



Characterization of Symbiotic Effectiveness of Rhizobia Nodulating Field Pea (*Pisum Sativum*)

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Abstract

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Field Pea is one of the most important Legumes plants and widely grown in Ethiopia. A study was made to re- isolate, characterize, and select best rhizobia for field pea. Results showed that all the 25 isolates exhibited typical colony characteristics and presumptive reactions of fast growing rhizobia. Out of the 25 isolates, 3(KL3, BR1 andCF5) relatively superior isolates were selected in sterilized sand. All isolates characterized their morphological and physiological characteristics. All isolates formed watery and mucoid colonies on YEMA medium, their mean growth time mostly between 2 &4 hours and failed to grow on peptone glucose agar medium and to solubilize inorganic phosphate. Almost all isolates were tolerated to pH 5to 9, 2% and 3% salt concentration, and at temperature of 15°C to 35°C. The isolates were also tolerant to erythromycin, streptomycin and ampicillin, and relatively sensitive to penicillin and chloroamphenicol at concentration of 50µg/ml. All isolates utilized to sucrose, glucanate, galactose and fructose as the sole source of carbon, and almost all isolates grow on YEMA medium containing galactose (90%), fructose (88.9%) and glucanate (76.7%) and the isolates utilized many amino acids as the source of nitrogen. BR1 was the most competitive inoculant with nodule occupancy of 75%; followed by KL3 and CF5 with nodule occupancy of 60 and 50% respectively. The mean nodule number, nodule dry weight, mean shoot dry weight and N content and of the host plants inoculated with different isolates showed variations. Particularly BR1 can be recommended as inoculants and good strain for field pea in the future.

Key Words

Field Pea
Nodule Occupancy
Rhizobium Symbiotic
Effectiveness

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1. Introduction

Biological nitrogen fixation (BNF) is an efficient source of nitrogen. It is estimated that 175 million tons of nitrogen yr⁻¹ is fixed by symbiotic association of which more than 70% is contributed by the legume-Rhizobium association [1]. It represents the most important renewable resource of nitrogen in the terrestrial ecosystem.

Field pea (*Pisum sativum*) 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 [2].

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 [3]. It represents a useful complement to cereal-based diets as a relatively inexpensive source of

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high quality protein. It contains 21-25% protein, 33-50% starch and amino acid [4].

Field Pea like other legumes is capable of fixing and utilizing atmospheric nitrogen through symbiotic relationship with Rhizobium bacteria at the root of the crop. Rhizobium inoculants significantly improves yield in many leguminous crops and can minimize the use of synthetic nitrogenous fertilizer, which is rather expensive and causes injury to soil properties [4]. This crop thus improves soil and economizes crop production not only for itself but also for the next cereals (non-legume crops) grown in the relation and thereby reducing the requirement of added nitrogen fertilizers [5].

Field Peas are used in crop rotation for improvement of soil fertility and yield of the succeeding crops. In crop rotation tests, spring wheat and durum wheat grown on pea stubble produced higher yields and a higher protein percentage compared to wheat grown on wheat stubble [4].

2. Materials and Methods

2.1. Collection And Isolation of Rhizobial Isolates

Nodules were collected from 5 major field pea growing area Kersa, Chefa, Munesa, Boru and Kulumsa of Arsi zone Ethiopia presented in Table (1). The collected nodules were surface sterilized with 95% ethanol for 10 seconds, and transferred to 3% (v/v) solution of sodium hypo chlorate for 2-minutes [6]. The surface sterilized nodules were then rinsed with sterile distilled water six times to completely remove the sterilizing chemicals. The nodules were crushed with sterile glass rods in 1 drop of sterilized 0.85% NaCl. The crushed nodules were then transferred to YEMA medium (DIFCO). They were then incubated at 28°C and periodically checked for colony formation.

2.2. Purification and Preservation

Colonies were picked with sterile inoculating loop and streaked repeatedly on sterile YEMA plates and incubated at 28°C. A total of 30 selected pure rhizobial isolates from each inoculant treated (KL3, BR1, and CF) nodules were selected and were preserved on YEMA slant tubes containing 0.3% (W/V) CaCO₃ at 4°C [7].

