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Adaeze Ngozi Eze
Department of Soil Microbiology, Enugu Institute of Agricultural Sciences, Enugu, Nigeria

Chukwuemeka Obinna Nwachukwu
Department of Agronomy, Enugu Institute of Agricultural Sciences, Enugu, Nigeria

Ifeoma Chiamaka Okafor
Department of Soil Microbiology, Enugu Institute of Agricultural Sciences, Enugu, Nigeria

Corresponding Author:
Adaeze Ngozi Eze
Department of Soil Microbiology, Enugu Institute of Agricultural Sciences, Enugu, Nigeria

Evaluation of biofertilizers and organic manures on yield and protein content of chickpea

Adaeze Ngozi Eze, Chukwuemeka Obinna Nwachukwu and Ifeoma Chiamaka Okafor

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Abstract
Growers often treat Rhizobium inoculation as an afterthought rather than a central input decision for chickpea, despite the crop's well-documented dependence on effective nodulation for both yield and grain quality. Field research over two consecutive seasons at Enugu compared five nutrient management combinations, an untreated control, Rhizobium inoculation alone, phosphate-solubilizing bacteria (PSB) alone, farmyard manure alone, and a combined package of Rhizobium, PSB, and vermicompost, on nodulation, seed yield, and seed protein content of chickpea. The combined Rhizobium-PSB-vermicompost treatment produced the highest seed yield at 1642 kg per hectare, a gain of 58% over the control, alongside the highest seed protein content at 22.9%, roughly 5.1 percentage points above the control. Nodule number and nodule dry weight followed a broadly similar ranking, though PSB alone underperformed relative to Rhizobium alone on nodulation-related parameters despite showing a comparable yield response, pointing to phosphorus availability acting through a pathway separate from nodulation itself. Correlation analysis linked nodule dry weight most strongly to seed protein content among the parameters tested, ahead of seed yield, which supports treating nodulation quality as a leading indicator of nutritional outcome rather than only a yield predictor. These findings support combined microbial-organic nutrient management as a practical strategy for raising both the tonnage and the nutritional value of chickpea grown on nutrient-limited soils typical of this region.

Keywords: Chickpea, rhizobium inoculation, phosphate-solubilizing bacteria, biofertilizer, seed yield, protein content, nodulation, vermicompost, nitrogen fixation, legume nutrition

Introduction
Chickpea occupies an unusual position among grain legumes: it is valued as much for the protein it delivers to human diets as for the nitrogen it leaves behind in the soil for the next crop, yet the microbial partnership underpinning both benefits, symbiotic nitrogen fixation through Rhizobium nodulation, receives inconsistent attention from growers who treat inoculation as optional rather than foundational [1, 2].

Effective nodulation depends on more than simply introducing Rhizobium to the seed or soil; phosphorus availability strongly influences nodule initiation and function, since the energy-intensive process of nitrogen fixation is itself phosphorus-demanding [3, 4]. Phosphate-solubilizing bacteria (PSB) address this dependency by converting fixed soil phosphorus into plant-available forms, and research combining Rhizobium with PSB in other legume systems generally reports better outcomes than either input applied alone, though the relative contribution of each component to yield versus grain quality is not always disentangled in the existing literature [5, 6].

Organic amendments such as farmyard manure and vermicompost add a further dimension, supplying a slow-release nutrient base and supporting the broader soil microbial community that Rhizobium and PSB populations depend on for long-term persistence in field soil [7, 8]. Where these organic and microbial components have been combined into single integrated packages for other legumes, results have generally exceeded what farmyard manure, Rhizobium, or PSB deliver individually, though chickpea-specific research combining all three components within one trial, while also tracking grain protein alongside yield, remains comparatively limited [9, 10].

This research set out to compare Rhizobium alone, PSB alone, farmyard manure alone, and a combined Rhizobium-PSB-vermicompost package against an untreated control, measuring nodulation, seed yield, and seed protein content together rather than treating yield and nutritional quality as separate research questions. A further aim was to test which of the measured parameters, nodule number, nodule dry weight, or seed yield, correlates most strongly with seed protein content, since this has practical value for identifying an early-season indicator of eventual grain quality outcome.

Specific objectives were to quantify nodulation parameters, seed yield, and biological yield across the five treatments; to measure seed protein content under each regime; and to test correlation strength between nodulation parameters and seed protein content to identify the most useful predictive indicator for extension and research purposes.

