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Role of bacteria, nitrogen, phosphorus and the herbicide imazethapyr on inhibiting *Orobanche crenata* Forsk infestation in *Vicia faba* L.

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Abstract: *Series of laboratory and greenhouse experiments were conducted to study the effects of bacteria, nitrogen + phosphorus and the herbicide imazethapyr, on O. crenata in faba bean. Laboratory experiments results showed that all treatments significantly inhibited O. crenata germination and haustorium initiation. The combinations of BMP+TAL1399 and BMP+USDA2478 with fertilizers dose (1N+1PμM) reduced germination in a range of 83.16-86.25%. The combinations of BMP+USDA2478 or BMP+TAL1399 with imazethapyr (100%) reduced haustoria in a range of 79.2-82.6%. In the two greenhouse experiments, all treatments delayed and significantly decreased O. crenata incidence. In the first experiment, combinations of strains BMP+USDA2478 with different fertilizers doses gave the highest reduction of O. crenata emergence as compared to the control. ISO44 alone or in combination with 1N+1P and BMP+TAL1399 gave the highest plant height as compared to the control. Inoculations with bacterial isolates ISO43 and ISO44 each alone significantly increased faba bean shoot and root dry weights. In the second experiment, ISO44 and the combination of BMP+USDA2478 with imazethapyr (0.21g/pot) reduced O. crenata emergence as compared to imazethapyr and the control. Application of imazethapyr (0.21g/pot) alone or in combination with ISO44 significantly increased faba bean plant height and root dry weight. Inoculation with BMP+USDA2478 sustained the highest shoot dry weight.*

Keywords: *Broomrape, biofertilizer, chemical fertilizer, faba bean, pursuit*

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I. Introduction

Orobanche infestation and its parasitism on host plants are generally more localized in low fertility soils. Application of nitrogen fertilizers during conditioning and germinating phases has been reported to reduce the germination, radical length and weed proliferation [1]. Phosphorus is a key element required for the growth of plants [2]. It is well known that major part of phosphorus in soil is usually present in the forms which are unavailable to plant [3]. Phosphorus precipitated to form insoluble salts such as iron and aluminum oxides or hydroxides in acidic soils and insoluble calcium salts in alkaline soils [4]. Use of phosphorus solubilizing bacteria as inoculants increases P uptake [5], because they produce organic and inorganic acids [6], secret acid and alkaline phosphatase [7] for solubilization of insoluble inorganic and organic phosphorus forms. Under phosphorus limiting conditions plants up-regulate the secretion of strigolactones in the rhizosphere to minimize excessive shoot branching [8]. At the same time, strigolactones act as seed germination stimulants for parasitic weeds such as *Striga*, *Orobanche* and *Phelipanche* [9]. Addition of phosphorus to deficient soil has been reported to reduce *O. crenata* infestation on clover [10]. There are differences between plant species in strigolactones exudation under P- and N-deficient conditions, which may possibly be related to differences between legumes and non-legumes. One of the important plant strategies for acquiring N and particularly P is to form relationships with microorganisms. The application of soil borne microorganisms can play a remarkable role in controlling parasitic weeds. Rugheim *et al*. [11] reported that bacterial isolates and strains inhibited *Phelipanche ramosa* seeds germination. Faba bean inoculated with mycorrhiza fungi (AM) alone or in

combination with *Rhizobium leguminosarum*, *Azospirillum brasilense*, and *Bacillus megatherium* var. *phosphaticum* completely inhibited *Orobanche* emergence [12].

Chemical strategies have been used to control broomrape either directly or indirectly. Measures such as soil fumigation, germination stimulants, and certain pre-plant or pre-emergence herbicides act directly on broomrape. Indirect control is aimed to suppressing growth of the parasite after attachment and penetration of the host roots. For this purpose foliage applied herbicides may be useful [13]. Several attempts and intensive research have been made in different countries to screen potential herbicides against *Orobanche* spp. In general, the most limiting factor in the use of promising herbicides is their degree of selectivity among the crops at the required rate for the parasite control, and the critical time of application, especially for foliar applied systematic herbicides. Glyphosate, was the first promising herbicide developed for *O. crenata* control in faba bean [14], and is still the most important herbicide in use. Iamzethapyr (R.S) -5-ethyl-2-(4-is opropyl-4-methy-5-oxo-2-imidazolin-2-yl) nicotinic acid, an imidazolinone (Pursuit), is selective systemic herbicide, it acts by inhibiting the enzyme acetohydroxy acid synthase (AHAS), also called acetolactate synthase (ALS) [15], which is key enzyme in the biosynthesis of the branched chain amino acids isoleucine, lecucin and valine in plant. It controls a wide spectrum of grass and broadleaved weeds, effective at low application rates, and has low mammalian toxicity [16]. Punia [17] reported that imazethapyr and imazapyr, effectively and selectively control broomrape on faba bean. This study was conducted to investigate the effect of soil borne bacterial isolates and strains, chemical fertilizers and imazethapyr (Pursuit**)** on *Orobanche crenata* on faba bean.

II. Materials And Methods

1.1, Laboratory experiments

1.1.1, *O. crenata* seeds collection: *O. crenata* seeds, used in this study, were collected from parasitic plants growing with faba bean at Shendi Research Station Farm, River Nile State, Sudan during the winter season, 2006.

