

## Enhancement of nitrogen use efficiency using *Tithonia diversifolia* green manure in lime-amended Ultisol: A $^{15}\text{N}$ isotope tracing study for sustainable chili Production

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**Abstract:** This research investigated the potential of *Tithonia diversifolia* as a sustainable nitrogen source for chili (*Capsicum annuum* L.) production in lime-amended Ultisol using  $^{15}\text{N}$  isotope tracing technology. A factorial pot experiment was conducted with two factors: lime application (0 and 2 ton ha<sup>-1</sup>) and nitrogen substitution levels (0%, 25%, 50%, 75%, and 100% replacement of synthetic N fertilizer with fresh *Tithonia* biomass). A pot experiment was conducted to evaluate the potential of *Tithonia diversifolia* green manure as a partial substitute for synthetic nitrogen fertilizer in chili (*Capsicum annuum* L.) production on acidic Ultisol soil. The study employed a factorial design with varying N substitution levels (0%, 25%, 50%, 75%, and 100%) and lime treatments (with and without lime application). *Tithonia* biomass was incorporated into the soil and incubated for 21 days before chili transplantation. Soil chemical properties, plant growth parameters, yield components, nutrient uptake, and  $^{15}\text{N}$  isotope analysis were measured throughout the 12-week growing period. The Ultisol soil exhibited typical constraints with pH 5.27, high Al saturation (36.85%), and low fertility status. Results demonstrated that *Tithonia* application significantly improved soil chemical properties by reducing exchangeable Al from 2.00 to 0.53 cmol kg<sup>-1</sup> and Al saturation from 36.85% to 7.48% without lime treatment. Soil organic carbon increased linearly with *Tithonia* application, reaching 2.47% at the highest substitution level with lime. Lime application enhanced soil pH and further reduced Al toxicity, creating synergistic effects with *Tithonia* amendments. Optimal chili performance was achieved with 25-30% N substitution, producing maximum straw dry mass (74.95 g pot<sup>-1</sup>), fruit fresh weight (397.53 g pot<sup>-1</sup>), and nutrient uptake efficiency. The  $^{15}\text{N}$  isotope analysis revealed that *Tithonia* contributed 1.78% to 28.40% of total plant nitrogen uptake, with higher substitution levels showing increased N contribution but reduced overall plant performance. Complete substitution (100%) significantly reduced plant growth and yield, indicating incomplete nutrient mineralization within the 21-day incubation period. The study concludes that *Tithonia diversifolia* can effectively substitute up to 50% of synthetic nitrogen fertilizer in chili production systems while maintaining optimal growth and yield parameters. This integrated approach offers a sustainable solution for reducing synthetic fertilizer dependency in tropical agriculture, particularly in acidic soils where lime application enhances the effectiveness of organic amendments.

**Keywords:** Nitrogen fixation, Isotopic tracing, Soil amendment, *Capsicum annuum*.

### Introduction

Nitrogen (N) deficiency remains one of the most critical constraints limiting crop productivity in tropical agricultural systems, particularly in highly weathered acidic soils such as Ultisols. These soils, which cover approximately 25% of global agricultural land and are predominant in Southeast Asia, are characterized by low pH, high aluminum toxicity, low cation exchange capacity, and poor nutrient retention capacity (Agegnehu et al., 2021). The inherent fertility challenges of Ultisols are further exacerbated by rapid N mineralization and leaching losses under tropical climatic conditions, necessitating frequent and often economically unsustainable synthetic fertilizer applications.

The intensive use of synthetic nitrogen fertilizers in tropical agriculture has raised significant environmental concerns, including groundwater contamination, eutrophication of aquatic ecosystems, and greenhouse gas emissions through N<sub>2</sub>O production (Tyagi et al., 2022). Moreover, the economic burden of synthetic fertilizers on smallholder farmers, coupled with their limited availability in remote rural areas, underscores the urgent need for sustainable and locally available nitrogen sources.

*Tithonia diversifolia*, commonly known as Mexican sunflower or tree marigold, is a promising green manure species from the Asteraceae family that offers exceptional potential for sustainable nitrogen management in tropical agroecosystems (Ajao and Moteetee, 2017). This

fast-growing perennial shrub can produce 15-20 tons of dry matter per hectare annually and demonstrates remarkable adaptability to various soil conditions and climatic zones, thriving in both humid and semi-arid environments with minimal external inputs. The species exhibits superior nitrogen accumulation capacity with foliar N concentrations ranging from 2.5% to 4.2% dry weight, significantly higher than most conventional green manure crops, while its extensive root system enables efficient nutrient scavenging from deeper soil layers, effectively recycling nutrients that would otherwise be lost through leaching (Murtaza et al., 2025)

The decomposition characteristics of *T. diversifolia* biomass are particularly favorable for sustainable N release, with studies reporting C:N ratios between 12:1 and 16:1, which facilitates rapid mineralization and subsequent N availability to crops (Gupta et al., 2023). However, the precise quantification of N contribution from *T. diversifolia* to crop uptake remains challenging using conventional methods, limiting our understanding of its true potential as an alternative N source. The application of stable nitrogen isotope ( $^{15}\text{N}$ ) tracing technology has revolutionized our ability to quantify N dynamics in soil-plant systems with unprecedented precision. The  $^{15}\text{N}$  labeling technique allows direct tracking of N transformation, movement, and uptake processes, providing quantitative estimates of fertilizer N use efficiency, soil N contribution, and organic matter N mineralization (He et al., 2020).

This isotopic approach is particularly valuable in green manure studies, where it enables differentiation between N derived from organic amendments versus soil indigenous N, thereby providing accurate assessments of green manure N contribution to crop nutrition (Lyu et al., 2024). Despite its proven effectiveness, the application of  $^{15}\text{N}$  tracing to evaluate *T. diversifolia* N contribution in tropical soil-crop systems remains limited, representing a significant knowledge gap in sustainable agriculture research. The management of soil acidity through lime application is fundamental to optimizing nutrient availability and crop performance in Ultisols (Rheinheimer et al., 2018). Soil acidification leads to increased aluminum and manganese solubility, reduced phosphorus availability, and impaired biological nitrogen fixation (Mao et al., 2017). Liming not only neutralizes soil acidity but also enhances cation exchange capacity, improves soil structure, and promotes beneficial microbial activity essential for organic matter decomposition and nutrient cycling (Wang et al., 2021). The interaction between lime application and organic matter decomposition is particularly complex, as pH modification can significantly alter microbial community structure and enzymatic activity, thereby influencing N mineralization rates from green manure materials (Lin et al., 2018). Understanding these lime-organic matter interactions is crucial for developing integrated nutrient management strategies that maximize N use efficiency while maintaining soil health.

