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Influence of bacteria on *Orobanche crenata* seed bank size, incidence and *Vicia faba* L. performance

[1]Esraa M Elabaied, [2]Ahmed M E Rugheim, [3]Mohammed M Hassan, [3]Magdoline M Ahmed, [2]Mahdi A Yahia, [3]Rania A Abakeer, [4]Rashida M A Abusin, [3]Awad G Osman, [3]Migdam E Abdelgani and [3]Abdel-Gabar E Babiker

[1]M.Sc. student, Sudan Academy of Sciences (SAS). Khartoum, Sudan
[2]Departmentof Landscaping and Arid Land Agriculture, Faculty of Agriculture, Omdurman Islamic University, Omdurman, Sudan.
[3]Environment, Natural Resources and Desertification Research Institute, the National Center for Research, Khartoum, Sudan.
[4]Pests and Plant Health, College of Agriculture, Bahri University, Khartoum, Sudan

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Address For Correspondence:
Ahmed M E Rugheim, Omdurman Islamic University, Department of Landscaping and Arid Land Agriculture, Faculty of Agriculture, Omdurman, Sudan.,
E-mail: arugheim@gmail.com

ABSTRACT

BACKGROUND: *Orobanche crenata* is a holoparasitic weed that seriously attacks legumes and depend entirely on their hosts for all nutritional requirements. A wide variety of approaches physical, cultural, chemical and biological have been explored against root parasite. Current means for controlling parasitic weeds are focusing on reducing soil seed bank and inhibiting early developmental stages. **OBJECTIVE:** Laboratory and greenhouse experiments were conducted to investigate the effects of bacterial strains and isolates cultures and filtrates, on early developmental stages of *Orobanche crenata* and to explore their potential in *O. crenata* control. **RESULTS:** Results of *in vitro* experiments revealed that germination of *O. crenata* significantly decreased after inoculation with bacterial cultures or filtrates. Bacterial isolates (ISO43 and ISO44) and strains of *Bacillus circulans*, *B. megatherium* var. *phosphaticum* (BMP) and the combination of BMP plus *Rhizobium leguminosarum* bv. *viceae* (TAL1399) significantly reduced germination as compared to other microbes and controls. With respect to the effects of bacterial cultures or filtrates on haustorium initiation, results displayed that *B. circulans*, BMP, BMP+*R. leguminosarum* bv. *viceae* (USDA 2478) and ISO44 significantly suppressed *O. crenata* haustoria factor as compared to the corresponding control. In the *in vivo* experiment, irrespective of faba bean cultivars and bacterial inoculation, *O. crenata* emergence progressively increased with seed bank size. Faba been inoculated with bacterial combinations of BMP+TAL1399 and BMP+USDA2478 significantly reduced *O. crenata* emergence and dry weight. In general, Basabeer cultivar invariably displayed better growth than Selaim. Faba bean height and dry weight invariably decreased with increasing seed bank size. The increase in faba bean growth parameters is consistent with the observed delay and decrease in *O. crenata* emergence in response to treatments. **CONCLUSION:** Improving soil fertility by using beneficial microorganisms appeared to decrease *O. crenata* infestation and its suppressiveness effects on host growth. Use of potential bacterial strains and isolates could be incorporated into existing *Orobanche crenata* management practices.

KEY WORDS
Broomrape, Faba bean cultivars, Soil borne bacteria, Suppression.

INTRODUCTION

Soil microorganisms interfering with early developmental stages of parasitic weeds were thought of as possible alternatives and/or viable supplements to other control methods [1].The symbiotic relationship formed between legumes and rhizobia plays an integral role in agriculture as bacteria fix atmospheric nitrogen (N_2). Rhizobia symbiosis with legumes produces 50% of 175 million tons of total biological N_2 fixation annually worldwide [2]. Therefore, inoculation of legumes with efficient rhizobia is one of the most important and

agronomically eco-friendly practices used for improvement of N fixation [3]. Many microorganisms possess an enzymatic system which enables them to mineralize phosphorus-containing organic compounds [4] or secrete acids to solubilize inorganic to solubilized compounds [5]. *Orobanche* spp., Orobanchaceae, is a major biological constraint to leguminous crops production in sub-Saharan [6]. A larger part of the damage is already done underground even before the parasite emerges from the soil thereby making weeding ineffective option in its control. Research findings have ascertained the link between poor soil fertility, strigolactones production and mycorrhizal infection [7]. Nutrient deficiency is conducive to strigolactones biosynthesis, reduction of shoot branching, maximization of the symbiotic interactions with arbuscular mycorrhizal fungi(AMF) and facilitation of nutrients uptake. Strigolactones have been shown to function as endogenous phytohormones that curtail shoot branching [8]. The present investigations were undertaken to evaluate the effects of bacterial strains and isolates cultures and filtrates, on early developmental stages of the parasite and to explore their potential for deployment as a component of an integrated management strategy.

