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EFFECTIVNESS OF SALT-TOLERANT *RHIZOBIUM* (E_1 & F_1) INOCULATION AND MINERAL NITROGEN FERTILIZATION ON FABA BEAN PLANTS AND WITHSTAND TEST OF ISOLATE F_1 TO PESTICIDAL TOXICITY

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ABSTRACT

Applicability of salt-tolerant bacterial isolates (F_1 and E_1) of *Rhizobium leguminosarum* biovar *viciae* was compared with the mineral Nitrogen fertilization on faba bean plants, under saline field conditions. Higher magnitudes of shoot length, dry weight of shoots and roots than control and N-fertilized plants were achieved. Results were more pronounced due to use dual combinations of F_1 or E_1 with 25 % N-supply. Parameters of N_2-fixation, seed yield and its crude proteins of both faba bean cultivars (Nubaria 1 and Sakha 1) were also, enhanced due to superiority of the dual treatments. So, utilization of salt-tolerant rhizobial isolates could be attributed to overcome the harmfull effect of soil salinity on growth, nodulation and seed productivity of faba bean plants. On the other hand, the largest diameter of inhibition zone reached 4.20 cm which representing 46.67 % growth inhibition of *Rhizobium* which was achieved via 1 g L^{-1} of Vitavax under laboratory conditions. Slope fitting data of the experimental values showed constant inhibitory effect of Ground-up, Malathion and Vydate with increasing their concentrations against Rhizobia. For Vitavax, sharp ascending slope was resulted; indicating induction of further inhibition with additional dose would be expected.

Keywords: faba bean, Rhizobium, salinity, N-supply, pesticides.

INTRODUCTION

Faba bean (*Vicia faba* L.) represents the main source of protein (25 – 40 %) for the majority of the Egyptian population, due to its high nutritive value (Farag *et al.,* 2005). Inoculation of faba bean plants with specific active rhizobial strains is the main factor for enhancing growth and productivity of these crops (Abo El-Soud *et al*., 2003). Plant growth, nutrient uptake, metabolism, and protein synthesis are all thought to be adversely affected under salt stress conditions (Almadini, 2011). Salinity is a serious threat affecting not only on faba bean but also on the symbiotic N_2-fixing bacteria at both free living stage and during the symbiotic process (Lloret, 1995). Rhizobia are soil-inhabiting bacteria that fix nitrogen from atmosphere to form ammonia via socalled biological N_2-fixation (BNF) process (Giller, 2001). However, salinity stress negatively affects the nodulation capacity of faba bean (Craig *et al*., 1991). Unsuccessful symbiosis under salt-stress conditions might be due to failure in the infection process, because salinity affects on establishment of rhizobia (Singleton and Bohlool, 1984).

Due to use of some pesticides during cultivation of faba bean, rhizobia showed varied responce under laboratory conditions. Some pesticides were not detrimental to the growth of rhizobia when applied at field rates, but others were found to be toxic when applied at low or at high rates (Martensson, 1992). Effect of various pesticides (insecticides, fungicides and herbicides) on growth and efficiency of symbiotic properties were investigated by Madhavi *et al.*(1993). Response of faba bean plants to certain salt-tolerant rhizobium isolates in comparison with mineral N-fertilization, was the main goal of the presented study. As well as, Toxicity of some commonly pesticids used with faba bean fields was also aimed.

