



EFFECT OF PLANT GROWTH REGULATORS ON SAFFLOWER OIL YIELD

Charles Mazereku^{1*}, Jerekias Gandure², Clever Ketlogetswe³

1. Student Engineering, University of Botswana, Notwane, Gaborone, Botswana
2. Professor Engineering, University of Botswana, Notwane, Gaborone, Botswana
3. Professor Engineering, University of Botswana, Notwane, Gaborone, Botswana

Abstract

Countries around the world have begun developing strategies to encourage the use of alternative fuels and mitigate the negative impacts of fossil fuel use. The current study examines the possibility of using safflower (*Carthamus tinctorius* L.) as an alternative biodiesel feedstock by exposing it to different types and amounts of plant growth regulators to optimize oil production. Four common plant growth regulators were applied in three doses; Kinetin: 10 mg/L, 20 mg/L, and 40 mg/L; Benzyl adenine (BA); 3mM, 6mM and 9mM; Maleic hydrazine was applied in three doses: 1m, 2m and 4m; 2,3,5-Triiodobenzoic acid (TIBA) was used at three dosages: 0.5mM, 1.0mM and 1.5mM and as a control water was mixed with sodium hydroxide. These rates represented 13 treatments, repeated three times and designed in a fully randomized design, and two experiments were performed, batch 1 and batch 2. For individual plant growth regulators, maleic hydrazide had a descending effect from a lower concentration of plant growth regulator to a higher concentration in treatments 1-3. Benzyl adenine had no discernible effect on the 1000 grain weight of safflower seeds. The effect of 2,3,5-triiodobenzoic acid had an ascending effect, treatment 7, which had the lowest concentration, had the lowest 1000 seed weight and treatment 9 had the highest seed weight and highest concentration. All plant growth regulator results significantly increased 1000 seed weight at lower concentration compared to control but the oil content of maleic hydrazide and TIBA applied rates did not exceed that of control in either batches 1 and 2.

Keywords: plant growth regulators, safflower, biodiesel, alternative energy, biomass

1. Introduction

Safflower (*Carthamus tinctorius* L.) belongs to the family Asteraceae or daisy family. The genus *Carthamus* consists of 16 species and is a member of the subtribe Centraureinae, tribe Cardueae (thistles), subfamily Tubuliflorae [1]. *Carthamus tinctorius* L. is the cultivated species and has a chromosome number of $2n = 24$ [2]. It is a thistle-like annual herb with spiny leaves and bracts. It also has multiple branches, each branch terminating in a spherical flower head that can have various colors such as yellow, orange and red flowers [3]. Safflower is a crop that is grown in a variety of climatic conditions and on different types of soil. Available information indicates that safflower is grown at altitudes below 900 m above sea level [4]. Germination occurs after 3-8 days of sowing and germination speed is strongly determined by temperature [5].

Growing seedlings require a cool temperature of 15-20°C for proper root growth and rosette development and 20-30°C during stem elongation, flowering and seed formation [6]. Safflower can be planted in summer and winter, winter plants are taller than summer plants [7]. The current study investigates the possibility of using safflower (*Carthamus tinctorius* L.) oil as an alternative biodiesel raw material. The plant was chosen for its good characteristics such as winter and summer cultivation, short maturation period, drought tolerance and minimal cultivation maintenance. There is also an increase in the accumulation of greenhouses and environmental pollution that cause climate change which has a bearing on crop productivity [8]. Therefore, crops have to be modified or their environment improved to maximise the yield. Plant growth regulators can play an important role in that endeavor.

Plant growth regulator (PGR) is a term that refers to naturally occurring hormonal substances (phytochromes) as well as their synthetic analogues [9]. The PGRs are divided into five classes; Auxin, cytokinins, gibberellins, abscisic acid and ethylene [10]. There are additional compounds such as jasmonate and brassinosteroids which also form two additional classes. Plant growth regulators promote, inhibit or retard metabolic processes such as nucleic acid and protein synthesis metabolism [11]. Growth inhibitors are known to decrease the intermodal distance, thereby improving the source-sink relationship and stimulating the

translocation of photo assimilations to seeds [12]. They bind to receptors and trigger a range of cellular changes that can affect the initiation or modification of tissues and organs [13].

2. Materials and methods

2.1. Site description

The study was carried out at the Morwa farming area in Kgatleng District near Gaborone, Botswana, on the northerly direction, along the famous A1 road. The area is located at latitude 24.33.40 S, longitude 25.56.37 E and an altitude of 992 m above sea level. Morwa farming area is categorized as semi-arid and receives an average annual rainfall of 457 mm ([14]. 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 [14].

