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Growth and bleaching response of yam (*Dioscoreaalata*L.) plant to foliar application of Fluridone under soil nitrogen fertilization

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Abstract:
Background*: It is not clear whether management of soil nitrogen (N) level can regulate the extensive expression of bleaching caused by spraying water yam (D. alata) leaves with Fluridone. Therefore, the objective of this study was to determine the effect of five levels of soil nitrogen (ammonium nitrate) fertilization on the expression of Fluridone inducedbleaching effect during yam growth and development.*
Materials and Methods*: The experiment was arranged in a Completely Randomized Design with five treatments: 0 kg N/ha + 0 Fluridone (FL) (experimental control), 0 kg N/ha + 10µM Fluridone (treatment control), 50 kg N/ha + 10µM Fluridone, and 150 kg N/ha + 10µM Fluridone, and with treatments replicated three times. Fluridone was applied daily for 6 days from the 8th day after the first split of nitrogen application. All treatments other than experimental control were given recommended levels of potassium and phosphorus for growing yam in Rivers State Nigeria.*
Results*: Bleached leaves were observed only on plants that received Fluridone. Early in the study (i.e at 4 weeks after Fluridone application) when Fluridone effect was strong, the application of 50 kg N/ha significantly (p>0.05) reduced the number of bleached leaves compared with the treatment control. Later in the study when the efficacy of Fluridone commenced the decline path, the application of 100 or 150 kg N/ha significantly (p>0.05) reduced the number of bleached leaves. The reverse was the case for number of green leaves and number of leaves.Tuber, root, vine, and leaf dry weights did not vary with nitrogen application.Chlorophyll a and b contents did not vary significantly with nitrogen levels at 4 weeks after Fluridone application (WAF). However, number of leaves related strongly (R^2> 0.75) and positively with carotene and chlorophyll (a and b) contents.*
Conclusion:*This study has shown that the number of bleached leaves of yam plants sprayed with 10µMFluridone can be reduced by the application of nitrogen but the rate depends on the activity of Fluridone in the leaf.*
Key Word*: dormancy, yam, Fluridone, abscisic acid,Dioscoreaalata*

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I. Introduction

Fluridone (1-methyl-3-phenyl-5-[3-trifluromethyl(phenyl)]-4-(1H)-pyridinone) is an aquatic herbicide that is largely used in water bodies to control weeds[1]. It is destroyed easily (over hours to days) by light and temperature in water bodies and thought to be nontoxic to humans[1]. Although its persistence in soil is longer (nine months or more) than in water, its effect has been shown to wean with time[2,3,4]. Today, Fluridone is gaining use as drugs for humans and as a compound for controlling seed/tuber dormancy in agriculture. Its use in dormancy studies is based on knowledge of its ability to inhibit the biosynthesis of abscisic acid (ABA) by inhibiting the activity of phytoene desaturase (PDS), which is responsible for the conversion of phytoene (the colorless/whitish carotene) to the colored C40 carotenes; the precursor of ABA[5].

In yam, tuber dormancy is the one physiological process responsible for the long (over one year) sprout to sprout cycles in yams[5,6,7,8,9,10]. With an increasing desire to achieve two or more sprout to sprout cycles per annum, several studies have been embarked on to understand the mechanism(s) that control dormancy and sprouting in yam in the hope to use the findings to bring about significant reduction in the duration of dormancy or to prevent it[11,12,10]. Abscisic acid is the endogenous plant growth hormone that has long been implicated to control of the length of tuber dormancy in yam and potato amongst others[13,14,9]. Invitro experiments with yam have proven that *D. rotundata* micro tubers can be induced to sprout during early development when they generate from plantlets grown in nutrient medium containing the herbicide fluridone[9,12]. Also, in pot studies where *D. alata* yam plants derived from minisetts that had just initiated new tubers were treated with Fluridone

in a hydroponics system, unusually early sprouting (i.e., during early tuber development) was induced on the new tubers[14]. Similar expression of early sprouting has been reported to occur on yam tubers generated from a soil growth media whether the Fluridone was applied to the soil or through the leaves[15,16].

