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Proceeding:

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Effect of phytase and citric acid supplementation in the feed quality of laying hen

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Abstract. *Nuningtyas YF, Huda AN, Marjuki, Ulfah SN. 2022. Effect of phytase and citric acid supplementation in the feed quality of laying hen. Asian J Agric 6: 55-60.* This study aims to determine and evaluate the combination of phytase and high dosage of citric acid on the feed quality based on seeds. This study used data experimental at Brawijaya University, Malang, Indonesia, with five treatments and four replications. This research analyzes the nutritional feed content in vitro digestibility and phosphorus content in concentrate. The result of the study was a basal feed of laying hens followed by a dry matter of 90.63%, organic matter of 81.92%, crude protein of 16.44%, crude fiber of 4.34%, and fat of 7.49%. The basal laying hen feed without adding phytase enzyme and citric acid had a relatively low phosphorus content of 0.43%. It would be limited due to the absence of phytic acid breakdown assistance from the phytase enzyme. The nutrient phosphorus content in the residual feed digestibility is sequent T0 2.2431%, T1 2.7809%, T2 1.6225%, T3 2.0717%, and T4 2.7199%, with T4 for the higher value and the lowest in T2. The addition of the phytase enzyme and citric acid to animal feed for laying hens with various levels has a significant difference ($P < 0.01$) when viewed at the end of the digestibility of phosphor. However, the T2 laying hens feed with the addition of 2% phytase enzyme + 2% citric acid had the lowest phosphorus content in residue feed digestibility in vitro. Therefore, further research should be carried out in-vivo testing, especially on laying hens, to determine the effectiveness of the added enzymes by using the in-vivo test and the amount of phosphorus.

Keywords: Citric acid, feed quality, laying hen, phytase enzyme

INTRODUCTION

Poultry feed is usually sufficient from feed ingredients in the form of grains because it contains many essential amino acids that are needed by poultry for growth. Essential amino acids in the form of lysine and sulfur-containing amino acids such as methionine and cysteine must be given in sufficient proportions to maximize poultry performance (Andri et al. 2020). One of the essential amino acids that layer hen needs are a substance in the form of phosphor. Phosphor is important in poultry's metabolic process and growth, with a fairly high economic value as a source of energy and amino acids. Phosphor (P), as much as 70% contained in raw materials, is stored in the form of Phytate. According to Vieira et al. (2016) and El-Hack et al. (2018), as much as two-thirds of phosphorus is found in grains in Phytate. Furthermore, Phytate, the main form of phosphorus in plants, has chelating properties (Kühn et al. 2016). Therefore, Phytate can be categorized as an antinutrient component (Pramita et al. 2008; Samtiya et al. 2020) in terms of binding proteins and ions of several minerals such as calcium, iron, zinc, magnesium, manganese, and copper.

Phytic acid (known as inositol hexakisphosphate (IP₆), or Phytate in a salt form, is the main phosphorus in various plant tissues, especially grains and legumes (Kumar et al. 2010). Monogastric livestock such as poultry have limitations in digesting Phytate due to the lack of endogenous phytase enzymes, so they cannot hydrolyze

phytase in digestion (Lamid et al. 2018). Phytases are chemically known as Myo-inositol (1,2,3,4,5,6) hexakisphosphate phosphohydrolase, which could catalyze the release of phosphate from Phytate and liberate proteins, and other bound minerals (Kumar et al. 2010; Bhavsar et al. 2012). The principle of phytase for the utilization of nutrients is to increase the absorption of nutrients by breaking the bonds of phytate compounds so that hydrolyzed minerals and proteins can be utilized optimally in subsequent metabolic and biosynthetic processes. In addition to phytase utilization, citric acid has also been shown to reduce phytic acid in plants. Research conducted by (Rodriguez et al. 2015) with the addition of phytase enzymes and citric acid in feed can reduce phytic acid (or Phytate, the salt form of phytic acid), which is characterized by increased digestibility and reduced excretion of the P and N.

The microbial phytase in Myo-inositol (1,2,3,4,5,6) hexakisphosphate phosphohydrolase in poultry positively affects growth performance, feed efficiency, protein digestibility, and good absorption of minerals, such as calcium and phosphorus (Lamid et al. 2018). Commercial use of phytase microbes has been used since the 1990s for poultry and pigs. Monogastric livestock's lack of ability to digest Phytate negatively impacts livestock growth and the economic side of the livestock business (Dersjant-Li and Dusel 2019). In addition, Phytate easily binds to proteins and minerals (especially calcium, Ca) in the stomach and intestinal pH (Rokhmah et al. 2009; Selle et al. 2012),

forming binary (phytate protein) or ternary complexes (phytate-mineral-protein), which will further affect the lack of availability of phytate-protein. As a result, p, minerals, protein, and other nutrients that livestock should absorb for growth will be wasted through feces.

Phytase activity is expressed as a phytase unit (FTU); one phytase unit is defined as the amount of enzyme that liberates one micromole of inorganic phosphorus per minute from 0.0051 mol/L sodium phytate at 37°C and pH 5.50 under test conditions (AOAC 2012). Studies have shown that a phytase dose of 500 FTU/kg of feed typically achieves 40 to 60% of phytate degradation in the late small intestine, compared to 10 to 40% in no dietary supplementation (Dersjant-Li et al. 2015). Growth performance increased with the addition of 500 or 1000 FTU, which could replace 1.9 g P of monocalcium phosphate. Phytate has been degraded in the digestive tract by adding microbial phytase, which can be seen from the digestibility of phosphorus, namely 32% and 49.4% in a low P diet without and with 500 FTU kg⁻¹ (Dersjant-Li et al. 2015). Generally, phytase microbes can increase growth performance, feed efficacy, and digestibility of monogastric animal feed.

The purpose of this study was to determine the effect of increasing the dose of phytase (%) and citric acid (%) to reduce the phytic acid content, reduce economic costs, improve growth performance, and improve the quality of animal feed in laying hens fed with a basal diet of wheat, maize, rice bran, fish meal, and MBM without added inorganic P, compared to a basal diet.

MATERIALS AND METHODS

Study area

This research was conducted in the Faculty of Animal Science, Universitas Brawijaya, Malang, East Java, Indonesia, and BPTP Karangploso Laboratory, Malang, East Java, Indonesia. The analysis of the nutritional content of feed and in vitro digestibility experiment was carried out at the Laboratory of Nutrition and Animal Feed, Faculty of Animal Science Universitas Brawijaya, while the phosphorus test on the concentrated sample was carried out in BPTP Karangploso Laboratory, Malang, East Java, Indonesia.

Procedures

Treatments-1

In treatment I in this study, five treatments was used, containing four replications. The use of basal feed in this study consisted of several ingredients: wheat, corn, bran, fish meal, and MBM. The treatment of laying hen's feed was based on the addition of doses of the phytase enzyme and citric acid levels, which were described as follows: (i) T0: basal diet without the addition of phytase enzyme and citric acid. (ii) T1: basal diet with the addition of 1% phytase enzyme + 1% citric acid. (iii) T2: basal diet with the addition of 2% phytase enzyme + 2% citric acid. (iv) T3: basal diet with the addition of 3% phytase enzyme + 3

% citric acid. (v) T4: basal diet with the addition of 4% phytase enzyme + 4% citric acid.

How to use a spectrophotometer for a phosphorus test-2

In a study by Xu et al. (2020), phosphorus contents were obtained using a spectrophotometer (UV-752 N, China). Next, phosphorus content was measured by a spectrophotometric method using a molybdate vanadate reagent (Wiyantoko et al. 2018). Finally, the phosphorus contents were obtained using a spectrophotometer (Genesys 10 UV, USA) on yellow molybdate reagent-vanadate. For the first procedure, prepare the sample until ready in a 10 mL volumetric flask. Then added, molybdate reagent-vanadate and aquadest to 10 mL (line of the flask) for color formation. Next, measured the absorbance was using a spectrophotometer (Genesys 10 UV, USA) with a length of 400 nm wave. The absorbance value is then related using linear regression with a standard solution.

Data analysis

The obtained data were tabulated using Microsoft Excel Program, then subjected to analysis of variance (ANOVA) from a Completely Randomized Design (CRD). Finally, it was continued with Duncan's Multiple Distance Test to see if there was a treatment effect.

RESULTS AND DISCUSSION

Proximate analysis of the basal diet of laying hens-1

Table 1 shows the result of the proximate analysis of the basal diet used in this study, namely the laying hens used. Based on research on the combination of citric acid and phytase enzyme on diet quality, the good quality of animal feed can be judged by its high digestibility value. The basal diet of laying hens presented in Table 1 shows the value of dry matter at 90.63%, organic matter at 81.92%, crude protein at 16.44%, crude fiber at 4.34%, and fat at 7.49%. The nutrient content of basal feed has value according to the nutritional standards of laying hens. However, without the addition of citric acid and phytase enzymes, the nutrient content cannot be digested properly by laying hen, especially protein and minerals, resulting in reduced nutrient digestibility.

Table 1. Proximate analysis of diets of laying hens

Treatments	DM (%)	OM* (%)	CP* (%)	CF* (%)	EE* (%)
T0	90.63	81.92	16.44	4.34	7.46
T1	90.42	81.87	16.47	4.25	7.35
T2	90.38	81.76	16.59	4.21	7.24
T3	90.34	80.72	16.63	4.19	7.19
T4	90.27	80.65	16.72	4.17	7.14

Note: Results of proximate analysis at the Laboratory of Nutrition and Animal Feed, Faculty of Animal Science, Universitas Brawijaya, Malang, East Java, Indonesia 2022; *: Based on 100% dry matter

Phosphorus content of basal feed of laying hens-2

The results showed that the basal laying hen feed without the addition of phytase enzyme and citric acid had a relatively low phosphorus content of 0.43% and would be limited due to the absence of phytic acid breakdown assistance from the phytase enzyme (Table 2).

Phosphorus content in residual feed digestibility-3

Figure 1 is a standard absorbance curve obtained in testing the phosphorus content using a spectrophotometer. The sample measurement principle is to convert all metaphosphate and pyrophosphate with nitric acid into orthophosphate using a wavelength of 400 nm. Those treatments will react with these reagents and form a yellow vanadic acid-molybdcic acid complex. The color intensity of the complex compound can be measured with a spectrophotometer at a wavelength of 400 nm and compared with a standard phosphor with a known concentration (Table 3).

The research results evaluating the phosphorus content in the remaining digestibility of laying hens showed that the highest phosphorus content was T1 with a level of $2.780 + 0.13$ % and the lowest value with a phosphorus content of $1.622 + 0.18$ %. The lower levels of phosphorus in the digestibility residue indicate that livestock can digest phosphorus well due to the help of phytase enzymes, so in this study, T2 with phytase enzyme levels of $2\% + 2\%$ citric acid was the feed treatment with the best results in laying hens (Table 4).

Table 2. A test result of phosphorus content of basal feed of laying hens

Feed	Water content (%)	Phosphorus content (%)
Laying hen	7.02	0.43

Note: Test results at the Soil Laboratory, BPTP, Indonesia

Table 1. The absorbance of the feed samples of laying hens

Treatments	Absorbance (nm)
T0	$0.322 + 0.22$
T1	$0.328 + 0.24$
T2	$0.313 + 0.05$
T3	$0.370 + 0.13$
T4	$0.452 + 0.14$

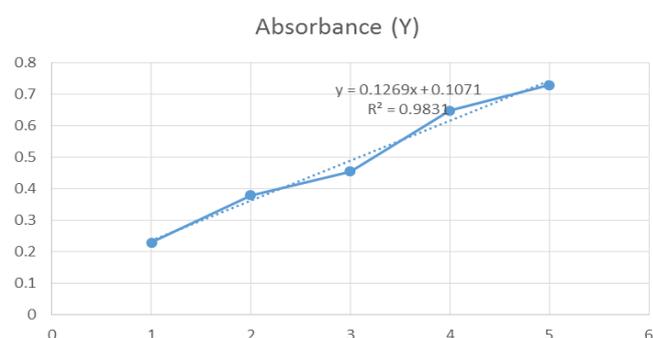


Figure 1. Standard absorbance curve

Table 4. Nutrient phosphorus content in the residual feed digestibility

Laying hen feed digestibility	
Treatments	Phosphorus content (%)
T0	$2.243 + 0.12^{bc}$
T1	$2.780 + 0.13^c$
T2	$1.622 + 0.18^a$
T3	$2.071 + 0.21^b$
T4	$2.719 + 0.17^c$

Note: a-c shows a very significant difference in each treatment ($P < 0.01$)

Discussion

Proximate analysis of laying hen feed ingredients

The proximate analysis of laying hen feed ingredients that have been formulated by adding various levels of phytase is tested for in vitro digestibility. Furthermore, the results of the residual feed digestibility of phosphorus content were tested using a quantitative spectrophotometer. Using phytases and citric acid in non-ruminant feeds is becoming increasingly relevant in conserving resources and minimizing the environmental impact of phosphorus (P) excretion. The feed's nutrient content must be of good quality to maximize productivity. Phytases are widely distributed in plants, microorganisms, and animal tissues used in animal feed to improve the nutritional value and reduce phosphorus pollution from animal waste (Cano et al. 2020). Microbial phytases are most promising for biotechnological applications to support feed digestibility (Jain et al. 2016). Intrinsic plant phytase activity varies widely among plant species and ranges from negligible in corn and soybean to very high in wheat and rye (Sommerfeld et al. 2020). Mucosal phytase activity is described for pigs and poultry (Selle and Ravindran 2007; Huber et al. 2015), but the data for ruminants are lacking (Haese 2017). The few studies describing InsP6 hydrolysis in the small intestine of ruminants, however, indicate that mucosal phytate activity seems to be physiologically irrelevant in ruminants (Humer et al. 2015).

Adding phytase enzymes and citric acid to basal feed can increase feed digestibility by meeting the nutrient requirements of laying hens. In addition to good feed digestibility, phosphorus digestibility is also very good, supporting the maximum digestibility of other nutrients is evidenced by the fulfillment of nutrient standards for laying hens in the treatment feed. Based on (SNI-01-3929-2006), the nutrient standards for layer hens are (min) CP 16%, (max) CF 7%, and (max) EE 7%. This study showed that the nutrient value of the treatment feed was by the standard nutrient requirements of laying hens. Table 1 shows the CP ranged from 16.47-16.72%, with the highest value at T4. The CF value is from 4.17-4.34%, with the lowest value at T4. And the EE value ranged from 7.14-7.46%, with the lowest score on T4. That shows that the increasing level of addition of phytase enzyme and citric acid will further affect the content of nutrients feed.

In addition to mineral substances in the form of phosphorus, could be digested properly due to phytase enzymes, there is also an increase in the digestibility of calcium (Ca) minerals. Harper et al. (1997) reported a 30%

improvement in P digestibility with grain sorghum-soybean meal based-diets supplemented with 500 FTU/kg dietary phytases. This report also indicated a 20% improvement in Ca digestibility but no response in Ca or DM digestibility due to phytase. Among the studies conducted, responses for the Ca and other nutrient digestibilities have been less consistent and small in magnitude than improvements in P digestibility.

In the present study, the nutrient content in basal feed showed the value of P according to the nutritional standards of laying hens. However, the nutrient content cannot be digested properly without adding phytase, especially crude protein and phosphorus. Phytic acid is described chemically as myo-inositol-1,2,3,4,5,6-hexakisphosphate. Phytic acid is an anti-nutritional substance contained in protein bonds, so that this protein will affect the digestibility of phytic acid (Dersjant-Li et al. 2015). The formation of insoluble phytate-mineral or phytate-protein compounds can cause a decrease in the availability of minerals and the nutritional value of proteins (Lamid et al. 2018). Therefore, the presence of phytase enzymes is very good for helping maximize the digestion of laying hens in digesting nutrients in the form of minerals. That is consistent with research showing that the use of phytase and citric acid can have a positive effect on phosphorus digestibility in laying hens.

Evaluation of phosphorus content of laying hen basal feed

Phytase use with the addition of citric acid is two complementary substances that play a significant role in digestion. These two substances are needed to maximize mineral and nutrient digestion, consequently supporting productivity. The role of phosphorus to be digested optimally benefits livestock in production and from an economic point of view because this can benefit farmers by saving high-priced feed ingredients. Usually, feed ingredients with high nutritional content can be obtained at a higher price than feed ingredients with standard nutrition, which is very beneficial in cutting the cost of animal feed. Phytate can bind to protein due to its rickets, which form insoluble salts. Although salts that are difficult to dissolve are formed because of low, neutral, or high pH, phytic acid is negatively charged, so it will form bonds with proteins that have positive groups. That is reinforced by Nisa and Yuanita (2014), that citric acid can form complexes with metals bound to Phytate, so soaking corn with citric acid will reduce the amount of Phytate. In addition to using citric acid, phytate levels can also be reduced by phytase.

The degradation of Phytate by phytase occurs through a hydrolysis reaction. Hydrolysis of Phytate by phytase occurs at C3 or C6 on the Myo-inositol hexakisphosphate form to the simpler; namely, D-inositol (1,2,4,5,6) P5 becomes inositol (2,4,5,6), T4 becomes inositol (2,4,6), T3 or inositol (2,4,5) T3 or inositol (1,2,6) T3 and finally become inositol-2-P (Greiner et al. 2002). Therefore nutrients bound to phytic acid are hydrolyzed and released from their bonds. Through the enzymatic technique, it is possible to completely degrade the Phytate of cereals and legumes. That is supported by Rodriguez et al. (2015) research results; namely, the best treatment for reducing

phytate levels was obtained by adding citric acid and phytase enzymes to hen's diets that, improve the digestibility and significantly reduce the excretion of P. That is to the results of the research in the form of residual phosphorus of feed digestibility value, with the best treatment being T2 1.622 + 0.18% compared to T0 2.243 + 0.12%, which has higher residual phosphorus. The lower the residual phosphorus is better because it shows that more phosphorus is being digested. A basal feed with 2% phytase enzyme and 2% citric acid (T2) compared to basal feed without the addition of phytase enzyme and citric acid (T0) was better because, with the addition of phytase enzyme and citric acid, nutrient phosphorus was digested better.

Phosphorus is one of the essential nutrients for livestock survival and optimum production. However, in livestock, there are limitations to digesting phosphorus, so it requires exogenous enzyme supplementation to help digest phosphorus. The phytase enzyme used for feed additives breaks down phytic acid in animal feed and liberates inorganic phosphorus and inositol. In addition, the phytase enzyme will work to free amino acids, proteins, minerals, and other materials attached to phytic acid. As a result, phosphorus and other nutrients such as trace minerals and protein become widely available and can be used to maintain health and promote the growth of the hen body. The addition of the phytase can also reduce and even eliminate the addition of inorganic phosphorus, which is expensive, resulting in savings in production costs (Pires et al. 2019).

Żyła (1993) proposed that acid phosphatase with optimum pH of 2.5 acts independently of phytase. The preponderance of the evidence shows the benefit of using phytase in non-ruminant animal feeding. The benefits of phytase are most manifest when feeding diets that are lower in P than required, thus making it possible to use less P in diets while meeting the P needs of the animals (Olukosi 2012). Increased understanding of the genetic makeup of the different microorganisms producing phytases has made it possible to produce newer generation phytases that can release more P per unit of enzyme supplementation and cope with high temperatures associated with some types of animal feed processing.

Evaluation of phosphorus content in residual digestibility of laying hens

Phosphorus is one of the mineral substances needed by poultry, especially laying hens, to support productivity in the form of eggs and bones (Alagawany et al. 2021). However, livestock has limitations in digesting minerals due to the presence of phytic acid in animal feed derived from plants, especially grains, because birds lack endogenous phytase (El-Hack et al. 2018). Phytic acid can interact with endogenous enzymes by rendering phytate-bound protein refractory to pepsin digestion, resulting in reduced nutrient digestibility (Dersjant-Li et al. 2015). Generally, phytic acid will be digested on the digestibility of the animal, and the rest will be disposed of in the form of residue. However, poultry productivity can be increased through the consumption of well-digested feed; therefore, it

is very important to pay attention to the content of poultry feed ingredients in the form of phosphorus, especially in laying hens. Using exogenous phytase enzymes can help the digestion in poultry by degrading phytic acid into positive substances that can be digested by livestock to maintain livestock productivity.

Long et al. (2017) suggested that providing atypically high doses of phytase (up to 7,600 FTU/kg) in diets for broilers may result in the release of 94.4% of Phytate bound P. This concept is known as "super dosing" in swine and poultry diets. The added benefits observed were a 131% increase in weight gain and a 59% increase in bone ash in laying hens super dosed with phytase compared with laying hens fed no phytase. In poultry diets, phytase is sometimes included at levels up to 2,500 FTU/kg diet DM (Simons and Versteegh 1990). One FTU is defined as the amount of phytase that liberates 1 μ mol of inorganic phosphate per minute from 0.0051 M Na-phytate solution at a pH of 5.5 and 37°C (Engelen et al. 1994).

The rumen microorganisms, such as *Actinobacillus* sp. and *Bacillus pumilus* in ruminants, are considered capable of naturally producing phytase as a feed additive to produce good quality with high protein and mineral (P, Mg, Mn, Fe, Zn, and Ca) availability (Lamid et al. 2014). In research, using phytase enzymes in non-ruminant animal feed resulted in more phosphorus absorption than many ruminants. Several factors, such as optimum pH for phytase activity, influence the difference in the effectiveness of the use of phytase enzymes. Dersjant-Li et al. (2015) stated that the optimum pH range for several phytase enzymes is 2.0 to 5.00. Therefore, the lack of phosphorus absorption in ruminants is indicated as a result of the rumen pH, which tends to be neutral, namely 6.7 to 6.9, and causes the ineffective performance of the phytase.

Table 4 shows the results of the percentage value of residual phosphorus feed digestibility. As a result, there was a significant difference in residual phosphorus content ($P < 0.01$), with the lowest post-digestion phosphorus levels, were found in the addition of laying hens concentrate + 2% phytase enzyme + 2% citric acid (T2) that has 1.622 + 0.18 % of residual feed digestibility of phosphorus. Therefore, the low phosphorus content in the digestibility residue indicates that phosphorus can be properly disassembled and absorbed in the digestion of non-ruminant livestock.

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Economic analysis of dairy production in Uganda, a case study on the performance of dairy cattle enterprises in Southwestern Uganda

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Abstract. Waiswa D, Günlü A. 2022. *Economic analysis of dairy production in Uganda, a case study on the performance of dairy cattle enterprises in Southwestern Uganda. Asian J Agric 6: 61-67.* The economic performance of dairy cattle enterprises in Southwestern Uganda was analyzed in this study. A survey was conducted on 100 dairy cattle enterprises in Mbarara, Kiruhura, Lyantonde, Ibanda, and Isingiro Districts using data compilation forms covering the 2019/20 production year. The unit production cost of milk was determined as US\$0.19L⁻¹. Veterinary expenses had the largest share of the production costs at 24.94%, followed by labor costs, depreciation of the inventory value, other expenses, and feed costs, which contributed 14.11%, 12.46%, 11.96%, and 11.41%, respectively. Additional costs included the depreciation of animals, electricity and water, buildings, equipment and machinery, maintenance-repair, and general administrative expenses, which contributed 9.95%, 7.86%, 2.54%, 2.29%, and 2.48% to the total production costs, respectively. As a result, while the net profit of the enterprises was determined as US\$1435.29, their financial profitability was 0.59, the profitability factor was 12.20, and the output-input ratio was 1.06. The overall profitability of the enterprises was affected mainly by the high veterinary expenses due to the high prevalence rates of tick-borne infections and the irrational distribution of capital elements. Therefore, measures to reduce the occurrence of tick-borne diseases are considered vital in lowering milk production costs, thereby increasing the profitability of enterprises.

Keywords: Dairy cattle enterprises, dairy production, economic analysis, economic performance

INTRODUCTION

Uganda's economy is estimated to have grown by 2.9% during the 2019/2020 fiscal year, with real Gross Domestic Product (GDP) standing at US\$35 billion (at 2016/2017 prices) (UBOS 2020). As among the largest foreign exchange earners, contributing to Uganda's export earnings through exports valued at more than US\$120.74 million as of 2019 (FAOSTAT 2021), the dairy industry contributes significantly to this GDP value. Furthermore, dairy production has significant potential to reduce hunger, malnutrition, and rural poverty, improve rural livelihoods, promote food security and nutrition, create employment opportunities, promote gender equality, and support the overall development of Uganda's economy (Herrero et al. 2014; FAO 2019; Waiswa and Akullo 2021).

