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ORIGINAL PAPER

Effect of Sulphuric Acid Amendments on Soil-Borne Pathogenic Fungi Associated with Root Rot of Two Dry Bean Cultivars

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ABSTRACT

Damping-off of common bean (*Phaseolus vulgaris* L.) is considered to be of paramount importance causing yield losses that exceed 50% and has been reported in many countries worldwide. In this study, damping off and root rot fungi and others associated with common bean root rot in Egypt were isolated and were identified as *Rhizoctonia solani* Kuhn, *Macrophomina phaseolina* (Tassi) Goid and *Sclerotium rolfsii* Sacc. Non-traditional control measures with chemicals as sulphuric acid were tried in literature. It has been shown in the present study that application of high rates of H_2SO_4 to bean plants have had indirect effect on disease(s) progress. Plants, 45 days after the treatment, were evaluated pathologically. Two dry bean Nebraska and Nahda cultivars reacted differently to the isolated pathogenic fungi. Pre and Post emergence damping off caused by mixture of the isolated fungi on Nahda cv. treated with sulphuric acid compared to Nebraska cultivar showed greater susceptibility to the disease. The results showed significant differences in shoot /root ratio(s) either on fresh or dry weights bases as influenced by H_2SO_4 strength, *i.e.*, the concentration. Different intensity values of nodulation in both varieties were found to follow the recorded difference in damping off susceptibility. The same conclusion may be applied to the inherent difference in varietal susceptibility to infection by nodule bacteria. Root rot severity was significant and also determination of microbial density, 45 days after application. The concentration (0.2N) of sulphuric acid resulted in the decrease of each of N, P and K in most cases. Further studies are needed to include the possible effect of induced resistance in common bean induced by sulphuric acid. Field trials may be advised.

Keywords: Bean, *Phaseolus vulgaris*, damping-off, root-rot complex, sulphuric acid amendments.

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important food crop worldwide (Wortmann et al., 1998) for its nutritive benefits, such as high content of protein, micronutrients, vitamins and dietary fiber (Widders, 2006). The commonly known bean (*Phaseolus vulgaris* L.) is an important legume that is widely grown in Latin America and Africa (Anon., 2014). Common bean has several market classes, which include dry beans and green beans. The cultivated land of dry bean in Egypt in the year 2021 was 216977 feddans produced 235293 tons, with an average yield 1.084 ton/fed.

The production of common bean is however, constrained by soil-borne pathogens especially those causing root rots. Common bean root rots cause significant yield losses and are wide spreading in Central and South America and Africa (Buruchara *et al*., 2015).

The most common soil-borne pathogens that cause common bean root rots were reported to include *Pythium* spp., *Fusarium* spp., *Rhizoctonia solani* and *Sclerotium rolfsii*, *Aphanomyces euteiches* f. sp. *phaseoli*. Stem diseases, are often found in the same areas and being erratically described as root-rot diseases such as Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *phaseoli* and charcoal rot, caused by *Macrophomina phaseolina* (Abawi *et al*., 1990) that varied in their pathogenic potentiality according to host susceptibility.

Sulphur is an important component of plant nutrition with demand varies with plant species. Sulphur fertilization has been shown to increase significantly sulphate ions in the nutrient solution, depends on many factors, as pH values, temperature, access need to energy, corresponding sulphate concentration, strength, and the presence of other ions, also the content of stress-related S-containing metabolites such as cysteine, GSH and H_2S (Salac *et al*., 2005). High sulphate concentrations may affect plant development and crop yield (Cerda *et al*., 1984). Sulphur is also an important constituent of some

compounds which may be involved in defense mechanisms against herbivorous pests, and pathogens (Bennett and Wallsgrove, 1994).

Higher content of sulphur in soil causes some soil disorders such as extra acidification. Sulphur is indirectly responsible for mobilization of phytotoxic chemicals, such as aluminum and other trace elements (Komarnisky *et al*, 2003).

Sulphur (S) is one of the components containing sulphur amino (cysteine and methionine) and many other compounds, as glutathione or Ferro dioxin (Kowalska, 2005). Klikocka *et al*. (2005) recorded that the increase in resistance against a variety of fungal pathogens on different crops under glasshouse and field conditions was found in soil applied with sulphur. Haneklaus *et al*. (2009) reported that sulphur metabolites such as cysteine, glutathione, gaseous S emissions, phytoalexins, glucosinolates, and elemental S depositions play an important role in plant defense or resistance to fungal pathogens.

Unfortunately, sulphur deficiencies became more frequent due to progressive emission of the respective gas to the natural environment and to the intensive use of S-free NPK fertilizers creating the necessity of sculpture application (Barczak *et al*., 2014).

The objectives of this work were an exploration of the effect of different sulphuric acid strength(s) in the nutrient solutions on plant growth, and their respective effects on root rot severity in some cultivated bean cultivars.

MATERIALS AND METHODS

Isolation and identification of the associated fungi:

Naturally infected bean plants showing typical root rot symptoms were collected from different farms located in Qaliobia Governorate. The associated fungi were isolated on potato dextrose agar (PDA) medium from the affected root tissues. Purification of the isolated fungi was done using the hyphal tip technique (Dhingra and Sinclair, 1995). Cultural properties, morphological and microscopical characteristics were determined as described by Dhingra and Sinclair, (1978), Carling and Summer (1992), Barnett and Hunter (1998). Stock cultures were maintained on PDA medium at 4±1°C till use.

Preparation of inoculum:

Inocula of the fungal isolates were prepared by growing each of them separately in bottles (500 ml) containing sterilized sand corn medium (25g washed sand, 75g corn and reasonable quantity of tap water), inoculated with 5 mm disc taken from the edge of 7 days old culture of the required fungus and incubated at 28±2°C for 15 days. Dhingra and Sinclair, (1995).

Pathogenicity tests:

Seeds of the local cultivars *i.e*., Nahda and Nebraska obtained from Veg. Res. Dept., Hort. Res. Inst., ARC., Giza, were planted in pots (12.5 cm) filled with disinfested sterilized soil (2:1 sand and peat moss). The pots, each was filled with one kg, soil infestation was made by transferring the fungal inoculum of each isolate *R. solani, S. rolfsii* and *M. phaseolina* as individually fungus or mixture fungi as 1:1:1 to the pots and mixed it well with the potted soil at the rate of 3% (w/w). Five seeds per pot were sown and three replicates were used for each treatment and were distributed in the greenhouse in complete randomized design. The percentage of pre- and post-emergence damping-off was determined 15, and 30 days after planting while root rot and surviving plants and the disease severity were determined after 45 days post planting.

Preparation of different concentrations of sulphuric acid tested:

Properties of sulphuric acid tested were:

Concentration of H_2SO_4 = 96% Density of H_2SO_4 = 1.840

Mass of H_2SO_4 present = 100 gm

Volume of H_2SO_4/l = Actual mass/molar mass (Density)

Volume of H_2SO_4 /l = 100/ (1.840×1000) = 0.054 l

Weight of H_2SO_4 = Volume of H_2SO_4 × Normality of H_2SO_4 × Equivalent weight

96 = 0.054 × N × 98.01

N = 18.14

To prepare 1000 mL of (0.1, 0.2, 0.3 N) solution,

M_1V_1 (before) = M_2V_2 (after)

18.14 × V_1 = 0.1 × 1000 mL

V_1 = 5.5 mL / l

H_2SO_4	Water	Normality
5.5 ml	994.5 ml	0.1
11.0 ml	989.0 ml	0.2
16.5 ml	983.5 ml	0.3

The solution(s) (0.1, 0.2, 0.3 N) each was added to distilled water, and was used at the rate of 3 cm/100 mL water/pot as soil drench just before sowing bean seeds and the second was applied one week after planting.

Treatments:

1- R. solani + H_2SO_4 (0.1N)

2- R. solani + H_2SO_4 (0.2N)

3- R. solani + H_2SO_4 (0.3N)

4- S. rolfsii + H_2SO_4 (0.1N)

5- *S. rolfsii* + H_2SO_4 (0.2N)
6- *S. rolfsii* + H_2SO_4 (0.3N)
7- *M. phaseolina* + H_2SO_4 (0.1N)
8- *M. phaseolina* + H_2SO_4 (0.2N)
9- *M. phaseolina* + H_2SO_4 (0.3N)
10- Mixture of fungi+ H_2SO_4 (0.1N)
11- Mixture of fungi+ H_2SO_4 (0.2N)
12- Mixture of fungi+ H_2SO_4 (0.3N)
13- H_2SO_4 (only 0.1N) control without pathogen
14- H_2SO_4 (only 0.2N) control without pathogen
15- H_2SO_4 (only 0.3N) control without pathogen
16- Control untreated acid and without pathogen
17- *R. solani* only (without treatment with acid)
18- *S. rolfsii* only (without treatment with acid)
19- *M. phaseolina* only (without treatment with acid)
20- Mixture of fungi only (without treatment with acid)
21- *R. solani* + fungicide (Tebuconazole 6%)
22- *S. rolfsii* + fungicide (Tebuconazole 6%)
23- *M. phaseolina* + fungicide (Tebuconazole 6%)
24- Mixture of fungi+ fungicide (Tebuconazole 6%)

Varietal reaction of bean cultivars to infection and the effect of sulphuric acid concentration:

Seeds of bean cultivars, Nahda and Nebraska were planted in pots and three concentrations of sulphuric acid were applied (0.1, 0.2, 0.3N) each alone to each of the tested treatments mentioned before, control treatments were soil artificially infested with any of the tested fungi or their mixture and drenched with fungicide Tebuconazole 6% or uninfested soil drenched only by different concentrations of sulphuric acid.

