Vol. 22(5), pp. 256-263, May, 2026
DOI: 10.5897/AJAR2025.16959
Article Number: 02F8A5674545
ISSN: 1991-637X
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African Journal of Agricultural Research

Full Length Research Paper

Integrated control package for *Orobanche crenata* in faba beans

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Received 28 April, 2025; Accepted 17 June, 2025

A series of experiments were conducted to study the effects of *Rhizobium leguminosarum*, *Bacillus megatherium* var. *phosphaticum*, *Trichoderma harzianum*, and the herbicide imimazethapyr on *Orobanche crenata* germination, incidence and faba bean plant growth and yield in Sudan. According to the study, *T. harzianum* + BMP + TAL1399 + imazethapyr together significantly slowed down germination and postponed *O. crenata* emergence and promoted plant growth. In field experiments, treating faba bean cultivars BB7 and MISR 3 with *T. harzianum* + imazethapyr + BMP + TAL1399 inhibited *O. crenata* emergence and boosted production. This study suggests the use of faba bean cultivar Giza in the Nile River State and MISR 3 in the Northern State with the combination of the microorganisms and the herbicide. Additionally, further research is recommended to look into alternative options for chemical herbicides.

Key words: *Bacillus*, faba bean, imazethapyr, *Orobanche*, *Rhizobium*, *Trichoderma*.

INTRODUCTION

Food legumes are very important for human nutrition and are produced mainly from grain (Mabrouk et al., 2010). Moreover, legume crops are important sources of income for farmers and play an important role in improving soil fertility and increasing the productivity of subsequent crops. Legumes have been planted in northern Sudan since ancient times along the fertile strip of the alluvial soils of the Nile valley extending north on both banks of the Nile, from Khartoum to Wadi Halfa on the Egyptian border. The total area of leguminous crops (Faba bean, Lentil and Chickpea) in Sudan is approximately 80 thousand hectares. Faba bean (*Vicia faba*) is the most important cool season food legume in Sudan, it planted in more than 70% of the country, yields approximately 70 thousand metric tons of bean lost annually and thus constitutes approximately 70% of the country's needs (Babiker et al., 2007).

Orobanche crenata (broomrape) is a root parasitic angiosperm that lacks chlorophyll and is completely dependent on its host for the supply of nutrients and organic compounds (Mabrouk et al., 2010; Gevezova et al., 2012). It is considered to be among the most serious agricultural pests of economic importance in many parts of the world. It was speculated that the parasite seeds

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were involuntarily introduced, as contaminations of faba bean seeds from Egypt have spread rapidly. At present, the parasite has become a national problem. Traditional control methods are rarely effective. Selective herbicides often do not differentiate between the crop and the parasite, except for herbicide-tolerant transgenic crops; solarization is costly and limited to the upper soil; and mechanical control is not feasible because of the physical connection between the weed and the host (Hassan et al., 2019). A range of strategies have been suggested or investigated for controlling parasitic plants in various crops. These include the use of resistant cultivars, cultural practices, chemical application and biological control. Control approaches applied as single means often are only partially effective, are inconsistent at times and are affected by environmental conditions (Joel et al., 2007).

The application of beneficial soil microorganisms, particularly bacteria, has demonstrated significant potential in enhancing plant performance and mitigating the detrimental effects of parasitic weeds and nutrient deficiencies. For instance, research on *O. crenata*, a parasitic weed affecting legumes, has shown that specific bacterial strains and isolates, such as *Bacillus circulans* and *Bacillus megatherium* var*. phosphaticum* (BMP), can significantly reduce the germination and haustorium initiation of the parasite, thereby decreasing its emergence and negative impact on faba bean growth (Elabaied et al., 2017a). This study highlighted that improving soil fertility through these beneficial microorganisms can suppress O. crenata infestation and its adverse effects on host plants (Elabaied et al., 2017b). Similarly, investigations into maize cultivation have revealed that inoculating seeds with bacterial combinations, such as *B. megatherium* var*. phosphorous* + *Azotobacter* species + *Flavobacterium* spp. (B2), particularly when supplemented with nitrogen fertilizer, can lead to substantial improvements in plant growth parameters, yield components, and the chemical constituents of maize, including nitrogen content, crude protein, and fiber. The synergistic effect of bacterial inoculation and nitrogen application underscores the role of these microorganisms in promoting nutrient uptake and overall plant vigor, suggesting that such bio-fertilizers can be an environmentally sound approach to enhance crop productivity (Abd El Magid et al., 2020).

