RESEARCH ARTICLE

Effect of *Trichoderma viride*, Sodium Benzoate (C_6H_5COONa) and Vitamin B2 against *Fusarium solani in vitro*

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ABSTRACT

A laboratory experiment was carried out according to the randomized complete design (RCD) during the season of 2020-21 to study the effect of *Trichoderma viride* , sodium benzoate C_6H_5COONa and vitamin B2. The results showed that the biological agent *T. viride* achieved an antagonistic ability against *Fusarium solani* amounted 1.33 according to the Bell scale, the concentration 100 mmol of sodium benzoate and vitamin B2 led to inhibiting of *F. solani* 100%, followed by the concentrations of 80, 60 and 40 mmol, which amounted (95.83, 65.27, 18.05%) and (100, 89.44, 58.33%) respectively, compared to the control treatment 0%. In contrast, the concentration of 100 and 80 mmol of sodium benzoate and vitamin B2 resulted in inhibiting of *T. viride* amounted (11.67 and 15%) and (91.66 and 87.77%) respectively, compared to the control treatment 0%. In comparison, the concentrations 40 and 60 mmol of sodium benzoate and vitamin B2 did not cause any inhibitory effect on *T. viride* 0%, the beltanol fungicide at concentrations (500, 1000, 1500, 2000) mg/L caused inhibition of *F. solani*, which reached 100, 78.51, 39.63 and 20.36% respectively compared to the control treatment 0%.

Keywords: *Trichoderma viride ,* sodium benzoate and vitamin B2, *Fusarium solani*

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INTRODUCTION

Sesame (*Sesamum indicum* L.) belongs to the pedaliaceae family and is one of the most important oil crops, it is cultivated in a wide environmental range which includes the tropics and subtropics of the continents of Asia, Africa and South America.[1] The crop gains importance in being the raw material for many foods and pharmaceutical industries,[2] its seeds contain a high percentage of oil ranging between 45–65% and a high percentage of protein up to 15%, as well as containing antioxidants such as sesamin and sesamolin.[3,4] Plant diseases significantly damage growth and development of plants, leading to reduction in yield and quality of produce.[5] One of the most important determinants of sesame production is soil diseases caused by fungal pathogens, including seed rot disease and seedling death, the traditional model of controlling fungal diseases is based on the use of chemical pesticides with their negative effects on humans, biodiversity and the environment, as well as the development of disease-resistant strains of pathogens as a result of their excessive use.[6] Therefore, it became necessary to search for integrated and sustainable management strategies based on non-traditional components in the control of pathogens, such as the use of microorganisms in the biological control of plant pathogens, especially those endemic to the soil, the genus Trichoderma has occupied a distinguished place in biological control programs due to its high ability to compete, parasitism and antagonism, as well as stimulate systemic resistance.[7] described the increase of yield and decreased *Fusarium oxysporum* growth by using *Trichoderma harzianum*. Some chemicals of biological origin such as sodium benzoate C6 H6CooNa and vitamin B2 contribute to stimulating the induced resistance in plants against root rot disease by stimulating of plants in increase secretion of inhibitory substances for pathogenic fungus growth, increasing the activity of defensive enzymes such as peroxidase and chitinase, increasing the thickness of the plant cell wall, and precipitation of callus, lignin and many secondary metabolites (phytoalexins and phenols) as well as its ability to increase the biological activity of fungi used in biological contro[7,9-13]. The objective of this study was to evaluate the antagonistic activity of sodium benzoate, vitamin B2 and *T. harzianum* against *F. solani in-vitro.*

MATERIALS AND METHODS

A laboratory experiment was conducted at plant pathology Laboratory in Diyala Agriculture Directorate during 2020- 21.

Sample Collection

Samples were collected from the fields of some provinces of Iraq, including Baghdad, Diyala, Salah Elden during the

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agricultural season for the year 2020. The sesame seedlings that showed symptoms of yellowing and wilting (damping off) were uprooted, and each sample was placed in a polyethylene bag containing a card which has the site and date of collection, then transferred to the laboratory for the purpose of isolation, purification and diagnosis.

