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The Effect of Plant Growth Regulators on Physico-Chemical Properties of Safflower (*Carthamus tinctorius* L.) Derived Biodiesel

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Abstract

Global concerns about the environmental impact of combustion emissions from petroleum fuels influence new research to seek for alternative energy sources. The current study investigates the possibility of using safflower (*Carthamus tinctorius* L.) as an alternative biodiesel raw material. Four plant growth regulators (PGR) were used to boost the production of safflower. Thirteen treatments were constituted from the four plant regulators and applied to the safflower crop arranged in completely randomised design, repeated three times. The results show that the effect of plant growth regulators was not more than that of the control. More studies have to be channelled towards the relationship between safflower and plant growth regulators.

Keywords

Safflower, Biodiesel Plant Growth Regulators

1. Introduction

The dwindling oil reserves and the environmental impact of burning fossil fuels that cause climate change are major global issues [1]. Countries around the world have developed biodiesel policies to increase energy security, promote rural development and reduce carbon emission [2]. The policies emphasis the use of oil bearing trees for the production of biodiesel. Biodiesel production comes with many challenges which includes: high initial cost of establishing the plantations [3]; the use of agricultural land and labour dedicated to food production [4] and well-known crops such as jatropha, safflower and croton have characteristically low seed and oil yields [5]. Research programs are needed to evaluate and improve crops suitable for biodiesel production. The current study investi-

gates the possibility of using safflower (*Carthamus tinctorius* L.) as an alternative biodiesel raw material. The plant was chosen for its good characteristics such as winter and summer cultivation, short maturation period and drought tolerance. However, the crop still has low oil quality and yield which needs to be improved using plant growth regulators.

2. Materials and Methods

2.1. Site Description

The project was carried in a farm in Morwa village, in Kgatleng district, Botswana. The village is on the northern side of capital city, Gaborone, along A1 road. The area is located at latitude 24.33.40S, longitude 25.56.37E and an altitude of 992 m above sea level. Kgatleng district is categorized as semi-arid and receives an average annual rainfall of 457 mm [6]. The temperature in the study area averages a maximum of 35˚C in summer and a minimum of 5˚C in winter. The area experiences occasional extreme weather conditions such as heat wave and frost [7].

2.2. Experimental Design

Thirteen (13) plots were planted with safflower and replicated three times and sets of experiments were planted and named as batch 1 and batch 2. The experiment was set up in triplicate in randomized complete design (CRD). Four plant growth regulators were used and each regulator had three rates or levels. Safflower was subjected to the following treatments: control (without plant growth regulator), PGR A1 represents the first class of plant growth regulator A; PGR A2 represents the second rate of plant growth A and PGR A3 represents the third rate of plant growth regulator A. (**Table 1**).

2.3. Formulation of Plant Growth Regulators

Four plant regulators, namely maleic hydrazine (MH), N6-benzyladenine (BA) 2,3,5-triiodobenzoic acid (TIBA),and kinetin were obtained. Three milliliters of 0.1 M sodium hydroxide was used to solubilize the PGRs before adding water and 2 ml of Tween 20 was added to act as a surfacant. MH had the following rates 1, 2, 4 µM, BA 3, 6 and 9 Mm, Triiodobenzoic acid 0.5, 1.0 and 1.5 Mm and kinetin 10, 20 and 40 mg/l. The plants were each fully sprayed with an equivalent solution and the control was treated with water treated with 0.1 M sodium hydroxide only. A hand sprayer was used to spray the plants. A clear plastic was used to cover other plants not being sprayed at the time to avoid chemical drift.

2.4. Planting of Safflower Crop

The seeds were simply drilled during planting and seedlings were thinned out to leave on plant per hole. Thirteen treatments were randomly administered to the plants. The treatments were applied during flowering to allow the effect of plant growth regulators to kick in as oil accumulates during seed formation [8].

Table 1. Presentation and description of the treatments.

Treatments codes	Description
T1 (PGR A1)	Meleic hydrazide at 1 μM
T2 (PGR A2)	Meleic hydrazide at 2 μM
T3 (PGR A3)	Meleic hydrazide at 4 μM
T4 (PGR B1)	Benzly adenine 3 mM
T5 (PGR B2)	Benzly adenine 6 mM
T6 (PGR B3)	Benzly adenine 9 mM
T7 (PGR C1)	2.3.5 Triidobenzoic acid 0.5 mM
T8 (PGR C2)	2.3.5 Triidobenzoic acid 1.0 mM
T9 (PGR C3)	2.3.5 Triidobenzoic acid 1.5 mM
T10 (PGR D1)	Kinetin 10 mg/l
T11 (PGR D2)	Kinetin 20 mg/l
T12 (PGR D3)	Kinetin 40 ml/l
T13	Control without treatment

2.5. Harvesting and Threshing of the Safflower

Harvesting was done, when plant reached physiological marurity, by cutting the branches with safflower capitulum and packaged in 50 kg bags. The bags were stored in an old greenhouse structure to allow the crop to dry completely. During harvesting, it is necessary to wear thick gloves to avoid being pricked by small thorns that are located throughout the body, including the leaves and capitulum of the safflower plant. Threshing was done with a short, thick stick by hitting the outer part of the sack until all the twigs and capitulum were broken open. Winnowing was done to separate seeds from the broken branches and leaves.

2.6. Extraction of Oil

The oil was obtained by chemical and mechanical extraction methods. Chemical extraction was mainly performed to determine oil yield in seeds while mechanical extraction was used to generate quantities of oil for later testing [9]. Oil yield was quantified using filter bag technology according to American Oil Chemists' Society (AOCS) standard method Am 5-04 and an Ankom extraction apparatus. At the beginning of the procedure, petroleum ether was charged as the solvent.

2.6.1. Chemical Oil Extraction

Dried safflower seeds were ground to powder form (<2 mm). A labelled filter bag was weighed, 1 - 2 g of ground seed samples were weighed into the labelled filter bags and the weight noted (W_1). The filter bags were heat sealed within 4 mm to encapsulate the sample. The sealed samples were placed in an oven set at 105˚C for 3 hours. After drying, the samples were cooled in a desiccant bag, then weighed (W_2). Samples were placed in a bag holder or carousel and placed in an

extractor. The extraction time was 60 minutes and the samples were then placed in the oven for 15 - 30 minutes, the samples were cooled in the desiccant bag, the weight (W_3) of the samples was taken. The oil yield was calculated using Equation (1).

$$\%\ \text{oil yield} = \frac{W_2 - W_3}{W_1} \times 100 \quad (1)$$

W_1 was original weight of the sample.

W_2 is the weight of the sample + weight of filter bag after oven drying.

W_3 is the weight of the sample after extracting + weight of filter bag after extracting.

