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EFFICACY OF BIOFERTILIZERS ON FUSILADE HERBICIDE ACTIVITY, GROWTH AND PRODUCTIVITY OF BROAD BEAN (*Vicia faba*).

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ABSTRACT

Two field experiments were performed at 2009/2010 and 2010/2011 to study the interaction effect between biofertilizers and fusilade herbicide at (1.5 L/fed. as a recommended dose) alone and in combination with additives at (1.0 L/fed.) to enhance narrow weeds control and broad bean productivity. During the two seasons, a positive correlation was found between biofertilizers and broad bean plant height, number of branches, number of nodules, fresh and dry weight, seed germination as well as number and activity of soil microbes. Inoculated broad bean seeds with *Rhizobium leguminosarum* or dual inoculation with (*Rhizobium leguminosarum* plus *Bacillus megaterium)* were encourage the capability of the plants to produce vigorous vegetable growth and increasing yield productivity of broad bean than non bio-fertilized treatment. The inoculation with *R. leguminosarum* as a single inoculants or dual inoculants with *B. megaterium* increased microorganism densities and activity which reflected on broad bean growth, yield and tolerability to the stress of fusilade herbicide. During1styear fusilade at micro rate (1.0 L/fed.) with mineral oil achieved the maximum inhibition in *Phalaris minor* fresh and dry weigh by 62.4 and 61.76 %, respectively comparing with untreated control. While in the 2nd year fusilade at the same micro rate with glue achieved the highest reduction in *P. minor* fresh and dry weight by 45.6 and 56.3% respectively comparing with the control. The highest reduction in *Staria* sp. fresh and dry weigh achieved from fusilade at micro rate with mineral oil by 65.31 and 71.18(1styear) and with rape seed oil 54.6 and 56.8% (2ndyear) respectively than its respectable control. There's no change in seed proteins types and percentage were detected after applying fusilade on broad bean in the presence or absence of biofertilizers. While the dual inoculation proved to be the most effective biofertilizers that increase protein components at the both seasons by (12.8% and 12.04%, respectively). Generally, a positive correlation was found between biofertilizers and fusilade on broad bean yield, so that to maximize broad bean productivity and reducing production costs as well as weed suppression, thus it is recommended for cultivating broad bean with dual inoculation (*R. leguminosarum* plus *B. megaterium*) as biofertilizers and using fusilade at (1.0 L/fed.) with spray tank additives for a narrow weed control which increased productivity of broad bean during the two years ranged from 324.01 to 349.27% (mineral oil) and with 296.06 and 340.32% (glue), respectively, and seed protein than untreated control.

Keywords; Biofertilizers, fusilade, additives, broad bean, productivity and Quality.

INTRODUCTION

Broad bean (*Vicia faba L.)* is an important legume crop grown for its green pods which considered as a good source of protein. Due to its high nutritive value, it is a primary source of protein in the diet of masses. Millions of people in Egypt, particularly those in the low and middle income brackets, depend on broad beans as a main staple food for both breakfast and dinner

Nassib *et al.,* (1991). To achieve the highest productively, chemical fertilizers were used intensively around the world. However, they started displaying their harmful effects to the environment Watson, (1981). Meanwhile, fertilizers such as phosphorus and nitrogen are very important nutrient for crop growth and high yield with good quality. In new cultivated sandy soils, may have some nutrient problems such as less fertility in general and less availability of some elements such as phosphorus in case of high pH value. The primary nutrients are nitrogen, phosphorus, and potassium. All plants need nitrogen to make amino acids, proteins and DNA, but the nitrogen in the atmosphere is not in a form that they can use. Phosphorus plays a key role in metabolic process such as the conversion of sugar into starch and cellulose. In the context, yield and its components showed a positive response to phosphorus fertilizers, As a result, phosphorus deficiency causes stunting, delayed maturity and shriveled seeds, Abd-Alla (2002), Mokhtar, (2001), El- Douby and Mouhamed, (2002). Therefore, biofertilizers were introduced as alternative tools to the farmers for reducing the usage of the chemical fertilizers and developed the number of branches, pods per plant, seeds per pod and seed weight to maximizing yield of a broad bean crop its yield components as well as preserving the environment in the long run. El Habbasha *et al.,* (2007) indicated that increasing phosphorous levels from zero 2 5 to 45 kg P O /faddan in combination with Rhizobium, Nitrobein or Rhizobium + Nitrobein increased significantly the most of studied characters compared to control treatment. El-Wakeil and El-Sebai, (2007) reported that bacterial total count was higher significantly in mixed inoculant's strains than in single inoculant. Either single or mixed inoculants strains showed positive response on seeds weight compared to NPK plots. The highest number of pods was achieved in treatment of rhizobia mixed with mycorrhizal or pseudomonas. Unfortunately, weeds represent a major obstacle of broad bean high yielding productivity. they are compete for nutrients, space, light and exert lot of harmful effects by reducing the quality, as well as quantity of the crop, if the weed populations are left un-controlled Halford *et al.,* (2001). To overcome this problem many suitable herbicides recommended Hassanein *et al.,* (1987). Also, to minimize requirements for cultivation multifaceted weed management strategies that are site specific and adopt a holistic approach aiming to optimize the whole herbicides efficiency are to be developed by using the spray tank additives. Additives can be especially effective in improving the biological activity of phenoxy herbicides specifically; the addition of a methylated seed oil or surfactants adjuvant to spray solutions can enhance spray retention, foliar absorption of the herbicide, and subsequent herbicide efficacy Bunting *et al.,* (2004); Hart *et al.,* (1992); Nalewaja *et al.,* (1995). The aim of this work was to study the interaction effect between using bio fertilizers nitrogen fixing bacteria (*R. leguminosarum*) and phosphate dissolving bacteria *B. megaterium* (PDB) and their dual inoculation (*R. leguminosarum* and *B. megaterium*) and fusilade as a narrow weed control herbicides without and with additives to enhancing its herbicidal efficacy for controlling weeds and for increasing broad bean productivity and quality which considered to be main crop for facing the big shortage of protein component in Egypt.

