Journal of
NUCLEAR
Technology in Applied Science
ISSN 2314-8209 e-ISSN 2314-8217

J. Nucl. Tech. Appl. Sci, Vol. 5, No. 3, PP. 131 : 141 (2017)

Contribution of ^{15}N-Labeled Organic Manures in Chickpea-Rhizobium Symbiosis Performance

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Received: 09/04/2017

Accepted: 14/05/2017

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KEYWORDS

BNF, Inoculation, Mesorhizobium, ^{15}N-labelling, Organic Additives.

ABSTRACT

A pot experiment was conducted in the greenhouse using chickpea as a test crop grown on sand soil. Organic farming concept was applied using wheat and faba bean dry residues as sources of nitrogen nutrient. These residues were labeled with ^{15}N in another separate pot experiment conducted before start of the main chickpea experiment. Residues were applied in two methods, one of them is as capsule and other one incorporated into the soil before planting. Different *Mesorhizobium ciceri* strains were used as inoculants either individually or in combination. Shoot dry matter yield was significantly affected by *Rhizobium* inoculation and frequently affected by type and methods of organic residues application. The mean average of inoculation indicated that B1 strain increased the shoot yield over the un-inoculated control by about 5.5%. *R. ciceri* strain (B2) causes relative increase in shoot yield by about 1.2 %, -7%; 24%, -19.3% over the un-inoculated control for capsulated and incorporated wheat and faba bean residues, respectively. Despite of organic additive type and method of application, mean average of dual inoculation (B1+B2) caused relative increase in shoots nitrogen uptake by about 15% over the un-inoculated control. The mean average of organic additives, despite of inoculation, reflected no significant variation in shoot-N uptake as affected by wheat residues either incorporated into the soil or in capsulated form. On the other hand, incorporation of faba bean residues into the soil was superior over those applied in capsulated

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form when shoots nitrogen uptake was considered. Percentages and absolute values of Ndfa by shoots were fluctuated according to type and method of application of organic additives and mesorhizobium inoculation treatments (individuals or dual inoculants). The superiority of one method over other seems to be related to type of organic additives. B2 strain either inoculated individually or in dual inoculants achieved the highest values of nitrogen derived from air by shoots. The percentages and absolute values of nitrogen derived from organic additives (Ndforg) were gradually decreased with bacterial inoculation treatments but still higher in case of faba bean residues than wheat residues. Incorporation method induced higher Ndforg by shoots of inoculated plants than those of capsulated one.

INTRODUCTION

Organic and inorganic fertilizers play an important role in improving the fertility of low fertile sandy soils. In the same time, increasing the depending on organic fertilization to reduce amounts of mineral fertilizers, is considered one of the new scientific approaches applied for safely and healthy yields with special emphasis on environmental pollution **(El- Ghanam *et al*., 2005).**

The organic residues application to agricultural soils had become an essential approach to increase soil fertility **(Pérez-Piqueres *et al*., 2006),** but also faced challenges, as the beneficial effects of these materials may be associated with risks to both the soil and the environment due to contaminants **(Bationo *et al*., 2007).** Another sort of organic fertilization is the Bio-fertilizers which are used to enrich soil fertility. Bio-fertilizer contains live microorganisms, colonizes the rhizosphere or the interior of the plant when applied to seed, plant surfaces, or soil, and thus enhances growth by different direct and indirect mechanisms **(Gupta *et al*., 2000).** Application of biofertilizers instead of chemicals is one of the strategies for improving crop production and protecting the environment. **(Alimadadi *et al*., 2010).** Therefore, bio-fertilizers offer a safe option to utilize renewable inputs to improve the fertility of land using biological wastes with those beneficial micro-organisms which impart organic nutrients to the farm produces.

Biofertilizers have emerged as potential environment friendly inputs that are supplemented for proper plant growth. They hold vast potential in meeting plant nutrient requirements while minimizing the use of chemical fertilizers. These, bio-inputs or bio inoculants, which on supply to plants improve their growth and yield, are the products containing living cells of different types of microorganisms which have an ability to mobilize nutritionally important elements from non-usable form through biological stress (**Khan and Naeem, 2011 & Mazid *et al*., 2012**).

