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Effects of Bacterial Strains and Chicken Manure on *Orobanche crenata* Infesting Faba Bean

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Abstract: A pot experiment was conducted to assess the effect of bacterial strains and chicken manure on broomrape in faba bean. Results displayed that all treatments reduced Orobanche emergence except the combinations of Rhizobial bacterial strain TAL 1399 and composted chicken manure at 35 g pot^{-1}. Among all treatments faba bean inoculated with TAL 1399 alone or in combinations with *Bacillus megatherium* var Phosphaticum (BMP) or *Azospirillum braziliense* (Ab) plus chicken manure at 35 g pot^{-1} displayed no Orobanche emergence (above the ground) until the end of the experiment. However, Orobanche attachment was observed only when faba bean was inoculated with TAL 1399 plus BMP. Moreover, all treatments increased faba bean plant height and dry matter as compared to the control. Faba bean inoculated with bacterial strain TAL 1399 alone or in combination with chicken manure at 30 g pot^{-1} sustained the highest plant height as compared to infested or non-infested control. They increased faba bean height by 17-19%. Furthermore, crop treated with TAL 1399 plus chicken manure at 30 g pot^{-1} was significantly higher in root, shoot and total dry weight as compared to the control and other treatments.

Key words: Chicken manure, bacterial strains, *Orobanche crenata*, feba bean, broom rapes, Sudan

INTRODUCTION

Parasitic weeds are a serious problem in agriculture causing sub-stantial crop losses in many parts of the world. Orobanchaceae, the family of the holoparasitic broomrapes includes a number of parasitic weeds that infect plants and which has a worldwide distribution. The broomrapes are chlorophyll lacking obligate root holoparasites that depend entirely on their hosts thereby depleting them of nutrients, minerals and water through a root connection. In Sudan, the holoparasitic family of broomrapes (Orobanchaceae) is represented by *Orobanche* sp. (Parker and Riches, 1993). Crops infected by broomrapes can be heavily damaged even before the parasites emerge above the soil. Intensive research is underway in order to achieve better management of these parasites, especially with environment-friendly approaches. In spite of the diverse methods of broomrapes control, the adoption of many of these methods is hampered by drawback in the subsistent farming systems (Abu-Irmaileh and Abu-Rayyan, 2006). Management of broomrapes is often difficult due to the high amount of seed produced and which remain viable in the soil over several years and even do not germinate in the absence of a chemical trigger from a suitable host (Linke and Saxena, 1991). Moreover, they have vigorous growth habit after emergence and close association with the host crop.

Traditional control methods have been tried on different crops but none have proved to be effective. The use of herbicides is not easy because of the damage they may cause to the host plant, except on transgenic crops with target site herbicide resistance (Aviv *et al*., 2002). Zonno and Vurro (2002) reported that some toxins produced by fungi of the genus Fusarium were able to inhibit germination of branched broomrape seeds and proposed their practical use in integrated strategies of parasitic plant management. Improved utilization of animal manure has a central role in efforts to decrease the undesirable environmental impacts of farming

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(University of California, 2001). Efficient utilization of nutrients in organic material amendments can reduce the need for applied mineral fertilizers.

Composting of manure also reduces weed seeds viability and other pests. Application of microbial herbicides for the management of agricultural weeds is an eco-friendly approach. A worldwide programme has been growing up to control the invasive weed species for the better crop production and stable ecosystem. Hence, the objective of this study was to assess the role of bacterial strains and chicken manure each alone or their combinations on *Orobanche crenata* infesting faba bean.

MATERIALS AND METHODS

Orobanche crenata seeds used in this study were collected from parasitic plants growing under faba bean in 2006 at Shendi research station farm during the Winter season. The materials used in this experiment included air dried freshly produced chicken manure obtained from Animal Farm Department, Sudan University of Science and Technology (Sudan). Three bacterial strains, namely Rhizobium leguminosarum {TAL 1399, *Bacillus megatherium* var Phosphatrium (BMB) and *Azospirillum brasilense* (Ab)} were obtained from the Bio-Fertilization Department, Environment and Natural Resources Research Institute (ENRRI), the National Centre for Research, Khartoum, Sudan. Meat peptone agar medium was used for growth of bacterial strains. Counts of the developing colonies were expressed as Colonies Forming Units (CFU) mL^{-1}.

