



# Nitrate Reductase, Chlorophyll Content Index and Yield *Zea mays sacharrata* Using Guano Waste Fertilizer

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# Abstract

Guano fertilizer is a type of fertilizer made from bat guano, which has been mixed with dirt and microorganisms that are decaying for a long time in the cave where it originally settled.

Research has been done on the effects of guano fertilizer doses on the development and production of maize in two populations of plants. The findings demonstrated that at a population density of 40000 plantsHa<sup>1</sup>, larger guano fertilizer doses resulted in increased chlorophyll content index (CCI), yield, and kernel length. The larger the guano fertilizer dose, the better the results were or cob height, plant height, Crop Growth Rate (CGR), and Nitrate Reductase Activity (NRA). Corn has a better density of 40000 tan Ha<sup>-1</sup> than 26666 tan Ha<sup>-1</sup>.

Keywords: CCI; CGR; NRA; Plant Height; Population

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# Introduction

Producers of corn, also known as maize (Zea mays L.), are continually examining factors affecting production and, consequently, their net returns, such as hybrids and plant populations. The ability of maize crops to adapt new reproductive structures to the availability of more resources per plant is also limited. The decrease in resource availability per plant in the time leading up to silking, however, causes a significant decrease in yield per plant if the plant density is too high, and this fall in yield per plant is not accompanied by an increase in the number of plants [1]. When populations are dense, many kernels might have greater planting densities decrease the amount of grain per ear, lengthen the time between female and male blooms, and promote plant sterility. Corn researchers' study on plant density is fascinating for at least two reasons. The grain yield, which is now pretty high for farmers can rise with the right populations. Furthermore, according to [2], environmental and genetic factors affect maize yields; or density, and damage to certain crop components can be demonstrated beyond a certain threshold. Stand population very growth and development plans, alter plant architecture, and impact carbohydrate production and partitioning [3]. In contrast to other grass families, corn is sensitive to variations in plant populations [4]. Different maize hybrids have recently produced one cob per plant pretty frequently and lost tillers efficiently at lower densities. In order to make up for the reduction. Maize does not have the most tiller-attributed grass because of the smaller reproductive components and smaller leaf area [5]. Guano, an organic fertilizer with high amounts of N, P, and other nutrients, can boost fertility. Guano fertilizer, according to [6], contains the following vital components for plants: 12% P, 13% N, 1% Mg, 2% K, and 5% S. When utilizing guano fertilizer to encourage growth, the appropriate dose for plants must be taken into account. Fertilizer made from guano has a 13% high N concentration. The use of organic fertilizer affects the soil's physical properties. Indicators of plant development had a positive connection with guano fertilizer dosage, with 15t Ha-1 being the ideal amount [7]. The usage of guano fertilizer may raise soil pH, soil CEC, and accessible N, P, K, and P levels, according to [8]. It is crucial to conduct research on how different sweet corn varieties (Zea mays L.) react to the application of guano fertilizer in a dry land, chemical without affecting dry land output, in order to develop straightforward, affordable, and sustainable improvements in sweet corn farming techniques that can reduce the need for fertilizers.

# **Materials and Methods**

The research was carried out on dry land designated for organic farming (Tabur Mas Organic Farming), Kenteng Village, Bandungan District, Semarang Regency, Longitude 110 036 42, South Latitude 7022 52. Every year there are 84 wet days and rainfall is 1,291 mm. The height of the place is 914 meters above sea level (Bandungan District Central Bureau of Statistics, 2021). The research began at the end of October 2018 until the beginning of March 2019. Starting with land preparation, namely cleaning and processing the soil, preparing seeds (Sweet boy variety), planting Soil characteristics before the study were pH 6.3; Total N 2.36%, P 340.14 mg.kg -1, K 12.60 mg.kg -1, organic C 4.16 %. In a Randomized Group Design (RAK) experiment, treatments are arranged factorially. The examination consists of two factors. Treatment levels P1 (population 40,000 plants ha-1) and P2 (population 26,666 plants ha-1) form the population factor (P). The second factor is guano fertilizer, with treatment levels G1: 0 tons/ha, G2: 2 tons/ha, G3: 4 tons/ha, and G4: 6 tons/ha. Because there were eight treatment combinations, each repeated three times, 24 experimental plots were required. The data obtained were analyzed by analysis of variance and followed by Duncan's multiple range test at the 5% level.

The land is processed until the structure is loose, then beds are made measuring 3 m x 3 m with a height of 20-30 cm with a distance between the beds of 30 cm and a distance between groups of 50 cm. The seeds used are the Sweet Boy variety of sweet corn. Plant with 1 seed per planting hole according to the planting distance attempted. Fertilization is done at the time of planting by making a hole next to the seed, then filling the hole with guano fertilizer according to the dosage.

