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Assessment of Nitrogen Availability to Wheat (*Triticum aestivum* L.) from Inorganic and Organic N Sources as Affected by *Azospirillum brasilense* and *Rhizobium leguminosarum* Inoculation

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THE BENEFITS of *Rhizobium* and / or *Azospirillum* to wheat were examined in a pot experiment containing virgin sandy soil and fertilized with either ^{15}N-ammonium nitrate or ^{15}N-labelled rice straw. Both inoculants were applied individually or in combinations (dual inoculation). Isotopic analysis and the isotope dilution technique were followed for quantification of nitrogen derived from fixation (Ndfa), nitrogen derived from fertilizer (Ndff) and ^{15}N-recovery. The dry matter accumulation of shoots and the grain yield were positively affected by inoculation comparable to those recorded in uninoculated treatments. The highest significant values were detected with dual inoculation followed by individual *Rhizobium and Azospirillum* Sp. 245, respectively. Similar trends were noticed for nitrogen accumulated by either shoots or grains. Generally, the dry matter accumulation and nitrogen uptake by shoots and grains were significantly higher with ammonium nitrate ($^{15}NH_4$ NO_3) than those recorded for rice residues applications. Similarly, the nitrogen derived from fertilizer and ^{15}N recovered by shoots and grains were increased by inoculation and followed the same trend when comparison was held between ^{15}N-ammonium nitrate and ^{15}N-rice straw. The obtained data showed a higher percentage of nitrogen derived from air and utilized by either shoots or grains of wheat plants inoculated with *Rhizobium leguminosarum* or dual inoculants as compared to *Azospirillum brasilense* Sp. 245, inoculation. These percentages were higher in case of soil incorporated with ^{15}N-rice straw than in soil fertilized with ^{15}N-ammonium nitrate, when dual inoculation was considered. On overall basis, the *Rhizobium* inoculation was superior over *Azospirillum* one. A good correlation was recorded between shoots and grains when ^{15}N-recovery was concerned in soil

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incorporated with ^{15}N-rice straw (r = 0.552*) and ^{15}N- ammonium nitrate (r = 0.602*). It is worthy to mention that the inoculation of wheat with *Rhizobium* seems to be promising and beneficial for enhancing wheat growth and production. Furthermore, the use of plant residues may act as an ideal slow release source of N because of its stability through the period of experiment.

Keywords: *Azospirillum* spp. ^{15}N dilution, N fertilizer, . N_2 fixation, ^{15}N recovery, Organic residues, *Rhizobium*, Wheat

Low input technologies are required for increasing food production from major crops and to sustain the productivity of the land that is regularly cultivated. These technologies offer a proper soil management approach aimed at preserving appropriate and sustainable levels of organic matter, available nutrients and soil microbial activity. Crop residues, as an organic N form, and biofertilization with either symbiotic or non-symbiotic bacteria are recently considered in sustainable agricultural ecosystems (Jordan *et al.*, 1996; Webster *et al.,* 1997; Yanni *et al.,* 1997; Sabry *et al.*, 1998).

The interrelationship between the microorganisms and the host plant can play an important role in improving and altering crop productivity through the manipulation of the rhizosplane or rhizosphere microorganism communities (Curl and Truelove, 1986). Recent work with *Azospirillum* spp., has shown that when the N metabolism of diazotrophic bacteria is altered, these bacteria are able to excrete N derived from N_2 fixation (Christiansen-Weniger, 1996; Christiansen-Weniger & Van Veen, 1991). Caballero-Mellado *et al.* (1992) showed that inoculation of wheat with various strains of *Azospirillum* spp., caused significant increases over the uninoculated controls in grain yield, N, P and K acquisition by the plant. The same author added that these increments might be attributed to process other than nitrogen fixation .

