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Full Length Research Paper

Competitiveness and symbiotic effectiveness of Rhizobial inoculants on Field Pea (*Pisum Sativum*) under field condition

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ABSTRACT

Field pea (*Pisum sativum*) is widely cultivated in Ethiopia as a source of protein and integrated in crop rotation to improve soil fertility because it fixes nitrogen with rhizobia. However, effectiveness in nitrogen fixation depends upon the selection of symbiotically effective rhizobia to enhance production. Thus, a study was conducted to evaluate the performance of three inoculants (isolates); *FBR 11*, *FBR 15*, *FBR 23* on growth, nitrogen fixation and yield under field conditions in relation to a commercial rhizobial strain *1018* at Kulumsa Agricultural Research Center during 2015-2016 growing seasons. Inoculation of field pea showed a highly significant ($p \leq 001$, $p \leq 0.01$ and $p \leq 0.05$) effect on all parameters compared to the un-inoculated plants in the field trial. Accordingly, plants inoculated with isolate *FBR15* and Strain *1018* showed a significant increase in nodule number 84-112 NN/plant (10 times), NDW 77-94 mg/plant (12 times) against the un-inoculated control plants, and they also showed a 20-25% increase in both parameters in comparison to *FBR11* and *FBR23* inoculated plants. The treatments with *FBR15* and the reference strain *1018* also showed significant difference in grain total nitrogen, N uptake and straw N uptake, and seed protein with 50-100% and 12-20% difference from the un-inoculated control plants and other Rhizobium treatments, respectively. Positive correlations were observed with respect to the number of nodule and shoot dry weight ($r=0.49$, $p<0.05$), number of nodule and number of pod ($r=0.59$, $p<0.01$), number of nodules and total grain yield ($r=0.56$, $p<0.05$), and shoot dry weight and N content ($r=0.73$, $p<0.001$). Although the MPN count of rhizobia in the soil at the experimental site was 1.5 × 104 and contained sufficient number of indigenous rhizobia, they were not effective that was indicated by the nodulation and yield parameters of the un-inoculated treatments. The data in general, showed that the rhizobial inoculants, particularly *FBR 15* isolate was nutritionally versatile, ecologically competent, and symbiotically effective rhizobia compared to the commercial inoculant (strain *1018*) that could be used as commercial inoculants for pea production after it is tested (validated) at different agro-ecological conditions.

Keywords: Biological yield, grain yield, inoculation, nodule occupancy, N uptake

INTRODUCTION

Field pea *(Pisum sativum L.)* is one of the cool season leguminous crops widely cultivated in Ethiopia at altitudes between 1800 and 3000 meters above sea level with annual average rainfall of 700-900 mm in the different regions of Oromia, Amhara, Tigray and Southern Ethiopia (EEPA, 2004). It is the second most important leguminous crops grown in the country after faba bean in terms of both area coverage and production. Field pea covers over 254,000 hectares with total production of 230,000 tons that accounts to 17% of the total grain legume production (IBC, 2008). It represents a useful complement to cereal based diets as a relatively inexpensive source of high quality protein. It contains 21-25% protein, 33-50% starch and amino acid (Lazanyi, 2002). Consequently, it is an important pulse in the daily diet of the society in urban and rural areas. It is eaten whole, spilt or milled usually fresh, fried, boiled or mixed with other cereals to make various types of stews and soups (EEPA, 2004). Field pea is integrated in different crop systems as a sole crop or an intercrop in crop rotation for its capability to fix nitrogen in symbiotic association with root nodule bacteria known as *Rhizobium leguminosarum biovar viciae* to the tune of 200-300 kg ha^{-1} yr^{-1} (Erman et al., 2008). For many years various studies have been carried out nation-wide to improve field pea cultivars in Ethiopia (Amare and Adamu, 1994). Many of these studies were restricted to soil plant nutrition and fertilizer trials in different agricultural research institutes (Tekalign and Asgelil, 1994). Recently few studies on taxonomic and symbiotic properties were undertaken on field pea rhizobia by collecting root nodules from different parts of Ethiopia (Kassa et al., 2015). Most of the studies were limited to laboratory and greenhouse based experiments and hence, further research must need to be worked on selected rhzobial strains under field conditions.

