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Effect of Borago officinalis extract and plant remains and the bio-agent *Chaetomium globosum* in reducing okra seed rot and seedlings death caused by *Rhizoctonia solani*

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Abstract

The objective of the study was to investigate the efficacy of *Borago officinalis* plant extract in the growth and pathogenesis of *Rhizoctonia solani* causing seeds rot and seedlings death of okra, and to evaluate the effectiveness of the bio-control fungus *Chaetomium globosum* in suppressing pathogenic *R. solani* where was used alone or incorporated with Borage plant remain powder at different rate in potting soil. Pathogenicity tests showed that *R. solani* caused completely seed rot and no seedlings at all, while germination rate was 96.6% from seeds inoculated with *Ch. globosum*.

The in vitro experiment showed that the culture media treated with Borage extracts at all tested concentrations (25, 50, 75 and 100%) were significantly effective and reduced R. solani radial growth compared to that of the control (PDA). Adversely, these media at same extract concentrations resulted in significantly higher radial growth of *Ch. globosum* compared to its growth on the control media.

The potting soil incorporation with Borage plant remain powder at all the ratios significantly increased the okra growth indicators, but did not affect R. solani pathogenicity. However, Borage remain incorporated soil did increase the effectiveness of the bio-controlling Ch. globosum in reducing the incidence of the pathogenic R. solani and resulted in much higher growth parameters values compared to all the other treatments.

INTRODUCTION

Okra, *Abelmoschus esculentus*, belongs to the malvaceae family. Okra or 'Bamiah' in Iraq is one of the important and widespread summer vegetable crops grown in all parts of the country. Okra is a high-value and nutrient vegetable crop. Each 100g of fresh okra pods contains 88.9g water, 2.4 fat, 7.6 g carbohydrate, 1 g fiber, 92 mg calcium, 51 mg phosphorus, 0.6 mg iron, 3 mg sodium and 41 mg Magnesium, 249 mg potassium, 31 mg ascorbic acid, 21 mg riboflavin, 1 mg niacin and a small amount of carotene and vitamin A (6). Borage *Borago officinalis* or star plant is locally called bee bread because of its richness in pollen and nectar (Rf), which is considered to be good food source for honeybees. Therefore, in the field of beekeeping, it is called brood. As a winter plant, Borage is considered Mediterranean native. It is origins in the Persian Mountains, Mosul Island, Damascus, and Alps. It is also grown in France, England, Spain and many other localities (2). The plant is made of herbaceous honey and all its parts are useful whether flowers, leaves, stems or roots. The plant contains volatile oils, salts, vitamins, minerals, amino acids and organic. It is used in many medical and therapeutic applications. Borage is used to treat people with depression, sadness, weak memory. And it also helps in the treatment of cough, urinary tract diseases, rheumatism, some skin infections and itching (14). *Rhizoctonia solani* is one of the main causes of seed rot and seedlings death diseases in Iraq. This pathogenic fungus attacks plants at different stages of life. It infects seeds in the soil and seedlings before and after emergence, ant it also infects the roots. The fungus attacks and affects the aboveground plant parts such as pods, fruits, leaves and stems (8). Regardless of their success, chemical control of such plant diseases is being less favorable applications due to their toxicity and environmental concerns. Beside application difficulties, fungicides may not be effective unless they reach or be close to the phytotoxic level (20). Many natural plant compounds are found to have antifungal activities. These compounds may be found in a particular plant parts or in the all parts of a plant. Such compounds always need to be at right concentration and applied in the right method to be effective. Another controlling method is using natural antagonists against those pathogens. The bio-agent fungus *Ch. globosum* is one of these antagonists and approved to have a broad spectrum activity against some pathogenic fungi including *R. solani* (7). Improvement of soil micro-fona diversity using natural methods will definitely increase microbial activities and decrease chemical pollution (11). Since most okra in Iraq is grown for subsistence in small private lands, using natural available and affordable methods to control plant pest and pathogens is the most reliable way to mitigate yield loss and increase productivity. This study, therefor, aimed to investigate possibility of using Borage plant remains alone or in combination with the bio-agent *Ch. globosum* to suppress pathogenicity of *R. solani* and to control seed rot and seedlings death in okra.