MATERIALS AND METHODS

Laboratory experiments:

A series of laboratory experiments were undertaken to investigate the effects of bacterial strains and isolates cultures and filtrates on germination and haustorium initiation of *O. crenata*. Treatments were arranged in a Complete Randomized Design (CRD) with 4 replicates. All experiments were repeated three times.

Bacterial isolates and strains inoculums:

Soil samples were collected between November and December, 2012 from Hudeiba Research Station, Agricultural Research Corporation (ARC), Sudan. The samples were collected from the top 10 cm soil layer in faba bean fields and incubated on rotary shaker (100 rpm) at 28ºC for 48 h to obtain microbial cultures. For reducing microbial population, one gram soil was dissolved in 9 ml sterile distilled water. Serial dilution was carried. Nutrient agar medium containing antifungal brilliant green was used for bacterial growth. The medium was sterilized by autoclaving at 120ºC for 15min. The spread plate method was used for isolation of bacteria. One ml of 10^{-5}- 10^{-7} dilutions were poured and spread over the media plates using sterile glass rod. The dishes were incubated at 28ºC for 48h.Microbial colonies were purified by sub-culturing and were morphologically characterized. Forty eight bacterial isolates were obtained from the soil samples. The isolated bacteria were preserved in nutrient agar slants and kept in a fridge at 4ºC for further studies. Every 3 months interval, bacterial isolates were refreshed. In addition, five bacterial strains, *Rhizobium leguminosarum bv. viceae* strains (TAL1399, USDA 2478 and ENRRI9), *Bacillus megatherium* var. *Phosphaticum* (BMP), *B. circulans*, *Azomonas* spp. and *Flavobacterium* spp. were obtained from the Biofertilization Department, Environment, Natural Resources and Desertification Research Institute (ENDRI), the National Centre for Research (NCR), Khartoum, Sudan.

Plant materials:

O. crenata seeds were collected from infested faba bean field in 2006 at Shendi Research Station Farm, Agriculture Research Corporation (ARC) during the winter season. Seeds were surface disinfected by immersion in 70% ethanol for 2-3 min., followed by washing three times with sterilized distilled water. Then seeds were immersed with swirling into a 1% sodium hypochlorite obtained by appropriate dilution of commercial sodium hypochlorite (Bleach) for 2-3 min. The sodium hypochlorite was drained off and the seeds were washed under suction system, with sterilized distilled water, several times, until the yellow color disappeared. The seeds, plotted dry on Whattman No. 1 filter papers, were air-dried under a laminar flow cabinet and subsequently stored at ambient temperature till used.

Chemicals preparation:

The strigolactone analogue GR24 was provided by professor Zwanenberg, University of Nimijhen, the Netherlands. A stock (10 ppm) of GR24 was prepared by dissolving 1 mg in 1 ml acetone and completed to volume (100 ml) with sterile distilled water. The solution was kept refrigerated at 4ºC for further use.

The haustorium inducer 2, 6 dimethoxybenzequinone synthetic stimulants (DMBQ) was provided by Prof. Sugimoto, from Kobe University, Japan. A stock solution (100 ml) was prepared by dissolving 1.68 gm in 1 ml acetone and completing to volume (100 ml) with sterile distilled water. The solution was kept refrigerated at 4ºC for further use.

Effects of bacterial strains and isolates cultures on O. crenata germination:

Glass fiber filter papers (GFFP) discs (8mm diameter) were cut, wetted thoroughly with water and placed in an oven (100 ºC for 1h.) to be sterilized just before use [9]. For pre-conditioning, sterilized discs, placed in 9 cm Petri dishes lined with glass fiber filter papers, were moistened with 5 ml distilled water, broth media yeast

extract mannitol, (meat peptone and nutrient broth) for the respective bacterial culture (TAL1399, USDA 2478, ENRRI9, BMP, *B. circulans*, *Azomonas* spp., *Flavobacterium* spp., ISO30, ISO33, ISO43 and ISO44). About 25-35, surface disinfected *O. crenata* seeds were sprinkled on each of the glass fiber discs. The Petri-dishes, sealed with parafilm and wrapped in black polythene, were incubated in the dark at 18°C for 11 days. Then each disc was subsequently treated with the synthetic germination stimulant GR24 (25µl /disc) at 5 and 10ppm, were re-incubated, and examined for germination after 168h using stereomicroscope.

Effects of bacterial strains filtrate on O. crenata germination:

Two bacterial strains (TAL1399 and BMP) and their combination were sub-cultured in yeast extract Mannitol broth medium and incubated for 24h at 30ºC. Bacterial suspension was centrifuged at 6000 rpm for 15 minutes and the supernatant was collected, filtered and diluted with sterile distilled water to the desired concentrations (25, 50, 75 and 100%). *O. crenata* seeds were moistened with 5-ml of either sterilized distilled water or undiluted or diluted bacterial filtrates and subsequently incubated and treated with GR24 as described above.

