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Effect of different doses of the dung of cow, human urine and their combination on water and vitamins contents of pineapple (*Ananas comosus* (L.) Merr.) in southern Benin

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ABSTRACT

Pineapple is a tropical fruit much appreciated and consumed throughout the world. The present study has determined the effects of different doses of excrements on fruit water and vitamins content in order to offer to South-Benin pineapple growers an efficient and inexpensive organic fertilization formula. Three-months old plants of the pineapple variety named "*Pain de sucre*" were submitted, every three months until physiological maturity, to organic fertilization consisting of two doses of dung of cow; two doses of human urine; two combinations of both organic fertilizers and control without any fertilization following a complete randomized block design with seven treatments and four replicates. Fruit water and vitamins contents were determined at fruit maturity using appropriate methods. The results revealed that the effect of excrement use is significant on fruit water, liposolubles (A, E, K1) and hydrosolubles (B3 and B6) vitamins contents ; also this effect varied according to the vitamin. Treatments T1-2 (the dung of cow 310.56 g), followed by T3-1 (dung of cow 77.64 g + human urine 14.05 g) induced the highest fruit water and vitamins contents. These results confirm the importance of cow's dung and human urine in soil fertilization. The use of these two best treatments (T1-2 and T3-1) will facilitate production of good nutritional quality pineapple suitable for exportation. © 2019 International Formulae Group. All rights reserved.

Keywords: Exportation, fertilization, nutritional quality, organic agriculture.

INTRODUCTION

Pineapple is a tropical fruit much appreciated and consumed throughout the world. It is one of the major tropical fruits traded internationally, with annual world production exceeding 15 million tons since 2005 (Loeillet, 2005). In Benin, pineapple production in 2006 contributed to 1.2% of the national Gross Domestic Product (GDP) and 4.3% of agricultural GDP. According to Cosinus Conseil (2016), the land surface occupied by this crop in 2008 was 3,049 ha

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with a production of 171,331 tons, rising to 6,719 hectares with production of 358,869 tons in 2013; which corresponds to an increase of more than 100%. About 50% of the annual production of Benin is sold on the national market (35% for self-consumption and 15% on processing) and the remainder is exported fresh or dried into Nigeria, Niger, Burkina Faso, Mali, the European Union, etc. (Sohinto, 2008). Pineapple is now one of the few most exported food crops in Benin and is produced in seven (07) of the twelve (12) provinces of the country, with the Atlantic Province being the top (Tossou, 2001). Because of its very high profitability, it is an important source of income for individual producers and it can ensure financial stability for households (MAEP, 2006). In Benin, pineapple provides an annual income of about 2 billion CFA per year on a regional scale, thus enabling producers to meet their needs (Aïvodji and Anasside, 2009). However, since 1994, the share of the Continent imports of fresh pineapples (the second tropical fruit imported into Europe behind bananas) in Europe has been steadily declining (Allagbe et al., 2012). One of the reasons for the decline in imports of pineapple produced on the African continent to the European market is the increasing demand for the standards of this market in relation to the presence of pesticide and chemical fertilizers residues in fruits (Allagbé et al., 2012). Tossou (2001) reported that the maximum residue limit (MRL) allowed passed from 2 per thousand in 2005 to 0.5 per thousand in 2008, according to the regulations of the Economic Commission of the European Union No. 889 / 2008 of September 05th, 2008. To maintain soil fertility without chemical residues in agricultural products mainly in vegetables, farmers use organic fetilizers including composts and dung of cow (Tchabi et al., 2012; Ognalaga et al., 2015; Biaou et al., 2017). In order to access the European market, some of the Benin pineapple producers have adopted the use of different types of organic fertilizers including poultry droppings and compost (Allagbe et al., 2012). The existence of a flourishing international market for

organic pineapple has also increased the search for other organic fertilizers for the production of pineapple, leading to the use of crop residues or different types of compost, poultry droppings, animal wastes, guano and human urine. Thus, the new ECOSAN concept, which considers human excreta as a source of nutrients in agriculture, may be a better approach for developing countries (Kiba, 2005). It is widely established that optimal fertilization ensures a good balance between nutrients to ensure high yields and good product quality by contributing to the provision of a varied, healthy and balanced diet (Bruulsema et al., 2012). Indeed, a balanced nutrition of the plant in all mineral elements produces foods of good nutritive value and high organoleptic quality. Vitamins are essential for the growth and maintenance of the body and are required in very small quantities (Ratnakar and Rai, 2013). Vitamins work in the body with carbohydrates, proteins and lipids for energy intake. They are functional components for different biochemical reactions regulated by enzymes that are realized to create energy (Sarkiyayi and Ikioda, 2010). They are brought to the body largely by fruits and vegetables.

