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Efficiency of Free and Encapsulated Yeast Strains as PGRs Producers on Faba bean Plants

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ABSTRACT

Twenty two yeast isolates from the rhizosphere of plants in different localities were tested to produce plant growth promoting substances and mobilized phosphate from insoluble tri-calcium phosphate. Three isolates have the ability to produce high amounts of plant growth regulators (PGRs) were selected and identified as *Candida middelhoveniana* 72, *Rhodotorula mucilaginosa* 151 and *Candida blankii* 375.

The encapsulation method described in this study can be effectively used to protect plant growth promoting yeast (PGPY) inoculum from adverse conditions of the soil for their successful establishment in the rhizosphere. Therefore, a field experiment was carried out at Giza Research Station, ARC, Giza, Egypt during winter season of 2013/2014 to study the effect of encapsulated growth promoting yeast species individual form or in a mixture ones compared to free cell inoculum on the growth and yield of Faba bean.

The obtained results recovered the highest total microbial, yeast, total asymbiotic and actinomycetes count with encapsulated and free cell of *C. middelhoveniana* 72 and *C. blankii* 375 during the two growth intervals, respectively. *C. middelhoveniana* 72 (encapsulated form) attained slightly more nodules number, nodules dry weight (g) and AM-colonization (%) than free cells form, whereas the other treatments recorded less efficiency. *C. blankii* 375 (encapsulated form) was the superior one to obtain more nitrogenase and dehydrogenase enzyme activities than other treatments. However, mixture of encapsulated yeasts recorded the highest activity of alkaline phosphatase enzyme. The maximum percentage of macronutrients (NPK) in plants obtained with *C. middelhoveniana* 72 free cells form except *C. blankii 375* which gave the highest K percentage only at 100 days. The highest percentage of NPK, crude protein and carbohydrates in Faba bean seeds were recorded by encapsulated form of *C. middelhoveniana* 72. The plant dry weight and consequently yield components were equal in value by encapsulated and free cells form of *C. middelhoveniana* 72 and *C. blankii* 375.

This study supports the trend to use yeasts as capsulated plant growth regulators for a new concept at different soils and temperatures for sustainable agriculture.

Key words: Plant growth regulators, yeast, encapsulation, phosphate solubilization

Introduction

Soil being a heterogeneous, predictable environment, the inoculated bacteria in a covenantal ways finds it often difficult to establish a niche for survival amongst the competitors and predators. The immediate response varies considerably depending on bacteria, some yeast species, plant species, soil type, inoculant density and environmental conditions resulting in a progressive decline in the inoculated microbial density thereby fails to elicit intended plant response (Young *et al.,* 2006).

Recently, several new dry inoculants formulations for agriculture, by encapsulation with various polymers, followed by drying, have been proposed including polyacrylamide based inoculants and sodium alginate where most of these inoculant carriers, which are delivered in powder form permit entrapment of living cells, their protection against various stresses during storage and their progressive release into the soil (Ivanova *et al.,* 2005).

Despite, the potentiality of these powders, as well as the liquid forms of inoculant are widely applied today they have some draw backs. To overcome the main drawbacks of the liquid or powdered inocula the use of encapsulated plant growth promoting some yeast species (macrocapsule) by using alginate as the encapsulating material as it forms micro beads instantaneously in the presence of polyvalent cations by binding the cation to guluronic acid units. Moreover, alginate beads are capable of entrapping sufficient numbers of yeast species (Zohar-perez *et al*., 2002).

The use of encapsulated cells for environment applications has several advantages over free cell formulation namely, protection from biotic and abiotic stresses, enhanced and survival and improved physiological activity (Weir *et al.,* 1995).

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The development of novel formulations is challenging task but regardless of whether the product is new or improved, the product must be stable during storage and transportation, easy to handle and apply, enhance the activity of the organisms in the field to be cost effective and practical (Young *et.al.,* 2006).

The present study demonstrates the improvement in the encapsulation of plant growth promoting yeast species using alginate as a carrier compared to free cell inoculum in a covenantal liquid form in promoting growth and yield of Faba bean under field trial.

