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Original Article

Impact of Compost and Microbial Inoculants on Nodulation, Growth and P-uptake of Faba Bean (*Vicia faba*) Grown on a Calcareous Sandy Soil

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Abstract

A pot experiment was conducted during season 2020/2021 to study the effect of compost application and microbial inoculants on growth and p-content of faba bean (*Vicia faba*) c.v. Giza-834 plants grown on sandy calcareous soil. The experimental treatments were arranged as split plots with 8 replications. The main plots were devoted to compost application at a rate 5 t/fed. (Without compost and with compost), whereas the subplots were assigned for microbial inoculants: 1, uninoculated; 2, inoculated with *Bacillus polymexia*; 3, inoculated with yeast strain *Saccharomyces cervisiae*; 4, inoculated with *Bacillus polymexia* + yeast strain *Saccharomyces cervisiae*. Faba bean Seeds of all treatment were inoculated with *Rhizobium leguminosarum*. The obtained results of shoots and roots, fresh and dry weights, N and P content in shoots, as well as nodules numbers were significantly increased by compost application compared with control (without compost). The microbial inoculation treatments (rhizobia + either Yeast or *Bacillus*) were also significantly affected the shoots and roots, fresh and dry weights, N and P content in shoots, as well as nodules numbers. Also, Signification positive interactions were recorded between compost application and the microbial seed inoculation treatment (R + Yeast) on nodules number and their dry weights, root and shoot fresh and dry weights, N and P content in shoot, seed yield and N and P content in seed and straw.

***Keywords**: Inoculation، Compost، Calcareous Soil، Phosphorus، Faba bean.*

Introduction

Calcareous soils are spread in arid and semiarid regions (Taalab *et. al* 2019). In Egypt, the calcareous soils constitute about 25-30% of the total area according to Ministry of Agriculture estimation (Wahba *et. al*., 2019). Calcareous soils are characterized by the presence of high content of calcium carbonate ($CaCO_3$) in the parent material and an accumulation of lime(FAO 2022, Wassif 2021). Phosphorus fertilizers are fixed by $CaCO_3$ through adsorption and precipitation, resulting in an efficiency of less than 20 % (Vassilev and Vassileva, 2003). P is an essential macroelement for plants, yet the total concentration of P in soils ranges from 0.02 % to 0.5 % and average of

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approximately 0.05 %., the variation being largely due to differences in the weathering intensity and parent material composition. Thus, to increase the availability of P for plants, large number of fertilizers are usually used on a regular basis, yet after application, a large proportion of the phosphorus fertilizer is quickly transferred to an insoluble form (Omer, 1998).

In the recent years many techniques for solubilization of insoluble P sources have been suggested, with increasing on application of P-solubilizing microorganisms (Selvakumar *et al*., 2013).The ability of phosphate-solubilizing microorganisms to solubilize P complexes has been attributed to production of organic or inorganic acids, acidification, ion chelation, and exchange reactions (Rajankar *et al*. 2007). Application of compost, organic manures, or plant residues improved plant growth and yield of crops grown on calcareous soils due to improving physico-chemical characteristics, solubilization of fixed phosphorus and activation of the microbial community of soil. (Nishanth and Biswas, 2008).

This research was conducted to assess the effect of compost application and microbial inoculants on nodulation, growth and P-content of faba bean grown on sandy calcareous soil.

Materials and Methods

A pot experiment was conducted during season (2020-2021) to study the effect of compost application and microbial inoculants on growth and p-content of faba bean (*Vicia faba*) c.v. Giza-834 plants grown on sandy calcareous soil. Experiment was conducted in polyethylene bag pots (25 cm in diameter and 30 cm higher) containing 5 kg of sieved sandy calcareous soil collected from El-Ghorieb farm , Faculty of Agriculture, Assiut University. Some physical and chemical characteristics of representative soil sample of the pot experiments are shown in Table (1). The experimental treatments were arranged as split plots on the basis of a randomized complete block design (RCBD) with 8 replications. The main plots were devoted to compost application at a rate 5 t/fed. (Without compost and with compost), whereas the subplots were assigned for microbial inoculants: 1, uninoculated; 2, inoculated with *Bacillus polymexia*; 3, inoculated with yeast strain *Saccharomyces cervisiae*; 4, inoculated with *Bacillus polymexia* + yeast strain *Saccharomyces cervisiae*. Faba bean Seeds of all treatment were inoculated with *Rhizobium leguminosarum*. Superphosphate (15.5% P) was added to surface layer of the soil at a rate 0.5 g/pot, and mixing them with soil before cultivation . Five seeds of fababean were placed in each pot, and then thinned to three plants after germination. Nitrogen fertilization, in the form of urea (46.5 % N) was applied

At rate of 20 kg N/feddan after 20 days from transplanting and the pot were irrigated every 2-4 days with a tap water.