MATERIALS AND METHODS

Source of Microorganisms:

Two active bacterial strains were used in this investigation; *R. leguminosarum* was obtained from Soil Microbiology and Fertility Dep. (DRC) and *Bacillus megaterium* which previously isolated from soil of faba bean field at Maryout Experimental Station.

Source of seeds:

Broad bean (*Vicia faba L*.) seeds (Roumi Kacere aspainy cultivar) were obtained from, Field Crops Res. Inst., ARC, Giza, Egypt.

Bio-fertilizers preparation:

B.megaterium and *R. leguminosarum* bacterial isolates were grown on modified Bunt and Rovira medium (Abd El-Hafez, 1966) and yeast extract mannitol agar (Allen, 1959) for 7 days at 28+ 2^{o}C, respectively. Two hundred fifty ml erlenmeyer flasks containing 100 ml of sterilized media were inoculated with one ml of standard inoculums from *R. leguminosarum* or *B. megaterium* and shaked with a rotary shaker (160 rpm) for 46 hours at 30 ^{0}C.

Biofertilizers treatment

Seeds of broad beans were successively washed and soaked in bacterial suspension either with 5% Carboxy methyl cellulose (CMC) containing about 10^{8} cells/ml for 20 minutes or in uninoculated medium to conserve as a control. Seeds were air dried for 12 hr. at 25-28 ^{0}C in shade place till dried.

Herbicide treatment:

Fusilade super (fluazifop-butyl) 12.5% EC, supplied by Syngenta Company, fusilade was used at the recommended rate (1.5 L/fed. as a recommended dose) alone or at (1.0 L/fed.) mixing with three additives by 20% (v/v) including crop seed oil (rape seed oil) (200 ml), mineral oil 200 ml (KZ company) and by 20% (v/w) for glue as sticking agent (200 gm). Application time was done at the 3-4 leaf stage (one month from emergence).

Field experiments

Two field experiments were carried out during two growing seasons *i.e*. 2009/2010 and 2010/2011 at Maryout Experimental Station, Desert Research Center (DRC) to investigate the effect of bio-fertilizers as single strains of *Rhizobium leguminosarum* and *Bacillus megaterium* or dual strains *R.leguminosarum* and *B. megaterium* on fusilade herbicide (with and without additives) activity for narrow weed controls as well as faba bean growth and productivity.

The experiment was established in Split- plot design with five replications. Where bio fertilizers treatments in the main plots and the herbicides treatments in the sub plot. The plot area was 4×1.5 m^2 and consisted of 5 rows, each row was 4 m length and 30 cm width. Two seeds were sown per hill at 1-5 cm a part. Twenty m^2 of organic manure was added to the soil, in addition to some mineral fertilizer (120 kg/ fed. ammonium sulphate and 150 kg.fed. super phosphate (15.5% P2O5) and 100 kg/fed sulphate potassium.divided at two times during the seed bed preparation and before the first irrigation such as Experimental soil texture is

sandy clay loam with pH 7.81 and organic matter 0.93 and with chemical properties was $CaCo_3$ 39.43% and the soluble cations (meq/100 g) 28.34 (Na^+), 1.02 for (K^+), 15.32 for (Ca^{++}), 9.56(Mg^{++}) and soluble anions (meq/100 g) 38.76 (Cl^-), 20.21 ($SO4^-$) and 1.43 (HCO_3^-). During the growing season, recommended cultural practices were followed and surface irrigation system was applied. After 30 days from seed sowing, the plants were sprayed with the herbicides and the remaining broad leaf weeds were hand pulling up.