2.2. Planting of safflower crop

The seeds were sown directly on the prepared site. The site was prepared with a disc plow as it is an area normally used for growing other crops. Planting took place in May in anticipation of flowering and harvesting before the rainy season. Rain spoils the safflower seeds and very cold temperatures can cause frostbite on the plant during the flowering period. Thirteen (13) plots were demarcated within the large field replicated three times.

2.3. Formulation and application of plant growth regulators

Four plant growth regulators (PGR) 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 rate for 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. The experiment was set up in triplicate in randomized complete design (CRD).

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 20 Merck was added to act as a surfactant. MH1, 2 and 4 µM; BA 3, 6 and 9mM; Triiodobenzoic acid 0.5, 1.0 and 1.5 mM; and kinetin 10, 20 and 40 mg/l. At flowering point (Figure 1b) the plants were each fully sprayed with an equivalent solution and the control was treated with water mixed 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.

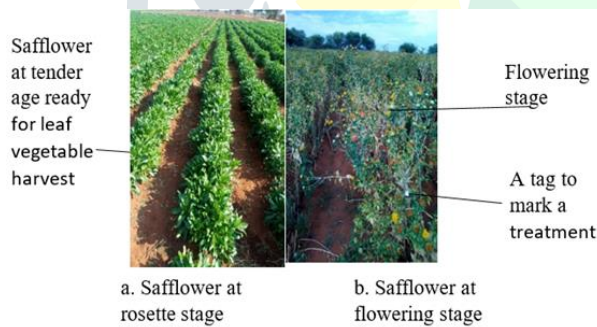


Figure 1 Safflower at rosette and flowering stage

2.4. Data collection

2.4.1. Number of branches, capitulum size, achne number, 1000 seed weight oil content.

At maturity safflower plants treated with plant growth regulators were harvested and sun dried before threshing. At least 4 plants were randomly picked and the number of branches were counted and the numbers were averaged to represent the number of branches per plant. In the same plants, number of capitulum and capitulum diameter were collected. The capitulum diameter were measured using a digital veneer calipers, at least 5 capitulum per plant. The capitulum were then opened to remove achenes for counting per capitulum. The seeds (achenes) were then counted with a seed counter and the seed weight was done with laboratory balance scale. The achenes were further bulked per plot for oil content quantification.

2.5. Oil extraction

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 [15]. Each plant was harvested from its seed and quantified prior to oil extraction to measure seed yield. 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.

The detail process specification ensued with grinding dried safflower achenes to powder form (<2mm). A labelled filter bag was then weighed and 1-2 g of ground achenes samples were weighed into the labelled filter bag and the weight noted (W1). The filter bag was heat sealed to close within 4mm of the top to encapsulate the sample. The sealed samples were placed in a drying oven set at 105°C for 3 hours. After drying, the samples were cooled in a desiccant bag, then weighed and the weight recorded (W2). 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 (W3) of the samples were taken. The oil yield was then calculated using equation 1 below.

$$\% \text{ oil yield} = \frac{W2-W3}{W1} \times 100 \quad 1$$

W1 is the weight of crushed seeds before oil extraction

W2 is the weight of the cake after oil extraction.

W3 is the weight of the dried sample and filter bag after extraction

3. Presentation of results

3.1. Number of branches per plant, capitulum per plant, number of achenes per capitulum, capitulum diameter, 1000 seed weight per plant and oil content.

The Number of branches per plant, capitulum per plant, number of seed per capitulum, capitulum diameter and 1000 seed weight per plant are presented in Table 1, 2, 3, 4 Figure 1 and Figure 2.

Table 1 An analysis of variance table for plant yield and yield attributes of batch1 safflower.

source		Sum of squares	DF	Mean squares	F value	Pr>F
Number of branches per plant	Model	371.7	12	30.97	2.02	0.06
	Error	398.7	26	15.3		
Capitulum per plant	Model	5965.4	12	497.1	2.09	0.06
	Error	6172.4	26	237.4		
Diameter	Model	723.6	12	60.3	1.74	0.1
	Error	900	26	34.7		
Achene	Model	1959.9	12	163.3	1.18	0.3
	Error	3612.5	26	138.9		
1000 achene weight	Model	376.0	12	31.3	12.30	0.0001
	Error	66.2	26	2.5		
Oil content	Model	656.7	12	50.5	6.7	0.0001
	error	90.7	26	7.6		

DF stands for degrees of freedom while Pr represents the p-value which ranges from P<0.05 to P<0.01. any value less than or equal 0.05 indicate that the treatments had made a significant difference.