2.3. Preliminary Screening of Rhizobia From Nodules (Presumptive Test)

2.3.1. Gram Staining Test

Gram staining was carried out to confirm that all isolates were gram negative and do not contain any gram positive bacteria or contaminants [6].

2.3.2. Congo Red Absorption

Colonies were tested for congo red absorption on Congo-Red (CR-YEMA) [7]. Stock solution of Congo Red (CR) prepared by dissolving 0.25gm of CR in 100ml sterile distilled water from which, 10ml was added to one liter of YEMA. Culture suspensions were inoculated into YEMA-CR medium, and the plates were wrapped with aluminum foil and incubated at 28±2 °C for 3-5 days.

2.3.3. Acid and Alkaline Production on BTB

Isolates were tested for the production of acid or alkaline by incorporating (0.5%) bromothymol blue (BTB) as reaction indicator on yeast extract manitol agar (YEMA) according to Somassegaran and Hoben, (1994). After 48 hours of growth, a loop full of *Rhizobium* culture (10⁵cells/ml) was streaked on YEMA-BTB plate, and incubated at 28±2 °C for 3-5 days.

YEMA	:	1 liter
BTB-(0.5 % w/v in 95% ethanol)	:	5ml
pH	:	6.8

2.3.4 Growth on Peptone Glucose Agar (PGA) Medium

Isolates were inoculated on PGA containing bromocresol purple dye (10µ g/ml) in order to check a change in pH of the medium associated with the presence of contaminants (Somassegaran and Hoben, 1994). The PGA composition was glucose 5gm, peptone 10gm, agar 15gm and BCP 10ml then adjusted pH at 6.8.

The BCP (Bromo cresol purple) was prepared as stock solution by dissolving 1gm/100ml of ethanol. The pH was adjusted to 6.8 by 1N NaOH and HCl. The bacterial culture suspension was inoculated on the medium and incubated at 28±2°C; to detect the presence/absence of bacterial colonies.

2.3.5. Authentication

Isolates were authenticated as root nodule bacteria according to Somassegaran and Hoben, (1994). Healthy seeds of field pea (Tegegnech) were surface sterilized as before and transferred into 0.75% water agar plates and incubated at 28±2°C five seedlings were then transferred into surface sterilized 3kg capacity plastic pots filled with river sand soaked in H₂SO₄ for 24 hours and extensively washed with tap water several times. After 3-5 day, each seedling was inoculated with 1ml actively grown rhizobial culture (10⁹cells/ml), and later were thinned down to three per pot.

The experiment was set up in randomized complete design in a greenhouse with a 12/12 light/ dark hour's cycle and average 25/18°C day/night temperature. All experiments were done in triplicates by including

Table1. Locations and codes of the new rhizobial isolates

Isolate No	Location	Cod	Isolate No	Location	Cod
FP1	Kulumsa	KL1	FP14	Boru	BR4
FP2	Kulumsa	KL2	FP15	Boru	BR5
FP3	Kulumsa	KL3	FP16	Kersa	KR1
FP4	Kulumsa	KL4	FP17	Kersa	KR2
FP5	Kulumsa	KL5	FP18	Kersa	KR3
FP6	Munesa	MN1	FP19	Kersa	KR4
FP7	Munesa	MN2	FP20	Kersa	KR5
FP8	Munesa	MN3	FP21	Chefa	CF1
FP9	Munesa	MN4	FP22	Chefa	CF2
FP10	Munesa	MN5	FP23	Chefa	CF3
FP11	Boru	BR1	FP24	Chefa	CF4
FP12	Boru	BR2	FP25	Chefa	CF5
FP13	Boru	BR3			

positive control pots fertilized with nitrogen 0.05% KNO₃ (W/V once a week) but without inoculation) and negative control pots neither fertilized nor inoculated). All treatments were fertilized with full strength N-free nutrient at a rate of 100ml/pot once a week (Broughton and Dilworth, 1970) and washed with tap water every 2 days to control salt accumulation in pots.