Although greenhouse screening of isolates is a preliminary work for symbiotic effectiveness of rhizobia, field trials are essential to assess their adaptive capability to field conditions and their competitiveness against the most recalcitrant, but often ineffective indigenous rhizobia in the soil (Theis et al., 1991; Evans et al., 1996). This necessitates a field trial in order to assess their field performance and select them for inoculant production to be used as biofertilizers.

MATERIAL AND METHODS

Description of Study Area

The experiment was conducted at Kulumsa Agricultural Research Centre (KARC), which is located in Tiyo wereda of Arsi zone in the Oromiya National Regional State, Asela, Ethiopia during the main cropping season of June-November, 2015. The experimental site is located within latitude 8001' 10" N and longitude 39009'11" E with altitude of 2200 m above sea level. The site receive 832 mm of rainfall with bimodal distribution (June to September and February-April) with average annual maximum temperatures of 22°C and minimum temperatures of 10°C.

Source of Rhizobia, Carrier and Seed

The selected rhizobia were *FBR 23*, *FBR 11* and *FBR15* obtained from culture collections of the Department of Microbial Cellular and Molecular Biology at Addis Ababa University (App. 5 and 6). They were selected based on their nutritional versatility and eco-physiological tolerance under laboratory test and symbiotic effectiveness on sand culture under greenhouse conditions. The commercially released *Rhizobium leguminasorium* strain (strain *1018*) obtained from Menagesha PLC was included as standard reference strain. Peat was obtained from Holeta Agricultural Research Center used as inoculants carrier. One of the seed of field pea improved varieties (*Pisum sativum* L., *Tegegnech*) was obtained from Kulumsa Agricultural Research Centre (KARC), EIAR.

Treatments and Experimental Design

A total of 6 treatments were arranged using the three selected rhizobia isolate, a standard reference commercial inoculants (strain), and 2 (1 positive and 1 negative control groups). A non nitrogen fixing reference crop (Barely variety, *Direbe*), released from KARC was also included to estimate the nitrogen derived from the atmosphere (Ndfa) using the N difference technique. The experiment was laid out in RCBD with factorial arrangement in three replications on one location. The plot size was 4 m by 3.6 m with a plot to plot spacing of 0.4 m and block to block spacing of 1 m.

Enumeration of Indigenous Rhizobia in the Soil

The rhizobial population from the soil (collected from the selected field site) was estimated using Most Probable Number (MPN) by inoculating soil dilutions on the host grown in Leonard Jar under greenhouse conditions after 28 days of growth (Somasegaran and Hoben, 1994). The MPN was calculated from the most likely number (m) found in MPN tables. The most likely numbers (m) were located from the table as MPN per gram of soils:

$$X=(m \times d)/V$$

Where X=rhizobia population per gram of soils, m=likely number from MPN table for the lowest dilution of series, d=lowest dilution (first unit used in tabulation) and V=volume of aliquot applied to plant.

Determination of Grain and Straw Yield, Total Nitrogen Content and N Uptake

Total nitrogen in grain and straw sub samples was quantitatively determined by Kjeldhal procedure (Page et al., 1982). Nitrogen up take in the grain and straw was determined after multiplying their N contents with their respective yields (Taye and Asfaw, 2010).

Estimation of Biological Nitrogen Fixation (Nitrogen Derived from Fixation) of Pea Plants using a Reference Crop

N-Difference Method: Field estimation of N2 fixation (nitrogen derived from fixation) was determined by measuring the total amount of N in the legume crop and nitrogen content in a non- fixing reference crop (barely, *Direbe*) (Beck et al., 1993). The amount of N2 fixed was calculated by subtracting the N yield of the reference crop from the N yield of legume as follows.

The quantity (Q) of N derived from N2 fixation was calculated as:

Q={(Total N of legume-Total N of reference crop)/Total N of legume} x 100

Determination of Seed Protein

In order to determine crude protein in seeds representative seed samples were taken from each treatment to determine total nitrogen in the seed through Kjeldahl Method (Page et al., 1982). Then the percentage of protein in seeds was calculated by multiplying the factor 6.25 (Morrison, 1956).

Plant and Seed Analysis

At physiological maturity five plants from each plot including the reference crop were harvested and separated into straw and grain. These samples were used to determine seed and straw N, total N, N derived from the atmosphere (Ndfa), and seed protein content. The sample materials were oven dried at 70°C to a constant weight and ground to pass through a 2 mm sieve. Plant tissue N was determined using Kjeldhal method (Page et al., 1982).