MATERIAL AND METHODS

Inoculums: Millet *Panicum miliacem* seeds were used as a reproductive storage medium for loading of fungi used in this study. Each 50 g of seeds were placed in 250 ml glass flask, the flasks then were sealed and autoclaved for one hour. Two days after the flasks were autoclaved again assuring sterilization (Dewan, 1989). The flasks contained the seeds were then saved to use in the preparation of fungal inoculums.

Okra seeds:

A local widely grown variety 'Husainawiya' okra seeds were used in all tests of this study. Seeds were washed with

tap water, bleached in 0.05% NaOCl for 4 min. then seeds were strained and washed with tap water for 2 min and rinsed with DW. This seed sterilizing procedure was used whenever seeds were needed.

Media:

Potato Dextrose Agar (P.D.A.): The PDA media was prepared according to name (18). The antibiotic (Chloramphenicol) was added at a rate of 250 mg/L and autoclaved at 121 C with 15 par pressure, then media was poured in 9 cm and saved until use.

Potato extract- B. officinalis extract-agar media (BexPA): Borage vegetative plants remain was used in combination with chopped potato and agar for preparing special media. The combinations rates were measured by weight w/w (g) of chopped potato:Borage shoot remains to be 75:25, 50:50, 25:75, 0:100 or 100:0 g, respectively. In one Liter glass flask, each combination were boiled in 500 cm3 distilled water for 15-20 min. the mix was then filtered using cheese cloth. The filtrate then was added to 20g dextrose dissolved with 17g agar in 500cm3 of distilled water and more DW was added to complete 1 Liter volume. The mix then was autoclaved and 250mg/L of the bacterial inhibiting Chloramphenicol was added and the mix was left to cool to the pouring point. The media then was poured in 9 cm Petri dishes, cooled and refrigerated until use (5).

Detection of Borage plant Bio-active compounds using gas chromatography---mass spectrometry (GC---MS) analysis: 50 g sample of Borage plant shoot parts dried and grinded was sent for gas chromatography-mass spectrometry (GC---MS) analysis to the GC-MS laboratory belongs to Dept. of Food sciences at the College of Agriculture/Univ. of Basrah.

Pathogenicity of the studied fungi: In 9 cm Petri dishes contained standard PDA media formerly mentioned (10), Both fungi *Rhizoctonia solani* and *Chaetomium globosum* individually or combined were tested for their pathogenic effects on okra seed germination and seed rot. Six dishes for each fungus and for the interaction treatment were planted with sterilized (as previously described) Okra seeds and incubated at 25C° ± 2. After seven days of incubation, percent germination and seed rotting rate were calculated and compared among treatments.

Effect of *B. officinalis* extract media (BexPA) on the growth of studied fungi: Growth of Rhizoctonia solani and Chaetomium globosum affected by *B. officinalis* extract media (BexPA) was also tested. In 9 cm Petri dishes contained BexPA media prepared as described above, 1cm diameter from each *R. solani* or *Ch. Globosum* cultured on PDA for 7 days was cut and inoculated in the center of each dish. The dishes were then incubated at 25 ± 2 C°. After 24h, the radial growth diameter for both fungi was measured daily until the plate was filled and the growth rate was calculated per day compared with the radial growth on the standard P.D.A. **Effect of Borage plant extract in germination of okra seeds:** 10 okra sterilized seeds with six replicates were planted in Petri dishes contained the BexPA at different extract concentrations. Another six dishes with standard PDA were planted with okra seeds and served as control. At 25 ± 2 C°, the dishes were incubated for 7 days and the percent germination was calculated and compared between treatments.

