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ORIGINAL ARTICLE

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Effects of 2,4,5-trichlorophenoxyacetic Acid, Gibberellic Acid and Potassium Nitrate on the Development of *Tangelo Orlando* (Citrus×tangelo) in the Sudano-Sahelian Area in Mali

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In Mali, citrus fruits play an important part in both human nutrition and the country's economy. They represent an important source of income for citrus growers. Citrus production in Mali is highly diversified, but the current trend is towards the Tangelo Orlando or *Citrus×tangelo* variety. The aim of this study was to determine the effects of two phytohormones (2,4,5-T acid and AG_3) and a mineral salt (KNO_3) on the production of Tangelo Orlando. The experiment was carried out in Koulikoro district, especially on the experimental plot of the IPR/IFRA of Katibougou. The plant material used was white-pulp Tangelo Orlando. Two phytophytohormones (2,4,5-T acid, and AG_3), a mineral salt (KNO_3) and distilled water (control) were used to treat the young plants. The experimental design was a Fischer block with three replications. Observations were made on the parameters of development. The results showed significant differences ($p<1\%$) between different development parameters. Both phytophytohormones significantly influenced early induction of flowering. The number of fruits per plant increased with the application of AG_3. The weight and diameter of the fruits improved with the application of phytophytohormones and mineral salt. The best yields were obtained with the application of the two phytophytohormones (27,685 kg/ha with 2,4,5-T acid and 25,835 kg/ha with AG_3). These yields are higher than the national average for Mali. In the Sudano-Sahelian zone of Mali, citrus growers can use these 2,4,5-T acid and AG_3 phytophytohormones in their orchards to induce early flowering and ripening in order to improve the yields of young Tangelo Orlando plants.

Key words: Phytohormone, development, citrus, Tangelo Orlando, Katibougou

Over the last thirty years or so, global citrus production has grown at extremely fast rates, making it the world's leading fruit crop (Eddine & Yamina, 2018). Taking all species together, citrus fruit production is over 110 million tonnes per year, over an area of around 7.5 million hectares. Oranges account for around 60% of total citrus fruit production. Tangerines, mandarins, clementines and satsumas account for 23% of the world volume (https://www.yara.fr). With a global production share of 36.5%, agrumiculture occupies an important place in Africa in general and in tropical and subtropical African countries in particular (FAOSTAT, 2018).

Fresh fruit for the market is produced mainly in subtropical climates (South Africa in particular) and Mediterranean climates (Tunisia, Egypt, Morocco and Libya) (Giz & FiBL. 2021). South Africa is the world's second largest exporter of citrus fruits after Spain and the largest in Africa (Agence Ecofin, 2023).

Citrus fruits are eaten fresh or after processing (juice, syrup, etc.). They are an important contributor to the nutritional balance of populations due to their high levels of vitamin C and B6, as well as being a source of fiber, ascorbic and folic acid, potassium and calcium (Eddine & Yamina, 2018).

There are four species of citrus fruit in Mali: orange, mandarin, lemon and pamplemousse. But the current trend in citrus production is towards the production of Tangelo, a hybrid of mandarin and pamplemosse. Over the last 5 years, new citrus plantations have been planted mainly with Tangelo. According to the citrus growers we met, the Tangelo produces twice as much as the orange tree, and its fruit is juicier, sweeter and milder than the orange. The actors in the citrus industry include nurserymen, growers and traders (Dolo, 2019).

Mali has many opportunities to produce citrus fruits (Traoré *et al.*, 2023). During the 2019/2020 growing season, producing areas were 6,950ha, with a total production of 118,589 tons (DNA, 2019). Citrus fruits are grown in all regions of the country (RGA, 2004).

One of the main challenges facing citrus growers is the problem of low fruit production. Mali therefore needs to find strategies to increase citrus production in general and Tangelo production in particular in order to maintain or boost its GDP. In other countries, such as Morocco, growers use phytohormones at different stages of plant development, particularly for citrus (Kaidi *et al.*, 2016). Phytohormones and mineral salts play a key role in the plant's development cycle. Phytohormones affect germination, embryogenesis, vegetative organ growth and fruit ripening (Maougal, 2015). These quantitative and qualitative transformations determine the different steps in the life of the plant, from planting to ripening.