Maintenance is watering, replanting, loosening, pest and disease control. Watering is done 2 times a day, depending on soil moisture and weather conditions. Replanting is carried out up to 7 days after planting, to replace plants that do not grow or grow abnormally. Cultivation is carried out simultaneously with loosening 4 weeks after planting. Pest control is carried out by spraying insecticide with the active ingredient dheltamethrin 2.5 EC at a dose of 0.5 ml liter-1 water, while disease control is carried out by spraying fungicide with the active ingredient Mankozep M-45 at a dose of 1.5 ml-1 liter water.

Harvesting is carried out 75 days after planting,

with the characteristics of brownish silk and the seeds have turned yellow. Harvesting is done by breaking the cob from the stem. Parameter measurements were carried out when the plants were 45 DAP. Plant height, ear height from the base of the plant/above the ground were observed at 65 DAP, nitrate reductase activity (observed at 55 DAP), plant growth rate (75 DAP/at harvest), and crop yield were all recorded in this study. Corn plant chlorophyll (CCI) was measured on plants aged 45 DAP.

$$CGR = \frac{W2 - W1}{T2 - T1} (gday^{-1})$$

\_ \_ \_ \_

Information:

W1 = weight of 1 plant seed

W2 = weight of the entire corn plant at harvest

T1 = day 1 (at planting)

T2 = harvest day (75 DAP)

Nitrate reductase activity [9] was measured using the ANR method; The third leaf from the top of the corn plant was picked at around 9-10 am as a plant sample. The leaves are washed with distilled water, finely sliced, then 200 mg of the leaves are taken. The weighed leaves were placed in a buffer solution of Na2HPO4 2H2O and NaH2PO4 2H2O at pH 7.5, 5 ml each in a polyethylene tube, then closed and soaked for 24 hours, the buffer solution was discarded and replaced with 5 mL of new buffer solution, then 0.1 mL, 5 M NaNO3 was added to each dark tube. The time for adding NaNO3 was expressed as incubation time 0, and incubation was carried out for 2 hours. Meanwhile, into another test tube, fill the reagent with 0.2 mL of 1% sulfalamide dissolved in 3 N HCl and 0.2 mL of 0.02% Napthylethylandiamide solution. Then 0.1 mL of filtration which had been incubated for 2 hours was put into a test tube containing HCl reagent and Napthylethylendiamide solution. The test tube is shaken to mix the filtrate to speed up the reaction. Leave it for about 15 minutes so that NO2- is reduced with the coloring reagent which will give a pink color. Next, 2.5 mL of distilled water was added to the test tube

as a color diluent. The solution in the test tube was put into a spectrophotometer cuvette to observe the absorbance at a wavelength of 540 nm.

# **Results and Discussion**

At the ear, *Zea mays saccharata* is tall. The interaction between guano fertilizer treatment and plant population was not found to have a significant impact on the cob height, plant height, CGR, or ANR parameters, according to the analysis of variance. Plant population and guano fertilizer both significantly affected plant height on their own, but neither factor was adequate to fully explain it.

#### Ear Heights

In comparison to 26666 plants Ha<sup>-1</sup> (63.08 cm), the plant population was higher at 40000 plants/ha (71.67 cm). The highest cob height in the guano fertilization treatment was generated at a fertilization rate of 6 tons/ha (79.67 cm).

Treatment	Cob boight	Plants haight	CCP	NID
	Cob height	r lants height	COK	INK
	(cm)	(cm)	(g.day <sup>-1</sup> )	(µmol NO2 g per jam)
Plants population				
40000	71.66a	117.92 a	97.59 a	69.90 a
26666	63.08 b	105.17 b	72.96 b	60.75 b
Guano Fertilizer(tons.ha <sup>-1</sup> )				
0	58.00 c	91.17 c	73.15 c	41.33 c
2	63.33 b	105.00 b	56.03 d	62.75b
4	60.50 bc	119.00 a	92.38 b	77.63 a
6	79.67 a	133.00 a	112.60 a	79.58a

Table 1: Average cob height, plant height, CGR, and ANR due to guano fertilizer application in different populations