Faba bean characters

Plant height, number of branches per plant and total fresh and dry weight of vegetative part of plant (leaves + stem were recorded after 35, 70 and 120 days from sowing. The third faba bean top leaf was taken after three weeks from treatment for total chlorophyll determination using chlorophyll meter (SPAD). Total protein was extracted according to Landry and Moureaux (1976) and fractionated by (SDS-PAGE) according to Weber and Osberne (1969).

During the harvest, total pods yield (green pods yields) was calculated and the total pods yield was calculated at the end of harvesting season. At the second harvest, random samples of green pods were taken from each plot and oven dried at 700^0C till constant, the dry matter.

Weeds characters

Fresh and dry weight, density, abundance, dominance and frequency of weeds were recorded after three weeks from treatment. Data was summarized and analyzed as relative abundance according to the formula used by Thomas, (1985). Also, coefficient of similarity percent was determined according to the methods described by Newsome and Dix, (1986).

Microbiological determinations:

Rhizosphere soil samples were taken during the experimental period and subjected to microbial determination. Total bacterial count was determined on soil extract agar Page *et al*., (1982). Phosphate dissolving bacterial count was determined on Bunt and Rovira agar medium after modification by Taha *et al*., (1970). CO_2 evolution (Yeast extract manitol agar (YMA) medium was used according to Allen, 1959) was determined as index to microbial activity in soil according to Atef and Nannipien, (1995).

Assessment of N_2- ase activity of root nodules:

Nitrogenase activity of root nodules was determined by acetylene reduction method (C_2H_2-> C_2H_4) according to Dart *et al.,* (1972). As soon as, the legume plants were uprooted, washed and the roots were separated and put in 600ml. serum bottles and then sealed with tide rubber stopper. The suitable volume of acetylene was then injected into the bottles using plastic syringe to give acetylene concentration of 10%of atmosphere. The bottles were left under ambient temperature for one hour. Then 0.125ml gas samples were withdrawn and assayed for acetylene concentration using a Hewlett-Packard model 5890 gas chromatograph equipped with a hp-plot Al_2O_3 capillary column (0.53 mm. by 50 m. and 15.0 um film thickens); a flame ionization detector and a Hewlett Packard model vactra 486/33 VL computer. Peaks were automatically integrated and acetylene amounts were calculated. The gas chromatographic parameters were as follows: carrier gas; ultrahigh

purity nitrogen, 10ml./Min. column temperature; 120 ^{o}C; injection port temperature 170 C$^{o.}$ Hydrogen and air for the flame were at rates of 30 and 300ml./min. respectively. The retention times of ethylene and acetylene were 1.3 and 1.9 minutes respectively. To calculate the nitrogenase activity of samples; the following equation was used:

$$C_2H_4.g^{-1} \text{ dry nod. } hr^{-1} = \frac{A \times V1}{V2 \times T}$$

Where; A= μl C_2H_4 calculated by computer, V2= Total volume of bottle,
V1= injected volume in G C. (0.125), T = Incubation time

Statistical analysis

Data were analyzed using ANOVA according to Snedecor and Cochran (1990). Effects were considered significant for P=0.05 from the F-test. Least significant differences LSD analysis and Duncan multiple range test were conducted for mean comparison.