Effects of bacterial strains and isolates cultures on O. crenata haustorial initiation:

Seeds conditioned in selected bacterial strains and isolates cultures (TAL1399, USDA 2478, ENRRI9, BMP, *B. circulans*, *Azomonas* spp., *Flavobacterium* spp., ISO30, ISO33, ISO43 and ISO44) were induced to germinate with GR24. Discs containing germinated seeds (germilings) were blotted dry on filter paper and placed top down on glass paper without seeds. Each pair of discs was treated with 40µl of DMBQ. Then the dishes were re-incubated. Seeds transferred to bacteria free media similarly incubated and treated with GR24 were included as control for comparison. The Petri dishes were sealed with Parafilm, wrapped in aluminum foil and incubated in the dark for 5 days. A seed was considered to have a haustorium when the radical tips were swollen and formed hair.

Effects of bacterial strains filtrate on O. crenata haustorial initiation:

O. crenata seeds were treated with the selected bacteria (TAL1399 or/and BMP) filtrates at 25, 50, 75 and 100%.Then each disc was treated with GR24 to induce germination. Then germilings were treated with 40 µl of DMBQ at 10 and 20µM and subsequently incubated and examined for haustorial initiation as described above.

Pot experiment:

The experiment was conducted in the greenhouse of the Faculty of Agriculture, Omdurman Islamic University, during December, 2015 - March, 2016. The experiment was conducted to study the effects of various combinations comprising bacterial strains and isolates, *O. crenata* seed bank and resistant varieties on *O. crenata* incidence and faba bean performance. Plastic pots (19 cm. diameter), with drainage holes at the bottom, were filled with soil mixture (9Kg/pot) of river silt and sand (1:1v/v). Artificial infestation of soil was accomplished by mixing *O. crenata* seeds (2g) with 1kg soil followed by subsequent dilutions with *O. crenata* free soil to give the required infestation level (4 and 8 mg/ pot), *O. crenata* infested and uninfested controls were included for comparison. Two bacterial combinations BMP+ TAL1399 and BMP+USDA2478 and two faba been cultivars (Selaim and Basabeer) were used. Faba bean seeds (5/pot) were sown at 2 cm soil depth. The pots were subsequently irrigated every 2 days. Faba bean seedlings were thinned to 2 plants per pot after 2 weeks of sowing. Treatments were arranged in a Randomized Complete Block Design (RCBD) with four replicates. Data collected for *O. crenata* emergences were measured at 4, 6, 8, 9, 10, 11 and 12 weeks after sowing (WAS). Data collected for faba bean growth parameters were plant height and dry weight.

Statistical analysis:

Prior to analyses data on percentage (germination or haustorium) were arcsine transformed, data on *O. crenata* emergence were square root transformed to fulfill ANOVA requirements. The analyses were performed across experiments using SAS Statistical package. Means separations were made by the LSD at 5% [10].

Results:

Laboratory experiments:

Effect of bacterial strains and isolates culture on O. crenata germination:

O. crenata seeds treated with water displayed negligible germination (data not shown). GR24 applied to *O. crenata* seeds conditioned in water induced the highest germination (58 - 69%) (Table1). Results revealed that conditioning in the growth medium had no adverse effect on germination in response to GR24 at the lower concentration. *O. crenata* seeds, previously conditioned in presence of bacterial strains, were comparable to that of the corresponding nutrient broth medium, irrespective to germination stimulant. In among all bacteria, *B. circulans* strain had the inhibitoriest effect as compared to other microbes. The combination of BMP + TAL1399 consistently showed significantly ($P \leq 0.05$) lower germination than the corresponding nutrient broth

control. The combination between *B. circulans* + ENRRI 9 induced *O. crenata* germination especially at the lowest concentration of GR24.

Table 1: Effects of bacterial strains and their combinations on *O. crenata* germination in response to GR24 (during conditioning) (Batch 1)

Treatments	Germination (%) GR24 conc. (ppm)		Means
	5	10	
Water	58.16* (72.00)**	69.97 (85.00)	63.97
Media (YEMB)#	51.12 (60.56)	54.16 (65.36)	52.60
ENRRI9	46.88 53.20)	44.22 (48.66)	45.55
TALL1399	38.98 (40.32)	46.17 (52.02)	42.57
USDA2478	42.62 (45.85)	42.53 (45.71)	42.57
Bacillus circulans	37.90 (37.96)	45.96 (51.57)	41.93
BMP+ ENRRI9	48.33 (55.72)	47.74 (54.25)	48.03
BMP+TAL1399	33.73 (31.27)	35.78 (34.71)	34.75
BMP+USDA2478	34.66 (32.84)	37.43 (37.05)	36.04
B. circulans + ENRRI9	67.17 (84.76)	57.44 (70.72)	62.30
B. circulans + TAL1399	60.57 (75.36)	71.37 (85.47)	48.28
B. circulans + USDA2478	42.21 (45.18)	45.69 (51.14)	43.95
LSD (Bacteria)			8.28
LSD (Interaction)			11.71

*Data out of brackets are arcsine transformed for analysis **Data between brackets are original data
YEMB: Yeast Extract Mannitol Broth

In the second batch, Seeds conditioned in distilled water and treated with GR24 at 5 and 10 ppm displayed 58.2 and 62.8% germination (Table 2). Results showed that conditioning in medium had no adverse effect on seed germination in response to GR24 at both concentrations. Seeds conditioned in BMP and similarly treated with GR24 displayed low and differential germination. The bacterial combinations of BMP plus *Flavobacterium* spp. and BMP plus *Azomonas* spp. had no adverse effects on *O. crenata* germination in response to GR24.

