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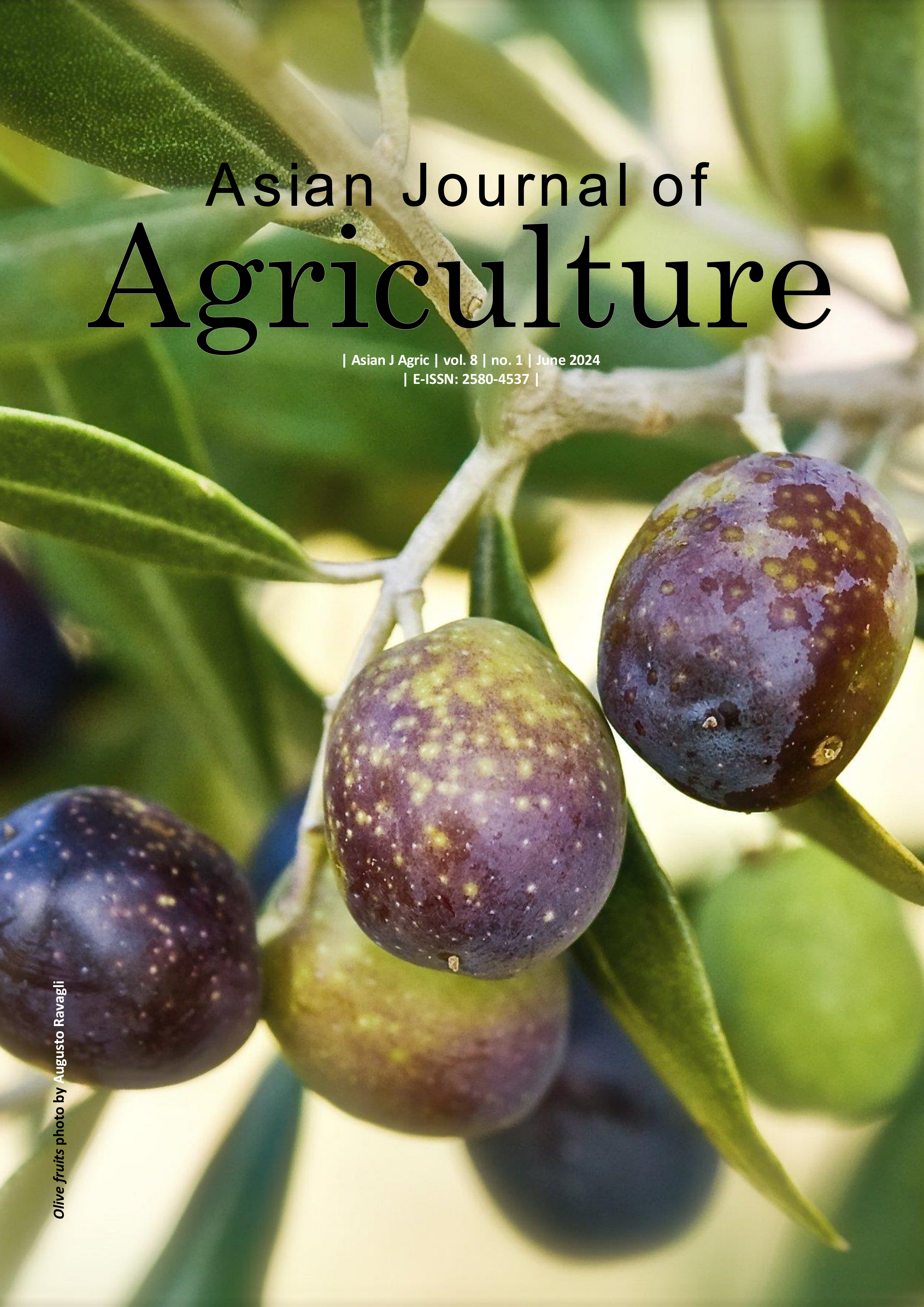

Olive fruits photo by Augusto Ravagli

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The usage of "et al." in long author lists will also be accepted:

Smith J, Jones M Jr, Houghton L et al. 1999. Future of health insurance. N Engl J Med 965: 325-329. DOI: 10.10007/s002149800025.

Book:

Rai MK, Carpinella C. 2006. Naturally Occurring Bioactive Compounds. Elsevier, Amsterdam.

Chapter in the book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds.). Tropical Forest Community Ecology. Wiley-Blackwell, New York.

Abstract:

Assaeed AM. 2007. Seed production and dispersal of *Rhazya stricta*. 50th annual symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds.). Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island. Universitas Sebelas Maret, Surakarta, 17-20 July 2000. [Indonesian]

Thesis, Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon. [Dissertation]. Universitas Brawijaya, Malang. [Indonesian]

Information from the internet:

Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. 2008. A synthetic *Escherichia coli* predator-prey ecosystem. Mol Syst Biol 4: 187. DOI: 10.1038/msb.2008.24. www.molecularsystembiology.com.

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Evaluating the impact of poultry manure variants and swine manure on soil chemical properties and growth of maize (*Zea mays*)

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Abstract. *Diri KH, Kedoneojo AT. 2024. Evaluating the impact of poultry manure variants and swine manure on soil chemical properties and growth of maize (*Zea mays*). Asian J Agric 8: 1-9.* A greenhouse experiment was conducted at the Niger Delta University Teaching and Research Farm, Nigeria, to assess the impact of various organic manure sources on soil chemical properties and maize growth. The treatments included three variants of poultry manure (broiler manure (PB), layers poultry manure (PL), point of lay poultry manure (POL)), swine manure (SW), and combined treatments of swine manure with each poultry manure variant. Three application rates (0 (L_0), 10 (L_1), and 20t/ha (L_2)) were employed for each treatment. The experiment followed a 7×3×3 factorial fitted into a randomized complete block design, with treatments replicated thrice; the test crop used was maize (*Zea mays* L.). Soil samples were collected before and after the experiment to evaluate the effect of each organic treatment on soil chemical properties. Statistical Analysis of Variance (ANOVA) was conducted on the collected soil and plant data, with mean separation performed using Tukey's t-test. The results showed significant differences ($p < 0.05$) between the experimental and control treatments, with notable effects on soil chemical properties. In soils, the SWPL (L_2) treatment presented the highest average values for pH (6.06), organic matter (2.95%), calcium (0.96 cmol/kg), magnesium (0.77 cmol/kg), potassium (0.54 cmol/kg), base saturation (63%), and effective cation exchange capacity (3.68 cmol/kg). The highest mean value for total soil nitrogen corresponded to the PL treatment (L_2). Plant parameters also exhibited significant differences ($p < 0.05$) compared to the control, with the most pronounced effects observed in the PL (L_2) and SWPL (L_2) treatments. These findings suggest that utilizing poultry manure from layers, combined with swine manure, positively influences soil quality, productivity, and maize growth. Thus, maximizing the use of these organic manure sources as alternatives for fertilizer application is recommended.

Keywords: Fertilizers, soil fertility, maize growth, organic manure, soil chemical properties

INTRODUCTION

Food production in Nigeria is limited due to challenges from using synthetic fertilizers, including availability, cost, and environmental concerns. While the use of chemical fertilizers plays a crucial role in maintaining short-term productivity in Agro-ecosystems, studies have shown that its excessive and indiscriminate application can lead to a decline in soil fertility, soil organic matter (SOM), increase in soil acidification, cause nutrient imbalances, negatively impact enzymatic activity, and pose risks to the copiotroph community (Ansari and Mahmood 2017).

The soils in Bayelsa State, situated in the southern region of Nigeria, attracted attention due to their lower fertility status, unique characteristics, and increased soil acidity resulting from consistent crop cultivation practices and mineral fertilizers (Diri and Joseph 2020). While applying mineral fertilizers can certainly enhance crop productivity within these soils, it is crucial to recognize the potential downsides, such as a decline in soil quality and fertility. This could pose challenges to the long-term sustainability of the soil systems, as highlighted in previous studies (Agegnehu et al. 2016; Srivastava et al. 2016). As a result, there is an immediate need to explore alternative approaches that emphasize sustainability in various aspects, including crop yield, efficient resource utilization, soil health, soil quality, and practicality for local farmers (Agegnehu et al. 2016).

Organic manures have been proven over many years to be more affordable. They can effectively replace their inorganic counterparts by enhancing soil structure and water retention capacity, improving soil fertility and crop yield. The growing attraction towards adopting organic nutrient sources and soil amendments is of paramount significance, primarily driven by the recognition that they represent a valuable reservoir of carbon (C) (Rayne and Aula 2020). This carbon content is pivotal in enhancing soil quality and assumes additional significance regarding its potential contribution to the amelioration of climate change implications.

Based on the research conducted by Agyarko and Adomako (2007), as cited by Afriyie et al. (2013), the utilization of organic manures is primarily attributed to their cost-effectiveness, easy accessibility, and efficacy. Compared to synthetic fertilizers, organic soil amendments have clear advantages and fewer adverse effects on soil structure, human health, and the environment. According to Rao and Padmaja (2016), proper organic matter management practices optimize the soil's biological processes, ensuring adequate crop nutrition. Furthermore, organic manures are crucial in improving soil structure by binding soil aggregates and reducing nutrient leaching and erosion risks. Adekiya et al. (2016) argued that utilizing indigenous and available organic nutrient sources can enhance fertilizer efficiency and reduce the amount of chemical fertilizers needed.

Following that, extended investigations into the impact of fertilizers derived from organic sources, as conducted by Blanchet et al. (2016), in a long-term study have demonstrated that the utilization of organic manure yields enhancements in soil chemical attributes and imparts notable increments in phosphorus (P) and potassium (K) content. In alignment with these findings, Achiba et al. (2010) reported increased soil nitrogen (N) content attributed to applying organic manure. Similarly, Gopinath et al. (2009) undertook a comprehensive two-year study, revealing that the incorporation of manure substantially contributed to the elevation of soil pH compared to using chemical fertilizers. Overall, incorporating organic residues and manures into soil management practices is essential as they improve soil physical, chemical, and biological properties.

Despite recognizing poultry manure's efficacy, gaps exist in understanding specific types, such as broilers, point-of-lay birds, or laying birds, and potential synergies with other organic sources like swine manure. Thus, this research investigates the impacts of different poultry manure sources, alone and in combination with other organic sources, on soil chemical properties and maize growth.

MATERIALS AND METHODS

Greenhouse experiment

The experiment was carried out at the Screen House of the Niger Delta University Teaching and Research Farm (NDUTRF), Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. The site is located at Latitude 4º38'38'N, Longitude 6º21'41'E Equator. The mean annual rainfall ranges from 2,000-4,000 mm per annum and spreads over 8-10 months between April and November, coinciding with the wet season having a mean rainfall of 2,500 mm per annum coupled with a fairly constant temperature of 30ºC. The soils in the study area are predominantly silty clay loam (Diri and Joseph 2020).

The organic manure was obtained from NDUTRF. The treatment consists of two different sources of organic manure: poultry manure from broilers (PB), point of lay birds (POL), and layers (PL) and swine manure (SW), as well as combined treatment of; swine manure + poultry manure from broilers (SWPB), swine manure + poultry manure from point of lay birds (SWPOL), and swine manure + poultry manure from Layers (SWPL). Point of lay birds (POL): pullets within the age range of approximately 16-20 weeks, positioned to enter the phase of egg production. Layers (PL): mature hens that have reached the age of consistent egg production, usually around 20 weeks and beyond and are actively laying eggs. The experiment was carried out in a 7×3×3 factorial randomized complete block design with three treatment levels, replicated three times, 0 t/ha, 10 t/ha, and 20 t/ha.

Soil from 0-15 cm depth was obtained from the teaching and research farm, air-dried, ground, and passed through a 2 mm sieve. Next, 2 kg of sieved soils were filled into the experimental pots and mixed with 10g and 20g of organic manure (w/w), following Rao and Padmaja (2016). The pots were then watered to field capacity and incubated for one week before planting. Hybrid Maize variety (Sam Max 44) obtained from the International Institute of Tropical Agriculture (IITA) was planted 2-3 cm deep and thinned to 2 plants per pot 10 Days after planting (DAP).

Soil and plant analysis

Soil collected was analyzed for its chemical properties before planting and after harvest. Soil pH was determined in 0.01M $CaCl_2$ salt solution at a 1:2 (w/v) ratio with a glass electrode pH meter. Organic carbon (Org C) was determined by the dichromate wet oxidation method of Walkley and Black. Organic matter (Org M) was calculated by multiplying the percentage of organic carbon (van Bemmelen factor) by 1.724. Total Nitrogen (Total N) was determined by the macro Kjeldahl method. Available phosphorus (Avail P) was determined by the Bray P-1 method of Bray and Kurtz (1945). Exchangeable bases (EB) were extracted using 1N Ammonium acetate ($NH_4CH_3CO_2$). Ca^{2+} and Mg^{2+} were analyzed using a PGI 990 Atomic Absorption Spectrophotometer (PG Instruments Ltd., UK), while K^+ and Na^+ were determined using ATS 200S Flame Photometer (ATS-Technology, Cyprus). The exchangeable acidity (EA) was determined by leaching the soil using 1N KCl and the extract titrated with 0.01N NaOH. Cation exchange capacity was determined using the 1N NH_4OAc method, and Effective Cation Exchange Capacity (ECEC) was evaluated by summing up the exchangeable bases and the exchange acidity. Data on plant parameters, including plant height, leaf number, leaf area, and stem girth, was taken to record growth attributes.

Data analysis

Data from plant and soil collected were subjected to statistical Analysis of Variance (ANOVA) using Minitab v17 software. The treatment means were separated for significant differences using the Tukey T-test at a 5% probability level.

RESULTS AND DISCUSSION

Characterization of the soil and organic materials used in the experiment

The chemical properties of organic manure and soil are shown in Table 1. The result of the selected properties indicates that the pH of the bulk soil sample was strongly acidic, while that of the organic materials was slightly to moderately acidic. The results and values obtained indicate that these organic materials could potentially enhance the fertility of the soil and impact positively on the growth and yield of maize. Furthermore, the swine and layer manure (SWPL) combination exhibited the best chemical properties. In addition, Hadas et al. (2004) also reported similar results.

Effect of organic amendment on soil chemical properties

Soil pH and exchangeable acidity

Before applying organic soil amendments, the results obtained from soil analysis at the experimental site indicated a strongly acidic soil with a pH value of 4.53 (0.04). However, the application of organic amendments at various levels led to a significant increase ($p < 0.05$) in soil pH, shifting it from strongly acidic 4.53 (0.04) to slightly acidic 6.06 (0.06). Among the treatments, SWPL (L_2) had the greatest impact on soil pH, resulting in a pH value of 6.06 (0.06). This can be attributed to the high pH value of the organic amendment (SWPL, 6.97) used. While both SWPL (L_2) and PL (L_2) treatments increased soil pH, no significant difference ($p > 0.05$) was observed between them. This suggests that both treatments can be used as organic amendments or liming materials to elevate soil pH levels when applied at the same rate.

On the other hand, the soil pH levels with the lowest mean were observed in soils subjected to the lower treatment application (L_1). Among the L_1 treatments, PB exhibited the lowest pH value of 5.03(0.08), and no significant difference was found between SWPB, PB, and POL treatments. In addition, the interaction between treatment and treatment levels on soil pH was significant ($p <0.05$).

This finding aligns with previous studies conducted by researchers such as Boateng et al. (2006), Busari et al. (2008), and Quansah (2010), who also reported similar increases in soil pH following the application of organic amendments. The pH elevation resulting from organic amendments, particularly poultry manure, is often attributed to the release and addition of basic cations during the decomposition and mineralization process (Ano and Agwu 2005; Melero et al. 2007), as well as the presence of organic anions in the manure which can neutralize hydrogen ions (Butterly et al. 2013). Ano and Ubochi (2007) also reported a consistent increase in soil pH for applying rabbit, swine, goat, chicken, and cow manures at different rates. This increase in the pH as a function of manure application has also been attributed to the calcium carbonate and bicarbonate found in manure (Eghball et al. 1996; Whalen et al. 2000).

Moreover, some authors, including Yaduvanshi (2003) and Balota et al. (2012), have reported decreases and no changes in soil pH following the application of swine manure; its effect on soil pH could be dependent on its specific characteristics, soil condition, and dietary composition. Furthermore, the capacity of the different manures to elevate soil pH aligns with the same sequence as their capacity to diminish exchangeable acidity. Consequently, it can be inferred that the manures' effectiveness in ameliorating soil pH is contingent upon their inherent capability to mitigate exchangeable acidity, encompassing both hydrogen (H^+) and aluminum (Al^{3+}) ions (Ano and Ubochi 2007). Following this observation, it could be observed that an increase in the application rate of these manures significantly decreased exchangeable acidity from control soil from 2.15 cmol/kg to 1.34 cmol/kg (Table 2). All organic amendments reduced EA below the critical limit (<2 cmol/kg -low-) given by Adamu et al. (2014). The Addition of SWPL (L_2) had a significant effect on EA, followed by PL (L_2). When organic materials in manure, compost, ash, biochar, and digestate are added to acid soils, they form strong bonds that chelate with H^+ and $Al^{3+,}$ reducing their solubility.

Organic carbon and organic matter

A significant setback in maintaining soil health arises from the gradual reduction of Soil Organic Matter (SOM) due to prolonged land cultivation. The pooled data analysis from this research revealed a significant increase in the organic carbon content of the soil. Initially, the soil had a low organic carbon content, increasing from 0.66% to 1.70% upon applying organic amendments. The combined treatment SWPL L_2 had the greatest impact on soil organic carbon (1.70%) and showed a statistically significant difference ($p <0.05$) compared to all other treatments. Furthermore, higher treatment levels were associated with increased organic carbon levels, indicating that L_2 treatment was more effective in enhancing soil organic carbon. In contrast, SWPOL, POL and PB exhibited the least effect on soil organic carbon at (L_1), but was significantly different ($p <0.05$) from control.

The SOM content increased concerning the increased treatment levels of all organic amendments and showed a statistically significant difference ($p < 0.05$) compared to the control. Incorporating organic manures into the soil resulted in a significant increase in organic matter, elevating it from 1.14% to 2.97%. Among the treatments, the combined treatment (SWPL) consistently exhibited a higher effect on SOM (2.97%) at L_2. Still, it did not show a statistically significant difference ($p >0.05$) compared to all other treatments at the 20 t/ha level (L_2), except for PL, SWPOL, and SW. Furthermore, the treatments, level of application of organic manure, and the interaction between treatment and its level were significant ($p<0.05$), bolstering that these amendments impacted SOC and SOM.

Table 1. Chemical properties of soil and organic manure used in this study

Sample	pH (water)	Org C (%)	Total N (%)	C/N Ratio	Avail P (mg/kg)	Ex. K (cmol/kg)
PB	5.60	1.13	1.83	1:1	3.81	0.32
SW	6.36	1.69	2.22	1:1	4.00	0.59
PL	6.66	1.79	2.27	1:1	4.27	0.47
POL	5.68	1.21	1.79	1:1	3.60	0.34
SWPB	6.17	1.48	2.26	1:1	4.23	0.50
SWPL	6.97	1.83	2.30	1:1	4.41	0.71
SWPOL	5.97	1.30	1.95	1:1	4.09	0.52
Soil	4.80	0.81	0.02	22:1	3.02	0.28

Note: PB: Broiler manure, SW: Swine manure, PL: Layer manure, POL: Point of lay manure, SWPB: Swine+Broiler manure, SWPL: Swine+Layer manure, SWPOL: Swine+Point of lay manure, Org C: organic carbon, Total N: total nitrogen, C/N Ratio: carbon/nitrogen Ratio, Avail P: Available phosphorus and Ex. K: extractable potassium

Studies conducted by Denton et al. (2020) also demonstrated an increase in organic carbon in soils when incorporated with organic manure. These results are consistent with Shi et al. (2009) and Deryqe et al. (2016), where poultry and swine were used as soil amendments. Furthermore, the rise in Soil Organic Carbon (SOC) can also be directly linked to the low C/N ratio found in different amendments used, as noted by Montemurro et al. (2010) and Stockmann et al. (2013). Therefore, it can be inferred that the combined treatment of swine manure and Layer manure can be considered effective in enhancing carbon sequestration and increasing the organic matter content in soils for agricultural purposes.

Total nitrogen

The preliminary assessment of the soil revealed that the concentration of Total N in the soils, before the incorporation of organic amendments, was generally deficient, falling below the critical threshold of 0.02% as determined by Chude et al. (2012). Previous research has also indicated low levels of Total N in these soils, attributed to a high C:N ratio and intensive mineralization caused by significant fluctuations in precipitation and temperature (Diri and Joseph 2020).

However, the soil N content increased in the amended plots after applying soil amendments compared to the unamended plots. Notably, utilizing the combined organic manure SWPL (L_2) and the manure from PL (L2) treatments resulted in a substantial elevation of soil N levels, reaching 0.70% and 0.78%, respectively. The increase observed in both treatments was statistically significant ($p < 0.05$) compared with other organic amendments, which can be attributed to the high N content in both PL and SWPL manure. Furthermore, the impact on soil Total N varied according to the treatment levels, with higher treatment levels (L_2) exhibiting a more pronounced effect than lower treatment levels (L_1). Additionally, the rise in soil Total N can be attributed to the subsequent decomposition and mineralization of the abundant organic matter content in all amendments. This finding aligns with the established knowledge that increased soil organic matter content is expected to enhance soil N status (Hadas et al. 2004). Similar results have been reported by Akbari et al. (2011).

It is well-established that releasing N and other essential nutrients from livestock manure depends on the mineralization rate. The quantity of a nutrient subject to mineralization within manure is contingent upon a confluence of factors, including manure composition, environmental variables, soil characteristics, and microbial enzymatic activity, as Eghball et al. (2002) outlined. In an empirical study conducted by Khan et al. (2007), which examined the influence of dairy manure application and tillage practices on maize cultivation, it was observed that the addition of 10 Mg/ha and 20 Mg/ha of dairy manure in conjunction with inorganic fertilizer yielded a notable increase of 24% and 27%, respectively, in soil N content, as compared to the utilization of inorganic fertilizer exclusively.

Available phosphorus and exchangeable potassium

Control soils indicated a deficiency in available phosphorus, with levels measuring 3.05 mg/kg, which fell below the critical threshold for phosphorus availability (Reddy et al. 2014). However, adding organic amendments did not significantly increase the soil's phosphorus status, although the mean values differed from those of the control group. Applying organic amendments at various treatment levels did not substantially impact the phosphorus status and availability. Phosphorus content in soil increased marginally from 3.05 mg/kg to 4.24 mg/kg, which remained below the critical threshold (Horneck et al. 2011). Poultry manure contains relatively high total and soluble P and low N:P ratios. Although PL, SW, SWPL, SWPB, and SWPOL had higher P content, the marginal increase of P in the soil could be a result of P-fixation by Al; Fe oxides linked primarily to the acidic nature of the soils as well as the EA concentration of the soils. This corroborates with studies by Boateng et al. (2006).

The increase in available P after manure application to soil is a function of various soil characteristics, including soil pH, organic matter content, and clay type (Chatterjee et al. 2014). Potassium (K) concentration in the soil before the addition of organic amendments was comparatively low (0.24 cmol/kg) concerning the critical rating for exchangeable K as specified by FAO (2006). SWPL (L_2) exhibited the highest K concentrations among the organic amendments, while the lowest values were observed for POL (L_1). These findings can be attributed to the relatively higher K contents in SWPL (0.71 cmol/kg) treatment than in POL (0.34 cmol/kg) as shown in (Table 1).

Calcium, magnesium, and effective cation exchange capacity

Calcium concentration in the soil was initially below the critical level (<2 cmol/kg), as indicated by FAO (2006) before the implementation of organic amendments. However, after applying organic amendments, a substantial increase in soil calcium content was observed for all treatment levels compared to the control, although it remained below the critical threshold. Notably, the combined SWPL treatment at L_2 significantly elevated soil calcium content, reaching 0.96 cmol/kg, followed by PL (L_2) at 0.84 cmol/kg, while POL (L_1) exhibited the least augmentation at 0.53 cmol/kg. These findings highlight the capacity of organic manures to enhance soil nutrient reserves, foster fertility development, and promote improved nutrient bioavailability (Brady and Weil 2005).

The magnesium (Mg) content of the soil (0.24 cmol/kg) was considered moderate, following the established range (0.3-0.1 cmol/kg). However, after applying organic amendments, a significant increase in soil magnesium content from 0.24 cmol/kg to 0.77 cmol/kg was observed for SWPL (L_2). Concerning sodium (Na) content, the baseline levels were low and below the critical limit of 0.10 cmol/kg. Applying organic amendments yielded significant distinctions ($p < 0.05$) compared to the control. Although there were significant differences ($p > 0.05$) detected among the various treatments, this can be negligible because manures generally have low sodium content.

Table 2. Effect of organic waste application on soil chemical properties

Treatment		pH ($CaCl_2$)	Org C	Org M	Total N	Avail P (mg/kg)	EA	Ca	Mg	Na	K	BS (%)	ECEC (cmol/kg)
			(%)				(cmol/kg)						
Control	L0	4.53 (0.04)e	0.66 (0.06)g	1.14 (0.11)f	0.01 (0.01)j	3.05 (0.18)f	2.15 (0.03)a	0.29 (0.03)e	0.24 (0.35)e	0.01 (0.00)d	0.24 (0.02)de	26 (0.95)h	2.93 (0.04)d
PB	L1	5.03 (0.08)de	1.30 (0.02)f	2.26 (0.03)e	0.23 (0.03)i	3.37 (0.11)de	1.56 (0.02)bcd	0.59 (0.02)cd	0.33 (0.02)de	0.04(0.01)c	0.26 (0.02)de	43 (1.38)g	2.78 (0.04)abcd
	L2	5.22 (0.09)bcd	1.53 (0.01)cd	2.66 (0.02)abcd	0.33 (0.03)h	3.44 (0.04)cde	1.55 (0.03)bcd	0.65(0.02)bc	0.37 (0.02)cde	0.04 (0.00)c	0.33 (0.01)cde	47 (0.84)	2.49 (0.07)abc
SW	L1	5.44 (0.06)abcd	1.49 (0.03)d	2.59 (0.05)bcd	0.48 (0.02)e	3.69 (0.04)bcd	1.83 (0.03)abc	0.55 (0.03)cd	0.44 (0.02)cd	0.06(0.01)abc	0.28 (0.01)de	42 (0.10)e	3.16 (0.05)bcd
	L2	5.70 (0.03)ab	1.61 (0.02)b	2.80 (0.03)ab	0.55 (0.02)d	3.65 (0.03)bcde	1.50 (0.04)bcd	0.63 (0.02)bc	0.66 (0.03)ab	0.07 (0.01)ab	0.35 (0.02)bcd	53 (0.75)c	3.21 (0.04)abcd
PL	L1	5.66 (0.03)abc	1.56 (0.00)bcd	2.71 (0.01)abcd	0.60 (0.02)c	3.91 (0.18)b	1.76 (0.02)acd	0.74 (0.02)ab	0.45 (0.01)cd	0.05 (0.01)c	0.37 (0.01)dcd	48 (0.49)d	3.37 (0.02)abcd
	L2	6.02 (0.09)a	1.61 (0.03)b	2.80 (0.06)abc	0.78 (0.04)a	4.24 (0.07)a	1.44 (0.01)cd	0.84 (0.03)ab	0.50 (0.02)bcd	0.06 (0.01)bc	0.45 (0.03)abc	56 (0.77)b	3.29 (0.06)ab
POL	L1	5.05 (0.09)cde	1.35 (0.02)ef	2.35 (0.04)de	0.27 (0.03)i	3.35 (0.07)ef	1.86 (0.04)ab	0.53 (0.03)d	0.32 (0.01)de	0.05 (0.01)c	0.19 (0.01)e	36 (0.94)f	2.95 (0.04)d
	L2	5.34 (0.04)bcd	1.51 (0.05)cd	2.62 (0.04)abc	0.37 (0.01)gh	3.56 (0.03)cde	1.72 (0.03b)cd	0.65 (0.02)cd	0.53 (0.02)bc	0.06 (0.00)bc	0.24 (0.01)de	46 (0.20)e	3.20 (0.04)cd
SWPB	L1	5.04 (0.13)cde	1.42 (0.02)e	2.47 (0.04)cde	0.40 (0.02)fg	3.34 (0.02)ef	1.84 (0.02a)bc	0.67 (0.03)bc	0.43 (0.01)cde	0.06 (0.01)bc	0.24 (0.02)de	43 (0.44)e	3.24 (0.06)abcd
	L2	5.25 (0.06)bcd	1.56(0.02)bcd	2.71 (0.03)abc	0.50 (0.02)e	3.53 (0.04)cde	1.65 (0.05)bcd	0.80(0.02)b	0.53 (0.02)bc	0.08(0.01)ab	0.36 (0.02)bcd	52 (0.48)c	3.42 (0.07)abcd
SWPL	L1	5.72 (0.03)ab	1.60 (0.02)b	2.78 (0.03)abc	0.51 (0.02)de	3.39 (0.05)de	1.65 (0.03bc)d	0.76 (0.01)ab	0.55 (0.03)bc	0.07 (0.01)ab	0.36 (0.01)bcd	51 (0.79)c	3.34 (0.01)abcd
	L2	6.06 (0.06)a	1.70 (0.02)a	2.97 (0.03)a	0.70 (0.02)b	3.76 (0.19)bc	1.33 (0.02)d	0.96 (0.07)a	0.77 (0.03)a	0.08 (0.02)ab	0.54 (0.02)a	63 (0.47)a	3.68 (0.00)a
SWPOL	L1	5.20 (0.16)bcd	1.38 (0.02)e	2.40 (0.04)de	0.35 (0.03)h	3.35 (0.03)ef	1.83 (0.01)abc	0.64 (0.03)cd	0.36 (0.01)cde	0.07 (0.01)ab	0.26 (0.03)de	42 (1.13)e	3.16(0.03)bcd
	L2	5.25 (0.03)bcd	1.58 (0.02)bc	2.74 (0.04)abc	0.44 (0.04)fg	3.53 (0.05)cde	1.66 (0.03)bcd	0.76 (0.03)ab	0.47 (0.01)bcd	0.09 (0.01)a	0.33 (0.00)cde	50 (0.05)cd	3.31 (0.05)abcd
T		**	**	**	**	**	**	**	**	**	**	**	**
L		**	**	**	**	**	**	**	**	**	**	**	**
T x L		**	**	**	**	**	ns	**	**	**	**	**	**

Note: Means (SD) with different letters are significantly different ($p<0.05$), ** significant, ns: not significant. Org C: Organic carbon, Org M: organic matter, Total N: Total Nitrogen, Avail P: Available phosphorus, EA: Exchangeable acidity, Ca: Calcium, Mg: Magnesium, K: Exchangeable potassium, %BS: Percent base saturation, ECEC: effective cation exchange capacity

The effective cation exchange capacity of the soil before the incorporation of organic manure was low (3.06 cmol/kg) but increased with the application of organic amendments. ECEC significantly increased from 2.93 to 3.68 cmol/kg, significantly different from the control. Applying SWPL was most effective in increasing the ECEC value of the soil (3.68 cmol/kg), followed by PL (3.37 cmol/kg). However, an increase in ECEC was more effective when soils were amended with the various organic sources at the (L_2) treatment level. Cation Exchange Capacity (CEC) encompasses quantifying the soil's capacity to retain positively charged ions on its particle surfaces, elucidated by Goldberg et al. (2020). It is well-established that the CEC of soil exhibits a positive correlation with elevated proportions of clay content and organic material. Empirical investigations have consistently indicated a discernible ascending pattern in CEC values concurrent with higher manure application rates. The observed trend can be attributed to organic matter within the applied manure and the concurrent increase in soil pH resulting from manure application (Magdoff and Amadon 1980). The ECEC values are also reflected in the base saturation of the soils. SWPL treatment at (L_2) showed the highest percent base saturation (63%), followed by PL (L_2) at 56% and SW (L_2) at 53%. The high percent base saturation SWPL (L_2) exhibited indicates its high concentration of basic cations needed for plant growth and development (Brady and Weil 2005).

Effect of organic amendments on maize growth

The effects of various organic amendments on maize growth were investigated, and the results are presented in Tables 3-6. Throughout the study, maize plant height significantly increased, reaching its peak at 8WAP, with a maximum height of 64.80 cm. Notably, all treatments applying organic amendments presented greater heights than the control. The organic amendment PL had the most pronounced impact on the vegetative growth of maize, resulting in the highest mean plant height. This was followed by SWPL and SW (Table 3.). The increase in plant height was found to be statistically significant ($p < 0.05$) with an elevation in treatment level. These findings align with the study conducted by Okoroafor et al. (2013), who also observed a similar trend in increased maize plant height as a result of single and combined application of poultry and swine manure. The higher plant height in PL, SW, and SWPL treatments may be attributed to its higher total N and avail P content than the other treatments, as supported by the study by Jjagwe et al. (2020).

The use of organic amendments led to an increase in the number of leaves, as indicated in Table 4. Nevertheless, not all organic amendments resulted in a noteworthy enhancement in number of leaves compared to the control group at 8 weeks after planting (8 WAP). Specifically, POL (L1) had the least impact on the number of leaves, while the application of SW (L2), SWPL (L2), and PL (L2) resulted in the highest leaf count.

Additionally, the leaf area of maize increased over time, peaking at 8 WAP (Table 5). The leaf area at this stage ranged from 127.23 cm² to 155.03 cm² for all treatment levels. These findings suggest that applying organic amendments at a higher rate positively contributes to an increase in the leaf area of maize. The stem girth of maize increased over time, reaching its highest mean value at 8 WAP. The mean stem girth ranged from 0.60 cm to 1.60 cm across all treatment and treatment levels (L_1, L_2) (Table 6). Similar to the observations in the leaf area, all treatments except POL and SWPOL exhibited greater girth than the control, and a pronounced effect on stem girth was observed in SW, PL, and SWPL treatment.

While plant height increased compared to the control group, the impact of organic amendments on other plant growth factors especially on the number of leaves, at the different stages of growth, was negligible, sometimes no different from the control. the reason for this can be attributed to slow release of nutrients from organic manures, unlike inorganic fertilizers that release nutrients quickly, organic amendments undergo slow mineralization, resulting in a gradual nutrient release. This observation corroborates with a similar finding reported by Kareem et al. (2017) and Rasool et al. (2023).

Table 3. Effect of organic amendments on plant height

Treatments		Plant Height (cm)			
		2WAP	4WAP	6WAP	8WAP
Control	L0	13.07±0.60i	20.00±0.10i	30.73±0.55f	43.87±0.41i
PB	L1	17.53±0.25fg	22.50±0.15h	37.63±0.32e	53.67±0.49de
	L2	18.73±0.25abc	24.70±0.43ef	37.96±0.15e	55.83±0.20cd
SW	L1	19.23±0.51ab	25.56±0.81cde	42.20±2.65c	56.03±1.20cd
	L2	19.70±0.30a	28.70±1.13a	43.06±1.00bc	50.33±1.20b
PL	L1	18.67±0.42abcd	25.20±0.98de	45.37±1.35a	50.50±0.87b
	L2	18.80±0.26abc	26.50±0.60bcd	46.26±0.86a	64.80±2.07a
POL	L1	16.13±0.20fg	23.90±0.85efg	39.60±0.79de	53.13±1.53def
	L2	17.77±0.25cde	25.20±0.98de	41.23±1.05cd	53.00±0.36def
SWPB	L1	17.00±1.00ef	23.36±0.49efg	38.90±1.11e	50.16±0.90fgh
	L2	17.36±0.35ef	23.20±0.98efg	42.33±1.10c	52.06±0.66efg
SWPL	L1	18.10±0.25bcde	27.03±2.15abc	41.37±0.96cd	54.13±1.00cde
	L2	19.73±0.25a	27.50±0.65ab	44.46±0.86ab	57.06±0.61bc
SWPOL	L1	17.10±0.26ef	24.57±0.66ef	41.53±1.56cd	53.23±0.75def
	L2	17.43±0.40de	25.37±0.71cde	41.20±0.00cd	54.13±0.40cde

Table 4. Effect of organic amendments on number of leaves

Treatments		Number of Leaves			
		2WAP	4WAP	6WAP	8WAP
Control	L0	5.00±1.00d	7.33±0.57d	11.33±0.57de	12±0.57d
PB	L1	6.00±0.00c	8.00±1.00cd	11.67±0.57de	13±1.00c
	L2	5.33±0.57d	7.67±0.57d	11.67±0.57de	13±0.57c
SW	L1	6.67±0.57bc	9.00±0.00ab	12.00±0.00cd	14±0.57b
	L2	6.33±1.15bc	8.67±0.57cd	12.67±0.57bc	15±0.00a
PL	L1	6.33±1.15bc	8.00±1.00cd	12.00±0.00cd	14±0.00bc
	L2	7.67±0.57a	10.33±0.57a	13.00±0.00b	15±0.00a
POL	L1	6.00±0.00c	8.67±1.15cd	11.00±0.00de	11±0.57e
	L2	5.67±0.57cd	8.00±0.00cd	11.00±1.00ef	12±0.57d
SWPB	L1	7.00±0.00ab	9.00±1.00ab	11.33±0.57de	12±0.00d
	L2	7.33±0.57ab	9.67±0.57ab	13.00±0.00b	13±0.00c
SWPL	L1	7.00±0.00abc	8.67±0.57cd	12.00±0.00cd	13±0.00c
	L2	7.67±0.57a	11.00±1.00a	15.33±0.57a	15±0.57a
SWPOL	L1	5.67±0.57cd	7.67±0.57d	11.00±0.00ef	12±0.00d
	L2	7.00±0.00ab	9.33±0.57ab	12.00±1.00cd	13±0.00c

Table 5. Effect of organic amendments on leaf area

Treatments		Leaf Area (m^2)			
		2WAP	4WAP	6WAP	8WAP
Control	L0	34.97±0.45i	50.90±0.36f	119.47±0.61gh	127.23±1.62i
PB	L1	39.43±0.81de	58.93±0.40de	135.43±0.61cdef	145.23±0.32ef
	L2	40.77±0.68c	60.03±0.95cd	133.53±0.45cdef	146.50±0.40def
SW	L1	42.77±0.49ab	64.03±1.99abc	140.83±1.33bcd	158.17±2.25bc
	L2	42.80±0.26ab	65.57±1.20ab	149.33±1.16ab	160.67±1.56b
PL	L1	41.77±0.25de	61.47±2.49abcd	141.73±1.60abcd	161.73±0.56b
	L2	41.83±0.20de	65.20±3.40ab	154.20±2.62a	169.93±0.15a
POL	L1	35.37±0.72hi	54.83±3.16ef	142.80±1.51abcd	149.97±0.37d
	L2	38.06±0.94ef	59.97±3.59cd	146.10±1.87abc	150.63±2.20d
SWPB	L1	36.20±0.36h	61.30±3.90abcd	122.37±1.07fgh	127.43±0.96i
	L2	38.96±1.00gh	61.40±3.64abcd	138.27±0.65bcde	143.40±0.78f
SWPL	L1	41.06±1.60de	64.00±1.67abc	141.47±1.89abcd	145.60±0.60ef
	L2	43.00±0.20a	67.10±1.51a	151.17±1.88ab	155.03±0.41c
SWPOL	L1	39.56±0.45de	62.43±4.76abcd	139.67±0.64bcde	144.17±0.55f
	L2	41.33±0.76de	63.60±3.92abcd	146.57±0.80abc	149.23±1.03de

Table 6. Effect of organic amendments on stem girth

Treatments		Stem Girth (cm)			
		2WAP	4WAP	6WAP	8WAP
Control	L0	0.60±0.05e	0.87±0.05f	1.26±0.05cde	1.36±0.05ef
PB	L1	0.70±0.00d	0.93±0.05e	1.33±0.11bcd	1.47±0.05cd
	L2	0.76±0.05cd	1.00±0.00d	1.36±0.05abc	1.56±0.05ab
SW	L1	0.83±0.00b	1.10±0.11bc	1.46±0.05ab	1.53±0.00abc
	L2	0.90±0.05ab	1.17±0.00abc	1.50±0.10a	1.60±0.05a
PL	L1	0.80±0.00bc	0.90±0.00e	1.30±0.00bcd	1.50±0.00bc
	L2	0.87±0.05b	1.10±0.00bc	1.50±0.00a	1.60±0.00a
POL	L1	0.76±0.00cd	1.00±0.05d	1.33±0.05bcd	1.30±0.00ef
	L2	0.80±0.05bc	1.06±0.00d	1.30±0.00bcd	1.30±0.00ef
SWPB	L1	0.70±0.00c	0.90±0.00e	1.20±0.00de	1.20±0.00g
	L2	0.90±0.00ab	1.10±0.00c	1.40±0.00abc	1.50±0.00bc
SWPL	L1	0.90±0.10ab	1.20±0.11b	1.40±0.00abc	1.50±0.10bc
	L2	0.97±0.05a	1.35±0.10a	1.50±0.00a	1.60±0.00a
SWPOL	L1	0.76±0.00bcd	1.00±0.00d	1.30±0.00bcd	1.30±0.00ef
	L2	0.80±0.05bc	1.10±0.00c	1.30±0.00bcd	1.30±0.00ef

The results demonstrated that all organic sources studied positively affected the soil chemical properties evaluated. Specifically, adding organic amendments increased various soil indices, including pH, Total N, organic matter, and carbon content. Notably, the application of manure layers and their combination with swine manure exhibited the most significant impact on soil chemical properties and maize growth parameters compared to other organic treatments. Moreover, applying these manures at varying quantities (10 to 20 t/ha) demonstrated promising effects on both soil chemical properties, soil fertility, and maize growth parameters. Consequently, based on these findings, employing a single application of poultry manure from layers and its combination with swine manure as an alternative fertilizer source is recommended to enhance soil fertility and promote optimal maize growth.