#### **Plant Height**

40 000 plants make up the population Compared to a population of 26 666 plants in  $Ha^{-1}$  (105.167 cm), sweet corn plants in Ha-1 were taller (117.917 cm). The guano fertilizer treatment resulted in the largest plant (133.00 cm) ever measured at a guano population of 6 tons/ha. 4 tons/ha of guano fertilizer were used, which was greater than the 0 and 2 tons/ha applications but less than the 6 tons/ha application. Due to increased plant density brought on by closer row spacing, photosynthesis is reduced, increasing plant height as a result of competition for light, and less optimal plant growth results [10]. Narrower row spacing can be used to enhance the distance between plants in a row, reducing density and further reducing competition among individual plants [11]. According to [4], root design is more important for increasing grain output in high punt populations than canopy architecture and light absorption. Rainfall and the capacity of plant roots to store soil determine the appropriate plant population in low rainfall areas. Narrower row spacing can be used to enhance the distance between plants in a row, reducing density and further reducing competition among individual plants [11]. The absence of guano fertilizer resulted in a 53.93% increase in crop growth rate. The population of 40000 plants Ha-1 (97.59 g.day-1) was greater than 26666 plants Ha-1 (72.9625 g.day-1). Plant growth rate and the impacts of organic matter and N dosage [12]. Guano is an additional source of nitrogen in addition to organic matter due to its 13% nitrogen content.In order to offer enough nutrients to speed up plant metabolism, 6 tons/ha of nitrogen fertilizer, or up to 780 kg N, might be used. The ability of guano fertilizer to both increase water retention capacity and enrich soil nutrients can be used to improve the water balance in the soil.

#### Activity Nitrate Reductase

In comparison to the plant population of 26666 plants Ha<sup>-1</sup> (60.750 mol NO<sub>2</sub>.g<sup>-1</sup>). ANR values for the plant population of 40000 plants Ha-1 (69.90 mol NO<sub>2</sub>.g-1) were higher. At a dose of 6 tons ha-1 of guano, the maximum NR value was 79,583 mol NO2.g-1, which did not statistically differ from a dose of 4 tons ha-1 of guano, which was 77,633 mol NO<sub>2</sub>.g-1.Nitric oxide (N0) is a secondary messenger gas found in humans, animals, plants, fungus, and bacteria. Growth, development, metabolism, leaf senescence, biotic and abiotic stress, defense mechanisms, and interactions with pathogens are only a few of the crucial physiological activities that NO mediates in plants [13]. Before recently, it was thought that NR created NO by directly reducing NO<sub>2</sub>, although a number of indirect processes, including NR, have since been postulated. According to this approach, NR acts through its diaphorase to catalyze the transfer of electrons from NAD(P)H to NO producing nitrite reductase (NOFNIR), which reduces NO<sub>2</sub> to NO [14]. Due to the complexity of this dependence, processes that regulate or compensate for it are triggered. The catalytic flux of NR is controlled by the presence of a substrate as well as the amount and activity of functional NR. The nitrate reduction capacity is regulated in relation to the plant's general metabolic rate through signal transduction pathways and metabolic sensors. The results of the analysis of variance showed a substantial correlation between the CCI, yields, lengths, and weights of the sweet corn kernels, as well as the treatment of plant population and rising guano fertilizer doses. The average CCI, yields, kernel length, and weight of sweet corn as a result of guano fertilizer application in diverse populations are shown in Figure 1 and Figure 2.

# Chlorophyll Content Index



Figure 1: Chlorophyll content index (CCI) and yield of Zea mays saccharate effect of guano fertilizer under two populations



Figure 2: Kernel longs and kernel weight of Zea mays saccharate effect of guano fertilizer under two populations

The higher the guano fertilizer dose, for both a population of 40000 plants.Ha<sup>-1</sup> and 26666 plants.Ha<sup>-1</sup>, the higher the CCL. The largest CCI value was at 6 tons/ha of guano fertilization in a population of 40000 plants Ha<sup>-1</sup> (22.5), which was significantly different from all treatments. Leaf chlorophyll is widely used as a crucial indicator of leaf greenness to assess leaf nutritional shortages and chloro-

phyll variations. The amount of chlorophyll in the canopy is one indicator of the environment's seasonal uptake of carbon [15]. In contrast to other plants' physiological and biochemical traits like the leaf area index (LAI) or crown chlorophyll content, the amount of chlorophyll in a plant's leaves directly indicates its nutritional health. Quantitative analyses of plant leaf chlorophyll concentration are particularly useful for developing a complete and accurate understanding of plant growth status and for precision agriculture management [16]. The CCI can also be used to support crop N fertilization in terms of relative chlorophyll content, and even more so to improve upyield and biomass estimates.