MATERIALS AND METHODS

***Rhizobium* inocula:**

Due to their salt withstand, two isolates (E_1 and F_1) of *Rhizobium leguminosarum* bv. *viciae*, formerly tested by El-Khateeb (2009), were used to achieve the objectives of this study. Pure cultures of both isolates were obtained using yeast extract manitol agar (YMA) media (0.5 g K_2HPO_4, 0.5 g $MgSO_4$, 0.1 g NaCl, 10.0 g mannitol, 1.0 g yeast extract and 15.0 g agar per one liter distilled water, pH 6.8 – 7.0, autoclaved at 121^oC for 30 min). To prepare *Rhizobium* inocula, one loopful of 10^8 CFU (Colony Forming Units) per 1 mL from each purified isolate was enriched using 250 mL YM liquid medium in 500 mL flask. Cultures were shaken incubated at 28-30 ^oC and 150 rpm (revolutions per minute) for 3-5 days. After that, number of bacterial cells of each culture was counted and adjusted at 10^8 cell ml^{-1}, using counting chamber (Haemocytometer specialized microscope slide). Cultures were used to impregnate sterilized peat (121°C for 30 min.) as the method described by Thao *et al*.,(2001), at the rate of 52 mL liquid culture per100 g peat. Inoculated peat was well mixed and maintained at room temperature for 48 h. Seeds of faba bean cultivars (Nubaria 1 and Sakha 1) were kindly supplied from Field Crop Res. Inst., Agric. Res. Center, Dept. of Legumes, Sakha Agric. Res. Station, Kafr El-Sheikh, Egypt**.** Seeds of faba bean varities were wetted with 10 % Arabic gum water solution as an adhesive agent and inoculated with rhizobial peat-based preparation (Hamdi, 1982) and were allowed to air drying in the shade for 30 min. and were sown immediately.

Field experiments:

Field trials were performed at two salt-affected locations at the northern stripe of Delta, Egypt (temperature: 18 °C, humidity: 72 %, wind speed: 7 Km h^{-1}). Trials were started at the first half of November, using two faba bean varities (Nubaria 1 and Sakha 1). Elhamoul (clay soil in texture, electrical conductivity (EC) 6.8 deci-Siemens/meter (dS m^{-1}) and Baltim (sandy soil, EC 8.5 dS m^{-1}) were the two experimental locations within Kafr El-Sheikh Governorate. Samples of soil were air dried, crushed, sieved (2 cm sieve) and homogenized with distilled water (1: 5 w/v) to estimate their EC-values (Conductance meter, Model YSI ®) according to Dewis and Freitas (1970) and Manual of Salinity Research Methods (1992). Fields were prepared, divided into plots (3 x 0.6 m) and sown with 60 inoculated seeds per plot. Here,

efficiency of rhizobia was further tested to salinity, but in comparison with the mineral N-supply. Plots were fertilized with 25 and 100 % N as 31 and 125 Kg urea (45-46% N) fed.$^{-1}$, respectively. For 25 % N-supply, plots received only one dose at the sowing time. For 100 % N-supply, 125 Kg urea fed.$^{-1}$ was divided into 4 equal doses, added to plots at 0, 15, 30, 45 days after sowing. Plots inoculated with rhizobia (E_1 or F_1) received also 25 % N-supply as an activation dose. All plots were also fertilized in one recommended doses before sowing with super phosphates (Ca $(H_2PO_4)_2$, $2CaSO_4$); 150 Kg fed.$^{-1}$). Potassium was added as potassium sulfate (K_2SO_4; 50 kg fed^{-1}) at flowering time. Plants were irrigated to field capacity (40 and 36% for El-Hamoul and Baltim, respectively). Trials were performed in three replicates. Irrigation and other practices were carried out as recommended.

Sampling and determinations:

Parameters of plant growth (shoot length, dry weight of roots and shoots) and of the N_2-fixation (number and dry weight of nodules, N % and N-content of the shoots) were determined 70 days after sowing. Yield parameters (number and dry weight of seeds plant^{-1}, weight of 100 seeds, N % and crude protein) were also determined at harvest (135 days after sowing). Dry weight values were determined using the oven at 70^{o}C till fix weight. For determining N % and total N-content, Kjeldahl methods (Barbano *et al.,* 1990) were applied. Samples of shoots or seeds were dried at 70^{o}C and then 0.2 g of each was digested in 5 mL concentrated sulphoric acid and 1 mL concentrated perchloric acid (in a conical flask as described by Chapman and Parker (1963). The digested samples were completed to 50 mL using distilled water. Distillation was carried out using 40 % NaOH, and ammonia was received in 4 % boric acid solution. The distillates were then titrated with 0.02 M H_2SO_4 using a mixture of methyl red-and bromocrysol green as an indicator according to Black *et al.,* (1965). Based on dry weight and total N-contents, crude proteins were also calculated according to El-Akhdar (2009) as follows:

Total nitrogen content = N % * dry weight of plants

Crude protein % = N % in seeds * 6.25

Pesticidal toxicity:

To test their toxicity against rhizobia, Vitavax (fungicide), Ground-up (herbicide), Malathion (insecticide) and Vydate (nematicide), widly used in faba bean fields, were selected. Trade and common names, active ingredient, chemical formula, recommended dose and manufacturer of the tested chemical pesticides were done in Table (1).

Table (1): Trade and common names, active ingredient, chemical formula, recommended dose and manufacturer of the applied chemical pesticides.

Trade Name	Common Name	Concentration of active ingredient	Chemical formula	Recommended dose	Manufacturer
Fungicide (Vitavax)	Thiram (37.5%)	75% WP	(a) 5,6-dihydro-2-methyl-1,4-oxathi-ine-3-carboxanilide (b) tetramethyl thirum disulfide	1 g / L	Kimewtora
Herbicide (Ground-up)	Glyphocide	48% SL	N-(phosphonomethyl) glycine, iosoprophylammonium salt	2.5 L / 125 L water	Vapco
Insecticide (Malathion)	Malaphion	57% E.C	Diethyl (Dimethoxytghiophosphos phorylthio) succinate	100 cm^3 / 20 L water	Vicum organics
Nematicide (Vydate)	Oxamyl	24% SL	Methyl N`N`-dimethyl-N-[(methyl carbamoyl) oxy]-1-thiooxaminidate	3-5 cm / L	Dupont

Pesticides were used at concentrations of 1.00, 0.50 and 0.25 of the recommended dose. For this test, disc diffusion method (Thornberry, 1950) was used in three replicates *in vitro*. In this method, filter paper disc (1 cm diameter) was impregnated with 0.05 mL portion of the applied concentration, and placed on the surface of YMA- medium subsequently inoculated with rhizobia (10^8 CFU mL^{-1}). Trials were carried out in three replicates. Discs impregnated with sterilized distilled water acted as control. Degree of the inhibitory action was estimated by measuring the diameter of the inhibition zone surrounding the discs for 48 h after incubation period at 28-30^oC. Diameter of inhibition zone was measured, and percentages of inhibition (I %) were calculated according to the formula suggested by Topps and Wain (1957) as follows:

$$I\ \% = \frac{A - B}{A} \times 100$$

Where: I % = Percentage of inhibition
A = Mean growth diameter of control.
B = Mean growth diameter of the treatment.

Statistical analysis:

Complete randomized block was the main design of these trials. Data were statistically tested for the analysis of variance using IRRISTAT, version 3/93. Means were compared using LSD methods according to Steel and Torrie (1980), and Duncan's multiple range tests were applied for comparing means (Duncan, 1955).

RESULTS AND DISCUSSION

Nodulation and growth parameters:

Biological impacts of two salt-tolerant *Rhizobium* isolates, with and without mineral N-supply, using two faba bean cultivars were investigated. Field trials were performed in two salt-affected fields (Elhamoul and Baltim) in Kafr El-Sheikh governorate. Data in Table (2) indicate that remarkable increases in all parameters in which plants were treated with dual combinations of F_1 or E_1 with 25 % N-supply, in comparisom with the other treatments within Elhamoul location. Data show also supereiority of the separate treatment by both rhizobial isolates in comparison with the separate supply of the mineral N. Accordingly, a great potential of the biological N_2-fixation (BNF) was obtained due to *Rhizobium* inoculation compared with N-supply. So, the significant increase of nodular numbers due to E_1 and F_1 comparing with N-supply could be explained by formation of large varied sized nodules due to the biological nitrogen fixation (BNF). Similar findings were stated by Gaballah and Gomaa (2005), who found a positive increase of nodulation due to *Rhizobium* inoculation, compared with control. These results were also in agreement with Matiru and Dakora (2004), who reported that rhizobia naturally produce auxins, cytokinins, absicic acids, rhiboflavin, lipo-chito-oligosaccharides and vitamins. These molecules promote cell division and cell elongation which could induce plant growth.