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Comprehensive characterization of Gerboui olive oil: Quality, composition and aromatic profile

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Abstract. *Manai-Djebali H, Nait Mohamed S, Madrigal-Martínez M, Martínez-Cañas MA, Sánchez-Casas J, Ben Youssef N. 2023. Comprehensive characterization of Gerboui olive oil: Quality, composition and aromatic profile. Asian J Agric 8: 10-17.* This study presents an in-depth analysis of Gerboui olive oil, a relatively lesser-known Tunisian variety. The investigation encompassed a thorough evaluation of key quality parameters, including acidity, peroxide value, ultraviolet absorbance, and fatty acid composition. The findings establish that Gerboui olive oil not only meets the stringent criteria set by the International Olive Oil Council for extra virgin olive oil but also exhibits remarkable qualities. It demonstrated a robust antioxidant capacity, with a DPPH (2,2-diphenyl-1-picrylhydrazyl) radical inhibition percentage of 94.93%. Furthermore, it boasts a substantial total phenolic content of 564.81 mg CA (caffeic acid)/kg and commendable oxidative stability, lasting 30.44 hours. The oil displayed exceptional resistance to oxidation, showcasing its potential as a premium product with potential health benefits. Additionally, the research delved into the fatty acid composition, highlighting the prevalence of unsaturated fatty acids, particularly oleic acid. The triacylglycerol profile revealed the presence of primary and secondary triacylglycerols, with notable triacylglycerols (TAGs) like Dilinoleoyl oleoyl glycerol (LLO) and Oleoyl linoleoyl oleoyl glycerol (OLO) found in substantial proportions at 5.04% and 17.84%, respectively. Trioleoyl glycerol (OOO) emerged as the most abundant TAG, constituting 30.07% of the TAG content, contributing to the oil's distinct characteristics. Furthermore, the analysis of volatile compounds unveiled a complex aroma profile, with (E)-2-hexenal (19.47%) and n-dodecane (15.66%) as the primary contributors. This comprehensive characterization enhances our understanding of Gerboui olive oil and underscores its potential as a unique and valuable olive oil variety.

Keywords: Antioxidant capacity, fatty acid composition, Gerboui olive oil, triacylglycerol profile, volatile compounds

Abbreviations: EVOO: Extra Virgin Olive Oil, FAs: Fatty Acids, TAGs: Triacylglycerols, LLL: Trilinolein, OOO: Triolein, PPP: Tripalmitin, SSS: Tristearin, LnLnLn: Trilinolenin, PoPoPo: Tripalmitolein, IOOC: International Olive Oil Council, DPPH: 2,2-diphenyl-1-picrylhydrazyl, CA: Caffeic Acid, OS: Oxidative Stability, SFA: Saturated Fatty Acids, UFA: Unsaturated Fatty Acids, MUFA: Monounsaturated Fatty Acids, PUFA: Polyunsaturated Fatty Acids, LLnLn: Dilinolenoyl linoleoyl glycerol, LLLn: Dilinoleoyl linolenoyl glycerol, OLLn: Oleoyl linoleoyl linolenoyl glycerol, LLL: Trilinoleoyl glycerol, PLLn: Palmitoyl linoleoyl linolenoyl glycerol, LLO: Dilinoleoyl oleoyl glycerol, OLnO: Dioleoyl linolenoyl glycerol, PLL: Dipalmitoyl linoleoyl glycerol, OLO: Dioleoyl linoleoyl glycerol, PLO+SLL: Palmitoyl linoleoyl oleoyl glycerol and Dilinoleoyl stearoyl glycerol, PPL: Dipalmitoyl linoleoyl glycerol, OOO: Trioleoyl glycerol, POO: Dioleoyl palmitoyl glycerol, PPO: Dipalmitoyl oleoyl glycerol, PPP: Tripalmitoyl glycerol, SOO: Dioleoyl stearoyl glycerol, SLS+POS: Distearoyl linoleoyl glycerol and Palmitoyl oleoyl stearoyl glycerol

INTRODUCTION

Renowned and savored worldwide for its role as a wholesome source of dietary fat, Extra Virgin Olive Oil (EVOO), renowned and savored worldwide for its role as a wholesome source of dietary fat, stands out as a culinary gem with profound global significance. Derived from freshly ripened olives, it holds a distinguished position as one of the cornerstone agricultural products in the Mediterranean region, a cradle of olive cultivation (Muzammil et al. 2021). The chemical composition of EVOO, this liquid gold of the culinary world, is intricately intertwined with a multitude of factors. These include the specific olive cultivar, the meticulous agronomic practices employed during cultivation, and the geographical locale in which these olive trees thrive (Hmida et al. 2022). Within the context of the Mediterranean diet, EVOO takes center stage as a dietary cornerstone celebrated not only for its exceptional taste but also for its well-documented health-promoting qualities (Jimenez-Lopez et al. 2020). Beyond its role on the plate, olive oil has found itself inextricably woven into the culinary heritage of many cultures, serving as an indispensable ingredient in a diverse array of traditional dishes. This dual role, both as a dietary staple and an essential culinary component, positions it as a vital commodity in the ever-evolving food industry (Fernández-Lobato et al. 2022). Moreover, the production and consumption of EVOO contribute significantly to the economic landscape of several countries, most notably Tunisia (Fernández-Uclés et al. 2020). Tunisia, renowned for its production of high-quality EVOO, has crafted a distinct reputation for its oil, characterized by a unique flavor profile and chemical composition. Additionally, the trend of producing monovarietal EVOOs, extracted from a single olive cultivar, is steadily gaining momentum in response to evolving culinary preferences and growing consumer demand for unique flavors (Hlima et al. 2017). Examining the composition of minor Tunisian olive oil holds considerable significance for a variety of compelling reasons. Firstly, it plays a pivotal role in the characterization and differentiation of diverse olive oil varieties, as underscored by Chtourou et al. (2021). This distinction is invaluable for both consumers and producers, serving as a safeguard to ensure the genuineness and excellence of the oil they encounter. By accurately identifying the unique chemical profiles of different monovarietal oils, consumers can confidently make informed choices while producers can maintain the integrity of their products. Secondly, the analysis of minor Tunisian olive oil composition contributes to the realm of nutrition studies, as highlighted by Manai-Djebali et al. (2012). The widely acknowledged health advantages of olive oil make it a subject of intense interest in the field of nutrition. By discerning the specific compositions of distinct olive oil varieties, researchers can delve deeper into the nutritional properties and potential health benefits associated with each, enhancing our understanding of how olive oil can positively impact human health. Furthermore, the knowledge surrounding the composition of minor Tunisian olive oil offers significant advantages in the realm of olive breeding projects, as indicated by Monasterio et al. (2013). For instance, understanding the precise sterol fraction of different olive varieties equips breeders with the tools to identify ideal parent trees for the cultivation of new olive cultivars. This selective breeding process can yield offspring with improved characteristics, both in terms of oil quality and other desirable traits, thereby advancing the olive industry and enriching the range of olive oil products available.

The aim of this research is to analyze and describe the chemical composition of Gerboui olive oils from Testour, located in the northern region of Tunisia. Through the analysis of olive oils from this specific variety, the study places its focus on critical chemical parameters, encompassing aspects such as free acidity, peroxide values, overall phenolic content, levels of chlorophylls and carotenoids, as well as tocopherols. Additionally, this investigation assesses the antioxidant potential of these olive oils, emphasizing the varying capabilities of both polar and lipid fractions in neutralizing DPPH radicals, while also scrutinizing the fatty acid and triacylglycerol profiles. The outcomes of this study offer valuable insights into the quality and nutritional properties of Gerboui olive oil, making significant contributions to the fields of scientific research and the olive oil industry.

MATERIALS AND METHODS

Olive oil and chemicals

The Gerboui olive oil was harvested from Testour, Béja, Tunisia (36° 32' 54.228'' N, 9° 25' 29.781'' E) (Figure 1).

Reagents and standards for spectrophotometric and titration analyses were sourced from Fluka (Buchs, Switzerland), Sigma-Aldrich (Saint Louis, MO, USA), and Merck (Darmstadt, Germany). Triacylglycerol standards, including trilinolein (LLL), triolein (OOO), tripalmitin (PPP), tristearin (SSS), trilinolenin (LnLnLn), and tripalmitolein (PoPoPo), each with a purity exceeding 98%, were obtained from Sigma-Aldrich (Saint Louis, MO, USA). A Fatty Acid Methyl Ester (F.A.M.E.) mix (CRM18918) was provided by Supelco (Bellefonte, PA, USA). α, β, and γ-tocopherol were acquired from Fluka (Buchs, Switzerland). All solvents used for chromatographic techniques adhered to high-performance liquid chromatography (HPLC) standards. Specifically, n-Hexane, diethyl ether, acetone, and acetonitrile were supplied by Panreac (Barcelona, Spain).

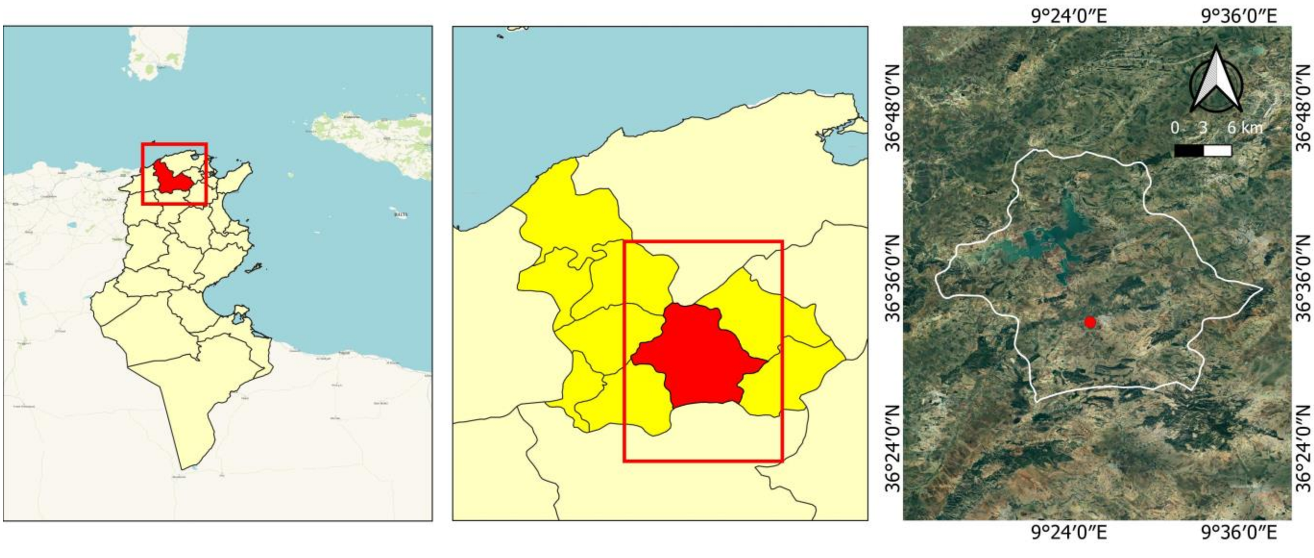

Figure 1. The Gerboui olive harvesting region: Testour, Béja, Tunisia

Sampling of Gerboui olive oil

For this study, we utilized three batches of extra virgin olive oils, each totaling 1000 mL, all derived from the Gerboui olive variety. These olive oils were sourced from a carefully selected farm located in Testour, within the Béja region of Tunisia. The Gerboui olives were harvested when they had reached their optimal ripeness stage, and the farm operators meticulously adhered to proper grove management and oil processing procedures. The extraction of the olive oils was carried out using a state-of-the-art continuous technological plant equipped with a three-phase centrifugal system. The freshly extracted oils were then stored in containers at a temperature of 4°C, shielded from light, until they were ready for analysis.

Determination of basic indicators of Gerboui olive oil quality

In this study, The free acidity was determined by dissolving 2 g of olive oil in a neutralized solvent mix, titrating it with a 0.1 M ethanolic solution of potassium hydroxide in the presence of phenolphthalein, and calculating the free acidity as a percentage of oleic acid by weight (IOOC 2017b). The peroxide value was determined by dissolving 1 g of olive oil in a mixture of chloroform, glacial acetic acid, and potassium iodide, followed by titration with a solution of sodium thiosulfate, expressed in meq O_2/kg of oil (IOOC 2017c). Specific extinction coefficients (K232 and K270) were determined by preparing a 1% oil solution in cyclohexane and measuring absorbances at 232 and 270 nm, and changes in specific extinction coefficients (ΔK) were calculated based on absorbance at wavelengths 266 and 274 nm (IOOC 2019).

Head space sampling

According to Flamini (2007), a 2g sample of olive oil was placed in a sealed 10 mL vial and allowed to equilibrate for 30 minutes. Subsequently, a solid-phase microextraction fiber (PDMS, 100µm, Supelco, Bellefonte, PA, USA) was exposed to the headspace for 50 minutes. The fiber was then retracted into a needle and transferred for analysis using a gas chromatography-mass spectrometry (GC-MS) system.

Volatile compound analysis by gaz chromatography

Gas chromatography analysis was performed using a Varian CP 3800 gas chromatograph equipped with a DB-5 Capillary column and a Varian Saturn 2000 ion trap mass detector. The injector was maintained at 250°C, the transfer line at 220°C, and the oven temperature gradually increased from 60 to 240°C at a rate of 3°C per minute. Helium served as the carrier gas at a flow rate of 1 mL/min. A splitless injection method was employed. The chemical constituents were identified by comparing retention times with authentic reference samples and using computer-assisted matching against the NIST 98 commercial library.

Oxidative stability of oils

According to Manaï et al. (2007), the assessment of oxidative stability in oil was conducted using a Rancimat 743 Metrohm apparatus (Herisau, Switzerland). This method is centered on the determination of the induction time, which signifies the duration required for the oil to undergo a specified level of oxidation, as indicated by a noticeable increase in the oil's electrical conductivity. The procedure involved heating the oils to 110°C and exposing them to a continuous flow of air. The electrical conductivity of the oil was systematically monitored over time, and the induction time was established as the moment when the conductivity exhibited a 2 mS/cm increase.

Antioxidant activity

The assessment of the oil samples' capacity to scavenge the DPPH radical was conducted in accordance with the procedure outlined by Kalantzakis et al. (2006). This involved the addition of 250 µL of an oil solution in ethyl acetate (10% weight/volume) to 1 mL of a freshly prepared DPPH solution (10 mM in ethyl acetate) within a 2 mL test tube. The mixture was vigorously shaken for 10 seconds using a Vortex apparatus and left in the dark for 30 minutes until it reached a stable state. Subsequently, the absorbance of the mixture was measured at 515 nm using a Cary 60 UV–vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) and compared to a blank solution devoid of the radical.

Evaluation of total phenolic content of the extra virgin olive oils

The total phenolic content of the olive oil was assessed using colorimetric analysis and the Folin-Ciocalteu reagent, following the protocols described by Psomiadou and Tsimidou (2002). This analysis focused on the polar oil fraction (2.5 g of each oil in 5 mL of n-hexane and then extracting it with a mixture of 5 mL of methanol and water (60:40, *v/v*)). To perform the assessment, a 20 µL aliquot of the appropriately diluted fraction was combined with 50 µL of distilled water and 20 µL of the Folin-Ciocalteu reagent in a tube. After thorough mixing and a 3-minute resting period, 125 µL of a 7% Na_2CO_3 solution was introduced, and the mixture was adjusted with an additional 100 µL of distilled water. The tube was then allowed to stand at room temperature in the dark for 90 minutes, following which the absorbance was measured at 760 nm. The results were expressed as the equivalent of caffeic acid (mg CA/kg of oil), and three replicates were performed for each sample.

Determination of chlorophyll and carotenoid contents

The determination of carotenoid and chlorophyll contents in the Extra Virgin Olive Oils (EVOOs) was carried out using a spectrophotometric method, following the methodology of Minguez-Mosquera et al. (1991). This involved measuring the absorbance at a wavelength of 470 nm for carotenoids and 670 nm for chlorophylls, respectively. Specific extinction coefficients were applied, with values of 613 L/(g·cm) for pheophytin A as the primary chlorophyll in EVOO and 2000 L/(g·cm) for lutein as the primary carotenoid. The pigment contents were calculated by dividing the absorbance at the specified wavelengths by the reference compound's extinction coefficients, the spectrophotometer cell thickness (1 cm), and a factor of 100. The results were expressed in milligrams of pheophytin A for chlorophylls and lutein for carotenoids per kilogram of oil. Three replicates were conducted for each sample.

Determination of tocopherol composition

The assessment of tocopherol composition was conducted in accordance with the method established by Manai-Djebali et al. (2012). This analytical procedure involved initially dissolving the oil sample in n-hexane. Subsequently, the solution was subjected to analysis using an Agilent 1200 High-Performance Liquid Chromatography (HPLC) system, featuring a silica gel Lichrosorb Si-60 column with a particle size of 5 µm. The column had dimensions of 25 cm in length and 4 mm in inner diameter and was provided by Agilent Technologies, based in Santa Clara, CA, USA. Elution was achieved by employing a solvent mixture of n-hexane and 2-propanol (99:1, *v/v*), while maintaining the flow rate was set at 1 mL/min. Detection was performed utilizing a fluorescence detector, with excitation and emission wavelengths configured at 290 and 330 nm, respectively. Individual tocopherols were quantified and expressed in milligrams per kilogram (mg/kg) of the oil. The identification process relied on comparing retention times for α-, β-, and γ-tocopherols. Quantitative analysis utilized a calibration curve for these three tocopherols, resulting in coefficients of determination (R^2) of 0.948, 0.975, and 0.996 for α-, β-, and γ-tocopherols, respectively. Each sample was subjected to three replicate analyses.

Determination of fatty acid composition of the EVOOs

The determination of the fatty acid composition of the olive oils involved the preparation of fatty acids methyl esters (FAs). This was achieved by vigorously shaking the oils in n-hexane along with 0.2 mL of a 2 M methanolic potassium hydroxide solution, following the procedure outlined by (IOOC 2017a). The determination of FAs was carried out using an Agilent 6890N gas chromatograph (Agilent Technologies, Wilmington, DE, USA) equipped with a Flame Ion Detector (FID). It featured an HP-1 (polydimethylsiloxane) fused-silica capillary column with a length (L) of 50 m, an Inner Diameter (I.D.) of 0.2 mm, and a film thickness of 0.33 mm. Helium was employed as the carrier gas, flowing at a constant rate of 1 mL/min. The oven temperature was programmed to rise from 60 to 250°C at a rate of 2°C/min, followed by an isothermal hold for 20 minutes. The FID temperature was set at 250°C. The identification of fatty acids was carried out by comparing the specific retention time of each compound with those from a Fatty Acid Methyl Ester standard (F.A.M.E. Mix, CRM18918-Supelco, Bellefonte, PA, USA). Three replicates were conducted for each sample, and the results were expressed in terms of area percentages.

Determination of triacylglycerol composition

The analysis of triacylglycerols (TAGs) was conducted by initially dissolving 0.12 g of olive oil in 0.5 mL of n-hexane. Subsequently, the triacylglycerol fraction was purified using Solid-Phase Extraction (SPE) with a Silica column and a mixture of n-hexane and diethyl ether (87:13, *v/v*). After purification, the triacylglycerols were reconstituted in 2 mL of acetone and subjected to analysis using an Agilent 1200 HPLC Series system (Agilent Technology, Palo Alto, CA, USA). The HPLC system was equipped with a refractometric detector and utilized a LiChrospher RP-18 column with a particle size of 5 µm, featuring dimensions of 25 cm in length and 4.6 mm in Inner Diameter (I.D). The elution solvent consisted of a mixture of acetone and acetonitrile (50:50, *v/v*), with a flow rate set at 1.2 mL/min. Standards for triacylglycerols, including LLL, OOO, PPP, SSS, LnLnLn, and PoPoPo, were used (Sigma-Aldrich, St Louis, MO, USA). To identify the TAGs, retention times were compared with reference chromatograms obtained from soybean oil, a 30:70 (m/m) mixture of soybean oil and olive oil, and pure olive oil, following the methodology outlined in (IOOC 2017d). It was assumed that the total area of peaks representing different TAGs summed up to 100%, and the proportional distribution of each TAG was subsequently calculated.

Data analysis

The data analysis was performed using JMP 14 software (© SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Phytochemical characteristics of Gerboui olive oil

Table 1 provides a comprehensive overview of the phytochemical characteristics of the analyzed olive oil sample, providing critical insights into its quality and composition. Notably, the oil exhibits low free acidity (0.58±0.07%), indicating a high-quality product. The peroxide value (11.5 meq O_2/kg) suggests that the oil is relatively stable concerning oxidation. Furthermore, the K232 (2.37) and K270 (0.201) values further indicate the oil's freshness and potential for degradation. A low ΔK (0.005) signifies stable oil composition. Remarkably, the oil demonstrates a strong antioxidant capability, as evidenced by a significant 94.93% inhibition of DPPH radicals. Furthermore, it presents a significant total phenolic content, measuring at 564.81 mg of caffeic acid equivalents per kilogram of oil (mg CA/kg). Lastly, the oil demonstrates commendable oxidative stability, with a substantial duration of 30.44 hours.

Table 1. Phytochemical characteristics of Gerboui extra virgin olive oils from Testour Region in Tunisia

Phytochemical Characterisation	Values
Free acidity (% oleic acid (w/w))	0.58±0.07
Peroxide value (meq O2/kg)	11.5±1.34
K232	2.37±0.01
K270	0.201±0.002
ΔK	0.005±0.0004
DPPH$^\bullet$ Inhibition (%)	94.93±0.46
Total phenolic content (mg CA/kg)	564.81±39.44
Oxidative stability (h)	30.44±0.14

Note: Data are expressed as average of a triplicates ± standard deviation

Fatty acid composition in Gerboui extra virgin olive oil

Table 2 provides a comprehensive overview of the fatty acid composition in Gerboui extra virgin olive oil originating from the Testour Region in Tunisia. This breakdown is crucial for gaining insights into the nutritional and sensory attributes of the olive oil. Notably, the oil is predominantly composed of high presence of oleic acid (C18:1), constituting a substantial 66.88% of the total fatty acids. This high presence of oleic acid is associated with the oil's excellent sensory qualities and potential health benefits. Furthermore, linoleic acid (C18:2), an essential polyunsaturated fatty acid, contributes 14.91% to the composition, enhancing the oil's nutritional value. The balance between saturated and unsaturated fatty acids is reflected in the sum of saturated (∑SFA) and unsaturated (∑UFA) fatty acids, comprising 16.7% and 83.3%, respectively. The ratio of oleic acid (C18:1) to linoleic acid (C18:2) (C18:1/C18:2) is 4.49, providing insights into the oil's fatty acid profile.

Phytochemical composition of the Gerboui extra virgin olive oil from the Testour Region in Tunisia

The data presented in Table 3 offers valuable insights into the phytochemical composition of the Gerboui extra virgin olive oil from the Testour Region in Tunisia. Notably, Table 3 shows a significant presence of α-tocopherol, with a content of 350 mg/kg, which are renowned for their potent antioxidant properties. This high α-tocopherol content enhances the oil's stability and potential health benefits. Furthermore, the presence of β-tocopherols and γ-tocopherols, at 7.42 mg/kg and 13.36 mg/kg, respectively, contributes to the oil's overall antioxidant capacity. The total tocopherol content of 370.86 mg/kg represents the combined antioxidant potential of these compounds. In addition to tocopherols, the oil contains chlorophylls (6.05 mg/kg) and carotenoids (2.71 mg/kg), which not only influence the oil's color but also add to its antioxidant profile.

Triacylglycerol (TAG) compositions in Gerboui olive oil from the Testour Region in Tunisia

The Table 4 presents a comprehensive overview of the triacylglycerol (TAG) compositions in Gerboui olive oil from the Testour Region in Tunisia. TAGs are a vital component of olive oil, influencing both its physical properties and potential health benefits. The table highlights the diversity of TAGs within this oil, with varying concentrations of different TAG species. Notable TAGs include Dilinoleoyl oleoyl glycerol (LLO) and Dioleoyl linoleoyl glycerol (OLO), which are present in substantial proportions at 5.04% and 17.84%, respectively. Trioleoyl glycerol (OOO) is the most abundant TAG, accounting for 30.07% of the TAG content. On the other hand, several TAGs, such as Dilinolenoyl linoleoyl glycerol (LLnLn) and Dilinoleoyl linolenoyl glycerol (LLLn), are present in comparatively minor amounts.

Table 2. Fatty acids composition (% total fatty acids) of the Gerboui extra virgin olive oils from Testour Region in Tunisia

Fatty Acids	% Total Fatty Acids
Palmitic acid (C16:0)	12.53±0.02
Palmitoleic acid (C16:1)	0.39±0.01
Stearic acid (C18:0)	3.65±0.08
Oleic acid (C18:1)	66.88±0.06
Linoleic acid (C18:2)	14.91±0.02
Alpha-linolenic acid (C18:3)	0.78±0.01
Arachidic acid (C20:0)	0.52±0.01
Gadoleic acid (C20:1)	0.35±0.01
Sum of Saturated Fatty Acids (∑SFA)	16.7±0.09
Sum of Unsaturated Fatty Acids (∑UFA)	83.3±0.09
Sum of Monounsaturated Fatty Acids (∑MUFA)	67.61±0.06
Sum of Polyunsaturated Fatty Acids (∑PUFA)	15.69±0.03
C18:1/C18:2	4.49±0.01

Note: Data are expressed as average of a triplicates ± standard deviation

Table 3. Phytochemical composition of the Gerboui extra virgin olive oil from the Testour Region in Tunisia

Phytochemicals	Contents (mg/kg)
α-Tocopherols	350.08± 1.52
β-Tocopherols	7.42±0.21
γ-Tocopherols	13.36±0.41
Total Tocopherols	370.86±2.15
Chlorophylls	6.05±0.23
Carotenoids	2.71±0.10

Note: Data are expressed as average of a triplicates ± standard deviation

Table 4. The triacylglycerol composition (% total triacylglycerols) of the Gerboui extra virgin olive oil from the Testour Region in Tunisia

Triacylglycerols	% total Triacylglycerols
Dilinolenoyl linoleoyl glycerol (LLnLn)	0.10±00
Dilinoleoyl linolenoyl glycerol (LLLn)	0.08±00
Oleoyl linoleoyl linolenoyl glycerol (OLLn)	0.37±00
Trilinoleoyl glycerol (LLL)	0.40±0.01
Palmitoyl linoleoyl linolenoyl glycerol (PLLn)	0.13±00
Dilinoleoyl oleoyl glycerol (LLO)	5.04±0.01
Dioleoyl linolenoyl glycerol (OLnO)	2.58±0.02
Dilinoleoyl palmitoyl glycerol (PLL)	0.85±00
Dioleoyl linoleoyl glycerol (OLO)	17.84±0.05
Palmitoyl linoleoyl oleoyl glycerol and Dilinoleoyl stearoyl glycerol (PLO+SLL)	10.09±0.02
Dipalmitoyl linoleoyl glycerol (PPL)	1.41±0.01
Trioleoyl glycerol (OOO)	30.07±0.09
Dioleoyl palmitoyl glycerol (POO)	21.46±0.04
Dipalmitoyl oleoyl glycerol (PPO)	3.84±00
Tripalmitoyl glycerol (PPP)	0.41±0.01
Dioleoyl stearoyl glycerol (SOO)	4.22±0.13
Distearoyl linoleoyl glycerol and palmitoyl oleoyl stearoyl glycerol (SLS+POS)	1.11±0.14

Note: Data are expressed as average of a triplicates ± standard deviation

Table 5. Volatile compounds of of the Gerboui extra virgin olive oil from the Testour Region in Tunisia

Volatile Compounds	Area %
(E)-2-penten-1-ol	0.52±0.03
(Z)-3-hexenal	6.24±0.31
(E)-2-hexenal	19.47±0.97
(Z)-3-hexen-1-ol	4.85±0.24
Heptanal	0.23±0.01
1-hexanol	3.42±0.17
(E.E)-2.4-Hexadienal	1.33±0.07
3-ethyl-1,5-octadiene*	1.79±0.09
3-ethyl-1,5-octadiene*	1.96±0.1
(Z)-2-Heptenal	0.61±0.03
3,7-decadiene	6.73±0.34
1-hexyl acetate	4.53±0.23
Limonene	1.33±0.07
(E)-β-ocimene	3.11±0.16
4,8-dimethyl-1,3,7-nonatriene	1.52±0.08
n-dodecane	15.66±0.78
Nonanal	0.66±0.03
(E)-2-dodecene	3.26±0.16
(E)-2-decenal	1.67±0.08
α-copaene	2.15±0.11
n-tetradecane	5.95±0.3
(E,E)-α-farnesene	4.03±0.2
Monoterpene hydrocarbons	3.11±0.16
Sesquiterpene hydrocarbons	6.18±0.31
Non-terpene derivatives	81.73±4.09
Total identified	91.02±4.55

Note: Data are expressed as average of a triplicates ± standard deviation, *: isomers

Volatile compounds of the Gerboui extra virgin olive oil

The volatile compound composition, as detailed in Table 5, reveals the intricate aroma profile of Gerboui extra virgin olive oil originating from the Testour Region in Tunisia. Among the most prominent constituents is (E)-2-hexenal, which contributes a significant 19.47% to the overall aroma. Another substantial component is n-dodecane, constituting 15.66%. Notable aromatic contributors also include 3,7-decadiene (6.73%), (Z)-3-hexenal (6.24%), and 1-hexyl acetate (4.53%). These compounds are known for their contributions to the fruity, green, and slightly spicy notes that are often associated with high-quality olive oils. Moreover, the presence of monoterpene hydrocarbons (3.11%) and sesquiterpene hydrocarbons (6.18%) adds complexity and depth to the aroma, while the non-terpene derivatives (81.73%) play a crucial role in providing a rich, well-rounded bouquet to the oil.

Discussion

Characterizing minor olive varieties is essential for several reasons. Firstly, it allows for the recovery and preservation of neglected germplasm, which may have unique genetic traits and contribute to the overall genetic diversity of olive species (Debbabi et al. 2020). Secondly, the characterization of minor compounds in olive oil, such as phenolic and triterpenic compounds, tocopherols, sterols, and free fatty acids, can help in identifying varietal markers and distinguishing the origin of olive oil samples (Olmo-García et al. 2019). This information is valuable for quality control, authentication, and traceability in the olive oil industry. Additionally, the characterization of minor components can contribute to the valorization of typical olive oil productions and aid in optimizing processing techniques (Amiri-Nowdijeh et al. 2018). Finally, the study of minor olive varieties can uncover their nutraceutical potential and identify varieties that may be superior in terms of their oil composition and active substances (Ilyasoglu et al. 2010).

The assessment of Gerboui olive oil, a lesser-known Tunisian variety, was conducted with respect to several critical quality markers, and the findings demonstrated that it complies with the criteria established for this category of olive oils. All the measured parameters conformed to the defined standards for "extra virgin olive oil" as outlined by the International Olive Oil Council (IOOC 2021). These standards include an acidity level of 0.8% or less, a peroxide value not exceeding 20 meq O_2/kg, K270 below 0.22, K232 under 2.5, and ΔK less than or equal to 0.01. Notably, lower values of these parameters typically signify that the olives used were fresh and in good condition, harvested at the ideal stage of ripeness, and promptly processed without prolonged storage. Our study's outcomes are in line with prior investigations on Gerboui olive oil in Tunisia (Hannachi et al. 2006), reinforcing the robustness of our results and contributing valuable insights to the existing knowledge base regarding Gerboui olive oil in Tunisia.

Impressively, the oil demonstrates a robust antioxidant capacity, with a DPPH radical inhibition percentage of 94.93%. Additionally, it boasts a substantial total phenolic content (564.81 mg CA/kg), which contributes to both its quality and potential health benefits. These results are consistent with several prior studies on Tunisian olive oil varieties (Manai-Djebali et al. 2017). Our findings align with the research conducted by Manai-Djebali et al. (2012), which focused on minor Tunisian olive varieties. In their study, they observed a similar range of chlorophyll content, varying from 6.22 to 1.15 mg/kg, and carotenoid content ranging from 3.82 to 1.07 mg/kg in these olive oils. Oxidative stability serves as a crucial parameter in the assessment of the quality of oils and fats. It provides a reliable estimate of their vulnerability to oxidative degradation, which is the primary cause of their deterioration (Carrasco-Pancorbo et al. 2005). Gerboui olive oil exhibits commendable oxidative stability (30.44 hours), a key factor for shelf life and resistance to spoilage. These findings collectively highlight the exceptional quality and promising attributes of the olive oil under examination, making it a noteworthy choice for culinary and health-conscious consumers. After an in-depth literature review, it is worth noting that this is the first report describing the characteristics of Gerboui olive oil.

As proposed by multiple authors, the tocopherol fraction in virgin olive oils is primarily composed of alpha-tocopherols, which exhibit both vitamin and antioxidant properties. Table 3 demonstrates a significant presence of alpha-tocopherol, with a content of 350 mg/kg. These findings align with previous research, emphasizing that tocopherol content exhibits substantial variability depending on the olive oil variety, especially in the case of minor Tunisian olive oils (Manai-Djebali et al. 2012).

Chlorophylls and carotenoids are the primary pigments found in vegetable oils (Yao et al. 2022). In olive oils, lutein and pheophytin are the predominant carotenoids and chlorophylls, respectively. It's important to note that both chlorophylls and carotenoids play roles in autoxidation and photooxidation processes (Borello and Domenici 2019). Our findings are consistent with the research conducted by Manai-Djebali et al. (2012), which focused on minor Tunisian olive varieties. In that study, they observed a similar range of chlorophyll content, varying from 6.22 to 1.15 mg/kg, and carotenoid content ranging from 3.82 to 1.07 mg/kg in these olive oils. This alignment between our results and their findings underscores the stability and reliability of the measurements and highlights the characteristic pigment profiles of minor Tunisian olive varieties, including Gerboui.

Fatty acids play a crucial role in human nutrition and exert diverse effects on health. They can be categorized into Saturated Fatty Acids (SFAs) and Unsaturated Fatty Acids (UFAs), which encompass Monounsaturated Fatty Acids (MUFAs) and polyunsaturated fatty acids (PUFAs). Existing research has demonstrated that SFAs tend to have adverse health implications, including an elevated cardiovascular disease risk. In contrast, UFAs have been associated with positive health outcomes, such as a reduced risk of cardiovascular disease, as highlighted by Islam et al. (2019). Additionally, the ratio of MUFAs to PUFAs is often regarded as an indicator of the overall fatty acid composition quality, as indicated by Chen and Liu (2020). Our findings align with prior investigations conducted by Ben-Temime et al. (2006), Youssef et al. (2010), Youssef et al. (2012), and Yahia et al. (2012).

Triacylglycerols (TAGs) are the predominant components found in olive oil (Torres-Cobos et al. 2023). These compounds comprise a glycerol molecule bonded to three fatty acid chains and play a pivotal role in determining various physical and chemical characteristics of the oil, including viscosity, stability, and oxidative characteristics, as highlighted in the study by (Sánchez and Harwood 2002). The analysis presented in Table 4 reveals a distinctive triacylglycerol profile in the olive oil under examination. Among these, three primary triacylglycerols, namely OOO (comprising three oleic acid chains), POO (with palmitic and oleic acid chains), and OLO (containing oleic and linoleic acid chains), dominate the composition. In addition to these primary triacylglycerols, the oils exhibit seven secondary ones, including PLO+SLL, LLO, SOO, PPO, OLnO, nPPL, and SLS+POS. It's noteworthy that these triacylglycerols are fundamental in shaping the oil's characteristics and properties. Furthermore, traces (less than 1%) of OLLn, PLLn, PLL, LLL, LLnLn, and PPP are also discernible across all the samples, contributing to the overall complexity of the oils' triacylglycerol composition. Our results are consistent with previous studies conducted by Manai-Djebali et al. (2012).

The intricate aroma profile of Gerboui extra virgin olive oil from the Testour Region in Tunisia, as revealed by the volatile compound composition in Table 5, showcases a diverse array of aromatic constituents. At the forefront of this aromatic symphony is (E)-2-hexenal, making a substantial contribution of 19.47% to the overall aroma. This compound is known for its characteristic fruity and green notes, adding a fresh and vibrant quality to the oil. Another noteworthy participant in the aroma is n-dodecane, which accounts for 15.66% of the composition, contributing a distinctive element to the oil's overall fragrance (Wang et al. 2023). In addition to these key players, several other compounds significantly influence the aromatic profile. Notable among them is 3,7-decadiene (6.73%), recognized for its role in enhancing the green and slightly spicy notes often associated with premium-quality olive oils. (Z)-3-hexenal (6.24%) is another essential component contributing to the oil's aroma, with a characteristic green and grassy scent (Manai-Djebali et al. 2023). The presence of 1-hexyl acetate (4.53%) introduces complexity to the aroma, imparting fruity and sweet undertones. Moreover, the inclusion of monoterpene hydrocarbons (3.11%) and sesquiterpene hydrocarbons (6.18%) adds depth and richness to the aromatic profile. These compounds can introduce floral, herbal, or woody notes, further enhancing the sensory experience. The majority of the aroma (81.73%) is attributed to non-terpene derivatives, which play a crucial role in providing a well-rounded and harmonious bouquet to the oil. This diverse group of compounds contributes to the overall complexity and balance of the oil's aroma, making it an appealing and sensory-rich experience for consumers (Manai-Djebali et al. 2023).

In conclusion, our study on Gerboui olive oil, a lesser-known Tunisian olive variety, demonstrates that this oil meets the stringent standards for "extra virgin olive oil" of the IOOC. It exhibits an exceptional antioxidant capacity with a DPPH radical inhibition percentage and a substantial total phenolic content. Furthermore, it boasts commendable oxidative stability. The fatty acid composition aligns with previous research on Tunisian olive oils, indicating its favorable nutritional profile. The distinctive triacylglycerol profile contributes to its unique properties, while the complex volatile compounds in the aroma enhance its sensory appeal. Our study adds valuable insights to the understanding of Gerboui olive oil and its potential as a high-quality olive oil option with promising health benefits and a unique sensory experience.

