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# The Effect of Soil amended with Organic Materials of Varying Qualities On the 6-Gingerol Content of Ginger (*Zingiber Officinale*).

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Abstract

The use of Organic Manures to meet the nutrient requirement of crops is likely to be an unavoidable practice in so many years to come for sustainable agriculture since it has been proven to generally improve soil physical, chemical and biological properties. It has also been used widely to dispose animal waste and reduce environmental pollution. This study evaluates the influence of different organic amendments on the 6-gingerol content of ginger with aim to identify the best organic material source for improved 6-gingerol content of ginger. The treatments used were sawdust, Cow dung, Poultry manure, rice mill waste and Absolute control (no manure or inorganic fertilizer). Five treatments were used and replicated three times using randomized complete block design (RCBD). 6-gingerol content of the harvested ginger was quantified in the laboratory using reverse phase high performance liquid chromatography. Data analysis was done using "R" statistical software version 4.1.0 and further mean separation was obtained by honest significant difference (HSD) at 5%.The result showed that the organic amendments enhanced the 6-gingerol concentration of ginger. The plot amended with cowdung gave the highest value of 489.1331 ug/ml which was significantly different from the absolute control and other organic amendments used in this experiment followed by poultry manure(476.3344 ug/ml), Rice mill waste (430.1956 ug/ml), saw dust(343.2329 ug/ml), and Absolute control(270.1886 ug/ml). The result showed that use of cow dung manure at the rate of 268g/plot of 2m should be applied to significantly enhance 6-gingerol content of ginger without compromising yield.

Keywords;6-Gingerol,Cowdung,Poultry manure.Rice mill waste.Sawdust,organic materials,Hplc

# INTRODUCTION

Ginger (Zingiber officinale) belongs to the Zingiberaceae family and has over the years been consumed as spice and herbal medicine (Han *e tal* .,2013). In recent times there has been growing interest all over the world in the use and application of ginger. According to (stoner 2013) most medicinal property of Ginger has been attributed to the presence of 6-gingerol (5-hydroxy – 1 – (4-hydroxy-3-methoxyphenyl)decan-3-one). In addition accumulating studies have demonstrated that ginger possess the potential to prevent and manage several diseases such as Cardiovascular disease(Akinyemi et al 2015), obesity(Suk et al 2017), neurodegenerative disease(ho et al 2013), respiratory disorder(Townsend et al 2015), and diabetes mellitus(Wei etal). In recent years, ginger has also been found to possess anti-inflammatory (Zhang etal2016), anticancer (Cintroberg et al 2013) and antioxidant (Nile et al 2015) activities. These days consumers are more concerned about exposure to agrochemical products more especially on those foods that are consumed fresh (Fortis-hernandiz et al, 2012), which implies that they prefer foods free from chemicals. Over the past decades consumers have become increasingly interested in a healthier diet by increasing the intake of ginger, fruits and vegetables (Jahansson et al, 2014).. It has been reported that inorganic/chemical fertilizer has high negative effects to the environment and human health(Rande et al 2013). Soil amendment using manure as alternative to chemical fertilizers is preferred because it reduces environmental pollution issues and enhances food quality. Studies on ginger with organic and inorganic sources of fertilization have been mainly focused on their productive characteristics. This study therefore aims at: identifying the potential effect of soil amended with organic manure of varying qualities on the 6-gingerol (5-Hyoroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one) content of seed ginger.