1.1.2, Isolation of bacteria: Forty eight bacterial isolates were isolated from soil of faba bean farms (infested and non-infested by *Orobanche*) in 2012. Isolates were sub-cultured in starch ammonium agar (SAA): (10g Starch, 2g ammonium sulphate, 1g dipotassium hydrogen phosphate, 1g $MgSO_4$-$7H_2O$, 3g NaCl and 20g Agar) then incubated for 7 days at 30^o C, then kept on SAA slant medium at 4^oC for further studies.

1.1.3, *Rhizobium* and *Bacillus* strains inoculum: A suspension of *Rhizobium leguminosarum* bv. *viceae* strains (TAL1399 and USDA2478) and *Bacillus megatherium* var. *phosphaticum* strain (BMP)were supplied by the Biological Nitrogen Fixation Laboratory, Department of Biopesticides and Biofertilizers, Environment, Natural Resources and Desertification Research Institute, the National Centre for Research, Khartoum, Sudan.

Nutrient Broth Medium (NB) and Yeast Extract Mannitol Broth Medium (YEMB) were used for the growth of bacterial isolates and strains, respectively.

1.1.4, *O. crenata seeds germination*

1.1.4.1, Effects of bacteria and chemical fertilizers on *O. crenata* germination: *O. crenata* seeds placed on glass fiber filter discs in Petri dishes were moistened with 5 ml of bacterial culture broth (ISO 43, ISO 44, BMP+TAL1399 and BMP+USDA2478) and/or nitrogen (1 and 0.5µM) + phosphorus (1 and 0.5 µM) solutions (1N+1P and ½N+½P). The Petri dishes were incubated for 11 days at 18°C and wrapped in black polythene. *O. crenata* seeds conditioned in water or broth medium were used as control. The seeds were subsequently treated with GR24 at 5 and 10ppm, re-incubated and examined for germination 7 days later as previously described by Hassan *et al*. [18].

1.1.4.2, Effects of bacteria and imazethapyr on *O. crenata* germination: *O. crenata* seeds, placed on glass fiber filter discs in Petri dishes, were moistened with 5 ml of the herbicide solution (1, 0.5 and 0.25µM). Dishes, wrapped in black polythene, were incubated for 11 days, subsequently treated with GR24, re-incubated and examined for germination as described above.

1.1.5, *O. crenata haustorium initiation*

1.1.5.1, Effects of bacteria and chemical fertilizers on *O. crenata* haustorial initiation: *O. crenata* seeds were conditioned in water, broth media, bacteria and N+P as described above. Then seeds were treated with GR24 at 10ppm, re-incubated and examined for germination 7 days later as previously described. The seeds were subsequently treated with DMBQ at 10 and 20µM, re-incubated and examined for haustorium 5 days later as previously described by Hassan *et al*. [15].

1.1.5.2, Effects of bacteria and imazethapyr on *O. crenata* haustorial initiation: *O. crenata* seeds were conditioned in water, broth media, bacteria and the herbicide as described above. Petri dishes were incubated and subsequently treated with GR24 and DMBQ, examined for haustoria as described above.

1.2, Greenhouse experiments

1.2.1, General: Two pot experiments were conducted at the greenhouse, Faculty of Agriculture, Omdurman Islamic University, during December, 2015 to March, 2016, to study the effects of i) bacterial strains and isolates and fertilizers (nitrogen and phosphorus) ii) bacterial strains and isolates and imazethapyr on *O. crenata*

in faba bean. A soil mixture was made of sandy-loam soil and sand (1:1 v/v), then 7 kg were placed in pots (19 cm diameter). *O. crenata* infested and un-infested controls were included for comparison. *O. crenata* infestation was accomplished by mixing 8 mg of sterilized broomrape seeds in the top 6 cm soil in each pot. Four faba bean seeds were sown per pot. Numbers of *O. crenata* shoots emerged per pot were recorded at 8, 9, 10, 11 and 12 weeks after sowing (WAS). Faba bean plant height was measured at 4, 6, 8, 9, 10, 11 and 12 WAS. Dry weight of faba bean shoots and roots were recorded at the end of the experiment (15 WAS). Treatments were arranged in randomized complete block design with four replicates.

1.2.2, The first experiment: Effects of bacteria and fertilizers on *O. crenata* in faba bean: This experiment was conducted to evaluate the effects of the bacterial strains BMP+TAL1399 and BMP+USDA2478, bacterial isolates ISO43 and ISO44 alone or in combinations with nitrogen as urea + phosphorus as triple super phosphate (1N+1P: 0.285+0.369 and ½N+½P: 0.142+0.184 g/pot, respectively).

1.2.3, The second experiment: Effects of bacteria and imazethapyr on *O. crenata* in faba bean: This experiment was conducted to evaluate the effects of the bacterial strains BMP+TAL1399 and BPM+USDA2478, bacterial isolates ISO43 and ISO44 alone or in combinations with the herbicide imazethapyr (0.21g/pot).

1.3, Statistical analysis: Prior to analyses, data on percentage (germination and haustorium) were arcsine transformed, data on *O. crenata* emergence were square root transformed to fulfill ANOVA requirements. The analyses were performed across experiments using Microsoft office excel 2010 software. Means separations were made by the LSD at 5% [19].