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Growth and yield potential of sludge-based organic fertilizers on bell pepper *Capsicum annum*

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Abstract. *Balkrishna A, Gautam AK, Sharma N, Arya V, Khelwade V. 2024. Growth and yield potential of sludge-based organic fertilizers on bell pepper* Capsicum annum. *Asian J Agric 8: 18-24.* This study investigated the effects of different organic fertilizers on bell pepper's growth and yield potential (*Capsicum annum* L.). A total of six fertilizer treatments and one control in three replications were analyzed using a Randomized Complete Block Design (RCBD). The effects of fertilizer application on plant parameters were evaluated on plant height, total number of leaves, leaf area, total number of branches, number of flowers, number of fruits, average fruit weight, average fruit diameter, total yield per plot, and total yield at every 30 days after sowing. All organic fertilizers positively impacted the growth and yield of the *Capsicum* crop. The experimental results revealed that treatment T1 (Jaivik Prom), T4 (Jaivik Poshak), and T6 (Jaivik Prom+ Jaivik Khad) displayed significant performance in all parameters, where T5 (Jaivik Khad) performed well in improving leaf area, fruit weight and total yield per plot. In addition, other treatments also exhibited a notable effect on different plant parameters. Based on these results, it was found that all organic fertilizers can potentially improve the growth and yield of *Capsicum*. From this, it was concluded that the sewage sludge processing into organic fertilizers highlighted the safer and environmentally friendly way of managing Ganga sludge. Further research should be conducted on different aspects of sludge-based organic fertilizers like processing, production and field application on more field crops.

Keywords: Agriculture, *Capsicum*, growth parameters, organic fertilizers, sludge management, yield

INTRODUCTION

The production of wastes from agriculture, food industries, and city compost is a big problem that represents an environmental and health challenge. These challenges are generated due to excess waste production; the non-availability of any eco-friendly permanent solution also contributed to raising this problem. The waste generated from food industries and city compost is generally called sewage. It produces Sewage Sludge (SS) as a by-product upon going through various treatment methods, including incineration, sanitary burial and composting. Besides treatment, sewage sludge management is still a big deal in economic and environment-friendly ways. However, one of its management solutions is hidden in its composition, which contains high Organic Carbon (OC), Nitrogen (N), and Phosphorous (P) content. The conversion of sewage sludge into organic fertilizers and their use in agriculture is the perfect way of management (Tchounwou et al. 2012; Camargo et al. 2016; Velasco-Munoz et al. 2021).

Organic fertilizers are now becoming much more popular due to the increased interest in organic farming all around the globe. Several synthetic fertilizers have come into existence that are being used in the agriculture system. These chemical fertilizers were mainly introduced during the implementation of various Green Revolution initiatives and are still in use. The long-term excess uses of these fertilizers have spoiled the soil structure and fertility (Jote 2023), and farmers are now moving towards organic farming using natural and organic fertilizers and pesticides. There is an increasing interest in organic farming as a trail to compensate for decreased soil fertility. Organic fertilizers not only help in improving soil fertility but also aid in achieving sustainable agriculture without compromising the soil health and its essential structure. Adding organic fertilizers also improves biological activity, soil exchange capacity, water retention capacity, and soil structure (Bastida et al. 2008; Hargreaves et al. 2008). Their application can gradually preserve the nutritional balance necessary for crop plants to thrive by releasing nutrients into the soil solution. In addition, they also serve as a productive source of energy for soil microorganisms, which enhance soil structure and crop development (Graham et al. 2017).

Organic fertilizers cannot only be considered free from harmful chemicals but their quality was also found superior to inorganic produce. Some studies depicted those vegetables grown in organic fertilization treatments performed better and yielded higher with significantly higher concentrations of sugars and vitamin C than chemical-fertilized vegetables. A significant enhancement in the yield of leafy vegetables was observed when the leaf-picking harvest was adopted to extend the growth and harvest period (Xu et al. 2003; Mohamad et al. 2022). Organic farming has been described as the best practice with long-term potential to improve and sustain soil quality and productivity (Ahmad et al. 2013; Shaheen et al. 2014). Several organic products are now available in the market, contributing to achieving the mission of organic farming worldwide, including in India. Due to the increasing concern about sludge management, Patanjali Organic Research Institute (PORI) has initiated a pilot project for sludge management by producing organic fertilizers and their application in agriculture.

Capsicum is a genus of flowering plants in the nightshade family Solanaceae. It has many subspecies, varieties, and cultivars and is one of the oldest cultivated crops. This genus is important in agricultural production because it contains both pungent (hot pepper) and non-pungent (sweet pepper) species. Among sweet peppers, bell pepper (*Capsicum annum* L.) is the most commonly known cultivar produced mainly in greenhouse conditions and used mostly as a vegetable (Tripodi and Kumar 2019). Like other field and vegetable crops, nutrition is important in increasing the yield of bell peppers and the availability of nutrients in the soil plays an important role in fulfilling the plant's nutritional requirements (Hapsoh et al. 2019). The fruits of bell peppers are rich in vitamin C and a good source of vitamin A and fiber. Their antioxidant properties help to protect consumers from cardiovascular disease, some cancers inflammatory conditions, such as arthritis (Tripodi and Kumar 2019).

Organic fertilizers are reported to be a good source of nutrition for vegetable crops that help them improve crop health and yield. As sewage sludge is rich in all essential nutrients required for the growth and development of any crop/plant, the present study was carried out to evaluate the effects of Ganga-sludge-based organic fertilizers on the growth, yield parameters, and fruit quality of bell pepper plants.

MATERIALS AND METHODS

Study Site

The present study was conducted from September to December 2022 to May 2023 at experimental farms of Patanjali Research Institute (PRI), Haridwar, India. The experimental area is located at 29° 54′ 49″ N and 77°59′ 51″ E and 314 meters above sea level (m asl.) (1,030 ft). Total precipitation during the study was recorded as 16.5 mm with 27°C maximum and 7°C minimum temperature. Soil samples were collected (0-20 cm depth) before the implementation of the field trial to determine the properties of soil like pH, EC, organic carbon, available nitrogen, phosphorus, and potassium as per the standard procedures (Jackson 1973).

Collection and processing of Ganga sludge samples

The Ganga sludge samples were collected from the Sludge Treatment Plant (STP) at the Jagjeetpur, Haridwar district, Uttarakhand, India. These sludge samples were then processed further for the five different organic fertilizer products based on the patented technology of Patanjali (Patent application number: 202211069280) at Patanjali Organic Research Institute (PORI). Five major organic fertilizer products, Jaivik Prom, Pori Potash, Dharti ka Chaukidar, Jaivik Poshak, and Jaivik Khad, were prepared after a series of processing procedures. The final products were evaluated singly or in combination, and their effects were assessed on the growth and yield parameters of the bell pepper *Capsicum* crop.

Experimental section

The experimental design was framed with six fertilizer treatments replicated in a Randomized Block Design (RBD) three times. The seed varieties, namely Namdhari, were used for the field experiments. Five different organic fertilizer levels evaluated in this experiment under eight treatment combinations were as T0 (Control), T1 (Jaivik Prom @ 100 kg/ac), T2 (Pori Potash @ 100kg/ac), T3 (Dharti ka Chaukidar @10kg/ac), T4 (Jaivik Poshak @ 7 kg/ac), T5 (Jaivik Khad @ 80kg/ac), T6 (Jaivik Prom+ Jaivik Khad @ 50+ 40kg/ac), T7 (Pori Potash + Jaivik Poshak @ 50+ 3.5kg/ac) (Balkrishna et al. 2023, 2024). Treatment of *Capsicum* seedlings with bio-pesticide (*Trichoderma* and *Pseudomonas* @ 5ml/liter each) was carried out by dipping their roots for about 20-25 minutes to avoid the attack of any soil-borne pathogen. The test fertilizer treatments were used at each 30-day interval after sowing. The *Capsicum* seedlings were planted with 60 cm spacing between rows and 30 cm between plants in a plot size of (2×4 m) $8m^2$. Therefore, 117 plants were used in this experiment for each fertilizer treatment. The irrigation of the crop was carried out crop once every 30-35 days (4-5 in total). Similarly, weeding the undesired weeds was carried out about three times, and 2 to 3 sprays of neem oil were utilized to eradicate the attack of many insects and pests.

Growth, yield, and fruit quality analysis

To study the effects of fertilizer application on plant parameters, five plants per plot were selected randomly and evaluated throughout the study for plant height (cm), leaf area (cm^2), total number of branches, number of flowers, number of fruits, average fruit weight (g), average fruit diameter (mm), total yield per plot (g) and total yield (g). The plant height was measured from the soil level to the tip of the shoot. *Capsicum* fruits were harvested at the full-sized lush green stage around once every two weeks, and the final yield in every 30 days till the last harvesting; the final yield was expressed as the total of all yields. The number of fruits per plant, average fruit diameter per plant, and fruit yield per plant and plot were evaluated under measured yield parameters. The percentage increase over control in plant height, leaf area, weight, and yield of fruits was also calculated.

Statistical analysis

The obtained experimental data for growth and yield parameters is presented as mean ± Standard Deviation (SD). Analysis of results with ANOVA (one-way) and Dunnett's multiple comparisons test was performed using GraphPad Prism version 8.02 for Windows.

RESULTS AND DISCUSSION

Effect of organic fertilizers on growth parameters

Organic fertilizers used in agriculture are now considered an eco-friendly and economical approach that can be considered the best and safest alternative to chemical fertilizers. Even at later stages of the bell pepper *Capsicum* crop, the positive effects on growth and yield revealed their long-term efficacy. Results of the present study were expressed in terms of the effects of organic fertilizers on growth (plant height, leaf area, and total number of flowers) parameters. After a comparison of results, it was observed that all fertilizer treatments could enhance the growth of *Capsicum* crop (Figure 1).

Plant height

The plant height in the present study was measured and expressed as shoot length only. The results indicate noteworthy trends in plant height increase over control for different treatments at various intervals. At 30 days, T1 (organic P fertilizer) demonstrates a significant early positive impact on growth, maintaining consistent positive effects throughout. At 60 days, T6 exhibits the highest increase, emphasizing its sustained positive effect. At 90 days, T4 surpasses others, suggesting the crucial role of mycorrhiza in promoting plant growth. This positive impact continues at 120 days, where T4 leads, closely followed by T1. Overall, the combined application of Mycorrhiza (T4) and the synergistic effects of K fertilizer and JaviK Khad (T6) consistently yield the best results across all time intervals, highlighting their significance in promoting sustained plant growth. The performance of other treatments was also found notable, and all posed potential effects on plant height (Table 1 and Figure 2).

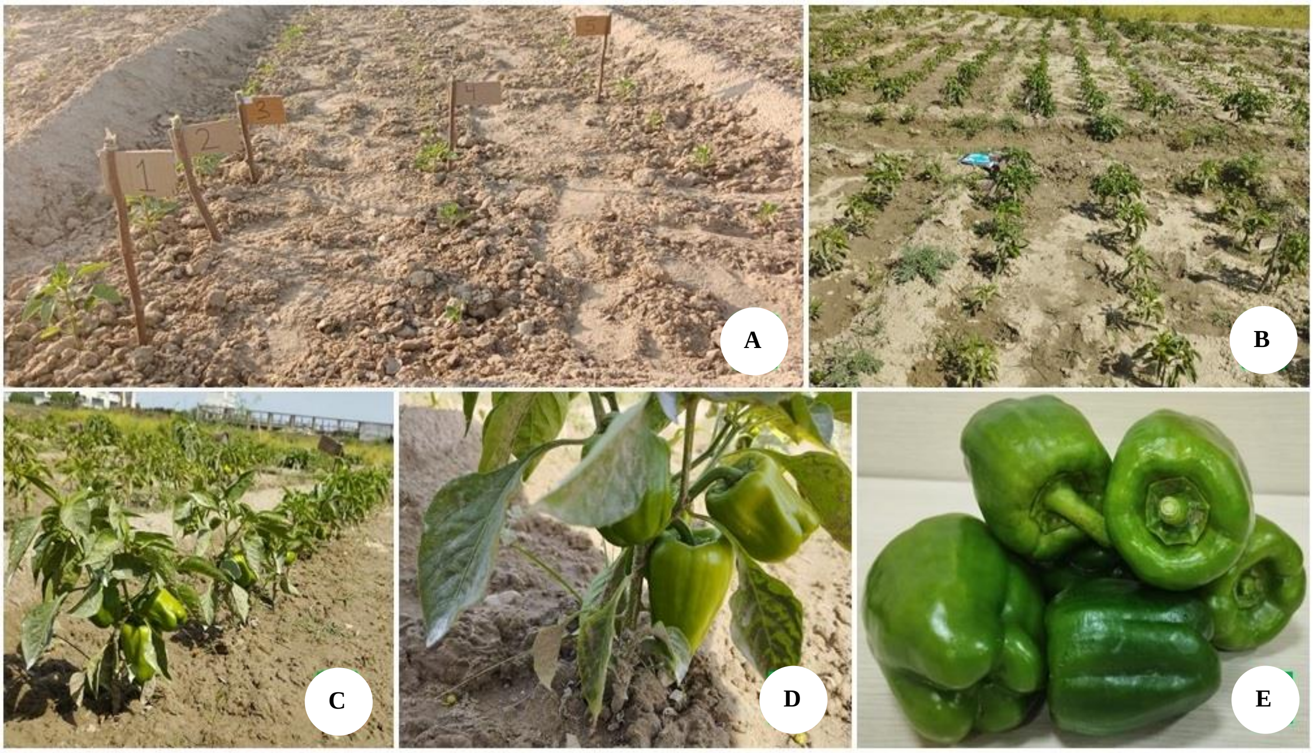

Figure 1. Comparative account on the effects of different treatments on plant parameters of bell pepper *Capsicum* at 30 and 60 days after sowing

Table 1. Effect of sludge-based organic fertilizers on plant height of bell pepper *Capsicum* at different time points

Treatments	Plant Height (cm)			
	30 Days	60 Days	90 Days	120 Days
T_0	10.600±1.778	21.133±3.139	29.267±4.793	36.000±4.400
T_1	14.800±1.562**	24.533±1.858*	37.200±8.697*	43.933±6.955**
T_2	12.000±1.744	23.200±2.623	31.800±2.778	38.800±1.249
T_3	11.400±1.058	23.667±3.449	32.800±4.303	37.533±4.206
T_4	13.733±0.503**	24.067±3.717	38.800±7.910**	45.733±3.765**
T_5	13.267±1.629**	25.200±1.458*	36.067±3.558*	42.533±1.605*
T_6	14.667±1.007**	26.133±1.514**	34.200±4.303	41.067±3.921*
T_7	12.400±0.872*	22.267±2.663	32.33±1.963	39.600±1.587

Note: Mean ± standard deviation of nine replicates. Means under each column with (*) and (**) show Significant ($p \leq 0.050$) and highly significant ($p \leq 0.001$) differences, respectively, in comparison to control

The efficacy of organic fertilizers on plant growth, even at later stages, reflects their slow release, which ensures nutrient availability up to the maximum duration of the crop (Diacono and Montemurro 2010; Jannoura et al. 2013; Lin et al. 2023). This property of organic fertilizers has been reported to have significant positive long-term effects on many crops. Based on this assumption, the basic composition of organic products used in the present study was prepared in such a way as to provide a complete nutritional balance of Nitrogen (N), Phosphorous (P), and Potassium (K) to the crops either through mycorrhiza or organic based materials (Balkrishna et al. 2023). The balanced nutritional approach enables the plants to regulate the opening and closing of the stomata, the exchange of water vapor, oxygen, and carbon dioxide, and overall growth and yield (Amanullah et al. 2016; Xu et al. 2020; Lin et al. 2023).

Leaf area and total number of flowers

Applying organic fertilizers could impact the formation of leaves, branching, and flowers. The percentage increase in leaf area compared to the control group in *Capsicum* plants exposed to different treatments at various time points reveals distinctive patterns. Throughout all stages, T5 consistently emerges as the most effective treatment, demonstrating the highest increments in leaf area. T6 also consistently showcases positive effects, particularly in the early stages, indicating a synergistic impact on leaf expansion. Notably, T1 significantly increases at 30 days, signifying an early positive influence on leaf area. T2 displays a noteworthy surge at 60 and 90 days, indicating a delayed yet positive effect on leaf expansion. Although T3 shows a substantial increase at 30 days, its impact diminishes later. T4, on the other hand, exhibits a substantial increase at 120 days, highlighting the role of mycorrhizal association in prolonged leaf development. The considerable performance of T5 establishing its effectiveness in promoting continuous leaf area growth. Furthermore, T7 manifests a notable increase at 30 days, indicating a positive effect on early leaf development. The results underscore the importance of specific treatments, such as Javik Khad and mycorrhizal association, in fostering consistent and prolonged leaf growth in *Capsicum* plants (Table 2). Therefore, the ample supply of N, P, and K by organic fertilizers can enhance nutrient uptake, including phosphorus, which is crucial for chlorophyll synthesis (Sharma and Agarawa 2009; Sardans and Peñuelas 2021; Aishwarya et al. 2022). The higher leaf area obtained in the case of treatments compared to control during the present study is perhaps associated with improved photosynthetic efficiency. Many earlier studies also demonstrated the influence of organic fertilizers on leaf area index as the result of improved chlorophyll content and influence on the growth of different crops, which also support our present findings (Hamblin et al. 2014; Nagaraj et al. 2019; Manjula et al. 2022; Ye et al. 2022).

Moreover, examining the number of branches in *Capsicum* plants under different treatments at 60 and 90 days highlights intriguing trends. However, it is crucial to note that no substancial increase in branches was observed at 60 and 90 days. Moreover, statistical analysis indicates no significant difference in branch numbers among treatments; these findings collectively suggest that the treatments did not significantly affect branching in *Capsicum* plants. At 60 days, T1 and T6 demonstrate a slight increase in branch number compared to the control (T0), indicating an early positive impact. However, by 90 days, T4 surpasses others, displaying the highest number of branches. T1 and T6 maintain respectable branch numbers, suggesting their continuous positive influence. Notably, T5 and T7 consistently demonstrate branch development, further highlighting the efficacy of Javik Khad and the combined application of phosphorus fertilizer and mycorrhiza (Table 2).

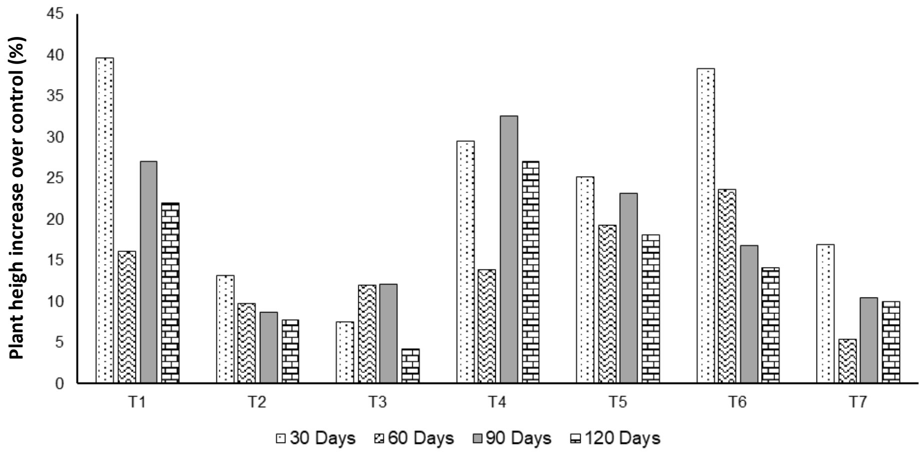

Figure 2. Percent increase over control in plant height at 30, 60, 90, and 120 DAS

A notable correlation between number of branches and number of flowers was found. At the sampled time (60 days), T4, T6, and T3 produced a considerable number of flowers (18.583±2.529, 16.267±7.814 and 15.333±8.353 respectively); other treatments also found potential in producing a respected number of flowers (Table 2, Figure 3). It also depicted the efficacy of organic fertilizer and underscored their overall significant effects on *Capsicum* growth characteristics like formation of leaves, branches, and leaves production. The credit for this improvement also goes to the balanced composition of nitrogen (N), phosphorous (P), and potassium (K) available in organic fertilizers used in the present study that can be helpful to enhance crop growth and development (Balkrishna et al. 2023). Although organic fertilizers can also lead to over-fertilization or nutrient deficiency in the soil when used improperly, their controlled release was useful to overcome such situations to neutralize these impacts and maintain sustainable agriculture yield. Their application can decline the repeated application of synthetic fertilizers. Their release of nutrients gradually into the soil can serve as a productive source of energy for soil microorganisms, which can enhance crop development and soil structure (Shaji et al. 2021).

Effect of organic fertilizers on yield parameters

Like in plant growth, increased fruit weight and total yield per plot of *Capsicum* plants were also observed continuously in all plants treated with different organic fertilizer treatments at 90 and 120 days after sowing. The yield parameters included were expressed in terms of the effects of organic fertilizers on the weight and diameter of fruits of selected plants, and total fruits yield per plot (Table 3). A positive effect of all treatments on yield parameters of *Capsicum* crops offers valuable insights into the effects of organic fertilizers on growth and overall crop yield. The yield regarding fruit weight and total yield per plot was also effectively increased in treatments compared to control. Notably, T5 consistently emerged as the most effective treatment, showcasing the highest average fruit weight (62.755±18.41 g) and total yield per plot (1839.72±355.88 g) at 90 days and maintaining its superiority at 120 days with a fruit weight of 69.24±8.17 g and a total yield of 1409.81±384.88 g. T6 also demonstrated promising results, displaying a fruit weight of 63.850±29.22 g and a total yield of 1792.22±349.70 g at 90 days and remaining competitive at 120 days with a fruit weight of 61.32±8.26 g and a total yield of 1385.11±196.99 g. Among the other treatments, T3 stands out, showcasing a significant increase at 90 days and a further at 120 days over the control. Notably, T4 also demonstrates positive effects, with a significant percentage increase at 90 days and 120 days over the control. The results emphasize the absolute yield and the substantial percentage improvements achieved by specific treatments, particularly T5 and T6, in enhancing *Capsicum* yield over the control conditions. A slight increase in average fruit was observed with the increase in days after sowing, possibly due to nutrition composition and the long-term effects of organic fertilizers. However, no significant variation in average fruit diameter was observed between treatment and control (Table 3, Figure 4). Based on the present study, organic fertilizers positively impacted crop yield; similar observations have been observed in several previous studies. Similar observations on crop yield were also observed on different crops like *Brassica*, lettuce, red chili, and other agricultural commodities (Verma and Verma 2012; Khandaker et al. 2017; Mohammed et al. 2019; Zandvakili et al. 2019; Zhang et al. 2023). Some earlier studies carried out by many researchers also concluded from their studies that the application of organic fertilizers not only enhances growth but also improves the yield in agriculture (Verma and Verma 2012; Zhou et al. 2022) and vegetable crops (Berova et al. 2010; Khandaker et al. 2017; Raturi et al. 2019). The nutrition composition of organic products produced by Patanjali Organic Research Institute (PORI) and their long-term effects contributed mainly to attaining and maintaining crop yield (Balkrishna et al. 2023). Many such findings, which revealed that suitable organic nutrient management practices could enhance the yield of field crops (Parewa et al. 2019; Jote et al. 2023), also validated the results of the present study. Such findings highlighted the utility of organic fertilizers in improving the growth and yields of crops in a safer and environmentally friendly way.

Table 2. Effect of sludge-based organic fertilizers on leaf area, number of branches, and number of flowers in bell pepper *Capsicum* at different time points

Treatments	Leaf area (cm^2)				Number of branches		Number of flowers
	30 Days	60 Days	90 Days	120 Days	60 Days	90 Days	60 Days
T_0	12.610±5.986	23.212±8.392	31.550±14.973	36.943±6.434**	5.000±0.872	6.067±1.361	13.867±7.614
T_1	19.241±4.211*	35.863±7.205*	39.317±4.276	52.940±11.244**	5.467±0.643	7.600±0.529	13.467±4.997
T_2	14.253±4.197	36.040±5.850*	40.833±8.410	53.303±11.743	5.000±2.030	7.533±1.858	12.267±0.902
T_3	17.990±5.990	29.447±4.343	40.973±15.673	44.097±4.195	5.400±1.929	7.267±2.641	15.333±8.353
T_4	17.125±2.365	32.580±9.591	38.633±7.910	38.579±0.364	5.200±1.311	8.133±1.528	18.583±2.529
T_5	19.567±2.043*	40.980±16.915**	48.930±10.266*	49.247±7.710*	5.067±1.206	7.400±1.587	12.533±2.996
T_6	20.207±3.443*	38.132±9.406*	39.133±3.855	48.030±2.920*	5.533±0.643	7.283±2.168	16.267±7.814
T_7	19.557±4.149*	34.880±2.156*	39.487±4.868	51.850±13.010*	5.267±0.503	7.200±0.872	12.533±4.692

Note: Mean ± standard deviation of nine replicates. Means under each column with (*) and (**) show Significant ($p \leq 0.050$) and highly significant ($p \leq 0.001$) differences, respectively, in comparison to control

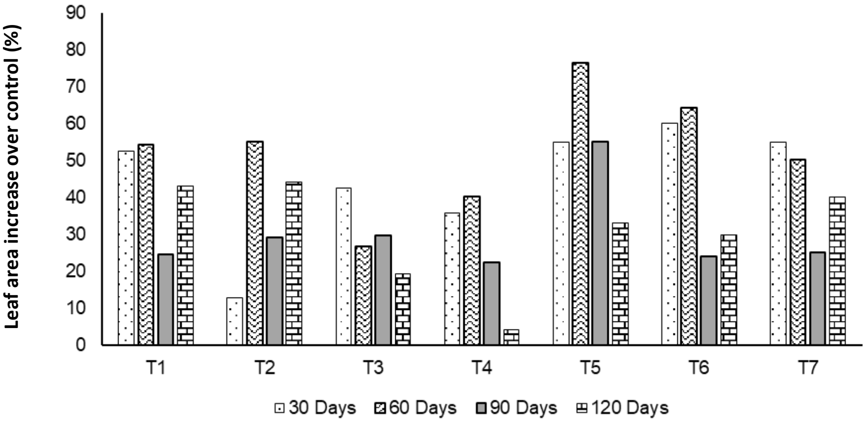

Figure 3. Percent increase over control in Leaf area at 30, 60, 90, and 120 DAS

Table 3. Effect of sludge-based organic fertilizers on weight, diameter of fruits, and total yield of bell pepper *Capsicum* at different time points

Treatments	Fruit weight (g)		Fruit diameter (mm)		Total yield per plot	
	90 days	120 days	90 days	120 days	90 days	120 days
T_0	36.514±7.042	46.607±8.559	2.0856±0.07	2.745±0.08	724.960±187.162	577.507±211.360
T_1	63.152±7.435*	60.985±12.855*	2.445±0.11	2.791±0.11	960.173±172.575	803.513±280.160
T_2	63.456±9.689*	60.024±8.859*	2.459±0.11	2.706±0.21	999.423±549.652	1047.640±135.202
T_3	55.540±18.737	66.386±10.997**	2.381±0.33	2.860±0.20	1359.490±106.062	1187.180±446.368
T_4	50.042±5.744	62.691±10.307*	2.058± 0.21	2.676±0.12	1723.027±429.965	1355.053±421.260*
T_5	62.755±18.407*	69.237±8.171**	2.460±0.49	2.791±0.04	1839.715±355.879*	1409.813±384.877*
T_6	63.850±29.224*	61.322±8.262*	2.525±0.38	2.869±0.19	1792.220±349.702*	1385.107±196.987*
T_7	61.864±19.162*	60.995±12.302*	2.653±0.29	2.829±0.18	1391.610±876.930	1164.253±223.932

Note: Yield/ plot is represented as Mean ± standard deviation of three replicates. Means under each column with (*) and (**) show Significant (p ≤0. 050) and highly significant (p ≤0.001) differences, respectively, in comparison to control

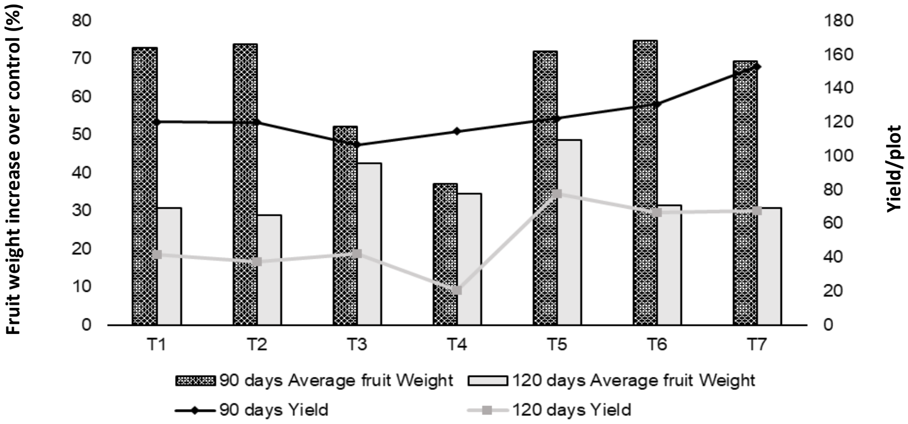

Figure 4. Percent increase over control in average fruit weight and yield/plot at 90 and 120 DAS

The overall conclusion drawn from the present study is that processing sewage sludge into organic fertilizers highlighted the safer and environmentally friendly way of managing Ganga sludge. Moreover, the importance of organic fertilizers concerning their long-term effects on growth and yield also advocated this approach of conversion of sludge into organic fertilizers. Therefore, more research should be conducted on different aspects of sludge-based organic fertilizers like processing, production and field application on more field crops.

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Effect of brown algae as biofertilizer materials on pepper (*Capsicum annuum*) growth, yield, and fruit quality

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Abstract. *Baroud S, Tahrouch S, Hatimi A. 2024. Effect of brown algae as biofertilizer materials on pepper (*Capsicum annuum*) growth, yield, and fruit quality. Asian J Agric 8: 25-31.* Our study aims to evaluate the effect of three brown algae, *Cystoseira gibraltarica* (Sauvageau) P.J.L.Dangeard, *Bifurcaria bifurcata* R.Ross, and *Fucus spiralis* L., as biofertilizers on pepper (*Capsicum annuum* L.). These three algae were applied in two forms and at different concentrations: amendment (C1, C2, and C3) and concentrations of aqueous extract (0.5%, 1%, and 2%). Generally, the studied parameters were significantly improved using the aqueous extracts or the amendment at low concentrations. Indeed, the extract of *F. spiralis* at 1% shows a maximum length of the aerial and root parts (75.8 and 33.2 cm, respectively) and the dry weight of the plants (9.88 g). Aqueous extracts of *F. spiralis* at 1% show the maximum values of organic matter and nitrogen content of pepper leaves (88.3% and 4.4%, respectively). In addition, the 0.5% *B. bifurcata* extract presented significant values for the number of flowers (8.8 flowers/plant), and the 1% *F. spiralis* extract showed a better result for the number of fruits (7.1 fruits/plant). The alga *C. gibraltarica* at 1% showed a maximum fruit weight (82.65 g). On the other hand, *F. spiralis* at 1% showed maximum values of fruit diameter (48.33 mm). Similarly, *F. spiralis* at 0.5% showed maximum values in brix and sugar content (5.33% and 5.4%, respectively). These three algae were found to be effective and good candidates for the efficient development of biostimulants to improve growth and yield as well as the fruit quality of pepper. This study provides important information on identifying and utilizing Moroccan algal resources in agriculture.

Keywords: Algal fertilization, extract and amendment, fruit quality, growth, yield

INTRODUCTION

Greenhouse cultivation is the most common type of cultivation because it can increase agricultural production in Morocco (Harbouze et al. 2019). It can also create optimal climatic conditions for plant growth and grow more per square foot than growing crops in the field. In addition to the optimal conditions provided by the greenhouse, algae fertilization could significantly improve the growth and yield of greenhouse crops.

In Morocco, sea currents and hydroclimatic conditions rapidly favor the development and expansion of marine algae such as brown seaweed (Riadi 1998), and many species of algae grow rapidly and efficiently, especially compared with terrestrial plants (Kindleysides et al. 2012). Brown algae are very abundant along the Atlantic coast, especially in the Cap Ghir region. This calls for better management of this biomass while promoting research aimed at exploiting these brown algae, particularly *Cystoseira gibraltarica* (Sauvageau) P.J.L.Dangeard, *Bifurcaria bifurcata* R.Ross, and *Fucus spiralis* L., in several fields, particularly agriculture. However, few studies have been conducted to identify the various molecules in the brown algae harvested on Morocco's double seafront.

Potting is the practice of growing plants, including vegetables, exclusively in pots instead of planting them in the ground (Mills 2012). Farmers use pot growing in areas where the soil or climate is unsuitable for the crop. Thus, this method is useful for scientific trials before growing in the ground. In addition to the optimal conditions of the greenhouse, fertilization with algae could significantly improve the growth and yield of greenhouse crops.

Using polar solvents, phenolic compounds, organic acids, tannins, and salts can be extracted from algae (Sabeena and Jacobsen 2013). Some studies indicate that algal extracts can partially substitute fertilizers (Hong et al. 2007; Zodape et al. 2010) because they contain both minor and major mineral elements. Saccharides in algal extracts can act as elicitors of plant defensive mechanisms (Khan et al. 2009). Algae-based fertilizers contain various plant growth-promoting substances such as auxins, cytokinins, and betaines (Khan et al. 2009). These substances can influence the development of the aerial and root plant parts of *Arabidopsis thaliana* (L.) Heynh. (Durand et al. 2003). In addition, macronutrients (N, P, K, Ca, and Na) and micronutrients (Fe, Zn, Mn, and Cu) promote fruit growth and yield (Hamouda et al. 2016). These algal extracts also increase phytochemical parameters (Lola-Luz et al. 2014). Positive responses include improved plant growth, fruit quality, plant vigor, and pathogen resistance (Khan et al. 2009). Whapham et al. (1993) showed that applying aqueous extracts of *Ascophyllum nodosum* (L.) Le Jol. algae increased omato plants' chlorophyll 'a' and 'b' content.

The field of agriculture has had a remarkable improvement in recent years as it is characterized by the diversity of crops, including pepper (*Capsicum annuum* L.)

(Harbouze et al. 2019). Therefore, to maintain the position of our country in this field, it is essential to use more effective biological fertilizers to produce significant and good-quality productive activity. Several researches indicate algal extracts can partially substitute conventional fertilizers (Dhargalkar and Pereira 2005; Hong et al. 2007; Zodape et al. 2010). Moreover, algal extracts are among the methods used to improve plant yield, fruit quality, and mineral composition (Elansary et al. 2016). Previous studies have shown that algal extracts positively affect the growth and yield of vegetable plants (Sharma et al. 2014).

In this context, we aim to conduct a greenhouse pot experiment on pepper growth parameters, yield, and fruit quality to test the effect of three brown algae: *B. bifurcata*, *C. gibraltarica,* and *F. spiralis*.

MATERIALS AND METHODS

Plant materials

Three species of brown algae, *C. gibraltarica*, *B. bifurcata*, and *F. spiralis*, were collected at low tide in the coastal area of Cap Ghir (30°38'37 "N, 09°53'20 "W), located about 43 km northwest of Agadir (Morocco). The collected seaweed species were washed on-site and put in a seawater box. In the laboratory, the samples were sorted, identified, and then rinsed thoroughly with fresh water to eliminate all impurities: sand, salt, shell debris, and some epiphytes; for each wash, electrical conductivity measurements were conducted. The algae were then dried at room temperature and protected from sunlight by spreading the samples on sieves for 10 days. After drying, the samples were ground to a powder and stored in a dry place.

Preparation of treatments

Amendments

Three amendments were prepared based on 25 kg/100 m^2 concentrations in organic agriculture. Each pot contained 5 kg of substrate consisting of a mixture of 75% soil and 25% peat. Three amendments were determined: C1 (2.5 g/pot), C2 (5 g/pot) and C3 (10 g/pot).

Algal extracts

Five grams of powder of each algal species were added to 100 ml of distilled water under magnetic stirring for 24 h. The recovered supernatant was filtered, and the obtained aqueous extracts were stored in a cool place. These extracts were designated as stock solution and coded according to genus and species: *C. gibraltarica* (CG), *B. bifurcata* (BB), and *F. spiralis* (FS). The stock solution of each alga was diluted with water to make three concentrations (0.5, 1, and 2%).

Greenhouse growth bioassay

Certified pepper seeds (*C. annuum*) of the Roldan variety were germinated in peat blister plates. After 25 days of germination, 200 plants were selected at the four-leaf stage and transplanted into pots containing 5 kg of a mixture of 75% soil and 25% peat. For each algal treatment, ten pots were used, with one plant per pot (10 replicates per treatment). Each pot receives 50 ml/week of the algal extract, represented by three concentrations (0.5, 1, and 2%). For the amendment, the treatment was also represented by three increasing concentrations: C1 (2.5 g/pot), C2 (5 g/pot), and C3 (10 g/pot). At the same time, we used a witness that received only water and the control treated with a chemical fertilizer (Maxi Greene: N:20, P:20, K:20).

Aqueous extracts were sprayed two weeks after sowing at a 50 ml/week rate for three months. Fertilization by amendment was done when transplanting the plants with the three different concentrations determined previously. All pots were irrigated with 50 ml of water every other day during cultivation.

The number of flowers per plant was counted at the time of flowering. After 90 days of cultivation, the pepper fruits were harvested, and the plants were carefully removed and washed. Then we measured the various growth parameters (length of the aerial part, length of the root and total length, fresh weight and dry weight of the plant), the mineral analyses of the leaves, the analyses of the fruits such as the yield (number of leaves, number of fruit/plant and weight of fruit) and the quality of the fruits (diameter, firmness, brix, sugar content, and maturity index).

Determination of growth parameters

Plant growth was measured based on the plant's length, fresh and dry weight, number of leaves, and number of flowers per plant. The dry weight was determined after drying at 80°C until the weight stabilized. The dry weight was expressed in grams.

Determination of the leaves' mineral elements

The mineral content of pepper leaves was determined based on the method of Page et al. (1982).

Determination of fruit yield

Determination of fruit yield was done at the end of the crop at the time of harvest; we also determined the number of leaves, number of flowers, number of fruit/plant, and fruit weight/plant.

Determination of fruit quality

We performed fruit quality parameters such as diameter, firmness, brix, sugar content, and fruit maturity index.

Fruit diameter

The caliper determined the diameter and measured the equatorial and polar diameters of the fruit.

Fruit firmness

Fruit firmness was measured with a penetrometer (dynamometer), which measures the force necessary to make a calibrated tip penetrate a certain depth in the fruit. The firmness of the fruit depends, among other things, on its maturity level and development stage. The results were expressed in kg/cm^2.

Brix

Brix is the number of grams of soluble dry matter per 100 g of product. This soluble dry matter consisted of about 80 to 85% sugars, 10% citric acid and salts, and nitrogenous and other soluble substances (Ting and Rouseff 1986). The Brix was determined according to the AFNOR NNFX standard (1988) and done by sufficiently pouring a few drops on the refractometer's prism and turning the device towards a light source to measure the refractometric index. The reading was done on the piece scale at the intersection of the light and dark areas.

Sugar content

The sugar content was determined based on the optical property of a sugar solution to reflect light. The percentage of dry matter measured is the refractometric index, expressed in degree brix (°Brix).

The value read in °Brix must be corrected for the temperature of the juice according to the following relationship:

Soluble Dry Extract = °Brix lu ± z 0.08 x (T°C - 20°C)

Where:

z : + if the temperature is above 20°C; - : if the temperature is below 20°C.

