Iraqi Journal of Market Research and Consumer Protection
(2025) 17(2): 177-191.
DOI:
AL-Mayahi & Hassan

المجلة العراقية لبحوث السوق
وحماية المستهلك

SENSITIVITY ASSESSMENT OF SOME ROSELLE PLANT VARIETIES TO SEED ROT AND DAMPING OFF INFECTION AND DISEASE BIOCONTROL

Hussein, S. M. AL-Mayahi [1] , Aalaa, K. Hassan [2]

[1]Higher Diploma. Ministry Of Agriculture, Baghdad, Iraq, Hussein.Musa2104m@coagri.uobaghdad.edu.iq
[2]Assistant Professor PhD. Department of plant protection, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq, alaa.khuder@coagri.uobaghdad.edu.iq

Received 1/ 10/ 2023, Accepted 29/ 11/ 2023, Published 30/ 9/ 2025

ABSTRACT

Roselle (*Hibiscus sabdarifa L.*) Family Malvaceae is an important crop used in food, cosmetics, and pharmaceutics industries. This study was carried out to evaluate the susceptibility of four different cultivars of Roselle plants against seed rot and damping off. The highest percentage of infection 56.7% was recorded in red cultivar. whereas the lowest 16.7% was recorded in white cultivar. *Fusarium nygamai* scored 42.5% incedence, and this is the first recored of this pathogen on the Roselle crop in Iraq. The treatment of *T.harzanium* + *B. subtilis* + NAA combination showed highely effective scoring disease severity (0.00 ,0.00)% disease infectivity and severity compared to control treatment (pathogen) (73.30,69.67)%. respectively. *T. harzanium* + *B. subtilis* + NAA combination treatment showed the highest antagonistic activity against *F. nygamai*. and improved growth parameters of Roselle when increased fresh and dry weight of plant up to (9.50,3.21) g/plant respectively. Based on the change in light absorbance/ min/g plant fresh weight. The activity of poly phenol oxidase enzyme increased in plants treated with the combination *T. harzanium* + *B. subtilis* + NAA or *T. harzanium* +NAA or *B. subtilis* + NAA produced after 15 and 21 days of treatment. scoring (74.73, 47.92) and (68.76, 45.39) and (68.44, 45.06) respectively, compared to control treatment (pathogenic fungus) the activity of poly phenol oxidase was (35.49,32.09) respectively.

Keywords: varieties sensitivity, *Hibiscus sabdarifa* L, Biological control *Fusarium nygamai*.

تقييم مدى حساسية بعض اصناف الكجرات للاصابة بمرض تعفن بذور وموت البادرات ومكافحته احيائياً

حسين صادق موسى المياحي [1] ، الاء خضير حسان [2]

1. باحث، وزارة الزراعة، بغداد، العراق. Hussein.Musa2104m@coagri.uobaghdad.edu.iq
2. الأستاذ المساعد الدكتور، قسم وقاية النبات، كلية علوم الهندسة الزراعية، جامعة بغداد، بغداد، العراق. alaa.khuder@coagri.uobaghdad.edu.iq

الخلاصة

يعد محصول الكجرات (*Hibiscus sabdariffa* L.)، التابع للعائلة الخبازية مهم، اذ يستعمل في مجال الصناعات الغذائية ومستحضرات التجميل والصيدلانية ، لذلك اجريت هذه الدراسة لتقييم حساسية اربع اصناف مختلفة من نبات الكجرات للاصابة بمرض تعفن بذور وموت بادرات الكجرات تحت ظروف الاصابة الطبيعية، اذ سجلت اعلى نسبة اصابة على الصنف الأحمر والتي بلغت 56.7%، بينما كانت اقل نسبة اصابة على الصنف الأبيض والتي بلغت 16.7%، وبينت نتائج العزل وجود الفطر *Fusarium nygamai* وقد سجل تواجداً بلغ 42.5% وهذا يعد التسجيل الاول من نوعه على محصول الكجرات في العراق ، في حين اظهرت نتائج دراسة مكافحة الفطر الممرض تحت ظروف الاصص تفوق معاملة التكامل بين العوامل الاحيائية *T.harzanium* + *B.subtilis* + NAA على باقي المعاملات بتحقيقها اقل نسبة وشدة اصابة اذا بلغت (0.00 و 0.00)% على التتابع ، قياساً بمعاملة المقارنة (فطر ممرض بمفرده) والتي بلغت (73.3 و 69.67)% على التتابع، وكذلك تفوقت على باقي المعاملات بتحقيقها اعلى زيادة في معدل الوزن الطري والجاف والتي بلغت (9.50 و 3.21) غم/ نبات على التتابع، وبينت النتائج بعد 15 و 21 يوم تفوق معاملات التكامل بين المقاوم الاحيائي *T.harzanium* + *B.subtilis* + NAA و معاملة *T.harzanium* + NAA ومعاملة *B.subtilis* + NAA بزيادة فعالية انزيم Polyphenol oxidase مقدراً على اساس التغير في الامتصاص الضوئي/ دقيقة/ غم وزن طري من نباتات الكجرات ليبلغ (74.73 و 47.92)، (68.76 و 45.39)،(68.44 و 45.06) على التتابع قياساً بمعاملة المقارنة (فطر ممرض بمفرده) والتي بلغت (35.49 و 32.09) على التتابع.

