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EFFICIENCY OF BIOLOGICAL AND CHEMICAL AGENTS IN INHIBITING THE FUNGUS *FUSARIUM SOLANI* CAUSING COWPEA DAMPING–OFF

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SUMMARY

The study intended to find out the main cause of cowpea damping-off and root rot disease. Separating a group of fungi comprised *Fusarium solani*, *Rhizoctonia solani*, and *Macrophomina phaseolina*. Findings of the field survey revealed fungus *F. solani* appeared in all isolated samples. The testing of pathogenicity of all isolated fungal isolates led to choosing the *F. solani* (Fs2) isolate as the most pathogenic in the experiment's implementation. The biological resistance of *Bacillus subtilis* and fungus *Trichoderma harzianum* bore testing, with their high efficiency observed in inhibiting the pathogenic fungus isolate. Biological bacteria with the highest concentration of 10^{-7} appeared with an inhibition rate of 82.20%. The results showed effectiveness of the chemical pesticide in all the concentrations and were highly successful. The wooden canopy showed the effectiveness of the biological resistance in inhibiting the pathogenic fungus. Results showed interaction of the fungus with bacteria reduced the rate and severity of infection to zero. A significant superiority of the biological resistance elements was notable in reducing severity of infection compared with pathogenic fungus alone. The field results confirmed the woody canopy for the efficiency of the biotic resistance elements in reducing the rate and severity of infection, raising the growth of cowpea plants.

Keywords: *F. solani*, *T. harzianum*, *B. subtilis*, cowpea damping-off

Key findings: This study aimed to reveal the effectiveness of biological and chemical resistance elements in inhibiting the pathogenic fungus *F. solani,* which causes the death of cowpea plants, as well as, improving the growth parameters of the plants.

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INTRODUCTION

Cowpea, *Vigna unguiculata* (L.), belongs to the Leguminaceae group. Cowpea growing is to obtain their dry seeds and green pods, cooked as food for humans or used as green fertilizer to improve soil properties and increase fertility. Cowpea seeds are nutritionally important because their dry seeds contain 10.5% water, 22.8% protein, 61.7% carbohydrates, 1.5% fat, and 3.5% ash. Characteristically, it contains substantial salts, such as phosphorus, iron, and calcium, plus some vitamins, such as, vitamins A, B1, B2, and B5 (Mahgoub and Heba, 2020).

The cowpea crop experiences exposure to many soil-inhabited pathogens causing the growing of ''damping-off diseases, root rot, and stem canker'' (Yang *et al.*, 2017 and Ludwig 2021). *F. solani* produces toxins belonging to the naphthazarin group. Baker *et al.* (1981) were able to purify the toxins Fusarubin, Javanicin, and Anhydrofusarbin from *F. solani* filtrate isolated from the roots of infected citrus trees. These toxins have a role in causing disease by affecting the cell membranes or inhibiting the action of enzymes. Then, they block the enzymatic reactions responsible for oxidative phosphorylation, or the toxins work as a metabolic antagonist, causing a deficiency of one of the necessary growth factors for plants.

Agrios' (1997) findings indicated the fungus *F. solani* is a kind, which basically causes root rot disease of broad bean plants in Babylon City (Al-Kif, 2015). Many studies showed the capability of the *Trichoderma* fungus to generate many antibiotics like Trichodermal, Trichodermin, Gliotoxin, Pachypus, and Imodium chrysophanol. These antibiotics reduced the fungal growth of many fungal pathogens, such as, *Fusarium*, *R. solani,* and *Sclerotinia sclerotiorum* (Radhi, 2022). The fungus *T. harzianum* colonizes the root surfaces, causing different biochemical and morphological modifications in the plant, which guides to effectuate systemic production and resistance of pathogen-related proteins.

However, Howell *et al.* (2000) found the biological resistance agent *T. harzianum* interacted significantly with the activity of phenol oxidation enzymes secreted by the pathogenic fungus *F. solani.* It leads to increase the activity of peroxidase and polyphenol oxidase enzymes related to plant defense mechanisms (Chakraborty and Chatterjee, 2007; Radhi, 2022). *T. harzianum* also produces enzymes B. glucanase, chitinase, protease, and xylanase in culture media (Jayalakshmi *et al*., 2009). In this regard, this study sought to find the main cause of cowpea damping-off and root rot disease by separating a group of fungi (*Fusarium solani*, *Rhizoctonia solani*, and *Macrophomina phaseolina*) and testing the pathogenicity of all isolated fungal isolates.