0.08: correction index of the refractometer prism.

Maturity index

The maturity index, which is the ratio of sugars to acidity of the juice, allows us to determine the state of maturity of the fruitsThe following formula calculated it:

$$\textbf{Maturity index} = \frac{\textbf{Soluble dry extract}}{\textbf{\% of citric acid in the juice}}$$

Statistical analysis

The data were processed by STATISTICA software, version 6.0. Analysis of variance was performed to determine the level of significance. The means were compared using Duncan's tests at the probability threshold (P <5%).

RESULTS AND DISCUSSION

Effect of algal fertilization on growth parameters of greenhouse pepper

The results (Table 1) show that the growth parameters improved significantly overall through aqueous extracts or amendments. The algal extracts of *B. bifurcata*, *C. gibraltarica*, and *F. spiralis* significantly improved the length of the aerial part compared to the control.

Indeed, the 1% *F. spiralis* extract gave statistically the maximum length of the aerial part (75.8 cm), followed by the 0.5% *B. bifurcata* extract (70.85 cm) and finally the 1% *C. gibraltarica* extract (68.40 cm). The amendments *B. bifurcata* and *F. spiralis* were also statistically effective in improving the length of the aerial part compared to the control. Indeed, *F. spiralis* at C2 gave a statistically significant length (68.00 cm), followed by the amendment of *B. bifurcata* at C1 (67.20 cm) and finally, the amendment of the same alga at C2 (61.1 cm) (Table 1).

All the algal extracts of *B. bifurcata*, *C. gibraltarica*, and *F. spiralis* significantly improve the length of the root part compared to the control and even the chemical fertilizer. Indeed, *F. spiralis* at 1% is significantly the most effective (33.2 cm), followed by *B. bifurcata* extract at 1% (31.5 cm), and finally, *C. gibraltarica* extract at 2% (31.20 cm). Regarding the amendments, *B. bifurcata*, *C. gibraltarica*, and *F. spiralis* were also statistically effective in improving the length of the aerial part compared to the control. Indeed, *F. spiralis* at C2 gave a statistically significant length (31.10 cm), followed by the amendment of *B. bifurcata* at C2 (29.30 cm) and finally, the amendment of *C. gibraltarica* at C2 (28.80 cm) (Table 1).

Regarding the fresh weight, all the algal extracts and all the amendments at all concentrations improve it or make it equivalent to the controls. We note that, in general, *F. spiralis* is more effective than either the extract or the amendment, but at medium concentrations (1% for the extract and C2 for the amendment). Indeed, *F. spiralis* at 1% gives statistically the best result (50.47 g), followed by the extract of *C. gibraltarica* at 2% (50.06 g) and finally the extract of *B. bifurcata* at 0.5% (40.04 g). Regarding the amendments, *B. bifurcata*, *C. gibraltarica*, and *F. spiralis* were also statistically effective in improving the fresh weight of the plant compared to the control. Indeed, *F. spiralis* at C2 was significantly effective (50.64 g), followed by the amendment of *C. gibraltarica* at C3 (47.57 g) and finally the amendment of *B. bifurcata* at C2 (36.31 g).

Regarding dry weight, all algal extracts and all amendments at all concentrations improve it or make it equivalent to the controls. We notice that *F. spiralis* is more efficient than the extract or the amendment, but at medium concentrations (1% for the extract and C2 for the amendment). Indeed, *F. spiralis* at 1% is significantly effective (9.88 g), followed by the extract of *B. bifurcata* at 0.5% (8.32 g) and finally the extract of *C. gibraltarica* at 2% (8.21 g). Concerning the amendments, *B. bifurcata*, *C. gibraltarica* at C1 and C3, and *F. spiralis* at C2 were also statistically effective for improving the plant dry weight compared to the control. Indeed, the alga *F. spiralis* at C2 gave statistically the best result (11.75 g), followed by the amendment of *C. gibraltarica* at C3 (9.54 g) and finally, the amendment of the same alga at C1 (7.38 g) (Table 1).

Fertilization by amendment also showed a clear improvement in the leaves of these elements (OM, N, and P). Indeed, amendment C2 of *B. bifurcata* positively affected organic matter content (86.68%), while amendment C2 and C3 of *F. spiralis* significantly improved nitrogen and phosphorus content (3.30% and 0.27%, respectively).

Table 1. Effect of the three algae *Cystoseira gibraltarica* (CG), *Bifurcaria bifurcata* (BB), and *Fucus spiralis* (FS) by both treatments (Spraying and amendment) at different concentrations on the growth parameters of pepper

Spraying	Aerial part (cm)	Root part (cm)	Fresh weight of the plant (g)	The dry weight of the plant (g)
Witness	54.15±1.59 e	23.15±0.78 f	31.05±1.26 f	5.11±0.24 h
Control	64.70±0.94 d	22.70±0.82 f	45.03±3.09 b	9.44±0.24 b
BB 0,5 %	70.85±4.21 b	25.95±2.45 e	40.04±1.23 c	8.32±0.33 c
BB 1 %	68.30±2.32 c	31.50±2.49 b	31.79±0.89 f	6.00±0.24 f
BB 2%	65.20±1.61 d	30.30±1.18 bcd	35.43±1.49 e	6.94±0.43 e
CG 0,5%	64.50±2.27 d	29.70±0.85 cd	37.38±2.92 d	7.61±0.68 d
CG 1 %	68.40±1.83 c	29.40±1.84 d	31.19±1.29 f	6.16±0.41 f
CG 2 %	65.55±1.49 d	31.20±1.7 b	50.06±0.97 a	8.21±0.3 c
FS 0,5 %	55.85±1.10 e	30.90±0.87 bc	37.87±1.2 d	7.29±0.24de
FS 1 %	75.80±1.54 a	33.20±0.91 a	50.47±1.37 a	9.88±0.2 a
FS 2 %	55.70±1.35 e	24.90±1.02 e	30.00±0.85 f	5.80±0.77 g
Amendment				
Witness	54.15±1.59 f	23.15±0.78 h	31.05±1.26 g	5.11±0.24 f
Control	64.70±0.94 b	22.70±0.82 h	45.03±3.09 c	9.44±0.24 b
BB C1	67.20±2.34 a	24.14±0.57 g	33.54±1.08 f	6.51±0.5 e
BB C2	61.10±1.02 c	29.30±0.71 b	36.31±0.65 d	6.55±0.3 e
BB C3	58.25±2.09 ed	28.10±0.87 d	33.04±1.28 f	6.55±0.23 e
CG C1	59.18±3.6 d	28.40±0.65 cd	35.95±0.57 ed	7.38±0.29 d
CG C2	52.90±2.07 f	24.55±0.76 fg	26.93±0.44 k	5.17±0.18 f
CG C3	56.75±1.31 e	28.80±0.78 bc	47.57±0.92 b	9.54±0.28 c
FS C1	51.10±1.28 f	25.54±0.70 e	34.51±0.54 ef	6.69±0.26 e
FS C2	68.00±3.02 a	31.10±0.73 a	50.64±0.56 a	11.75±0.58a
FS C3	57.60±3.57 ed	25.05±0,6 ef	28.56±0.49 h	5.05±0.23 g

Note: Values show the mean ± standard deviation (n=10). Values indicated by a different letter differ significantly (P ≤0.05)

Table 2. Effect of *Cystoseira gibraltarica* (CG), *Bifurcaria bifurcata* (BB), and *Fucus spiralis* (FS) algae by both treatments (Spraying and amendment) at different concentrations on the mineral element content of leaves

Spraying	OM (%)	P (%)	N (%)	K (ppm)	Ca (ppm)	Na (ppm)
Witness	80.78±0,43 e	0.24±0.01 f	2.3±0.13 f	340.80±0.3 d	106.13±0.6e	18.93±0.45c
Control	86.48±0.76 c	0.26±0.02 f	3.3±0.24 c	344.13±1.08c	98.74± 0.26h	33.76±0.4 a
BB 0.5%	85.90±0.3 d	0.36±0.03 b	2.6±0.12 e	393.80±1.03b	109.20±0.5c	17.96±0.98c
BB 1%	84.53±0.67 d	0.39±0.02 a	2.5±0.15 e	408.50±0.7 a	102.63±0.85f	31.10±0.6 b
BB 2 %	86.65±0.65 c	0.36±0.01 b	2.6±0.13e	340.25±0.85d	107.93±0.4d	14.00±0.23e
CG 0.5 %	86.78±0.62 c	0.35±0.01 c	2.8±0.16 d	315.90±0.94f	124.83±0.3b	14.04±0.7 e
CG 1 %	86.94±0.62 c	0.36±0.01 b	2.8±0.18 d	328.80±0.41e	100.80± 0.6g	15.20±0.4de
CG 2 %	86.73±1.07 c	0.34±0.02d	2.8±0.25 d	302.90±0.64g	133.60±0.23a	14.23±0.55e
FS 0.5 %	88.10±0.78 a	0.33±0.01 d	4.00±0.24 b	235.25±0.8 k	107.93± 0.5 d	14.63±0.6 e
FS 1 %	88.30±0.79 a	0.32±0.01 d	4.4±0.14 a	269.85±0.85h	100.40±0.52g	16.00±0.36d
FS 2 %	87.30±0.55ab	0,31±0.01 e	3.5±0.17 c	327.60±0.96e	109.00±0.79c	11.30±0.34f
Amendment						
Witness	80.78±0.01 f	0.24±0.02 d	2.30±0.13 d	340.80±1.8 b	106.13±0.15a	18.93±0.45g
Control	86.48±0.01 a	0.26±0.01 b	3.30±0.18 a	344.13±1.15a	98.74±0.20 c	33.76±0.4 c
BB C1	85.93±0.02 b	0.21±0.02 f	1.90±0.13 f	327.33±1.46d	75.23±0.1 d	18.73±0.9 g
BB C2	86.68±0.02 a	0.23±0.02 e	1.90±0.19 f	251.16±0.47k	49.30±0.30 k	16.56±0.37h
BB C3	85.18±0.03 b	0.21±0.01 f	2.00±0.35 e	306.96±1.45f	63.13±0.34 f	16.23±0.49h
CG C1	83.49±0.01 d	0.26±0.02 b	2.10±0.14 e	320.23±1.44e	101.83±0.55b	38.93±0.55b
CG C2	83.86±0.02 d	0.19±0.02 g	2.10±0.26 e	237.10±0.96k	55.56±0.51 h	30.80±0.5 d
CG C3	81.16±0.01 e	0.23±0.03 e	2.10±0.15 e	286.36±1.93h	65.53±0.7 e	28.70±0.17e
FS C1	83.98±0.02 d	0.23±0.02 e	3.00±0.15 b	341.00±1.99b	103.60±1.33a	42.00±0.52a
FS C2	84.49±0.02 c	0.25±0.01 c	3.30±0.15 a	317.90±1.02e	65.86±0.73 e	33.46±0.5 c
FS C3	81.69±0.02 e	0.27±0.01 a	2.60±0.18 c	317.53±2.47e	57.93±0.34 g	25.70±0.83f

Note: Values show the mean ± standard deviation (n=3). Values indicated by a different letter differ significantly (P ≤0.05)

The plants treated with the aqueous extracts (spraying) or by amendment show, in general, an insignificant improvement of the content of potassium (K), sodium (Na), and calcium (Ca) in the leaves (Table 2). this study shows that the fertilization with the aqueous extracts of the three brown algae showed a significant improvement in the calcium content, with a maximum value obtained by the aqueous extract of *C. gibraltarica* at 2% (133.60 ppm). On the other hand, the 1% aqueous extract of *B. bifurcata* showed highly significant potassium and sodium content

(408.50 and 31.10 ppm, respectively). Amendment fertilization generally shows an improvement in leaf sodium content with a maximum value for C1 of *F. spiralis* (42.00 ppm). On the other hand, this treatment showed no significant effect on potassium (Table 2).

Effect of algal fertilization on fruit yield of pepper

The study shows that the plants cultivated in pots in a greenhouse, treated by aqueous extract (spraying) or amendment, generally significantly improved fruit yield (Table 3). Nevertheless, we notice that the contribution of algal fertilizer in the form of aqueous extracts gives significantly more important results than the amendment.

The algal extracts of the three algae *B. bifurcata, C. gibraltarica*, and *F. spiralis* significantly improved the number of leaves compared to the control, with a maximum obtained by the extract of *B. bifurcata* at 1% and 2% (32.10 and 31.40 leaves/plant, respectively). The amendments were also statistically effective in improving the number of leaves compared to the control, with a maximum number obtained by *F. spiralis* at C2 (32.00 leaves/plant) (Table 3).

All the algal extracts of the three algae improved the number of flowers and fruits significantly compared to the control. Indeed, the extracts of *B. bifurcata* at 0.5% and 1% showed significant values for the number of flowers (8.80 and 8.60 flowers/plant, respectively). In comparison, the extract of *F. spiralis* at 1% showed a better result for the number of fruits (7.10 fruits/plant). On the other hand, amendments with *B. bifurcata* and *C. gibraltarica* were also statistically effective in improving the number of flowers and fruits compared to the control. Furthermore, *B. bifurcata* at C3 statistically gave the best result for flowers and fruits (8.80 flowers/plant and 6.10 fruits/plant).

The algal extracts of the three brown algae *B. bifurcata, C. gibraltarica*, and *F. spiralis* significantly improved fruit weight over the control, with a maximum value being obtained by *C. gibraltarica* at 1% (82.65 g), followed by *B. bifurcata* extract at 1% and *F. spiralis* at 2% (72.66 and 72.38 g, respectively). As with the algal extracts, amendments with *B. bifurcata* (49.82 g) and *F. spiralis* (44.61 g) were statistically effective in improving fruit weight compared to the control (58.34 g); Table 3 shows the highest weight in the control; hence, it is not effective in improving fruit weight. Likewise, all amendments by *C. gibraltarica* (48.79 g) positively affected fruit weight but were lower than the control at 58.34 g (Table 3).

Effect of algal fertilization on fruit quality of pepper

Fertilization with algae, aqueous extracts, or amendment significantly improved the quality of fruits grown in greenhouse pots (Table 4). Table 4 shows that Brix % aqueous extracts (5.33%) are higher than amendment (5.10%) and also the quantity of sugars (%) and MI that aqueous extracts are higher than their amendments.

The extracts of *B. bifurcata*, all concentrations combined, the 2% extract of *C. gibraltarica*, and 1% *F. spiralis* significantly improved the firmness of the fruits compared to the control. The same algal extracts at 1% showed maximum fruit diameter values of 47.83 mm for *B. bifurcata* at 1% and 48.33 mm for *F. spiralis* at 1% (Table 4). Regarding firmness, the amendment by *B. bifurcata* to C2 recorded maximum values for firmness and diameter (3.62 kg/cm and 53.50 mm, respectively). The *F. spiralis* amendment C1 also presented maximum values for a fruit diameter of 52.66 mm.

Table 3. Effect of *Cystoseira gibraltarica* (CG), *Bifurcaria bifurcata* (BB), and *Fucus spiralis* (FS) algae by the two treatments (spraying and amendment) at different concentrations on pepper yield parameters

Spraying	Number of leaves	Number of flowers	Number of fruits/plant	Fruit weight (g)
Witness	20.30±0.94 g	5.40±0.69 f	3.10±0.73 d	42.07±2.38 g
Control	33.60±0.84 a	12.20±0.91 a	6.00±0.47 b	58.34±2.32 e
BB 0.5 %	28.50±1.85 d	8.80±0.91 b	3.70±0.94 cd	59.29±0.88 d
BB 1 %	32.10±1.02 b	8.50±0.70 b	3.80±0.91 cd	72.66±1.01 b
BB 2%	31.40±1.17 b	6.50±0.52 e	6.10±1.79 b	63.46±0.88 c
CG 0.5%	30.40±0.96 c	8.20±0.42 c	5.80±0.63 b	63.52±1.01 c
CG 1%	21.80±0.78 f	4.60±0.69 gh	6.30±0.82 b	82.65±0.63 a
CG 2%	22.70±0.94 f	4.00±0.66 h	6.10±0.56 b	63.53±0.71 c
FS 0.5%	24.20±0.78 e	7.40±0.51 d	4.00±1.05 c	57.95±0.78 d
FS 1%	22.50±0.52 f	5.00±0.81 gf	7.10±0.87 a	56.18±0.72 f
FS 2%	19.60±0.84 g	6.40±0.51 e	5.80±0.63 b	72.38±1.16 b
Amendment				
Watering	20.30±0.94 h	5.40±0.69 f	3.10±0.73 d	42.07±2.38 d
Control	33.60±0.84 a	12.20±0.91 a	6.00±0.47 a	58.34±2.32 a
BB C1	26.50±0.84 e	8.20±0.42 c	4.80±0.78 c	49.82±0.72 b
BB C2	24.90±0.56 f	7.80±0.42 cd	5.60±0.51 b	39.16±0.94 f
BB C3	25.80±0.63 e	8.80±0.78 b	6.10±0.56 a	42.58±1.15 d
CG C1	22.20±0.78 g	7.60±0.51 cd	6.20±0.63 a	48.79±2.19 b
CG C2	25.80±0.91 e	6.50±0.52 e	5.30±0.48 b	47.73±2.53 b
CG C3	27.70±0.82 d	6.40±0.51 e	3.40±0.69 d	45.73±0.48 c
FS C1	29.70±0.82 c	6.30±0.48 e	5.40±0.51 b	44.61±3.06 c
FS C2	32.00±0.66 b	7.40±0.51 d	4.70±0.67 c	42.21±1.37 d
FS C3	29.60±1.07 c	7.70±0.48 cd	5.90±0.73 ab	38.81±0.94 f

Note: Values show the mean ± standard deviation (n=10). Values indicated by a different letter are significantly different ($P \leq 0.05$)

Table 4. The effect of the algae *Cystoseira gibraltarica* (CG), *Bifurcaria bifurcata* (BB), and *Fucus spiralis* (FS) at different concentrations on fruit quality by both treatments (spraying and amendment). The values are presented as mean ± standard deviation (n=6)

Spraying	Firmness (kg/cm)	Diameter (mm)	Brix %	Quantity of sugars (%)	MI
Witness	2.11±0.06 d	40.50±1.00 f	4.90±0.62 b	4.98±0.59 b	53.39±0.95 b
Control	3.50±0.61 a	52.50±0.50 a	4.66±0.23 bc	4.68±0.23 c	43.45±2.20 d
BB 0.5 %	3.03±0.56 b	46.33±1.25 c	4.36±0.05 bd	4.48±0.05 d	46.49±0.52 c
BB 1 %	3.23±0.16 b	47.83±3.21 b	4.46±0.23 cd	4.54±0.23 c	69.58±3.57 a
BB 2 %	2.95±0.18 b	45.50±0.50 c	3.73±0.15 ef	3.79±0.15 f	41.84±1.75 e
CG 0.5 %	2.03±0.11 d	42.33±1.01 d	4.26±0.15 d	4.33±0.15 e	18.30±0.64 h
CG 1 %	2.03±0.07 d	43.00±1.00 d	3.46±0.15 f	3.51±0.16 g	31.07±1.48 f
CG 2 %	2.64±0.12 c	45.00±1.00 c	4.23±0.20 d	4.29±0.20 e	43.16±2.09 d
FS 0.5 %	1.86±0.1 d	40.83±1.52 f	5.33±0.05 a	5.40±0.05 a	24.42±0.24 g
FS 1 %	2.56±0.23 b	48.33±1.25 b	3.93±0.15 e	3.98±0.16 f	46.05±1.93 c
FS 2 %	1.88±0.10 d	41.16±1.25 e	4.33±0.2 d	4.37±0.19 e	40.20±1.79 e
Amendment					
Witness	2.11±0.06 e	40.50±1.00 d	4.90±0.62 a	4.98±0.59 b	53.39±0.95 a
Control	3.50±0.61 b	52.50±0.50 a	4.66±0.23 b	4.68±0.23 c	43.45±2.20 d
BB C1	2.77±0.18 d	48.50±1.00 b	3.90±0.10 d	4.04±0.01ab	44.51±0.05 c
BB C2	3.62±0.07 a	53.50±1.80 a	3.76±0.32 d	3.88±0.31 f	35.22±2.90 f
BB C3	3.50±0.61 b	44.33±3.21 c	4.13±0.46 c	4.24±0.48 d	46.43±0.76bc
CG C1	3.23±0.16 c	48.5±2.17 b	4.03±0.55 c	4.12±0.55 e	40.19±1.12 e
CG C2	2.95±0.18 d	45.16±1.04 c	4.23±0.25 ab	4.32±0.25 d	37.75±2.19 ef
CG C3	2.03±0.11 e	40.00±2.64 d	3.80±0.45 d	3.87±0.46 f	30.37±1.49 g
FS C1	2.03±0.07 e	52.66±1.52 a	4.63±0.05 b	4.72±0.06 c	36.01±0.45 f
FS C2	2.64±0.12 d	46.66±0.57 c	4.73±0.11 b	4.81±0.10 b	45.83±1.03 c
FS C3	1.86±0.10 f	47.33±2.08 b	5.10±0.10 a	5.18±0.11 a	48.45±1.04 b

Note: Values indicated by a different letter differ significantly ($P \leq 0.05$). Witness: water control: chemical fertilizer (Maxi Greene) MI: Maturity Index

The organoleptic qualities of pepper fruits (Brix, quantity of sugars, maturity index), grown in greenhouse pots, were significantly little affected by the two treatments, aqueous extracts and amendment (Table 4); However, the algal extracts significantly improved the organoleptic qualities of the fruits. Indeed, *F. spiralis* at 0.5% showed maximum values in brix and amount of sugar (5.33% and 5.40%, respectively). Similarly, *B. bifurcata* at 1% significantly improved the maturity index (69.58).

Furthermore, we noted that the amendment treatment had no significant effect on the organoleptic qualities of the fruits except for the amendment with *F. spiralis* at C3, which showed a significant difference in sugar content (5.18%) compared to the control treatment (Table 4).

Discussion

Moreover, to evaluate the effect of algal fertilization on plant growth and productivity, we tested increasing concentrations of extracts and amendments of the three brown algae (*B. bifurcata, C. gibraltarica,* and *F. spiralis*) on the pepper crop.

The results obtained after three months of cultivation in pots under greenhouse conditions are generally very satisfactory. The root and aerial growth and the fresh and dry weight of the pepper plants are clearly improved after treatment with the aqueous extracts and the amendments, particularly *B. bifurcata.* Similar results showed that treatment with the alga *A. nodosum* as an extract effectively affected the growth of several crops (Danesh et al. 2012; Bozorgi 2012). In addition, algal extracts improve nutrient uptake by roots, especially Mg, K, and Ca (Yassen et al. 2018). This stimulates root activity by increasing the uptake of water and mineral elements, which improves plant growth and vigor. These results are consistent with those obtained by Zermeno-Gonzalez et al. (2015), who showed that applying organic fertilizers derived from algal extracts to the soil on maize crops increased plant height, stem diameter, and plant dry weight. Our study clearly shows that the mineral contents of pepper leaves are improved in the presence of extracts and amendments of the three algae. This could increase the water uptake by the roots of the pepper plants, which would increase the fresh weight of the crop. At the same time, their dry weight increment can be explained by the increase in their protein and organic matter content. Similar results showed that treatment with algal extracts increased the leaves, fresh weight, and dry weight (Xu and Leskovar 2015). These results can be explained by the constituents of algae that contain nutrients, namely vitamins, amino acids, auxins and cytokinins, and macro and micro minerals that affect the cellular metabolism of treated plants, resulting in increased growth of these plants (Khan et al. 2009; Spann and Little 2010; Craigie 2011; du Jardin 2015).

The yield of pepper fruits increased following treatment with the extracts or amendment, especially the aqueous extract of *B. bifurcata* at low concentrations. Similar results showed that the aqueous extract of *Sargassum wightii* recorded an increase in yield and quality of *Vigna radiata* fruits (Kumar et al. 2012). According to Sarhan and Ismael (2014), algal extracts significantly increase cucumbers' number of flowers and fruits. This can be explained by algal extracts containing high amounts of auxins, cytokinins, and betaines, which affect cell division in the early stages of growth and consequently increase fruit yield (Roussos et al. 2009). The reproductive phase has higher nutrient requirements, which are met by applying aqueous extracts of *B. bifurcata* and *C. gibraltarica,* which contain both macro and micronutrients. Fertilization (extracts and amendments) by the three brown algae improved the fruit quality (brix, soluble sugars, diameter, firmness, maturity index) of pepper, especially the alga *B. bifurcata*, which showed higher efficiency on the fruit quality of both crops. It can also be noted that the low concentrations of the three brown algae showed maximum values of pepper fruit quality.

Similarly, according to Ali et al. (2016), plants treated with *A. nodosum* alga in extract form significantly improved fruit quality. This study revealed clearly that fertilization with extracts or amendment improves the soluble sugar content of pepper fruits. Increasing soluble sugar content also contributes to the fruit's nutritional value, except for MI on the amendment (FS at 48.45) that shows a lower result than Witness (53.39).

In conclusion, fertilization with the three brown algae improved all studied parameters. In particular, the two algae, *B. bifurcata* and *F. spiralis* highly efficiently increased pepper's growth, yield, and fruit quality. The three algae used in our study effectively develop biostimulants to improve the vegetable plant's yield and fruit quality.

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Effects of organic fertilizers on growth and yield of field crop cowpea

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Abstract. *Balkrishna A, Gautam AK, Sharma N, Arya V, Khelwade V, Arya D. 2024. Effects of organic fertilizers on growth and yield of field crop cowpea. Asian J Agric 8: 32-40.* The present study was carried out to evaluate the efficacy of organic fertilizers on the growth and yield parameters of an annual herbaceous legume crop, cowpea (*Vigna unguiculata* (L.) Walp.). Randomized Complete Block Design (RCBD) with five fertilizer treatments was used in the Factorial experiments, and Farm Yard Manure (FYM) was used as a base fertilizer in the experimental field. Five different organic fertilizers in six treatment combinations as T0 (Control), T1 (Jaivik Prom), T2 (Pori Potash), T3 (Jaivik Khad + Poshak), T4 (Jaivik Poshak), T5 (Jaivik Khad) were evaluated during the present study. The plant parameters like plant height, shoot biomass, leaf area, total chlorophyll, and yield of pods and seeds parameters were investigated. The results revealed that all fertilizer treatments can enhance crop growth from the initial vegetative phase to the late production stage. A positive impact of all fertilizers on growth and yield was observed on plants at all-time points of 30, 60, and 90 days after sowing. The application of Jaivik Poshak in combination with Jaivik Khad proved significant. Their application also enhanced concentrations of total and available nutrients (NPK) in all treatments. The present study provides important information on the organic fertilizers used in agriculture and concludes that these fertilizers can efficiently enhance the growth and yield of crops, including cowpea.

Keywords: Cowpea, effects on growth and yield, field crop, FYM, organic fertilizers

INTRODUCTION

Using organic fertilizers is an environment-friendly initiative that can help in improving soil fertility and conservation and increase and produce quality food grains (Bisht and Chauhan 2021). While chemical fertilizers benefit the crop at a faster pace in comparison to organic fertilizers, they deteriorate the fertility of the soil and render the soil susceptible to erosion. On the other hand, organic fertilizers make soil nutritionally rich, restore fertility over time, and are comparatively safer (Uddin et al. 2023). Organic fertilizers provide balanced nutrition to crops and enrich microbial diversity in the soil, making nutrient availability to crop plants easy. In addition, these are not only safe to use but also more effective for plant growth and yield (Kleinman et al. 2002; Gezahegn 2021). Their use helps to improve soil fertility and modifies soil properties like biological activity, exchange capacity, nutritional balance, structure, and water retention capacity (Bastida et al. 2008; Hargreaves et al. 2008). The improved soil properties provide a productive energy source for soil microorganisms and crop development (Graham et al. 2017). It is now proven that organic fertilizers have a long-term potential to improve and sustain the soil quality and productivity of field crops (Ahmad et al. 2013; Shaheen et al. 2014).

Cowpea (*Vigna unguiculata* (L.) Walp.) is an annual summer season crop generally grown in arid and semiarid regions. It is used as a vegetable and remains in consumption throughout the year. The seeds contain proteins, dietary fibers, carbohydrates, iron, magnesium, and various vitamins and are a rich source of essential amino acids: lysine, leucine, and phenylalanine (Bhat et al. 2013). It is a legume crop that produces root nodules and contains nitrogen-fixing *Rhizobium* bacteria that fix atmospheric nitrogen into biologically useful ammonia that the host plant can assimilate. The ammonia produced in excess is expelled into the soil and plant tissues surrounding the nodule (Negi et al. 2006). As legume crops need a high phosphorous requirement for nodule development and optimal growth (Madukwe et al. 2010; Zhang et al. 2023), organic fertilizers can be a beneficial option in acid-aluminum-rich soils to fulfill this requirement to promote crop growth.

India imports most of its cowpeas from Brazil, Madagascar, and Thailand, which shows the need for improvement in crop productivity. Although inorganic fertilizers can enhance production, their application can destroy soil structure and pollute air and water (Lin et al. 2019; Bisht and Chauhan 2021). In contrast, organic fertilizers are reported to mitigate several harmful effects on crop plants (Bi et al. 2009; Yan and Gong 2010; Lin et al. 2019). A study carried out by Masarirambi et al. (2010) on red lettuce, Abbas et al. (2011) on mungbean as well as Jannoura et al. (2013) and Wazir et al. (2018) on peas displayed the advantageous effects of organic fertilizers. The enhanced growth and yield characteristics of food crops like wheat and vegetable crops like capsicum and carrot were recently investigated and proven (Balkrishna et al. 2024a,b,c). It is now documented that the vegetables growing through organic farming depicted better growth and yield with significant nutritional components. Enhanced growth of leafy vegetables was observed in organic farming practices with extended growth and harvest periods (Xu et al. 2003; Mohamad et al. 2022). Based on the advantages of organic farming on the growth and yield potential of different crops, the present study was conducted to study the effects of organic fertilizers on the growth and yield of cowpeas, considering the health of the environment, producers, and consumers.

MATERIALS AND METHODS

Study site and experimental material

The study was conducted from June to September 2023 at the experimental farms of Patanjali Organic Research Institute (PORI), Haridwar, Uttarakhand, India (29.83° N and 78.13° E, and 314 m (1,030 ft) ASL). Over the experiment, the temperature was varied between 23°C to 30°C, while precipitation varied widely between 2.5-20 mm. The whole experimental field was ploughed well to ensure the distribution and mixing of soil. The Ankur VU-5 cowpea variety was used for ploughing in this study. Soil properties like pH, EC, organic carbon, available nitrogen, phosphorus, and potassium were analyzed as per standard procedures (Jackson 1973).

Experimental design

The experimental field was prepared in Randomized Complete Block Design (RCBD) with five fertilizer treatments with a single control replicated three times. The plots of (5 × 3.5 m) 17.5 m^2 were prepared in the field for the experiment and treated with organic fertilizers alone or in combination. Five different organic fertilizer levels were evaluated in this experiment in 6 treatment combinations as T0 (Control), T1 (100% RFD) (Jaivik Prom @100 kg/ac), T2 (100% RFD) (Pori Potash @100 kg/ac), T3 (50% RFD) (Jaivik Khad + Poshak @40 + 3.5 kg/ac), T4 (Jaivik Poshak @7 kg/ac), T5 (Jaivik Khad @80 kg/ac) (Balkrishna et al. 2023). The Farm Yard Manure (FYM) was used as a base fertilizer in the experimental field.

Jaivik Prom is an organic manure high in phosphate and a vital source of nutrients for bio-farming. It is composed of rock and bio-residue and has a phosphorus content. Similarly, Pori Potash is a green chemistry potash fertilizer (K_2O) made of potash extracted from molasses. Jaivik Khad is derived from organic waste and contains nutrients, organic matter beneficial microorganisms that promote the growth and development of field crops. Jaivik Poshak is a mycorrhiza-based granular biofertilizer with a combination of micronutrient nano compounds that are extremely potent. Humic acid, amino acids, seagrass, natural nutrients, and a variety of significant and important herbs and remedies are the ingredients of its composition (Balkrishna et al. 2023).

The sowing of seeds was carried out with the dimensions as the distance between seeds (S-S) of 10 cm, between rows (R-R) 40 cm, and an average of 96 seeds in 6 lines per plot. Seed treatment of bio-pesticide (*Trichoderma* + *Pseudomonas*) @5 mL/liter each was carried out by soaking them overnight before sowing to avoid the attack of any soil-borne pathogen. The trial consists of 18 ploughed plots arranged as a randomized block design. Light irrigation was done immediately after sowing and later when required. Organic fertilizers were applied every 30 days after sowing.

Observation and analysis of plant growth parameters

Moreover, 9 plants were randomly selected for each treatment (three from each replicate) to observe and analyze growth parameters. The experimental data on plant height (cm), total biomass (root and shoot) per plant (gm), nodules count, leaf area (cm^2), number of pods per plant, pods weight (gm) per plant, pods length per plant, pods weight per plant, number of seeds per pod, seed weight, and seed yield per plot were measured at 30, 60 and 90 days after sowing. The total chlorophyll content was measured with the help of the SPAD meter and expressed as the SPAD meter value. The Relative Growth Rate (CGR), Net Assimilation Rate (NAR), and Agronomic Efficiency (AE) were calculated using the following formula:

Net Assimilation Rate (NAR) = (W 2-W 1/Ti 2-Ti 1) (InLA 2-InLA 1/A 2-A 1)

Where:
W 2 and W 1 : Total dry matter at successive stages,
Ti 2 and Ti 1 : Time interval
LA2 and LA1 are the natural logs of leaf areas A1 and A2 at times Ti 1 and Ti 2.
W2: Total dry matter at time Ti 2
W1: Total dry matter at time Ti 1
ln(LA2): Natural log of leaf area at Ti 2
ln(LA1): Natural log of leaf area at Ti 1
A2: Leaf area at Ti 2
A1: Leaf area at Ti 1

Agronomic Efficiency (AE) (%) = (Increase in Yield due to Fertilizer /Amount of Fertilizer Applied) ×100

Increase in yield due to Fertilizer = Yield with Fertilizer - Yield without Fertilizer (Control)

Statistical analysis

The obtained experimental data is presented as mean ± Standard Deviation (SD) and analyzed by ANOVA (one-way) and Dunnett's multiple comparisons test using GraphPad Prism version 8.02 for Windows.

RESULTS AND DISCUSSION

The application of organic fertilizers significantly impacted the growth and yield parameters of the Cowpea crop (Figures 1-7 and Tables 1-2). Their application led to an increase in the soil's Available Nitrogen (AN), Available

Phosphorus (AP), and Available Potassium (AK). The enhanced concentrations of total and available nutrients (NPK) in all treatments over control reflected the effect of organic fertilizers on the chemical properties of the soil.

Effects of organic fertilizers on growth parameters

Shoot fresh weight and percentage biomass content

The shoot fresh weight and its biomass percentage for cowpea plants subjected to different treatments (T0-T5) at 30 days, 60 days, and 90 days are presented in Table 1 and Figure 3. In terms of shoot fresh weight (Figure 3), the results indicate that treatment T3 consistently showed the highest values across all time points, notably reaching 165.7±48.0 g at 60 days and 201.4±25.5 g at 90 days, suggesting a positive impact on cowpea plant growth attributed to the combination of VA Mycorrhiza and Javik Khad. Treatment T2 also demonstrated significant growth at 90 days, recording 195.0±54.3 g. At the 30-day time point, T3 displayed the most substantial percentage increase over the control, registering a noteworthy augmentation of 58.3% in fresh shoot weight. T2, characterized by applying organic K fertilizer, also exhibited a remarkable increase of 44.2%. Simultaneously, T1, T4, and T5 demonstrated increments of 41.8%, 39.3%, and 19.8%, respectively. Advancing to the 60-day interval, T3 sustained its preeminence by exhibiting a 68.6% increase over the control, highlighting the enduring efficacy of the combined application of VA Mycorrhiza and Javik Khad. T2 continued to manifest commendable performance, recording a 28.7% increase over the control. Subsequently, at the 90-day juncture, T3 again manifested the highest percentage increase over the control, registering an elevated value of 80.7%. Meanwhile, T2 maintained a robust performance with a 79.5% increase, and T5 demonstrated noteworthy advancement with a 36.0% increase.

Considering the correlation with moisture content and biomass percentage (Table 1), T3 consistently stood out by maintaining higher shoot biomass percentages at all time points. This observation suggests that the increase in shoot fresh weight is not solely attributable to higher moisture content. The positive correlation between shoot fresh weight and biomass percentage further implies that the observed weight increment is not merely a consequence of elevated moisture content, underscoring the significance of the treatment effects on cowpea plant biomass.

Figure 1. Field trial on cowpea crop after 30, 60, and 90 Days of Sowing (DAS)

Figure 2. Field trial on cowpea crop after 30 Days of Sowing (DAS): comparison of parameters (*upper*) and root nodules production (*lower*)

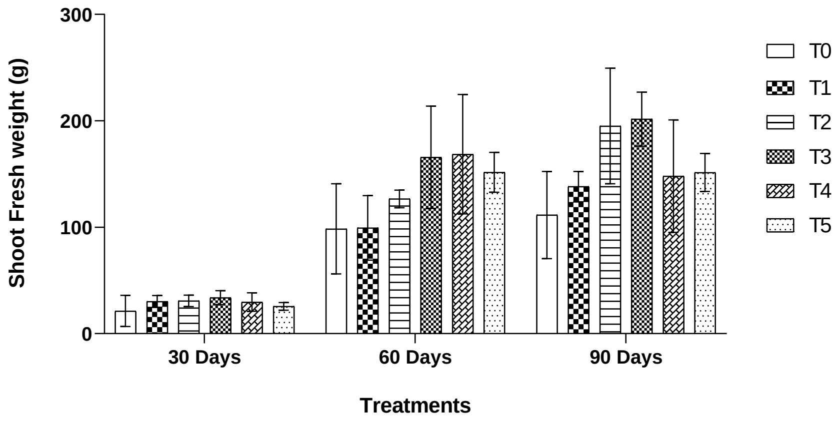

Figure 3. Comparative effects of different treatments on shoot fresh weight (g) of cowpea crop

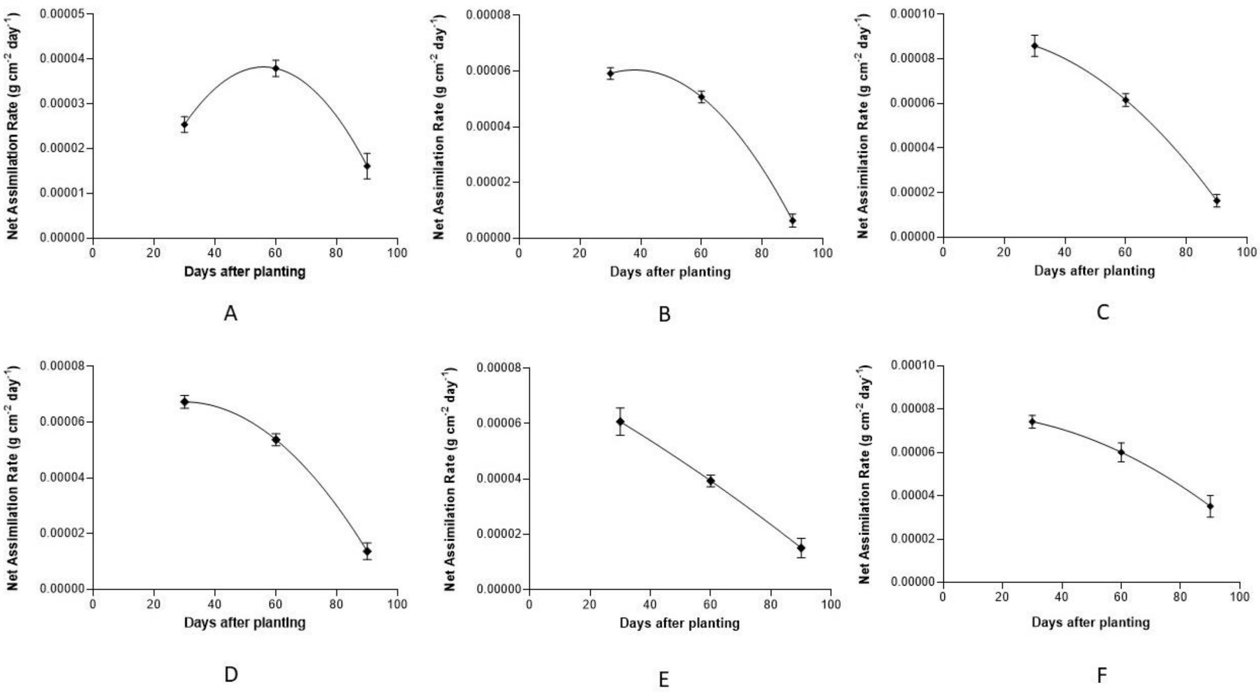

Figure 4. Net assimilation rate of cowpea plants at 30 days, 60 days, and 90 days under various treatments. A: T0, B: T1, C: T2, D: T3, E: T4, F: T5

Plant height

The shoot length or plant height data of cowpea plants reveals diverse growth patterns. At 30 days, T2 exhibited the highest shoot length (45.222±2.502 cm) and the highest increase over the control, indicating early and robust growth promotion. However, at 60 and 90 days, T3 consistently outperformed other treatments, reaching 164.222±1.359 cm and 207.556±26.492 cm, respectively, at the respective time points. T3 exhibited the highest percentage increase, indicating that this treatment has a sustained positive effect on plant growth. T5 also demonstrated promising results at 30 days and maintained competitive growth throughout the experiment (Table 1).