Table 1. Some physical and chemical characteristics of soil sample of the pot experiments

Soil property	Value
Particle size distribution	
Sand %	92.01
Silt %	4.72
Clay %	3.27
Texture	Sandy
EC (1:1) d S/ m	1.12
pH (1:1)	8.50
Total calcium carbonate %	16.95
Organic matter %	0.12
Soluble cation and anions (1:1) meq/100 g soil:	
Ca^{++}	0.26
Na^{++}	0.54
K^{+}	0.05
Cl^{-}	0.52
$CO_3^{--} + HCO_3^{-}$	0.46
Total nitrogen %	0.02
$NaHCO_3$-extractable p (ppm)	4.86

The organic compost used in the treatment:

The organic compost used in this investigation is commercial named: (Al-Zahraa Vegetarian Compost 100%) produced by Beni Suef Organic Fertilizer Company.

Some chemical characteristics of compost used in the pot experiment are shown in Table (2).

Table 2. Chemical composition of the compost used in this experiment

Properties	Value
pH (1:10)	7.50
E.C dS/m(1:10)	5.50
C%	11.26
N%	0.96
P%	0.80
K%	1.30
O.M%	19.41
C/N ratio	11.73
Fe (ppm)	1500
Cu (ppm)	160
Mn (ppm)	100
Zn (ppm)	60

Microbial inoculants used

Bacillus polymyxa strain was obtained from Desert Research Center, Egypt that is used in large-scale production of biofertilizer called "phosphorine".

Soil yeast strain (*Saccharomyces cervisiae*) that was previously isolated from composite sample of the clayey soil (Mohamed, 2006).

Rhizobium leguminosarum isolated from root nodules of faba bean, was used in this study.

Preparation of microbial cultures and seeds treatment

The broth cultures of the microbial strains used were prepared as follow;

Rhizobium leguminosarum was cultured on yeast extract-mannitol broth (Trinick and Parker, 1982), for 5 days at 28 ºC on a rotary shaker (150 rpm).

Bacillus polymyxa strain was grown on nutrient broth (NB) medium for 5 days at 28 ºC.

Yeast strains *Saccharomyces cervisiae* was grown for 5 days at 25ºC on the malt extract broth medium, , of the following composition: Malt extract, 30 g; Peptone, 5 g , and 1000 ml distilled water (Lodder and Kreger-van Rij,1967).

For inoculant preparations sterilized peat moss was used as a carrier. The dry pulverized peat moss was neutralized to pH 7 with calcium carbonate and calcium hydroxide, and divided in batches of 30g each in polyethylene bags and autoclaved for 30 minutes at 121 ºC on three successive days. Broth culture was used per 30 g of the sterilized carrier as aliquots of 15 ml of *Rhizobium*, *Bacillus polymexia* or yeast strain *Saccharomyces cervisiae*. Before seed inoculation, in case of mixed inoculation, the single peat inoculant was mixed in equal weights just.

The seeds of each treatment were inoculated by adding 5 ml of carboxymethyl cellulose (CMC) solution and after mixing; the peat inoculant was added and thoroughly mixed with the seeds. The inoculant was added to seeds at a rate of 15 g/100g seeds. Peat inoculant contained 10^7 viable cells of yeast strain/g and 10^8 viable cells of *Bacillus polymexia* /g.

Plant sampling and growth measurements

Four replicates pots from eight were taken after 45 days from planting; the number of nodules, their fresh and dry weights, fresh and dry weights of shoots and roots were recorded. The other four replicates were taken after 110 days from planting and total, dry and seeds yield per pot were determined. Dried shoots at 70 ºC were ground and submitted to the acid-digestion using a 2:1 H_2SO_4:H_2O_2 acid mixture for the determination of nitrogen and phosphorous content. Phosphorus was determined by the chlorostannous phosphor-molybdic acid method using "Spectronic 20, Bauch & Lomb" colorimeter. The N content of the dried shoots and seed was measured by the semi-micro-Kjeldahl technique (Bremner and Mulvaney, 1982).