Isolation of Pathogenic Fungi: The roots were thoroughly washed under tap water to remove dust and other impurities and the isolation process was done by cut the roots to small pieces then sterilized with sodium hypochlorite NaOCl (1%) for 3 minutes and using sterilized distilled water to remove sterilization trace , the roots pieces were placed on sterilized paper to remove excess water, then transferred to potato-dextrose agar medium after sterilizing at 121°C at a pressure of 1.5 kg cm² for 20 minutes in an autoclave. The petri dishes were kept at 25 ± 1o C for 7 days, pieces of mycelium were transferred by a sterile needle to PDA medium in petri dishes to purpose of fungi purification, then incubated at 25 ± 2°C for four days. The fungi were diagnosed to the level of genus and species based on the characteristics of the fungal colony and the nature of the mycelium and spores by using the approved taxonomic keys.[14,15] The percentage of the presence and frequency of fungi in each sample was calculated according to the following equation:

% Frequency = A number of pieces which fungus appeared in the dishes/Total number of pieces used in the sample x 100 (Table 1).

Pathogenicity Test of Pathogenic Fungi

The pathogenicity of forty four fungi isolates was tested on sesame seeds, which included the preparation of 9 cm Petri dishes containing 15–20 mL of 2% water agar medium, consisting of (20 g Agar in 1 liter of distilled water), the medium was sterilized with the autoclave at 121°C at a pressure of 1.5 kg, cm² for 20 minutes, the agar disk 6 mm from the tip of each fungus colony at age of 4 days was transferred in the center of the dish and incubated at 25 ± 2°C for 3 days, sesame seeds (local variety) were sterilized with sodium hypochlorite solution NaOCl (1%), then washed with sterile water, then transferred and arranged in a circular pattern parallel to the edge of the dish, at a rate of 10 seeds / dish with three replicates, as well as to the control treatment (without fungi), the dishes were incubated at a temperature of 25 ± 1°C for 3 days, then the results were taken by calculating the percentage of germination and according to the following equation:

% Germination = Number of germinated seeds/ Total number of seeds x 100 (Table 2 and Figure 1)

Where isolate No. (4) Fusarium solani was selected to perform the rest of the tests because it is more frequent 55% and more pathogenicity 0.0%, and the isolate No. 4 was diagnosed as fusarium solani by the technique of polymerase chain reaction (PCR) under accession number MW730742.

Effectiveness of *T. viride* against *F. solani* on PDA medium: *Trichoderma viride* was obtained from the Plant Diseases Laboratory, Ministry of Science and Technology. The antagonistic ability of *T. viride* was tested against the selected fungus isolate *F. solani* by the dual culture method. Petri dishes 9 cm containing PDA medium (Potato Dextrose Agar) was divided into two equal parts, an agar disk 0.6 cm from the tip of *F. solani* colony at age of 4 days was transferred to the first part of the dish and an agar disk 0.6 cm from the tip of *T. viride* colony at age of 5 days to the second part of the dish, the experiment was carried out with 3 replications and the dishes were incubated at 25 ± 1°C for 5 days. The antagonistic ability was estimated according to the Bell scale[16,] which consisted of 5 degrees as follows:

- The anti-fungus covers the entire area of the dish without allowing the pathogenic fungus to grow.
- The anti-fungus covers two-thirds of the dish, and pathogenic fungi cover the remaining third of the dish.
- The anti-fungus covers half the area of the dish, and the pathogenic fungus covers the other half of the dish.
- The anti-fungus covers one-third of the dish, and pathogenic fungi cover the remaining two-thirds of the dish.
- The pathogenic fungus covers the entire area of the dish.

The biological agent is effective when it shows a degree of antagonism equal to 2 or less with the pathogenic fungus.