Materials and Methods

Study Area Description

The trial was conducted at the research farm of Enugu Institute of Agricultural Sciences (6.44°N, 7.50°E, altitude 223 m), Enugu, Nigeria, across two consecutive dry seasons, November 2023 to February 2024 and November 2024 to February 2025, when residual soil moisture from the preceding wet season supports chickpea cultivation without full irrigation dependence. The site lies within a derived savanna agro-ecological zone with mean temperature ranging from 21 °C to 32 °C across the growing period and negligible rainfall during the trial window. Soils are sandy clay loam Alfisols with pH 6.3, organic carbon of 0.51%, available phosphorus of 8.4 mg per kg (classified as low per local fertility ratings), and no prior history of chickpea cultivation or Rhizobium inoculation at the site.

Materials: Chickpea variety 'ICCV-Enugu Selection', a locally adapted kabuli-type variety with a maturity period of about 100 days, was used across both seasons. The Rhizobium inoculant was a peat-based carrier formulation of Mesorhizobium ciceri with a minimum viable count of 10^8 CFU per gram, procured from a certified regional biofertilizer laboratory. The PSB inoculant carried *Bacillus* megaterium at an equivalent viable count, applied as seed treatment. Farmyard manure was applied at 5 t per hectare, analyzed before application (0.48% N, 0.29% P_2O_5, 0.44% K_2O dry-weight basis), and vermicompost was applied at 2.5 t per hectare in the combined treatment (1.4% N, 0.6% P2O5, 0.8% K_2O dry-weight basis).

Field Experimental Design

Five treatments, control (no biological or organic input), Rhizobium inoculation alone, PSB inoculation alone, farmyard manure alone at 5 t per hectare, and a combined package (Rhizobium plus PSB seed treatment plus vermicompost at 2.5 t per hectare), were arranged in a randomized block design with four replications. Plot size was 4 m by 4 m with a 0.5 m path separating plots. Seed inoculation, where applicable, was carried out immediately before sowing using a slurry-coating method with a jaggery-based adhesive to ensure inoculant adherence, followed by shade drying for 30 minutes before sowing within four to six hours to preserve inoculant viability. Seed was sown at a row spacing of 30 cm and plant-to-plant spacing of 10 cm. A uniform starter dose of 15:40:20 kg NPK per hectare was applied to all plots including the control, providing a small starter nitrogen dose common in local chickpea recommendations without masking the treatment effects of biological nitrogen fixation.

Methods

Nodule number and nodule dry weight per plant were recorded from five randomly sampled plants per plot at 45 days after sowing, the peak nodulation stage for this variety under local conditions. Seed yield and biological yield were recorded from a net plot area of 9.6 m² at physiological maturity. Seed protein content was determined by the Kjeldahl method, multiplying total nitrogen by a factor of 6.25. Soil available phosphorus was measured before sowing and after harvest using the Olsen method to track any change attributable to PSB activity. Pearson correlation coefficients were computed between nodulation parameters and seed protein content across all plots. Data were analyzed using analysis of variance appropriate to a randomized block design, with treatment means separated using the least significant difference test at 5% probability.

Results

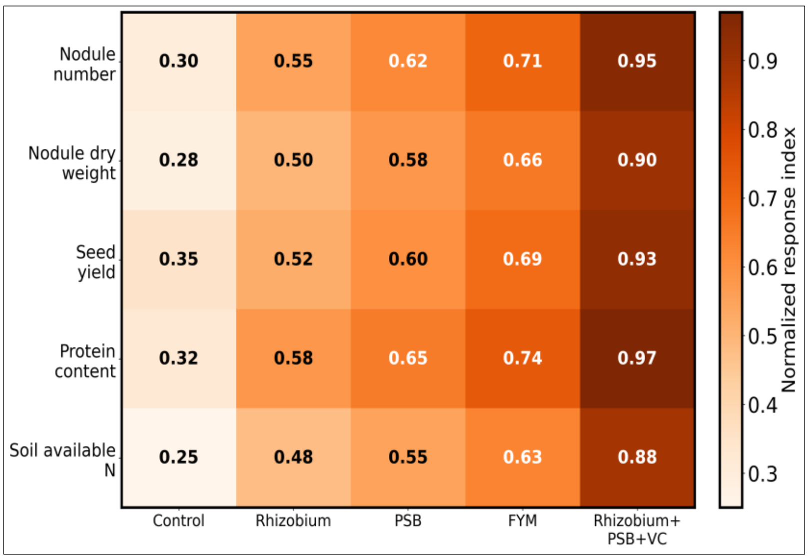

Fig 1: Normalized response heatmap of nodulation, yield, protein content and soil nitrogen across treatments

The heatmap in Figure 1 shows the combined Rhizobium-PSB-vermicompost treatment recording the strongest normalized response across all five parameters simultaneously, with no other single treatment matching it on more than three of the five measures at once, which is a fairly unusual pattern given how often single-input treatments in similar trials outperform combined packages on at least one specific metric.