# MATERIALS AND METHOD

## Materials, Reagents and Equipment

High performance Liquid chromatography equipment

Mulching material

Ginger seed

Hplc grade Methanol

6-gingerol standard

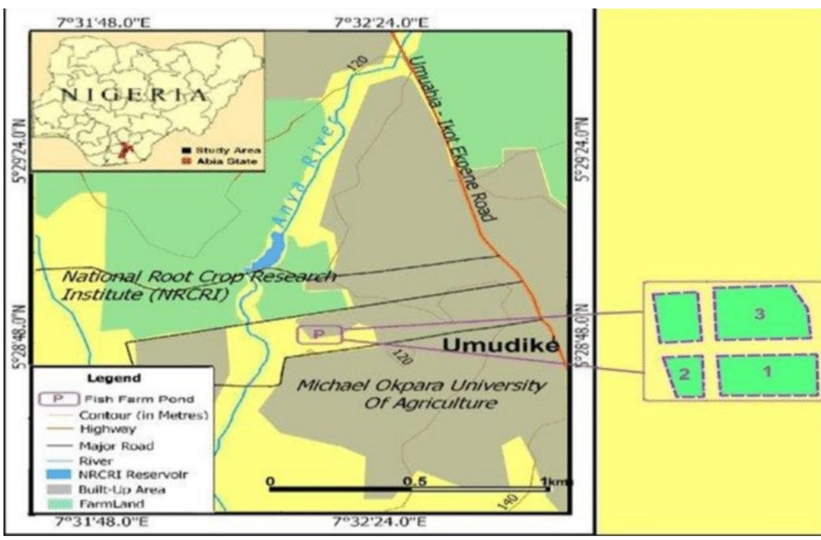
Acetronitrile

Mulching materials

**Methodology**

**The Study Site**

The field trial was executed at the National Root Crops Research Institute's Umudike field, which is located on the Umuahia Ikot-Ekpene Road at kilometer 7. It is at an altitude of 122 meters above sea level. The minimum temperature in the area is 22°C, while the maximum temperature is 32°C. The rainfall ranges between 2500 and 3000 mm.




**Experimental Design and Treatments**:T he field experiment was carried out in a randomized complete block design with manure rate of 100%(quantity of manure required to supply 45kg Nitrogen/hectare)replicated three times with a plot size of 1mx2m,inter plot distance of 50cm and inter replication distance of 1m.Absolute control treatment(without amendment material) was also included. The manure source are Poultry manure, Cow dung, Rice mill waste and Saw dust at the rate of 100%( of manure required to supply 45kg Nitrogen/ ). The Nitrogen content of the organic materials used in this experiment were analyzed in the lab using kjedahl method and found to be Cow dung (3.36% N), Poultry Manure (2.87% N), Rice Mill Waste (1.33% N), and Saw Dust (0.56% N) and applied at the rate of 268g/plot of 2m, 314g/plot of 2m, 677g/plot of 2m, 1607g/plot of 2m respectively which is the quantity of each of the manure source required to supply 45kg Nitrogen needed for ginger production.

**Sample processing Extraction and Quantification:** After harvesting, the ginger rhizome was dried and grinded. 5g weighed and refluxed with methanol (100ml) for 15 minutes in a water bath and filtered through whatman filter paper (No.41). The residue left out was refluxed again three times with 70ml of methanol for 15 minutes and filtered. The solvent was then evaporated using a rotary vacuum evaporator. This procedure was performed for all the 15 different sample treatments. The 6-gingerol concentration of the extract was quantified using reverse phase high performance liquid chromatography. Data analysis was statistically done in an open source R environment version 4.1.0. Further mean separation was done using HSD at 5%. Mobile Phase used was Acetronitrile:Water 55:45,Column: C18 – ODS(Octadeciylsilane) (Lichrocart 250-4,Lichrospher RP-18e-5m(merck)Art No:1.50216.The column size is150 X 4.6mm and at wavelength of 280nm

## RESULTS AND DISCUSSION

Reverse phase high performance liquid chromatography was used to quantify the quantity of 6-gingerol content of the ginger extract. The gingerol showed a UV absorption maximum at 280nm and retention time of 3.8 minutes. The result is shown in table 1.

Table 1.Concentration of 6-gingerol in the harvested ginger

| Treatments | Rep 1(ug/ml) | Rep 2(ug/ml) | Rep 3(ug/ml) | Mean(ug/ml) |
|---|---|---|---|---|
| Cow dung | 493.9488 | 485.3584 | 488.0920 | 489.1331 |
| Poultry Manure | 472.4045 | 475.7917 | 480.8071 | 476.3344 |
| Rice mill waste | 432.7749 | 426.0523 | 431.7597 | 430.1956 |
| Sawdust | 345.8530 | 342.0937 | 341.7519 | 343.2329 |
| Absolute control | 269.7359 | 270.8640 | 269.9660 | 270.1886 |
| Stantard control | 273.4033 | 273.4030 | 273.1302 | 273.3122 |

CD=Cow dung, PM=Poultry manure, SD=Sawdust, RMW=Rice mill waste

Table 2 shows the statistical information of each of the treatments compared with the absolute control. From the statistical information ,each of the treatments had significant effect on the 6-gingerol concentration of ginger when compared with the absolute control