Evaluation of the efficiency of sodium benzoate C_6H_5COONa in inhibiting the growth of *F. solani* and *T. viride* on PDA medium: Four concentrations of the sodium benzoate viz 40,60,80 and 100 mmol were prepared with PDA medium (Potato Dextrose Agar), then poured separately into petri dishes (9 cm in diameter), two control treatments included only PDA medium, an agar disks 0.6 cm from the tip of *F. solani* colony at age of 4 days and an agar disks 0.6 cm from the tip of *T. viride* colony at age of 6 days were transferred separately

Table 1: The percentage of the fungi frequency in the sesame roots

Fungi	*% Frequency*
Fusarium solani	55
Fusarium oxysporum	7.08
Rhizoctonia solani	13.75
Macrophomina phaseolina	5
Alternaria alternata	2.08
Aspergillus flavus	3.75
Aspergillus niger	2.08
Pencillium sp.	5.41
Rhizopus sp.	5.83

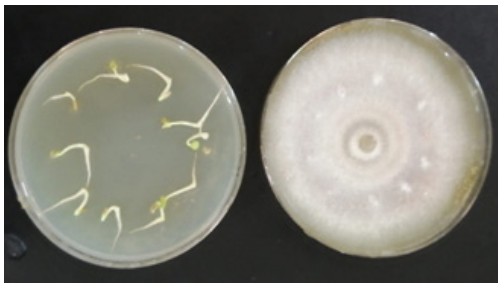


Figure 1: Pathogenicity test of the isolate No. (4) *F. solani* on sesame seeds in vitro, A- control, B- *F. solani*

Table 2: Pathogenicity test of fungi isolates on sesame seeds *in vitro*

	Fungi isolates	*% Germination*		*Fungi isolates*	*% Germination*
1	*F. solani*	26.67	24	*F. oxysporum*	3.33
2	*F. solani*	33.33	25	*F. oxysporum*	6.67
3	*F. solani*	36.67	26	*F. oxysporum*	23.33
4	*F. solani*	0.00	27	*F. oxysporum*	3.33
5	*F. solani*	3.33	28	*F. oxysporum*	33.33
6	*F. solani*	23.33	29	*R. solani*	26.67
7	*F. solani*	3.33	30	*R. solani*	16.67
8	*F. solani*	0.00	31	*R. solani*	0.00
9	*F. solani*	46.67	32	*R. solani*	3.33
10	*F. solani*	26.67	33	*R. solani*	13.33
11	*F. solani*	63.33	34	*R. solani*	3.33
12	*F. solani*	3.33	35	*R. solani*	0.00
13	*F. solani*	10.00	36	*R. solani*	3.33
14	*F. solani*	20.00	37	*R. solani*	33.33
15	*F. solani*	20.00	38	*M. phaseolina*	0.00
16	*F. solani*	3.33	39	*M. phaseolina*	16.67
17	*F. solani*	0.00	40	*M. phaseolina*	46.67
18	*F. solani*	30.00	41	*M. phaseolina*	66.67
19	*F. solani*	56.67	42	*M. phaseolina*	0.00
20	*F. solani*	43.33	43	*M. phaseolina*	13.33
21	*F. oxysporum*	33.33	44	*M. phaseolina*	3.33
22	*F. oxysporum*	40.00		Control	100.00
23	*F. oxysporum*	0.00			
	L .S.$D_{0.05}$				9.146

into all petri dishes, and incubated at 25 ± 1°C for 4 days for *F. solani* and 6 days for *T. viride*, the test was performed in three replications for each concentration and the diameters mean of fungi growth was calculated, then the inhibition percentage of fungi growth was calculated as:

% Inhibition = (1- T/ C) X 100

Where, C = Colony growth of control

T = Colony growth of treatment[17]

Evaluation of the efficiency of vitamin B2 in inhibiting the growth of *F. solani* and *T. viride* on PDA medium: Same protocol of evaluation of the efficiency of sodium benzoate C_6H_5COONa in inhibiting the growth of *F. solani* and *T. viride* on PDA medium was followed , but the only difference is using vitamin B2 with the same concentrations instead of sodium benzoate.

