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Meta-analysis of the effects of *Rhizobia* inoculants and phosphorus fertilizer on soybean nodulation in Africa

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Soybean has the potential to bring significant benefits in diversified cropping systems, which could help restructuring soil fertility and allow smallholders to increase grain yield. *Rhizobium* inoculation improves the biological nitrogen fixation (BNF) in legume crops and assists resource-poor farmers to increase grain yield at lower financial costs. The efficacy of symbiotic bacteria on legumes can also be improved through supplementation of phosphorus fertilizer. In this work, a meta-analysis of 29 peer-reviewed studies was performed to understand the effects of various *Rhizobium* strains and phosphate fertilizer application on soybean nodules. Results showed that *Rhizobium* inoculation was highly effective in increasing the number of soybean nodules, nodule dry weight, and shoot dry weight. Application of phosphorus fertilizer increased the overall nodule number due to improved BNF processes by *Rhizobia*. The main effects of both *Rhizobium* inoculation and phosphate fertilizer resulted in moving grain yields to 1.67 t ha^{-1} and 1.95 t ha^{-1}, respectively. Furthermore, the interaction of *Rhizobium* inoculants and phosphorus led to relatively higher grain yield (2.51 t ha^{-1}). Therefore, African smallholders were advised to adopt *Rhizobium* inoculation in soybean fields concomitantly to phosphate fertilizer application, to improve soybean productivity at lower costs.

Key words: Phosphorus application, nodule number, nodule dry weight, shoots dry weight, grain yield.

INTRODUCTION

The African population was expected to double in the next 40 years (Cleland, 2013), raising food insecurity especially in the sub-Saharan region where 239 million people are experiencing dire undernourishment (FAO, 2020). Sustainable intensification and integrated approaches are therefore needed to increase the

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agricultural productivity of smallholders, improve household food security, and reduce poverty at the country level (Peoples et al., 1995; McNamara, 2009). Integrating legume crops, especially soybean, is an important approach in many cropping systems, as they can perform biological N_2 fixation (BNF), thus reducing N fertilizer requirements and improving grain yield (Peoples et al., 1995; Giller, 2001). Soybean was first domesticated in China and has been grown in other Asian countries like Japan and Korea for more than 3000 years as a primary source of vital proteins and vegetable oil (Giller, 2001; Herridge et al., 2008; Nishinari et al., 2014). Globally, soybean (*Glycine max* (L.) Merr.) is the world's largest grown legume crop (Giller, 2001), accounting for 50% of the worldwide legume crop area and 68% of global crop production (Herridge et al., 2008). However, there is no clear evidence of when soybean was first introduced to Africa (Mpepereki et al., 2000), but its nodulation with indigenous *Rhizobia* in African soils was first ascertained by Corby (1967).

The ability of symbiotic rhizobial bacteria to fix atmospheric nitrogen in legume plants can improve grain yield without applying nitrogen fertilizer (van Heerwaarden et al., 2017). Herridge et al. (2008) reported that soybean can fix more than 16 million tons of N annually, which is 77% of the N fixed by legume crops. Soybean has been reported to fix 80% of its nitrogen requirements (Smaling et al., 2008). *Bradyrhizobium* strains are commonly used in soybean inoculation worldwide (Chianu et al., 2011; Chen et al., 2015). In Africa, *Rhizobia* inoculants have been used to control the effects of debilitating soil fertility and high fertilizer costs incurred certainly by smallholders. They became an affordable and effective agronomic approach in improving yield and promoting sustainable agriculture (Dakora and Keya, 1997; Paynel et al., 2008). Ronner et al. (2016) found that *Rhizobia* inoculants increase soybean yield at a lower financial cost compared to chemical N fertilizers, thus benefiting the resource-poor farmers. Despite a rapid expansion of soybean production in many African countries (Mpepereki et al., 2000) and wide use of inoculants, legume yields in the smallholder farming sector generally remain far below their potential (Ronner et al., 2016). The effectiveness of *Rhizobia* inoculants can be affected by factors like soil nutrient status, organic matter content, pH, salinity, temperature, drought, and managerial practices (Thilakarathna and Raizada, 2017). However, Ronner et al. (2016) mentioned that the soybean yields in Africa could be improved through the use of adaptive technologies like phosphate fertilizer and improved varieties to aid the *Rhizobium* inoculation approach. Phosphorus (P) is the second most important macronutrient required by the legume plants in the BNF among other crucial processes (Uchida, 2000). Symbiotic *Rhizobium* bacteria need P as the energy storage and transfer component (adenosine diphosphate (ATP) and adenosine triphosphate (ATP) for the conversion of free N_2 to ammonium (NH_4), a N usable form by legumes (Dakora and Keya, 1997). Furthermore, P increases nodule number and size, and it promotes general root growth. Legumes need optimum P levels for maximum nitrogen fixation and to achieve high grain yield (Bashir et al., 2011). Since 1980, no meta-analysis was conducted to determine the extent to which *Rhizobium* inoculation and phosphorus fertilizer technologies have influenced soybean productivity under field conditions in Africa. This study aimed to review various researches conducted in Africa to understand the effectiveness of rhizobial inoculants, P-fertilizer, and their interaction on soybean performances. The following conceptual model (Figure 1) was suggested to predict the effects of *Rhizobium* inoculation, phosphate fertilizer, and other adaptive technologies that could further improve soybean yield.

METHODOLOGY

Data collection

An extensive literature synthesis was performed based on robust published research articles in 1980-2020 downloaded from the ScienceDirect databases (https://www.sciencedirect.com) and Web of Science (http://apps-webofknowledge-com.vpn.cau.edu.cn). The search terms used as the main topics in both databases were *Rhizobium*, Phosphorus, Soybean OR *Glycine max*, Nodulation, and Grain yield. A total of 441 research articles were obtained from ScienceDirect and 170 from Web of Science. Google Scholar provided additional articles. Only 86 articles were retained after excluding duplicates and exploitation of the titles and abstracts' relevance to the subject of the work for further screening. A study had to meet six requirements for its consideration in the dataset. They included: being conducted under rainfed or irrigated field conditions; assessing the effect of any strain of commercial *Rhizobium* inoculant or chemical phosphorus fertilizer, and/or both on nodulation characteristics and grain yield; presence of a control to either *Rhizobium* inoculation or phosphorus application; having every treatment being repeated at least three times; being conducted in an African country; having been published between 1980 and 2020.

The different characteristics of the nodulation consisted of nodule number, nodule dry weight, and shoot dry weight. A database with 396 data points extracted from 29 qualified peer-reviewed articles based on the aforementioned criteria was compiled. Studies with a sample size of less than 2 were excluded from analyses because they would have resulted in small size effect (Viechtbauer, 2010). 26 out of the 29 researches retained were conducted in the sub-Saharan-Africa region (SSA) and the other 3 in the Saharan region (Figure 2). Treatment variances, standard deviations, or standard errors were disregarded as they were only presented in a few studies. In fact, only treatment mean values of nodule number, nodule dry weight, shoot dry weight, and grain yield were collected. Experiment details recorded include location/country, latitude, longitude, annual mean temperature and rainfall, soil type, pH, organic matter content, total available nitrogen, available phosphorus, inoculant strain, nodule number, nodule dry weight, shoot dry weight, grain yield, phosphorus application rates, to name a few. Control and experimental treatments' data were recorded as well as data on the interaction effect that was assessed in 4 studies only. The available data showed that the minimum and maximum

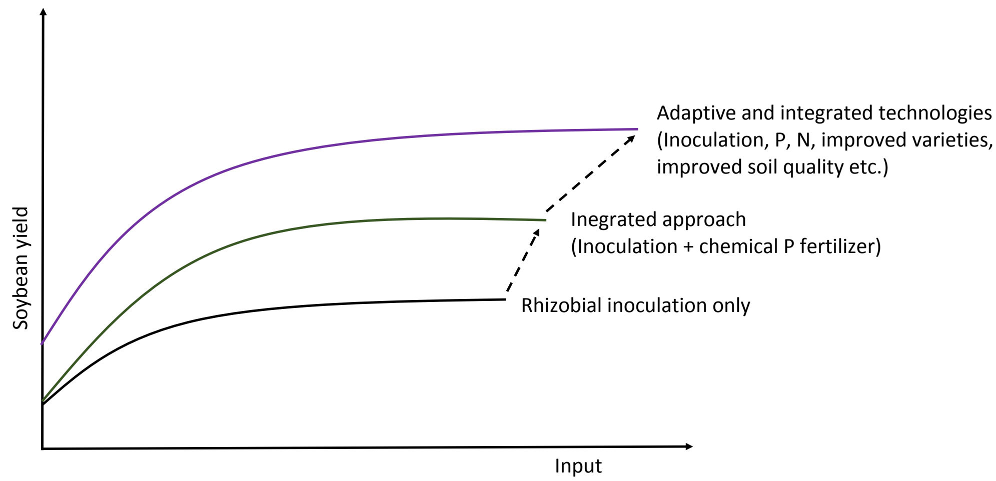

Figure 1. A conceptual model for anticipating the effect of integrating *Rhizobium* inoculation with phosphorus fertilizer and other adaptive technologies.