#### Maize Yield

The maximum yield value was at 6 tons Ha<sup>-1</sup> of guano fertilization in a population of 40.000 tons, which was significantly different from all treatments (61.403 kg plot<sup>-1</sup>). Many modern maize cultivars usually only produce one cob per plant and have difficulty producing tillers at low densities. In reality, using large populations makes plants compete more fiercely for nutrients, water, and light. This can reduce yield by encouraging apical dominance and sterility, which in turn lowers the number of cobs and sets of kernels produced per plant and ear. The findings are consistent with earlier studies [17]. The amount of water, nitrogen, and photosynthesis available to the growing ear can be constrained by dense plant populations. Furthermore, it was found that the competition for light, air, and nutrients caused by towering stands caused the plants to experience impaired regenerative development and seed filling. Grain yield was also significantly impacted by plant population [18]. Hybrids and plant densities, with low plant density plots displaying the least amount of logging, had an impact on stem fall variations, according to [19].

#### **Enormous Kernels**

The largest kernel longs were produced by applying 6 tons/ha of guano fertilizer to either a population of 40000 tons.Ha<sup>-1</sup> or 26666 tons.Ha<sup>-1</sup>, which was comparable to applying 4 tons.Ha<sup>-1</sup> of guano fertilizer to a population of 26666 tons.Ha<sup>-1</sup>. High plant densities can limit the amount of water, nitrogen, and photosynthesis available to the developing ear. High plant densities can limit the amount of water, nitrogen, and photosynthesis available to the developing ear. Large kernels are conducive to the formation of well-developed roots, while sowing at the right depth is conducive to downward growth and distribution of roots in the middle and late stages, thereby increasing the water absorption capacity and drought resistance, ultimately achieving an increase in yield [20].

#### The Kernel Weight

As a result of competition for light, air, and nutrients, tall stands have also been demonstrated to cause plants to experience reduced reproductive growth and seed filling [18]. The application of guano fertilizer at a rate of 6 tons Ha<sup>-1</sup> on a population of 26666 plants. Ha<sup>-1</sup> (93,0g) resulted in the highest kernel weight and was noticeably different from all other treatments.

## Discussion

This measurement produces the Chlorophyll Content Index (CCI) as the relative chlorophyll content. This chlorophyll measuring device usually measures the transmission of two wavelengths of radiation through plant leaves, namely the red spectrum at a wavelength of 650 nm and near infrared (NIR) at a wavelength of around 900 nm. Increasing chlorophyll concentration increases the absorption of red radiation. According to [21] the energy used in the photosynthesis process is in the form of photons originating from the light spectrum. Solar radiation energy is captured by chlorophyll and then converted into chemical energy through the process of photosynthesis [22]. In comparison to the plant population of 26666 plants Ha-1 (60,750 mol NO2.g-1). ANR values for the plant population of 40000 plants Ha-1 (69.90 mol NO2.g-1) were higher. This high population causes an increase in ANR which is thought to be significantly related to water content in the tissue [23]. As plant populations increase, there is an increase in the number of potential seeds, which is reflected in greater yields. With the number of kernels that maximizes plant population being higher than the average kernel weight, consequently higher yields are associated with more kernels per unit area of land. This occurs because grain yields increase as plant populations increase. The decrease in grain yield due to changes in the number of plants from 26666 plants to 4000 plants was caused by a decrease in the average kernel weight. For at least two reasons, research on plant density is of interest to corn experts. Corn yield density also depends on environmental impacts and genotype, and damage to certain plant components may be demonstrated above certain thresholds. Because these changes alter growth and development strategies, as well as carbohydrate partitioning and production, stand population has an influence on plant architecture [24]. Increasing harvested areas, using high-yielding cultivars, balanced fertilization strategies, and other technological advances can be used to increase corn production. Yields increased when N application was delayed until side-dress, according to [4] analysis of the relationship between row spacing and timing of N. organic fertilizers with nitrogen sources such as guano. The chemical and biological properties of soil are influenced by organic fertilizer with a nitrogen source such as guano fertilizer, which makes the physical and chemical properties of the soil easier to digest, holds water stronger, and has a smoother surface [25]. Organic fertilizer can improve the biological properties of soil by encouraging the proliferation of microorganisms there [26].

# Conclusion

The population of 40000 plants.Ha<sup>-1</sup> produces

ANR, CGR, ear height, and plant height. The fertilization with 6 tons. Ha<sup>-1</sup> of guano resulted in the highest ear height, plant height, CGR, and ANR. Additionally, with a population of 40000 plants.Ha<sup>-1</sup>, the higher the dose of guano fertilizer, the higher the CCI, kernel yield, kernel length, and kernel weight.

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# **Conflict of Interest**

The authors declare there are no conflicts of interest regarding the publication of this manuscript.

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