الكلمات المفتاحية: حساسية اصناف، الكجرات، مكافحة احيائية، *Fusarium nygamai*

INTRODUCTION

Roselle plant *Hibiscus sabdariffa* L. is a medicinal plant belonging to the family Malvaceae. It is cultivated for the red calyx and\ or fiber production, Its cultivation season is during the month of April and continues until the fruits ripen until December. It grows at temperatures of 30 and may reach 45°C. **(Fantini *et al.*, 2015; Al-Sayed *et al.*, 2020)**. Roselle plant has many medical and pharmaceutical uses. The calyx leaves contain phenolic and glycoside compounds with a high content of vitamin C, Ca and Na **(Ismail *et al.*, 2008)**. Besides, they contain large quantities of ascorbic and malic acids the main source of sour teste in the calyx leaf extract. It was found Porocatenic acid (PCA) extracted from the calyx leaves has antioxidant and anti-cancer activities. PCA can reduce the carcinogenic effect of Diethylntro sarline in liver and 4-methyl oxide in oral cavity and epithelial gastric glands. Damping off diseases are one of the major factors limiting crops worldwide. Losses caused by these diseases may vary depending on the pathogen. physical and chemical properties of the soil and soil temperature **(Hashem *et al.*, 2017)**. The fungus *Fusarium nygamia* is one of the major seed rot and damping off pathogens as it infects plants at deferent growth stages. This fungus attacks seeds. pre and post emergence and roots in soil. Loses due to the infection with this fungus are mainly correlated to the inoculum size of this fungus available in soil, growing season and biotic factors availability **(Eslaminejad & Zakaria, 2011)**. Fungal diseases can be a major problem on different crops, so chemicals have extensively been used to handle it. Fungicides have been used for 200 years to protect plants against fungal diseases. Usually, most of recommended fungicide treatments can control 90% or more of targeted diseases. However, frequent use and abusing of chemical fungicides resulted in the emergence of pesticide resistance and microbial imbalance in soil. The high completion in agriculture market along with the mentioned limitation have led to search for new approaches to control the disease and eco-friendly fungicide alternatives to minimize the extensive use of chemical fungicides **(Lamichhane *et al.*, 2017; Sahu *et al.*, 2018)**. Among those, the resistance varieties and bio-control have been used as natural controlling approaches and can be introduced into IPM programs against plant pathogens. The bio-agents *Trichoderma* spp and *Bacillus* sp. have widely been used against fungal pathogens of plants in soil including *Fusrium spp* **(Vinale *et al.*, 2006; Dubey *et al.*, 2007; Joshi *et al.*, 2019)**. Due the high importance of seed rot and damping off diseases and the absence of local studies investigating diseases on Roselle plant in Iraq, this study was initiated to assess the sensitivity of some Roselle plant varieties to seed rot and damping off disease along with the biocontrol of this disease.

MATERIAL AND METHODS

Isolation and identification of fungi contaminating Roselle seedling roots

Symptomatic Roselle seedling samples were collected, transferred to Plant Pathology Lab. /Plant Protection Dept./ College. Of Agricultural. Engineer. Sc./Univ. of Baghdad and cleaned to remove soil. Infected parts (crown and roots) were separated from each sample, washed with tap water for 30 min and let to dry. Infected plant parts were cut into small pieces of about 0.5 cm, sterilized with sodium hypochlorite (1% free chlorine) for 2 min, washed with distilled water for 2 min and placed on filter paper to dry of excess water.

The plant pieces were moved into Petri-plates containing Potato Dextrose Agar (PDA) medium (autoclaved for 20 min at 121 °C and 105 Kg/cm^2) and including tetracycline antibiotic at 200 mg/L. Four pieces per plate were added and plates were incubated for 3 days at 25 ± 2°C. Growing fungi were examined using compound microscope to identify the genera based on spores, sexual and asexual structures. Fungal colonies were counted and purified by taking small cuts from the tip of each colony and placing them in the middle of PDA plates, then plates were incubated for 4 days at 25 ± 2°C. The purified fungi were identified up to genus and species based morphology using approved taxonomic keys **(Parameter& Whitney, 1970; Ellis, 1971; Booth, 1977; Veverka, V. *et al.*, 2008; Mc-Govern, R.J., 2015; Hassan, A. K. *et al.*, 2021; Alheety, A. M. S. *et al.* 2022).**

Fungus frequency percent of each sample was calculated as follows:

Fungus frequency per sample % = $\frac{F}{N}$ X100

Where F= Number of plant pieces showing fungal growth in plates

N= Total number of plant pieces used for each sample

Seed viability testing of Roselle plant varieties

Seeds were sterilized by sodium hypochlorite (25 free chlorine) for 2 mints, washed with distilled water and transferred into 9 cm petri plates containig soaked filter paper. Seeds were covered with another filter paper and incubated for 4 days at 25 ± 2°C to calculate seed viability percent.

