Biological control of Fusarium solani and Rhizoctonia solani caused of root rot disease on pepper

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Abstract

The study aimed to evaluation of Trichoderma spp. and the effective microorganism preparation EM1 against some fungi that are pathogenic to pepper plants infected with root rot disease. The results of the field survey showed the spread of pepper root rot disease in all areas in Babylon Governorate. The results of isolation and diagnosis showed the emergence of pathogenic fungal species, the most frequent of which was the Fusurium solani, Rhizoctonia solani and Macrophomina phaseolina these fungi caused a significant reduction in the percentage of pepper seed germination.The results showed that all treatments involving the microbial preparation (EM-1), the biocontrol fungus Trichoderma viride, the chemical inducer salicylic acid, and the fungicide Baltanol had a significant effect in inhibiting the growth of pathogenic fungi and providing protection to pepper plants against root rot disease, especially when combined together. The integrated treatment of biological agents (EM-1, T. viride, and salicylic acid) was the most effective in reducing the incidence and severity of infection by R. solani and F. solani, with infection rates and severity recorded at 8.33% and 3.33%, respectively, compared with R. solani and F. solani alone were 100%, with severity levels reaching 75% and 80%, respectively. Additionally, all treatments used to combat pepper root rot pathogens led to an increase in growth parameters. The integration of all biological agents significantly improved growth fresh shoot weight, dry weight, root fresh weight, root dry weight, root volume, plant height and root length.

Key words: pepper, disease, root rot, biological control, Salicylic acid.

Introduction:

The chili pepper crop Capsicum annuum L. belongs to the Solanaceae family and is mainly grown for its food consumption and other purposes, as it contains a large amount of wonderful vitamin C, which is found in cooking[31,26].pepper contain multiple nutrients of great importance, calcium, phosphorus, iron, manganese and zinc) as the calories of a pepper weighing 148 g are about 30 calories and 2 g dietary fiber and 7 g carbohydrates and 1 g protein and 4 g sugar wonderful about 2% and 18% of vitamins Fe and A on lemon fruits also contain vegetables B2, B1 and B3 [22,27] Chili pepper is grown in Iraq at the beginning of spring in the protected farm at the beginning of the open farm, as the total area planted with pepper crops in general is about 21,189 dunums in 2020 and the total production capacity is about 46,498 tons [15]. It has obtained a productivity rate per unit area due to several reasons, the most important of which is infection with fungi, including Pythium spp. Rhizoctonia spp. and Macrophomina phaseolina [21,25]. Therefore, they resorted to a method using chemical pesticides to combat infection from diseases that cause diseases such as fungal pathogens as a result of the failure of pesticides due to these pathogens to selective pressure, which led to the emergence of a method of resistance against them that does not cause many harms [33]. This encouraged

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workers in the field of agricultural pest control to divide the sectors, including the use of biological factors through the use of microscopic microbes, such as bacteria that promote the growth of the experiment Rhizobacteria Plant Growth Proping (PGPR) such as Azotobacter spp. and the fungus Trichoderma spp. [7]. The fungus Tricoderma spp. also has For this reason, several anti-pathogens have been added and its importance in improving production, growth and mechanical resistance to industrial plants is quickly eliminated [18,34,2]. The use of microbiology practitioner (EM1) can play an important role in enhancing and increasing pepper under the effects of salt stress and increasing effective photosynthesis by facilitating pistachios with many nutrients including phosphorus, nitrogen, potassium, zinc, calcium, copper and manganese, reducing cadmium and sodium [1]. It has a role in maintaining soil fertility, and many of them have been proven to be toxic plants and negatively affect their growth. [4]. Chemical inducers stimulate resistance against many pathogens as their efficiency has been proven in the laboratory and in the field, in addition to being safe for disposal and animals and having no transparent effects, they are dangerous and low-cost, but they are good profits, the most important of which is salicylic acid and proving its ability to control fungi and bacteria in the plant's bed by inducing resistance in the host [22]. Due to the necessity of downy mildew disease and to introduce safe methods to combat its causes, the study aims to: detect some fungi isolated from herbaceous plants infected with mold viruses, diagnose them and their pathogenicity, and try to combat pathogens using types of Trichoderma fungi and EM1 microbiology techniques and the completely induced salicylic acid and do otherwise.