This study was carried out to evaluate an integrated management approach for controlling *O. crenata* infesting faba beans.

MATERIALS AND METHODS

A series of laboratory and field experiments were conducted at the laboratories and at the Soba Experimental Farm, Biopesticides and Biofertilizers Department (BBD), Environment, Natural Resources and Desertification Research Institute (ENDRI), National Centre for Research (NCR), Khartoum, Sudan; at the experimental field of Shendi Research Station, Agricultural Research Corporation (ARC), River Nile State, Sudan, in the 2015-2016 and 2016-2017 seasons; and at farmers' fields at Shendi and Dongola, Northern State, Sudan, in the 2018-2019 season to study the effects of *Trichoderma harzianum* (T.h), the herbicide imazethapyr, *B. megatherium* var. *phosphaticum* (BMP) and *the Rhizobium leguminosarum* strain (TAL1399) on *O. crenata* germination, incidence and faba bean plant height and yield.

Experimental site

The Soba area is a semidesert situated in the lower region of the Blue Nile, and the mean temperatures are 22 and 40°C in January and April, respectively. The wintertime is from November to March and is relatively cool and dry. The soil is a silty clay loam. The Shendi area is a semidesert situated in the lower region of the river Nile, and the mean temperatures are 40.7 to 13.6°C from October to March. The winter season extends from October to March and is relatively cool and dry.

The soil is a silty clay loam. The Dongola area is a semidesert situated in the lower region of the River Nile, and the mean temperatures are 30.7 to 10°C from October to March. The winter season extends from September to March and is relatively cool and dry. The soil is a silty clay loam. The bacterial strains *R. leguminosarum* (TAL1399) and *B. megatherium* var. *phosphaticum* (BMP) were obtained from the Biopesticides and Biofertilizers Department (BBD), ENDRI, NCR. *T. harzianum* fungi were obtained from the Department of Plant Protection, Faculty of Agriculture, Omdurman Islamic University. *O. crenata* seeds were obtained from BBD. Seeds of the Faba bean cultivars BB7 and MSRI 3 were obtained from Shendi Research Station.

Laboratory experiments

O. crenata seeds were germinated as described by Yahia et al. (2018, 2020). Briefly, discs containing *O. crenata* seeds conditioned in distilled water for 10 days were dapped on Whatman filter paper to remove excess water and subsequently transferred to sterile Petri dishes. Each disc was treated with distilled water, medium or the appropriate treatments. The dishes were sealed with parafilm, placed in black polythene bags, incubated at 18°C±2 for 3 days and subsequently treated with distilled water or GR24 at a concentration of 30 ml of GR24 (10 ppm). The treated seeds in Petri dishes were sealed with parafilm, wrapped in aluminium foil and subsequently incubated in the dark at 18°C±2 for 7 days prior to measurement of germination and radicle extension using a stereomicroscope.

Field experiments

Field preparation and sowing were performed as described by Yahia et al. (2019). In total, fields (Soba, Dongla and Shendi) were disc ploughed, harrowed, levelled, ridged and divided into subplots (7×6 m). All the fields were normally infested with *Orobanche* seeds. Faba bean (cv: BB7, Giza and MSRI 3) seeds were coated with BMP, and TAL 1399 was carried out on charcoal powder when applicable. Five grams of *T. harzianum* inoculum was added to each well at sowing where applicable. The herbicide imazethapyr was applied at a rate of 47.6 g/ha at sowing where applicable. Weeds other than *O. crenata* were removed 2 times at biweekly intervals, starting 2 weeks after crop emergence, using a hand hoe. The data collected for faba bean growth attributes included the following: i) plant height; ii) plant biomass; iii) number of pods; iv) 100-seed weight; and v) grain yield (kg ha^{-1}). The data for *O. crenata* included the following: i) the day of *O. crenata* emergence and ii) Number of *O. crenata* plans/m^2 and biomass.