Response of some Roselle plant varieties to seed rot and damping off disease under field conditions

The field experiment was conducted in the 1st of May, 2022 at College of Agricultural Engineering Sciences\University of Baghdad. to test relative sensitivity of Roselle plant varieties to natural infection of seed rot and damping off disease. Experimental field was prepared based on Randomized Complete Block Design (RCBD). Field layout was 80 cm between blocks, 20 plants per replicate and 30 cm between plants. Fertilization was applied following the Iraqi Ministry of Agriculture recommendations. NPK fertilizer (15-10-15) was applied at 30 Kg\ Donam 10 days before cultivation. The plants were irrigated each 5 days depending on plant growth stage and temperature during the season. Four varieties grown were kindly provided by Medicinal Plants Propagation Program\Agriculture Research Directorate\Iraqi Ministry of Agriculture (Table 1).

Table (1): Roselle plant varieties tested.

No.	Var. name
1	Red
2	Dark red
3	White
4	Stripy

The sensitivity of varieties to seed rot and damping off infection was tested. Data were collected 8 days of cultivation for 4 weeks and infectivity percent was calculated as follows:
Infectivity% = (No. of infected seedlings) / (Total No of seedlings) X 100
Fresh and dry plant weights were measured 5 months of plant cultivation. Plant samples were incubated for 3 days at 50 °C or up to full dryness and stability of weight.

Sensitivity test of Roselle plant varieties to *Fusarium nygami* infection in pots

An isolate of *F. nygami* (Fn3) was selected based on field experiment, pathogenicity test, morphological and molecular identification, and deposited in NCBI (**OQ572738**). Soil and Peat Moss mixture was prepared, autoclaved and added to 2.5 cm pots at 3 Kg/pot rate. Four varieties were planted (Table 1) in the autumn growing season 2022. The experiment was performed based on CRD using 8 treatments with 3 replicates each. Data were analyzed based on L.S.D. at probability value 0.05. The inoculum of pathogenic fungus, previously loaded on local millet seeds for 3 days, was added to pots at 1% rate. Roselle plant seeds of each variety were sown at 10 seeds\pot rate. Autoclaved millet seeds were used for control treatment. Infection percent was calculated 10 days of seed sowing based on equation above. Whole plants were collected 40 days of cultivation to calculate disease severity based on the following disease index:

0=Healthy plants
1=1-25% of root is rotten
2=26-50% of root is rotten
3=51-75 of root is rotten
4=76-100% of root is rotten or plant death

The disease severity percent (DSP) was calculated based on McKinney (1923) as follows:

DSP = ((No. of plants in class 0×0) +⋯+ (No. of plants in class 4×4))/(Total no. of plants × thighest class) × 100

Fresh and dry plant weights were measured to determine the best var. resistance against the infection of pathogenic fungus isolate.

Efficacy assessment of bio-agents to control *F. nygamai* in pots.

An isolate of the fungus *T. harzianum* provided by Plant Pathology Dept., Agricultural Research Directorate, Ministry of Agriculture was used. and The bacterium *Bacillus subtilis* was kindly provided by Central Lab./Dept. of Soil and Water Resources/Coll. of Agri. Eng. Sc./Univ. of Baghdad.

The experiment was performed in pots as previously described. The following treatments were used:

1. *F. nygamai* (Fn) only treatment
2. Pathogen free control

3. Sterilized soil (SS)+ *B. subtilis* (Bs)
4. SS+*T.harizanium* (Th)
5. SS+ Naphthalen acitic acid (NAA)
6. SS+ Bs + Th
7. SS +Bs + NAA
8. SS+ Th + NAA
9. SS+ Bs + Th + NAA
10. Fn contaminated soil (FnCS) + Bs
11. FnCS + Th
12. FnCS + NAA
13. FnCS + Bs + Th
14. FnCS + Bs + NAA
15. FnCS + Th + NAA
16. FnCS + Bs + Th +NAA
17. FnCS +Uniform

Treatments were performed as previously described. Pots were covered with pitted polyethylene bags for 3 days. Roselle plant var. dark red seeds were sterilized for 1 min using 3% of sodium hypochlorite, washed by sterile distilled water and sown at 5 seeds\pot rate. Treatments were added during seeding. *T. harzianium* treatment was added and mixed with the soil at 0.6 g\pot rate and 1 $\times 10^9$ concentration. NAA treatment was added at 300 mg/L concentration and 50ml\pot rate **(Al-Masri & Barakat, 2003)**. Uniforme fungicide was added to soil at 1ml\L concentration and 59 ml\pot rate following the manufacturer company recommended dose. Bacterial inoculum was prepared from 48h *B. subtilis* culture at 5×10^6 CFU\ml concentration and added at 100 ml\pot rate. Similar concentrations were used with pathogenic fungus free treatments. Ahalf of each concentration was used for combination treatments. CRD was applied using 17 treatments with 3 replicates each. Data were analyzed based on L.S.D. at probability value 0.05. Seed rot and pre-emergence damping off percentages were calculated after seed germination of control treatment completed. While post-emergence damping off calculation continued up 30 days of sowing. The infection of pathogenic fungus was confirmed through pathogen isolation from roots on PDA and Microscope examination was performed to confirm the pathogen. Plants from each replicate were collected two months of cultivation to estimate the disease severity based on 5 scale disease index mentioned. Disease severity percent was calculated based on McKinney's formula. Seedling roots were surface sterilized, cultured on PDA and examined by microscope to confirm the pathogen. Three plants from each replicate were taken to measure the fresh and dry weights. The root system of each plant was washed with water to remove soil remaining, put in a paper bag and fully dried in electric oven at 40 °C up to weight stabilization. Poly Phenol Oxidase activity was estimated in plant leaves treated with the factors mentioned, and pathogen, 15 and 30 days of seed sowing to test resistance induction in plant against the disease (**Sadasivam & Manickam, 1996; Ojha & Chatterjee, 2012)**.