For N_2-fixing parameters, number of nodules reached to 154.44 and 153.11 per plant due to mixing 25% N with either F_1 or E_1, respectively of Nubaria 1. The corresponding values of Sakha1 plants were 156.22 and 147.55 nodules per plant, respectively. These were positive reflected on accumulation of great amounts of nodular dried tissues. Due to combination between F_1 and 25 % N, nodular dry weight reached 1.00 and 0.94 g plant^{-1} for Nubaria 1 and Sakha 1, respectively. These could be attributed to accumulation of great percentages of nitrogen (data not shown) and total N-content in the shoot tissues. Similar positive effect was observed for Baltim but with less magnitude, indicating the suppressing role of high salinity of Baltim soils on indigenous rhizobia in plant rhizosphere. Resalts are in agreement with Ghazi (2006), who reported that number and dry weight of nodules and dry weight of shoots were increased due to *R. leguminosarum* and its dual with15 kg N fed^{-1} as ammonium sulfate. Reduction in N_2-fixing activity by salt stress is usually attributed to a reduction in cytosolic protein production, specifically leghemoglobin by nodules (Kapulmik *et al.,* 1989). So, the harmfull effect of soil salinity reported by Cordovilla *et al.* (1995) and Cordovilla *el al.,* (1999) against growth, nodulation and N-accumulation of faba bean was also reduced by inoculation with salt-tolerant rhizobium isolates.

Table (2): Effect of *Rhizobium leguminosarum* bv. *viciae* (isoates F_1 and E_1) and N-supply on nodulation, growth parameters and total N-content of faba bean cultivars (Nubaria 1 and Sakha 1) in Elhamoul and Baltim fields.

Treatment	N-supply %	No. of nodules plant^{-1}	Dry weight g plant^{-1}			Total N-content mg plant^{-1}
			Nodules	Shoots	Roots	
		Nubaria 1 (Elhamoul)				
Uninoculated	0	19.44 ab	0.18 a	4.85 a	1.99 a	78.09 a
F_1	0	130.33 c	0.85 bc	11.35 de	3.75 c	246.29 de
E_1	0	124.11 c	0.86 bc	11.89 e	4.25 cd	263.96 e
Uninoculated	25	30.78 b	0.23 a	9.04 bc	2.79 b	165.43 b
F_1	25	154.44 d	1.00 e	15.55 f	6.04 e	360.76 fg
E_1	25	153.11 d	1.01 e	16.17 f	6.12 e	388.08 g
Uninoculated	100	23.22 ab	0.19 a	11.15 de	3.01 b	224.12 cd
		Sakha 1 (Elhamoul)				
Uninoculated	0	17.44 a	0.18 a	5.42 a	2.11 a	83.47 a
F_1	0	129.22 c	0.84 b	9.84 bcd	4.50 d	206.64 c
E_1	0	125.00 c	0.90 bcd	11.24 de	4.25 cd	239.41 de
Uninoculated	25	28.44 ab	0.23 a	8.55 b	2.92 b	146.21 b
F_1	25	156.22 d	0.94 cde	16.54 f	5.95 e	370.49 fg
E_1	25	147.55 d	0.96 de	15.04 f	5.91 e	342.91 f
Uninoculated	100	23.89 ab	0.20 a	10.45 cde	3.12 b	204.82 c
		Nubaria 1 (Baltim)				
Uninoculated	0	13.44 a	0.15 ab	4.18 a	2.27 ab	64.79 a
F_1	0	136.78 c	0.63 cd	10.19 ef	4.42 cd	216.03 fg
E_1	0	137.11 c	0.64 cd	10.39 efg	4.64 cd	227.54 g
Uninoculated	25	34.11 b	0.21 b	7.13 bc	2.79 ab	124.78 bc
F_1	25	177.45 e	0.74 e	13.45 hi	5.92 e	305.32 i
E_1	25	178.11 e	0.75 e	14.22 i	5.75 e	331.33 j
Uninoculated	100	10.55 a	0.11 ab	8.47 cd	3.05 b	158.39 d
		Sakha 1(Baltim)				
Uninoculated	0	16.55 a	0.15 ab	3.67 a	2.14 a	52.48 a
F_1	0	129.00 c	0.56 c	9.03 de	3.84 c	187.82 e
E_1	0	129.11 c	0.58 c	7.53 bcd	4.75 d	158.88 ef
Uninoculated	25	29.11 b	0.20 ab	6.51 b	2.77 ab	106.76 b
F_1	25	165.67 d	0.72 de	11.93 gh	5.95 e	262.45 h
E_1	25	173.67 de	0.75 e	11.98 gh	5.75 e	265.96 h
Uninoculated	100	10.44 a	0.10 a	7.94 bcd	2.84 ab	141.33 cd

