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TRICHODERMA HARZIANUM AND BACTERIAL STRAINS AS BIOAGENTS FOR SUPPRESSING OROBANCHE CRENATA GROWTH AND PARASITISM IN FABA BEAN

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ABSTRACT

A series of field, laboratory and greenhouse experiments were conducted at the Bio-pesticides and Bio-fertilizers Department, Environment, Natural Resources and Desertification Research Institute (ENDRI), National Centre for Research (NCR), Khartoum, Sudan and College of Agricultural Studies, Sudan University of Science and Technology at Shambat, Khartoum North Sudan to study the effects of *Trichoderma harzianum*, herbicide imazethapyr, *Bacillus megatherium* var. *phosphaticum* (BMP) and *Rhizobium leguminosarum* biovar *viceae* strain (TAL1399) on *O. crenata* germination, incidence and faba bean growth, nodules number and plant height. Results of germination experiment showed that application of GR24 to *O. crenata* seeds conditioned in water induced germination to 80%. Application of *T. harzianum* significantly reduced *O. crenata* germination by 82% compared to control. The combination BMP + TAL1399 significantly reduced *O. crenata* germination by 32-42% as compared to control. Destructive sample experiment results showed that *T. harzianum* alone gave the highest nodules number and nodules dry weight compared to the control and the herbicide. The bacterial strains BMP and TAL1399 each alone or in combination significantly increased nodules number and dry weight as compared to the control. Extract of nodules collected from roots treated with *T. harzianum* and imazethapyr each alone or in combination, BMP and TAL1399 each alone or in combination significantly decreased *O. crenata* seed germination as compared to the control. Greenhouse results showed that *T. harzianum* and imazethapyr alone or in combination significantly decreased nodules numbers and *O. crenata* numbers as compared to the infected control and significantly increased faba bean biomass and plant height as compared to control. BMP, TAL1399 each alone or in combination significantly increased plant height, faba bean biomass and nodules numbers and decreased *O. crenata* number as compared to the infested control.

Keywords: BMP, Broomrape, Imazethapyr, *Rhizobium*, *Trichoderma*.

1. INTRODUCTION

Orobanche spp. are root parasitic flowering plants devoid of chlorophyll that cause important yield losses in several crops especially in food and feed legumes. *Orobanche crenata* is a

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holoparasite causes significant damage to the culture of leguminous plants particularly faba bean (*Vicia faba* L.). An intensive hyphal branching in response to host recognition can be induced by a group of chemicals called ‘‘strigolactones’’ (SLs) that are released from plant roots. 5-Deoxystrigol was the first SL isolated as a branching factor from root exudates of *Lotus japonicus* (Akiyama *et al.*, 2005).

The structures of orobanchol and orobanchyl acetate have been revised recently as shown by (Ueno *et al*., 2011). To date, most research regarding acquisition by plants has been focused on the association of legume root nodules with bacteria, whereas the importance of arbuscular mycorrhizal (AM) fungus–plant symbiosis has been mainly remarked on in the context of phosphorus uptake. However, AM fungi supply nitrogen as well as phosphorus to host plants (Ames *et al.*, 1983; Johansen *et al*., 1996). *Trichoderma* spp. are free-living fungi commonly widespread in soil and root ecosystems. Root colonization frequently results in enhancing of growth and development, crop productivity or induction of resistance to abiotic and biotic factors (Hohmann *et al*., 2011). Guzm´an-Guzm´an *et al*. (2018) reported that the mechanisms employed by *Trichoderma* include secretion of effector molecules and secondary metabolites that mediate the beneficial interaction of *Trichoderma* with plants, providing tolerance to biotic and abiotic stresses. The objective of this study is developing a control method for *O. crenata* using soil born fungi and bacteria and herbicide imazethapyr.