Chili (*Capsicum annum* L.) represents one of the most important horticultural crops in tropical and subtropical regions, with global production exceeding 38 million tons annually (Olatunji and Afolayan, 2018). The crop's high economic value and nutritional significance make it an attractive option for smallholder farmers seeking to improve their livelihoods. However, chili production is highly responsive to nitrogen nutrition, with N deficiency severely limiting fruit yield and quality parameters including capsaicin content, fruit size, and post-harvest shelf life (Delai et al., 2024). The nitrogen requirement of chili varies significantly across growth stages, with peak demand occurring during flowering and fruit development phases. Conventional N management relies heavily on synthetic fertilizers, but the integration of organic N sources through green manure systems offers promising alternatives for sustainable chili production, particularly in resource-constrained smallholder farming systems.

This study addresses critical knowledge gaps in sustainable nitrogen management for tropical horticulture by employing  $^{15}\text{N}$  isotope tracing technology to quantify the contribution of *T. diversifolia* as an N source for chili production in Ultisol conditions. The research specifically investigates how lime application influences the efficiency of N transfer from green manure to crop, providing essential information for developing integrated nutrient management strategies. The findings will contribute to the growing body of evidence supporting ecological engineering approaches in agriculture, where natural processes are harnessed to enhance ecosystem services while maintaining productive capacity. This research aligns with global sustainability goals by promoting reduced dependence on synthetic inputs, enhanced soil health, and improved resource use efficiency in tropical agricultural systems.

Understanding the precise mechanisms of N cycling between *T. diversifolia*, soil, and chili plants will inform the development of site-specific recommendations for green manure management, ultimately supporting the transition toward more sustainable and resilient agricultural systems in tropical regions.

## Result and Discussion

### pH H<sub>2</sub>O, Al-exchangeable and Al saturation

Ultisol soil used in this study has a low level of fertility. This is indicated by nutrient levels and exchangeable bases in low to medium criteria. In addition, this soil also reacts acidic with a pH of 5.27, exchangeable-Al: 2 cmol.kg<sup>-1</sup> and Al saturation is quite high at 36.85%. The treatment without liming was deliberately designed with the intention of seeing whether the green manure applied in this study could replace or reduce the need for lime to reduce Al solubility and increase the pH of Ultisol.

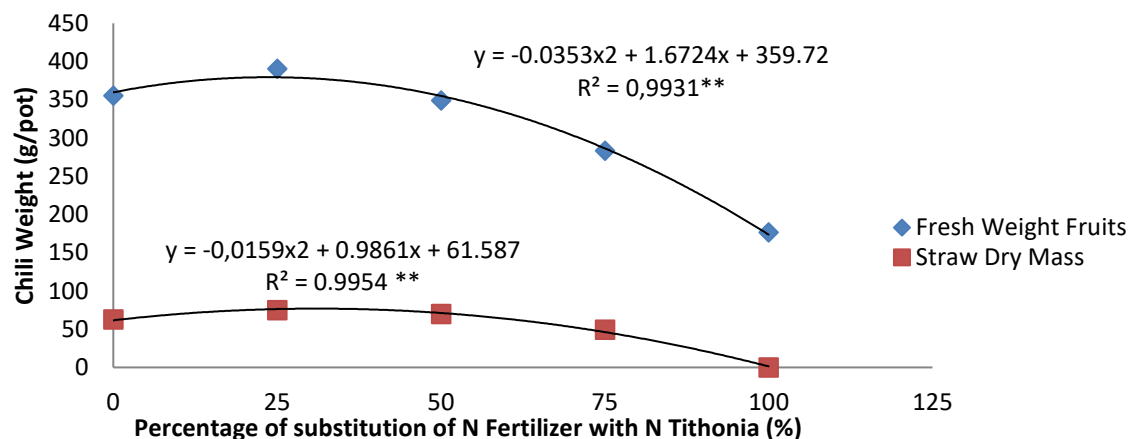
Based on Table 1, it can be stated that the treatment of lime 2 ton.ha<sup>-1</sup> at various doses of tithonia able to raise the pH and reduce soil exchangeable-Al. Increasing the dosages of fresh tithonia from 400 g/pot to 1600 g/pot has also decreased soil exchangeable-Al from 2 cmol.kg<sup>-1</sup> to 0.53 cmol.kg<sup>-1</sup> in the treatment no lime, while in the treatment of soil lime Al-exchangeable content which was originally 0.53 became unmeasurable. Likewise, the Al saturation decreased as the dose of tithonia increased, both in the soil without lime and in 2x Al-exchangeable lime.

Increasing the dosages of fresh tithonia from 400 g pot<sup>-1</sup> to 1600 g pot<sup>-1</sup> has also reduced soil Al-exchangeable from 2 cmol.kg<sup>-1</sup> to 0.53 cmol.kg<sup>-1</sup> in the treatment without lime, while in the treatment of soil lime Al-exchangeable content which was originally 0.53 became unmeasurable. Likewise, Al saturation decreased with the increase in tithonia dose. In the treatment without lime, the increase in tithonia dose can reduce Al saturation from 36.85% to 7.48%. For the treatment of liming 2 tons ha<sup>-1</sup>, an increase in the dose of tithonia decreased Al saturation from 0.76 to not measurable.

However, the increase in the dose of Tithonia is not always followed by an increase in soil pH and even tends to lower soil pH. This situation is thought to be due to Tithonia which in the process of weathering will produce organic acids. Some of these organic acids in the soil show more acidic influence than the capacity to form complexes by freeing H<sup>+</sup> ions. Changes in pH in the soil that is calcined show an increase as the dose of tithonia increases. This increase in pH indicates that the provision of tithonia green fertilizer on Ultisol will be more influential when liming is done first. Ca + elements added through lime will be absorbed by the soil, then organic acids produced from weathering tithonia will bind Al and H to reduce its solubility in the soil.