Means number in the same column ,followed by the same letters are not significantly different according to DMRT at 0.05 levels.

Seed productivity and crude proteins:

At harvest, number of seeds per plant, dry weight of seeds (data not shown), dry weight of 100 seeds, N_2 % and crude proteins of seeds were shown in Table (3). It shows that the deleterious effect of soil salinity was reduced with either F_1 or E_1 inoculations, indicating higher efficiency of the symbiotic N_2-fixation process. Data are in accrordence with Praxedes *et al.* (2010), who stated that N-concentration of the soil strongly affects on growth and productivity of the plants under salt-stress conditions. Dual combinations of F_1 or E_1 with 25 % N resulted in higher values of seed index parameters and N_2 % in both faba bean varities within Elhamoul fields. It is positive reflect on protein levels of the seeds. Similar behaviour was also observed in Baltim, but with fewer magnitudes. The present results were previously supported by

Delgado *et al*., (1993) who obtained higher seed yield and protein contents of faba bean via the dual supply of N-fertilizer and *R. leguminosarum* inoculation. Also, Atwa *et al*., (2009) found reduction in weight of 100-seeds and seed protein content of faba bean with increasing soil salinity. Crude proteins were significantly increased by inoculation with salt-tolerant isolates of *R. leguminosarum* bv. *viciae* under high level of salinity stress (Hussain *et al*.,2002). Therefore, salt-tolerant rhizobial isolates used in this study were successfully established within the salt-affected soils with great potential of biological N_2-fixation (BNF) of faba bean plants.

Table (3): Effect of *Rhizobium leguminosarum* bv. *viciae* (isoates F_1 and E_1) and N-supply on seed productivity and crude proteins of faba bean cultivars (Nubaria 1 and Sakha 1) in Elhamoul and Baltim fields.

Treatments	N-supply %	Dry weight of 100 seeds (g)	N_2 (%)	Crude protein (%)	Dry weight of 100 seeds (g)	N_2 (%)	Crude protein (%)
		Nubaria 1 (Elhamoul)			Nubaria 1 (Baltim)		
Uninoculated	0	56.07 b	2.75 a	17.17 ab	51.72 b	2.55 a	15.96 a
F_1	0	80.88 e	3.34 de	20.87 cd	79.15 gh	3.27 cd	20.42 cde
E_1	0	81.57 e	3.40 e	21.25 cd	80.47 h	3.35 d	20.94 de
Uninoculated	25	71.90 d	3.08 bc	19.26 abc	69.47 f	2.78 b	17.38 abc
F_1	25	94.31 f	4.11 fg	25.69 e	87.36 i	3.88 fg	24.25 f
E_1	25	95.45 f	4.21 g	26.33 e	88.94 i	3.96 g	24.75 f
Uninoculated	100	80.14 e	3.23 d	20.19 bc	79.64 h	3.18 c	19.88 bcd
		Sakha 1 (Elhamoul)			Sakha 1 (Baltim)		
Uninoculated	0	48.92 a	2.69 a	16.81 a	45.10 a	2.46 a	15.36 a
F_1	0	70.57 d	3.25 de	20.31 de	66.16 de	3.18 c	19.88 bcd
E_1	0	71.53 d	3.28 de	20.50 bc	67.31 ef	3.21 cd	20.06 bcd
Uninoculated	25	64.48 c	2.99 b	18.69 abc	60.86 c	2.69 b	16.81 ab
F_1	25	80.59 c	3.99 f	24.96 e	76.058 g	3.71 e	23.19 ef
E_1	25	82.43 e	4.04 f	25.25 e	77.59 gh	3.76 ef	23.52 ef
Uninoculated	100	66.53 c	3.21 cd	20.06 bc	63.79 cd	3.12 c	19.52 bcd