Effectiveness of beltanol fungicide in inhibiting the growth of *F. solani* on PDA medium: The fungicide beltanol produced by the Spanish Probelta company with the active ingredient Chinosol SL 50% was used, the toxicity of the fungicide was tested by using poisoned food media, with four concentrations 500, 1000, 1500, 2000 mg/L calculated on the basis of the active ingredient, the concentrations were added separately to the PDA medium, then poured separately into petri dishes (9 cm), the control treatment included only PDA medium, an agar disks 0.6 cm from the tip of *F. solani* colony at age of 4 days was transferred into the center of petri dishes, and incubated at 25 ± 1°C for 4 days, the test was performed in three replications for each concentration, the inhibition percentage of fungi growth was calculated as:

% Inhibition = (1- T/ C) X 100

Where, C = Colony growth of control

T = Colony growth of treatment

The experimental design: The results were analyzed using the SAS statistical program and the averages were compared according to Duncan's test at a probability level of 0.05.[18]

RESULTS AND DISCUSSION

The results showed that the existence of a high antagonistic ability of the biological agent *T. viride* against the pathogenic fungus *F. solani*, as the biological agent achieved an antagonistic ability amounted 1.33 according to the Bell scale, where the activity of *T. viride* continued to inhibit the progression of the pathogenic fungus *F. solani*. It was also observed the direct contact between the colony of the biological agent *T. viride* and the colony of the pathogenic fungus, as the growth of *F. solani* was limited to the edge of the dish due to the antagonistic ability of the biological agent (Figure 2), it was also seen that hyphae of *T. viride* covered the hyphae of *F. solani* under the light microscope, which may indicate to the parasitic activity of the biological agent on the pathogenic

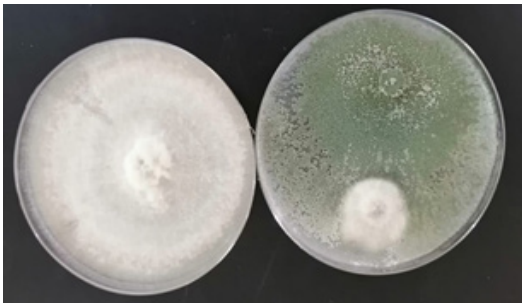


Figure 2: Effect of *T. viride* against *F. solani* on PDA medium, A- *F. solani*, B- *F. solani* with *T. viride*

Control only *F.solani* *F. solani* with sodium benzoate

Figure 3: Effect of sodium benzoate C_6H_5COONa in inhibiting the growth of *F. solani* on PDA medium

Table 3: Effect of sodium benzoate C_6H_5COONa and vitamin B2 in inhibiting the growth of *F. solani* and *T. viride* on PDA medium

Fungi	*Concentration mmol*	*% inhibition*	
		Sodium benzoate	*Vitamin B2*
F. solani	0	0	0
	40	18.05	58.33
	60	65.27	89.44
	80	95.83	100
	100	100	100
L.S.D 0.05		2.73	1.16
T. viride	0	0	0
	40	0	0
	60	0	0
	80	11.67	87.77
	100	15	91.66
$L.S.D_{0.05}$		1.73	0.74

fungus. This characteristic of the biological agent may be due to the fungal hyphae has small diameters relatively , which enables it to wrap around the hyphae of the pathogenic fungus with the help of some decomposing enzymes such as Protease, Cellulase and Chitinase, which able to degrade the fungi cells walls, then penetration and parasitism , also the competition for nutrients, or production of some volatile organic compounds such as ethyl hexadecanoate, azetidine and 2-phenyl ethanol, as well as the production of some enzymes that destroy the walls of fungal cells, such as chitinase enzymes and B-1,3-glucanase, which affect on the growth of pathogenic fungi and limit their reproduction[19].