Materials and Methods

Yeast isolates:

Samples from rhizosphere of tomato, maize, clover and Faba bean were collected from different localities, i.e. Giza, Minufia and El-fayom governorates, Egypt. A number of twenty two yeast isolates were obtained. These isolates were classified according to their biochemical and morphological characteristics.

The yeast species were isolated and maintained on malt extract agar medium (MEA) (Difco, 1985).

In-vitro screening of the yeast isolates:

All yeast isolates were tested for their quantitative capabilities to produce auxins and gibberellins by the methods described by Glickmann and Dessoux (1995) and Udagwa and Kinoshita (1961), respectively. Mineral phosphate solubilization (MPS) activity of the isolates was detected on tricalcium phosphate agar medium (Nautiyal, 1999).

Identification of the isolated yeast:

The most efficient yeast isolates that able to produce auxins, gibberellins and phosphate solubilization were selected and identified according to Barnett *et al*., (2000) using CBS database software.

Preparation of yeast strains culture:

Yeast strains were grown on glucose peptone yeast extract medium, (GPY) (Difco, 1985), the flasks (250ml) were inoculated with a loop full of yeast growth and incubated at 28ºC for 48h and used as preculture for standard inoculants.

The conical flasks containing 100 ml of GPY medium were inoculated with 5 ml of pre culture of each yeast strain and incubated at 28ºC for 48h on a rotary shaker at 150 rpm. The cultures were centrifuged for 30 min at 5000 rpm/min. The cell pellets were washed three times with saline solution (0.85% NaCl, w/v) and then used for the validation work.

Encapsulation process:

Sodium alginate solution (2%) was prepared by dissolving the powder in distilled water with agitation using magnetic stirrer at room temperature and autoclaved at 121ºC for 20 min. The cell pellets were suspended in 25ml of alginate and mixed thoroughly. This suspension was introduced in a syringe and extruded drop by drop through the needle by acting the syringe pump into a pre-cooled sterile 1.5% (w/v) $CaCl_2$ under mild agitation. The water soluble sodium alginate was converted into water insoluble calcium alginate beads. Thus instantaneously formed beads were allowed to harden for 3-6 h. at room temperature. Beads were collected by sieving and were washed several times with sterile water and stored at 4°C in 0.85% (w/v) saline solution for further studies. Alginate beads were prepared according to the method described by Bashan (1986) with modifications.

Enumeration of encapsulated yeast cells:

100 mg of encapsulated beads were suspended in 100 ml of phosphate buffer solution (pH, 7.0) followed by homogenization. The total number of released yeast was determined on GPY ager medium (Difco, 1985) by standard plate count method after incubating at 28ºC for 48h.

Field trial and experimental design:

A field experiment was conducted on 25th October, 2013/2014 (winter season) at the experimental research station ARC, Giza, Egypt. To evaluate the response of Faba bean to inoculation with yeast strains as PGRs either encapsulated or free cells culture forms.

The experimental treatments were arranged in complete randomized plots designed with three replicates as following:

1- NPK full (control) (T1)	2- *C. middelhoveniana* 72 capsule (T2)	3- *Rh. mucilaginosa* 151 capsule (T3)
4- *C. blankii* 375capsule (T4)	5- Mixture of tested yeasts capsules (T5)	6- *C. middelhoveniana* 72 free cells culture (T6)
7- *Rh. mucilaginosa* 151 free cells culture (T7)	8- *C. blankii* 375 free cells culture (T8)	9- Mixture of tested free yeasts (T9)

Each experimental plot area was 6 m^2 (3 m in length × 2m in width). The crop variety was Giza 843. The soil texture was clay loam having the following characteristics: coarse sand 11.2%, fine sand 19.8%, silt 35.6%, clay 33.4%, pH 7.3, EC 2.6 ($dS.m^{-1}$).

Each experimental plot consisted of 6 lines each of 3m long and 50cm width, the sowing was carried out on the two sides of each line, two seeds were placed on each hole, the distance between each hole 20cm apart.

Fertilization:

Nitrogen was applied in the form of urea (46% N) as an activity dose at a rate of 15 kg/fed. phosphorus (15.5% P_2O_5) was added as full recommended dose (150 kg/fed), treatments inoculated with yeast strains received half dose of phosphorus. Potassium was added as K_2SO_4 (48%) at a rate of 50 kg/fed into two equal doses, once after 35 days from sowing to help plants to tolerate the severe cold conditions and the other dose was applied directly before flowering stage.