Leaf area and chlorophyll content

Results showed that treatments T1 and T2 showed consistent and significant increases. T1 particularly stands out as it exhibits the highest leaf area at 60 days. This suggests that the application of organic P and K fertilizers has a positive impact on the cowpea plants' leaf development. In contrast, treatments T3 and T4 elicit disparate effects on leaf area, with T3 exhibiting superior performance at the 90-day interval. It was observed that T3 exhibited highest chlorophyll content on the 30th day, T1 on the 60th day, and T2 on the 90th day; however, T2 and T4 also showed a notable increase in chlorophyll content, especially at 60 days. T1 and T5 display relatively moderate effects on chlorophyll content (Table 1).

Net assimilation rate

Moreover, it is found that the NAR values vary across treatments and time intervals. At 30 days, T2 shows the highest NAR, followed closely by T3. This suggests that treatments involving organic K fertilizer or a combination of VA Mycorrhiza and JaviK Khad may enhance the assimilation rate early in the growth period. As time progresses to 60 days, the NAR for most treatments decreases, with T2 still maintaining a relatively high rate. Interestingly, T4 (Fertilizer with VA Mycorrhiza) and T5 (JaviK Khad with organic NPK) show improvements in NAR at this stage. At 90 days, T2 and T3 continue to exhibit relatively higher rates, showcasing the sustained positive effects of these treatments (Figure 4).

Nodule count

The nodule count increased for all treatments as the duration progressed, and T3 consistently exhibited the highest nodule counts across all time points, peaking at 39.33±13.29 at 90 days. This suggests that T3 has a significant positive impact on root nodule development in cowpea plants. T1 shows a significant increase in nodule count, while T2, T4, and T5 consistently and positively impact nodule count (Figure 5).

Effects of organic fertilizers on yield parameters

Table 2 showed a positive correlation between the number of pods per plant and pod weight per plant. The T3 displayed the highest number of pods per plant (9.667±2.963), followed by T4 (8.444±4.526) and T5 (7.222±4.018). These treatments exhibited significantly greater pod numbers than the control, T0 (2.111±1.171). Moreover, T3 also showcased the highest pod weight per plant (60.111±15.420), closely followed by T4 (59.861±22.280 in comparison to the control, T0 (7.978±2.422). T3 and T4 also exhibit longer pod lengths, i.e., 27.889±0.509 and 27.500±0.167, respectively, compared to the control T0 (17.556±0.255). Aditionally, T5 also performs well in pod number but has a significant lower pod weight and length on T5 than T3 and T4. The combination of VA Mycorrhiza and Javik Khad (T3) or using a fertilizer with VA Mycorrhiza (T4) appears to be the most effective treatment for maximizing cowpea yield (Table 2).

At the harvest stage (90 DAS), a positive correlation is observed between the weight of 100 seeds and the seed yield per plot. The treatment T4 is noteworthy for presenting a relatively elevated number of seeds per pod (15.778±0.192) alongside a 100 seed weight of 8.937±0.550 g than T5 which has 15.111±0.509 seeds per pod along with a moderate 100 seed weight (8.953±0.898 g). This implies that the individual seeds exhibit a lower mass than certain alternative treatments despite a higher seeds-per-pod count. Conversely, Treatments T1, T2, and T3 showcase an elevated 100 seeds weight, signifying that each seed demonstrates greater mass while the number of seeds per pod may be comparatively lower. Treatment T2 demonstrates a notable seed yield per plot (439.363±42.590), accompanied by a substantial 100 seeds weight (9.703±0.472) and a moderate quantity of seeds per 100 grams (1130.000±45.826). Similarly, Treatment T3 exhibits a heightened seed yield per plot (444.113±52.329), along with a relatively elevated 100 seeds weight (9.327±0.265) and a comparable quantity of seeds per 100 grams (1136.667±56.862) (Table 2 and Figure 6).

Agronomic Efficiency (AE)

The study revealed the distinct treatments exhibited varying degrees of AE, providing insights into their efficacy in promoting crop productivity. Notably, Treatment T4 showcased the most notable agronomic efficiency, registering an AE of 2384.66%, indicative of a highly efficient yield response to the applied fertilizer. T3 also demonstrated significant agronomic efficiency, yielding 296.85%, an impressive result achieved with a moderate fertilizer application rate of 87 kg/acre. Additionally, Treatment T5 displayed a commendable AE of 244.70%, while T1, T2, and T7, though featuring lower agronomic efficiency values, exhibited positive responses to the applied fertilizers (Figure 7).

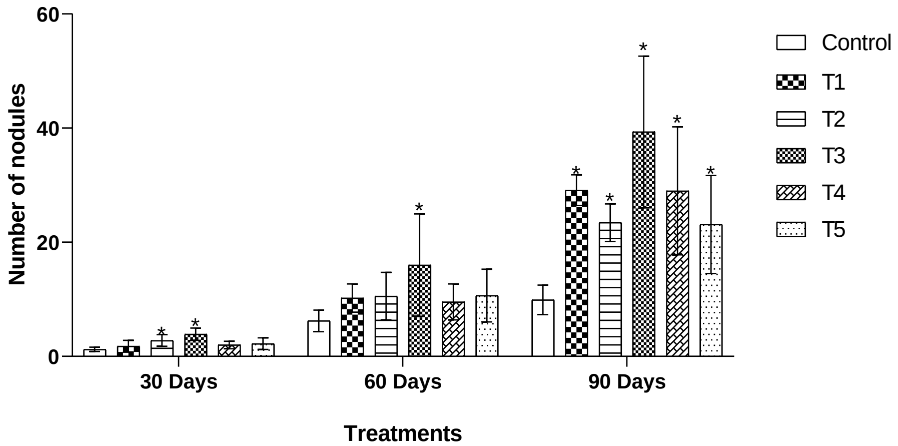

Figure 5. Comparative effects of different treatments on Nodule count of cowpea crop

Table 1. Effect of organic fertilizer treatments on growth attributes of cowpea

Treatments	30 Days				60 Days				90 Days			
	Shoot Biomass (%)	Plant Height (cm)	Leaf Area (cm^2)	Chlorophyll	Shoot Biomass	Plant Height (cm)	Leaf Area (cm^2)	Chlorophyll	Shoot Biomass	Plant Height (cm)	Leaf Area (cm2)	Chlorophyll
T0	13.76±1.30	38.67±7.84	47.67±23.10	36.54±3.75	18.36±2.05	128.78±29.64	48.22±8.6	51.42±1.54	21.297±5.336	136.67±22.85	45.0±5.77	54.82±1.36
T1	16.22±1.48*	41.67±2.91	59.22±6.88*	38.86±2.20	22.13±3.02*	154.0±16.1*	64.78±8.53**	53.92±4.67	27.439±1.386*	191.12±25.68*	46.89±1.01	60.19±3.56
T2	15.74±1.72*	45.22±2.50**	71.55±6.31*	39.69±1.60	19.94±0.43	148.0±3.59*	53.22±7.183	48.89±5.45	32.528±7.514*	204.44±40.84**	47.22±1.84	63.47±0.62
T3	16.21±0.74*	42.78±1.84	62.44±9.34**	77.60±65.65	20.72±1.07*	164.22±1.3**	62.44±9.968*	47.96±3.0	26.496±2.838*	207.56±26.49**	52.56±2.37*	62.82±1.96
T4	16.29±2.28*	43.5±5.27	58.0±6.94**	37.63±2.63	20.03±0.43	160.22±3.43**	59.667±7.219*	50.9±0.15	24.832±2.311	174.11±33.36	51.39±4.28*	57.99±0.84
T5	16.046±0.893*	44.89±0.96*	55.89±0.84*	41.08±2.71	20.66±1.98*	154.56±4.67*	56.333±1.764	50.38±5.22	24.373±1.265	160.56±40.01	50.67±12.22*	58.18±1.77

Note: Mean ± standard deviation of nine replicates. * Significant (p≤ 0. 50); ** Highly significant (p≤ 0.01)

Table 2. Effect of organic fertilizer treatments on yield attributes of cowpea

Treatments	Pods			Seeds			
	Pods No. per Plant	Weight per Plant (g)	Length per Plant (cm)	Seeds No. per Pod	100 Seeds Weight (g)	No. per 100 g of Weight	Yield per Plot
	60 Days			90 Days			
T0	2.111±1.171	7.978±2.422	17.556±0.255	6.444±0.962	8.257±0.436	1206.667±20.817	185.857±67.916
T1	3.333±2.887	22.780±19.896	23.278±15.218	14.333±1.453	9.670±0.956*	1090.000±36.056	417.910±72.263*
T2	5.222±1.836	34.411±17.035*	26.000±2.728*	14.111±1.836	9.703±0.472**	1130.000±45.826	439.363±42.590*
T3	9.667±2.963**	60.111±15.420**	27.889±0.509*	13.778±2.143	9.327±0.265*	1136.667±56.862	444.113±52.329*
T4	8.444±4.526**	59.861±22.280**	27.500±0.167*	15.778±0.192	8.937±0.550	1153.333±113.725	352.783±43.170*
T5	7.222±4.018*	39.333±14.295**	25.111±1.644	15.111±0.509	8.953±0.898	1083.333±75.056	381.620±24.449*

Note: Mean ± standard deviation of nine replicates. * Significant (p≤ 0. 50); ** Highly significant (p≤ 0.01)

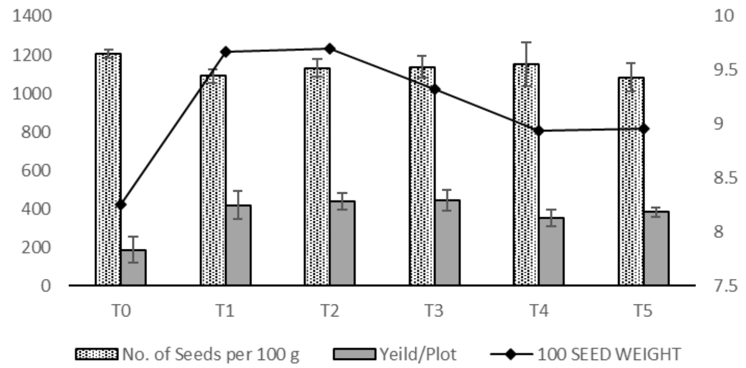

Figure 6. Comparative account on the effect of organic fertilizers treatments on yield attributes (seeds) of cowpea

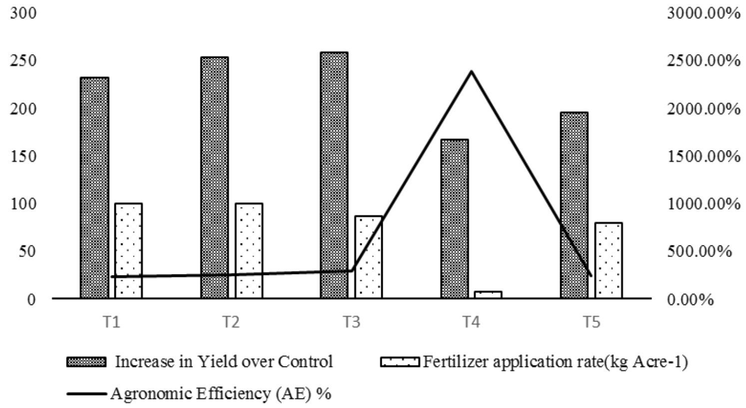

Figure 7. Agronomic efficiency of organic fertilizers used in the present study

Discussion

The positive effects of organic fertilizers on the growth and yield of field trial crops were observed during the present study. A considerable alteration in soil chemical properties to increase in Available Nitrogen (AN), Available Phosphorus (AP), and Available Potassium (AK) was observed. The enhanced concentrations of total and available nutrients (NPK) in all treatments over control reflected the effect of organic fertilizers on the chemical properties of the soil. The changes in chemical characteristics of the soil were also observed in the growth and yield of cowpea. The treatment T3, involving a combination of VA Mycorrhiza and Javik Khad, consistently outperforms others, exhibiting the highest percentage increase in fresh shoot weight and promoting root nodule formation. This synergistic effect enhances plant growth and nutrient assimilation, making T3 the most effective strategy over 90 days. Both fertilizers are fortified with microbial consortium and are a rich source of N, P, and K. The presence of microorganisms enhances the nutrient uptake of the plants (Solomon et al. 2012). While organic fertilizers foster consistent growth and yield, the ample supply of nutrients N, P, and K through organic fertilizers can enhance nutrient uptake, including phosphorus, a crucial element for chlorophyll synthesis (Surya et al. 2022). The treatment T2, featuring organic K fertilizer that enables the plants to regulate the opening and closing of the stomata, the exchange of water vapor, oxygen, and carbon dioxide and consistently demonstrates positive effects on plant development and yield (Amanullah et al. 2016; Xu et al. 2020). The observed weight increment is not merely a consequence of elevated moisture content, underscoring the significance of the treatment effects on cowpea plant biomass. Here also, T3 consistently stood out by maintaining higher shoot biomass percentages at all the time points. The combination of Jaivik Poshak and Javik Khad again justified a significant effect. Earlier studies also demonstrated significant effects on the growth and yield of hot pepper and tomato (Akande et al. 2018; Felföldi et al. 2022; Sadek et al. 2023; Gao et al. 2023) on mixed fertilizer application. Moreover, Mycorrhiza-rich fertilizer Poshak is known to improve the nitrogen content of the soil (Wang et al. 2023; Balkrishna et al. 2023). The application of combined fertilizers contributes to the higher chlorophyll content, which may be due to the ample supply of NPK (Sharma and Agarawal 2009; Aishwarya et al. 2022; Manjula et al. 2022; Balkrishna et al. 2024a). A similar reason may apply to nodule count. The availability of nitrogen by application of organic fertilizers also proved that T3 consistently exhibited the highest nodule counts. This observation was supported by the studies of Wamalwa et al. (2019) and

Khalofah et al. (2022). Moreover, mycorrhiza-based fertilizers performed well because of their ability to absorb micronutrients, even if they are available in trace amounts (Aishwarya et al. 2022).

The observation on pods and seeds yield parameters also supports the results of growth parameters of plants grown on T3. The application of organic fertilizers supplies directly available nutrients such as nitrogen to the plant and improves the proportion of water-stable aggregates in the soil (Khetran et al. 2017; Yin et al. 2018; Rashid et al. 2021; Balkrishna et al. 2023). Moreover, applying mixed fertilizers promoted the absorption of essential nutrients from the surrounding soil. Similarly, organic fertilizers ensure the long-term availability of essential nutrients to plants (Mohamed et al. 2019; Olaniyan et al. 2022; Zhou et al. 2022; Balkrishna et al. 2024a,b,c). Adding organic K fertilizer in T2 consistently demonstrated favorable outcomes in cowpeas. These results highlight the importance of organic fertilizers in influencing different aspects of seed development and yield in crops (Zhou et al. 2022).

In summary, the results underscore the importance of organic fertilizers to enhance cowpea plants growth and development. The general observations during this study revealed that applying organic fertilizers is a healthy practice as they release nutrients in the soil at a controlled rate. As a result, they do not disturb the ratio of nutrients already present in the soil and maintain the soil's microbial population. Therefore, the present study concludes that applying organic fertilizers significantly affects the overall growth and yield of the cowpea crop.

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Status of basal stem rot disease on areca nut palm plantations in Kubu Raya District, West Kalimantan, Indonesia

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Abstract. *Supriyanto, Sulistyowati H, Arifin N. 2024. Status of basal stem rot disease on areca nut palm plantations in Kubu Raya District, West Kalimantan, Indonesia. Asian J Agric 8: 41-49.* Areca nut (*Areca catechu* L.) is one of the leading plantation commodities with high economic value in West Kalimantan Province, Indonesia. One of the problems that affects the productivity of areca nut plantations is the presence of pathogen attacks, especially attacks by the fungus *Ganoderma* sp. which is the main pathogen in Arecaceae (palmae) plants. *Ganoderma* cause basal stem rot disease which is still difficult to control. The objective of this research was to determine the incidence level of basal stem rot disease and the diversity of fungi which cause disease in areca nut plantations in Kubu Raya District. The experiments were conducted using plant census methods and the collection of fruiting bodies of pathogenic fungi. The collected fungi were isolated, morphologically identified, and then confirmed it pathogenicity by Koch's postulates. The results showed that the incidence of areca nut basal stem rot disease in Kubu Raya District was relatively low (1.99%). The incidence of disease was found to be higher (4%) in poorly maintained plantations. A total of 5 types of fungi were found associated with areca nut basal stem rot disease, namely 3 types of *Ganoderma* sp., and 2 types of *Trametes* sp. However, only the type of *Ganoderma* has proven capable of causing disease in areca nut palm plants.

Keywords: Areca nut**,** basal stem rot, *Ganoderma*, Kubu Raya, *Trametes*

Abbreviations: BPS: *Badan Pusat Statistik*, Kalbar: *Kalimantan Barat*, PDA: Potato Dectrose Agar

INTRODUCTION

The areca nut (*Areca catechu* L.) is one of the leading plantation commodities which has high economic value in West Kalimantan Province, Indonesia. The largest areca nut plantations in West Kalimantan Province are in Kubu Raya District with an area of 1,524 ha managed by around 3,875 farmers. In the last 5 years, areca nut production in Kubu Raya has increased rapidly, from 608 tonnes in 2017, to 1,849 tonnes in 2021 (67%). This is due to the ever-increasing demand and increasingly promising selling price offers. Currently, areca nut plants are the plantation commodity with the highest area and production in Kubu Raya District after oil palm, rubber, and coconut (BPS Kubu Raya 2022). Areca nuts are currently also a leading export commodity from West Kalimantan and are included as one of the five leading export commodities from West Kalimantan, and are widely export to India, Bangladesh, Iran, Afghanistan, China, and Myanmar with a total export volume of 3,643.21 tons (Ministry of Agriculture 2022).

Considering the high economic opportunities, the West Kalimantan Provincial Government tried increasing areca nut production. One of the programs that was being promoted was increasing the areca nut planting area in all districts in West Kalimantan. This program was contained in the West Kalimantan Provincial Government's Strategic Development Plan Document for 2019-2024, where it is targeted to realize new plantation crops covering an area of around 728,000 ha, including areca nut plants (Disbunnak Kalbar 2023). This program targets areca nut plants that can be developed in several districts, namely Kubu Raya, Mempawah, Sambas, Sanggau, Sekadau, Sintang, and Kapuas Hulu. For the province of West Kalimantan, the areca nut commodity was considered strategic because it could be an alternative income for plantation farmers. Apart from that, areca nut was also considered an alternative commodity when there was a decline in the prices of other plantation commodities such as palm oil, rubber, and coconut. This is because, in the last two decades, plantation commodity prices have always experienced relatively high fluctuations. Based on BPS records, the highest fluctuations in plantation commodity prices in West Kalimantan occurred in pepper, rubber, and palm oil (BPS West Kalimantan 2022). Thus, the development of areca nut commodities in West Kalimantan has strategic value to support the stability of the welfare of plantation farmers.

One of the factors that influences the yield and productivity of areca nut plantations is the presence of pathogen attacks, especially by the fungus *Ganoderma* sp. which is the main pathogen in palmae plants (Lim and Fong 2005; Paterson 2007; Flood et al. 2010; Prasetyo and Simanjutak 2017). As in other palmae plantations, such as oil palm and coconut, *Ganoderma* attacks which cause stem rot disease are a serious problem that is still difficult to control (Rakib et al. 2015; Supriyanto et al. 2022). In addition, considering that areca nut plantations in West Kalimantan are generally converted the land from the secondary forest, usually, these pathogens are naturally present in the land, so the potential for areca nut plants to be attacked is also relatively high (Supriyanto et al. 2021).

In a cursory field observation, in areca nut plantations in Kubu Raya District, relatively many diseased plants were found, which were believed to be basal stem rot disease caused by *Ganoderma* sp. However, until now no in-depth observations have been carried out either to quantify the value of the losses or as an effort to find out how to control them. Even though there is a research report based on interviews that states that the areca nut plantations owned by farmers in Kubu Raya District have not yet shown any pest or disease attacks, this still requires further research to ensure the truth based on actual data in the field (Sulistyowati et al. 2023).

Considering the growing interest of the people of Kubu Raya District and the support of the West Kalimantan provincial government in areca nut cultivation, potential losses and yield losses that may arise in the future need to be anticipated early on. One of them is the need for further observation regarding suspected symptoms of *Ganoderma* sp. attacks, by observing the plantation in more detail. The aim of this research was to find out the status of *Ganoderma* attack which causes basal stem rot disease on areca nut plants in Kubu Raya District, and collecting the basic data relating to the diversity of disease-causing pathogens, patterns of disease spread, and environmental factors that influence it. This is very important basic data, especially in relation to disease control and management efforts in areca nut plantations.

MATERIALS AND METHODS

Study area

The research was conducted in May-November 2023. The research covered the entire administrative area of Kubu Raya District (108° 35'-109° 58' East Longitude, 0° 44' N-1° 01' South Latitude), West Kalimantan Province, Indonesia. The study covered 9 sub-districts, namely Batu Ampar, Terentang, Kubu, Teluk Pakedai, Sungai Kakap, Rasau Jaya, Sungai Raya, Sungai Ambawang, and Kuala Mandor B (Figure 1).

Determination of areca nut plantation samples

The plantation site samples were determined based on the representativeness of the sub-district area. The number of samples for each sub-district was determined based on the proportion of plantation area based on BPS data from Kubu Raya District. Plantation site samples were taken randomly based on the proportion of garden area in each sub-district. The initial database used is the latest plantation area data released by the BPS Kubu Raya District (2022). The sample population taken was around 2.5% of the population based on the officially recorded plantation area. The population sample distribution was carried out based on data on the area of the plantation area in each sub-district. The site of areca nut palm plants was categorized as plantations if they are planted in one area of at least 0.5 ha, or consist of at least 200 plants.

Areca nut plants census

The research included two stages, namely plant census stage and collecting samples of fungal fruiting bodies infected with stem rot disease in the field. Data collection on diseased plants was carried out using the plant population census method. A population census was carried out on a sample site selected in a structured random manner. The plant population census was carried out using the cruise method in which, the population was counted for each sample garden and the number of plants experiencing stem rot disease was counted. Quantification of disease incidence was carried out by comparing diseased plants with the total number of plants in the plantation in each sample site. Diseased plants were determined based on morphological symptoms on the plant and the presence of signs of disease in the form of fungal fruiting bodies.

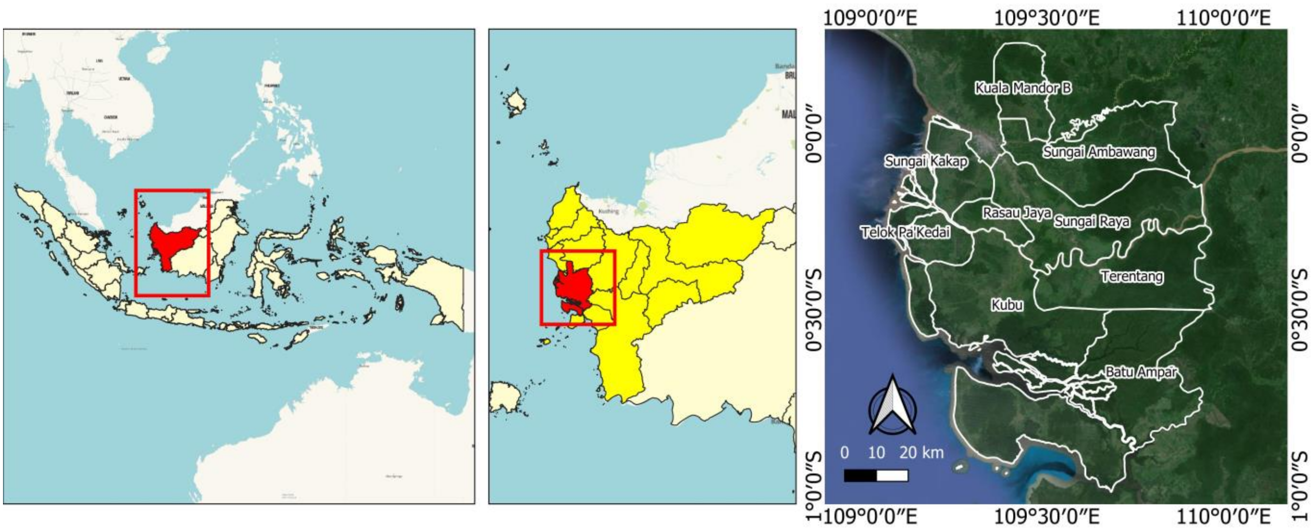

Figure 1. The map of study site in Kubu Raya District, West Kalimantan Province, Indonesia

Table 1. Criteria for scoring disease symptoms on areca nut seedling leaves

Score	Symptoms
0	Healthy seedlings, green leaves, no visible fungal mass on the seedlings.
1	1-3 chlorotic leaves without showing any fungal masses on the seedlings
2	Seedlings show a mass of fungus anywhere
3	More than 3 chlorotic leaves, necrotic leaves with or without fungal masses on seedlings
4	At least 50% of leaves had chlorosis or necrosis with or without fungal masses on seedlings
5	Seedlings die with or without the appearance of fungal masses on the seedlings

Collecting samples of fruiting bodies of disease-causing fungi

Samples of pathogenic fungi were collected from the field by taking fungal fruit bodies found on the stems of diseased arecanut palm plants. Three samples of macroscopic fruiting bodies were taken from each diseased plant, placed in a plastic bag, and taken to the laboratory for isolation and identification. Identification of pathogenic fungus was carried out by observing the morphology, such as shape, color, size, and special characteristics of the fruiting body and matching them with the characteristics in guidebooks (Alexopoulos et al. 1996; Minarsih et al. 2011).

Fungal isolation and inoculation test on areca nut seedlings

Isolation of fungus and transmission test on areca nut seedlings were performed according to the method of Supriyanto et al. (2023). After identification, each group of fungi was isolated on a Potato Dextrose Agar (PDA) medium. Isolation was carried out by using a sterile scalpel to take a 0.5 × 0.5 × 0.5 cm piece of the inside of the fungal fruiting body. Before cutting, the outer surface of fungal fruiting body was washed thoroughly in tap water, followed by wiping using cotton wool moistened with 96% alcohol, and left to dry. The sections were placed on the PDA surface in a Petri dish and incubated for 6 days. After the mycelium grew on the surface of the PDA, mycelium was transferred to a new PDA until a pure isolate was obtained. After obtaining a pure isolate, the fungus was grown on 6 × 6 × 6 cm rubber wood blocks. Before use, the rubber wood block was soaked overnight, washed thoroughly, wrapped in a heat-resistant plastic bag, and autoclaved for 45 minutes. After cooling, rubber wood was inoculated with a fungal mycelium culture previously grown in a corn-water medium (Supriyanto et al. 2011). The rubber wood was then incubated at room temperature for 4-8 weeks. Rubber wood blocks were ready for use when the entire surface of the wood was covered with fungal mycelium. The infection test on areca nut seedlings was carried out in polybags in the following way. Areca nut seedlings in the form of shoots were planted on rubber wood blocks in which pathogenic fungi were grown and then covered with soil. The seedlings were then cultivated for 6 months in 50% para net shade. Maintenance included watering to keep the media within field capacity and controlling weeds around the seedlings. To determine the success of disease transmission, disease symptoms were observed in the seedlings. Observation of disease symptoms was carried out by scoring following the method of Rakib et al. 2015 (Table 1), and was carried out once a month. Disease intensity was calculated using the formula of Arwiyanto et al. (1994):

$$DI = \frac{\sum_{i=1} k \times nk}{Z \times N} \times 100\%$$

Where:
DI : Disesase intensity
nk : Number of plants with a score of k (k:0,1,2,3,4)
k : Score used
Z : Highest score
N : Number of plants observed

Observation of disease spread patterns

Observation of disease spread patterns was carried out in the following way. In each plantation where there was more than one diseased plant, the distance between the diseased plants was measured. Based on this distance, the distribution pattern was determined using the nearest neighbor analysis method (Clark and Evans 1954; Kamu et al. 2015, 2016).

RESULTS AND DISCUSSION

Condition of areca nut plantations in Kubu Raya District

Based on observations, areca nut palm plants are spread throughout all sub-districts in Kubu Raya District. However, each sub-district had a different area of areca nut plantations. In Sungai Ambawang District, there are many areca nut palm plants, both in home gardens and in community gardens. In general, the characteristics of areca nut plantations in Kubu Raya District were relatively varied, both in distribution, area, cultivation methods, and plant conditions. In this study, each plantation site sample was categorized into two groups of characteristics, namely cultivation method (monoculture/mixed) and maintenance status (maintained: not/maintained). These characteristics were taken considering that environmental conditions greatly influence the level of emergence of basal stem rot disease.

Based on the method of cultivation, areca nut plantations in Kubu Raya District were generally cultivated using a polyculture or mixed method. Areca nut palm plants cultivated in polyculture are more than 78.12% higher than those planted in monoculture (21.88%). Generally, areca nut palm plants were planted together with other plants, both plantation crops and fruit crops such as coconut, oil palm, rubber, durian, sugar cane, langsat, cempedak, jackfruit, banana, coffee, cassava, lemongrass, jabon, etc. In Teluk Pakedai District and Sungai Raya District, it was almost planted in polyculture (Table 2).

Areca nut palm plants planted in polyculture were generally planted in rows between other plants, or planted in ditch embankments to strengthen the soil structure so that it did not collapse easily. In Sungai Kakap District, in several places, it was found that areca nut plants were also planted between rice plants.

The method of cultivating areca nut plants in Kubu Raya District, both polyculture and multiculture, was generally also related to plantation conditions. In areca nut plantations planted in monoculture, they were generally in a well-maintained condition. Meanwhile, areca nut plantations planted in polyculture were generally found to be in conditions that were poorly maintained. In general, based on the samples observed, almost half of the areca nut plantations in Kubu Raya District were in poor condition, up to 46.88% of the total sample plantations (Table 3). The unmaintained sample plantations studied were found to have uncontrolled weeds, plantation cleanliness was not maintained, never fertilized, and plant pest organisms were not controlled. Apart from that, in general, areca nuts, which were the main crop in some sample plantations, were not harvested and post-harvest processed properly. In some locations, areca nuts were even left unpicked for years. Generally, this may be related to fluctuations in the price of areca nut. If the price increases, the fruit could usually be harvested and processed properly.

These results indicate that the management of areca nut plantations in Kubu Raya District were not being carried out well. Especially in mixed plantations, maintaining for areca nut palm plants only depends on maintaining for the other plants around them. In Sungai Kakap District, the well-maintained areca nut plantations were mixed plantations with langsat and durian plants. When farmers maintain their durian trees and langsat trees, they also maintain other trees, including areca nut palm trees on the plantation. In contrast to areca nut plantations which were planted together with rubber plantations, when the price of rubber decreased, the plantations were generally not maintained, so the areca nut palm plants around them were also not maintained.

Status of areca nut basal stem rot disease in Kubu Raya District

Basal stem rot disease on areca nut can be identified by the presence of symptoms that appear on the leaves, such as paleness, wilting, yellowing, drooping of the leaf, and the appearance of spear leaves (young leaves that did not open). Apart from that, it can also be seen by the appearance of signs of disease in the form of fruiting bodies of pathogenic fungi at the base of the areca nut stem or trunk (Figure 2). These symptoms were very similar to the symptoms of basal stem rot disease in oil palm (Idris et al. 2000; Rakib et al. 2015; Supriyanto et al. 2020). Every plant that showed these symptoms were considered a diseased plant. Plants that die and showed signs of disease were also considered as diseased plants.

Based on the population census and basal stem rot disease census conducted in each sample site, data were obtained that basal stem rot disease occured evenly in almost all sample sites and was the main cause of areca nut plant death. Basal stem rot disease occurs both in areca nut plants grown in monoculture, polyculture and both in well-maintained and unmaintained plants . In monoculture plantations, basal stem rot disease was not found only in Teluk Pakedai District and Sungai Raya District. However, this may be because the plants were young and not yet fully grown. Generally, basal stem rot disease is found in old plants because it is associated with the development of pathogenic fungi that are relatively slow to develop (Naher et al. 2015; Lisnawita and Tantawi 2016; Muniroh et al. 2019). These results indicate that all areca nut plantation locations in Kubu Raya District have the potential to be infected with basal stem rot disease so the future development of areca nut plantations requires more serious attention.

Table 2. The cultivation methods of areca nut palm in Kubu Raya District, West Kalimantan Province, Indonesia

Sub-districts	Cultivation methods				Number of sample sites
	Monoculture	Percent	Policulture	Percent	
Kubu	1	33.3	2	66.7	3
Teluk Pakedai	0	0	3	100	3
Sungai Kakap	2	16.67	10	83.3	12
Rasau Jaya	1	50	1	50	2
Sungai Raya	0	0	2	100	2
Kuala Mandor B	3	30	7	70	10
Kubu Raya	7	21.88	25	78.12	32

Table 3. Conditions of areca nut plantations in Kubu Raya District, West Kalimantan Province, Indonesia

Sub-districts	Plantation conditions				Number of sample sites
	Well-maintained	Percent	Poorly-maintained	Percent	
Kubu	2	66.67	1	33.3	3
Teluk Pakedai	2	66.67	1	33.33	3
Sungai Kakap	7	58.33	5	41.67	12
Rasau Jaya	1	50	1	50	2
Sungai Raya	1	50	1	50	2
Kuala Mandor B	4	40	6	60	10
Kubu Raya	17	53.12	15	46.88	32

Table 4. The incidence of basal stem rot disease in areca nut based on cultivation methods in Kubu Raya District, West Kalimantan Province, Indonesia

Sub-districts	Disease Incidence				Average (%)
	Monoculture	Percent	Polyculture	Percent	
Kubu	2/274	0.73	12/513	2.34	1.54
Teluk Pakedai	0	0	24/780	3.08	1.54
Sungai Kakap	34/671	5.07	187/3960	4.72	4.90
Rasau Jaya	5/344	1,45	0/260	0	0.73
Sungai Raya	0	0	9/681	1.32	1.32
Kuala Mandor B	8/688	1.16	56/2121	2.64	1.90
Kubu Raya	49/1977	1.40	288/8315	2.35	1.99

Table 5. The incidence of basal stem rot disease in areca nut based on plantation conditions in Kubu Raya District, West Kalimantan Province, Indonesia

Sub-districts	Disease incidence				Average (%)
	Well-maintained	Percent	Poorly-maintained	Percent	
Kubu	2/274	0.73	12/513	2.34	1.535
Teluk Pakedai	4/572	0.69	20/208	9.62	5.155
Sungai Kakap	57/2472	2.31	164/2159	7.59	4.95
Rasau Jaya	5/344	1.45	0/260	0	0.725
Sungai Raya	2/301	0.66	7/380	1.84	1.25
Kuala Mandor B	10/971	1.029	54/1838	2.94	1.98
Kubu Raya	80/4934	1.14	257/3524	4.06	2.59

Table 6. The incidence of basal stem rot disease in areca nut based on plantation conditions in Kubu Raya District, West Kalimantan Province, Indonesia

Sub-districts	Isolated species									
	Ganoderma sp. 1		*Ganoderma* sp. 2		*Ganoderma* sp. 3		*Trametes* sp. 1		*Trametes* sp 2.	
	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%
Kubu	1	0.29	3	0.87	9	2.62	1	0.29	0	0
Teluk Pakedai	1	0.29	20	5.81	2	0.58	1	0.29	0	0
Sungai Kakap	4	1.16	214	62.20	3	0.87	4	1.16	1	0.29
Rasau Jaya	1	0.29	4	1.16	0	0	0	0	1	0.29
Sungai Raya	0	0	7	2.03	0	0	1	0.29	2	0.58
Kuala Mandor B	5	1.45	58	16.86	1	0.29	0	0	0	0
Kubu Raya	12	3.49	306	88.95	15	4.36	7	2.03	4	1.16

Figure 2. Disease symptoms of areca nut basal stem rot disease

Based on the cultivation method, the intensity of basal stem rot disease in areca nut was higher in polyculture than in monoculture plantations (Table 4). On average, disease intensity in polyculture was 2.35%, while in monoculture it was only 1.4%. This result is likely related to the origin of the areca nut plantations in Kubu Raya District, which were generally former forests, so the pathogen was already in the land before the plantations were built. As was already known, the pathogenic fungus that causes basal stem rot generally comes from *Ganoderma* sp., a fungi inhabiting tropical rainforest habitats (Paterson 2007). This is also related to the wide host range of *Ganoderma* (Hasan et al. 2005; Hushiarian et al. 2013). Coconut, rubber, langsat, durian, jackfruit, and cempedak, are also hosts for *Ganoderma* sp. that cause stem rot disease (Susanto 2009).

Based on the condition of plantations, basal stem rot disease in areca nut was known to occur more often in areca nut plants that were not well maintained. In areca nut plantations that were not well maintained, the incidence of disease was much higher (4.06%) than in those that were well maintained (1.14%) (Table 5). Unmaintained plantation conditions are very favorable for the development of the disease (Alizadeh et al. 2011; Kok et al. 2013; Widiastuti et al. 2018; Puspita et al. 2022). In plantations that are unmaintained, generally, the environmental conditions of the plants may be very humid because the weeds are not controlled, there is a lot of organic material available that can support the pathogen's survival, and the plants are less vigorous, so they are more susceptible to pathogen attacks. Meanwhile, in relatively well-maintained plantations, with relatively clean conditions, not a lot of organic material, and more vigorous plants, the disease will develop less, so the incidence is also low.

These results indicate that well-maintained plantations were able to prevent the development of basal stem rot disease, both on areca nuts planted in monoculture or planted in a mixture with other plants. Thus, plantation maintenance was one of the important factors that must be carried out in efforts to control this disease.