Statistical Analysis

The data were analyzed using a two-way analysis of variance (ANOVA) using the Co-Stat Version 6.4 software for statistics (2008). Significant and non-significant differences means were compared using the least significant difference (L.S.D) test at P = 0.05 (StatSoft 1995).

Result and Discussion

Main effects of the compost application and microbial inoculants on growth, yield, N and P content of faba bean

Data in Table (3) show the main effects of the compost application and microbial inoculants on growth, nitrogen and phosphorus content of faba bean. The obtained results of shoots and roots, fresh and dry weights, N and P content in shoots, as well as nodules numbers were significantly increased by compost application compared with control (without compost). Whereas compost application induced the following increases in shoot dry weight, root dry weight, nodule numbers: 8.78 %, 8.94 %, and 25.57% respectively. Also, compost application induced the following increases in N and P content in shoots 18.13 % and 19.66 % respectively.

Table 3. Faba bean growth on sandy calcareous soil in pots as affected by soil compost application and seeds inoculations by different microbial strains*

Tested factor	Treatments	Root nodules /pot			Root wt. (g/pot)		Shoot wt. (g/ pot)		Total content in shoots (g/p)	
		No.	Fresh	Dry	Fresh	Dry	Fresh	Dry	N	P
compost application	Without	18.58	0.64	0.10	40.96	5.93	35.13	4.01	10.92	1.17
	25 (g/pot)	23.33	0.66	0.11	42.73	6.46	37.18	4.37	12.90	1.40
L.S.D. 0.05		2.71	ns	ns	1.39	0.53	1.19	0.22	1.41	0.13
Seeds inoculation treatments	Rhizobium	18.00	0.7	0.10	39.58	5.78	36.17	3.98	10.63	1.06
	R+Yeast	22.67	0.71	0.11	45.55	6.75	37.57	4.42	12.58	1.31
	R+Bacillus	22.00	0.52	0.09	42.10	6.17	35.47	4.16	11.38	1.36
	R+Y+ B	21.17	0.68	0.12	40.15	6.08	35.40	4.19	12.05	1.4
L.S.D. 0.05		1.6	0.1	0.01	1.41	0.65	1.3	0.53	ns	0.15

* Each value is a mean of 3 replicates, ns: not significant

As to plants macronutrients N and P content, they were affected positively by the addition of compost comparing to the control. This finding is in agreement with Roghanian *et al*. (2012). Compost contains macro and micronutrients, which are required for plant growth. Nitrogen, phosphorus and potassium are the nutrients, which are utilized, in relatively big quantities by plants. The addition of organic matter to soil, especially in the form of compost, results in increased mineralization of nitrogen and micronutrients (Dick and McCoy, 1993).

The microbial inoculation treatments (*rhizobia* + either *Yeast* or *Bacillus*) also significantly affected the shoots and roots, fresh and dry weights, N and P content in shoots, as well as nodules numbers (table3). The mixed inoculation treatments containing *Rhizobium* + yeast produced further promotive effects on vegetative plant growth, nodule number, and nitrogen and phosphorous contents in shoots as compared with the single inoculation with *Rhizobium*. The improvements by microbial inoculation are maybe due to yeast hormonal secretion like indole acetic acid (IAA) on root growth and lateral roots leading to increased nodulation and nutrient uptake and subsequently increased yield. Also, by increasing the solubilization of mineral nutrients like P and Fe (Mohamed and

Metwally, 2014). Tuladhar and Subba Rao (1985) found that mixed inoculation of *Rhizobium trifolii* with *S. cerevisiae* bring about significant increases in the nodules number, length of plants and dry weight of Egyptian clover (*T . alexandrinum*). *Co*-inoculation of common bean with *R. leguminosarum* and *S. cerevisiae* caused significant increases in nodule numbers, nodule dry weight, N-uptake, straw and seed yield compared with the single inoculation with *Rhizobium* alone (Mohamed and Metwally, 2014). Metabolic activities of the genus *Bacillus* are known to result in acid production, but do not produce hormons or vitamins or chelating agents that affect P or N uptakes (Abo-Bakre , 2003).