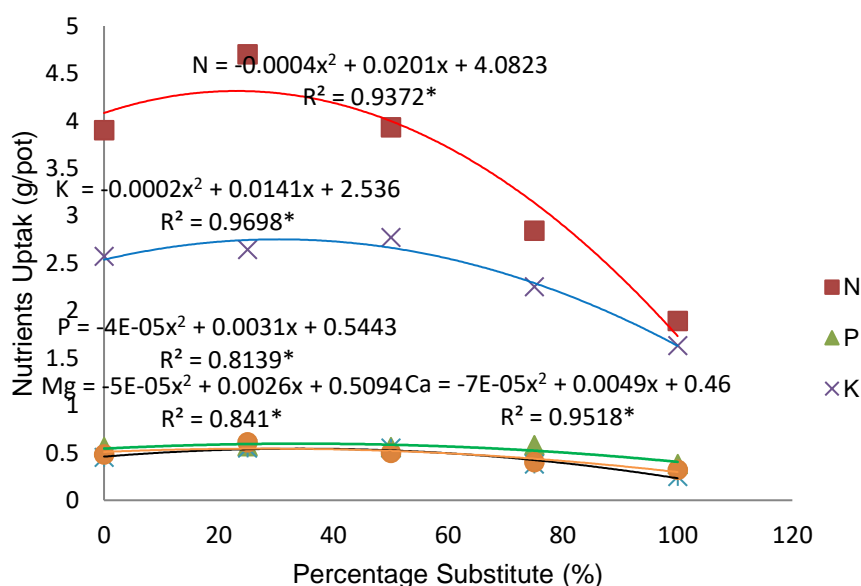
**Table 1.** Effect of fresh Tithonia dosage and lime on pH (H<sub>2</sub>O), Al-exch and Al saturation.

Fresh Tithonia (g/10 Kg soil)	pH (H <sub>2</sub> O)		Al-exch (cmol.kg <sup>-1</sup> )		Al-Saturation (%)	
	No Lime	Lime 2 t.ha <sup>-1</sup>	No Lime	Lime 2 t.ha <sup>-1</sup>	NoLime	Lime 2 t.ha <sup>-1</sup>
0	5.27	5.51	2.00	0.53	36.85	10.76
400	5.18	5.49	1.06	Unmeasured	20.77	Unmeasured
800	5.18	5.56	1.06	Unmeasured	18.04	Unmeasured
1200	5.21	5.61	0.53	Unmeasured	7.63	Unmeasured
1600	5.22	5.60	0.53	Unmeasured	7.48	Unmeasured

**Figure 1.** Correlation parameters relating Percentage of substitution of N Fertilizer with N Tithonia. \*\* = significant at P ≤ 0.05.**Table 2.** Effect of fresh Tithonia treatment and lime on C-organic Content, N-total and P-available Soil.

Fresh Tithonia (g/10 Kg soil)	SOC (%)		Total N (%)		C:N Ratio		Available P (ppm)	
	No Lime	Lime 2 ton.ha <sup>-1</sup>	No Lime	Lime 2 ton.ha <sup>-1</sup>	No Lime	Lime 2 ton.ha <sup>-1</sup>	No Lime	Lime 2 ton.ha <sup>-1</sup>
0	2.13 b	2.19 a	0.21c	0.21c	10.14a	10.43a	13.42a	12.26a
400	2.22 a	2.28 a	0.25b	0.25b	8.88a	9.12a	14.80a	12.26a
800	2.23 a	2.35 a	0.28a	0.27a	7.96b	8.70b	11.84b	11.94a
1200	2.26 a	2.47 a	0.29a	0.30a	7.79b	8.23b	11.84b	11.42a

Note: Numbers with different lowercase superscripts in the same column are significantly different at the 5% level according to the LSD test.

**Figure 2.** Correlation parameter relating chili nutrients uptake (Dry Straw and Fruit) on percentage substitution of N fertilizer with N Tithonia.

### **Levels of c-Organic, N-total and P-available**

The effect of lime soil organic carbon (SOC) increased both in the treatment without tithonia and its given tithonia. Without tithonia soil SOC levels was increased by 2.8% or about 560 mg pot<sup>-1</sup>. Increasing the dose of tithonia increased the percentage of SOC linearly from 0.45% to 4.2% at each increase in the dose of tithonia 400 g plant<sup>-1</sup>. The highest increase was obtained in the treatment of lime with a dose of tithonia (1600 g plant<sup>-1</sup>) which amounted to 8.9% compared with C-organic levels without tithonia.

The increase in soil C-organic levels due to the addition of tithonia doses after 3 weeks of incubation clearly comes from tithonia green fertilizer. Tithonia incubated with 20 kg of soil for 3 weeks has begun to decompose and produce organic compounds that are mostly composed of the element Carbon. The more the amount of fresh tithonia added, the more organic compounds produced from the decomposition process. Soil N-total levels also increased along with the addition of tithonia doses, while the effect of lime alone did not show an increase in soil N-total levels. Table 2 shows the soil N-total levels were previously 0.21% increased to 0.25% due to the provision of fresh tithonia as much as 400 g pot<sup>-1</sup>, until it reached 0.32% at the rate of 1600 g pot<sup>-1</sup> tithonia. N-total increase ranged from 3.5% - 19% each addition of 400 tithonia g pot<sup>-1</sup>. Giving lime equivalent to 2 tons ha<sup>-1</sup> on Ultisol soil has not increased the P-availability. This can be seen from the low levels of P-available in the treatment without adding tithonia in Table 4, even a decrease of 1.16 ppm after the incubation period with lime. However, the changes in P-available levels are still within the same criteria. The same thing also happens in the treatment of increased doses of tithonia which tends to reduce soil available-P. The same thing also occurs in the treatment of increased doses of tithonia which tends to decrease the available P every addition of 400 g pot<sup>-1</sup> of fresh tithonia. This is caused by the incomplete weathering process of Tithonia, resulting in the production of organic acids. According (Wang et al., 2016), humic acid can react directly with phosphate ions without the process of metal bridging or co-adsorption. Because phosphates will react rapidly with exposed OH groups of clay minerals, it is more than conceivable to expect the phosphate to also react directly with hydroxyls (OH) present in the functional groups of humic matter. Likewise, (Mwangi et al., 2020), assert tithonia inputs in combination with phosphate rock treatments are feasible alternatives for sustaining soil phosphorus compared to sole phosphate rock and sole *Tithonia diversifolia* in acidic humic nitisols. (Scrase et al., 2019) is comparable to that mycorrhizae in binding P was higher when phosphorus plus tithonia was given compared to the sole phosphate addition.