The aim of this study was to investigate the effects of two phytophytohormones (2,4,5-T acid and AG_3) and a mineral salt (KNO_3) on the production of citrus Tangelo in Mali.

MATERIAL AND METHODS

Experimental site

The orchard of the Rural Polytechnic Institute for Training and Applied Research (IPR/IFRA) in Katibougou (12°54'86''N; 07°31'85''W), located in the Koulikoro district, was used as the study site (Figure 1). It covers an area of 400 ha. It has a Sudano-Sahelian climate with a short rainy season of 4 to 5 months (June to September), followed by a long dry season of 8 to 9 months (October to June). Annual rainfall is low to begin with, reaching a peak in August with an annual total of 600-900mm. The average temperature is 39°C in the hot season and 21°C in the cool season (Katibougou weather station, 2020). The vegetation is characterized by an arboreal savannah. The main soil types are tropical ferruginous soils with a silty to silty-sandy texture at the surface and a silty texture at depth (Diarra *et al.*, 2022).

Plant material

The plant material used is the white-fleshed Tangelo Orlando. It is an interesting productive variety, giving fruit of fairly good quality. The fruit is spherical with a flattened base and a peduncle at the bottom of a crater surrounded by an irregular collar. Slightly ribbed fruit (Rey, 1982). The Tangelo Orlando grafted seedlings were obtained from the Genetic Resources Unit (URG) of the Rural Economy Institute (IER) in Mali. The plants were grafted five months before being transplanted. The grafted rootstocks were eight-month-old bigarade saplings grafted onto Tangelo Orlando branches averaging 30 cm in height from mother plants that had fruited well.

Hormones and salt used

Two phytohormones 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), gibberellic acid (AG_3) and a mineral salt, potassium nitrate (KNO_3) were used.

- 2,4,5 - trichlorophenoxyacetic acid is a synthetic auxin belonging to the clorophenoxyalkanoic acid family (Pichaad *et al.*, 2005).

- Gibberellins (GAs) are phytohormones that regulate various developmental processes, including stem elongation, germination, dormancy, flowering, flower development and leaf and fruit senescence (Khlelil *et al.*, 2013).

- Potassium nitrate (KNO_3) is a mineral salt containing potassium, the third most important plant nutrient after nitrogen and phosphorus. Potassium can be combined with phosphorus and nitrogen (NPK) (AGRIMAROC, 2017).

Treatment and application methods

To improve the development and reproduction of Tangelo Orlando, a trial was carried out using three products: 2,4,5-T acid, AG_3, KNO_3 and distilled water (H2O) used as a control. There was a total of four treatments, coded T0, T1, T2 and T3. The three products and distilled water were applied at different concentrations (Table 1) by spraying (Technoma 15 sprayer) to the leaves of the seedlings 110 days (3 months) after planting.

Experimental design

The experiment was carried out in a Fischer block design with three blocks. Each replication was made up of four individual plots, each 20 m long and 5 m wide, covering an area of 100m².

The planting holes were identified by picketing the plot using the 3-4-5 method to identify right angles. Holes 50 cm in diameter and 50 cm deep were made. Each hole was fertilized using 30 kg of organic fertilizer, i.e., 12 tons per hectare. In addition to the organic fertilizer, 30 g of carbofuran was used per hole to control nematodes and insects.

The grafted seedlings were planted in the holes so that the tree neck was 10 cm above the soil surface. The seedlings were allocated to the holes in the elementary plots by random drawing. A basin with a radius of 50 cm was made around each plant after planting. Immediately after planting, the seedlings were watered with 20 liters of water per plant. When the rain stopped, they were watered three times a week with the same amount of water.

Trial management

Additional irrigation was needed to avoid water stress on the plants. To achieve this, the principle of the double basin was adopted: a small basin 50 cm in diameter to prevent direct contact between the water and the stem of the tree (risk of disease, in particular citrus gum disease or phytophtora) and a large basin 100 cm in diameter to retain the irrigation water.

An initial uniform dose of 100 g of urea, cereal complex and K_2SO_4 was spread per Tangelo plant across the entire area of the leaf canopy on the ground. Subsequent doses of fertilizer were applied: 44 g ammonium phosphate, 40 g potassium sulphate and 92 g urea when irrigation was resumed.