Organic pineapple has been developed in Benin for a few years. However, no fertilization formula exists for this crop. In addition, very few studies have examined the influence of different doses of organic fertilizers available in Benin on the nutritional quality of pineapples. At the global level, studies are also very rare. In a previous study, we showed that different doses of the dung of cow, human urine and their combination improved plant and fruit growth with a difference between treatments (Kpéra et al., 2017). Our hypothesis is that some of the seven treatments will improve nutritional vitamins contents of pineapple fruit. The present study on the effects of the same treatments on water and vitamins contents of pineapple fruits will make up for the lack of data and identify the best organic fertilizers for the production of pineapples of good nutritional quality according to organic agriculture standards in Benin.

MATERIALS AND METHODS Plant material

The plant material consists of homogenous rejections of pineapples from the variety « *pain de sucre* ». These rejections were provided by the Agro-System farm located in Zinvié.

Experiment conditions

The study was conducted in Zinvié, city of Abomey-Calavi in the southern part of the Republic of Benin and the Atlantic Province at an average altitude of 48 m from the level of the Atlantic Ocean and between 06 $^{\circ}$ 62 'and 06 $^{\circ}$ 37' north latitude then 02 $^{\circ}$ 35 'and 02 ° 21' longitude east. The climate is a subequatorial type with two rainy seasons and two dry seasons. The average annual rainfall is about 1200 mm, of which 700 to 800 mm for the first rainy season and 400 to 500 mm for the second rainy season (CENAGREF, 2010). There are two agricultural seasons of unequal importance (CENAGREF, 2010). The larger goes from March to July and the smaller one takes place between September and November. In recent years, there has been a marked change in the alignment of seasons as a result of climate change disturbances. The average monthly ambient temperatures range from 25.79 to 33.98 °C. As August is the coldest month, March is however the warmest month with a thermal deviation of up to 8 °C in northern Benin (CENAGREF, 2010). According to these authors, the relative humidity of the air varies between 65% (January-March) and 95% (June-July). The absolute maxima reached are 100% and are recorded during the rainy months. Absolute minima revolve around 20% and correspond to the great dry season when the harmattan is in full swing. The experimental site consists of ferralitic soils.

Determination of the quantities of fertilizers to be brought

The method of collection and the methods of determining the quantities of each fertilizer supplied and the preliminary analysis of the experimental soil are those described in our previous publication (Kpéra et al., 2017). Urines were collected in schools near the experimental site based on the level of hygiene and the number of schools. In each school, sterile 25-liter cans were deposited, the contents of which are recovered at the end of each day. Well-dried storage cans are carefully closed to avoid volatilization of nitrogen and the urine is stored under the same conditions at room temperature for a minimum of one month before use. The dung of cow comes from a herd located about 1 km from the field of experimentation. The fresh droppings deposited by the animals are collected without the addition of sand with the consent of the shepherd and then brought back to the site in a pit of 2 m x 1 m x 1 m (Length-Width-Depth) provided for this purpose, for a stay of 3 months for mineralization. The composition of samples of human urine and cow dung in organic matter, nitrogen, organic carbon, phosphorus and potassium was determined at the Laboratory of Soil Sciences of the Faculty of Agricultural Sciences (FSA / UAC) in order to determine the quantity of each fertilizer to be brought to the plants. The chemical composition of cow dung and urine is reported in Table 1. Doses of fertilizers were calculated from the indications of DPQC (1999) which state that pineapples require a fractional intake of 1.04 g of N, 0.65 g of P and 2.6 g of K per plant every two months. It was therefore necessary to determine the composition in nutrients from cow dung and urine in order to evaluate the quantity of each fertilizer to be fed to plants.

Sampling and preliminary analysis of experimental soil

Before fertilizers application, soil samples were taken at a depth of 15 cm at various points on the plot along the diagonals using an auger. Samples were mixed and soil composition in organic matter, nitrogen, organic carbon, phosphorus and potassium was determined at the Soil Science Laboratory of the Faculty of Agronomical Sciences of the University of Abomey-Calavi (Benin Republic) (Table 2).