### **Exchangeable soil cations**

The content of soil bases (K, Ca, and Mg) showed an increase in the form of exchangeable due to increased doses of tithonia in both lime and non-lime soils. Based on the data presented in Table 3, the effects of fresh Tithonia and lime treatment on exchangeable soil cations, specifically exchangeable potassium (K<sup>+</sup>), calcium (Ca<sup>2+</sup>), and magnesium (Mg<sup>2+</sup>), can be interpreted as follows:

The application of fresh Tithonia significantly influences the levels of exchangeable cations in the soil. As the amount of Tithonia increases from 0 to 1600 g pot<sup>-1</sup>, there is a noticeable increase in exchangeable K<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup>, regardless of lime treatment. For instance, at 1600 g pot<sup>-1</sup> of Tithonia, the exchangeable K<sup>+</sup> reaches 1.20 cmol.kg<sup>-1</sup> without lime and 1.40 cmol.kg<sup>-1</sup> with lime, indicating that Tithonia contributes to higher potassium availability in the soil. Similarly, the exchangeable Ca<sup>2+</sup> levels also show an increase with the addition of Tithonia, with values rising from 1.26 cmol.kg<sup>-1</sup> at 0 g/pot to 1.31 cmol.kg<sup>-1</sup> at 1600 g/pot without lime, and from 2.01 cmol.kg<sup>-1</sup> to 2.83 cmol.kg<sup>-1</sup> with lime treatment. This suggests that lime may enhance the availability of calcium in the presence of organic amendments like Tithonia. Moreover, the exchangeable Mg<sup>2+</sup> levels also exhibit an upward trend, increasing from 0.67 cmol.kg<sup>-1</sup> at 0 g pot<sup>-1</sup> to 1.31 cmol.kg<sup>-1</sup> at 1600 g pot<sup>-1</sup> without lime, and from 0.85 cmol.kg<sup>-1</sup> to 1.44 cmol.kg<sup>-1</sup> with lime. This indicates that both Tithonia and lime treatments positively affect magnesium availability in the soil. In summary, the data suggest that the incorporation of fresh Tithonia into the soil enhances the availability of essential cations, with lime treatment further improving the levels of calcium and magnesium. These findings highlight the potential of using organic amendments like Tithonia in soil management practices to improve soil fertility and nutrient availability.

The addition rate of tithonia from 400 to 1600 g/ha in the treatment without lime has increased the exchangeable K content from 0.36 to 1.2 or an increase of 0.84 me 100 g<sup>-1</sup> or equivalent to 6.55 g K pot<sup>-1</sup> from 0.36 to 1.2 or an increase of 0.84 me/100g or equivalent to 6.55 g K pot<sup>-1</sup>. The increase in exchangeable K was due to the increase in tithonia dose in the treatment of 2 t.ha<sup>-1</sup> lime is 1.07 cmol.kg<sup>-1</sup> or 8.35 g/pot. The content of Ca-exchangeable and Mg-exchangeable also showed an improvement with increasing doses of tithonia added. Increased bases that can be exchanged in addition to tithonia origin also due to increased solubility of bases in the soil. The content of these bases is on average higher in the soil that is calcined. This is clearly due to the increased levels of Ca and Mg that are carried along with the lime. Chemically, organic matter can increase the cation exchange capacity and water holding capacity so as to increase the solubility of soil nutrients. This description confirmed that lime and tithonia green manure can improve soil chemical properties characterized by increased nutrient availability and decreased soil Al solubility and saturation. Thus, it is expected to provide higher growth and production of red chili.

### **Chili straw dry mass (g/10 Kg soil)**

Straw dry mass is a reflection of the vegetative growth of plants quantitatively. The results of the analysis of variance on the dry weight of chili straw showed that the dose of tithonia green fertilizer gave a significant effect, while the lime treatment and the interaction of these two factors did not significantly affect the dry mass of chili straw. The data presented in Table 4 illustrates the effect of varying percentages of Tithonia substitution and lime application on the straw dry mass of chili plants, measured in grams per pot. The results indicate that the percentage of Tithonia substitution significantly influences the straw dry mass, with the highest values observed at 25% substitution. At 0% substitution, the straw dry mass is recorded at 53.64 g/pot without lime and at 71.35 g pot<sup>-1</sup> with lime, resulting in an average of 62.50 g pot<sup>-1</sup>. The addition of lime appears to enhance the straw dry mass, likely due to improved nutrient availability. As the percentage of Tithonia substitution increases to 25%, the straw dry mass further increases to 70.79 g pot<sup>-1</sup> without lime and 79.11 g pot<sup>-1</sup> with lime, yielding an average of 74.95 g pot<sup>-1</sup>. This suggests that a moderate substitution of Tithonia positively impacts the growth of chili plants. However, at 50% substitution, the straw dry mass decreases slightly to 63.67 g pot<sup>-1</sup> without lime and 75.93 g pot<sup>-1</sup> with lime, averaging 69.80 g pot<sup>-1</sup>. This decline may indicate that excessive substitution could lead to competition for nutrients or other growth factors. The trend continues at 75% substitution, where the straw dry mass drops to 53.79 g pot<sup>-1</sup> without lime and 45.05 g pot<sup>-1</sup> with lime, averaging 49.42 g pot<sup>-1</sup>. Finally, at 100% substitution, the straw dry mass reaches its lowest point, with values of 37.75 g pot<sup>-1</sup> without lime and 44.91 g pot<sup>-1</sup> with lime, resulting in an average of 41.33 g pot<sup>-1</sup>.

This highlights that the 25% substitution treatment is significantly more effective than the higher substitution levels, suggesting that a balanced approach to Tithonia incorporation is crucial for optimizing chili plant growth. In conclusion, the data suggest that moderate substitution of Tithonia, particularly at 25%, combined with lime application, enhances the straw dry mass of chili plants. This finding underscores the importance of optimizing organic amendments in agricultural practices to improve crop yield. The numbers in the "Main

**Table 3.** Effect of fresh Tithonia and lime treatment on Exchangeable Soil Cations (Exchangeable K<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup>).

Fresh Tithonia (g/10 Kg soil)	Exchangeable K <sup>+</sup> (cmol.kg <sup>-1</sup> )		Exchangeable Ca <sup>2+</sup> , (cmol.kg <sup>-1</sup> )		Exchangeable Mg <sup>2+</sup> (cmol.kg <sup>-1</sup> )	
	No Lime	Lime 2 t.ha <sup>-1</sup>	No Lime	Lime 2 t.ha <sup>-1</sup>	No Lime	Lime 2 t.ha <sup>-1</sup>
0	0.36	0.33	1.26	2.01	0.67	0.85
400	0.41	0.47	1.60	2.58	0.83	0.89
800	0.74	0.73	1.30	2.37	0.96	1.00
1200	1.17	1.13	1.26	2.64	1.27	1.37
1600	1.20	1.40	1.31	2.83	1.31	1.44

**Table 4.** Effect of percentage substitution of Tithonia and lime on straw dry mass of chili plants (g/10 Kg soil).

Percentage substitution	Dosages of Lime		Main effect of substitution
	No Lime	Lime 2 ton.ha <sup>-1</sup>	
0%	53,64	71,35	62,50 <sup>a</sup>
25 %	70,79	79,11	74,95 <sup>a</sup>
50 %	63,67	75,93	69,80 <sup>a</sup>
75 %	53,79	45,05	49,42 <sup>ab</sup>
100 %	37,75	44,91	41,33 <sup>b</sup>

Note: Numbers with different lowercase superscripts in the same column are significantly different at the 5% level according to the LSD test.