Effect of Borage plant remain Powder incorporation on Growth of okra Planted in Plastic pots: This experiment used potting soil mix (V/V) contained heat sterile river sand incorporated with dry grinded *B. officinalis* plant remains powder (BPRP) at five different rates (1:3, 2:2, 3:1, 0:4 and 4:0 respectively). Each soil mix was inoculated with 5 g/kg soil inoculum of the pathogenic *R. solani* or the bio-agent *Ch. Globosum* individually or combined. The inoculated and non-inoculated (control) soil was used to fill 11 cm diameter plastic pots with 1 kg of soil with 5 replicates. 3-5 okra seeds were sewn in each pot and seedlings were thinned to 1 plant/pot after germination. Pots were irrigated as needed and maintained on 20 cm height benches in shade house condition After 48 days of planting, the fresh and dry weight of the shoot and root system of each plant was recoded to be compared among different soil mix and inoculation.

Statistical analysis: All the study experiments were conducted twice. Data were subjected to Analyses of variance (ANOVA) using GenStat package 2009, (12th edition) version 12.1.0.3278 (www.vsni.co.uk). Least significant difference LSD was performed to test differences between treatments at $p \leq 0.05$ (21).

RESULTS AND DISCUSSION

Pathogenicity test of studied fungi: Pathogenicity of *Ch. globosum* and R. solani and their effects on okra seed germination rates found to be different as fungus differs. It can be seen from the figure 1. That the highest germination rates were in the control (media only) and *Ch. globosum* treatments that of 96.67% and 96.2% respectively, while in the case of seed treated with R. solani there was no germination at all. R. solani also resulted in significantly ($P \leq 0.05$) the highest (100%) seed rot rate compared to 3.3% seed rot in case of the control and *Ch. globosum* treatments figure 1. High rates of seed germination were found in the presence of some certain fungi but not with the others. This is mostly due to the fact that these fungi are not pathogenic and their secretions are non-toxic or ineffective on seed germination (13). The reason for the increase in the percentage of seed germination may be due to these fungi produce some substances that helps in breaking down the seed shell and consequently facilitate seed germinating. These compounds may include enzymes such as Cellulase, or the secretion of germination-helper substances such as Indol Acetic Acid (IAA) (1, 4, 16) or 2-Carboxymethyl-3-n-hexyl malic acid (19).

Effect of *B. officinalis* extract media (BexPA) on the growth of pathogenic *R. solani*:*R.solani* radial growth was highly affected by Borage extract concentration and incubation period. There were significant differences between the days and the highest radial growth (5.02) appeared on the fourth day and the lowest (1.33) was on the first. As for the treatments, the highest radial growth (5.59) occurred in the treatment of only P.D.A. while the lowest radial growth (0.94) in the treatment contained only Borage extract. Interaction between incubation period and media type showed that the highest radial growth occurred in the

treatment of complete PDA on the fourth day and the lowest radial growth of the pathogenic fungus was in the treatment of 100% borage extract media with significant differences Table (2). Secretion of some compounds by the pathogenic fungus R. solani might be toxic and negatively affect seed germination and resulting in seedling death. This pathogenic fungus on the other hand was also affected by the Borage plant extract. This plant contains chemical compounds that have a negative effect on the growth of R. solani. These compounds may have antagonistic activities against the pathogenic fungus or their properties may have been changed where added to the medium and consequently made the medium toxic or unfavorable for the growth of the pathogenic fungus .

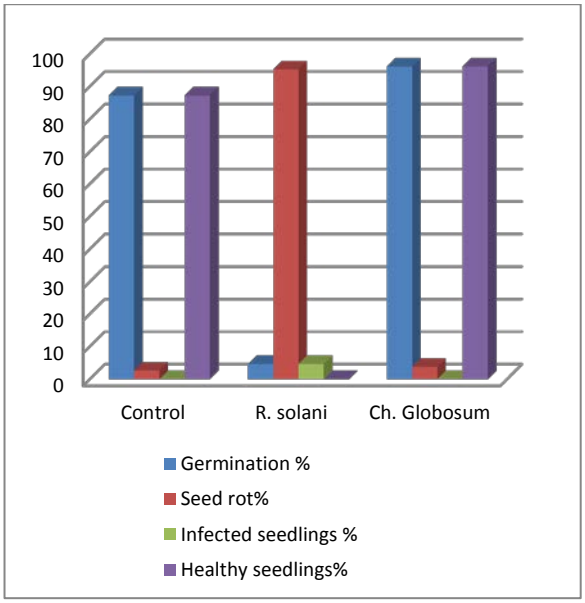

Figure1. In vitro pathogenicity test of the pathogenic *R. solani* and the bio-control agent *Ch. globosum* on seeds rot and seedlings death in okra planted in P.D.A. media