RESULTS AND DISCUSSION

In Egypt, Chemical fertilizers beside biofertilizers were used to full the need of nutrients on new cultivated land. Also, herbicide application and pulling up with hand are important methods of weed control in broad bean productivity. During the two years, broad bean seeds were inoculated with biofertlizers compared with uninoculated treatments beside assessment of soil microbiology component, in addition to weed species, relative abundance and coefficient of similarity between plots were implemented under the field conditions. Also, the role of additives in fusilade activity against narrow leaved weeds and its impact on broad bean productivity were conducted. Data presented in (Table 1) demonstrate that total bacterial count affected in the presence of fusilade and bio fertilizers by decreasing and increasing trend respectively during the three stages at the two seasons. The addition of dual inoculation (*B. megaterium* and *R. leguminosarum*) without herbicides increased total bacterial count at 35, 70 and 120 days by 472.1, 512.5 and 570.0 % (1styear) and 570.0,656 and 457.1 % (2ndyear) as compared with un inoculated control. Application of fusilade alone or with additives resulted reduction in the total bacterial count on the state of the first period 35 days from broad bean sowing. However, inoculation with biofertilizers increased microbe numbers. Meanwhile the highest microbial total count achieved from the double inoculation during the three stages. It was noted that the addition of rape seed oil to fusilade at (1.0 L/fed.) clearly reduced the total bacterial count at 1st and 2nd year which caused the maximum decreasing in both biofertilizers and non biofertilizers plots as compared with its respectable control. According to PDB densities (counts x 10 ^{4} CFU/g dry soil), PDB affected regardless of the presence or the absence of both herbicide and biofertilizers at both seasons depending on the state of inoculation and broad bean stage (Table 1). Seed inoculation with *B. megaterium* or the dual inoculation induced PDB densities during the three stages in both seasons than untreated control, while the highest induced in PDB densities was recorded at 70 days then at 35 and 120 day which achieved from dual

inoculation. Application of mixed inoculation without herbicides increased PDB densities at 35, 70 and 120 days by 200, 266.7 and 262.5 % (1st year) and 238.2, 320and 247.8 % (2nd year) as compared with un inoculated control.

Table (1): Total bacterial count and PDB densities in the rhizosphere of broad bean affected by biofertilizers inoculation and fusilade herbicide application during 2009/2010 and 2010/2011 seasons, at Maryout Experimental Station.

Biofertilizers	Herbicide treatments	1st. Year (2009/2010)			2st. Year (2010/2011)			1st. Year (2009/2010)			2st. Year (2010/2011)		
		Total bacterial counts (counts x 10^6) CFU)/g dry soil*						PDB densities (counts x 10^4 CFU/g dry soil)			PDB densities (counts x 10^4 CFU/g dry soil)		
		Days after sowing											
		35	70	120	35	70	120	35	70	120	35	70	120
Non Inoculation	Control	0.19	0.40	0.30	0.25	0.70	0.42	35	45	40	34	50	46
	Fusilade alone	0.16	0.36	0.23	0.21	0.58	0.30	30	35	32	30	35	32
	Fusilade+ glue	0.15	0.35	0.22	0.19	0.60	0.36	32	36	34	32	40	35
	Fusilade+ mineral oil	0.14	0.32	0.21	0.20	0.51	0.33	32	37	35	30	37	34
	Fusilade+ rape seed oil	0.12	0.30	0.20	0.17	0.40	0.19	30	35	32	30	35	32
R .leguminosarum	Control	0.42	1.90	1.40	0.95	2.38	2.12	70	100	80	75	110	85
	Fusilade alone	0.32	1.30	1.10	0.69	2.01	1.60	50	70	65	50	78	70
	Fusilade+ glue	0.30	1.34	1.15	0.70	2.02	1.70	54	75	67	54	80	70
	Fusilade+ mineral oil	0.34	1.24	1.00	0.58	1.89	155	55	70	60	55	75	67
	Fusilade+ rape seed oil	0.25	1.04	0.70	0.51	1.40	1.04	40	65	55	46	70	60
B. megaterium	Control	0.40	1.70	1.24	0.90	2.20	1.55	80	115	100	85	115	105
	Fusilade alone	0.35	1.40	1.04	0.70	1.65	1.30	57	105	70	70	110	85
	Fusilade+ glue	0.36	1.55	1.15	0.72	1.95	1.34	60	110	78	75	110	95
	Fusilade+ mineral oil	0.32	1.40	1.05	0.70	2.00	1.40	55	100	75	70	105	90
	Fusilade+ rape seed oil	0.30	1.15	1.00	0.48	1.35	1.04	46	80	70	65	100	80
Dual inoculants	Control	1.60	2.45	2.01	1.89	3.90	2.85	105	165	145	115	210	160
	Fusilade alone	1.55	1.89	1.55	1.55	2.85	2.11	78	145	110	100	160	145
	Fusilade+ glue	1.40	1.95	1.60	1.60	2.65	2.10	85	155	115	105	165	150
	Fusilade+ mineral oil	1.30	1.65	1.30	1.40	2.75	2.02	75	115	100	105	160	150
	Fusilade+ rape seed oil	1.04	1.40	1.15	1.24	2.10	1.84	70	110	90	90	145	115

***- Initial total bacterial count was 60 x 10^2 (CFU/g dry soil).**