Therefore, we aimed to trace the release of nitrogen from different organic plant residues added to inoculated chickpea in different application methods in order to achieve the organic farming (low cost) concept.

MATERIALS and METHODS

A pot experiment was conducted under greenhouse conditions at Soil and Water Research Department, Nuclear Research Center, Atomic Energy Authority using chickpea as a test crop. Seeds of chickpea (*Cicer arientinium* cv Giza 195) was provided by the Agricultural Research Center (ARC), Ministry of Agriculture, Giza, Egypt.

Quantities of ^{15}N-labeled organic residues of faba bean and wheat previously grown in a separate pot experiment and fertilized with ^{15}N labeled urea salt were used. Seeds were sowed in pots (30-cm height, 30-cm diameter) containing 9 kg sand soil. Nitrogen fertilizer (^{15}N-urea salt with 10% atom excess) was applied after sowing at recommended rates for the two plants. Faba bean and wheat plants were uprooted after 60 days of sowing, dried and then ground to fine powder. Residues of both were separately added to soil on the basis of its N content at rate of 20 kg N fed^{-1} for chickpea plant. Measured ratios of nitrogen in wheat and faba bean residues are 2.1% and 1.8 % ^{15}N atom excess, respectively.

Microbial inoculations

Symbiotic nitrogen fixers *Mesorhizobium ciceri (1148 ICARDA)* and *Sinorhizobium sp. (36 ICARDA)* were propagated on yeast extract mannitol medium **(Vincent, 1970).** It contain**:** Yeast extract mannitol (YM) agar medium **(Vincent, 1970)**. YM medium contained of (g/l): Mannitol, 10.0; yeast extract, 1.0; K_2HPO_4, 0.5; $MgSO_4.7H_2O$, 0.2; and NaCl, 0.1; pH was adjusted to 7.0, for YM, agar 20 (g/l) was added. YM medium was used also for culture maintenance and examinations of rhizobia. These two inoculants were applied to seeds (seed inoculation) either individually or in combination.

Experimental soil samples

Sand soil samples were collected from surface layer (0 – 30 cm) of Soil and Water Research Department farm, Nuclear Research Center. Some physical and chemical properties of experimental soil sample were presented in Table (1). Physical and chemical determinations of experimental soil samples were carried out according to **Carter and Gregoreish (2008)**.

Table (1) *Some of physical and chemical characteristics of the experimental soil.*

Soil Texture	Particle size distribution %			Bulk density g/cm^3			
	Sand	Silt	Clay				
Sand	91.5	2.7	5.8	1.75			
Soluble ions (meq L^{-1})							
Cations (meq 100 g^{-1} soil)				Anions (meq 100 g^{-1} soil)			
K^+	Na^+	Ca^{++}	Mg^{++}	Cl^-	HCO_3^-	CO_3^-	SO_4^-
0.09	0.20	1.45	1.18	0.66	1.90	0.00	0.36
pH (1:2.5)	O.M %	EC dS m^{-1}	$CaCO_3$ %	Avail Nmeq $100g^{-1}$		Avail Pmeq $100g^{-1}$	
7.80	0.043	0.65	1.21	0.01		0.020	

Plant residues addition (capsulated and incorporation) methods

^{15}N-labeld faba bean and wheat plant residues were applied at rate of 20 kg N fed^{-1} to soil under chickpea plants in two methods. One of them is in the form of capsules containing the labeling organic residues and the other one incorporated into the soil before planting.

Experiment conditions

Chickpea seeds were sown in pots with 30cm height, 30cm diameter, containing 9 kg soil, thinned after emergency to 5 seedlings per pot. Pots including different treatments were arranged in completely randomized block design (CRBD) under greenhouse condition. All agricultural practices were kept into consideration and pots were watered when needed. After 120 days, plants were harvested. After harvest, shoot was dried at 70 °C until constant weights, then the dry weight were recorded. Total nitrogen uptake by shoots was determined according to **Estefan *et al*. (2013)**.