In the first year, some periodic weeding of the basins, spraying with discs or superficial tilling were carried out to avoid destroying the topsoil roots. This work was followed by scarifying to improve the looseness and cleanliness of the soil. In the second and third years, the same management work was carried out.

Data collection

Observations were focused on the following development parameters:

- The delay between planting and flowering; this consisted in noting the date on which 50% of the plants had produced a flower. Which enables to be determined the plant's vegetative cycle;

- The number of fruiting branches per plant was determined by counting. This involved counting the number of branches with at least one flower;

- The number of flowers per plant was determined by counting the number of flowers present in the plot after 50% of the flowers had appeared in each treatment;

- The number of fruits per plant at harvest was determined by counting;

- The mean diameter of a fruit was measured using a caliper on 3 randomly selected fruits per plant in each elementary plot just after picking

- The average weight of a fruit was determined using an electronic balance with a 0.01mg precision;

- The yield was determined after harvest using an electronic balance with a 0.1mg precision;

Data analysis

The statistical analysis involved a comparison of the means of the different treatments using an analysis of variance with STATITCF software, followed by a comparison of the means using the Newman-Keuls test with a threshold of 5%. The results obtained were then presented in the form of graphs using Excel software.

RESULTS

Time in months between planting and emergence of flowers on the plants

The analysis of variance showed a highly significant difference between the growing phases (number of months between planting and flowering) with a $p < 1\%$ between products. The Newman-Keuls test with a threshold of 5% produced two distinct groups (A and B).

The T1 and T2 treatments produced flowers early (after 12 months) and formed the homogeneous group B (Figure 2). Plants in these treatments received the hormones AG_3 and 2,4,5-T acid. Late flowering was observed in treatments T0 (control treated with distilled water) and T3 (treated with KNO_3), with respective durations of 28 and 30 months between planting and the appearance of flowers on the plants. These two treatments form homogeneous group A (Figure 2).

Number of fruiting branches per plant

The results of the analysis of variance of the number of fruiting branches per plant around 12 months (360 days) after the application of the products showed a highly significant difference with a $p < 1\%$ between the treatments. Using the Newman-Keuls test at the 5% significance level produced three distinct homogeneous groups (A, B and C).

Plants that were sprayed with the hormones 2,4,5-T acid (T2) and AG_3 (T1) produced more fruiting branches, with 8.5 and 8.3 fruiting branches per plant, respectively (Figure 2). They were followed in decreasing order of the number of fruiting branches by plants sprayed with KNO_3 salt (T3) and those sprayed with distilled water (T0), with 5.6 and 3.2 fruiting branches, respectively (Figure 3).

Number of flowers per plant

As was the case for the number of fruiting branches, analysis of variance revealed a highly significant difference in the number of flowers per plant after treatment, with a critical probability of $p < 1\%$. Plants receiving gibberellic acid (AG_3) had the highest number of flowers per plant (30.4 flowers), followed in descending order by plants treated with 2,4,5-T acid (24 flowers) and KNO_3 salt (19.8 flowers) (Figure 4). The lowest number of flowers was observed on plants in the distilled water treatment (5.1 flowers). The four treatments (T0, T1, T2 and T3) formed four (04) distinct homogeneous groups (A, B, C and D) based on the Newman-Keuls test with a threshold $p < 5\%$ (Figure 4).

Number of fruits per plant

The results of the analysis of variance of the number of fruits per plant 16 months (492 days) after the products were sprayed revealed a highly significant difference with a $p < 1\%$ between the treatments (Figure 5). Treatment T1 (plants treated with AG_3) formed the homogeneous group A with the highest number of fruits (26.7 fruits). Treatments T2 and T3 formed the homogeneous group B with numbers of fruits per plant of 21 and 18.2 fruits, respectively. The control treatment (T0) had the lowest number of fruits per plant (4.5 fruits).

Mean fruit diameter (cm)

A highly significant difference ($p < 1\%$) was observed between the mean diameters. Two distinct homogeneous groups (A and B) were identified using the Newman-Keuls test at the 5% significance level. The control (T0) which forms homogeneous group B was significantly different from the other three treatments which form group A (T1, T2 and T3). Average fruit diameter values ranged from 3.3 to 5.4 cm (Figure 6).