RESULTS AND DISCUSSION

Seeds viability test of Roselle plant varieties

Roselle seeds of varieties showed viability scoring up to 100% germination (Table 2). The high viability of embryo may be related to short storage period of seeds.

Table (2): Seed viability assessment of Roselle plant varieties.

No.	Var.	Seed viability%
1	Red	100
2	Dark red	100
3	White	100
4	Mixed	100
	L.S.D $_{0.05}$	N.S.

Sensitivity test of some Roselle plant varieties to seed rot and damping off - disease under field conditions:

Roselle plants are infected by several pathogens worldwide, among them those causing seed rot and damping off disease (SRDD). This most significant disease is caused by the fungi *Fusarium*, *Rhizoctonia* and *Sclerotina* and the protists *Pythium* and *Phytophthora*. SRDD caused by the fungus *F. nygamai* can limit Roselle plant production. In this study, Roselle varieties varied in their sensitivity to the infection of soil fungi (Table 3). Amongst others, the white variety was highly tolerant against SRDD pathogens scoring 18.3% infection. In contrast, red var. was highly sensitive scoring 58.3% infection. Similarly, **Eslaminejad (2011) and Hassan *et al.*, (2014)** indicated significant variations among Roselle varieties. Variation of infection among varieties may be related to the structure of plant cell walls or other types of incompatibility (**Eslaminejad** ,**2011** ;**Hassan *et al.*, 2014** ;**Sultan and Khorshid 2018**).

Table (3): The response of some Roselle varieties to SRDD infectivity percent in field conditions.

No.	Var.	% Infectivity	(Fresh weight g/plant)	Dry weight (g/plant)
1	Red	58.3	487.5	165.7
2	Dark red	28.3	495.3	172.3
3	White	18.3	517.2	181.3
4	Mixed	20.0	504.2	174.4
	L.S.D $_{0.05}$	19.41	30.96	14.52

- Each number is an average of 3 replicates.

These pathogens decreased growth parameters in tested varieties including fresh and dry weights which varied among varieties (Table 3 and Fig 2). White var. scored 517.2 and

181.3 g/plant highest fresh and dry weights, respectively. Whereas, red var. scored 487.5 and 165.7 g/plant lowest fresh and dry weights, respectively. These differences among varieties may be related to their activity to produce many enzymes involved in plant defense and hypersensitivity reaction at fungal penetration sites through epidermis and cortex tissues, and metabolites accumulation in outer tissues. restricting fungal advancing to xylem vessels **(Hassan *et al.*, 2014).**

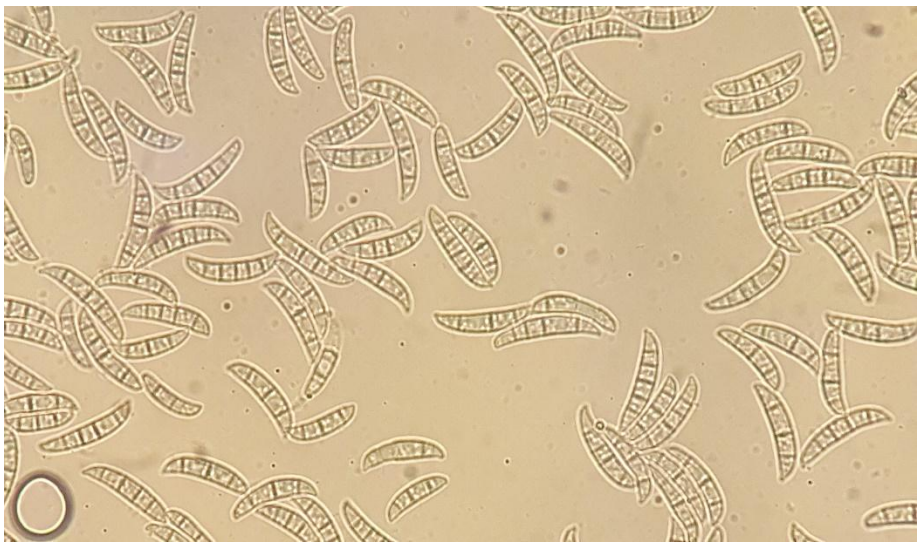

Figure (1): Identification and characterization of *F. nygamai*.