Recently, agricultural scientists have long wished to extend the host range of rhizobia with their excellent nitrogen fixing ability and as the cheapest source of nitrogen, to the cereals but yet there is little hard evidence of the mechanisms to induce the nodule-like structures or precisely how these structures may be formed (Ridge *et al.,* 1992). In this respect, Cocking *et al.* (1993; 1995) in experiments on wheat inoculated with rhizobial strains found that the wheat roots would occasionally [and with higher frequency upon application of 2,4-D] develop "pseudonodules" that were actually hypertrophies of modified, short-thick lateral roots rather than true nodules, and that the rhizobia had colonized within numerous cells of the cortex of these modified swollen roots and this invasion was achieved via a pathway of crack-entry at the point of emergence of lateral root primordia. Subsequent studies using improved protocols of specimen preparation for plant microbiology and a wide variety of microscopies (brightfield microscopy using *Lac*Z reporter strains, laser scanning confocal microscopy, scanning and transmission electron microscopy) confirmed that the portal of entry of rhizobia into the cereal root [in this case, rice] was via crack entry at junctions between epidermal cells and at lateral root emergence, and that the rhizobia within the invaded root cortex would colonize intercellular spaces and within dead cortical cells only (Reddy *et al.*, 1997). Application of rhizobia by seed pelleting caused an increase of shoot dry matter yield of winter wheat by 8% in a pot experiment. Also, root development and tillering were stimulated (Höflich, 1989).

With respect to plant residue application with microbial inoculation, Halsall and Gibson (1989) reported that rice straw yielded the highest nitrogenase activities with *Azospirillum* than sawdust and sugar cane trash. Similarly, wheat residues maintained high populations of *Rhizobium* spp. in the soil (Kucey and Hynes, 1989). In most soils where microbial activity is limited by a lack of carbon, cereal stubble represented a substantial potential source of energy, and the incorporation into the soil was more effective than mulching on the surface. Seed inoculation with *Azospirillum* spp. in combination with organic amendments induced stimulation of wheat growth and nitrogenase activity (Ishac *et al.*, 1986). Comparative studies in relation to organic manuring have shown that morphologically and physiologically distinguishable types of bacteria, particularly *Pseudomonas* types, have been stimulated in the rhizosphere of wheat and rye like those found after incorporation of straw in the soil (Höflich, 1989).

This work aims at the confirmation of the possibility of *Rhizobium* inoculation of wheat for promoting the growth and yield. In the same time, to follow the effect of crop residues, as source of nitrogen, on wheat growth and N_2 fixation by the introduced bacteria in soil.

Material and Methods

A pot experiment was carried out in the greenhouse of the Soil and Water Research Department, Nuclear Research Center, Cairo, Egypt. Five-kilogram of an air-dried and sieved (< 2 mm) virgin sandy soil (N, 0.07%; OC, 0.02%; pH (H_2O), 7.9) were placed in plastic pots with capacity of 5.2 L. Six seeds of wheat (*Triticum aestivum* cv. Sakha 69), which was obtained from Field Crops Research Institute, Agriculture Research Center (ARC), Giza, Egypt, were sown per pot and the seedlings were thinned to four per pot after emergence. Wheat seeds were coated with the peat-based inoculum of *Rhizobium leguminosarum* bv. trifolii strain ARC 101, kindly provided by the Agricultural Microbiology Department, ARC, at 10^7 cells g^{-1} peat, and/or *Azospirillum brasilense* Sp. 245 at 10^8 cells g^{-1} peat, before sowing. Treatments were 1) uninoculated control; 2) inoculated with A. brasilense; 3) inoculated with *R. leguminosarum;* 4) inoculated with a mixture of both bacteria in the ratio of 1 *R.l.t* :10 A.b. These treatments were arranged in completely randomized blocks with three replications. Phosphorus and potassium fertilizers were applied at rate of 250 mg and 125mg pot^{-1}, respectively when preparing soil for cultivation. All inoculated pots, including the controls, were divided into two batches, one was given 125 mg N pot^{-1} as ^{15}N-labelled ammonium nitrate (5% atom excess) and the other was given 10 g pot^{-1} of ^{15}N-labelled rice straw (3.1% atom excess) that have 42% C; 0.46%N; and a C: N ratio of 91. The plants were harvested at maturity stage (150 days after emergence) and the dry weight of straw and grain were recorded separately. The finely ground samples of plant components were analyzed for total N and ^{15}N . Total N was determined by a micro-kjeldahl method (Bremner and Mulvaney, 1982) and the distillates were concentrated for ^{15}N isotope ratio analysis using an emission spectrometer (NOI-6PC, Fischer). Nitrogen derived from fertilizer, air and ^{15}N recovery were estimated as described in previous work (Galal *et al.*, 2001).