Table 2 An analysis of variance table of plant yield and yield attributes of batch 2 safflower.

	source	Sum of squares	DF	Mean squares	F value	Pr>F
Number of branches per plant	Model	303.5	12	25.3	1.57	0.2
	Error	417.7	26	16.1		
Capitulum per plant	Model	3762	12	313.5	1.0	0.5
	Error	7855.5	26	314.2		
Diameter	Model	543.1	12	45.3	0.7	0.7
	Error	1618.2	26	64.7		
Achene	Model	2161.3	12	180.1	0.71	0.7
	Error	6326.9	26	253.1		
1000 achene weight	Model	188.4	12	15.7	1.39	0.2
	Error	294.4	26	11.3		
Oil content	Model	936.5	12	78	13.26	0.0001
	error	152.9	26	5.9		

DF stands for degrees of freedom while Pr represents the p-value which ranges from $P < 0.05$ to $P < 0.0001$. any value less than or equal 0.05 indicates that the treatments had made a significant deference.

Table 3 Mean groupings of first batch of the thirteen treatments on the 1000 seeds weight of safflower achenes

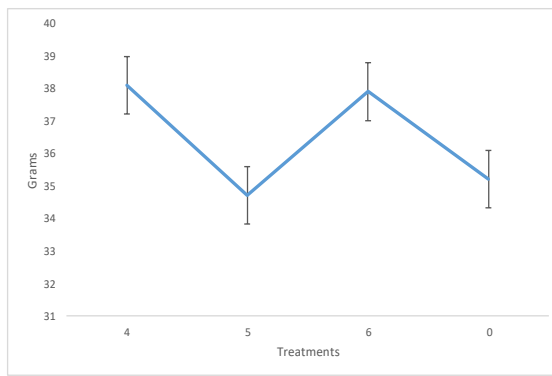
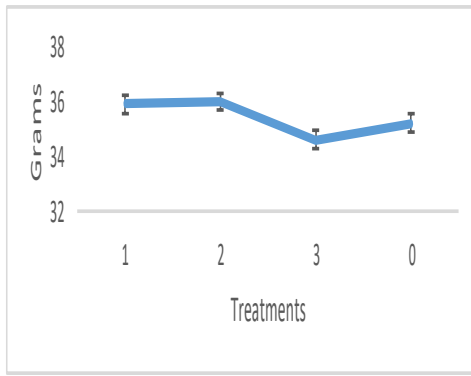
MEAN	TREATMENT	N	DUNCAN GROUPING
35.3	2	3	A
35.2	1	3	A
34.8	10	3	A
34.6	9	3	A
33.3	11	3	AB
32.3	5	3	AB
31.3	13	3	BC
30.9	8	3	BCD
28.6	3	3	ECD
28.2	7	3	ED
28.1	4	3	ED
27.3	6	3	E
26.6	12	3	E

*Treatments with similar letters are not significantly different. The Treatments are arranged in descending order.

Table 4 Mean groupings of second batch of the thirteen treatments on the 1000 seeds weight of safflower achene

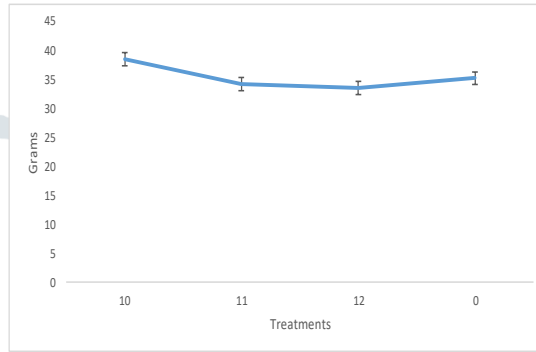
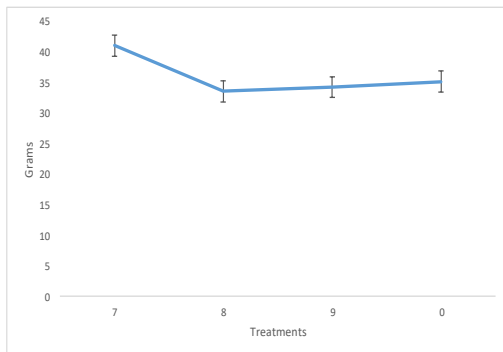
MEAN	TREATMENT	N	DUNCAN GROUPING
41.1	7	3	A
38.5	10	3	AB
38.1	4	3	AB
37.9	6	3	AB
36.0	2	3	AB
35.9	1	3	AB
35.2	13	3	AB
34.7	5	3	AB
34.6	3	3	AB
34.3	9	3	B
34.2	11	3	B
33.6	8	3	B
33.5	12	3	B