Fungal diversity associated with areca nuts basal stem rot disease in Kubu Raya District

Based on observations of the shape and color of the fruiting bodies of fungi collected from plantation samples, there were five different groups of fungi. The first group was fungi with stalkless, oyster-shaped fruiting bodies, thick and hard, rough and shiny upper surfaces, dark brown, with a diameter of 5-16 cm (Figures 3.C-D). The second group was fungi with oyster-shaped fruiting bodies with stalks, thick and tend to be soft, the upper surface was smooth and shiny, light brown, and 3-8 cm in width (Figure 3.A). The third group was fungi with fruiting bodies of indeterminate shape and mostly branched, tending to be thin and hard, the upper surface was rough and shiny, light brown to dark brown, with varying sizes (Figure 3.B). The fourth group was fungi with fruiting bodies shaped like oysters but in layers, thick and soft, with a rough, dull white upper surface with varying sizes (Figure 3.E). The fifth group was fungi with semicircular to oyster-shaped fruiting bodies, thin and tough, bright red rough upper surface, 2-7 cm in diameter (Figure 3.F). Based on the appearance of the fruiting bodies, the first to third groups were thought to belong to the *Ganoderma* genus which is characterized by the presence of shiny upper skin (Loyd et al. 2017), while the fourth and fifth groups were thought to belong to the *Trametes* genus (Olou et al. 2020).

Based on the collection of fungal fruiting bodies found on diseased areca nut palms, it was observed that in each sampling site, more than one type of fungus was found associated with basal stem rot disease. However, all types of fungi were not found in the same frequency. The most frequently found fungus was *Ganoderma* sp. 2nd type, where the percentage of occurrence was more than 88%. Meanwhile, other fungi were found with relatively low frequency, with below 5 percent. *Ganoderma* type 1 was 3.49%, *Ganoderma* type 3 was 4.36%, *Trametes* type 1 was 2.03%, and *Trametes* type 2 was 1.16%. Based on the percentage of presence of fungi that was collected, it can be assumed that the most likely cause of the main basal stem rot disease of areca nut in Kubu Raya District was the *Ganoderma* type 2 fungus (Table 6).

To ensure that the fungi associated with areca nut basal stem rot disease were the cause of disease or not, a disease transmission test was carried out. The test results showed that all the three types of *Ganoderma* tested were able to cause disease in areca nut seedlings by showing symptoms, such as leaves wilted and died, and the presence of *Ganoderma* fruiting bodies at the base of the stem, which were similar to the field symptoms. Based on observations, each fungus from the *Ganoderma* group was capable of causing disease in areca nut seedlings as indicated by the high intensity of the disease (Table 7). Meanwhile, two other types of fungus, namely *Trametes* sp., until the 6th month of observation, did not cause disease in test areca nut seedlings, as indicated by the zero intensity of disease. *Trametes* type 1, was even able to form fruiting bodies on the soil surface, but the plant showed no symptoms of disease (Figure 4). This means that the plant remains healthy even though pathogens also develop around the plant. Thus, this indicates that *Trametes* fungus tested in this study may be saprophytic and not a pathogen of areca nut plants. *Trametes* sp. known that it was not a pathogen (Hasan et al. 2005). This result was the same as previous studies, where the fungus that causes areca nut basal stem rot disease generally comes from the *Ganoderma* group, especially *G. boninense* (Surbakti et al. 2010; Goh et al. 2014; Wong et al. 2021; Castillo et al. 2022).

Table 7. Results of fungal transmission tests on areca nut seedlings

Fungi	Disease intensity (%)	Remark
Ganoerma sp. 1	91b*	Pathogenic
Ganoerma sp. 2	93b	Pathogenic
Ganoerma sp. 3	89b	Pathogenic
Trametes sp. 1	0a	Non Pathogenic
Trametes sp. 2	0a	Non Pathogenic

Note: *The same letters following the numbers in the same column indicate there is no significant difference in the 5% level DMRT test

Table 8. Distribution pattern of basal stem rot disease on areca nut.

Sub-districts	Distribution Patterns (Number of Plantations)		
	Random	Clustered	Random and Clustered
Kubu	2	0	0
Teluk Pakedai	2	0	1
Sungai Kakap	3	2	6
Rasau Jaya	1	0	0
Sungai Raya	1	1	0
Kuala Mandor B	4	0	4
Kubu Raya	13	3	11

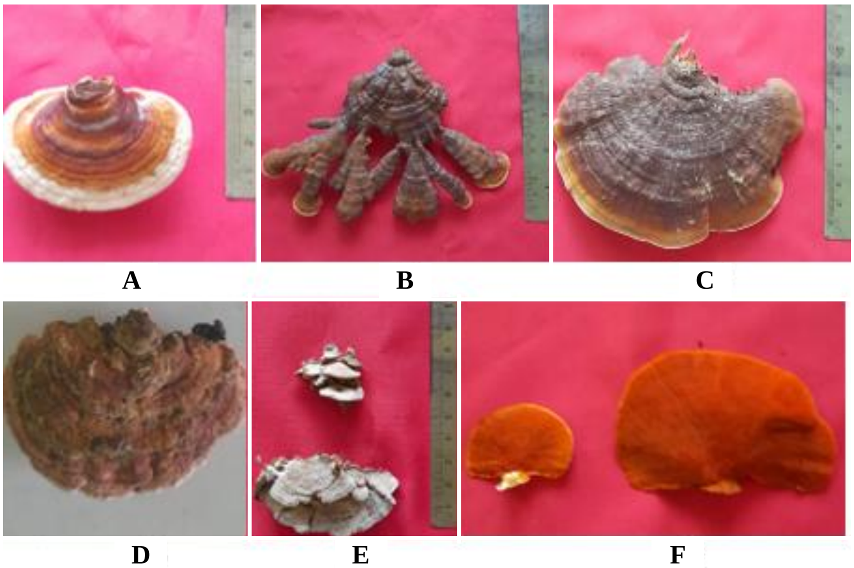

Figure 3. Five types of fungal fruiting bodies associated with areca nut basal stem rot disease. A-D. *Ganoderma* sp. groups, E-F. *Trametes* sp groups

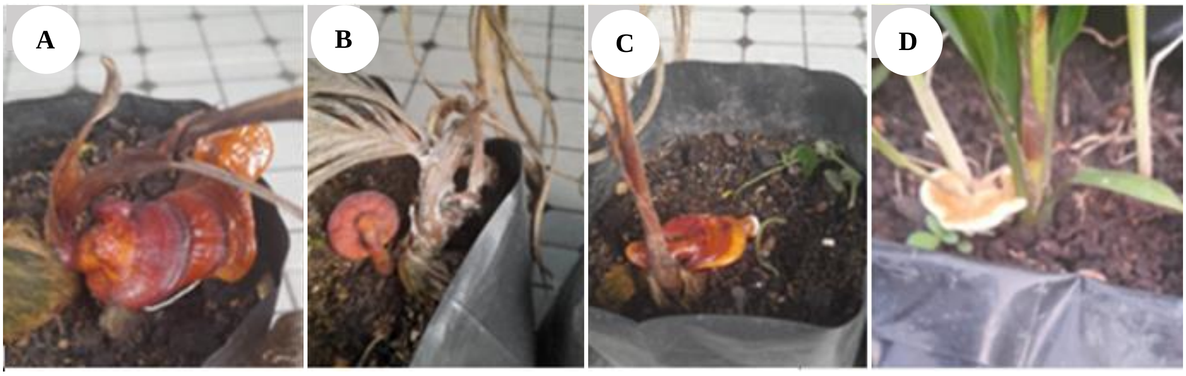

Figure 4. Test results of fungal inoculation on the areca nut seedling: A-C. All the three fungi *Ganoderma* genus were caused areca nut basal stem rot disease. D. 2 other types of fungi, namely *Trametes* sp. did not cause disease in areca nut seedlings

Pattern of distribution of areca nut basal stem rot disease in Kubu Raya District

Based on observations in the field, there were two types of distribution patterns of areca nut basal stem rot disease in Kubu Raya District, namely a random distribution pattern and a clustered pattern. Each pattern can be found in the observed sample site. In each sample site, some were found spreading randomly, in groups, but some were found spreading randomly and also in groups. Random distribution patterns were found in around 40.63% of the sample sites, while group distribution patterns were found in around 9.38% of samples. The random or group distribution patterns were found in around 34.38% of samples (Table 8). This result is different from the distribution pattern of basal stem rot disease in oil palms where it is generally found in groups (Sanderson 2005; Rakib et al. 2017; Hamzah et al. 2020). This may occur for several reasons. Most likely, it is related to the level of disease progression. In plantations where the disease was found to spread randomly, generally, the incidence was very low below 1%. Meanwhile, in plantations with a higher incidence, there is a pattern of disease spread that tends to be clustered. Thus, if the disease spreads further, it is also possible that it may spread into clusters.

In conclusion, the results of this research show that in Kubu Raya District, basal stem rot disease has been found on areca nut plantations caused by the fungus *Ganoderma* sp. In the future, even though the incidence is still low, the development of areca nut plantations in Kubu Raya District needs to be aware of the potential spread of this disease.

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Evaluation of the yield and agronomic trait performance of BC2F2 common bean lines at Hawassa, Ethiopia

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Abstract. *Ebrahim MS, Bisetegn KB, Tedla YR, Tufa MT. 2024. Evaluation of the yield and agronomic trait performance of BC2F2 common bean lines at Hawassa, Ethiopia. Asian J Agric 8: 50-56.* The common bean is the most important legume in Ethiopia and is grown mainly for domestic consumption, export and as a source of protein. Farmers in the study area are still producing old varieties; therefore, the production decreases in the area, even though there are suitable environments for common bean production. Bean anthracnose is one of the major constraints that challenged the production of common bean in the study area. To address the above gap, to identify introgressed bean lines for anthracnose resistance with improved agronomic traits that were developed through backcross breeding program using pot experiments under screen house conditions. Twelve common bean genotypes, including eight developed BC2F2 lines, two parental lines, and two cultivars, were used to evaluate agronomic performance. Data on seven quantitative traits and three qualitative traits were collected from each tagged tested material. Analysis of variance revealed a highly significant difference ($p<0.001$) in the agronomic traits of the BC2F2 line under screen house conditions. As a result, four BC2F2 lines (Plant-3, Plant-5, Plant-9, and Plant 15) exhibited significantly greater differences in the most important agronomic traits than the remaining studied plant materials. In the end, two lines identified to have better agronomic performance (Plant-3 and Plant-15) were selected and promoted for the future to be used for production after verifying in multiple locations for the study and similar agro-ecologies. The results will contribute to an increase in the production of this important crop under farmer conditions. They will significantly benefit and impact Ethiopia's regional and national common bean improvement programs.

Keywords: Better performance, developed lines, old variety, quantitative traits

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is an autogamous (2n=2x=22) diploid legume most commonly grown in East and Central Africa (Gichangi et al. 2012). The common bean is the most important legume in Ethiopia and is grown mainly for domestic consumption and export and as a source of protein (Asfaw et al. 2009; Dejene et al. 2016). It is also used in crop rotation because it binds atmospheric nitrogen. According to FAOSTAT (2019), India, Myanmar, Brazil, the United States, China, Mexico, the United Republic of Tanzania, Uganda, Kenya, and Ethiopia are among the top 10 bean-growing countries globally, with India at the top. The area of the world was 329,824 hectares, with a production of 543,984 tons and a productivity of 1.64 tons/ha (FAOSTAT 2019). In Ethiopia, the acreage of common bean was 140,541.74 hectares, with a production of 242,773.54 tons. The national average was 1.75 tons/ha (CSA 2020). In the SNNPR, the area covered by common beans was 45,424.78 hectares, with a production of 7,095.28 tons and a productivity of 1.7 tons per hectare (CSA 2020).

Bean anthracnose is one of the major constraints on the production of common bean plants in Ethiopia. Developing more disease-resistant varieties is the most important goal of common bean breeding programs in Ethiopia. However, disease resistance is not the only trait that needs improvement in a breeding program. Other factors, such as yield, plant architecture, and grain appearance, are critical to the success of common bean cultivars (Cunha et al. 2005; Menezes-Júnior et al. 2008; Mendes et al. 2009). Yield is the most important indicator of crop performance, but disease resistance is costly on the plant and thus has commercial significance because it may impede the most important goal of increasing yield (Brown 2002). Genetically diverse plants are critical for future improvements in meeting societal demands for food security under climate change scenarios (Bellucci et al. 2014). Previously, variability between developed common bean lines obtained by marker-assisted backcrossing breeding for different agronomic traits has been reported (Rahman et al. 2002; Tar'an et al. 2002; Rezene et al. 2018; and Okii et al. 2019). Moreover, marker-assisted backcrossing facilitated progeny selection by combining good agronomic traits with resistance loci and testing under screening house conditions.

Farmers in the study area are still producing old varieties; however, the pathogen changes occasionally. As a result, the production decreases in the area, even though there are suitable environments for common bean production. Durable, resistant varieties of too many disease-causing pathogens are receiving increased priority from farmers in Ethiopia. Again, in most cases, small-scale farmers in Ethiopia have not been using genetically resistant common bean cultivars, and they grow infected seeds. It would be very critical for farmers in the study area to have genetically resistant common bean cultivars. Therefore, identifying varieties that combine resistant loci to anthracnose and good agronomic traits is highly desirable. Even though developing bean varieties with higher disease resistance levels and good agronomic performance is the most important goal of common bean breeders in Ethiopia. However, the development of superior common bean varieties in the study area is few compared to the changing nature of the pathogen. Little, if any, information exists about these lineages that developed in Ethiopia. The results will contribute to an increase in the production of this important crop under farmer conditions. They will have a significant benefit and implication for Ethiopia's regional and national common bean improvement programs. To adresss the challenges of bean anthracnose in the study area, we have been done an experiment to introgress the $Co\text{-}1^4$ resistance gene through marker assited backcrossing experiments. The lines were developed through crossing the resistance lines with the seccuptible lines and backrossed up to two generations to fix the desired alleles. The resistance line selction is in such away that the developed lines were tested for their marker presence and severity score using three anthracnose races. As a result we have been identified four lines were having the resistance reactions (two of them with marker presence and the remaining two is with marker absence) and developed backcross lines with resitance gene (Ebrahim et al. 2023). However, the agronomic performance thus lines were not studied; since disease resistance is not the only trait that could be improved in resistance breeding program. Eventhouh, developing more disease-resistant varieties is the most important goal of common bean breeding programs in Ethiopia. Therefore, this study was conducted to identify introgressed bean lines for anthracnose resistance with improved agronomic traits.

MATERIALS AND METHODS

Description of study area

The study was conducted in the Molecular Biotech Lab and green house (with average temperature of 29^0C and average relative humidity of 65%) at the Southern Agricultural Research Institute (SARI), located 7° 4' N latitude and 38° 31' E longitude and an altitude of 1700 m.a.s.l in Hawassa, Ethiopia during 2019/20 (Figure 1).

Experimental treatments, design and trial management

Eight BC2F2 lines, two parental lines, and two released varieties were grown under greenhouse conditions. A Completely Randomized Design (CRD) with four replications was employed, and the pots were grown in greenhouse conditions (Figure 3). The present study was conducted in the Molecular Biotech Lab and green house (with average temperature of 29°C and average relative humidity of 65%) at the Southern Agricultural Research Institute (SARI). Two seed of each indicated genotypes were planted in plastic pots filled with forest soil, sand and animal manure at ratio of 2:1:1. The trial was put in the screen house untile all the desired parametrs is collected i.e. up to three and half month starting from date of planting to harvesting.

Data collected

Quantitative traits

Days to emergence (DTE), days to flowering (DTF), days to maturity (DTM), pod per plant (PPP), seed per pod (SPP), seed yield per plant (SYPP) and hundred seed weight (HSW) were recorded on individual tagged plant.

Qualitative traits

The qualitative characteristics of common bean genotypes, such as seed size, seed color, and growth habit, were scored according to the descriptor (based on the IBPGR 1982). These morphological characters were scored through visual observations on an individual base using the descriptor list developed by International Board for Plant Genetic Resources (IBPGR).

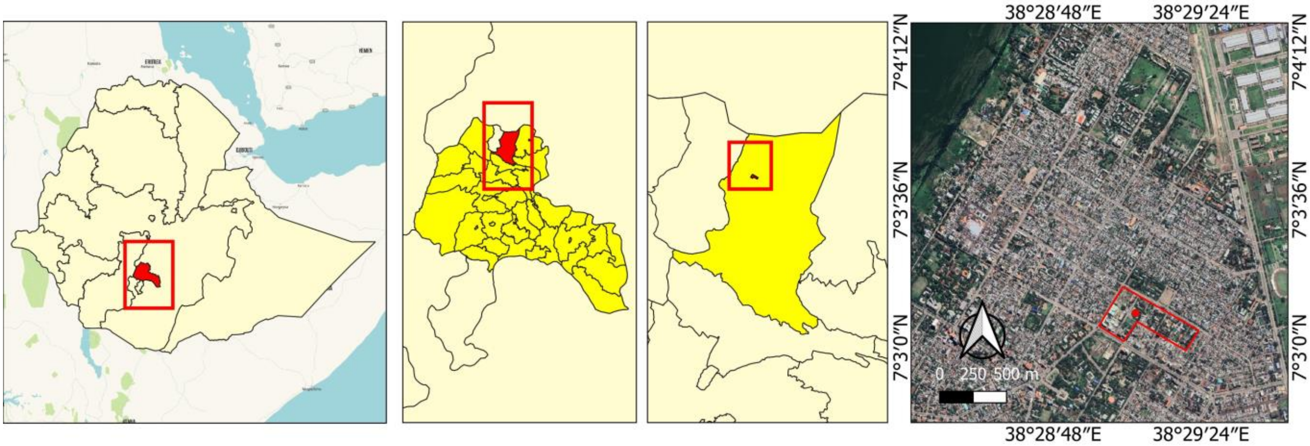

Figure 1. Study area location map in Southern Agricultural Research Institute (SARI), Hawassa, Ethiopia

Statistical analysis

Analysis of variance (ANOVA)

Analysis of Variance (ANOVA) was computed following the procedures for a Completely Randomized Design (CRD) using SAS 9.3. Mean comparisons among treatment means were conducted using the Least Significant Difference (LSD) test at 5% significance. The model used for CRD for evaluating agronomic traits is as follows:

$$X_{ij} = \mu + \alpha_i + \varepsilon_{ij},$$

Where:

μ : Grand mean,

α_i : Treatment effect

ε_{ij} : Random error

RESULTS AND DISCUSSION

Performance of the BC2F2 lines yield and yield related traits

The BC2F2 lines showed significant differences ($p<0.001$) in all quantitative traits analyzed (Table 1). Thus, the findings demonstrate the relative importance of selecting and promoting superior lines among segregants via future selection to increase the desired traits' yield. The presence of this variation in the backcrossed common bean lines tested maight be due to the genetic heterogeneity between developed BC lines. It has been reported that extreme phenotypes between segregates are due to genetic recombination of alleles at multiple loci, epistasis, and reduced genotypic developmental stability (Schwarzbach et al. 2001; Hallauer et al. 2010). Pereira et al. (2013) found significant differences ($P<0.05$) between developed common bean progenies for all assessed traits.

The average data of the eight selected BC2F2 lines revealed that Plant-12 was an early emergent line, while Plant-8 and Plant-15 were identified as late emergent lines (Table 2). Plant-12 had the lowest number of days to flower, while Plant-15 had the highest (Table 2). Among the BC2F2 lines, Plant-17 was observed to be an early maturing line compared to the recurrent parental line KT-IBMV4 and the released varieties Ibbado and Tatu, while Plant-15 was observed to be a late maturing line based on its average performance for this trait (Table 2). Ferreira et al. (2012) reported significant variability among introgressed common bean lines regarding trait days to maturity. Plant-15 had the greatest number of seeds per pod, while Plant-12 had the lowest number of seeds per pod, according to Table 2.

Regarding to number of pods per plant, Plant-15 had the highest number of pods per plant, followed by Plant-5, Plant-17, Plant-2, and Plant-3, whereas Plant-12 had the lowest number of pods per plant (Table 2). It is important to note that plant 9 was identified as having a resistance reaction to the three tested races. Similarly, Mulanya et al. (2014) reported multiple resistance to anthracnose in snap beans. They reported four lines with multiple resistance to three diseases that had better pod yields than did existing commercial varieties in Kenya, the findings of which support the present findings. Among the developed BC2F2 lines, Plant-15 had the highest seed yield per plant, followed by Plant-5 and Plant-9, while Plant-12 had the lowest seed yield per plant (Table 2). Eventhough, Plant-12 showed better performance in the trait seed yield per plant than the released variety (Ibbado), it does not performed best than the remaining developed lines based on this trait. Interestingly, these two specific lines, which presented higher seed yields per plant, were identified using a master marker as a linkage of $Co1^4$ resistance loci. Thus, these results suggest that future improvements in selecting unique plants from evolved lines of common beans are crucial (Singh and Ocampo 1997). An equivalent result was reported by Moses (2017), who observed differences between developed lines in terms of anthracnose resistance and seed yield per plant, supporting this claim. Based on the average hundred-seed weight data, the highest hundred-seed weight was recorded for Plant-15, followed by Plant-9, while the lowest hundred-seed weight was recorded for Plant-2, as shown in Table 1. According to Singh and Schwartz (2010), common bean seed weight can range from 15-90 g per 100 seeds. Genotypes are divided into small-seed common beans with 100 seed weight <25 g, medium up to 40 g, and large-seed over 40 g (Singh and Schwartz 2010). Genotypes weighing greater than the seed weight are preferred for common beans because size is considered a quality parameter (Ejigu et al. 2017).

Performance of the BC2F2 lines in terms of qualitative traits

There was a difference between the developed BC2F2 lines regarding seed size, seed color, and growth habit (Table 3). Based on the effect of the BC2F2 lines on seed size, five lines (Plant-3, Plant-5, Plant-8, Plant-9, and Plant-15) were categorized as large-seeded plants. However, two BC2F2 lines (Plant-2, and Plant-17) were categorized as medium-seeded plants (Table 3). Plant-12, was categorized as small seeded similar to the donor parent (Table 3). The variability may be due to the parental lines used, as crosses were made between the large-seeded and the small-seeded lines. Intermaturely seeded beans weighing 25 g to 40 g per 100 seeds were used to characterize introgression between the gene pools through breeding programs (Gepts and Bliss 1988), confirming the current evidence. Consistent with the present findings, Musango et al. (2016) also noted that the observed difference between the tested common bean plants could be due to parental breeds between the two groups: Andean and Mesoamerican origin.

Five BC2F2 lines (Plant-3, Plant-5, Plant-8, Plant-9, and Plant-17) with red mottled seeds were identified, whereas three BC2F2 lines (Plant-2, Plant-12, and Plant-15) were identified by light red mottled seeds (Table 3 and Figure 2). The variability observed in these lines may be due to the difference in seed color between the parental lines used. Although bean seed size and color are of particular interest to consumers (Stoilova and Pereira 2013), these dominant properties of the seeds may reflect the preferences of farmers and consumers. Thus, this discovery highlights the best lines for these specific traits that can be exploited for future common bean breeding in Ethiopia. Differences in seed color have been reported previously, confirming the results of previous studies (Beninger and Hasfied 1999; Musango et al. 2016). Looking at the growth habit of the BC2F2 lines, two lines (Plant-2 and Plant-8) were categorized as "type V", while six lines were categorized as "type I" (Table 3). Hence, this variation in growth habits among the developed BC common bean lines may be due to differences in the parental lines used. Similar results were observed when growth habit differences in the studied common bean genotypes were observed (Musango et al. 2016).

Table 1 Mean square values of twelve common bean genotypes evaluated for seven agronomic traits at Hawassa, Southern Ethiopia during 2020

SV	DF	DEM	DTF	DTM	PPP	SPP	SYPP	HSW
Genotypes	11	4.429	2.886	5.369	20.339	0.49	656.869	309.653
Error	36	0.354	0.763	1.645	1.354	0.069	8.062	2.558
P values	-	<0.001**	0.0012**	0.0035**	<0.001**	<0.001**	<0.001**	<0.001**

Note: * represents a highly significant difference, DEM: Days to emergence, DTF: Days to flower, DTM: Days to mature, SPP: Number of seeds per pod, PPP: Number of pods per plant, SYPP: Seed yield per plant in grams and HSW: Hundred-seed weight in grams

Table 2. Summary of twelve common bean genotypes evaluated for yield and agronomic traits at Hawassa, South Ethiopia during the year 2020

Genotype	DTE	DTF	DTM	SPP	PPP	SYPP	HSW	DTE
KTIBMV4	6.5[e]	38.25[abc]	78.75[cde]	4.0[b]	9.75[de]	43.75[cd]	41.15[c]	6.5[e]
KTRWA77	6[e]	37.5[bcd]	78.5[de]	4.0[b]	10.55[d]	33.00[e]	20.5[g]	6[e]
Plant-2	7.5[dc]	37.50[bcd]	78.25[de]	4.0[b]	13.00[b]	45.50[c]	28.32[e]	7.5[dc]
Plant-3	8[abc]	37.50[bcd]	78.5[de]	4.0[b]	12.75[b]	41.50[cd]	42.12[c]	8[abc]
Plant-5	8[abc]	37[cd]	80.5[abc]	4.0[b]	13.75[b]	59.50[b]	43.12[c]	8[abc]
Plant-8	8.75[a]	38.25[abc]	79.75[abcd]	4.0[b]	11.00[cd]	39.75[d]	43.05[c]	8.75[a]
Plant-9	6.75[ed]	38.25[abc]	79.75[abcd]	4.0[b]	12.50[bc]	57.25[b]	45.47[b]	6.75[ed]
Plant-12	6[e]	36.25[d]	79.75[abcd]	3.5[c]	8.5[e]	32.25[e]	26.02[f]	6[e]
Plant-15	8.75[a]	39.5a	81.25[a]	4.75[a]	16.50[a]	67.00[a]	51.02[a]	8.75[a]
Plant-17	7.75[bc]	37.5[bcd]	77.5[e]	4.0[b]	13.25[b]	43.75[cd]	34.05[d]	7.75[bc]
Ibbado	8.75[a]	38.75[ab]	78.75[cde]	3.25[c]	10.25[d]	29.75[ef]	42.77[c]	8.75[a]
Tatu	8.5[ab]	38.25[abc]	81[ab]	4.0[b]	9.50[de]	32.25[e]	35.05[d]	8.5[ab]
CV (%)	7.82	2.30	1.61	6.65	9.88	6.69	4.0	7.82
LSD (5%)	0.85	1.25	1.83	0.37	1.66	4.07	2.29	0.85

Note: DTE: Days to emergence (No.), DTF: Days to flower (No.), DTM: Days to mature (No.), SPP: Seed per pod (No.), PPP: Pod per plant (No.), SYPP: Seed yield per plant (gram) and HSW: Hundred seed weight (gram)

Table 3. Summary of twelve common bean genotypes evaluated for three qualitative traits at Hawassa, Southern, Ethiopia during the year 2020

Genotype	SC	SZ	GH
KTIBMV4	LRM	LS	I
KTRWA77	R	SS	V
Plant-2	LRM	MS	V
Plant-3	RM	LS	I
Plant-5	RM	LS	I
Plant-8	RM	LS	V
Plant-9	RM	LS	I
Plant-12	LRM	SS	I
Plant-15	LRM	LS	I
Plant-17	RM	MS	I
Ibbado	RM	LS	I
Tatu	DRM	MS	I

Note: SC: Seed colour, SZ: Seed size, GH: Growth habit, LRM: Light red mottled, R: Red only, RM: Red mottled, DRM: Dull red mottled, LS: Large seeded, MS: Medium seeded and SS: Small sized

Correlation of grain yield with other traits

The analysis of the relationship among characters and their association with yield is essential to establish selection criteria (Singh et al. 1990). Therefore, understanding of interrelationships of seed yield and of the magnitudes of phenotypic correlations of seed yield and its components among yield related traits are highly crucial to utilize the existing variability through selection. Phenotypic correlation estimates between the various characters are indicated in Table 4. Seed yield had positive and significant correlation with number of seed per pod, and number of pod per plant (Table 4). These results are in accordance with the findings of Salehi et al. (2010). The finding of Daniel et al. (2015) showed that common bean grain yield was positively and significantly correlated with pod per plant and seeds per pod. Akhshi et al. (2015) also reported a strong positive correlation of seed yield with seed number per plant. However, Daniel et al. (2015) reported a negative and significant correlation of days to harvest maturity with seed yield across locations and over stress regimes.

Figure 2. Sample photo of BC2F2 common bean lines showing seed color variability

Figure 3. Common beans genotypes plants grown in the screen house

Grain yield showed significant and negative correlation with days to flowering and days to maturity (Table 4). Negative correlation was indicated inverse relationship between earliness characters and grain yield that is desirable if stresses such as terminal heat and drought are expected. This negative correlation between grain yield and days to flowering is in harmony with the finding of Daniel et al. (2015).

Disease resistance is not the only trait that must be improved in a breeding program. Other factors, such as yield, plant architecture, and grain appearance, are critical to the success of bean varieties (Cunha et al. 2005; Menezes-Júnior et al. 2008; Mendes et al. 2009). Yield is the single most important indicator of crop performance. However, disease resistance is reportedly costly for the plant and has commercial significance because it may hinder the most important objective of increasing yield (Brown 2002). Liebenberg et al. (2005) reported the successful gene pyramiding of three rust resistance genes, Ur-3+, Ur-5, Ur-11, and other uncharacterized genes in advanced common bean lines. Field testing for agronomic performance of the lines showed that resistance was considerably improved without yield loss.

Previously, variability between developed common bean lines obtained by marker-assisted backcrossing breeding has been reported in terms of traits, flowering days, maturity, seeds per pod, pods per plant, seed weight (Tar'an et al. 2002) and hundred-seed weight (Rezene et al. 2018), confirming the present study. Additionally, Reddy and Singh (1990) and Rahman et al. (2002) reported the occurrence of intergenerational variation in the trait of backcrossing to pods per plant in common bean lines, suggesting that the current results are confirmed. Several agronomic traits evaluated significantly differed between the developed common bean lines (Okii et al. 2019). Hybrids between the Mesoamerican and Andean gene pools are highly important for plant breeding, where there is often a need to recombine Mesoamerican and Andean traits (Johnson and Gepts 2002). According to Blair et al. (2010), hybridization can result in the emergence of novel genotypes and phenotypes (e.g., resistance to pathogens, nutritional quality, and seed size) that are absent in either parental taxa. Alternatively, hybrid traits predominating over parental phenotypes (transgressive segregation) can lead to evolutionary novelty (Allendrof and Luikart 2007). The selection must consider the growers' and consumers' demands (Ramalho et al. 1998). Therefore, this study considered the three most important qualitative traits (seed size, seed colour and growth type). Therefore, the selected BC2F2 lines can improve agronomic performance because of their red-mottled seed color, large-seeded nature, and type I growth habits.

Table 4. Correlation analysis of grain yield with other traits at Hawassa, Southern Ethiopia in the year 2022

	DTE	DTF	DTM	SPP	PPP	SYPP	HSW
DTE	1	-0.108	-0.180	0.077	0.321*	0.266	0.344*
DTF	-0.108	1	0.329*	-0.325*	-0.474**	-0.647**	-0.121
DTM	-0.180	0.329*	1	-0.251	-0.520**	-0.386**	-0.224
SPP	0.077	-0.325*	-0.251	1	0.545**	0.505**	0.156
PPP	0.321*	-0.474**	-0.520**	0.545**	1	0.758**	0.262
SYPP	0.266	-0.647**	-0.386**	0.505**	0.758**	1	0.154
HSW	0.344*	-0.121	-0.224	0.156	0.262	0.154	1

Note: DTE: Days to emergence (No.), DTF: Days to flower (No.), DTM: Days to mature (No.), SPP: Seed per pod (No.), PPP: Pod per plant (No.), SYPP: Seed yield per plant (gram) and HSW: Hundred seed weight

Generally, a highly significant difference was observed for days to emergence, days to flowering, days to maturity, pod per plant, seed per pod, 100-seed weight, and yield per plant. Regarding the present experiment, genotypic variation in grain yield and yield components (Emishaw 2007) has been reported for common beans. Data for the number of pods per plant, seeds per pod, seed yield, and hundred seed weight showed highly significant ($P<0.01$) differences among varieties. The current variations in yield components among varieties consent with previous reports (Daniel et al. 2014). In line with the findings (Shahid 2013; Fahad et al. 2014), it was reported that significant variability was observed in days to flowering, days to maturity, pods per plant, seed yield per pod, hundred seed weight, and yield. This study followed the works of Zelalem (2014) regarding qualitative traits of the tested genotypes showing differences in seed size, color, and growth habit.

In conclusion, the analysis of variance results revealed highly significant differences ($p<0.001$) among the BC2F2 lines for all the quantitative traits considered. Finally, four BC2F2 lines (Plant-3, Plant-5, Plant-9 and Plant-15) were identified as better for most of the agronomic traits studied than were their parental lines and the two released common bean varieties based on the trait days to emergence, flowering, maturity, number of seed per pod, number of pod per plant, seed yield per plant and hundred seed weight. Therefore, based on these results, the two lines that exhibited better agronomic performance and having $Co\text{-}1^4$ anthracnose R-gene background (Plant-3 and Plant-15) should be improved and promoted as potential parental lines for the next crossing.

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Environmental impact of biochar and wheat straw on mobility of dinotefuran and metribuzin into soils

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Abstract. *Fouad MR, El-Aswad AF, Aly MI, Badawy ME-T. 2024. Environmental impact of biochar and wheat straw on mobility of dinotefuran and metribuzin into soils. Asian J Agric 8: 57-63.* The environmental effect of 5% biochar and wheat straw on the mobility of dinotefuran (DNF) and metribuzin (MBZ) in alluvial soil (A) and calcareous soil (B) was studied. The breakthrough (BTC) curves of DNF and MBZ were delayed in soil columns compared to that of iodide. The amendment of soil A or B columns with biochar and wheat straw increased the leaching of DNF collected in leachates, consequently reducing the breakthrough time. Biochar amendment increased the leaching of MBZ in soil A, whereas it significantly reduced the leaching of MBZ in soil B. Wheat straw amendment also significantly reduced the MBZ cumulative percentage. It is indicated that the downward of DNF in soil A is more rapid than that in soil B, while MBZ was faster in soil B compared to soil A; the leaching rate of MBZ is higher than DNF in all soil columns. The calculated GUS indices of DNF and MBZ were lower than 2.8, which means these pesticides are non-lechers. DNF required more water to leach from soil B than from soil A. In contrast more water was needed to leach MBZ from soil A compared to soil B. In addition, the DNF required more water for leaching from soil A or B than MBZ.

Keywords: Dinotefuran, metribuzin, mobility, soil amendments

INTRODUCTION

The pesticide leaching potential in soil is described by the pesticide sorption characteristics. The attenuation factor and retardation factor are two indexes frequently used in predicting the environmental risk assessment of pesticide groundwater contamination (Rao et al. 1985; Mulla et al. 1996; Abdel-Raheem et al. 2023; Abd-Eldaim et al. 2023; El-Aswad et al. 2023a). The attenuation factor thus estimates the mass emission of pesticides in groundwater. The retardation factor is a numerical representation of the slowing down of pesticide leaching to the water flow within the soil. The leaching delay is caused by the soil's sorption and degradation of pesticides (El-Aswad et al. 2024a, b) and the soil diffusion of pesticides in gaseous and aqueous forms. The graph of the breakthrough curve represents the relationship between the relative concentration and time evolution concentration (Paraiba and Spadotto 2002; El-Aswad et al. 2023b). The mobility of the pesticides has been related to the total OM content, with the nature of the OM having little apparent influence on sorption processes (Bekbölet et al. 1999; Fouad 2023a). Conventional pesticide formulations are applied at rates exceeding the minimum threshold concentration to mitigate losses from sorption (Fouad et al. 2023a, 2024a, b) photodecomposition, chemical and microbial degradation, volatilization (Fouad et al. 2023b), and leaching (Fouad 2023b, c).

Leaching is one of the important factors affecting the herbicidal activity of soil-applied herbicides. Some herbicides may disappear from the soil's upper layers where most weed seeds are located by excessive leaching when heavy rainfall occurs or when large amounts of irrigation water are applied. Leaching data is valuable for predicting and comprehending pesticide behavior in diverse soil types under varying rainfall conditions. There was a direct correlation between the pesticides' water solubility and the depths of leaching in mineral soils; the leaching depth decreased as the soil clay content increased. Leaching depth also decreased as OM increased (Gray and Weierich 1968). A set of column experiments was conducted to assess the leaching potential of dinotefuran (DNF), thiamethoxam, and imidacloprid insecticides in two experimental conditions: mixed and individual pulse modes. In both cases, the pesticide breakout pattern in the predominantly acidic to neutral vineyard soil illustrates their medium to high leachability. DNF has shown a high tendency to leach and has passed through the column with fewer pore volumes, while imidacloprid was retained for a longer period, indicating lower leachability (Kurwadkar et al. 2014). The potential for leaching into surface water associated with the widespread use of neonicotinoids, particularly near water bodies, is a significant concern (Anderson et al. 2015; Fouad 2023d).

Metribuzin (MBZ) is one of the most important contaminants in ground and surface waters (López-Piñeiro et al. 2013). MBZ is characterized by its high water solubility (1,050 mg/L) and low soil adsorption (Koc = 60); therefore, it has a high potential for soil profile movement. The US EPA maximum advisory concentration for MBZ in drinking water is 175 μg/L (Singh 2009). The mobility of MBZ within the soil profile is of concern because crop phytotoxicity levels depend on the quantity of MBZ absorbed by the root system and possible groundwater contamination. The movement of herbicides through soil columns is analogous to chromatography, and the extent of movement in soil could be predicted from the distribution or adsorption coefficient, the void volume, water content, and OM content (Savage 1976). MBZ is readily leached in sandy soils low in OM content, but leaching potential is reduced on finer textured soils (Milburn et al. 1991).

MATERIALS AND METHODS

Tested pesticides

Dinotefuran

IUPAC name: (RS)-1-methyl-2-nitro-3-(tetrahydro-3-furylmethyl) guanidine. Structure: Shown in Figure 1. Solubility (20°C): Water 54.3 g/L, methanol 57.2 g/L and dichloromethane 60.9 g/L. Chemical class: Neo-nicotinoid. Pesticide type: Insecticide.

Metribuzin

IUPAC name: 4-amino-6-terbutyl-3-methylsulfanyl-1,2,4-triazin-5-one. Structure: Shown in Figure 1. Solubility (20°C): Water 1,200 mg/L, methanol (several hundred times greater than in water). Chemical class: Triazinone. Pesticide type: selective systemic herbicide.

Tested soils

This study tested two types of Egyptian soil: clay loam and sandy loam. The samples were collected from the topsoil profile layers from different locations. The physical and chemical properties were determined at the Department of Soil and Water Sciences, Faculty of Agriculture, University of Alexandria, Egypt, and the data are presented in Table 1.

Tested soil amendments

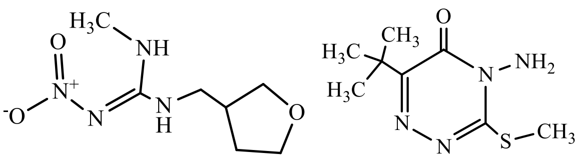
The study tested four amendment substances. The commercial substances' forms were obtained from the Faculty of Agriculture, University of Alexandria, Egypt.

Mobility study

The bench-scale soil columns were employed to assess the potential mobility of the pesticides tested in both soil and amended soil with 5% biochar or wheat straw. Each column was uniformly filled with 3 kg of soil to achieve a known bulk density (Weber et al. 1986; Fouad 2023b, c). The bottom end cap supported a porous stainless-steel plate, and the control column soil was similarly mixed for consistency. The columns were pre-treated with 0.01 M $CaCl_2$ before applying the pesticide and KI (Thanos and Maniatis 1995). The flow rate was chosen to give saturated flow conditions and to limit leaching experiment timescale, thereby reducing potential pesticide degradation. Next, 10 mL of KI solution with a concentration of 0.2 molar was used as a water tracer. Dinotefuran or metribuzin was then added to each column to achieve a soil concentration of 10 µg/g.