Table 2.Statistical Table of the treatments compared with Absolute control

| Treatment | Treatment - Absolute control | contrast | SE | df | lower.CL | upper.CL | p-value |
|---|---|---|---|---|---|---|---|
| CD | CD - Absolute control | 252.13 | 2.55 | 24.00 | 243.28 | 260.97 | <.0001 |
| PM | PM - Absolute control | 239.33 | 2.55 | 24.00 | 230.48 | 248.18 | <.0001 |
| Rmw | Rmw - Absolute control | 193.19 | 2.55 | 24.00 | 184.34 | 202.04 | <.0001 |
| SD | SD - Absolute control | 106.23 | 2.55 | 24.00 | 97.38 | 115.07 | <.0001 |

Table 3 shows the statistical information when the treatments were compared with standard control(NPK 15:15:15).From the results, each of the treatments had significant effect($p<0.05$)when compared with standard control.

Table 3: Statistical Table of the treatments compared with Absolute control



| Treatment | Treatment - Standard control | contrast | SE | df | lower.CL | upper.CL | p-value |
|---|---|---|---|---|---|---|---|
| CD | CD - Standard control | 215.82 | 2.55 | 24.00 | 206.97 | 224.67 | <.0001 |
| PM | PM - Standard control | 203.02 | 2.55 | 24.00 | 194.17 | 211.87 | <.0001 |
| Rmw | Rmw - Standard control | 156.89 | 2.55 | 24.00 | 148.04 | 165.73 | <.0001 |
| SD | SD - Standard control | 69.92 | 2.55 | 24.00 | 61.07 | 78.77 | <.0001 |

| Treatment | Means | |
|---|---|---|
| Absolute control | 270.1886 | e |
| CD | 489.1331 | a |
| PM | 476.3344 | b |
| Rmw | 430.1956 | c |
| SD | 343.2329 | d |
| **HSD** | **9.490309** | |

Table 4. Chemical composition of manure used

| MANURE | N (%) | P(mg/kg) | K(Cmol/kg | OC(%) | OM (%) | Na(Cmol/kg) |
|---|---|---|---|---|---|---|
| CD | 3.36 | 7.5 | 1.205 | 6.30 | 23.94 | 0.725 |
| PM | 2.87 | 19.0 | 1.295 | 7.40 | 28.12 | 2.300 |
| RMW | 1.33 | 5.8 | 0.15 | 8.60 | 32.68 | 1.025 |
| SD | 0.56 | 2.0 | 0.08 | 8.4 | 31.92 | 12.700 |

**The Effect of Sawdust Amended Soil (at 100%) on 6-Gingerol Concentration of Ginger**

Using the reverse phase HPLC–UV technique with gradient elution, the 6 – gingerol in the extract of seed ginger cultivated on soil amended with sawdust at a rate of 100 percent was quantified and qualitatively evaluated. A typical HPLC-UV chromatogram with a clean baseline and excellent resolution was obtained, allowing all of the marker peaks to be recognized and quantified. The wavelength at which 6-gingerol absorbs the most ultraviolet light is 280nm.

Table 5: 6-gingerol concentrations of ginger from sawdust-amended soil at 100%

| Treatment | Rep 1 Ug/ml | Rep 2 Ug/ml | Rep 3 Ug/ml | Mean Ug/ml |
|---|---|---|---|---|
| Cowdung | 345.8530 | 342.0937 | 341.7519 | 343.2329 |
| Standard control(N:P:K) | 273.4033 | 273.1302 | 273.1322 | 273.3122 |
| Absolute control | 269.7359 | 270.8640 | 269.9660 | 270.1886 |

The results showed that ginger harvested from soil amended with sawdust at a rate of 100% (i.e. 1607g) per plot of 1m x 2m yielded a mean gingerol content of 343.2329 ug/ml, which is the quantity required to give 45 kg N per hectare.

Among all the organic amendments and combinations utilized in this study, ginger harvested from soil treated with sawdust at 100% yielded the lowest 6-gingerol concentration. This could be because it has the largest C:N ratio among the organic amendments tested, favoring slower residue decomposition, release, and mineralization of organic matter and other nutrients in Sawdust. This means that a change in the release of nutrients could lead to a difference in 6-gingerol formation.

