Effect of Herbicide-treated soil inoculum on Nodulation in Cowpea (*Vigna unguiculata* L. Walp.) and Soybean (*Glycine max* L. Merr.) planted under Glasshouse Conditions

Alban K N Kpemuonye[1,2], Titus Y Ngmenzuma[1], R Oteng Frimpong[3] and Felix D Dakora[1,4].

[1]Department of Crop Sciences, Faculty of Science, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa; 232243775@tut4life.ac.za , NgmenzumaTY@tut.ac.za & Dakorafd@tut.ac.za

[2]Department of Science Education, McCoy College of Education, Nadowli, P.O. Box ND12, Ghana; akkpemuonye@mccoy.edu.gh

[3]Crop Improvement Programme, CSIR-Savanna Agricultural Research Institute, Tamale, P.O. Box TL52, Ghana; ro.frimpongg@csir.org.gh

[4]Department of Chemistry, Faculty of Science, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa; Dakorafd@tut.ac.za

INTRODUCTION & AIM

The application of herbicides in agricultural systems is a common practice to control weeds and enhance crop productivity. However, their non-target effects on soil microbial community, particularly symbiotic nitrogen-fixing rhizobia, remain a concern. In the Upper West Region of Ghana, where legumes such as cowpea (*Vigna unguiculata*) and soybean (*Glycine max*) are vital for soil fertility and food security, understanding the impact of herbicides on rhizobial nodulation is crucial. Rhizobia-legume symbiosis plays a key role in nitrogen fixation, reducing reliance on chemical fertilizers. Globally, cowpea fixes 35 million tons of Nitrogen (Saxena, Rathi & Tilak, 1996) with significant fixation potential (200 kg N/ha under optimal conditions) and soybean fixes more than 200 kg N/ha per year. Cowpea and soybean rely on rhizobial symbiosis for nitrogen fixation, reducing dependence on synthetic fertilizers particularly among smallholder farmers. Yet if left unchecked, herbicide residues may inhibit nodulation, affecting crop yields and soil health. The aim of the study was to evaluate the effect of herbicide-treated soil inoculum on rhizobial nodulation in cowpea and soybean under glasshouse conditions.

METHOD

Herbicide-treated and untreated soil samples were collected from 15 farmers' fields in the Upper West Region, Ghana between September and October, 2023 and transported to South Africa and used as inocula. The soil samples for soil suspensions were collected from paired farmers' fields/locations in the Upper West region where similar/same climatic and soil conditions exist, with the herbicide-treated fields having a long-term history (3+ years) of herbicide use and uncultivated field (≥500 m away) with no such inputs for at least 6 years. The study was conducted at the TUT Skill Centre (25°44'03.9"S 28°09'50.8"E) between January 2025 – April 2025 using sterilized sea sand as growth medium and herbicide-treated and untreated soil suspensions as inocula, commercial inoculant for legumes and zero treatment for each crop. Two legume species (Cowpea (Kirkhouse) and Soybean (Favour). were used as the host plants. Seeds were surface-sterilized (70% ethanol and 3.5% sodium hypochloride) and sown at 2–3 cm depth. Seedlings were inoculated with herbicide-treated and untreated soil suspensions (5–10 g in 300 mL of distilled water and 100 mL was used to inoculate each pot) 10 days after planting (DAP), with three replicates per treatment. Plants were irrigated with N-free solution when necessary and harvested at flowering, 65 DAP and separated into shoots and roots. The roots were washed and nodules detached and manually counted. The shoots, roots, and nodules were oven-dried (65–70 °C, 72 h) to determine their dry matter (DM) yield as well as the N-fixed, total N-fixed and relative symbiotic effectiveness (%RSE).

Statistical Analysis

The data on nodule number, nodule DM, shoot DM, root DM, shoot-root ratio and whole plant DM, N-fixed, total N-fixed and relative symbiotic effectiveness were subjected to analysis of variance (ANOVA) using Statistica software version 10.1 and means with significant difference at $p < 0.05$ were separated using Duncan Multiple Range Test (DMRT).

RESULTS & DISCUSSION

The results revealed significant ($p \leq 0.05$) differences in nodulation, plant growth, and nitrogen fixation in cowpea treated with herbicide-treated soil inoculum compared to untreated controls, with variability influenced by crop species and soil origin. Untreated soil inolculum enhanced whole-plant dry matter (DM), particularly in cowpea from locations Nator (6.05 g/plant) and McCoy (5.87 g/plant), reinforcing the notion that herbicide-free inoculums optimize overall biomass (Dos Santos *et al.*, 2018). Soybean exhibited notable nodule reduction with herbicide-treated soil inocula, except in location Dapuori, suggesting potential herbicide tolerance in indigenous rhizobial strains. Conversely, untreated soil inoculum from Lambussie recorded an increase in soybean nodulation (19.00 nodules/plant). Cowpea exhibited greater sensitivity to herbicide residues, with pronounced declines in nodule formation, while soybean demonstrated moderate resilience. Untreated soil inoculum promoted superior nodulation and biomass, emphasizing the need for herbicide selection based on soil-microbe compatibility. Site-specific variations suggest the existence of herbicide-tolerant rhizobial strains in certain soils.