MATERIALS AND METHODS

The field source

Ten cowpea cultivation areas became study sites in Babil and Baghdad governorates for the period from April 1, 2022, to May 10, 2022, as shown in Table 1. Detection of unhealthy and healthy plant occurred at the study site. The calculation determined the quantity of plants infected by noting the case and symptoms on the plants. The extraction of infection rate of the studied plants employed the following equation.

$$Disease\ \text{incidence} = \frac{\text{infected plants number}}{\textit{tested}\ \text{plants total number}} * 100$$

Infected plants proceeded to the laboratory after placing them in polyethylene bags and labeling them, afterward, putting the specimens in the refrigerator at 4 °C before examining them the next day.

Isolation and identification

Isolation procedure continued from each of the infected cowpea samples on the day after collecting plants, polishing the roots of the infected plants with water for 15 min to remove the soil attached to them. Then, cutting them into small pieces with a length of 0.5–1 cm, proceeded to sterilization with

Table 1. The date and location of sampling from surveyed cowpea fields.

Samples	Site	Sampling date
1	Babylon / Mahaweel	1/4/2022
2	Babylon / Jableh	5/4/2022
3	Babylon/Mahnawiyah	10/4/2022
4	Babylon / Musayyib Project	21/4/2022
5	Babylon / Hashemite	25/4/2022
6	Baghdad / Radwaniyah	28/4/2022
7	Baghdad / Abu Ghraib	1/5/2022
8	Baghdad / Al-Rasheed district	3/5/2022
9	Baghdad / As-Siyafiyah	6/5/2022
10	Baghdad / Taji	10/5/2022

Table 2. Fungus *F. solani* isolates' pathogenicity tested using cabbage seeds.

Sample number	Site	Sample symbol
1	Babylon / Mahaweel	F.s1
2	Babylon / Jableh	F.s2
3	Babylon/Mahnawiyah	F.s3
4	Babylon / Musayyib Project	F.s4
5	Babylon / Hashemite	F.s5
6	Baghdad / Radwaniyah	F.s6
7	Baghdad / Abu Ghraib	F.s7
8	Baghdad / Al-Rasheed district	F.s8
9	Baghdad / As-Siyafiyah	F.s9
10	Baghdad / Taji	F.s10

sodium hypochlorite solution for 3 min. Drying them with filter paper occurred before transferring to sterile agricultural medium, pouring into Petri dishes, and adding with the antibiotic tetracycline at a concentration of 200 mg per liter, using four pieces for each plate. The plates remained in the incubator at a temperature of 1 °C ± 25 °C for three days. After the growth of fungi, purification and classification ensued to the genus level according to the approved taxonomic keys (Booth, 1971, Leslie and Summerell, 2006). Then, *F. solani* isolates remained in test tubes containing sterilized mixed soil with an autoclave device for an hour for two consecutive times. The sterilized soil's contamination utilized adding three pieces (0.5 cm in diameter) of the specimen taken from near the colonies' edge of 5-day-old *F. solani* isolates. Placing them in the incubator followed at a temperature of 1 °C ± 25 °C for 15 days, after which, transferring to the refrigerator at a temperature of 4 °C until subsequent tests continue.

Pathogenicity tests of the cabbage seeds

The pathogenicity testing of 10 isolates of *F. solani* (Table 2) began by preparing 9-cm diameter Petri dishes. The dishes contained 15–20 ml of water agar culture medium formulated by dissolving 20 g of an acre soil in a liter of distilled water, sterilized with an autoclave for 15 min. Then, adding the antibiotic Tetracycline had a concentration of 200 mg/liter. After the medium solidified, the plates' inoculation on the center disc with a 0.5-cm diameter isolate taken from near the edges of the *F. solani* fungus colony at the modern growth. The plates sustained a temperature of 1 °C ± 25 °C for three days. Then, sowing local Cabbage seeds (with their germination rate previously tested) followed, sterilized superficially with sodium hypochlorite solution (1% free chlorine) in a circular motion near the side of the plate, at 25 seeds/plate. Four plates for each isolate served as replicates in addition to the comparison treatment without pathogenic fungus. Putting the plates