Materials and methods

Isolation and diagnosis of fungi associated with pepper plant roots

The isolation process was carried out from pepper plant samples that showed symptoms (represented by root rot, poor plant growth, yellowing and wilting of leaves). Sterile pieces of infected roots were transferred at a rate of 4 pieces in each plate (9 cm in diameter) (containing sterile Potato Dextrose Agar (PDA) medium using an autoclave and the plates were incubated in an incubator at a temperature of 25±1 °C for three days. The fungi were purified and identified at the genus and species level using taxonomic criteria based on the characteristics of the fungal colonies and the nature of the fungal mycelium, as well as the spores and structures they form[17,14,29]. The percentage of appearance of the fungi that appeared was calculated according to the following equation:

Fungi appearance = (Number of root pieces in dishes in which pathogenic fungi appear / The total number of root pieces used for each sample) * 100

Detection of pathogenic isolates of the fungus R.solani , F. solani using cabbage seeds on the PDA culture medium

The pathogenicity test of8 isolates of the fungus F. solani, 8 isolates of the fungus R. solani and 5 isolates of the fungus M. phaseolina were tested according to the method of [13]. The plates were inoculated from After solidification of the PDA medium with a 0.5 cm disk taken from the edges of the fungal colony of R. solani , F. solani and M. phaseolina, the plates were incubated in the incubator at a temperature of 25±1°C for three days. The seeds of the cabbage were planted

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after sterilization with sodium hypochlorite solution (1% free chlorine) in a circular manner near the edge of the plate at a rate of 10 seeds per plate. The plates were incubated in the incubator at a temperature of 25±1°C and after seven days the results were taken by calculating the percentage of germination.

Preparation of the fungal inoculum of R. solani and F. solani

Local millet seeds were used for the purpose of preparing fungal inoculums according to the method of [16]. Millet seeds were washed with water to remove impurities and dust, soaked in water for six hours, then left on a piece of gauze for 30 minutes to remove excess water. 50 g of seeds were placed in 250 ml glass flasks and sterilized for half an hour in an autoclave. After 24 hours, they were sterilized again and left to cool. The glass flasks were inoculated by placing 5 discs (0.5 cm in diameter) of the PDA medium containing R. solani and F. solani fungi, each separately. Then the flasks were incubated at a temperature of 25±1°C for 14 days, stirring every two days for the purpose of ventilation and distributing the fungal inoculum to all millet seeds.

Effect of F. solani and R. solani fungi isolates on the germination of pepper seeds under wood canopy conditions.

This experiment was conducted at the Technical College / Al-Musayyab. The fungal inoculum of F. solani isolates (2-F.s, 4-F.s, and -6 F.s) and R. solani isolates (-1 R.s, R.s-3, and R.s-5) loaded on local millet seeds was added to sterilized mixed soil with an autoclave and distributed in 1 kg plastic pots at a rate of 1% (weight / weight). 10 pepper seeds were planted in each pot, superficially sterilized with sodium hypochlorite solution. Each treatment was repeated 3 times, and 3 replicates were left without adding the pathogenic fungus as a comparison. The pot was watered. The percentage of germination was calculated after the seeds of the comparison treatment were fully germinated

Evaluation of the efficacy of the bio-fungus Trichoderma spp. and the Effective Microorganisms (EM-1) product, and the chemical inducer Salicylic Acid in combating the fungi responsible for root rot in pepper plants and assessing certain growth parameters in pots under shaded conditions.

The experiment was conducted in the shade house of Al-Musaib Technical College on September 3, 2024, using 4 kg plastic pots. The soil, a mixture of sand and peat moss (1:2), was sterilized with commercial formalin. The formalin was applied at a concentration of 20 ml per liter of water, where 3 liters of water were used per cubic meter of soil. The sterilized soil was mixed,. covered with plastic, and left under sunlight for seven days, followed by three days of ventilation before planting the sterilized soil into the pots[10.[

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Table 1: Treatments Used in the Shade House Experiment

