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Research Article

Evaluation of Nitrogen-fixing Inoculants for Enhanced Production of Groundnut (*Arachis hypogea L.*) in Pawe District, North Western Ethiopia

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Abstract

Groundnut (Arachis hypogea L.) cultivation in Ethiopia is dominated by smallholder farmers who use little or no inputs, often resulting in low yields. The use of effective rhizobia isolates was considered an ecologically and environmentally sound approach for groundnut production. A field experiment was conducted in the Pawe district in Metekel zone, Benishangul Gumuz National Regional State (BGNRS) during the 2022 cropping season on five farmers' field to evaluate the effectiveness of nitrogen-fixing inoculants of groundnut, namely, Dibate-modermo. The experiment consisted of four treatments; T1=negative control (without chemical fertilizer and inoculant), T2= NPS fertilizer at a rate of 100kg ha^{-1}, T3=NEM isolate, and T4=Dibate-modermo isolate. It was laid out in a randomized complete block design with five replications. Five composite soil samples were collected from 0-20 cm depth before planting and analyzed for selected soil properties. The treatments were not replicated per farm; instead, farmers were used as a replicates. Results of the analysis of variance (ANOVA) indicated that plant height, number of nodules, branches, seeds, and pods per plant, haulm yield, and grain yield were significantly affected by the application of treatments. The highest grain yield was obtained with inoculation of Dibate-modermo isolate, which increased grain yield by 49.9% as compared to the untreated plots. Similarly, this isolate resulted in the highest net benefit (75,460.8ETB ha^{-1}) with acceptable MRR (2667.7%). Hence, mass production and utilization of Dibate-modermo isolate is recommended for enhanced production and improved productivity of groundnut in the area.

Keywords

Dibate-modermo, Grain Yield, Groundnut, Inoculants, Isolate, NEM

1. Introduction

Groundnut (*Arachis hypogaea L.)* is among the main oil seeds in the world. China, India, and the USA are the main producers of groundnuts [17]. It was introduced to Africa, North America, Asia, and Europe between the 16th and 17th centuries [27]. It is grown in tropical and subtropical countries and in the continental parts of temperate countries for its seeds, which contain up to 50% of nondrying oil and about 35% protein, and is used in the oil and feed industries [24]. It is the major oil crop grown in Ethiopia, mainly cultivated in the eastern, southern, western, and northwestern parts of the

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country, and occupies about 41 thousand hectares of land with a corresponding gross annual production of about 468,872 quintals [8].

Nevertheless, productivity of pulses especially groundnut in the country is extremely low compared to the potential yield of the crop in other developed countries. This could be attributed to low agricultural input supply, limited use of improved varieties, and poor soil fertility status, particularly deficiency of nitrogen [12, 14]. One of the most important soil factors adversely affecting crop production in most parts of Ethiopia is nitrogen (N) deficiency [11]. With regard to this, seed inoculation with rhizobium strains was found to be effective to alleviate the problem and has improved the yield of legume pulses in Ethiopia [3]. Hence, biological nitrogen fixation (BNF) appears to be economically attractive and ecologically sound means, which would improve crop yields, reduce external N inputs, and enhance the quality of soil resources that consequently reduces the dependence on mineral fertilizers that could be costly and unavailable to smallholder farmers [18].

It has been reported that inoculation is a major strategy for the development of sustainable nitrogen supply into agricultural soils [16], and inoculation of legume seeds is an efficient and convenient way of introducing highly efficient rhizobia to the soil to improve productivity of crops like peanut [9]. The practice of inoculation is particularly important in areas where the target legume is planted for the first time or the soils lack a sufficient number of effective rhizobia to allow efficient nodulation of the plant.

Hence, BNF appears to be important for smallholder farmers as it is a relatively cheaper source of nitrogen as compared to inorganic fertilizers and less prone to losses through leaching and denitrification. With regard to this, some previous studies have shown that inoculation of groundnut seeds with Dibate-modermo isolate resulted in higher grain yields than did chemical fertilizers [19]. Therefore, this study was carried out to evaluate the effectiveness of rhizobial isolate-Dibate-modermo for yield improvement and enhanced nitrogen fixation of groundnut (*Arachis hypogea L.*) in pawe district.