After the determination of plant nitrogen, the captured ammonia as NH_4Cl, had been dried and converted to N_2-gas, following the method used by **Buresh *et al*. (1982)**. Then, the Emission Spectrometer (NOI-6PC) had been used for the $^{15}N/^{14}N$ ratio analysis.In this regard, the Dumas dry combustion method can be also used, and the total plant nitrogen could be directly converted to N_2-gas in the closed discharge tubes, using the Cu-mixture and high temperature (450°C) according to **IAEA (2001)**. Percentages of N-derived from air (%Ndfa), and N-derived from organic plant residues (%Ndforg) were calculated according to the following standard equations:

$$\% \text{ Ndfa} = \left(1 - \frac{^{15}\text{N\% atom excess in inoculated plant}}{^{15}\text{N\% atom excess in un-inoculated plant}}\right) \times 100$$

$$\% \text{ Ndforg} = \left(1 - \frac{^{15}\text{N\% atom excess in treated with plant residues}}{^{15}\text{N\% atom excess in untreated with plant residues}}\right) \times 100$$

All the obtained data were subjected to ANOVA analysis followed by Duncan's multiple range test (DMRT) for comparison between means using **SAS software program (2002)**.

RESULTS and DISCUSSION

Shoot dry matter yield

Capsulated wheat residues in the soil under un-inoculated plants resulted in, to somewhat extent, higher shoot dry matter yield than those recorded with incorporation of wheat residues (Table 2). In this respect, the relative increase was accounted for 6.5% over the incorporation treatment. Another trend was noticed with application of faba bean residues in un-inoculated treatment, where the shoot dry matter yield in incorporation method was relatively higher (27.5%) than the capsulated treatment. Inoculation with B1 strain had enhanced shoot dry matter yield as compared to the un-inoculated one. Inoculation performance was more vigorous under wheat residues especially when capsulated. Relatively, B1 inoculation caused increments in shoot dry matter yield by about 7%, 13%; 5%, -2.7% over or under the un-inoculated control for capsulated and incorporated wheat and faba bean capsulated residues, respectively. In this treatment, capsulated wheat residues was more effective on shoot dry matter yield than incorporated one while reversible trend was noticed with faba bean residues. The mean average of inoculation indicated that B1 strain increased the shoot dry matter yield over the un-inoculated control by about 5.5%. *R. ciceri* strain (B2) causes relative increase or decrease in shoot dry matter yield by about 1.2 %, -7%; 24%, -19.3% over or under the un-inoculated control for capsulated and incorporated wheat and faba bean residues, respectively.

Considering the mean average of B2 inoculation, data showed that shoot dry matter yield was quite similar to the un-inoculated control. Significant increases in shoot dry matter yield were observed with incorporated wheat residues and capsulated faba bean residues in combination with dual B1+B2 inoculant as compared to the un-inoculated control. Dual inoculation caused relative increase in shoot dry matter yield by about 14.4% over the un-inoculated control for incorporated wheat residues. Despite of organic additive type and method of application, mean average of dual inoculation (B1+B2) caused relative increase in shoot dry matter yield by about 1.3% over the un-inoculated treatment, but increments still insignificant.

Togay *et al*. (2008); Saini *et al*. (2004) and **Ogutcu *et al*. (2008)** reported that inoculation with *Rhizobium* significantly increases the plant height, first pod height, number of branches, pods and seeds per plant, grain and dry matter yield of chickpea. **Namvar *et al*. (2011)** stated that the greatest number of primary and secondary branches per plant was recorded in inoculated plants. Inoculation with *Rhizobium* increased the number of primary and sec-

Table (2) *Effect of ^{15}N-labeled wheat or faba bean residues added as capsulated or incorporated materials and Rhizobium inoculation on shoots dry matter yield (g pot-1) of chickpea plant.*