Table 2: Effects of bacterial strains and their combinations on *O. crenata* germination in response to GR24 (during conditioning) (Batch 2)

Treatments	Germination (%) GR24 conc.(ppm)		Means
	5	10	
Water	58.22* (71.79)**	62.83 (78.76)	60.52
Media (MPB)#	52.99 (63.45)	59.47 (73.75)	56.23
BMP	40.05 (41.66)	42.57 (45.83)	41.31
Flavobacterium spp.	48.84 (56.41)	51.07 (60.27)	49.96
Azomonas spp.	48.49 (55.93)	53.00 (57.04)	50.74
BMP + *Flavobacterium* spp.	42.31 (45.47)	51.63 (61.25)	46.97
BMP + *Azomonas* spp.	51.83 (61.53)	56.43 (68.58)	54.13
LSD (Bacteria)			10.06
LSD (Interaction)			14.23

*Data out of brackets are arcsine transformed for analysis **Data between brackets are original data
MPB: Meat Peptone Broth

Results of batch 3, showed that germination rate of seeds treated with GR24 was 56%. *O. crenata* seeds, previously conditioned in presence of bacterial isolates (ISO30, ISO44, ISO43 and ISO33), showed variable response to GR24 (Table 3). In among all bacterial isolates, ISO43 and ISO44 induced only 33.9 and 33.4% germination, respectively.

Table 3: Effect of bacterial isolates on *O. crenata* germination in response to GR24 (during conditioning) (Batch 3)

Treatments	Germination (%) GR24 conc.(ppm)		Means
	5	10	
Water	53.65* (57.26)**	58.84 (60.18)	56.24
Media (NB)#	52.24 (66.73)	53.74 (54.40)	52.99
ISO30##	38.07 (38.13)	46.64 (52.84)	42.35
ISO33	36.60 (35.71)	38.74 (39.55)	37.67
ISO43	34.19 (31.65)	33.65 (32.33)	33.92
ISO44	32.85 (30.10)	34.09 (30.45)	33.47
LSD (Bacteria)			6.15
LSD (Interaction)			8.69

*Data out of brackets are arcsine transformed for analysis **Data between brackets are original data
#NB: Nutrient Broth ##ISO: Bacterial isolate

Effects of bacterial filtrates on O. crenata germination (during conditioning):

The present investigation was set to study the effects of bacterial filtrates on *O. crenata* germination (Table 4). Results displayed that seeds conditioned in the medium had no adverse effect on *O. crenata* germination in response to GR24. The filtrate of TAL1399 irrespective to concentrations, significantly (P≤0.5) inhibited *O. crenata* germination to 31.17-34.91% and 37.70-39.20% in response to GR24 at 5 and 10 ppm, respectively.

The filtrates of the combination of TAL1399 plus BMP reduced *O. crenata* germination by 5.05 -35.44% and 39.65-45.31% in response to GR24 at 5 and 10 ppm, respectively, compared to the control. While the BMP filtrate at concentration of 75 and 100% significantly (P≤0.5) inhibited *O. crenata* germination by 44.3 and 37.7%, respectively.

Table 4: Effect of bacterial strains filtrates on *O. crenata* germination in response to GR24 (during conditioning)

Filtration	Filtrate concentration	Germination (%) GR24 conc. (ppm) 5	10	Means
Water		66.45* (83.64)**	67.40 (84.68)	66.92
Media (YEMB)#		67.57 (84.97)	69.23 (86.63)	68.40
BMP	100	36.38 (35.50)	39.10 (39.97)	37.74
	75	44.80 (49.63)	43.84 (48.00)	44.32
	50	50.76 (59.80)	53.33 (64.05)	52.04
	25	55.78 (68.32)	57.25 (69.93)	56.51
TAL1399	100	40.40 (42.03)	41.99 (44.77)	41.19
	75	43.25 (46.99)	47.06 (53.48)	45.15
	50	46.19 (52.06)	47.55 (54.42)	46.87
	25	54.70 (66.38)	56.19 (69.02)	55.44
BMP + TAL1399	100	36.34 (35.18)	40.67 (42.49)	38.50
	75	42.90 (46.45)	43.77 (47.99)	43.33
	50	47.54 (54.32)	50.48 (59.49)	49.01
	25	49.11 (56.87)	52.77 (63.09)	50.94