Available statistics reveal that Uganda's annual milk production stands at 2.04 million tons (UBOS 2020), obtained from an estimated 4.14 million heads of milked cows. Each cow was estimated to produce 492.8 liters of milk per lactation (FAOSTAT 2021). Out of the total milk produced, 80.2% is marketed for an estimated US\$835.9 million (2019), and the remaining 19.8% is consumed at farms and household levels. Of the marketed milk, 34% is processed into a wide range of products such as powdered milk, butter, UHT milk, yogurt, cream, ice cream, and

cheese, while the remaining 66% is sold in raw form (DDA 2020).

Regarding dairy products' export and import, Uganda exported 0.16 million tons of dairy products worth over US\$120.74 million in 2019, while 412 tons worth US\$1.69 million were imported in the same year (FAOSTAT 2021). The high level of dairy exports over imports implies low reliance and expenditure on imported dairy products, indicating the industry's growth. The increase in Uganda's dairy export value is attributed to improved compliance of Uganda's dairy products to regional and international market standards and the annual increase in dairy processing capacities (DDA 2020).

Despite these developments, studies analyzing the economic performance of dairy cattle enterprises in Uganda are still scanty. Therefore, this study was conducted to determine the profitability of dairy cattle enterprises while calculating the costs involved in the production and establishing the unit cost of milk production. The information generated is intended to draw the attention of dairy producers, international and voluntary organizations, government agencies, and other private stakeholders to implement technical, economic, and structural policies to improve dairy cattle production. These improvements will be essential in rural development and in alleviating poverty and malnutrition in the country.

MATERIALS AND METHODS

Face-to-face interviews were conducted with the enterprise owners on 100 randomly sampled dairy cattle in Southwestern Uganda. The interviews are from January to April 2021, located in Mbarara, Kiruhura, Lyantonde, Isingiro, and Ibanda Districts. The data was collected using data compilation forms covering 2019 to 2020. Production costs on the enterprises included feed, labor, veterinary, maintenance and repair, general administrative costs, depreciation in the capital elements (machinery, equipment, and animals), and other expenses.

Feed costs included the production and purchase of roughage and concentrate feeds. Labor costs were divided into family and hired labor. For hired labor, the labor cost was calculated using the amount of money directly paid to workers. While for unpaid family labor, the labor cost was calculated by multiplying the number of family workers by the average amount of money paid to a hired worker to perform the same task in the same region. Veterinary expenses covered veterinary examination fees, treatment and vaccination costs, and acaricides and disinfectants. Artificial insemination costs were not included in the veterinary expenses because any interviewed farmers didn't use it. Instead, they relied on the natural breeding system. Maintenance and repair costs covered expenses incurred in maintaining and repairing fixed capital investments (animal shelter, equipment, and machinery) used in production. Finally, general administrative expenses were calculated as 3% of the total costs of dairy enterprises (Mat and Cevger, 2020).

The straight-line method was used to calculate the depreciation values of buildings, machinery, and equipment because of its simplicity and the assumptions that the assets were expected to render uniform service throughout their estimated economic life and the amount of depreciation was a function of time only. These were calculated by subtracting the item's scrap value from its new value or value at initial purchasing and dividing the result by the item's economic life. For example, the depreciation value of animals used in milk production (lactating cows) was arrived at by subtracting its value at slaughter from the breeding value and dividing the resultant figure by the economic life of the animals (Mat and Cevger, 2020). The average economic life of cattle was taken to be six years. Since depreciation was calculated for lactating cows, the change in inventory was calculated using the values of heifers, calves older than seven months, and bulls on the enterprise. Calves less than seven months old were considered under calf income. A negative value indicates a depreciation in the inventory. Therefore, the resultant value was considered as a production cost. In contrast, a positive value indicated an increase in the inventory, and the consequent value was regarded as a secondary income of the enterprise (Mat and Cevger 2020).

Income from products other than milk, considered the main product in dairy farming, was considered secondary income. These included the sum of income from the sale of calves and manure and the positive value of the change in inventory (Günlü 1997; Mat and Cevger 2020). The total

costs of the enterprises were obtained by subtracting the total secondary incomes from the total general expenses. For example, income from milk sales was obtained by multiplying the amount of milk produced at the end of the production period by its selling price, while the production cost of 1 Liter of milk was obtained by dividing the total cost by the total amount of milk received at the end of the production period (Mat and Cevger 2020).

Financial profitability or profitability of equity capital is an important criterion used to measure how efficiently the enterprise uses its capital and, therefore, its success. It was obtained as a percentage of net profit and equity capital quotient. While economic profitability, an indicator of how effectively all the economic resources involved in the dairy production activities of the enterprise are utilized, was obtained by dividing the sum of net profit and passive capital by the value of active capital. The profitability factor obtained a percentage of the quotient of the net product of the enterprise and the gross product (Günlü 1997). The elements included in the calculation of the gross product were the values of all products obtained from the enterprise and inventory value increases. The value of the products given out by the enterprise in kind, while the net product was obtained by subtracting the total costs (variable + fixed costs) from the value of the gross product (Gül and Gürbüz 2016; Göçoğlu and Gül 2019). The Output-Input ratio was obtained by dividing the enterprises' total revenue by the total expenses (Cicek and Tandogan 2008). All expenses and revenues were converted to United States dollars at the 2019/20 financial year-end period's average exchange rate of US\$1 = 3730.14 UGX (BoU 2021).

RESULTS AND DISCUSSION

This study was conducted on 30, 29, 14, 14, and 13 randomly selected dairy cattle enterprises in Kiruhura, Lyantonde, Mbarara, Ibanda, and Isingiro districts, respectively, as presented in Table 1. The most significant proportion of dairy producers (41%) had attained primary education at the highest level, followed by 24% with tertiary or vocational training and 21% with secondary education. The ages of the interviewed dairy producers ranged from 30 to 78 years, and the average age was 49 years. A significant portion of dairy producers (88%) practiced animal farming as their main economic activity.

These findings corroborate well with Sikawa and Mugisha (2011), Rutaro (2015), Tibeziinda et al. (2016), Wangalwa et al. (2016), and Byaruhanga et al. (2020)'s findings from their studies conducted in Southwestern Uganda. These studies revealed that the average age of dairy producers in the region ranged from 47 to 54 years, more than 50% of the dairy producers had attained above the primary level of education, and the largest percentage of the population in this region is engaged in livestock farming as the main economic activity. Therefore, the education level of farmers may have a significant influence on several factors as far as dairy production is concerned, such as managerial competencies, successful implementation of improved production, processing, and

milk marketing, and the rate of adoption of innovations in dairy production, which could enhance increased milk production and the profitability of the enterprises (Sikawa and Mugisha 2011; Sisay et al. 2018).

Dairy producers in the study area owned large pieces of land for dairy activities such as grazing and growing fodder; none hired land for any dairy farming activities. The land is a great requirement for dairy producers in the study area because they practice extensive dairy farming. The size of land owned ranged from 20 to 680 acres, with the average land size being 177 acres. The results further revealed that, on average, 2.3 acres of land were used by each grazing animal. That exceeds the recommended 1.5 to 2.0 acres of land per cow (Virginia 2020), indicating that land is not a problem for dairy producers in the study area. However, despite the availability of enough land, only 11% of the interviewed dairy producers planted pastures, mainly Napier grass, for their animals moreover on small pieces of land (4 acres on average). That shows a high level of reliance on rain-fed natural pastures as fodder, which explains why dairy producers are significantly affected during dry seasons. During such seasons, dairy producers are constrained by pasture scarcity, leading to drastic milk yield reductions (Balikowa 2011; Waiswa et al. 2021).

Considering that there is enough land on which to establish pastures, the low levels of planting pastures reported could be attributed to factors such as lack of planting materials (pasture seeds), high costs of the available planting materials, insufficient farmer training opportunities about pasture establishment and conservation and/or the bad attitude of farmers towards planting pastures. Therefore, this calls for efforts to be invested in encouraging and training farmers on pasture establishment and conservation and providing the necessary planting materials (either freely or at subsidized costs) to ensure the availability of animal feeds throughout the year.

The average number of cattle raised was 78 heads, with some enterprises having as low as 19 heads while others had as high as 450 heads of cattle (Table 2). Most cattle raised in the study area were crossbreeds between Ankole cattle and exotic dairy breeds, the most common being the Holstein Friesian breed; 84% of the respondents raised only crosses, while 16% raised both crosses and local Ankole breeds. The number of cows milked ranged from 5 to 85, with 22 heads as the average number. The ratio of lactating cows to the total number of cattle raised in the enterprises was 28.2%. Each milked cow produced an average of 6 liters per day. On average, 109.2 liters of milk were produced per enterprise per day, 6.8 liters were consumed per day, and 97 liters of milk were sold majorly to dairy cooperatives at an average selling price of US\$0.23L⁻¹.

These results are comparable with other studies conducted in Southwestern Uganda. Their studies revealed that the majority of dairy producers in the region kept crossbreeds of cattle for dairy production. The average herd size ranged from 81 to 96 heads of cattle, which is higher than this study's finding, and the average daily milk production per cow ranged from 5.4 to 7.4 liters depending on the breed, values that are closer to this study's finding

(Sikawa and Mugisha 2011; Rutaro 2015; Tibeziinda et al. 2016; Vudriko et al. 2016; Wangalwa et al. 2016; Vudriko et al. 2018; Byaruhanga et al. 2020). This study's daily milk yield (6 L) is higher than that obtained from studies in Rwanda, which obtained a daily milk yield of 4.6 liters from Ankole and Friesian crosses (50% Friesian) under similar management conditions, i.e., extensive grazing on natural pasture with minimal supplementation (Manzi et al. 2020). The average selling price was higher than the national average farmgate price of US\$0.20L⁻¹ but lower than the national average retail price of US\$0.39L⁻¹, reported by the Dairy Development Authority (DDA) (DDA 2020).

The number of cows milked differed from other studies as well. While this study revealed that each farmer milked 22 heads of cattle on average, which makes 28.2% of the total number of animals owned. Tibeziinda et al. (2016)'s study revealed that the average number of milked cows was 25 (30.9% of the entire herd), while Byaruhanga et al. (2020)'s study showed that the percentage of milked cows was 19.8% of the total herd. The study's differences may be attributed to the different years in which the studies were conducted and the method of selecting the study area and samples. The average daily milk production per milked cow found in this study is higher than estimates from FAO (FAOSTAT 2021; Ndambi et al. 2008). That is attributed to the fact that this study's results were from Uganda's highest milk-producing region, unlike FAO and Ndambi et al.'s findings, which are estimates of the total national milk production.

Table 1. Socio-demographic characteristics of enterprises

Variable	Number	%
Address		
Kiruhura	30	30.0
Mbarara	14	14.0
Isingiro	13	13.0
Ibanda	14	14.0
Lyantonde	29	29.0
Education level of dairy producers		
Primary Education	41	41.0
Secondary Education	21	21.0
Tertiary/Vocational institution	24	24.0
University Education	10	10.0
No Formal Education	4	4.0
Main economic activity of dairy producers		
Animal Farming	88	88.0
Crop Farming	4	4.0
Civil Servant	5	5.0
Others	3	3.0
Breeds of cattle raised		
Crosses (Ankole x Exotic breeds)	84	84.0
Both crosses and Ankole breeds	16	16.0
Age of dairy producers		
Mean	49.17	
Std. Deviation	10.64	
Minimum Value	30	
Maximum Value	78	

Table 2. Production characteristics of enterprises

Variable	Mean	Std. Deviation	Minimum value	Maximum value
Size of land (Acres)	176.74	164.62	20	680
Total number of cattle (heads)	78.38	56.79	19	450
Number of milked cows (heads)	22.32	15.40	5	85
Daily milk production (liters)	109.25	76.21	30	561
Daily milk consumption (liters)	6.83	5.4	1.5	30
Daily milk sales (liters)	97.05	67.67	26	556
Selling price of milk (US\$)	0.23	0.02	0.16	0.26
Milk production per milked cow per day (liters)	6.27	1.67	1.83	9.82

Calculation of milk production costs on enterprises

The cost elements and their proportional distributions that makeup milk production cost in dairy cattle enterprises examined are presented in Table 3.

While the average selling price of milk was determined as US\$0.23L⁻¹, the unit cost of one liter of milk was calculated as US\$0.19. The most significant proportion (24.94%) of this was veterinary expenses. This production cost is lower than the average production cost of US\$0.26L⁻¹, which was reported by Mugambi et al. (2015). Some studies note that the cost of milk production in Uganda ranges from US\$0.13 to US\$0.16L⁻¹ (Waiswa et al. 2021). Others show that the average cost of milk production in Uganda varies according to the size of the herd. Large-scale enterprises cost less than US\$0.20L⁻¹, and small-scale enterprises have values ranging from US\$0.2 to US\$0.35L⁻¹ (Ndambi and Hemme 2009).

Studies conducted in other Sub-Saharan African countries show variations in milk selling prices, production costs, and profits obtained. For example, studies in Kenya reveal that while the average selling price of milk ranges from US\$0.25 to US\$0.39L⁻¹, the average production cost ranges from US\$0.25 to US\$0.38L⁻¹ of milk depending on the production system (Mugambi et al. 2015; TIAPD et al. 2016). In Zambia, the average milk production cost was reported to be US\$0.18L⁻¹, while the average selling price was US\$0.43L⁻¹ of milk (Mumba et al. 2011). Compared to this study's results in other countries, it clearly shows that dairy cattle farming has significant potential to achieve rural development and solve food security and nutritional problems in Uganda, as long as economic, political, and infrastructural improvements are considered.

On the contrary, studies conducted in other countries show that feed costs cover the largest share of the expenses in milk production (Hemme et al. 2014; Semerci et al. 2014; Rashid et al. 2015; Yilmaz et al. 2016; Oğuz and Yener 2017, 2018a,b; Mat et al. 2021; Mat and Cevger 2022). In this study, veterinary expenses had the largest share of production costs at 24.94%. Feed expenses constituted 11.41% of the total production costs. That owes to the fact that dairy producers in the study area rely on the extensive grazing system, which involves grazing cattle on large tracts of land with natural pastures. Under such a system, low feed costs are an advantage because dairy producers spend on feeds only when they supplement animals' diets with concentrate feeds and silage (Hanson et al. 1998; White et al. 2002; Waghorn and Hegarty 2011).

Table 3. Proportional distribution of cost elements that make up milk production costs on enterprises

However, the biggest challenge in this system is the high levels of infestation of animals with ticks while grazing, which increases the risk of tick-borne infections in enterprises (Hezron et al. 2012; Kasaija et al. 2021). Prevention and control of these diseases require extra costs in implementing preventive measures such as acaricide spraying, vaccination, and treatment costs, resulting in high veterinary expenses.

Therefore, measures to reduce the occurrence of tick-borne diseases should be vital in lowering milk production costs. Among these measures can be the use of an integrated tick-borne disease control system, which includes vaccinating cattle against infections such as Theileriosis and applying acaricides to control ticks (Ginsberg and Stafford III, 2005; Gachohi et al. 2012; Vudriko et al. 2018). In addition, training programs for dairy producers need to include good practices such as acaricide rotation, mixing the right acaricide concentrations, using the right equipment to spray cattle, applying adequate amounts of acaricide on cattle while spraying, and establishing appropriate cattle health management programs (Vudriko et al. 2016; Vudriko et al. 2018). Additionally, offering veterinary drugs and services at subsidized costs can be a good strategy for reducing production expenses.

Technical and economic analysis of enterprises

The results obtained from the calculation of the technical and economic analyses of the enterprises are presented in Table 4.

The gross product value of enterprises was contributed mainly by revenue from the sale of milk (76.69%). That was followed by income from calves sales (14.06%), an increase in inventory value (6.87%), and income from the sale of manure (2.38%). The active capital of enterprises was contributed mainly by real estate capital (88.99%), livestock capital (7.76%), and lastly, working capital (3.25%). The largest share of real estate capital was land (87.97%), while buildings contributed 12.03%. The distribution of active capital elements is essential in the effective management of enterprises. In a rational working enterprise, it is recommended that capital be distributed so that 50% is real estate capital, 25% is livestock capital, and 25% is working capital (Oğuz and Yener 2017). This study indicated that capital elements in the enterprises were irrationally distributed, suggesting an area that requires improvement.

Production cost elements		Value (US\$)	%
1	Feed costs	1178.27	11.41
2	Labor costs	1457.97	14.11
3	Veterinarian, drugs, acaricides, and vaccination costs	2576.21	24.94
4	Electricity and water costs	812.46	7.86
5	Inventory Depreciation	1287.39	12.46
6	Other expenses	1235.22	11.96
A	Total of Costs	8547.52	
7	Livestock depreciation	1027.66	9.95
8	Buildings, equipment, and machinery depreciation costs	262.25	2.54
9	Buildings, equipment, and machinery maintenance-repair costs	237.05	2.29
10	General administrative expenses (A × 3%)	256.43	2.48
B	Overall Total Costs	10330.91	100.00
C	Total Secondary Income	2742.41	
i	Income from the sale of calves	1654.36	
ii	Income from the sale of manure	280.05	
iii	Inventory Value Increase	808.00	
D	Total cost (B - C)	7588.50	
	Total Produced Milk (Liters)	39600	
	Milk Price Paid to Producer	0.23	
E	1-liter milk cost (D ÷ Total milk produced)	0.19	
F	Milk Sales Revenue	9023.79	
G	Net Profit or Loss (US\$) (F - D)	1435.29	

Note: * Average exchange rate of 2019/20 financial year: US\$1 = 3730.14 UGX (BoU 2021)

Table 4. Findings from technical and economic analysis of enterprises

Element	Value (US\$)
Overall total costs	10330.91
Income from the sale of calves	1654.36
Income from the sale of manure	280.05
Milk sales revenue	9023.79
Total Revenue	10958.20
Real estate capital	215771.47
Livestock capital	18819.67
Working capital	7884.12
Active capital	242475.26
Passive capital	0.00
Equity capital	242475.26
Gross Product (GP)	11766.20
Net Product (NP)	1435.29
Net profit	1435.29
Financial Profitability	0.59
Profitability factor	12.20
Output-Input ratio	1.06

Note: * Average exchange rate of 2019/20 financial year: US\$1 = 3730.14 UGX (BoU 2021)

In conclusion, dairy cattle farming is essential in transforming Uganda's economy, creating employment opportunities, alleviating poverty, and improving livelihoods. This paper presents an outlook on the economic viability of dairy cattle enterprises in the study region. It also revealed a generally low level of profitability of the enterprises. That was mostly attributed to the high production costs, especially the veterinary expenses, and the irrational distribution of capital elements. Implementing

strategies to reduce the prevalence of tick-borne infections will be key to reducing production costs. Among these strategies are using an integrated tick-borne disease control system, incorporating good practices such as acaricide rotation, mixing the right acaricide concentrations, using the right equipment to spray cattle, and applying adequate amounts of acaricide on cattle while spraying into dairy producers' training programs. Subsidizing veterinary drugs and services for farmers can also play a role in reducing production costs.

Furthermore, the irrational distribution of capital elements can be improved by increasing the number of lactating cows in a production period, reducing the number of unproductive animals by replacing them with high-yielding animals and integrating extensive grazing with the intensive production system to reduce the amount of land required for grazing and increase the overall efficiency of the enterprises. Therefore, implementing the recommendations presented in this paper will not only aid in improving overall profitability at the farm level but will also aid in improving the overall productivity of the dairy industry nationwide. Furthermore, improvements in the productivity of the dairy industry come with a wide range of benefits, such as contribution to growth in national income, reduction of dependency on imported dairy products, creation of employment opportunities, improved livelihoods, and poverty reduction.

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A daylength-neutral winged bean (*Psophocarpus tetragonolobus*) for Southern Australian latitudes

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Abstract. Eagleton GE. 2022. A daylength-neutral winged bean (*Psophocarpus tetragonolobus*) for Southern Australian latitudes. *Asian J Agric* 6: 68-78. In the summer of 2019, on the central coast of NSW, Australia (at Latitude 34°S), an early-maturing genotype of the tropical legume crop, winged bean (*Psophocarpus tetragonolobus* (L.) DC.), was detected among a range of late-maturing accessions. The performance of this accession, MYO-01 from Bago in Myanmar, was evaluated in staked plots alongside one other accession from Myanmar and two from the island of New Guinea planted on three successive occasions between October and late November 2020 in a split-plot experiment. Across the three planting dates, the mean number of days from planting to the first open flower for MYO-01 ranged from 68 to 82 + s.e. 3.2 compared with a range of 119 to 167 for the other three accessions. The mean accumulated seed yield of MYO-01 obtained from the October planting equated to 3.1 t ha⁻¹, but by the third planting in late November, the yield was only half as much. Among the four accessions, MYO-01 was second in the amount of lower stem branching and tuber yield, with the smallest pods and hardest seeds. Hard seededness creates difficulties for germination and plant establishment and is a limitation in MYO-01, as are its small pods, which lowers its potential for vegetable production. Investigating genetic control of photoperiod insensitivity in MYO-01 and combining the ability for pod and seed characteristics is relevant to sub-tropical latitudes and for developing stable early maturity cultivars for the tropics.

Keywords: Daylength, genotype, photoperiod, *Psophocarpus tetragonolobus*, winged bean

INTRODUCTION

The winged bean (*Psophocarpus tetragonolobus* (L.) DC.) is a minor leguminous crop of the tropics, particularly in Asia and Melanesia (Khan 1982; Eagleton 2020). All parts of the plant, including the root tubers of certain ecotypes, are high in protein (NAS 1975). Nevertheless, from the beginning of focussed research efforts to explore this potential, it was recognized that the phenology of most genotypes is profoundly influenced by sensitivity to photoperiod. Burkill (1906) reported that when planted outside the tropics in a north Indian summer, winged beans did not usually flower early enough to enable mature pods to ripen and return good yields of mature seed. That suggested photoperiod inhibition in the summer months. Detailed investigations by Wong and Schwabe (1980) concluded that the Malaysian variety they studied would only set flowers when the day length fell below the critical day length of between 11.25 and 12.25 hours. Moreover, at these short, potentially inductive day lengths, flowering was delayed if the ambient temperature fell outside the optimum around 26°C: a constant temperature of 32°C or 18°C in the day or 14°C at night inhibited flowering. Similar studies with a wider range of germplasm have confirmed that the phenology of winged bean accessions is inhibited by long day lengths and have demonstrated the equally important role of temperature in influencing the time taken from planting through to initiation of flower buds, the opening of first flowers and development of mature seed-bearing pods (Tanzi et al. 2019a). The dual

role of day length and temperature in controlling phenological development has been investigated in many flowering plant species and is particularly relevant to legume crops of economic importance (Lawn et al. 1995; Nguyen et al. 2016; Zhang et al. 2020; Gonzalez et al. 2021).

In Japan, researchers identified a few winged bean accessions with reduced sensitivity to photoperiod and developed varieties that could flower and produce green pod yield over 15,000 kg ha⁻² at latitudes up to 40°N (Abe et al. 1988; Okubo et al. 1989; Endo et al. 1993). However, in Perth, Western Australia (latitude 31.96°S), four years of research (Eagleton 1985) failed to find any winged bean genotype that flowered earlier than 80 days from among a large number of introduced accessions or the progeny of hybrids between select accessions. Even in Kununurra's potentially more favorable environment, in northern WA (latitude 15.78°S), an early summer planting of 66 diverse accessions and hybrid progeny found only one that flowered in 64 days, one other in 78 days, and most others in over 100 days. Since then, more than twenty years of sporadic experimentation by the author, exploring a diversity of germplasm in the frost-free locality of Wagstaffe, north of Sydney, NSW (latitude 33.52°S), has produced similar results. At least that was the case up until two years ago, when by chance, a seed of an introduced accession from Zin Myo Than's family in the city of Bago, Myanmar (latitude 17.32°N) produced plants that consistently flowered in 80 days less from early summer plantings.

