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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

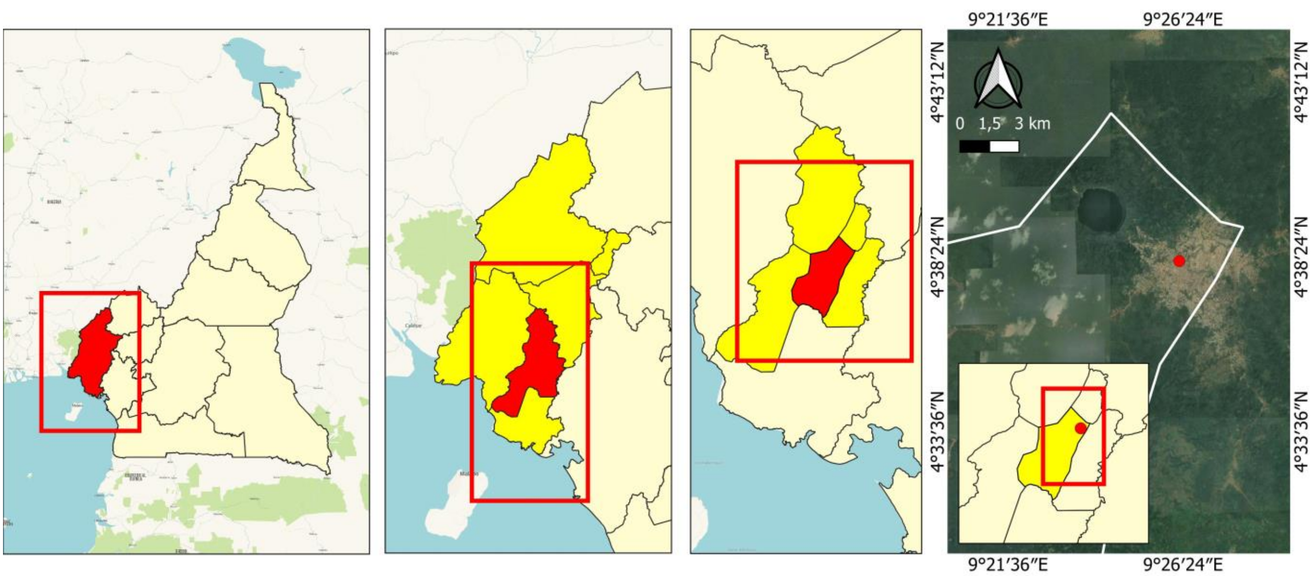

Figure 1. Map of Kumba showing the study site, Cameroon

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).

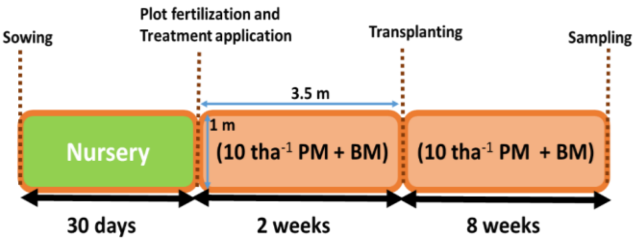

Figure 2. Timeline of the experiment from nursery to sampling. PM: Poultry Manure, BM: Bone Meal (0, 143, 286, 429, or 572 $kgha^{-1}$)

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.

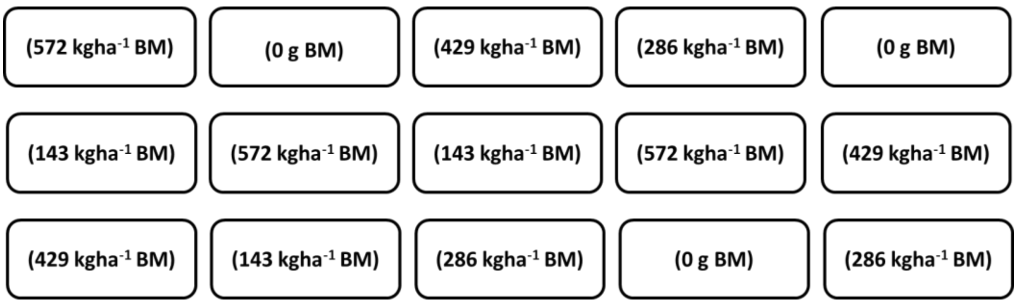

Figure 3. Plot layout and random allocation of the treatments in the field. BM: Bone Meal applied per plant

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 $\alpha = 0.05$. 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.