Data Collection and Analysis

Data on number of nodules, nodule dry weights and shoot dry weight was recorded after two months of planting. After having excavated selected plants from central rows, nodulation was scored as positive with at least one nodule, and counted as mean value of nodules plant^{-1}. At physiological maturity, five plants from central rows of each plot were randomly harvested to measure plant height, number of pods plant^{-1}, and number of seeds pod^{-1} (Birhanu and Pant, 2012). Thousand seeds weight, grain yield and above ground dry matter (biological yield) were recorded on net harvestable plot. Grain yields were adjusted to 10% moisture content and the yield per plot was converted to kg hectare^{-1} for statistical analysis. Field data were analyzed using SAS analytical software. Effects were considered significant if P values are <0.05 and means were separated using Duncan multiple range test (DMRT). Correlation analysis was carried out to study the nature and degree of relationship between numbers of nodule and selected parameters using the same software.

RESULTS AND DISCUTION

Estimation of Indigenous Rhizobia (MPN) in the Soil at the Experimental Site

The MPN count of rhizobia in the soil at the experimental site was 1.5 x 104, indicating that the soil contained sufficient number of indigenous rhizobia but they were not effective that was indicated by the nodulation and yield parameters of the un-inoculated treatments (Zahran, 2001).

Table 1. Effects of inoculation of the rhizobia on nodulation and growth parameters of field pea under field conditions. Means in the same column followed by the same letter are not significantly different at the 5% probability level by Duncan test. ***=significant at P=0.001; CV=coefficient of variation.

Treatment	No of nodule/plant	Nodule DW(mg)	Shoot DW(gm)	Plant height(cm)
Control	10.7^{c}	8.7^{c}	13.7^{c}	169.3^{b}
Recommended N	13.7^{c}	10.7^{c}	28^{a}	200^{a}
FBR 15	107^{a}	94.3^{a}	28.3^{a}	200^{a}
FBR 11	84^{b}	77^{b}	25^{b}	198.5^{a}
FBR 23	86^{b}	73.7^{b}	23.3^{b}	198^{a}
Strian *1018*	112^{a}	93.7^{a}	28.7^{a}	202.3^{a}
F Value	***	***	***	***
CV	7.6	7.8	3.6	0.7

Effects of Rhizobial Isolates on Nodulation, Shoot Dry Weight and Plant Height of Field Pea under Field Conditions

Nodule dry weight per plant: The field pea plants also displayed differences in nodule dry weight ranging from 8.7 mg plant^{-1} (un-inoculated negative control) to 94.3 mg plant^{-1} recorded from plants inoculated with rhizobial isolate *FBR 15* (Figure 1a). The nodule dry weight of plants induced by the standard strain *1018* and isolate *FBR15* (94.3 and 93.7 mg plant^{-1}) was significantly higher than the nodule dry weight obtained from other treatments with isolates *FBR 11* and *FBR 23* (73.7 and 77 mg plant^{-1}). The inoculated plants increased nodule dry weight by 8-10 times more than the un-inoculated control plants. This is much higher than the increase by 57% reported by Brkic et al., 2004.