**Table 5.** Effect of Tithonia as a substitute for N fertilizer and liming on fresh weight fruits of chili (g/10 Kg soil).

Percentage substitute	Dosage of Lime		Main effect of substitution
	No Lime	Lime 2 ton.ha <sup>-1</sup>	
0%	291.57 <sup>ab</sup> A	418.71 <sup>a</sup> B	355.14 <sup>a</sup>
25%	380.18 <sup>a</sup> A	400.95 <sup>a</sup> A	390.57 <sup>a</sup>
50 %	325.23 <sup>ab</sup> A	373.11 <sup>a</sup> A	349.17 <sup>a</sup>
75%	297.55 <sup>ab</sup> A	268.80 <sup>ab</sup> A	283.18 <sup>ab</sup>
100%	166.02 <sup>b</sup> A	186.73 <sup>b</sup> A	176.38 <sup>b</sup>

Note: Numbers with different lowercase superscripts in the same column and different uppercase letters in the same row are significantly different at the 5% level according to the LSD test.

Effect of Substitution" column of Table 4 are obtained by calculating the arithmetic mean (average) of the two corresponding values for each percentage substitution level—one from the "No Lime" treatment and one from the "Lime 2 ton ha<sup>-1</sup>" treatment. All values are expressed in grams per 10 kg of soil [g (10 kg soil)<sup>-1</sup>] and rounded to two decimal places for consistency. For instance, at 0% substitution, the mean is derived from (53.64 + 71.35) / 2 = 124.99 / 2 = 62.50; for 25% substitution, (70.79 + 79.11) / 2 = 149.90 / 2 = 74.95; at 50% substitution, (63.67 + 75.93) / 2 = 139.60 / 2 = 69.80; for 75% substitution, (53.79 + 45.05) / 2 = 98.84 / 2 = 49.42; and at 100% substitution, (37.75 + 44.91) / 2 = 82.66 / 2 = 41.33.

The percentage increase in tithonia substitution from 0% to 50% resulted in plant dry mass that was not significantly different. Instead, there was a significant decrease in dry mass when the substitution of artificial fertilizer N with tithonia N was raised to 75% to 100%. The relationship between these two variables is also quadratic (Figure 1) with the equation  $Y = -0.0064x^2 + 0.3721x + 65.13$  with  $R = 0.87$ . Based on this equation, the maximum dry mass was obtained, amounting to 70.54 g pot<sup>-1</sup> at the substitution of fertilizer with tithonia by 29.07%. The addition of tithonia green fertilizer more than 29.07% substitution will reduce the dry mass of chili straw.

A significant decrease in dry weight in plants that are given tithonia to substitute artificial fertilizer 75 - 100% indicates a growth disorder that is thought to reduce the available nutrients, especially the element N. Although each pot gets fertilizer with the same dose of N, but the higher the N sourced from tithonia, the less available the element for the plant due to weathering that is not yet completed. It is suspected that the incubation period of 3 weeks is not enough to release all nutrients from tithonia, especially N. This means that the percentage of fertilization that is more than the green fertilizer cannot provide sufficient nutrients so that vegetative growth. According to (Pypers et al., 2005). The release of ammonium by green manure from *Flemingia congesta*, *Mucuna pruriens*, *Pueraria phaseoloides* occurs after 2 weeks of incubation in the soil, while *Tithonia diversifolia* is more gradual because it has the highest lignin content. However, for a longer time Tithonia was highest in reducing extractable- Al (70%) and increasing the solubility of soil N and P of Vietnam's Ferralsol and Acrisol soils.

#### **Fresh weight of chili fruit (g/10 Kg soil)**

Analysis of variance revealed that the main effect of lime application was not significant on chili fruit fresh weight, while the percentage of N fertilizer substitution with Tithonia and its interaction with lime significantly affected fruit fresh weight in Ultisol. The interaction effect demonstrated that lime application substantially enhanced chili fruit fresh weight only in the control treatment (0% substitution with 100% synthetic fertilizer), increasing yield from 291.57 g/10 Kg soil to 418.71 g/10 Kg soil, representing a 44% improvement. However, in treatments involving N fertilizer substitution with Tithonia, lime application showed no significant effect on fruit weight, although values remained slightly higher than non-limed treatments.

As shown in Table 5, the data reveal that optimal fruit fresh weight was achieved with 25% Tithonia substitution (390.57 g/10 Kg soil), while complete substitution (100%) resulted in the lowest fruit weight (176.38 g/10 Kg soil). The results in Table 7 indicate that partial replacement of synthetic fertilizer with Tithonia biomass maintains superior chili productivity compared to complete organic substitution. Furthermore, Table 7 demonstrates a declining trend in fruit fresh weight as Tithonia substitution percentage increased beyond 25%, suggesting that excessive reliance on organic nitrogen sources may compromise fruit production in the short term.

**Table 6.** Effect of *Tithonia* as substitute material N fertilizers and lime on the nutrients uptake of chili plants (g/10 Kg soil).

The Main Effect of Treatment	Nutrients uptake in straw and dried fruit (g/10 Kg soil)			
	N	K	Ca	Mg
<b>Dosages of Lime</b>				
No Lime	3.17	2.17	0.40	0.45
Lime 2 ton.ha <sup>-1</sup>	3.76	2.57	0.47	0.47
<b>Percentage of Substitute</b>				
Substitute 0%	3.90 <sup>a</sup>	2.57 <sup>a</sup>	0.45 <sup>ab</sup>	0.48 <sup>b</sup>
Substitute 25%	4.70 <sup>a</sup>	2.64 <sup>a</sup>	0.55 <sup>a</sup>	0.61 <sup>a</sup>
Substitute 50%	3.93 <sup>a</sup>	2.77 <sup>a</sup>	0.55 <sup>a</sup>	0.50 <sup>a</sup>
Substitute 75%	2.84 <sup>b</sup>	2.25 <sup>a</sup>	0.38 <sup>b</sup>	0.40 <sup>c</sup>
Substitute 100%	1.89 <sup>c</sup>	1.63 <sup>b</sup>	0.25 <sup>b</sup>	0.32 <sup>c</sup>

Note: Numbers with different lowercase superscripts in the same column are significantly different at the 5% level according to the LSD test.