Measurements:

Biological parameters

The population dynamics of total microbial count, yeast count and total actinomycetes in the rhizospheric zone of Faba bean roots were determined by the plate count technique according to (Reinhold *et.al.,* 1985). While total asymbiotic N_2-fixer populations in the rhizospheric zone of Faba bean were determined using most probable number (CFU/g rhizosphere) method described by Cochran (1950) at the two growth intervals 50 and 100 days of planting.

At 50 and 100 days after planting, nodulation was estimated by counting the number of nodules in plant roots and dry weight of nodules was also determined after drying at 60°C to constant weight. Moreover, the colonization percentage of native AM mycorrhizal fungi in plant root tissues was estimated according to Philips and Hayman (1970).

Enzyme activities

The activities of nitrogenase (µmole C_2H_4/g dry nod/h), dehydrogenase (µg TPF/g dry soil/day) and alkaline phosphatase (mg PNP/g dry soil/day) were determined according to Somasegaran and Hoben, (1994), Skujins (1976) and Tabatabai (1982), respectively, after 50 and 100 days.

Morphological parameters

Plant height (cm) and plant dry weight (g/plant) were measured after 50 and 100 days of sowing, respectively.

Physiological parameters

The percentages of NPK in Faba bean shoots were determined after 50 and 100 days, respectively, whereas the N, P and K percentages and crude protein (%) were also determined in Faba bean seeds according to Jackson (1973). Moreover, total carbohydrates (%) was determined according to (Dubois *et al*., 1956).

At harvest, Faba bean seeds yield (g/plant) as well as ton / feddan and number of 100 seeds were determined.

The significance of various treatments was evaluated by Duncan's multiple range tests at P value 0.05 (Duncan, 1955). Statistical analysis was made using a software package "Costat," a product of cohort software Inc. Berkley, California.

Results

In vitro screening of yeast isolates

Yeast isolates were evaluated in vitro for producing plant growth regulators (auxins and gibberellins) and assaying phosphate solubilization. Data presented in Table (1) revealed that varying levels of auxins and gibberellins were produced with different yeast isolates. The isolates Y72, Y151 and Y375 were able to produce high amounts of growth promoting substances. These isolates produced auxins being 30.67, 29.56 and 51.67 µg/ ml, respectively, gibberellins being 61.9, 46.5 and 56.8 µg/ ml, respectively. Only 9 yeast isolates showed positive reaction for solubilize tri-calcium phosphate on agar plate medium.

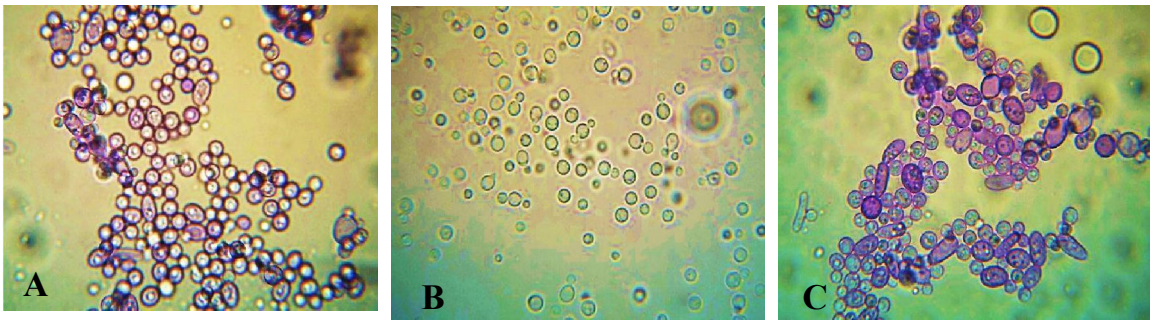
Identification of the selected yeast isolates

Yeast isolates that exhibited highly production of growth regulators and observed more efficient for solubilization of tri-calcium phosphate were selected and identified using morphological, biochemical and physiological methods using CBS database software. The three efficient isolates were identified as *Candida middelhoveniana* 72*, Rhodotorula mucilaginosa* 151 and *Candida blankii* 375.