2. Materials and Methods

2.1. Description of the Study Area

The experiment was conducted in Pawe district of Metekel zone in the northwestern part of the country (Figure 1). The area is located at 570 km away from Addis Ababa and is part of the Upper Blue Nine Basin, Ethiopia. It located 11°17'46'' - 11°20'0'' North latitude and 36°27'22'' - 36°28'20'' East longitude with an elevation range of 1071-1158 meters above sea level (*m.a.s.l)*. The area is characterized by gently undulating to undulating plain with an average slope of 5% [28].

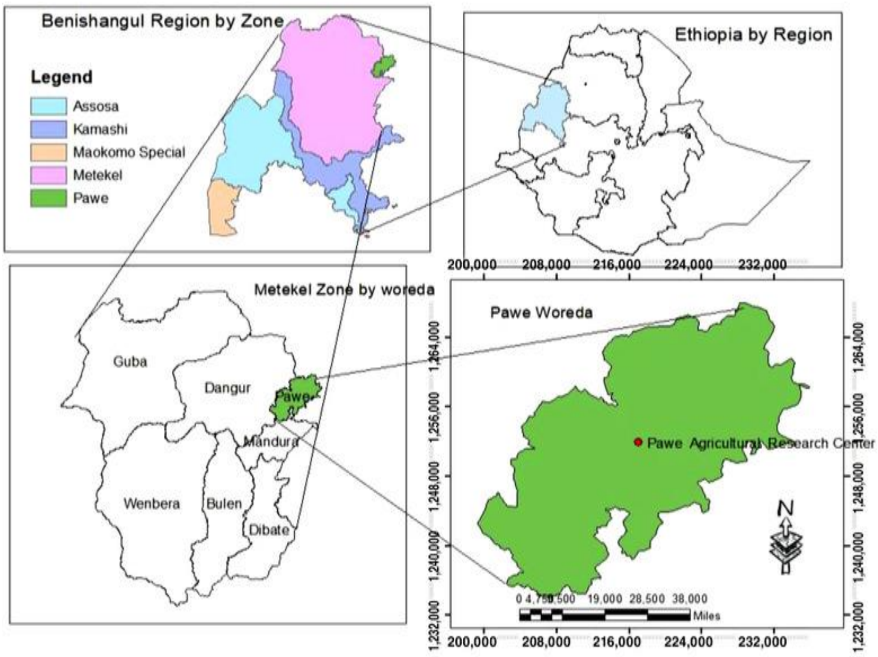

Figure 1. *Location map of the study area.*

The study area has a unimodal rainfall pattern with a peak rainy season is from June to August (Figure 2). According to the meteorological records of 2022, the annual rainfall is 1254 mm. It is characterized as a warm sub-humid and moist lowland area with very high rainfall. The mean annual maximum and minimum temperatures are 37.7℃ and 13.1℃, respectively. The coldest months are December and January, whereas February to May is hottest.

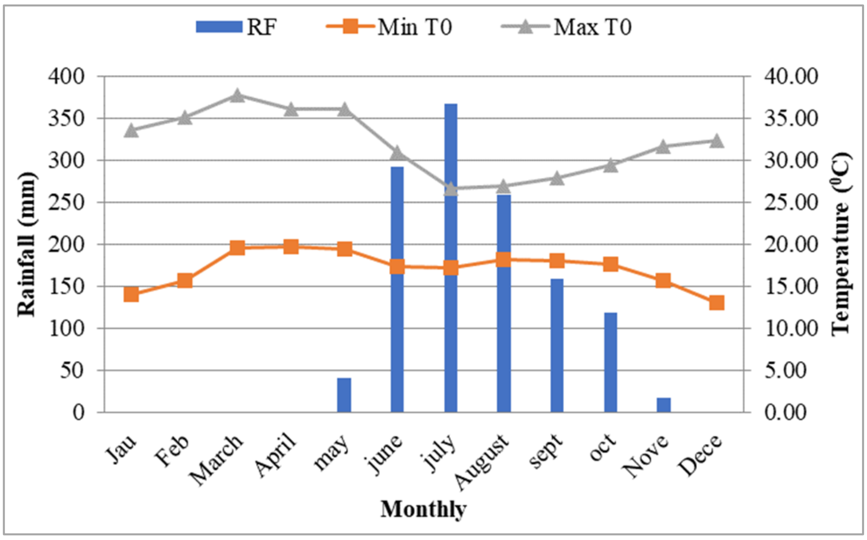

Figure 2. *Monthly rainfall (mm), minimum and maximum temperature of the study area.*