Each pot was treated just before sowing and at seven days after sowing with the required concentration of H_2SO_4 (0.1, 0.2, 0.3N) at the rate of 3 mL/100 mL water/pot as soil drench, other pots were treated separately with the fungicide Hattrick (Tebuconazole 6%) at the rate of 1.25mL/l water as soil application (100mL/pot) as the mentioned before. Percentages of pre- and post-emergence damping-off were calculated at 15 and 30 days after sowing while percentages of root rot severity and survived plants were determined at 45 days after planting. Moreover, growth readings of bean plants, *i.e*., plant fresh and dry weights (g/plant), were also determined 45 days after planting. Plants were dried in an air circulation oven 70°C for 72 hours and weighed. These plant parameters were:

Shoot /root fresh weight ratio =
fresh weight of shoot / fresh weight of root (g)
Shoot /root dry weight ratio =
dry weight of shoot / dry weight of root (g)

Diseases assessment:

Percentages of pre- and post-emergence damping-off as well as healthy surviving plants and root rot plants in each treatment were determined 15,30 and 45 days after sowing respectively using the formula reported by Muhanna *et al*. (2018).

$$\textbf{Pre-emergence damping-off\%} = \frac{\textbf{No. of non-germinated seeds}}{\textbf{Total no. of planted seeds}} \times 100$$

$$\textbf{Post-emergence damping-off \%} = \frac{\textbf{No. of post emerged dead seedlings}}{\textbf{Total no. of planted seeds}} \times 100$$

$$\textbf{Survived plants \%} = \frac{\textbf{No. of survived plants}}{\textbf{Total No. of planted seeds}} \times 100$$

$$\textbf{Root rot incidence \%} = \frac{\textbf{No of rotted roots plants}}{\textbf{Total No. of planted seeds}} \times 100$$

Disease severity:

The plants were scored for root rot disease severity using the root damage scale from 0-5 proposed by Shahzad and Gahffar (1992), cited after Abd-Elghany *et al*. (2021) following the formula:

Where:
0 = healthy (without any damage)
1 = weak damage (0≥10%)
2 = medium damage (more than10≥25%)
3 =medium strong damage (more than 25≥50 %)
4 = strong damage (more than50≥75%)
5 = very strong damage to total destruction (more than 75≥100%)

$$\textbf{Disease severity} = \Sigma\,(fv) / nX \times 100$$

Where:
F = number of roots tested in each grade.
V = numerical rating of the scale (1-5), grade.
nX = Total number of roots tested multiplied by (5) *i.e*., the highest grade.

Soil Microbial counts:

Soil samples (100 g) were collected from Nebraska bean root zones of different treatments to determine the microbial densities, 45 days after the first application. Counts of total microorganisms were made by adding 10 g of soil to 90 ml of sterile water and shaking on an orbital shaker (200 rpm) for 2 hrs. Soil extract agar and peptone dextrose agar media (Martin, 1950 and Allen,1957) were used to estimate populations of bacteria and fungi, following the serial dilution count technique. Incubation was carried out at 28-30°C for 2-3 days. The mean number of colony forming units (CFU) on plates

was calculated and converted to densities per/1 gram soil sample (Waksman and Fred 1922).

CFU/g soil = number of colonies × dilution factor/ volume of culture plat

Soil analysis:

Acid treated soil samples (0.2N) were collected from Nebraska bean root rhizosphere, each equal one kg in paper bag, from the artificially infested soil at the end of the experiments. Soil samples were air-dried at a temperature of 25°C to 35°C and relative humidity 20 to 60% or completely dried in an oven at a temperature 105°C for 24 h. (Jackson, 1958). Soil samples were collected for determination the levels of the essential nutrients (Table, 1). Total nitrogen was determined by the modified macro-Kjeldahl technique as outlined by Jackson (1958). Phosphorus was analyzed according to the method described by Bingham (1949), determined by spectrophotometer (Jenway 6305 UV-VIS at 470 nm). Potassium was determined by Distillation Unit Velp UDK 129 at 450 nm wavelength (Rich, 1965). The determinations were run in the laboratory (Central Lab for Water, Soil Analyses), the general assembly of the executive body for projects and land reclamation, Ministry of Agriculture and land reclamation, Dokki, Egypt.

Table (1): Nutrient levels approved arranged in three classes.

Nutrient ranges	Nutrient level range (in ppm)		
	Nitrogen	Phosphorus	Potassium
Low	0-40	0-10	0-200
Medium	40-80	10-15	200-400
High	80+	15+	400+

ppm= part per million

Statistical analysis:

Data were compared by the analysis of variance according to the procedures of Snedecor and Cochran (1980). Means of treatments were compared by the least significant difference LSD at 5% level.

RESULTS

Isolation and identification of the associated fungi:

Several fungi were isolated from rotten roots of bean plants collected from Qalubia governorate (Table, 2). The isolated fungi were identified according to their cultural and morphological characters as *Rhizoctonia solani* Kühn, *Macrophomina phaseolina* (Tassi) Goid and *Sclerotium rolfsii* Sacc. [*Athelia rolfsii* (Curzi) Tu Kimbrough]. The frequency percentage of these fungi was calculated, *Sclerotium rolfsii* showed the highest percentage followed by *Rhizoctonia solani*, and *Macrophomina phaseolina,* being, 53.33, 25.33 and 14.07%, respectively. Very low frequency of minor fungi (6.67 %) was also scored and neglected.

Table (2): Frequency (%) of fungi isolated from the rotten roots of bean plants.

Isolate fungi	No of isolates	Frequency %
Rhizoctonia solani Kühn	19	25.33
Macrophomina phaseolina (Tassi) Goid	11	14.67
Sclerotium rolfsii Sacc.	40	53.33
Other fungi (neglected)	5	6.67
Total No. of isolates	75	-

Pathogenicity test:

Results in Table (3) clearly indicate that all the tested fungi were able to infect the two bean cultivars. The highest percentage of pre-emergence damping off in seedlings of Nebraska cv. occurred by *Sclerotium rolfsii* (33.3%) followed by those recorded from infection by *Rhizoctonia solani* (26.7%) and *Macrophomina phaseolina* (20.0%), respectively. Meanwhile, in the case of Nahda cv. the highest percentage of pre-emergence damping off was recorded due to soil infestation with *S. rolfsii* (46.7%) followed by *R. solani* (33.3%) and *M. phaseolina* (26.7 %), respectively.

Concerning post-emergence damping-off incidence, data in Table (3) show that soil infestation with *R. solani* and *S. rolfsii* caused the highest percentages of infection (13.3%) followed by *M. phaseolina* (6.7%). Meanwhile, in the case of Nahda cv. the highest percentage of post-emergence damping off was recorded from soil infested by *R. solani* (13.3%) followed by each of *M. phaseolina* and *S. rolfsii* (6.7%). The percentages of surviving plants of Nebraska cv. were higher than those of Nahda cv.

However, percentage of root rot 45 days after sowing, data in Table (3) indicate that infection, by *R. solani* was (26.7%) followed by *S. rolfsii* and *M. phaseolina* (13.3%) on Nahda cv. While, in the case of Nebraska cv. root rot incidence due to treatment with each of *S. rolfsii* and *R. solani* was (13.3%), followed by that caused by *M. phaseolina* (6.7%).

Table (3): Pathogenicity tests of the three tested fungi expressed as percentages of pre and post – emergence damping off and root rot in two dry bean cvs. grown under greenhouse condition.

Pathogens	Bean cultivars							
	Nebraska cv.				Nahda cv.			
	Damping-off %		% Root rot	% plant survival	Damping-off %		% Root rot	% plant survival
	% Pre-emergence	% Post-emergence			% Pre-emergence	% Post-emergence		
Rhizoctonia solani	26.7	13.3	13.3	46.7	33.3	13.3	26.7	40.1
Macrophomina phaseolina	20.0	6.7	6.7	66.6	26.7	6.7	13.3	53.3
Sclerotium rolfsii	33.3	13.3	13.3	40.1	46.7	6.7	13.3	33.3
Control	0.0	0.0	0.0	100	0.0	0.0	0.0	100
LSD at 5%	2.88	1.87	1.12	1.91	1.99	1.27	1.72	1.75

Varietal reaction of bean cultivars to infection and effect of sulphuric acid concentration:

Results in Table (4) show that the tested fungi were able to cause pre-and post-emergence damping-off on the two bean cultivars (Table, 4). Infection by the recovered fungi, applied singly to the potted soil showed high pre-emergence damping off on Nebraska cv. caused by *S. rolfsii* (26.7%) followed by *R. solani* (20.0%) and *M. phaseolina* (13.3%). On the other hand, Nahda cv. was also affected, pre-emergence damping-off caused by *S. rolfsii* recorded (33.3%), followed by *R. solani* (26.7%) and *M. phaseolina* (13.3%). Meanwhile, high pre-emergence damping-off (33.3%) was recorded using the mixture of the tested fungi in infesting soil used for growing Nahda cv.