No	Treatments	No	Treatments
1	*R.solani*	*14*	Fu+Tv+EM
2	Rs+Tv	15	Fu+Tv+Sa
3	Rs+Em	16	Fu+EM+Sa
4	Rs+Sa	17	Fu+Tv+EM+Sa
5	Rs+Tv+EM	18	Betle+Fu
6	Rs+ +Tv+Sa	19	Tv
7	Rs+ +Sa+EM	20	EM
8	Rs+TV+Sa+EM	21	Sa
9	Betle+Rs	22	Tv+EM
10	*F.solani*	23	Tv+Sa
11	Fu+Tv	24	Tv+EM+Sa
12	Fu+EM	25	Control
13	Fu+Sa		

The experiment was conducted using a Randomized Complete Design (R.C.D). Pathogenic fungal inocula (R. solani and F. solani) were applied to millet seeds at 10g/pot for treatments requiring inoculation. The bio-product EM-1 was applied 5 days prior to the fungal inoculation, with 10 ml per pot. Salicylic acid was added at a concentration of 100 mg/L for soil drenching, 2-3 ml per pot, immediately after sowing the seeds. The bio-control agent T. viride was added at 1% wheat bran 3 days before fungal inoculation. The chemical fungicide Baltanol was applied at 1 ml/L, one day after fungal inoculation. Results were evaluated after three months by determining the infection rate and severity of root rot caused by R. solani and F. solani isolates using a disease scale. Additionally, fresh and dry plant weights, shoot length, root length, root volume, and chlorophyll content were measured for pepper plants.

Results and Discussion

Isolation and diagnosis of fungi associated with the roots of infected pepper plants

The results (table 2) of isolation and diagnosis of several types of fungi associated with the roots of pepper plants infected with root rot disease showed symptoms of disease represented by rotting of the entire root or part of it, and its brown color with wilting and yellowing of the leaves of the plants. The most frequently occurring pathogenic fungi were Fusurium solani, which was isolated from 8 areas with an incidence rate of 56.12% and the highest incidence rate was 100%, followed by Rhizoctonia solani, which was isolated from 8 areas with an incidence rate of 45.5% and the highest incidence rate was 80% (Figure 1), followed by Macrophomina phaseolina, which was isolated from 5 areas with an incidence

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rate of 25.2% and the highest incidence rate was For its appearance 32% and these results agree with what[8]. found that the fungus Fusurium solani and Rhizoctonia solani are the most important causes of pepper root rot. These results agree with what several studies have shown about the presence of pathogenic fungi and their spread on various crops[3,6,28]

The results of the examination showed the appearance of many fungi accompanying the roots of pepper plants at lower rates of recurrence, such as the fungus Trichoderma spp., Aspergillus niger, Pencillium spp., Mucor spp., Chaetomium globosum, and Alternaria alternate.

Table 2 The percentage of fungi appearing in the roots of pepper plants infected with root rot disease

Names of fungi	Imam	Al-Sabbaghiya	Al-Mahawil	Al-Himyari	Al-Azzawiya	Al-Dulaimi	Al-Nil	Muwailiha	Al-Haidari	Al-sayahi	Sinjar	Rate (%)	Highest presence
Fusurium solani	45	40	58	68	100	50	38	50	-	-	-	56.12	100
Rhizoctonia solani	-	-	-	80	36	56	48	25	40	43	36	45.5	80
Macrophomina phaseolin	-	-	32	24	25	22	23	-	-	-	-	25.2	32
Trichoderma	-	-	-	-	14	-	-	-	-	-	-	14.00	14
Aspergillus niger	36	25	-	-	8	22	-	-	-	17	38	24.2	38
***Pencillium* spp.**	23	-	14	26	15	33	-	-	-	16	-	21.1	33
***Mucor* spp.**	-	-	24	25	12	-	-	-	-	16	14	2. 18	25
Chaetomium globosum	-	-	14	-	-	-	-	14	-	-	-	14.00	14
Alternaria altrnate	10	-	-	-	-	-	53	-	-	-	-	31.5	53

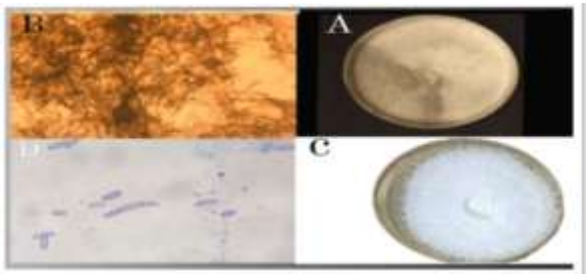

Figure (1). Morphological and microscopic diagnostic characteristics of some fungi causing pepper root rot disease. 7-day-old fungal culture A = represents the fungus R. solani culture on PDA medium B = sclerotia of R. solani, C = the fungus F. solani culture on PDA medium, D = the large and small conidia of F. solani.