Pot experiment: In this experiment, a soil mixture made of river silt and sand (1:1 v/v) was placed in plastic bags (19 cm diameter) with drainage holes at the bottom. Row Chicken Manure (CM) at different levels (25, 30 and 35 g pot^{-1}) and *Orobanche crenata* seeds were added to the soil mixture in pots 2 weeks prior to sowing date of faba bean. Orobanche infestation was accomplished by mixing 6 mg of seeds in the top soil in each bag. Pots were then wetted by water and then sealed to allow manure composting for 2 weeks and kept in a greenhouse.

After the end of the 2 weeks, surface sterilized faba bean seeds (7 seeds pot^{-1}) were planted and irrigated immediately. Aliquots of the respective bacterial suspensions (15 mL each) were injected within the root zone in each pot. Subsequent irrigations were made every 2 days. Germinated Faba bean was thinned to two plants per pot at 10 days after planting. Seventeen treatments including chicken manure (3), bacterial strains (3) and their combinations were used in this study plus Orobanche infested and un-infested controls for comparison. The treatments were arranged in a factorial randomized complete block design with four replicates and two factors. Factor one was bacterial strain while factor two was chicken manure.

Parameters measured: Numbers of Orobanche shoots emerged per pot were recorded (5, 6 and 7 WAS) whereas numbers of tubercle (attached under soil) were recorded at 12 WAE. Faba bean plant height was measured at 2, 4 ad 6 Weeks after Emergence (WAE). Data collected on numbers of nodules, pods and the dry weights of faba bean shoot, roots and nodules were recorded at the end of the experiment (12 WAE). Data from the greenhouse experiments were transformed to log (x + 0.5) in which x is the number of Orobanche plants/pot and then subjected to Analysis of Variance (ANOVA). Means were tested for significance by LSD at 5%. Data on faba bean height, number of nodules and dry weight were subjected to analysis of variance. Means were tested for significance by LSD at 5% (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Effects on Orobanche: Generally, Faba bean treated with chicken manure and bacterial strains alone or in combinations displayed various effects. At 5 Weeks after Sowing (WAS), Orobanche emergence was very low as only 0.63 Orobanche plants emerged on the untreated control. At the same time, all treatments showed reduced emergence of the parasite.

Faba bean treated with the combination of bacterial strain TAL 1399 plus Ab, TAL 1399 plus BMP, TAL 1399; TAL 1399 plus Ab plus 25 g pot^{-1}; TAL 1399 plus Ab plus CM 35 g pot^{-1}; 25 g pot^{-1} displayed no Orobanche emergence (Fig. 1).

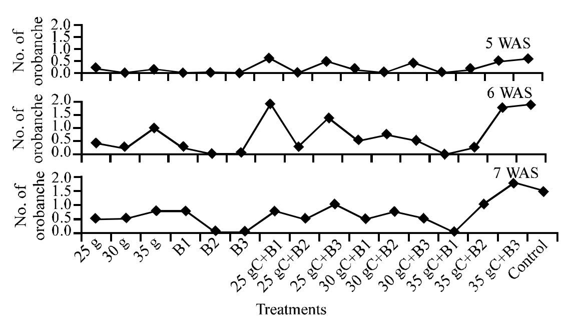

Fig. 1: Effects of bacterial strains and chicken manure on *Orobanche crenata* infested faba bean. Vertical bar indicates LSD (B1: TALL 1399+Ab (*Azospirillum brasilense*); B2: TALL 1399+BMP (*Bacillus megatherium* var. Phosphatrium (BMB)); B3: TAL 1399