Thus, in the spring/summer of 2020, a small, replicated planting-date trial was carried out to determine the characteristics of MYO-01. The trial's objective was to compare its phenology and seed yield with three other winged bean accessions and the responsiveness of the four accessions to a difference in planting date across late spring and early summer. This paper reports the trial results and discusses its implications for producing well-adapted winged bean cultivars for southern Australia. The results also have implications for developing early maturing cultivars for equatorial latitudes (Eagleton 2019).

MATERIALS AND METHODS

The trial compared the performance of four accessions of winged bean (*P. tetragonolobus*), each of which was planted at three different planting dates within a split-plot experimental design.

Trial area

The trial was located on the coastal fringe of Wagstaffe in NSW, Australia (Kourung Gourung Point, 33.52°S, 151.34°E, 50 m asl). It was set out on a residential plot of land at 10 m x 5 m that had previously been under a buffalo grass lawn for several decades. The soil underlay of the area is a yellow-brown podzol with topsoil enriched by millennia of leaf fall. The climate of the Wagstaffe is mild and frost-free (Figure 1). From October 2020 to July 2021, the total rainfall was just over 1300 mm, with the lowest monthly total of 40 mm recorded in April.

In August 2020, the trial area was sprayed with glyphosate herbicide and rotary hoed to kill the buffalo grass lawn. In early September, all grass remnants were removed by raking and vigorous hand-hoeing to a depth of

35 cm. The area was fertilized with Dynamic-LifterR pellets (containing NPK 3.6: 1.1: 1.7 plus traces of S, Fe, Mg, Mn, Zn) at a rate of 80 g m⁻², Blood-and-Bone at 140 g m⁻² and cow manure at 0.4 L ha⁻² deeply incorporated. The area was then harrowed into four length-wise ridges spaced 1 m apart.

Trial design

The experiment was planned as a randomized block design with three replications of a split-plot arrangement with three planting dates as main plots and winged bean accessions as randomized subplots. Subplots were 100 cm x 75 cm in size, each containing a single plant.

The four-winged bean accessions (detailed in Table 1 and Figure 2) were chosen for contrasting characteristics based on preliminary observations in the 2019 season.

Details of the three planting dates between October and December 2020 are listed in Table 2. Seeds of the accessions were scarified by abrasion with sandpaper applied to the back of each seed on each planting date. The seed batches were then placed in a film of water for 12 hours. Next, the individual seeds were planted into a commercial soil mix in jiffy pots and grown in a glasshouse until the full unfolding of the two seed leaves. At that time, the jiffy pots were planted in the appropriate positions in the field. For planting date 1, sufficient viable seedlings were available for two seedlings to be placed at each planting point, but later on, the smallest seedling was removed to leave just one plant per plot. Because of seed shortages, only one seedling per point was available for planting for the other two planting dates. On the third planting date, for accession MYO-01, the seedling in replicate one did not survive through flowering and was treated as a missing plot for analysis purposes.

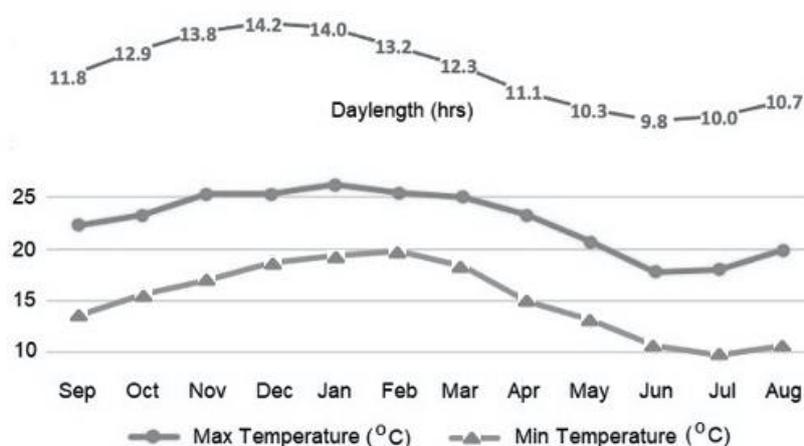


Figure 1. The monthly mean maximum and minimum temperature between September 2020 and August 2021 in the vicinity of Wagstaffe, NSW, Australia, together with the mean day length in hours for each month at latitude 34°S

Table 1. Origins and general characteristics of the four-winged accessions based on initial observation at Wagstaffe in 2019/2020

Name	Origin	Coordinates	General characteristics
MYO-01	Home garden, Bago, Myanmar Collected 15 Oct 2019	17.33°N, 96.49°E; 10 m asl	Habit-branching; Leaves-small sized; Flowers-calyx green, standard blue; Pods-rectangular, fully green, mean pod length 11 cm; Seeds-round, tan with black hilum ring, mean seed weight 314 mg.
CHIMBU	Chimbu Province, Papua New Guinea, via USDA PI-477148-03 Received June 2018	6.02°S, 144.96°E; 1560 m asl	Habit-moderate branching; Leaves-large sized; Flowers-calyx purple, standard mauve; Pods-semi-flat, purple-on-green body, purple wings, mean pod length 25 cm; Seeds-oval, dark purple, mean seed weight 337 mg.
MYAN-05	Town Market, Nawngkhio, Myanmar Collected 18 Feb 2017	22.33°N, 96.80°E; 860 m asl	Habit-branching, tuberous; Leaves-medium sized; Flowers-calyx green, standard white; Pods-semi-flat, fully green, mean length 23 cm; Seeds-oval, light cream, mean seed weight 356 mg.
WAM-07	Yetni, Wamena, Papua Province, Indonesia Collected 30 Dec 2016	4.19°S, 139.02°E; 1600 m asl	Habit-non-branching, non-tuberous; Leaves-large sized; Flowers-calyx green, standard pale-blue; Pods-semi-flat rough-textured yellow body, green wings, mean pod length 12 cm; Seeds-round, brown, mean seed weight 299 mg.

**Figure 2.** Pod and seed characteristics of the four-winged bean (*Psophocarpus tetragonolobus* (L.) DC.) accessions included in the trial

Management

When planting, seedlings were watered, and a layer of sugarcane mulch was spread across the plot. Subsequently,

plants were watered by hand at intervals of two weeks without rainfall. Once plants were well established, rainfall was sufficient to sustain growth without additional watering. The application of sugarcane mulch meant that only sporadic hand-weeding was needed for the trial duration. In mid-December, a tripod of canes was erected around a central metal stake to provide climbing support for the single plant per plot. At that time, all plots received a sprinkling of broad-spectrum, slow-release fertilizer, and snail-bait pellets. That was repeated on 9 Apr 2021. From the first week of February through to mid-April, damage to growing shoots from broad mite (*Polyphagotarsonemus latus*) was controlled by Stealth_R acaricide (active ingredient *abamectin BI*) at a rate of 0.75 mL L⁻¹, sprayed on six occasions. To control insect larvae attacking buds, flowers, and young pods, Confidor_R insecticide (active ingredient *imidacloprid*) was sprayed at a rate of 0.25 mL L⁻¹ on three occasions. Beginning in late December, some plants began to reach the top of the 2 m support posts, and by mid-February, all plants had done so. As a result, from 15 Jan onwards, all plant shoots exceeding 2.2 m in height were pruned, while lateral branches extending beyond the tripod's limit into adjacent plots were also pruned back at regular intervals. Despite this, excessive vegetative growth in several plots meant that additional cane support was required to prevent the plants from collapsing. In accession MYO-01, fully mature pods began to be harvested by hand from late February onwards. Once all mature pods had been harvested from a plot, the plant was dug up with roots intact and was partitioned for measurement. That began from mid-May onwards, according to the maturity of respective plots. The trial was terminated on 30 Jun when it was judged that no viable seed bearing-pods remained to be harvested, and the last remaining plots were dug up for partitioning and measurement.

Table 2. Details of the three planting dates

Planting No.	Date of seed imbibition	Date of transfer to field	Details of planting
PD 1	5 Oct 2020	24 Oct	On 5 Oct, seed of all four accessions was scarified with sandpaper and soaked for 12 hours to imbibe water before planting them directly into jiffy pots in a glasshouse. On 24 Oct, the jiffy pots of CHIMBU and MYO-1 were planted in the field. The jiffy pots of MYAN-05 and WAM-07 were somewhat behind in their emergence and were planted in the field three days later, on 27 Oct.
PD 2	3 Nov 2020	11 November	After scarification and soaking in water on 3 Nov, followed by planting in jiffy pots, emerged seedlings of all four accessions were transferred to the field plots on 11 Nov.
PD 3	24 Nov 2020	11 December	After scarification and soaking in water on 24 Nov, jiffy pots of all four accessions were incubated in the glasshouse. After emergence, jiffy pots were transferred to the field on 11 Dec. Unfortunately, only two healthy jiffy pots of MYO-1 emerged, so replicate 1 of MYO-1 was treated as a missing plot.

Observations and measurements

The date of the first open flower was recorded for all plots. For those plots that produced mature pods before 30 Jun 2021, the date of the first mature pod was recorded. For each plot, the number plant open flowers were recorded every second or third day from the date of the first open flower up until the date of harvest of the whole plot. Likewise, the number of pods attaining full maturity and hand-harvested for their seed was recorded every second or third day until the harvest date of the whole plot.

On each date of pod harvest, the following data were recorded: the number and weight (g) of mature pods harvested in the plot; the length (cm) of each pod; the number of seeds in each pod; and the total weight (g) of mature seed extracted from the pods.

At the time of the final harvest of each whole plot, the following measurements were taken. First, the internode lengths (cm) and the number of lateral branches (longer than 10 cm) per node were recorded for the first ten nodes of the main stem (not including the initial unifoliate leaf node; i.e., the first trifoliate leaf was taken as node 1). Second, the diameter of the main stem at ground level and a point just below node ten was measured (mm) using Vernier calipers, as was the maximum diameter of each of the three widest roots. Third, the number of green pods remaining on each plant was counted. Finally, each plant was partitioned into four components: (1) green pods; (2) roots-plus-tubers; (3) leaf laminae (the three leaflets plus their petiolules); and (4) stem, branches plus leaf petioles. The four components were weighed fresh, and a random sample of up to 250 g of each component was oven-dried and weighed to convert fresh component weights to dry weights.

Analysis

Split-plot analyses of variance on measured traits for planting date \times accession combinations were carried out using the "sp.plot" function of "agricolae" (Mendiburu 2021) developed within "R: A language and environment for statistical computing" (R Core Team 2021). Shapiro-Wilk tests to check for normality of distributions and Levene's tests to check for homogeneity of variances across the planting date accession combinations were carried out before the analyses of variance. Tests of significance for treatment comparisons were performed at the 5% level and

corrected for false discovery rate (FDR) by the Bonferroni procedure (Benjamini and Hochberg 1995).

RESULTS AND DISCUSSION

Phenology

For all three planting dates, the accession MYO-01 was markedly earlier to flower than the other three accessions (Table 3, Figure 3). Whereas accessions CHIMBU, MYAN-05, and WAM-07 all produced their first open flowers in March, irrespective of planting date, all three plantings of MYO-0 produced their first open flower before 12 Feb.

There was a highly significant difference ($p < 0.001$) between accessions and a significant difference ($p < 0.01$) between the effect of planting dates on the number of days from planting to the first open flower and the number of days to the first mature pod (Table 4, Figure 4). Irrespective of planting date, accessions CHIMBU, MYAN-05, and WAM-07 produced a negligible number of mature-seed-bearing pods before termination of the experiment in the depths of winter on 30 Jun. In contrast, accession MYO-01 produced an accumulated mean of 93 mature pods per plant from the 5 Oct planting, 68 pods from the 3 Nov planting, and 44 pods from the 24 Nov planting (Figure 5).

When the last mature pods were harvested from MYO-01 (Figure 5), the plants and the entire MYO-01 plots, above and below ground, were harvested and partitioned into component parts. At this time, accessions CHIMBU and MYAN-05 had a significant number of full-length green pods but a negligible number of mature seed-bearing pods (Figure 4C). Likewise, accession WAM-07 had almost no mature pods or full-length green pods at the time of termination of the experiment on 30 Jun (Table 4).

Vegetative characteristics of accessions

Apart from these differences in phenological characteristics, analysis of vegetative characteristics revealed highly significant differences ($p < 0.001$) between accessions, particularly in their tendency to form tubers and in their branching behavior (Table 5). For stem height to the tenth node, there was a trend with each successive planting date from a mean of 39 cm in Planting 1 up to 65

cm in Planting 3, but the planting date did not affect the number of branches from the first ten main stem nodes.

Of the four accessions, accession MYAN-05 from north-eastern Myanmar demonstrated the greatest tendency to form tubers, as indicated by the mean diameter of the

three largest roots. MYO-01 from central Myanmar and CHIMBU from Papua New Guinea also showed some tendency to form tubers in the environment of eastern NSW, but WAM-07 from Wamena in Indonesian New Guinea produced no tuberous roots (Table 6).

Table 3. The range (across replications) in date of first open flower in 2021 for four accessions of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) planted on three successive dates in late 2020

Planting No.	Date seed first soaked in water (imbibition)	Date transfer to field	Accession	Date of first open flower (range across replications)
1	5 Oct 2020	24 Oct	MYO-01	21 Dec-31 Dec
			CHIMBU	3 Mar-5 Mar
			MYAN-05	7 Mar-18 Mar
			WAM-07	18 Mar-26 Mar
2	3 Nov 2020	11 Nov	MYO-01	16 Jan-20 Jan
			CHIMBU	5 Mar-20 Mar
			MYAN-05	7 Mar-18 Mar
			WAM-07	18 Mar-28 Mar
3	24 Nov 2020	11 Dec	MYO-01	19 Jan-11 Feb
			CHIMBU	21 Mar-24 Mar
			MYAN-05	11 Mar-20 Mar
			WAM-07	24 Mar-1 Apr

Table 4. The number of days to first flower, number of days to the first mature pod, accumulated number of mature pods harvested, and number of green pods per plot remaining at the time of final plot harvest before termination of the trial on 30 Jun 2021, for four-winged bean accessions planted on three successive dates in late 2020. The values for the planting date x accession comparisons are the means determined across three replicate plots

Planting no.	Date seed first soaked in water (imbibition)	Date of transfer to field	Accession identity				Planting date means across accessions	Standard error of the means
			MYO-01	CHIMBU	MYAN-05	WAM-07		
Days from planting (imbibition) to first flower (mean across reps)								
1	5 Oct	24 Oct	82	150	157	167	139 d	10.1
2	3 Nov	11 Nov	76	131	130	141	120 e	7.8
3	24 Nov	11 Dec	68	119	112	123	109 f	6.5
Accession means across plantings			76 c	133 b	133 b	143 a		
Standard error of the means			3.2	4.8	6.6	6.5		
Days from planting (imbibition) to first mature pod (mean across reps)								
1	5 Oct	24 Oct	150	235	249	249	221 j	12.6
2	3 Nov	11 Nov	152	229	225	228	209 k	9.8
3	24 Nov	11 Dec	143	213	213	212	200 k	9.0
Accession means across plantings			149 i	225 h	229 h	230 h		
Standard error of the mean			4.4	3.8	5.4	5.3		
Number of mature seed-bearing pods up to termination of the trial on 30 Jun 2021								
1	5 Oct	24 Oct	93	5	1	1	25 o	12.0
2	3 Nov	11 Nov	68	1	1	1	18 op	8.9
3	24 Nov	11 Dec	44	2	1	2	9 p	5.6
Accession means across plantings			68 m	3 n	1 n	1 n		
Standard error of the mean			8.2	1.0	0.2	0.4		
Number of green pods remaining at final plot harvest on or before 30 Jun 2021								
1	5 Oct	24 Oct	0	23	14	4	10 u	3.5
2	3 Nov	11 Nov	3	17	20	4	11 u	3.4
3	24 Nov	11 Dec	1	11	6	4	6 u	1.6
Accession means across plantings			2 t	17 r	13 s	4 t		
Standard error of the mean			1.1	3.7	3.7	1.4		

Note: Within a single row or a column, mean values followed by the same letter do not differ at the p<0.05 level of significance with Bonferroni correction for multiple comparisons

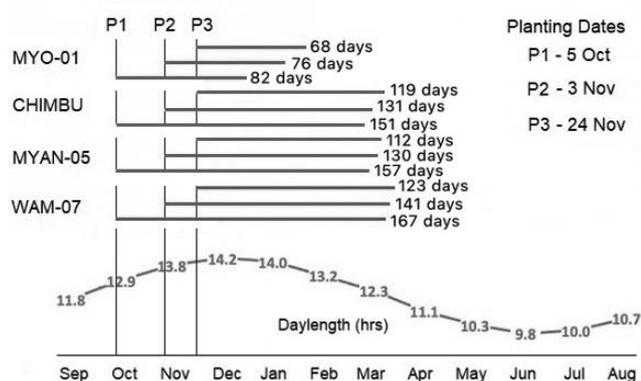


Figure 3. Number of days to first flower of four-winged bean (*Psophocarpus tetragonolobus* (L.) DC.) accessions (MYAN-05, WAM-07, CHIMBU, MYO-01) in response to the difference in planting date in late 2020 at Wagstaffe, NSW, Australia (Latitude 33.5°S). Each value is the mean of three replicate plots



Figure 4. Winged bean trial (A) overview of trial area on 6 Feb 2021 (support stakes were 2 m in height); (B) a plot of accession MYO-01 with mature pods on 27 Feb; (C) from left to right, plots of accessions MYAN-05, CHIMBU and WAM-07 with only immature pods on 4 May

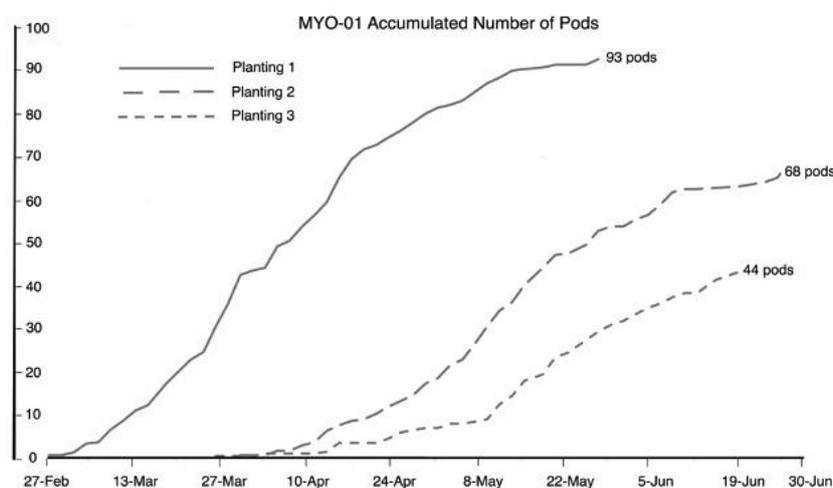


Figure 5. The accumulated number of mature seed-bearing pods per plant harvested in 2021 from winged bean accession MYO-01 from three successive plantings in late 2020. Each point is the mean of three replicate plots (RBD)

Table 5. Analysis of variance of vegetative characteristics of four-winged bean accessions planted on three successive planting dates

	Planting Date (PD)	Accession (A)	Interaction (PD x A)	Overall Mean	Subplot CV %
Mean diameter of 3 largest roots (mm)	NS	***	NS	11.6	22.5
Stem diameter at ground (mm)	NS	***	NS	8.8	19.8
Stem diameter at tenth node (mm)	**	***	*	5.5	24.5
Height to tenth node (cm)	*	***	NS	52	25.2
Number of primary branches to tenth node	NS	***	NS	4	19.7

Note: NS = variance component not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$

Table 6. Vegetative characteristics of the four-winged bean accessions. Values are the accession means determined across three planting dates x three replicate plots

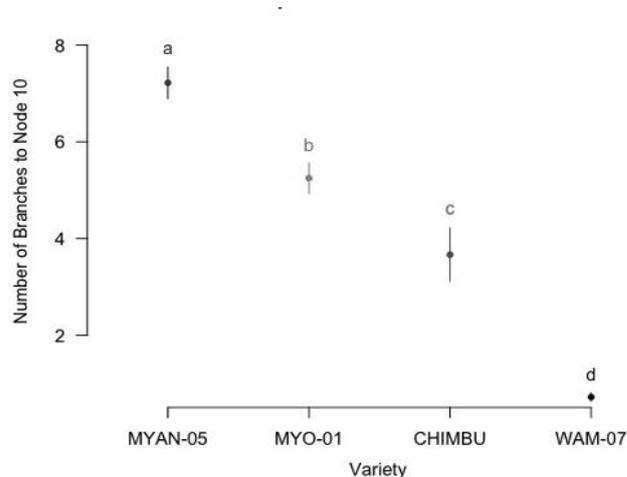
	MYO-01	CHIMBU	MYAN-05	WAM-07
Mean diameter of 3 largest roots (mm)	13.4 b	10.4 b	18.2 a	4.7 c
Stem diameter at ground (mm)	5.7 c	9.8 ab	11.1 a	8.3 b
Stem diameter at tenth node (mm)	2.6 b	5.5 a	6.4 a	7.3 a
Height to tenth node (cm)	39 b	49 b	72 a	47 b
Number of primary branches to tenth node	5 b	4 c	7 a	1 d

Note: Values within the same row followed by the same letter do not differ significantly at the $p < 0.05$ level of significance with Bonferroni correction for multiple comparisons

The most notable characteristic separating the four accessions was the number of primary branches from the first ten main stem nodes (Table 6 and Figure 6). Accessions MYAN-05 and MYO-01 from Myanmar had prolific branching from the lowermost nodes. CHIMBU was intermediate in branching tendency. WAM-07 produced negligible branching from the first ten nodes; its branches appeared much higher in the plant canopy, above the tenth mainstem node (Figure 4C). MYO-01, the early flowering accession, had a much thinner main stem at ground level and node ten than the other accessions.

Dry matter partitioning

All plots were harvested on a date before 30 Jun 2021 when no other pods could be expected to dry down to yield mature seeds. For convenience in harvesting and measurement, this date varied somewhat between accessions, with MYO-01 being harvested a few days earlier than the others and MYAN-05 plots being the last to be harvested. The plots were partitioned into their component parts which were weighed fresh with samples taken to determine moisture content. The results of this partitioning are presented in Tables 7 and 8 and Figure 7).

**Figure 6.** Comparison of the number of primary branches in the first ten mainstem leaf nodes between the four-winged bean accessions. Accession means and standard errors were determined across planting dates. The accession means differed from one another at the 5% level of significance with Bonferroni correction for multiple comparisons**Table 7.** Analysis of variance of the partitioning of dry matter at harvest for four-winged bean accessions across three planting dates

Parameters	Planting Date (PD)	Accession (A)	Interaction (PD x A)	Overall Mean	Subplot CV %
Root-plus-tuber dry matter yield (g m^{-2})	NS	***	NS	46.4	57.4
Stem dry matter yield (g m^{-2})	NS	***	*	277.9	34.5
Leaflet dry matter yield (g m^{-2})	NS	***	NS	103.2	41.6
Green pod dry matter yield (g m^{-2})	NS	**	NS	64.0	103.4
Mature pod yield (g m^{-2})	*	***	*	110.8	48.7
Seed yield (g m^{-2})	*	***	**	59.1	44.4

Note: NS = variance component not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$

Table 8. Comparison of dry matter partitioning by four-winged bean accessions. Values are the accession means determined across three planting dates x three replicate plots

Parameters	MYO-01	CHIMBU	MYAN-05	WAM-07
Root-plus-tuber dry matter yield (g m^{-2})	54.8 b	26.1 bc	94.8 a	10.7 c
Stem dry matter yield (g m^{-2})	98.0 c	340.5 b	490.1 a	155.0 c
Green pod dry matter yield (g m^{-2})	5.4 b	123.0 a	107.9 a	13.2 b
Leaflet dry matter yield (g m^{-2})	12.0 b	150.4 a	193.8 a	46.4 b

Note: Values within the same row followed by the same letter do not differ significantly at the $p < 0.05$ level of significance, with Bonferroni correction for multiple comparisons

Because of the split-plot design, the differences between planting dates were determined with lower precision than between accessions. As a result, the difference between planting dates in total plot dry matter yield at harvest did not reach statistical significance. However, there was a general trend from heaviest to lightest yields from the first to third planting dates for all components except for green pod dry matter yield.