After 6 weeks of planting, the whole plants were uprooted to count the number of nodules, and measure of nodule dry weight and shoot dry weight after drying at 70 °C for 48hrs until constant weight. Effectiveness of isolates in accumulating plant shoot dry weight was calculated according to the equation.

$$SE = \frac{\text{Inoculated plant DM}}{N - \text{fertilized plant DM}} \times 100$$

DM =dry matter

SE = symbiotic effectiveness

Nitrogen fixing effectiveness classified as ineffective, <35%; lowly-effective, 35-50%; effective, 50-80%; and highly effective, >80%.

2.4.Cultural and Growth Characteristics of Authenticated Rhizobial Isolates

2.4.1 Cultural conditions

All experiments were done in triplicates by growing the isolate for 48 hours and adjusted to inoculum size of 106 /ml. They were inoculated on YEMA medium, incubated for 3-5 days at 30oC unless stated otherwise.

2.4.2. Mean Generation Time

Each isolate was inoculated in to 10ml of YEMB test tube and shaken on orbital shaker at 120 rpm (revolution per minute) for 48hrs at room temperature. One ml of each culture was transferred into 250ml Erlenmeyer flasks containing 100ml of YEMB and placed on rotary shaker at 120 rpm (revolution per

minute). After calibrating spectrophotometer to zero with sterile uninoculated YEMB (3.5 ml blank), 3.5ml of culture samples were was transferred into cuvette to read optical density (UV-7804C-Ultraviolet-Visible spectrophotometer) at 540 nm beginning from time of inoculation (0hr) and at every 6hrs interval for 72hours. The generation time (g) was calculated from the logarithmic phase [19].

The formula;

$$g = \frac{\log_2 (t)}{\log X - \log X_0}$$

Generation time (g) =t/n

Where: g = generation time

t = time elapsed

XO = First OD,

X = second OD reading

OD = optical density

n = number of generations

2.4.3. Colony Morphology

The cultural characteristics of the isolates were performed after having grown them for 3-5 days on YEMA medium according to (Lupwayi and Haque, 1994). Each single colonies of each isolate was characterized based on colony appearance (texture), diameter, color, shape and extra cellular polysaccharide production.

2.5. Nutritional Characteristics

All isolates were checked for the following nutritional and eco-physiological characteristics as selective markers for the identification of the original inoculants (KL3, BR1 and CF5) in order to determine their nodule occupancy in comparison to the local rhizobia in the soil.

2.5.1. Carbohydrate Utilization

Isolates were checked for their ability to utilize different carbohydrate sources i.e. fructose, galactose and Gluconate. The test was carried out according to Somasegaran and Hoben, (1994). Each carbohydrate (w/v but v/v for glycerol) was prepared (10%) and mixed with basal medium containing $MgSO_4 \cdot 7H_2O$ (0.2gm), KH_2PO_4 (0.5gm), NaCl (0.2gm), manitol (10gm), yeast extract (0.5gm) and agar (15gm). Heat stable carbohydrates (fructose) were autoclaved together with the medium, but heat labile carbohydrates (galactose and gluconate) were filter sterilized using disposable membrane filter of 0.22 μ m and added to the basal medium (YEMA). After sterilization when the medium temperature was reduced to 50 °C. Finally, a loop full of 72 hours old YEM broth culture was separately streaked on the plates and incubated at 28 °C for 3 to 5 days and growth was recorded as (+) for positive growth and (-) for no growth in relation to the positive control YEMA plates.

2.5.2. Amino Acid Utilization

The isolates were streaked on different nitrogen source including: Glutamine, DL- β -Phenylalanine, Peptone and Arginine in order to determine their ability to utilize them. Each nitrogen source was added to a basal medium at concentration of 0.5gm/l [7]. Finally 72hr old rhizobial culture was inoculated in to these medium and incubated at 28 \pm 2 C for 3-5 days.