Means number in the same column, followed by the same letters are not significantly different according to DMRT at 0.05 levels.

Pesticidal toxicity:

To test their toxicity, effect of Vitavax, Ground-up, Malathion and Vydate were *in vitro* evaluated against growth of *R. leguminosarum* bv. *viciae* F_1, wherase E_1 was excluded because of the similarity of their behaviour. Table (4) showed large toxicity of the recommended doses of all tested pesticides against colonization of rhizobia, in comparison with the other concentrations. Large diameter of inhibition zone reached 4.20 cm and was obtained due to use 1 g L^{-1} Vitavax with strongly inhibitory effect reached 46.67 %. On the other hand, utilization of 0.625 L^{-1} of Ground-up has the lowest inhibitory effect (18.56 %) against rhizobia with little inhibition zone of 1.67 cm diameter.

Table (4): Toxicity of different concentrations of the tested pesticides against growth of *Rhizobium leguminosarum* bv. *viciae* isolate F_1 under laboratory conditions.

Pesticide	Concentration	Diameter of inhibition zone (cm)	Inhibition (%)
Control	0.00	0.00 a	0.00
Vitavax (g L^{-1})	1.00 *	4.20 i	46.67
	0.50	3.03 h	33.67
	0.25	2.40 ef	26.67
Ground-up (L 125 L^{-1})	2.50 *	2.83 gh	31.44
	1.25	2.20 cde	24.44
	0.625	1.67 b	18.56
Malathion (mL 20 L^{-1})	100.00 *	2.60 fg	28.89
	50.00	2.30 def	25.56
	25.00	2.00 cd	22.22
Vydate (mL L^{-1})	5.00 *	2.90 gh	32.22
	2.50	2.37 ef	26.33
	1.25	1.93 bc	21.44

Means numbers in the same column followed by the same letters are not significantly different according to DMRT at 0.05 levels. * Recommended dose.

To test performance of theses pesticides, expermintal data were fitted and slopes were araised using Microcal Origin Program (Fig. 1).