In contrast to planting date effects, the difference between accessions was highly significant in the total plot dry matter yield and in each component that comprised the total dry matter. Figure 7 reveals the substantial difference graphically in the way total dry matter yield was distributed across the component parts. In the case of the early maturing accession MYO-01, by far, the greatest proportion of the dry matter at final harvest was in the accumulated yield of mature seed-bearing pods. The other three accessions had negligible dry matter in the form of mature seeds. Accessions MYAN-05 and CHIMBU had the greater component of their total dry matter as vegetation (i.e., stems, branches, and leaves). For all four accessions, but particularly WAM-07, there had been a substantial loss of dry matter from the standing biomass due to leaf drop, which became particularly pronounced in late autumn and early winter. WAM-07 plants at the time

of harvest had very low-standing biomass, stringy roots, and no tubers, whereas MYAN-01, in addition to a significant root-plus-tuber yield of 95 g m⁻² still retained a substantial yield of biomass in the form of stem branches and leaves, as did CHIMBU.

The yield of mature pods and seed

In addition to a root-plus-tuber dry matter yield of 55 g m⁻² averaged across the three planting dates, MYO-01 produced an accumulated mature pod yield of 435 gm⁻², of which 240 g m⁻² was the seed (Table 9). For MYO-01 planted in October, the total yield of mature pods and seeds was equivalent to 5.6 t ha⁻¹ and 3.1 t ha⁻¹, respectively.

Accession MYO-01 had a pod length of 10.8 + s.e. 0.2, compared with 24.9 + s.e. 1.9, 23.0 + s.e. 2.0, and 11.7 + s.e. 1.7 for Chimbu, MYAN-05 and WAM-07, respectively, determined across planting dates. For MYO-01, there was no significant difference between planting dates for the number of seeds per pod (8.3 + s.e. 0.2), 100-seed weight (31.4 g + s.e. 0.9 g), and shelling percentage (55.6% + s.e. 0.7 %). For the other three accessions, these characters could not be accurately estimated because of the small number of pods producing mature seeds before the termination of the trial on 30 Jun 2021.

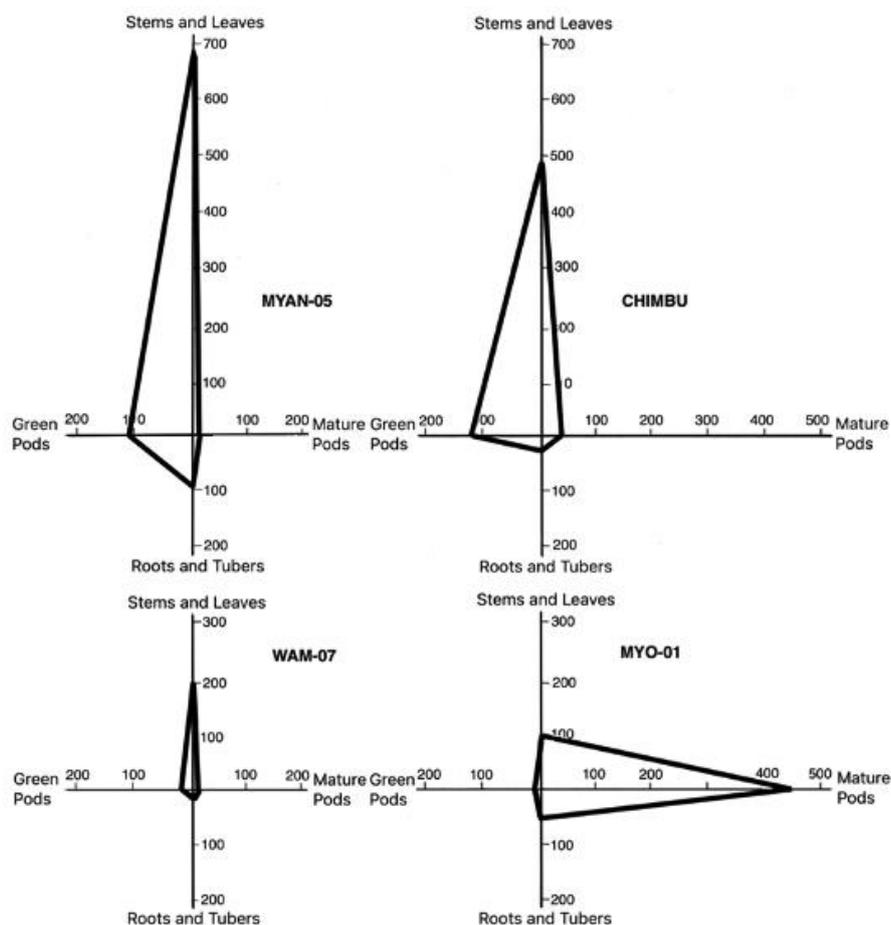


Figure 7. Partitioning of dry matter (g m⁻²) at plot harvest between vegetation (stem, branches, and leaves), roots-plus-tubers, green pods, and mature seed-bearing pods, compared between four accessions of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) averaged across three planting dates

Table 9. Mature pod yield and seed yield of four varieties within three planting dates. The values for the planting date x accession comparisons are the means determined across three replicate plots

Planting No.	Date seed first soaked in water (imbibition)	Date of transfer to field	Accession identity				Planting date means across accessions	Standard error of the means
			MYO-01	CHIMBU	MYAN-05	WAM-07		
Mature pod yield (g m⁻²)								
1	5 Oct	24 Oct	564 a	81 cd	0 d	4 d	162	71.3
2	3 Nov	11 Nov	467 ab	14 d	9 d	0 d	107	52.4
3	24 Nov	11 Dec	281 bc	12 d	5 d	9 d	58	36.7
Accession means across plantings			435	36	5	4		
Standard error of the means			49.1	15.0	3.2	3.2		
Seed yield (g m⁻²)								
1	5 Oct	24 Oct	308 f	34 h	0 h	1 h	85.7	39.3
2	3 Nov	11 Nov	227 fg	4 h	1 h	0 h	57.9	29.5
3	24 Nov	11 Dec	157 g	5 h	2 h	4 h	31.4	20.4
Accession means across plantings			240	14	1	2		
Standard error of the mean			25.9	6.9	0.6	1.3		

Note: Values for the planting date x accession comparisons followed by the same letter do not differ significantly at the $p < 0.05$ level of significance, with Bonferroni correction for multiple comparisons

Discussion

At sub-tropical and temperate latitudes, most accessions of winged bean (*P. tetragonolobus*) are very late flowering and produce low yields of vegetable pods, mature seeds, and edible tubers due to the inhibition of phenological development by the long day-lengths summer when temperatures are at their optimum for vegetative growth (Wong and Schwabe 1980; Eagleton 1985; Okubo et al. 1989).

Day-length sensitivity delays phenological development in sub-tropical and temperate regions and equatorial latitudes (Eagleton 2019). That was well demonstrated by Sinnadurai and Nyalemegbe (1979) in Ghana. They planted out the well-known IITA accession, TPt-1, in Accra at Latitude 5°30' N each month for a year. In Accra, there is little seasonal variation in day length (from 11 hours 50 minutes in December to 12 hours 25 minutes in June) and in monthly mean daily temperature (from 25.7°C in August to 29.6°C in March), yet the effect of planting date on the number of days to flowering is profound (Figure 8).

Thus, the identification of accessions with reduced sensitivity to day-length conditions, such as MYO-01 in this study and the Urizun and KUS selections in Japan (Abe et al. 1988; Okubo et al. 1989), holds promise not only for producing winged bean varieties adapted to high latitude summer plantings but also for producing varieties in tropical zones that flower and set pod early, irrespective of the difference in planting date.

One research task for the future, prompted by the findings of this study, is to investigate the genetic control of reduced photoperiod sensitivity in MYO-01 and the Japanese selections and to determine whether the genetic factors are the same for each of these independently identified daylength-neutral genotypes. In other legume crops such as common bean (*Phaseolus vulgaris* L) and soybean (*Glycine max* (L.) Merr.) that share the short-day

habit of winged bean, genetic pathways with a central role in florigen activation and induction of flowering is the subject of detailed investigation (Lin et al. 2020; Gonzalez et al. 2021). It remains to be seen whether the failure of most winged bean accessions to flower in day lengths exceeding 13 hours is determined by genes with functions homologous to those that inhibit flowering in other short-day legume species.

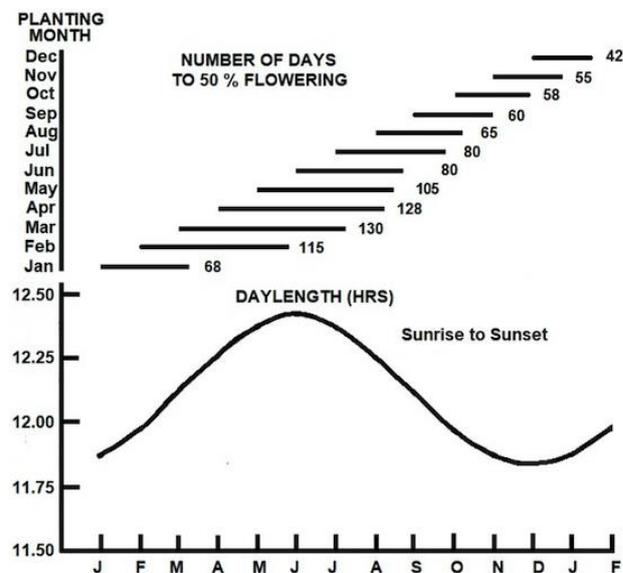


Figure 8. Effect of planting date on the number of days to flowering of winged bean accession TPt-1 in Accra, Ghana (adapted from Sinnadurai and Nyalemegbe (1979))

To produce a really successful winged bean vegetable variety for southern Australian conditions, other characteristics, in addition to stable early maturity, would need to be incorporated into the genome. For example, accession MYO-01 has quite small pods (a mature pod length of around 11 cm, half that of accessions CHIMBU and MYAN-05), but studies elsewhere have shown that high vegetable pod yields are correlated with pod length and weight more so than with pod number (Kesavan and Erskine 1980, in Papua New Guinea; Yulianah et al. 2020, in Indonesia). Similar germplasm evaluations in various locations have shown considerable diversity in pod characteristics, including pod length, width and weight, and pod color, shape, and texture (Khan 1982; Kuswanto et al. 2016; Kant and Nandan 2018; Sarode Hemal and Dodake 2019; Adegboyega et al. 2019, 2021; Laosatit et al. 2021; Chankaew et al. 2022) that could provide nutritional diversity and novelty in competitive commercial vegetable markets.

Plant architecture is another trait of importance in optimizing crop management and maximizing vegetable pod yields. This study reconfirmed the significant difference in branching habit between accessions from the highlands of the island of New Guinea and accessions from mainland Southeast Asia, as shown in previous genetic studies by the author (Eagleton 1985). Plants of Myanmar accessions MYO-01 and MYAN-05 have, on average, five or more branches from the first ten main stem nodes, whereas CHIMBU has a mean of 4 and WAM-07 just one. Tanzi et al. (2019b) demonstrated the importance of a branching habit for maximizing vegetable pod yields on trellised production systems. On the island of New Guinea, winged bean plants are supported on upright, thin stakes rather than on trellises. That no doubt explains the selection of genotypes for climbing rather than strongly branching habits. In considering the possibility of a winged bean cultivar for vegetable production in Southern Australia, likely, the moderately branching habit of MYO-01 supported on a trellis-like system will be preferable to the vigorous branching habit of MYAN-01, which has evolved specifically for a root-tuber production system in central Myanmar without any trellis or pole support (Burkill 1906). It remains to be seen whether genotypes with a determinate architectural form, such as represented in the selection KUS-101 identified by Okubo et al. (1989), will play a greater role in the future development of winged beans.

Finally, a trait that poses a significant constraint to the development of winged beans, especially for the use of its seed protein, but also more generally for agronomic convenience, is the hardness of its seed. MYO-01 has a particularly tough seed coat. The longer the seed is held in the cold, dry conditions that ensure long-term viability in storage, the greater the imperviousness of the seed to uptake by water when planted in normally optimum conditions for germination (Ellis et al. 1985). That is a major obstacle to the increased utilization of winged beans (Alex et al. 2010, Kumar and Rajalekshmi 2021). Unraveling the physiology and genetics of hard-seededness

in the winged bean is a significant task for future research (Rudrapal et al. 1992).

CONCLUDING REMARKS

The detection of a genotype with the ability to flower early in the long days of a high latitude summer has been a goal of the few researchers with interest in the possible development of a winged bean cultivar for vegetable pod production in southern Australia. Three decades ago, scientists in Okinawa and at Kyushu University in Japan achieved just such a milestone, but in Australia, the goal had proved elusive until the identification in this study of the accession MYO-01, an apparently day-length neutral variety under the climate conditions of the NSW central coast. The pursuit of this goal has not been to facilitate the development of an adapted vegetable cultivar for home- and market gardeners in Australia but also to support R&D efforts in the tropics where daylength-sensitivity normally delays and destabilizes the phenological development of most cultivars, thus reducing greater utilization of this valuable high-protein legume crop.

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Physical and chemical properties of soil on selected sugarcane farms in Mt. Nebo, Valencia City, Bukidnon, Philippines

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Abstract. Labajo JRN, Pabiona MG. 2022. *Physical and chemical properties of soil on selected sugarcane farms in Mt. Nebo, Valencia City, Bukidnon, Philippines. Asian J Agric 6: 79-86.* The study was conducted to determine soil's physical and chemical properties on selected sugarcane farms in Mt. Nebo, Valencia City, Bukidnon, Philippines. Soil samples were collected from 24 different sugarcane farms planted with sugarcane following Sloven's Formula for farm selection. Soil samples were collected from August 2018 to April 2019, and the physical and chemical properties of the soil were determined at SPAL, CMU, Musuan, Maramag, Bukidnon, Philippines. Soil texture and percent Water Holding Capacity (%WHC) soil of the sugarcane farms were clayey textured, with %WHC ranging from 54.24-76.30% and bulk Density (BD) and Particle Density (PD) values ranging from 1.05-1.31 Mg m⁻³ (BD) and 2.28-2.41 Mg m⁻³, which is ideal for growing sugarcane. Soil samples collected were categorized as medium acidic, marginal in soil organic matter content (SOMC), extractable phosphorus (P) was very deficient, and low on exchangeable potassium (K). That implies these sugarcane farms need soil amendments to meet sugarcane's desired nutrient and pH requirements. Therefore, the sugarcane farms in Mt. Nebo, Valencia City, Bukidnon are required to apply 1.64-7.04 Mg ha⁻¹ of lime and a recommendation rate of 80-200 kg ha⁻¹ Nitrogen (N), 200-500 kg ha⁻¹ of Phosphorus (P), and 170-200 kg ha⁻¹ potassium (K). Moreover, a soil fertility map is necessary to classify and group the delineated soils into map units and capture soil property information to interpret and depict soil spatial distribution on a map.

Keywords: Assessment, Bukidnon, physical-chemical properties, *Saccharum officinarum*, soil mapping, Valencia City

Abbreviations: BD: Bulk Density, PD: Particle Density, SOMC: Soil Organic Matter Content, WHC: Water Holding Capacity

INTRODUCTION

The soil's physical and chemical characteristics regulate the number of nutrients available to plants. Their relevance in nutrient supply to crops must be regularly checked for long-term development and increased crop production to achieve optimal crop production (Suleiman et al. 2017). Soil nutrient availability is the adequacy with which soil supplies the plant needed nutrients. With the complex availability of the nutrient and incompletely understood, plant response remains the only reliable basis for diagnosing nutrient deficiencies. To maintain the sustainability of sugarcane production, soil management practices that can increase soil fertility and quality should be employed (Wakgari et al. 2020; Abdullah et al. 2022). Sugarcane (*Saccharum officinarum* L.) belongs to the family Gramineae and is a raw material for everyday use in sugar and bioethanol production. Like most crops, they cannot grow without the important natural resources, soil, and water. As a medium for plant growth, the soil must be properly managed to attain a higher production yield.

To understand the growth requirement of sugarcane and how to achieve the desired yield, the soil's chemical and physical properties must be considered. Fertile soil is critical to our ability to achieve food security, but inadequate management exacerbates soil degradation (such as acidification). Liming affects all soil elements, resulting

in several simultaneous changes in soil processes that affect plant nutrient uptake (Holland et al. 2018). The application has benefited humans for centuries in ameliorating acidic soil conditions. One of the major keys to food security for future food production is healthy soil for the plant to grow. The efficient use of inorganic and organic fertilizers is the most important aspect of a sustainable agriculture farming system. Ensuring the health of the soil and correct agricultural practices and preventing the loss and damage to the soil, correct fertilizer management of 4R Principles (right source, right rate, right time, and right place), nutrient stewardship, increased crop production, and sustainable farming will be achieved.

The rising soil erosions, land degradation, and nutrient depletion in the Philippines are problems to the region, with yearly visits by typhoons and hurricanes a serious threat to the agriculture sector in the country. The soil nutrient maps and physical property maps are effective strategies for ensuring precise evaluation of the area. The development of maps is crucial in site-specific soil physical and chemical management for sustainable agricultural practices (Verma et al. 2018).

The Philippines is a sugar-producing country, growing mainly on the Island of Negros, Western Visayas, Bukidnon in Northern Mindanao, and the CALABARZON Region (PSA 2019). Although in Northern Mindanao, sugarcane can only be found in Bukidnon, the sugar

industry in the province formally started in 1975 when it was discovered that Bukidnon's wide lands are suitable for sugarcane. Recently its contribution to the national production was 3,068,055 metric Mg in 2018. This study was conducted with the following objectives: (i) to determine the chemical and physical properties of soil on selected sugarcane farms in Mt. Nebo, Valencia City, Bukidnon; (ii) to formulate lime and fertilizer recommendations based on the data gathered and results of analysis; and (iii) to generate nutrient fertility map based on soil pH, soil organic matter content, extractable phosphorus, and exchangeable potassium.

MATERIALS AND METHODS

Study area

The soil quality assessment was conducted in Mt. Nebo, Valencia City, Bukidnon, on the Island of Mindanao, Philippines (Figure 1). With an altitude of 896.5 meters above sea level, located at the foot of Mt Kalatungan Range. Its latitude is 7.9728, 124.9866 (7°58' North, longitude 124°59' East), which has a diversity of plantation crops and is identified as adtuyon clay soil series.

Survey and farm selection procedure

Each sugarcane farm in Mt. Nebo, Valencia City, Bukidnon had an equal probability of being selected in the sampling selection. Due to the numerous sugarcane farms, a survey questionnaire was formulated and distributed to the farmers, including interviews done during the study. Sloven's formula was used to secure a randomly selected

sugarcane farm. The following criteria were imposed to minimize the number of farmers. (i) The farmer must have at least 1.5-4 ha of sugarcane farm; (ii) must have at least five years of sugarcane operation; and (iii) did not practice burning sugarcane residue. If one of the farmers cannot meet at least two of the following criteria, the farm will be disregarded for being part of the study.

$$n = N/(1+Ne^2)$$

Where: n = sample size, N = population size, e = margin of error

Given: N = 26 total number of sugarcane farms fit the criteria

$$e = 0.05 \text{ or } 5\%$$

$$n = 26/(1+26 \times 0.05^2)$$

Furthermore, a total number of 24 sugarcane farms from the 26 sugarcane farms fits the criteria used for the survey.

Collection and preparation of soil sample

A survey questionnaire was provided to the randomly selected farmer in the area. The area was inspected first, and the land feature, coordinates, and farmer's profiles were collected before sampling the area for analysis. The was collected in a zigzag pattern, with a composite sample of 15-20 per area with a 10-15cm soil depth. Soil samples were air-dried and pulverized. Then sieved and stored in a clean plastic container.

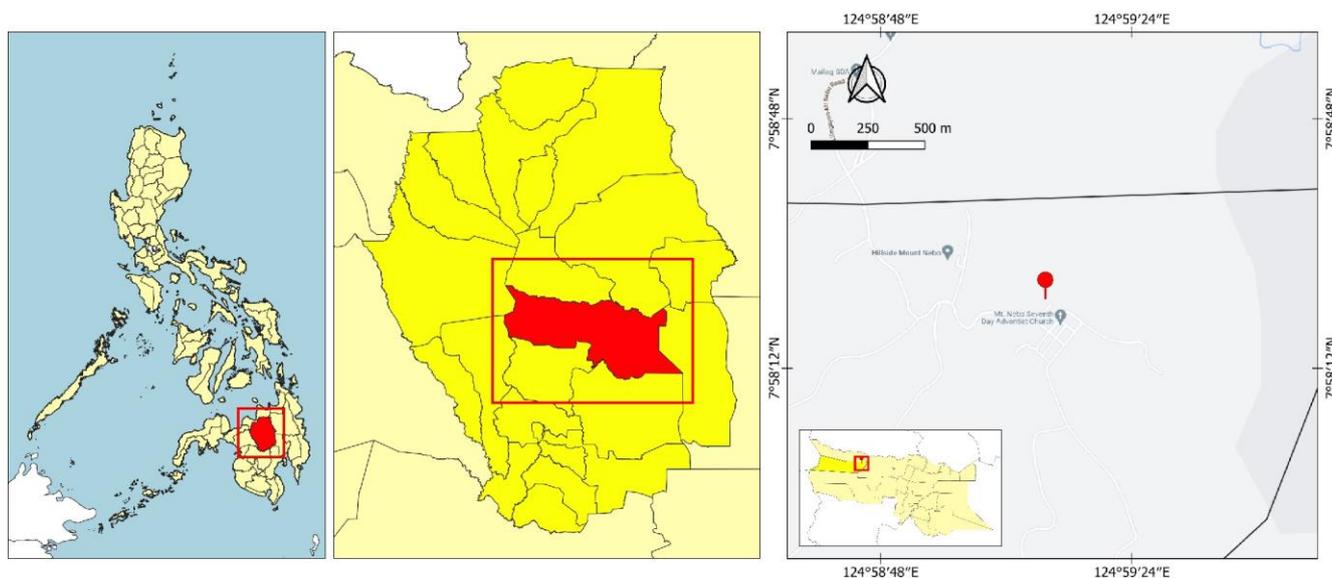


Figure 1. Location map of Mt. Nebo, Valencia City, Bukidnon, Philippines

Table 1. Soil test values and a nutrient recommendation rate of sugarcane

Category	Soil test values			Recommendation (kg ha ⁻¹)		
	% Nitrogen	Phosphorus (ppm)	Potassium (ppm)	N	P ₂ O ₅	K ₂ O
Very Low	<0.05	<5	<25	200	200	600
Low	0.05-0.09	5-9	25-49	170	170	500
Medium Low	0.10-0.14	10-14	50-74	140	140	400
Average	0.15-0.19	15-19	75-99	110	110	300
Medium High	0.20-0.24	20-24	100-124	80	80	200
High	0.25-0.29	25-29	125-149	40	40	100
Very High	>0.29	>29	>149	20	20	50

Table 2. Methods used in the analysis of the physical and chemical properties of soil

Property	Methods of analysis	Reference
Physical properties		
Soil Texture	Pipette Method	(PCARRD 1991)
Water Holding Capacity	Wire gauze method	(Lal and Shukla 2004)
Bulk Density	Core method	(Blake 1965)
Particle Density	Pycnometer method	(Blake 1965)
Chemical properties		
Soil pH	Potentiometric method (1:5 soil water ratio)	(Biddle 1997)
Organic Matter Content	Walkley-Black method	(FAO GLOSOLAN 2020)
Extractable Phosphorus	BrayP ₂ (0.1N HCl + 0.03N NH ₄ F)	(Landon 1984)
Exchangeable Potassium	1 N NH ₄ OAc extraction/Flame photometer	(Landon 1984)
Lime Requirement	Veitch Method	(Hoskins 1997)

Preparation of soil physical and chemical fertility map

Mapping of each sugarcane farm in Mt. Nebo, Valencia City, Bukidnon was the first to categorize using the soil test values for pH, exchangeable phosphorus, and extractable potassium (Hoskins 1997). Also, tests for soil organic matter and soil text values of PCARRD (1991) were assigned with designated colors for identification, as presented in Figure 2. The development of soil fertility, soil texture, pH, organic matter content, extractable phosphorus, and exchangeable potassium was mapped using ArcGIS software by Esri.