LSD (Bacterial filtrate) 2.59
LSD (Filtrate concentration) 2.59
LSD (Interaction) 8.18

*Data out of brackets are arcsine transformed for analysis **Data between brackets are original data
YEMB: Yeast Extract Mannitol Broth

Effect of bacterial strains and isolates on O. crenata haustorium initiation:

Results revealed that *O. crenata* germilings resulting from seeds conditioned in water or nutrient broth medium showed similar response to DMBQ (Table 5). In among the bacterial strains, the inhibitory effect was highest in *B. circulans* as compared to other treatments. However, conditioning in USDA2478 resulted in a noticeable increase in haustorium initiation, albeit not significant compared to the broth medium. Conditioning in BMP + USDA2478 reduced haustorium initiation and the observed reduction increased with DMBQ concentration.

Table 5: Effects of bacterial strains and their combinations on *O. crenata* haustorium in response to DMBQ (during conditioning) (Batch 1)

Treatments	Haustorium (%) DMBQ conc. (µM) 10	20	Means
Water	44.04* (48.33)**	45.98 (51.70)	45.01
Media (YEMB)#	41.83 (44.48)	45.25 (50.43)	43.54
ENRRI9	41.92 (44.72)	33.04 (30.23)	37.48
TALL1399	49.53 (57.51)	41.34 (43.69)	45.43
USDA2478	47.36 (54.18)	48.97 (56.76)	48.16
Bacillus circulans	27.11 (21.33)	39.86 (41.17)	33.48
BMP+ ENRRI9	43.64 (47.76)	35.25 (33.53)	39.44
BMP+TAL1399	33.04 (29.85)	33.23 (30.18)	33.13
BMP+USDA2478	29.32 (42.45)	31.30 (27.77)	30.31
B. circulans + ENRRI9	38.59 (39.07)	45.68 (51.56)	42.13
B. circulans + TAL1399	43.04 (46.21)	43.22 (46.96)	43.13
B. circulans + USDA2478	39.84 (41.59)	37.83 (38.12)	38.83

LSD (Bacteria) 8.47
LSD (Interaction) 11.98

*Data out of brackets are arcsine transformed for analysis **Data between brackets are original data
#YEMB: Yeast Extract Mannitol Broth

O. crenata germlings arising from seeds conditioned in distilled water and broth medium and subsequent treated with GR24 displayed 51.9% and 43.6%, haustorium initiation in response to DMBQ respectively (Table 6). Conditioning in BMP culture significantly (P≤0.05) reduced haustorium initiation and the observed reduction

increased with concentration. The combinations of the bacterial strains significantly (P≤0.05) inhibited haustorium induction compared to the corresponding water control.

Table 6: Effect of bacterial strains and their combinations on *O. crenata* haustoriumin response to DMBQ (Batch 2)

Treatments	Haustorium (%) DMBQ conc.(µM) 10	20	Means
Water	48.90* (56.65)**	54.92 (66.83)	51.91
Media (MPB)#	42.65 (46.18)	44.60 (49.31)	43.62
BMP	37.64 (37.49)	32.59 (30.50)	35.11
Flavobacterium spp.	50.17 (58.54)	42.14 (45.07)	46.15
Azomonas spp.	46.09 (51.80)	41.29 (43.58)	43.69
BMP + *Flavobacterium* spp.	34.41 (32.46)	34.22 (31.65)	34.31
BMP + *Azomonas* spp.	29.95 (25.29)	34.67 (32.50)	32.31
LSD (Bacteria)			7.00
LSD (Interaction)			9.89

*Data out of brackets are arcsine transformed for analysis **Data between brackets are original data
#MPB: Meat Peptone Broth

DMBQ at 10 and 20 µM applied to *O. crenata* germilings resulting from seeds previously conditioned in water and treated with GR24 induced 55.7% and 59.3% haustoria, respectively (Table 7). Seed conditioned in nutrient broth medium, stimulated to germinate with GR24 and similarly treated with DMBQ displayed 52.4% and 53.6% haustoria, respectively. In among the bacterial isolates, ISO44 significantly (P≤0.05) reduced haustorial initiation, irrespective to haustorium inducing factor.

Table 7: Effects of bacterial isolates on *O. crenata* haustoriumin response to DMBQ (Batch 3)

Treatments	Haustorium (%) DMBQ conc.(µM) 10	20	Means
Water	55.70* (68.00)**	59.25 (73.26)	57.47
Media (NB)#	52.44 (62.77)	53.62 (64.58)	53.03
ISO 30##	34.36 (32.22)	38.10 (38.33)	36.23
ISO 33	34.67 (32.41)	39.39 (40.34)	37.03
ISO 43	33.16 (30.41)	34.05 (31.63)	33.60
ISO 44	28.90 (23.71)	30.31 (25.56)	29.60
LSD (Bacteria)			5.87
LSD (Interaction)			8.31

*Data out of brackets are arcsine transformed for analysis **Data between brackets are original data
#NB: Nutrient Broth ##ISO: Bacterial isolate

Effects of bacterial filtrates on O. crenata haustorium initiation:

Filtrates from the combination of TAL1399 + BMP significantly (P≤0.5) inhibited the haustorium initiation in response to DMBQ at 10 and 20 µM, it reduced haustorium by 44.80% and 41.68%, followed by BMP which reduced haustorium initiation by 58.61%and 59.03% respectively, irrespective to the filtrate concentrations, compared to control (Table 8).