*Treatments with similar letters are not significantly different. The Treatments are arranged in descending order. N represents number of repeats per treatment.



a. The effect of maleic hydrazide on 1000 seed weight of batch2 safflower

d. The effect of benzylhydrazide on 1000 seed weight of batch2 safflower



b. The effect of 2,3,5 triiodobenzoic acid on 1000 seed weight of batch2 safflower

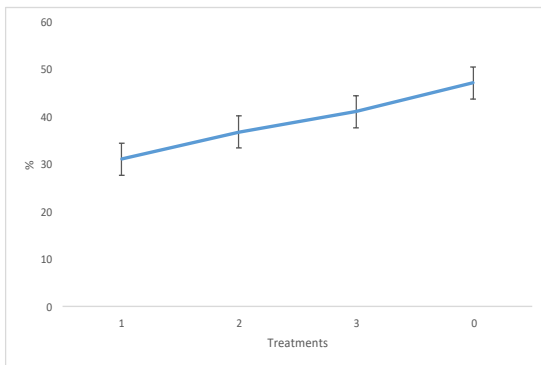
c. The effect of kinetin on 1000 seed weight of batch2 safflower

Figure 2 The effect of maleic hydrazide, benzyl adenine, triiodobenzoic acid and kinetin on the 1000 seed weight of batch 2

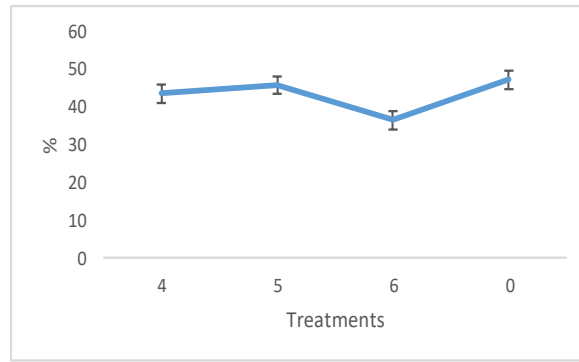
Table 5 Mean groupings of first batch of thirteen treatments on the oil content of safflower

MEAN	TREATMENT	N	DUNCAN GROUPING
48.8	12	2	A
47.8	10	2	AB
47.2	13	2	AB
45.8	5	2	ABC
45.5	11	2	ABCD
43.5	4	2	ABCDE
42.0	9	2	BCDEF
41.1	3	2	BCDEF
39.5	8	2	CDEF
39.0	7	2	DEF
36.8	2	2	EFG
36.5	6	2	FG
31.1	1	2	I

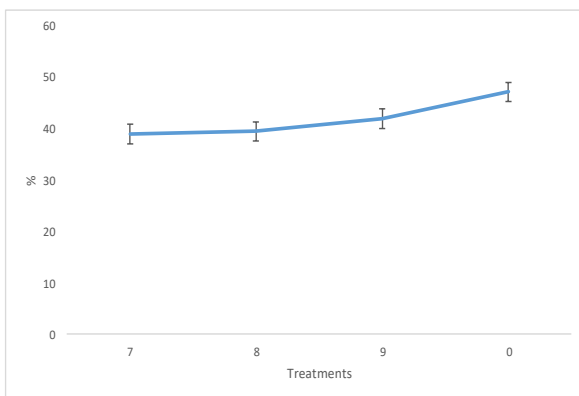
* Treatments with similar letters are not significantly different. The Treatments are arranged in descending order. N represents number of repeats per treatment.