2.6.2. Mechanical Oil Extraction

Fully dried seeds were cold-pressed using an oil extraction machine model BGC-T15. A hopper was filled with the dried seeds and the machine cold pressed the seeds to extract the crude oil. The machine was able to separate oil from the seed kernel and what was left was seed cake. The extracted oil was used in other analyses and the cake was used as animal feed.

2.7. Determination of the Fatty Acid Methyl Esters (FAME)

Safflower oil was converted into biodiesel through a process called transesterification [9]. The biodiesel was then analysed for fatty acids methyl esters using Gas Chromatography-Mass Spectrometry (GC-MS) following test method ASTM D6584 as described by [10] [11] [12]. Helium was used as the gas to a pressure of 72 kpa at a flow rate of 64 ml/min as specified by the manufacturer. 1 µl of the FAME was injected into an automated injector and the injector was set to 325˚C. The GC-MS was allowed to run for 36 minutes for each sample.

2.8. Transesterification of Safflower Seed Oil

Transesterification is a process in which reactions between organic classes result in one ester being converted into another by exchanging the alkoxy moiety [13]. Transesterification of oil from the safflower plant followed a method proposed by [9] [14]. Sodium hydroxide (7.5 g) was dissolved in 300 mL of methanol to produce a solution called methoxide, and the methoxide corresponds to 1 L of preheated (105˚C) safflower oil. The reaction was carried out in a Pyrex bottle with a capacity of 500 ml; 250 ml of safflower oil was preheated to 105˚C for 10 minutes, then cooled to 50˚C, the methoxide was poured into the oil and the solution was placed onto a heater, Corning PC-620D, which has a magnetic stirrer control mode. The reaction was carried out under a magnetic stirrer and the Pyrex bottle was connected to a condenser, the temperature was kept at 60˚C for one hour. The condenser was connected to a water pump placed in a cool box with ice blocks. The reaction apparatus is shown in **Figure 1**.

After the reaction, the solution was poured into a separator funnel and the formation of two layers started **Figure 2**. The top layer consisted of crude biodiesel, residual catalysts, water, unreacted alcohol, free glyceryl acids, and soaps,

while the bottom layer consisted of alcohol phase and glycerine. The upper layer was distilled at 60˚C to produce methanol through a condenser. The process was continued until alcohol stopped dripping from the condenser (Figure 3). The diesel layer was further washed with warm water or soft water (slightly acidic) to remove the impurities. Washing was done by gently stirring with a plastic spatula. The water was separated from the diesel with a separator funnel. The washing step was repeated until the water phase was clear and then separated from the biodiesel. The remaining water was removed by air drying the biodiesel.

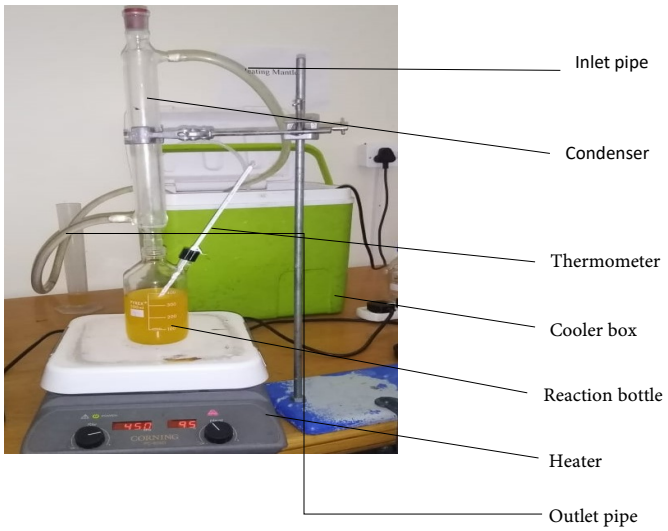


Figure 1. Transesterification apparatus set up.

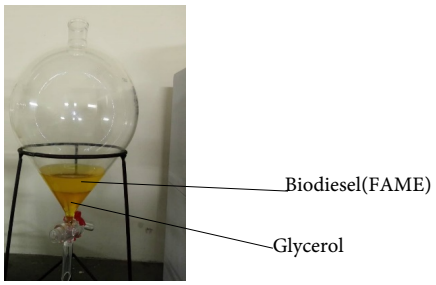


Figure 2. Separating funnel for separating biodiesel and glycerol.

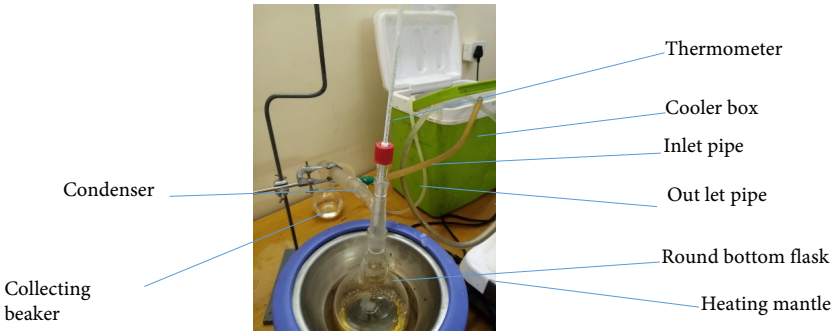


Figure 3. Distillation process for separating methanol from biodiesel.

2.9. Quality Analyses of Biodiesel Fuel

The safflower derived biodiesel was analysed to test for compliance with key international biodiesel standards ASTM and EN14214. Analysis of the fuel properties were carried out on selected physico-chemical properties which included the flash point, cloud point, water content, viscosity, density and pour point [15].

2.10. Determination Flashpoint (FP)

The flash point of a safflower biodiesel was determined by an automated closed up tester method, ASTMD92, ISO 13736, ISO 1516/1523, IP170 [16] as described by [16] and is as follows, automated Pensky-Martens closed cup flash point tester APM-8fc was used. The test cup was filled with 75 mL of oil sample and the cup was closed with a test cover and placed in the assembly, ensuring that the locking groove was engaged. The temperature of the test cup and test specimen was kept at least 18˚C below the expected flash point. The test flame was switched on and the oil was heated at a rate of 5 - 6˚C/minute. The machine displayed a green screen and a temperature figure to indicate the flash point of a sample and the temperature was then recorded.

2.11. Determination of Cloud Point (CP) and Pour Point (PP)

Cloud point and pour point were determined using Huazheng Electric Manufacturing, Baoding, Hebei, China machine according to ASTM D2500 and ASTM D97 respectively. A sample was injected into the dry and clean test tube to a mark. A thermometer was fixed in the center of the test tube with a plug, ensuring that the thermometer and the test tube were on the same axis, and the mercury ball of the thermometer just contacts the bottom of the test tube. The test tube was then put in a casing which was cooled for 10minutes. The test tube in a casing was placed in a testing hole. When the thermometer reading of the observation tube droped by 1˚C, the test tube was taken out of the hole quickly without stirring the sample, cloud point was checked.