Computation for fertilizer and lime recommendations

The fertilizer recommendation of sugarcane was based on the nutrient recommendation rate developed by PCARRD (1991), presented in Table 1. Soil test values result from the conducted analysis of OM, extractable phosphorus, and exchangeable potassium. The result of OM was multiplied by the conversion factor of 0.05 to convert OM to (%) Nitrogen, and extractable potassium was multiplied by 390 to convert mg dm⁻³ to ppm potassium. The lime recommendation was based on the Veitch method (Hoskins 1997) with the amount of lime on the x-axis and pH on the y-axis and was computed based on the desired pH for sugarcane being 6.5.

Laboratory work

Soil samples collected in the sampling sites were replicated during the analysis to ensure the correct analysis of the samples. Analysis of Physical and chemical properties of the selected sugarcane farms in Mt. Nebo, Valencia City, Bukidnon, Philippine soil was performed at the Soil and Plant Analysis Laboratory, Department of Soil

Science, College of Agriculture, Central Mindanao University, University Town, Musuan, Maramag, Bukidnon, Philippines. Methods used in the Analyses of Soil's Physical and Chemical properties are presented in Table 2.

RESULTS AND DISCUSSION

Soil chemical properties

Soil pH values of farms grown to sugarcane in Mt. Nebo, Valencia City, Bukidnon is presented in Table 3. Fifteen (15) sugarcane farms (62.50%) were identified as medium acidic with soil pH values of 5.6-6.0. Seven (7) farms (29.16%) farms were categorized as strongly acidic, having pH values of 5.1-5.5. One (1) sugarcane farm (4.17%) was slightly acidic with soil pH values of 6.1-6.9, and only one (1) sugarcane farm (4.17%) was found to be very strongly acidic with soil pH values of 4.5-5.0. Therefore, it was observed that most and the optimum soil pH are about 6.5. Considerably, sugarcane can tolerate a degree of soil acidity and alkalinity. Soil reaction refers to the degree of acidity or alkalinity and is expressed in terms of pH units (Braddy and Weil 1999), as cited by Daquiado and Pabiona (2017).

The soil organic matter content of sugarcane is presented in Table 3. The analysis showed seventeen (17) or 70.83% of sugarcane farms have a marginal amount of organic matter (Walkley-Black 1934). Four (4) or 16.67% of sugarcane farms are deficient in organic matter. Lastly, three (3) or 12.50% are adequate in organic matter content (FAO GLOSOLAN 2020). The results show that sugarcane farms' percent organic matter content is marginal.

The extractable phosphorus of sugarcane farms is presented in Table 3. Twenty-four (24) or 100%, whose

extractable phosphorus ranged from $<4.1 \text{ mg dm}^{-3}$, were classified as very deficient. Based on the result, the extractable phosphorus of sugarcane farms is very low, and it is recommended to apply phosphorus-containing fertilizer. Proper management of phosphorus on the farm will lead to higher crop production and sustainability for sugarcane production (Arruda et al. 2016). P application, especially in P-deficient soils, promotes root growth, stimulates tillering, influences better growth, and thus increases yield and juice quality. P availability in the soil is influenced by various factors such as soil texture, clay content, clay type, and soil organic matter (Gichangi et al. 2009).

The exchangeable potassium (K) of sugarcane farms is presented in Table 3. There were eight (8) farms 33.33% categorized as a medium in exchangeable K content with values ranging from 78.0 to $109.59 \text{ mg dm}^{-3}$, and seventeen (17) or 70.83% farms were categorized as low in exchangeable K with values less than 78.0 mg dm^{-3} (FAO GLOSOLAN 2020). Plant requirement for this element is quite high, and increased potassium availability in the soil promotes higher absorption of nutrients by plants (Pancelli et al. 2015). Therefore, sugar utilizes a large amount of K. Deficiencies are commonly observed on well-drained, coarse, sandy soils. Furthermore, compared to other nutrients, sugarcane's response to potassium fertilization is usually the most immediate (Rice et al. 2010). Therefore, K often increases the percentage of sugar in the cane and juice recovery even when there is no increase in cane yield, particularly when the harvest is delayed. The nutrient map

for pH, organic matter content, phosphorus, and potassium of the sugarcane farms is presented in Figure 2.

The soil quality assessment will help the farmers apply the 4R nutrient stewardship to their soil. Rajendran and Shanmuganathan (2019) observed diverse roles that soils provide as the cornerstone for sustainable land management were demonstrated using evaluation tools for indexing soil quality at various scales. Shivanna and Nagendrappa (2014) investigated the soil fertility status in Selected Command Areas of Three Tanks in Tiptur Taluk of Karnataka, India. The factors examined were pH, OM, N, P, and K. The pH of the soil samples ranged from 7.07 to 7.87, indicating that they were somewhat alkaline but within the ideal range of 6.5-7.5 for crops. The OM concentration of the samples ranged from 0.86 percent to 1.15 percent, all rated as medium. Nitrogen availability ranged from 54.825 kg/ha to 54.825 kg/ha . Available phosphorous ranged from 5.33 kg/ha to 10.79 kg/ha ; the samples were nitrogen and phosphorus deficient. Potassium levels ranged from 156.18 kg/ha to 434.38 kg/ha , except for one sample with a high potassium level. A similar study was conducted by (Wagh et al. 2013) in Mula, Mutha, India. Moreover, because most farmers use excessive chemical fertilizers, the amount of phosphorus and potassium in the soil is higher than the usual range, and inferior drainage characteristics in this area, making the soil alkaline. As a result, Wagh et al. (2013) suggested that the additional fertilizers during crop formation may be responsible for varying concentrations of various parameters and irregular macronutrient distributions.

Table 3. Physical and chemical properties of the soil in the selected sugarcane farms in Mt. Nebo, Valencia City, Bukidnon, Philippines

Farm code	Soil pH	% OM	Exchangeable phosphorus mg dm^{-3}	Extractable potassium mg dm^{-3}	Bulk density Mg m^{-3}	Particle density Mg m^{-3}	Water holding capacity (%)	Soil texture class
F1	5.33	2.32	1.48	67.47	1.22	2.31	70.54	Clay
F2	5.38	3.30	2.02	78.00	1.11	2.36	65.96	Clay
F3	5.42	2.70	2.87	106.47	1.05	2.33	71.08	Clay
F4	5.67	4.58	4.91	65.91	1.17	2.38	73.68	Silty Clay
F5	5.89	4.88	1.59	104.91	1.21	2.32	65.23	Clay
F6	5.77	4.05	1.43	65.91	1.28	2.40	67.34	Clay
F7	5.86	2.85	1.79	108.03	1.22	2.39	59.47	Clay
F8	5.82	4.58	3.25	69.03	1.23	2.31	69.58	Clay
F9	5.72	1.05	1.70	113.88	1.10	2.30	82.90	Clay
F10	5.73	3.15	1.42	49.53	1.23	2.33	63.55	Silty Clay
F11	6.38	3.00	1.42	79.56	1.19	2.28	71.66	Clay
F12	5.28	3.45	6.58	51.09	1.31	2.38	69.69	Clay
F13	5.68	3.00	1.87	72.15	1.21	2.32	76.30	Clay
F14	5.82	1.95	3.37	64.35	1.29	2.28	63.68	Clay
F15	5.29	1.72	1.14	55.38	1.26	2.30	75.94	Clay
F16	5.59	4.20	1.79	55.38	1.23	2.31	68.12	Clay
F17	5.77	1.58	5.18	90.09	1.31	2.28	70.35	Clay
F18	5.64	2.85	1.70	61.62	1.21	2.35	65.00	Clay
F19	5.08	3.00	4.25	73.32	1.25	2.24	65.48	Clay
F20	5.49	3.22	1.53	56.94	1.13	2.39	74.44	Clay
F21	5.86	4.42	2.12	55.38	1.22	2.31	69.74	Clay
F22	5.62	3.15	2.25	52.65	1.15	2.31	65.27	Clay
F23	5.47	3.00	2.24	109.59	1.18	2.37	70.26	Clay
F24	5.71	3.82	1.31	61.62	1.23	2.41	54.24	Silty Clay



Figure 2. Soil physical and chemical fertility map of selected sugarcane farms in Mt. Nebo, Valencia City, Bukidnon, Philippines. A. Soil pH, B. Organic matter content, C. Exchangeable phosphorus, D. Extractable potassium, E. Soil texture

Soil physical properties

The bulk density of sugarcane farms in Mt. Nebo, Valencia City, Bukidnon showed in Table 3. There were twenty-two (22), or 91.67%, whose values ranged from 1.0-1.3 Mg m⁻³, and only two (2), or 8.33%, with values greater than 1.3 Mg m⁻³. This area was a well-drained, deep, loamy soil with a bulk density of 1.1 to 1.2 Mg m⁻³ (and 1.3-1.4 Mg m⁻³ in sandy soils). The critical value of Bulk density for restricting root growth varies with soil type (Hunt and Gilkes 1992). Still, bulk densities greater than 1.6 Mg m⁻³ tend to restrict root growth (McKenzie et al. 2004). Sandy soils usually have higher bulk densities (1.3-1.7 Mg m⁻³) than fine silts and clays (1.1-1.6 Mg m⁻³) because they have larger but fewer pore spaces. In clay soils with good soil structure, there is a greater amount of pore space because the particles are very small, and many small pore spaces fit between them. Soils rich in organic matter (peaty soils) can have densities of less than 0.5 g Mg c⁻³. Most of the sampling area planted with sugarcane is considered favorable for planting sugarcane.

Particle density values ranging from 2.24 Mg c⁻³ to 2.40 Mg m⁻³ were determined from the sugarcane farms, as shown in Table 3. Typical particle densities for soils range from 2.60 to 2.75 Mg m⁻³ for mineral particles. However, they can be as high as 3.0 Mg m⁻³ for very dense particles and as low as 0.9 Mg m⁻³ for organic particles. Particle density measurements provide information about the kinds of material present in the soil. If the particle density is high, we know that the soil's parent material consists of high-density minerals. This information provides insight into the geologic history of the soil. On the other hand, a low particle density (<1.0 Mg m⁻³) indicates high organic matter content. It also provides information about the potential release of carbon from the soil into the atmosphere as the organic matter decomposes over time (Globe 2014).

Twenty (20), or 87.50%, sugarcane farms were classified as optimal soil in terms of water holding capacity, with values ranging from 60-80%. Only two (2), or 8.33%, have values ranging from 55-to 60%. And only one (1) or 4.17% whose value is greater than 80% and categorized as saturated. Available water holding capacity of 15% or more (15 cm per meter depth of soil is considered ideal for sugarcane cultivation (Gliński and Lipiec 2018). Daquiado (2018) states that the higher the percentage of silt and clay-sized particles, the higher the water-holding capacity. As presented in Table 3, Twenty (20), or 87.5% of sugarcane farms, have clayey textured soil, and three (3), or 12.50%, are silty clay textured soil. Most sugarcane farms in Mt. Nebo, Valencia City, Bukidnon had clayey soil and less silty clay soil texture suitable for growing sugarcane. The soil texture will affect the yield since it influences nutrient and water retention (Gerpacio et al. 2004).

An important finding was the investigation by Campos et al. (2020), which established the soil's physical properties in plantations of lacustrine soils in Venezuela. These location does not favor the rapid movement of water;

such is the case of the high values of BD observed in some plots associated with their lake origin, which can affect the normal development of plant roots. Likewise, Rondon et al. (2021) indicated that the physical variables of these soils of lacustrine origin, such as texture class, apparent density, porosity, and moisture retention capacity, were related to banana yield and productivity.

The results of Olivares et al. (2022) agree with our results, which indicate that bananas show a high sensitivity to cycles of fertilization and a decrease in yields due to soil compaction; thus, the weight of the roots and shoots of the banana decreases significantly as the BD of the soil increases.

Lime and fertilizer recommendation

The lime recommendation of the sugarcane farm in Mt. Nebo, Valencia City, Bukidnon is presented in Table 4. All twenty-four (24) or 100% sugarcane farms must apply lime. Fourteen (14), or 58.33%, required up to 3-6 Mg ha⁻¹. Another six (6), or 25% of farms, required 6-9 Mg ha⁻¹, while only four (4), or 16.67% of farms, required 1-3 Mg ha⁻¹. Lime is usually added to acid soil to increase soil pH. Adding lime can eliminate most of the major problems associated with acid soils by replacing hydrogen ions and raising soil pH. It can also provide calcium and magnesium to the soil. Lime also makes phosphorus more available for plant growth and increases nitrogen availability by hastening the decomposition of organic matter in the soil.

The lime recommendation was based on the result of the Veitch method. The lime mounted on x-axis and pH on y-axis was computed based on the desired pH for sugarcane which is 6.5. There were twelve (12) different sets of recommendation rates distributed on different sugarcane farms. The recommended rate for nitrogen ranges from 80 to 140 kg ha⁻¹, which needs 0-5.29 bags of urea (46-0-0) ha⁻¹. The recommended rate of phosphorus ranges from 200-500 kg ha⁻¹, which needs 8.70 to 21.74 bags of ammonium phosphate (16- 20-0) ha⁻¹. For potassium, the recommendation rate ranges from 170-200 kg ha⁻¹, which needs 4 to 5.67 bags of muriatic potash (0-0-60) ha⁻¹. Fertilizers used for recommendations were based on the farmer's choice of fertilizers.

Also, the studies by Olivares et al. (2021) and Paredes et al. (2021) establish that the crops with an important demand for water and chemical products for conventional management, with the majority of plantations of more than ten years planted, generate inconveniences in the filtration and movement of water in the soil coupled with the absence of soil and water conservation practices in the plots, with the improvement of irrigation depending on the planting age of the banana plantation strictly necessary to avoid infiltration problems of the underground water. Finally, these studies in tropical territories (Olivares and Hernandez 2020; Olivares 2022) imply that these farms of sugarcane, bananas, and other tropical crops need soil amendments to meet the desired nutrient requirement and good physical soil conditions.

Table 4. Fertilizer and Lime recommendation of sugarcane farms in Mt. Nebo, Valencia City, Bukidnon, Philippines

Farmers code	N (kg ha ⁻¹)	P ₂ O ₅ (kg ha ⁻¹)	K ₂ O (kg ha ⁻¹)	Lime recommendation (mg ha ⁻¹)
F1	140	400	200	6.28
F2	110	300	200	6.27
F3	140	200	200	6.46
F4	80	400	200	4.71
F5	80	200	200	1.84
F6	80	400	200	3.96
F7	140	200	200	1.87
F8	80	400	200	3.57
F9	200	200	200	4.51
F10	110	500	200	3.68
F11	110	300	200	1.64
F12	110	400	170	6.96
F13	110	400	200	4.64
F14	140	400	200	3.29
F15	170	400	200	5.99
F16	80	400	200	5.95
F17	170	300	170	3.62
F18	140	400	200	4.46
F19	110	400	200	8.68
F20	110	400	200	4.79
F21	80	400	200	2.14
F22	110	400	200	4.34
F23	110	200	200	7.06
F24	110	400	200	4.15

In conclusion, the result of the study indicated that most of the selected sugarcane farms were affected by heavy cultivation over the past decades without proper management and are expected to affect the yield. The soil texture in the sampling sites is clayey soil. Bulk density and particle density values were recommended for optimum sugarcane cultivation sugarcane. This location was within the ranges of (BD) and (PD) values for clayey textured soil, and the water-holding, capacity was at the optimum values. The medium acidic pH values in the selected sugarcane farm Mt. Nebo are required to apply liming material to achieve the required pH values for growing sugarcane. The marginal organic matter, very deficient extractable phosphorus, and low exchangeable potassium contents indicate that soil fertility is among the major constraints for sustainable sugarcane production in the area. Moreover, to maintain and ensure sustainable sugarcane production, good soil management should be practiced to ensure good soil pH, increase soil organic matter content, extractable phosphorus, and exchangeable potassium.

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Factors influencing smallholder cardava banana farmers' participation in collective marketing in Southern Philippines

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Abstract. Orejudos RD, Duka JU, Baladjay AA. 2022. Factors influencing smallholder cardava banana farmers' participation in collective marketing in Southern Philippines. *Asian J Agric* 6: 87-96. Cardava banana farming is a good source of living for smallholder rural farmers in Cotabato province in the Southern Philippines, but they are often faced with constraints in finding the right buyers and good prices. This research examined the factors influencing smallholder cardava banana farmers' participation in collective marketing in the Southern Philippines. The data from 172 respondents were gathered using a pre-tested survey questionnaire. Means, Percentages, and linear regression analysis were used to address the study's objectives. The study results established that smallholder cardava banana farmers' participation in collective marketing is predominantly determined by household size, price, and payment scheme. In addition, they are also determined by the delivery schedule, distance to the market, access to extension services, access to production inputs, access to credit assistance, access to market information, and membership in farmers' organizations. This study's findings offer empirical evidence that socio-economic, market,- and institutional factors can influence the participation of smallholder cardava banana farmers in collective marketing.

Keywords: Group marketing, institutional market, linkSfarM project, market participation, poverty alleviation

INTRODUCTION

The Philippines, with some 2.85 million metric tons shipped out in 2017, is one of the top five highest banana exporters worldwide. In 2018, the nation harvested some 9.36 million metric tons of bananas from 447,889-hectare plantations, with cardava banana accounting for about 27% of the overall production (Vézina and Van den Bergh 2020). Cotabato province in the Southern Philippines considers the banana as one of the major contributors to its robust agricultural economy and other high-valued commercial crop sectors, including sugarcane, coconut, rubber, and oil palm. Today, there are 14,787.7 hectares of land occupied by banana plantations, of which 34.40% are planted with cardava bananas. Cotabato province is harvesting 30 MT per hectare per year of cardava banana (Province of Cotabato 2020). That implies cardava banana farming is a good means of living for rural farmers in Cotabato province.

However, they are faced with the harrowing fact concerning the low prices of commodities. Banana prices depend on fundamental variables, such as supply and demand, world prices, dollar barter rates, and other variables. These affect farmers who, after ensuring that the bargain amount would be acceptable to offset their production costs, embrace the risks and costs of production. Insufficient market facilities, such as drop-off points, bargain markets, and aggregate marketing facilities, abide by the local or domestic business of bananas. Domestic/local trading is about agitated out by small

traders with minimal aggregate activities that are inefficient and difficult to manage in terms of quality and price.

One important action to alleviate smallholder farmers' abjection and poverty is access to markets (Gyau et al. 2016; Tanielian 2020). Increased market access can drive sustainable product growth and increase food security. However, owners of infant industries and enterprises cannot participate in those markets and charge collective activity to strengthen their negotiating accommodation and abate transaction costs (Tefera et al. 2016; Mango et al. 2017).

Collective market participation also increases farmers' negotiating power as market sellers; according to Mango et al. (2017), they are likely to get better prices for their produce than individuals collectively. Farmers perceive that collective marketing helps them achieve better value, decreases input and output market transaction costs, increases negotiating powers, and improves revenue (Mugwe et al. 2019).

Hence, the Department of Agrarian Reform (DAR), the country's lead agency in the implementation of the comprehensive agrarian reform program, in partnership with the Catholic Relief Services, a non-government organization, piloted the "Linking Smallholder Farmers to Markets (LinkSFarM) Project" in Tulunan, Cotabato in 2016. DAR has introduced the LinkSFarM project to reduce the impact of declining prices on agricultural products and increase the negotiating power of emerging farmers by giving them a voice in cooperation.

However, the market involvement of smallholder farmers is still limited (Mugwe et al. 2019). Therefore, there is a need to look at the factors influencing farmers' participation in the collective marketing of cardava bananas. In addition, Kehinde and Adeyemo (2017) emphasized that the usefulness of technology is not permanent. Therefore, farmers' interest may decline, contributing to technical disappointment and rejection. That requires that farmers' decisions to adopt or innovate be considered.

Theoretical background

The study's main arguments were based on the Social Capital Theory and Transaction Cost Economics Theory. Bourdieu (1985) conceptualizes social capital based on recognizing that capital is not just economic, social exchanges are not solely self-interested, and capital and benefits in all forms need to be included. The Social Capital Theory also underscores the importance of networks and connections in providing access to resources that can be leveraged to identify and exploit entrepreneurial opportunities. In the context of this study, cardava banana farmers, as social beings, are equipped with the emotional prowess to establish and maintain relationships. Therefore, social capital can substantially contribute to the empowerment of small-scale farmers by providing access to information and reducing communication and contracting costs in circumstances where transaction costs are high. Leenders (2014) underscored that people benefit from their own social networks at the individual level as well as from the relations maintained by collectives that they are part of, while organizational groups derive the outcomes from both their own contact with other groups and the relationships maintained by some of their members.

Cardava banana farming is also considered an agri-enterprise where some smallholder farmers depend on their income and sustenance. However, as a business, there are market factors that they need to consider to take advantage of the opportunities to earn more. In cardava banana farming, farmers are engaged in various activities, from land preparation to the delivery of their products to the market, which entails a lot of costs. Hence, this study was also anchored on Ronald Coase's transaction costs economics theory. Coase (1960) explains that it is important to figure out who one wants to meet with to carry out a market agreement, to perform negotiations leading up to a bargain, to draw up a contract, to carry out the necessary checks to ensure that the terms of the contract are respected, and so on. The theory suggests that farmers aim to avoid markets that incur high transaction costs such as fare, travel time, waiting time, and delays in transactions, which should significantly impact the cost of transactions, leading to greater uncertainty and greater cost of transactions (Carlson and Bitsch 2019).

MATERIALS AND METHODS

Research design

A descriptive causal research design was used in this study because it can evaluate the factors influencing smallholder cardava banana farmers' participation in collective marketing in the Southern Philippines. Descriptive research was used because its purpose is to describe and analyze the current situation of individuals, locations, conditions, or events or to explain the features of the sample (Mertler 2014). Data may be collected intuitively in such studies, but it is frequently examined quantitatively, with frequencies, percentages, averages, and other statistical analyses used to discover associations (Nassaji 2015). Causal analysis was used to determine if the socio-economic, market, and institutional factors significantly influence smallholder cardava banana farmers' participation in collective marketing. Causal research, also known as explanatory research, is conducted to identify the extent and nature of cause-and-effect relationships.

Locale and respondents of the study

The study was conducted in Cotabato Province in the Southern Philippines (Figure 1). The province's most extravagant land assets are over 656,590 hectares, representing 36% of the district's territory zone (1,815,500 hectares). It positions topographically first in the four provinces of Region XII. Cotabato Province comprises 17 municipalities and one city, with Kidapawan City as its city, and is divided into three legislative districts (Province of Cotabato 2020).

The study involved three different municipalities of Cotabato province where the LinkSFarM project was implemented. These municipalities include Aleosan, highlighted in yellow, and belong to the first legislative district, Pres. Roxas is in blue and allied with the second legislative district; Tulunan is in red and affiliated with the third legislative district.

Cotabato Province in the Southern Philippines has planted some 14,787.7 hectares of different cultivars of banana, where 34.40% is cultivated for cardava banana (Province of Cotabato 2020). The majority of banana production in the Philippines comes from its northern and southern portions due to its highly suitable and marginally suitable climate conditions. Among the banana cultivars planted in the country, the cardava banana accounts for 28% of the total production (Solpot et al. 2016).

The study's respondents were smallholder cardava banana farmers who are members of the cooperatives from the three municipalities of Cotabato Province, where the collective marketing of cardava bananas was implemented. A total of 172 smallholder cardava banana farmers were selected using the proportionate sampling, where 98 were taken from the members of the Bla'an Klayag Amda De Du Sansato Agrarian Reform Beneficiaries (ARB) Cooperative in Tulunan; 42 from Sto. Niño Farmers ARB Cooperative in Pres. Roxas; and 32 from New Leon Multi-purpose Cooperative in Aleosan. Smallholder Farmer refers to a farmer owning farmland with an area of not more than three hectares.