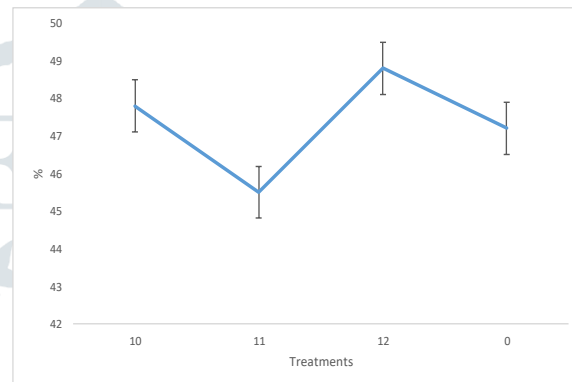
a. The effect of maleic hydrazide on the oil content of safflower batch1



d. The effect of benzyl adenine on the oil content of safflower batch1



b. The effect of 2,3,5 tridobenzoic acid on the oil content of safflower batch1



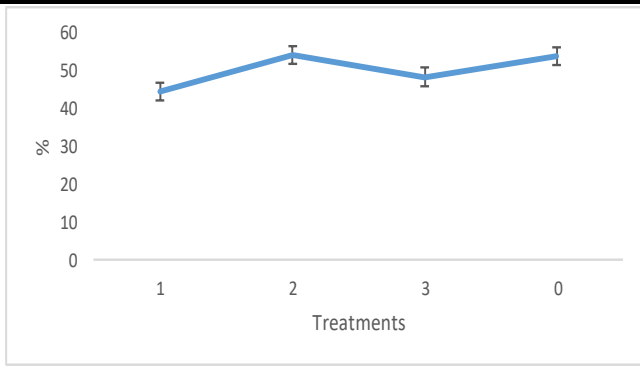
c. The effect of kinetin on the oil content of safflower batch1

Figure 3 The effect of maleic hydrazide, benzyl adenine, triiodobenzoic acid and kinetin on the oil content of batch 1 safflower

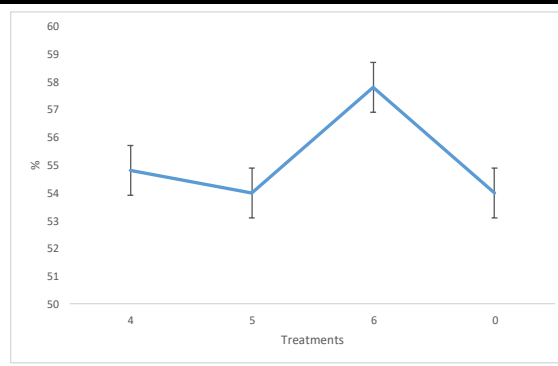
Table 6 Mean groupings of second batch of 13 treatments on the oil content of safflower

Mean	Treatment	N	Duncan grouping
57.8	6	2	A
57.1	11	2	AB
56.8	10	2	AB
54.8	4	2	AB
54.3	2	2	ABC
54.0	13	2	ABC
54.0	5	2	ABC
53.1	8	2	BC
49.9	9	2	CD
48.4	3	2	DE
44.8	7	2	EF
44.4	1	2	EF
43.0	12	2	F

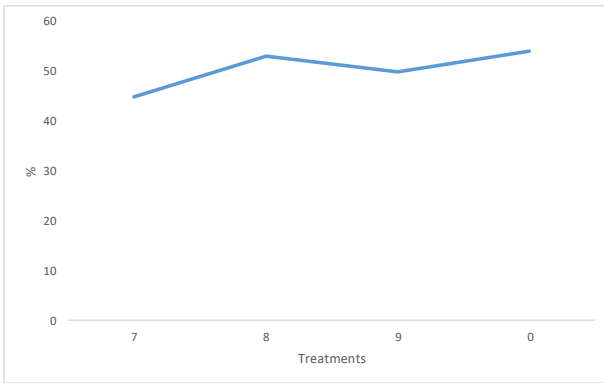
Treatments with similar letters are not significantly different. The Treatments are arranged in descending order. N represents number of repeats per treatment.



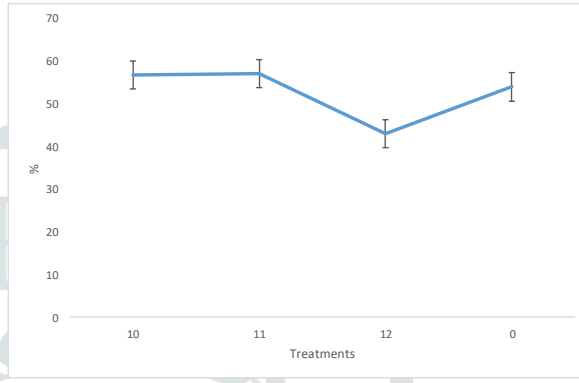
a. The effect of maleic hydrazide on the oil content of safflower batch 2



b. The effect of benzyl adenine on the oil content of safflower batch 2



c. The effect of 2,3,5 triiodobenzoic acid on the oil content of safflower batch 2



d. The effect of kinetin on the oil content of safflower batch 2

Figure 4 The effect of maleic hydrazide, benzyl adenine, triiodobenzoic acid and kinetin on the oil content of batch 2 safflower