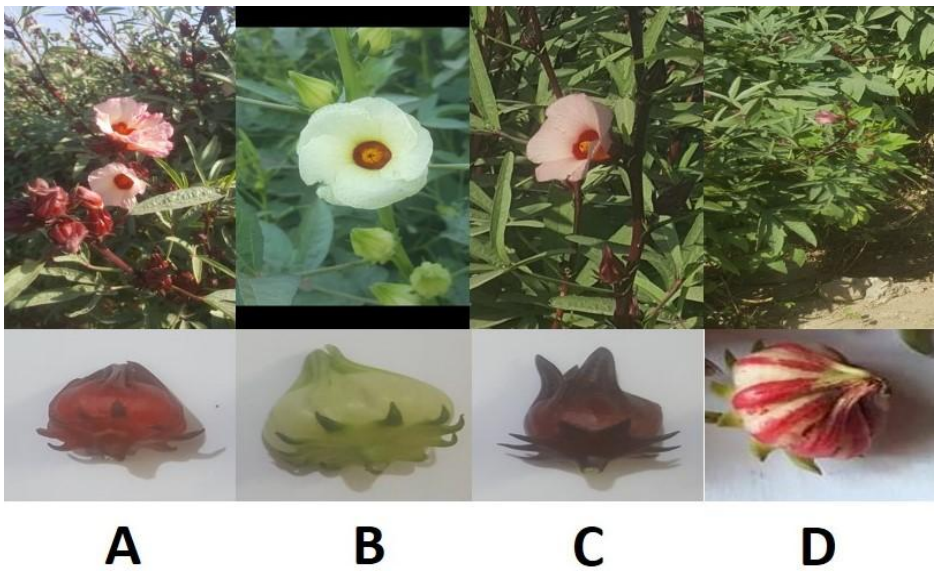

Figure (2): Roselle varieties tested.
A: Red; **B:** White; **C:** Dark red; **D:** Mixed

Sensitivity test of Roselle plant varieties to *Fusarium nygami* infection in pots

The sensitivity to SRDD showed a significant variation among varieties tested (Table 4). The white was highly resistant among other varieties when scored 16.7 and 13.3% lowest infectivity and disease severity, respectively. While, disease resistance in red var. was the lowest scoring 63.3 and 57.5% maximum infectivity and disease severity, respectively. In mixed and dark red varieties, the infectivity and disease severity scored 26.7 and 36.7%, between moderate resistant and infect-able. The variation in varieties response to pathogenic fungus infection may be related to genotype, morphological, anatomical and biochemical characteristics of each variety **(Hassan *et al.*, 2014)**. Similarly, the fungal infection affected growth parameters including fresh and dry weights of the 4 varieties. The white var. scored 7.170 and 3.707 g, compared to red var. which scored 6.677 and 2.967 g/plant lowest fresh and dry weights, respectively.

Table (4): Infectivity and disease severity of Roselle plant varieties inoculated with *Fusarium nygami.*

No	Var.	Infectivity%	Disease severity %	Resistance level	Fresh weight g/plant	Dry weight g/plant
1	Red	0	0	Non	7.907	3.873
2	Dark red	0	0	Non	7.857	3.910
3	White	0	0	Non	7.840	3.827
4	Mixed	0	0	Non	7.853	3.897
5	*F. nygamai* + White	16.7	13.3	Resistant	7.170	3.707
6	*F. nygamai* + Red	63.3	57.5	Sensitive	6.677	2.967
7	*F. nygamai* + Dark red	36.7	33.3	Infectible	6.967	3.180
8	*F. nygamai* + Mixed	26.7	26.7	Moderate resistant	6.953	3.057
	L.S.D $_{0.05}$	9.35	10.48		0.15	0.36

The increase of fresh weight in white var. infected with the pathogenic fungus may be related to its content of some compounds with antimicrobial activity against many plant pathogens. including *F. nygami* that provide plants with nutrients, resulting in the increase in the fresh and dry weights **(Sultan & Khorshid, 2018).**

Biological control of seed rot and damping off disease caused by *Fusarium nygamai* in pots:

All treatments could reduce the negative effect of the pathogenic fungus *F. nygamai* and protect Roselle plants significantly, against SRDD (Table 5 and Figure 3). Roselle plants treated with the combination *T. harzianum* + *B. subtilis* + NAA + *F. nygamai* scored 0.00 and 0.00% infectivity and disease severity, compared to fungus only control treatment scoring 73.3 and 69.67%, respectively. This treatment did not show a significant difference to *T. harzianum* +*B. subtilis* and *B. subtilis* + NAA (with *F. nygamai*) when scored 6.7 and 4.00, 6.7 and 2.67 % infectivity and disease severity, respectively, followed by other treatments. The absence of infection and disease severity in *T. harzianum* + *B. subtilis* + NAA combination may be related

to the efficacy of the *T. harzianum*. The hyphae of this bio-agent can enter through seedling roots and grow within intercellular spaces, resulting in the induction and increase the activity of peroxidase and polyphenol oxidase in treated plants **(Mousa & Hassan, 2023)**. Moreover, *T. harzianum* can parasitize directly on the pathogenic fungus growth, produce antifungal compounds and enzymes breaking the pathogenic fungus cell walls. including B – 1,3-glucanase and chitinase or inhabit the fungus through food and space competition in rhizosphere **(Yao *et al* 2023)**. *B. subtilis* can inhibit other plant pathogens through production of antibiotics including bacillomycin, iturin A, surfactin, difficidin, subtilin, oxydifficidin and mycosubtilin in addition to lytic enzymes cleaving polymeric compounds, including amylase, lipase, protease, chitinase, catalase, lecithinase and superoxide dismutase **(Mageshwaran *et al.*, 2022)**. Additionally, this bacterium can induce the systemic resistance through increasing the activity of peroxidase and B – 1,3-glucanase **(Chen *et al.*, 2016)**. *B. subtilis* can produce cytokinins that increase leaf content of chlorophyll, and auxins that increase leaf area and metabolic synthesis **(Yao *et al.*, 2006; Cao *et al.*, 2012)**. Infection reduction in Plants treated with NAA may be related to auxin role in protein and enzyme synthesis involved in cell division and elongation which resulted in improving plant growth parameters.