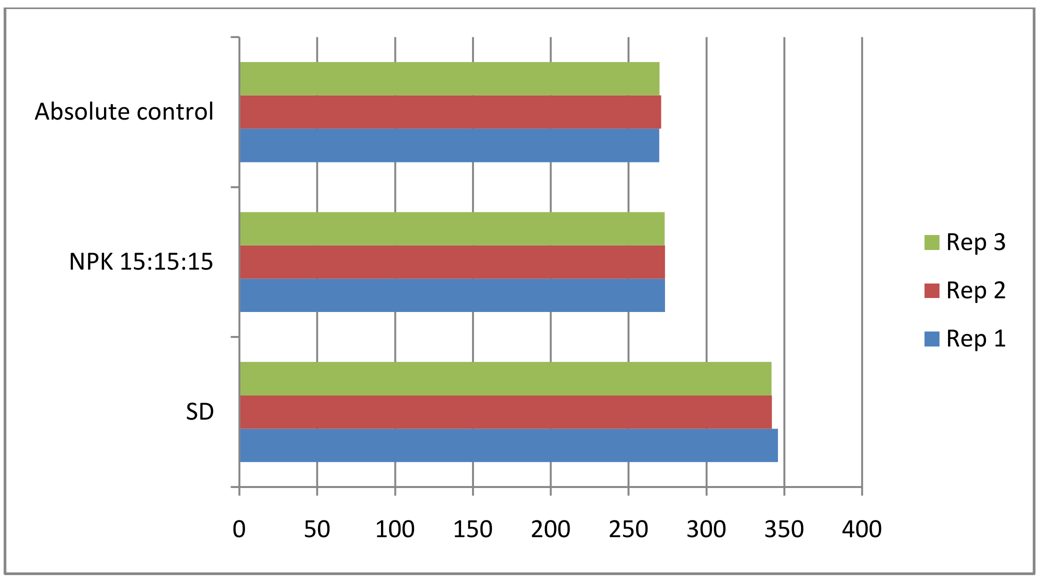



Figure 1.The bar graph 6-gingerol content of ginger collected from 100 percent sawdust-amended soil

**Effect of Cow dung Amended Soil (at 100%) on 6-Gingerol Concentration of Ginger.**

The 6 – gingerol in the extract of seed ginger cultivated on soil amended with cow dung at a rate of 100 percent was quantitatively and qualitatively evaluated using the reverse phase HPLC–UV technique with gradient elution.

Table 6.Concentration of 6-gingerol of ginger harvested from soil amended with cowdung

| Treatment | Rep 1 | Rep 2 | Rep 3 | Mean |
|---|---|---|---|---|
| | Ug/ml | Ug/ml | Ug/ml | Ug/ml |
| Cowdung | 493.9488 | 485.3584 | 488.0920 | 489.1331 |
| N:P:K 15:15:15 | 273.4033 | 273.1302 | 273.1322 | 273.3122 |
| Absolute control | 269.7359 | 270.8640 | 269.9660 | 270.1886 |

The result obtained showed that ginger harvested from the soil amended with cow dung at rate of 100% (i.e 268g) per plot of 1m x 2m which is the quantity required to supply 45 kg N per hectare yielded mean gingerol concentration of 489.1331 ug/ml.

It had a significant effect ($p<0.05$) on the 6 gingerol content when compared with the Absolute Control (No amendment material) and standard control.It was also observed that gingerol content of ginger grown on soil amended with cowdung at the rate of 100% was significantly different from the other different organic amendments used in this study.