Application of different H_2SO_4 concentrations showed high pre-emergence damping off on Nebraska cv. in the presence of the tested fungi at (0.1N) concentration, *S. rolfsii* caused (20.0%) damping-off followed by *R. solani* and *M. phaseolina* (13.3%). On the other hand, on Nahda cv. *S. rolfsii* recorded 26.7%, followed by *R. solani* and *M. phaseolina* (20.0%). When sulphuric acid (0.2N) concentration was used as soil treatment, in the presence of the tested fungi, pre-emergence damping-off in Nebraska cv. caused by each of *R. solani, S. rolfsii* recorded (13.3%) while *M. phaseolina* recorded (6.7%). Meanwhile, for Nahda cv., *S. rolfsii* caused (20.0%), each of *R. solani* and *M. phaseolina* caused (13.3%) infection. On the other hand, suphuric acid (0.3N) concentration showed pre-emergence damping off in Nebraska cv. due to *S. rolfsii* (13.3%) followed by *R. solani* and *M. phaseolina* (6.7%). On the other hand, infection on Nahda cv. caused by each of *S. rolfsii*, *R. solani* recorded (13.3%) followed by *M. phaseolina* (6.7%).

After 30 days seedlings of both cultivars developed in soil treated with different concentrations of H_2SO_4, showed that post-emergence damping off, caused by the mixture of fungi as soil infestation treatment of Nahda cv. was high at (0.1 N) concentration of H_2SO_4, being (20.0%) infection followed by concentration (0.2,0.3N) where infection reached 13.3 %. While percentage of post-emergence damping-off due to the mixture of fungi treatment on Nebraska cv. was (6.7%) with greater sulphuric acid concentration (0.3 N).

The percentage of post-emergence damping-off of seedlings of both cultivars developed in the presence of H_2SO_4 (0.3 N) (without pathogen) was 0.0 %. Post-emergence damping-off did not occur after treatment of both cultivars by the fungicide. The surviving plants of Nebraska cv. were higher than those of Nahda cv after treatment with the fungicide.

Percentage of root rotted plants after 45 days on both cultivars grown in soil treated with different concentration of H_2SO_4 showed that root rot caused by the mixture of fungi used as soil infestation treatment of Nahda cv. was high at (0.1 N) concentration of H_2SO_4 being (26.7%) infection followed by concentrations (0.2,0.3N) where infection reached 20.0%. While root rot incidence due to the mixture of fungi treatment of Nebraska cv. was (13.3%) with greater sulphuric acid concentration (0.3 N).

Results in Table (5) and Fig (1) concerning disease severity show significant differences among the results of using each fungus alone and the mixture of fungi treatments. The highest disease severity was due to infection by *S. rolfsii* (25.3%) and *R. solani* (24.0%) followed by *M.*

phaseolina (20.0%) on Nebraska cv. but for Nahda cv however, the highest disease severity was occurred due to *S. rolfsii* and *R. solani* (25.3%) followed by *M. phaseolina* (24.0%). The disease severity was decreased in case of increasing concentrations of H_2SO_4 as the severity incited by *R. solani* was decreased from (20.0 to16.0%), meanwhile *M. phaseolina* was decreased from (20.0 to13.3%), and in infection by *S. rolfsii* was disease severity decreased from (24.0 to17.3%) on Nebraska cv. With Nahda cv however, disease severity was decreased with increase concentrations of H_2SO_4 as that caused by infection by *R. solani* was decreased from (20.0 to18.7%), meanwhile that recorded from infection by *S. rolfsii* ranged from (25.3 to 20.0%) and in case of infection by M. *phaseolina* it was ranged from (24.0 to17.3%).

Table (4): Effect of soil treatment with different sulphuric acid concentration (normalities) on percentage of pre-and post-emergence damping off, root rot caused by the tested fungi and plant survival of two dry bean cultivars under greenhouse conditions.

No	Soil treatments	Concentrations *	Bean cultivars							
			Nebraska				Nahda			
			% pre-emergence damping off	% Post- emergence damping off	% root rot	%Plant survival	% pre-emergence damping off	% Post- emergence damping off	% root rot	%Plant survival
1	H_2SO_4 only (control) ***	0.1	0.0	0.0	0.0	100	0.0	0.0	0.0	100
2	*R. solani*		13.3	6.7	13.3	66.7	20.0	13.3	13.3	53.4
3	*S. rolfsii*		20.0	13.3	20.0	46.7	26.7	20.0	20.0	33.3
4	*M. phaseolina*		13.3	6.7	13.3	66.7	20.0.	13.3	20.0	46.7
5	Mixture of fungi		26.7	13.3	20.0	40.0	26.7	20.0	26.7	26.6
6	H_2SO_4 only (control) ***	0.2	0.0	0.0	0.0	100	0.0	0.0	0.0	100
7	*R. solani*		13.3	6.7	6.7	73.3	13.3	13.3	13.3	60.1
8	*S. rolfsii*		13.3	13.3	13.3	60.1	20.0	13.3	20.0	46.7
9	*M. phaseolina*		6.7	6.7	13.3	73.3	13.3	6.7	13.3	66.7
10	Mixture of fungi		20.0	13.3	20.0	46.7	20.0	13.3	20.0	46.7
11	H_2SO_4 only (control) ***	0.3	0.0	0.0	0.0	100	0.0	0.0	0.0	100
12	*R. solani*		6.7	6.7	6.7	79.9	13.3	6.7	13.3	66.7
13	*S. rolfsii*		13.3	6.7	13.3	66.7	13.3	13.3	13.3	60.1
14	*M. phaseolina*		6.7	6.7	6.7	79.9	6.7	6.7	13.3	73.3
15	Mixture of fungi		13.3	6.7	13.3	66.7	13.3	13.3	20.0	53.4
16	Control (without pathogen) ****	0.0	0.0	0.0	0.0	100	0.0	0.0	0.0	100
17	*R. solani*		20.0	13.3	20.0	46.7	26.7	20.0	20.0	33.3
18	*S. rolfsii*		26.7	20.0	20.0	33.3	33.3	20.0	20.0	26.7
19	*M. phaseolina*		13.3	13.3	20.0	53.4	13.3	20.0	20.0	46.7
20	Mixture of fungi		26.7	20.0	20.0	33.3	33.3	26.7	20.0	20.0
21	*R. solani*	Fungicide **	6.7	0.0	6.7	86.6	6.7	6.7	6.7	79.9
22	*S. rolfsii*		13.3	0.0	6.7	80.0	20.0	0.0	6.7	73.3
23	*M. phaseolina*		0.0	6.7	6.7	86.6	6.7	0.0	6.7	86.6
24	Mixture of fungi		13.3	0.0	13.3	73.4	20.0	6.7	13.3	60.0
	LSD at 5%									
	Treatments (T)		0.61	0.59	0.54	0.77	0.91	0.93	0.64	0.86
	Concentrations (C)		0.61	0.59	0.54	0.77	0.91	0.93	0.64	0.86
	T × C		1.35	1.33	1.21	1.72	2.05	2.10	1.43	1.92

*Concentration of sulphuric acid according to acid normality; **Fungicide (Tebuconazole 6%) 1.25mL/l water; *** H_2SO_4 only (control) without pathogen; **** Control (without pathogen and without H_2SO_4).

Table (5): Effect of soil treatment with different sulpheric concentrations (normalities) on root rot severity of bean Nebraska and Nahda cvs. grown in soil artificially infested by the tested fungi under greenhouse conditions 45 days after planting.

No.	Soil Treatments	Conc. (Normality) *	Disease severity	
			Nebraska cv.	Nahda cv.
1	H_2SO_4 only (control)***	0.1	0.0	0.0
2	*R. solani* + H_2SO_4		20.0	20.0
3	*S. rolfsii* +H_2SO_4		24.0	25.3
4	*M. phaseolina*+ H_2SO_4		20.0	24.0
5	Mixture of fungi+ H_2SO_4		24.0	25.3
6	H_2SO_4only(control)***	0.2	0.0	0.0
7	*R. solani* + H_2SO_4		18.7	18.7
8	*S. rolfsii* + H_2SO_4		20.0	24.0
9	*M. phaseolina* + H_2SO_4		17.3	20.0
10	Mixture of fungi +H_2SO_4		21.3	24.0
11	H_2SO_4only (control)***	0.3	0.0	0.0
12	*R. solani* + H_2SO_4		16.0	18.7
13	*S. rolfsii* + H_2SO_4		17.3	20.0
14	*M. phaseolina* + H_2SO_4		13.3	17.3
15	Mixture of fungi + H_2SO_4		18.7	20.0
16	Control (without pathogen) ****	0.0	0.0	0.0
17	*R. solani*		24.0	25.3
18	*S. rolfsii*		25.3	25.3
19	*M. phaseolina*		20.0	24.0
20	Mixture of fungi		25.3	16.0
21	*R. solani*	Fungicide**	9.3	10.7
22	*S. rolfsii*		10.7	12.0
23	*M. phaseolina*		8.0	9.3
24	Mixture of fungi		12.0	13.3
	LSD at 5%			
	Treatments (T)		0.60	0.61
	Concentrations (C)		0.60	0.61
	T × C		1.35	1.37