2.12. Determination of Water Content (WC)

The presence of water in biodiesel fuel promotes biological growth in storage tanks, which can lead to corrosion of some metals such as copper, iron and steel [17]. Water content was measured according to ASTM D-2709 and was also limited to 0.05% by volume [18]. The water content was measured using the HI 904 kilometric Karl Fischer titrator machine.

2.13. Determination of Density

Density was measured according to ASTM-D1298, limited to 860 - 900 kg/m^3. Density was measured with an instrument called KEM Kyoto electronics density meter. Density measurement was carried out by filling the cell with sample then recording the reading from the display screen. Three repeats were carried out for each sample then calculating the average value. The cell was frequently cleaned

using ethanol before measuring a different sample then allowed to dry for 30 minutes as specified by the manufacturer.

2.14. Viscosity

Viscosity of biodiesel was determined using a manual viscometer in accordance to ASTM D445 IP 71) [19]. A Tamson TV 2000 visual bath was filled with water and set at 40˚C. A viscometer was placed in the water bath to match the temperature of the bath. For testing, the viscometer was filled with a biodiesel sample. The sample was allowed to flow and the time required for the sample to flow through the viscometer was measured in seconds. The measurement was repeated three times and an average was calcculated. The mean and the calibration constant of the viscometer were used to calculate the viscosity of the samples.

2.15. Energy Content (EC)

Energy content is a description of the potential of a chemical substance to undergo a chemical reaction and transform into other substances. A standard ASTM D240 test method was developed to measure the energy content of liquid fuels by burning a weighed sample of the fuel in the presence of oxygen in a calorimeter. The bomb calorimeter used was 3k-1. The energy content was measured in mass units, mega joules per kilogram (MJ/Kg) [20].

3. Results and Discussions

3.1. Fatty Acids Composition of Batches 1 and 2 Safflower Oil

The fatty acids results were derived from the two batches of safflower which were subjected to 13 treatments.

The results presented in **Table 2**, **Table 3** and **Figure 4**, **Figure 5** show the effect of plant growth regulators on the fatty acid composition of biodiesel derived from two batches of safflower. The fatty acid composition is an important characteristic in biodiesel production. Biodiesel properties are determined by the amount of each fatty acid present in the biodiesel fuel sample [21]. The results presented in **Table 2**, and **Table 3** show that the sample contains a greater amount of polyunsaturated fatty acids than monounsaturated. High levels of polyunsaturated fatty acids tend to show poor oxidization stability and can affect fuel properties such as viscosity [22].

The fatty acids of the safflower batches were analysed and the results presented in **Table 2**, **Table 3** and **Figure 4**, **Figure 5** indicate that linoleic acid dominates, followed by oleic acid. Linoleic acid is a polyunsaturated fatty acid that can affect the properties of biodiesel. It is one of the fatty acids responsible for the poor oxidation stability of oil, which can lead to deposit formation and corrosion in engines [23]. In treatment 11 there is a maximum of 80% linoleic acid, in treatment 2 of batch 1 there is a minimum of 60.2%. In Batch 2 shown in **Table 3**, Treatment 8 had the highest linoleic acid content at 79.6% and Treatment 6 had the lowest at 60.3%. Fatty acids can affect fuel properties in a variety of

Table 2. Batch 1 of Fatty acid composition of crude safflower treated with various levels and types of plant growth regulators.

	Linoleic acid $C_{19}H_{34}O_2$	Oleic acid $C_{19}H_{36}O_2$	Palmitic $C_{17}H_{34}O_2$	Stearic acid $C_{19}H_{38}O_2$	Vaccenic acid $C_{18}H_{34}O_2$	Eicosadienoic acid $C_{20}H_{36}O_2$
Treatments						
1	65.2	17.1	9.1	3.6		
2	60.2	7.5	2	0.9		
3	66.8	15.7	8.7	3.4		
4	67.6	10.3	8.4	2.3		
5	68.7	14.2	9.2	3		
6	71	9.2	15.5	4.5		
7	67.2	14.5	8.9	3.5		
8	65.6	11	6.4	1		
9	8.8	13	8.6	2.7		63.6
10	1.6	15.8	9.5	3.3		68
11	80	-	6.4	1.7	11.9	
12	42.3	8	4.7	1.6		
13	62	13.8	8.2	13.8		

Table 3. Batch 2 of Fatty acid composition of crude safflower treated with various level and types of plant growth regulators.

Fatty acid with their	Linoleic acid $C_{19}H_{34}O_2$	Oleic acid $C_{19}H_{36}O_2$	Palmitic $C_{17}H_{34}O_2$	Stearic acid $C_{19}H_{38}O_2$	Vaccenic acid $C_{18}H_{34}O_2$	Eicosadienoic acid $C_{20}H_{36}O_2$
Treatments						
1	61.6	28	8.4	2		
2	52.1	-	-	2.1		
3	73.6	16.4	8.6	1.2		
4	61.5	28.2	8.5	0.9		
5	66.1	25.4	5.5	0.5		
6	60.3	7.7	8.6	0.1		
7	65.6	25	8	0.1		
8	79.6	12.7	8.1	2.1		
9	64.8	12.5	7.3	0.2		
10	66.1	14.3	6.7	2.4		
11	73.6	18.1	8.3	0.2		
12	61.8	-	8.1	2.7		
13	64.8	14.9	6,2	3		

ways, including their energy content, viscosity, and combustion characteristics [24]. The effect of maleic hydrazide, benzyl adenine, 2,3,5-triiodobenzoic acid and kinetin has shown a steady improvement on the linoleic fatty acid (**Figure 4**). In Batch 2, **Figure 5** shows that both the four growth regulators increased the