2.6. Eco-Physiological Characteristics

2.6.1. PH Tolerance

The ability of isolates to grow at different pH was tested on YEMA adjusted to pH levels 4.0, 4.5, 5.0, 5.5, 8.0, 8.5, 9.0 and 9.5 with sterile 0.1N HCl and 1N NaOH [8]. The results were recorded qualitatively as + for presence or – for absence of growth after 3-5 days of incubation at 28 \pm 2°C.

2.6.2. Temperature Tolerance

The ability of all isolates to grow at varying temperatures was assessed on YEMA plates incubated at the temperatures of, 4,10, 15, 35 and 40°C [6]. Growth was qualitatively recorded as (+) for growth and (-) for no growth .

2.6.3. Salt Tolerance

Tolerance of all isolates to sodium chloride (NaCl) evaluated through determining growth on YEMA solid medium supplemented with 2, 3, 5 and 6 % (w/v), NaCl concentration. Growth was evaluated qualitatively as (+) for growth and (-) for no growth after 3-5 days (Lupiwaiyi and Haque, 1994).

2.6.4. Heavy Metal Resistance

The resistance of isolates to heavy metals was tested by streaking them on solid YEMA medium containing different concentrations of heavy metals as described [6]. The heavy metals $ZnCl_2$ 25; $HgCl_2$ 5; $CuCl_2$ 100; $CrCl_2$ 100; $CdCl_2$ 20; Ni Cl_2 60 and Pb $(CH_3CO)_2$ 100 and 500 were filter sterilized using sterile 0.22 μ m pore size membrane filter (μ g ml^{-1}) and added to the media (YEMA) after autoclaving and cooling to approximately 50 °C and mixed thoroughly [6].The isolates were streaked on the plates and incubated at 28 \pm 2°C for 3-5 days. The result was recorded qualitatively either as +/- for growth and no growth, respectively.

2.6.5. Intrinsic Antibiotic Resistance (IAR)

The resistance of isolates to antibiotics was tested by streaking them on solid YEMA medium containing freshly prepared filter sterilized antibiotics using 0.22 μ m sized membrane filters: Tetracyclin, Erythromycin, Streptomycin, Penicillin, Chloroamphenicol, Neomycin, Ampicillin and Nalidixic acid and five concentrations (5, 10, 20, 30 and 50). The stock solution of each antibiotic was first prepared by dissolving 2gm of each antibiotic in 100ml of water as described [6]. Erythromycin was dissolved in ethanol and Nalidixic acid was dissolved in 1M NaOH, whereas the other was dissolved in sterilized distilled water. Each filter sterilized antibiotic solution was added to sterile YEMA cooled to 50°C and mixed thoroughly. The isolates were then streaked on the plates and incubated at 28 \pm 2°C for 3-5 days. The result was recorded qualitatively either as +/- for growth and no growth, respectively.

2.7. Nodule Occupancy of Rhizobial Inoculants From Nodules

Nodule occupancy of the three inoculants was determined by comparing the biochemical profiles of the three inoculants (KL3, BR1 and CF5). The major biochemical markers were Inherent Antibiotic Resistance (IAR), pH tolerance, carbohydrate and amino acid utilization, and tolerance to heavy metals. Isolates with more than 80% similarity with the original characters (markers) were considered similar (identified as KL3, BR1 and CF5).

2.8. Data Collection and Analysis

Data on number of nodules, nodule dry weights and shoot dry weight was recorded. The green house data was analyzed using SAS analytical software. Effects were considered significant if P values are \leq 0.05 and means were separated using Duncan multiple range test (DMRT).

3. Results and Discussion

3.1. Authentication and Characterization Of Rhizobia from Nodules to Estimate Nodule Occupancy Of the Test Inoculants

Nodule bacteria were re-isolated from nodules to estimate the nodule occupancy (%) of the three rhizobial inoculants (KL3, BR1 and CF5) and the indigenous (local) rhizobia in the soil of the field site using different eco-physiological markers.