Mean weight of fruit (g)

The mean fruit weight also varied significantly ($p<1\%$) between treatments (Figure 7). Fruit from plants that were treated with hormones and salt had the highest mean fruit weight and were statistically equal, constituting homogeneous group A. The lowest mean fruit weight (13.6 g) was observed in plants treated with distilled water, forming group B.

Yield (kg/ha)

A highly significant difference was observed in yields ($p < 1\%$) between the four treatments 16 months (492 days) after product application. The highest yield was obtained by plants treated with both types of phytohormones (AG_3 and 2,4,5-T acid). These two treatments form the homogeneous group A, and are therefore not significantly different from each other according to the Newman-Keuls test at the critical threshold $p < 5\%$. Treatment T2 provided the highest yield at 27,685 kg/ha, whereas treatment T1 produced a yield of 25,835 kg/ha (Figure 8). A yield of 19,856 kg/ha was obtained by the plants that received the KNO_3 salt (T3) and formed the distinct homogeneous group B. The lowest yield was (10,861.33 kg/ha) observed when distilled water was applied (T0) (Figure 8). This treatment also forms another distinct homogeneous group C. Thus, significantly different from all the other treatments according to the Newman-Keuls test at the p < 5% threshold.

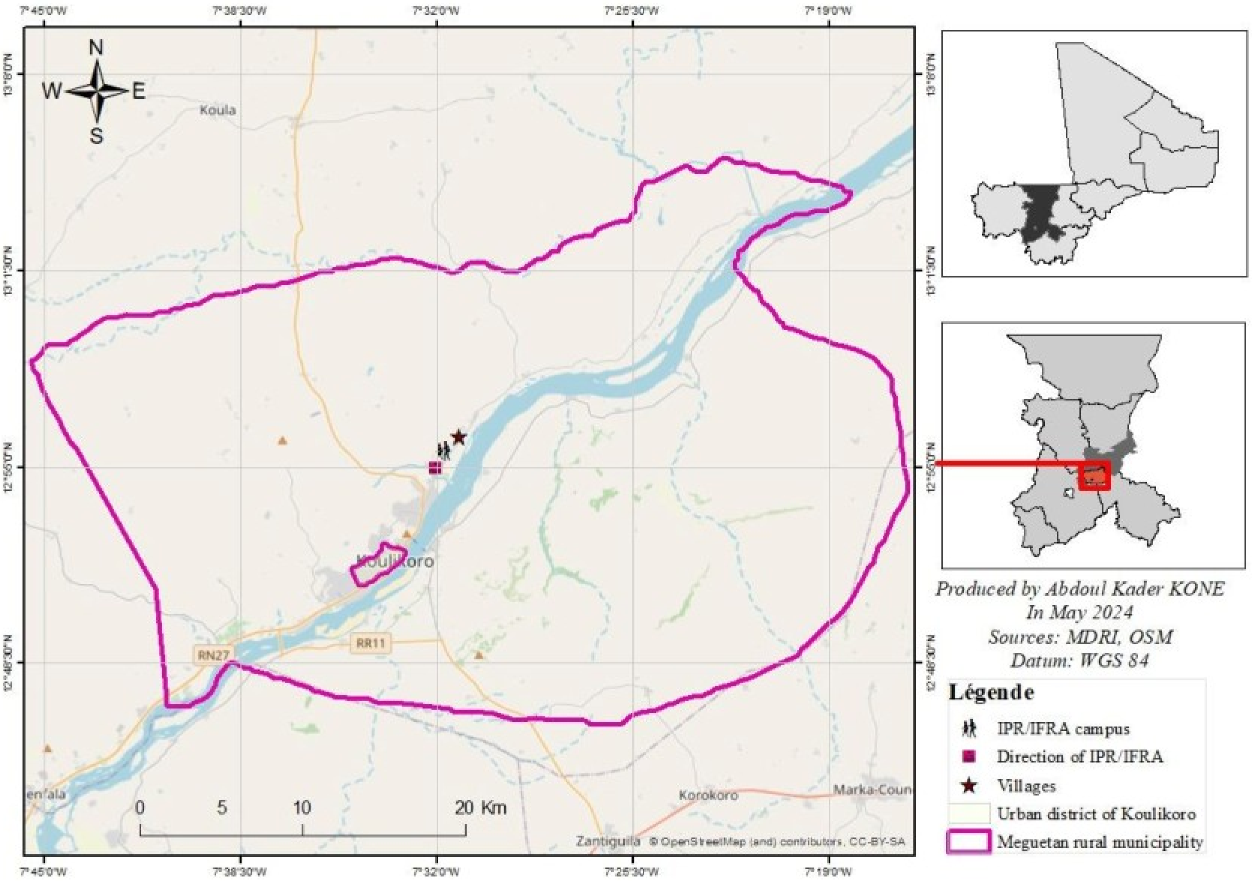

Figure 1: Geographical location of the IPR/IFRA Katibougou site in Mali

Table 1. Treatments and their concentrations

Treatment or product code	Product	Concentration
T0	Water	Distilled water
T1	AG3	2mg/l water
T2	2,4,5-T	5mg/l water (dissolved in 10ml of 70° alcohol)
T3	KNO_3	80g/l water

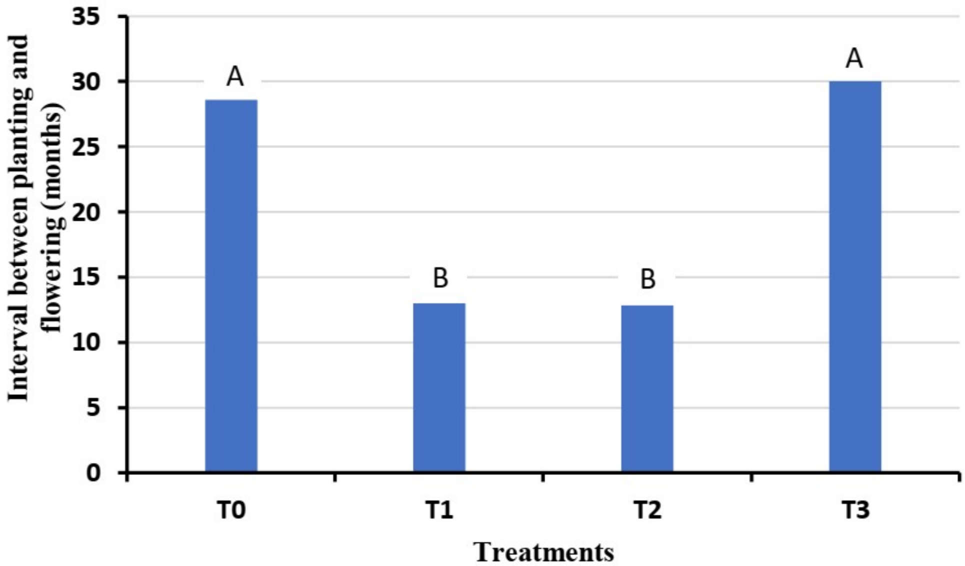

Figure 2: Variation in the length of the growing season according to treatments. (A, B, C, D etc.) on each histogram, the batons affected by different letters, are significantly different based on the Newman-Keuls test with a threshold $p < 5\%$.