Sampling procedure

A multi-stage sampling technique was used in selecting the respondents for the study. The first stage used the purposive selection of the three recipient municipalities. Purposive sampling was used to concentrate on people with particular characteristics who will provide unique and rich information of value to the study (Etikan et al. 2016). In the second stage, purposive sampling was also used by selecting one barangay in each municipality where a huge number of cardava banana farmers involved in the LinkSFarm project could be found.

In the third stage, stratified random sampling was used so that the participating farmers in the collective marketing of the LinkSFarm project would have an equal chance of being selected to represent the population properly. Finally, a simple random sampling was used to administer the questionnaire based on the required number of respondents on each site to represent the population. Then, using stratified random sampling, adequate samples could be obtained from all strata in the population. In contrast, simple random sampling is used when the whole population is accessible and the researchers have a list of all the respondents in the target population (Elfil and Negida 2017).

In determining the sample size from the total number of participating farmers, Slovin's formula was used:

$$n = \frac{N}{1 + Ne^2}$$

Where, n = is the sample size, N = is the population size, and e = is the level of precision (for a confidence interval of 95%, equal to 5%). When the formula was applied, the study's sample size was specified at 172.07 and rounded up to 172.

Research instrument

In this study, a researcher-made questionnaire and informal or oral interviews of the respondents were used to elicit primary data related to this study. The factors used in this study were adopted from previous research that also dealt with farmers' market participation and collective marketing. The socio-economic factors used in this study were adopted from the studies of Amao (2017), Kyaw et al. (2018), Melesse (2018), and Mutonyi (2019). The market factors were patterned from the studies of Mbitsemunda and Karangwa (2017), Maponya et al. (2018), and Abate et al. (2019). The institutional factors were in line with the studies of Kyaw et al. (2018), Abate et al. (2019), Mugwe et al. (2019), and Ssajakambwe et al. (2019).

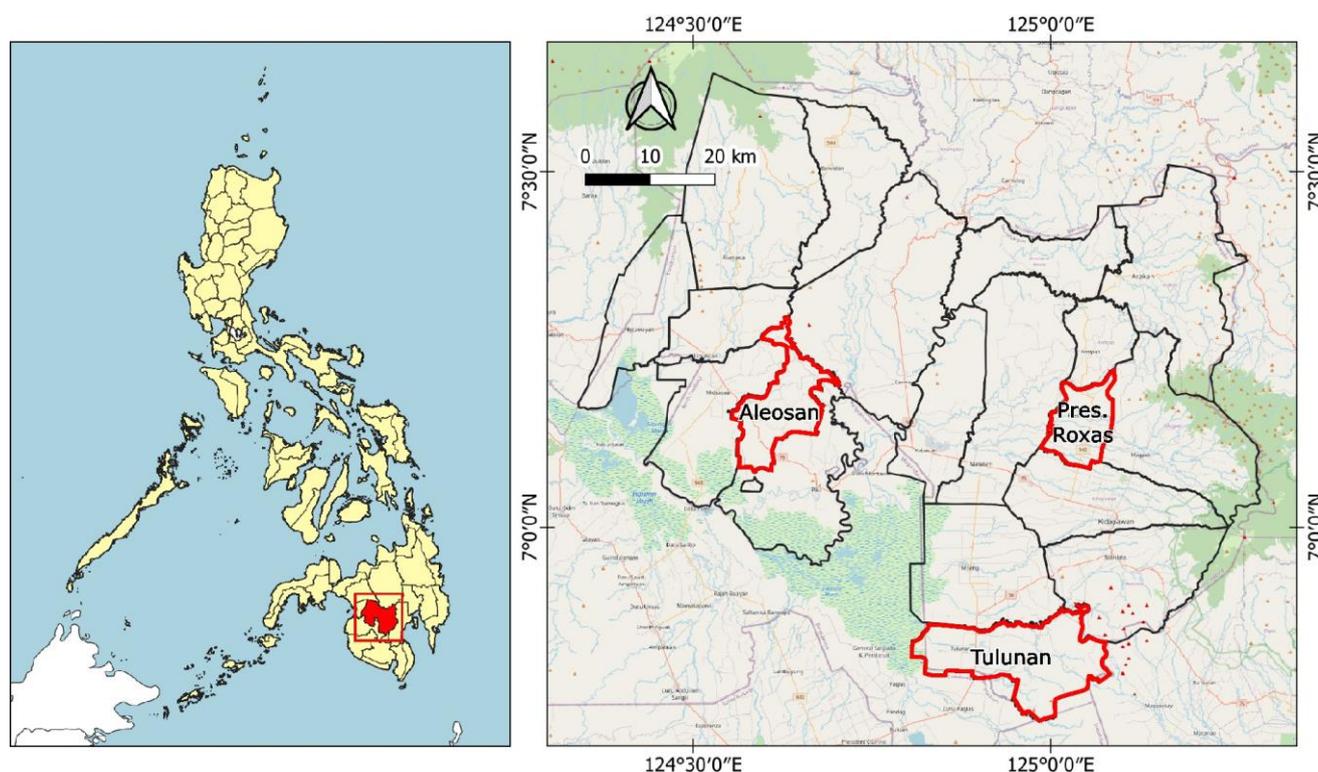


Figure 1. The provincial map of Cotabato in the southern part of the Philippines highlights the three municipalities where the study was conducted

In measurement validation, the questionnaire was pre-tested on 20 farmers to verify the validity of the items. Pre-testing is a method of examining the items, addressing any misunderstanding of the wording of the questions as intended and understood by those individuals who are likely to respond to them (Hilton 2015).

In determining the validity of the questionnaire, Cronbach's alpha was used. Cronbach's alpha is used to establish that tests and scales used in the constructed or adopted research instruments are reliable and fit to be used for their intended purpose (Taber 2018). The base proposed composite unwavering quality worth is 0.80. In addition, a base alpha of 0.803 was received. That implies the items and scales used in this study were reliable.

Research ethics

Before survey administration, a request letter was sent to the implementing agency informing them about the study. Permission was also sought through written consent from all the Barangay Chairmen and the heads of the cooperatives involved. They were also informed of the specific dates for the enumerators to gather the data in the community. Consent of the participants was also sought before proceeding with the interview. All respondents gave their affirmative responses and agreed to participate in the interview. The participants were assured that their answers would be treated with the utmost confidentiality and that the data would be used purposely for the study (Surmiak 2018). The enumerators were also reminded to observe safety and health protocols during the data gathering, such as physical distancing (Nilsen et al. 2020), wearing a face mask (Matuschek et al. 2020), and hand hygiene (Głabska et al. 2020). A face mask is provided for the respondent when they are not wearing one during the face-to-face interview.

Data gathering procedures

In conducting the study, the researcher closely coordinated with the Department of Agrarian Reform as a project implementer. The researcher also sought the assistance of the said agency, especially in field coordination. The researcher also employed eight enumerators with at least a college level of education to assist in data gathering. The enumerators were briefed and oriented to the questionnaire content and the process of interviewing the respondents before proceeding with data gathering from 30 April to 9 May 2021. The respondents were randomly selected and interviewed in their respective barangays or the comfort of their homes. Since the questions were written in English, the enumerator translated the long sentences into the vernacular. The enumerators were urged to examine the questionnaire before leaving the area to ensure that all items were properly filled-out and all information was completely supplied.

Data analysis

The researcher coded the data by assigning a numerical value to facilitate easier workability on the statistical

software. Two types of statistical methods were used to analyze the data. First, descriptive statistical methods such as frequency counts, means, and percentages were used to analyze the respondents' socio-economic characteristics and describe the market and institutional factors in the participation of smallholder cardava banana farmers in collective marketing. Descriptive Statistics is used to give a picture by summarizing the samples and measures included in the study (Sharma, 2019). A four-level Likert scale was used to describe the factors influencing the smallholder cardava banana farmers' participation in collective marketing (Table 1). The extent of market participation (Y) was also interpreted using a four-level Likert scale as an indicator (Table 2).

Second, the multiple regression model was used to analyze whether the socio-economic, market, and institutional factors significantly influence smallholder cardava banana farmers' participation in collective marketing. Burton (2021) opined that researchers could use regression modeling to look at the specific impacts that variables have on one another while also controlling for other factors effects. An Ordinary Least Squares (OLS) linear regression process generates a line of best fit, which is the most accurate way of representing the distribution of data points on a single line.

The OLS model is given by:

$$Y = \beta_0 X_0 + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_n X_n + e \dots$$

Where: Y denotes the extent of market participation, β_0 is a constant, $\beta_1 \dots n$ are parameters to be estimated X_{is} are a vector of explanatory variables. Hypotheses were tested at a 5% level of significance. The statistical analysis for the study was carried out using statistical software.

Table 1. A four-level Likert scale

Mean	Verbal interpretation
3.51-4.00	Strongly agree
2.51-3.50	Agree
1.51-2.50	Disagree
1.00-1.50	Strongly disagree

Table 2. The extent of market participation (Y) was also interpreted using a four-level Likert scale as an indicator

Mean	Verbal interpretation
3.51-4.00	Always
2.51-3.50	Often
1.51-2.50	Sometimes
1.00-1.50	Rarely

RESULTS AND DISCUSSION

Socio-economic characteristics of the smallholder cardava banana farmers engaged in collective marketing

The analysis using the descriptive statistics, as shown in Table 3, on the socio-economic profile of the respondents revealed that collective marketing in the study areas is mostly participated by men. That implies that men still dominate the decision to participate in collective marketing. The result is consistent with Mango et al. (2017) and Yaméogo et al. (2018) that men mostly participate in collective marketing. Furthermore, Mishra et al. (2014) emphasized that family units led by men are bound to obtain innovation. That can reinforce the workforce that is always needed when fostering innovation today. On the contrary, Mugwe et al. (2019) claimed that female-led households are more active in collective market transactions than male-led households. However, a closer look at the data revealed that there was only a slight difference between the number of men and women participants. Hence, the study's results also showed that women were actively engaged in cardava banana farming and collective marketing.

Farmers with higher educational marks are more likely to acquire agricultural ideas and innovations for advancement, thus, easily increasing market access (Kiprop et al. 2020). However, the findings of the study show otherwise. The result revealed that the study's populations possessed lower levels of educational attainment. That implies the participants of collective marketing are less educated, receiving an elementary level of education. It is interesting to note that a large portion of the population was taken from an indigenous community of the B'laan tribe at Bacong, Tulunan, where several respondents only reached the elementary level of education. The result is consistent with Khapayi and Celliers's (2016) findings that many farmers remained less educated. Thus, they are more prone to deception and financial abuse.

Regarding the landholdings planted for cardava bananas, the respondents had an average land size of 1.64 hectares. Almost all respondents have small landholdings, which translates into one hectare or less (89.5%). That implies the participants of collective marketing have easier disposal of their produce on the market, given the minimum production area. The findings support the claim of Nwafor et al. (2020) that minimal land area cultivation entails a manageable yield volume, meaning that respondents do not require multiple market channels for selling their produce.

The average household size of cardava banana farmers involved in collective marketing was 4. The result implies that the participants of collective marketing have strong support systems regarding farming activities. That is consistent with the claim of Oh and Kim (2019), Simtowe and Mausch (2019), and Kiprop et al. (2020) that farming families with a high number of members are more likely to engage in higher production. Thus, the propensity to participate in collective marketing is also high. On the contrary, Kyaw et al. (2018) and Nwafor et al. (2020)

claimed that family size greatly influenced the flow of production and consumption of households. They argue that the higher the number of persons in a family, the higher the consumption level. That entails a smaller market surplus, lowering the likelihood of participation in collective selling.

Table 3. Socio-economic profile of the smallholder cardava banana farmers engaged in collective marketing

Variables	Frequency (n=172)	Percentage
Sex		
Male	99	57.6
Female	73	42.4
Highest educational attainment		
Elementary Level	70	40.7
Elementary Graduate	29	16.9
High School Level	5	2.9
High School Graduate	33	19.2
College Level	19	11.0
College Graduate	16	9.3
Post Graduate		
Farm size		
1 hectare & below	154	89.5
1.1-3.0 hectares	16	9.3
3.1-5.0 hectares	2	1.2
5.1 hectares & above		
Household size		
2 & below	11	6.4
3-4	90	52.3
5-6	51	29.7
7-8	16	9.3
9 & above	4	2.3
Number of years in farming		
1-2	39	22.7
3-4	73	42.4
5-6	42	24.4
7-8	12	7.0
9 & above	6	3.5
Average monthly quantity produced and sold		
100 kg. & below	27	15.7
101-300 kg.	104	60.5
301-500 kg.	32	18.6
501-700 kg.	6	3.5
701-900 kg.	1	0.6
901-1100 kg.	2	1.2
1101 & above		
Average monthly income		
1, 000 & below	42	24.4
1,001-3,000	90	52.3
3,001-5,000	28	16.3
5,001-7,000	8	4.7
7,001-9000	2	1.2
9001 & above	2	1.2

As to farming experience, the results revealed that the participants in collective marketing had an average of 3.9 years of cardava banana farming. That implies many respondents were new to the cardava banana agri-business. It also showed that the respondents' number of years in cardava banana cultivation was almost the same as the number of years that the LinkSFarM project has existed in the study areas. The result is consistent with the findings of Mango et al. (2017) and Mugwe et al. (2019), which stated that farmers with fewer experiences are more likely to participate in collective marketing than those farmers with more experiences because they tend to dwell in the traditional ways that they used to. On the other hand, Abebe et al. (2016) also confirmed that farmers honed by time are more inclined to transact with wholesalers because they can meet the required volume, and dealing with them causes no delays.

Out of 172 respondents included in the study, 60.5% produce and sell an average monthly cardava banana volume, ranging from 101 kg to 300 kg or 256.97 kilograms a month. The result implies that farmer-respondents produced a minimal number of cardava bananas every month. Therefore, smallholder farmers could not directly penetrate the available markets to get good prices for their produce with a limited viable harvest volume. Collective action is one way to address the gap in low production volume to penetrate the market. Institutional buyers prefer transacting with suppliers with great harvest volumes (Abate et al. 2019).

The average monthly income of the respondents from selling cardava bananas is P2,131.71, as depicted in Table 2. That implies farmers still live below the poverty line as far as income from their main crop is concerned. According to Balogbog et al. (2019), most farmers continue to be impoverished, earning less than Php10,000 for every cropping of their main crop. As a result, farmers should make the most of their property by cultivating various crops, particularly cash crops, to supplement their income and boost farm production.

The extent of participation of smallholder cardava banana farmers in collective marketing

The extent of participation of smallholder cardava banana farmers in collective marketing was measured using four factors which were rated as rarely, sometimes, often, and always. The factors include the delivery of products as scheduled, patronization of organizations' services, attendance to meetings, participation in training sessions, and putting up capital build-up.

Table 4 reflects the results of farmers' behavior regarding the extent of participation in collective marketing. The weighted mean score of 3.79 or Always showed that the respondents were highly engaged in the collective marketing of their cardava banana. However, further evaluation of the data collected showed that the average mean score of the first four factors used was consistently rated as "Always," while the factor about putting up capital build-up was only rated as "Often." That implies the members of the organizations, as a strong social

capital, were not actively and consistently contributing to their capital build-up. The cooperative must empower and convince the members to strengthen the pooling of capital through the capital build-up to harness economic opportunities. Previous research findings by Yaméogo et al. (2018) presented that combining funds when farmers in each sector come together will provide informal financial outlets with a mechanism to ease credit limitations. It additionally allows producers to carry out bundled income while keeping off wholesalers and intermediaries.

The study included some socio-economic, market, and institutional factors hypothesized to influence farmers' decision to participate in collective marketing and included in the linear regression model. The pseudo-R² was found to be 0.506, which suggests that the specification fits the model well. It further suggests that the variables included in the model explain a 50% variation in the extent of market participation in collective marketing, signifying the model's goodness of fit.

Influence of socio-economic characteristics on the participation of smallholder cardava banana farmers in collective marketing

Table 5 revealed that using the linear regression analysis, only household size (p-value of 0.043) out of the seven factors assumed to have significant influence emerged to have significantly influenced smallholder cardava banana farmers' participation in collective marketing. However, it also revealed a significant positive influence on smallholder cardava banana farmers' participation in collective marketing, having a t-value of 2.040*. That implies an increase in household members will potentially encourage smallholder farmers' participation in the collective marketing of cardava bananas. The significant positive influence further entails that families with more members engaged in farming will also maximize the potential land area for production, thus, increasing the economic opportunity to gain greater yield and income through collective marketing. The result of the study concurs with the previous work of Oh and Kim (2019), Kiprop et al. (2020), and Nwafor et al. (2020), who presented that the number of family members significantly impacts farmers' deliberate choices in selecting market options. They further stressed that the number of individuals in every family has tremendous encouragement in deciding to join the collective action initiatives.

Table 4. The extent of participation of smallholder cardava banana farmers engaged in collective marketing

Extent of participation	Frequency (n=172)	Percentage	Weighted mean
Always	136	79.1	3.79
Often	36	20.9	-
Sometimes	-	-	-
Rarely	-	-	-

Note: 1.0-1.50: Rarely, 1.51-2.50: Sometimes, 2.51-3.50: Often, 3.51-4.0: Always

Table 5. Influence of socio-economic characteristics on the participation of smallholder cardava banana farmers engaged in collective marketing

Model	Standardized coefficients	t	Sig.
(Constant)		-1.062	0.290
Sex	-0.036	-0.562	0.575
Education	-0.027	-0.389	0.698
Farm size	0.081	1.216	0.226
Household size	0.123	2.041*	0.043
Years in farming	-0.088	-1.350	0.179
Average monthly produce & sold	0.000	0.000	1.000
Average monthly income	0.023	0.325	0.745

Note: ***, **, * means significant at $P \leq 0.001$, $P \leq 0.01$, $P \leq 0.05$, respectively. a. Dependent Variable: Extent of Participation. b. Predictors: (Constant) sex, highest educational attainment, household size, farm size, years in farming, average monthly quantity produced and sold, and average monthly income. c. $R = 0.711^a$, $R^2 = 0.506$, Adjusted R Square = 0.448, Std. Error of the Estimate = 0.21425

Table 6. Influence of market factors on the participation of smallholder cardava banana farmers engaged in collective marketing

Model	Standardized coefficients beta	t	Sig.
(Constant)		-1.062	0.290
Price	-0.557	-3.903***	0.000
Payment Scheme	-0.231	-2.410*	0.017
Delivery Schedule	0.644	6.195***	0.000
Distance to the Market	0.353	3.605***	0.000
Transportation Cost	-0.092	-0.982	0.328

Note: ***, **, * means significant at $P \leq 0.001$, $P \leq 0.01$, $P \leq 0.05$, respectively. a. Dependent Variable: Extent of Participation. b. Predictors: (Constant) price, payment scheme, delivery schedule, distance to the market, and transportation cost. c. $R = 0.711^a$, $R^2 = 0.506$, Adjusted R Square = 0.448, Std. Error of the Estimate = 0.21425

Kiprop et al. (2020) further expounded that the more family members participate in farming activities, the higher the chances of partaking in collective marketing. Hence, when there is high available labor in the family, the probability of producing a greater volume of the agricultural crop is also high. Therefore, the high production volume will also increase the likelihood of contributing to group selling.

On the contrary, the result of the study is inconsistent with the studies of Kyaw et al. (2018), which noted that households with a greater number of members are more likely to hold back from participating in group marketing or selling their produce. They further argue that the bigger the size of the family, the higher the degree of setting some volume of yield for the family's consumption, which can result in a very low surplus.

On the other hand, socio-economic factors such as sex, highest educational attainment, farm size, number of years in farming, average quantity produced and sold, and

average monthly income were found to be statistically insignificant and do not necessarily influence the participation of smallholder cardava banana farmers' participation in collective marketing.

Influence of market factors on the participation of smallholder cardava banana farmers in collective marketing

As to the influence of market factors, linear regression analysis, as shown in Table 6, revealed that four out of five market factors were found to have a significant influence on smallholder cardava banana farmers' participation in collective marketing. These include price, payment scheme, delivery schedule, and distance to the market.

Table 6 indicates that the coefficient of price has shown a significant influence on the collective marketing participation of smallholder cardava banana farmers at a p-value of 0.000. The results also showed a significant negative influence, having a t-value of 3.903 ***. That implies the lower the price offered for farmers' produce, the probability of participation in collective marketing will also be reduced. That is because farmers are persistently and consistently looking for a higher price and gain to defray expenses for transaction costs from making the trade. Higher prices will provide sufficient revenue for farmers to finance their farming activities and will eventually encourage them to produce more. Therefore, cooperatives need to find institutional buyers that offer higher prices to sustain the participation of smallholder farmers in collective marketing. Yaméogo et al. (2018) discussed that farmers would only be interested in producing more and selling it through collective marketing if they can get a good offer for their harvest. The findings of Anh and Bokelmann (2019), Ssajakambwe et al. (2019), and Nwafor et al. (2020) noted that price is a strong factor that would influence farmers' decision in choosing a market channel for their agri-produce. Hence, market options offering higher prices for their commodities are vital to influencing the volume sold and encouraging smallholder farmers' participation in group marketing (Khapayi and Celliers 2016).

The payment scheme was also found to have a significant negative influence with a p-value of 0.017 and a t-value of 2.410*, which implies that the delays in receiving the expected profit can discourage farmers from selling their products through collective marketing. Farmers' daily subsistence and sustenance depend mainly on the revenue of their produce. Therefore, payment delays will affect their future decisions about whether to continue or withdraw their participation in collective marketing detrimentally. Previous research findings by Nandi et al. (2017), Poku et al. (2018), and Ssajakambwe et al. (2019) also found that payment mechanism has a negative influence on smallholder farmers' decision to participate in collective marketing. The result of the study also coincided with the findings of Poku et al. (2018); and Nwafor et al. (2020), which emphasized the importance of direct payment and concluded that direct payment schemes must be strengthened by farmers' organizations engaged in collective marketing.

The delivery schedule was found to positively influence smallholder cardava banana farmers' participation in collective marketing, with a p-value of 0.000 and a t-value of 7.117***. The results only showed that smallholder farmers' participation in collective marketing is directly associated with the delivery schedule. The result implies that when the delivery time and schedule are strictly followed, the participation of smallholder farmers in collective marketing also increases. Buyers must show up on the agreed time and schedule to avoid wasting farmers' time waiting, which will cause dissatisfaction and discouragement. Anh and Bokelmann (2019) noted that setting a delivery date and time is necessary, especially when there is an understanding between a buyer and a seller. Scheduling the collection and distribution of perishable products must be thoroughly considered. Proper coordination must be taken into consideration before the delivery schedule.

Distance to the market also positively influences smallholder cardava banana farmers' participation in collective marketing, having a p-value of 0.000 and a t-value of 3.605***. The result implies that the nearer the farm is to the market or consolidation center, the higher the chance of farmers' participation in collective marketing. The significant positive influence was attributed to the fact that farmers' produce was picked up at a certain collection point near their farm gates, or at least at a lesser distance to the consolidation center, which would not incur high transportation costs. Melesse (2018) and Simtowe and Mausch (2019) affirmed that the market proximity of farmers' farms plays a critical role in reducing transportation costs and accessing market information from various sources. Previous research findings of Melesse (2018), Yaméogo et al. (2018), Simtowe and Mausch (2019), and Ssajakambwe et al. (2019) underscored that the distance absolutely defines farmers' visibility and actual participation in the market to the most accessible market where they could sell their produce.

Influence of institutional factors on the participation of smallholder cardava banana farmers in collective marketing

As presented in Table 7, five out of six variables used in the study were found to have a significant influence on the participation of smallholder cardava banana farmers in collective marketing. The result suggests that five out of six model parameters were strongly significant in elucidating the dependent variable.

The results indicated that explanatory variables such as access to extension services, access to market information, and membership in farmers' organizations have positively and significantly influenced the participation of smallholder cardava banana farmers in collective marketing. While access to production inputs and access to credit assistance had a significant negative influence on the participation of smallholder cardava banana farmers in collective marketing.