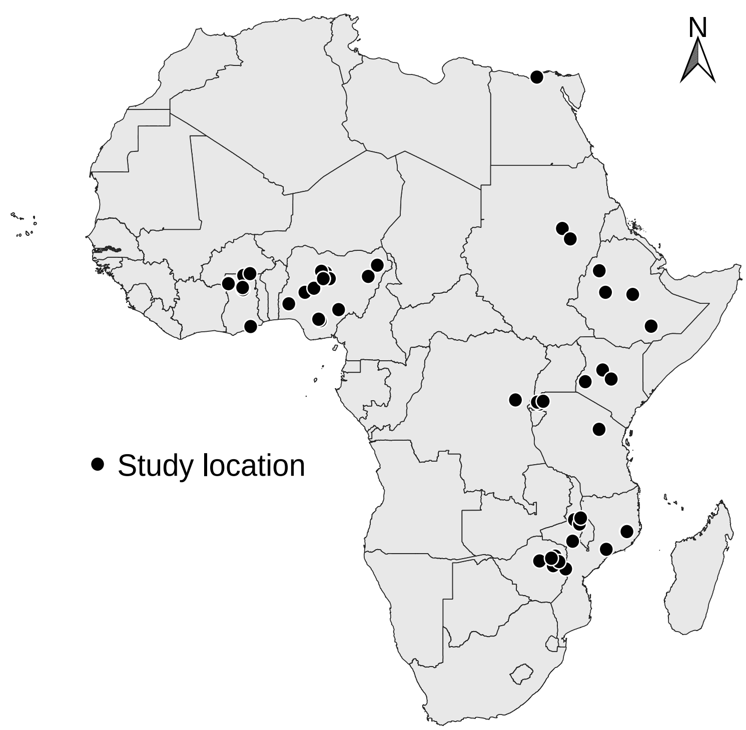

Figure 2. Distribution of the experimental locations of the studies included in the meta-analysis.

mean annual temperatures were 22 and 29°C, minimum and maximum mean annual rainfall were 552 mm and 1300 mm, respectively. Data reported in one study but conducted in more than one country/location or in different years were considered derived from different studies. Tabular and graphical data were collected and in the latter case, Engauge Digitizer software version 12.1 was used for data extraction. Detailed information of the selected peer-reviewed studies is given in Table 1.

Data processing

Data on nodule number, nodule dry weight, shoot dry weight, and grain yield were pooled on per variable basis. Means, and standard deviations (STDEV.P) were calculated using Microsoft Excel package and the data were exported to R software for statistical analyses. The author's name, publication year, and sample sizes for each study were recorded for corresponding means and STDEV.P.

Statistical analyses

The effects of *Rhizobium* inoculation and phosphorus fertilizer on soybean performances were estimated using the random-effects model (REM) described by Viechtbauer (2010). The statistical analyses in this study were all performed in R version 4.0.2 (R Core Team, 2020), using the R program Metafor (Viechtbauer, 2010; Schwarzer et al., 2015). The *Escalc()* function contributed to calculate the standardized mean differences (SMD), which measure effect sizes that allowed comparing treatments to the controls (Viechtbauer, 2010). The REM was used to estimate the average true effect and the total amount of heterogeneity among the true effects (Viechtbauer, 2010; Schwarzer et al., 2015). Results were presented in form of forest plots with the *Forest()* function (Lewis and Clarke, 2001) and boxplots using the *Plot()* function (Viechtbauer, 2010). Heterogeneities within-study and between-study were assessed using the I^2 statistic (Higgins and Thompson, 2002). The presence of publication bias and/or heterogeneity was determined by creating Funnel plots for both the inoculation and phosphorus variables (Sterne et al., 2001; Rothstein et al., 2006). A Funnel plot is a simple scatter plot for the study's estimated treatment effects (x-axis) against the measure of study size on the y-axis (Sterne et al.,, 2001). Trim and fill method using the *Trimfill()* function (Viechtbauer, 2010; Schwarzer et al., 2015) was applied on asymmetrical Funnel plots to determine the effect of missing studies on the overall outcome. The standardized mean difference for the study was plotted on the horizontal axis against the standard error on the vertical axis. A box plot using the *Plot()* function was realized to show the overall effects of rhizobial inoculation, phosphate fertilizer, and the interaction on soybean grain yield (Figure 15). Bootstrapping was iterated 1000 times (95% CI) with R package Mosaic (Pruim et al**.,** 2015) to improve the probability that the confidence interval was calculated around the relative yield mean. The frequency distribution plots were then plotted with the *Ggplot*() function.

RESULTS

Effects of *Rhizobium* inoculation on soybean

Response of nodule dry weight to inoculation

The effects of inoculation on nodule dry weight are shown in Figure 3. The heterogeneity (I^2) of the combined studies was 92% (P=0.0001). The study by Argaw (2014) clearly showed that inoculation significantly favored nodulation contrasting seven other studies, which had 95% confidence interval (CI) lines touching or crossing no effect line, indicating that inoculation did not influence nodulation. The position of the diamond symbol on the graph, which showed the overall effect of inoculation, testified that inoculation significantly favored nodulation. Furthermore, the overall effect of *Rhizobium* inoculation had 95% CI of -2.02[-2.95, -1.08] (Figure 3). The purpose of the funnel plot was to indicate the level of bias in this study. Twenty studies showed an asymmetric distribution pattern, indicating the presence of bias (Figure 4). However, after trim and fill (white dots), the overall result was not significantly affected.

Soybean nodule number

The studies had an I^2 of 75 % (P=0.0001) indicating that they were heterogenous. *Rhizobium* inoculation favored high nodule numbers (Argaw, 2014) (Figure 5). The overall effect of soybean inoculation is shown by the diamond which is on the left side of the no effect line, indicating that inoculation significantly favored a high nodule number compared to no inoculation (Figure 5). The overall effect of inoculation had 95% CI of -1.62[-2.17, -1.07] (Figure 5). There was a little publication bias in 23 studies analyzed as shown by an asymmetric distribution pattern on the Funnel plot (Figure 6). Trim and fill did not result in a significant change to the overall result.

Shoot dry weight response to rhizobial inoculants

An I^2 of 54 % (P=0.0001) resulted from the 13 studies showing the presence of heterogeneity. Two studies clearly demonstrated that *Rhizobium* inoculation increased shoot weight (Pulver et al., 1982; Okereke et al., 2001) (Figure 7). The overall effect of soybean inoculation significantly increased shoot dry weight compared to the control treatment, according to the position of the diamond, which is on the left side of no effect line. A 95% CI of -1.31[-1.74, -0.88] was produced for the overall effect of inoculation. The studies showed a slightly asymmetric distribution pattern on the Funnel plot, indicating very limited bias in the 13 studies (Figure 8). Trim and fill of the missing studies did not bring meaningful change to the overall result.

Grain yield

The analyzed studies were heterogenous with an I^2 of 64 % (P=0.0001). *Rhizobium* inoculation significantly favored high grain yield compared to non-inoculated control with a symmetrical distribution pattern, indicating the absence of publication bias (Figure 10). a 95% CI of -1.05 [-1.39, 0.72] (Figure 9).

Studies within the inverted funnel of the Funnel plot had Response of soybean to phosphorus fertilizer application

Nodule number

The heterogeneity of the all the analyzed studies (I^2) was

Table 1. Summary of the 29 studies in the meta-analysis on the effects of *Rhizobium* inoculation and P-fertilizer on soybean.

References	Area/Country	Soil type	*Rhizobia* strains	Phosphorus (kg/ha)	Key findings
Dadson et al. (1984)	Legon, Ghana	Sandy-loam (0.05% TSN)	-*B. japonicum* (Nitragin S)	Triple superphosphate (TSP) -0 (control) - 30 kg P/ha - 60 kg P/ha - 90 kg P/ha	- Medium to high rates of phosphorus and *Rhizobia* treatments significantly increased nodule number, total dry matter, and grain yield compared to controls.
Okogun and Snginga (2003)	Fasola, Mokwa and Zaria, Nigeria	Sandy-loam (0.06% TSN)	- Control (local *Rhizobia*) - *Bradyrhizobium* isolate R25B - *B. japonicum* IRj 2180A + R25B		- The mixture of introduced R25B+IRj 2180A increased nodule number by 34%, while R25B formed only about 24% of the nodules but did not influence biomass yield. -Inoculation by foreign strains failed to significantly affect grain yield.
Ronner et al. (2016)	Kaduna and Kano, Nigeria	Luvisols	*B. japonicum* strain: - USDA 532c	Single Superphosphate (SSP, 18% P_2O_5) - 20 kg P/ha	- Phosphorus and *Rhizobia* inoculation increased soybean yields by 452 and 447 kg/ha respectively over control treatment. - The combined effect of phosphorus and inoculations resulted in highly significant yield averaging 777 kg/ha
Argaw (2014)	Shinille, Ethiopia	Sandy-clay (0.25% TSN)	- *B. japonicum* (TAL-379 isolate) - *Bradyrhizobium* sp. (UK-Isolate) - *Bradyrhizobium* sp. (local- isolate)		- Inoculation improved nodulation characteristics, plant growth and productivity over uninoculated treatment. - Local and UK isolates significantly increased grain yield (P=0.05) as compared to the control and TAL-379 treatments.
Okereke et al. (2000)	Awka, Nigeria	Sandy-loam (0.08-0.1% TSN)	*B. japonicum* strains -USDA 136 -USDA 138 -USDA 110 -USDA 122		- Nodule number and dry weight, shoot dry weight and grain yield significantly increased against uninoculated treatments.
Zengeni and Giller (2007)	Goromonzi, Zimbabwe	Sandy soil (0.05% TSN)	- Soybean isolates from nodules of the Magoye variety (M1-M5) - Soybean isolates from nodules of the Hernon variety (H1-H5) - Commercial inoculants (MAR 1491 and 1495)		- High variations in the nodule numbers and yields were considered unreliable indicators of effectiveness. - Different *Rhizobia* strains resulted in strong harvest index effects which directly related to grain yield.
Chowdhury et al. (1983)	Morogoro, Tanzania	Rhodustult (0.11% TSN)	*Rhizobia* strains: -IRj 2101 -IRj 2114 -IRj 2111 -IRj 2123		- Inoculation significantly increased nodule number and grain yields in the first year. - There was huge decrease in nodule number (about 10-folds) and grain yield in the subsequent years after inoculation evidenced by no significant differences between inoculated and uninoculated treatments.
Gyogluu et al. (2016)	Nampula, Ruace and Mutequelesse, Mozambique	-Sandy clay loam -Clayey loam	*B. japonicum* strain: -WB74		- *Rhizobia* inoculation effectively improved nodulation of TGx and non-TGx soybean varieties and yield was increased by 12% as compared to uninoculated control treatments.
Akpalu (2014)	Bolgatanga, Ghana	- 0.03% TSN	*Rhizobium* strain	Triple Superphosphate (TSP)	- *Rhizobia* inoculant plus phosphorus fertilizer treatment showed highly significant increase in