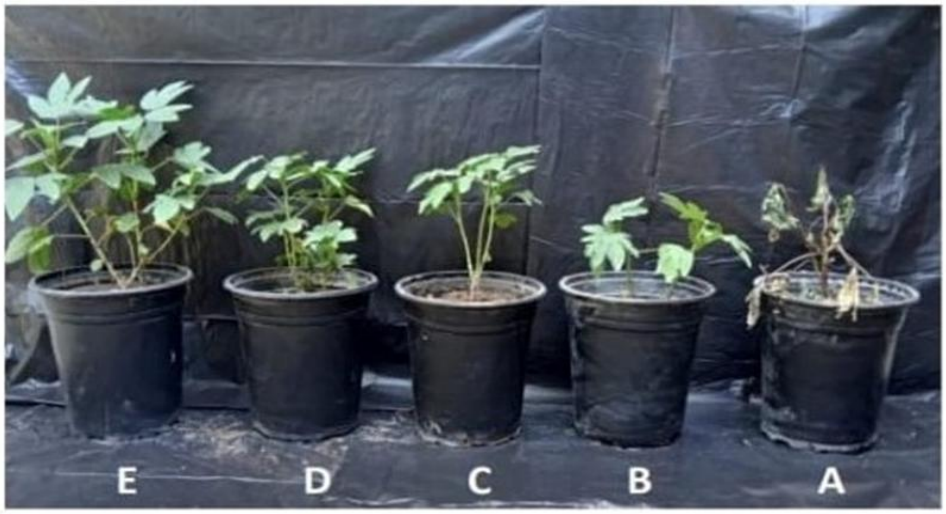

Figure (3): The effect of biotic factors on the growth of the Gujarat plant.
A: Pathogenic fungus *F. nygamai* (Fn3)
B: Sterilized soil (control)
C: Fn3 + *B. subtilis* + *T.harizanium*
D: Fn3 + NAA + *T.harizanium*
E: Fn3 + *B. subtilis* + *T.harizanium* + NAA

Table (5): % Infectivity and **%** disease severity percentages to test the effect of biological control agents against pathogenic fungi in pots. (5 seeds\pot).

No	treatments	% Infectivity	%Disease severity %
1	Control	0.00	0.00
2	*F. nygamai* (Fn3)	73.30	69.67
3	*T.harizanium*	0.00	0.00
4	*B. subtilis*	0.00	0.00
5	NAA	0.00	0.00
6	*B. subtilis* + *T.harizanium*	0.00	0.00
7	NAA + *T.harizanium*	0.00	0.00
8	NAA + *B. subtilis*	0.00	0.00
9	NAA + B. subtilis + *T.harizanium*	0.00	0.00
10	Fn3 + *T.harizanium*	13.30	12.67
11	Fn3 + *B. subtilis*	13.30	11.33
12	Fn3 + NAA	20.00	25.33
13	Fn3 + *B. subtilis* + *T.harizanium*	6.70	4.00
14	Fn3 + NAA + *T.harizanium*	13.30	10.67
15	Fn3 + NAA + *B. subtilis*	6.70	2.67
16	Fn3 + NAA + *B. subtilis* + *T.harizanium*	0.00	0.00
17	Fn3 + Uniform	46.70	36.67
	L.S.D 0.05	14.70	5.31

- Each number is an average of 3 replicates.

All treatments improved plant growth significantly, through increasing fresh and dry weights (Table 6). The combination *T. harzianum* + *B. subtilis* + NAA + *F. nygamai* scored highest 9.50 and 3.21 g/plant fresh and dry weights compared to 5.01 and 1.25 g/plant for fungus only control treatment, respectively. The high efficacy of the combination treatments in improving plant growth parameters may be related to the activity of factors used to increase the availability of nutrients in Roselle leaves which resulting in improving growth indicators (**Al-masri & Barakat, 2003 ;Dhaher, 2016; AL.Awabid & Yass, 2023**).

Table (6): Fresh weight and dry weight to test the effect of biological control agents against pathogenic fungi in pots. (5 seeds\pot).

No	treatments	Fresh weight g/plant	Dry weight g/plant
1	Control	8.07	2.95
2	*F. nygamai* (Fn3)	5.01	1.25
3	*T.harizanium*	9.85	3.22
4	*B. subtilis*	10.15	3.53
5	NAA	10.08	3.12
6	*B. subtilis* + *T.harizanium*	10.39	3.47
7	NAA + *T.harizanium*	10.04	3.38
8	NAA + *B. subtilis*	10.15	3.74
9	NAA + B. subtilis + *T.harizanium*	10.48	3.82
10	Fn3 + *T.harizanium*	8.06	2.92
11	Fn3 + *B. subtilis*	8.07	2.96
12	Fn3 + NAA	7.82	2.71
13	Fn3 + *B. subtilis* + *T.harizanium*	8.14	2.47
14	Fn3 + NAA + *T.harizanium*	8.10	2.43
15	Fn3 + NAA + *B. subtilis*	8.30	3.03
16	Fn3 + NAA + *B. subtilis* + *T.harizanium*	9.50	3.21
17	Fn3 + Uniform	6.65	2.02
	L.S.D $_{0.05}$	0.31	0.19

- Each number is an average of 3 replicates.