III. Results

1.1, Laboratory experiments

1.1.1, Effects of bacteria and chemical fertilizers on *O. crenata* germination: Generally, nitrogen+phosphors applied at different doses (½N+½P) and (1N+1P) alone or in combination with bacteria significantly (P≤0.5) inhibited *O. crenata* seeds germination in response to GR24 at 5 and 10ppm (Table 1). The combinations of 1N+1P with BMP+TAL1399 and BMP+USDA2478 inhibited germination by (86.25 – 83.16%) and (83.97 – 83.86) in response to GR24 at 5 and 10ppm respectively, compared to YEMB medium control.

Table 1 Effects of bacteria and chemical fertilizers on *O. crenata* germination in response to GR24 (during conditioning)

Treatments	Fertilizer	Germination (%) GR24 (ppm) 5	Germination (%) GR24 (ppm) 10	Means
Distilled water		62.33* (78.55)**	66.18 (82.03)	64.25
Medium (NB)	**0N+0P**	53.45 (60.92)	55.69 (64.77)	54.57
Medium (YEMB)		51.34 (63.93)	53.83 (67.35)	52.58
Control	**½N+½P**	32.55(29.21)	38.08 (38.18)	35.31
	1N+1P	28.64 (23.22)	30.55 (26.17)	29.59
ISO 43	**0N+0P**	28.46 (22.73)	33.75 (31.33)	31.10
	½N+½P	35.93 (34.80)	36.20 (35.02)	36.06
	1N+1P	25.05 (18.24)	23.27 (15.81)	28.66
ISO 44	**0N+0P**	34.86 (32.78)	37.35 (36.96)	36.10
	½N+½P	30.52 (25.98)	30.74 (26.46)	30.63
	1N+1P	19.03 (10.88)	22.07 (14.69)	20.55
BMP+USDA2478	**0N+0P**	26.89 (20.68)	31.09 (27.19)	28.99
	½N+½P	27.61 (21.72)	29.56 (24.80)	28.58
	1N+1P	18.45 (10.25)	19.19 (10.87)	18.82
BMP+TAL1399	**0N+0P**	37.46 (37.07)	39.23 (40.05)	38.34
	½N+½P	24.10 (16.94)	22.08 (14.18)	23.09
	1N+1P	17.14 (8.79)	19.30 (11.34)	18.22
LSD (Bacteria)	**4.09**	**LSD (Bacteria*N+P)**		**5.36**
LSD (N+P)	**2.68**	**LSD (Interaction)**		**10.02**

* For the analysis, data were arcsine transformed, **Data between brackets were original data, YEMB**:** Yeast Extract Mannitol Broth, NB**:** Nutrient Broth

1.1.2, Effects of bacteria and imazethapyr on *O. crenata* germination: All treatments significantly (P≤0.5) inhibited germination in response to GR24 at 5 and 10ppm, as compared to corresponding control (Table 2). Combination of the highest concentration of herbicide (100%) with ISO 43 gave the highest reduction of germination in response to GR24 at 5 and 10ppm by 78.9–79.1% respectively, compared to corresponding control. The combination of ISO 44 with the 50% imazethapyr concentration significantly (P≤0.5) inhibited germination in response to GR24 at 10ppm by 75.7%.

Table 2 Effects of bacteria and the herbicide imazethapyr on *O. crenata* germination in response to GR24

Treatments	Imazethapyr (µM)	Germination (%) GR24 conc. 5	Germination (%) GR24 conc. 10	Means
Distilled water	**0**	49.11* (57.08)**	55.43 (66.31)	52.57
Medium (NB)	**0**	58.85 (72.50)	66.88 (83.50)	62.86
Medium (YEMB)	**0**	53.79 (64.98)	66.47 (84.08)	60.13
Control	**25**	43.93 (48.14)	44.90 (49.94)	44.41
	50	31.82 (28.07)	35.69 (34.33)	33.59
	100	27.44 (21.35)	28.57 (23.44)	28.00
ISO 43	**0**	35.11 (33.19)	38.45 (39.32)	36.78
	25	31.54 (27.68)	35.64 (34.30)	33.59
	50	26.13 (19.85)	26.69 (20.30)	26.41
	100	22.83 (15.32)	24.12 (17.45)	23.47
ISO 44	**0**	32.79 (29.37)	37.64 (37.42)	35.21
	25	36.69 (36.09)	39.66 (40.96)	38.17
	50	29.90 (25.17)	32.62 (30.94)	31.26
	100	23.52 (16.08)	25.39 (18.96)	24.45
BMP+USDA2478	**0**	39.34 (40.22)	38.33 (38. 80)	38.83
	25	36.67 (35.70)	38.55 (38.89)	37.61
	50	26.67 (20.30)	29.64 (25.82)	28.15
	100	21.30 (13.37)	25.82 (19.67)	23.56
BMP+TAL1399	**0**	35.53 (34.59)	36.48 (35.39)	36.00
	25	40.91 (42.98)	36.58 (35.72)	38.74
	50	29.55 (24.47)	31.25 (27.07)	30.40
	100	23.94 (16.92)	26.86 (21.22)	25.40
LSD (Bacteria)	**3.27**		**LSD (Bact.* imaz.)**	**6.53**
LSD (imazethapyr)	**2.47**		**LSD (Interaction)**	**9.24**