Table 1: In vitro screening of yeast isolates for producing plant growth regulators and phosphate solubilization

No of	Gibberellins	Auxins	Phosphate	No of	Gibberellins	Auxins	Phosphate
Isolates	µg/ml		solubilization	Isolates	µg/ml		Solubilization
Y 72	61.9	30.67	+++	Y 376	47.2	5.45	++
Y 151	46.5	29.56	+	Y P6R	23.4	29.11	+
Y 375	56.8	51.67	+++	Y 66	41.1	8.45	-
Y 153	52.4	1.67	-	Y W1	41.0	3.33	-
Y 154	40.2	1.45	-	Y W2	31.4	9.78	-
Y 155	23.1	21.20	++	Y 150	8.3	8.50	-
Y 22	42.3	10.20	-	Y LB5	22.8	20.00	+
Y 43	29.7	11.22	-	Y 57	22.9	10.89	-
AMY 1	24.2	13.80	-	Y 47	9.0	6.85	-
AMY 2	36.1	6.45	++	Y 34	44.3	29.33	++
Y 152	31.2	7.86	-	Y 51	29.5	9.89	-

High: +++ Moderate: ++ Low: + Non: -



Candida middelhoveniana 72 *Rhodotorula mucilaginosa* 151 *Candida blankii* 375.

Fig 1: Microscopic morphology of the three identified plant growth promoting yeast species

Microbial population of Faba bean after inoculation:

Table (2) shows microbial populations with different applicable forms of yeast strains either encapsulated form or cell free cultures after 100 days of planting. Results clearly showed that the total microbial count in the rhizosphere of faba bean plants increased by the inoculation with *C. middelhoveniana* 72 more than all other yeast strain treatments after 50 and 100 days. The total microbial flora did not show a clear variation between other encapsulated yeasts and non-capsulate ones. The least total microbial count obtained with control treatment.

Table 2: Effect of encapsulated and free cells of yeast strains on different microbial populations in the rhizosphere of Faba bean plants at two growth intervals

Treatments	Total microbial count CFU × 10^6/g soil		Yeast count CFU × 10^5/g soil		Total asymbiotic N_2-fixers CFU × 10^6/g soil		Total actinomyctes CFU × 10^5/g soil	
	50 d	100 d	50 d	100 d	50 d	100 d	50 d	100 d
Control (T1)	21	36	2	5	0.14	0.20	2	7
C. middelhoveniana 72 (T2)	66	107	18	28	0.47	2.80	19	31
Rh. Mucilaginosa 151 (T3)	39	79	9	24	0.21	0.39	11	22
C. blankii 375 (T4)	49	84	16	35	0.26	2.80	16	27
Mix. of tested yeast (T5)	54	77	11	24	0.31	1.54	10	24
C. middelhoveniana 72 (T6)	68	90	20	25	0.62	2.50	16	28
Rh. Mucilaginosa 151(T7)	50	70	12	22	0.39	0.54	15	20
C. blankii 375 (T8)	75	91	19	31	0.40	1.80	19	24
Mix. of tested yeast (T9)	50	68	13	19	0.39	1.40	15	18

Encapsulated form (T2, T3, T4 andT5) Free cell form (T6, T7, T8 and T9)

For the yeast populations in Faba bean rhizosphic soil, it was obvious that *C. blankii* 375 treatments whether encapsulated or free cell forms recorded total yeast count higher than all other treatments especially after 100 days of planting. After 50 days *C. middelhoveniana* 72 recorded the highest populations and its value in free cells form was more than in encapsulated form whereas at 100 days where the values of total yeast were higher encapsulated form than free cells with *C. blankii* 375 where it exhibited 35 × 10^5 CFU/ml with encapsulated form and 31 × 10^5 CFU/ml with free cells, respectively.

In concern, the count of asymbiotic nitrogen fixers in faba beans rhizosphere, it was found that the inoculation with *C. middelhoveniana* 72 enhanced other asymbiotic nitrogen fixers that colonize the roots of Faba bean plants to exist in populations higher than those in soil and the populations increased with

encapsulated form especially at 100 days (2.8×10^6 CFU/ml) more than free cells form (2.5×10^6 CFU/ml), respectively.