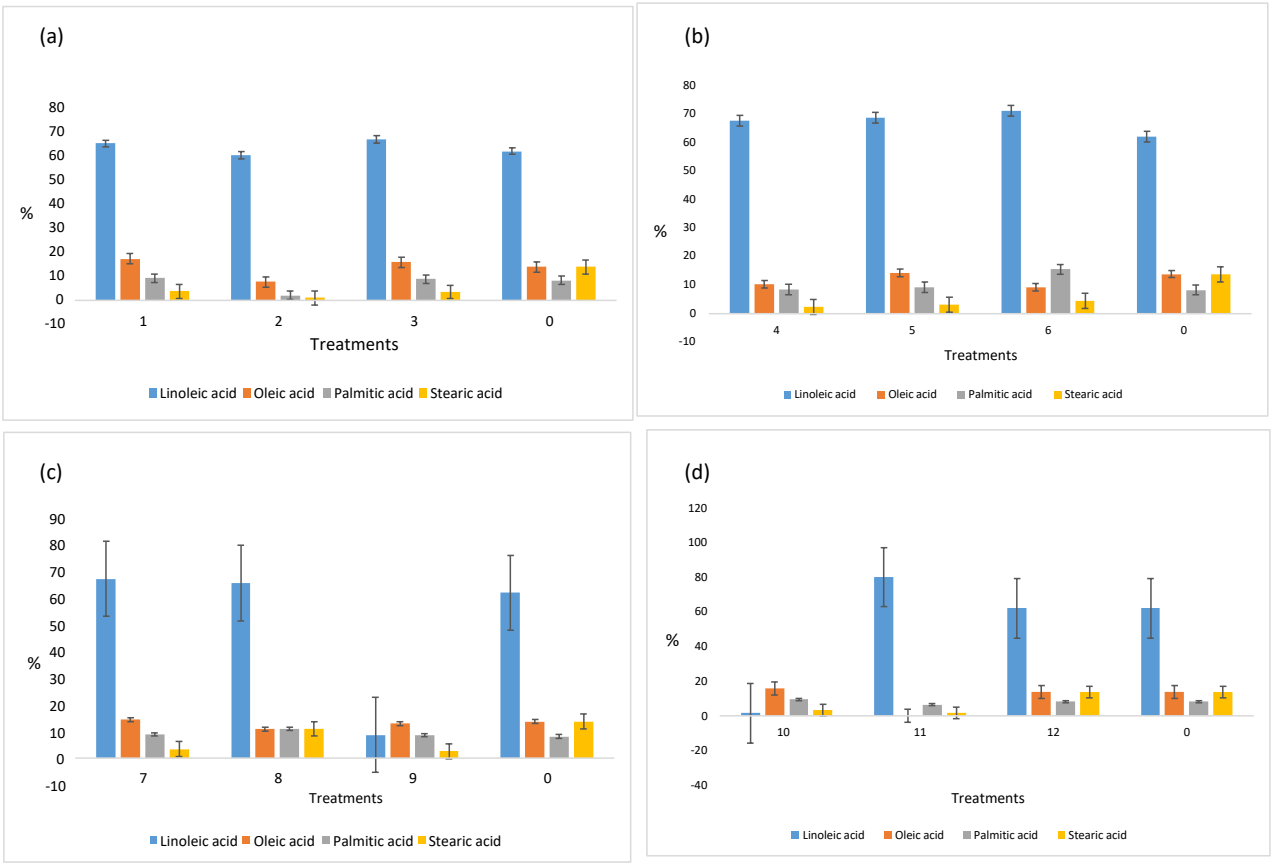
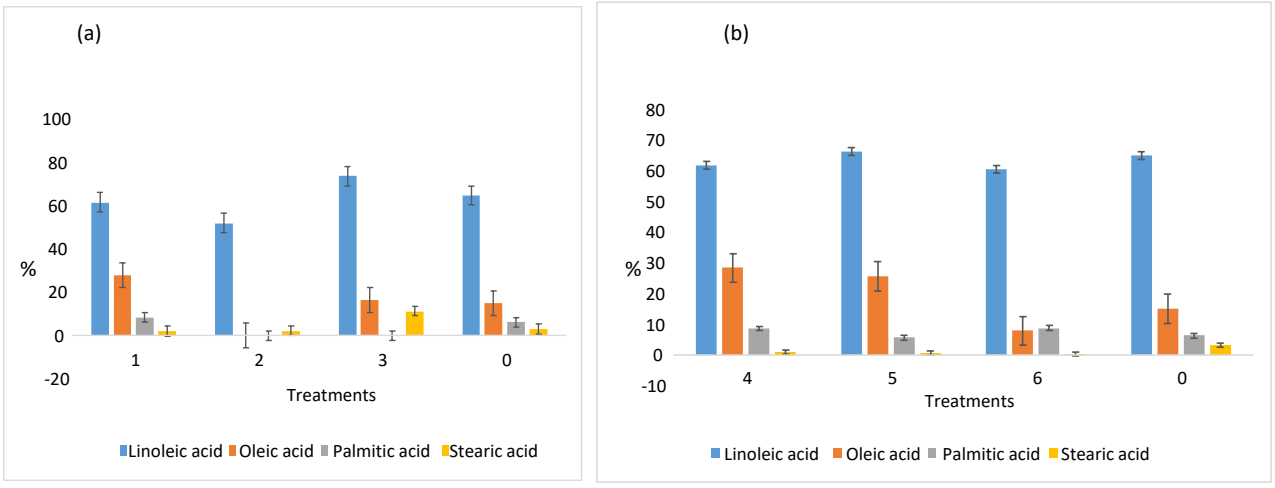


Figure 4. The effect of maleic hydrazide, benzyl adenine, triiodobenzoic acid and kinetin on the fatty acid compositionof batch 1 safflower. (a) The effect of maleic hydrazide on the fatty acids composition of safflower batct1; (b) The effect of benzyl adenine on the fatty acids composition of safflower batct1; (c) The effect of 2,3,5 triiodobenzoic acid on the fatty acids composition of safflower batct1; (d) The effect of kinetin on the fatty acids composition of safflower batct 1.



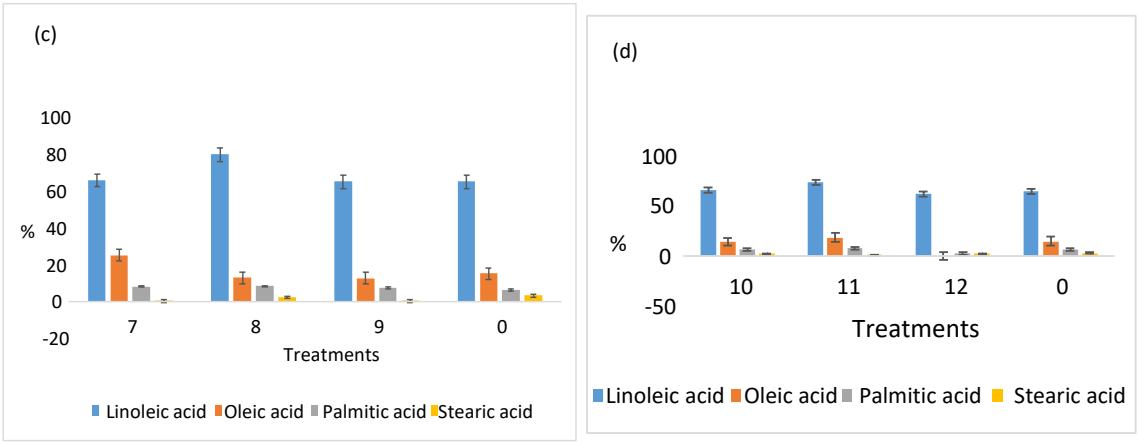


Figure 5. The effect of maleic hydrazide, benzyl adenine, triiodobenzoic acid and kinetin on fatty acid compositionbatch 2 safflower. (a) The effect of maleic hydrazide on the fatty acids composition of safflower batch 2; (b) The effect of benzyl adenine on the fatty acids composition of safflower batch 2; (c) The effect of 2,3,5 triiodobenzoic acid on the fatty acids composition of safflower batch 2; (d) The effect of kinetin on the fatty acids composition of safflower batch 2.

linoleic acid present in the safflower oil. The effect of plant growth regulators has been observed by other researchers such as [25] who observed that the application of auxins to the leaves significantly affects the fatty acid composition of safflower. Exogenous application of plant growth regulators has been observed to improve metabolic pathways in plants, which helps them with drought and stress tolerance [26].