Experimental design

The experimental setup is the same as that described in our previous publication (Kpéra et al., 2017). Using a machete, a hoe and a rake, the land was cleaned and underwent a flat and deep plowing of 20 cm. After delimitation of the plots (5 m by 10), they were planted with rejects of more or less uniform sizes according to a density of 30 cm x 30 cm. Moreover, the strips are spaced 90 cm apart. An aisle of 0.5 m wide separates each plot from the other in the direction of the length and the width. Using a load cell and a syringe, the spreading means were determined and then the fertilizers were distributed at the feet of the plants in pockets according to their nature. The cropping system is a pure culture of pineapple. From the results of the analysis of the samples recorded in the Table 1, we defined the quantity of fertilizers to be applied. For example, 155.28 g of dung of cow corresponded to the application of 3.14 g of N; 0.76 g of P and 4.05 of K ; and 310.56 g of dung of cow corresponded to 6.28 g of N; 1.52 g of P and 8.1 g of K.

The application rates per treatment are as follows:

- Treatment T0: control: no intake of fertilizer
- Treatment T1-1: 155.28 g of dung of cow per plant
- Treatment T1-2: 310.56 g of dung of cow per plant
- Treatment T2-1: 28.1 g of human urine per plant
- Treatment T2-2: 56.2 g of human urine per plant
- Treatment T3-1: 77.64 g of dung of cow + 14.5 g of human urine per plant
- Treatment T3-2: 155.28 g of dung of cow + 28.1 g of human urine per plant.

The experimental design is a Randomized Complete Block Design (RCBD) with the previous seven (7) treatments and four (4) replications. The experimental plot was plowed flat to a depth of 20 cm. The dimensions of the elementary plots are 5 m by 10 m. An aisle of 0.50 m wide separated the elementary plots and an aisle of 0.90 m between the blocks. Pineapple rejections (discharges) of similar size were planted with a spacing of 0.30 m on the line and 0.90 m between the lines. The quantities of each fertilizer corresponding to each of the treatments were placed at the feet of the plants. Fertilizer application began three (3) months after planting and was repeated every three months until the carbide induction treatment occurred in the 12^{th} month after planting. No spraying of chemicals was done. Weeds were eliminated by manual weeding at the number of six (6) carried out during the entire production cycle (15 to 16 months).

Determination of fruit water contents

The water content content is defined as the sudden loss of mass in the measurement conditions. The method of Audigié et al. (1978) was applied to pineapple juice obtained.

Extraction and determination of vitamins

Vitamins were extracted from ripe pineapple fruits from various treatments. Fatsoluble vitamins (vitamins A, E and K1) were extracted using a combination of the methods developed by Jedlicka and Klimes (2005) and Kini et al. (2008) using hexane and methanol. Water-soluble vitamins: Vitamins **B**3 (Niacin) and B6 (Pyridoxine) were extracted using a combination of the methods developed by Gregory (1954) and Benmoussa et al. (2003). The separation and determination of the vitamins were carried out by Agilent Technologies 1120 Compact LC High Pressure Liquid Chromatograph (HPLC), equipped with an isocratic pump (Isopump G4286A), a loop injector (Rheodyne P/N 5067-4102) of 20 µL capacity and a UV-Visible detector (G4286A) according to the method used by Ba et al. (2016).

Statistical analysis

The effect of treatments on the different parameters was studied using a oneway ANOVA analysis. Means were compared using the Tukey test with four replicates. The analysis were done using the JMP Pro software (JMP Pro SAS Institute, 2015).

Elements	Dung of cow	Urine
Total Nitrogen (N)	1.61	8.89
Organic matter	17.69	-
Organic Carbon	10.26	-
Total Phosphorus total (P)	0.47	0.205
Total Potassium (K ⁺)	2.635	2.16
P/N	0.292	0.023
K/N	1.637	0.243

Table 1: Composition (%) of urine and dung of cow used.

Table 2: Chemical composition of the experimental soil.

Parameters	Content
Organic matter (%)	1.92
Organic carbon (%)	1.12
Total nitrogen (%)	0.09
C/N	12.44
Assimilable phosphorus (ppm)	11.35
Potassium (K ⁺) (%)	0.714

RESULTS

Initial chemical composition of the soil

The initial chemical composition of the soil at the experimental site revealed that the average contents of organic matter, carbon, total nitrogen and potassium were 1.92%, 1.12%, 0.09% and 0.714%, respectively; that of the assimilable phosphorus amounts was 11.35 ppm (Table 2).