BIOFERTILIZER	WHEAT GREEN RESIDUES		FABA BEAN GREEN RESIDUES		MEAN
	Capsule	Incorporation	Capsule	Incorporation	
Un-inoculated (B0)	8.1	7.6	5.8	7.4	**7.2**
Rh. Spp.(B1)	8.7	8.6	6.1	7.2	**7.6**
Rh. ciceri (B2)	8.2	7.1	7.2	6.2	**7.2**
B1 +B2	7.9	8.7	5.8	6.7	**7.3**
MEAN	**8.2**	**8**	**6.2**	**6.9**	
LSD 0.05	**A, 0.93 B, 0.93 AB, 1.9**				

***A**, organic residues, **B**, inoculation, **AB**, interaction*

ondary branches by 8.50 and 14.70%, respectively, compared to non-inoculated plants.

Nitrogen uptake by shoots

Incorporation of wheat residues in the soil under un-inoculated plants resulted in, to somewhat extent, higher shoots nitrogen uptake than those recorded with capsulated wheat residues (Table 3). In this respect, the relative increase was accounted for 19% over the capsulated treatment. Similar trend was noticed with application of faba bean residues but the relative increase was higher than those calculated for wheat residues which accounted for 62% over the capsulated treatment. Inoculation with B1 strain had enhanced shoots nitrogen uptake as compared to the un-inoculated one. Inoculation performance was more vigorous under wheat residues especially when capsulated. Relatively, B1 inoculation caused increments in shoots nitrogen uptake by about 45%, 31%; 20%, and -11% over the un-inoculated control for capsulated and incorporated wheat and faba bean residues, respectively. In this treatment, capsulated wheat residues were more effective on shoots nitrogen uptake than incorporated one while reversible trend was noticed with faba bean residues. In spite of organic additives and method of application, the mean average of inoculation indicated that B1 strain increased the shoots nitrogen uptake by about 21% over the un-inoculated control. Similar trend, but to somewhat lower extent, was noticed with plants inoculated with *R. ciceri* strain (B2). In this regard, it causes relative increase in shoots nitrogen uptake by about 31.3%, -9%; 33%, -26.4% over the un-inoculated control for capsulated and incorporated wheat and faba bean residues, respectively. Considering the mean average of B2 inoculation, data showed that shoots nitrogen uptake was relatively increased by about 6.7% over the un-inoculated control.

Another picture was drawn with dual inoculation treatment (B1+B2) as interacted with organic additives where capsulated wheat residues plus B1+B2 induced high relative increase (40.9%) over the un-inoculated plants when shoots nitrogen uptake was concerned. Similar trend, but to somewhat lower extent, was noticed with incorporated wheat residues (12%). Low increases in shoots nitrogen uptake (7%) were observed with capsulated faba bean residues in combination with B1+B2 inoculation as compared to the un-inoculated control. Despite of organic additive type and method of application, mean average of dual inoculation (B1+B2) caused relative increase in shoots nitrogen uptake by about 15% over the un-inoculated control. The mean average of organic additives, despite of inoculation, reflected no significant variation in shoot-N uptake as affected by wheat residues either incorporated into the soil or in capsulated form. On the other hand, incorporation of faba bean residues into the soil was superior over those applied in capsulated form when shoots nitrogen uptake was considered.

Adding N increases the production of dry matter in plants which can increase the potential of plant to produce more plant height, branches, pods and seeds that ultimately results in high grain and biological yield (**Salvagiotti *et al*., 2008 and Erman *et al*., 2011).**

Inoculation of legumes with rhizobia for the purpose of enhancing N_2 fixation and yield in legume crops is possibly the oldest and most common method of voluntary release of microbes into the environment **(Ogutcu *et al*., 2008)**. The influence of *Rhizobium* bacteria on promoting legumes growth is documented in some researches **(Saini *et al*., 2004; Erman *et al*., 2011; Namvar *et al*., 2011).**

Table (3) *Effect of ^{15}N-labeled wheat or faba bean residues added as capsulated or incorporation materials and Rhizobium inoculation on shoots nitrogen uptake (mg pot^{-1}) by chickpea plant.*