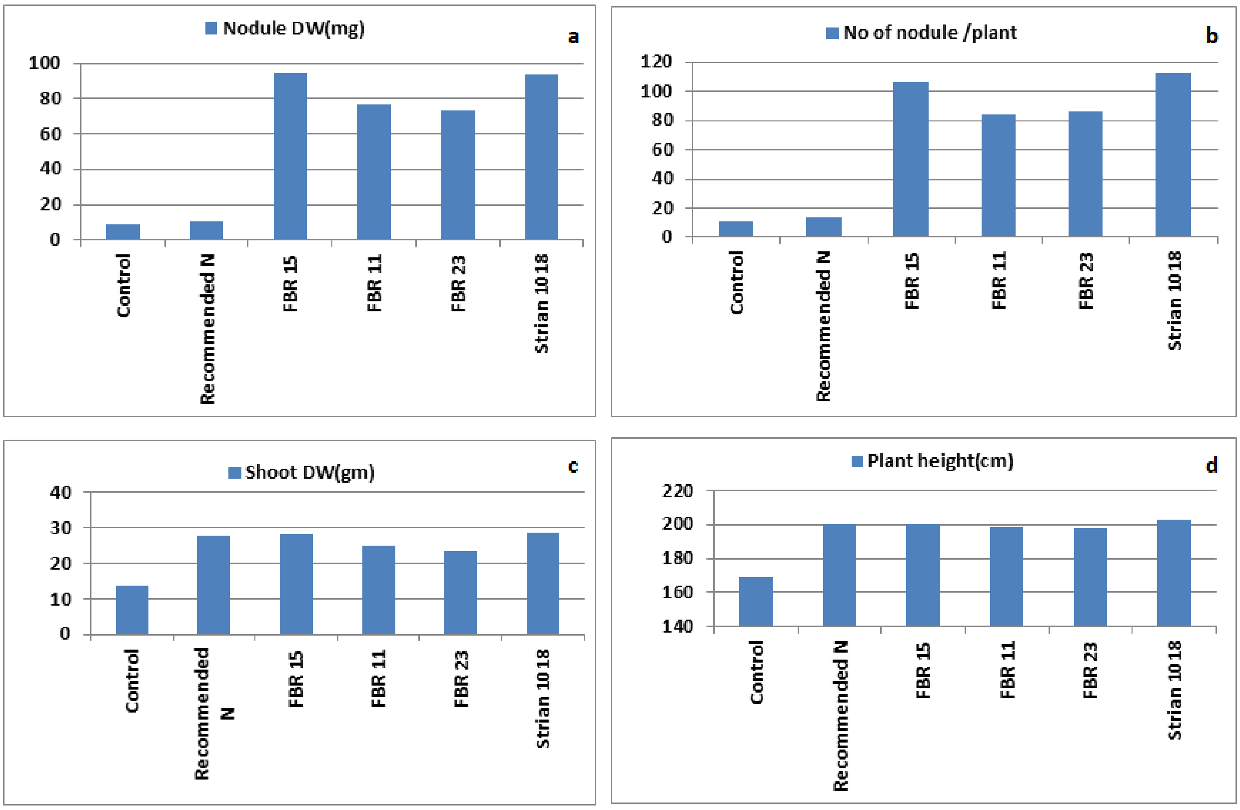

Figure 1. Means of effect of inoculation of the rhizobia on nodulation and growth parameters of field pea under field conditions

Number of nodules per plant: The field pea plants showed significant variation in nodulation and phenotypic characters (P<0.001) with inoculation and fertilizer treatments under field conditions (Table 1). Accordingly, the *FBR 15* and the commercial strain *1018* treated plants induced the highest number of nodules plant^{-1} (107 nn plant^{-1}) and the (112 nn plant^{-1}) that was significantly higher than the number of nodules produced (84/86 nn plant^{-1}) by isolates *FBR11* and *FBR 23*. On the contrary, the lowest number of nodules plant^{-1} (13.7 and 10.7) was recorded from N-fertilized pea plants and un-inoculated and non-fertilized plants respectively (Figure 1b). Number of nodule was higher in inoculated plants compared to the non-inoculated ones by the range of 20.3%-27.1%. Erman et al., 2009 also reported increase in nodule number by 11% compared to un-inoculated control plants.

Shoot dry weight: The inoculation of field pea with Strain *1018* and the indigenous isolate *FBR15* significantly (P<0.001) enhanced shoot dry weight of field pea comparable to the N-fertilized plants (28 g plant^{-1}) (Figure 1c). Thus, the field plants inoculated with the most effective isolates *FBR15* increased shoot dry weight as much as N-fertilized plants which was 50% higher than the un-inoculated (negative control) plants a (13.7 g plant^{-1}). This indicates inoculation by the most effective inoculants could increase shoot dry matter significantly. This finding similar to the finding of Erman et al., 2008 who reported the highest shoot dry weight from inoculated field pea plant was greater than 14.4% of non-inoculated field pea plant shoot dry weight.

Plant height: The different treatments showed a significant difference in plant height ranging from 169.3 cm (negative control) to that of 202.3 cm recorded from the inoculation of the standard strain *1018* (Figure 1d). The variation in plant height did not show significant difference amongst all treatments, except the un-inoculated and non-fertilized treatments. The inoculated plant height was increased by 22% over the un-inoculated control plants. This result also obtained by Erman et al., 2008 which documented inoculated field pea plant height 11% greater than from non-inoculated field pea plant.