All the isolates recovered from the nodules were gram negative fast growing with doubling time between 2 and 4 h, colony diameters within the range of 2.5mm and 5.5mm and formed mucoid, convex and white colonies after 3-5 days incubation on YMA plates, and changed the YEMA-BTB medium into yellow. They did not absorb red color from CR-YEMA medium and failed to grow on peptone glucose agar (PGA) medium, and re-nodulated field pea host indicating that they were fast growing rhizobia [9] and *Rhizobium leguminosarum* var *viciae* [10].

Isolates were utilized glucanate, galactose and fructose as the sole source of carbon, and almost all isolates grow on YEMA medium containing galactose (90%), fructose (88.9%) and glucanate (76.7%) (Table 2). Isolates were able to metabolize arginine (73.3%), glutamine (67.8%), peptone (63.3%), DL-phenylalanine (57.8%) and 65.5% of the isolates were able to utilize all amino acid tested this result greater than the findings [11] where 48% of the isolates utilized all amino acids sources.

The different isolates displayed antibiotic resistance to different types of antibiotics (Table 3). The data on inherent antibiotic resistance of isolates showed that they were resistant to erythromycin, streptomycin and ampicillin, and relatively sensitive to penicillin and chloramphenicol at concentration of 50µg/ml.

This pattern of resistance of the isolates was higher than the report [12] which showed pea rhizobia from soils of eastern Washington grow well on the same type of antibiotics with concentration of 5-20µg/ml. It also reported on resistant faba bean rhizobia, from Wollo, Northern Ethiopia [13]. It is reported that 90% and 88% of *Rhizobium leguminosarum* biovar *viciae* from field pea were resistant to chloramphenicol at concentrations of 5 and 10µg/ml respectively [14].

3.2 Ecophysiological Characteristics of Isolates

Most of the rhizobial isolates were tolerant to temperatures 35°C (95.5%) with optimum growth at 30°C (100%), but some of them were able to grow at 15°C (22.2%), but failed to form colonies at 4°C, 10°C

and 40°C (table 4). This was contrary to the report [14] who showed 50% and 64% of field pea rhizobia were tolerant to 5 and 10°C respectively.

Almost all of the isolates were tolerant to pH 5.5, 8 and 8.5 (100%) and some of the isolates were grown at pH 4.5 (6.3%), pH 5 (60%) and pH 9 (50%) however no growth was observed at pH 4 and 9.5 this result was similar to the result [10] that showed field pea rhizobial isolates did not grow at pH 4.

All isolates were tolerant to 2% NaCl concentration, but show diversity as the salt concentrations increased to 3 % (85.6%), 5 % (50%) and 6 % (25.6%). Field pea rhizobia from Ethiopia also showed tolerance up to 6% NaCl concentrations [14].

Evaluation of the intrinsic resistance to heavy metals showed that all tested isolates shown that high resistance to ZnCl₂, Pb (CH₃CO)₂, CrCl₂ and CuCl₂ at concentrations of 25, 100, 100 and 100 µg ml⁻¹, respectively and most of the isolates exhibited an intrinsic resistance to CoCl₂ 100 µg ml⁻¹ (77.8%), ZnCl₂ 50 µgml⁻¹ (74.4%), NiCl₂ 60 µgml⁻¹ (74.4%) and Pb (CH₃CO)₂ 500 µgml⁻¹ (54.3%). However, all isolates were sensitive to HgCl₂ 10 µgml⁻¹ (table 5) but some of the isolates were tolerate HgCl₂ 5 µgml⁻¹ (44.3%) this result was different with the finding [14] who reported 54% tolerant field pea rhizobial isolates to HgCl₂ 10 µgml⁻¹.