**Table 7.** Interaction between lime rates and prcentage of *Tithonia* substitution on Chili P uptake.

Percentage of Substitute	Dosage of Lime	
	No Lime	Lime 2 ton.ha <sup>-1</sup>
0%	0.39 <sup>bA</sup>	0.73 <sup>aB</sup>
25%	0.52 <sup>abA</sup>	0.62 <sup>aA</sup>
50 %	0.48 <sup>bA</sup>	0.64 <sup>aA</sup>
75 %	0.76 <sup>aA</sup>	0.49 <sup>bA</sup>
100 %	0.36 <sup>bA</sup>	0.40 <sup>bA</sup>

Note: Numbers with different lowercase superscripts in the same column and different uppercase letters in the same row are significantly different at the 5% level according to the LSD test.

### ***Chili nutrient uptake (g/10 Kg soil)***

Nutrient uptake in this study is the amount of nutrients contained in straw and dried fruit. The main effect of tithonia green fertilizer rate as a substitute for N fertilizer is high significant on the uptake of N, P, K, Ca and Mg of chili, while the lime treatment has no significant effect on all nutrient uptake. The effect of the interaction of the two factors is seen in the P uptake. The results of the Least Significant Difference test of the effect of the main treatment of tithonia substitution on the nutrients uptake are shown in Table 6.

Table 6 reveals a decreasing trend in the fresh weight of chili fruit on media treated with 2 tons ha<sup>-1</sup> of lime. Likewise, the main effect of the percentage of tithonia substitution shows a trendline of decreasing fruit fresh weight (Figure 1). The highest fruit fresh weight was obtained in the tithonia substitution treatment of 23.71% at 397.53 g/10 Kg soil. The decrease in the fresh weight of chili fruit was due to the main effect of the percentage of substitution along with the data on the dry weight of straw and nutrient transport of chili plants. The greater the contribution of tithonia substitute fertilizer, the more adverse the effect on the growth and yield of chili, especially the percentage exceeds the average of 50%. N needs of artificial fertilizer for chili plants can be substituted with N tithonia up to 50%. If you have to substitute N artificial fertilizer by 50% it takes fresh tithonia as much as 800 g plant<sup>-1</sup> or equivalent to about 23 tons of fresh tithonia per hectare when the populace of chili are 27.700 plants ha<sup>-1</sup>.

*Tithonia* as a green fertilizer given to substitute artificial fertilizer N as much as 25% to 50% resulted in plant N uptake that is not significantly different from the provision of 100% fertilizer or without tithonia except Mg elements. In general, further increase in substitution from 50% to 75% and 100% decreased nutrient uptake significantly. It can be said that tithonia green fertilizer is able to substitute fertilizer up to 50% of the recommended dose. The interesting thing about the results of this study is that green manure is able to replace or reduce the need for lime in terms of nutrient uptake in ultisol, where the uptake of N, P, K, Ca, Mg is relatively the same in both lime and non-lime media. The effect of the lime dosing factor was significantly different in each Table 7.

### ***Interaction between lime rate and percentage of Tithonia substitution on chili P uptake factor of percentage substitution on P uptake***

The interaction effect of lime and tithonia factors explained that the P uptake of chili was significantly higher in the media given lime without green fertilizer. The increase in P uptake in lime media is 87%. The effect of lime is seen clearly when without the provision of tithonia as an artificial fertilizer N substitute. When tithonia is applied, the effect of lime becomes reduced and inconsistent on P uptake, especially in media without lime. However, an increase in tithonia N substitution up to 75% significantly reduces P transport in media that are lime.

Green fertilizer tithonia in the process of decomposition is not completed will produce organic acids that at low concentrations can help nutrient uptake, but at high concentrations can interfere with plant growth. As it is known that organic acids in addition to functioning as a source of nutrients also function as hormones that at certain concentrations can stimulate growth, and at high concentrations can interfere with enzymatic reactions that ultimately disrupt the absorption of nutrients. Figure 1. is the relationship between nutrient uptake of N, P, K, Ca, and Mg chili with the percentage of substitution of N fertilizer with N tithonia. Figure 1 shows that the relationship between all nutrient transport of chili with the percentage of substitution is quadratic according to each equation, respectively.

Based on the regression equation in Figure 2, the highest N uptake of chili was produced by a substitution percentage of 25.13% (4.33 g pot<sup>-1</sup>), K uptake at 35.25% substitution (2.78 g pot<sup>-1</sup>), P uptake at 32% substitution (0.595 g pot<sup>-1</sup>), Ca uptake at 35% substitution (0.546 g pot<sup>-1</sup>) and Mg uptake at 26% substitution (0.543 g pot<sup>-1</sup>). Looking at the uptake of the five elements, the contribution of tithonia green fertilizer as a substitute for N fertilizer is 25 - 35%. Application of tithonia in excess of this amount reduces nutrient uptake linearly. Apart from being caused by incomplete weather, it is thought that there may be a loss of dissolved by volatilization and leaching. This case has shown that (Liang et al., 2022), (Lou et al., 2024), green manure substitution with 30% of chemical N fertilizer significantly reduced 31.1% of ammonia volatilization loss without reducing the crop yield. The use of green manure legumes reduced the need for chemical nitrogen fertilizer by 31% and decreased the risk of nitrogen leaching for subsequent winter wheat in the Loess Plateau of China.

**Table 8.** Contribution of N Tithonia to chili plants from several treatments of N fertilizer substitution with N Tithonia.

Percentage of Substitute	% <sup>15</sup> N a.e Chili Plant		% N Chili from Tithonia	
	Straw	Fruit	Straw	Fruit
0%	0	0	0	0
25%	0,05	0,03	2,96	1,78
50 %	0,08	0,05	7,69	2,96
75%	0,23	0,18	13,61	10,65
100%	0,48	0,36	28,40	21,3

Notes: %<sup>15</sup>N in Tithonia = 1.69 /1.568.

However, it was observed that nitrogen loss increased with higher application amounts of green manure, specifically when the application exceeded 30 tons ha<sup>-1</sup>. This indicates a potential trade-off between the benefits of reduced chemical nitrogen fertilizer and the risk of increased nitrogen loss associated with higher green manure application

There are many factors that influence nutrient mineralization in soil. One of them is the level of decomposition of organic material which will determine the presence of functional groups and carbon fractions in the soil. As a result (of this), (Ndung'u et al., 2021) (Laub et al., 2023) showed that employing organic fertilizer either independently or in conjunction with inorganic fertilizer over an extended period leads to higher corn yields and an enhanced capacity for soil carbon absorption. This boost can be attributed to the elevated levels of Alkyl and O-Alkyl carbon functional groups in soil organic matter.