The data were subjected to an analysis of variance followed by least significant test (LSD) and Duncan's multiple range test (DMRT) using SAS software. ^{15}N recovery in shoots and grains as affected by N sources were subjected to linear regression analysis and the correlation coefficient was estimated .

Results

Dry matter and N yields

Table 1 indicates the accumulation of a high dry matter and N in shoots and grain of inoculated wheat under ^{15}N-ammonium nitrate application. Single inoculation with *A. brasilense* or *R. leguminosarum* had a significant positive general effect on dry matter yield and N uptake by shoot and grain as compared to the uninoculated control. *Rhizobium*-wheat relation resulted in higher dry matter accumulation and N uptake than those recorded with *Azospirillum brasilense.* The *Rhizobium*-induced relative increase accounted for 52.5% and 65% higher than *A. brasilense,* for dry matter and N uptake by shoot, respectively. Low relative increments of 17% and 44% were estimated for grain in the same experiment. Shoot dry matter and N yields indicated the superiority of dual inoculation over the individual inoculation. The dual inoculation has the same effect on grain yield and grain N acquisition as did the *Rhizobium* sole inoculation. Similar trends, but to a lower extent, were noticed with ^{15}N-rice residue incorporated into the soil followed with the same inoculation treatments (Table 2). Superiority of dual inoculation was more pronounced with the addition of the ^{15}N-rice residue, when N uptake and grain yields were considered. In this respect, dual inoculation had increased the shoot dry matter and N accumulation by about 92% and 208% over the control, respectively. Grain yield and N uptake, as affected by dual inoculation, were increased by two-fold and four-fold over the uninoculated control, respectively .

N derived from fertilizer or residue and ^{15}N recovery

Amounts of N derived from fertilizer and ^{15}N recovery by shoots and grain were presented in Table 3. Shoots gained more N from fertilizer as compared to grain-N. Amounts of N derived from fertilizer were significantly increased by inoculation. Dual inoculation by *Rhizobium* and *Azospirillum* was the best treatment followed by *Rhizobium* and then *Azospirillum* alone. Similar trends were observed with ^{15}N recovery. Lower amounts of N derived from rice residues and uptake by shoots and grains were recorded (Table 4). These portions were also affected by inoculation. In this regard, the dual inoculation was still superior over individual inoculations. Similar trends were noticed with ^{15}N recovered by both plant organs from labeled rice residue. A linear correlation of shoot/root for ^{15}N recovery of fertilizer and residue showed a moderately significant correlation ($y = 0.065 + 0.162x$; $r = 0.602$, and $y = 0.011 + 0.750x$; $r = 0.552$, respectively).

N derived from air

Amounts of plant N derived from atmospheric N_2 in shoots and grain were significantly affected by inoculation and N fertilization (Table 5). It is worthy to mention that there was no N_2 fixation detected in uninoculated control, therefor it was excluded from the table. This is logical since the examination of the virgin sandy soil proved that it lacks native *Rhizobium* and *Azospirillum*. Portions (%) of N_2 fixed in shoots and grain were higher with dual inoculation and *Rhizobium* alone than with *Azospirillum* inoculation alone. Dual inoculation was superior over the individuals in soil treated with rice straw. Grain N derived from N_2 was, to some extent, higher than those in shoots. The inoculants were frequently affected by N sources. It seems that the microorganism's activity was rapidly affected by the available N source. At the same time, the positive effect of combined N on nitrogen fixation activity was also recognized .

TABLE 1. Effect of inoculation on dry matter and N yields of wheat plants fertilized with ^{15}N-ammonium nitrate.

Inoculation	Dry matter yield (g pot^{-1})	N concentration (%)	N accumulation (mg pot^{-1})
	Shoots		
Uninoculated	1.2 b	0.41 d	4.9 c
Azospirillum	1.58 ab	0.43 c	6.8 bc
Rhizobium	2.41 a	0.46 b	11.2 ab
Azosp. +Rhiz	2.47 a	0.53 a	13.0 a
LSD (0.05)		0.006	4.758
C.V. (%)	65.14	2.75	26.52
	Grains		
Uninoculated	0.44 ab	1.56 c	6.8 b
Azospirillum	0.47 ab	1.50 d	7.0 b
Rhizobium	0.55 a	1.81 b	10.1 a
Azosp. +Rhiz	0.56 a	1.95 a	10.7 a
LSD (0. 05)	0.14	0.006	1.859
C.V. (%)	17.79	0.64	10.76

Means in each column followed by the same letter are not significantly different at $P \leq 0.05$.