* Concentration of sulphuric acid according to acid normality; **Fungicide (Tebuconazole 6%) 1.25mL/l water; *** H_2SO_4 only (control) without pathogen; **** Control (without pathogen and without H_2SO_4)

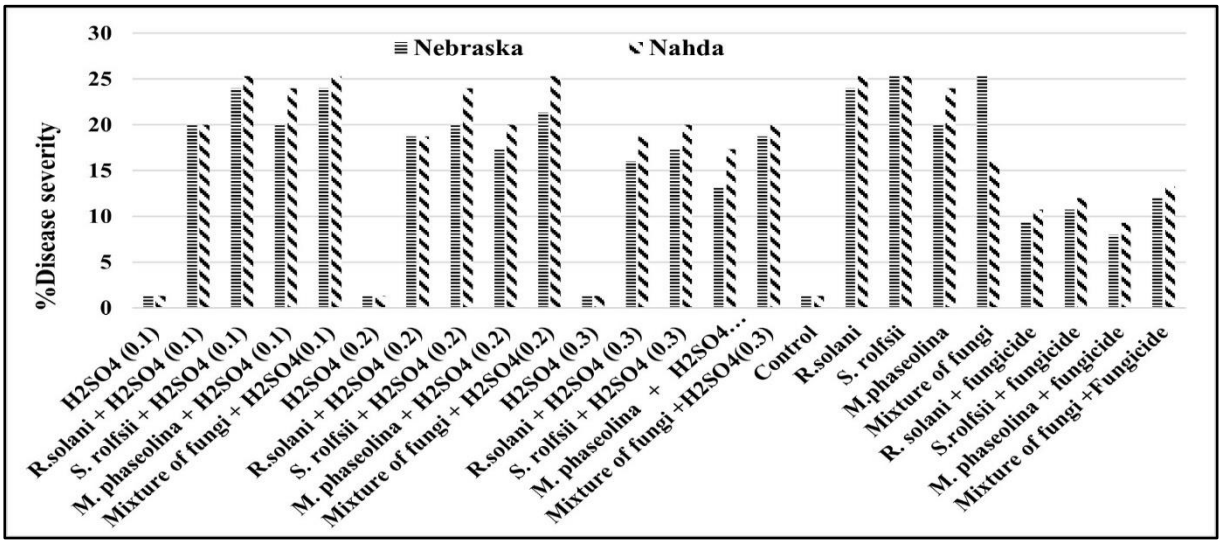


Fig. (1): Disease severity of bean root rot in Nebraska and Nahda cultivars grown in soil infested with various fungi and treated with different sulphuric acid normalities.

Effect of sulphuric acid concentrations on some growth parameters of two dry bean cultivars grown in soil infested with the tested fungi:

Influence of different sulphuric acid concentrations (0.1, 0.2, 0.3N) on some plant growth parameter of two bean cultivars (Nebraska and Nahda) *i.e.,* fresh, and dry weights, in addition to the effect on ratio between, the shoot and the root fresh and dry weights of bean cultivars grown in soil infested with any of the tested fungi and exposed to different doses of sulphuric acid were taken into consideration.

1-Effect on fresh and dry weights:

Results in Table (6) show significant differences between the averages of fresh and dry weights (shoot and root) due to the tested treatments**.** Treatment by H_2S0_4 (0.1, 0.2, 0.3N) concentrations significantly gave good effect on bean vigor compared to pathogen-free control. In most cases, differences were found between both shoots and roots of plants grown in the presence of H_2SO_4 and between plants grown in the infested soil and those grown in uninfested soil (control). Moreover, significant differences between fresh weights and dry weights of roots and shoots were found to be due to the high strength application with H_2SO_4 0.3N as well.

2- Effect of the ratio between Nebraska cv. shoot /root on fresh weight:

Results concerning the effects of H_2SO_4 treatments are shown in Tables (6 and 7) and Fig (2, a-b). Ratio in case of plants grown in artificially infested soil with *R. solani* treatments ranged from 4.1 to 5.1 for high and low concentrations compared with that recorded from plant grown in infested soil with *R. solani* treatment without acid that was 6.1. Moreover, for *S. rolfsii,* the ratio ranged from 3.8 to 6.1. for high and low acid strength(s) compared with *S. rolfsii* treatment only that gave 7.5. Meanwhile *M. phaseolina* ratio ranged from (2.9 to 6.3) for high and low concentrations, compared to *M. phaseolina* treatment only this ratio was (5.9) as well as the mixed fungi treatment ratios which ranged from (3.5 to 4.6) while plant grown in infested soil with the mixture of fungi alone showed approximately 5.7 compared to control without any pathogen that ranged between (3.2 and 5.2). Fungicide treatment, in the presence of the tested pathogens ratio ranged between (4.1 and 5.7) compared to control grown in uninfested soil (5.5.).

3- Effect of the ratio between Nebraska cv. shoot /root on dry weight:

Results in Tables (6, 7) and Fig (2, a-b) show the effects of H_2SO_4 with different concentrations 0.1, 0.2 and 0.3 N. on bean plants grown in soil infested any of the tested fungi in case of soil infestation with *R. solani* recorded ratios ranged from (4.7 to 4.9), while *R. solani* without additive acid gave (4.4), but in case of *S. rolfsii* it was ranged between (4.9 to5.8) while *S. rolfsii* without acid gave (9.6).The recorded ratios for *M. phaseolina* ranged from (4.2 to 5.3) compared to *M. phaseolina* alone without acid (9.2). The mixture of fungi treatment however, recorded (5.4 to 6.2) while the mixture of fungi alone without sulphuric acid showed (6.8), compared to control without the pathogen that ranged from (2.5 to 3.9). Fungicide treatment, in the presence of the tested pathogens ratio ranged between (3.9 and 4.7) compared to control grown in uninfested soil (4.7).

4- Effect of the ratio between Nahda cv. shoot /root on fresh weight:

Shoot /root ratio in Tables (6, 7) and Fig (2, a-b) was influenced by the H_2SO_4 treatments at different strengths, 0.1, 0.2 and 0.3N. Infested treatments with *R. solani* showed a ratio ranging from (4.1 to 5.1) while *R. solani* only without acid treatment showed (5.5.). *S. rolfsii* ratios ranged on the other hand, showed similar trend (3.2 to 5.0) compared to *S. rolfsii* only that gave (5.5). Moreover *M. phaseolina* showed ratios ranging from (4.7 to 8.5) while *M. phaseolina* alone showed (5.7). Moreover, the mixture of fungi treatment recorded ratios ranged from (2.6 to 5.5) compared to mixture of fungi alone showing approximately (5.9), compared to the control without pathogen that ranged from (5.4 to 6.3). The fungicide treatment, however, showed ratios ranging between (4.1 to 5.5) compared to plant control (5.7).

5- Effect of the ratio between Nahda cv. shoot /root on dry weight:

The ratio between shoot and root dry weight in Tables (6 and 7) and Fig (2, a-b) was influenced by H_2SO_4 strengths, 0.1, 0.2 and 0.3N, and by *R. solani* stress that was ranged from (4.2 to 5.0) compared to *R. solani* alone without acid treatment (17.8.). Meanwhile, *S. rolfsii* treatments showed ratios ranging from (4.9 to 5.4) compared to *S. rolfsii* only (16.4). Greater effect could be recognized with *M. phaseolina,* being, between (4.4 and 5.4) compared to *M. phaseolina* alone (19.5) while

the mixture of fungi recorded ratio between (4.3 and 5.4) compared to the mixture of fungi treatment without acid (21.6), as compared to control without the pathogen where ratios ranged from (3.2 to 4.6). The pathogen treated with fungicide treatment ratios ranged between (3.2 and 4.2) compared to plant free pathogen treatment, being 4.6.

6- Effect of soil drench with different concentrations of sulphuric acid on counted N2-fixing nodule bacteria:

Data in Table (8) and Fig (3, a-b) show the averages of counted N2-fixing nodule bacteria on Nebraska cv**.** bean plants grown in soil treated with H_2SO_4 at different strength(s). The nodule bacteria due to different strengths of H_2SO_4 (0.1, 0.2 and 0.3N) under the stress of *R. solani* ranged from 16.0 to 36.0 compared to treatment of *R. solani* alone (2.0). In most cases, similar trend was observed for each of *S. rolfsii,* ranged from 5.0 to 12.0 compared to treatment of *S. rolfsii* alone 9.0 *M. phaseolina* ranged from 4.0 to 14.0 compared to treatment of *M. phaseolina* 17.0 and the mixture of fungi treatments ranged from 3.0 to 37.0 compared to treatment of the mixture of fungi alone (46.0). While within plants grown in the presence of the fungicide the number of nodules was between 2.0 and 8.0 compared with those of the control (5.0).