#### **Nitrogen Contribution of Tithonia to chili plants based on <sup>15</sup>N isotope tracing**

<sup>15</sup>N analysis conducted is <sup>15</sup>N contained in straw and chili fruit. Based on dry weight and N-total content of plants, the total N uptake of plants can be calculated. Based on % <sup>15</sup>N a.e in tithonia which is used for substitution of N fertilizer and which is tracked in chili plants, it can also be determined the amount of N contributed by tithonia to chili plants (Table 8).

Table 8 explains that the amount of N from Tithonia that can be absorbed by chili plants ranges from 1.78% to 28.40%. This means that around 98% to 72% of N comes from soil and fertilizers. The more tithonia added to the growing medium, the higher the N contributed to the chilies. The low nutrient contribution of tithonia to chilies indicates that most of the tithonia N has not been released and is still remaining in the soil. It is recommended to increase the incubation time for tithonia in the soil more than 21 days before crop cultivated. An interesting thing found in the results of this research was that nutrient availability increased when the tithonia substitution percentage was applied which was incubated for 21 days, which was not in line with the nutrient uptake of chili plants. This is certainly true in the case of (Kiboi et al., 2020). The nitrogen and phosphorus release rates from *Tithonia diversifolia* were notably elevated under conventional tillage by 29% - 28%, respectively, in comparison to minimum tillage during the eighth week incubation.

## **Materials and Methods**

### **Description of location and research design**

The research was conducted in the Experimental Garden of the Faculty of Animal Husbandry, Andalas University Limau Manis Padang - West Sumatra with an altitude of 350 m d.p.l Soil and plant analysis was carried out at the Laboratory of the Center for Research and Utilization of Nuclear Science and Technology, Andalas University. This research was conducted in the form of a pot experiment using a 2 x 5 factorial design and placed according to a split-plot design in the field. The first factor is the provision of lime which is the main plot with two levels, namely No Lime (K0) and lime equivalent to 2x exchangeable Al (Al-Exch). The second factor which is a sub-plot is the percentage of fertilizer N (Urea) substitution with 5 levels, namely: S0: 0% substitution (100% N fertilizer, without Tithonia), S25: 25% substitution (75% N fertilizer + 25% N tithonia), S50: 50% substitution (50% N + 50% N tithonia), S75: 75% substitution (25% N fertilizer + 75% N tithonia) and S100: (100% substitution (no fertilizer + 100% N tithonia). Each treatment was repeated 3 times so that the number of experimental units was 30 polybags. The reference dose of fertilizer for chili plants is 180kg/ha N, 100kg/ha P, and 150 kg ha<sup>-1</sup> K. Based on the planting distance of 60 x 60 cm and a population of 27,700 plants per hectare, the fertilizer requirement for chili plants per pot is 6.5 g N, 3.61 g P, and 5.4 g K.

The dose of tithonia for each treatment is adjusted to the N content contained in the tithonia analyzed previously. Calculation of the treatment dose of the need for tithonia per polybag diperoleh and substitute 100% N fertilizer dose of 6.5 g plant<sup>-1</sup> and the determination of the need for P and K fertilizer is adjusted to the content of P, K which is in the amount of tithonia 100% substitution of N. The need for tithonia per polybag as a substitute for N is determined by calculation:

$$\text{Tithonia Needed (100\%)} = \frac{\text{Amount N Needed/polybag}}{\% \text{ N tithonia}} \times (\text{MCF})$$

Note: MCF (Moisture Corection Factor)

### **Preparation of Tithonia as green fertilizer**

Tithonia prunings used as green fertilizer is the result of propagation of stem cuttings of 80 days old tithonia. Planting these cuttings is done in a polybag that is given 3 grams of ZA fertilizer labeled <sup>15</sup>N containing 10% <sup>15</sup>N a.e as treacer. A total of 50 polybags of tithonia cuttings were planted to meet the number of green manure pruning needs, each polybag containing 4 stem cuttings. Tithonia harvested when 80 days old by cutting along 70 cm from the top then chopped with a chopper machine to produce 3-5 cm size and ready to be used as green fertilizer material per treatment. Some examples of tithonia were taken, then analyzed for moisture content and N content to get a dose of tithonia in accordance with the provisions of the treatment.

### **Fertilizer treatment description**

The experimental design consisted of five substitution treatments that systematically replaced conventional nitrogen fertilizer with fresh Tithonia biomass to evaluate their combined effects on chili plant growth. The control treatment (S0) received 100% conventional fertilization, comprising 6.50 g N g/10 Kg soil (equivalent to 14.44 g urea g/10 Kg soil), with no Tithonia supplementation. Progressive substitution treatments were established at 25% intervals, where S25 treatment combined 75% conventional fertilizer (4.888 g/10 Kg soil or 10.83 g urea g/10Kg soil) with 25% nitrogen contribution from fresh Tithonia applied at 400 g g/10 Kg soil (11.08 ton ha<sup>-1</sup>). The S50 treatment provided equal contributions from both sources, with 50% conventional fertilizer (3.25 g N g/10 Kg soil or 7.22 g urea



g/10 Kg soil) and 50% from Tithonia biomass applied at 800 g pot<sup>-1</sup> (22.16 ton ha<sup>-1</sup>). The S75 treatment further reduced conventional fertilizer input to 25% (1.63 g N per g/10 Kg soil or 3.61 g urea g/10 Kg soil) while increasing Tithonia application to 1200 g pot<sup>-1</sup> (33.24 ton ha<sup>-1</sup>), representing 75% of the nitrogen requirement. Finally, the S100 treatment completely substituted conventional fertilizer with fresh Tithonia biomass applied at 1600 g/10 Kg soil (44.32 ton/ha), providing 100% of the nitrogen requirement through organic sources. This systematic substitution approach allowed for comprehensive evaluation of the potential for organic Tithonia biomass to replace conventional nitrogen fertilization in chili cultivation systems.

For physical and chemical characteristics of tithonia please refer to the results of the analysis conducted by Hakim et.al 2003, namely fresh weight 2.26 kg.m<sup>-2</sup>, dry weight 0.39 kg.m<sup>-2</sup>, moisture content 82.74%, lignin content 10.16%, C-total 54.69%, N-total 2.5%, P-total 0.25%, K-total 4%, Ca-total 1.46%, Mg-total 0.64%, C/N ratio 21.88. The results of the analysis of tithonia samples obtained tithonia N content of 2.5% N with a moisture content of 515%. Material that has been chopped weighed in accordance with the provisions of the treatment and its N content.