3.2. Physicochemical Properties Results of Safflower Derived Biodiesel for Batch 1

Physico-chemical properties include flash point, moisture, viscosity, density and energy content. The results presented in **Table 4**, **Table 5** and **Figures 6-9** show that most properties meet international standards for biodiesel. The flash point shown in **Table 4** is between 101.6˚C and 133.4˚C. The action of plant growth regulators lowered the flash point compared to the control (133˚C). The international standard for the flash point of biodiesel is between 100˚C (D93) and 55˚C (TS EN 590) [27]. A fuel's flammability hazard is quantified by its flash point, which is the lowest temperature at which the fuel can vaporize and form an ignitable mixture in air [28]. It is measured according to international standard methods such as ASTDM and EN 590. This flash point test aims to ensure that the fuel is safe to handle [29].

The international standard for biodiesel density is between 860 and 900 kg/m^3 (ASTM D1298) [30]. There was no significant difference between the treatments in their effect on density at 900 kg/m^3 (**Table 4**). The density of biodiesel depends on the methyl ester concentration and the contamination of the biodiesel [31]. The energy content in batch 1 showed no significant difference between the treatments, but the energy content is within the international biodiesel standard, which is between 39 and 43.33 mJ/kg, while the petroleum diesel is at 49.6 MJ/kg [32]. Viscosity differed significantly between treatments, ranging from 4.3 mm^2/s

to 4.7 mm^2/s for treatments 2, 12 and 10. Viscosity results from all treatments are within the international standard for biodiesel, which is between 3.5 and 5.0 mm^2/s (ASTM D445). Viscosity was more pronounced with 2,3,5-triiodobenzoic acid and kinetin treatment (Figure 7(a), Figure 7(d)). Another notable effect of plant growth regulators was their influence on moisture content. Treatment 5 had the highest moisture content, 1.3%, and treatments 12, 10 had the lowest moisture content, 0.5%.

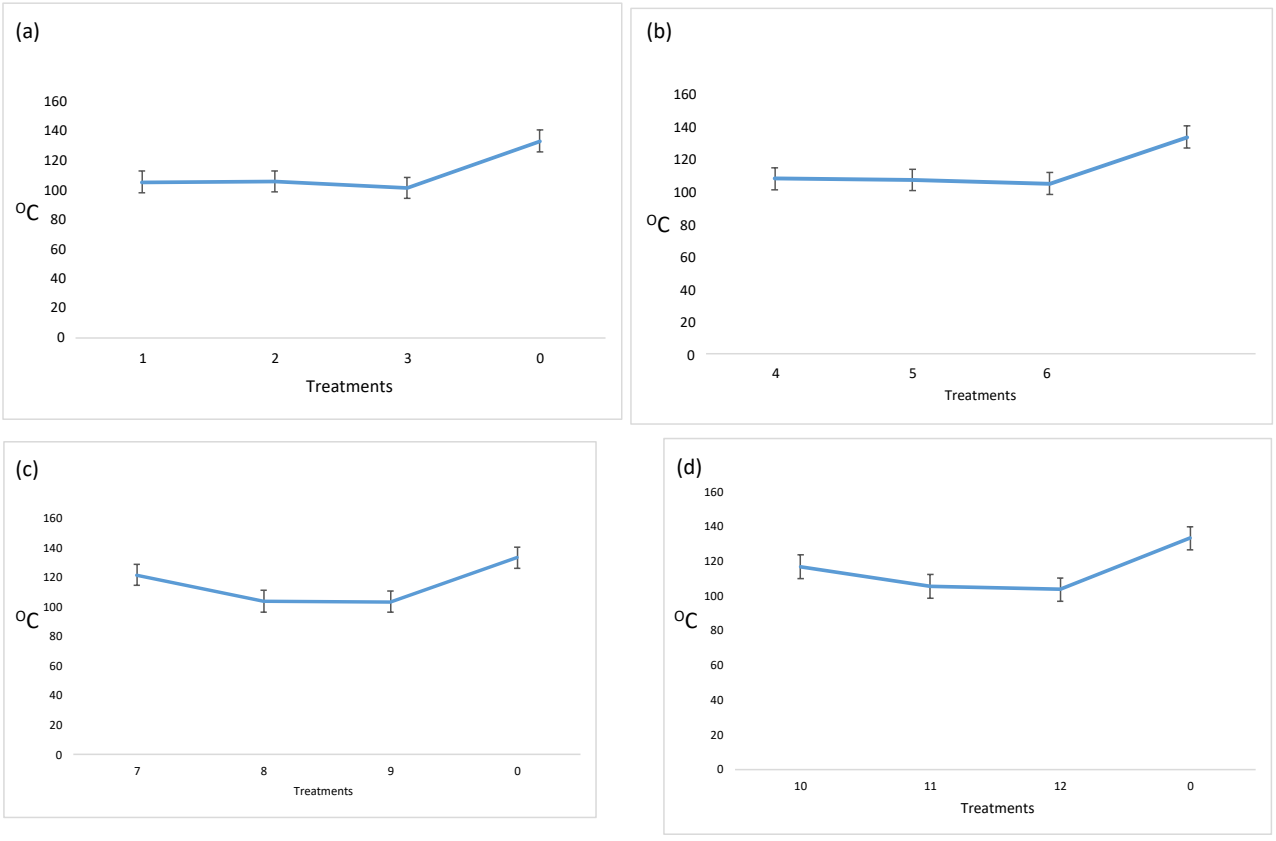
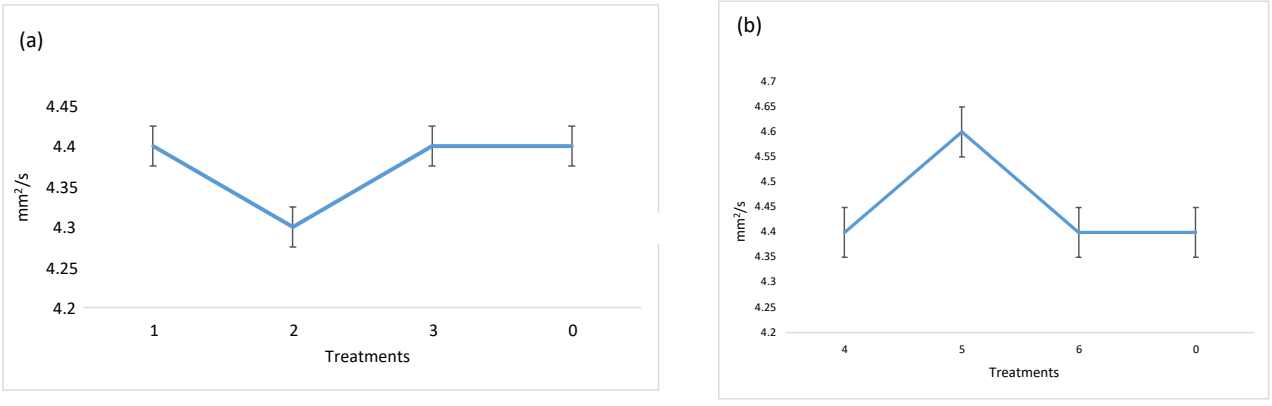