TABLE 2 . Effect of inoculation on dry matter and N yields of wheat plants fertilized with ^{15}N-rice residues.

Inoculation	Dry matter yield (g pot^{-1})	N concentration (%)	N accumulation (mg pot^{-1})
		Shoots	
Uninoculated	0.82 c	0.3 d	2.5 c
Azospirillum	1.24 b	0.39 c	4.8 b
Rhizobium	1.51 a	0.44 b	6.7 ab
Azosp. + Rhiz.	1.58 a	0.48 a	7.7 a
LSD (0. 05)	–	0.006	2.247
C.V. (%)	23.06	3.90	20.73
		Grains	
Uninoculated	0.27 b	1.02 d	2.8 c
Azospirillum	0.37 b	1.38 c	5.1 b
Rhizobium	0.42 b	1.45 b	5.9 b
Azosp. + Rhiz.	0.81 a	1.51 a	12.3 a
LSD (0. 05)	0.179	0.006	1.359
C.V. (%)	24.18	1.34	10.43

Means in each column followed by the same letter are not significantly different at $P \leq 0.05$.

TABLE 3. Nitrogen derived from fertilizer (Ndff), ^{15}N % atom excess and 15N recovery as affected by inoculation[a].

Inoculation	Ndff (%)	Ndff (mg pot^{-1})	^{15}N % atom excess	^{15}N recovery[b] (mg pot^{-1})
		Shoots		
Uninoculated	33.0 a	1.62 b	1.65 a	0.08 b
Azospirillum	26.6 b	1.81 b	1.33 b	0.09 b
Rhizobium	25.4 b	2.85 ab	1.27 c	0.14 ab
Azosp. + Rhiz.	25.2 b	3.28 a	1.26 c	0.16 a
LSD (0. 05)	1.304	1.34	0.06	0.06
C.V. (%)	2.36	28.02	2.36	31.24
		Grains		
Uninoculated	25.8 a	1.75 ab	1.29 a	0.09 ab
Azospirillum	20.4 b	1.43 b	1.02 b	0.07 b
Rhizobium	16.8 d	1.70 ab	0.84 c	0.08 ab
Azosp. + Rhiz.	18.0 c	1.93 a	0.90 c	0.10 a
LSD (0. 05)	1.123	0.44	0.06	0.02
C.V. (%)	2.76	12.69	2.76	11.20

[a] Means in each column followed by the same letter are not significantly different at $P \leq 0.05$.
[b] Determined by multiplication of total N (mg pot^{-1}) x (atom % ^{15}N excess).

TABLE 4 Nitrogen derived from rice straw residue (Ndfr), 15N % atom excess and ^{15}N recovery as affected by inoculation.

Inoculation	Ndfr (%)		^{15}N % atom excess	^{15}N recovery (mg pot^{-1})
	(%)	(mg pot^{-1})		
		Shoots		
Uninoculated	33.2 a	0.83 b	1.03 a	0.02 c
Azospirillum	25.8 b	1.24 ab	0.80 b	0.04 b
Rhizobium	25.1 b	1.68 a	0.78 c	0.05 a
Azosp. +Rhiz.	22.6 c	1.74 a	0.70 d	0.05 a
LSD (0.05)	0.99	0.56	0.019	0.006
C.V. (%)	1.87	20.51	1.87	22.44
		Grains		
Uninoculated	31.3 a	0.88 c	0.97 a	0.03 c
Azospirillum	21.9 b	1.12 bc	0.68 b	0.03 b
Rhizobium	20.3 c	1.20 b	0.63 c	0.04 b
Azosp. +Rhiz.	19.0 d	2.34 a	0.59 d	0.07 a
LSD (0.05)	0.723	0.30	0.019	0.006
C.V. (%)	1.55	10.77	1.59	13.54

Means in each column followed by the same letter are not significantly different at $P \leq 0.05$

TABLE 5. Nitrogen derived from air (Ndfa) and utilized by inoculated wheat plants amended with ^{15}N- ammonium nitrate or 15N labelled rice straw.