The pathogenicity of fungal isolates using cabbage seeds on PDA medium

The results of the table 3 showed that all the tested R.solani and F.solani isolates led to a significant reduction in the germination percentage, compared to the comparison treatment in which the seed germination percentage reached 100%, as the R.solani isolate-1 (Al-Hamri isolate) outperformed the rest of the isolates in reducing the germination percentage, as the germination percentage rate reached 0.00, followed by R. solani-3 (Al-Dulaimi isolate) had a germination percentage of 10.00, while the germination percentage of the remaining isolates ranged between (13.33-43.33) (Figure 2). The results also indicated that all tested F. solani isolates caused a

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significant reduction in the germination percentage of cabbage seeds compared to the comparison treatment, in which the germination percentage reached 100%. The isolates varied among themselves in reducing the germination percentage, as the isolate F. solani-2 (Al-Sabaghiya isolate) excelled, as the seed germination percentage reached 0.00, followed by F. solani-4 (Al-Hamri isolate), as the germination percentage reached 10.00, and the germination percentage of the remaining isolates ranged between (13.33-50.00 .(

Table 3 Detection of pathogenic isolates associated with the roots of infected pepper plants using cabbage seeds

No.	Site	Isolate	No. of seed germinated	% Germination
1	-	control	10.00	100.00
2	Al-Himyari	R1	0.00	00.00
3	Al-Azzawiya	R2	4.33	43.33
4	Al-Nil	R3	1.00	10.00
5	Al-Nil	R4	3.00	30.00
6	Muwailiha	R5	1.33	13.33
7	Al-Haidari	R6	4.00	40.00
8	Al-sayahi	R7	3.00	30.00
9	Sinjar	R8	2.66	26.67
10	Imam	F1	2.66	26.66
11	Sabbaghiya	F2	0.00	0.00
12	Al-Mahawil	F3	3.33	33.33
13	Al-Himyari	F4	1.00	10.00
14	Al-Azzawiya	F5	4.33	43.33
15	Al-Dulaimi	F6	1.33	13.33
16	Al-Nil	F7	5.00	50.00
17	Muwailiha	F8	2.66	26.67
18	Al-Mahawil	Mp1	4.33	43.33
19	Al-Himyari	Mp2	3.33	33.33
20	Al-Azzawiya	Mp3	2.33	23.33
21	Al-Dulaimi	Mp4	4.33	43.33
LSD (0.05)		-	1.2811	12.811

Each number in the table represents the average of three replicates, Fs = F. solani, Rs = R. solani, Mp = M. phaseolina The number near the isolate symbol represents the isolate number.

These results are consistent with what[24] found that these fungi are among the main causes of root rot of many crops, and most of the tested fungi cause For the root rot disease of the tested plants, it caused a significant reduction in the germination rate of cabbage seeds in the PDA culture medium compared to the control treatment. This is attributed to the secretion of secondary toxic metabolites by fungi that led to the death of embryos, in addition to the production of many

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decomposing enzymes responsible for seed rot and thus preventing them from germinating. From the results of this test, the isolates that most reduced the germination of cabbage seeds were selected, namely isolate-1 R.solani, R.solani-3, F. solani-2, and F. solani-4 to conduct subsequent experiments. As for the isolates of the fungus M. phaseolina, they did not cause a significant reduction in the germination of cabbage seeds.

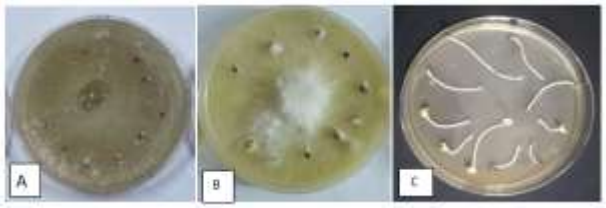

Figure (2) represents the high pathogenicity of the fungi isolated from the roots of pepper plants A= pathogenicity of the fungus R. solani, B = pathogenicity of the fungus F. solani, C =control .