in the incubator ensued at a temperature of 1 °C ± 25 °C, then obtaining the findings after seven days by counting the percentage of germination and following the formula below:

$$Germination\ \%\ (GP) = \frac{Seeds\ gerinated}{total\ seeds} \times 100$$

Effect of *F. solani* isolates on cowpea plants

This test proceeded based on a completely randomized design. The fungal inoculum preparation for *F. solani* isolates included Fs1, Fs2, Fs5, Fs7, and Fs9, which are the isolates with the strongest pathogenicity among the currencies tested (Dewan, 1989). As for the soil, its sterilization employed an autoclave device, thereafter, its distribution continued in plastic pots with a diameter of 25 cm and a capacity of 2 kg. Then, the added pathogenic fungus to each pot by 1% (w/w) of the fungus inoculum continued to load on millet seeds. Each treatment comprised four replicates, with the control treatment adding only sterilized millet seeds. After three days, sowing cowpea seeds consisted of five seeds per pot. After germination, thinning the plants to three, noting the severity of cowpea wilt disease caused by the pathogenic fungus *F. solani* after 45 days of cultivation using the following satisfactory criterion.

0 = healthy roots,
1 = the root coloration in light brown by 1%–25%,
2 = the root coloration in dark brown by more than 25%, and
3 = the root coloration in dark brown by more than 50%.
4= the root is colored in a dark brown by more than 75%''

The counting of percentage of disease severity depended on the formula (Mckinney, 1923):

$$Disease\ severity = \frac{(number\ of\ plants\ in\ grade\ 0*0)}{tested\ plants\ number*4} + \frac{(number\ of\ plants\ in\ grade\ 1*1) \ldots + (number\ of\ plants\ in\ grade\ 4*4)}{tested\ plants\ number*4} * 100$$

Antibiotic tests of fungi

The antagonistic potential of *T. harzianum* fungus bore testing by following the double culture technique, with the plates placed in the incubator at a temperature of 1 °C ± 25 °C for seven days. The -antagonism estimation used the five-point scale prepared by Bell *et al*. (1982) as follows:

Grade 1 – the growth of the bio-resistance fungus covering the entire area of the plate without allowing the growth of the pathogenic fungus *F. solani*.
Grade 2 – the growth of the bio-resistance fungus covers two-thirds of the plate area, and the growth of the pathogenic fungus covers the remaining third.
Grade 3 – the growth of the bio-resistance fungus covering half of the plate and the growth of pathogenic fungi covering the other half, with no separating area between the two colonies.
Grade 4 – the growth of the bio-resistance fungus covers one-third of the plate area, while the growth of the pathogenic fungus covers the other two-thirds.
Grade 5 – no growth of the bio-resistance fungus, and the growth of the pathogenic fungus covers the entire plate area.

The bio-resistance fungus is effective from the point of view of antagonism when it shows a degree of antagonism (2 or less) with the isolate of the pathogenic fungus, *F. solani*'.

Antibiotic tests for bacteria

A sequence of dilutions of *B. subtilis* suspension ensued by picking up 1 ml of the liquid medium (NB), where the bacteria grow, with a sterile pipette and added to a test tube containing 9 ml of sterile distilled water. All tubes' inoculation proceeded by taking 1 ml from the first tube with a sterile pipette. Transferring to the second tube continued, carrying out this process to obtain a series of dilutions, starting from 10^{-1} to 10^{-10}. Then, the dish containing the culture medium received inoculation by taking one of each dilution and placing on the medium in four points. Placing a disc with a diameter of 0.5 mm on its center

came from the fungus colony. The pathogen was five days old, with four dishes remained untreated by simply adding distilled and sterile water (Hassoun, 2005). Placing the dishes in the incubator, where these remained for three days. Then, calculating the inhibition rate employed the following equation:

$$Inhibition\ rate\% = \frac{control\ colony\ diameter - treated\ colony\ diameter}{control\ colony\ diameter} * 100$$

After obtaining the minimum inhibitory dilution of 10-6 from the bacterial inoculum of the pathogenic fungus *F. solani* from the previous step, preparing four Petri dishes continued to contain the PDA. Then, the dishes' inoculation with bacterial suspensions of 10-6 dilutions had 1 ml/plate. After the plates' incubation for 48 h, calculating the number of colonies in each plate had the rate of bacterial colonies multiplied by the effective dilution inverse (Clark 1965). The number of colonies is 45 ×107 (CFU/ml).