BIOFERTILIZER	WHEAT GREEN RESIDUES		FABA BEAN GREEN RESIDUES		MEAN
	Capsule	Incorporation	Capsule	Incorporation	
Unino.(B0)	47.6	56.7	32.7	53.1	**47.5**
Rh.sp.(B1)	69.3	74.3	39.3	47.4	**57.6**
Rh.ciceri (B2)	62.5	51.6	43.5	39.1	**50.7**
B1 +B2	67.1	63.5	35.1	52.7	**54.6**
MEAN	**61.6**	**61.5**	**37.6**	**48.1**	
LSD (0.05)	**A= 5.5 B=5.5 AB=11**				

In addition, **El Hadi and Elsheikh (1999)** found that Rhizobium inoculation or N fertilization significantly increased the total nodule number per plant, 100 seed weight, yield and protein content of seeds. Their results indicated that the three Rhizobium strains were infective and effective in nitrogen fixation. Rhizobium inoculant has the potential to increase N in legumes plant and percentage nitrogen derived from the atmosphere (Ndfa) **(Beck, 1992; Peoples and Baldock, 2001 Clayton *et al*., 2004 & Bambara and Ndakidemi, 2010).**

Nitrogen derived from organic sources (Ndforg)

It was clear that the un-inoculated plants accumulated more nitrogen from organic resources in shoots than those of inoculated plants (Table 4). This holds true with wheat or faba bean residues either applied in capsule or incorporated into the soil. In this regard, Ndforg by shoots from incorporated faba bean was higher than those of capsulated one. Similar trend, but to somewhat lower extent was noticed with wheat residues.

In general, the portions and absolute values of Ndforg were gradually decreased with bacterial inoculation treatments but still higher in case of faba bean residues than wheat residues. Incorporation method induced higher Ndforg by shoots of inoculated plants than those of capsulated one.

On the other hand, **Tena *et al*. (2016)** reported that inoculation had a pronounced effect on grain yield, yield component, total N uptake, grain protein content, percentage N derived from the atmosphere (%Ndfa) for the seed, and amount of seed N fixed compared to non-inoculated treatments. In contrast, in a pot experiment, they recorded significant difference among the mesorhizobial strains used. In a pot experiment, **Rizkalla *et al*. (2014)** indicated that nitrogen derived from organic residue by inoculated chickpea including percentages and absolute values were very low as compared to nitrogen derived from air (Ndfa) and N derived from mineral fertilizer (Ndff). Their results proved that Mesorhizobium inoculated plants were more dependent on proportion of N derived from air rather than those derived from mineral fertilizer or organic residue. In other turn, rhizobial inoculation compensated remarkable amounts of nitrogen requirements via biological nitrogen fixation process. They added that low contribution of organic additives in nitrogen nutrition of chickpea could be attributed to slow release and decomposition of organic residues. They recommended residues to be incorporated in the soil a long time before cultivation to give a period needed for degradation and digestion or turnover of organic-N.

Table (4) *Nitrogen derived from organic sources by shoot of chickpea as affected by addition methods and bacterial inoculation treatments.*

Organic sources and application methods	Wheat Residues				Faba Bean Residues			
	Capsule		Incorporation		Capsule		Incorporation	
Inoculation Treatments	Shoot							
	%	mg	%	mg	%	mg	%	mg
Un-inoculation (B0)	45.9	10.3	46.5	12.8	54.8	13.5	55.4	16.8
Rh.sp.(B1)	13.7	4.2	11.9	4.0	16.6	5.0	16.7	6.2
Rh.ciceri (B2)	12.2	4.3	11.6	4.5	13.6	4.9	16.4	8.2
B1 +B2	11.7	2.3	11.1	5.1	13.2	6.3	15.9	6.4

Nitrogen derived from air (Ndfa)

Chickpea plants inoculated with *Mesorhizobium* strain (B1) and treated with wheat residues reflected portions of Ndfa by shoots ranged from 70% to about 74% according to application method of residues (Table 5). It seems that incorporation of wheat residues resulted in higher portion (73.8%) and absolute value (24.6 mg pot^{-1}) of Ndfa than those recorded with capsulated wheat residues (70.2% and 21.6 mg pot^{-1}). Plants treated with faba bean residues showed nearly closed Ndfa% (about 70%), when comparison was held between the two methods of application, but the absolute value tended to increase with incorporated faba bean residues as compared to capsulated one (26.1 against 21.1 mg N pot^{-1}).