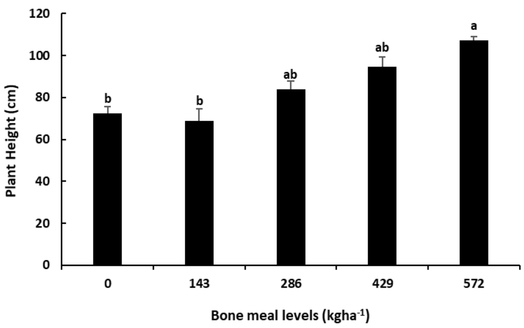

Figure 5. Effect of BM on the height of tomato plants. Bars represent means ± SE (n = 9), and different letters indicate significant differences among means ($p \leq 0.05$)

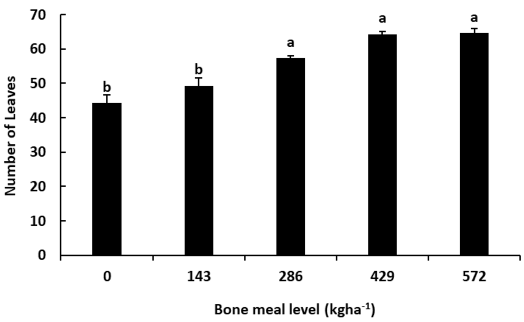

Figure 6. Effect of BM on the number of leaves of Cobra F1 tomato plants. Bars represent means ± SE (n = 9), and different letters indicate significant differences among means ($p \leq 0.05$)

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.

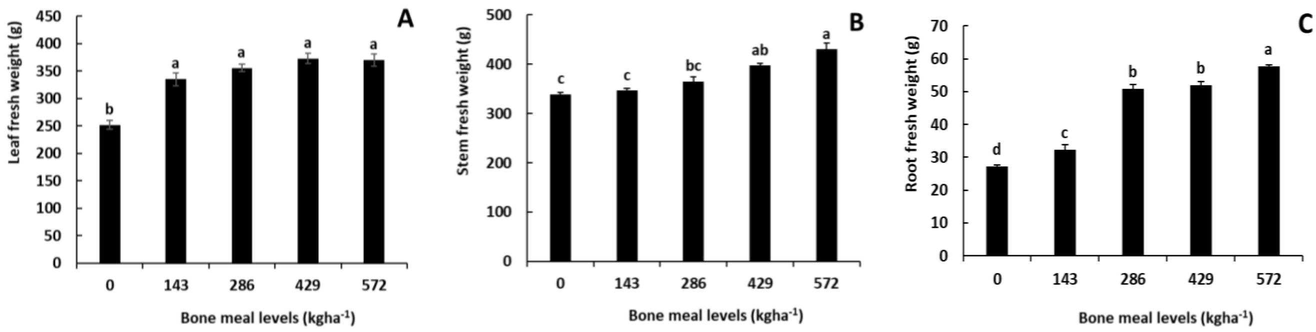

Figure 7. The effect of BM treatments on the fresh biomass of tomato plants. A. Leaf, B. Stem, C. Root, and D. Fruit. The bars represent means ± SE (n = 9), and bars with the same letter are not statistically different ($p \leq 0.05$)