Table 1. Contd.

				-139.4 g P per 9 square meter	nodulation and root growth while phosphorus along failed to effect root growth. - Grain yield was significantly high in inoculant plus phosphorus fertilizer and phosphorus fertilizer only treatments (7.61 t/ha and 7.30 t/ha respectively) and lowest in *Rhizobia* inoculation only and control treatments (4.41 t/ha and 3.80 t/ha respectively).
Ahiabor et al. (2016)	Nyankpala, Ghana	Loamy-sand (0.50 g/kg TSN)	*Bradyrhizobium* strain: - USDA 532c	0, 22.5 and 45 kg P_2O_5/ha	- *Bradyrhizobium* inoculation had no effect on nodule number, nodule dry weight, shoot dry weight plant height and grain weight. - Phosphorus applied at 22.5 and 45 kg P_2O_5/ha significantly improved grain yield by 35.4 and 33.9% respectively and nitrogen fixation 49.39 and 69.82% respectively as compared to untreated control. - Application of inoculant plus phosphorus had no effect on the investigated parameters except phosphorus fertilizer which increased the growth and grain yield of soybeans.
Lamptey et al. (2014)	Nyankpala, Ghana	Loamy sand	*Rhizobium* strain	Triple Superphosphate (TSP) - 30 kg P/ha	- Both inoculation and phosphorus fertilizer significantly increased nodule number, nodule dry weight, shoot dry weight and grain yield.
van Heerwaarden et al., (2017)	DR Congo, Ethiopia, Ghana, Kenya, Malawi, Mozambique, Nigeria, Rwanda, Uganda and Zimbabwe	N/A	*Rhizobia* strains: - USDA 110 - 532c - WB74 - TAL379 - MAR1391		- Across all the countries, average yield of inoculated and uninoculated treatments was estimated at 1343 and 1227 kg/ha respectively. - Different varieties across different countries had no significant differences in uninoculated yields in contrast to high yield increase in inoculated soybean.
Fituma et al. (2018)	Metahara, Ethiopia	Calcaric Cambisols (0.12% TSN)	*Bradyrhizobia* strains: - SB6B1 - 532c (Legumefix)	Triple Superphosphate (TSP) - 0 (control) - 23 kg P/ha - 46 kg P/ha - 69 kg P/ha	- Nodule number, plant height, pods per plant, dry biomass yield and grain yield were significantly increased by SB6B1 inoculation over control. - Legumefix inoculation significantly increased nodule dry weight, nodulation rating and nodule volume. - Phosphorus applied at 69 kg P_2O_5/ha improved nodule number and nodule volume while 23 kg P_2O_5/ha increased 100-seeds weight. - Overall, inoculation with *Bradyrhizobia* strains significantly increased nodulation and grain yield.
Solomon et al. (2012)	Bako, Western Ethiopia	Nitisols (0.14% TSN)	*B. japonicum* strains: - TAL 378 - TAL 379		- Inoculation by *B. japonicum* strains increased all the nodulation characteristics (nodule number per plant, nodule dry weight, nodulation rating, and nodule volume per plant) compared to uninoculated soybean. - Yield was significantly higher ($P \leq 0.01$) by 53.2% to the soybean inoculated by TAL 379 over uninoculated control.
Savala (2020)	Ntengo, Ruace and Muriaze, Mozambique	- 0.10% TSN - 0.12% TSN - 0.05% TSN	*B. japonicum* (USDA 110)	0 and 40 kg P_2O_5/ha	-*Bradyrhizobium* inoculation improved nodulation and yield ranging from 37% to 95% over control. The effect of phosphorus on nodulation was inconsistent across study locations and different varieties.
Kamara et al. (2014)	Miringa and Azir, Nigeria	Alfisols - Loamy		0, 20, and 40 kg P/ ha	- Phosphorus fertilizer increased soybean total dry weight and grain yield among other parameters

Table 1. Contd.

		(0.08% TSN) - Clay loam (0.15% TSN)			tested though there was no significant difference between the effects of 20 and 40 kg P/ha rates.
Pulver et al. (1982)	Mokwa and Yandev, Nigeria Tanzania	Coarse-textured Paleustaff soils (low N soil) Sandy soil (low N)	*B. japonicum* strains - 110 - 110-M - 61A76 - SM-31 - SM-35 *Rhizobium* strain (Nitragin),		- Nodule mass increased significantly, but seldomly yield, in response to inoculation by several *R. japonicum* strains as compared to U.S. varieties. - Yield of U.S. varieties increased with inoculation than without inoculation. - The same results were noticed in Tanzania. - Overall, the response of nodule and yield characteristics were variety depended though inoculated performed better than uninoculated soybean.
Pule-Meulenberg et al. (2011)	Wa, Ghana	Ferric Luvisols	*B. japonicum* strain: - WB74		- *B. japonicum* strain WB74 significantly improved nodule number, shoot and whole plant dry weight, and nodule mass as compare to control treatments though it differed with soybean variety.
Okogun et al. (2005)	Kaya, Nigeria	N/A	*Bradyrhizobia* strains: - R25B - IRj 2180A		- Nodulation, shoot dry weight, percentage nitrogen derived from the air (%Ndfa), grain yield, and nutrient uptake varied across and within farmers' fields as affected by the variations in soil fertility and field management. - The shoot dry matter varied among farmers ranging from 2.4 to 166.3 g/plant with an average of 30.7 g/plant, and inoculated improved soybean variety (TGx 1448-2E outperformed uninoculated improved variety (Samsoy-2) though not statistically different. - Inoculation increased grain yields in both tested soybean varieties over uninoculated soybean.
Rurangwa et al. (2018)	Bugesera and Kamonyi and Kayonza, Rwanda	1.7-1.8 g/kg TSN	*B. japonicum* strain: - USDA 110	Triple Superphosphate (TSP): - 0 and 30 kg P/ha	- Inoculation, phosphorus and manure increased grain yield ranging from 1 to 3.8 t/ha in inoculated soybean plots as compared to 1 to 1.7 t/ha in untreated control plots.
Waswa et al. (2014)	Nyabeda, Kenya	Red clay loam (0.21% TSN)	- *B. japonicum* USDA 110 - *Rhizobia* isolates - NAK84 - NAK89 - NAK 115 - NAK 117 - NAK 128 - NAK 135		- NAK 128 outperformed USDA110 by 29% and 24% on both promiscuous and specific soybean varieties. - NAK 128 significantly increased nodule number by producing up to 2.4 million nodules (334 kg) per hectare more that USDA 110. - Overall, many *Rhizobia* isolates increased nodule number, nodule biomass and grain yield compared to uninoculated crop.
Muhammad (2010)	Minna, Nigeria	Alfisol (0.38% TSN)	*Rhizobia* strains: - R25B - IRj 2180A - IRc 46 - IRc291	Single Superphosphate (SSP): - 0, 25 and 50 kg P_2O_5/ha	- *Rhizobia* inoculation increased nodule number, shoot dry biomass and grain yield over uninoculated control. - Phosphorus also improved nodule number, shoot dry weight, and grain yield over control treatment.
Mulambula et al. (2019)	Meru South, Kenya	Clay (0.23%	*Rhizobia* strain rates: - 0, 100 and 200 g	Triple Superphosphate (TSP):	- *Rhizobia* inoculation and phosphorus fertilizer significantly (P=0.05) increased plant height, nodule
		TSN)	/ha	- 0, 20 and 30 kg P/ha	number, nodule dry and fresh weight, mean number

Table 1. Contd.