Statistical analysis

The data collected from the laboratory and first field experiments (Soba and Shendi) were calculated as a percentage of the corresponding control, and the data collected from the Shendi second field experiment were subjected to statistical analysis (analysis of variance (ANOVA)) using the SPSS 22 statistical package.

RESULTS

Laboratory experiments

Effects of T. harzianum, bacteria and imazethapyr on reducing O. crenata germination

As shown in Table 1, all the treatments effectively suppressed *O. crenata* germination by 45.20 to 89.62%. The combination of *T. harzianum* + BMP + TAL1399 + imazethapyr had the most substantial and statistically significant (P≤0.05) reduction in seed germination (89.62%), followed by *T. harzianum* + imazethapyr (87.67%), *T. harzianum* (86.30%), and imazethapyr (80.82%).

Field experiments

First field experiments

Effects of *T. harzianum*, bacteria and imazethapyr on delaying the emergence of *O. crenata*: All the treatments resulted in a delay in the emergence of *O. crenata* at both the Shendi and Soba sites compared to the corresponding controls, as shown in Table 2. At the Shendi site, the treatments caused a delay in emergence ranging from 3.08 to 12.31%, while at the Soba site, the delay ranged from 8.06 to 16.13%. The combination of BMP + TAL1399 + imazethapyr or imazethapyr alone produced the highest percentage of delay at the Shendi site, while *T. harzianum* followed by imazethapyr resulted in the highest percentage of delay at the Soba site.

Effects of *T. harzianum*, bacteria and imazethapyr on *the* emergence of *O. crenata* shoots: All the treatments resulted in a decrease in the number of O. crenata that emerged at both the Shendi and Soba sites compared to the corresponding controls, as indicated in Table 3. The reduction in emergence due to the application of treatments at the Shendi site ranged from 43 to 81%, while at the Soba site, the reduction ranged from 32 to 81%. At the Shendi site, the highest percentage of reduction was achieved by *T. harzianum*, followed by the combinations of T. *harzianum* + BMP + TAL1399, *T. harzianum* + imazethapyr, and *T. harzianum* + imazethapyr + BMP + TAL1399. Moreover, at the Soba site, the highest percentage of reduction was obtained for *T. harzianum* + imazethapyr + BMP + TAL1399, followed by *T. harzianum* + imazethapyr, imazethapyr + BMP + TAL1399, *T. harzianum*, and *T. harzianum* + imazethapyr + BMP + TAL1399.

Effects of *T. harzianum*, bacteria and imazethapyr on increasing faba bean plant height: All the treatments led to an increase in the plant height percentage at both the Shendi and Soba sites compared to the corresponding controls, as shown in Table 4. The increase in plant height percentage due to the treatments at the Shendi site ranged from 4 to 20%, while at the Soba site, the increase ranged from 14 to 31%. The highest percentage of plant height at the Shendi site was achieved by imazethapyr + BMP + TAL1399, followed by imazethapyr and *T. harzianum*. At the Soba site, the highest percentage of plant height was attained by *T. harzianum*, followed by *T. harzianum* + BMP + TAL1399, *T. harzianum* + imazethapyr, and *T. harzianum* + imazethapyr + BMP + TAL1399.

Effects of *T. harzianum*, bacteria and imazethapyr on increasing faba bean yield: All the treatments significantly boosted **the** faba bean yield at both the Shendi and Soba sites (Table 5). Yield increases ranged from 12 to 124% in Shendi and from 19 to 113% in Soba. Notably, the *T. harzianum* + BMP + TAL1399 combination was the most common performer at both sites, followed by *T. harzianum* alone and imazethapyr + BMP + TAL1399.

Second field experiment

Effects of microbes and herbicides on *O. crenata* infesting faba bean varieties: All the treatments applied to the three faba bean varieties in Table 6 significantly delayed the emergence of *O. crenata* shoots ($p \leq 0.05$).

The most effective combinations were Microbes + Imazethapyr 200 ml/fed + Pendinour 1000 ml/fed and Microbes + Imazethapyr 100 ml/fed, leading to the longest delays compared to those of the controls. All the treatments significantly reduced O. crenata shoots in all three faba bean varieties (BB7, MSRI 3, and Giza) compared to their respective controls ($p \leq 0.05$).