Effect oF Rhizobia Isolates Inoculation on Yield and Yield Components of Field Pea

Number of pods per plant: The inoculated plants with FBR15 and the standard strain *1018* increased the number of pods plant^{-1} by the number 14.7 plant^{-1} compared to the number of 7.7 pods plant^{-1} (negative control).

The inoculation gave the same pattern of pods counted from N fertilized plants. They showed significant increase of more than 50% pods per plant compared to the un-inoculated treatments and produced 13% more pods than the other rhizobial treatments *FBR 11* and *FBR 23* (Figure 2a). Seed inoculation might have increased nitrogen supply to crop plants, which ultimately resulted in more number of pods plant^{-1}. This result was similar with the findings of Ahmed et al., 2007 who conducted experiment with field pea and reported that Rhizobium inoculant significantly increased number of pods compared to inoculated (14.45 pods plant^{-1}) and un-inoculated control (9.25 pods plant^{-1}).

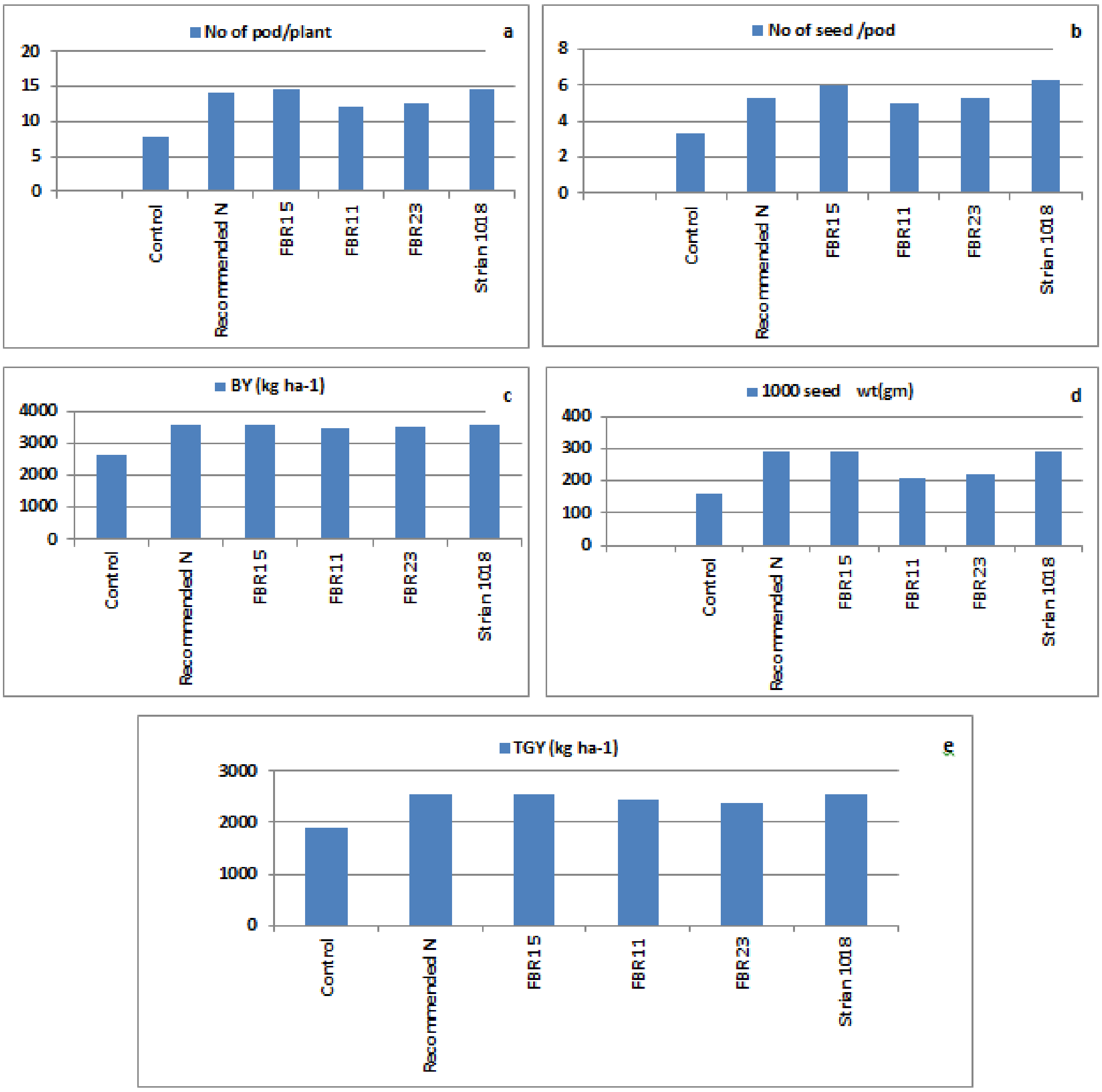

Figure 2. Means of effect of rhizobial inoculation on yield parameters of field pea under field conditions

Number of seeds per pod: Inoculation of different rhizobial strains showed significant difference in number of seeds pod^{-1}. The maximum number of seeds pod^{-1} was 6.3 and 6.0 obtained from the plant inoculated with strain *1018* and isolate *FBR 15* respectively, showing significance difference from other treatments. Accordingly, these effective inoculants produced twice as much number of seeds per pod over the negative control (3.3 seed pod^{-1}) and more than 20% of number of pods produced with other treatments (Figure 2b). Ahmed et al., 2007 reported that rhizobial inoculation produced 5.14 seeds pod^{-1} in pea plants whereas Solaiman and Rabbani, 2005 observed that rhizobium inoculant alone produced 6.3 seeds per pod of pea than the un-inoculated control (3 seeds pod^{-1}).

Total biomass or biological yield (BY) production (kg/ha): Analysis of variance indicated that total biomass (biological yield) of the inoculated and N-fertilized treatments were within the range of 3491 and 3584 kg ha^{1} without showing any significant difference among the treatments. However, the inoculated plants showed a 35% increase in BY compared to the 2656 kg ha^{1} BY obtained from un-inoculated negative control plants (Figure 2c) indicating that the different treatments significantly ($P<0.001$) improved pea production. Interestingly, Erman et al., 2008 showed a significant difference (33% in pea production of BY) of 3616.7 kg ha^{1} compared to the 1866.7 kg ha^{1} obtained from un-inoculated pea plants.