* For the analysis, data were arcsine transformed, **Data between brackets were original data, YEMB**:** Yeast Extract Mannitol Broth, NB**:** Nutrient Broth

1.1.3, Effects of bacteria and chemical fertilizers on *O. crenata* haustorium: All treatments significantly (P≤0.5) inhibited *O. crenata* haustorium initiation in response to DMBQ at 10 and 20µM (Table 3). However, the combination of bacterial strains BMP+USDA2478 with 1N+1P significantly (P≤0.5) inhibited haustorium initiation of *O. crenata* by 79.45–74.92%, followed by the combination of ISO 43 with 1N+1P by 77.44–78.22% in response to DMBQ at 10 and 20 µM, respectively compared to the corresponding control.

Table 3 Effects of bacteria and chemical fertilizers on *O. crenata* haustorium in response to DMBQ

Treatments	Fertilizer	Haustorium (%) DMBQ conc. (µM) 10	Haustorium (%) DMBQ conc. (µM) 20	Means
Distilled water		69.89*(88.09)**	71.41 (89.59)	70.65
Medium (NB)	**0N+0P**	66.70 (85.29)	69.63 (88.36)	68.16
Medium (YEMB)		67.71 (84.14)	70.18 (87.32)	68.94
Control	**½N+½P**	40.03 (41.58)	43.03 (46.68)	41.53
	1N+1P	29.34 (24.30)	37.62 (37.56)	33.48
ISO 43	**0N+0P**	39.92 (41.18)	42.82 (46.22)	41.37
	½N+½P	34.06 (31.50)	36.71 (35.86)	35.38
	1N+1P	25. 92 (19.24)	25 92 (19.24)	25.92
ISO 44	**0N+0P**	38.70 (39.14)	42.23 (45.17)	40.46
	½N+½P	34.77 (32.57)	36.26 (35.18)	35.51
	1N+1P	29.04 (23.62)	29.94 (25.00)	29.49
BMP+USDA2478	**0N+0P**	35.26 (33.54)	38.41 (38.90)	36.83
	½N+½P	32.58 (29.16)	33.09 (29.83)	32.83
	1N+1P	24.48 (17.29)	27.65 (21.90)	26.06
BMP+TAL1399	**0N+0P**	37.01 (36.18)	43.55 (47.49)	40.28
	½N+½P	33.04 (29.77)	35.35 (33.52)	34.19
	1N+1P	26.80 (20.41)	27.80 (22.08)	27.30
LSD (Bacteria)	**3.63**		**LSD (Bacteria*N+P)**	**4.76**
LSD (N+P)	**2.38**		**LSD (Interaction)**	**8.90**

* For the analysis, data were arcsine transformed, **Data between brackets were original data, YEMB**:** Yeast Extract Mannitol Broth, NB**:** Nutrient Broth

1.1.4, Effects of bacteria and imazethapyr on *O. crenata* haustorium initiation: Table 4 shows that all treatments significantly (P≤0.5) inhibited haustorium initiation in response to DMBQ at 10 and 20µM, as compared to the corresponding control. Combination of the high concentration of herbicide (100%) with bacterial strains and isolates BMP+USDA2478 and BMP+TAL1399, gave the highest reduction of haustoria in response to DMBQ at 10 and 20µM by 79.2–82.6% and 79.8–82.1%, respectively, compared to corresponding

control. However, the 100% herbicide concentration alone in response to DMBQ at 10 and 20µM, inhibited haustorium initiation by 78.8-73.2% as compared to untreated control.

Table 4 Effects of bacteria and the herbicide imazethapyr on *O. crenata* haustorium in response to DMBQ

Treatments	Imazethapyr (µM)	Haustorium (%)		Means
		DMBQ conc.		
		10	20	
Distilled water	**0**	64.22* (81.02)**	66.00 (83.36)	65.11
Medium (NB)	**0**	62.11 (78.03)	63.56 (80.02)	62.83
Medium (YEMB)	**0**	54.25 (65.59)	62.30 (78.17)	58.27
Control	**25**	42.46 (45.65)	47.31 (54.01)	44.88
	50	32.51 (29.19)	32.53 (28.94)	32.52
	100	24.39 (17.20)	28.18 (22.38)	26.28
ISO 43	**0**	29.12 (23.98)	37.90 (37.78)	33.51
	25	32.06 (28.60)	31.73 (27.72)	31.89
	50	28.96 (23.61)	29.76 (25.31)	29.36
	100	24.55 (17.30)	25.74 (19.10)	25.14
ISO 44	**0**	38.82 (39.58)	38.61 (52.22)	38.71
	25	35.39 (33.67)	38.63 (39.09)	37.01
	50	27.34 (21.15)	32.83(29.96)	30.08
	100	22.97 (15.08)	26.08 (19.40)	24.52
BMP+USDA2478	**0**	38.78 (39.46)	38.84 (39.46)	38.81
	25	34.69 (32.43)	33.37 (30.37)	34.03
	50	26.92 (20.54)	27.97 (22.00)	27.44
	100	21.40 (13.61)	21.42 (13.90)	21.41
BMP+TAL1399	**0**	34.33 (32.30)	34.60 (33.01)	34.46
	25	31.69 (27.65)	34.54 (32.17)	33.11
	50	24.99 (17.90)	28.61 (23.03)	26.80
	100	21.14 (13.23)	21.94 (14.29)	21.54
LSD (Bacteria)	**2.99**		**LSD (Bact.* imaz.)**	**5.98**
LSD (imazethapyr)	**2.26**		**LSD (Interaction)**	**8.46**