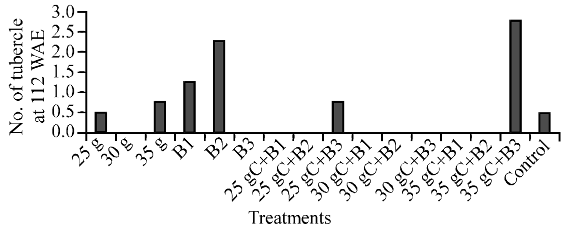

Fig. 2: Effects of bacterial strains and chicken manure on Orobanche incidence and tubercle numbers at 12 WAE. Vertical bar indicates LSD (B1: TALL 1399+Ab (*Azospirillum brasilense*); B2: TALL 1399+BMP (*Bacillus megatherium* var. Phosphatrium (BMB)); B3: TAL 1399

At 6 WAS, faba bean treated with bacterial strain TAL 1399 plus BMP; TAL 1399; TAL 1399 plus Ab plus 35 g pot^{-1} displayed no Orobanche emergence. Crop treated with chicken manure at 25; 30 g pot^{-1} or combinations of TAL 1399 plus BMP plus 35 g pot^{-1} sustained the low Orobanche emergence as compared to the control. Results showed that all treatments except the combinations of TAL1399 plus Ab plus 25 g pot^{-1} reduced emergence of the parasite (Fig. 1). At 7 WAS, the number of emergent Orobanche on the control showed a considerable decrease. Combinations of compost and bacterial strains were invariably more suppressive to the parasite emergence than each treatment alone. Results showed that all treatments except the combination of TAL 1399 plus 35 g pot^{-1} reduced emergence of the parasite (Fig. 1). With respect to tubercle number, results displayed that faba bean inoculated with most of the combinations of bacterial strains and chicken manure displayed no Orobanche attached root while the combination of bacteria TAL1399 and chicken manure at 35 g pot^{-1} showed the highest Orobanche attached as compared with control (Fig. 2).

Effects on faba bean plant height: The data on plant height are shown in Fig. 3 and Table 1. Results displayed that all treatments increased faba bean height albeit not significantly compared to infested control. At 2 WAE, untreated Orobanche free crop displayed a height of 28.5 cm.

However, unchecked Orobanche infestation reduced plant height by 33.3% (Fig. 3 and Table 1). Faba bean inoculated with the bacterial strain TAL 1399 alone or in combination with chicken manure at 30 g pot^{-1} gave the highest plant height as compared to infested or non-infested control. They increased faba bean height by 24-26%, respectively. Application of compost at 25 g pot^{-1} alone or in combination with bacterial strain

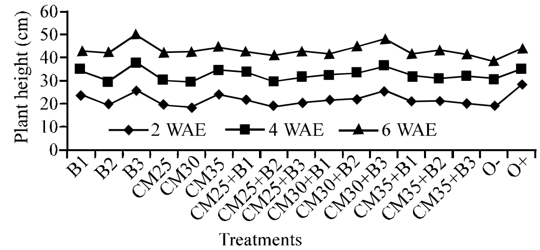

Fig. 3: Effects of bacterial strains and chicken manure on faba bean plant height. Vertical bar indicates LSD (B1: TALL 1399+Ab (*Azospirillum brasilense*); B2: TALL 1399+BMP (*Bacillus megatherium* var. Phosphatrium (BMB)); B3: TAL 1399

Table 1: Effects of bacterial strains and chicken manure on faba bean dry matter

No. of treatments	Treatments	Root dry weight	Shoot dry weight W	Total dry matter
1	CM25	7.038	12.560	19.410
2	CM30	6.918	12.530	19.450
3	CM35	7.558	9.850	17.400
4	B1	7.946	16.060	22.060
5	B2	6.373	10.180	16.550
6	B3	6.210	13.880	20.090
7	CM25+B1	6.752	10.780	17.710
8	CM25+B2	8.987	17.580	24.740
9	CM25+B3	7.581	10.820	18.420
10	CM30+B1	6.898	13.590	20.490
11	CM30+B2	6.983	14.090	21.080
12	CM30+B3	9.569	16.410	23.690
13	CM35+B1	7.108	11.730	18.840
14	CM35+B2	4.553	11.540	16.090
15	CM35+B3	6.213	12.550	18.760
16	O+	6.735	5.920	12.660
17	O-	7.253	9.090	16.460
LSD for chicken manure		±1.095	±2.180	±2.952
LSD for bacteria		±1.095	±2.180	±2.952
LSD for chicken manure x bacteria		±2.190	±4.360	±5.905