Meanwhile, Nahda cultivar showed lower averages of counted N2-fixing nodule bacteria, in general due to different strengths of H_2SO_4 (0.1, 0.2 and 0.3N) the number of nodules under the stress of *R. solani was ranged* from 0.0 to 1.0 compared to treatment of *R. solani* alone (0.0). In most cases a similar trend was observed for each of *S. rolfsii* as the nodule numbers ranged from 1.0 to 2.0 compared to treatment of *S. rolfsii* alone 0.0. *M. phaseolina* nodule numbers ranged from 0.0 to3.0 compared to treatment of *M. phaseolina alone* 0.0 and for the mixture of fungi treatments it was ranged from 1.0 to3.0 compared to treatment of the mixture of fungi alone 1.0. While the number of nodules with plants grown in the presence of the fungicide was between 0.0 and 1.0 compared with those of the control (1.0).

Soil microbial densities:

Data in Table (9) show the total counts of bacteria and fungi in soil ,45 days after soil application. Application of H_2SO_4 resulted in a pronounced increase in bacterial densities compared to untreated soil. The rate of H_2SO_4 concentrations with pathogenic fungi, caused considerable increase in bacterial densities, in the presence of *R. solani* treated with H_2SO_4 bacterial counts ranged from 2.5 to 7.2×10^6/g compared to 1.4×10^6/g in soil treated with *R. solani* only, Similar trend was recognized with each of *S. rolfsii* treated with H_2SO_4 ranged from 2.0 to 2.7×10^6/g compared to 1.6×10^6/g treated with *S. rolfsii* only while for, *M. phaseolina* treated with H_2SO_4 the counts ranged from 2.1 to 3.4×10^6/g compared to 1.1×10^6/g treated with *M. phaseolina* only, as well as mixture of fungi treatments ranged from 2.4 to7.3×10^6/g compared to 1.5×10^6/g treated with mixture of fungi only. Meanwhile, in soils treated with H_2SO_4 only the counts ranged from 2.4 to 4.8×10^6/g, the treatment with the fungicide ranged from 1.0 to 2.4×10^6/g compared to control, 0.0. This was accompanied with decrement in fungi counts, *i.e., R. solani* treated with H_2SO_4 ranged from 1.2 to 1.5×10^6/g compared to 3.0×10^6/g in soil treated with *R. solani* only, Similar trend was recognized with each of *S. rolfsii* treated with H_2SO_4 ranged from 0.7 - 1.6×10^6/g compared to 2.3×10^6/g treated with *S. rolfsii* only, for *M. phaseolina* treated with H_2SO_4 ranged from 0.8 to 1.2×10^6/g compared to 3.3×10^6/g treated with *M. phaseolina* only, as well as mixture of fungi treatments the counts ranged from 1.4 to 2.7×10^6/g compared to 3.5×10^6/g treated with mixture of fungi only. Meanwhile, treatments with H_2SO_4 only ranged from 0.2 to 0.4×10^6/g, where for the fungicide the counts ranged from 0.2 to 1.2×10^6/g compared to control, 0.0.

Soil analysis:

The limit of concentrations was found to be below the sufficiency range for nitrogen, phosphorus and potassium (Table, 10) indicating the low fertility as stress factor in addition to biotic stress. The soil analysis in most cases showed low levels of nitrogen (9.33-18.0), phosphorus (1.7-3.3), and potassium (128.0-150.0) in treatments with H_2SO_4, indicating poor nutrition for the plants. While treatment with any pathogen alone or their mixture showed N% between (17.0-25.0), P% ranged between (1.0-4.0), and K% ranged between (125.0-150.0) compared to control.

Table (6): Effect of soil treatment with different acid concentrations (normalities) on shoot, root fresh and dry weights of Nebraska and Nahda cultivars grown under soil infestation by any of the tested fungi and their mixture 45 days from planting in greenhouse experiment.

No.	Soil treatments	*Concentrations (normality)	Nebraska cv.				Nahda cv.			
			Ave. fresh weight(g)		Ave. dry weight (g)		Ave. fresh weight(g)		Ave. dry weight (g)	
			shoot	root	shoot	root	shoot	root	shoot	root
1	H_2SO_4 only (control) ***	0.1	8.79	1.69	0.99	0.25	9.26	1.59	1.89	0.41
2	*R. solani* + H_2SO_4		5.57	1.10	1.13	0.23	6.66	1.30	0.95	0.19
3	*S. rolfsii* + H_2SO_4		6.45	1.06	0.81	0.14	4.90	0.98	0.97	0.18
4	*M. phaseolina* + H_2SO_4		7.87	1.24	0.85	0.16	7.16	0.84	1.03	0.19
5	Mixture of fungi + H_2SO_4		6.76	1.47	0.99	0.16	5.15	1.01	0.65	0.12
6	H_2SO_4 only(control)***	0.2	8.29	2.41	1.05	0.29	12.9	2.06	1.32	0.40
7	*R. solani* + H_2SO_4		6.02	1.18	0.96	0.20	6.89	1.37	1.19	0.25
8	*S. rolfsii* + H_2SO_4		7.52	1.48	0.99	0.19	5.92	1.31	0.96	0.19
9	*M. phaseolina* + H_2SO_4		7.99	2.07	1.02	0.21	8.18	1.73	0.91	0.19
10	Mixture of fungi + H_2SO_4		8.05	1.82	1.02	0.18	8.78	1.96	1.72	0.32
11	H_2SO_4 only(control)***	0.3	8.60	2.72	0.83	0.33	12.9	2.39	1.74	0.54
12	*R. solani* + H_2SO_4		7.58	1.84	0.99	0.21	5.50	1.35	1.44	0.34
13	*S. rolfsii* + H_2SO_4		7.26	1.92	0.84	0.17	6.39	1.99	0.97	0.20
14	*M. phaseolina* + H_2SO_4		6.30	2.11	1.10	0.26	8.19	1.64	1.24	0.28
15	Mixture of fungi + H_2SO_4		8.12	2.29	1.14	0.21	8.89	3.46	1.13	0.26
16	*R. solani*	0.0	4.46	0.73	0.70	0.16	5.59	1.02	0.89	0.05
17	*S. rolfsii*		6.43	0.86	0.77	0.08	4.55	0.83	0.82	0.05
18	*M. phaseolina*		5.93	1.01	0.83	0.09	4.87	0.85	0.78	0.04
19	Mixture of fungi		4.47	0.79	0.61	0.09	3.99	0.67	0.65	0.03
20	Control (without pathogen) ****		6.65	1.20	0.99	0.21	5.72	1.01	0.97	0.21
21	*R. solani* + fungicide	Fungicide **	6.06	1.49	1.05	0.27	6.85	1.24	0.99	0.28
22	*S. rolfsii* + fungicide		6.76	1.41	0.99	0.21	4.93	1.19	0.84	0.22
23	*M. phaseolina* +fungicide		8.09	1.65	1.04	0.23	6.08	1.44	0.87	0.27
24	Mixture of fungi + fungicide		7.47	1.32	1.12	0.28	6.15	1.17	0.93	0.22
	LSD at 5%									
	Treatments (T)		0.36	0.09	0.06	0.02	0.61	0.16	0.07	0.02
	Concentrations (C)		0.31	0.07	0.05	0.01	0.51	0.13	0.05	0.02
	T × C		0.73	0.17	0.13	0.03	1.21	0.32	0.13	0.04

* Concentration of sulphuric acid according to acid normality; **Fungicide (Tebuconazole 6%) 1.25mL/l water; *** H_2SO_4 only (control) without pathogen; **** Control (without pathogen and without H_2SO_4)

Table (7): Effect of soil treatment with different acid concentrations (normalities) on shoot/root ratio (fresh and dry weights) of Nebraska and Nahda cultivars grown under soil infestation by any of the tested fungi and their mixture 45 days from planting in greenhouse experiment.

No.	Soil treatments	* Concentration (normality)	Shoot/Root ratio			
			Nebraska cv.		Nahda cv.	
			Fresh weight	Dry weight	Fresh weight	Dry weight
1	H_2SO_4 only (control) ***		5.2	3.9	5.8	4.6
2	*R. solani* + H_2SO_4		5.1	4.9	5.1	5.0
3	*S. rolfsii* + H_2SO_4	0.1	6.1	5.8	5.0	5.4
4	*M. phaseolina* + H_2SO_4		6.3	5.3	8.5	5.4
5	Mixture of fungi + H_2SO_4		4.6	6.2	5.1	5.4
6	H_2SO_4 only(control)***		3.4	3.6	6.3	3.3
7	*R. solani* + H_2SO_4		5.1	4.8	5.0	4.8
8	*S. rolfsii* + H_2SO_4	0.2	5.1	5.2	4.5	5.1
9	*M. phaseolina* + H_2SO_4		3.9	4.9	4.7	4.8
10	Mixture of fungi + H_2SO_4		4.4	5.7	5.5	5.4
11	H_2SO_4 only(control)***		3.2	2.5	5.4	3.2
12	*R. solani* + H_2SO_4		4.1	4.7	4.1	4.2
13	*S. rolfsii* + H_2SO_4	0.3	3.8	4.9	3.2	4.9
14	*M. phaseolina* + H_2SO_4		2.9	4.2	4.9	4.4
15	Mixture of fungi + H_2SO_4		3.5	5.4	2.6	4.3
16	*R. solani*		6.1	4.4	5.5	17.8
17	*S. rolfsii*		7.5	9.6	5.5	16.4
18	*M. phaseolina*	0.0	5.9	9.2	5.7	19.5
19	Mixture of fungi		5.7	6.8	5.9	21.6
20	Control (without pathogen) ****		5.5	4.7	5.7	4.6
21	*R. solani* + fungicide		4.1	3.9	5.5	3.5
22	*S. rolfsii* + fungicide	Fungicide **	4.8	4.7	4.1	3.8
23	*M. phaseolina* +fungicide		4.9	4.5	4.2	3.2
24	Mixture of fungi + fungicide		5.7	4.0	5.3	4.2
	LSD at 5%		0.61	0.64	0.55	0.65