Figure 6. The effect of maleic hydrazide, benzyl adenine, triiodobenzoic acid and kinetin flashpoint of batch 1safflower. (a) The effect of maleic hydrazide on flashpoint of safflower biodiesel batch 1; (b) The effect of maleic hydrazide on flashpoint of safflower biodiesel batch 1; (c) The effect of 2,3,5 triiodobenzoic acid on flashpoint of safflower biodiesel batch 1; (d) The effect of kinetin of safflower biodiesel b on flashpointbatch 1.



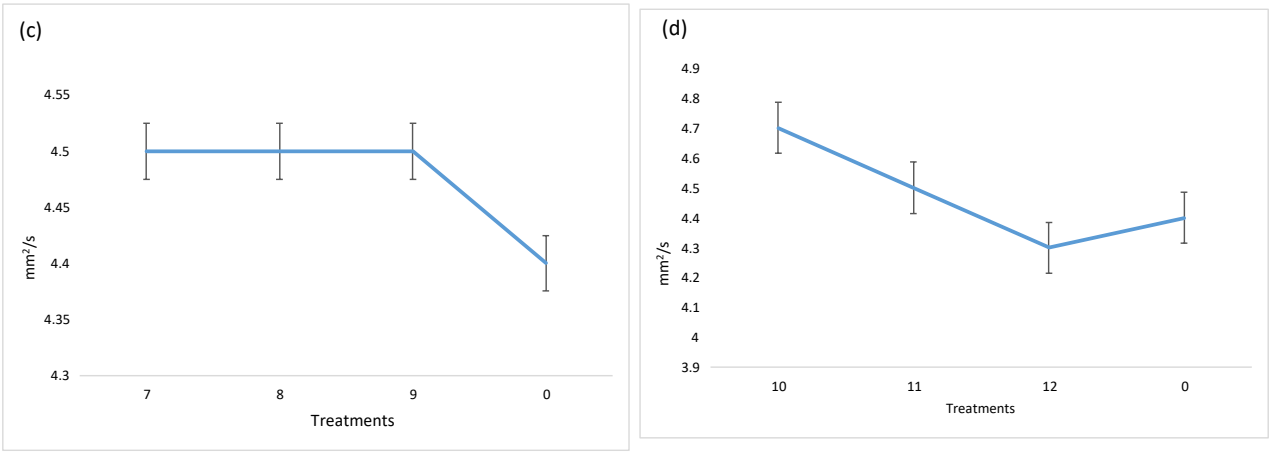


Figure 7. The effect of maleic hydrazide, benzyl adenine, triiodobenzoic acid and kinetin on viscosity of batch 1 safflower. (a) The effect of maleic hydrazide on viscosity of safflower biodiesel batch 1; (b) The effect of benzyl adenine on viscosity of safflower biodiesel batch 1; (c) The effect of 2,3,5 triiodobenzoic acid on viscosity of safflower biodiesel batch 1; (d) The effect of kinetin on viscosity of safflower biodiesel batch 1.

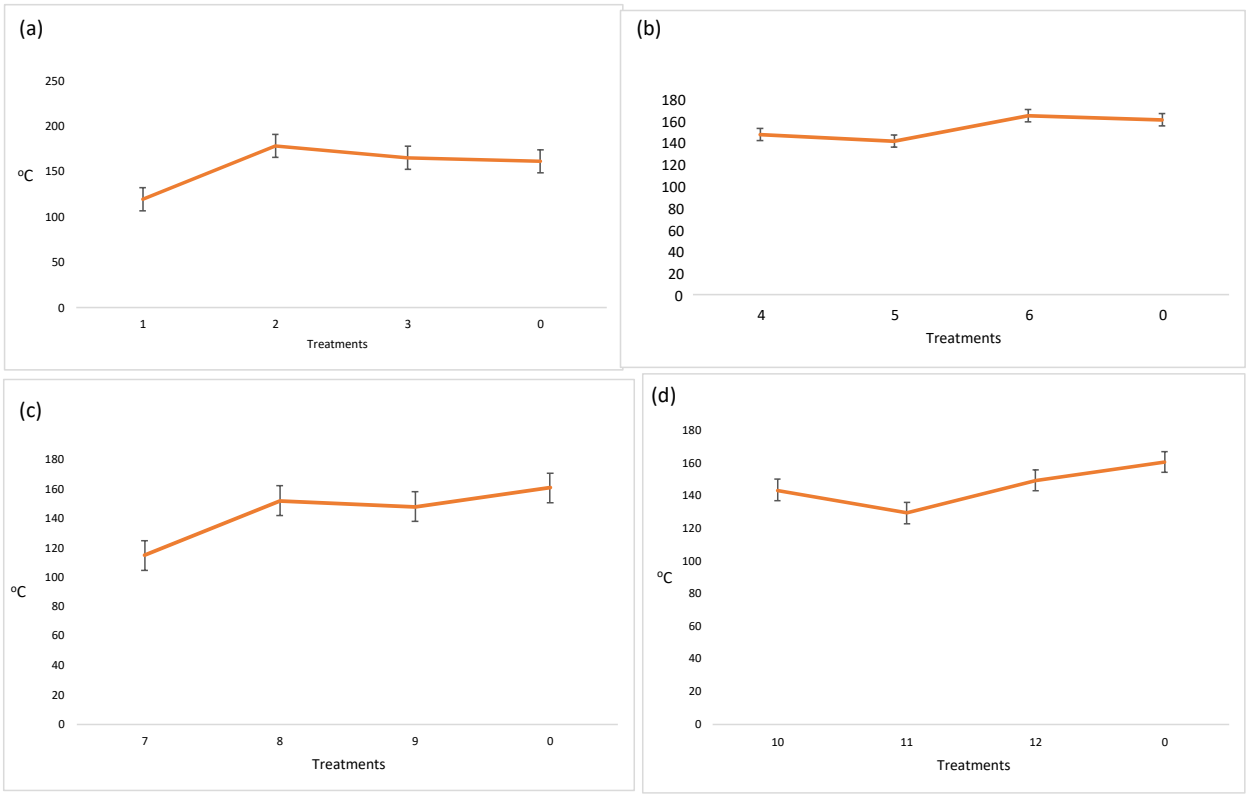


Figure 8. The effect of maleic hydrazide, benzyl adenine, triiodobenzoic acid and kinetin on the flashpoint of batch 2 safflower. (a) The effect of maleic hydrazide on flashpoint of safflower biodiesel batch 2; (b) The effect of benzyl adenine on flashpoint of safflower biodiesel batch 2; (c) The effect of 2,3,5 triiodobenzoic acid on flashpoint of safflower biodiesel batch 2; (d) The effect of kinetin on flashpoint of safflower biodiesel batch 2.