Inoculation	^{15}N-ammonium nitrate		^{15}N – rice straw	
	Ndfa		Ndfa	
	(%)	(mg pot^{-1})	(%)	(mg pot^{-1})
		Shoots		
Azospirillum	19.4 b	1.32 b	22.3 b	1.07 b
Rhizobium	23.0 a	2.57 a	24.3 b	1.63 b
Azosp. +Rhiz.	23.6 a	3.07 a	32.0 a	2.46 a
LSD (0.05)	2.74	0.94	2.58	0.84
C.V. (%)	6.31	18.03	4.97	21.62
		Grains		
Azospirillum	20.9 c	1.46 b	29.9 c	1.52 b
Rhizobium	34.9 a	3.52 a	35.0 b	2.06 b
Azosp. +Rhiz.	30.2b	3.23 a	39.2 a	4.82 a
LSD (0.05)	5.45	0.73	2.14	0.73
C.V. (%)	9.58	11.80	3.10	11.52

Means in each column followed by the same letter are not significantly different at $P \leq 0.05$.

Discussion

The promotion effect of *Rhizobium* inoculation either alone or in combination with *Azospirillum brasilense* is the most interesting reaction released from the present work. This bacteria was isolated from the Egyptian berseem clover and showed ability to nodulate (Nod$^+$) the root system and very effective in symbiotic N_2 fixation (Fix$^+$) Naturally, *Rhizobium*, in general, can be survived over many decades in soil without its host. The concentration of biovar *Trifolii* in the soil may reached 10^2 cells g^{-1} soil. In fields intensively cultivated with legumes the concentration was higher. For example, with monoculture of *Trifolium, R. leguminosarum* bv. *Trifolii* reached 7 x 10^5 cells g^{-1} soil. The populations of these bacteria are influenced by groups of abiotic, microbiotic and biotic factors (Werner, 1992). Also, this bacteria can occupy another endophytic niches inside different cereal crops as we will discuss later, and via different mechanisms can benefits these cereal hosts. Some of the responsible mechanisms, *i.e.* N_2 fixation and plant growth promoting (PGP) have been approved through ^{15}N techniques followed in the present study. These finding lead us to focus on the synergistic effect of both bacteria on each other and gave us the opportunity to suggest further research in this area on field scale with different cereal crops and different soil types. Noteworthy, some positive findings with dual inocula of *Rhizobium* and *Azospirillum* were previously detected by author under field conditions (Galal *et al.*, 2001).

Application of rhizobia with non-legumes proved the significance of these bacteria that have the ability to invade the lateral roots and multiply in cellular or intercellular root structure of rice, wheat and maize plants through the crack-entry mechanism (Reddy *et al.* 1997; Webester *et al.*, 1997; Yanni *et al.*, 1997; 2001; Galal *et al.*, 2001). Yanni *et al.* (2001) demonstrated that a few *Rhizobium* strain-variety combinations significantly increased rice grain yield, agronomic fertilizer N-use efficiency and harvest index. The benefits of this association leading to greater production of vegetative and reproductive biomass likely involve rhizobial modulation of the plant's root architecture for more efficient acquisition of certain soil nutrients. They showed that these bacteria could produce auxin and gibberellins representing two major classes of plant growth regulators. In this respect, Antoun (1998) explained that the beneficial effects of *Azorhizobium* are related not only to its N_2-fixing proficiency but also to the ability of producing antibacterial and antifungal compounds, growth regulators and siderophores. Saleh *et al.* (2000) confirmed the promotion of maize growth by *Azorhizobium caulinodans* inoculation .