Therefore, it is clear that Fluridone causes significantly early sprouting (by reducing the duration of dormancy or by preventing it) in yam, which has promising potentials to lead to more planting times per year and higher tuber production and increase in the availability of seed tubers for planting and hence lower cost of production. Apparently, the absorption and metabolism of Fluridone in plant results in bleaching of many leaves leaving stems whitish to pinkish-purple stems and whitish roots (that suggest lack or low suberin accumulation) upon. Although indications show that the characteristic bleaching effect of Fluridone on leaves wear out over time[10,12] in yam, a study[15] show that the bleaching effect on leaves is more intense when Fluridone is foliar-applied than via soil application, with corresponding effects on number of sprouting tubers. This effect on leaf colour and function is however undesirable since such bleached leaves look unusual, lack adequate chlorophyll and are unable the undertake photosynthesis. In this study, the ensuing questions were asked. Was the number of bleached leaves significantly affected by nitrogen fertilization? Would the application of nitrogen regulate the bleaching effect of Fluridone on yams growing in coastal plain soils of the Niger Delta? Nitrogen is one of the macronutrients required by plants in large quantity for growth and development[17]. It is a major component of nucleic acid[18] and chlorophyll[19] with the concentration of chlorophyll being approximately proportional to leaf tissue nitrogen content[20,21]. Also, nitrogen fertilization is well known to increase the rate of chlorophyll synthesis in many plant species[22,23].

However, little is known about the effect of nitrogen fertilization on the expression of Fluridone induced bleaching of yams leaves. Also, it is not clear whether the inherent low soil nitrogen levels (often below critical level of 0.11%) of coastal plain sands of the Niger Delta[24] contributed to the bleaching of leaves under a soil growing condition than in a nutrient rich hydroponics system[15,16]. This study therefore hypothesized that the bleaching effect caused by foliar application of Fluridone reduces with increasing level of soil nitrogen. Thus, the objectives of this study were to: (1) determine the effect of five levels of soil nitrogen fertilization on growth and the expression of Fluridone effect (bleaching) during growth and development of yam (*Dioscoreaalata*).

II. Material And Methods

Study environment

This study was conducted in a screen house in the Department of Crop and Soil Science, Faculty of Agriculture, University of Port Harcourt with geographical coordinates of latitude 4°47´ N and longitude 70°E. The screen house was constructed with plastic mesh sheet and transparent plastic roofing sheets. Thus, the screen house allowed for ventilation. Temperature and relative humidity in the screen house were taken using a temperature /relative humidity sensor. Photosynthetic active radiation (PAR) level in the screen house was monitored using a quantum PAR meter (Hydro farm product, USA); this is important because rate of photosynthesis in plants is proportional to the number of photons absorbed in 400 to 700 nm band of the light spectrum.

Planting material

Tubers of *Dioscoreaalata*(var*TDa* 98/01166) was used in this study. This variety has been used in many studies in which the leaves have been shown to bleach in response to Fluridone application and the emerging tuber commence sprouting.

Preparation of minisetts

Minisetts weighing approximately 50 g ±3 g were obtained from the proximal region of the non-dormant tubers by cutting longitudinally through the head region; ensuring that every minisett had a measure of the head region since the head region of tubers tend to sprout faster than the middle or distal regions[25]. Prior to cutting, the tuber-head, which is the corm-like structure attached to the proximal/head region of the tuber was severed from the tuber (where present) to remove apical dominance and hence encourage shoot growth from other parts. The cut ends of all the minisetts were treated by dabbing them in wood ash to minimize rotting and infestation of micro-organism[26].

Treated minisetts were air-dried for 24 h then planted in baskets lined with a 2 mm net and filled with moist sawdust by placing the bark firmly in sawdust. Pre-sprouting was essential to obtain uniformly sprouted minisetts. Pre-sprouting was done on the 20th of April 2018 followed by daily observation of the minisetts for vine emergence was done. Irrigated was done as necessary by sprinkling 100 ml of water. Most of the minisetts sprouted by 10 d after pre-sprouting (30th of April, 2018), had initiated sprouts.

Transplanting of rooted plants to treatment pots

Pots were filled with 8 kg of soil from the farm of Department of Crop and Soil Science, University Port Harcourt and then air-dried to constant weight. The soil was then brought to field capacity two days before the start of the study. A soil moisture probe instrument was used to monitor soil moisture levels.