T. harzianum + *B. subtilis* + NAA + *F. nygamai* combination was high efficient to induce systemic resistance, 15 and 21 days of plant cultivation (Table 7). The activity of Polyphenol oxidase (PPO) in Roselle plants treated with this combination, scored 74.73 and 47.92 increase in absorbance/min/g fresh weight, compared to 35.49 and 32.09 increase in absorbance/min/g fresh weight in fungus only treatment.

Table (7): The effect on the activity of the polyphenol oxidase (PPO) enzyme after 15 and 21 days of plant cultivation.

No	treatments	15 days of plant cultivation	21 days of plant cultivation
1	Control	21.27	21.77
2	*F. nygamai* (Fn3)	35.49	32.09
3	*T.harizanium*	45.97	45.18
4	*B. subtilis*	46.81	45.88
5	NAA	46.32	41.38
6	*B. subtilis* + *T.harizanium*	48.52	45.39
7	NAA + *T.harizanium*	47.04	41.83
8	NAA + *B. subtilis*	48.41	45.04
9	NAA + B. subtilis + *T.harizanium*	49.57	46.20
10	Fn3 + *T.harizanium*	56.86	42.45
11	Fn3 + *B. subtilis*	59.33	46.48
12	Fn3 + NAA	57.83	41.02
13	Fn3 + *B. subtilis* + *T.harizanium*	63.85	43.98
14	Fn3 + NAA + *T.harizanium*	68.76	45.39
15	Fn3 + NAA + *B. subtilis*	68.44	45.06
16	Fn3 + NAA + *B. subtilis* + *T.harizanium*	74.73	47.92
17	Fn3 + Uniform	18.39	19.04
	L.S.D 0.05	2.292	2.018

- Each number is an average of 3 replicates.

CONCLUSION

Based on the results observed in this study, we concluded that the most resistant variety to rot seed and damping off seedling Roselle was the white variety. Whereas, the best way to control the disease is to treate plants with a combination of biological agents with naphthalene acetic acid.

REFERENCES

1. AL-Awabid,A.K., & Yass, S.T. (2023). Evaluation the activity of Hirsutella sp Fungus and the Nematicide Verox to Control Root knots Nematode Meloidogyne spp. On Fig seedlings, Iraqi journal of market research and consumer protection, 15(1), 147-156.
2. Alheety, A.M.S., Faarhan, M.A., & Hassan, A.K. (2022). Evaluation of the efficiency of some plant extracts in inhibiting the growth of Fusarium solani that causes pepper root rot. Int. J. Agricult. Stat. Sci., 18, (1): 2085-2090.
3. Al-Masri, M. I. & Barakat, R. (2003). Role of the auxin, Naphthalene Acetic Acid (NAA) in the pathogenicity of Sclerotinia sclerotiorum**,** causative organism of white mold disease. J. Phytopathol. 150,481-487.
4. Al-Sayed, H. M., Hegab, S. A., Youssef, M.A., Khalafalla, M.Y., Almaroai, Y.A., Ding, Z. & Eissa, M.A. (2020). Evaluation of quality and growth of roselle (Hibiscus sabdariffa L.) as affected by bio-fertilizers. Journal of Plant Nutrition. 43(7), 1025-1035.
5. Booth, C. (1977). Fusarium. Laboratory guide to the identification of the major species. Commonwealth Mycological Institute, Kew, Survey, England, 58 pp.
6. Cao, y., Zhang, Z.H., Ling, N., Yuan, Y.J. & Shen, Q.R. (2012). Bacillus subtilis SQR9 can control Fusarium with in Cucumber by colonizing plant roots. Biology and Fertility of Soils, 47(5), 495-506.
7. Chen, L.A., Peng, J., Zhang, Q.D. & Wang, S. (2016). Rsiadb, collective resource for genome and transcriptome analyses in Rhizoctonia solani AGI IA. Biology, Medicine, Computer Science. 31(1), 1-8.
8. Dhaher, H.R., (2016). Evaluation of efficiency of some plant growth regulators, Pseudomonas fluorescens and pleurotus ostreatus in induction of resistance of cucumber plant (cucumis sativus) against Sclerotinia sclerotiorum, MSC. Thesis, university of Kufa, Al-kufa, Iraqi, 102pp.
9. Dubey, S.C., Suresh, M. & Singh, B. (2007). Evaluation of Trichoderma species against Fusarium oxysporum f. sp. ciceris for integrated management of chickpea wilt, Volume 40, Issue 1, January, Pages 118-127.
10. Ellis, M. B. (1971). Dematiaceous Hyphomycetes. Commonwealth Mycological Institute. Kew, survey England .608 pp.
11. Eslaminejad, T. & Zakaria, M. (2011). Morphological characteristics and pathogenicity of fungi associated with Roselle (Hibiscus Sabdariffa) diseases in Penang, Malaysia. Microb Pathog. Nov, 51(5), 325-37.
12. Fantini, M., Benvenuto, M., Masuelli, L., Frajese, G., Tresoldi, I., Modesti, A. & Bei, R. (2015). In vitro and in vivo antitumoral effects of combinations of polyphenols, or polyphenols and anticancer drugs: Perspectives on cancer treatment. Int. J. Mol. Sci., 16, 9236–9282.
13. Hashem, H.A.E.A., El-Hadidy, A.E.A. & Ali, E.A. (2017). Impact of some safe agricultural treatments on insect pests, vascular wilt disease management and Roselle