1000 seed weight (gm): The response of inoculation to rhizobial inoculants was significant on weight of 1000 seeds of field pea ($P<0.001$) compared with the control. The maximum mean weight of 292 for 1000 seeds was recorded from plants inoculated with the isolate *FBR 15*, strain *1018* and the plants fertilized with Nitrogen (Figure 2d). The treatments with the most effective inoculants significantly increased up to 20% and 50% of 1000 seed weight (g) of the other inoculants (*FBR11, 23*) and the negative control plants, respectively. This finding was similar with 1000 seed weight obtained with rhizobial inoculation (47.2% increases from un-inoculated control pea plants) (Rabbani et al., 2005). However, it was more than twice higher than the seed weight of 161 g and 131.9 g obtained from the inoculated and un-inoculated plants, respectively (Ahmed et al., 2007).

Total Grain Yield (kg/ha): The different treatments also significantly increased grain yield of field pea ($P=0.01$) (2389.8 kg ha^{-1}-2544.9 kg ha^{-1} compared to the negative control (1879.4) (Figure 2e). Although the treatments increased total grain yield by 35% compared to the un-inoculated control plants, the different treatments did not show significant difference amongst one another. The increase in yield may be due to effective nodulation and nitrogen fixation of different rhizobial isolate inoculation on total grain yield of field pea. These results have similarity with those of Erman et al., 2009 find significant difference among the rhizobial treatments although they increased total grain yield (TGY) of 2583.7 kg ha^{-1} in comparison with total grain yield obtained 2269.7 kg ha^{-1} obtained from un-inoculated control.

Effect of Rhizobia Isolates Inoculation on Total N and N Uptake from Grain and Straw

Grain total N and N up take: The field pea plants inoculated with the most effective rhizobial inoculants; strain *1018* and isolate *FBR15* significantly increased total N content and N uptake of grain up to 4% and 102 kg ha^{-1}, respectively ($P<0.001$) which was more than 32% and 46% of the total N content and N uptake recorded from the un-inoculated negative control plants (Figures 3a and 3b). The data also showed that these inoculants were more effective (12-14% increases) in accumulation of grain nitrogen content and grain N uptake than the other inoculants (*FBR 11* and *FBR 23*). Rather et al., 2010 reported that rhizobial inoculation significantly increased total N content (3.8%) compared to control (2.6%) in field pea plants.