2. MATERIALS AND METHODS

Series of field, greenhouse and laboratory experiments were conducted at the Bio-pesticides and Bio-fertilizers Department, Environment, Natural Resources and Desertification Research Institute (ENDRI), National Centre for Research (NCR), Khartoum, Sudan. The field experiments were conducted at the College of Agricultural Studies, Sudan University of Science and Technology at Shambat, Khartoum North, Sudan during November to January, 2017/18.Seeds of the faba bean cultivars cv: BB7 was obtained from Shendi Research Station, Agricultural Research Corporation, River Nile State, Sudan. Greenhouse experiments were conducted at the greenhouse, Faculty of Agriculture, Omdurman Islamic University, to study the effects of *Trichoderma harzianum*, herbicide imazethapyr, *Bacillus megatherium* var. *phosphaticum* (BMP) and *Rhizobium leguminosarum* strain (TAL1399) on *O. crenata* germination, and faba bean growth, nodules number and plant height. The strigolactone analogue synthetic stimulant (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 completing to volume (100 ml) with sterile distilled water. The solution was kept in refrigerated at 4ºC for further use.

Laboratory experiments

Collection of treated faba bean nodules: Seeds of the faba bean cultivars BB:7 were obtained from the (ARC), Shendi Research Station. Seeds were sown and treated with treatments mentioned above, at the farm of College of Agricultural Studies, Sudan University of Science and Technology at Shambat during November to January, 2017/2018. The nodules were collected at 40, 50 and 60 days after sowing.

Faba bean nodules (1g each) were crushed in 10 ml of sterilized distilled water in a mortar. The nodules extract, filtered through Whatman No. 1 filter paper, was kept in a refrigerated at 5ºC.

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Effect of nodules extract on *O. crenata* germination in response to GR24 during conditioning: *O. crenata* seeds were conditioned in water for 11 days were treated with nodules extracts. Seeds were dapped on normal filter paper (No. 1) to remove excess water and then transferred to sterile Petri dishes. Each disc was treated with diluted filtrate of nodules extracts (30 µl) GR24 (10 ppm). Then seeds were re-incubated and examined for germination. Then seeds were re-incubated and examined for germination.

Greenhouse experiment: Greenhouse experiments were conducted at the greenhouse, Faculty of Agriculture, Omdurman Islamic University, during December, 2018 to March, 2019, to study the effects of *T. harzianum*, herbicide imazethapyr, BMP, TAL1399 Bacterial and combinations 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 (20 cm diameter). *O. crenata* infested and un-infested controls were included for comparison. *O. crenata* infestation was accomplished by mixing 5 mg of sterilized broomrape seeds in the soil in each pot. Three faba bean seeds were sown per pot. Seventeen days after sowing, plants were thinned to one plant per pot and the pots were irrigated twice every week. Experiments were terminated when the host plants in the control treatments stopped growing due to *O. crenata* infection. 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 and dry weight of faba bean shoots and roots were recorded at the end of the experiment.

Statistical analysis: Data collected from laboratory and field experiments were subjected to statistical analysis (ANOVA), using SPSS 22 statistical package and means were separated for significance using the (DMRT) at $P \leq 0.05$.

3. RESULTS

Laboratory experiments

Effect of *T. harzianum* and bacterial strains on *O. crenata* seeds germination

Results in table (1) showed that application of GR24 at 10 and 5ppm to *O. crenata* seeds conditioned in water induced germination by 69% and 82% respectively. Application of all *T. harzianum* densities gave the highest significant ($P \leq 0.05$) inhibition on *O. crenata* germination. It reduced germination by 82 - 62% compared to both controls. Application of the bacterial strains combination BMP + TAL1399 significantly ($P \leq 0.05$) reduced germination by 31- 42.47% as compared to the control.