=					of branches and pods, shoot fresh and dry weight and seed weight averaging 29.35 cm and 26.79 cm, 38.71 and 35.14, 0.51 and 0.38, 5.5 g and 12.54g, 49.13 and 59.18, 77.65 and 90.91, 56.99 and 69.33, 168.9 and 148.13g for SB19 and SB24 soybean varieties respectively.
Rechiatu (2015)	Kpongu, Nyankpala and Manga, Ghana	Loamy sand (0.02 and 0.06% TSN) Sandy loam (0.04% TSN)	*Rhizobium* strain: - 532c (Legumefix)		- Soybean nodule dry weight responded significantly ($P<0.05$) to Legumefix inoculation over control, though varied with location. - Inoculation increased grain yield by 22.43% and 135.54% across two study locations, outperforming uninoculated control.
Tarekegn and Kibret (2017)	Pawe, Ethiopia	N/A	*B. japonicum* strain: - TAL-379	Triple Superphosphate (TSP): - 0, 23, 46 kg P_2O_5/ha	- Nodule number (80.26), fresh and dry weight (3.77 and 0.99 gm/plant respectively) were recorded following application of 46 kg P_2O_5/ha, *B. japonicum* and 11.5 kg N/ha. - Rhizobial inoculation and phosphorus fertilizer (46 kg P_2O_5/ha) increased seed yield by 11.91 gm/ plant and 15.97 gm/plant respectively. - Phosphorus applied at 23 kg P/ha resulted in highest plant biomass of 27.25 gm/plant. - 100-seed weight of 16.96 gm and grain yield of 3151.88 kg/ha were brought by the application of 46 kg P_2O_5/ ha, *B. japonicum* and 11.5 kg N/ha.
Okereke et al. (2001)	Igbariam, and Awka, Nigeria.	Loamy sand (Igbariam) and sandy loam (Awka) (0.14-0.18% TSN)	*B. japonicum* strains: -USDA136 - TAL 122		- *Bradyrhizobia* strains have increased nodule number, nodule dry weight, shoot dry weight, and grain yield compared to the uninoculated crop.
Khalid et al. (2011)	Shambat, Sudan	0.05% TSN	*Bradyrhizobium* - TAL 109		- Inoculation improved shoots and roots dry weight, nodulation, yield components and grain yield.
Mukhtar et al. (1987)	Gezira, Sudan	N/A	*R. japonicum* strains: - 2R-210-3A - 2R-210-2A - 2R-210-3 - PRC-201 - I 1110 Tn5 - 5PRC (ut) - (SR) RJ	Phosphorus fertilizer - 50 and 100 kg P_2O_5/ha	- Inoculation increased plant dry matter, nodule dry matter, yield and yield components and seed protein over control. - Applied phosphorus (50 and 100 P_2O_5/ha) plus starter nitrogen (10 kg N/ha) and inoculation gave unreliable results.
Youseif et al. (2014)	Giza, Egypt	Sandy loam (0.018% TSN)	*Rhizobia* strains: - NGB-SR3 - NGB-SR4 - NGB-SR7 - NGB-SR14		- Tested *Rhizobia* strains increased nodulation with the nodule masses of 265-362 mg/plant compared to 15-31 mg/plant of the uninoculated control. - Inoculants NGB-SR4 and NGB-SR7 out-performed other tested strains in terms of seed yield, N-yield and crude protein content.

N/A, not available; TSN, total soil nitrogen.

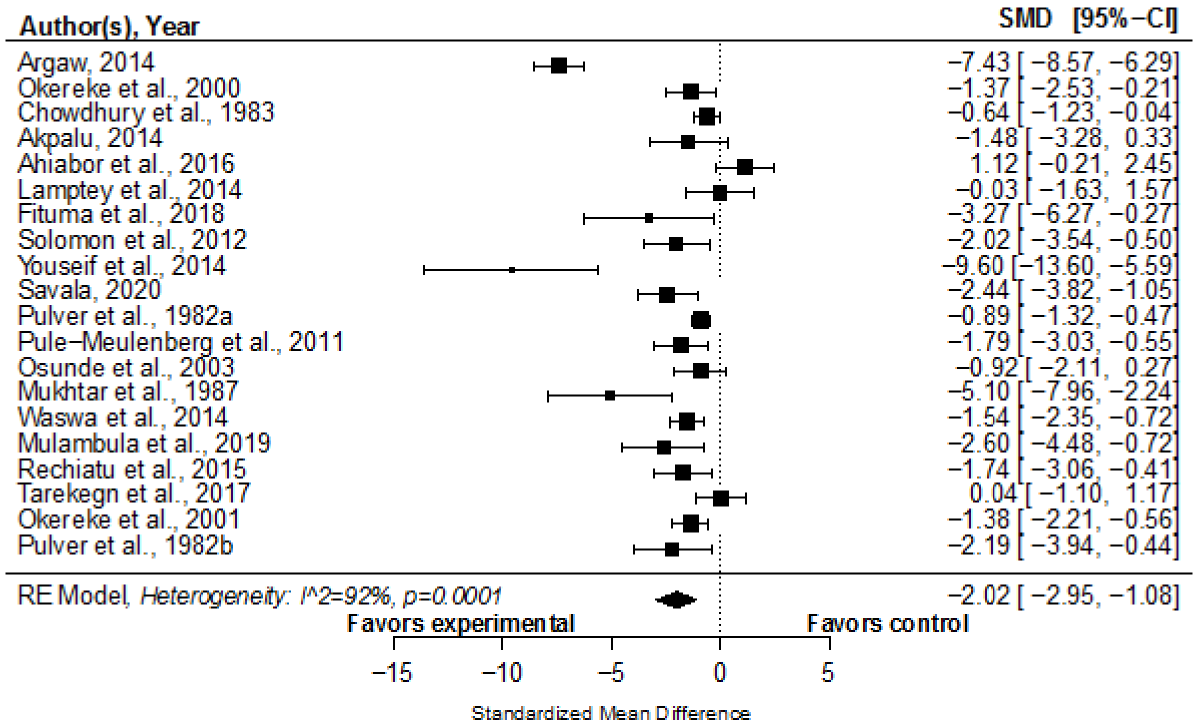

Figure 3. The overall effect of soybean inoculation on nodule dry weight after doing a meta-analysis on 20 studies.

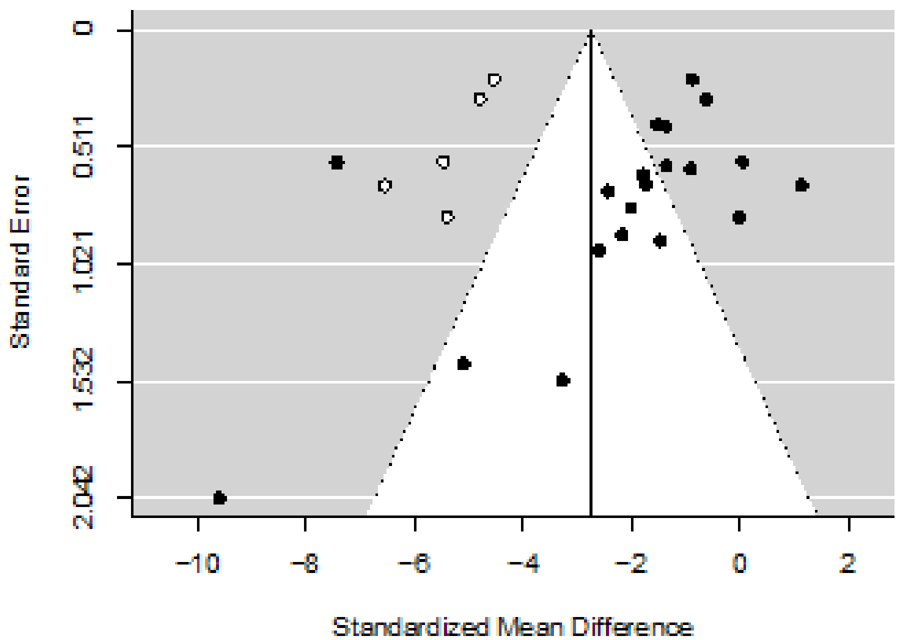

Figure 4. A funnel plot on nodule dry weight for 20 studies.