Table 1. Physical properties and chemical properties of tested soils

Properties	**Soil A**	**Soil B**
Soil type	Alluvial	Calcareous
Texture class	Clay loam	Sandy loam
Water holding capacity (mL/100g)	46	35
EC (m mohs/cm) at 25°C	1.32	2.34
Soil pH	8.25	8.20
Organic matter content (%)	3.31	1.32
Total carbonate (%)	7.87	40.09
Soluble cations conc. (meq/L):		
Ca^{++}	3.8	8.8
Mg^{++}	5.0	7.0
Na^{+}	9.4	15.3
K^{+}	0.5	2.4
Soluble anions conc. (meq/L):		
CO_3^{-}	1.6	1.4
HCO_3^{-}	2.6	3.4
Cl^{-}	8.5	16.5
SO_4^{-}	0.6	1.8



Dinotefuran Metribuzin

Figure 1. Chemical structure of dinotefuran and metribuzin

Next, we applied the $CaCl_2$ solution and collected the leachates (25 mL leachate). The KI was determined in all leachate samples using the iodimetric method (Mendham et al. 2000). Additionally, the tested pesticides were identified by analyzing all leachates.

Statistical analysis

Experimental data are presented as mean ± standard error, and the statistical analysis was performed by the SPSS program (ver. 21.0, USA).

RESULTS AND DISCUSSION

Figure 2 represents DNF's breakthrough (BTC) curves compared to iodide as a water tracer in columns of unamended soil A and B and 5% of biochar and 5% of wheat straw amended soil A and B. The BTC curves of DNF were delayed in soil A and soil B columns compared to that of iodide; the top of the BTC corresponding to the maximum concentration of DNF was obtained after percolating 8.2 L of leachates. All BTC of DNF needed about 1.1 L of cumulative volume. The amendment of soil A or B columns with biochar or wheat straw increased DNF's leaching, reducing the breakthrough time. The maximum concentration of DNF was recorded after percolating 1,000 mL and 3,000 mL of leachates cumulative volume of biochar amendment soil A and soil B columns, respectively. Also, the top of the breakthrough peak of DNF was obtained after percolating 3,000 mL leachates cumulative volume of wheat straw amended soil A and soil B columns. All BTC need about 6 L of leachates. Accordingly, there was no decrease in the DNF leachate obtained from biochar and wheat straw amended columns. The BTC curve is a specific form that depends on the chemical's soil solids, physicochemical properties, and the soil structure through which the chemical moves (Shipitalo et al. 1990).

In addition, 97.2 % and 91.5 % of applied DNF in the unamended soil A and B columns were recovered in the leachate after percolating about 12 L of $CaCl_2$ solution. About 89.2%, 98.0%, 93.2%, and 89.3% of the applied DNF were recovered in the leachates of biochar-amended soil A and B columns and wheat straw amended soil A and B columns, respectively. However, adding soil A and B with biochar or wheat straw significantly increased the cumulative percentage of DNF collected in leachates (Figure 3). It was observed that the data indicated that the leaching of DNF was low in the soil columns. Furthermore, adding soil columns with 5% biochar or wheat straw increased the DNF leachability.

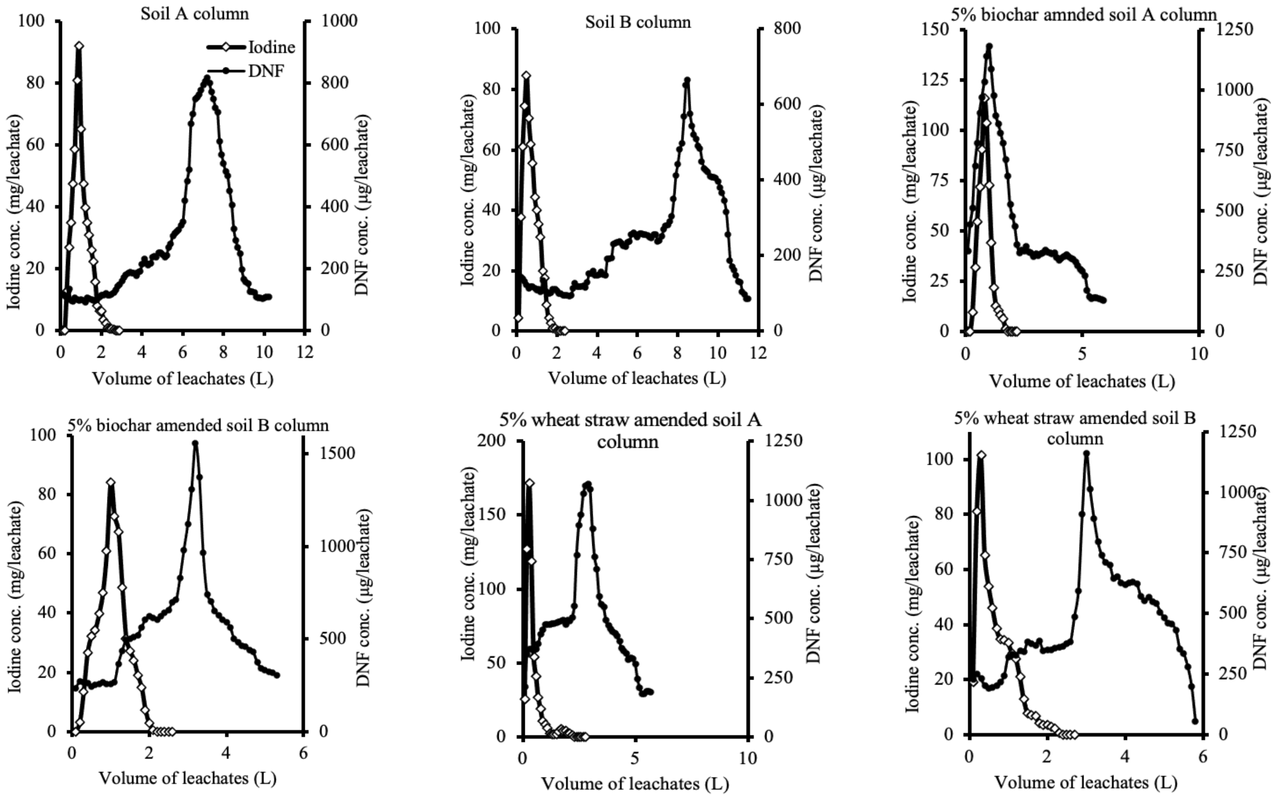


Figure 2. Breakthrough curves of insecticide DNF and water tracer I- in unamended and amended soil columns

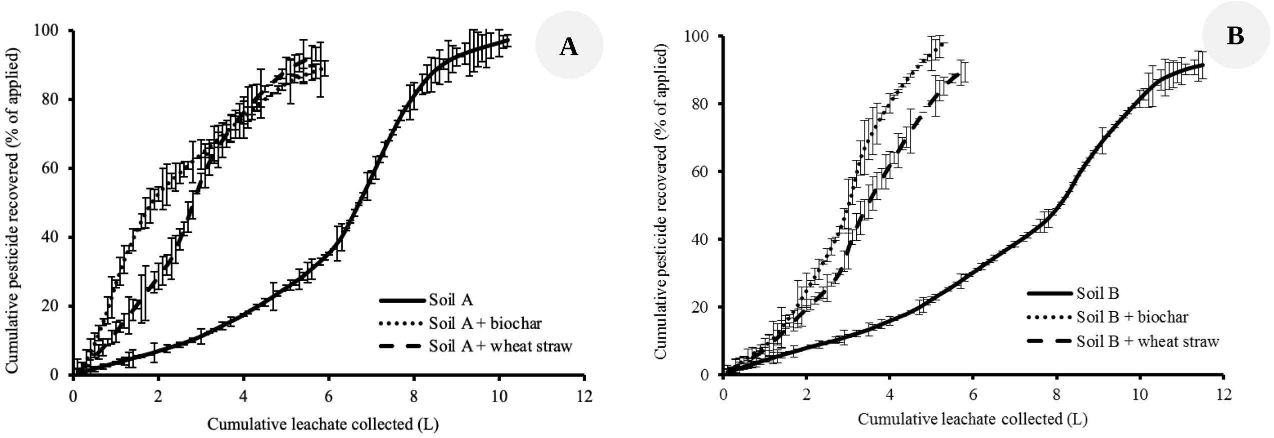


Figure 3. Cumulative leachate curves of DNF in unamended and amended soil A (upper) and B (lower) columns

Therefore, the leaching of pesticides in the soil depends on the sorptive characteristics of the compound and soil. Sorptive characteristics significantly affect the physical leaching of pesticides within a soil-water matrix (Perry et al. 1988). Dinetofuran is highly water soluble but exhibits high percent sorption (Kurwadkar et al. 2013). Also, this observation is similar to the findings reported by Kinney et al. (2006), who reported that acetaminophen with higher water solubility tended to accumulate more in the soil than other compounds with low water solubility (Kinney et al. 2006). Moreover, DNF is highly leachable compared to the other neonicotinoids (Kurwadkar et al. 2014).

Figure 4 exhibited the breakthrough curves of metribuzin in unamended soil A and B and 5% biochar and wheat straw amended soil columns. The iodide BTCs observed from the release of these compounds started with the first leachates. The accumulated volume of the leachates until the top of BTC was about 2.6 L with a concentration of 120 µg/mL for soil A column, whereas about 0.6 L with 190 µg/mL for soil B column. It is indicated that the downward of MBZ in sandy loam soil is more rapid than in clay loam soil. The BTCs of MBZ for soil A and B columns required nearly 5 L accumulation volume. The BTCs of MBZ were retarded relative to the I^- movement and exhibited a flatter peak in soil A and tailing in soil B column. The highest concentration of MBZ was delayed by about 500 mL to the highest concentration of iodide in biochar and wheat straw soil A columns. The highest concentration of MBZ in biochar soil A column was 148 µg/mL of leachate, corresponding to 1.5 L cumulative volume. The concentration decreased to 57 µg/mL of leachate, corresponding to 2.3 L, which remains constant until the 5.5 L cumulative volume. Almost the same shape of BTC was obtained in wheat straw amended soil A columns but with broader. The highest concentration of MBZ in the wheat straw column was 100 µg/mL of leachate. Then, the concentration of MBZ decreased to a minimal value after the release of 5.5 L leachates.

The breakthrough curves of MBZ in biochar and wheat straw amended soil B showed two peaks. The first peak in each case was small and approximately identical to that of iodide, while the second peak was much larger. The second peak in biochar-amended soil B was symmetrical, and its maximum concentration of MBZ was 68 µg/mL, corresponding to about 4.5 L cumulative volume. The second peak in wheat straw amended soil B was flatter, and its maximum concentration of MBZ was 82 µg/mL, corresponding to a 2.5 L cumulative volume. The BTC of MBZ contained two peaks needs 5.5 L leachates cumulative volume neither in biochar amended soil B nor in wheat straw amended soil B. El-Aswad et al. (2002) suggested that in the case of BTC contained two peaks, some pesticide molecules might be leached through macropores without interference with soil matrix, giving rise to a small peak and leaching of pesticide in the soil matrix was occurred by diffusion through micropores, giving a greater peak.

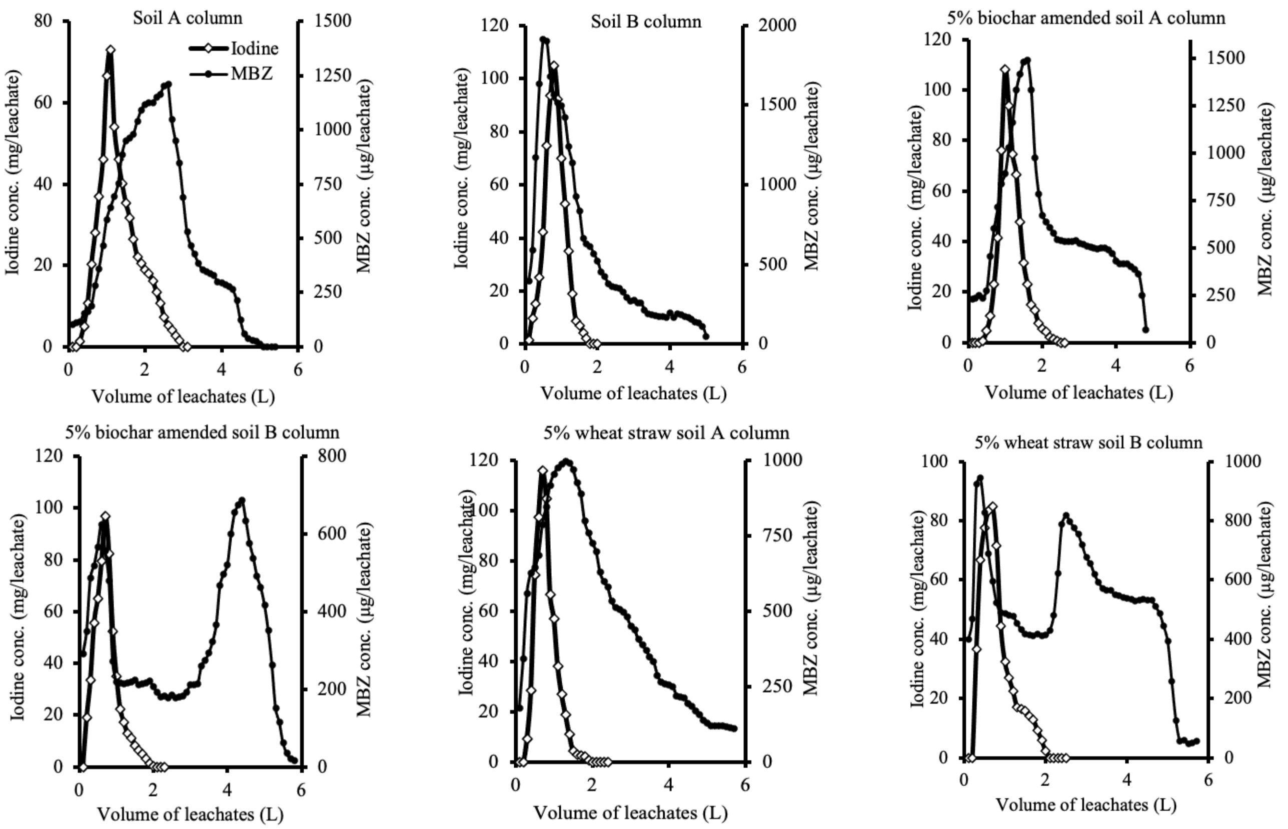


Figure 4. Breakthrough curves of insecticide MBZ and water tracer I- in unamended and amended soil columns

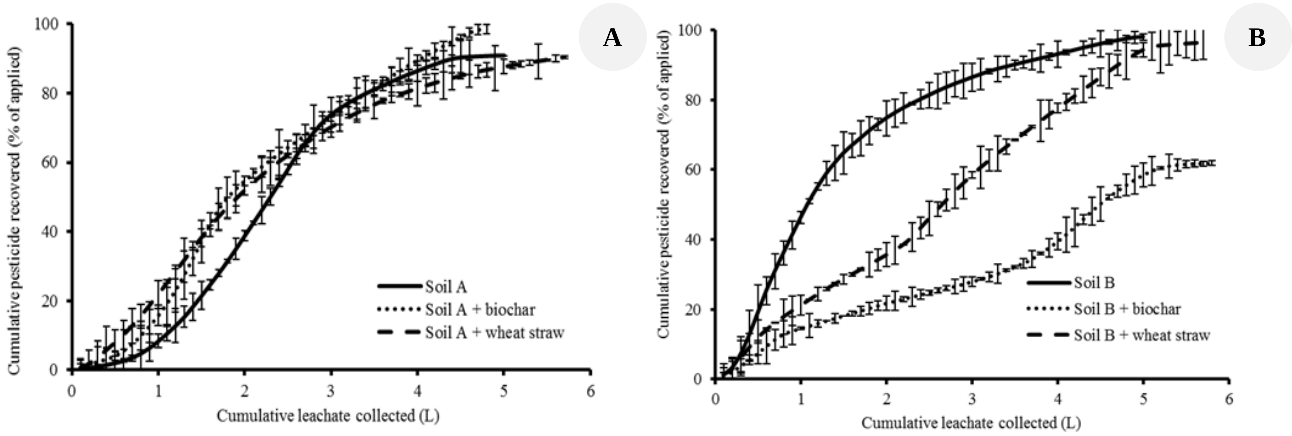


Figure 5. Cumulative leachate curves of MBZ in unamended and amended soil A (upper) and B (lower) columns.

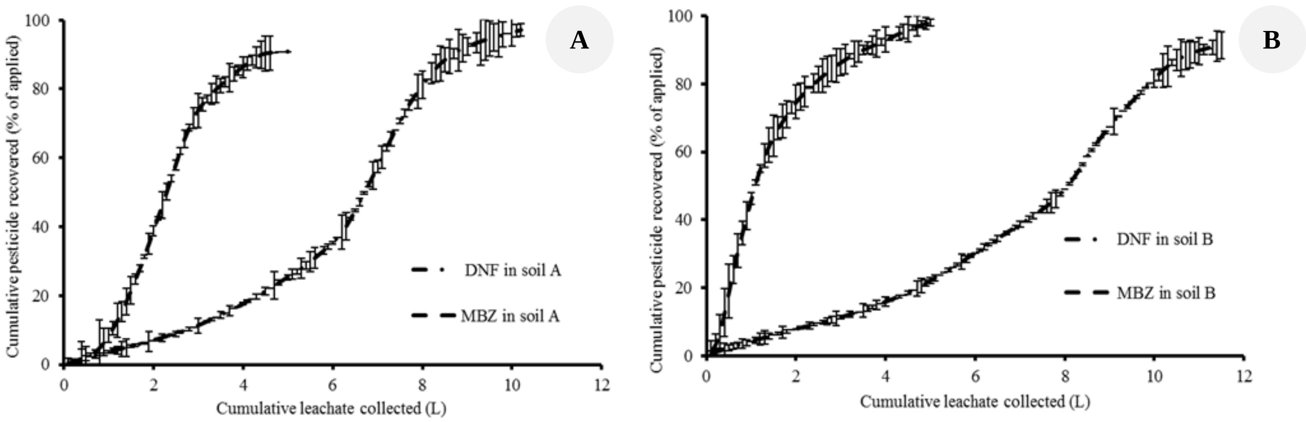


Figure 6. Comparison of tested pesticides cumulative leachate curves in unamended and amended soil A (upper) and B (lower) columns

The cumulative percentages of MBZ collected in leachates of unamended soil A and B columns were 90.8% and 98.0%, respectively. Biochar amendment increased the leaching of MBZ in soil A and significantly reduced the leaching of MBZ in soil B (Figure 5). Compared to 98.0% leaching losses of MBZ from unamended soil B columns, leaching losses of MBZ were significantly reduced to 61.8% after biochar amendment. The amendment of soil A with wheat straw increased the cumulative percentage of MBZ until 3 L percolated, then it was amended, and unamended soils were symmetrical. BTCs of MBZ and I indicated that the cumulative percentage of herbicide decreased until 6 L percolated. Therefore, 90.4% of the initially applied MBZ was recovered from the wheat straw amended soil A column leachates. The wheat straw amendment also significantly reduced the MBZ cumulative percentage; about 96.6% of the applied MBZ was recovered in the wheat straw amended soil B column leachates. A previous study showed that both animal manure and fly ash are highly effective in decreasing the downward movement of metribuzin in packed soil columns of sandy loam soil (Majumdar and Singh 2007). Jones et al. (2011) also demonstrated that biochar decreased the leaching of simazine from the soil due to the strong binding capacity of biochar. Peter and Weber (1985) found that 82.3% of the applied metribuzin was recovered in the leachate, and they observed that MBZ is highly mobile in soil. Consequently, the activity of MBZ increased, in which leaching was prevented, indicating that leaching may be important in the loss of activity of MBZ (Peek and Appleby 1989).

Moreover, comparing the leachability of tested pesticides in columns of soil (A) and soil (B), the recovered amounts were standardized to 100% to compare the tested pesticides (see Figure 6) directly. The leaching rate can be arranged in the following order in soil A and B columns: MBZ > DNF. The differences detected in pesticide leaching were more significant in the columns containing soil A than in those containing soil B, except for DNF, which had the lowest leachability. There were notable variations found in the cumulative percentages of DNF and MBZ. It was observed that the arrangement of the pesticides according to their cumulative percentage in soil A columns corresponding to the arrangement depends upon their water solubility, DNF (54.3 g/L) > MBZ (1.2 g/L). Harris (1966) and Rodgers (1968) studied several herbicide leaching and found water solubility is crucial, but adsorption data provided a better indicator of mobility.

Savage (1976) and Sharom and Stephenson (1976) found that MBZ mobility in soils was inversely related to the soil sorption capacity of the metribuzin molecules. Greater movement of MBZ was observed in soil, particularly in coarser textured column soil (Peek and Appleby 1989; Beck et al. 1993). In addition, DNF has exhibited high leaching potential in soil columns (Kurwadkar et al. 2014). Morrissey et al. (2015) indicated that DNF has high leaching and runoff potential. Therefore, due to the high water solubility, DNF risks water contamination higher, particularly by leaching into the groundwater (Sánchez-Bayo and Hyne 2014).

Generally, data from leaching are valuable for predicting and comprehending the behavior of pesticides in various soil types (Gray and Weierich 1968). Moreover, the drinking water directive (98/83/EC) requires pesticide concentration in drinking water not to exceed 0.1 μg/L for a single pesticide and 0.5 μg/L for total pesticides. Not only does groundwater pollution harm human health since it is used for drinking, but when used for irrigation, it contaminates the food chain (Navarro et al. 2007). The study by Si et al. (2006) suggested that organic amendment could be an efficient strategy for regulating pesticide leaching. Incorporating organic amendments into calcareous and sandy soils may gradually diminish the risk of groundwater contamination by pesticides, as evidenced by research from Cox et al. (1997), Tatarková et al. (2013) and Fouad et al. (2024c, d).

The Gustafson model is based on the pesticide partition coefficient between soil organic carbon and water (Koc) and pesticide soil disappearance (half-life DT50, required for 50% dissipation of initial concentration) (Gustafson 1989). The relationship between these two parameters is called the Groundwater Ubiquity Score (GUS) index.

$$\text{GUS index} = [\log DT_{50}] \times [4 - \log Koc]$$

Higher values of the GUS index indicate the pesticide will have higher mobility, posing a greater threat to groundwater resources. Gustafson has ranked many tested pesticides into leachers, non-leachers, and transient, which have intermediate properties (Bottoni et al. 1996). Accordingly, the calculated GUS indices of DNF and MBZ were lower than 2.8, meaning the tested pesticides in soil A and B are non-lechers. After calculating Kd values for the tested pesticides, indicating that the Kd value of DNF = 1.972 and 2.438 mL/g and MBZ = 0.505 and 0.105 mL/g in soil A and B, respectively. These results illustrated that MBZ required more water to leach from soil A than from soil B; in contrast, more water was needed to leach DNF from soil B than from soil A. In addition, the compound DNF required more water for leaching from soil A or B than MBZ (El-Aswad et al. 2024c; Fouad et al. 2024e).

In conclusion, the water tracer I^- was leached fast, and their BTCs in unamended soil A and B and biochar and wheat straw amended soil columns are symmetrical. The BTC curves of DNF were delayed in soil A and soil B columns compared to that of iodide. The amendment of soil A or B columns with biochar or wheat straw increased DNF's leaching, reducing the breakthrough time. The downward of MBZ in sandy loam soil is more rapid than in clay loam soil. The BTCs of MBZ in biochar and wheat straw amended soil B showed two peaks. The first was small, it attributed to leaching through macropores, while the second peak was much larger, it attributed to leaching through micropores. The soil A and B column's leaching rates can be sorted as follows: MBZ>DNF. The evaluated pesticides' computed GUS indices were less than 2.8. This indicates that the tested soils' DNF and MBZ are not leachers. The Kd values for the pesticides tested in soil columns showed that more water was needed for MBZ to leach from both soils. Conversely, compared to soil A, more water was required for soil B to drain DNF. Furthermore, compared to MBZ, the compound DNF needed more water to leach from soil A or B.

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Soil arthropod pests associated with groundnut (*Arachis hypogaea*) in Golinga, Northern Ghana

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Abstract. *Kyerematen R, Issifu I, Adu-Acheampong S. 2024. Soil arthropod pests associated with groundnut (*Arachis hypogaea*) in Golinga, Northern Ghana. Asian J Agric 8: 64-69.* Groundnut (*Arachis hypogaea* L.) production is one of the main livelihood activities in the northern part of Ghana consisting of the five main regions, Upper West, Upper East, Northern, Savannah and North East regions and the principal source of protein for mostly, the rural people. Notwithstanding that, not much research has been conducted on soil arthropod pests associated with the crop in the study area. To fill this gap, this research documented soil arthropod pest diversity of the crop and the damage they cause as baseline data for pest management decisions in the study area. Pitfall traps were set up on twenty-four (24) 5 m × 5 m plots close to harvest time after raising groundnut plants on them in a Randomized Complete Design. The traps were emptied on four occasions from each plot at two-week intervals. Results from the field trials revealed that beetles, termites, wireworms, false wireworms and millipedes were the dominant pest groups in the study area. The results further showed that these key pests caused nearly 90% damage to groundnut pods which goes a long way to impact negatively on the livelihood of farmers in the study area. The study recommended environmentally friendly pest control methods such as the use of botanical extracts and other biorational means in the study area. This was based on findings from our preliminary survey which revealed that the current pesticides that farmers use are largely ineffective in the study area.

Keywords: Damage, diversity, farmers, pesticide, production

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is a leguminous crop that supplies people especially those in the northern part of Ghana with the needed proteins, vitamins, and essential oil (Adjepong et al. 2018; Boadi et al. 2022). It is a staple seed and forms a substantial part of various local diets in Ghana (Adjepong et al. 2018; Boadi et al. 2022). It is estimated that more than 70% of farmers in the five regions in the northern part of Ghana namely; Upper West, Upper East, Northern, Savannah and North East, cultivate groundnut and together account for over 90% of the total production in Ghana (Owusu-Adjei et al. 2017). Groundnut is cultivated in all 16 regions of Ghana although production is relatively higher in Oti, Upper West, Northern, Volta and Upper East regions (Oteng-Frimpong et al. 2015). According to USDA (2023), about 370,000 ha of groundnuts were cultivated across the 16 regions of Ghana in the year 2022, resulting in a yield of a little over 600,000 metric tons. Besides playing an important role in income generation and consumption within households (Ramakrishna et al. 2006; Patil et al. 2015), groundnut improves fertility and moisture of the soil and so improves soil conservation, thereby increasing farmer resilience against climate change (Feldman et al. 2019; Mayes et al. 2019). According to research there are more than 25 million ha. of cultivated groundnut which produces more than 45 million tons of seeds worldwide (Simtowe et al. 2009; Konate et al. 2020). Groundnut is a crop that is produced especially in warm temperate zones as well as tropic and subtropics and can only do well in soil of high humidity and temperature range of 20°C and 30°C, and annual rainfall within the range of 200 and 1200 mm (Singh and Nigam 2016; Infonet Biovision 2017; Kadiyala et al. 2021). Total groundnut cultivation in sub-Saharan regions of Africa makes up about 40% of the total world production area of land although the total seed production in these areas is about 26% of the world production (Angelucci and Bazzucchi 2019).

The groundnut crop is attacked by several pests resulting in rising production cost and yield loss to farmers (Agoyi et al. 2019; Okello et al. 2013). For instance, it has been reported that the crop is attacked by some 360 insect pest species worldwide and that some of the ground-dwelling pests cause higher yield losses even more than foliage feeders (Wightman and Amin 1988; Nataraja et al. 2014). Because farmers are not able to detect the presence of soil pests early enough for prompt management, the pests often cause huge yield losses (Wightman and Amin 1988). Research has shown that jassids, thrips, aphids, whiteflies, leaf miners etc. are among the most notorious pests of groundnuts for which *Amrasca biguttula biguttula,* Ishida (Hemiptera: Cicadellidae), *Helicoverpa armigera,* Hübner (Lepidoptera: Noctuidae), *Aproaerema modicella,* Deventer (Lepidoptera: Gelechiidae), *Spodoptera litura,* Fabricius (Lepidoptera: Noctuidae) and *Aphis craccivora,* Koch (Hemiptera: Aphididae) are reported to be the most destructive (Amin and Mohammad 1980). At the early stages of growth, the main pests of groundnut are leaf miners and then afterwards tobacco caterpillars followed by pod borers and sucking pests with most of the sucking pests also being vectors of groundnut diseases (Amin and Mohammad 1980; Panse 2021; Maheshala et al. 2023).

Despite these studies on pests of groundnuts, studies on soil arthropod pest profile of the crop in traditional farming communities in Golinga in the northern region of Ghana remain relatively understudied. This notwithstanding, preliminary observations within the study area coupled with the results of previous survey conducted in nearby farming communities suggest that soil pests do cause huge damages to the crops in the study area (Tanzubil 2016). Additionally, conventional pesticides applied as a control measure have become less effective within the study area.

As a result, this study aims to document soil pests of groundnut, determining the level of destruction of these pests and the efficacy of the main control methods and recommending alternative effective methods for their management. This is because farmers complained about lack of effectiveness of conventional pesticide application as pest management in the study area. This study also investigates the validity or otherwise of the results of the pest survey records of previous studies in neighbouring communities with similar production dynamics.

MATERIALS AND METHODS

Experimental site and choice of groundnut variety

The research was conducted at Golinga in the Tolon district of the Northern Region of Ghana in August 2022. The experimental site is located on longitude 0^{0} 53 and 1^{0} 25 W, latitude 09^{0} 15 and 10^{0} 02 N and 14.5 km southwest of Tamale and 12 km away from the University for Development Studies, Nyankpala campus (Abagale et al. 2014; Sayibu et al. 2015). The area has an unimodal rainfall pattern with an annual average of 1060 mm and a relative humidity of 20% to 82% in January and August respectively (Sayibu et al. 2015). The annual average minimum and maximum temperatures at Golinga are 20°C and 30°C respectively (Bekoe et al. 2021) (Figure 1). The Chinese groundnut variety was used for the study because it is the most preferred variety for cultivation by farmers in the study area.

Experimental design and data collection

The study was conducted using Completely Randomized Design (CRD). The experimental field was divided into 24 plots for cultivated groundnuts and 12 plots of uncultivated groundnuts with each plot measuring 5 m × 5 m and 2 m alley in between plots and rows. Two 14 x 8 cm pitfall traps (a total of 48 traps for the cultivated plots and 24 traps for the uncultivated plots) were fitted into holes dug out on each of the plots. Each of the plots was fitted with 2 of the pitfall traps randomly at approximately 3 meters intervals to each other. The main factors investigated were the differences in pest records (diversity) between the cultivated and uncultivated plots and pest damage between plots. The containers were filled with clean water and little drops of detergent to break the surface tension to prevent the escape of trapped arthropods. This was done after the containers were firmly fixed to the soil with the top of the container level with the ground. The traps were emptied four times at 12 days, 8 days, 4 days and 1 day before harvesting and stored with 70% alcohol for further identification. In other words, the traps were emptied on 4 occasions at 4 days intervals. The collected insect specimens were identified using reference collections from the laboratory of the Department of Animal Biology and Conservation Science, University of Ghana with guidance from Gullan and Cranston (2014).

Holes and scarification created by pests were observed on pods after harvest and used as criteria to categorize pods into highly damaged versus less damaged per plot. The percentage of pod damage was calculated by subjecting the highly damaged versus less damaged seeds to the formula by Quitco and Quindoza (1986):

$$\%\ \text{damage} = \frac{\text{Total number of highly damaged Pods x 100}}{\text{Total number of Pods}}$$

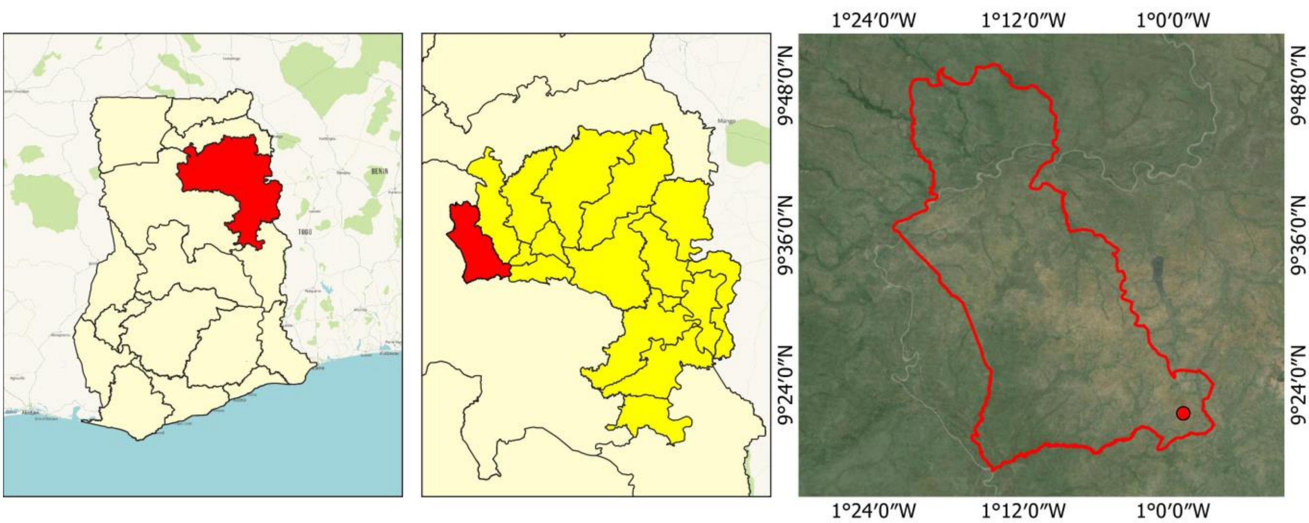

Figure 1. A picture showing Tolon District where Golinga is located on the map of Ghana

Data analysis

Student t-test was employed to measure differences between pest infestation of cultivated versus uncultivated lands and damaged versus undamaged pods per each plot in the study using SPSS software. This was after log transforming the data (count data) and subjecting the transformed data to a Shapiro-Wilk test for normality (W= 0.94, p > 0.05).

Preliminary survey on pest management

A preliminary survey was conducted for 30 farmers who already belonged to a farmer group in the study area to ascertain their preferred means for controlling soil arthropod pests of groundnuts, and perceptions of the effectiveness of the methods employed, and whether they would want a change as evidence of potential resistance build up within the pest population for pesticides used in general in the area during the 2022 rainy season. This was achieved through random administration of stratified questionnaires (hard copies in person) within the farmer group. The participants were mostly led by the researchers to answer the questions after seeking their consent to participate in the survey. Ethical clearance was received from the Department of Crop Science of the University for Development Studies.

RESULTS AND DISCUSSION

Diversity of soil arthropods

This study recorded a total of 1366 individual arthropods belonging to the orders Coleoptera, Myriapoda, Isoptera and Araneae. The most abundant arthropod group was the class Insecta with Isoptera and Coleoptera being the most abundant orders (Table 1).

There were similar but slight differences in arthropod diversity as reported by surveys conducted by Tanzubil (2016). This is because this study recorded natural enemies, but respondents in the previous study did not have knowledge of any natural arthropod enemy within the groundnut farms (see Table 1). The other difference recorded in the diversity of soil arthropods between this study and that of Tanzubil (2016) could be assumed to be that the current study only focused on soil arthropods and even near harvest time while the previous study considered responses from farmers at all stages covering both foliage and soil arthropods as well as varietal (only Chinese variety was used for this study but the previous study used more than one variety of groundnut) differences between the two studies as reported elsewhere (War et al. 2013; Krishna et al. 2015; Srinivasan et al. 2018). It has been shown that varietal differences affect arthropod preferences and so this may be a factor in the differences in the arthropod records in this study and the previous study (Tooker and Frank 2012; Ebeling et al. 2018)

Soil arthropod pests

The soil arthropod pests recorded for the study included Termites: *Macrotermes* spp. and *Microtermes* spp. and *Odontotermes* spp., Wireworms: *Heteroligus claudius*, Millepedes; *Eurymerodesmus* spp., False wireworms: *Gonocephalum* spp.; *Holotrichia* spp. while Aranae was the main natural enemy recorded for the study (Table 1) and the wireworms, and false wireworms and scarab beetles were the most destructive of them all. Also, the t-test results for the study showed that there was a significantly high pest infestation of groundnuts on the cultivated lands (M = 88.7, SD = 12.21) compared to the uncultivated lands (M = 15.0, SD = 9.3); (t (34) = 27.9, p = 0.0). This result agrees with that of the study by Umeh et al. (2001) for their research conducted in five West African countries that reported that termites, millipedes, wire worms and scarab beetles were the key arthropod pests of groundnuts. Other similar studies (Panse 2021; Maheshala et al. 2023) in other groundnut production areas have also reported similar ground-dwelling pests, however, there were other pests recorded in those other studies which were not recorded in this study. Such species as *S. litura*, *A. modicella* and *Frankliniella schultzei* were recorded in a previous study conducted elsewhere but were not encountered in the present study (Amin and Mohammad 1980; Panse 2021; Maheshala et al. 2023). There were also slight differences in pest records between the study by Tanzubil and Baba (2017), who reported similar arthropod pest groups except for beetles which were the second most dominant groups, and the former study compared to the latter which beetles dominated. By comparing this study with that of Tanzubil and Baba (2017), which was mainly through farmer interviews, it may be that the respondents in the latter study could not have encountered some of the nocturnal beetles and this might have accounted for their inability to confirm or list some of the soil-dwelling arthropods and nocturnal beetle pests. This is because farming activities do not occur at night in the study area and possible misidentification of pests by respondents could also have played a role in the findings. The insect pest guild recorded in this study agrees with results from other similar research works done in other parts of the world (Okello et al. 2013; Biswas 2014; Harish et al. 2015).

Table 1. A table showing abundance of arthropods recorded for the study

Arthropod	Order	Key pests	Abundance
Beetle	Coleoptera	*Holotrichia* spp., *Conoderus* spp., *Gonocephalum* spp.	797
Millipedes	Myriapoda	Odontopygidae, *Eurymerodesmus* spp.	99
Spider	Aranae	*Latrodectus* spp., *Nephilingis* spp., *Menemerus* spp., *Cyrtophora* spp., *Caerostris* spp.	50
Termites	Isoptera	*Macrotermes* spp., *Microtermes* spp.	420

Figure 2. A picture showing exit and or entry holes of soil arthropods on groundnut pod and a pod filled with frass of arthropod pests

Damage assessment of groundnut pods

All pods collected at harvest per plot were used for the computation of the damage assessment. It was revealed that all the experimental plots were infested with arthropod pests with various degrees of percentage damage to pods ranging from 65% to 100% damage on the various plots with an average of 89% (Figure 2).

The results further showed that the mean values (per each plot) of the damaged pods (*M* = 88.7, *SD* = 12.2) were significantly higher than the undamaged pods from a paired two-sample t-test for means (*M* = 12.17, *SD* = 12.7); (t (24) = 15.13, p = 0.0). The study further revealed that damages was caused by live insects detected within the pods, creating holes and scarification on pods and dead plants with severely damaged roots and or pods, which confirms reports of the type of damage by soil pest on groundnuts (Culliney 2013). Studies show that groundnut pod borers such as wireworms, termites, scarab beetles, and other pod-destructive pests could cause up to 100% of yield loss as was observed on some plots in this study (Naawe and Angyiereyiri 2020; Pawar et al. 2023) (Figure 3).

According to Okello et al. (2013) and Ajeigbe et al. (2014), soil arthropods contribute to yield loss by damaging pods, kernels and plant roots causing death and loss of up to 100% which was the case with some of the studies plots. Some of the most common damages caused were related to feeding activities of centipedes and termites as reported by Okello et al. (2013) and Tanzubil (2016) although they were not as devastating as damages caused by the coleopteran pests.