Table 8: Effect of bacterial strains filtrates on *O. crenata* haustorium in response to DMBQ

Filtration	Filtrate concentration	Haustorium (%) DMBQ conc.(µM) 10	20	Means
Water		68.11* (86.03)**	68.82 (86.80)	68.46
Media (YEMB)#		64.82 (81.68)	70.71 (88.75)	67.76
BMP	100	34.06 (31.45)	35.56 (33.81)	34.81
	75	36.96 (36.81)	42.82 (46.21)	39.89
	50	43.61 (47.57)	44.35 (48.87)	43.98
	25	48.31 (55.76)	49.69 (58.01)	49.00
TAL1399	100	37.70 (37.41)	39.11 (39.79)	38.40
	75	39.73 (40.87)	41.22 (43.41)	40.47
	50	41.65 (44.17)	44.70 (49.47)	41.65
	25	45.91 (51.58)	48.20 (55.56)	47.05
BMP + TAL1399	100	28.19 (22.37)	28.19 (22.58)	28.19
	75	37.59 (37.31)	40.13 (41.59)	38.86
	50	41.97 (44.73)	44.06 (48.36)	43.01
	25	44.75 (49.56)	46.14 (51.98)	45.44
LSD (Bacterial filtration)				1.40
LSD (Filtrate concentration)				1.40
LSD (Interaction)				4.44

*Data out of brackets are arcsine transformed for analysis. **Data between brackets are original data.
YEMB: Yeast Extract Mannitol Broth.

Green house experiment:

Effects of bacterial strains, cultivars and O. crenata seeds level on O. crenata incidence in faba bean:

At 8 WAS, a few emergences of *O. crenata* was observed, irrespective of seed bank size and faba bean cultivars (Table 9). At 9 WAS, irrespective of the seed bank size, results showed considerable increase in comparison to that recorded at 8 WAS. At 10 WAS, *O. crenata* emergence on faba bean cultivars inoculated with BMP+TAL1399 and BMP+USDA2478 sustained the lowest emergence, irrespective to seeds bank size as compared to the control. At 11 WAS, a further increase in *O. crenata* seed bank to 8 mg per pot increased the parasite emergence.

Table 9: Effects of bacterial strains and faba bean cultivars on *O. crenata* incidence

Treatments			*O. crenata* count					
Faba bean cultivars	Bacteria	*O. crenata* seeds conc. (mg/ pot)	Weeks after sowing					Means
			8	9	10	11	12	
Selaim	Control	4	0.71*(0.00)**	1.56 (2.00)	1.93 (3.25)	2.28 (4.75)	2.81 (7.50)	1.86
		8	0.97 (0.50)	2.00 (3.50)	2.11 (4.00)	2.34 (5.00)	2.59 (6.25)	2.00
	BMP+TAL 1399	4	0.71 (0.00)	1.73 (2.50)	1.73 (2.50)	1.56 (2.00)	2.06 (3.75)	1.56
		8	0.93 (0.50)	1.82 (3.00)	2.10 (4.50)	1.86 (3.75)	1.86 (3.75)	1.71
	BMP+USD A2478	4	0.71 (0.00)	1.68 (2.50)	1.68 (2.50)	1.59 (2.25)	1.59 (2.25)	1.45
		8	0.84 (0.25)	1.19 (1.25)	1.58 (2.75)	1.84 (3.25)	1.84 (3.25)	1.46
Basabeer	Control	4	0.93 (0.50)	1.98 (3.50)	2.10 (4.00)	2.27 (4.75)	2.81 (7.50)	2.02
		8	1.06 (1.00)	2.11 (4.00)	2.27 (4.75)	2.48 (5.75)	2.66 (6.75)	2.12
	BMP+TAL 1399	4	0.84 (0.25)	1.64 (2.50)	1.57 (2.25)	1.57 (2.25)	1.57 (2.25)	1.44
		8	0.71 (0.00)	1.27 (1.20)	1.50 (2.00)	1.64 (2.25)	1.64 (2.25)	1.35
	BMP+USD A2478	4	0.71 (0.00)	1.86 (3.00)	1.86 (3.00)	1.89 (3.25)	2.39 (5.25)	1.74
		8	0.71 (0.00)	1.54 (2.25)	1.70 (3.00)	1.92 (3.50)	1.92 (3.50)	1.56
Means			0.82	1.70	1.84	1.94	2.14	
LSD			0.50	0.73	0.95	0.88	0.88	

* indicates square root transformed data (√x+0.5 x: variable) **Data between brackets are original data