* Concentration of sulphuric acid according to acid normality; **Fungicide (Tebuconazole 6%) 1.25mL/l water; *** H_2SO_4 only (control) without pathogen; **** Control (without pathogen and without H_2SO_4)

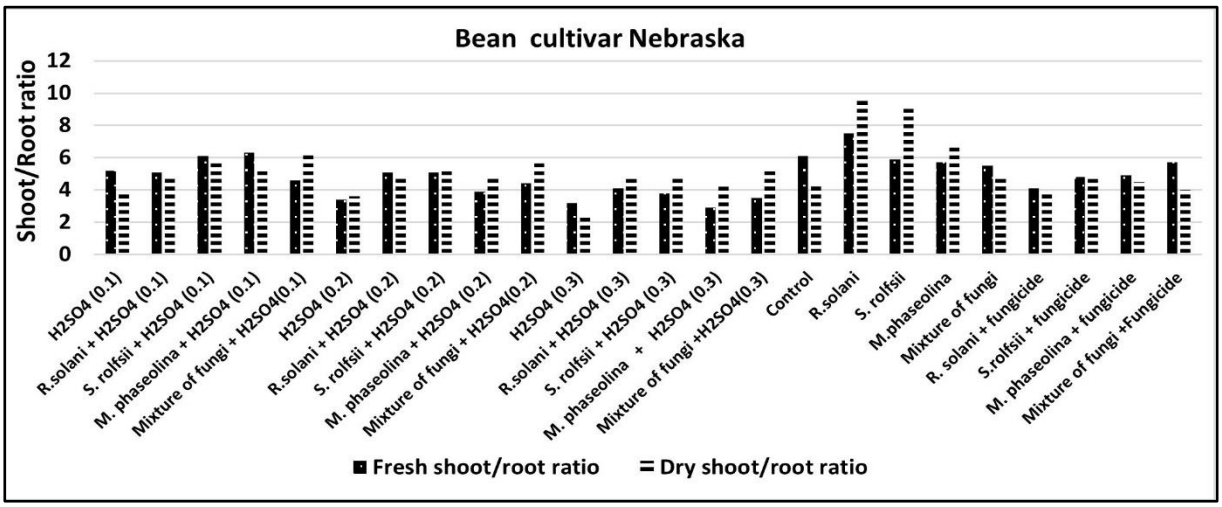
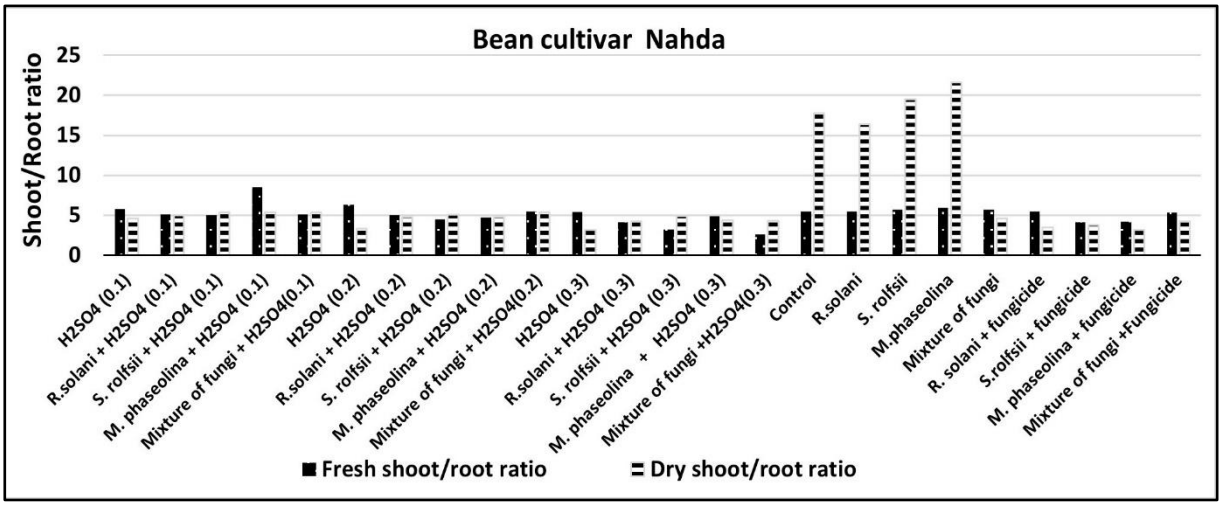


Fig (2 -a): Effect of soil treatment with different acid concentrations (normalities) on shoot/root ratio (fresh and dry weights) of Nebraska cultivar grown under soil infestation by any of the tested fungi and their mixture 45 days from planting in greenhouse experiment.



Fig(2-b): Effect of soil treatment with different acid concentrations (normalities) on shoot/root ratio (fresh and dry weights) of Nahda cultivar grown under soil infestation by any of the tested fungi and their mixture 45 days from planting in greenhouse experiment.

Table (8) Effect of drenching soil artificially infested with the tested fungi with different concentrations of sulphuric acid on the nodule numbers formed in the roots of Nebraska and Nahda cvs. plants grown in these soils.

No	Treatments	C*.	No of nodules in Nebraska cv	No of nodules in Nahda cv
1	H_2SO_4 only (control) ***	0.1	5.0	0.0
2	*R. solani* + H_2SO_4		16.0	0.0
3	*S. rolfsii* + H_2SO_4		5.0	2.0
4	*M. phaseolina* + H_2SO_4		4.0	0.0
5	Mixture of fungi + H_2SO_4		3.0	3.0
6	H_2SO_4 only(control)***	0.2	5.0	0.0
7	*R. solani* + H_2SO_4		26.0	0.0
8	*S. rolfsii* + H_2SO_4		8.0	1.0
9	*M. phaseolina* + H_2SO_4		7.0	0.0
10	Mixture of fungi + H_2SO_4		17.0	1.0
11	H_2SO_4 only(control)***	0.3	14.0	6.0
12	*R. solani* + H_2SO_4		37.0	1.0
13	*S. rolfsii* + H_2SO_4		12.0	1.0
14	*M. phaseolina* + H_2SO_4		14.0	3.0
15	Mixture of fungi + H_2SO_4		37.0	3.0
16	*R. solani*	0.0	2.0	0.0
17	*S. rolfsii*		9.0	0.0
18	*M. phaseolina*		17.0	0.0
19	Mixture of fungi		46.0	1.0
20	Control (without pathogen) ****		5.0	1.0
21	*R. solani* + fungicide	Fungicide **	5.0	0.0
22	*S. rolfsii* + fungicide		2.0	1.0
23	*M. phaseolina* +fungicide		8.0	0.0
24	Mixture of fungi + fungicide		2.0	1.0
	LS D at 5%			
	Treatments (T)		0.15	0.18
	Concentrations (C)		0.12	0.15
	T × C		0.30	0.37

* C=Concentration of sulphuric acid according to acid normality; **Fungicide (Tebuconazole 6%) 1.25mL/l water; *** H_2SO_4 only (control) without pathogen; **** Control (without pathogen and without H_2SO_4)