Source of NPK fertilizers and Fluridone

The major nitrogen source was ammonium nitrate which contains 34.98% N while phosphorus and potassium were applied in the form of single super phosphate (P_2O_5) and potassium nitrate (KNO_3) respectively. Nitrogen was applied at the rate of 100 kg N/ha, which is within the recommended rate for tubers in the region. The P and K were applied at rates that correspond to the recommended fertilizer formulation of 15-10-10 N:P:K for tuber crops in Rivers State (National Council on Agriculture and Rural Development, 2016).

Fluridone was purchased from ChemServices Ltd, USA and 10 µM concentration of it was used in this study. The rate of 10 µM Fluridone solution was chosen because past studies have consistently reported the expression of bleaching at this concentration.

Treatments and treatment application

The experiment consisted of five treatments: a) 0 Kg N/ha + 0 µMFluridone(FL) (experimental control), b) 0 Kg N/ha + 10 µM Fluridone (treatment control), c) 50 Kg N/ha + 10µM Fluridone, d) 100 Kg N/ha (recommended rate) + 10 µM Fluridone, and e) 150 Kg N/ha + 10 µM Fluridone.

Potassium and phosphorus were applied at planting, while nitrogen was applied in two splits; at 35 and 56 days after planting (DAP). Because potassium nitrate (the K source) contains 13.84% nitrogen, the quantity of ammonium nitrate applied was estimated to ensure that plants in each treatment received the half dose of N by the first split N application date. Fluridone was applied at a rate of 50 ml per plant at a pressure of 40 si using a pressure sprayer fitted with a cone nozzle. Fluridone was sprayed on each plant daily for six consecutive days. The test solution was sprayed on plant leaves while ensuring that the solution did not drip onto the soil or drift on to the control plants. To achieve this, the soil was covered with a black polyethylene film during spraying while the plants in the complete control were screened out during a spraying operation. Fluridone application was timed to occur just before tuberization because Fluridone is known to induce whitening of leaves that is associated with induction of sprouting on developing tubers[15].

Nitrogen application was synchronized with Fluridone application because nitrogen is known to enhance soil and plant tissue N status limiting under soil N conditions, which in turn allowed for the evaluation of the response of yam leaves to foliar-applied Fluridone under enhanced tissue N statue. Second split N application was so timed in order to encourage and maintain increased tissue nitrogen levels during the first three weeks after Furidone application, when Fluridone is most active.

Data collection

Vegetative growth data collection

Baseline data was collected three days prior to Nitrogen application, *i.e.*, at 32 days after sprouting/planting. Thereafter, data was collected weekly on three randomly selected plants per treatment per replicate.

The following data were collected using nondestructive methods. Number of green and bleached leaves per plant were counted weekly on all six plants per treatment per replicate. Leaf length and width were measured weekly on fifteen randomly selected pre-labelled leaves on each of the three pre-selected plants per treatment per replicate.Indexof chlorophyll content was measured from 2 WAT till 8 WAT using a rapid and non-destruction method of chlorophyll measurements. Two readings per leaf (upper and lower part of the leaf) were taken on fifteen representative leaves (five from the lower, middle and top regions of the plant) per plant and there were three plants per treatment per replicate. Thus, there were 270 data points per treatment and a total of 1,350 data points. The meter was produced by FT Green LLC, USA[27]. The *at*LEAF® meter estimates chlorophyll content based on the algorithms derived from the transmissions of red light at 660 nm (where chlorophyll absorbs light) and infrared light at 940 nm wavelength (where no light is absorbed by chlorophyll). The meter compares the transmission of light in red and near infrared wavelengths to give a measure of chlorophyll content in leaves. Chlorophyll value of 35 and above suggests that a plant is healthy with adequate nitrogen content.

Plants were sampled at 4 WAF to determine chlorophyll a and b contentsand leaf carotene content. One plant/treatment/replicate was sampled, and fifteen green leaves were taken from each of the plants for the analyses of chlorophyll a and b, and leaf carotene following methods by Quadruplicate[28]. Pigment measurements were quantified spectrophotometrically using a GENESYS TM 10 Series Spectrophotometer by Thermo Electron Corporation. Absorbances of chlorophylls a and b and carotenoids (x + c) extracts were determined at wavelengths of 662, 645 and 470 nm, respectively: where (x + c) = Total carotene. Readings at each wavelength was taken in quadruplet. The whitish leaves on Fluridone treated plants were not used for the analysis since the procedure required green leaves.