Table 1 Effects of treatments on *O. crenata* germination

Treatments	Germination (%)	
	GR24 10ppm	GR24 5ppm
Water	80.21^{d}	69.73^{e}
Medium	77.45^{d}	70.42^{e}
T. harzianum	13.41^{a}	8.63^{a}

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½ *T. harzianum*	14.31[a]	13.53[ab]
⅓ *T. harzianum*	28.92[b]	27.76[bc]
¼ *T. harzianum*	29.25[b]	26.46[bc]
BMP + TAL1399	48.55[c]	46.46[d]
½ BMP + TAL1399	50.69[c]	40.44[cd]
⅓ BMP + TAL1399	57.14[c]	49.64[d]
¼ BMP + TAL1399	55.29[c]	53.52[d]
p≤ 0.05	**	**
SE±	4.29	3.90
SD±	22.30	20.27

Means followed by the same letter(s) are not significantly different according to DMRT at P≤0.05. According to LSD test. **= p ≤ 0.01

Effects of faba bean nodules extract on *O. crenata* seeds germination in response to GR24, *T. harzianum* +Imazethapyr

Results in figures (Fig.1, 2 and 3) showed that application of GR24 at 10 and 5ppm to *O. crenata* seeds conditioned in water induced germination by 91.48 - 77.56%. Application of nodules extract collected at 40 DAS from all treatments decreased seeds germination of *O. crenata* during conditioning as compared to the control. The low concentration of all treatments gave the higher germination compared to the high concentration **(**Fig.1**).** Extract of nodules collected at 50 DAS and treated with *T. harzianum* and imazethapyr each alone or in combination decreased *O. crenata* seeds germination (Fig.2**).** Extract of nodules treated with *T. harzianum*, imazethapyr each alone or in combination and collected at 60 DAS decreased seed germination of broomrape compared to the control (Fig.3).

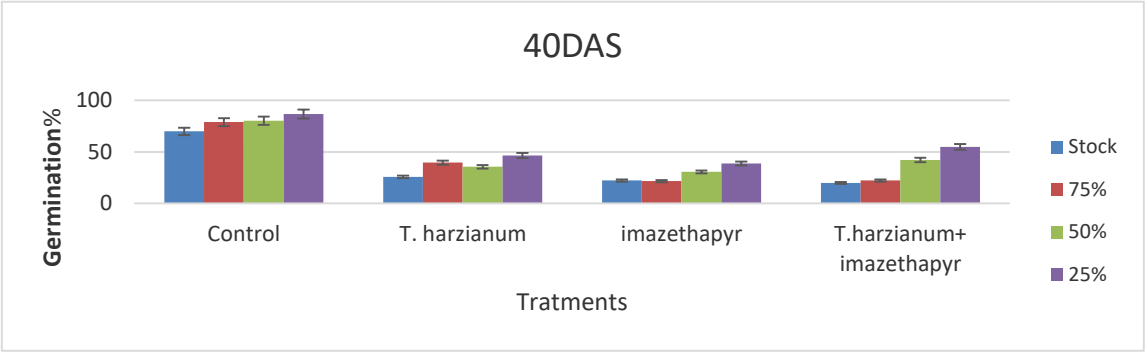

Fig. 1 Effects of nodules extract (collected at 40 DAS) on *O. crenata* seeds germination in response to GR24 (during conditioning) as *T. harzianum* +Imazethapyr

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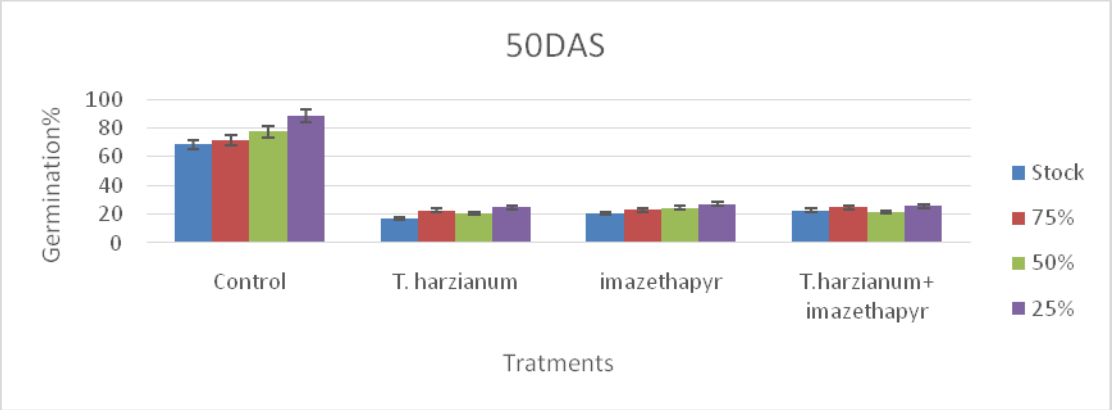