3.3. Nodule Occupancy of Isolates KL3, BR1 and CF5

Based on the original ecophysiological markers, the inoculants KL3, BR1 and CF5 were identified from each nodule sample. Accordingly, KL3 was characterized by its resistance to Nalidixic acid, Chloramphenicol, heavy metal lead and chromium, utilization of fructose, and phenylalanine; whereas BR1, was characterized on its resistance to neomycin, tetracycline, streptomycin, and heavy metals lead and nickel; utilization of glucose and peptone. CF5 was also detected based on its resistance to ampicillin and penicillin, mercury; utilization of phenylalanine, and tolerance to pH 4.5 (Table 6). Accordingly, BR1 was the most competitive inoculant with nodule occupancy of 75%; followed by KL3 and CF5 with nodule occupancy of 60 and 50% respectively. It is known that high competitiveness is a precondition of successful inoculation of a plant seed by rhizobium. This is the way how to add selected rhizobia isolate or strains to legume seeds effectively and consequently prevent the formation of nodules by low effective indigenous soil rhizobia [15].

Table 2. Pattern of carbon and amino acid utilization by rhizobial isolates collected from nodules of host pea plants inoculated with the three inoculants KL3, BR1 and CF5 incubated at 30°C for 3-5 days.

Details	Carbohydrate sources			Nitrogen sources			
	Glucanate	Galactose	Fructose	Glutamine	Phenylalanine	Peptone	Arginine
KL3	76.7	83.3	86.7	76.7	56.7	80.0	83.3
BR1	50.0	96.7	90	80.0	90.0	66.7	86.7
CF5	76.7	90	90	46.7	56.7	83.3	50.0
Average	67.8	90%	88.9	67.8	57.8	63.3	73.3

Table 3. Pattern of antibiotic of rhizobial isolates collected from the inoculants treated field pea plants grown on YEMA containing 50µg/ml and incubated at 30°C for 3-5 days

Inoculant	Amp	Nal	Tet	Chl	Pen	Ery	Neo	Str
KL3	90	43	70	51	70	73	67	100
BR1	83	80	45	86	73	90	43	57
CF5	67	50	60	77	55	80	53	90
Average	83	57	58	71	66	81	54	82

Table 4 Ecophysiological tolerance of the rhizobial isolates grown on YEMA medium and incubated at 30°C for 3-5 days

Inoculants	pH				ToC				NaCl (%)				
	4	4.5	5	9	9	4	10	15	35	40	3	5	6
KL3	-	-	60	50	-	-	-	22.4	94	-	96	46.7	23.3
BR1	-	20	63.3	46.7	-	-	-	24	97	-	80	50.0	26.7
CF5	-	-	56.7	50	-	-	-	20.2	95.5	-	79	53.3	26.7
Average	-	6.3	60	50	-	-	-	22.2	95.5	-	85.6	50	25.6

Table 5 Pattern of heavy metals resistance of rhizobial isolates collected from the inoculants treated field pea plants grown on YEMA containing different concentration

Isolates	Zn		Pb		Cu		Hg	Co	Ni	Cr
	25	50	100	500	100	5	10	100	60	100
KL3	96.7	73.3	100	53.3	93.3	43.3	0	76.7	76.7	93.3
BR1	100	76.7	100	56.7	100	46.7	0	80	73.3	100
CF5	100	73.3	100	53.3	96.7	43.3	0	76.7	73.3	93.3
Average	98.9	74.4	100	54.3	96.7	44.3	0	77.8	74.4	95.5

Table 6 Nodule occupancy of the inoculants on the basis of their specific ecophysiological characters originally identified during their prescreening activities.

Isolates	Markers (IAR)	Markers (pH) tolerance	Markers Carbon utilization	Markers Nitrogen utilization	Markers (HM) tolerance	Nodule occupancy (estimation)
KL3	Nal, Chl		Fru	Phen	Pb, Cr	60%
BR1	Neo, Tet		Glu	Peptone	Pb, Ni	75%
CF5	Amp, Pen	pH 4.5		Phen	HgCl ₂	54%

Table 7 Effect of rhizobia inoculants on degree of nodulation, shoot dry weight (symbiotic effectiveness), and N content of field pea on sand culture grown for 45 days under greenhouse conditions