49 % (P=0.0001). The application of phosphorus fertilizer increased the number of nodules compared to controls (no phosphorus applications) (Figure 11). Phosphorus application resulted in a 95% CI of -1.73[-2.51, -0.94] as

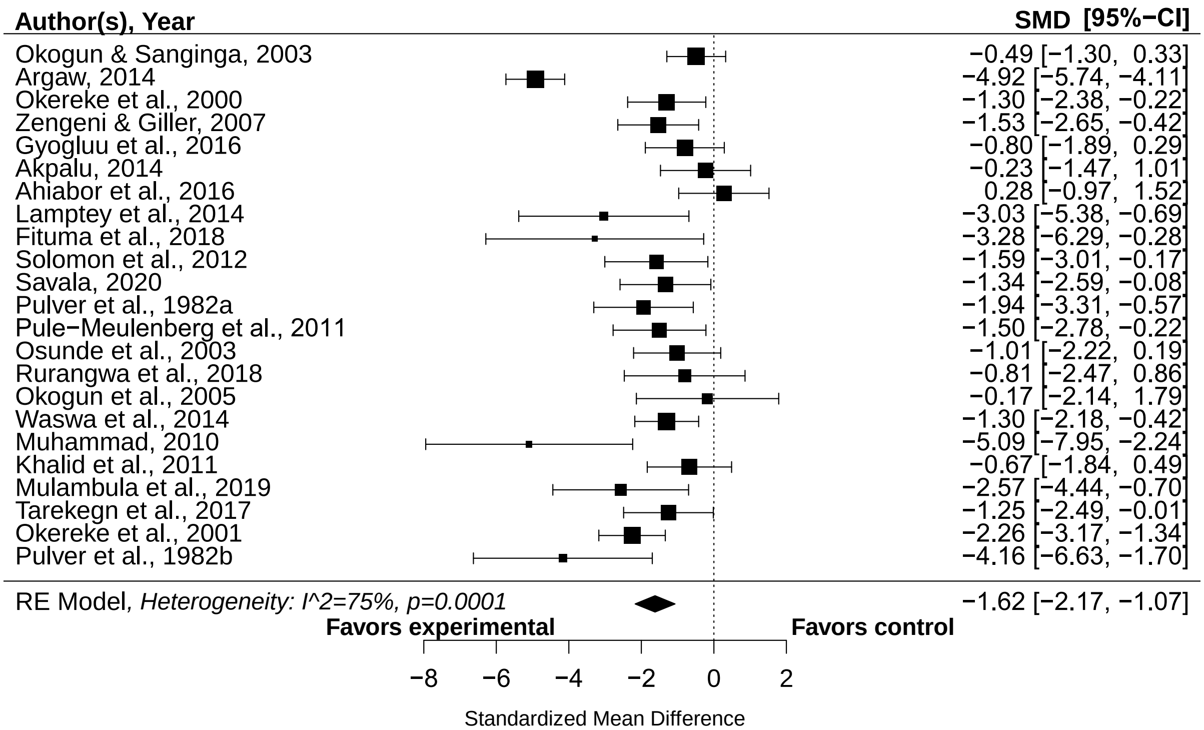

Figure 5. The effect of soybean inoculation on nodule number.

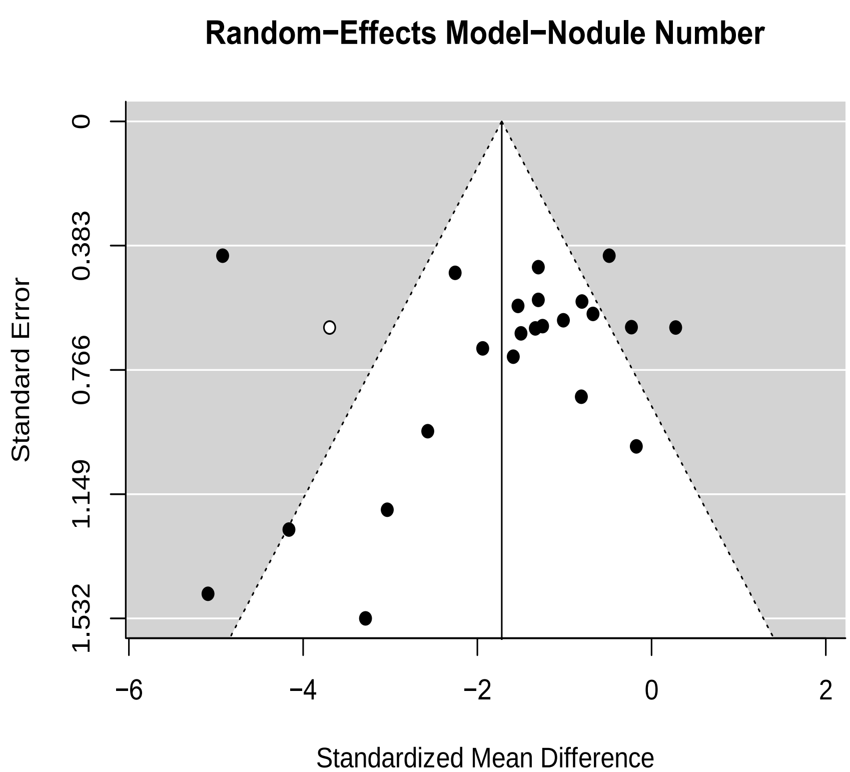

Figure 6. A funnel plot on nodule number response to inoculation for 23 studies.

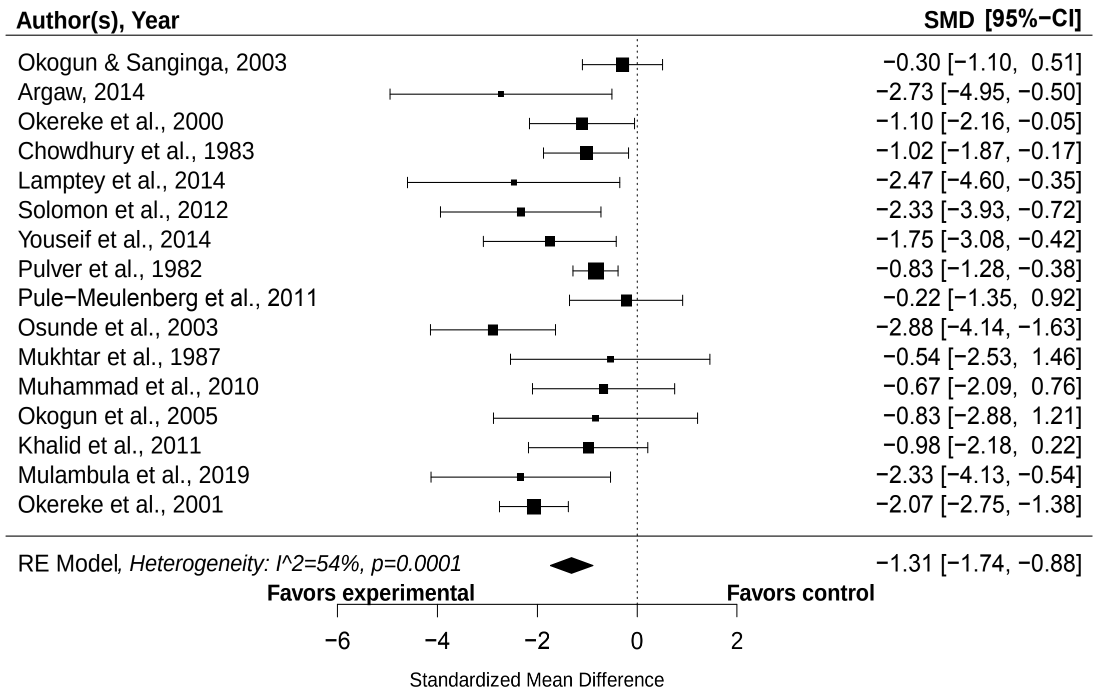

Figure 7. Shoot dry weight as affected by *Rhizobium* inoculation.

the total effect. The studies were asymmetrically distributed on the Funnel plot (Figure 12), showing the presence of bias. However, no significant changes were brought to the final result by trim and fill of the missing studies.

Grain yield

The 10 studies were less heterogenous (I^2 = 20%) (P=0.0001), and the overall effect of phosphorus application on standardized differences had 95% CI of -1.55[-2.14, -0.96] (Figure 13). The overall addition of phosphorus increased the grain yield compared to no phosphorus controls. The Funnel plot showed an asymmetrical distribution of the 10 studies (Figure 14), hence the presence of bias. The overall result remained unchanged after trim and fill.

Grain yield variations as influenced by *Rhizobium* inoculation and P fertilizer

The interaction of inoculation and phosphorus resulted in high grain yield (2.51 t ha^{-1}) compared to the main effects of inoculation and phosphorus (1.67 t ha^{-1} and 1.95 t ha^{-1}, respectively) (Figure 15). However, the main effects of phosphorus and inoculation also contributed to high grain yields.

Relative yield increase

The relative yield increase of the inoculated treatments over non-inoculated controls for the combined studies ranged from 74 to 87% (mean = 80%). The median, first and third quartiles were 80, 78.6, and 81.4%, respectively (Figure 16a). Phosphorus-treated plants had a mean relative yield increase of 73.4% over control treatments with median, first and third quartiles of 73, 71.5, and 75%, respectively (Figure 16b).