At the end of the study (9 weeks after Fluridone application), dry matter content and total leaf nitrogen contents were determined. Sampled plants were separated into their component parts and then, fresh and dry weights were recorded after drying in a forced air oven at 80^{o}C. Total nitrogen content of leaves was determined using the Kjeldahl methods where samples were digested in sulfuric acid and N quantified by titration.

Experimental design and data analysis

The experiment was designed as a Completely Randomized Design (CRD) with five treatments, six plants per treatment per replicate and three replicates. The pots were arranged on benches and treatments were randomly assigned.

Data analysis was run on GENSTAT 12th Edition software using one-way ANOVA program. All count data were transformed using square root transformation prior to data analysis. Means were separated using Least Significant Difference (LSD) at 5% probability level.

III. Result

Growing environment

The average temperature at 7am, 2pm, and 6pm in the screen house were 26.95° C, 28.19° C and 27.38° C respectively, while the average relative humidity at the same time were 92.6, 85.8 and 89.0% respectively. On average, the photosynthetic active radiation (PAR) in the screenhouse ranged from 21.50 µmoles to 290.46 µmoles/m^2 depending on the weather condition of the day. Soil pH and total nitrogen content at the start of the study were 6.5 and 0.03% respectively. Thus, the soil was slightly acidic and low in nitrogen while growing temperature and relative humidity were within the for normal crop growth. Light level was however generally low for normal growth.

Effect of treatments on leaf morphology

Plants treated with Fluridone expressed observable color change by the sixth day of treatment application (Fig. 1). Some of the leaves showed extensive/complete bleaching with a white stem/vine while others were either partly bleached or green. In contrast, all leaves of plants growing in the Complete control expressed no bleaching.

Fig. 1.Bleached leaves of *D.alata*plants in response to foliar Fluridone

Effect of nitrogen rates on number of Fluridone induced bleached leaves

By the 6th day of Fluridone application (1 WAF), there were no bleached leaves in the complete control (0 Kg N/ha + 0µM Fl) (Table 1). In contrast, a combination of fully bleached, partially bleached and fully green leaves was observed on plants given Fluridone in the absence of N (treatment control) or with N. This indicates a role of Fluridone in the development of bleached leaves.

Table 1. Effect of nitrogen rates on the number (square root transformed) of Fluridone induced bleached leaves per plant at one week after Fluridone application (WAF) to 8 WAF

Treatments	1WAF*	2WAF	4WAF**	5WAF	6WAF	7WAF	8WAF	9WAF
0 Kg N/ha + 0µM Fl	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0 Kg N/ha + 10µM Fl	1.21	2.93	3.85	3.65	3.28	3.22	2.98	2.59
50 Kg N/ha + 10µM Fl	0.85	2.57	3.91	3.88	3.14	2.79	2.01	1.79
100 Kg N/ha + 10µM Fl	1.14	3.40	4.06	4.03	3.65	3.65	3.01	2.42
150 Kg N/ha + 10µM Fl	1.50	3.31	4.01	3.05	2.44	1.99	1.43	0.68
LSD($p \leq 0.05$)	0.75	0.79	0.79	0.73	0.41	0.74	0.90	0.99**

*=2 week after 1st split nitrogen application; **= 2 weeks after 2nd split nitrogen application date

The application of nitrogen both enhanced and reduced bleaching depending on the time space between N application and bleach count. Two weeks after the application of the 1st split of N (1 WAF; when N level in soil was only slightly raised), nitrogen treatments did not differ in the number of bleached leaves they induced with the highest number of bleached leaves observed under 150 kg N/ha treatment. Two weeks after the application of the full N rates (4 WAF), more bleached leaves were observed in all treatments with or without N indicating that some leaves take longer time to get bleached than others. Also, under the prevailing high N and Fl condition, there were more bleached leaves under higher N rate treatments (100 and 150 kg N/ha) than lower N rate treatments (50 kg N/ha). In contrast, at 5 WAF or later, high N rates (particularly 150 kg N/ha) significantly reduced the number of bleached leaves compared to lower N rate (0 or 50 kg N/ha) treatments. Also, the number of bleached leaves per treatment declined with every progressing week, with 150 kg N/ha giving the most significant decline in number of bleached leaves compared to either 0 or 50 kg N/ha.