Data in Table (4) show the main effects of the compost application and microbial inoculants on seeds and straw yield, N and P content in seeds and straw. The obtained results of seeds yield were significantly increased by compost application compared with control (without compost). Also, at harvest highly significantly increases in seed yield were obtained by mixed inoculations of faba bean seeds with (rhizobia + either Yeast or *Bacillus*) compared with rhizobia alone (Table4). But straw yield was not significantly increases by any microbial inoculation treatment. At harvest, both N and P content in straw and seeds were significantly increased by compost application table (4) and Fig. (1).

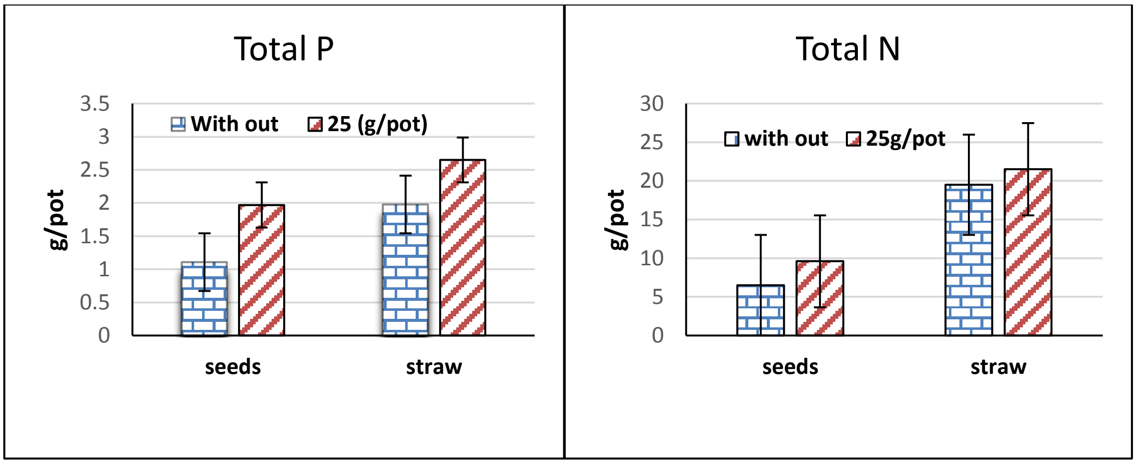

Fig. 1. Effect of compost application on total P and N contents in Seeds and straw of faba bean at harvest

Nitrogen and phosphorus contents in straw and seeds yields were significantly increased by the different microbial inoculation treatments. The highest positive effects were recorded in the treatment inoculations of (*rhizobia* + Yeast) followed by (*rhizobia* +*Bacillus*), and the lowest effect in (*rhizobia* + Yeast + *Bacillus*) (Table 4) and Fig.(2).

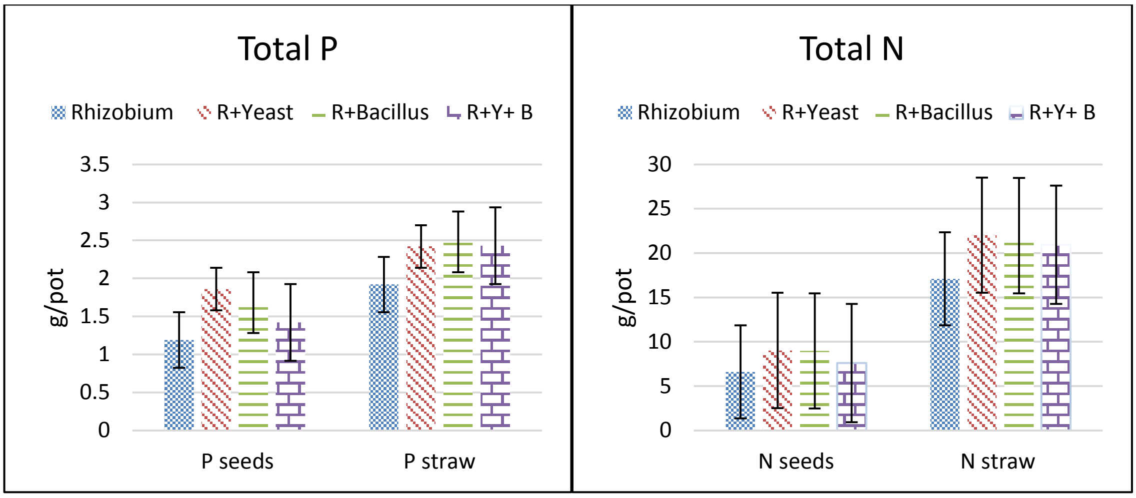

Fig. 2. Effect of microbial seed treatments on total P and N contents in straw and seeds of faba bean (g/pot) at harvest of plant.