The cloud and pour points of safflower in batch 1 derived biodiesel in control treatment (treatment 13) are −3.3˚C and −12˚C respectively (**Table 4** and **Figure 10**). The results are different from those found by [33], their cloud and point

was −14˚C and −23˚C respectively. Unlike the current study [33] found that a two-step transesterification process of biodiesel resulted in high quality of safflower derived biodiesel with good fuel properties, including a low pour point and cloud point.

Table 4. Batch 1physicochemical properties of safflower derived biodiesel.

Treatment	Flashpoint ˚C	Moisture content %	Viscosity mm^2/s	Density g/cm^2	Energy MJ/KG	Cloud point ˚C	Pour point ˚C
1	105.6EF	0.6DCE	4.4AB	0.9A	39.4A	−2.6C	−11.0C
2	106.0D	0.8DC	4.3B	0.9A	39.4A	−7.3F	−12C
3	101.6C	0.8DC	4.4AB	0.9A	38.3B	−5.6D	−12C
4	107.9G	1.0B	4.4AB	0.9A	39.3A	−3.3C	−9.3B
5	107.9EF	1.3A	4.6AB	0.9A	39.5A	−1.3B	−8A
6	104.8EF	1.0B	4.4AB	0.9A	39.3A	0.0A	−8.6AB
7	121.7B	0.6DE	4.5AB	0.9A	39.5A	−2.6BC	−7AB
8	103.8EF	0.8DC	4.5AB	0.9A	39.3A	−4.6D	−9AB
9	103.5GF	0.8C	4.5AB	0.9A	39.4A	−5.3D	−11.6C
10	116.9C	0.5E	4.7A	0.9A	39.3A	−4.6D	−8.6AB
11	105.6EF	0.7DC	4.5AB	0.9A	39.4A	−6.3EF	−11.6C
12	103.8EF	0.5E	4.3B	0.9A	39.4A	−5D	−11.6C
13	133.4A	0.6DCE	4.4AB	0.9A	39.3A	−3.3C	12C

Treatments with similar letters are not significantly different. The Treatments are arranged in chronological order. Each figure was obtained after an average of three replicates.

Table 5. Batch 2 physicochemical properties of safflower derived biodiesel.

Treatment	Flashpoint ˚C	Moisture content (%)	Viscosity mm^2/s	Density g/cm^2	Energy Mj/kg	Cloud point ˚C	Pour Point ˚C
1	119.6D	0.7CB	4.6A	0.9A	39.1B	−4.6FG	−11.3AB
2	178.2A	1.4A	4.4A	0.9A	39.1B	−4.6FG	−11.3AB
3	164.8AB	0.5BC	4.6A	0.9A	38.4A	−4.3EFG	−9.3AB
4	147.4ABCD	0.8B	4.6A	0.9A	39.5A	−0.6AB	−8.6AB
5	141.3ABCD	0.7BC	4.7A	0.9A	39.2AB	−1.6C	−8AB
6	124.7BCD	0.7BC	4.4A	0.9A	39.2AB	−0.3A	−9AB
7	114.9D	0.6BC	4.5A	0.9A	39.3AB	−3.3DE	−11.6B
8	152.3ABCD	0.6BC	4.5A	0.9A	39.2AB	−2.6D	−12B
9	148.2ABCD	0.7BC	4.4A	0.9A	38.49D	−3.0D	−12B
10	143.7ABCD	0.5BC	4.5A	0.9A	38.6DC	−5.3G	−11.6B
11	129.8BCD	0.7BC	4.3A	0.9A	38.5DC	−3.6DEF	−12.3B
12	149.7ABCD	0.5BC	4.5A	0.9A	38.5D	−1.3BC	−11.6B
13	161.0ABCD	0.7BC	4.5A	0.9A	38.8C	−3.3DE	−9AB