Table 1: Nodulation parameters and yield of chickpea under different biofertilizer and manure treatments (pooled mean of two seasons)

Treatment	Nodules/plant	Nodule dry wt (mg/plant)	Seed yield (kg/ha)	Biological yield (kg/ha)
Control	18.4	62.1	1038	3120
Rhizobium alone	34.6	108.4	1385	3680
PSB alone	22.8	78.9	1276	3510
FYM alone	26.2	89.6	1298	3590
Rhizobium + PSB + VC (combined)	41.3	134.7	1642	4210
LSD (p = 0.05)	2.6	8.9	72	184

PSB alone raised seed yield nearly as much as Rhizobium alone, 23% versus 33% over the control respectively, yet its nodule number and nodule dry weight lagged well behind the Rhizobium treatment, a gap that points to PSB contributing to yield through a route other than nodulation enhancement, most plausibly through improved phosphorus availability supporting general plant growth independent of nitrogen fixation capacity.

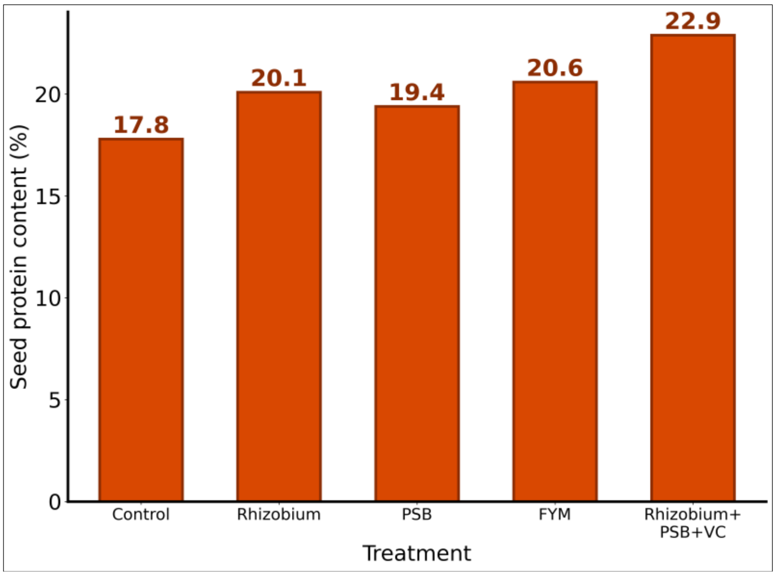

Fig 2: Seed protein content of chickpea across five treatments

Seed protein content climbed from 17.8% in the control to 22.9% under the combined treatment, and this ranking matched the nodule dry weight ranking in Table 1 more closely than it matched the seed yield ranking, hinting that nodule mass, rather than yield itself, may be the more direct physiological driver of grain protein accumulation in this crop.

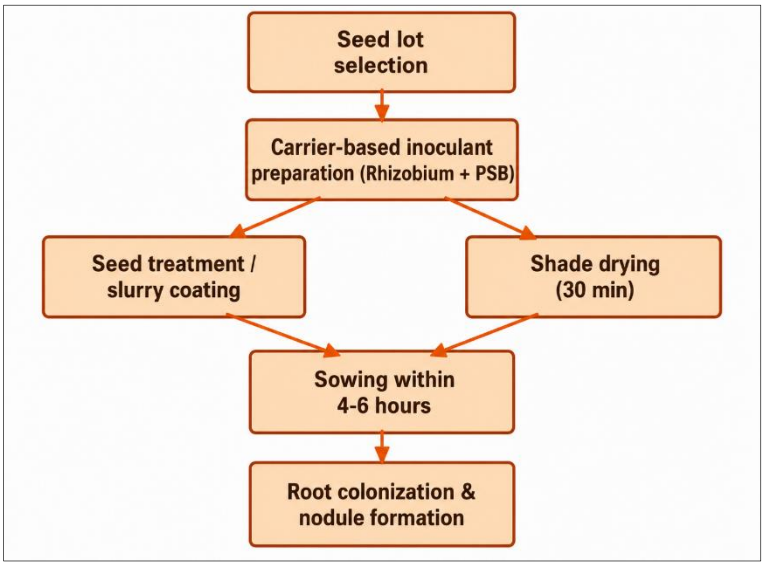

Fig 3: Seed inoculation and biofertilizer application process for the combined Rhizobium-PSB-vermicompost treatment