Testing the pathogenicity of the pathogenic fungi Fusarium solani and Rhizoctonia solani in the germination of pepper seeds under the conditions of the wooden canopy

The results (Table 4) indicated that all the tested isolates of the fungi F. solani and R. solani caused a significant reduction in the percentage of germination of pepper seeds, as the isolate R. solani-1 achieved the highest percentage of reduction in the percentage of germination, which reached 0.00%, and the percentage of germination for the rest of the isolates ranged (16.7-46.7) with a significant difference with the comparison treatment without adding the pathogenic fungus, in which the percentage of germination was 100%. As for the isolation of the fungus F. solani-2, it caused a significant reduction in the percentage of seed germination, which reached 0.00%, and the remaining isolates ranged between (23.3-26.7 .(

Table 4 The effect of pathogenic fungal isolates on the germination of pepper seeds .

No.	Isolate	No. of seed germinated	% Germination
1	Control	10	100
2	*F.solani*-2	0.00	0.00
3	*F.solani*-4	2.67	26.7
4	*F.solani*-6	2.33	23.3
5	*R. solani*-1	0.00	0.00
6	*R. solani-3*	4.67	46.7
7	*R. solani* -5	1.67	16.7
LSD (0.05)		1.011	10.11

Evaluation of the Efficiency of the Biocontrol Fungus Trichoderma spp., Effective Microorganisms (EM-1), and the Chemical Inducer Salicylic Acid in Reducing the Incidence and Severity of Infection by the Pathogenic Fungi R. solani and F. solani and

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Some Growth Parameters of Pepper Plants under Shade Conditions

The results in Table (5) show that all treatments involving the microbial preparation (EM-1), the biocontrol fungus Trichoderma viride, the chemical inducer salicylic acid, and the fungicide Baltanol had a significant effect in inhibiting the growth of pathogenic fungi and providing protection to pepper plants against root rot disease, especially when combined together. The combined treatment of biological agents (EM-1, T. viride, and salicylic acid) was the most effective in reducing the incidence and severity of infection by R. solani and F. solani, with infection rates and severity recorded at 8.33% and 3.33%, respectively. These values were not significantly different from the healthy control plants, which had an infection rate and severity of 0.00%. In contrast, the infection rate and severity for the treatments involving R. solani and F. solani alone were 100%, with severity levels reaching 75% and 80%, respectively.

Table (5): Evaluation of the Efficiency of the Biocontrol Fungus Trichoderma spp., Effective Microorganisms (EM-1), and the Chemical Inducer Salicylic Acid in Reducing the Incidence and Severity of Infection by the Pathogenic Fungi R. solani and F. solani

Treatments	Disease Incidence %	Severity %
R.solani	100	75
Tv+R.s	41.67	18.33
Em+R.s	33.33	15
Sa+R.s	50	23.33
R.s+Tv+Em	25	8.33
R.s+Tv+Sa	33.33	16.67
R.s+Em+Sa	25	10
R.s+Tv+Em+Sa	8.33	3.33
R.s+Belte	0.00	0.00
F. solani	100	80
Tv+*F.s*	41.67	20
Em+*F.s*	33.33	16.67
Sa+*F.s*	50	26.67
F.s+Tv+Em	25	6.67
F.s+Tv+Sa	25	18.33
F.s+Em+Sa	25	11.67
F.s+Tv+Em+Sa	8.33	3.33
F.s+Belte	0.00	0.00
Tv	0.00	0.00
Em	0.00	0.00
Sa	0.00	0.00

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Tv+Em	0.00	0.00
Tv+Sa	0.00	0.00
Tv+Em+Sa	0.00	0.00
Control	0.00	0.00
L.S.D = 0.05	12.52	7.80