2.2. Experimental Design and Source of Materials

The experiment was laid out in a randomized complete block design (RCBD) with five replications (farmer's as a replication). It consisted of four treatments: unfertilized and un-inoculated control (negative control), NPS fertilizer at a rate of 100 kg ha^{-1}, NEM isolate, and Dibate-modermo isolate. Dibate-modermo isolate was first identified by Holeta Agricultural Research Center (HARC) from groundnut root nodules around the Metekel zone, while NEM is a promising exotic isolate included for comparison in the study. Lignite-based inoculants of each isolate were prepared at HARC microbial laboratory and applied at a rate of 10 g per kg of seed, which was moistened with 10% sucrose [21]. Seeds were allowed to air dry under shade for a few minutes and were then sown at the rate of 70 kg per ha. Un-inoculated plots were planted first, followed by inoculated treatments to avoid cross-contamination. An improved groundnut (*Arachis hypogaea L.)* variety *Maniputer* was used as a test crop.

2.3. Field Management

The experimental field was prepared by a pair of oxen following the conventional tillage practice (three times tillage before planting), then leveled for plot preparation. During field layout, space between plots and blocks was separated by paths of 1 m and 2 m, respectively. The crop was planted in rows with recommended inter- and intra-row spacing of 60 cm and 10 cm, respectively. All recommended agronomic practices were timely applied in the course of the experiment.

2.4. Soil Sampling and Analysis

A composite soil sample (0-20 cm depth) was collected diagonally before treatment application (five composite soil samples) to evaluate the nutrient status of the study area. Each composite soil sample was analysed for soil pH, organic carbon, total nitrogen, available phosphorous contents, and soil texture. The soil samples were properly labeled with the appropriate information, and transported to the soil laboratory. Soil samples were air-dried, ground using mortar and pestle, and crushed to pass through a 2 mm diameter sieve for analysis. The samples were passed through a 0.5 mm sieve for determination of organic carbon and total nitrogen contents. Soil pH was determined by potentiometric method at a 1:2.5 soil-to-water ratio as described by [5]. Soil organic carbon content was determined by the Walkley-Black oxidation method [26]. Total nitrogen (TN) was determined by the Kjeldahl digestion method [22]. Available phosphorous was determined by the Bray II methods [4]. The analysis was carried out in the Soil and Water Laboratory Analysis at Pawe Agricultural Research Center (PARC).

2.5. Agronomic Data Collection

All agronomic data, such as nodule number, plant height, and number of branches, pods and seeds per plant, number of pods without seed, haulm weight and grain yield, were collected at the recommended time. All plant growth and yield-related traits were recorded for ten (10) randomly sampled plants from the harvestable rows in each plot. The haulm yield of each net plot was harvested close to the ground surface with sharp sickles and sun-dried in open air to attain a constant weight, and recorded by weighting using a sensitive balance. The grain yield of each net plot was estimated by adjusting moisture content to 12.5% and converted to a hectare basis [10] using the following formula.

$$Adjusted\ grain\ yield = \frac{100 - actual\ grain\ moisture\ content}{100 - 12.5\%} * grain\ yield$$

2.6. Statistical Analysis

All agronomic data were subjected to analysis of variance (ANOVA), as outlined for randomized completed block design (RCBD), using the General Linear Model (GLM) of the statistical analysis software [23] version 9.4. Whenever the ANOVA detected significant differences between treatments, mean separation was conducted using the least significant difference (LSD) test at P<0.05.

2.7. Partial Budget Analysis

Economic analysis was done to investigate the economic feasibility of the treatments. The partial budget analysis was carried out based on the formula developed by [7].

Variable costs, gross field benefit, adjusted yield, net benefit and marginal rate of return were calculated for each treatment. The prices of inorganic fertilizer, bio-fertilizer, grain and haulm yields and labor costs were estimated based on the current market condition in the area at the time of harvesting.