Effect of *B. officinalis* extract media (BexPA) on the growth of the bio-agent *Ch. globosum*

The study of the effect of the plant extract on the bio-agent Ch. globosum showed there were significant differences between the treatments and the number of incubation days. The highest radial growth (8.39) was observed on the sixth day and the lowest (1.43) was observed on the first day. Among treatments, the highest radial growth (6.00) occurred in the treatment of 100% BexPA and the lowest radial growth (3.54) was recorded in the complete PDA treatment. In the case of interaction between media type and incubation period, the highest radial growth increased as the BexPA increases. Generally, all the Borage plant extract media significantly supported higher radial growth of the bio-agent Ch. globosum compared that of the same fungus where grown on standard PDA (control treatment) as shown in Table 3. This may be due to the high ability of this fungus to grow on the remaining plant material in the soil, or to its high ability to colonize the medium with its antifungal or toxic secretions that reduce or inhibit the growth of other fungi (12, 13). This also may be due to its ability to saprophyte or release toxic substances under various environmental conditions (9, 14). The reason why certain substances in the studied plant extracts did not affect the *Chaetomium globosum* is may be due to its complex enzymatic system, which may play a role in biodegradation of some toxic compounds. However, encouragement of some plant extracts to Chaetomium globosum may be due to the containment of these extracts on growth regulators such as hormones or due to containing quantities of nutrients that promote the growth of the fungus.

Effect of Borage plant remain Powder incorporation on Growth of okra Planted in Plastic pots: The effect of borage plant remains powder on okra growth indicators (fresh weight of shoot and root systems) where added to either Rhizoctonia solani or Chaotomium globosum or combination of both fungi varied among treatments and showed significantly different effect on these indicators. Shoot weight was the highest in the treatment of Chaetomium globosum and the lowest values for shoot and root weights resulted in the presence of Rhizoctonia solani (table 3).

Generally 100% Borage plant remains potting resulted in significantly higher values of the okra growth indicators while the lowest values were always in 100% pure soil. All Borage plant remains rates used in potting soil resulted in significantly in much higher plant growth where interacted with the bio-agent *Ch. globosum* compared to the control (fig.2 and 3) and the lowest values resulted from all the same potting mix rates in the presence of pathogenic *R. solani*(table 3).

The effect of these residues may be due to the accumulation of allelopathic compounds and active substance (table 5) that negatively or positively affect germination rates and seedlings health (5). Plant extracts were also indicated by (3) to have an effect on seeds germination and growth parameters. The increase in shoot and root fresh weight may due to the presence of such bioactive compounds contained in the Borage plant as shown in table 5. Or this may also due to the interaction between the Borage bioactive compounds with other compounds secreted by the bio-agent CH. globosum, and consequently releasing some sort of growth promote compounds. It was reported by several studies that substances produced from chemical and biological breaking down of plant remain of a crop will have an effect on the following crop. The mechanism by which allelophathic compounds affect germination and growth may be due to their effect increasing seed water absorption and thus increasing cells elongation and division, and they may also play some roles in certain intercellular metabolic activities (17).

Table1. In vitro bioassay evaluating the effect of Borage plant extract media at different rates on radial growth of the pathogenic fungus *R. solani*

Medium type	Colony diameter of pathogenic *R. solani* (mm)				
	Day 1	Day 2	Day 3	Day 4	Average
PDA	22	44	67	90	60
25% BexPA*	16	32	46	59	51
50% BexPA	15	23	35	46	30
75% BexPA	10	20	30	46	25
100% BexPA	3	7	12	16	<10
Avrage	13	25	38	50	

*Percentage of Borage plant extract mixed with potato agar. Values are average of three replicates. At confidence of 95% (P≤0.05) the L.S.D. values are 0.10, 0.09 and 0.21 for treatments, days and interaction, respectively.