Fig. 2 Effects of nodules extract (collected at 50 DAS) on *O. crenata* seeds germination in response to GR24 (during conditioning) as *T. harzianum* +Imazethapyr

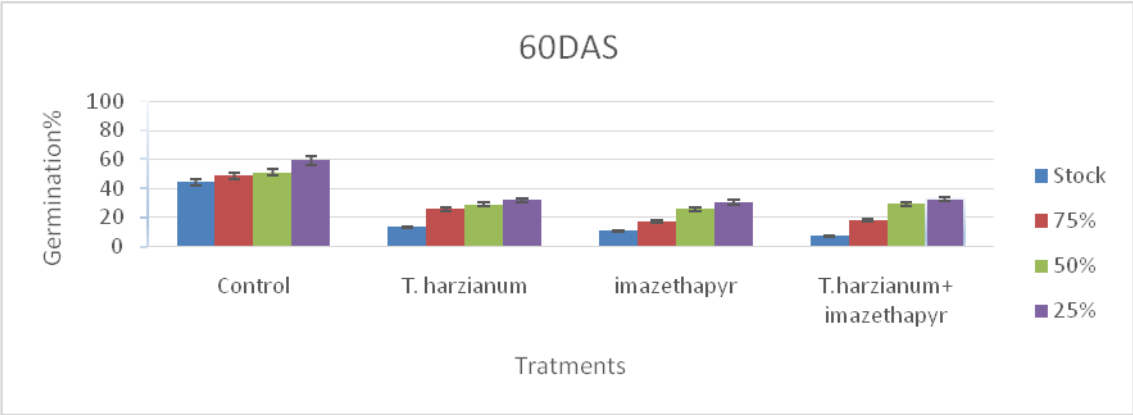

Fig. 3 Effects of nodules extract (collected at 60 DAS) on *O. crenata* seeds germination in response to GR24 (during conditioning) as *T. harzianum* +Imazethapyr

Effects of faba bean nodules extract on *O. crenata* seeds germination in response to GR24, bacterial strains

Results in figure4, 5 and 6 showed that application of GR24 at 10 and 5 ppm to *O. crenata* seeds conditioned in water induced germination by a rage (91.48 - 77.56%). Application of nodules extract collected at 40 DAS from all treatments decreased *O. crenata* seeds germination during conditioning compared to the control, the higher extract concentration gave less germination percentage compared the low concentration **(**Fig.4**).** The highest extract concentration of nodules treated with BMP and TAL1399 each alone or in combination and collected at 60 DAS decreased *O. crenata* seed germination as compared to the control (Fig.6).

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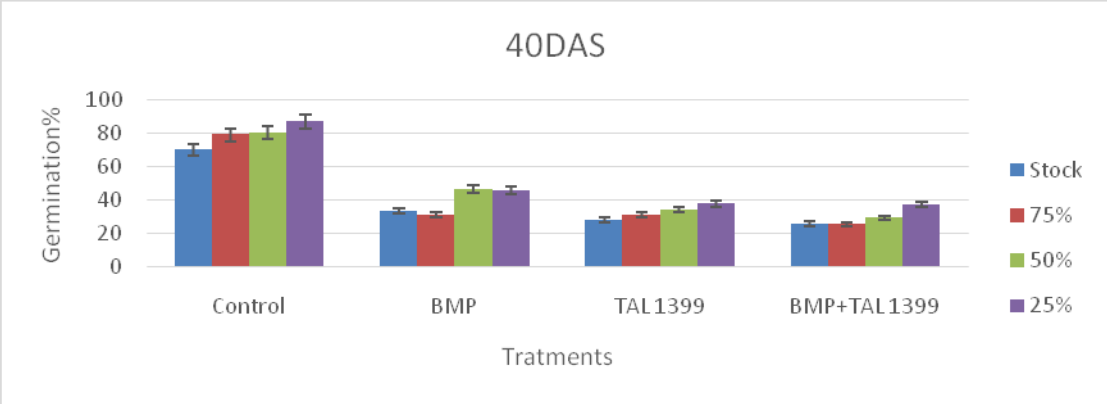