Adjusted yield (AjY): the average yield was adjusted downward by 10% to reflect the difference between the experimental yield and the yield of farmers. AjY = AvY- (AvY x 0.1).

Net financial benefit (NFB): was calculated by subtracting the total costs that vary from gross field benefits for each treatment. NFB = GFB – TVC.

Marginal rate of return (MRR%): was calculated by dividing the change in net benefit by a change in total variable cost.

$$\text{MRR (\%)} = \frac{Change\ in\ NFB}{Change\ in\ TVC} * 100\%$$

3. Results and Discussion

3.1. Soil Physico-Chemical Properties Before Treatment Application

Soil physicochemical properties of the experimental site before treatment application are summarized and presented in (Table 1). The laboratory result indicated that the soil has clay texture [20] and its soil pH ranges from extremely to very strongly acidic soils condition [13]. Available phosphorous content of the soil is in a very low range [15]. Its total nitrogen and organic carbon contents are classified as low [15]. In general, physicochemical properties of the soil are in low range due to continuous removal of essential plant nutrients by high runoff.

Table 1. *Soil physico-chemical properties before treatment application.*

Replication	pH (1:2.5 H_2O)	Available Phosphorous (mg kg^{-1})	Organic Carbon (%)	Total N (%)	Texture (%)			
					Clay (%)	Silt (%)	Sand (%)	Textural Class
1	4.9	2.66	2.55	0.17	52	6	42	Clay
2	4.39	2.24	2.25	0.21	50	10	40	Clay
3	4.48	2.78	2.60	0.21	46	12	42	Clay
4	4.46	2.63	2.50	0.19	50	12	38	Clay
5	4.66	2.24	2.53	0.25	44	16	40	Clay
Mean	4.58	2.51	2.49	0.21	48.4	11.2	40.4	Clay

3.2. Yield and Yield Components of Groundnut

Results of the study showed that plant height, number of nodules, branches, pods, seeds per plant, haulm yield and grain yield were significantly affected by the treatments, but number of pods without seeds and pegs without pods don't show a significant difference among treatments (Table 2).

The highest number of nodules per plant (121.9) was recorded for plot treated with Dibate-modermo isolate, while the lowest (64.8) was recorded for the untreated control plot. As a result, inoculation of groundnut seeds with Dibate-modermo isolate increased the number of nodules per plant by 88.1% as compared to the untreated control. The presence of nodules in the uninoculated plots confirms the presence of indigenous rhizobia that can nodulated groundnut in the area. Groundnut has been previously considered to be a highly promiscuous species, nodulated by rhizobia from a diverse group of legumes and a large group of unrelated rhizobia [1]. In the present study, variations in between the inoculated and uninoculated plots for number of nodules per plant could be attributed to the effectiveness of introduced isolates. The number of nodules per plant recorded in the present study was higher than that reported in some previous studies with exotic isolates [25] and for soybean inoculation with two exotic and one local isolates [2, 29].

Both inoculation and application of inorganic fertilizer (NPS) enhanced the growth of groundnut as evidenced by the improvement in plant height of the crop. The tallest plant (39.42 cm) was recorded for plots that received the inorganic fertilizer, which was statistically comparable with those inoculated with Dibate-modermo and NEM isolates. However, the shortest plant (32.26 cm) was recorded for the control plot. The increase in plant height could be attributed to the pronounced vegetative growth of groundnut due to increased nitrogen supply from biological nitrogen fixation by inoculants as well as sourced from inorganic fertilizer NPS.

The analysis of variance showed that treatments had highly significant ($P < 0.01$) effect on the number of branches per plant (NBPP). The highest number of branches per plant (7.4) was recorded for application of Dibate-modermo isolate, but it was statistically comparable with those plots treated with NEM isolate and the chemical fertilizer. Whereas, the smallest number of branches per plant (5.68) was recorded for the control plot. Number of seeds per plant (NSPP) was also significantly affected by application of different treatments. The highest number of seeds per plant (23.9) was recorded for application of Dibate-modermo isolate. Conversely, the lowest value (16.24) was recorded for the control plot. The number of seeds per plant increased by 47.2% due to inoculation of groundnut by Dibate-modermo isolate as compared to the untreated control plot.