Faba bean cultivars inoculated with both bacterial combinations decreased *O. crenata* emergence albeit not significantly in presence of the two *O. crenata* seed bank size, compared to the control. At 12 WAS, Basabeer cultivar inoculated with both bacterial combinations significantly (P≤0.5) reduced *O. crenata* emergence at the two seed bank size. While Seliam cultivar treated with BMP+TAL1399 sustained the lowest *O. crenata* emergence at the lower seed bank size (4mg/pot) compared to the control. In general, Selaim cultivar sustained the lowest *O. crenata* infestation as compared to Basabeer, irrespective to *O. crenata* seed bank and bacterial inoculation. Inoculation with the bacterial combinations BMP+TAL1399 and BMP+USDA2478 reduced *O. crenata* emergence, irrespective to faba bean cultivars and the parasite seed bank.

Effects of bacterial strains, cultivars and O. crenata seed bank on faba bean growth:

In all treatments, faba bean height progressively decreased with increase in *O. crenata* seed bank size and increased with time (Table 10). Basabeer cultivar, irrespective to bacterial inoculation or *O. crenata* seed bank size displayed better growth than Selaim cultivar. Moreover, faba bean inoculated with bacteria BMP+TAL1399 and BMP+USDA2478, irrespective to cultivars and seed bank size, displayed better growth than un-inoculated control. At 4 WAS, *O. crenata* seed bank at 4mg/pot inflicted insignificant decrease in Basabeer plant height. Increasing *O. crenata* seed bank size to 8 mg per pot significantly (P≤0.05) reduced faba bean height by 20.02 %. At 6 WAS, Selaim cultivar inoculated with BMP plus TAL1399 in presence of the *O. crenata* seed bank at 8 mg seeds/pot increased faba bean height albeit not significantly compared to the control. However, Basabeer cultivar treated with the same combination gave the highest growth at lower level of *O. crenata* seed bank. At 8 WAS, inoculation with BMP+TAL1399 at the lower *O. crenata* infestation seed bank size (4mg/pot) significantly (P≤0.5) increased Selaim plant height compared to the corresponding control. At 9 WAS Selaim cultivar inoculated with the bacterial combination of BMP+TAL1399 displayed the highest growth as compared to the control, irrespective to *O. crenata* seeds bank. At 10, 11 and 12 WAS faba bean height, irrespective to cultivars and bacterial inoculation, displayed a progressive decrease with *O. crenata* seed bank size. Inoculation with BMP+USDA2478 significantly (P≤0.5) increased Selaim cultivar plant height at the lower seed bank size at 10 and 11 WAS compared to the corresponding control.

Table 10: Effects of bacterial strains and *O. crenata* on faba bean growth

Treatments			Plant height (cm)							
Faba bean cultivars	Bacteria	*O. crenata* seeds level (mg/ pot)	Weeks After Sowing (WAS)							Means
			4	6	8	9	10	11	12	
Selaim	Control	0	22.08	30.50	34.45	38.00	45.66	46.12	47.33	37.73
		4	23.66	33.41	35.85	38.33	40.08	38.58	40.46	35.77
		8	22.49	32.62	38.87	40.99	41.66	42.25	37.75	36.66
	BMP+ TAL1399	4	19.75	32.45	37.75	39.99	40.16	42.66	41.12	36.27
		8	24.41	35.29	42.58	44.41	43.08	45.50	39.58	39.26
	BMP+ USDA2478	4	22.16	33.58	40.16	41.16	44.50	43.75	43.54	38.41
		8	21.66	34.08	39.00	40.66	41.75	44.70	40.25	37.44
Basabeer	Control	0	24.58	34.91	42.50	44.58	52.99	53.66	56.25	44.21
		4	21.54	33.04	41.83	43.20	44.08	44.37	40.29	38.34
		8	19.66	32.83	41.58	43.16	43.66	44.50	40.75	38.02
	BMP+ TAL1399	4	26.12	36.50	43.08	43.75	45.08	43.83	40.04	39.77
		8	23.50	34.50	39.25	40.00	42.00	41.75	38.00	37.00
	BMP+ USDA2478	4	20.62	32.95	39.25	39.95	40.87	41.08	41.21	36.56
		8	20.41	32.20	39.62	40.87	41.79	42.45	38.33	36.52
Means			22.33	33.49	39.70	41.36	43.38	43.94	41.78	
		I	3.55	3.56	4.15	3.99	4.10	3.84	4.01	

Effects of bacterial strains, cultivars and O. crenata seeds bank on faba bean dry weight:

Faba bean dry weight irrespective to bacterial combinations and cultivars, decreased with increasing *O. crenata* seeds bank size (Table11). *O. crenata* seed bank size at 4 and 8 mg/pot reduced faba bean biomass by 8.07 and 16.95% respectively as compared to uninfested control. Results showed that inoculation of Selaim cultivar with the bacterial combinations did not affect the plant dry weight. Inoculation with BMP+USDA2478 significantly ($P \leq 0.5$) increased Basabeer cultivar root dry weight at the lower seed bank size. While inoculation with BMP+TAL1399 significantly ($P \leq 0.5$) increased Basabeer shoot dry weight, in presence of *O. crenata* seed bank at 4 mg/pot compared to infested control.