Preliminary survey of effectiveness of pest management

Results of the preliminary survey of 30 farmers in a farmers' group at the study area revealed that there is a very high reliance of pesticides for the control of arthropod pests with close to 50% (13 respondents) of the respondents relying solely on synthetic pesticides and the remaining approximated half (17 respondents) preferring a combination of pesticides and other traditional methods of control with only 2 (approximately 7%) respondents opting solely for traditional control methods. Also, when it comes to the efficiency of the control method used, only 3 out of the 30 respondents, representing 10% said that the method they use is good with the rest (90%) showing dissatisfaction with the effectiveness of method they use in controlling arthropod pests of groundnut in the study area (Figure 4).

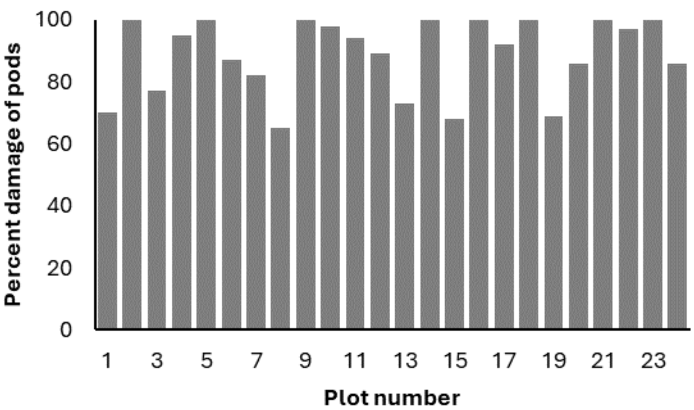

Figure 3. A graph showing percentage damage of groundnut pods on the various study plots

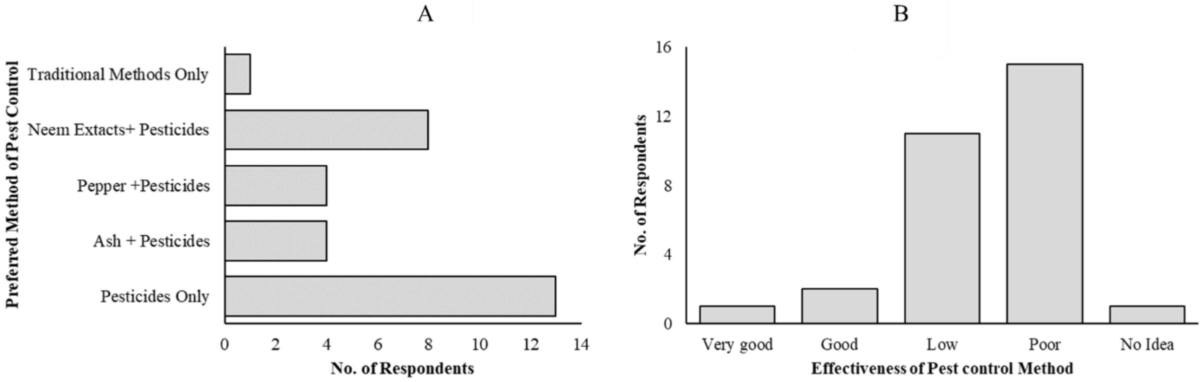

Figure 4. Response of farmers to their preferred method and its effectiveness in controlling arthropod pests in the study area

The finding from the preliminary survey in this study has revealed that there could be a heavy reliance on synthetic pesticide for the cultivation of groundnut in the study area. This may be a worrying sign as it was shown that farmers in the study area rely heavily on synthetic pesticides and such a situation will likely lead to environmental pollution. This is even more serious, especially in a rural area like Golinga where farmers are less educated to know the implications of misapplication of the required dosage of the pesticides they use (Abhilash and Singh 2009; Plianbangchang et al. 2009; Onwona-Kwakye et al. 2019). Under such circumstances, farmers are more likely to abuse the pesticides they use by increasing concentrations above the required level when they realize the required amount is no longer effective (Özkara et al. 2016; Tang et al. 2021). This seems to be the situation in the study area where majority of the respondents claim they could not rely solely on synthetic pesticides for the control of soil pests with most of them complaining of lack of effectiveness of their control method which is mostly synthetic pesticides as reported elsewhere (Sharifzadeh et al. 2018; Khayatnezhad and Nasehi 2021).

In conclusion, the study concludes that close to 90% of pods harvested from the study plots were heavily infested with pests and hence damaged. That could be translated to mean that the soil within Golinga can be said to be heavily infected with soil arthropod pests which will eventually lead to drastic yield reduction in groundnut production. The study further shows that beetles, termites, wireworms, false wireworms and millipedes were the key soil arthropod pests with beetles being dominant among the pest guilds within the farming communities in the study area. Based on the results of the previous studies by Tanzubil (2016) and Tanzubil and Baba (2017) and the preliminary survey of this study, The study recommended the use of biorational methods such as biopesticides from plant extracts to help farmers acquire easy and environmentally friendly control means to manage soil arthropod pest attacks on groundnuts at Golinga. This can help reduce the reliance on conventional pesticides and the problems associated with them as has already been listed. The results of the preliminary survey further show that if farmers use a specific group of pesticides in controlling soil pests amidst indiscriminate application, that might have conferred on them some form of resistance to those pesticides in the study area which require further investigation.

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Bone meal enhances the growth and yield of the tomato cultivar Cobra F1 by increasing fruit Ca content and alleviating blossom-end rot

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Abstract. *Assaha DVM, Petang LY. 2024. Bone meal enhances the growth and yield of the tomato cultivar Cobra F1 by increasing fruit Ca content and alleviating blossom-end rot. Asian J Agric 8: 70-77.* Bone Meal (BM) is a cheap organic fertilizer rich in Ca, which can be important for tomato production to counter the fruit's Blossom-End Rot (BER) disorder. The present study evaluated the effect of cow bone meal on the growth and yield of the Cobra F1 tomato variety. Therefore, to accomplish this, field plots randomly treated with BM (0, 143, 286, 429, and 572 $kgha^{-1}$) were sown with 30-day-old uniform-sized tomato seedlings. At the end of 8 weeks of treatment, plant height, number of leaves, fresh and dry vegetative biomass, and yield (fruit weight) were measured. The results obtained revealed that BM, at especially 572 $kgha^{-1}$, significantly elevated plant height (48%), leaf number (45%), root fresh weight (2.1-fold), stem fresh weight (27%), leaf and stem dry weight (27 and 51%, respectively), fruit weight per plant (2.1-fold), and up to 14% increase in fruit Ca concentration. BER affected control plants but did not affect BM-treated plants. Together, these results indicate that BM can significantly increase vegetative growth and yield of tomato void of BER; hence, it should be very suitable to enhance tomato production.

Keywords: Biomass, Ca deficiency, cow bone meal, fruit weight, *Solanum lycopersicum*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important fruit vegetables in the world because of its rich nutritional and medicinal value. Tomatoes are a valuable source of vitamins A and C and other minerals. It is equally rich in carotene antioxidants, especially lycopene, responsible for the fruit's bright red color, and phenolic compounds (Collins et al. 2022). These antioxidants have been associated with reduced risk of various cancers, cardiovascular diseases, and hypertension and constitute additives for industrial applications, such as in the meat industry (Dominguez et al. 2020; Collins et al. 2022). In addition, tomatoes and their by-products have been observed to be very important additives for meat quality enhancement in animal production (Biondi et al. 2020; Mohammed et al. 2021; Kirlan and Ketenoglu 2022).

Despite these invaluable benefits of tomatoes, their production is very challenging due to pests, diseases, and physiological disorders such as Blossom-End Rot (BER). BER is responsible for huge losses in tomato production, especially in developing countries such as Cameroon, where some of its production areas have low soil Ca concentrations (Aghofack-Nguemezi et al. 2014). This disorder is brought about by the inability of the plants to distribute Ca to the internal organs, especially to the fruits where concentrations are 3.08-16 mg per 100 g, effectively reducing BER incidence (Mazumder et al. 2021). The condition is further aggravated by water deficit in the soil for Ca mineralization and absorption, implying that plants can still suffer from Ca deficiency even in high Ca concentrations (Topcu et al. 2022). Therefore, for optimal tomato production, in addition to NPK and other nutrients, high concentrations of Ca and sufficient water availability are imperative in the production system.

Although global tomato production has increased (Collins et al. 2022; Karki and Dawadi 2022), local production in some countries is declining. Such is the case with Cameroon, where tomato production has declined since 2016 (Tabe-Ojong et al. 2020). This decline may be due largely to the socio-political crisis, pests and diseases, and climate change effects, such as rainfall variability, that have greatly affected some major tomato-producing areas, including Santa and Buea, reputed for tomato production (Malyse 2021; Wanie et al. 2020; Afanga et al. 2022). In addition, the major tomato cultivation areas have low soil fertility, and Ca deficiency is often pronounced, leading to losses due to BER. To cope with this, farmers spend a lot on fertilizers to boost production, thereby significantly raising the cost of production. Therefore, looking for cheaper alternative sources of Ca fertilizers is crucial for the cost-effective production of tomatoes.

Cow bone meal is an alternative organic source of Ca that could be exploited for this purpose since huge deposits of cow bones from slaughterhouses are under-exploited. Those cow bones are exploited and used primarily for animal feed production, but their application in crop production is yet to be embraced. Cow bone ash contains phosphorus (18%), calcium (30%), K (2.46), S (1.77), iron (0.46%), magnesium (0.79%), and zinc (0.06%), Cu

(0.02%) and Si (0.01%) (Deydier et al. 2005; Adetayo et al. 2021). The high Ca content in BM can improve root growth and help prevent BER in tomato plants. Therefore, supplemental Ca applied as BM during the cultivation of tomatoes could significantly enhance production and ensure the availability of this important fruit vegetable all year round. However, there is no empirical data to ascertain the effect of bone meal on tomato production, especially for the Cobra F1 cultivar. Therefore, the present study seeks to identify the most suitable level of BM as a soil amendment that will impact the growth and yield of the Cobra F1 tomato variety and curb BER incidence.

MATERIALS AND METHODS

Location and characteristics of the study site

The experiment was conducted in the experimental station of the Department of Agriculture, Higher Technical Teachers' Training College, Kumba, University of Buea in Barombi, Kumba, Meme Division in the South West Region of Cameroon (Figure 1). According to Tabot et al. (2018), Kumba has the following geographical Coordinates: 4°38′N 9°27′E and 4.63°N 9.45°E with an average elevation of 240 m above sea level (masl). Kumba lies in the Humid Forest, Agro-ecological Zone IV, with a monomodal rainfall regime. The climate of Kumba is typically equatorial with two seasons, the dry season (November to March) and the rainy season (April to October), with an average annual rainfall of 2,200 mm. The mean annual relative humidity and temperature are between 70% to 84% and 24℃ to 35ºC, respectively, and are characterized by hot days with high intensity of sunlight. It has sandy clay soil, which is brownish.

Soil sampling

Soil samples were randomly collected from different points at the experimental site from the top 30 cm (Tabot et al. 2020), air-dried, and thoroughly mixed to make a composite soil sample. Then, a portion of the composite soil sample was sent to analyze soil physico-chemical properties at the Soil Science Laboratory of the Faculty of Agronomy and Agricultural Science of the University of Dschang, Cameroon. The properties analyzed were soil texture, soil pH, organic matter (organic carbon, total nitrogen), exchangeable cations (Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}), cation exchange capacity, and available phosphorus (Table 1).

Plant material

Cobra F1 26 tomato variety seeds, TECHNISEM France, were purchased from a local Agriculture Shop in Muea, Cameroon. The seeds' indicated germination rate was 95%.

Table 1. Soil chemical characteristics of the study site

Property	Value	Property exchangeable cations (meq/100g)	Value
Texture	Sandy clay		
pH (H_2O)	6.12	Ca^{2+}	1.39
pH (KCl)	4.75	Mg^{2+}	1.11
Organic carbon (%)	4.61	K^{+}	2.57
Total nitrogen (gkg^{-1})	0.85	Na^{+}	0.60
C/N ratio	16.01	CEC	16.05
Available phosphorus ($mgkg^{-1}$)	9.25		

Note: CEC: Cation Exchange Capacity; Ca^{2+}: Calcium; Mg^{2+}: Magnesium; K^{+}: Potassium; Na^{+}: Sodium; KCl: Potassium Chloride; H_2O: Water

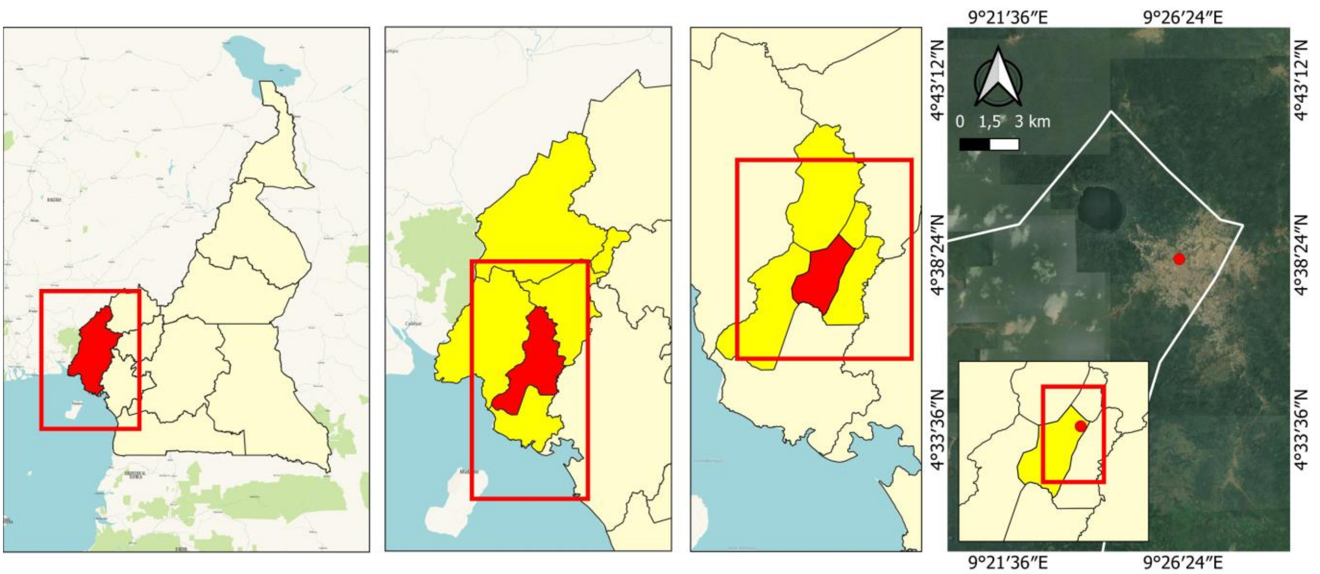

Figure 1. Map of Kumba showing the study site, Cameroon

Nursery establishment

The 3 x 1 m nursery beds were prepared under a shed to reduce sun intensity. Poultry manure was incorporated in the bed to increase the soil fertility at a rate of 20 tha^{-1}, and the beds were watered thoroughly for a week before sowing the seeds for proper decomposition of the manure. The nursery bed was disinfected according to Tambe et al. (2024) by watering beds with a mixture of 5.3 gL^{-1} Mancozep (fungicide) and 2 mLL^{-1} Parastal (insecticide) in a 15-L knapsack sprayer. Then, sowing was done at a depth of 1.5 cm.

Plot layout and treatment application

Fifteen plots of 3.5 x 1 m were planted in the field with 70 cm between plots. During tilling to prepare the plots, the plots were disinfected by watering beds with a mixture of 5.3 gL^{-1} Mancozep (fungicide) and 2 mLL^{-1} Parastal (insecticide) in a 15-L knapsack sprayer, followed by poultry manure incorporation at the rate of 10 t/ha, to ensure a good rate for tomato growth and yield (Ilodibia and Chukwuma 2015; Tambe et al. 2024). Then, bone meal was prepared from cow bones collected at a local slaughterhouse. The bones were oven-dried and crushed into a fine powder. Then the meal 0 (Control), 5, 10, 15, and 20 g per plant corresponding respectively to 0, 143, 286, 429, and 572 $kgha^{-1}$ (Table 2) was then randomly applied as treatments to the different plots within row spacing of 50 cm, around where the tomato seedlings would be transplanted. This was done two weeks before transplanting to ensure nutrient release and availability when the plants were transplanted (Figure 2).

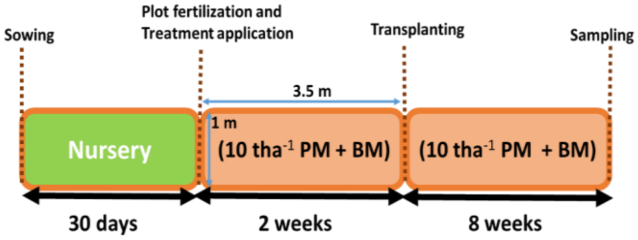

Figure 2. Timeline of the experiment from nursery to sampling. PM: Poultry Manure, BM: Bone Meal (0, 143, 286, 429, or 572 $kgha^{-1}$)

Table 2. The different treatments used in this study

S/N	Treatment Bone meal ($kgha^{-1}$)	Replicates (plot number)
1	0	3
2	143	3
3	286	3
4	429	3
5	572	3

The treatments were randomly assigned to the different plots after preparation, as shown in Figure 3. After two weeks of treatment application, the seedlings were uprooted from the nursery with soil around the root, transported to the field, transplanted in the evening, and irrigated. The seedlings were transplanted at a spacing of 50 cm (within rows) by 70 cm (between rows). Then, leaves were used to provide shade over the plants to reduce death from excessive sunlight. The shade was removed after 4 days when the plants were acclimated to the field conditions.

Agronomic practices

Moreover, the beds were weeded every 2 or 3 weeks. Before the onset of rains, the plants were irrigated twice daily (morning and evening) using a watering can. All the plants were treated with insecticide and fungicide fortnightly from 4 weeks after transplanting to prevent pest and disease infestation, using the same chemicals above. The plants were staked using wood stakes (Figure 4) in a similar way to that described by Tambe et al. (2024) to prevent infections of the branches and fruits when they touch the ground, as well as for improved yield.

Data collection

Three plants from each experimental unit were randomly selected and used to collect various growth and yield data.

Growth measurement

The growth parameters included plant height, number of leaves, and biomass partitioning. These were recorded at the end of the experiment. For the measurements, 3 plants were selected from each plot to 9 plants sampled per treatment since there were 3 replicate plots per treatment.

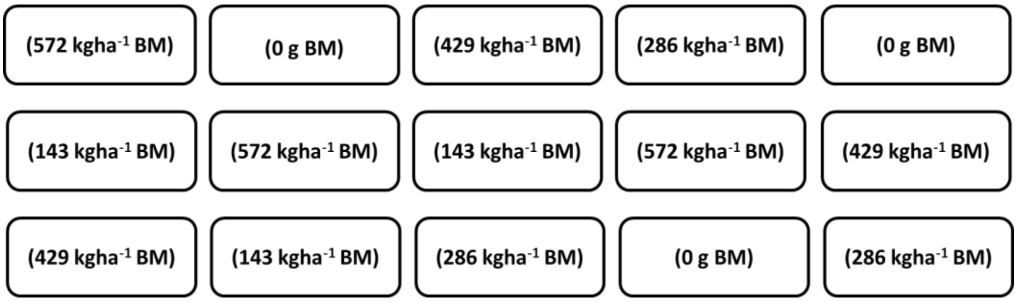

Figure 3. Plot layout and random allocation of the treatments in the field. BM: Bone Meal applied per plant

Figure 4. The tomato (Cobra F1 26) plants in the experimental field

Height (cm): Plant heights were measured from the soil surface to the apex of the terminal leaf, according to Zhang et al. (2020). This was done using a meter rule.

Number of leaves: According to Tabot et al. (2020), fully opened leaves were counted on all selected plants.

Biomass measurements (g): This was assessed according to Tabot et al. (2020). The plants were separated into leaf, stem, root, and fruit, and their fresh weights were recorded. Then, the parts (except fruits) were oven-dried for 72 h at 70ºC, and their dry weights were recorded. The total fresh biomass was the sum of the root, stem, and leaf fresh weights, while dry weight was the sum of the root, stem, and leaf dry weights.

Measurement of fruit Ca concentration

Fruit Ca concentration was measured following a modified method described by Reitz et al. (2021). Samples of whole fresh tomato fruits (3 per treatment) were harvested and sent to the Laboratory of Soil Analysis and Environmental Chemistry, Faculty of Agronomy and Agricultural Sciences, University of Dschang, Dschang, Cameroon, for analysis. Furthermore, fruits from similar degrees of maturity and ripening were harvested from the same position on the plants for this analysis.

Yield measurement

Tomato yield parameters included fresh fruit weight of single fruits, fresh fruit weight per plant, and total yield per hectare (Zhang et al. 2020). All fruits from each selected plant in all treatments (n = 9) were harvested, and the weight of the fruits per plant was recorded. Then the yield per hectare was extrapolated from fruit weight per plant and the area of one plot.

Symptoms of BER

The fruits of BM-treated and non-treated plants were visually inspected for signs of BER from the onset of fruiting till harvesting, and photographs of infected fruits were taken according to Coulibaly et al. (2023).

Data analysis

All collected data were subjected to One-Way Analysis of Variance (ANOVA) using the IBM SPSS statistical package version 21 (IBM Corp., Armonk, NY, USA). Tukey HSD was used to separate the means (n = 3 for Ca concentration and n = 9 for all other parameters) at α = 0.05. Pearson correlation (two-tailed) was used to verify the relationship between fruit yield and calcium concentration.

RESULTS AND DISCUSSION

Effect of Bone Meal (BM) on growth of tomato plants

Effect on plant height

Figure 5 presents the effect of BM amendment on the height of cobra F1 tomato plants. The lowest bone meal level (143 kgha-1) did not significantly alter the plants' growth, but 286 - 572 kgha^{-1} significantly increased it, with 572 kgha^{-1} inducing the most growth effect (48% increase from control).

Effect on leaf number

The effect of BM treatment on the number of leaves of Cobra F1 tomato plants is presented in Figure 6. The application of 143 kgha^{-1} BM did not significantly alter the leaf number compared to control plants. However, 143-572 kgha^{-1} BM significantly increased the leaf number (up to 45% increase under the 2 g BM treatment), with no significant difference among the 3 treatments.

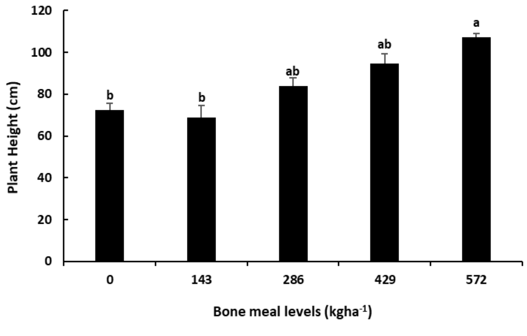

Figure 5. Effect of BM on the height of tomato plants. Bars represent means ± SE (n = 9), and different letters indicate significant differences among means (p≤0.05)

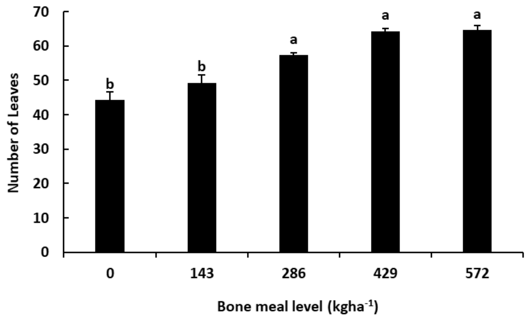

Figure 6. Effect of BM on the number of leaves of Cobra F1 tomato plants. Bars represent means ± SE (n = 9), and different letters indicate significant differences among means (p≤0.05)

Effect on fresh biomass

The effect of BM treatments on the fresh biomass of the different parts of the plants (root, stem, leaf and fruit) is shown in Figure 7. All the BM treatments significantly increased the root fresh mass ($p<0.05$), with 572 kgha^{-1} per plant producing the highest mass (2.1 folds of the control, Figure 7.A). Concerning the stem, while 143 kgha^{-1} BM showed no difference with the control ($p>0.05$), the rest of the treatments significantly raised the stem fresh mass, with the 20 g BM raising the mass by 27% when compared with controls (Figure 7.B). All BM treatments significantly enhanced the leaf fresh mass compared to the control, and the increase was similar among all the treatments (Figure 7.C).

Effect on dry biomass

The effect of BM treatments on the dry mass of vegetative plant parts is presented in Figure 8. The BM treatments did not significantly alter the dry mass of the root (Figure 8.C) but significantly increased that of the stem by up to 51% in the stem (Figure 8.B) and 27% in the leaf (Figure 8.A) except for 143 kgha^{-1} BM which did not change leaf dry mass from control levels. Thus, in dry mass, BM enhanced growth by exerting its action more on the stem and leaves than on the roots.

Effect of BM on yield

Figure 9 shows the effect of different levels of BM on the yield of tomato plants. The weight of a single fruit per treatment was measured and shown to increase with the increased bone meal level, with the highest level recording the greatest weight (45% higher than the control fruit, Figure 9.A). The yield per plant presented as fresh fruit weight equally significantly increased under the BM treatments, with the 572 kgha^{-1} BM recording the highest fruit mass (2.7-fold increase from the control level, Figure 9.B). The extrapolated yield on a hectare shows an approximately 3-fold increase under the 572 kgha^{-1} treatment from control (Figure 9.C). This indicates that BM, especially the 572 kgha^{-1} per plant rate, is very suitable for improving tomato production.

Effect of BM amendment on fruit Ca concentration

Figure 10 presents the concentration of Ca in tomatoes grown with or without BM. All BM treatments significantly increased the fruit Ca concentration, with the 572 kgha-1 BM producing the highest concentration, 14% higher than the control. This indicates that BM enhances Ca accumulation in tomatoes' fruits.

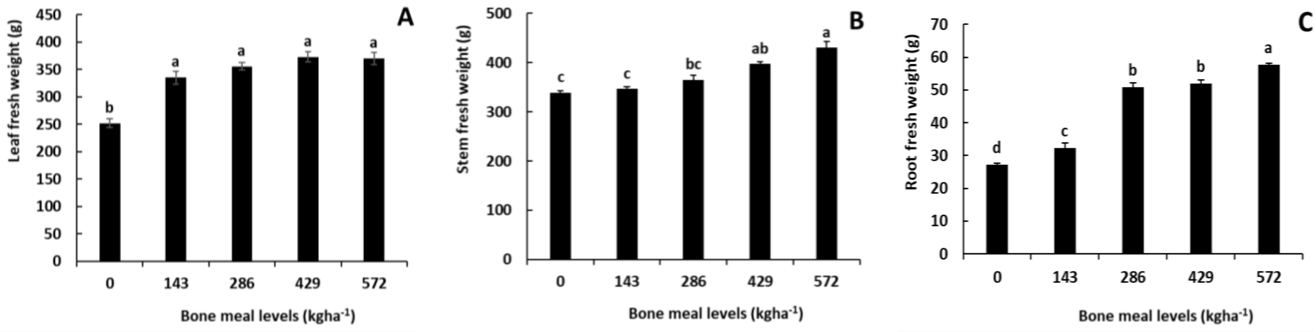

Figure 7. The effect of BM treatments on the fresh biomass of tomato plants. A. Leaf, B. Stem, C. Root, and D. Fruit. The bars represent means ± SE (n = 9), and bars with the same letter are not statistically different ($p \leq 0.05$)

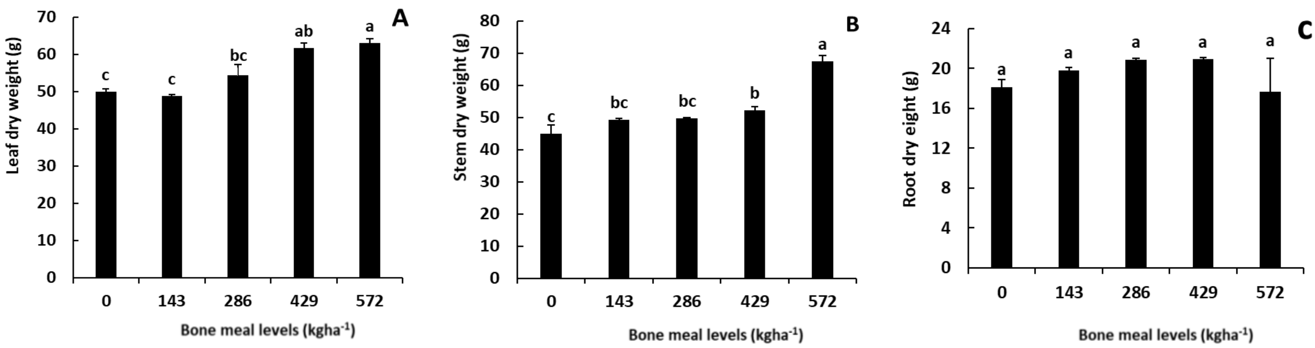

Figure 8. The effect of BM treatment on tomato plants' root, stem, and leaf dry biomass. The bars represent means ± SE (n = 9), and bars with the same letter are not statistically different ($p \leq 0.05$)

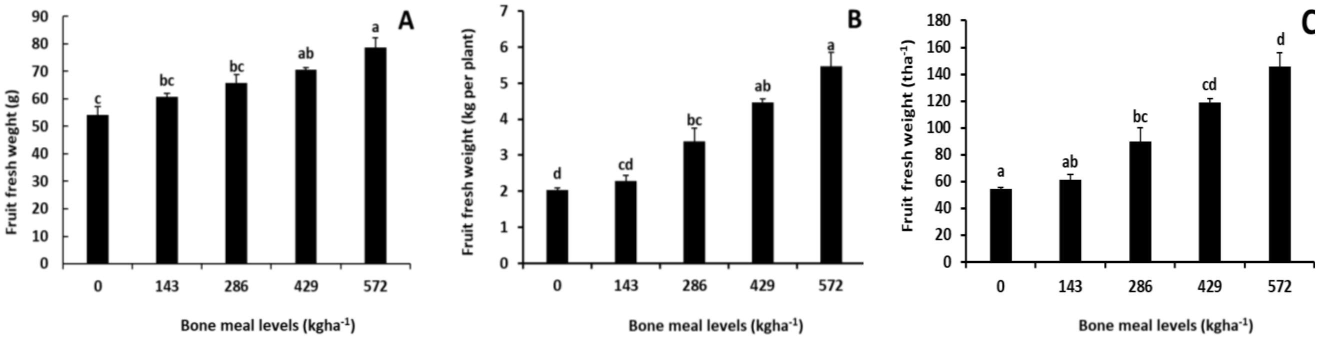

Figure 9. Effect of BM on single fruit weight (A), total fruit weight per plant (B), and extrapolated yield per hectare (C) of Cobra F1 tomato. Bars represent Means ± SE (n = 9), and different letters indicate significant differences among means (p≤0.05)

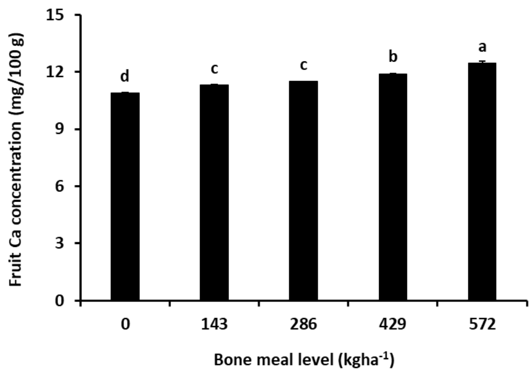

Figure 10. Effect of BM on the Ca concentration of tomato fruits. Bars represent means ± SE (n = 3), and different letters indicate significant differences among means (p≤0.05)

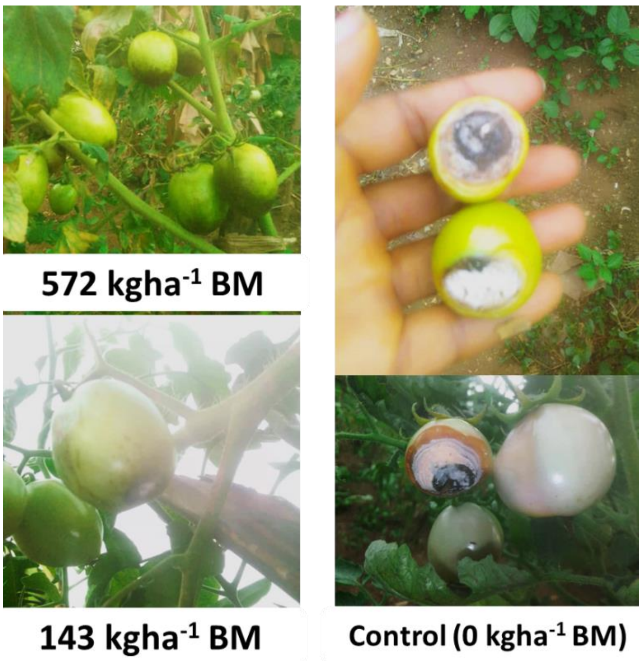

Figure 11. BM inhibits BER in tomato fruits

Effect on BER incidence

Tomato plants that received no BM showed BER symptoms, whereas those grown with BM were unaffected (Figure 11). The 143 $kgha^{-1}$ BM treatment showed signs of the disease at the onset of fruit, but the signs disappeared as the fruits matured, while no sign of infection was noticed on the fruits of the rest of the treatments. Hence, only plants that did not receive BM (control) were damaged by BER, while no BM-treated plant had BER-damaged fruits; this indicates that BM can control BER infection.

Discussion

The present study evaluated the effect of cow Bone Meal (BM) on the growth, yield, and incidence of Blossom-End Rot (BER) of the Cobra F1 26 variety of tomatoes in the field. The results clearly indicate that BM significantly enhanced the vegetative growth of the plants, concomitantly with inhibited BER incidence, which resulted in markedly enhanced yield of tomato fruits. This could be attributed to the characteristics of BM, a rich source of Ca and P (Adetayo et al. 2021), whose application has been shown to improve soil physico-chemical characteristics and promote the growth and yield of many plants. For example, Unagwu et al. (2023) evaluated the growth and yield performance of cucumber on degraded soils with and without Cow Bone Meal (CBM) and NPK; they found that the CBM significantly improved soil bulk density, organic matter content, and N, P and Ca contents. This increase in the soil characteristics by CBM led to a more significant increase in growth and plant yields than NPK and control conditions. Similarly, Atemni et al. (2023) studied the effect of BM on soil chemical characteristics; BM elevated soil pH, EC, Ca, and N but decreased heavy metal concentrations, creating a favorable environment for improved growth and yield of *Pelargonium graveolens* (Thunb.) L'Hér. This superior growth and yield were further attributed to enhanced photosynthetic pigments. The present study revealed that the tomato plants subjected to BM showed a significant increase in plant height and biomass accumulation, suggesting its role in improving soil characteristics that favored growth. The yield of the tomato plants was significantly improved by the BM treatments, similar to observations in other plants (Atemni et al. 2023; Unagwu et al. 2023).

The enhanced growth and biomass accumulation under BM treatment may be related to enhanced carbon assimilation in photosynthesis, which correlates with increased leaves and leaf area (Weraduwage et al. 2015; Sarkar et al. 2021). Therefore, increased height leading to initiation of new leaves (increase in leaf number) and the expansion of the leaves will increase the plant's ability to intercept more radiation for optimal photosynthetic output and a resultant increase in biomass, and hence the growth of the plant (Walne and Reddy 2022). The current study showed the BM significantly increased leaf number, with a significant increase in biomass and height of the plant, indicating that photosynthesis was enhanced and effective biomass partitioning for the observed enhanced yield.

One of the most important causes of low tomato production is BER, a physiological disorder caused by the inability of the plant to regulate the translocation of Ca to the fruit (Mazumder et al. 2021). This inability arises because of low transpiration and rapid growth of organs such as fruits, for increased fruit transpiration is correlated with increased fruit Ca accumulation (Mazumder et al. 2021; Topcu et al. 2022). In addition, it has been shown that low Ca and excess Ca in the plant will induce BER (Reitz et al. 2021), implying that moderate amounts will solve the problem. Therefore, the increased availability of moderate amounts of Ca in the soil and favorable conditions for its translocation to the fruit, such as increased transpiration, especially under low moisture conditions, will lead to increased fruit Ca accumulation and, hence, reduced incidence of BER. In the present study, the fruit Ca concentration significantly increased under the BM treatments, especially in the 572 $kgha^{-1}$ BM treatment that had the highest concentration, which strongly correlated with enhanced fruit yield per plant ($R = 0.914$, $p<0.01$) and inhibited blossom-end rot (Figure 11). In contrast, control plants with no BM had fruits affected by BER and low yield. This could contribute to reduced yield in plants under control conditions. Therefore, to reduce BER and increase the yield of the BER-sensitive tomato variety, Beorange, Karlsons et al. (2023) sprayed the fruits of the plants with Ca solutions. They found that the treatments did not alleviate BER, with no yield difference compared to control plants, suggesting that uptake and internal distribution might have been ineffective. The present study revealed that the Ca role in alleviating the condition was very obvious from the lowest BM level (143 $kgha^{-1}$), under which the signs of BER appeared at the onset of fruiting but disappeared towards fruit maturity; this indicating that increased release from the BM over time and its uptake helped alleviate the condition, while its complete absence under higher BM treatments indicates high Ca availability. Although recent findings have indicated that Ca is not the cause of BER but a combination of stress factors (Saure 2014), the present study's observations support the widely accepted Ca-deficiency cause.

Blossom-end rot incidence was inversely related to soluble Ca concentration in the distal part of tomato fruit, whereby high concentrations will inhibit it; in contrast, low concentrations (<0.2 $\mu mol.g^{-1}$) will induce it (Yoshida et al. 2014). The concentration of Ca in the fruit depends on the amount of Ca in the growth medium and on solar radiation (Yoshida et al. 2014), while the apoplastic Ca in the distal part of the fruit and not total Ca regulates BER incidence (Riboldi et al. 2020; Topcu et al. 2022). In the current study, the use of cow BM significantly enhanced the Ca concentration of tomato fruits, which inhibited BER, whereas lack of BM induced BER. In another study, egg, snail, and oyster shell meals applied to tomatoes also reduced the incidence of BER and enhanced growth and yield, and eggshells produced the lowest BER incidence (Coulibaly et al. 2023). These studies indicate that Ca-rich sources such as BM and shell meals are important for enhanced tomato production. Reducing leaf number has also been shown to be important in reducing the incidence of BER, where reduction of leaf number to 12 per plant significantly increased soluble Ca concentration in the distal parts of tomato fruits and a resultant reduction of BER incidence when compared with plants having higher leaf numbers (Indeche et al. 2020). This suggests that the Ca supplied by BM in the current study was sufficient to meet the needs of the vegetative parts and the fruits of Cobra F1 tomatoes. However, it will be interesting to investigate whether the application of BM on defoliated tomato plants will have an added effect on growth and yield.

In conclusion, the BM amendment, especially the 572 $kgha^{-1}$ level, markedly increased the growth of cobra F1 tomato plants. The BM equally significantly increased the yield of tomato fruits, as well as the fruit Ca concentration. This increased fruit Ca concentration possibly increased the resistance of the fruits to BER infection, unlike the control plants, without BM, that were affected by BER. Hence, we recommend that BM (especially at 572 $kgha^{-}1$) be adopted as a rich source of Ca and P to enhance tomato production by potentially altering soil characteristics that favor plant growth and yield. However, the physiological and molecular basis of BM-induced growth and yield enhancement are yet to be understood and will be a worthwhile undertaking. It will equally be of interest to determine whether BM application rates beyond 572 $kgha^{-1}$ will have additive effects on the growth and yield of tomatoes.

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