The number of pods per plant (NPPP) showed similar trend as did number of pod per plant. Seed inoculation with Dibate-modermo isolate resulted in the highest number of pods per plant (15.6). But, it was statistically at par with application of chemical fertilizer and plots treated with NEM isolate, which exhibited 14.1 and 13.9 pods per plant, respectively. On the other hand, the lowest number of pods per plant (11.06) was recorded for the control plot. The increase in number of pods per plant due to inoculation of Dibate-modermo isolate was 41% when compared with the control plot. This could be due to increases in the supply of nitrogen from nitrogen fixation, which increased plant vigor and the number of pod bearing branches. The increase in the number of pods per plant due to inoculation of groundnut by Dibate-modermo isolate in this study was higher than the 21.4% increase reported by [19].

The effect of inoculation showed significant variation among treatments for haulm yield (kg ha^{-1}). The highest haulm yield (3680 kg ha^{-1}) was obtained from plots that were inoculated with Dibate-modermo isolate, but it didn't show statistically significant difference from those plots treated with NEM isolate and chemical fertilizer. Conversely, the lowest haulm yield (2153kg ha^{-1}) was recorded for the control (no input) plot. As a result, seed inoculation with Dibate-modermo isolate increased haulm yield by 70.9% over the control plot. Increase in haulm yield due to inoculation of effective rhizobium isolates could be attributed to improved vegetative growth of plant parts, such as increases in leaf area index, due to improved soil physicochemical properties and increased nitrogen supply to the plants.

The analysis of variance showed a significant difference ($P<0.01$) among treatments for grain yield of groundnut. The highest grain yield (887.7 kg ha^{-1}) was obtained from plots that were inoculated with Dibate-modermo isolate, but it was statistically at par with those plots inoculated with NEM isolate and treated with NPS fertilizer. Conversely, the lowest grain yield (592.3 kg ha^{-1}) was recorded for the untreated plot. Seed inoculation with Dibate-modermo isolate increased grain yield by 49.9% over the untreated control plot. The effect of inoculation with indigenous rhizobial isolates in enhancing nodulation of groundnut and improving the number of seeds per plant and grain yield has been previously reported by [19]. Similar results obtained from legume fix, a rhizobia inoculant that helps to increase natural population of beneficial nitrogen-fixing bacteria to form effective nodules that are responsible for effective biological nitrogen fixation (BNF) and yield increase as compared to uninoculated [6].

Table 2. *Effects of nitrogen-fixing inoculants on yield and yield components of groundnut in Pawe district.*

Treatment	NN	PH (cm)	NBPP	NPPP	NSPP	PDWOS	PG WOP	HY (kg ha^{-1})	GY (kg ha^{-1})
Negative control	64.8^{b}	32.26^{b}	5.68^{b}	11.06^{b}	16.24^{c}	4.28	2.62	2153.3^{b}	592.3^{c}
NPS 100 kg ha^{-1}	65.24^{b}	39.42^{a}	7.04^{a}	14.1^{a}	20.9^{ab}	4.36	2.82	3333.3^{a}	823.5^{ab}
NEM isolate	65.84^{b}	38.02^{a}	7.2^{a}	13.9^{a}	19.56^{bc}	5.14	3.52	3160^{a}	691.8^{bc}
Dibate modermo	121.9^{a}	38.62^{a}	7.4^{a}	15.6^{a}	23.9^{a}	3.88	2.9	3680^{a}	887.7^{a}

Treatment	NN	PH (cm)	NBPP	NPPP	NSPP	PDWOS	PG WOP	HY (kg ha^{-1})	GY (kg ha^{-1})
isolate									
CV (%)	18.8	8.13	10.46	12	14.6	24.1	23.7	19.6	15.5
LSD (%)	20.6	4.15	0.98	2.26	4.05	1.28	0.96	833.2	160.14
Significant level	***	**	**	**	**	Ns	Ns	**	**

Means followed by the same letter(s) with in a column are non-significantly different at p<0.05. *** Significant at P < 0.001, ** significant at P < 0.01, * significant at P < 0.05, ns – non-significant difference. NN= Nodule number, PH= Plant height, NBPP= Number of branch per plant, NPPP= Number of pod per plant, NSPP= Number of seed per plant, PD WOS= number of Pods without seed, PG WOP= Pegs without pod, HY = haulm yield, GY= Grain yield.