The study also revealed that membership in farmers' organizations significantly positively influences

The study's results revealed that access to extension services positively influences smallholder cardava banana farmers' participation in collective marketing with a p-value of 0.000 and a t-value of 5.675***. The result indicates that the higher the access of smallholder cardava banana farmers to extension services, the higher the probability of market participation in collective marketing. Study results also showed that farmer respondents have access to agricultural skills and technology training, technical coaching, and mentoring services since they have direct access to Agricultural Extension Workers (AEWs) in their respective areas. The result of the study is consistent with the previous findings of Kyaw et al. (2018), Mugwe et al. (2019), and Ssajakambwe et al. (2019), which concluded the impact of extension services offered by extension workers could enhance farmers' understanding of the technologies and various market opportunities that could encourage farmers' participation in collective marketing.

Access to market information was also found to positively influence smallholder cardava banana farmers' participation in collective marketing, with a p-value of 0.041 and a t-value of 2.059*. The result implies that the more farmers have access to market information, the more they participate in collective marketing. The study also confirmed that smallholder cardava banana farmers have access to market information such as price information, the delivery time and schedule, and information on the demand and supply of the cardava banana industry that helped their decision-making in joining the collective marketing. The result of the study was consistent with the findings of Kyaw et al. 2018, Ssajakambwe et al. (2019), and Muzemil (2020); they found that market data and information have a significant positive influence on encouraging farmers to participate in collective marketing.

Table 7. Influence of institutional factors on the participation of smallholder cardava banana farmers engaged in collective marketing.

Model	Standardized coefficients beta	T	Sig.
(Constant)		-1.062	0.290
Access to extension services	0.654	5.675***	0.000
Access to production inputs	-0.289	-2.926**	0.004
Access to credit assistance	-0.137	-2.023*	0.045
Access to icts	0.132	1.542	0.125
Access to market information	0.287	2.059*	0.041
Membership in farmers' organization	0.171	2.614**	0.010

Note: ***, **, * means significant at $P < 0.001$, $P < 0.01$, $P < 0.05$, respectively. a. Dependent Variable: Extent of Participation. b. Predictors: (Constant) price, payment scheme, delivery schedule, distance to the market, and transportation cost. c. $R = 0.711^a$, $R^2 = 0.506$, Adjusted R Square = 0.448, Std. Error of the Estimate = 0.21425

smallholder cardava banana farmers' market participation in collective marketing, with a p-value of 0.010 and a t-

value of 2.614**. The result implies a farmer is a member of a group, an association, or a cooperative, the probability of participation in collective marketing is higher than those who do not belong to a group. That is true because farmers are social beings with social networks that have access to various sources and resources that they could use to advance their trade. The result of the study is supported by the findings of Mango et al. (2017), Maspaitella et al. (2017), Kyaw et al. (2018), and Nwafor et al. (2020), which presented that having an alliance with farmers' organizations has a significant positive effect on the optimal selection of market channels among smallholder farmers.

Access to production inputs was found to have a significant negative influence on smallholder cardava banana farmers' participation in collective marketing, having a p-value of 0.004 and a t-value of 2.926**. That implies that smallholder farmers who do not have enough access to production inputs are less likely to participate in collective marketing. It implies further that in the absence of production inputs, smallholder farmers will have difficulty producing more yield, restricting them from participating in the market. The result of the study is in line with the findings of Simtowe and Mausch (2019), who believed that expensive agri-inputs and lack or late provision of production supplies had an insurmountable impact on the rejection of smallholder farmers to any agricultural innovations.

Access to credit assistance was also found to have a significant negative influence on smallholder cardava banana farmers' participation in collective marketing, having a p-value of 0.045 and a t-value of -2.023*. That implies a lack of insufficient opportunity to avail the credit schemes can lead to a higher likelihood of farmers not participating in collective marketing because agricultural cultivation depends greatly on input availability. When accessing agri-inputs is not feasible for smallholder farmers due to lack or limited financing, farmers will have difficulty producing marketable surpluses for a certain market. Sanou et al. (2017) emphasized the importance of credit accessibility for smallholder farmers who frequently face financial constraints.

In conclusion, collective marketing in Southern Philippines, specifically in Cotabato province, is always participated in and patronized by smallholder cardava banana farmers where household size showed the only triggering socio-economic factors that influenced their participation. That implies the number of household members who also serve as the workforce in cardava banana farming plays a crucial role in joining the collective marketing. The more family members are involved in cardava banana farming, the higher the chances of participation in collective marketing. Other socio-economic factors used in the study do not necessarily influence the participation of smallholder cardava banana farmers in collective marketing. Transportation costs and access to information and communication technologies were no longer issues in the areas where collective marketing was implemented. These imply that the smallholder cardava banana farmers are no longer spending significant amounts

on fares for transporting their produce owing to a strategic drop-off point established near their farm gates. The availability of functional ICT infrastructures was also accessible in the project areas. Other market and institutional factors greatly influenced smallholders' participation in collective marketing. Hence, the findings suggest the need for the government to provide strategic interventions to the factors that significantly influence the participation of smallholder cardava banana farmers in collective marketing. Policymakers consider enacting policies to strengthen the roles of farmers' organizations in rural development, the provision of extension services in areas where collective marketing is being implemented, and the improvement of infrastructures and development modalities for faster diffusion of information, particularly in the marketing aspect. In light of opportunities for future research, it is also recommended to explore the factors used in a larger group of respondents since the study was only limited to 172 smallholder cardava banana farmers. Qualitative research on collective marketing is also recommended.

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In-vitro efficacy of *Trichoderma* isolates on *Sclerotium rolfsii* causing collar rot of chili

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Abstract. Yadav D, Adhikari A, Dhuingana B, Gurung H, Khatri N, Pandit S. 2022. In-vitro efficacy of *Trichoderma* isolates on *Sclerotium rolfsii* causing collar rot of chili. *Asian J Agric* 6: 97-102. The experiment was conducted in the Nepal polytechnic institute plant pathology laboratory to study the in-vitro efficacy of *Trichoderma* isolates on *Sclerotium rolfsii* Sacc. collar of chili, Bharatpur, Chitwan, Nepal by dual culture technique. The experiment was carried out in a completely randomized design (CRD) with four replications. The *Trichoderma* isolates, namely Kapilvastu isolate, Kavre isolates, Salyan isolates, Lalitpur isolates, and Taplejung isolates, were used in the experiment. The mycelium growth was measured at 2 DAI, 4 DAI, 6 DAI, 8 DAI, and 10 DAI. Also, the number of sclerotia, days to sclerotia, and width of the browning area at the interception region of interception were measured in 10 DAI. All the *Trichoderma* isolates significantly affect mycelium growth and the number of sclerotia formed. Among all the *Trichoderma* isolates, Kavre isolates show a good result with (74.44%) followed by Salyan isolates (74.22%) and Lalitpur isolates (73.55%) inhibition in the mycelium growth and several sclerotia (9.6~10) also formed. The lowest number of sclerotia was observed in Salyan isolates, which was three days, followed by Kapilvastu isolate, i.e., 20 days. The antagonist Kavre isolate can be used as a bio-control agent against *S. rolfsii* of chili in Nepal.

Keywords: Sclerotia, *Sclerotium rolfsii*, *Trichoderma* isolates

INTRODUCTION

Chili (*Capsicum annum* L.) is mainly grown in almost all planes throughout the country of Nepal, whereas the green pepper is grown relatively at little high elevations, also where the climate is relatively mild. Chili belongs to the family Solanaceae, locally known as Khursani. Chili is a critical vegetable commodity with future business opportunities, containing excessive calories, protein, fat, carbohydrates, calcium, and vitamins A, B1, and C (Piay et al. 2010; Yanti et al. 2016). The chilies are used in green as well as dry in powdered form. It's a wealthy supply of vitamins A and C, among most vegetables, for spices, pickles, and sauces. In Nepal, the green chili production was 120,462 tons under a 12,134-ha cultivated area yielding 9,927 kg/ha (FAO 2019). The dry production of chili was 67,167 tons under a 10,690-ha cultivated area yielding 6,283 kg/ha (FAO 2019). *Sclerotium rolfsii* Sacc. is an important disease that has spread globally and causes diseases in many economically important crops (Khan et al. 2020; Jabeen et al. 2021). The soil-borne fungus is a subgroup of Deuteromycotina, which contains more than 500 species of cultivated and wild plants in tropical and subtropical areas (Javid et al. 2022). Many fungal, bacterial, and viral diseases affect the crop, resulting in huge economic losses. Among fungal infections, *S. rolfsii*, responsible for southern blight in chilies, is the most common (Javid et al. 2020; Sharf et al. 2021). The *S. rolfsii* causing collar rot in chili may occur in any plant

growth stage (Haque et al. 2001). Allowing the leaves and the growth of dark brown sores in the collar area along the soil line are symptoms of the disease, which leads to the complete drying of the plant (Mahadevakumar et al. 2018). The rot-affected plant has a fungal infection in the column region, just above the soil level, in the form of a griddle. With white mycelium, the grilling rises to the top. Wilting begins within 3-5 days, and when the green canopy is removed, the entire plant will dry out (Daunde et al. 2018).

The *S. rolfsii*, the polyphagous fungus, has a host range of more than 500 species in about 100 families, including nuts, green beans, lima beans, onions, garden beans, peppers, potatoes, sweet potatoes, tomatoes, and tomatoes watermelons worldwide, causing great loss (Aycocock 1966). The *S. rolfsii* grows well in highly active areas and thus thrives well near the underground. Natural conditions conducive to fungus and disease growth are high temperatures (27 to 35°C), humid conditions, and acidic soils (Mullen 2000). Initially, this fungal infection could cause a decrease of up to 53.4% in yield quality and some pepper plants (Fery and Dukes 2011). Most of the diseases, collar rot or pepper rot, due to *S. rolfsii* occur in almost all pepper growing regions. It reasons a decrease in yield to 16-80% (Daunde et al. 2018). The pathogen is found to cause various symptoms like crown rot, root rot, stem rot, rotting of pseudo bulbs, wilt, and the gradual death of the plants, etc. (Agrios 2005). The pathogen is very common in tropical, subtropical, and warm temperate regions. Collar rot is prominent in one-month-old seedlings. Under field

conditions, the pathogen has been reported to cause a 30 to 60% reduction in the yield of chickpeas. Because of the prolific growth of and ability to produce persistent sclerotia, it is contributing to a high degree of economic losses. Under conducive conditions, it can cause 55-95% mortality of the crop at the seedling stage (Gurha and Dubey 1982). Since chemicals are hazardous to soil and users, biological control agents are the best alternative to toxic chemicals. Among the biocontrol agents, *Trichoderma* spp. was the most effective against many soil-borne pathogens (Eziashi et al. 2006). Dennis and Webster (1971) described the antagonistic properties of *Trichoderma* in antibiotic production and hyphal interactions. The species of *Trichoderma* are capable of hyper-parasitizing pathogenic fungi and are highly efficient antagonists (Iqbal and Mukhtar 2020). Sanchez et al. (2006) reported that *Trichoderma* species could inhibit the growth of plant pathogens, especially fungi, through competition for nutrients, enzymes, substrate, oxygen, and space.

Trichoderma is a fungus found in all soils and is the most commonly occurring fungus. Many species of this genus can be identified as opportunistic virulent plant symbionts (Harman et al. 2004). That refers to the capability of several *Trichoderma* species to form harmonious endophytic relationships with numerous plant species (Bae et al. 2011). *Trichoderma* spp. is known as mycoparasites of some plant pathogens. For example, the *T. harzianum* Rifai colonizes *S. rolfisii* hyphae, disrupts mycelial growth, and kills the organism (Ali et al. 2020). *Trichoderma* can potentially suppress the boom of pathogenic fungi (Jegathambigai et al. 2010; Khan and Javaid 2020; Khan et al. 2021). It has also been reported that *Trichoderma* species with different mechanisms, such as lysis of sclerotia, inhibit the growth of mycelial *S. rolfisii* with flexible metabolites that produce and parasites hyphal styles of disease agent (Shaigan et al. 2008). The genome of many *Trichoderma* spp. edited and publicly available at the Joint Genome Institute (Mycocosm 2021). Biological control of plant diseases has been the subject of extensive research in the last two decades. *Trichoderma* spp. is well-documented as an effective biological control agent for plant diseases (Sain and Pandey 2016). Therefore, the present research was carried out to evaluate the native *Trichoderma* spp. against *S. rolfisii*, causing collar rot of chili.

MATERIALS AND METHODS

Sample collection

Diseases of plants infected with *S. rolfisii* of chili wilt were identified in the field by specific symptoms, i.e., the development of browning discoloration in the root surface of chili. First, the pure culture of *S. rolfisii* was acquired from the AFU Rampur, Chitwan, Nepal, and Laboratory.

Then the samples were preserved at 4°C centigrade in the refrigerator for isolation of *S. rolfisii*.

Media preparation

Potato dextrose agar media was prepared with the composition of 200 g peeled potato agar, 20 g of dextrose, and 20 g for one liter of the final volume of water. The media was autoclaved at 121°C and 15 psi for 15 to 20 minutes and allowed to cool to bring around 50 to 60°C at room temperature before pouring sterilizing the glass wares under the hot air oven.

Prepared PDA powder was also used with the composition of 42 g for one liter of the final volume of water. The media was sterilized in an autoclave at 15 psi (125°C) for 15-20 minutes. The sterilized media was allowed to cool at 50 to 60°C, pouring the sterilized media into the Petridis.

Collection and isolation of the pathogen

Diseases plants infected with *S. rolfisii* of chili wilt were identified in the field by specific symptoms. Pure culture of *S. rolfisii* was acquired from the AFU Rampur, Chitwan, and Laboratory. Then the samples were preserved at 4°C centigrade in the refrigerator for isolation of *S. rolfisii*. Potato Dextrose Agar media was prepared and autoclaved at 121°C and 15 psi for 15 to 20 minutes and allowed to cool to bring around 50 to 60°C to room temperature before pouring sterilized the glass wares under the hot air oven. The pure culture of the segregated areas was repaired following hyphal tip methods and later transferred to a new PDA site.

Dual culture technique

Culture discs (5 mm) of *Trichoderma* and the pathogen taken from the edges of the cultures grow vigorously and are evenly spaced approximately seven inches from 9 mm Petri plates containing a 20 mL PDA. With each treatment, a minimum of four responses were maintained, and controls were maintained by placing a pathogen disc only in the center (Singh et al. 2019). Petri plates are then heated to 24 ± 2°C. The pathogen's growth and *Trichoderma*'s ability to prevent the pathogen were documented from time to time. The reduction of the growth percentage (Pi) of the experimental pathogen is calculated when the growth of *S. rolfisii* is filled with control plates using the following formula (Vincent 1947).

$$Pi = \frac{C-T}{C} \times 100$$

Where

Pi : Percent growth reduction of test pathogen

C : Radial growth of test pathogen in control (mm)

T : Radial growth of test pathogen in treatment (mm)

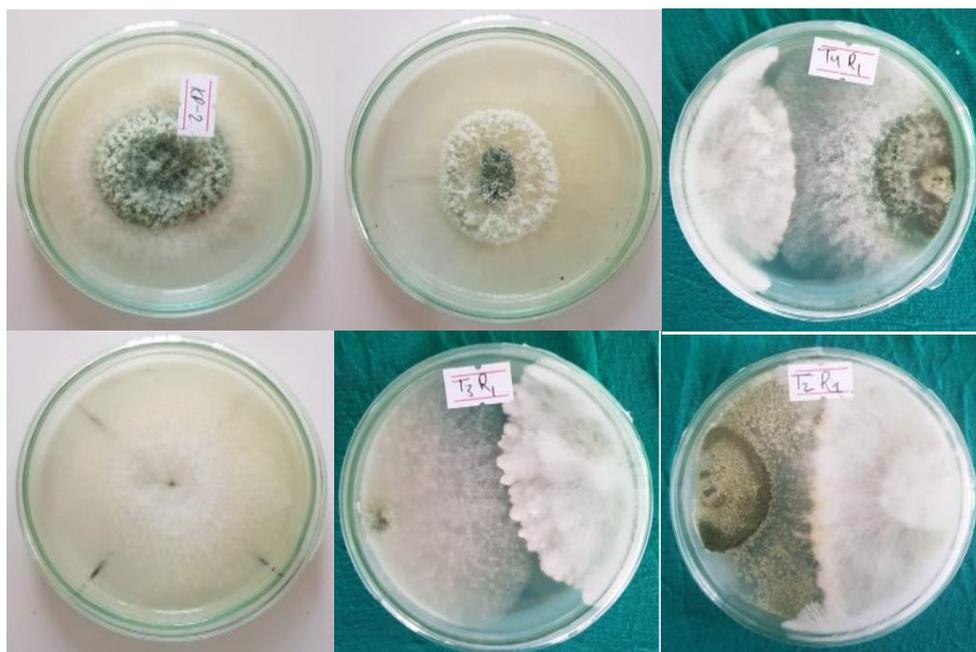


Figure 1. *Trichoderma* isolates and species selected for study

Statistical analysis

All recorded information was processed into the R-studio, and analysis was performed using R 4.0.4 (R Core Team (2013) and the Agrícola 1.1-8 version package (De Mendiburu (2014)). The data entry was done to develop an ANOVA table, and different treatments were compared by Duncan's multiple range test and the least significant difference at a 5% significance level. All the figures and graphs were prepared using Microsoft excel 2013.

RESULTS AND DISCUSSION

The radial mycelium growth of *S. rolfii* for all treatments varied significantly at 2, 4, 6, 8, and 10 DAI ($P < 0.001$) (Figure 1). The highest inhibition percentage of mycelial growth was found in Kavre isolate, i.e., 52.41, 69.11, 69.77, 72.00, 74.44 after 2, 4, 6, 8, and 10 days of incubation, respectively. Whereas Salyan isolates, Taplejung isolates, Kavilvastu isolates, and Lalitpur isolates were found at par (Table 1).

This result is supported by Elad et al. (1984), and Bastakoti et al. (2017) stated *Trichoderma* species could produce extracellular lytic enzymes responsible for their antagonistic activity. They also reported a similar positive effect on *Trichoderma* species. Therefore, it can be assumed that *T. harzianum* attacks the pathogen's mycelium by penetrating its cell wall in a certain location (Chet et al. 1981). In field condition, *Trichoderma viride*

Pers. reduce disease incidence by 75.54% (Jegathambigai et al. 2010). It is supported by Pandey and Gaire (2019), Rampur rice field isolate (0.42) and Tarhara, Sunsari isolates (0.55) were found to be better performance against *S. rolfii* under the width of the browning region at the point of interaction. The present findings corroborate the results of Rasu et al. (2013). They screened different *Trichoderma* spp. against *S. rolfii*, among all *T. asperellum* (TTH1) exhibited 64.40% of mycelial growth inhibition. Further, Darvin et al. (2013) recorded 56.25% of mycelial growth inhibition of *S. rolfii* through *T. harzianum*.

The days of sclerotia formed in the dual culture of *S. rolfii* for all treatments varied significantly at 10 DAI ($P < 0.001$) (Table 2). Lalitpur isolate takes a long period to form sclerotia, i.e., 9.6~10 days statistically at par with Salyan isolate (9.6~10 days), Kavre isolates (8.8~9 days), Kapilvastu isolates (8.8~9 days), and Taplejung isolate (8.8~9 days). In contrast, early sclerotia formation was observed in control with 5.8~6 days. *Trichoderma* isolates killed 62-100% of the sclerotia within 25 days of inoculation (Dos and Dhingra 2011). This result is supported by Ordóñez et al. (2015); the process of forming sclerotia ends on the 9th day when they were easy to detach from the culture medium and had a black coloration. Mycelium was hyaline, thin-walled, sparsely septate hyphae. Several others showed similar morphological characteristics of the pathogen's sclerotia (Singh and Thapliyal 1998; Swart et al. 2003; Jeeva et al. 2005).

Table 1. Inhibition percentage of *Sclerotium rolfii* against *Trichoderma* isolates

Treatment (<i>Trichoderma</i> isolates)	Inhibition percentage (%)				
	2DAI	4DAI	6DAI	8DAI	10DAI
Taplejung isolate	50.9 ^a (45.50)	65.33 ^a (53.94)	67.55 ^a (55.29)	69.33 ^a (56.38)	70.44 ^a (57.08)
Salyan isolate	51.25 ^a (45.69)	69.11 ^a (56.25)	69.55 ^a (56.54)	71.78 ^a (57.95)	74.22 ^a (59.51)
Lalitpur isolate	50.52 ^a (45.28)	67.78 ^a (55.40)	68.66 ^a (55.95)	71.78 ^a (57.92)	73.55 ^a (59.08)
Kavre isolate	52.41 ^a (46.36)	69.11 ^a (56.24)	69.77 ^a (56.67)	72 ^a (58.09)	74.44 ^a (59.67)
Kapilvastu isolate	50.89 ^a (45.49)	68.22 ^a (55.66)	68.66 ^a (55.94)	70.44 ^a (57.04)	71.55 ^a (57.75)
Control (<i>Sclerotium rolfii</i> Sacc.)	0 ^b (2.56)	0 ^b (2.56)	0 ^b (2.56)	0 ^b (2.56)	0 ^b (2.56)
Mean	42.69(38.48)	56.62(46.68)	57.40(47.16)	59.25(48.32)	60.74(49.27)
CV	3.15	4.15	4.35	4.51	4.25
LSD	1.58***	2.53***	2.68***	2.85***	2.73***
SEm(±)	0.77	1.22	1.29	1.38	1.32

Note: CV: coefficient of variation, LSD: Least significant difference, SEm: Standard error of the mean. Figures denoted by the same letter do not differ significantly

Table 2. Days to sclerotia formed in dual culture

Treatment (<i>Trichoderma</i> isolates)	Days to sclerotia formation
Taplejung isolate	(8.8 ^a)
Salyan isolate	(9.6 ^a)
Lalitpur isolate	(9.6 ^a)
Kavre isolate	(8.8 ^a)
Kapilvastu isolate	(8.8 ^a)
Control (<i>Sclerotium rolfii</i> Sacc.)	(5.8 ^b)
Mean	8.57
CV	11.07
LSD	1.24***
SEm(±)	0.6

Note: CV: coefficient of variation, LSD: Least significant difference, SEm: Standard error of the mean. Figures denoted by the same letter do not differ significantly

Table 3. Number of sclerotia formed in dual culture

Treatment (<i>Trichoderma</i> isolates)	No. of sclerotia
Taplejung isolate	24.8 ^c (5.01)
Salyan isolate	2.8 ^d (1.80)
Lalitpur isolate	62 ^b (7.84)
Kavre isolate	27 ^c (5.26)
Kapilvastu isolate	20 ^c (4.56)
Control (<i>Sclerotium rolfii</i> Sacc.)	501 ^a (22.38)
Mean	106.43(7.81)
CV	8.78
LSD	0.89***
SEm(±)	0.43

Note: CV: coefficient of variation, LSD: Least significant difference, SEm: Standard error of the mean. Figures denoted by the same letter do not differ significantly

The number of sclerotia formed in the dual culture of *S. rolfii* for all treatments varied significantly at 10 DAI ($P \leq 0.001$)(Table 3). The lowest number of sclerotia was observed in the Salyan isolate (2.8~3), followed by the Kapilvastu isolate (20), statistically at par with the Taplejung isolate (24.8~25) and Kavre isolates (27), followed by Lalitpur isolate (62) whereas the maximum number of sclerotia observed in control (501). The ability of *T. viride* to control *S. rolfii* infection has been attributed to the ability of *Trichoderma* to parasitize the sclerotia (Jegathambigai et al. 2010).