Fig.4 Effects of nodules extract (collected at 40 DAS) on *O. crenata* seeds germination in response to GR24 (during conditioning) as bacterial strains

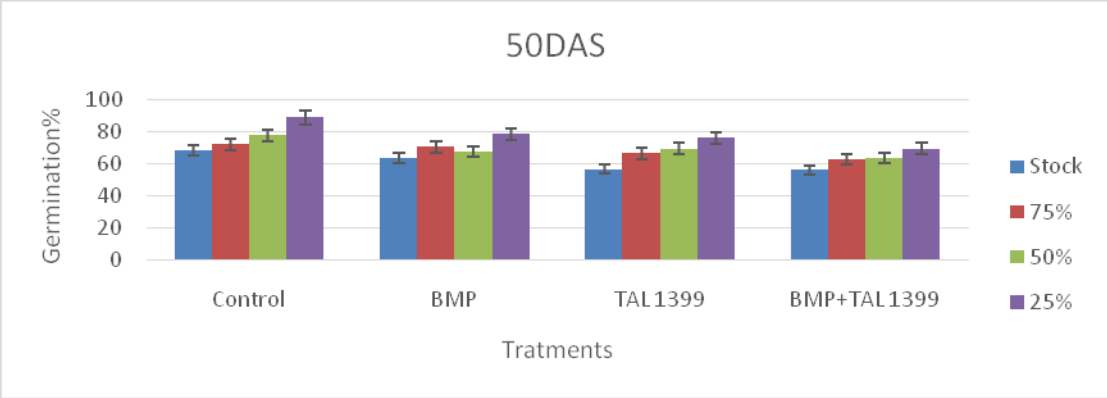

Fig.5 Effects of nodules extract (collected at 50 DAS) on *O. crenata* seeds germination in response to GR24 (during conditioning) as bacterial strains

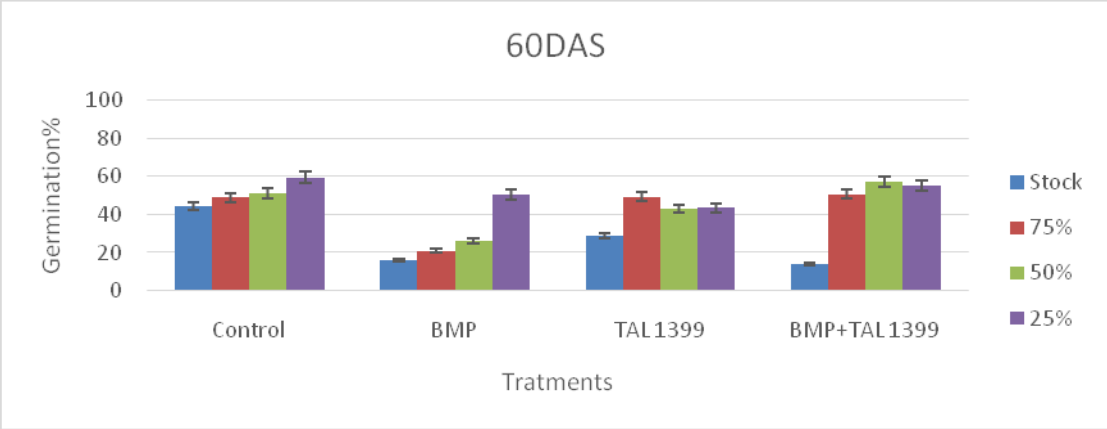

Fig.6 Effects of nodules extract (collected at 60 DAS) on *O. crenata* seeds germination in response to GR24 (during conditioning) as bacterial strains