DISCUSSION

Response of soybean to rhizobial inoculation

The results of the meta-analysis confirmed that the inoculation of soybean with *Rhizobia* strains in African soils has a highly significant influence on nodule number, nodule dry weight, shoot dry weight, and yield. The performance of *Rhizobia* inoculants varies with strain species/isolates (*Bradyrhizobium/ Sinorhizobium*) and/or indigenous/introduced), soybean genotype, and soil underlying characteristics (pH, soil organic matter, nutrients, salinity, temperature) (Mapope and Dakora, 2016; Thilakarathna and Raizada, 2017). Many of the studies meta-analysed concluded that rhizobial inoculation effectively increased nodule number per plant. However, Thilakarathna and Raizada (2017) mentioned

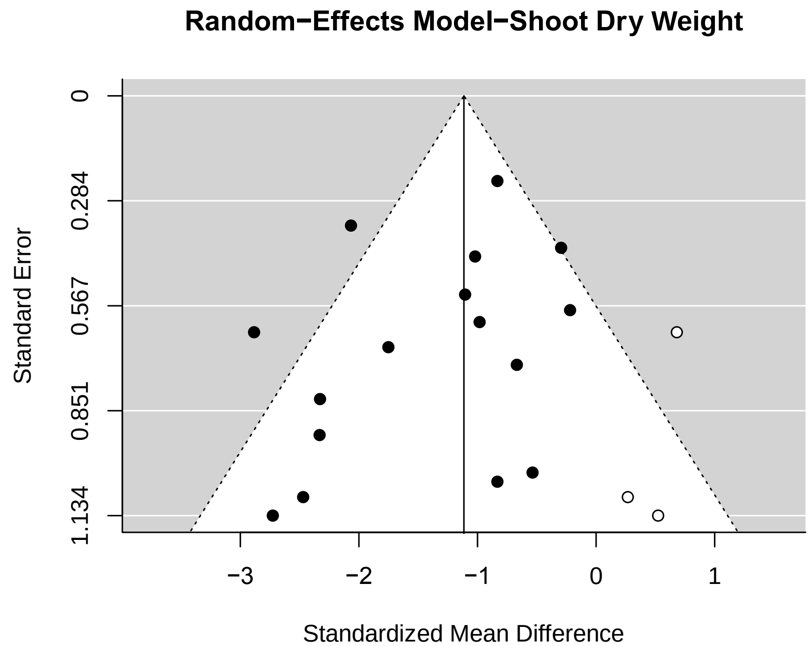

Figure 8. A funnel plot for the 16 studies analyzed for shoot dry weight response to inoculation.

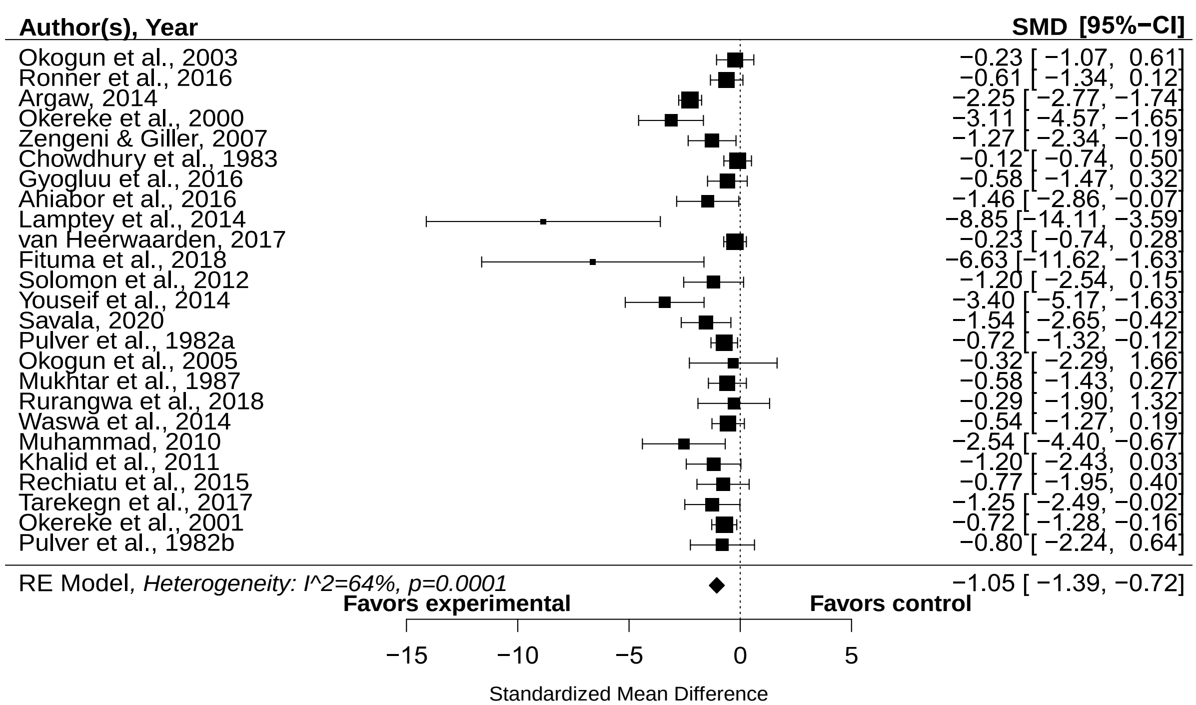

Figure 9. Effect of *Rhizobium* inoculation on soybean grain yield after doing meta-analysis on 25 studies.

that the efficacy of inoculants (*Bradyrhizobium and Sinorhizobium*) for nodule number varied from -28 to +178 nodules in contrast to the non-inoculated controls.

According to the authors, the highest nodule number

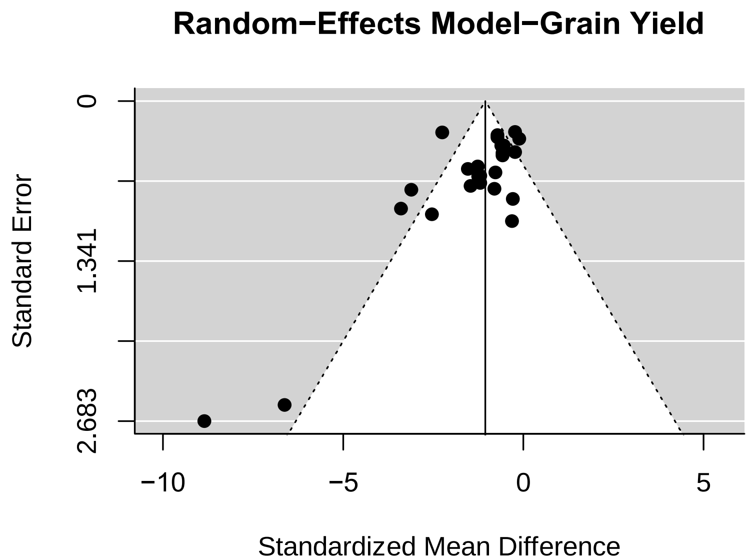

Figure 10. A funnel plot on soybean grain yield response to inoculation.

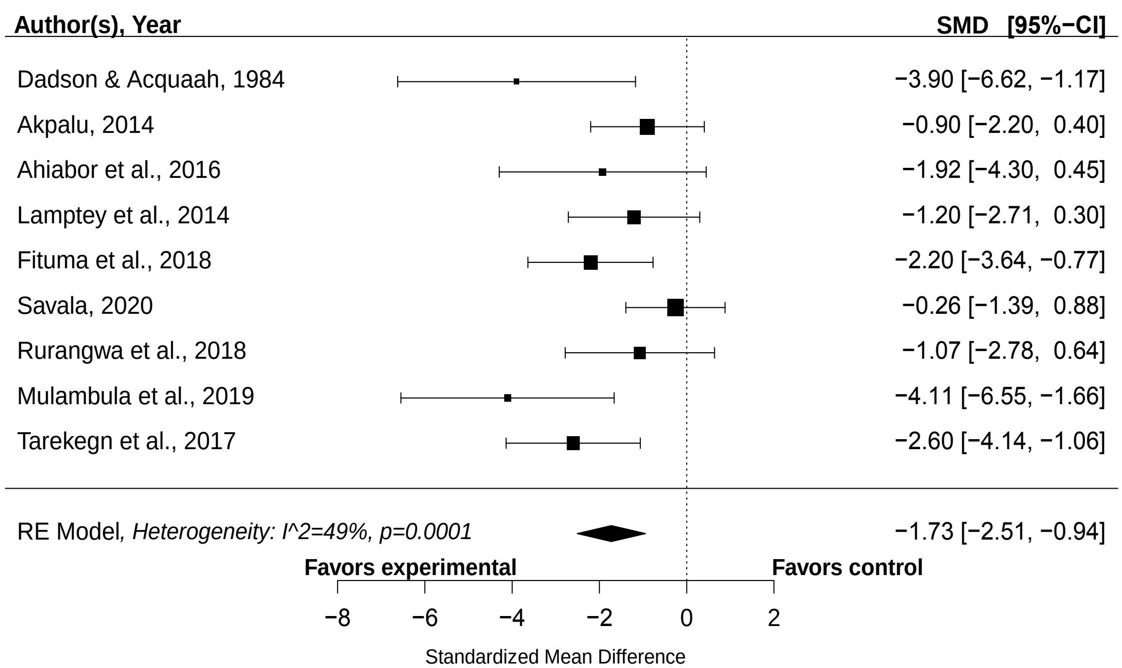

Figure 11. Effect of phosphorus fertilizer to soybean on nodule number after doing meta-analysis on 9 studies.

occurs in soils where indigenous *Rhizobia* are absent or extremely low. This could be probably due to less competition between the commercial *Rhizobia* and indigenous strains. A recent field research conducted across three sites found that inoculation increased nodulation of different soybean genotypes ranging from 37-95% against the non-inoculated treatments (Savala and Kyei-Boahen, 2020). Okereke et al. (2000) also found that soybean inoculation with *Bradyrhizobia* strains significantly increased nodule number but with huge variability at 84 days after planting (DAP); and this was attributed to the variations in the ability to nodulate the