The same trend was obtained for the total actinomycetes where *C. middelhoveniana* 72 recorded the highest count and the encapsulated form recorded 31×10^5 CFU/ml, whereas the non-capsulated form gave 28×10^5 CFU/ml at 100 days, respectively.

Data presented in Table (3) showed the inoculation with *C. middelhoveniana* 72 as a plant growth promoter followed by *C. blankii* 375 enhanced both native rhizobia and AM-fungi to colonize the roots of Faba bean plants represented on increase of both nodules number and dry weight besides the percentage of Arbuscular mycorrhizal fungi that colonized Faba bean roots. The encapsulated *C. middelhoveniana* 72 exhibited 115 nodule, 0.77g dry nodule weight and 92% mycorrhizal colonization at 100 days, respectively.

The same trend was recorded with *C. blankii* 375 in both time intervals except the higher nodule dry weight 0.79g at 100 days was observed with free cells form than all other treatments. The mixture treatment in both inoculation forms and periods recorded less figures whereas the control treatment gave the least results.

Table 3: Effect of encapsulated and free cells of yeast strains on nodulation and colonization of native mycorrhizal fungi of Faba bean plants at two growth intervals

Treatments	No. nodules (g/plant)		D. W of nodules (g/plant)		AM colonization (%)	
	50 d	100 d	50 d	100 d	55 d	100 d
Control (T1)	16	33	0.23	0.31	45	60
C. middelhoveniana 72 (T2)	88	115	0.61	0.77	83	92
Rh. Mucilaginosa 151 (T3)	66	82	0.43	0.52	70	75
C. blankii 375 (T4)	80	101	0.56	0.76	83	90
Mix. of tested yeast (T5)	78	98	0.52	0.69	73	81
C. middelhoveniana 72 (T6)	80	90	0.62	0.76	83	90
Rh. Mucilaginosa 151(T7)	75	79	0.47	0.53	60	70
C. blankii 375 (T8)	60	100	0.57	0.79	70	85
Mix. of tested yeast (T9)	56	85	0.54	0.66	63	70
LSD 0.05	4.952	4.919	0.036	0.034	4.539	4.75

Encapsulated form (T2, T3, T4 andT5) *Free cell form (T6, T7, T8 and T9)*

Enzyme activity:

The increase of the nitrogenase enzyme activity relys on the ability of plant growth promoting yeasts either free cells or encapsulated to enhance the native *Rhizobia* to fix atmospheric nitrogen inside the nodules. Results in Table (4) showed that encapsulated *Candida blankii* 375 attained more activity than free cells form and recorded the highest activity than all other treatments during the two growth intervals 50 and 100 days, respectively, where it recorded 20.42 and 48.75 µmole, respectively.

With respect to the activity of dehydrogenase enzyme, the same trend recorded as encapsulated *C. blankii* 375 still the unique one and gave the highest activity in both 50 and 100 days, respectively.

In the contrary, the mixture of encapsulated yeasts possessed the highest activity of alkaline phosphatase and its values were 46 mg at 50 days whereas at 100 days it increased to 53.50 mg, respectively.

Table 4: Effect of encapsulated and free cells of yeast strains on enzyme activity in rhizosphere of Faba bean plants at two growth intervals

Treatments	N_2 ase (µmole C_2H_4/h/nod D.W.)		Dehydrogenase (µgTPF/g dry soil/day)		Alk. Phosphatase (mg PNP/g dry soil/day)	
	50 d	100 d	50 d	100 d	50 d	100 d
Control (T1)	6.23	29.14	52.22	66.95	21.50	30.50
C. middelhoveniana 72 (T2)	19.33	42.67	116.60	133.44	33.39	42.80
Rh. Mucilaginosa 151 (T3)	13.77	35.81	76.84	92.28	29.77	36.62
C. blankii 375 (T4)	20.42	48.75	127.70	140.21	30.54	39.10
Mix. of tested yeast (T5)	16.56	39.19	98.19	112.80	46.00	53.50
C. middelhoveniana 72 (T6)	15.44	38.39	101.30	127.42	30.39	39.40
Rh. Mucilaginosa 151(T7)	18.22	32.28	89.42	108.81	27.85	34.56
C. blankii 375 (T8)	17.34	37.91	116.61	114.10	35.62	40.26
Mix. of tested yeast (T9)	16.60	36.26	86.69	101.00	42.69	50.50
LSD 0.05	0.25	0.154	0.856	3.383	3.235	3.334