Treatment	NN/p	NDW(mg plant ⁻¹)	SDW(g plant ⁻¹)	SE %	SE Rate	% N in shoot
Isolate KL3	198 ^b	105.0 ^b	2.3 ^b	77	E	1.7 ^a
Isolate BR1	230. ^a	121.3 ^a	2.9 ^a	97	HE	2.0 ^a
Isolate CF5	197. ^b	103.7 ^b	2.3 ^b	77	E	1.7 ^a
Strain EAL110	232 ^a	121.3 ^a	3.0 ^a	100	HE	2.0 ^a
(+) control	0	0	3.0	-	-	1.9 ^a
(-) control	0	0	1.4 ^c	-	-	1.3 ^b
CV	8.3	4.2	8.1	-	-	4.3
F value	208.8***	812.6***	54.7**	-	-	41.7*

Means in the same column followed by the same letter are not significantly different at the 5%. Probability level by Duncan test. ***, ** and * = significant at P= 0.001, 0.01 and 0.05 respectively; CV = coefficient of variation, HE = highly effective; E= effective; NN= nodule number; NDW=nodule dry weight and SDW= shoot dry weight.

3.4. Authentication and Symbiotic Effectiveness Test of Re-Isolated Rhizobial Isolates on Sand Culture

All the three rhizobial isolates (KL3, BR1 and CF5) and commercially released strain (strain 1018) nodulated pea plants on sand culture under greenhouse conditions (Table 7). Accordingly, they showed prolific nodulation (196-229 N/plant) with mean nodule number of 224; with nodule dry weight of 104-121mg/plant (mean weight of 113mg/plant), and shoot dry weight (2.3-2.9gm/plant) (mean 2.6g/plant) showing that the inoculants did not lose their nodulation capacity.

The data showed that isolate BR1 and Standard strain EAL110 showed significant variations in all parameters from the other two local isolates. Thus, isolates BR1 and the standard strain EAL110 isolates displayed high effectiveness (HE) measured by percentage shoot dry matter accumulation (80-100%) in relation to the shoot dry weight obtained from the Nitrogen-fertilized control plants; whereas isolates KL3 and CF5 were effective with SE of 50-80%) (Table 7) [16]. However, the field pea plants did not show significant variations in shoot nitrogen content (1.7-2.0%) and N at $p \leq 0.05$ (Table 7).

Field pea plants inoculated with isolate BR1 and strain EAL110 produced the maximum nodule number of (232.3 and 229.7 per plant), nodule dry weight (121.3mg/plant) that were significantly different from plants treated with isolates KL3 and CF5 (103-105mg/plant). Similarly, the inoculated plants with the most effective inoculants (BR1 and strain EAL110) accumulated the highest shoot dry weight of 2.9/3.0g plant⁻¹ equivalent to N-fertilized plants that was statistically different from plants inoculated with the other inoculants (Table 7).

The average nodule number counted from the inoculants was 151.2 nodules per plant were

significantly higher than 108 nodules per plant recorded from pea plants [17]. The pattern of nodule number and nodule dry weight was similar and to the shoot dry weight displayed by the good performing inoculant and standard inoculum of BR1 and strain EAL110 was similar in that the plants displayed the higher nodule number also showed.

Based on dry matter accumulation of inoculated plants with nitrogen fertilized (N⁺) control plants, BR1 and strain EAL110 were rated as highly effective whereas the others were effective N-fixers. The fact the inoculated plants did not show significant variation in % N content (1.7%-2%) , indicated that shoot dry matter is a good indicator of relative effectiveness of isolates (Somasegaran and Hoben, 1994). The %N content of the inoculated plants was similar to % N content of PR3 inoculated field pea plants reported [18].

4. Conclusion and Recommendation

The tolerance of rhizobial isolates to different pH levels, temperature, salinity, carbon and nitrogen utilization, phosphate solubilization 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 data in general, showed that the rhizobial inoculant, particularly BR1 was nutritionally versatile, ecologically competent, and symbiotically effective rhizobia and can be recommended as bio-fertilizer for better field pea production in the future.

5. Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

6. Conflict of Interest

The authors declare that they have no conflicts of interest.

7. Acknowledgement

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