Evaluation of pesticide effectiveness

After preparing the plates of the agricultural medium with the adding of the pesticide Beltanol, inoculation with the pathogenic fungus followed, and then, placed in the incubator. After a week, recording the results of inhibition was according to the following equation.

$$Inhibition\ rate\% = \frac{control\ colony\ diameter - treated\ colony\ diameter}{control\ colony\ diameter} * 100$$

Woody canopy conditions' experiment

The lath house experiment commenced on a private farm in Babil Governorate, Al-Mahaweel District, within the district 3/ Bada'a Al-Nasiriyah, and plot number 1/2 on February 25, 2023. The pathogenic fungus *F. Solani* (R.s2) reached culturing on the seeds of local millet, as previously mentioned. The growing of *B. subtilis* occurred on the Nutrient broth and the bio-resistant fungus *T. harzianum* on the seeds of the local millet plant. The experiment proceeded depending on a completely randomized design (CRD), using sterile plastic pots with a diameter of 20 cm, a height of 20 cm, and a capacity of 5 kg, with four replicates per treatment engaging the treatments below: a) *Fusarium solani* (F.s2) alone, b) *Trichoderma harzianum* (T.h) alone, c) *Bacillus subtilis* (B.s) alone, d) *B. subtilis* (B.s) + fungus *T. harzianum* (T.h), e) *T. harzianum* (T.h) + *Fusarium solani* (F.s2), f) *B. subtilis* (B.s) + *Fusarium solani* (F.s2), g) *B. subtilis* (B.s) + *T. harzianum* (T.h) + *Fusarium solani* (F.s2), and h) Beltanol + *Fusarium solani* (F.s2)

A comparison is uncontaminated with the pathogenic fungus, to which sterilized millet seeds were added alone.

The *T. harzianum* fungus, added to the potting soil, proceeded loading on millet seeds at 10 g/kg, then, mixed well with the soil a week before the addition of the pathogenic fungus. Likewise, the biological resistance bacteria *B. subtilis* placed on a concentration of 45 × 107 CFU/ml preceded a week before the addition of pathogenic fungi by one ml per liter. Three days after planting the cowpea seeds, adding the pathogenic fungus inoculum had a rate of 1% w/w and watered. As for the Beltanol herbicide treatment, it occurred a day after adding the pathogenic fungus inoculum at 1 ml/liter (Hassoun, 2005). The results' calculation ensued 30 days after the conduct of the experiment, with the disease incidence and severity estimated, as well as, the fresh and dry weight, the length of the shoot, and the root of cowpea plants.

Field experiment

The field experiment layout used the randomized complete block design (RCBD), with four replicates for each treatment, preparing the land into furrows with a length of 1.5 m. The distance between the furrows is 50 cm, with each furrow containing four holes. Three seeds' planting in each hole commenced. The field experience included the same as the exchange of the flourishing experience list. The process of adding the fungal inoculum continued by making an incision along the furrow, lifting the soil, with its weight calculated. Then, its infection followed, mixing

it with the inoculum of the fungus *F. solani*. Applying *T. harzianum* to the soil started a week before the soil contamination with the pathogenic fungus. As for the suspension of *B. subtilis*, its combination to the irrigation water was 1 ml/L at a concentration of 45 × 107 (CFU/ml), a week before contamination with the pathogenic fungus. As for the Beltanol herbicide treatment, its treatment began one day after adding the pathogenic fungus (Hassoun, 2005). However, the control treatment without the pathogenic fungus comprised only sterile millet seeds added to it, while the control treatments were *T. harzianum* alone, with *B. subtilis* bacteria added to the soil alone. Recording the findings occurred after 90 days of cultivation, calculating the disease incidence and severity of cowpea plants, fresh and dry weight, and the length of the shoot and root.