The decreases ranged from 59 to 68% in BB7, 17 to 83% in MSRI 3, and 54 to 72% in Giza. Microbes + imazethapyr (200 ml/fed) + pendinour (1000 ml/fed) emerged as the most effective treatment for minimizing shoot count across all varieties. Microbes + 100 ml imazethapyr/fed and microbes + 200 ml imazethapyr/fed also performed well.

All the treatments, except for microbes alone at BB7, significantly reduced O. crenata shoot biomass in all three faba bean varieties (BB7, MSRI 3, and Giza) compared to that in the controls ($p \leq 0.05$). The reductions ranged from 21 to 54% in BB7, 18 to 93% in MSRI 3, and 40 to 61% in Giza. The microbes + imazethapyr (200 ml/fed) + pendinour (1000 ml/fed)

Table 1. Effect of treatments on reducing *O. crenata* seed germination.

Treatment	Germination reduction (%)
T. harzianum	86.30
T. harzianum + imazethapyr	87.67
Imazethapyr	80.82
BMP	45.20
BMP + imazethapyr	56.16
TAL1399	53.42
TAL1399 + Imazethapyr	57.53
T. harzianum + BMP + TAL1399 + Imazethapyr	89.62
Significant (P≤ 0.05)	**
SE ±	6.43

Table 2. Effect of treatments on delaying the emergence of *O. crenata.*

Treatment	Shendi (%)	Soba (%)
T. harzianum	8.15	16.13
T. harzianum + Imazethapyr	4.60	12.90
Imazethapyr	12.31	14.52
T. harzianum+ Imazethapyr + BMP + TAL1399	10.76	8.06
T. harzianum + BMP+ TAL1399	3.08	12.90
BMP + TAL1399	3.08	8.06
Imazethapyr + BMP+ TAL1399	12.31	12.90
Significant (P≤ 0.05)	**	**
SE ±	1.576	1.160

Table 3. Effects of treatments on the emergence of *O. crenata* shoots.

Treatment	Shendi (%)	Soba (%)
T. harzianum	80.60	77.80
T. harzianum + Imazethapyr	72.20	80.23
Imazethapyr	60.59	53.78
T. harzianum + Imazethapyr + BMP + TAL1399	71.45	81.28
T. harzianum + BMP + TAL1399	78.45	73.08
BMP + TAL1399	43.47	31.55
Imazethapyr + BMP + TAL1399	48.24	77.99
Significant (P≤ 0.05)	**	**
SE ±	5.523	7.034

treatment consistently achieved the lowest shoot biomass of all the varieties, followed by the microbes + imazethapyr (100 ml/fed) and microbes + imazethapyr (200 ml/fed).

Effects of microbes and herbicides on the yield of faba bean varieties: All the treatments except for microbes alone significantly boosted the faba bean yield across all the varieties (BB7, MSRI 3, and Giza) compared to that of their respective controls ($p \leq 0.05$) (Table 7).

The yield increases ranged from 72 to 80% in BB7, 12 to 19% in MSRI 3, and 12 to 36% in Giza. Notably, the microbes + Imazethapyr 200 ml/fed + Pendinour 1000 ml/fed combination emerged as the top performer, followed by the microbes + Imazethapyr 100 ml/fed and the microbes + Imazethapyr 200 ml/fed.

Table 4. Effects of treatments on increasing faba bean plant height.

Treatment	Shendi (%)	Soba (%)
T. harzianum	13.69	31.46
T. harzianum + Imazethapyr	11.36	28.19
Imazethapyr	14.09	14.15
T. harzianum + Imazethapyr + BMP + TAL1399	8.21	26.64
T. harzianum + BMP + TAL1399	7.08	28.59
BMP + TAL1399	3.70	19.43
Imazethapyr + BMP + TAL1399	20.05	20.42
Significant (P≤ 0.05)	*	**
SE ±	2.041	2.349

Table 5. Effects of treatments on increasing faba bean yield.