* For the analysis, data were arcsine transformed, **Data between brackets were original data, YEMB**:** Yeast Extract Mannitol Broth, NB**:** Nutrient Broth

1.2, Green house experiments

1.2.1, Experiment 1

1.2.1.1, Effects of bacteria and chemical fertilizers on *O. crenata* emergence: All treatments delayed and decreased *O. crenata* incidence significantly (P≤0.5) at all observation periods, compared to the control (Table 5). At 9 weeks after sowing (WAS), the combination of strains BMP+USDA2478 with 1N+1P gave the highest reduction of *O. crenata* emergence, followed by the combinations of BMP+USDA2478with ½N+½P, BMP+TAL1399 with ½N+½P and isolate ISO 43with ½N+½P. At 10, 11 and 12 WAS, the highest reductions of *O. crenata* emergence were obtained by the combination of BMP+USDA2478 with 1N+1P followed by 1N+1P alone and the combination of BMP+TAL1399 with ½N+½P.

Table 5 Effects of bacteria and chemical fertilizers on number of *O. crenata* (plant/pot)

Treatments		*O. crenata* count					
Treatments	Fertilizers	Weeks after sowing					Means
		8	9	10	11	12	
Control	**0N+0P**	1.54*(2.25)**	2.53(6.00)	2.47(6.00)	2.99(8.75)	3.31(11.00)	2.57
	½N+½P	0.97(0.50)	1.51(2.00)	1.54(2.25)	2.43(5.50)	2.43(5.50)	1.77
	1N+1P	0.71(0.00)	1.40(1.50)	1.31(1.25)	1.92(3.25)	2.04(3.75)	1.48
ISO43	**0N+0P**	0.71(0.00)	1.64(2.25)	1.97(3.75)	2.32(5.25)	2.36(5.25)	1.80
	½N+½P	0.71(0.00)	1.18(1.00)	1.56(2.00)	2.21(4.50)	2.38(5.25)	1.61
	1N+1P	1.41(2.00)	1.89(4.00)	1.76(3.75)	2.45(6.25)	2.21(5.25)	1.95
ISO44	**0N+0P**	0.71(0.00)	1.40(1.50)	1.40(1.50)	2.09(4.00)	1.99(3.50)	1.52
	½N+½P	0.71(0.00)	1.54(2.25)	1.92(3.25)	2.47(5.75)	2.44(5.50)	1.82
	1N+1P	0.97(0.50)	1.31(1.25)	1.87(3.25)	2.53(6.00)	2.28(4.75)	1.79
BMP+USDA2478	**0N+0P**	0.71(0.00)	1.40(1.50)	1.48(1.75)	1.98(3.50)	2.11(4.00)	1.54
	½N+½P	0.71(0.00)	1.22(1.00)	1.27(1.25)	2.03(3.75)	2.04(3.75)	1.45
	1N+1P	0.71(0.00)	1.10(0.75)	1.10(0.75)	1.92(3.25)	2.17(4.25)	1.40
BMP+TAL1399	**0N+0P**	0.71(0.00)	2.24(5.25)	1.86(3.75)	2.47(6.00)	2.47(6.00)	1.95
	½N+½P	0.71(0.00)	1.14(1.00)	1.70(2.75)	2.38(5.50)	2.52(6.25)	1.69
	1N+1P	0.84(0.25)	1.25(1.50)	1.85(3.75)	2.35(5.50)	2.43(5.75)	1.74
LSD		**0.49**	**0.86**	**1.06**	**0.90**	**0.86**	

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

1.2.1.2, Effect of bacteria and chemical fertilizers on faba bean plant height: Results presented in table (6) show that at 4 and 5 WAS, application of ISO 44 significantly (P≤0.5) increased faba bean plant height, followed by BMP+TAL1399 compared to the infested control. At 8 WAS, all treatments significantly (P≤0.5) increased faba bean plant height, the highest plant height was obtained by ISO 43. At 9 and 10 WAS, application of ISO 44 with 1N+1P followed by BMP+TAL1399 and ISO 44 significantly (P≤0.5) increased faba bean plant height compared to the infested control. At 11 WAS, ISO 43 and ISO 44 gave a significant (P≤0.5) increment in plant height. At 12 WAS, the only significant (P≤0.5) plant height increase was obtained by the combination of BMP+TAL1399 compared to the infested control. The overall mean showed the best results on plant height were obtained by ISO 44 alone or in combination with 1N+1P and BMP+TAL1399.