Table 11: Effects of bacterial strains and *O. crenata* on faba bean dry weight

Treatments			Dry weight (g)		
Faba bean cultivars	Bacteria	*O. crenata* seeds conc. (mg/ pot)	Root	Shoot	Total biomass
Selaim	Control	0	2.95	15.40	9.18
		4	2.82	14.05	8.44
		8	1.87	13.37	7.62
	BMP+TAL1399	4	2.62	9.57	6.10
		8	2.00	11.87	6.94
	BMP+USDA2478	4	1.85	7.47	4.66
		8	1.65	7.70	4.68
Basabeer	Control	0	3.95	17.07	10.51
		4	1.32	8.10	4.71
		8	1.80	14.52	8.16
	BMP+TAL1399	4	1.12	12.02	6.57
		8	2.50	10.95	6.73
	BMP+USDA2478	4	2.55	11.27	6.91
		8	1.07	6.97	4.02
Means			2.26	12.11	
LSD			0.91	3.64	

Discussion:

Orobanche crenata Forsk is a major constraint to faba bean (*Vicia faba*L.) production introduced recently into the Sudan and widely distributed and became a national problem. The study showed that in laboratory and pot experiments, bacterial strains and isolates have the potential to reduce *O. crenata* infestation and mitigate at least in part its negative effects on growth of faba bean [11]. The present study revealed that germination of *O. crenata* increased with increasing GR24 concentration. Germination of *O. crenata* decreased significantly after inoculation with bacterial cultures or filtrates of ISO43 and ISO44 isolates and *B. circulans***,** BMP, BMP+TAL1399 strains as compared to other microbes and controls. Hassan and Abakeer [9] in similar study reported that the combinations of BMP plus TAL1399 and BMP plus USDA2478 inhibited *Orobanche* germination. Gafar *et al*. [12] reported that isolate ISO20 (*Gluconacetoacter* spp.) had the potential to inhibit *Striga* germination and radical elongation. The decline in germination of *O. crenata* with the bacteria could be due to a possible phytotoxic effect on the embryo thus leading to reduction of its outwards thrust on the surrounding tissues. With respect to the effects of bacterial cultures or filtrates on haustorium initiation, results

displayed that *Bacillus circulans*, BMP, BMP+USDA2478 and isolate ISO 44 suppressed *O. crenata* haustoria factor significantly as compared to the corresponding control. Barghouthi and Salman [13] reported that bacteria may control weeds by interrupting signals required for radical elongation, haustorium formation, rhizotropism or attachment.

In the greenhouse experiment, results displayed that seed bank size influenced parasitism and growth parameters of both the host and the parasite. Infestation of *O. crenata* to faba bean, invariably, increased with increasing size of the seed bank. In among the two faba bean cultivars tested, basabeer showed the highest *O. crenata* emergence as compared to Selaim. *O. crenata*, irrespective of the seed bank size and cultivars reduced plant height and dry matter. These findings are consistent with those obtained by Mesa-Garcia and Garcia-Torres [14] who reported that *O. crenata* infection resulted in significant decrease in faba bean. The present study showed that bacteria have the potential to reduce *O. crenata* parasitism and damage to faba bean growth. This result is ongoing with Esra *et al*. [15] who reported that inoculation cowpea with *Rhizobium leguminosarum* delayed and repressed *Striga* emergence. Moreover, faba bean inoculated with bacterial BMP+TAL1399 and BMP+USDA2478, irrespective to cultivars and *O. crenata* seed bank size, displayed better growth than un-inoculated crop. Basabeer cultivar, irrespective to bacterial inoculation or *O. crenata* seed bank size displayed better growth than Selaim cultivar. Ahonsi *et al.* [16] reported that co-inoculation of legumes with ethylene-producing pseudomonads and N_2-fixing bradyrhizobial strains as supplements to legume rotation, reduced *Striga* infestation in maize The reductions in shoot and root biomass are consistent with those previously reported by Frost *et al.* [17] and could be attributed to a multitude of factors related to dry matter production and partitioning including siphoning of nutrients, water and photosynthate by the parasite. It is known that rhizosphere bacteria are capable of producing compounds, which if taken up by plants, can stimulate defense responses against deleterious pathogens [18]. Musyoka [19] showed that inoculating cowpeas with *Rhizobium* in soils supplied with P has the potential to increase soil available N. Improving soil fertility as reported by Jain and Foy [20] appears to decrease *Orobanche* infestation and its enhancement on host growth.

Conclusion:

The results clearly showed the adverse effects of *O. crenata* on its host and the need for an integrated approach for *O. crenata* management. However, these results need to be verified in field experiments and the cost effectiveness of the treatments needs to be considered.

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