Inoculation and fertilizer-N sources significantly affected the availability of nitrogen to wheat plants grown in pots under the experimental conditions of this study. Inoculation with *A. brasilense* strain as one of the common diazotrophic bacteria, promoted wheat growth and grain yields either with N_2 from organic residue or inorganic N fertilizer. Pinhiero *et al.* (2002) stated that recent microscopic evidence acquired using strain-specific monoclonal antibodies and specific gene probes confirms earlier claims that some strains of *Azospirillum lipoferum* and *A. brasilense* are capable of infecting the interior of wheat roots. At the same time, the Scanning Electron Microscopy (SEM) images showed that at the root tip the *Azospirillum* cells were principally located in cracks between epidermal cells. In the root hair zone the bacteria were more numerous but again principally located in the depressions. In a pot experiment conducted by Saubidet *et al.* (2002), *A. brasilense* bacteria were reported to improve yield of wheat plants. However, the mechanisms through which this effect is induced is still unclear. At the booting stage, the inoculated roots showed a similar colonization by *Azospirillum* spp. that was not affected by N addition. The plants grown in the disinfected soil showed a higher biomass, N content and N concentration than those in the non-disinfected soil, and in both soils the inoculation stimulated plant growth, N accumulation, and N and NO_{3-} concentration in the tissues. The same authors found that at maturity, the inoculated plants showed a higher biomass, grain yield, higher grain protein concentration and N content than the uninoculated ones. They concluded that *A. brasilense* increased plant growth by stimulating nitrogen uptake by the roots. In a field experiment, the results obtained by Kaushik *et al.* (2002) clearly demonstrated that strains of *A. brasilense* capable of growing and producing PGP substances as a suggested mechanism for promoting plant growth and nutrient acquisition are better inocula for wheat at sub-optimal temperature, 22°C. Under greenhouse controlled conditions, Dobbelaere *et al.* (2002) explained that the effect of inoculation with wild type *A. brasilense* Sp 245 was most pronounced at low to intermediate N fertilization levels, while the organic matter (OM) content of the substrate had no effect. Inoculation was found to affect early plant and root development, plant and root dry weight, grain yield and the N-uptake efficiency of plants. However, inoculation did not change the N concentration in shoots or grains. In addition, a difference in the ability of both strains to stimulate plant growth and N uptake of wheat and maize was observed, with *A. brasilense* Sp245 having a better effect on spring wheat and *A. irakense* KBC1 being more effective on grain maize

Applications of organic ^{15}N-rice residue was found to have a positive effect on all estimated parameters, but still in lower values than those obtained with the labelled inorganic fertilizer N. These lower values of wheat growth parameters and N uptake could be attributed to its low content of N and high C/N ratio that lead to N-immobilization in the unavailable organic fraction. Seligman *et al.* (1986) suggested that most of the N in organic residues with a wide C/N ratio is rapidly incorporated into a stable organic N fraction and is only moderately available to plants. They added, however, that some nitrogen was mineralized and taken up by the plants despite the wide C/N ratio of the ^{15}N-labelled residues. Consistently, during the decomposition of plant residues low in N (*e.g.* wheat and rice straw), soil N may be transformed into highly stable and complex organic matter components, rendering the soil N unavailable to the subsequent crop for a long time. Azam *et al.* (1990) demonstrated that the decreased availability of soil N was responsible for the reduced plant growth in organic N amended to soil. This trend was more pronounced when ^{15}N-recovery was considered, where the lower recovery from labelled rice straw was noted as compared to mineral-N. In harmony with this finding, Norman *et al.* (1990) reported that the minimum estimate of N mineralized from rice residue from the time of incorporation until rice harvest was 9% and only 3% of this was recovered in the subsequent rice crop. Similarly, Bremer & van Kessel (1992) concluded that recovery of added N in wheat top was lower for green manure (*i.e.* lentil and wheat) than for $(NH_4)_2SO_4$ fertilizer (19 vs. 34%) because the proportion of green-manure N mineralized was less than the proportion of fertilizer N immobilized. In a comparative study, Soon & Arshad (2002) found that wheat straw decomposed more slowly than canola or pea straw loosing an average of 12%, 24% and 25%, respectively, of initial dry matter of residues

Despite the good linear correlation in this work between ^{15}N-residue and ^{15}N-mineral fertilizer indicating the possibility to use the organic residues (direct method) for quantifying N derived from residue as well that of ^{15}N-mineral fertilizer (indirect method), we are in agreement with Hood *et al.* (1999) and

Hood (2001) who stated caution when using the direct or indirect method. They explained that pool substitution appeared to be responsible for the discrepancy between the direct and indirect techniques. Hood (2001) concluded that the modified soil labeling approach allows reasonable measurement of N derived from residues, because it is assumed that the technique overcomes the problems associated with pool substitution.

Considerable amounts of N_2 fixed by shoot and grains with the application of crop residue indicated the promotion effect on diazotrophic and symbiotic bacteria. An excellent review by Dommergues (1980) revealed that at different levels, the incorporation of organic matter into the soil increased and promotes both symbiotic and asymbiotic nitrogen fixation depending on nature and chemical composition of the organic source .