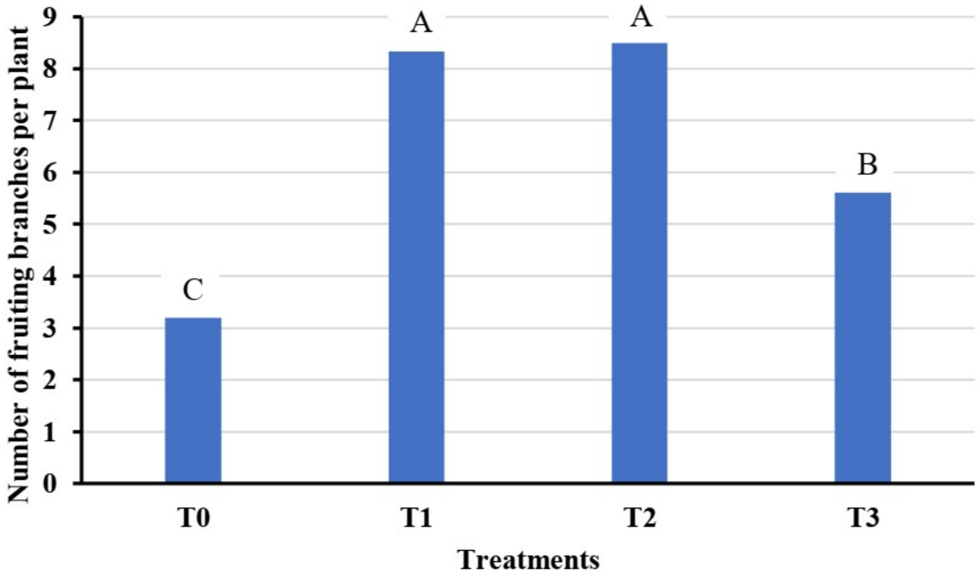

Figure 3: Variation in the number of fruiting branches per plant according to treatments. (A, B, C, D etc.) on each histogram, the batons affected by different letters, are significantly different based on the Newman-Keuls test with a threshold $p < 5\%$.

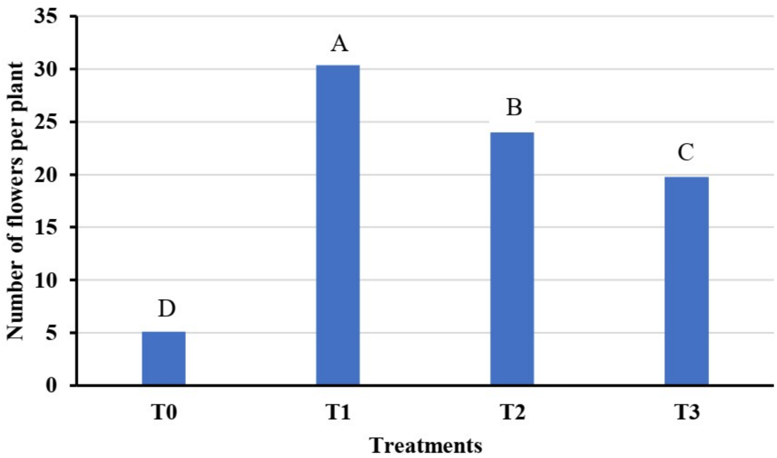

Figure 4: Variation in the number of flowers per plant according to treatments. (A, B, C, D etc.) on each histogram, the batons affected by different letters, are significantly different based on the Newman-Keuls test with a threshold p < 5%.

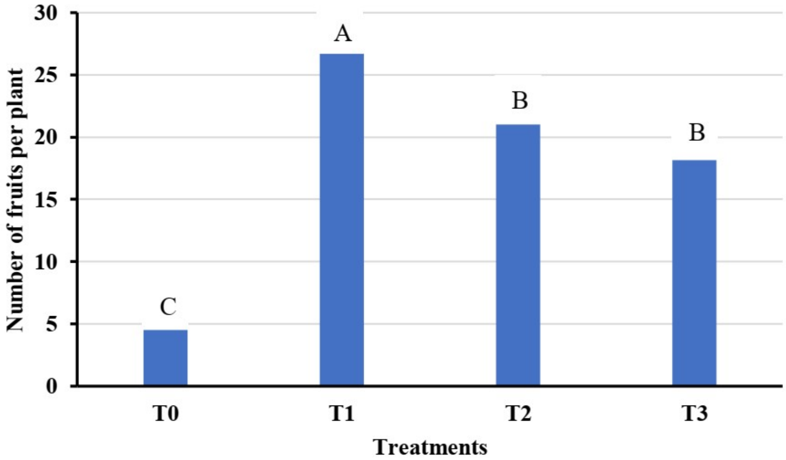

Figure 5: Variation in the number of fruits per plant according to treatments. (A, B, C, D etc.) on each histogram, the batons affected by different letters, are significantly different based on the Newman-Keuls test with a threshold p < 5%.