B1: TALL 1399+Ab; B2: TALL 1399+BMP; B3: TAL 1399

TAL 1399+BMP had no effect on infested faba bean growth in comparison to the control. In general, results at 4 WAE followed the same trends as at 4th WAE. Faba bean inoculated with bacterial strain TAL 1399 alone or in combination with 30 g pot^{-1} displayed 38 and 37 cm as compared to the control (Fig. 3 and Table 1). While at 6 WAE, all treatments increased faba bean height as compared to the control. Among all treatments faba bean inoculated with bacterial strain TAL 1399 alone or in combination with 30 g pot^{-1} displayed the maximum height as compared to the control.

Effects on dry matter: The data recorded on dry matter accumulation in stem and root at the end of the experiment (12 WAE) is shown in Table 1. The dry weight of faba bean plants parasitized by Orobanche was lower than that of uninfested control plants (Table 1). Results displayed

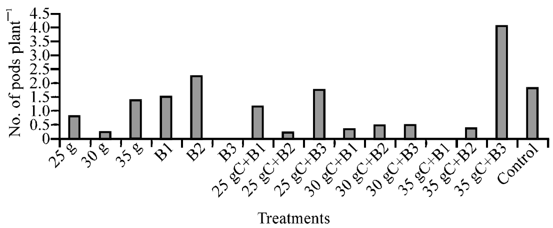

Fig. 4: Effects of bacterial strains and chicken manure on average pod numbers faba bean plants. Vertical bar indicates LSD (B1: TALL 1399+Ab (*Azospirillum brasilense*); B2: TALL 1399+BMP (*Bacillus megatherium* var Phosphatrium (BMB)); B3: TAL 1399

that all treatments increased faba bean dry matter as compared to the control. The higher root, shoot and total dry weight were obtained when faba bean plants were inoculated with bacterial strain TAL 1399 plus compost at CM 30 g pot^{-1} as compared to the control and other treatments. Results showed that the shoot dry weight in the combinations between TAL 1399 plus BMP plus 25 g pot^{-1} was found to be 3 times greater than that of the control.

With respect to root:shoot ratio results displayed that all treatments reduced the ratio as compared to the infested control (Table 1). Faba bean inoculated with B2 plus compost (25 g pot^{-1}) sustained the highest percent reduction of *O. crenata* emergence (85%) and increased total dry matter of faba bean over control by 52% (Table 1).

Effects on pod numbers and weight: The pod weight per treatment was an indicator of the efficacy of the combination between bacteria and chicken manure. Among the yield component studied, all treatments increased pods number except bacterial strain TAL 1399 treatment. Inoculation with bacterial strain TAL 1933 plus compost at 30 g pot^{-1} had an average 7.8 g pot^{-1} while non-inoculated control had an average weight of 4.7 g pot^{-1} (Fig. 4).

Effects on nodule numbers: Results showed that faba bean treated with CM at 30 g pot^{-1} plus TALL 1399 displayed the highest nodule numbers (98) followed by CM 35 g pot^{-1} plus TALL 1399 plus *A. brasilense* as compared to the control (Table 2). However, faba bean inoculated with CM at 25 g pot^{-1} plus *A. brasilense* sustained the lowest nodule numbers (12) as compared to the control. Effects of compost and bacterial strains were evaluated during the faba bean growth infested with

Table 2: Effects of bacterial strains and chicken manure on faba bean nodule numbers

No. of treatments	Treatments	Nodule No.
1	CM25	47.63
2	CM30	28.78
3	CM35	66.90
4	B1	16.52
5	B2	39.86
6	B3	29.04
7	CM25+B1	12.42
8	CM25+B2	38.00
9	CM25+B3	23.63
10	CM30+B1	22.58
11	CM30+B2	56.67
12	CM30+B3	98.22
13	CM35+B1	71.38
14	CM35+B2	58.33
15	CM35+B3	35.78
16	O+	32.58
17	O-	21.42
LSD for chicken manure	±19.61	
LSD for bacteria	±19.61	
LSD for chicken manure x bacteria	±39.23	