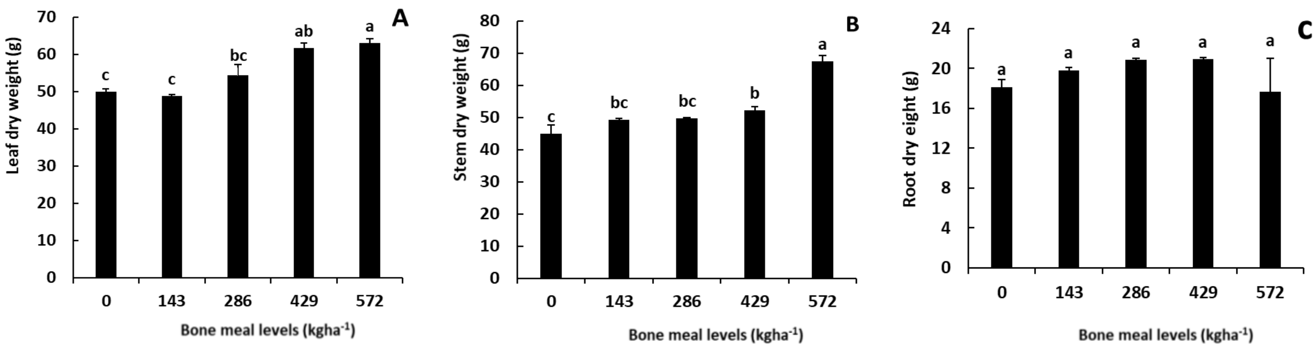

Figure 8. The effect of BM treatment on tomato plants' root, stem, and leaf dry biomass. The bars represent means ± SE (n = 9), and bars with the same letter are not statistically different ($p \leq 0.05$)

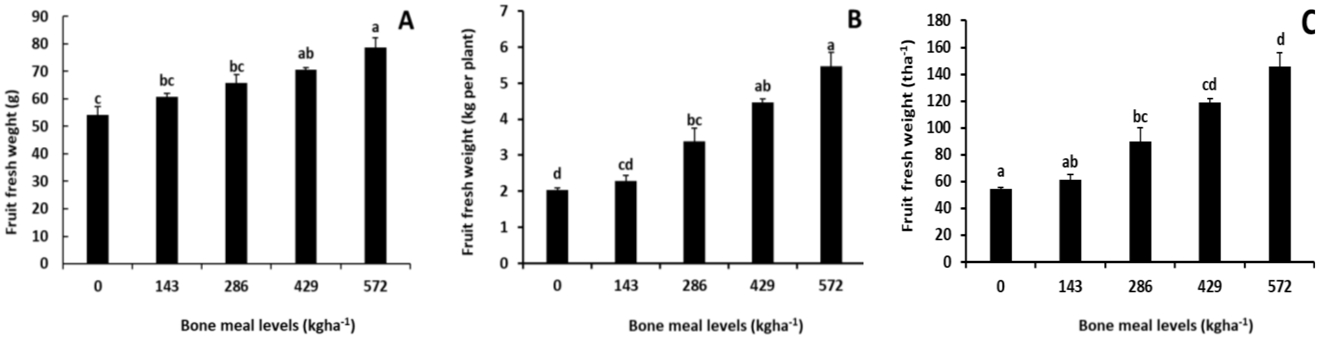

Figure 9. Effect of BM on single fruit weight (A), total fruit weight per plant (B), and extrapolated yield per hectare (C) of Cobra F1 tomato. Bars represent Means ± SE (n = 9), and different letters indicate significant differences among means (p≤0.05)

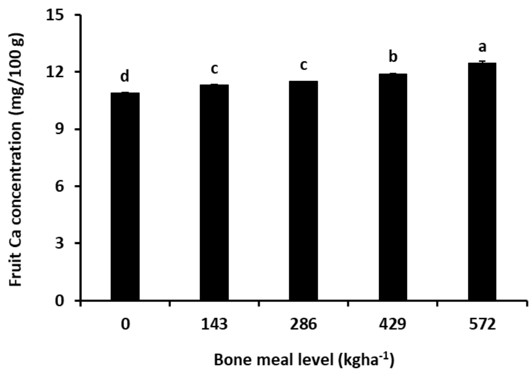

Figure 10. Effect of BM on the Ca concentration of tomato fruits. Bars represent means ± SE (n = 3), and different letters indicate significant differences among means (p≤0.05)

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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