Highly significantly increases in seed yield by compost application could be attributed to the supplying energy and carbon sources for growth and multiplication of the microorganisms around the germinated seeds and formation of metabolites and organic acids that increase root growth and promote the vegetative growth of the formed seedling, and consequently increase the content of mineral and organic metabolites produced by the activated microbial community around the germinated seeds. Application of compost leads to formation of strong root deeply penetrating the sandy textured soil and absorb the available nutrients, P and other elements released in available forms by the stimulated microbial community (Hargreaves *et al*., 2008; Lakhdar *et al*., 2009; Castillejo and Castello, 2010).

Table 4. seed and straw yields of faba bean grown on sandy calcareous soil in pots as affected by soil compost application and seeds inoculation treatments*

Tasted factors	Treatments	Total yield/pot(g/p)		N content in straw	N content in seeds	P content in straw	P content in seeds
		seeds	Dry straw				
Compost application	With out	2.06	7.28	19.5	6.5	1.98	1.11
	25g / pot	2.91	7.54	21.52	9.6	2.65	1.97
L.S.D 0.05		0.23	0.23	1.07	1.05	0.11	0.16
Seeds inoculation treatments	Rhizobium	2.22	6.77	17.09	6.6	1.92	1.19
	R+Yeast	2.65	7.65	22,04	9.02	2.48	1.86
	R+Bacillus	2.96	7.64	21.97	8.98	2.43	1.68
	R+Y+ B	2.31	7.30	20.94	7.60	2.42	1.42
L.S.D 0.05		0.38	Ns	5.01	2.05	0.41	0.43

* Each value is a mean of 3 replicates.

Interaction effects of the compost application and microbial inoculants on growth, yield, N and P content of faba bean

Data in Table 5 and 6 show the interaction effects of the compost application and microbial inoculants on growth, nitrogen and phosphorus content, seeds and straw yield, N and P content in seeds and straw. Signification positive interactions were recorded between compost application and the microbial seed inoculation treatment (R + *Yeast*) on nodules number and their dry weights., root and shoot fresh and dry weights., N and P content in shoot, seed yield and N and P content in seed and straw. The Signification positive interaction of compost application with (R + *Yeast*) during the vegetative plant stage continued till end of plant harvest at 110 days from sowing. It resulted as shown in Table (6) in production of highest straw and seed yield of faba bean ; as well as total N content in straw and seeds yields, and total P content in seeds and straws yield . Also, the inoculation treatment (R + *Bacillus*) recorded lower positive interaction effects with compost application on; straw and seed yields and total content of P in seed and straw Table (6).

Table 5. Interaction effects of soil compost application and seed inoculations by different microbial species on faba bean growth on sandy calcareous soil in pots*

Tested factors / compost application	Seeds inoculation treatments	Root nodules / Pot			Roots wt. (g/pot)		Shoot wt. (g/pot)		Total content in shoots (g/p)	
		No.	Fresh	Dry	Fresh	Dry	Fresh	Dry	N	P
Without compost	Rhizobium	17.67	0.58	0.10	37.73	5.59	36.08	3.84	9.77	0.98
	R+Yeast	17.67	0.67	0.09	45.06	6.7	37.13	4.31	12.13	1.21
	R+Bacillus	19.00	0.41	0.07	42.25	5.93	33.48	3.88	11.53	1.15
	R+Y+ B	20.00	0.63	0.11	38.81	5.51	33.83	4.04	11.24	1.34
25 (g/pot) Compost	Rhizobium	18.33	0.56	0.11	41.43	5.96	36.27	4.12	11.48	1.38
	R+Yeast	27.67*	0.73*	0.13*	46.03*	6.8*	38.01	4.52	13.03	1.42*
	R+Bacillus	25.00	0.62	0.11	41.95	6.4	37.45	4.41	13.23	1.57
	R+Y+ B	22.33	0.73	0.12	41.5	6.66	36.98	4.34	12.86	1.46
L.S.D.0.05		2.27	0.14	0.021	1.99	1.06	1.84	0.45	2.8	0.26

* Each value is a mean of 3 replicates.