Effect of fertilizers on fruit water and vitamins contents

The effect of treatments on pineapple fruit water content was shown in Figure 1. Water contents was 86.19; 89.31; 92.68; 94.9; 90.78; 93.75 and 94.77% respectively for the treatments T0, T1-1, T2-1, T3-1, T1-2, T2-2 and T3-2. Fruit water contents increased under the effect of the treatments compared to the control treatment T0 with a very highly significant difference (p < 0.001) among

treatments. The highest water content was observed in treatments T2-2, T3-2 and T31 (greater than 93.7%) while the lowest water contents was obtained for T0 control treatment (about 86%). Water is the major constituent of most foods. Although it does not provide any energetic value to foods, its existence plays a very important role. It affects the structure, appearance, taste and touchiness of food to degradation. The water contents of pineapple is a particularly important parameter because it makes it possible to assess the ability to yield a high amount of juice. Our results showed that fertilizers formulas used lead to an increase in the water contents of the fruit compared to the control with the best water contents obtained in treatments including urine alone or the combination of urine with the dung of cow (T2 and T3).

Effect of fertilizers on fruit vitamins contents

Table 3 showed the effect of treatments on the fat-soluble vitamins content of ripe pineapple fruits. The vitamin A contents varied between 1986.89 and 2002.13 µg / 100 mL. A very significant difference (p <0.001) was observed among treatments. The highest vitamin A contents was observed in the treatment T1-2 while the lowest vitamin A contents was obtained for the T3 treatment (T3-1 and T3-2): control treatment T0 has an intermediate value. For vitamin E, the contents varied from 235.69 to 253.32 µg / 100 ml. A very significant difference (p <0.001) was observed among treatments. The highest vitamin E contents was observed in T3-1 treatment while the lowest vitamin E contents is obtained for T0. T1-1. T1-2 and T3-2 treatments; The treatments T2-1 and T2-2 have intermediate values. The vitamin K1 contents varied from 433.125 to 438.602 µg / 100 mL. A very significant difference (p <0.001) was observed among treatments. The

highest vitamin K1 contents was observed in treatment T1-2 and T3-1 while the lowest vitamin K1 contents were obtained for T2-2 and T0 treatments, treatments T1-1, T2-1 and T3-2 have intermediate values. Table 4 showed the effect of treatments on the watersoluble vitamin contents of ripe pineapple fruits. The vitamin B3 contents varied from 14.8128 to 20.0850 µg/100 mL. A very significant difference (p < 0.001) was observed among treatments. The highest contents of vitamin B3 was observed in treatment T2-2 while the lowest vitamin B3 contents was obtained for treatment T1 -1; the other treatments have intermediate values. For vitamin B6, the contents varied from 11180 and 33129 µg/100 mL. A very significant difference (p <0.001) was observed among treatments. The highest vitamin B6 content was observed in the T3-2 treatment while the lowest vitamin B6 content was obtained for T0 treatments and T1-1; the other treatments have intermediate values.

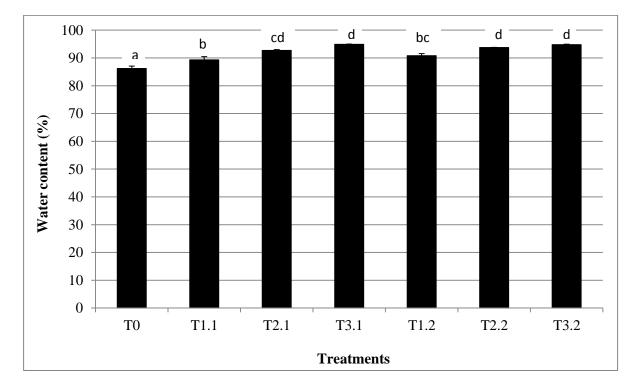


Figure 1: Water content (%) of pineapple fruit as affected by organic fertilization treatments (n = 4, means \pm standard error). Means with different letters are significantly different (p<0.001).

Treatments	Vitamin A	Vitamin E	Vitamin K1
Т0	1998.45±0.05d	237.28±0.07a	433.243±0.031a
T1-1	2000.84±0.07e	235.69±0.04a	435.039±0.221b
T2-1	1991.21±0.6b	242.01±0.47b	435.271±0.15b
T3-1	1998.60±0.15d	253.32±1.29c	438.602±0.302c
T1-2	2002.13±0.03f	235.73±0.13a	437.949±0.4c
T2-2	1992.36±0.66c	241.30±0.30b	433.125±0.034a
T3-2	1986.89±0.28a	237.33±0.16a	434.508±0.047b

Table 3: Effect of different organic fertilization treatments on liposolubles vitamins content (μ g 100 ml⁻¹) of pineapple fruit at maturity.

n=4; means ± standard error; Means in the same column with different letters are significantly different (p<0.001).