Table 4. Width of the browning area at the region of interception in dual culture

<i>Trichoderma</i> isolates	Width of the browning area at the region of interception in dual culture (<i>Sclerotium rolfii</i>)
Kapilvastu isolates	0.78 ^c
Kavre isolates	0.96 ^b
Lalitpur isolates	1.14 ^a
Salyan isolates	0.56 ^d
Taplejung isolates	1.06 ^{ab}
Mean	0.9
CV	13.52
LSD	0.16***

Note: CV: coefficient of variation, LSD: Least significant difference, SEm: Standard error of the mean. Figures denoted by the same letter do not differ significantly

Decreased concentrations were less inhibitory to the growth of *S. rolfii*. Kapil and Kapoor (2005) tested the volatile and non-volatile metabolites of bioagents viz. *T. harzianum*, *T. viride*, and *T. atroviride* significantly reduced the mycelial growth and germination of *Sclerotinia sclerotiorum*. This result is supported by Mishra et al. (2011) reported the efficacy of culture filtrate of *T. viride* Tr 8 against *M. phaseolina* and other soil-borne pathogens under in vitro conditions that also suppressed the growth of test pathogen.

The number of sclerotia formed in the dual culture of *S. rolfii* for all treatments varied significantly at 10 DAI ($P \leq 0.001$)(Table 4). The maximum width of the browning area at the interception region in dual culture was observed in the Lalitpur isolate (1.14 cm). Followed by Lalitpur isolate (1.14 cm), Kavre isolates (0.96 cm), Kapilvastu isolates (0.78 cm), and the minimum width of the browning area at the region of interception in dual culture was observed in Salyan isolate (0.56 cm). Rampur rice field isolate (0.42 cm) and Tarhara, Sunsari isolate (0.55 cm) were found to be better performing against *S. rolfii* under the width of the browning region at the point of interaction, Supported by (Pandey and Gaire 2019). A similar result was shown by Pandey and Devkota (2020) as they stated

that the average growth of mycelium of *S. rolfsii* on PDA plates treated with liquid culture filtrate (LCF) of *Trichoderma* species was maximum. The width of the area was 0.775 cm. (Darvin et al. 2013) reported that *T. viride* isolate completely inhibited the mycelial growth of *S. rolfsii* through poisoned food technique.

In conclusion, *S. rolfsii*, the polyphagous fungus, has a wide host range of vegetables. Therefore, it is an important pathogen to be controlled by integrated pest management. Using botanicals and bio-agents provides an alternative to synthetic pesticides with the advantage of minimizing the cost of cultivation and avoiding health hazards. *Trichoderma* is an eco-friendly fungus, nonhazardous to soil and human beings. From the in vitro findings, it can be suggested that among all the *Trichoderma* isolates, Kavre isolates, on average, shows a good result in controlling this. Therefore, it is recommended that the biological agent Kavre isolate be suggested to the farmers to manage the collar rot of chili.

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Saline irrigation management in field conditions of a semi-arid area in Tunisia

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Abstract. *Daghari I, Daghari H, Ben Khalifa N., Mahmoud M. 2022. Saline irrigation management in field conditions of a semi-arid area in Tunisia. Asian J Agric 6: 103-108.* Tunisian saline soils occupy about 25% of the total irrigated area. The irrigated area of "Diyar-Al-Hujjej" in Tunisia was considered because seawater intrusion took place and salinization of the aquifer reached a very high water electrical conductivity value of 15 dS/m in the 90s, which caused many local farmers to abandon land parcels and wells. In addition, salinity has reduced plant growth and water quality, leading to lower crop yields and degradation of stock water reserves. Excess salt affects overall soil health, reducing productivity. It kills plants, leaving bare soil prone to erosion. In this study, the electrical conductivity of the soil under several irrigated crops was evaluated for two soil layers (0-20 cm and 20-40 cm). Follow-up surveys of agricultural practices have shown that the irrigation water's quality has a considerable effect on the soil's electrical conductivity and, therefore, on the choice of crops. We also showed that crop rotation is a practice done by farmers to maintain and improve soil fertility by utilizing soil nutrient losses due to salinization. This study can be the basis of a valuation to allow farmers to have the best cultural practices to fight the salinization of their soils.

Keywords: Farmers practices, irrigation, seawater intrusion, semi-arid, soil salinization

INTRODUCTION

Several recent publications on the Mediterranean show that agriculture in the Maghreb will be affected by the impacts of climate change (Chebil et al. 2022) mainly through the depletion of available water (Chebil et al. 2019) and degradation of local ecosystems (Ferjani et al. 2013) including soil salinization. According to the FAO, 7.3 million hectares of salinized irrigated area are located in Morocco, Tunisia, Spain, and Turkey, for a total of 27.3 million hectares for the entire Mediterranean region (Yacoubi et al. 2020). Within the irrigated area of Skhirat in Morocco, due to the use of high, very saline groundwater, an increase in soil salinity was observed, affecting yields, so farmers are left with the only choice of growing salt-tolerant crops (El Hamdi et al. 2022). In Algeria, in the fertile oasis of Biskra, an increase in soil salinity has led to the abandonment of several lands by farmers or the adoption of other more resistant crops to salinity (Abdennour et al. 2020). Tunisia has about 0.47 million hectares of irrigated land. Irrigation resulted in the deterioration or total sterilization of a considerable part of its soils (Khawla and Mohamed, 2020).

Indeed, in Tunisia, the main source of salinization in irrigated areas is water irrigation (Louati et al. 2018); nonetheless, agriculture is important in Tunisia (Ben Nouna et al. 2016a), it accounts for over 11% of the gross domestic product and employs about 22% of the workforce (Ben Nouna et al. 2016b). The irrigated sector accounts for

over 35% of national agricultural production. Furthermore, some regions of Tunisia suffer from high salinity in the groundwater, as in Diyar-Al-Hujjej. This coastal region has a large depression of its aquifer over 12 m under sea level, and seawater intrusion is the origin of several abandoned wells (Bani et al. 2021). High salinities were measured in several wells leading to their abandonment (Closas and Molle 2016), cessation of agricultural activity in these areas, and migration of farmers to outlying areas less affected by salinity (Yacoubi et al. 2018). On the road of Korba-Menzel-Temime, several abandoned well are seen on both sides. As an emergency, the Tunisian government did an expensive surface water transfer from the northwest of Tunisia via the Cap Bon-Medjerda Channel (CBMC) for almost a hundred kilometers. The volume of surface water supplied from the state supply network for this irrigated area varies yearly, depending on rainfall. The volume was 1,569,467 m³, 1,714,603 m³ and 1,714,421 m³ between 2000, 2011, and 2012, respectively. No water transfer was done in 2016 and 2017 (Daghari et al. 2019) due to a shortfall of rain in Tunisia. In Diyar-Al-Hujjej irrigated area, farmers resorted to blending surface fresh water and saline aquifer water. Other farmers injected this surface water with an EC lower than 1.4 dS m⁻¹ into wells to be blended with saline aquifer water. Drip irrigation was adopted throughout all the study areas. Drip irrigation is considered a water-salt regulation technology (Brahim et al. 2021). In this region, tomatoes and peppers are the most important in Tunisia. The main processing tomatoes and

peppers factories are also located in this region. It is also the main strawberry-producing region in Tunisia. The area cultivated with summer tomatoes decreased from 450 to 210 ha between 1998 and 2011 (Daghari et al. 2021). A new crop combination of strawberry and pepper has become more and more widespread in this area. The Korba region is the main strawberry-producing area in Tunisia. Strawberry has become very popular and sought after by the agri-food industry for producing strawberry juice at all Tunisian festivities. Pepper is used in the culinary harissa process. Both crops are kept together in the same land parcel (on the same crop row) for two years, strawberry from September to May and pepper from June to August.

Usually, the largest consumption is recorded during April, May, and June, corresponding to the full growth period of tomatoes and strawberries. The CROPWAT model crop's water requirements calculation is 2.545 million m³. According to the farmers' association, pumping saline water from the aquifer filled the gap between the surface water and the water requirement. All farmers are aware of the salinity problem in the irrigated area of Dyyar-Al-Hujjej. That is reflected in their practice of abandoning several wells and introducing rainfed crops. Although farmers' behavior as part of salinity management has not been studied before, it is only recently that attention has been given to this aspect in this irrigated area (El Zarroug et al. 2021). Our objectives are (i) to see the impact of irrigation with salt water on the electrical conductivity (EC) of the land parcels, (ii) to assess the feasibility of using different sources of irrigation water, (iii) to study whether cultural practices can be a reliable tool for controlling salinity in irrigated crops.

MATERIALS AND METHODS

Experiment and field measurements

Dyyar-Al-Hujjej is located in the delegation of Korba in the northeast of Tunisia, about 66 km southeast of the city of Tunis in the Cap Bon peninsula (Figure 1), having coordinates 36°35' North and 10°52' East.

According to Ouelhazi et al. (2013), the most important geological outcrops in the studied area are mainly Mio–Plio–Quaternary deposits. The lower part of the Middle Miocene forms the detrital deposits known as the Beglia Formation. The Korba aquifer system comprises three geological units of marine Quaternary, Pliocene, and Late Miocene ages. The Tyrrhenian (Quaternary) is a 1.2-km-wide strip parallel to the coastline.

Investigating the history of the aquifer in Dyyar-Al-Hujjej, in 1969, the EC varied from 3 dS/m to 5 dS/m. In 2007, the measured EC varied between 4 dS/m to 15 dS/m, averaging 9 dS/m (Daghari 2016). The area is characterized by a semi-arid climate with an average annual precipitation of 420 mm (INM 2001). It has been observed that. Agricultural practices depend on the soil texture and structure. The remarkable thing in this perimeter is that the crops predominantly grown are vegetables (tomato, pepper, cabbage, etc.) or crops whose roots do not exceed 30 cm. Farmers in the discussions said it was impossible to grow other crops because the crust is very close to the ground. Indeed, soil profiles showed that the crust is often present from 60 cm depth, restricting farmers' choice of crops; therefore, no trees can be grown. After granular analysis, clay, silt, and sand percentages were 9.57%, 35.7%, and 55.35% (Daghari and Gharbi 2014).

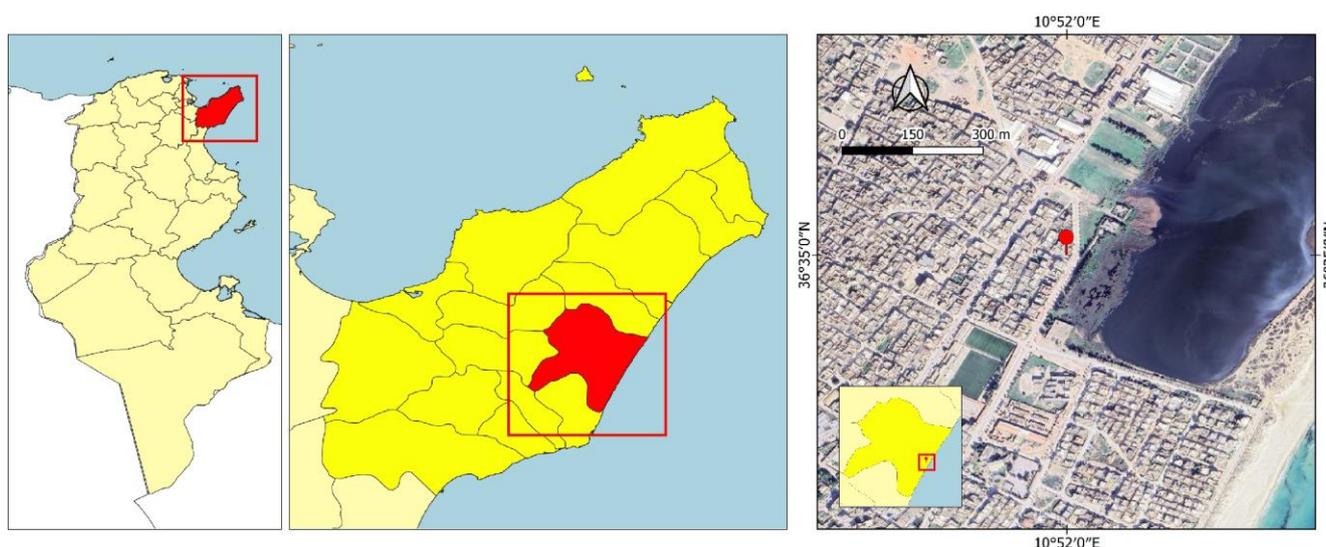


Figure 1. The geographic position of Dyyar-Al-Hujjej, Tunisia (36°35' North and 10°52' East)

Table 1. Farmer analysis questionnaire template

Distribution of crops			Irrigation water quality			Soil's EC
Sowing date	Crop	Area (ha)	Irrigated or not	Water sources	Irrigation water's EC	Aquifer depth
-	-	-	-	-	-	-

Thanks to the availability of surface water from the state network supply, a strawberry high-return crop's growth are increasing yearly. That explains the increase in the area occupied by strawberries from 55 ha in 2000 to 160 ha in 2011. During almost the same period, the cultivated areas dedicated to tomatoes, a summer crop with high water requirement, decreased from 450 in 2000 to 210 ha in 2011. Sandy soils are ideal for growing strawberries (Daghari et al. 2020).

About 20% of irrigated area is allocated for strawberries. Transplanting is done in late September or early October, and harvest lasts until May but is only kept for two years. Crops' choices vary depending on available water resources. Farmers having only water from the state network supply and renting small land parcels do not practice rainfed crops. Farmers with both surface and well water blend the two water. These farmers grow a succession of irrigated summer crops (tomato, pepper) and winter-spring crops (strawberry, cabbage, cauliflower, lettuce, spices), but they are forced to leave their land to follow eventually. In the case of farmers that do not have access to surface water, they grow only rainfed crops in summer. In the winter, they lean on the preseason industrial tomato, sold at a good price, to take advantage of the rain for their water needs.

This study conducted a series of meetings with farmers and farmer associations, and in situ soil samples were taken. In addition, analysis questionnaires were provided to the farmers (Table 1) to answer certain questions for a full cropping season.

Statistical analysis

The statistical analysis was done by the Statistical Analysis Software SAS/STAT. The one-way ANOVA was performed using the F-test at a significance level of 5%. The data were obtained on a sample of 7 land parcels separated into three groups (1: use of land parcels irrigated with dam water/2: land parcels irrigated with a blending of dam water and groundwater/3: land parcels irrigated with only dam water). Fourteen measurements were taken to measure the salinity of water sources (0: 1st month of the agricultural season, 1: the end of the agricultural season). Thirty-six measurements were taken to measure the salinity of water sources (0: 1st month of the agricultural season, 1: the end of the agricultural season).

RESULTS AND DISCUSSIONS

Three sources of water are available: surface water (less than 1.5 dS/m) from the state supply network discharged into a local dam called Lubna (Lubna Dam), water source from the saline aquifer up to 5.37 dS/m and blended water source (surface water and saline aquifer water blended).

Experiment and field measurements

The water requirements of a crop do not vary if it is grown under the same weather conditions, so the same amount of water will be supplied to the crop. Thus, the salinity measurement under different crops is a good indicator of the correlation between the amount of irrigation water and the EC of the soil. In our study area, tomato cultivation is the most widespread. However, Strawberry cultivation has increased too if only surface water is available.

Electrical conductivity under tomato

The salinities were measured from soil profiles under tomato cultivation on different dates (Table 2) to know the temporal evolution of the salinity.

As F1, These are usually farmers with only surface water of good quality and do not invest in the creation of well. F1 was visited on 13 July 2021, having a small land parcel of two hectares. This farmer is a tenant that grows only vegetable crops with high income (strawberry - pepper in mixture and tomato) and no rainfed crops. In the whole irrigated area of Diyar Al Hujjej, this is a unique case where low EC was observed under tomatoes with an average of 1.8 dS/m (Table 2). A low EC under the tomato was possible using only surface water from the state supply network.

Farmer F2's land is too close to the sea, so the well has a high EC due to seawater intrusion. The farmer' has a single well (depth = 14 m as of 09/11/2021. Surface water was injected into the well and pumped out after blending with water from the aquifer. The EC of the blended water was three dS/m. This farmer's land parcel is about 30 Hectares, the largest in this area, so we found several crops in this land parcel. The average EC is 5.315 dS/cm under the tomato crop, an EC greater than F1 (1.8 dS/m), which only uses surface water.

Table 2. Soil electrical conductivity (dS/m) at different depths under different crops for different farmers

Date	F1	F2	F3	F4	F5	F6
	12 July, 2021	11 September 2021	11 September 2021	11 September 2021	11 September 2021	11 September 2021
access to surface water	Yes	Yes	No	No	No	No
EC water	1.5	3	5.37	3.72	5.2	5.7
EC soil overground	-	-	-	-	19.5	-
EC soil (0-20)	2	6.65	5.3	4.71	5.3	9.24
EC soil (20-40)	1.6	3.98	5.6	4.27	Crust	Crust

For farmer F3, his land is located close to the sea and is not supplied with surface water. On 09/11/2021, the well's EC was 5.37 dS/m. Given this high salinity, the farmer grows industrial winter tomato, which has a good economic profit during the rainy season and can be harvested before the summer. However, soil solution dilution occurs, and salinity decreases due to the rain. Therefore, EC measurements were made in a land parcel where the tomato was almost harvested. EC values of 5.3 dS/m, and 5.6 dS/m, respectively, for the depth of 0-20 and 20-40 cm layers.

For farmer F4, he has a well close to farmer F5's well. His well is less saline (3.72 dS/m) and is localized in a low area that accumulates runoff water. This farmer cultivated tomato and pepper, having good yields. Measured salinities were lower than in the previous case (F3), which demonstrates the importance of rainwater harvesting. For F4, EC under harvested tomato was 4.71 dS/m and 4.27 dS/m for the layers 0-20 and 20-40 cm, respectively. For F2, EC under tomato was 5.35 dS/m and 5.6 dS/m for the layers 0-20 and 20-40 cm, respectively. For farmer F5, he has only one well to irrigate his crops. His well has an EC of 5.2 dS/m. A value of 19 dS/m was measured on a soil sample collected at the surface of cultivated soils with white spots, evidence of salts following a tomato crop that had just been harvested (Figure 2). Salinity under tomato was 5.35 dS/m for the layer 0-20 cm. For farmer F6, the measured EC in the well is 5.7 dS/m. EC reached 9.24 dS/m under finished irrigated tomato. Only rainfed crops or fallow are grown during the summer because of the high aquifer water EC. Irrigated pepper grown in the summer was abandoned because of the unmarketable and low small size of pods. Average EC was 1.8 dS/m and 5.26 dS/m, respectively, in the case of farmers F1 and F2 when only surface water or blended water was used. These values are lower compared to measured values for other Farmers.

Electrical conductivity under other crops

We also took soil profiles under other crops for more samples for statistical analysis. The F2 land parcel is about 30 hectares, which is the largest in this area, so we found several crops in this land parcel. Strawberry is a high-added value crop that all farmers want to grow on their land parcels. However, without a surface water source, it is impossible to have agricultural production. In our study area, two types of strawberries are grown. The 'Carmella' strawberry is sold at a high price but is very sensitive to water salinity, and the 'Tilda' strawberry is cheaper but more resistant to water salinity. For F2, EC measured from the soil profiles is 5.17 dS/m and 5.32 dS/m of soil under Strawberry "Tilda" for 0-20 cm and 20-40 cm, respectively. For another farmer, the EC measured for the "Carmella" strawberry is 3.24 dS/m (0-20 cm) and 3.82 dS/m for the "Tilda" strawberry. From 20 cm, there is a crust in this farmer's land parcel. Profiles were taken on 11 July 2021. The EC of the blended irrigation water is 3 dS/m and 5.37 dS/m for the latter two farmers.

Statistical analysis

For the layer 0-20 cm, the ANOVA results give an F value equal to 8.49, which means that there is an intergroup variation (between Water EC and Soil EC) 8 times greater than an intragroup variation. In other words, the differences in soil CE that we observe are indeed linked to the differences in the irrigation water EC and not to chance. The value of p is less than 0.05, which shows that the ANOVA test is significant and that the differences in intragroup means are not due to chance.

For the layer 20-40 cm, the value of F is 1.73, which shows that the difference in soil EC is not due to irrigation water quality. The value of p is more than 0.05, which confirms that the ANOVA test is not significant and that the differences in intragroup means are due to chance.

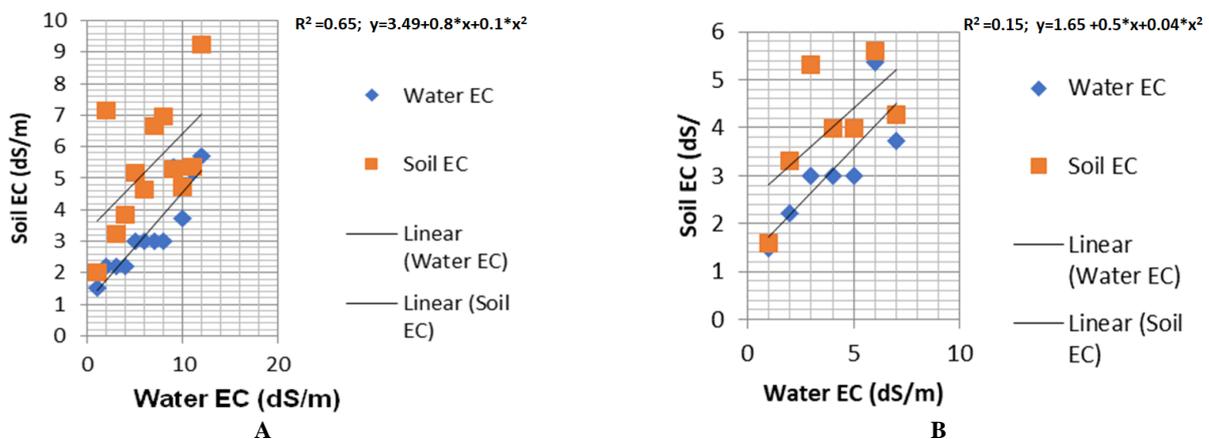


Figure 2. Effect of the water quality on the soil salinity at 0-20 cm layer (a), on the soil salinity at 20-40 cm layer (b)

We can deduce that irrigation in this irrigated area degrades the upper layer of the soil and that agriculture must be aware of these anthropogenic effects. With a soil depth of only 60 cm, farmers will soon be forced to leave this perimeter to practice agriculture. The presence of salt in this last layer is due to the accumulation of salts from previous irrigations. The leaching dose of salts is always taken into account by Tunisian farmers to get rid of salts in their land parcels (Saidi et al. 2018; Hammami and Zayani 2016). However, from a practical point of view (Besser et al. 2022), farmers cannot calculate the leaching dose and do so according to their intuitions (Dhaouadi et al. 2021a). In a future study, the leaching fraction must be calculated considering the interactions between the soil and the solution (Dhaouadi et al. 2021b). The geochemical approach is based on calculating the chemical balances between the solution and the soil minerals.

Discussions

In this study, a field experiment involving the dynamics of saline solutes observed the importance of irrigation water quality for farmers. ANOVA for measured values of salt concentrations was reliable for a root depth of 0-20 cm ($R^2 = 0.65$) compared to a root depth of 20-40 cm ($R^2 = 0.15$). These values showed the ability of agricultural practices to control salt levels and subsequently reduce the effects of irrigation with brackish water on the soil and the crop of tomatoes and strawberries. That was confirmed by Uddin (Uddin and Dhar 2007), who discussed the interest in introducing crop rotation. In other words, keeping the soil under rainfed crops reduces soil salinity and other benefits. In addition, crop rotation breaks the cycle of pests affecting crops by limiting pathogens and weeds.

For F2, irrigation with saline water with $EC_w = 3.5$ $dS \cdot m^{-1}$ may be tolerable for the crop and induces a low risk of soil salinization. However, for F6, saline water with $EC_w = 5.7$ $dS \cdot m^{-1}$ increases soil salinity in the short term and could be disastrous for the crop after several irrigation cycles. Using a drip irrigation system combined with appropriate management practices such as increased irrigation frequency or controlled deficit irrigation can mitigate these effects on soil and crops. Indeed, the sensitivity of crops to salinity depends on the vegetative stage; the effect of irrigation with saline water at the developmental stages is great and will surely harm the crop (Ashraf and Harris 2004).

In conclusion, our study area represents an exemplary case study of the effect of irrigation with saline water on soil and crops. Our studies have shown the importance of irrigation water quality for sustainable agriculture. However, repeating these experiments over at least three years can provide a solid statistic. The modeling approach can be considered, too, for the simulation of global salinity.

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