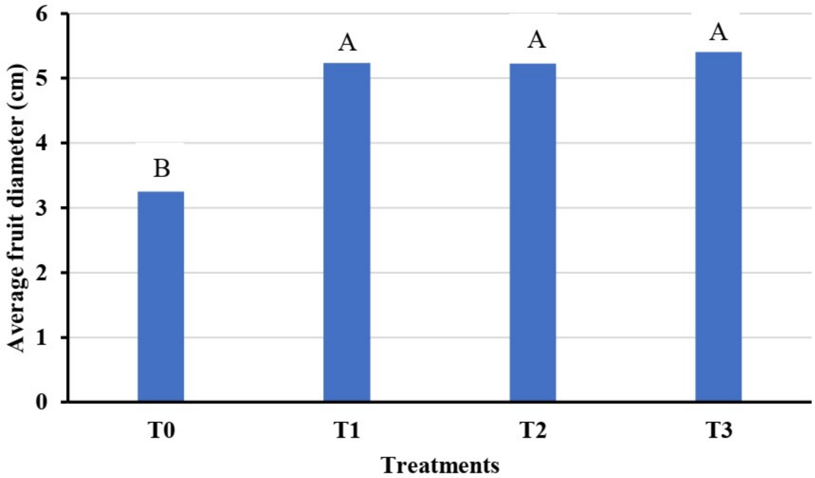

Figure 6: Variation in mean fruit diameter according to treatments. (A, B, C, D etc.) on each histogram, the batons affected by different letters, are significantly different based on the Newman-Keuls test with a threshold p < 5%.

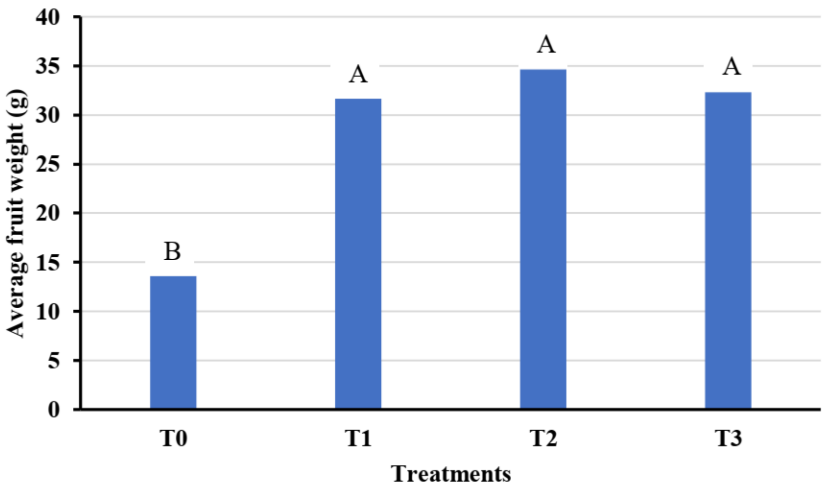

Figure 7: Variation in mean fruit weight according to treatments. (A, B, C, D etc.) on each histogram, the batons affected by different letters, are significantly different based on the Newman-Keuls test with a threshold $p < 5\%$.

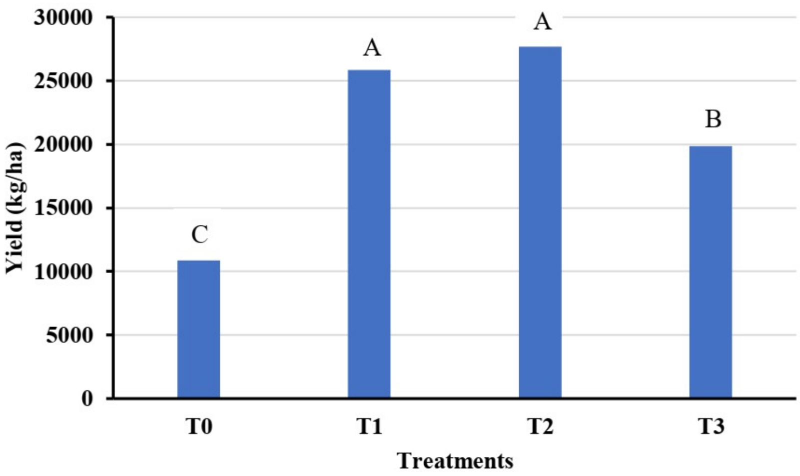

Figure 8: Variation in yield according to treatments. (A, B, C, D etc.) on each histogram, the batons affected by different letters, are significantly different based on the Newman-Keuls test with a threshold $p < 5\%$.