Table 6 Effect of bacteria and chemical fertilizers on faba bean plant height (cm)

Treatments	Fertilizers	Plant height (cm.) Weeks after sowing (WAS)							Means
		4	6	8	9	10	11	12	
Control without *O. crenata*	**0N+0P**	19.83	35.25	45.08	48.75	53.08	55.91	55.75	44.81
Control	**0N+0P**	18.08	32.83	38.00	40.58	40.58	40.66	40.41	35.88
	½N+½P	18.49	34.33	42.41	44.33	43.91	41.83	41.66	38.14
	1N+1P	17.91	29.16	42.58	45.87	46.70	44.33	41.75	38.33
ISO 43	**0N+0P**	18.58	35.25	46.16	46.00	44.66	45.33	41.49	38.33
	½N+½P	16.50	31.50	44.08	43.20	44.16	42.08	40.37	37.41
	1N+1P	19.25	33.75	41.08	41.25	41.41	38.58	39.58	36.41
ISO 44	**0N+0P**	22.04	37.50	46.41	46.16	46.66	44.41	42.25	40.78
	½N+½P	19.00	34.08	42.91	42.66	43.75	41.12	42.25	37.97
	1N+1P	21.41	36.41	44.91	47.25	47.66	44.16	43.25	40.72
BMP+USDA2478	**0N+0P**	18.33	31.33	40.16	43.58	46.91	44.25	38.00	37.51
	½N+½P	16.75	31.50	40.83	42.91	43.50	39.91	39.16	36.37
	1N+1P	16.74	30.50	45.75	43.91	44.50	41.83	41.91	37.88
BMP+TAL1399	**0N+0P**	21.83	37.08	42.75	46.16	47.25	44.25	44.25	40.51
	½N+½P	16.04	32.33	42.75	43.79	45.04	42.00	41.20	37.59
	1N+1P	17.66	34.50	42.33	41.66	43.20	44.29	41.37	37.86
LSD		**3.08**	**3.24**	**3.59**	**3.96**	**3.71**	**3.68**	**2.99**	

1.2.1.3, Effects of bacteria and chemical fertilizers on faba bean dry weight: Inoculation with strains BMP+TAL1399 in combination with ½N+½P insignificantly increased root dry weight by 25% compared to the infested control (Table 7). All treatments significantly (P≤0.5) increased shoot dry weight except the combination of ISO 43 with both doses of fertilizers and the combinations of ½N+½P with ISO 44 and BMP+USDA2478. The highest shoot dry weight was obtained by ISO 43 and ISO 44.

Table 7 Effects of bacteria and chemical fertilizers on faba bean dry weight (g) at 15 WAS

Treatments	Fertilizers	Dry weight (g)		Average biomass
		Root	Shoot	
Control without *O. crenata*	**0N+0P**	3.29	20.97	12.13
Control	**0N+0P**	2.22	6.95	4.59
	½N+½P	1.30	8.32	4.81
	1N+1P	1.43	10.77	6.10
ISO 43	**0N+0P**	1.70	14.95	8.33
	½N+½P	1.58	8.32	4.95
	1N+1P	1.42	8.75	5.09
ISO 44	**0N+0P**	1.39	14.52	7.96
	½N+½P	1.42	9.30	5.36
	1N+1P	1.70	12.55	7.13
BMP+USDA2478	**0N+0P**	1.05	12.60	6.83
	½N+½P	1.13	9.72	5.43
	1N+1P	1.62	12.70	7.16
BMP+TAL1399	**0N+0P**	2.40	10.92	6.66
	½N+½P	2.77	11.60	7.19
	1N+1P	1.58	11.02	6.30
LSD		**0.67**	**3.46**	

1.2.2, Experiment 2

1.2.2.1, Effects of bacteria and the herbicide imazethapyr on *O. crenata* emergence: All treatments significantly (P≤0.5) decreased the number of *O. crenata* compared to infested control (Table 8). At 9 WAS, the highest emergence reduction was obtained by ISO 44 and BMP+USDA2478 in the absence of herbicide and by ISO 43 and BMP+TAL1399 in the presence of herbicide. At 10 WAS, the highest emergence reduction was

obtained by ISO 44 in the absence of the herbicide and by BMP+USDA2478 and BMP+TAL1399 in the presence of the herbicide. At 11 WAS, the highest emergence reduction was obtained by ISO 44 in the absence of herbicide. At 12 WAS, the highest emergence reduction was obtained by ISO 44, BMP+USDA2478 and BMP+TAL1399 in the presence of herbicide.

Table 8 effect of bacteria and chemical herbicide (imazethapyr) on number of *O. crenata* (plant/pot)

Treatments		***O. crenata* count**					
Treatments	**Imazethapyr**	**Weeks after sowing**					**Means**
		8	**9**	**10**	**11**	**12**	
Control	**0**	0.84*(0.25)**	2.34(5.00)	2.34(5.00)	2.53(6.00)	2.71(7.00)	2.15
	1	0.84(0.25)	1.41(2.00)	1.52(2.50)	1.69(3.50)	1.82(3.75)	1.46
ISO43	**0**	0.84(0.25)	1.27(1.25)	1.48(2.00)	1.73(2.75)	1.93(3.50)	1.45
	1	0.71(0.00)	1.06(0.75)	1.35(1.50)	1.70(2.50)	1.92(3.25)	1.35
ISO44	**0**	0.71(0.00)	1.10(0.75)	1.26(1.25)	1.39(1.50)	1.90(3.25)	1.27
	1	0.71(0.00)	1.26(1.25)	1.39(1.50)	1.63(2.25)	1.76(2.75)	1.35
BMP+USDA2478	**0**	0.71(0.00)	1.10(0.75)	1.32(1.50)	1.59(2.25)	2.01(3.75)	1.34
	1	0.71(0.00)	1.13(1.00)	1.26(1.25)	1.48(1.75)	1.80(2.75)	1.27
BMP+TAL1399	**0**	1.19(1.25)	1.48(2.00)	1.54(2.25)	1.92(3.25)	1.99(3.50)	1.62
	1	0.71(0.00)	1.06(0.75)	1.27(1.25)	1.64(2.25)	1.79(2.75)	1.29
LSD		**0.41**	**0.76**	**0.91**	**0.87**	**0.85**	