Encapsulated form (T2, T3, T4 andT5) *Free cell form (T6, T7, T8 and T9)*

Macroelements in plants:

The increase of the uptake of macroelements represented in nitrogen, phosphorus and potassium is a sign of the healthy state of the plants. *C. middelhoveniana* 72 in both encapsulated and free cells attained optimum percentages of nitrogen and *C. middelhoveniana* 72 in liquid form gave 2.76% at 50 days whereas encapsulated *C. middelhoveniana* 72 recorded 2.81% at 100 days.

For K percentages (free cells of *C. middelhoveniana* 72) attained the highest percentage 2.81% at 50 days while at 100 days whereas encapsulated *C. blankii* 375 recorded 2.96% better than encapsulated *C. middelhoveniana* 72 and gave more figures than the control and all other treatments. We should take in our consideration the usefulness of some yeast strains as phosphorus and potassium solubilizers to adopt more studies in this field.

Table 5: Percentages of macronutrients (NPK) in Faba bean plants grown in soil inoculated with encapsulated and free cells of yeast strains.

Treatments	Macroelements in plants (%) after					
	50 day			100 day		
	N	P	K	N	P	K
Control (T1)	2.04	0.143	2.05	2.15	0.186	2.15
C. middelhoveniana 72 (T2)	2.6	0.219	2.66	2.81	0.212	2.94
Rh. Mucilaginosa 151 (T3)	2.25	0.168	2.46	2.55	0.198	2.90
C. blankii 375 (T4)	2.51	0.175	2.71	2.70	0.211	2.96
Mix. of tested yeast (T5)	2.34	0.198	2.44	2.59	0.205	2.83
C. middelhoveniana 72 (T6)	2.76	0.205	2.81	2.71	0.241	2.85
Rh. Mucilaginosa 151(T7)	2.35	0.186	2.43	2.44	0.187	2.56
C. blankii 375 (T8)	2.65	0.198	2.63	2.62	0.221	2.77
Mix. of tested yeast (T9)	2.45	0.197	2.51	2.48	0.199	2.60
LSD 0.05	0.149	0.013	0.258	0.105	0.017	0.134

Encapsulated form (T2, T3, T4 andT5) *Free cell form (T6, T7, T8 and T9)*

Encapsulated *C. middelhoveniana* 72 recorded the highest available phosphorus percentage 0.219 % whereas in free cells of it exhibited the highest P being 0.241% at 100 days

Macronutrients, crude protein and total carbohydrates percentages as shown in Table (6) revealed that, there was a significant increase in NPK values in Faba bean seeds especially with encapsulated *C. middelhoveniana* 72 which recorded more values than control and other treatments even the mixture ones. This treatment recorded 4.85% nitrogen, 0.59% phosphorus and 3.50% potassium.

The increase of crude protein percentages was correlated with the percentages of total nitrogen where T2 significantly exhibited the highest value of crude protein 30.31% whereas control treatment gave 27.56% (the least value). The mixture of all treatment (T5 and T9) obtained less value than T2 but more than the control.

The same trend recorded with total carbohydrates as this treatment significantly obtained higher carbohydrates 52.04% more than all other treatments. Encapsulated *C. middelhoveniana* 72 is considered as a well preserved viable yeast strain and its ability to produce some growth regulators without any loss of its populations positively reflected on all growth parameters of faba bean plants.