Table 1: Effect of Herbicide-treated-soil inoculums on nodulation, Growth, and N-Fixed in Cowpea

Treatment	Nodule no.	Nodule DM	Shoot DM	Root DM	Shoot/root ratio	Whole-plant DM	N-fixed	Total N-fixed	RSE
Treated soil	per plant	g/plant	g/plant	g/plant	-	g/plant	mg/plant	m/plant	%
Nawuli	21.67±2.73	0.20±0.03	2.73±0.58b	0.88±0.58a	2.65±0.47bcd	3.41±0.44b	1.83±0.42b	2.78±0.47abc	60.84±10.8
Nator	21.00±0.58c	0.14±0.06bcd	4.66±0.71a	1.08±0.71a	4.32±0.34abc	5.88±0.80a	2.82±0.42ab	3.92±0.34ab	88.64±9.06
McCoy	20.00±4.61c	0.11±0.04bcd	3.59±0.73ab	0.79±0.71a	4.53±1.04abc	4.50±0.91ab	2.43±0.54ab	3.20±0.58ab	76.41±13.9
Lambussie	26.67±5.17bc	0.18±0.05bc	3.16±0.58ab	1.01±0.73a	3.24±0.63bc	4.35±0.30ab	2.66±0.36ab	3.72±0.30ab	82.50±9.34
Gbierung	20.33±3.28c	0.14±0.04bcd	3.73±0.75ab	0.82±0.29a	6.12±2.39ab	4.69±1.01ab	2.57±0.12ab	3.39±0.41ab	80.07±3.25
Nanga	44.67±6.22ab	0.18±0.06bc	3.32±0.75ab	0.80±0.73a	4.20±0.19abc	4.29±0.60ab	2.48±0.20ab	3.33±0.33ab	77.89±5.19
Saan	22.67±1.45bc	0.19±0.07b	2.76±0.39b	0.51±0.71a	6.51±1.43ab	3.46±1.16b	2.39±0.25ab	2.97±0.46abc	75.46±6.51
Kaleo	25.00±1.52bc	0.18±0.05bc	4.18±0.41a	0.88±0.58a	5.90±1.95b	5.25±0.99a	3.01±0.10a	3.95±0.39ab	91.73±2.59
Chaang	16.67±2.19d	0.20±0.03b	3.60±0.40ab	1.41±0.39a	2.53±0.10bcd	5.22±0.54a	2.77±0.09ab	4.26±0.21a	85.38±2.32
Dakpaa	22.00±2.12bc	0.17±0.01bc	1.77±0.58b	0.60±0.38a	2.60±0.68bcd	2.54±1.28c	2.15±0.39ab	2.79±0.64abc	69.10±10.1
Dakyie	19.33±0.67bc	0.26±0.08ab	3.78±0.58ab	0.81±0.11a	4.72±0.49abc	4.86±0.41ab	2.95±0.04ab	3.89±0.09ab	90.08±1.14
Nanvile	30.67±5.92abc	0.17±0.01bc	2.14±0.58b	0.68±0.80a	3.24±0.37bc	2.99±0.47c	1.64±0.34ab	2.37±0.47abc	55.96±8.92
Takpo	25.33±2.66bc	0.27±0.04a	4.01±1.52a	0.97±0.58a	4.15±0.36abc	5.20±0.20a	3.17±0.09a	4.23±0.15a	95.91±2.42
Dapuori	15.33±4.41de	0.19±0.06b	2.85±0.58b	0.75±0.58a	3.87±0.15bc	3.78±0.66b	2.35±0.47ab	3.15±0.66ab	74.33±12.3
Kunzokalaa	36.67±7.67abc	0.18±0.05bc	3.13±0.58ab	0.86±0.11a	3.42±1.36bc	4.17±1.42ab	2.97±0.44ab	3.88±0.57ab	90.51±11.7
Com.In.	46.66±2.85a	0.09±0.01e	2.92±0.91b	0.32±0.18a	9.36±1.36a	3.33±0.30b	2.42±0.28ab	2.70±0.30abc	76.15±7.24
Zero	0.00±0.00f	0.00±0.00f	0.63±0.58c	0.51±0.13a	3.22±1.90bc	1.14±0.28d	0.00±0.00c	0.00±0.00d	0.00±0.00e
F-Statistics	**2.41***	**3.85*****	**2.82****	**1.74ns**	**2.43***	**2.25***	**5.54*****	**5.80*****	**7.48*****

Table 2: Effect of Herbicide-treated-soil inoculums on nodulation, Growth, and N-Fixed in Soybean