RESULTS AND DISCUSSION

Field survey

The findings of the field survey, as seen in Table 3, revealed 10 regions, five from the province of Babil and five from the regions of Baghdad. The results showed the prevalence of the phenomenon of deterioration and damping-off of cowpea plants in all the sites included in the survey, as damping-off and deterioration of plants were prevalent in all these fields. The disease incidence and severity ranged between 10%–70% and 10%–60%, respectively. The outcomes showed the highest disease incidence and severity was in the Jableh Region of the Babil Governorate, by 70% and 60%, respectively. One of the most prominent reasons for this percentage was the repetition of crop cultivation in the same field and irregular irrigation, in addition to the spread of weeds and irregular fertilization of the crop. Meanwhile, the lowest disease incidence and severity are 10% and 10%, respectively, in the Al-Rasheed District of Baghdad Governorate.

Isolation and identification

Table 4 shows isolates of fungi connected with the root of infected cowpea plants from the surveyed regions. Three fungal species reached isolation, namely, *Fusarium solani*, *Rhizoctonia solani*, and *Macrophomina phaseolina*. The table shows the fungi of each surveyed region and the symbol of each isolate of these fungi.

Table 4 further shows the fungus *Fusarium solani* appeared in all samples. As for the fungus *Rhizoctonia solani*, it appeared in three samples, while the fungus *Macrophomina phaseolina* occurred in only one sample. Therefore, the results indicate the pathogenicity is due to the fungus *Fusarium solani*. The conducted morphological identification of the fungi isolated from roots of the infected cowpea plants relied on the morphological characteristics and the growth of mycelium. Either the *Fusarium solani* colony was white to gray or the formation of microconidia, macroconidia, and chlamydospores was with a rough wall. Meanwhile, the *Macrophomina phaseolina* colony was distinctive with the formation of black stone objects. Similarly, the colony of the fungus, *Rhizoctonia solani* was light brown and diverse in its growth speed and formation of dark-colored stone objects, and the density of the mycelium branching at right angles (Booth. 1971).

Pathogenicity tests of the cabbage seeds

The findings of Table 5 indicate the whole examined fungal separates signify to a remarkable decrease in the percentage of germination of cabbage seeds, as the rate ranged between 0%–35%. These further revealed a differentiation to the control treatment of 100%, as the rate of germination was zero in the pathogenic fungus isolates, F.s1, F.s2, F.s5, and F.s9, as they were highly pathogenic. The highest germination was 35% in *M. phaseolina*. This test showed all fungal isolates were pathogenic, but a discrepancy

Table 3. Field survey of damping-off and root rot disease of cowpea plants.

Sample number	Site	Incidence %	Disease severity
1	Babylon / Mahaweel	65	52
2	Babylon / Jableh	70	60
3	Babylon/Mahnawiyah	30	26
4	Babylon / Musayyib Project	42	31
5	Babylon / Hashemite	40	30
6	Baghdad / Radwaniyah	15	12
7	Baghdad / Abu Ghraib	40	30
8	Baghdad / Al-Rasheed district	10	10
9	Baghdad / As-Siyafiyah	50	48
10	Baghdad / Taji	65	50

Table 4. Fungi related to the roots of infected pepper plants in the surveyed areas.

Sample number	Site	Fungus	Isolate symbol
1	Babylon / Mahaweel	*Fusarium solani*	F.s1
2	Babylon / Jableh	*Fusarium solani*	F.s2 + R.s1
3	Babylon/Mahnawiyah	*Rhizoctonia solani* + *Fusarium solani*	F.s3
4	Babylon / Musayyib Project	*Macrophomina phaseolina, Fusarium solani*	F.s4 و M.p
5	Babylon / Hashemite	*Fusarium solani*	F.s5
6	Baghdad / Radwaniyah	*Rhizoctonia solani , Fusarium solani*	F.s6+ 2R.s
7	Baghdad / Abu Ghraib	*Fusarium solani*	F.s7
8	Baghdad / Al-Rasheed district	*Fusarium solani*	F.s8
9	Baghdad / As-Siyafiyah	*Rhizoctonia solani , Fusarium solani*	F.s9 + 3R.s
10	Baghdad / Taji	*Fusarium solani*	F.s10

Table 5. Pathogenicity of cabbage seeds.