Table 4: Effect of different organic fertilization treatments on hydrosoluble vitamins content (μ g 100 ml⁻¹) of pineapple fruit at maturity.

Treatments	Vitamin B3	Vitamin B6
то	19.38±0.02f	12981.78±242.61a
T1-1	14.81±0.01a	11180.18±49.1a
T2-1	18.11±0.05d	28186.10±758.74d
T3-1	16.64±0.03ab	18699.73±343.31c
T1-2	18.66±0.01e	26366.30±422.43d
T2-2	20.08±0.01g	16097.89±269.63b
T3-2	17.83±0.01c	33128.87±1525.76e

n=4; means \pm standard error; Means in the same column with different letters are significantly different (p<0.001).

DISCUSSION

Initial chemical composition of soil

Values obtained for the soil composition can be considered as high for organic matter, organic carbon, assimilable phosphorus and potassium according to Tossou et al. (2006). The nitrogen contents of the soil is low whereas the C/N ratio (12.44) corresponds to a less or more decomposed organic matter, reflecting a degraded soil according to soil classification based on the C/N ratio presented by Wild et al. (2013) and Gentsch et al. (2015).

Effect of fertilizers on fruit water contents

Our results showed that the different fertilizer formulas used lead to an increase in the water contents of the fruit compared to the control with the best water contents obtained in treatments including urine alone or the combination of urine with the dung of cow (T2 and T3). This can be explained by a quick absorption of the mineral elements contained in the urine increasing the osmotic pressure of the juice, which results in a strong attraction of the water molecules. This explanation is supported by the report of Jansson et al. (2004), quoted by Yahaya (2009), who stated that most of the nutrients needed by plants and contained in human excreta are found in the urine in ionic form and their availability to the plant competes seriously with chemical fertilizer.

Effect of fertilizers on fruit vitamins contents

Vitamins A (retinol), E (tocopherol) and K1 (phylloquinone) are fat soluble vitamins. They are indispensable because of their multiple physiological roles. Vitamin A is involved in the vision, growth and differentiation of epithelia; vitamin E is an excellent antioxidant that intervenes in protection against oxidative stress and vitamin K1 is an antihemorrhagic vitamin that intervenes in blood clotting (Guilland, 2009). Vitamins B including vitamin B3 (niacin or vitamin PP) and vitamin B6 (pyridoxine) are essential precursors of metabolic cofactors (Hanson et al., 2016). They are involved in several essential reactions of cellular metabolism including the metabolism of carbohydrates, proteins, amino acids and lipids.The main importance of fruits in the diet lies on their richness in vitamins. Our results show that except vitamin A and vitamin B3, all the fertilizing treatments used improved vitamins contents of the fruits (compared to the control treatment) with a great variability according to the vitamin. Treatments T1-2 consisting of the dung of cow alone (310.56 g of the dung of cow per plant) and T3-1 treatment combining urine with the dung of cow (77.64 g of the dung of

cow + 14.05 g of human urine per plant) are the best as both treatments induced the best grades for two of the five vitamins studied: vitamins A and K1 for treatment T1-2 and K1 and E for treatment T3-1. Both treaments induced also a very good content in the other vitamin: B6 for treatment T2-1 and vitamin A for treatment T3-1. These treatments were followed by treatments T2-2 (urine alone at 56.2 g per plant) and T3-2 (dung of cowurine combination, 155.28 g the dung of cow+ 28.1 g of human urine per plant) with the best contents for only one of the six vitamins (vitamin B3 for T2-2 and vitamin B6 for T3-1) in addition to a very good contents for vitamin E (treatment T2-2) or vitamin K1 (treatment T3-2). It is important to note the particularly stimulatory effect of treatments with the dung of cow alone (T1-1 and T1-2) on vitamin A contents which gave the best grades. Treatments T1-2, T2-2, T3-1 and T3-2 which gave the best vitamin contents also have higher water contents than the control treatment. This shows that the increase in the water contents did not lead to a reduction in the vitamin contents due to a possible dilution. There is therefore a real stimulation of the synthesis of vitamins, not an increase in their concentration (by reduction of the water contents) under the effect of these fertilizing treatments.