Treatment	Nodule no.	Nodule DM	Shoot DM	Root DM	Shoot/root ratio	whole-plant DM	N-fixed	Total N-fixed	RSE
Treated Soil	per plant	g/plant	g/plant	g/plant		g/plant	mg/plant	mg/plant	%
Nawuli	6.00±2.08c-g	0.06±0.02a	0.90±0.03cde	0.28±0.01def	3.63±1.60a	1.24±0.27c	1.18±0.09ab	1.19±0.09ab	58.24±3.26
Nator	2.33±0.33c-j	0.03±0.01a	0.50±0.01de	0.22±0.01def	2.28±0.67a	0.75±0.22d	1.32±0.08ab	1.25±0.11ab	63.57±2.81
McCoy	2.33±0.33c-j	0.04±0.02a	0.43±0.01de	0.25±0.02def	1.90±0.68a	0.72±0.12d	1.17±0.13ab	1.14±0.06ab	57.99±2.74
Lambussie	8.33±0.33cde	0.09±0.03a	1.97±0.02c	0.62±0.01abc	3.40±0.94a	2.68±0.31b	1.58±0.32ab	1.97±0.31ab	73.11±11.7
Gbierung	4.67±0.67c-h	0.11±0.02a	1.05±0.06cd	0.42±0.02d	2.20±0.34a	1.62±0.22c	1.37±0.12ab	1.61±0.10ab	64.93±4.42
Nanga	11.00±0.58c	0.12±0.02a	1.05±0.03cd	0.35±0.03de	3.08±0.68a	1.52±0.18c	1.16±0.08ab	1.31±0.09ab	57.74±3.12
Saan	8.33±0.88cde	0.09±0.01a	2.07±0.04b	0.70±0.03ab	3.11±0.43a	2.86±0.11b	1.68±0.03a	2.15±0.11a	77.08±1.08
Kaleo	3.67±0.66c-i	0.32±0.03a	1.21±0.05cd	0.40±0.02d	2.99±0.50a	1.93±0.27c	1.82±0.26a	2.22±0.27a	82.16±9.49
Chaang	3.00±0.58c-i	0.04±0.01a	1.16±0.06cd	0.42±0.02d	2.74±0.46a	1.62±0.40c	1.24±0.08ab	1.38±0.12ab	60.59±2.81
Dakpaa	10.33±1.78cd	0.08±0.02a	0.81±0.07cde	0.38±0.01de	2.25±0.62a	1.27±0.15c	1.28±0.12ab	1.43±0.08ab	62.21±4.34
Dakyie	7.67±0.88c-f	0.12±0.03a	1.15±0.05cd	0.50±0.02bc	2.19±0.46a	1.78±0.48c	1.16±0.05ab	1.47±0.20ab	57.62±1.87
Nanvile	9.00±0.57cde	0.07±0.02a	1.74±0.06bc	0.51±0.02bc	3.50±1.07a	2.32±0.46b	1.48±0.39ab	1.75±0.36ab	69.64±14.4
Takpo	6.00±0.01c-g	0.03±0.01a	1.12±0.03cd	0.47±0.01d	2.50±0.70a	1.69±0.21c	1.40±0.12ab	1.64±0.13ab	66.41±4.35
Dapuori	22.33±7.35a	0.24±0.02a	3.15±0.06a	0.81±0.03a	4.05±0.53a	4.21±0.75a	1.77±0.03a	2.50±0.17a	80.42±1.22
Kunzokalaa	11.33±7.88c	0.04±0.01a	1.91±0.08c	0.75±0.03ab	3.66±0.66a	2.67±0.28b	1.52±0.36ab	1.96±1.79ab	70.88±13.3
Com.In.	14.33±0.33b	0.18±0.02a	2.11±0.03b	0.52±0.02bc	3.97±0.56a	2.81±0.56b	1.05±0.20ab	1.43±0.27ab	53.53±7.26
Zero	0.00±0.00k	0.00±0.00a	0.39±0.02f	0.32±0.01de	1.35±0.30a	0.71±0.12de	0.00±0.00b	0.00±0.00b	0.00±0.00c
F- Statistics	**3.08****	**1.76ns**	**3.17****	**2.01***	**1.15ns**	**4.05*****	**5.00*****	**4.57*****	**7.21*****

CONCLUSION

The study highlights the effects of herbicide-treated soil inoculum (HTSI) on symbiotic nitrogen fixation and growth in cowpea and soybean plants, demonstrating the adaptive potential of indigenous rhizobial populations in herbicide-exposed environments. While herbicide residues impose subtle constraints on microbial-plant interactions, the overall symbiotic efficacy remains largely preserved, supporting the preservation of tolerant indigenous/native rhizobial strains for sustainable agricultural practices.

FUTURE WORK / REFERENCES

Further research should investigate the direct impact on rhizobial viability, as well as the potential for isolating herbicide-resistant strains to improve growth and nodulation in herbicide-exposed soils.

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