The data of ^{15}N outcome from the present study may gave us the chance to answer some questions raised about the mechanisms responsible for improveing *Rhizobium* and/or *Azospirillun*-cereal association. Considerable amounts of N_2-fixed revealed the possibility of wheat to benefit from this portion to compensate a part of N requirement. This finding may give evidence about the potential importance of biological N_2 fixation in the particular plant-microbe association which drive Yanni *et al.* (1997) to suggest the requirement of further investigation including studies using ^{15}N labelled techniques. Also, the combination between *Rhizobium* and *Azospirillum* has a synergistic effect on plant growth and N_2 fixation. The hormones excreted by both bacteria may have a potential to modulate the growth physiology of the host enabling the plant root system to utilize the existing resources of available nutrients and water more efficiently in ways that may be independent of biological N_2 fixation (Yanni *et al.,* 1997). This alternative mechanism is confirmed by the data of ^{15}N recovery that revealed enhancement of fertilizer N uptake as affected by dual inoculation. Such PGP mechanisms appear to be largely responsible for the ability of the endophytic diazotroph *Azospirillum* brasilense to enhance growth of various cereal crops (Okon & Labandera-Gonzalez, 1994; Galal, 1997; Yanni *et al.,* 1997; Galal *et al.* 2001; Galal & Thabet, 2002).

Our results further indicate the potential of exploiting the plant-rhizobia association as an alternative agrobiotechnology approach that may assist low-income farmers in increasing cereal production with less fertilizer N in sustainable agriculture and environmentally safe farming .

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تقييم إتاحة الازوت من مصادره المعدنية والعضوية لنبات القمح تحت تأثير التلقيح بالريزوبيا والازوسبيريللم

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أختبر تأثير التلقيح بالريزوبيا والازوسبيريللم لنباتات القمح المنزرعة فى أصص تحتوى على تربة رملية تم تسميدها إما بنترات الأمونيوم الموسومة أو بقايا قش الأرز الموسوم بالازوت المستقر (^{15}N) من تجربة سابقة . تأثر تراكم المادة الجافة في السيقان وكذلك محصول الحبوب بالتلقيح البكتري مقارنة بمعاملة الشاهد غير الملقحة، وكانت أعلي القيم المتحصل عليها من التلقيح المختلط يليها التلقيح بالريزوبيا ثم الازوسبيريللم على التوالى . نفس الاتجاه تم رصده فى حالة امتصاص الآزوت بواسطة كل من السيقان والحبوب . وعموما كان تراكم المادة الجافة في السوق ومحصول الحبوب وكذا امتصاص الآزوت أعلى فى حالة التسميد بنترات الأمونيوم عنها فى حالة إضافة بقايا الأرز . بنفس الاتجاهات كان حال الآزوت المنفرد من السماد المعدنى أو ذلك المنفرد من البقايا العضوية . التلقيح بواسطة الريزوبيا منفردة أو مع الآزوسبيريللم أعطت نسب عالية من الآزوت المثبت مقارنة بمعاملة الآزوسبيريللم المنفردة وقد كانت تلك النسب أعلى فى حالة إضافة البقايا العضوية منها فى حالة التسميد المعدنى وتحت ظروف التلقيح المختلط . عند إخضاع كميات الآزوت الممتص والنسبة المئوية للآزوت المستقرالمزود (^{15}N% atom excess) والكمية الممتصةمن السماد والبقايا العضوية (%Ndff& % Ndfr) وتلك المأخودة من الهواء الجوى(%Ndfa) لتحليل الارتباط وجد ارتباطا معنويا عاليا بين مصدري الآزوت . وكذلك وجد ارتباطا معنويا فيما بين السوق والحبوب عند قياس كمية الآزوت المستقر المستعاض تحت ظروف إضافية البقايا العضوية ($r = 0.552$) . أو السماد المعدنى($r = 0.602$). يشير هذا البحث إلى إمكانية استخدام التلقيح بالريزوبيا مع النباتات غير البقولية كما هو الحال مع نبات القمح . كما أن استخدام البقايا النباتية فى التسميد الآزوتى والتى تعمل كأسمدة بطيئة التحلل يمكنها أن تكون مفيدة فى دراسات التثبيت الحيوى للآزوت لثباتها خلال فترة التجريب مع الاستفادة التامة من الآزوت المنفرد منها خلال عمليات المعدنة .