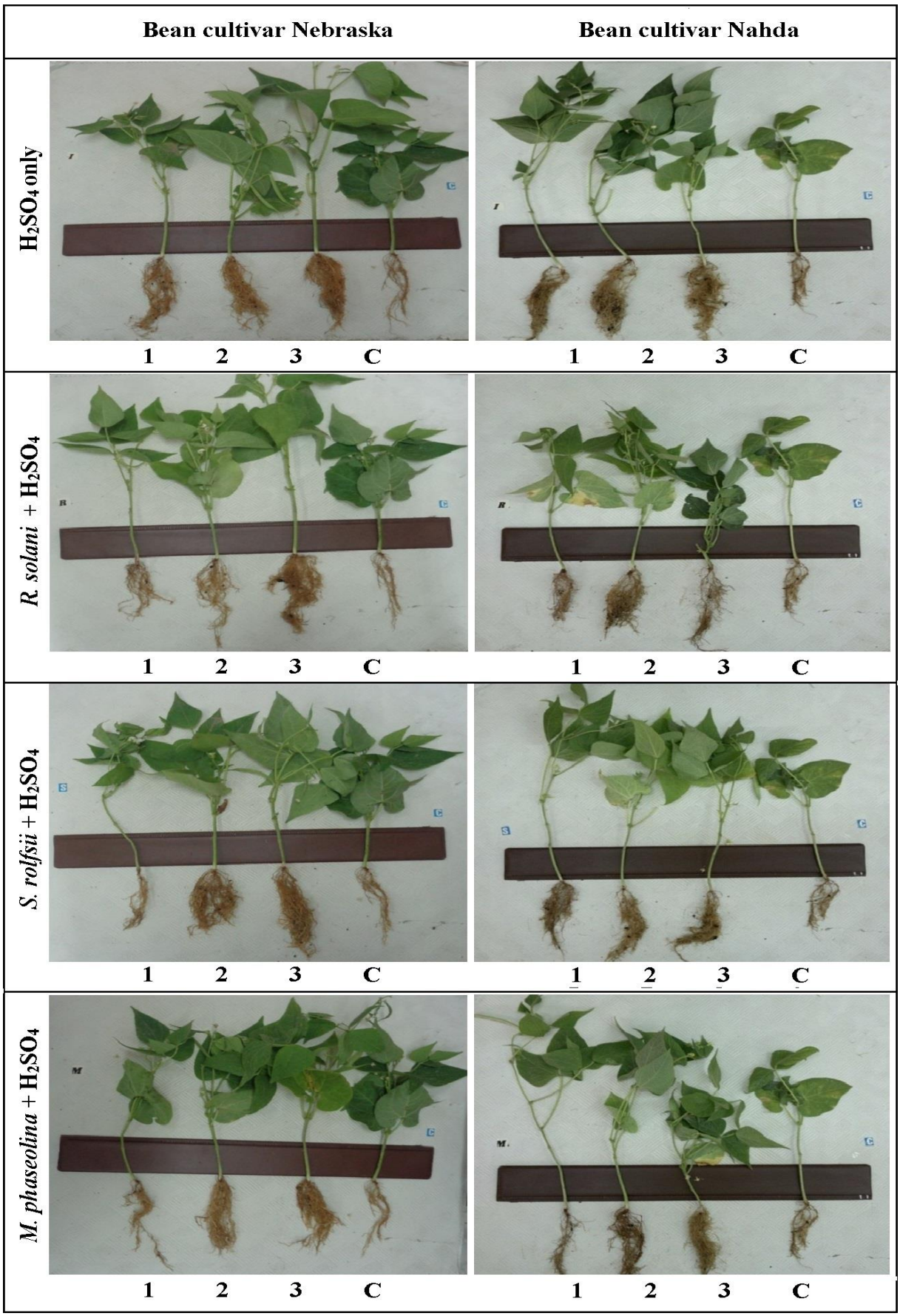


Fig (3-a): Effect of drenching soil artificially infested with the tested fungi with different concentrations of sulphuric acid on the nodule numbers formed in the roots of Nebraska and Nahda cvs plants grown in these soils. (Concentration H_2SO_4: 1=0.1N; 2=0.2N; 3=0.3N).

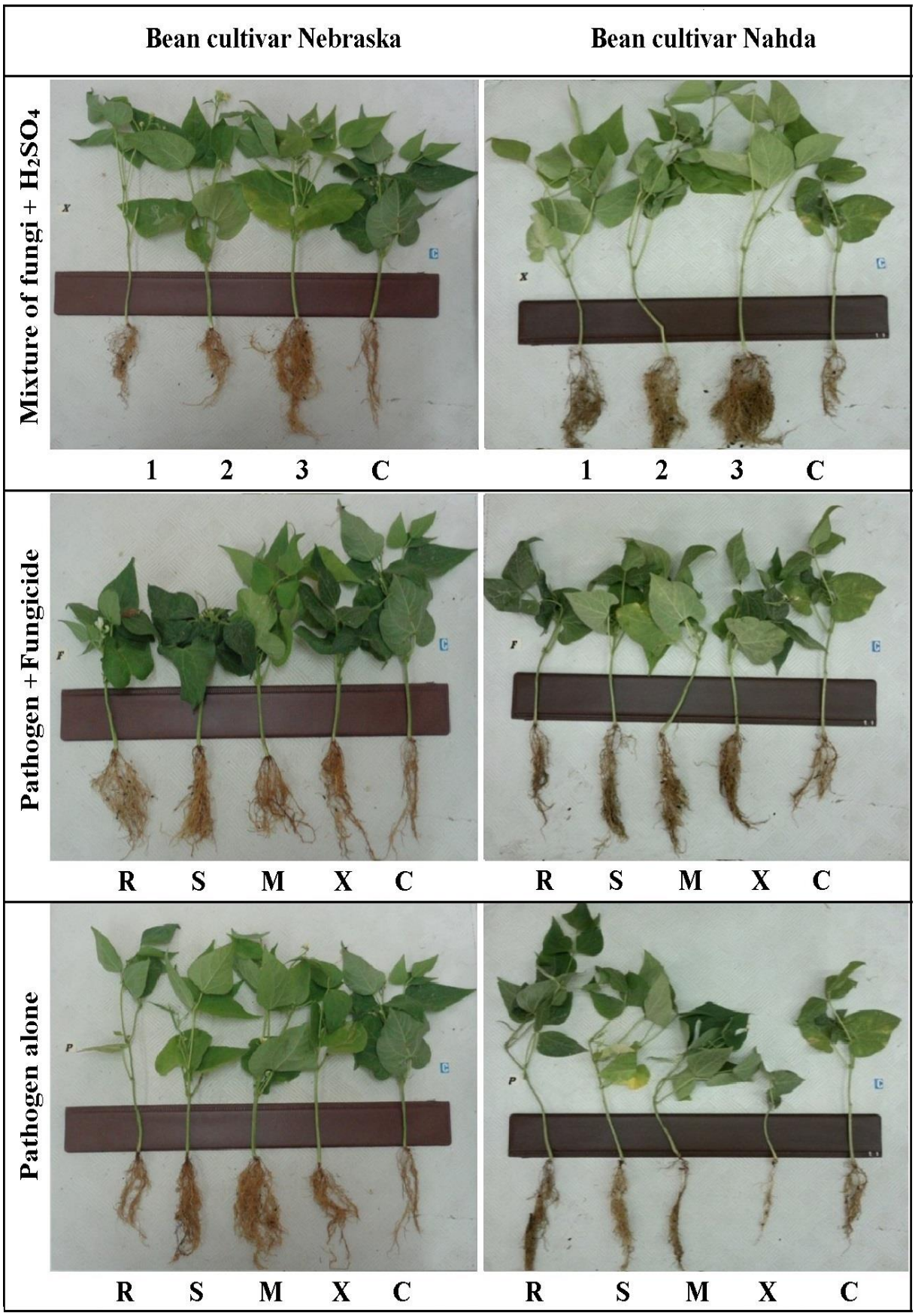


Fig (3-b): Effect of drenching soil artificially infested with the tested fungi with different concentrations of sulphuric acid on the nodule numbers formed in the roots of Nebraska and Nahda cvs. plants grown in these soils. [(Concentration H_2SO_4: 1=0.1N; 2=0.2N; 3=0.3N) (Pathogens: R= *R. solani*; S = *S. rolfsii*; M = *M. phaseolina*; X = Mixture of fungi; C = control)].

Table (9): Total microbial densities in different soil treatments.

No	soil treatments	*Concentration normality	Count × 10^6 /g soil	
			Bacteria	Fungi
1	H_2SO_4 only (control) ***	0.1N	2.4	0.4
2	*R. solani* + H_2SO_4		2.5	1.5
3	*S. rolfsii* + H_2SO_4		2.0	1.6
4	*M. phaseolina* + H_2SO_4		2.1	1.2
5	Mixture of fungi + H_2SO_4		2.4	2.7
6	H_2SO_4 only(control)***	0.2N	4.5	0.3
7	*R. solani* + H_2SO_4		3.0	1.4
8	*S. rolfsii* + H_2SO_4		2.0	1.2
9	*M. phaseolina* + H_2SO_4		2.8	1.0
10	Mixture of fungi + H_2SO_4		6.3	2.1
11	H_2SO_4 only(control)***	0.3N	4.8	0.2
12	*R. solani* + H_2SO_4		7.2	1.2
13	*S. rolfsii* + H_2SO_4		2.7	0.7
14	*M. phaseolina* + H_2SO_4		3.4	0.8
15	Mixture of fungi + H_2SO_4		7.3	1.4
16	*R. solani*	0.0	1.4	3.0
17	*S. rolfsii*		1.6	2.3
18	*M. phaseolina*		1.1	3.3
19	Mixture of fungi		1.5	3.5
20	Control (without pathogen) ****		0.0	0.0
21	*R. solani* + fungicide	Fungicide **	2.4	0.2
22	*S. rolfsii* + fungicide		2.2	0.4
23	*M. phaseolina* +fungicide		1.6	0.7
24	Mixture of fungi + fungicide		1.0	1.2
	LSD at 5%		0.73	0.52

* Concentration of sulphuric acid according to acid normality; **Fungicide (Tebuconazole 6%) 1.25mL/l water; *** H_2SO_4 only (control) without pathogen; **** Control (without pathogen and without H_2SO_4)

Table (10): Available nutrients in ppm of soils surface.