Table 6: Percentages of macroelements (NPK), crude protein and carbohydrates in Faba bean seeds grown in soil inoculated with encapsulated and free cells of yeast strains

Treatments	Macroelements in seeds (%) after			Crude Protein (%)	Carbohydrates (%)
	N	P	K		
Control (T1)	4.41	0.31	3.15	27.56	47.41
C. middelhoveniana 72 (T2)	4.85	0.59	3.50	30.31	52.04
Rh. Mucilaginosa 151 (T3)	4.59	0.49	3.12	28.69	49.24
C. blankii 375 (T4)	4.71	0.56	3.30	29.63	51.04
Mix. of tested yeast (T5)	4.63	0.50	3.10	28.94	49.77
C. middelhoveniana 72 (T6)	4.73	0.55	3.20	29.56	51.25
Rh. Mucilaginosa 151(T7)	4.51	0.47	3.08	28.19	48.83
C. blankii 375 (T8)	4.77	0.52	3.10	29.81	51.98
Mix. of tested yeast (T9)	4.55	0.47	3.00	28.44	49.53
LSD 0.05	0.089	0.042	0.236	0.167	0.121

Encapsulated form (T2, T3, T4 andT5) *Free cell form (T6, T7, T8 and T9)*

Data presented in Table (7) revealed the encapsulated *C. middelhoveniana* 72 obtained an increase in dry weight as a single encapsulated form of inoculation. It recorded 8.3 and 55.7 g/plant at both the two growth intervals 50 and 100 days, respectively. In free cell cultures form of *C. middelhoveniana* 72 inoculation gave slightly less Faba bean shoot dry weight, showing 7.1 and 52.1 g/plant, respectively. Whereas, *C. blankii* 375 in the form of encapsulation obtained moderate higher dry weight than those in free cell cultures particularly at 100 days of sowing. Its value was 5.8 and 56.7 (encapsulated form) and 6.1 and 55.1 (free cell cultures form) at both 50 and 100 days, respectively.

In concern the weight of 100 seeds, encapsulated *C. middelhoveniana* 72 and *C. blankii* 375 in free cell cultures form obtained higher weight of 100 seeds as a single form of inoculation more than all other treatments even the mixture ones. They gave nearly the same values 93.8 and 93.65 g/plant, respectively.

The yeast strain *C. blankii* 375 in encapsulated form recorded the optimum yield being 1.834 ton/fed almost the same treatment in free cells form being 1.82 ton/fed.

Table 7: Plant dry weight and yield parameters of Faba bean plants grown in soil inoculated with encapsulated and free cells of yeast strains.

Treatments	Plant Dry Weight (g/plant)		Weight of 100 seeds (g/plant)	Yield (g/plot)	Yield (ton/fed)
	50 d	100 d			
Control (T1)	3.8	40.2	78.6	1.393	0.975
C. middelhoveniana 72 (T2)	8.3	55.7	93.8	2.500	1.750
Rh. Mucilaginosa 151 (T3)	3.7	51.6	85.9	1.987	1.390
C. blankii 375 (T4)	5.8	56.7	87.4	2.62	1.834
Mix. of tested yeast (T5)	4.0	54.3	87.65	2.032	1.423
C. middelhoveniana 72 (T6)	7.1	52.1	91.05	2.460	1.722
Rh. Mucilaginosa 151(T7)	3.9	48.9	80.25	1.852	1.300
C. blankii 375 (T8)	6.1	55.1	93.65	2.600	1.820
Mix. of tested yeast (T9)	4.9	53	87.1	2.230	1.630
LSD 0.05	0.172	1.715	1.618	0.105	0.153

Encapsulated form (T2, T3, T4 andT5) *Free cell form (T6, T7, T8 and T9)*

Discussion

It is well known successful of the use of immobilized microorganisms in different applications (industrial, fermentation, environmental and agricultural). The main goals of the use of encapsulated microorganisms, as they enable slow release, control the cells in beads and protection from biotic and abiotic stress under applications (Vassilev *et al.,* 2001, Young *et al*., 2006 and Rekha *et al.,* 2007).

Several studies used encapsulated bacterial as asymbiotic nitrogen fixers and producing plant growth promoting substances in agricultural applications, but few studies were carried on the use of yeast in encapsulated form on soil application.

For the choice of polymer for encapsulation of beneficial microorganisms is very important. The increase and maintain of microbial cells density in alginate beads than free cells form depended upon the fact that survival of microbes referred to the enrichment of the beads with nutrients (Young *et al*., 2006).

