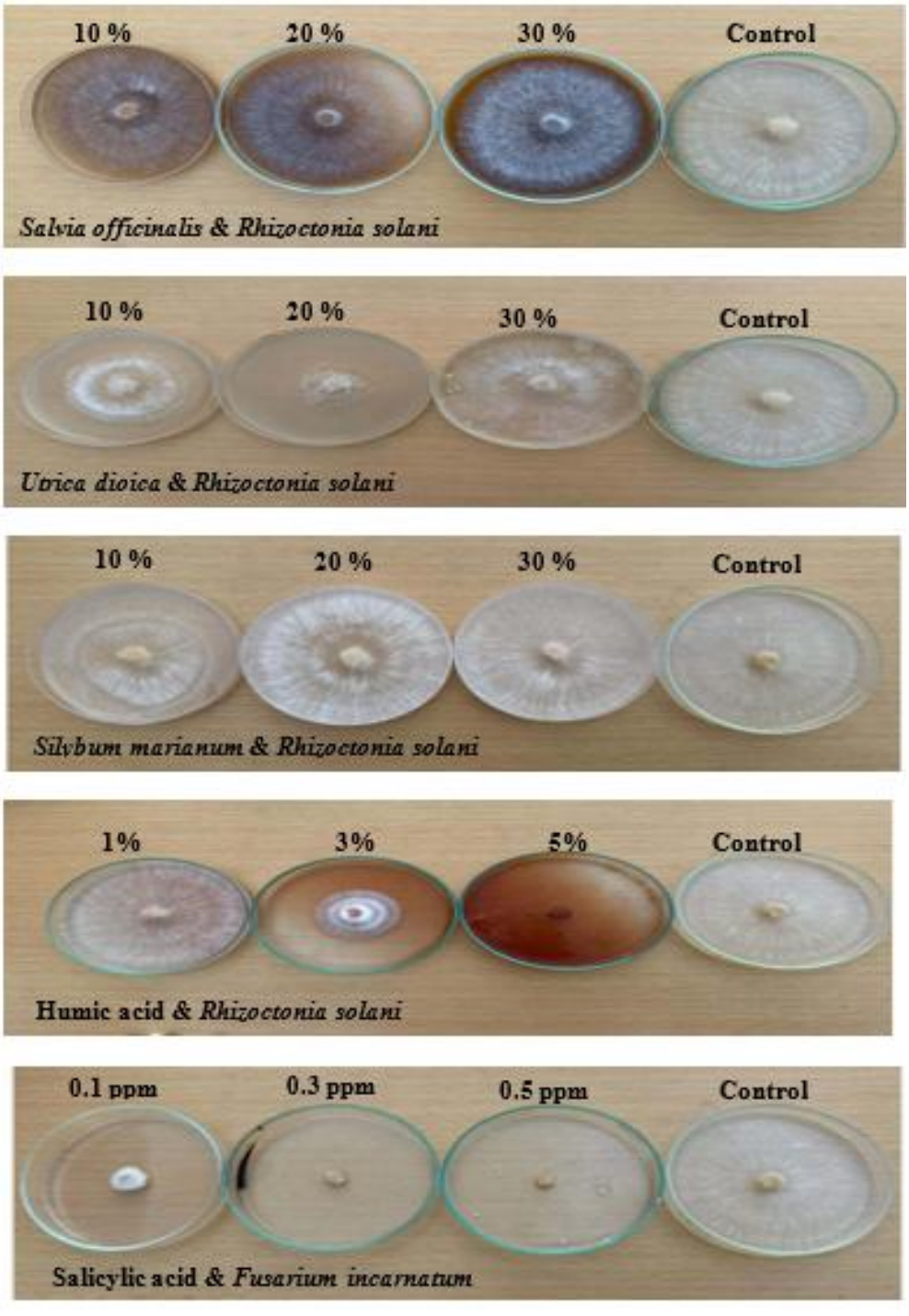


Figure 8. Effect of various concentrations of *S. officinalis, U. dioica, S. marianum* extracts, Humic acid and Salicylic acid on growth inhibition of *R. solani in vitro*

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