Percentages and absolute values of Ndfa by shoots as affected by interaction between *mesorhizobiumciceri* strain (B2) and organic additives were higher than those recorded with B1 strain. Incorporated wheat residues induced a slightly higher Ndfa% and absolute values than those of capsulated one. Reversible trend, but only with Ndfa%, was noticed with faba bean while the absolute value induced by incorporated faba bean residues seems to be higher than those of capsulated residues.

Dual inoculation (B1+B2) showed nearly closed portions of Ndfa, to those recorded with B2 strain. This holds true with both two organic additives and methods of application. Concerning the absolute values, incorporated wheat residues resulted in higher Ndfa by shoots than those of capsulated one, while reversible trend was noticed with faba bean residues where capsulation enhanced Ndfa rather than incorporation method.

In conclusion, proportion and absolute values of Ndfa by shoots were fluctuated according to type and method of application of organic additives and mesorhizobium inoculation treatments (individuals or dual strains). The superiority of one method over other seems to be related to type of organic additives. B2 strain either inoculated individually or in dual strains achieved the highest values of nitrogen derived from air by shoots.

Table (5) *Nitrogen derived from air by shoot of chickpea as affected by organic sources, addition methods and bacterial inoculation treatments.*

Organic sources and application methods	Wheat Residues				Faba Bean Residues			
	Capsule		Incorporation		Capsule		Incorporation	
Inoculation Treatments	Shoot							
	%	mg	%	mg	%	mg	%	mg
Un-inoculation (B0)	-	-	-	-	-	-	-	-
Rh.sp.(B1)	70.2	21.6	73.8	24.6	69.8	21.1	69.9	26.1
Rh.ciceri (B2)	73.5	26.0	75.1	29.2	75.3	27.0	70.4	35.0
B1 +B2	74.6	18.9	76.1	34.9	75.9	36.1	71.3	28.5

Different *Mesorhizobium* strains were evaluated by **Tena *et al.* (2016a)** where they recorded varied range of %Ndfa by chickpea crop ranged from 51.5% to 67.9%. These portions were estimated using ^{15}N isotope dilution method and nearly closed to our results. Their results were in agreement with **Yadav *et al.* (2011)**, that a Mesorhizobium strain indigenous to the growing field locality proved to be a highly effective symbiotic nitrogen fixer for uptake of nutrient content and grain yield of chickpea. On the other hand, **Evans (2005)** found indigenous Rhizobium strains to be more highly effective symbiotic nitrogen fixers for the uptake of nutrient content and grain yield than introduced commercial inoculants. The current result was also similar to the recent report (**Tena *et al.*, 2016b**) where evaluations of rhizobial strains isolated from Ethiopian soils revealed higher rates of N fixation on lentil than the introduced commercial inoculant.

In the same way, **Elias (2009)** demonstrated that individual competitive strains in naturalized population of chickpea rhizobia in the cropping soils in the northern grains belts, vary significantly in their effectiveness. Of most concern is the 41% of evaluated rhizobia strains of reduced effectiveness that are successfully gaining occupancy of nodules thus posing a potential threat to the N-fixing capacity of these chickpea crops.

On line, **Lee *et al.* (2011)**, at the field scale, found that percent N derived from N fixation (%Ndfa) was ranged from 51 to 93 % and related to the amount of soil-derived N in the plant. Similarly, **Aslam *et al.* (2003)** indicated that chickpea N_2 fixation assessed using the natural ^{15}N abundance method, showed varied percentage of chickpea N derived from N fixation (%Ndfa) according to experimental sites (58% (Mandra), 65% (Taxila) and 86% (Islamabad)).