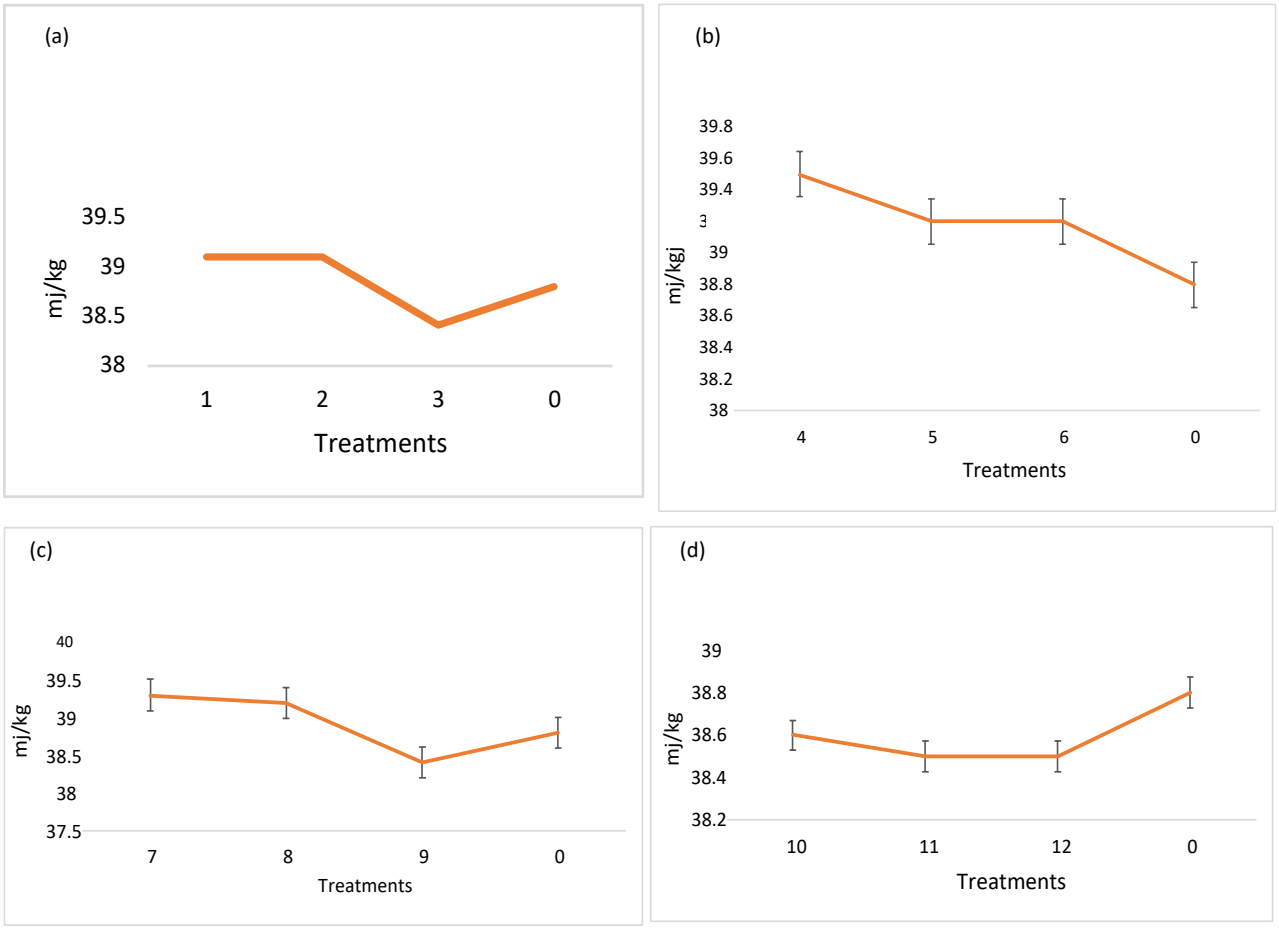


Figure 9. The effect of maleic hydrazide, benzyl adenine, triiodobenzoic acid and kinetin on the energy content of batch 2 safflower. (a) The effect of maleic hydrazide on energy content of safflower biodiesel batch 2; (b) The effect of benzyl adenine on energy content of safflower biodiesel batch2; (c) The effect of 2,3,5 triiodobenzoic acid on energy content of safflower biodiesel batch 2; (d) The effect of kinetin on energy content of safflower biodiesel batch 2.

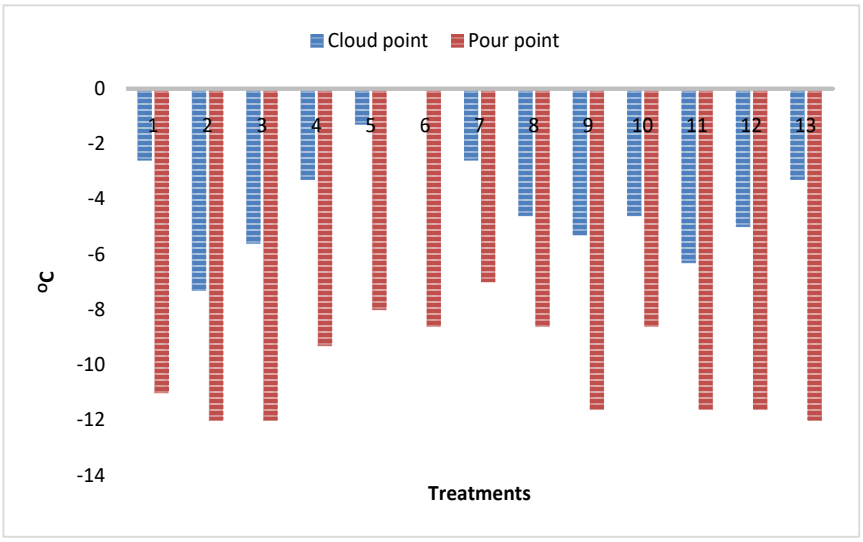


Figure 10. The effect of growth maleic hydrazide, benzyl adenine, 2,3,5 triidobenzoic acid and kinetin on cloud and pour points of safflower derived biodiesel in batch 1.

The effect of plant growth regulators on cloud and pour point were less than those of the control except for some few treatments such as treatment 2, 3, 9, 10 and 12 with their cloud points. However, all the results of the pour points are less than of the control excepts for few treatments which are equal to the control, Treatment 2 and Treatment 3 (**Table 5**). The effect of maleic hydrazide represented by treatment 1 to 3 is at par with the control. Cloud and pour points of biodiesel are largely influenced by many factors such as the feed stock, impurities in the vegetable oil, alcohol used and the amount of residual glycerine in the biodiesel [34]. In case of safflower oil, it is predominantly unsaturated fatty acids and the presence of unsaturated fatty acids results in the reduction of cloud and pour points of the biodiesel [35]. The results presented in **Table 4** and **Figures 3-8** of cloud and pour point derived safflower biodiesel batch 2 were lightly different, of notable difference is the pour point of treatment 13 which is the control it was up to −9˚C. The cloud point is similar to that one of batch 1. Just like the first batch the effect of treatment 1 to treatment 3 had a lower cloud and pour points. Treatment 7 to treatment 9 showed a lower pour point (**Table 4**). Treatment 1 to treatment 3 represents the effect of maleic hydrazide from lower concentration to higher concentration. There is not significant difference among those rates for both cloud and pour points in batch 2 though in batch 1 there was a significant difference in cloud point but not in pour point. Compared to other biodiesel fuels from different feed stocks, safflower has lower cloud and pour points, petroleum diesel has 6˚C pour point and jatropha biodiesel has 3˚C pour point [36]. Other feed stock such as sunflower, mustard and linseed oils have 7, −11 and −10 cloud point respectively, their pour points are as follows −8, −14 and −12 respectively [37].

In the safflower batch 2, some physico-chemical properties differed, e.g. viscosity and density, did not have a significant difference (**Table 5**). The biodiesel production process and the level of contaminants in the final product might have caused this inconsistence of the results [38]. Flash point, moisture content and energy content differed significantly (**Table 5**). The highest flash point was measured at 178.2˚C for treatment 2 and the lowest at 114˚C for treatment 7. The results show that all treatments were lower than the control (**Figure 8**). The international standard for the flash point of biodiesel is between 100˚C (D93) [27].

The treatments had a significant difference in their effect on the energy content of safflower biodiesel in batch 2 (**Table 5**). The energy content of biodiesel is determined by several factors, including the raw material used to make the biodiesel, the production process, and the level of contaminants in the fuel [39]. The energy content ranged from 38.4 to 39.5 in treatments 3, 9 and 4. The energy content results are within the international biodiesel standard, which ranges from 39 to 43.33 MJ/kg, while petroleum diesel is at 49.6 MJ/kg [32]. The energy content of all treatments was mostly lower than the control, with the exception of the effect of benzyl adenine (**Figure 9(b)**).

4. Conclusion

Plant growth regulators, particularly benzyl adenine and 2,3,5-triidobenzoic acid, increased the amount of linoleic acid in both batch 1 and batch 2. Further research can be conducted to include other plant growth regulators which may increase oil yield and quality.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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