Table2. In vitro bioassay evaluating the effect of Borage plant extract media at different rates on radial growth of the Bio-agent *Ch. globosum*

Medium type	Colony diameter of bio-agent *Ch. globosum* (mm)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Average
PDA	1.06	1.96	2.93	4.26	5.10	5.96	3.54
25% BexPA*	1.46	3.03	4.4	6.00	7.50	9.00	5.23
50% BexPA	1.46	3.00	4.50	6.10	7.50	9.00	5.26
75% BexPA	1.53	3.43	5.03	7.30	9.00	9.00	5.88
100% BexPA	1.66	3.40	5.30	7.66	9.00	9.00	6.00
Avrage	1.43	2.96	4.43	6.26	7.62	8.39	

*Percentage of Borage plant extract mixed with potato agar. Values are average of three replicates. At confidence of 95% (P≤0.05), the L.S.D. values are 0.09, 0.103 and 0.23 for treatments, days and interaction, respectively.

Table3. Effect of Borage plant remain powder in potting soil at different rates in presence of Ch. Globosum and R. solani in on okra plants growth parameters 45 days after planting

	Ch. Globosum		*R. solani*		*Ch. Globosum+R. solani*		
Potting soil*	Sh W	R W	Sh W	R W	Sh W	R W	Average
100%BRP	30.33	26.00	21.00	16.66	25.00	19.00	16.72
75%BRP	29.00	25.33	19.00	15.00	22.00	16.33	15.44
50%BRP	26.66	23.00	17.00	13.00	20.00	14.00	13.94
25%BRP	25.00	21.33	15.00	12.00	18.00	12.66	12.83
100%Soil	22.00	17.00	0.00	0.00	16.00	11.00	11.00
Avrage	26.59	22.53	14.46	11.33	20.20	14.59	

*Percentage of Borage plant remains powder in potting soil mix. Values are average of five replicates. At confidence of 95% (P≤0.05), the L.S.D. values are 1.578, 1.23 and 2.75 for treatments, growth indicator and interaction, respectively.

Table 4. Showing 17 bioactive compounds detected in Borage plant extract using GC-MS analysis

Peak	R.Time	Area	Area%	Name
1	4.416	180635	0.43	Butane, 1,1-diethoxy-3-methyl-
2	14.558	181727	0.43	Dodecanoic acid
3	14.945	106154	0.25	Dodecanoic acid, ethyl ester
4	16.867	221722	0.53	Tetradecanoic acid
5	**18.947**	**6821728**	**16.17**	**l-(+)-Ascorbic acid 2,6-dihexadecanoate**
6	20.420	1780699	4.22	Gamolenic Acid
7	**20.554**	**5539367**	**13.13**	**9,12-Octadecadienoic acid (Z,Z)-**
8	**20.612**	**8869680**	**21.03**	**6-Octadecenoic acid, (Z-(**
9	20.833	1980820	4.70	Octadecanoic acid
10	23.659	1530064	3.63	Tricosyl heptafluorobutyrate
11	23.881	231806	0.55	1,2-Benzenedicarboxylic acid, diisooctyl ester
12	25.185	2734411	6.48	Hexatriacontyl trifluoroacetate
13	26.100	3389058	8.04	.gamma.-Sitosterol
14	26.570	1364422	3.24	Triacontane
15	26.608	1567071	3.72	Cyclohexane, [6-cyclopentyl-3-(3-cyclopentylpropyl)hexyl-[
16	27.014	294850	0.70	Lupeol
17	27.255	5381993	12.76	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-
	42176207	100.00		

Figure2. (A) 1. Root growth in soil contained 25% Borage plant powder, 2. Root growth in soil only (control), B) vegetative growth of okra plants 45 days after planting in pots contained soil mixed with Borage plant powder at different rates (25, 50 or 57%).

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