In another study by **Bambara and Ndakidemi (2010)**, the percentage of Ndfa increased significantly with rhizobium inoculation in roots, shoots, pods, and whole plant in both the glass house and field experiment compared with un-inoculated control treatment. For example the estimation per ha basis of the amount of N fixed amounted from 8.6 to 33 kg ha^{-1} in the un-inoculation control and Rhizobium inoculation, respectively.

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مجـلة
التقنيــات النـــوويـــة
فى العلوم التطبيقية
مجلد 5 ، عدد 3 ، ص 131 : 141 ، (2017)

ESRSA
الجمعية المصرية للعلوم الإشعاعية وتطبيقاتها
The Egyptian Society of Radiation Science and Applications

مساهمة الأسمدة العضوية المرقمة ـ ن^{15} في أداء العلاقة التكافلية بين الحمص والريزوبيا

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أقيمت تجربة أصص في الصوبة الزراعية باستخدام الحمص النامي على الرض الرمل كنبات اختبار. تم تطبيق مفهوم الزراعة العضوية من خلال استخدام بقايا القمح والفول البلدي الخضراء كمصادر للنيتروجين التغذوى. تم ترقيم تلك المتبقيات بالنيتروجين المستقر (ن١٥) من خلال تجربة أصص احريت قبل البدء في تجربة الحمص الرئيسية. أضيفت المتبقيات العضوية المرقمة بطريقتين احداهما في صورة كبسولات مغطاة والأخرى من خلال الدمج في التربة قبل الزراعة. استخدمت سلالات مختلفة من الريزوبيوم (ميزوريزوبيوم) كملقحات اما منفردة أو في مخلوط منهما. محصول المادة الجافة للسيقان تأثر معنويا بالتلقيح البكتيري بينما تفاوت تأثير نوع المادة العضوية وطريقة الاضافة. اشار المتوسط العام للتلقيح البكتيري الى أن السلالة B1 أدت الى زيادة محصول المادة الجافة للسيقان مقارنة مع المعاملة غير الملقحة بقيمة نسبية ٥,٥٪. بينما السلالة B2 أحدثت زيادة نسبية في محصول المادة الجافة للسيقان بحوالي ١,٢٪، -٧٪ و ٢٤٪، -١٩,٣٪ اعلى من المعاملة غير الملقحة لكل من الكبسولات ومعاملة الدمج لمتبقيات القمح والفول البلدي على الترتيب. بغض النظر عن نوعية المتبقيات العضوية وطرق اضافتها، فان المتوسط العام لمعاملة التلقيح المزدوج (B1+B2) أدت الى زيادة نسبية في امتصاص النيتروجين بالسيقان بحوالي ١٥٪ أعلى من معاملة الشاهد غير الملقحة. المتوسط العام للنيتروجين الممتص متأثرا بنوع المادة العضوية، بغض النظر عن التلقيح لم يعكس فروقا معنوية في وجود متبقيات القمح سواء دمجت في التربة أو اضيفت في كبسولات. من جهة أخرى، كانت معاملة دمج متبقيات الفول البلدي متفوقة على معاملة الكبسولات. النسب المئوية وكذا القيم المطلقة للنيتروجين المثبت من الهواء الجوي والممتص بالسوق تباينت طبقا لنوع وطريقة اضافة المتبقيات العضوية ومعاملات التلقيح البكتيري. تفوق أحدى طريقتي الاضافة على الخرى بدى مرتبطا بنوع المتبقيات العضوية المضافة.

السلالة B2 سواء لقحت منفردة أو في مخلوط مزدوج مع السلالة B1 حققت أعلى قيم للنيتروجين المثبت من الهواء الجوي بواسطة السوق. النسب المئوية والقيم المطلقة للنيتروجين المنساب من المصادر العضوية تناقص تدريجيا في وجود التلقيح البكتيري ولكنه استمر عاليا في حالة متبقيات الفول البلدي عنها في حالة متبقيات القمح. طريقة الدمج في التربة اسفرت عن امتصاص أعلى للنيتروجين المنساب من المصادر العضوية بواسطة السوق للنباتات الملقحة عنها في حالة الاضافة في شكل كبسولات.

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