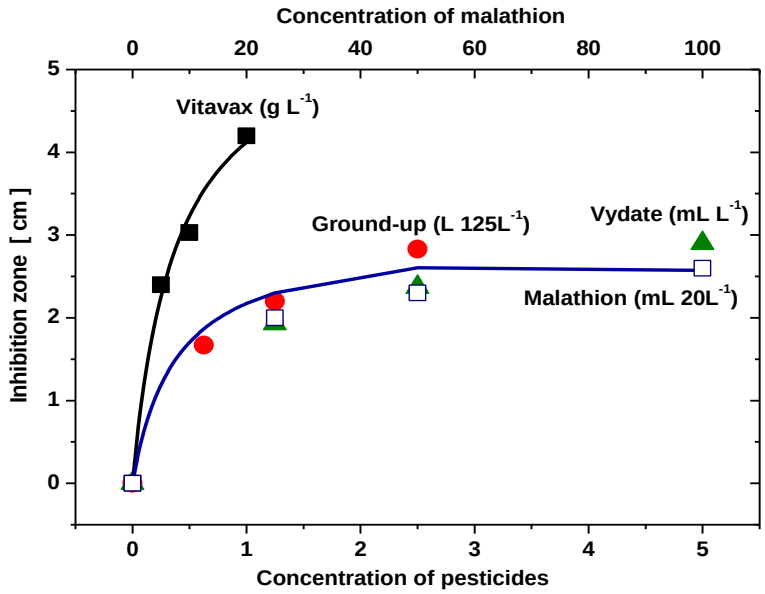

Fig. (1): Fitting slope of the experimental data of inhibition zone recorded by Vitavax, Ground-up, Malathion and Vydate against *R. leguminosarum* bv. *viciae* (F_1). Symbols = experimental data & Lines = Fitting data

Results indicate that Vitavax did not reach yet its maximum inhibitory effect against rhizobia. Therefore, higher dose of Vitavax is not recommended to avoide its harmfull effects against the symbiotis N_2-fixation process. Similar slope of Ground-up, Malathion and Vydate was obsearved, indicating constant effect and no further inhibition will be induced with increasing their doses (dose limitation). These results are in agreement with Fisher and Hayes (1981), who found that Captan (fungicide) has dangerous effect against *Rhizobium*-legume symbiosis and nodulation and N_2-fixation were reduced. Mallik and Tesfai (1985) and Rennie *et al*. (1985) indicated that Thiram and Captan are harmful to nodulation and N_2- fixation of several grains and forage legumes**.** Lal (1990) found that Captan was highly toxic for rhizobia even at low concentrations, followed by Mancozeb, Thiram and Vitavax, but Benlate, Dorsal and Topsin were reported to be less toxic.

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فعالية التلقيح بعزلات ريزوبيوم (E_1 & F_1) تتحمل الملوحة والتسميد بالنيتروجين المعدنى على نباتات الفول البلدى وإختبار تحمل العزلة F_1 لسمية بعض مبيدات الأفات

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قورنت قابلية العزلات البكتيرية المتحملة للملوحة (F_1 و E_1) من *Rhizobium leguminosarum* biovar *vicea* للتطبيق مع التسميد بالنيتروجين المعدنى على نباتات الفول البلدى تحت ظروف ملوحة التربة بالحقل. وقد أوضحت النتائج المتحصل عليها ان هناك فروقا معنوية لكل من طول الساق، الوزن الجاف للمجموع الخضري والجذري مقارنة بالنباتات المعاملة بالنيتروجين وكذلك بالكنترول. وكانت النتائج أكثر وضوحا بإستخدام تركيبات مزدوجة من F_1 أو E_1 مع 25٪ تسميد نيتروجينى. وقد تحسنت معايير التثبيت النيتروجينى و محصول البذور و مكوناتها من البروتين الخام لكل من صنفى الفول (نوبارية 1 و سخا 1) نظرا لتفوق المعالجات المزدوجة. وعليه، فإن إستخدام عزلات ريزوبيا متحملة للملوحة يمكن أن يعزى إليه قدرة نباتات الفول فى التغلب على التأثيرات الضارة لملوحة التربة على نمو والعقد الجذرية و إنتاجية بذور نباتات الفول. من ناحية أخرى، وصل أكبر قطر لمنطقة تثبيط النمو إلى 4.20 سم وهو ما يمثل إعاقة لنمو الريزوبيوم بما يعادل 46.67٪ بأستخدام 1 جرام لتر$^{-1}$ من Vitavax تحت الظروف المعملية. وأظهرت نتائج تناسب ميل القيم التجريبية تأثير كابح ثابت لكل من Ground-up و Malathion و Vydate مع زيادة تركيزها نحو الريزوبيوم. وقد نتج ميل تصاعدى حاد بسبب المعاملة بـ Vitavax , دلالة على أن إحداث تثبيط أخر عند زيادة الجرعة المضافة من المبيد سيكون أمرا متوقعا.

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