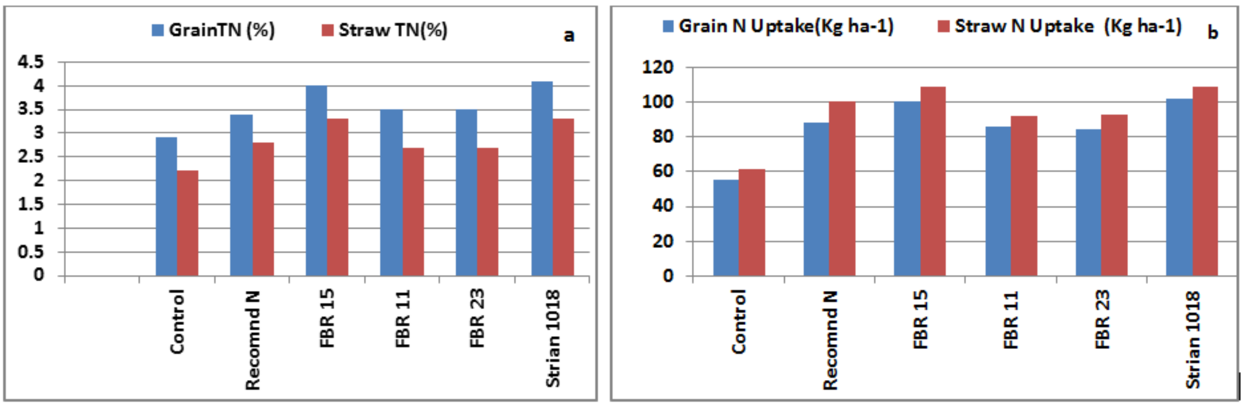

Figure 3. Means of effect of rhizobial inoculation on Ndfa (BNF), Total N contents of grain and straw **(a)** and Grain and straw N uptake **(b)** seed protein of field pea under field conditions

Straw total N and N uptake: The highest straw N (3.3%) and N uptake (108.6 and 108.4 kg ha-1) of the field pea plants was recorded from plants inoculated with strain *1018* and isolate *FBR 15* (Figures 3a and 3b). This was significantly different in N content (33%) and N uptake (45%) compared to the un-inoculated plants (negative control plants) and 12% more than the other inoculated plants (*FBR 11* and *FBR 23*) in both parameters. The lowest straw N (2.2%) and N uptake (61.6 kg ha^{-1}) was recorded from the non-inoculated control plant. This result is similar to the finding of Rather et al., 2010 who recorded (30-35%) of straw N content and (47%) of N uptake compared to the un-inoculated field pea plants.

The result indicated positive correlations between number of nodule and shoot dry weight ($r=0.49$, $p<0.05$), number of nodule and number of pod ($r=0.59$, $p<0.01$), number of nodules and total grain yield ($r=0.56$, $p<0.05$), and shoot dry weight and N content ($r=0.73$, $p<0.001$). Khondaker et al., 2003 also showed strong positive correlation between nodule dry weight and N content ($r=0.563$, $p<0.01$) for pea plant.

Seed Protein

The inoculated field pea plants with the effective inoculants (*FBR 15* and standard reference strain) showed the highest seed protein content (25%) which was higher by 28% compared to the negative control plants, and indicating a significant difference of 12-16% ($p<0.001$) from other inoculated treatments (Figure 4). The result was similar to the finding of Solaiman and Rabbani 2005 that showed a difference between the highest seed protein content (24.6%) of inoculated plants and the lowest protein content (17.4%) recorded from un-inoculated pea plants.