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

1.2.2.2, Effects of bacteria and the herbicide imazethapyr on faba bean plant height: Application of the herbicide, BMP+TAL1399 and the combination of ISO 44 with herbicide significantly (P≤0.5) increased faba bean plant height in all observation intervals except 8 WAS compared to infested control (Table 9). At 8 WAS, the significant (P≤0.5) increment of plant height was obtained by ISO 43.

Table 9 Effects of bacteria and the herbicide imazethapyr on faba bean plant height (cm)

Treatments	**Imazethapyr**	**Plant height (cm.)**							
		Weeks after sowing							
		4	**6**	**8**	**9**	**10**	**11**	**12**	**Means**
Control without *O. crenata*	**0**	20.66	36.83	42.41	46.00	50.66	51.08	52.04	42.81
Control	**0**	17.75	31.58	40.08	42.58	43.17	41.83	40.83	36.83
	1	23.91	39.08	44.33	51.08	50.66	46.75	46.83	43.23
ISO 43	**0**	20.37	35.33	48.41	41.08	42.16	40.50	40.87	38.39
	1	15.87	28.37	35.95	41.45	42.91	40.50	40.62	35.10
ISO 44	**0**	16.24	29.08	37.08	40.50	41.41	37.37	37.75	34.40
	1	21.41	36.33	45.74	49.25	50.54	46.58	45.83	42.24
BMP+USDA2478	**0**	20.25	34.66	40.41	43.25	42.16	41.75	40.16	37.52
	1	17.66	26.67	35.66	38.74	36.08	36.79	36.75	32.62
BMP+TAL1399	**0**	20.58	36.41	43.25	47.91	47.66	46.25	44.41	40.92
	1	14.66	27.50	32.17	37.16	37.83	35.83	34.33	31.35
LSD		**2.76**	**3.28**	**5.99**	**4.61**	**4.25**	**3.17**	**3.57**	

1.2.2.3, Effects of bacteria and the herbicide imazethapyr on faba bean dry weight: Application of the herbicide alone or in combination with ISO 43 and ISO 44 significantly (P≤0.5) increased root dry weight compared to infested control (Table 10). Strains USDA2478+BMP followed by the combination of herbicide with ISO 44 significantly (P≤0.5) increased shoot dry weight compared to infested control.

Table 10 Effects of bacteria and the herbicide imazethapyr on faba bean root and shoot dry weight (g)at 15 WAS

Treatments	**Imazethapyr**	**Dry weight (g)**		**Average biomass**
		Root	**Shoot**	
Control without *O. crenata*	**0**	3.65	19.50	11.58
Control	**0**	1.00	8.60	4.80
	1	1.92	12.32	7.12
ISO 43	**0**	1.07	8.77	4.92
	1	1.92	7.17	4.55
ISO 44	**0**	1.52	9.32	5.42
	1	1.77	13.37	7.57
BMP+USDA2478	**0**	1.37	14.00	7.69
	1	1.02	10.37	5.70
BMP+TAL1399	**0**	1.30	11.47	6.39
	1	0.90	11.00	5.95
LSD		**0.61**	**4.28**	

IV. Discussion

Improving soil fertility is one of the main factors to increase yields and productivity. The importance of plant growth promoting rhizobacteria (PGPR) comes from their potential role in improving soil fertility and enhancing crop yield and their nutrients content. All treatments significantly inhibited *O. crenata* germination. Furthermore, the combinations of BMP+TAL1399 and BMP+USDA2478 with the higher fertilizers dose (1N+1µM) reduced germination in the range of 83.16 - 86.25%. Similar results were obtained by Bouraoui *et al*. [20] and Mabrouk *et al*. [21] who reported that inoculation with *Rhizobium* strain significantly decrease *O. crenata* germination. *O. aegyptiaca* and *O. cernua* seeds treated with *B. subtilis*, significantly reduced germination and radicle elongation in the presence of GR24 [22]. Nitrogen as urea, ammonium nitrate and ammonium sulfate were found to be most effective in reducing *Orobanche* parasitism [1]. Indirect effects of nitrogen and phosphorus have also been described by affecting the production and exudation of germination stimulants by the host and therefore the ability of parasitic plants to germinate [23].

All treatments significantly suppressed *O. crenata* haustorium initiation. In addition, the combination of bacterial strains BMP+USDA2478 and isolate ISO 43 with the higher fertilizers dose significantly reduced haustorium initiation of *O. crenata* in the range of 74.92 – 79.45%. In a previous study, bacterial strains and isolates inhibited *O. crenata* haustorium initiation in response DMBQ [24]. This may be attributed to phytotoxic substances, inhibitors or extracellular enzymes that degrade and/or curtail release of the haustorium factor from the host root.