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Greenhouse experiments

Effects of *T. harzianum* and imazethapyr on faba bean growth and *O. crenata* development

Results of the experiment showed that plant height increased with all treatments compared to the infected control. *T. harzianum* and uninfected control gave the highest plant height (33-35) (Table 2). *T. harzianum* and imazethapyr alone or in combination significantly ($P \leq 0.05$) decreased nodules numbers (Table.6) and *O. crenata* numbers as compared to the infected control (85-86.6) (Table 3). *T. harzianum* + imazethapyr significantly ($P \leq 0.05$) increased faba bean biomass as compared to the control with *O. crenata* (Table 4).

Table 2. Effects of *T. harzianum* and imazethapyr on faba bean plant height

Treatments	Plant height (cm)		
	60 DAS	**70 DAS**	**80 DAS**
Control without *O. crenata*	31.33	33.83	34.83
Control with *O. crenata*	33.67	31.83	24.00
T. harzianum	31.50	34.75	35.33
T. harzianum+ Imazethapyr	26.33	28.33	33.33
Imazethapyr	25.00	26.33	26.67
SE±	0.20	0.20	1.09
SD±	0.92	0.91	5.34

Table3. Effects of *T. harzianum* and imazethapyr on nodules number and faba bean biomass

Treatments	Nodules number/plant	Faba bean biomass
Control without *O. crenata*	12.00	5.67
Control with *O. crenata*	11.00	2.67
T. harzianum	8.67	5.33
T. harzianum+ Imazethapyr	7.67	6.67
Imazethapyr	5.33	4.00
SE±	0.95	0.42
SD±	4.67	2.05

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Table 4. Effects of *T. harzianum* and imazethapyr on *O. crenata* number/pot

Treatments	*O. crenata* No/pot	
	70 DAS	**80 DAS**
Control with *O. crenata*	2.17*(5.00)**	2.57 (6.67)
T. harzianum	0.47 (0.67)	0.80 (1.00)
T. harzianum+ Imazethapyr	1.14 (2.00)	1.62 (3.00)
Imazethapyr	0.91 (1.33)	1.24 (2.33)
SE±	0.28	0.27
SD±	0.98	0. 94

*Data out of brackets are square root transformed for analysis

**Data between brackets are original data.

Effect of bacterial strains BMP and TAL1399 on faba bean growth and *O. crenata* development

The BMP+TAL1399 and unifected control gave the highest plant height at 80DAS (Table 5). The BMP and TAL1399 alone or combination increased nodules number, decreased *O. crenata* number and significantly ($P \leq 0.05$) increased faba bean biomass as compared to the infected control (Tables 6 and 7).

Table 5. Effect of bacterial strains BMP and TAL1399 on faba bean plant height

Treatments	Plant height (cm)		
	60DAS	**70DAS**	**80DAS**
Control without *O. crenata*	31.33	33.83	34.83
Control with *O. crenata*	25.67	26.00	32.08
BMP	26.33	27.67	34.00
TAL1399	28.33	29.83	33.33
BMP+ TAL1399	25.67	34.00	34.33
SE±	0.87	1.09	1.40
SD±	3.03	5.34	4.83

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Table 6.Effect of bacterial strains BMP and TAL1399 on nodules number and faba bean biomass

Treatments	Nodules number/plant	Faba bean biomass
Control without *O. crenata*	12.00	5.67
Control with *O. crenata*	11.00	2.67
BMP	9.67	5.67
TAL1399	14.67	6.00
BMP+ TAL1399	14.33	5.33
SE±	0.95	0.42
SD±	4.67	2.05