Isolate symbol	Germination %	Isolate symbol	Germination %	Isolate symbol	Germination %	Isolate symbol	Germination %
F.s1	00.00	F.s5	00.00	F.s9	00.00	R.s3	28.00
F.s2	00.00	F.s6	18.00	F.s10	12.00	M.p	35.00
F.s3	15.00	F.s7	00.00	R.s1	10.00	Control	100
F.s4	21.00	F.s8	27.00	R.s2	30.00	LSD	1.53

* Each number represents 4 replicates. * F.s = *Fusarium solani,* R.s = *Rhizoctonia solani,* M.p = *Macrophomina phaseolina*

exists in the percentage of their effect on germination. The reason for this may be due to the toxic substances secreted from the fungal isolates (Lozovaya *et al*., 2006).

Effect of *Fusarium solani* isolates on cowpea plants

The results of Table 6 revealed the tested *F. solani* isolates showed a remarkable difference in the disease incidence and severity of the cowpea plant. The isolate R.s2 reached the maximum disease incidence and severity of cowpea seedlings, with a rate of 100% and 100%, respectively. This affected the prevention of germination and growth of cowpea seeds when compared with the control process, where the disease incidence and severity were 0% and 0%, accordingly. Moreover, the fresh and dry weights were 11.30 and 3.85 g, respectively. The cause for the dissimilarity in the disease incidence and severity between the isolates is due to a difference in the proportion of enzymes and toxins secreted by these isolates.

Table 6. Primary infection test of some *F. solani* isolates on pepper plants.

Seq.	Isolate symbol	Incidence %	Disease severity %	Fresh weight (g)	Dry weight (g)
1	F.s1	80.00	70.00	3.65	1.27
2	F.s2	100.00	100.00	0.00	0.00
3	F.s5	90.00	85.00	3.15	1.09
4	F.s7	70.00	60.00	4.50	1.63
5	F.s9	90.00	75.00	3.40	1.13
6	Control	0.00	0.00	11.30	3.85
$LSD_{0.05}$		1.4	1.9	0.51	0.50

* F.s=*Fusarium solani*

Antibiotic tests of fungi

The findings of the antagonistic potential (Table 7) of the biological fungus *T. harzianum* indicated the fungus is highly effective in inhibiting pathogenic fungus *F. solani*. The inhibition reached 85.5% because the fungus possesses mechanisms enabling it to resist the pathogenic fungus. These results were consistent with the study results of Radhi (2022).

Antibiotic tests for bacteria

The outcomes of the laboratory examination (Table 8) showed the bacteria *B. subtilis* possessed a high antagonistic potential against the *F. solani* (F.s2) isolate, with an inhibition of 100% when differentiated with the control treatment. The rate of hindering was zero, and these results were similar to what Radhi (2022) found.

The findings of laboratory examination (Table 9) revealed the antagonistic potential of a series of *B. subtilis* dilutions against the isolate of the pathogenic fungus *F. solani* (F.s1) showed the highest inhibitory dilution reached 10-7, with an inhibition rate of 82.2%. Meanwhile, the inhibition rate was zero in the control treatment.

Evaluation of pesticide effectiveness

The results of Table 10 indicate all concentrations of the Beltanol used in the experiment (0.25, 0.50, 1.00, and 1.50 mm/l) led to the inhibition of the pathogenic fungus *F. solani* (F.s2) by 100% for all concentrations. These results agree with the study of Radhi (2021).

Woody canopy conditions experiment

The findings of the lath house experiment (Table 11) disclosed a highly significant difference in the *B. subtilis* interaction with the biological fungus *T. harzianum* against the pathogenic fungus *F. solani* in reducing the incidence and severity of infection to 23% and 15%, respectively. When measuring with the treatment of pathogenic fungus alone, the disease incidence and severity is the same at 100%. This manifested in the plant length and the fresh and dry weight of the shoot and root, with values of 5.60 and 13.20 cm, 2.10 and 27.10 g, and 0.59 and 5.5 g, respectively. Meanwhile, the plant length and the fresh and dry weight of the shoot and root in the treatment of the pathogen alone was 0.00. The cause for this may be because both the bacteria *B. subtilis* and the fungus *T. harzianum* have worked to inhibit the pathogenic fungus, as well as, its competition for food and place. They worked to analyze the Hypha through the secretion of several enzymes, such as, protease, lipase fats, amylase, and chitinase. This result agreed with the results of many researchers who used the control agent *B. subtilis* (Farah and Sahera, 2016; Radhi, 2022). The two agents of biological resistance exceeded when they interacted together without the presence of the pathogenic fungus in raising the growth parameters. These comprised plant length, fresh and dry weight of the root and shoot, reaching 13.40 and 28.5 cm, 6.90 and 57.30 g,