No	Soil treatments	Ave. ppm		
		N	P	K
1	H_2SO_4 only (control)*	22.0	2.0	155.0
2	*R. solani*+ H_2SO_4 ***	18.0	3.3	150.0
3	*S. rolfsii* + H_2SO_4 ***	13.7	2.3	150.0
4	*M. phaseolina* + H_2SO_4	9.33	1.7	135.0
5	Mixture of fungi + H_2SO_4 ***	15.0	2.7	128.3
6	*R. solani* alone	17.0	3.0	130.0
7	*S. rolfsii* alone	25.0	1.0	130.0
8	*M. phaseolina* alone	21.0	4.0	150.0
9	Mixture of fungi alone	17.0	1.0	125.0
10	*R. solani* + Fungicide **	21.0	3.0	170.0
11	*S. rolfsii* + Fungicide **	17.0	1.0	240.0
12	*M. phaseolina* + Fungicide **	17.0	4.0	150.0
13	Mixture of fungi + Fungicide **	17.0	1.0	115.0
14	Control (without pathogen and without H_2SO_4)	25.0	3.0	250.0
	LSD at 5%	1.56	0.92	3.19

* Concentration of sulphuric acid (0.2 N) without pathogen; **Fungicide (Tebuconazole 6%) 1.25 mL/l water; ***Pathogen + Concentration of sulphuric acid (0.2 N).

DISCUSSION

The present study demonstrated that root diseases caused by *R. solani, M. phaseolina*, and *S. rolfsii* could be decreased by growing bean plants in acidic soil conditions. Based on experimentations using three concentrations of sulphuric acid (0.1, 0.2, 0.3N), 3mL/100 mL water from each strength, were applied as soil drench in potted soil infested separately with *R. solani, M. phaseolina*, and *S. rolfsii* and observing the developed disease symptoms compared to control plants.

Pathogenicity test clearly indicated that all the tested fungi showed high percentages of pre- and post-emergence damping-off of cv. Nebraska and Nahda seedlings, the highest value was obtained due to using *S. rolfsii* followed by those caused by *R. solani*, and *M. phaseolina*, respectively. The surviving plants of Nebraska cv. were higher than those of Nahda cv.

The present study showed a significant difference in pathogenic potentials of the tested fungi applied singly. The highest disease severity was recorded due to infection by *S. rolfsii*, *R. solani* and *M. phaseolina* on cv. Nebraska, however, on Nahda cv. the highest disease severity was occurred by *S. rolfsii, R. solani* followed by *M. phaseolina*, and the effect of soil drench using different concentrations (different normalities) of sulphuric acid on pre-, post-emergence damping off and root rot diseases of bean caused by the three tested fungi. It is obvious from data that percentages of damping off and root rot diseases were significantly reduced by the previously mentioned treatments with different concentrations of sulphuric acid. This was more pronounced by increasing acid concentration in drenched solution to 0.3N as the lowest percentages of pre and post emergence damping off and root rot were recorded from bean plants grown in soil artificially infested with the three tested fungi and their mixture in case of Nebraska cv. On the other hand, treatment with high concentration of sulphuric acid in case of Nahda cv. made a deficiency close to that occurred in case of Nebraska cv. The decreases in the incidence of both diseases were reflected in increasing the percentage of surviving plants in both the tested cvs.

Moreover, the two bean cultivars showed differences in their susceptibility to different fungi and different treatments. Nahda cv. seedlings were comparatively the most disease-susceptible and Nebraska cv. seedlings were the lasting in this regard.

Sasaki-Sekimoto *et al*., (2005) found that S-related genes were even more up-regulated due to methyl JA treatment than stress-related genes and that more than one pathway is involved in plant stress response. Gene expression of the ascorbate and glutathione metabolic pathways was increased in response to JA as well as the synthesis of indole glucosinolates. In this regard, Huckelhoven, (2007) reported that plants have three major strategies to combat pathogens: cell wall strengthening, apoplectic defense for inhibition of microbial enzymes and poisoning of the pathogen by toxic compounds like phytoalexins cysteine displays a regulatory function in pathogen defense. Alvarez *et al*. (2012) showed that a specific cytosolic cysteine content is mandatory for the initiation of the plant immune response to pathogens and a link to the hypersensitive response (HR).

The interrelation between root diseases and sulphuric acid concentrations was investigated and revealed that the high strength of sulphuric acid (0.3N) had provoked the fast growth in bean cultivars under investigation and showed less disease severity. Therefore, agricultural methods for controlling plant diseases that include the previous method of acidification may be considered to limit various plant diseases.

Randle *et al*. (1999) reported that plants grown under conditions of high sulphate, and relation to the needs, accumulate the surplus of sulphate in vacuoles as S-SO4 in case of some species, *e.g*., cabbage. Caracuel *et al*. (2003) found that pH 6 was the best for *F. oxysporum* growth and aggressiveness. In this regard, Garrett *et al*. (2006) recorded that environmental conditions and soil reactions (pH) are very important different factors for severity of plant diseases. Zhao (2008) reported that sulphur plays an important role in physiology and protection of plants against environmental stresses and pests through its antioxidative protective functions.

Accordingly, in the present study significant differences were found between the averages of each of fresh and dry weights values (shoot and root) of plants with elevated strength of H_2SO_4 applied Moreover, differences in fresh and dry weight ratios of shoots and roots were almost clear at hyper acidic conditions, as 0.3N strength. Moreover, significant differences in fresh and dry weights of shoots and roots were found at different levels of H_2SO_4 between inoculated plants, or under disease stress and/or the control plants. In general, significant differences between the values of shoot/root

ratios (fresh and dry weight) ratio(s) were influenced by elevation of H_2SO_4 strengths.

The two bean cultivars under investigation indicated genetic differences in susceptibility to infection to the respective pathogenic fungi. Moreover, the highest nodulation values were reported from plants grown in infested soil with *R. solani* (37.0*)*, *S. rolfsii* (12.0*)*, *M. phaseolina* (14.0) treatments and the mixture of fungi (37.0) at 0.3N in comparison with sulphuric acid treatment only (without the pathogen), being (5.0), while Nahda cultivar that showed the least nodulation numbers in treatments of *R. solani*, *S. rolfsii*, and the mixture of fungi.

Scherer and Lange (1996) recorded positive effects of sluphur on growth and yield of leguminous plants, stimulation of biological N_2 fixation expressed by developed larger number of nodules on the roots.

Scherer *et al.* (2008) found that root and nodule development on the roots of legumes are promoted by sulphur fertilization. Szulc *et al*. (2012) reported that sulfate promotes legume nodulation and activating some enzymes and vitamins of plant metabolism such as biotin and thiamine.

The results of soil analysis showed that application of (0.2N) H_2SO_4, in most cases significantly reduced the levels of nitrogen, phosphorus and potassium compared to treatments by each pathogen alone indicating poverty for nutrients.

Eriksen *et al*. (1998) indicated that losses of inorganic sulphur in soils, leaching, after the first cultivation, occur through the adsorption of sulfate on Fe, Al oxides and clays, Volatilization losses may be more relevant in flooded soils due to the microbial reduction of oxidized sulphur forms to volatile H_2S it is worth noting, however that the bacterial population was increased in soils amended with sulphuric acid contrary to fungi population which was decreased in soils treated with sulphuric acid.

Fuentes-Lara, (2019) reported that soil sulphur exists as organic sulphur compounds, sulphide (S^{2-}), elemental sulphur (S^0), and sulphate (SO_4^{2-}). It is transformed between these forms via processes of mobilization, mineralization, immobilization, oxidation, and reduction. Jamal *et al*. (2010) found that up to 98% of the total soil sulphur occurs in the form of organic sulphur compounds and comprises a heterogeneous mixture of plant residues and soil microbes.

Kertesz *et al*. (2007) mentioned that sulphur can be mineralized by the activity of sulphates, such as aryl sulphates, which is produced by a wide variety of heterotrophic microorganisms, especially *Pseudomonas*. Li *et al*. (2010) reported that bacteria are more efficient in oxidizing sulphur than fungi in soil treated with sulphur and identified 18 fungal isolates belonging to genera *Penicillium*, *Aspergillus*, *Paecilomyces*, *Fusarium*, *Bipolaris,* and *Pleosporales* with ability to oxidize sulphur *in vitro*.

Goodwin *et al.* (2000) found that during the early stages of infection, aryl sulphates gene expression was higher of the biotrophic and necrotrophic phases of growth, indicating that S was limiting to the fungus during all stages of growth in plant.

Sluphur nutrition plays a role in stress tolerance and defense mechanisms, by formation of sulfhydryl (S-H) and disulphide bonds (S-S). These bonds are important for the stabilization of protein structures (Saito, 2000). Massalha *et al*. (2017) reported that plant root exudates contain components such as flavonoids, strigolactones, or terpenoids were used in below ground chemical communication strategies.

In general, the reasonable sluphur level significantly decreased disease of damping off and root rot incidence on cultivars of dry bean. The effect on yield components should be seriously managed in further studied. Additional open field studies are necessary. Pilot experiment with other agricultural practices may be advised in addition.

CONCLUSIONS

Sulphur plays key roles in the primary metabolism of plants, conferring antioxidative and protective physiological functions against numerous abiotic stresses. Sulphur may play a central role, just like other macronutrients, in sustainable soil fertility management, improving crop productivity, sulphur interactions with other nutrients and exploring the role of soil rhizospheric microbes in plant sulphur transformations.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

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