Numerous scientific results have demonstrated that the relevant management of fertilizer application can both increase the productivity and market value of fruits and vegetables as well as their health effects. In muskmelon (Cucumis melo. reticulatus group), Lester et al. (2005) showed that an additional supply of potassium by foliar spraying could improve the ascorbic and β carotene contents of plants well fertilized by soil application of K. According to Bruulsema et al. (2012), foliar application of potassium and sulfur improves sweetness, texture, color, vitamin C and beta-carotene and folate contents of cantaloupe melon. For pink grapefruit, additional foliar application of potassium increases the concentration of betacarotene and vitamin C. Generally, a contents of powerful antioxidants such as lutein and

raised beta-carotene up with nitrogen fertilization. Other authors have compared the effect of chemical fertilizers with that of organic fertilizers. Thus, Premuzic et al. (2002) reported that in lettuce, compost (organic fertilizers) leads to a greater accumulation of vitamin C compared to an inorganic fertilizer (nitrates), which in turn results in a greater accumulation of vitamin C in comparison with a non fertilized control. Similar results were found in cherry tomatoes (Premuzic et al., 2001): vitamin C contents was higher for organic fertilization or fertilization combining mineral and organic inputs than for mineral fertilization alone. On the other hand, according to Veit-Kohler et al. (1999), high nitrogen fertilization tends to decrease the concentration of vitamin C in citrus or tomato fruits, or in potatoes. These authors justify this observation by the existence of competition for the assimilation between the plant growth and the processes of synthesis and storage of secondary metabolites; the biosynthesis pathway of vitamin C being related to carbohydrate metabolism. As a result, anything that would tend to slow plant growth would be beneficial to the synthesis and accumulation of nutritional compounds. Moreover, it has been reported that quercetin contents of the onion is not influenced by nitrogen fertilization while exhibiting a strong interannual variation (Mogren et al., 2006). Similarly, the contribution of phosphorus to greenhouse tomato plants did not significantly affect lycopene or carotene contents (Oke et al., 2005). It is also important to note that from the two treatments T1-2 and T3-2 which gave the best results in terms of plant growth and fruit weight (Kpéra et al., 2017), only T1-2 gave the best results with respect to the fruit vitamin contents. These results indicated that fertilizers that induced the best yields did not necessary induce the best quality products.

The use of human excreta in agriculture, however, poses risks and requires precautions. For Calvert et al. (2004), urine is generally sterile and is a danger only in some cases. The most common pathogens in the urine can cause typhoid, paratyphoid and bilharzia. For Adissoda et al. (2004), urine is recommended for use in food crops, provided that there is no direct contact between eatable parts and urine (eg. cereals, fruits). In addition, urine can be used on crops for animal feeding regardless of conditions (Adissoda et al., 2004). According to these authors, in the general case, especially in cases where there is a possibility of contact between the edible parts and the urine, the recommended storage time for the urine is two months before use; after one month of storage, only viruses survive and after six months of storage, there is probably no more virus in the urine. In our experiment, the urine was kept for a minimum of one month before use. In addition, it was brought directly to the foot of plants and fertilization was stopped at the time of the floral initiation treatment, which excludes any possibility of contamination of pineapple fruits by urine. On the physiological level, Ganrot et al. (2007) reported that the use of urine alone as fertilizer can be a source of trouble for dryland producers in that it could cause salt stress for crops because of its high NaCl contents up to 8.8 g/L. Since the main pineapple production areas in Benin are rather humid areas, the risk of salinization of land due to the use of human urine in the fertilization of pineapple remains negligible.

Conclusion

In this study, we discussed the effect of animal excreta on the nutritional quality of pineapple. Our results indicate that the dung of cow and urine, alone or in combination, improve the water contents and especially the water-soluble and fat-soluble vitamin contents in pineapple. However, treatment T1-2 (the dung of cow alone at 310.56 g / plant) and T3-1 (combination of both fertilizers with 77.64 g of the dung of cow + 14.05 g of human urine per plant) appeared as the best indicating that the hypothesis is accepted. Thus, fertilization by the dung of cow and urine can contribute to improve the quality of pineapple, organic or not, for export in the southern part of Benin. However, an in-depth study on the economic profitability of using these fertilizers for pineapple production is necessary for a better use of the results.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

AK and CBG designed the study, wrote the protocol, and wrote the first draft of the manuscript. AK, AGH and CBG managed the literature searches. AGH, FAG and SG contributed to the protocol writting and managed the analyses of the study. AK, AGH and CBG performed the statistical analysis. All authors read and approved the final manuscript.

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