4. Results analysis and discussion

4.1. Number of branches per plant, capitulum per plant, capitulum diameter, number of seed per capitulum, 1000 seed weight per plant and oil content

At maturity safflower plants treated with plant growth regulators were harvested and sun dried before thrashing. At least 4 plants were randomly picked and the number of branches were counted and the numbers were averaged to represent the number of branches per plant. In the same plants, number of capitulum per plant, capitulum diameter, seeds per capitulum, and 1000 seed weight were collected. There was no significant difference in all the collected data except 1000 seed weight and oil content (Table 1). The effect of plant growth regulators may not have been significant on the number of branches, capitulum size, number of seeds because the application was done during flowering when most of the plant had stopped growing save flowering only. The results were different from those found by Gabrel et al., 2018, who showed that benzyl adenine significantly increased inflorescence diameter of chrysanthemum plant. Kinetin has also been reported to have no effect on the growth of tomatoes and cucumber at lower concentration but inhibitory at higher concentration [17].

The effect of PGR was significant on the 1000 seed weight and oil content; the results are highly significant as shown in Table 1. Treatment 2 on safflower batch1 had a highest weight with 35.3g and treatment 12 had the lowest 26.6g (Table 3). In the second batch the results were not significantly different but some treatments have higher seed weights as shown in Table 2. In the second batch, treatment 7 had the highest 1000 seed weight of 41.1g while treatment 12 had the lowest 1000 seed weight of 33.5g (Table 4). Looking into the individual plant growth regulators presented in Figure 2, maleic hydrazide in treatment 1 to 3 had a descending effect from lower concentration of the plant growth regulator to higher concentration (Figure 2a). In Figure 2b benzyl adenine increased the seed weight in treatment 5 and reduced seed weight in treatment 6. The effect of 2,3,5 triiodobenzoic acid had an ascending effect, treatment 7 being the lowest concentration had the lowest 1000 seed weight and treatment 9 had the highest seed weight (Figure 2c). Figure 2d shows that the effect of kinetin had a descending effect from treatment 10 to treatment 12. Treatment 12 is even lower than the control. The second batch had a similar trend on the 1000 seed weight across the three growth regulators except on the benzyl adenine which was not conclusive (Figure 3). All the results of the plant growth regulators significantly increased the 1000 seed weight at lower concentration as compared to the control. The results were echoed by [18]. This increase of seed weight may be attributed to the enhanced translocation of assimilates from leaves to seeds [19].

There was a highly significant difference ($P < 0.0001$) among the treatments on the oil content of safflower in batch 1 (Table 5). Treatment 12 had the highest quantity of oil 48.8% and treatments 6 and 1 had the lowest oil content of 35.5%, far less than the control (47.2%) (Table 5). The individual comparisons of plant growth regulators presented in Figure 4 shows that maleic hydrazide had ascending effect on oil content and all the treatments did not exceed that one of control (Figure 4a). Benzyl adenine effect on oil content was higher in treatment 5 but lower in treatment 6 (Figure 4b). The effect of 2,3,5 triiodobenzoic acid shows an ascending order on the oil content of safflower (Figure 4c). Figure 4d shows the effect of kinetin to exponentially influence the oil content of safflower. Batch 2 safflower also had a significant difference among the treatments on oil content (Table 6). Maleic hydrazide and 2,3,5, triiodobenzoic acid had a significant effect at lower concentration and they had a little reduction of oil content at a higher concentration (Figure 5a and Figure 5c). This may be attributed to the ability of the plant growth regulators to accelerate cell enlargement as well as inhibiting cell division [20].

5. Conclusion

Plant growth regulators (PGR) can be used to improve the oil content of safflower. The PGRs, even at their lowest concentration, showed their effectiveness on the oil content of safflower. The plant growth regulators showed no effect on the main agronomic traits of safflower, which could indicate that the application should be done before flowering. The applied levels of maleic hydrazide and 2,3,5 triiodobenzoic acid did not increase the oil content of the safflower compared to the control in either batches 1 or 2. Further research can be conducted to determine optimal application rates.

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