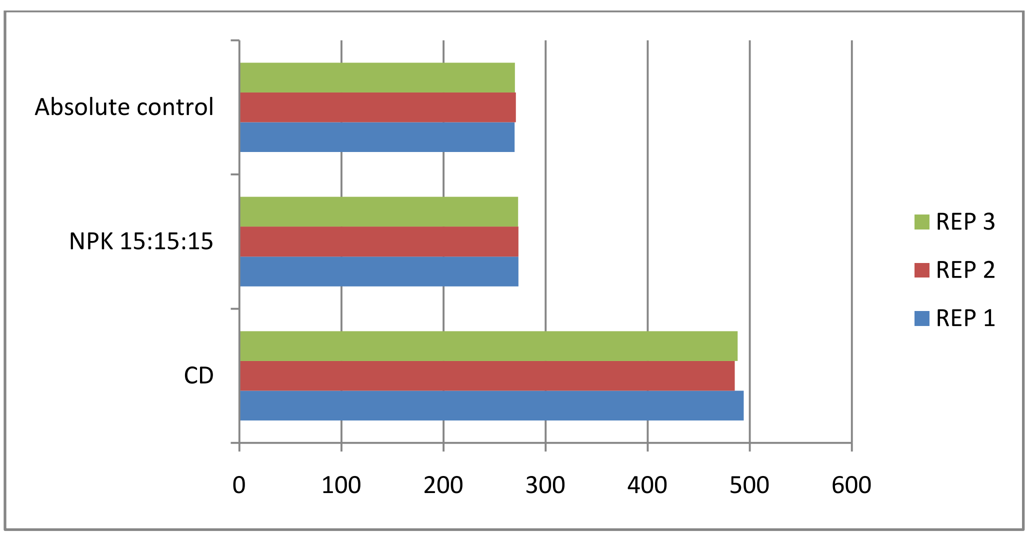



Figure 2..Bar graph for 6-gingerol content of ginger harvested from soil amended with cowdung at 100%

**Effect of Poultry manure Amended Soil (at 100%) on 6-Gingerol Concentration of Ginger.**

The 6 – gingerol content of a extract of seed ginger grown on soil modified with poultry manure was quantified and qualitatively assessed using the reverse phase HPLC–UV method with gradient elution.

Table 7. Concentration of 6-gingerol of ginger harvested from soil amended with poultry manure

| Treatment | Rep 1 Ug/ml | Rep 2 Ug/ml | Rep 3 Ug/ml | Mean Ug/ml |
|---|---|---|---|---|
| Poultry Manure | 493.9488 | 485.3584 | 488.0920 | 489.1331 |
| Standard control(N:P:K 15:15:15) | 273.4033 | 273.1302 | 273.1322 | 273.3122 |
| Absolute control | 269.7359 | 270.8640 | 269.9660 | 270.1886 |

The results indicated that ginger collected from soil supplemented with poultry manure at a rate of 100% (i.e. 314g) per plot of 1m x 2m, which is the amount needed to provide 45 kg N per hectare, gave a mean gingerol concentration of 476.3344ug/ml.

When compared to the standard control, it had a significant impact ($p<0.05$) on the 6-gingerol content. When compared to the Absolute Control, it had a significant impact ($p<0.05$).

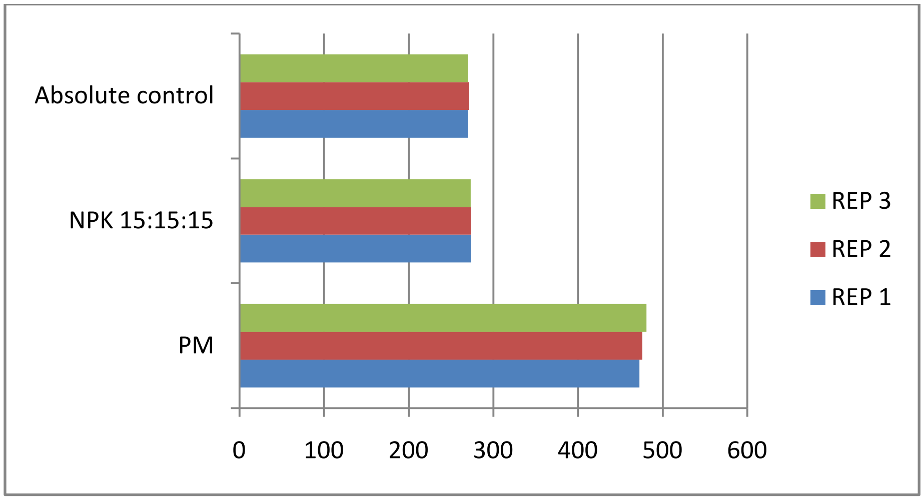



Figure 3..Bar graph for 6-gingerol content of ginger harvested from soil amended with poultry manure at 100%

**Effect of Rice mill waste Amended Soil (at 100%) on 6-Gingerol Concentration of Ginger.**

Using the reverse phase HPLC–UV method with gradient elution, the 6–gingerol in the extract of seed ginger grown on soil amended with rice mill waste at a rate of 100 percent was quantified and qualitatively assessed.A typical HPLC-UV chromatogram with a clean baseline and good resolution obtained is shown on the subsequent page. The wavelength at which 6-gingerol absorbs the most is 280nm.