### **Preparation of planting media and application of treatments**

Ultisol soil was taken as a composite in the 0-20 cm tillage layer from the experimental garden of the Faculty of Agriculture, Andalas University. Padang - West Sumatra. Soil as much as 20 kg equivalent fixed dry weight, put into each pot of 30 pots previously sieved with a 20 mm sieve. Half of the pots were given 100% CaCO<sub>3</sub> milled lime passing a 20 mesh sieve at a dose 2 ton.ha<sup>-1</sup> (20 g pot<sup>-1</sup>). Soil equivalent to 20 kg dry weight remains spread on plastic measuring 1 m x 1 m, sprinkled with lime evenly which is then sprinkled with tithonia according to treatment and stirred until evenly distributed. The soil was put back into polybags with a diameter of 50cm and a height of 60 cm, watered to field capacity and incubated for 3 weeks. Soil samples were taken to analyze some of the chemical characteristics after incubating for 3 weeks. Chili seedlings that are 4 weeks old are planted into each polybag that has been given fertilizer. Urea and KCl fertilizers were given in 3 stages, namely: at planting, 2 weeks and 4 weeks after planting, while P fertilizer at a dose of 3.61 g P pot<sup>-1</sup> (7.85 g TSP pot<sup>-1</sup>) was given at the same time at planting which was placed 10 cm below the soil surface.

The experimental design consisted of five substitution treatments that systematically replaced conventional nitrogen fertilizer with fresh Tithonia biomass to evaluate their combined effects on chili plant growth under the described soil conditions. The control treatment (S0) received 100% conventional fertilization, comprising 6.50 g N pot<sup>-1</sup> (equivalent to 14.44 g urea pot<sup>-1</sup>), with no Tithonia supplementation. Progressive substitution treatments were established at 25% intervals, where S25 treatment combined 75% conventional fertilizer (4.888 g N pot<sup>-1</sup> or 10.83 g urea pot<sup>-1</sup>) with 25% nitrogen contribution from fresh Tithonia applied at 400 g pot<sup>-1</sup> (11.08 ton ha<sup>-1</sup>). The S50 treatment provided equal contributions from both sources, with 50% conventional fertilizer (3.25 g N per pot or 7.22 g urea pot<sup>-1</sup>) and 50% from Tithonia biomass applied at 800 g pot<sup>-1</sup> (22.16 ton ha<sup>-1</sup>). The S75 treatment further reduced conventional fertilizer input to 25% (1.63 g N pot<sup>-1</sup> or 3.61 g urea pot<sup>-1</sup>) while increasing Tithonia application to 1200 g pot<sup>-1</sup> (33.24 ton ha<sup>-1</sup>), representing 75% of the nitrogen requirement. Finally, the S100 treatment completely substituted conventional fertilizer with fresh Tithonia biomass applied at 1600 g pot<sup>-1</sup> (44.32 ton ha<sup>-1</sup>), providing 100% of the nitrogen requirement through organic sources. This systematic substitution approach allowed for comprehensive evaluation of the potential for organic Tithonia biomass to replace conventional nitrogen fertilization in chili cultivation systems while potentially ameliorating the soil's inherent fertility constraints.

Harvesting chili peppers is done when the fruit is red, and is done twice according to the stage of fruit maturity. The first harvest was done when the plants were 70 days after planting, while the second (last) harvest was done when the plants were 95 days after planting. At the last harvest, most (75%) of the plants had red fruits.

### **Observations and data analysis**

Soil samples were analyzed before and after incubation for pH, Total-N, available-P, exchangeable Al, K, Ca, Mg, and C-organic, while chili plant observations included wet and dry mass of fruit and straw, with nutrient uptake (N, P, K, Ca, Mg) calculated as the sum of straw nutrient content multiplied by straw dry mass plus fruit nutrient content multiplied by fruit dry mass.

N contribution from tithonia to chili plants was calculated based on %<sup>15</sup>N a.e in tithonia and in the chili plants with the following formula (Zapata, 1990) :

$$\%NU_{fT} = \frac{\%^{15}Na.e\ chili\ plant}{\%^{15}Na.e.Green\ manure\ (tithonia)} \times 100\%$$

% N-UfT = Percentage of N absorbed by chili from Tithonia

%<sup>15</sup>N a.e = %<sup>15</sup>N-abundant - %<sup>15</sup>N natural (0.37%)

%<sup>15</sup>Nab = measured from the sample on the Emission Spectrometer

Nitrogen uptake efficiency of chili plants derived from tithonia was calculated by the formula :

$$Nitrogen\ absorption\ efficiency\ (\%) = \frac{N - uptake\ of\ Chili}{N\ applied} \times 100$$

N uptake = nitrogen in total plant

N applied = nitrogen applied as fertilizer.

Observation values were analyzed using a 2×5 factorial design in a split-plot arrangement through Analysis of Variance (ANOVA). Factors showing significant effects were further analyzed using the Least Significant Difference (LSD) test. Additionally, the relationship between the percentage substitution of N fertilization with Tithonia green fertilizer, N uptake, and chili production was analyzed through correlation analysis (Pearson correlation) and multiple regression prediction testing using Microsoft Excel analytical tools, with results displayed in scatterplots.

### **Conclusions**

This study demonstrates that fresh Tithonia biomass serves as an effective natural soil amendment for Ultisol, significantly reducing aluminum toxicity from 36.85% to 7.48% saturation without lime treatment while improving overall soil chemical properties. Using <sup>15</sup>N isotope tracing technology, researchers determined that substituting 25-30% of synthetic nitrogen fertilizer with Tithonia biomass achieves optimal crop performance, including maximum straw dry mass (74.95 g pot<sup>-1</sup>) and fruit fresh weight (397.53 g pot<sup>-1</sup>), while maintaining economic viability for farmers. The findings reveal that Tithonia can reduce synthetic nitrogen fertilizer requirements by up



to 50% while maintaining productivity, though excessive substitution beyond this level may compromise crop performance due to incomplete nutrient mineralization. This research offers practical solutions for smallholder farmers in tropical regions facing high fertilizer costs and contributes to sustainable agriculture practices by demonstrating how natural processes can enhance ecosystem services while maintaining productive capacity. Future research should focus on optimizing incubation periods, investigating long-term effects of repeated applications, developing region-specific recommendations, and conducting economic analyses to support the transition toward more sustainable and resilient farming practices that align with global sustainability goals.

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