Treatment	Shendi (%)	Soba (%)
T. harzianum	110.30	98.83
T. harzianum + Imazethapyr	59.65	53.29
Imazethapyr	47.94	68.20
T. harzianum + Imazethapyr + BMP + TAL1399	77.76	90.13
T. harzianum + BMP + TAL1399	124.00	113.06
BMP + TAL1399	11.69	58.06
Imazethapyr + BMP + TAL1399	89.65	19.34
Significant (P ≤ 0.05)	*	*
SE ±	14.510	12.016

Table 6. Effects of treatments on *O. crenata* emergence, count and biomass.

Variety	Treatment	Days of emergence	No. of shoots	Biomass (g)
BB7	Microbes* alone	80.33	5.93	12.57
	Microbes + Imazethapyr at 200 ml/fed	84.33	4.80	8.00
	Microbes + Imazethapyr 100 ml/fed	86.00	4.67	9.97
	Microbes + Imazethapyr 200 ml/fed + Pendinour 1000 ml/fed	87.00	5.70	5.77
	Control	71.67	14.43	12.60
	SE ±	1.55	3.93	1.06
MSRI 3	Microbes alone	100.05	2.83	5.17
	Microbes + Imazethapyr at 200 ml/fed	99.00	0.67	0.77
	Microbes + Imazethapyr 100 ml/fed	98.00	0.70	0.67
	Microbes + Imazethapyr 200 ml/fed + Pendinour 1000 ml/fed	95.33	0.60	0.43
	Control	89.33	3.43	6.30
	SE ±	1.47	0.37	0.70
Giza	Microbes alone	85.67	4.70	8.03
	Microbes + Imazethapyr at 200 ml/fed	90.00	2.90	6.43
	Microbes + Imazethapyr 100 ml/fed	86.33	3.03	5.37
	Microbes + Imazethapyr 200 ml/fed + Pendinour 1000 ml/fed	85.67	2.93	5.17
	Control	76.67	10.23	13.40
	SE ±	1.28	0.80	1.24

*Microbes: *T. harzianum* + BMP + TAL1399.

Table 7. Effect of treatments on faba bean yield.

Variety	Treatment	Yield (kg/fed)
BB7	Microbes* alone	954
	Microbes + Imazethapyr at 200 ml/fed	1056
	Microbes + Imazethapyr 100 ml/fed	1025
	Microbes + Imazethapyr 200 ml/fed + Pendinour 1000 ml/fed	1071
	Control	596
	SE ±	4.98
MSRI 3	Microbes alone	1126
	Microbes + Imazethapyr at 200 ml/fed	1160
	Microbes + Imazethapyr 100 ml/fed	1223
	Microbes + Imazethapyr 200 ml/fed + Pendinour 1000 ml/fed	1230
	Control	1036
	SE ±	0.38
Giza	Microbes alone	1130
	Microbes + Imazethapyr at 200 ml/fed	1131
	Microbes + Imazethapyr 100 ml/fed	1230
	Microbes + Imazethapyr 200 ml/fed + Pendinour 1000 ml/fed	1376
	Control	1013
	SE ±	3.64

*Microbes: *T. harzianum* + BMP + TAL1399.

Farmer field experiment

At local farmer fields in both Shendi and Dongola, the combined treatment of *T. harzianum*, imazethapyr, BMP, and TAL1399 significantly suppressed O. crenata and boosted faba bean yields, depending on the cultivar (Table 8). In Shendi, MSRI 3 completely eliminated emergence and increased yield by 75%, while BB7 delayed emergence by 25%, reduced shoot numbers by 82%, and yielded a remarkable 369% increase. Similar trends were observed in Dongola, in which MSRI 3 delayed emergence by 33%, reduced shoot yield by 89%, and yielded 145% more, and BB7 showed a 44% delay in emergence, 49% shoot reduction, and a 269% increase in yield. These results highlight the effectiveness of this integrated approach in managing O. crenata and achieving substantial yield gains in faba beans, with cultivar choice further influencing the degree of success.