Thus, under FL and enhanced N condition (*i.e.*, prior to or at two weeks after full N application), the number of bleached leaves per treatment was higher at high N rates compared to low N rate (0 and 50 kg N/ha). On the other hand, under weaning FL condition (5 WAF and later), number of bleached leaves declined significantly under high N rates than lower N rate, particularly at 150 kg N/ha.

Effect of nitrogen fertilization on number of green leaves before and after Fluridone application

Prior to treatment application, the average number of leaves per plant was 22±6.37 (SD) and there was no significant difference between the number of leaves on plants assigned to other treatment plots (Table 2). By 39 DAP (4 days after the first split N application), significant differences in leaf number was observed (Table 2). Compared with the controls, the low N rate treatment (50 kg N/ha) did not significantly increase leaf number. However, at higher nitrogen rates, number of leaves was significantly increased by the application of 100 kg N/ha compared with the controls, 50 or 150 kg N/ha. A similar pattern was observed at 11 days after first split N application, which also correspond to the 3rd Fl application day.

Table 2. Number (square root transformed) of leaves per plant before nitrogen application, four days after first split N application and three days after Fluridone application

Treatments	Initial number (32 DAP)	39 DAP (4 days after 1st N appl.)	46 DAP (11 days after 1st N appl.)
0 Kg N/ha + 0µM Fl	4.64	5.37	5.89
0 Kg N/ha + 10µM Fl	4.36	5.06	5.64
50 Kg N/ha + 10µM Fl	4.35	5.0	5.61
100 Kg N/ha + 10µM Fl	4.71	5.99	6.57
150 Kg N/ha + 10µM Fl	4.42	5.29	6.08
LSD ($p \leq 0.05$)	0.36^{ns}	0.38	0.37

Thus, the application of high N rates led to significantly more leaves observable as early as 3 and 11 days after N application with the number of leaves being highest at 100 and significantly higher than that at 150 kg N/ha. On the other hand, at lower N rate (50 kg N/ha), the number of leaves produced was not significantly different from the controls. The application of Fluridone for three consecutive days did not change the pattern of the effect of N application on number of green leaves.

Number of green leaves continued to increase through the study period in all treatments (Table 3). However, the application of Fluridone under a 0 kg N/ha condition (0 kg N/ha + 10µM Fl) caused a significant

reduction in number of green leaves produced compared with the complete control. The application of nitrogen at 100 and 150 kg N/ha, significantly increased the number of green leaves on the Fluridone treated plants compared to that in the treatment control and 50 Kg N/ha + 10µM Fl treatment (Table 3).

Table 3. Effect of different nitrogen levels on number of green leaves produced on Fluridone treated plants

Treatments	1WAF*	2WAF	4WAF**	5WAF	6WAF	7WAF	8WAF	9WAF
0 Kg N/ha + 0µM Fl	6.40	6.45	7.47	7.74	8.10	8.34	8.70	9.00
0 Kg N/ha + 10µM Fl	6.00	5.81	5.81	6.60	7.12	7.65	8.13	8.46
50 Kg N/ha + 10µM Fl	6.01	5.80	5.71	5.97	6.89	7.43	8.01	8.23
100 Kg N/ha + 10µM Fl	6.94	6.68	6.77	7.44	8.08	8.64	9.24	9.65
150 Kg N/ha + 10µM Fl	6.46	6.57	7.02	8.01	8.61	9.15	9.64	10.28
LSD ($p \leq 0.05$)	0.41	0.50	0.52	0.66	0.62	0.69	0.72	0.78

*=2 week after 1st split nitrogen application; **= 2 weeks after 2nd split nitrogen application date