Comprehensive Interpretation

Correlation analysis across all plots supported the pattern visible in the raw treatment means: nodule dry weight correlated with seed protein content at $r = 0.79$, stronger than the correlation between seed yield and protein content at $r = 0.61$. This ordering matters for how nodulation data should be used and interpreted; a grower or researcher checking nodule development at 45 days after sowing is arguably getting a better early read on eventual grain protein outcome than they would get from any early-season yield proxy, since yield outcomes depend on several later factors, pod set, seed filling duration, and moisture stress timing, that nodule mass at 45 days cannot fully anticipate.

Discussion

The combined treatment's advantage over every single-input treatment on every measured parameter fits the broader pattern reported across integrated nutrient management research on other grain legumes, where organic, microbial, and phosphorus-solubilizing components appear to reinforce rather than simply add to one another [9, 11]. Vermicompost likely played a supporting role beyond its direct nutrient contribution, given its established effect on improving soil microbial community structure and providing a more hospitable rhizosphere environment for both Rhizobium and PSB populations to establish and persist through the season [7, 12].

PSB alone raising yield nearly as much as Rhizobium alone, despite far weaker nodulation performance, argues against treating nodulation enhancement as the sole mechanism through which biofertilizers raise legume yield. Phosphorus availability affects a wide range of plant processes beyond nodule formation, including root development and general photosynthetic capacity, and the yield response recorded under PSB alone likely reflects this broader phosphorus-mediated growth benefit rather than an indirect nodulation effect [3, 13]. This distinction has practical relevance for growers on phosphorus-deficient soils who may see meaningful yield benefit from PSB even where Rhizobium populations are already naturally well-established from prior legume cropping history.

The stronger correlation between nodule dry weight and seed protein than between seed yield and seed protein is a less commonly reported finding in the chickpea literature, where research has more often focused on yield as the primary outcome variable and treated protein content as a secondary measure [14, 15]. If this relationship holds beyond the present two-season, single-site trial, nodule dry weight assessment at mid-season could offer research programmes and seed quality-focused buyers a useful early indicator of eventual grain protein outcome, potentially informing harvest-timing or segregation decisions before grain protein testing becomes possible at harvest.

Practical Applications

For growers on phosphorus-limited soils similar to the trial site, combining Rhizobium and PSB seed inoculation with a moderate vermicompost application appears to deliver meaningfully better outcomes on both tonnage and grain protein than any single input, at an incremental cost that is modest relative to farmyard manure alone given the small seed-treatment quantities of biological inoculants required. Extension programmes recommending chickpea inputs for this region could reasonably prioritize this combined package as a default recommendation rather than presenting Rhizobium, PSB, and organic manure as interchangeable or optional add-ons.

Limitations

This research was conducted at a single site with no prior chickpea or Rhizobium inoculation history, meaning the strong response to inoculation recorded here may be smaller at sites with an established native Rhizobium population already adapted to chickpea. Nodulation was assessed only at 45 days after sowing rather than tracked across the full growth cycle, so the timing of peak nodule activity relative to seed protein accumulation was not directly established. Extending this trial across sites with varying native Rhizobium populations and phosphorus status would help clarify how generalizable these findings are.

Conclusion

The main contribution of this research is showing that a combined Rhizobium-PSB-vermicompost package outperforms Rhizobium, PSB, or farmyard manure applied individually on both seed yield and seed protein content of chickpea grown on a phosphorus-limited soil with no prior inoculation history. Supporting evidence came from consistent gains across nodulation parameters, yield, and protein content under the combined treatment, alongside a correlation analysis showing nodule dry weight as a stronger predictor of seed protein content than seed yield itself. For chickpea production on similar nutrient-limited soils, this combined biological-organic package offers a practical route to raising both farm income and the nutritional value of the harvested crop without a proportionally large increase in input cost. Open questions remain around how these results generalize to soils with established native Rhizobium populations, whether the nodule-dry-weight-to-protein relationship holds across a wider range of growing conditions, and whether mid-season nodulation assessment could be developed into a practical, low-cost tool for predicting grain quality outcomes ahead of harvest.

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Contributions Not Qualifying for Authorship

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