DISCUSSION

Currently, population pressure and the need to increase food production have led, in most sub-Saharan African countries, to land degradation and the expansion of agriculture into marginal soils of low fertility and, consequently, to a flare-up of the *Orobanche* problem. Intensified research efforts have yielded several control technologies, including culture, chemical and biological methods (Yahia et al., 2019, 2020; Hassan et al., 2019). The situation is further compounded by socioeconomic and marketing conditions together with illiteracy, unavailability of inputs and high cost (Traore et al., 2011). Effective *Orobanche* control is hindered by the persistent seed bank, which rapidly regenerates due to the parasite's high fecundity, making seed depletion critical yet challenging (Yahia et al., 2018). Integrated strategies are essential, as single-season control methods fail to suppress infestations long-term. Combining *T. harzianum* with BMP, TAL1399, and imazethapyr reduced *O. crenata* germination by 89.62%, outperforming individual treatments like *T. harzianum* (86.30%) or imazethapyr alone (80.82%), aligning with studies showing microbial and herbicidal synergies suppress germination (Elabied et al., 2017a, b; Yahia et al., 2018). These findings underscore the necessity of multifaceted approaches to mitigate seed bank replenishment and achieve sustainable control.

With respect to field experiments, the results showed that the application of *T. harzianum* alone or in combination with BMP + TAL1399 reduced and delayed Orobanche emergence, respectively, as well as increased plant growth at both sites. Wu et al. (2005) and Azarig et al. (2020) reported that a mixture of four microbes, AMF (*Glomus mossae* or *Glomus intraradices*), N fixer (*Azotobacter chroococcum*), P-solubilizer (*B. megatherium* var. *phosphaticum*) and K-solubilizer (*Bacillus mucilaginous*), significantly increased the growth of *Zea mays*. The notable negative impact on faba bean growth attributed to unrestricted *Orobanche* parasitism is

Table 8. Effects of treatments on the emergence and number of *O. crenata* and faba bean yield.

Treatment		Days to *O. crenata* emergence		Number of *O. crenata* (m^2)		Yield (ton/ha)	
		Shendi	Dongola	Shendi	Dongola	Shendi	Dongola
Control	MSRI 3**	76	72	16	45	1.93	1.29
	BB7	72	52	343	237	0.54	0.83
Treated*	MSRI 3	0	96	0	5	3.38	3.17
	BB7	90	75	63	120	2.68	3.06

**T. harzianum* + imazethapyr + BMP + TAL1399. **Faba bean cultivars.

in line with several reports (Elabaied et al., 2017a; Yahia et al., 2018). However, Yahia et al. (2018) reported that, compared with the corresponding control, *T. harzianum* alone or in combination with bacteria or imazethapyr significantly increased the height of faba bean plants.

Field trials demonstrated that the BB7 cultivar and MSRI 3 line treated with *T. harzianum* + imazethapyr + BMP + TAL1399 suppressed *O. crenata* emergence by 100 and 82%, respectively, while boosting yields by 75 and 369% compared to controls, with cultivar Basabeer outperforming Selaim (Hassan and Abakeer 2013; Yahia et al., 2020). These results align with studies showing combined microbial treatments (e.g., *Trichoderma* + bacterial strains or AMF) reduce *Orobanche* seed banks via suppressed germination and delayed emergence (63.97–70% at 100 days), mitigating parasitism and enhancing host growth through induced resistance (Lendzemo et al., 2007; Yahia et al., 2018, 2019; Elabaied et al., 2017b). The study found that faba bean growth is inversely correlated with seed bank size and tolerance, defined as maintaining productivity under infection (Fernández-Aparicio et al., 2016), varied by genotype. Broomrape dry weight per plant strongly correlated with infestation severity, reinforcing integrated strategies for sustainable control (Garcia-Torres et al., 1996).

RECOMMENDATIONS

Based on the study's findings, two key recommendations are proposed:

1. The package consisting of *T. harzianum* + imazethapyr + BMP + TAL1399 is recommended for use with specific faba bean cultivars (Giza in the Nile River State and MISRI3 in the Northern State) due to its positive effects.
2. Further research should focus on exploring alternatives to chemical herbicides, investigating biological or organic options to promote sustainable agricultural practices.

ACKNOWLEDGEMENT

The authors appreciate the Department of Scientific Research and Innovation, Ministry of Higher Education and Scientific Research, Sudan, for providing funding for this research.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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