These results are consistent with the findings of[9] who indicated that T. viride reduces the incidence and severity of pathogenic fungi due to its production of various hydrolytic enzymes such as chitinase, cellulase, β-glucanase, and glucanase. These enzymes break down fungal cell walls, facilitating their penetration, colonization, and parasitism[19,32].Additionally, Table (6) shows that all treatments used to combat pepper root rot pathogens led to an increase in growth parameters. The integration of all biological agents (EM-1, T. viride, and salicylic acid*) significantly improved growth in plants infected with R. solani and F. solani. The combined treatment increased plant biomass significantly, with fresh shoot weight reaching 26.50 g and 25.43 g, and dry shoot weight at 9.50 g and 9.10 g for R. solani and F. solani, respectively. Root fresh weight was 19.43 g and 19.10 g, while root dry weight was 4.66 g and 4.40 g, respectively. Root volume reached 13 ml and 12.93 ml, plant height 41.43 cm and 39.47 cm, and root length 19 cm and 17.46 cm for R. solani and F. solani, respectively.These findings align with the results of[12] who reported that EM-1 increased growth parameters in faba bean plants. This effect is attributed to the presence of beneficial microorganisms in EM-1, which enhance soil fertility and nutrient availability. It contains lactic acid bacteria, which play a vital role in soil sterilization and organic matter decomposition, as well as actinomycetes, which suppress pathogenic fungi and bacteria while forming symbiotic relationships with photosynthetic bacteria. These microorganisms increase soil microflora diversity and enrich it with actinomycetes, bacteria, and fungi that produce growth regulators like indole acetic acid and gibberellins, which enhance root growth and nutrient uptake[20].The highest growth parameters were observed in treatments without the pathogenic fungi, where fresh shoot weight reached 41 g, dry shoot weight 18.96 g, fresh root weight 28.33 g, and dry root weight 8 g. Root volume was 19 ml, plant height 51.50 cm, and root length 28.34 cm. This is due to the role of biocontrol agents in improving nutrient availability, thereby enhancing vegetative growth, photosynthesis efficiency, and nutrient absorption[5,11.[

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Table (6): Evaluation of the Efficiency of the Biological Fungus Trichoderma spp., Effective Microorganisms (EM-1), the Chemical Inducer Salicylic acid, and the Fungicide Beltanol on Some Growth Parameters of Pepper Plants Under Shade Conditions.

Treatments	Dry Root Weight (g)	Fresh Root Weight (g)	Dry Shoot Weight (g)	Fresh Shoot Weight (g	Root Volume (ml)	Root Length (cm	Plant Height (cm)
R.solani	2.63	7.03	3.66	8	2	5.43	11
Tv+R.s	3.40	14.63	5.66	23.40	9.57	13	32
Em+R.s	3.56	15.57	7	25	10	17.80	36.53
Sa+R.s	3.30	13.83	5.50	23	9.43	10	27
R.s+Tv+Em	4.66	19.43	9.50	26.50	13	19	41.43
R.s+Tv+Sa	3.63	17.70	8.83	24	11	17.43	40
R.s+Em+Sa	3.86	18.77	8.96	25.50	12	18.87	40.47
R.s+Tv+Em+Sa	4.70	20	10.50	28.80	14	20.43	45
R.s+Belte	2.90	9.83	6.40	16.50	8	12	27
F. solani	2.50	6.70	3.33	7.47	2	4.76	9
Tv+*F.s*	3.50	14.43	5.50	20.50	9.50	11.50	30.50
Em+*F.s*	3.57	15.43	6	23.80	9.87	16.60	35.60
Sa+*F.s*	3.10	13.70	5.16	18	9.33	9.80	25.83
F.s+Tv+Em	4.40	19.10	9.10	25.43	12.93	17.46	39.47
F.s+Tv+Sa	3.56	17.57	8.63	23.50	10.83	16.40	37.47
F.s+Em+Sa	3.70	18.63	8.70	24.20	11.97	17.33	38.53
F.s+Tv+Em+Sa	4.50	19.63	10.33	27	13.47	18.77	44
F.s+Belte	2.83	9.63	6.16	16.63	7	12	27
Tv	5.46	22.03	14.16	27.87	15.63	21.87	45
Em	5.56	22.90	14.90	31	16.50	23.57	45.43
Sa	2.70	13.50	5.43	22	9.63	10.73	26.90
Tv+Em	6.60	24.30	17.83	33.50	18	25.47	49.47
Tv+Sa	6.23	23.90	16.43	31.30	17.63	24.60	47.63
Tv+Em+Sa	8	28.33	18.96	41	19	28.43	51.50
المقارنة	2.90	9.83	5.56	22.90	9	11	26
L.S.D = 0.05	0.58	0.75	0.88	1.36	1.37	1.11	1.25

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