(Hibiscus sabdariffa L.) productivity under Siwa Oasis conditions. International Journal of Environment, 6(4), 139-162.

14. Hassan,A.K., Abdul-Karim,E.K., AL-Kawaiti,N.A.S. & Saleh,N.M. (2021) .Efficacy assessment of black pepper and clove extracts against sunflower seeds black rot disease caused by Aspergillus spp. Int. J. Agricult. Stat. Sci. 17 (1): 2239-2244.
15. Hassan, N., Elsharkawy, M.M., Shimizu, M. & Hyakumachi, M. (2014). Control of root rot and wilt diseases of Roselle under field conditions. Mycobiology, 42(4), 376-384.
16. Ismail, A., Ikram, E.H.K. & Nazri, H.S.M. (2008). Roselle (Hibiscus sabdariffa L.) seeds nutritional composition protein quality and health benefits. Food, 2, 1–16.
17. Joshi, D., Gupta, J., Mishra, A. et al. (2019). Distribution, Composition and Bioactivity of Endophytic Trichoderma spp. Associated with Sugarcane. Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci. 89, 1189–1200.
18. Lamichhane, J.R., Dürr, C., Schwanck, A.A., Robin, M.H., Sarthou, J.P., Cellier, V., Messéan, A. & Aubertot, J.N. (2017). Integrated management of damping-off diseases, A review. Agron. Sustain. Dev. 37,10.
19. Mageshwaran, V. Gupta, R., Singh, S., Sahu, P.K., Singh, U.B., Chakdar, H., Bagul, S.Y. Paul, S. & Singh, H.V. (2022). Endophytic Bacillus subtilis antagonize soil-borne fungal pathogens and suppress wilt complex disease in chickpea plants (Cicer arietinum L.). Front. Microbiol. 13:994847.
20. Mc-Govern, R.J. (2015). Management of tomato diseases caused by Fusarium oxysporum. Crop Protection. 73:78-92.
21. Mousa, A.R. & Hassan, A.K. (2023). Inducing systemic acquired Resistancein pepper plants against against Rhizoctonia solani, Iraqi J. of Market Research and consumer protection, 15(1), 92-105.
22. Ojha, S. & Chatterjee, N.C. (2012). Induction of resistance in tomato plants against Fusarium oxysporum f. sp. lycopersici mediated through salicylic acid and Trichoderma harzianum. Journal of Plant Protection Research. 52(2), 220-225.
23. Parameter, J. R. & Whitney, H. S. (1970). Taxonomy and nomenclature of the imperfect state. In: Rhizoctonia solani: Biology and Pathology. Parmeter, J. R. (Editor). University of California Press, California.
24. Sadasivam, S. & Manickam, A. (1996). Biochemical methods. New Age International. 3(2), 124-126.
25. Sahu, B., Singh, J., Shankar G. & Pradhan, A. (2018). Pseudomonas fluorescens PGPR bacteria as well as biological agent: A review. International Journal of Chemical Studies, 6(2), 1-7.
26. Sultan, F.I. & Khorshid, A.CH. (2018). Separation and Identification of some Phenolic Compounds and Maleic Acid from Hibiscus sabdariffa L. and Study its Effect on the some Types of Pathogenic Bacteria, Al-Rafidain Science Magazine, Vol.,27, Issue 5/Botany/Special issue of the Third Scientific Conference for Biology, 169-180.

27. Veverka, V.; Crabbe, T.; Bird, I.; Lennie, G.; Muskett, F. W.; Taylor, R. J & Carr, M. D. (2008). Structural characterization of the interaction of mTOR with phosphatidic acid and a novel class of inhibitor: compelling evidence for a central role of the FRB domain insmall molecule-mediated regulation of mTOR. Oncogene, 27(5), 585-595.
28. Vinale, F., Marra, R., Scala, F., Ghisalbert, E.L. & Sivasithamparam, K. (2006). Major secondary metabolites produced by two commercial Trichoderma strains active against different photopathogens. Let. Appl. Microbiol. 43,143-148.
29. Yao, X., Guo, H., Zhang, k., Zhao, M., Ruan, J. & Chen, J. (2023). Trichoderma and its role in biological control of plant fungal and nematoda disease, Frontiers in microbiology, 14, https: // doi.org/ 10.3389/ fmict. 1160551.
30. Yao, A.V., Karimov, S., Bochow, H., Boturov, U. & Sharipov, A. (2006). Effect of FZB24 Bacillus subtilis as biofertilizer on cotton yields in Fields test. Archives of phytopathology and plant protection, 39(4), 323-328.