and 2.30 and 13.12 g, respectively, compared with the control treatment. The plant length and fresh and dry weight of the root and shoot for the control had values of 7.00 and 16.00 cm, 3.20 and 33.20 g, and 1.10 and 6.95 g, accordingly. The findings of the lath house experiment showed the treatment of the chemical herbicide Beltanol when interacting with the pathogen, reduced the disease incidence and severity to 16% and 10%, respectively. This influenced the growth parameters of plant length and fresh and dry weight for the shoot and root (6.00 and 14.10 cm, 2.70 and 29.30 g, and 0.62 and 6.10 g, respectively). The reason is that the herbicide is one of the fungicides affecting the fungus *F.*

Table 7. Antagonistic potential test of the fungus *T. harzianum* against the *F. solani* isolate on culture media.

Treatment Type	Radial growth of the fungus *F. solani*	Inhibition %
T. harzianum	1.30	85.5
Control	9.00	0.00
$LSD_{0.05}$	1.00	1.02

Table 8. Antagonistic potential test of *B. subtilis* bacteria against the *F. solani* (F.s2) isolate on culture media.

Treatment	Radial growth of the fungus *F. solani*	Inhibition %
B. subtilis	0.00	100
Control	9.00	0.00
$LSD_{0.05}$	1.41	2.45

Table 9. Testing series of dilutions of *B. subtilis* bacteria in inhibition of the pathogenic fungus *F. solani* (F.s2) on PDA culture medium.

Concentration	Radial growth of the fungus *F. solani*	Inhibition %
10-1	0.00	100
10-2	0.20	97.80
10-3	0.50	94.50
10-4	0.80	91.10
10-5	1.10	87.80
10-6	1.35	85.00
10-7	1.60	82.20
10-8	2.00	77.80
10-9	2.20	75.60
10-10	3.00	66.70
Control	9.00	0.00
$LSD_{0.05}$	0.71	0.62

Table 10. Examining the efficiency of the chemical herbicide in hindering the *F. solani* (F.s2) isolate on culture medium.

Herbicide concentration (ml/L)	Radial growth of the fungus *F. solani*	Inhibition %
0.25	0.00	100
0.50	0.00	100
1.00	0.00	100
1.50	0.00	100
Control	9.00	0.00
$LSD_{0.05}$	1.23	1.74

Table 11. Evaluating the effectiveness of biological agents in reducing pathogenic fungal infection under woody canopy conditions.

Seq.	Treatment type	Incidence	Disease severity (%)	Plant height (cm)		Fresh weight (g)		Dry weight (g)	
				Shoot	Root	Shoot	Root	Shoot	Root
1	*F. solani* (F.s)	100	100	0.00	0.00	0.00	0.00	0.00	0.00
2	*B. subtilis* (B.s)	0.00	0.00	9.10	20.30	4.10	40.50	1.80	9.10
3	*T. harzianum* (T.h)	0.00	0.00	12.2	26.30	5.70	51.30	2.00	10.90
4	T.h + B.s	0.00	0.00	13.40	28.50	6.90	57.30	2.30	13.12
5	B.s + F.s	45.00	30.00	3.10	7.50	1.50	16.40	0.33	4.31
6	T.h + F.s	31.00	20.00	4.40	11.60	1.90	23.40	0.51	5.46
7	T.h + B.s + F.s	23.00	15.00	5.60	13.20	2.10	27.10	0.59	5.81
8	Beltanol + F.s	16.00	10.00	6.00	14.10	2.70	29.30	0.62	6.10
9	Control	00.00	0.00	7.00	16.00	3.20	33.20	1.10	6.95
$LSD_{0.05}$		1.31	1.37	0.69	0.58	0.42	0.43	0.56	0.40

Table 12. Evaluating the effectiveness of biological agents in reducing pathogenic fungal infection under field conditions.