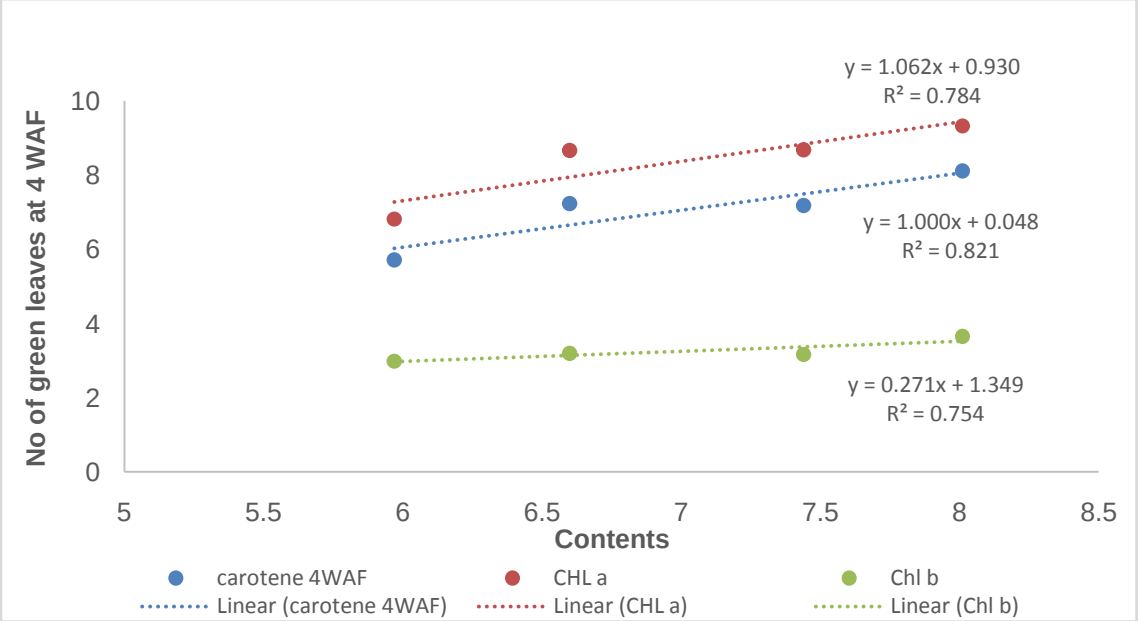
Effect of different nitrogen rates on chlorophyll and total carotene content of yam leaves at 4 weeks after 10 µM Fluridone application

A 4 WAF, which corresponded to 2 weeks after full N application, the total carotenes in green leaves from the experimental control was 8.07 µg/ml at 4 WAF while that in treatment control (0 Kg N/ha + 10µM Fl) was lower (7.23 µg/ml). This indicates that Fluridone caused a reduction in carotene level. To determine whether the application of nitrogen regulates Fluridone action through the control of leaf carotene content, only data from treatments that received Fluridone were included in the analysis. At low N (50 kg N/ha) rate, leaf carotene (µg/ml) content was significantly reduced by 1.52 compared to the control, indicating that implies that carotene production was blocked (**Table 4).** However, at higher N rates the reverse was the case. Chlorophyll a and b contents also increased at high N rates (particularly 150 kg N/ha) compared to lower N rate (50 kg/ha). Furthermore, number of green leaves at 4 WAF was highly (R^2= >0.75) and positively and related to leaf carotene content and chlorophyll a and b contents (Fig. 2)

Table 4. Green Leaf carotene content at four weeks after the application of 10µM**Fluridone in a nitrogen**

Treatments	Carotene content (µg/ml)	Chlorophyll *a*	Chlorophyll *b*
0 Kg N/ha + 10µM Fl	7.23	8.66	3.19
50 Kg N/ha + 10µM Fl	5.71	6.82	2.99
100 Kg N/ha + 10µM Fl	7.18	8.68	3.17
150 Kg N/ha + 10µM Fl	8.11	9.33	3.65
SED ($p \leq 0.05$)	0.684*	1.090^{ns}	0.300^{ns}

Thus, Fluridone alone and at low N rate reduced carotene, and chlorophyll *a*, content of the leaves compared to the experimental control while high N application rates (particularly 150 kg N/ha) increased the carotene and chlorophyll a b contents of green leaves. Also, a strong positive relationship between carotene, chlorophyll contents and number of green leaves at 4 WAF was observed.



Effect of treatments on*in situ* leaf chlorophyll content

There was no significant difference in the chlorophyll content of leaves of the experimental control and those of the treatment control. Also, among the N treatments, leaf chlorophyll content did not significantly vary. This indicates that the observed Fluridone induced bleaching response may not be related to alteration(s) of the pathway of chlorophyll in green leaves.