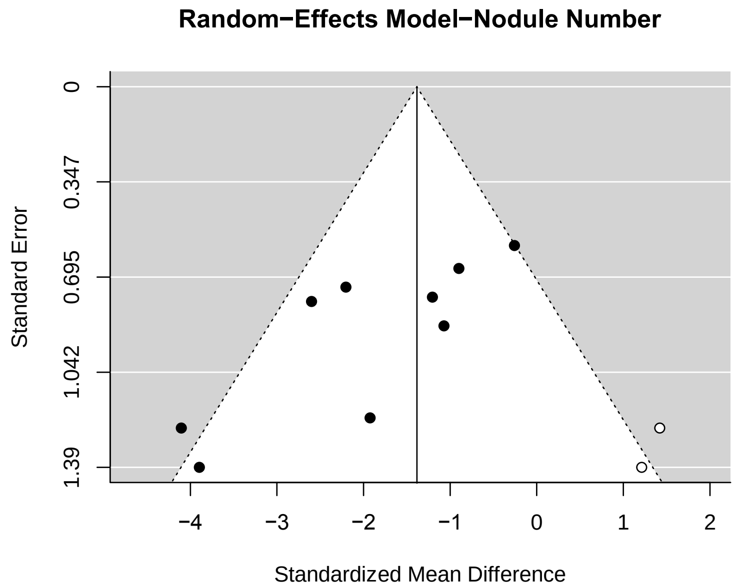

Figure 12. A funnel plot on nodule number response to P-fertilizer application for 9 studies.

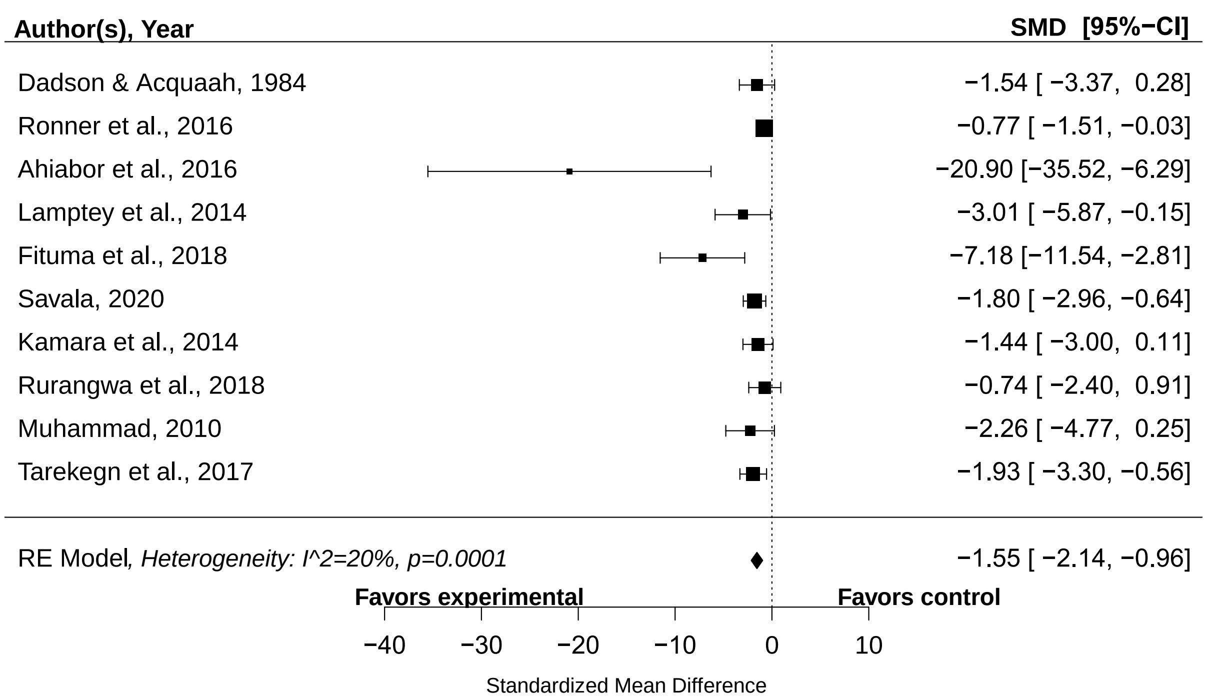

Figure 13. Effect of phosphorus application to soybean grain yield.

soybean variety used (TGX 536-02D). On the other hand, *Rhizobium* inoculation has failed to significantly increase nodule number as demonstrated by Ahiabor et al. 2016). These results implied that N might not be always the limiting factor to lack of nodulation but other nutrients like low phosphorus and molybdenum may impede the inoculation response; and also, indigenous *Rhizobia* could prevent the introduced *Rhizobia* from forming

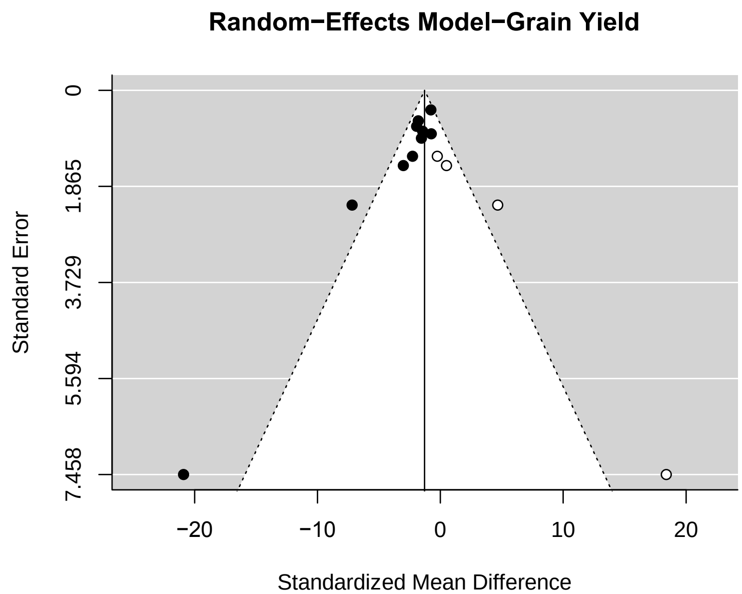

Figure 14. A funnel plot for the 10 studies analyzed for soybean grain yield response to P.

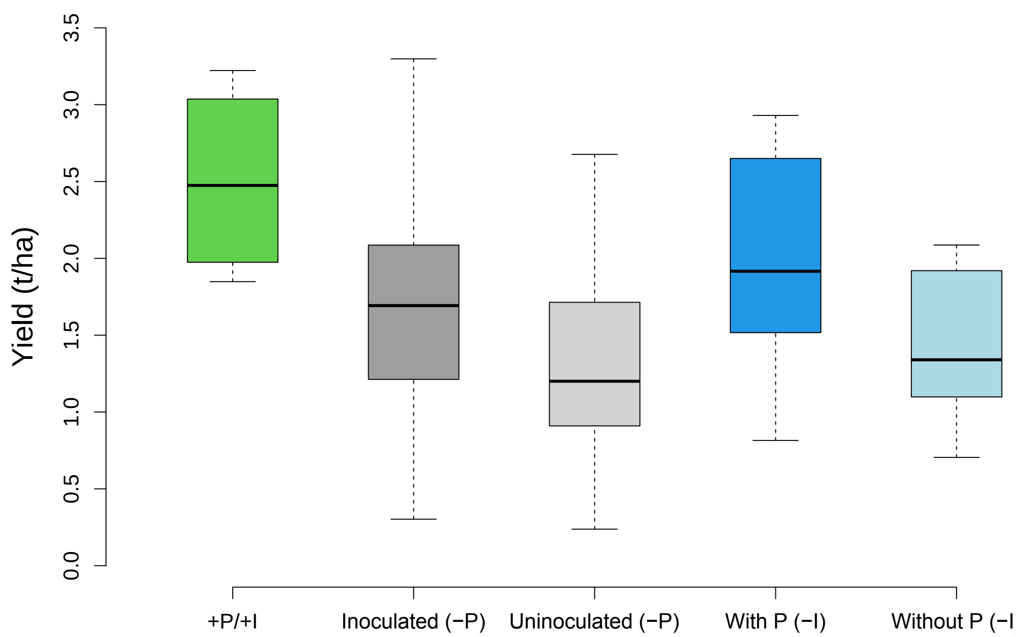

Figure 15. Effect of inoculation and phosphorus application on soybean grain yield. +P, phosphorus was applied; -P, no phosphorus applied; +I, inoculated; -I, non-inoculated.

nodules on soybean (Ahiabor et al., 2016).