Table 8.Concentration of 6-gingerol of ginger harvested from soil amended with rice mill waste

| Treatment | Rep 1 Ug/ml | Rep 2 Ug/ml | Rep 3 Ug/ml | Mean Ug/ml |
|---|---|---|---|---|
| Rice mill waste | 432.7749 | 426.0523 | 431.7597 | 430.1956 |
| Standard control(N:P:K 15:15:15) | 273.4033 | 273.1302 | 273.1322 | 273.3122 |

| | | | | |
|---|---|---|---|---|
| Absolute control | 269.7359 | 270.8640 | 269.9660 | 270.1886 |

A mean 6-gingerol concentration of 430.1956 ug/ml was found in ginger collected from soil treated with Rice mill waste at a rate of 100 percent (i.e. 677g) per plot of 1m x 2m, which is the amount needed to provide 45 kg N per hectare.

When compared to the standard control, it had a statistically significant effect ($p<0.05$) on the 6-gingerol content of the extract (N.P.K 15:15:15). When compared to the Absolute Control, it likewise exhibited a statistically significant effect ($p<0.05$) (No amendment material).

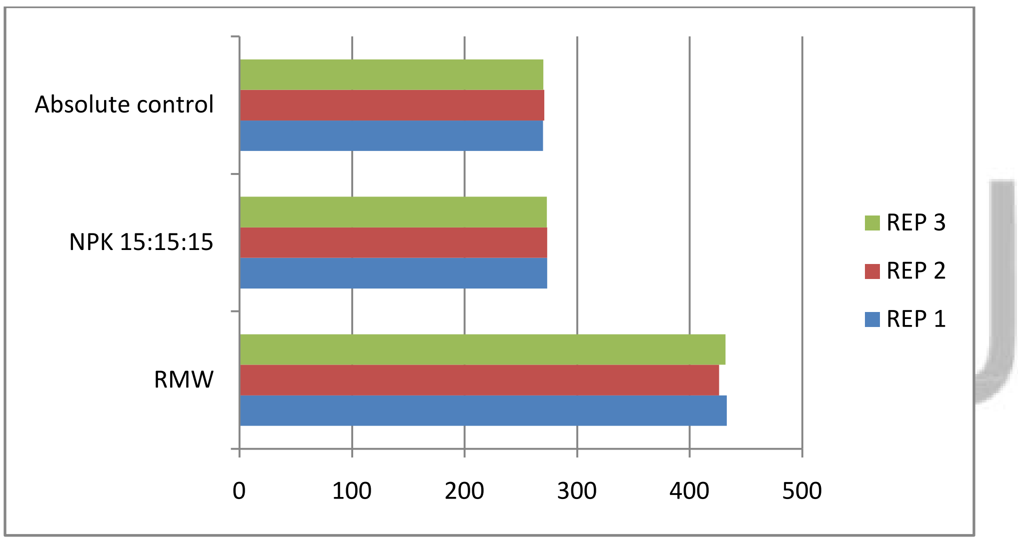



Figure 4. Bar graph for 6-gingerol content of ginger harvested from soil amended with rice mill waste at 100%

## CONCLUSION AND RECOMMENDATION

The organic manures used in this experiment enhanced the 6 gingerol concentration of ginger but at different degrees. Cowdung has the most significant effect when compared with the other organic manures used in this experiment though all the other organic manures used had significant effect ($p<0.05$) when compared with the absolute control and standard control.

The plot amended with cowdung gave the highest value of 489.1331 ug/ml which was significantly different from the absolute control and other organic amendments used in this experiment followed by poultry manure(476.3344 ug/ml), Rice mill waste (430.1956 ug/ml), saw dust(343.2329 ug/ml), and Absolute control(270.1886 ug/ml). The result showed that use of cow dung manure at the rate of 268g/plot of 2m should be applied to significantly enhance 6-gingerol content of ginger without compromising yield and is therefore recommended.

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