Phat *et al.*(2019) reported that compost supplemented effects with plant growth promoting rhizobacteria (Nitrogen-fixing *Bradyrhizobium japonicum* and Phosphate-solubilizing (*Bacillus subtilis*), on three vegetables (sweet cabbage, pakchoi and mustard green) cultivated on arenosols reached to 50% amount of the chemical fertilizers on both biomass yield and trade productivity. Farrag and Bakr (2021) reported that the application of acid producing bacteria and yeast or their mixture with farmyard manure (FYM) was more effective than using FYM alone in improving of calcareous soil properties and enhancing wheat growth.

Table 6. Interaction effects of soil compost application and microbial seeds inoculation treatments* on yield of faba bean grown on calcareous soil in pots*

Tested factor / Compost application	Seeds inoculation treatments	Yield /pot (g/p)		N content in straw	N content in seeds	P content in straw	P content in seeds
		Seeds	Dry straw				
Without compost	Rhizobium	1.93	6.4	15.81	5.64	1.67	0.87
	R+Yeast	1.99	7.6	20.73	6.17	2.09	1.38
	R+Bacillus	2.31	7.4	21.41	7.3	2.08	1.27
	R+Y+ B	1.99	7.3	20.04	6.9	2.07	0.89
25 g / pot compost	Rhizobium	2.51	7.13	18.38	7.58	2.16	1.51
	R+Yeast	3.31*	7.7	23.31*	11.86*	2.75*	2.34*
	R+Bacillus	3.18	7.8	22.54	10.67	2.87	2.09
	R+Y+ B	2.63	7.3	21.84	8.28	2.8	1.95
L.S.D 0.05		0.45	0.48	2.15	2.09	0.52	0.32

* Each value is a mean of 3 replicates.

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تأثير إضافة الكومبست والتلقيح الميكروبي على نمو وامتصاص الفسفور لنبات الفول البلدى من الاراضي الجيرية الرملية

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الملخص

أجريت تجربة اصص خلال الموسم الزراعي 2020/ 2021 لدراسة تأثير إضافة الكومبست واللقاحات الميكروبية على نمو ومحتوى الفسفور في نبات الفول البلدي صنف جيزة 834 في الاراضي الجيرية الرملية. تم ترتيب المعاملات التجريبية على شكل قطع بعدد 8 مكررات. تم تخصيص القطع الرئيسية لاستخدام الكومبست بمعدل 5 طن / فدان (بدون سماد وبإضافة سماد) في حين تم تخصيص القطع الفرعية بإضافة معاملات التلقيح الميكروبي

1- بدون اضافة

2- اضافة باسيلس *Bacillus polymexia*

3-اضافة الخميرة سلالة *yeast strain Saccharomyces cervisiae*

4-اضافة *Bacillus polymexia + yeast strain Saccharomyces cervisiae*

وتم إضافة *Rhizobium leguminosarum* لجميع المعاملات

وقد أظهرت النتائج ما يلي بالنسبة للجذور والساق، الوزن الجاف والرطب ومحتوى النيتروجين والفسفور في الساق، وعدد العقد الجذرية زادت بشكل معنوي عند إضافة الكومبست

مقارنة بدون إضافة. كما اثرت معاملات التلقيح الميكروبي بالريزوبيا مع الخميرة او الباسيلس تأثيراً معنوياً على السيقان والجذور، والوزن الرطب والجاف، ومحتوى النيتروجين والفسفور في السيقان وعدد العقد الجذرية.

أيضا كان التأثير معنوي عند التفاعل بين إضافة الكومبست ولقاح الريزوبيا مع الخميرة على عدد العقد الجذرية ووزنها الجاف، والوزن الجاف والرطب للجذور والسيقان وغلة المحصول ومحتوى النيتروجين والفسفور في السيقان والبذور ومحتوى النيتروجين والفسفور في البذور والقش.