The significant increase in the nodule dry weight was not surprising given the effective response of the nodule number to inoculation. This result concurs with the recent works conducted in Ethiopia and Kenya which demonstrated that rhizobial inoculation resulted in increased nodule dry weight per plant (Fituma et al., 2018; Mulambula et al., 2019). Different *Rhizobia* strains also showed significant effects on nodule dry weight, ranging from 0.33 to 0.44 g plant^{-1} in contrast to the non-inoculated controls (Argaw, 2014). Similarly, a field research demonstrated a prolific nodule dry response to

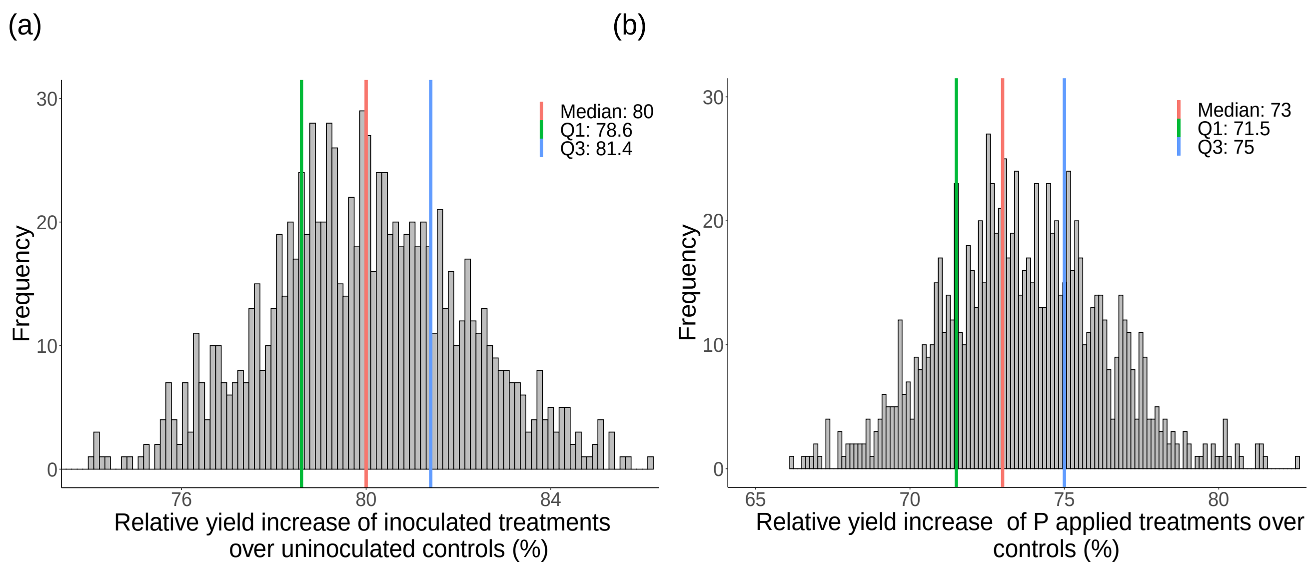

Figure 16. Relative yield increase of soybean in response to inoculation (a) and P fertilizer (b).

Rhizobium inoculation, which ranged from 0.27-0.36 g plant^{-1} versus 0.02-0.03 g plant^{-1} for non-inoculated treatments (Youseif et al., 2014). The results indicated the importance of soybean *Rhizobium* inoculation in African soils. Soybean shoot dry weight increase was highly significant in response to inoculation with *Rhizobia* strains as compared to the non-inoculated control. This result concurs with Ibrahim et al. (2011) who reported a significant increase of shoot dry weight in inoculated treatment over non-inoculated treatment. However, Lamptey et al. (2014) also reported the highest shoot fresh and dry weight following application of 30 kg P ha^{-1}. Inoculation of soybean with *Rhizobia* strains improves nodulation leading to higher nitrogen fixation which subsequently increases the vegetative growth as well as dry matter formation from the inoculated soybean (Lamptey et al., 2014). Interestingly, the results showed a highly significant grain yield of inoculated soybean increased by 25.5% over non-inoculated, confirming the benefits from *Rhizobium* inoculation to soybean in Africa. A global meta-analysis also reported the efficacy of various *Rhizobia* inoculants on soybean yield ranging from -34% to +109% over non-inoculated controls (Thilakarathna and Raizada, 2017). Another farmer-managed field research conducted across 10 sub-Sahara African countries estimated mean grain yield at 1.34 t ha^{-1} and 1.23 t ha^{-1} for inoculated and non-inoculated treatments, respectively, indicating a very narrow margin (van Heerwaarden et al., 2017). They mentioned huge varietal and spatial variations across the region as major contributing factors to their results. Ulzen et al. (2018) also found that *Rhizobium* inoculation increases soybean yield, hence improving the livelihood of smallholders.

Effect of applied P on soybean productivity

Phosphorus is one of the irreplaceable nutrients (Giller and Cadisch, 1995), and its deficiency in many tropical regions is limiting legume performance (George et al., 1995). The BNF process in legumes is substantially driven by phosphorus, which functions as the energy storage and transfer component for the symbiotic bacteria (Dakora and Keya,1997), and increases tissue-%N as well as uptake of N derived from fertilizer (Thomas, 1995; cited by Giller and Cadisch, 1995). This study demonstrated that the supplementation of P-fertilizer on soybean at rates between 20 to 60 kg P ha^{-1} across African soils has a higher significant effect on nodule number. In their findings, Ahiabor et al. (2016) found that applying 22.5 kg and 45 kg P_2O_5 ha^{-1} also effectively increased the number of nodules in soybean by 12 and 22%, respectively, as compared to untreated control. Despite the benefits of this technology, about 24% of farmers were reportedly applying P-fertilizer on soybean in Western Kenya (Franke and Wolf, 2011), which remains true for the majority of smallholders especially, in the SSA (Sheahan and Barrett, 2017). The significant response of grain yield to applied P was not surprising because 11 out of 12 studies that reported on phosphorus fertilizer in this meta-analysis found concurring results. The yield was increased by 36.4% as compared to the control treatment (Figure 15). A field experiment conducted in Nigeria demonstrated that P supplementation increased soybean yield by 452 kg ha^{-1} under smallholder farming (Ronner et al., 2016). Another recent study concluded that 23-46 kg P_2O_5 ha^{-1} of P-fertilizer applied together with a lower level of N (11.5 kg N ha^{-1}) as starter fertilizer potentially increases yield (Tarekegn and Kibret, 2017).

Rhizobium inoculation and P-fertilizer interaction effect on soybean grain yield

The combined application of *Rhizobia* inoculants and P-fertilizer on soybean has resulted in 50.3% and 28.7% yield increase, respective of the independent effects of the two technologies. Servani et al. (2014) reported that P plays a critical role in nodulation processes in legumes; hence its deficiency can limit the yield. Supplementing P-fertilizer will however enhance the BNF process in soybean through improved nodulation processes by the rhizobial bacteria. Ekeleme *et a*l. (2009) mentioned that phosphorus is mostly deficient in many soils, and its optimum application improves the shoot weight and yield of legumes. Another field study also found that applying 30 kg P ha^{-1} of phosphorus together with *Rhizobium* inoculant significantly increased soybean grain yield (Lamptey et al., 2014). Relative yield increase from inoculated treatments over non-inoculated controls was 80%, on average, attesting the effectiveness of rhizobial inoculation in soybean grain yield's improvement. In this regard, van Heerwaarden et al. (2017) obtained an average yield response of 88 kg ha^{-1} from inoculated plants over non-inoculated controls. Phosphorus-treated plants had also a high relative yield increase over control plants, averaging 73.4%. Eleven studies reported that supplementation of P fertilizer resulted in improved BNF with direct impacts on grain yield. However, it was hard to conclude that rhizobial inoculation and P fertilizer result in higher relative yield increase given the huge variability in agronomic, climatic, and edaphic factors across African countries which could affect the biological nitrogen fixation process of soybean.

Although the meta-analysis ascertained the general effect of rhizobial inoculation on soybean, it should be noted that the efficacy of the commercial inoculants varies with the underlying soil factors like indigenous rhizobial level, soil available nutrients, soil pH, organic matter content, temperature, and precipitation. Unfortunately, the effects of the above-mentioned factors were not analyzed due to the huge variation of the data and scarcity of valid studies. It was also clear that P-fertilizer notably between 20 kg and above 60 kg P ha^{-1} had varying effects on soybean's nodulation and grain yield. Furthermore, it was not established that the rates between 20 or above 60 kg P ha^{-1} could not affect soybean productivity.

Conclusion

Meta-analyses of the effects of *Rhizobia* inoculants and phosphorus fertilizer on soybean nodulation in Africa revealed rhizobial inoculation has, in absolute terms, highly significant effects on nodulation characteristics, shoot dry weight, and grain yield of soybean on African soils that may vary with the underlying soil characteristics, *Rhizobium* strain, climatic conditions, to name a few. Application of phosphate fertilizer at rates of 20-60 kg P ha^{-1} proved to increase the nodule number per plant and most importantly, soybean grain yield. Phosphorus showed a slightly higher effect on grain yield as compared to rhizobial inoculation, in absolute terms. Finally, the application of both inoculant and P-fertilizer on soybean greatly increased grain yield by 50.3% compared to a simple *Rhizobium* inoculation and 28.7% compared to P application alone. Therefore, it was recommended to African farmers to adopt this sustainable approach of combined application of both *Rhizobium* inoculants and phosphate fertilizer for reduced financial costs of production and increased yield.

CONFLICT OF INTEREST

The authors have not declared any conflict of interest.

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