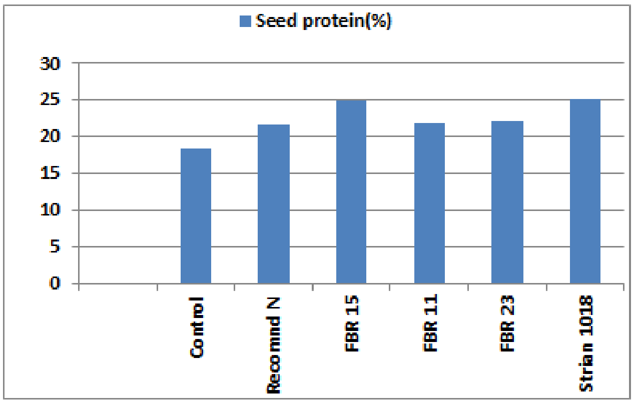

Figure 4. Means of effect of rhizobial inoculation on seed protein of field pea under field conditions

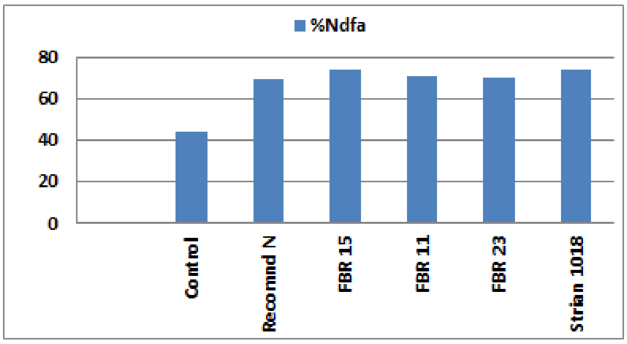

Figure 5. Means of effect of rhizobial inoculation on Ndfa (BNF) of field pea under field conditions

Nitrogen Derived from Fixation (Biological Nitrogen Fixation)

Rhizobium inoculation of field pea showed significance variation ($p<0.05$) in %Ndfa (Figure 5). The highest Ndfa (74.4%) was recorded in plants inoculated with strain *1018* followed by plants inoculated with isolate *FBR 15*, whereas non inoculants resulted in the lowest Ndfa (44.8%). Seed inoculation significantly influenced the amount of N fixed, which increased and significantly difference between inoculated and the control. The best criteria for a rhizobium used as bio-fertilizer is that it must be highly effective in nitrogen fixing ability forming symbiotic association with the host plants (O' Hara et al., 2002). This result similar to the result of Clayton et al., 2004 documented that the highest nitrogen fixation 76.2% from rhizobial inoculation and the lowest nitrogen fixation 59% from the control field pea plant.

CONCLUSION AND RECOMMENDATION

The tolerance of rhizobial isolates to different pH levels, temperature, salinity, carbon and nitrogen utilization, phosphate solublization and antibiotics and heavy metal resistance is an important quality of rhizobial strains to screen and develop inoculants that are capable with ecological competitiveness. In the presence of different environmental stresses, the tolerant isolates would survive, occupy nodules, and fix nitrogen and provide the host to boost plant production.

The result of this experiment indicated that inoculation of selected rhizobial isolate *FBR 11*, *FBR 15*, *FBR 23* and commercially released rhizobial strain *1018* improved number of nodule, nodule dry weight, shoot dry weight, number of pod $plant^{-1}$, number of seed pod^{-1}, grain yield, N content, seed protein and nitrogen fixation as compared to recommended N and the control. Also from the results of the correlation analysis showed that the nodule number was directly and highly significantly ($p \leq 0.01$) correlated with shoot dry weight ($r=0.49$), number of pod ($r=0.59$), number of seed ($r=0.63$), total grain yield ($r=0.56$) and N up take ($r=0.80$). The result indicated that there was no significant difference between rhizobial isolate *FBR15* and commercial rhizobial strain 1018 but there was significant difference with rhizobial isolate FBR 11 and *FBR 23*. Also selected rhizobial isolates was shown that highly effective and competitive. Based on the findings of this study, inoculation with selected rhizobial isolates improved nitrogen uptake and symbiotic nitrogen fixation efficiencies in field pea.

The data in general, showed that the rhizobial inoculant, particularly *FBR 15* was nutritionally versatile, ecologically competent, and symbiotically effective rhizobia comparable to the commercial inoculant Strain *1018*.

Based on the findings of this study the following recommendations are forwarded

Rhizobial isolate *FBR 15* can be used as commercial inoculants for pea production after it is tested (validated) at different agro-ecological conditions.

Selected rhizobial isolate *FBR 15* can be recommended as bio-fertilizer for better field pea production in the future.

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CONFLICT OF INTEREST

The author of this article declares that there are no conflicts of interest or financial interests regarding the publication of this article.

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