Seq.	Treatment type	Incidence	Disease severity %	Plant height (cm)		Fresh weight (g)		Dry weight (g)	
				Shoot	Root	Shoot	Root	Shoot	Root
1	*F. solani* (F.s)	87.00	76.00	11.25	26.50	17.60	36.70	4.11	8.71
2	*B. subtilis* (B.s)	0.00	0.00	35.60	100.75	60.31	160.31	14.70	35.12
3	*T. harzianum* (T.h)	0.00	0.00	38.70	115.50	68.30	176.20	15.30	38.73
4	T.h + B.s	0.00	0.00	43.20	125.30	76.21	195.31	17.25	46.26
5	B.s + F.s	40.00	34.00	16.75	40.50	24.80	73.23	6.10	13.34
6	T.h + F.s	30.00	25.00	18.30	47.25	29.63	79.15	7.50	14.51
7	T.h + B.s + F.s	27.00	22.00	20.25	55.30	34.76	90.60	8.90	17.61
8	Beltanol + F.s	18.00	10.00	24.00	64.50	43.31	110.50	9.73	20.11
9	Control	00.00	0.00	28.00	70.75	57.67	126.70	11.43	24.61
$LSD_{0.05}$		1.19	1.24	0.54	0.11	0.42	0.07	0.11	0.12

solani. The herbicide worked on the formation of chelating compounds with copper in the host tissues, facilitating its passage into the cells of the pathogen (Meister, 2000). This result is similar to several studies (Hassoun 2005; Radhi 2021). The biological resistance elements alone and in their interaction with each other showed a high superiority of the biological resistance agents in the growth parameters when compared with the control treatment.

Field experiment

The results of the lath house experiment (Table 12) revealed a highly significant difference when the *B. subtilis* interacted with the biological fungus *T. harzianum* against the pathogenic fungus *F. solani* isolate. It reduced the disease incidence and severity (27.00% and 22.00%, accordingly), compared with the process of the pathogenic fungus alone. Its disease incidence and severity were 87% and 76%, respectively, as reflected in the plant length and the fresh and dry weight of the shoot and root. Their values were 20.25 and 55.30 cm, 34.76 and 90.60 g, and 8.90 and 17.16 g, respectively. Meanwhile, the plant length and the fresh and dry weight of the shoot and root in the treatment of the pathogen alone were 11.25 and 26.50 g, 17.60 and 36.70 g, and 4.11 and 8.71 g, accordingly. The two agents of biological resistance excelled when they interacted together without the pathogenic fungus treatment. They raise the growth parameters, such as, plant length, fresh and dry weight of the root and shoot (43.20 and 125.30 cm, 76.21 and 195.31 g,

and 17.25 and 46.26 g, respectively). When compared with the control treatment, lower values emerged for the plant length and fresh and dry weight of the root and shoot (7.00 and 16.00 cm, 3.20 and 33.20g, and 1.10 and 6.95 g, accordingly).

The findings of the lath house experiment disclosed the treatment of the chemical herbicide Beltanol, when it interacted with the pathogen, reduced the disease incidence and severity to 16% and 10%, respectively. This affected the growth parameters of plant length and fresh and dry weight for the shoot and root (28.00 and 70.75 cm, 57.67 and 126.70 g, and 11.43 and 24.61 g, respectively). This is due to the herbicide as one of the fungicides altering the fungus *F. solani*. The herbicide worked on the formation of chelating compounds with copper in the host tissues, and this facilitates its passage into the cells of the pathogen (Meister, 2000; Onwubiko, 2020; Qassim *et al*., 2023). This result is analogous to Hassoun (2005) and Radhi (2021). Meanwhile, the biological resistance elements alone and in their interaction with each other showed a high superiority of the biological resistance agents in the growth parameters when compared with the control treatment.

CONCLUSIONS

A significant superiority of the biological resistance elements was notable in reducing severity of infection compared with pathogenic fungus alone. The field results confirmed the woody canopy for the efficiency of the biotic resistance elements in reducing the rate and severity of infection, raising the growth of cowpea plants.

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