B1: TALL 1399+Ab ; B2: TALL 1399+BMP; B3: TAL 1399

Orobanche crenata. The present study showed that mixing of chicken manures with the broomrape contaminated potting soil resulted in less broomrape infestation as compared to the control. This result was expected to occur as composting with different organic matters reduces the viability of broomrape seeds (Abu-Rayyan and Abu-Irmaileh, 2004). Mixing chicken manures with the potting soil improved the growth of faba bean plants as compared to soil without manure treatment (Fig. 1, 2 and Table 1). This effect could be due to that the manure compost during the growing season might have adverse effect on broomrape infestation. Phytotoxicity of biodegradation products is known to occur during compost formation (Baker and Bryson, 2002).

Abu-Irmaileh and Abu-Rayyan (2006) reported that Broomrape seeds viability was 0 and 2% in soils amended with poultry and sheep manure or compost, respectively. Fermentation of different organic matters can also reduce seeds viability of many plant species through the effect of the produced heat and the resulting toxic compounds such as certain organic acids, ammonia and ammonium salts (Simpson, 1986). Ammonia is known to be a product of manure fermentation and could reduce plant growth at high concentrations (Simpson, 1986) and it is used as a defoliant in many crops (Foster *et al*., 1995).

It was reported that the reduction of infestation of broomrape on some crops was probably due to the volatile compounds evolving from manure fermentation (Simpson, 1986; Abu-Irmaileh and Abu-Rayyan, 2004; Abu-Rayyan and Abu Irmaileh, 2004). Esilaba *et al*. (2000) reported that combined application of 40 kg N ha^{-1} and 30 ton ha^{-1} manure (FYM) significantly reduced Striga emergence. Moreover, Keyes *et al*. (2000) reported that auxin-like compounds were inhibiting Striga seeds germination. *Azotobacter* sp., *P. putida*, *A. brasilense* and *Klebsiella* sp. are known to produce auxin and auxin-like compounds in plants rhizosphere (Frankenberger and Muhammed Arshad, 1995). There have been previous reports of increased nodulation and yield of legumes co-inoculated with *Bradyrhizobium japonicum* and non-pathogenic *Pseudomonas* sp., particularly *P. fluorescens*, *P. putida* complex (Shabayev *et al*., 1996; Singh *et al*., 1995). Such enhancement of root nodulation by *Pseudomonas* sp., may be due to the production of plant growth-promoting substances (Shabayev *et al*., 1996). Ahonsi *et al*. (2003) reported that co-inoculation of legumes (selected for capacity to induce germination of *S. hermonthica* seeds) with ethylene-producing, non-pathogenic rhizosphere *Pseudomonas* sp., isolates and *B. japonicum* isolates is worth developing as a biological control option for *S. hermonthica* in maize. Abdel-Kader and El-Mougy (2009) reported that *Trichoderma harzianum* (T1 and T3) and *T. viride* (T3) have a potential for development as mycoherbicides for control of *O. crenata* on pea plants. Furthermore, the present study clearly indicated that the rhizobacteria employed are plant growth promoters. They relieve a portion of the growth retardation caused by the parasitic infestation of *O. crenata*. Hassan *et al*. (2010) reported that the bacteria increased sorghum plant height and biomass. Also, the present result displayed that parasitism by *O. crenata* reduced faba bean pod number as compared to untreated control (Grenz *et al*., 2005). Adoption of an integrated approach encompassing chicken manure and bacterial inoculation may provide a novel, cheap and easy method to apply for *Orobnanche crenata* control under subsistence low-input farming systems.

CONCLUSION

Results displayed that faba bean growth was improved, especially if soil was mixed with chicken manure. These treatments could offer a new environmentally safe procedure to manage broomrapes using farm resources and hence, improve the sustainability of crop management. It would also be an effective asset in organic farming.

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