Effect of treatments on plant dry matter content and leaf nitrogen content at 9 WAF

Data at the end of the study showed that dry matter content of the vine (petioles inclusive), leaves, tubers and root did not vary significantly across treatments indicating that Fluridone did not affect dry matter content of different parts of the yam plant. Also, leaf tissue nitrogen content did not vary significantly across treatments with the values ranging from 2.9% (in the experimental control) to 3.5% (in the Fluridone treatments). The values suggested that Fluridone alone encouraged increase in tissue N content while N application to soil before leaf foliar application Fluridone slightly increased leaf tissue N content.

IV. Discussion

The characteristic bleaching effect of Fluridoneon leaves of treated plants was observed as earlier as the 6th day following daily spraying of *D. alata* yam plants with Fluridone. This bleaching effect affirms that the test plants in this study absorbed the Fluridone and responded to it. The bleaching response of plants, including *D. alata*, to Fluridone is well documented[30,12,16]. Bleaching is purported to result from the accumulation of the whitish substance phytoene that accumulates overtime as a result of the inhibition of the action of the enzyme known as phytoene desaturates (PDS) whose function is to convert phytoene to carotenoids[1]. The significant reduction in leaf carotene content observed at 4 weeks after Fluridone application in this study, supports the existing theory on the mode of action of Fluridone. Therefore, this study has shown that daily application of 50 ml of 10 µM Fluridone causes bleaching of leaves of *D. alata* plants as early at six days after its application with observable reduction in leaf carotene content.

Enhancing soil and in turn leaf tissue nitrogen status through the application of ammonium nitrate to the slightly acidic sandy loam soil of the Niger Delta of Nigeria was found to both increase and reduce the number of bleached leaves on the Fluridone treated plants depending on the time space between N application and leaf (bleached) count. Between the second week after first split N application and the second week after the second split/full N application, the number of bleached leaves on Fluridone treated plants was significantly more when the plants grew on soil given high N rates (100 and 150 kg N/ha) than on soils given low N rates (0 and 50 kg N/ha). The reverse was however the case on later dates; in which high N application rates (particularly 150 kg N/ha) led to significantly fewer bleached leaves than at lower N rates. This was a major finding of this study. Although it isn't fully clear why the bleaching response of leaves to different nitrogen levels varied with time, the fact that the number of bleached leaves in all Fluridone treatments increased until 4 WAF application and then declined progressively thereafter provides some explanation. The progressively declining number of bleached leaves indicates that the effect of Fluridone began to wear off by about the 5 WAF application. The wearing off of Fluridone effect as well as decline in its concentration over time have been reported in plants,

soils or sediments[30,12,31]. Therefore, this study proposes that high N rates reduces the number of bleached leaves on treated plants asFluridoneeffect weans while the reverse is the case under highFluridoneefficacy. The increase inleaf N and chlorophyll (an N rich pigment) contents observed in this study following the application of Fluridone alone indicates that it encouragesincreasedN uptake from low N soils. Thus, additional supply of 100 or 150 kg inorganic N/ha in the face of active 10 µMFluridone with its attendantaccumulation of phytoene and reduced carotenes may have combined to facilitate increased bleaching through possible photooxidation of the accumulated photosensitizers on the exposed chlorophyll biosynthetic pathway. Photooxidation of photosensitizers such as chlorophyll and protoporphyrin IX has been reported[32,1]. This also shows that Fluridone may not have a direct effect on chlorophyll content as also suggested by other researchers[1].

The presence of many green leaves (on all Fluridone treated plants) with atLeafreadings of 35 or higher indicates chlorophyll and leaf nitrogen contents were withinacceptable ranges[27](https://download.atleaf.com/mainp). It also indicates thatsome growth compensations (e.g., increased N uptake and continued dry matter accumulation) may have occurred to maintain normal functioning of some leaves and hence their ability to respond normally to increase N supply. The observed increase in number of green leaves per plant level with increase in N up to 100 kg N/ha may be explained bythe strong (R^2= >0.75) and positive relationship found between number of green leaves per plant andcarotene and chlorophyll a and b contents. Many reports have also shown similar relationship between increased chlorophyll content and number of leaves[22,23].

V. Conclusion

In conclusion, in order to withstand the bleaching effect of Fluridone, the application of 50 kg N/ha is advised when the soil is low in nitrogen content and N is applied before Fluridone application. However, high N levels (100 or 150 kg N/ha) may be applied when the bleaching effects of Fluridone begins to wean. This action would cushion the effect of Fluridone and promote fast growth.

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