3.3. Partial Budget Analysis

The prices of groundnut at the local market during harvesting time (80 ETB kg^{-1}), bio-fertilizer (55 ETB per sachet), haulm yield (4.70 ETB per kg), and the official price of NPS fertilizer (38 ETB per kg) were used for partial budget analysis. Results of the partial budget analyses revealed a maximum net benefit of 75,460.8 ETB ha^{-1} with an acceptable MRR (2667.7%) due to inoculation with Dibate-modermo isolate. The treatment produced 23,706.7ETB ha^{-1} more than control. The next maximum net benefit of 69,591.9ETB ha^{-1} with an acceptable MRR of 469.42% was recorded for the treatment that received 100 kg ha^{-1} NPS fertilizer. According to [7], treatment recommendation is not necessarily based on the highest marginal rate of return, but rather based on the lowest cost, the highest net benefit, and the highest grain yield. Hence, applications of Dibate-modermo isolate were found to be the best economically feasible option for groundnut production in the study area, which provided the net benefit of (75,460.8 ETB ha^{-1}) with acceptable MRR (2667.7%). This finding also suggests that, in the absence of Dibate-modermo isolate, application of 100 kg NPS ha^{-1} has a good economic return in the research area and other places with similar agro-ecology.

Table 3. *Economic analysis for the effect of nitrogen-fixing inoculants on groundnut production in Pawe district.*

Trt	Treatments	GY	AGY	HY	AHY	GFBGY	GFBHY	TGB	TVC	TNB	MRR (%)
1	Negative control	592.3	533.07	2153.3	1937.97	42645.6	9108.46	51754	0	51,754.1	-
2	NPS 100 kg ha^{-1}	823.5	741.15	3333.3	2999.97	59292	14099.86	73392	3800	69,591.9	469.42
4	Dibate-Modermo isolate	887.7	798.93	3680	3312	63914.4	15566.4	79481	4020	75,460.8	2667.7
3	NEM isolate	691.8	622.62	3160	2844	49809.6	13366.8	63176	4020	59,156.4	D

GY= Grain yield, HY= Haulm yield, AGY=Adjusted grain yield, AHY=Adjusted haulm yield, GFBGY=Gross field benefit grain yield, GFBHY= Gross field benefit haulm yield, TGB=Total gross benefit, TVC=Total variable cost, TNB=Total net benefit, MRR=marginal rate of return, D = dominated.

4. Conclusion and Recommendation

The soils of the experimental sites are strongly acidic, deficient in available phosphorous, organic carbon, and total nitrogen, justifying the need for application soil fertility management practices to boost crop yield. Hence, application of nitrogen-fixing rhizobial inoculants has a significant role in the improvement of soil fertility and crop productivity. In line with this, results of the present study showed that seed inoculation with Dibate-modermo isolate significantly improved most agronomic parameters of the crop. Economically, application of Dibate-modermo isolate was found to be the best option, as it resulted in the maximum net benefit with an acceptable marginal rate of return (MRR). Hence, it is concluded that Dibate-modermo isolate should be mass produced and distributed to farmers for enhanced groundnut production in Pawe district.

Abbreviations

CIMMYT International Maize and Wheat Improvement

	Center
TSP	Triple Super Phosphate
MRR	Marginal Rate of Return
BNF	Biological Nitrogen Fixation

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Author Contributions

Asresach Addisu: Conceptualization, Proposal writing, Data collection, Investigation, Methodology, Software analysis, Validation, Visualization, Writing – original draft, Writing – review & editing

Mesfin Kuma: Conceptualization, Investigation, Methodology, Supervision, Validation, Visualization, Writing-review & editing

Wubayehu Kidanemariam: Investigation, Methodology, Supervision, Validation, writing review & editing

Misganew Andualem: Investigation, Methodology, Supervision, review & editing

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Conflicts of Interest

The authors declare no conflicts of interest.

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