The controlled release of the yeast cells as plant growth promoting organisms helped in the long term survival and establishment of the inoculated PGPR in the soil and led to the stimulation of the levels of microbial groups represented in total microbes, total yeast, total asymbiotic and total actinomycetes in the rhizosphere of Faba bean plants during growth periods especially with encapsulated *C. middelhoveniana* 72 (Vassilev *et al.*, 2001). The increase of microbial communities in the soil after inoculation with encapsulated *C. middelhoveniana* 72 was explained by the increases in level of inorganic nutrition after decomposition of yeast inoculum and enhanced biologically CO_2 derived production. It was generally believed that microorganisms exert their beneficial effect by producing metabolic activities such as production of vitamins, amino acids and phyto-hormones (Barea *et al*., 1997 and Morsy *et al.,* 2014).

The enhancement of native rhizobia and consequently the increase of nodules number, nodules dry weight and Arbuscular mycorrhizal colonization rely on the abundance and activity of encapsulated *C. middelhoveniana* 72 which as plant growth promoter was very active and viable when used in encapsulated form as it could produce plant growth regulating substances and yet the contribution of biological nitrogen fixation ability and consequently an alteration in root morphology thought increased number of lateral roots and root hairs enlarges the root surface available for nutrients. So the inoculated roots created a symbiosis relation with other beneficial microorganisms like native rhizobia and Arbuscular mycorrhizal fungi. This symbiosis resulted in a higher nutrient uptake particularly nitrogen, phosphorus and potassium and led to an improvement in water statues of the plant which in turn could be the main factor enhancing plant growth (Young *et al*., 2006).

The yeast application could enhance its role in cell division, cell elongation producing more leaf area and thus increasing photosynthesis, producing bioactive substances such as phyto-hormones and enzymes (phosphatase and dehydrogenase) (Hussain *et al.*, 2002). The increase of nitrogenase activity with encapsulated *C. blankii* 375 stimulated the growth of Faba bean by producing phytohormones that stimulated the native rhizobia to nodulate the plant roots and consequently fixation of atmospheric nitrogen in nodules whereas the abundant of viable yeast cells in rhizosphere led to the increase of dehydrogenase activity (Nassar *et al*., 2005).

Alonso *et al.,* (2008) stated that, some yeast strains like *Candida* and *Saccharomyces* species could solubilize phosphate by producing phosphatase that help in the production of some beneficial compounds which could directly enhance the growth and productivity of Faba bean and other crops such as sugar beet (Agamy *et.al.,* 2013).

The unique role of both *C. middelhoveniana*72 and *C. blankii* 375 as plant growth promoting substances whether in free cells or encapsulated form in improving the uptake of macroelements in both shoots and in Faba bean seeds as observed in Tables (5 and 6), relied mainly on the ability of these strains rather than others to produce more plant growth promoting substances like gibberellins, auxins and cytokinines besides their

efficiency in phosphates compounds solubilization. Yeast and bacterial phytohormone production is assumed to cause the detected changes in root morphology after inoculation which in turn may be related to enhancing mineral uptake like some macro (N, P and K) and micro (Fe, Zn, Mn, Cu…..etc) elements (Vassilev *et al.,* 2001).

Also, Kucey *et al.,* (1989) stated that, microbially mediated solubilization of mineral like phosphorus and potassium was examined by bacteria and filamentous fungi in fermentation and soil conditions. The application of growth promoters from yeast strains led to improve soil physical, chemical and biological properties result in more release of available nutrient elements to be absorbed by plant roots. This can affect the physiological process such as photosynthesis activity as well as the utilization of carbohydrates and proteins in addition to water use efficiency by different plants (Metin *et al.,* 2010).

Direct stimulation on plant growth was observed in this study where plants inoculated with encapsulated yeast strains mainly *C. middelhoveniana* 72 and C. blankii 375 increased shoot growth represented in dry weight and thus led to an increase in yield components though in the bead inoculated plants, the initial growth was slower, subsequent growth was more vigorous.

In general, basic and the most important requisite of encapsulation of cells are to maintain high cell density with maximum survival even after prolonged storage. It is a challenging task to incorporate a suitable additional material along with the active ingredient causing cell death.

This study demonstrates the feasible technology of microbial inoculants formulation for soil applications, as there is a world wide demand for biofertilizers and organic fertilizers as ecofriendly to reduce the excessive use of chemical fertilizers input and to achieve environmental sustainability.

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