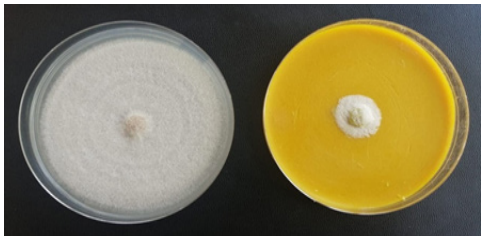
The results of Table 3 and Figures 3 and 4 showed that the poisoned food media with sodium benzoate in all its concentrations (40,60,80,100) mmol led to increase in inhibition of colony diameter of *F. solani* between 18.05-100% compared with the control treatment, which recorded an inhibition rate of 0%, while the concentration of 100 mmol achieved a highly significant difference in inhibiting the pathogenic fungus reached 100% compared to other treatments followed by the concentrations of 80,60 and 40 mmol, which amounted 95.83, 65.27, 18.05%, respectively. As for the effect of sodium benzoate on *T. viride*, some of its concentrations had a negative effect on the growth of biological fungus, where the concentrations 80 and 100 mmol recorded an inhibition rate reached 11.67 and 15%, respectively, while the concentrations of 40 and 60 mmol did not cause any inhibitory effect on *T. viride*.

The poisoned food media with vitamin B2 in all its concentrations (40,60,80,100) mmol achieved a significant increase in inhibition percentage of the colony diameter of *F. solani* compared with the control treatment, which recorded an inhibition rate of 0%, while the concentrations of 100 and 80 mmol were significantly superior in inhibiting the pathogenic fungus, which amounted 100% compared to other treatments followed by the concentrations of 60 and 40 mmol, which reached 89.44 58.33% respectively. Whereas the concentrations 100 and 80 mmol of vitamin B2 resulted in high inhibition to biological fungus amounted 91.66 and 87.77%, respectively, while the concentrations of 40 and 60 mmol did not cause any inhibitory effect on *T. viride*.

The explanation of an effect of sodium benzoate in inhibiting the growth of pathogenic fungi on PDA media is due to the inhibition of a number of enzymes in the fungal cell such as amylase, as well as inhibition a number of enzymes of the citric acid cycle, where sodium benzoate is characterized by its ability to inhibit enzymes responsible for cellular reactions which leads to inhibiting the ability of microorganisms to grow[20, 21]. Or it may work to destroy the cell walls of pathogenic fungi, which is reflected in its effect on the protein content of the fungal cell [22]. The reason for the tolerance of the biological fungus to sodium benzoate may be due to that the biological fungus is a mutagenic isolation, so it has the ability to tolerate high concentrations of benzoate, while the pathogenic fungus is sensitive to the same concentrations.

The explanation of an effect of the vitamin B2 in its high concentrations in the biological agent is attributed to its direct effect as an inhibitor of the vital processes for the growth of pathogens, thus impeding their growth and death. As for the reason for the tolerating of the pathogenic fungus to the high concentrations of vitamin used in the study, due to the vitamin B2 is an inducing factor that stimulates of plant defenses when used in the field[23].

The results of Table 4 showed that the use of the beltanol fungicide at concentrations (500, 1000, 1500, 2000) mg/L caused the inhibition of the growth of the pathogenic fungus *F. solani* in varying proportions, where the concentration of 2000 mg/L achieved a highly significant difference in inhibiting the pathogenic fungus reached 100% compared to other treatments



Control only F.solani benzoate F.solani with vitamin B2

Figure 4: Effect of vitamin B2 in inhibiting the growth of *F. solani* on PDA medium

Table 4: Effect of beltanol fungicide in inhibiting the growth of *F. solani* on PDA medium

Fungi	Concentration ml/L	% inhibition
F. solani	Control	0.00
	500	20.36
	1000	39.63
	1500	78.51
	2000	100.00
$L.S.D_{0.05}$		7.41

followed by the concentrations of 1500, 1000 and 500 mg/L, which amounted 78.51, 39.63 and 20.36%, respectively.

CONCLUSION

These results confirm the outstanding effectiveness of this fungicide in control the pathogens, as the active substance of the beltanol (Chinosol), which has the ability to bind with heavy elements and form a complex compound, which it's difficult to absorb by pathogen, or may be attributed to the fact that the fungicide forms chelating compounds with the copper element in the host tissues, which facilitates its passage into the cells of the pathogen, and then it is released and kills the pathogen (24, 25).[26] showed that application of beltanol fungicide with concentration 1-ml/L led to inhibition of Fusarium solani on the PSA medium.[27] reported that using of beltanol resulted in significant reduction in the growth of Fusarium solani and Rhizoctonia solani in vitro that causes root rot of eggplant.

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