In the greenhouse experiment, all treatments delayed and significantly decreased *O. crenata* incidence at all observation periods, compared to control. Based on overall means, results showed that the combinations of strains BMP+USDA2478 with full and half dose of fertilizers followed by 1N+1P alone gave the highest reduction of *O. crenata* emergence, compared to control. Mabrouk *et al.* [25] reported the beneficial effect of *Rhizobium* strains in controlling *O. crenata* parasitism. Phosphate solubilizing bacteria are able to affect the solubility of low dissolvable inorganic compounds, by producing phosphatase and other biological compounds like hormones such as auxin, gibberellic acid, vitamins and organic acids [26]. Ngwene *et al*. [27] reported that N fertilization enhanced phosphorus uptake by cowpea plants since the same Strigolactone (SL) being down-regulated for *Striga* suppression. Previous studies have correlated Strigolactones production with levels of *Striga* parasitism especially in susceptible lines, and have also shown that SLs production is enhanced under P deficiency conditions while an *in vitro* P micro dosing significantly down regulated SLs production [28]. It has been demonstrated that P deficiency enhances the exudation of SLs in red clover and tomato [23, 29].

The overall mean of plant height showed that ISO 44 alone or in combination with 1N+1P and BMP+TAL1399 gave the highest plant height as compared to infested control. Inoculation with bacterial isolates ISO 43 and ISO 44 each alone significantly increased shoot dry weight and total biomass as compared to the infested control. The increment effects of the bacterial strains on faba bean height could be attributed to a direct effect of the bacteria on releasing minerals to the plants or indirectly through production of chemical (s) that inhibited infestation. Karasu *et al.* [30] observed that inoculation of chickpea seeds with *R. ciceri* isolate had a significant effect on plant growth.

Combinations of the imazethapyr (100%) with ISO 43 and herbicide (50%) with ISO 44 reduced germination by 79% and 76%, respectively. Furthermore, the combinations of imazethapyr (100%) with BMP+USDA2478 or BMP+TAL1399 reduced haustoria in the range of 79.2-82.6%. Kleifeld *et al.* [31] reported that application of imazethapyr was effective in inhibiting *Orobanche* germination and attachment when applied directly to the soil.

In the greenhouse experiment, all treatments significantly decreased the number of *O. crenata* at 9, 10, 11 and 12 WAS. In overall means, results showed that ISO 44 and the combination of BMP+USDA2478 with imazethapyr (0.21g/pot) gave the highest emergence reduction as compared to the herbicide alone and control. Many species of microorganisms degrade a variety of organic carbon substances including herbicides to derive energy and nutrients for their metabolism [32]. Furthermore, all plant growth promoting rhizobacteria (PGPR) inoculated plants caused a high number of tubercle deaths of *Orobanche* when compared with non-inoculated controls [33]. Miche *et al*. [34] reported that inoculation of the groundnuts by isolated bacteria significantly reduced *O. crenata* infestation. Herbicides as well, reduced the broomrape seeds germination. Similar results were obtained by Garcia-Torres and Lopez-Granados [35] who showed that imazaquin and chlorsulfuron applied to broad bean at 40 - 80 $g \cdot ha^{-1}$ (a.i.) and 6 $g \cdot ha^{-1}$ (a.i.), respectively, considerably reduced the number of broomrape spikes and their dry weights.

Application of herbicide imazethapyr alone or in combination with ISO 44 significantly increased faba bean plant height. Application of imazethapyr (0.21g/pot) alone or in combination with ISO 43 or ISO 44 significantly increased root dry weight. However, inoculation with USDA2478+BMP sustained the highest significant faba bean shoot dry weight followed by the combination of imazethapyr plus ISO 44. Similar results were obtained by Demissie [36] who reported that co-inoculation of *Vicia faba* with *Pseudomonas* and rhizobia increased shoots and roots dry weights in comparison to the un-inoculated plants. However, the herbicide alone

gave the best plant height than its combinations with bacterial strains and isolates. Soil persistence and ecotoxicity of imidazolinones and imazaquin were reported by Milanova and Grigorov [3]. O'Keefe *et al*. [38] reported that inhibitors of ALS adversely affect prokaryotic microbes. Martensson and Nilsson [39] reported that one of the sulfonylureas, chlorsulfuron inhibited the normal development of nitrogen-fixing *Rhizobium* nodules on alfalfa and red clover. On the other hand, Debnath *et al.* [40] reported that application of oxyflourfen increased the yield of grain and haulms crop. The increment may be due to the enhancing of soil microbial populations by the chemical.

V. Conclusion

All treatments significantly reduced *O. crenata* germination, haustorium initiation and incidence. In among all treatments, the combinations of BMP+USDA2478 with 1N+1P gave the best result. The combination of imazethapyr (100%) with BMP+USDA2478 reduced haustorium initiation as well as emergence of *O. crenata*. Application of imazethapyr and ISO 44 each alone or in combination significantly increased faba bean plant height and root dry weight. Appropriate N and P fertilizers or herbicides doses in combination with the potential microbes might be a promising and affordable strategy for combating *O. crenata*.

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