DISCUSSION

According to Carter *et al*. (2022), hormones have an important effect on the growth, flowering and maturation processes of plants. The results of an analysis of the delay between planting and flowering showed that plants treated with the hormones AG_3 and 2,4,5-T acid produced flowers early. The result of this analysis is in contrast to the work of Mehraj et al (2013), where gerbera seedlings that were not sprayed with any phytohormone (GA_3) took fewer days for the initiation of the first floral bud compared to seedlings that were sprayed with one spray at 15 days after transplanting, two sprays at 15 and 30 days after transplanting, and three sprays of GA_3 at 15, 30 and 45 days after transplanting. The GA_3 sprays were therefore interpreted as delaying flowering. But the result is confirmed by the work of other researchers on other crops. Nitsch (1977) states that auxins have a clear influence on plant flowering, and the results of his work show that foliar spraying of young figs with 2,4,5-T acid (25 mg/l) brings forward ripening and harvesting by one month. A simple spray of naphthalene acetic acid (5-10 mg/1) at a rate of 60 g of pure product per hectare triggers flowering two months later. This practice has completely revolutionized pineapple growing, making it possible to regulate flowering at will and stagger the harvest throughout the year. According to Py (1952), by spraying very dilute solutions of hormones onto the pineapple's foliage, it is possible to induce flowering within a specific period of time, thus moving the natural flowering date forward, which obviously has the effect of moving the harvest date on the treated plants. The analysis did not reveal any significant difference between plants treated with KNO_3 and distilled water with regard to the delay between planting and the emergence of flowers on the plants. This suggests that salt does not affect the earliness of flowering in Tangelo Orlando. In contrast, Afiqah *et al.* (2014) obtained a high rate of flower induction on five-year-old mango trees sprayed with KNO_3 at 2% and 5%. The best results were observed in response to KNO_3 at 2% in the spray solution, on shoots of five-year-old trees. Traoré et al (2023) reported that application of 2,4,5-T to Tangelo at the seedling stage improved vegetative growth which induced early flowering.

The greatest number of fruits was observed on plants treated with the hormone AG_3. Gibberellin has been identified in previous studies as a fruit set promoter (Hield *et al.*, 1958; Krezdorn, 1969).

This study revealed that fruit weight and diameter increased with the application of both hormones (AG_3 and 2,4,5-T acid) and salt (KNO_3). Hield et al (1958) and Krezdorn (1969) have shown that the increase in fruit weight and diameter depends partly on the application of hormones and salt, which the plant needs very much. Ontario (2021) improved fruit size by stimulating cell division shortly after flowering in the early stages of fruit formation and development with MaxCel or Cilis Plus (1.9% or 2.0%, respectively, of 6-benzyladenine (6-BA). El-Otmani et al (1992) as well as Rensburg et al (1996) showed that the use of AG_3 improved fruit weight resulting from the delay or inhibition of premature fruit drop and consequently an increase in fruit set. On the other hand, Py (1952), in a study of hormones in pineapple cultivation, reported that the weight of fruit obtained following hormone treatment was generally lower than that of fruit that would have been harvested several months later if no treatment had been applied.

These findings also show that the plants treated with the two hormones (AG_3 and 2,4,5-T acid) produced the highest yields per hectare: 27 685 kg/ha for plants treated with 2,4,5-T acid and 25 835 kg/ha for AG_3. These yields are higher than the national average for Mali, which was 19,300 kg/ha in the field during the 2018 - 2019 season (DNA, 2019), and also higher than those for Burkina Faso, according to Sanou (1993).

CONCLUSION

Phytohormones and mineral salts are chemical substances that can affect plant growth and development, even at low concentrations. This study aimed to identify the effects of phytohormones (AG_3 and 2,4,5-T acid) and salt (KNO_3) on development and reproduction in Tangelo Orlando citrus fruit. Phytohormones (AG_3 and 2,4,5-T acid) had a greater influence on the early induction of flowering in Tangelo plants than salt (KNO_3) and distilled water.

The application of gibberellin (AG_3) increased the number of fruits per plant. However, the effects of the 2,4,5-T acid in KNO_3 were less considerable but greater than those of distilled water. Fruit weight and diameter improved with the application of phytohormones and salt, compared with the use of distilled water. The best yields were obtained with the application of both phytohormones.

Citrus growers in the Sudano-Sahelian zone of Mali have the ability to employ 2,4,5-T acid and AG3 in their orchards to induce early flowering and ripening, thereby enhancing the yields of young Tangelo Orlando plants.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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