Table 7. Effect of bacterial strains BMP and TAL1399 on *O. crenata* number/pot

Treatments	*O. crenata* No/pot	
	70 DAS	80 DAS
Control with *O. crenata*	2.17* (5.00)**	2.57 (6.67)
BMP	0.47 (0.67)	0.94 (1.33)
TAL1399	0.66 (0.67)	0.80 (1.00)
BMP+ TAL1399	0.67 (1.33)	1.00 (1.67)
SE±	0.29	0.29
SD±	1.01	0.99

*Data out of brackets are square root transformed for analysis

**Data between brackets are origin data.

4. DISCUSSION

The world faces two enormous challenges that can be met, at least in part and at low cost, by making certain changes in agricultural practices. There is need to produce enough food for a growing population in the face of adverse climatic trends. Improving photosynthetic efficiency of crop plants can help meet the challenges. Fortuitously, when crop plants' roots are colonized by certain root-endophytic fungi of the genus *Trichoderma*, this induces up-regulation of genes and pigments that improve the plants' photosynthesis.

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Seeds conditioned in water or yeast extract mannitol broth medium displayed high germination (69.7-80.2%) in response to GR24. Seeds treated with nodule extract came up from faba bean plants treated with *Trichoderma* or bacterial strains showed differential germination. *Trichoderma* irrespective to their concentrations effected negligible germination in comparison to the corresponding control. The observed reductions in germination, the key phase in development of parasitic weeds, by *Trichoderma* is in line with several reports on reduction of seed germination and viability by *Trichoderma* spp. (Abbasher and Babiker, 2012).

In the greenhouse experiments, the parasite, when not checked, reduced faba bean height by 8.57- 45.12%. *Trichoderma* considerably, improved faba bean height. The lack of significant reduction in dry weight in the *Trichoderma* inoculated *Orobanche* free faba bean indicates that, the fungus had no significant adverse effects on faba bean growth and that the fungus has the potentials to ameliorate the depressive effects of the parasite on faba bean growth.

Faba bean dry weight, determined at harvest, indicated the high efficacy and potentials of *Trichoderma* and bacterial strains, as a bio agent for suppressing *Orobanche* growth and parasitism. Count results showed that *Trichoderma* affected considerable reductions in the parasite emergence.

Vinale *et al*. (2008) reported that effective bio-inoculant should penetrate the roots not only to directly antagonize root pathogens, but also to stimulate plant growth and vigor through various mechanisms such as nutrient mobilization, nitrogen use efficiency, induction of host defence as well as the involvement of growth phytohormones from both plant and fungal origins. Application of *T. harzianum*, the herbicide and their combinations promoted plant growth and reduced *Orobanche* infestation.

Trichoderma is not only a good biocontrol agent, but also a general fertility promoter. In the absence of pathogens, application of appropriate *Trichoderma* formulations (following solarisation and/or preceding fumigation with authorized and environmentally-friendly chemicals) can also serve to promote plant growth and crop precocity, increase fruit production and reduce chemical treatments.

Trichoderma spp. is considered as widely studied fungal agent as microbial bio-control agent in agriculture and are marketed in the form of bio-pesticide, bio-fertilizer, growth promoter and natural resistance inducer.

Louarn *et al*. (2012) reported that Broomrape seeds are less capable to recognize crop roots colonized by arbuscular mycorrhizal fungi, *Rhizobium leguminosarum* or *Azospirillum brasilense* due to change in the composition of the root exudates in colonized.

Karasu *et al*. (2009) observed that inoculation of chickpea seeds with *R. ciceri* isolate had a significant effect on seed yield, plant height, and number of pods per plant, number of seeds per plant, harvest index and 1000 seed weight. The present study supports the hypothesis that inoculation of roots with bacterial combination or *Trichoderma* enhances a host defence mechanism in faba bean. A decrease of stimulant production by inoculated faba bean could explain the reduced proportion of parasite germination observed in the presence of inoculated plants. Further research in this area is needed.

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