Current Applied Science and Technology e-ISSN: 2586-9396

Curr. Appl. Sci. Technol. 2025, Vol. 25 (No. 3), e0261218

Research article

Effects of Nitrogen Fertilizer Rate with Urease and Nitrification Inhibitors on Certain Morphological Traits and Quality of Sugarcane (*Saccharum officinarum* L.)

Phatchariya Welutung[1], Patthra Pengthamkeerati[1]*, Boonlue Kachenchart[2] and Saowanuch Tawornpruek[3]

[1]*Department of Environmental Technology and Management, Faculty of Environment, Kasetsart University, Bangkok, Thailand*
[2]*Faculty of Environment and Resource Studies, Mahidol University, Nakhon Pathom, Thailand*
[3]*Department of Soil Science, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand*

Received: 15 November 2023, Revised: 25 April 2024, Accepted: 22 October 2024, Published: 9 December 2024

Abstract

In this study, we investigated the effects of the N fertilizer rates (commonly applied by farmers compared to rate based on soil analysis) with an appropriate N fertilizer rate associated with urease inhibitors (UIs), nitrification inhibitors (NIs) and their combinations (UINIs) on sugarcane (*Saccharum officinarum* L.) growth, morphological traits, and quality. The treatments consisted of 2 UIs (N-(n-butyl) thiophosphoric triamide (NBPT) and garlic (*Allium sativum* L.)) and 3 NIs (dimethylpyrazole phosphate (DMPP), ground neem seed (*Azadirachta indica* A. Juss. var. siamensis Valeton) and praxelis (*Praxelis clematidea* (Griseb.) R.M. King & H. Rob)). The results showed that increasing the N fertilizer rate encouraged sugarcane growth by up to 4.5% and increased N content by 16.2% in the cane yield. Adding inhibitors produced positive responses in plant growth and yield, possibly due to prolonging N fertilizer in the soil and extending the supply of N to the plant. Compared to biological inhibitors, the synthetic inhibitors resulted in longer stalk lengths but lower stalk diameters. The inhibitor treatments significantly enhanced the aboveground biomass and N content in plants by up to 41.5 and 41.9%, respectively, compared to only fertilizer. The inhibitor treatments in commercial cane sugar (CCS) increased by up to 13.6%. However, decreasing the N fertilizer rate with addition of inhibitors assisted in keeping mineral N in the soil, which further enhanced N uptake and led to improved plant growth and yield. Added DMPP showed the potential to slow down N loss from soil, which enhanced rapid growth and resulted in higher aboveground biomass.

Keywords: nitrogen content; nitrification inhibitors; morphological traits; sugarcane; urease inhibitors

*Corresponding author: E-mail: fsciptp@ku.ac.th
https://doi.org/10.55003/cast.2024.261218

1. Introduction

Sugarcane (*Saccharum officinarum* L.) is a valuable cash crop in the tropics. Thailand has been the second largest sugar exporter in the world after Brazil for over a decade and is the fourth largest sugar producer behind Brazil, India, and the European Union (USDA, 2022). Currently, in order to produce high sugarcane yields, excessive N fertilizer application is widespread in many sugarcane areas in Thailand. It is necessary to apply an optimum N rate to promote plant development and productivity as well as allowing farmers to reduce production cost (Zeng et al., 2020; Kandhro et al., 2021).

The application of chemical fertilizer recommendations based on soil analysis is one of the most efficient ways to enhance fertilizer use, promote crop yield, and help farmers reduce their production costs (de Castro & Franco, 2019). Integrated use of urease inhibitors (UIs) and nitrification inhibitors (NIs) may improve crop growth and productivity, while decreasing fertilizer expenses. Numerous synthetic UIs and NIs have been researched for their ability to slow down urea hydrolysis and the nitrification process. Commercial UIs (such as N-(n-butyl) thiophosphoric triamide or NBPT) are most frequently adopted to interact with the urease enzyme, including complex formation with the nickel (Ni) atom at the enzyme's active site (Volpi et al., 2017). NIs, including dicyandiamide (DCD), dimethylpyrazole phosphate (DMPP), and nitrapyrin are used to block the enzyme that converts ammonium (NH_4^+-N) to hydroxylamine (NH_2OH) by suppressing the activity of ammonia-oxidizing bacteria (AOB) (Subbarao et al., 2006). However, little research has been focused on the response to UIs and NIs on plant growth and development processes. For example, Zhu et al. (2023) reported that the application of DMPP with urea significantly promoted photosynthesis, which improved the N uptake and the root and above ground dry weight of sweet sapphire.

Nonetheless, many researchers have investigated the use of natural inhibitors to reduce environmental impact. As a consequence, natural inhibitors have become a preferred alternative to synthetic inhibitors for improving plant N uptake because they are cost-efficient. Moreover, they are not harmful to the environment, are chemically stable at low concentrations, and are thus effective (Arora & Srivastava, 2013). Allicin, flavonoids, polyphenols, quercetin, and humic acids have been shown to be capable of inhibiting urease activity (Salehuddin et al., 2019; Matczuk & Siczek, 2021). Some compounds, including fatty acids, phenylpropanoids, flavonoids, and isothiocyanates have been reported as biological nitrification inhibitors (BNIs). Examples include neem cake, neem oil, mint, and aromatic herbs (Upadhyay et al., 2011). Therefore, it was of interest to examine the ability of praxelis (*Praxelis clematidea* Griseb.) to suppress the nitrification process using its content of phenylpropanoids, flavonoids, and other substances (Yang et al., 2020).

In general, nitrification is an important process for upland crops because it contributes to N availability in various forms, including NH_4^+-N and nitrate (NO_3^--N) that are taken up by plants (Nasholm et al., 2009) and are incorporated into the dry matter in plants, and thus increases productivity (Muratore et al., 2021). Many researchers have focused on the impact of good management practices on crop productivity, greenhouse gas (GHG) emission, N contained in soil, nitrogen use efficiency, and N loss through the environment (Abalos et al., 2014; Fan et al., 2022), with less focus on the effects on plant growth and physiology, which may result in differing yields. Therefore, the current study was focused on the use of UIs and NIs in improved N fertilizer transformation and their influence on N soil availability and effects in terms of sugarcane growth and quality.

2. Materials and Methods

2.1 Study site and experimental details

The study was located at the Lopburi Research Station, Faculty of Agriculture, Kasetsart University, Khok Charoen district, Lopburi province, Thailand (15°21' N, 100°55' E). The study area had an average annual temperature of 30.3ºC, and annual precipitation of 994.4 mm. The soil classification was Sop Prap soil series (fine, smectitic, isohyperthermic Lithic Haplustolls), which was slightly acid to neutral and characterized by a very dark grayish brown or very dark brown clay loam or clay. The soil samples used in this research were collected from a topsoil depth of 0-20 cm. The soil sample was air-dried, passed through a 2 mm sieve, and analyzed to determine its physical and chemical properties. The soil was neutral (pH 6.7), organic matter content was high (2.93%), available potassium was high (394 mg kg^{-1}), available phosphorus was high (87 mg kg^{-1}) and the soil texture was clay (29% sand, 24% silt and 47% clay).

The tested sugarcane (*Saccharum officinarum* L.) cultivar Khon Kaen 3 was planted on individual plots, in rows of 4 m by 7 m (28 m^2 in total area), with 1.5 m × 0.5 m row spacing. Four replications of the experiment were set up in a randomized complete block design. The method of planting involved choosing healthy sugarcane plants, splitting each stem into 3-4 joints, and laying each piece horizontally into the furrow. Watering was applied as necessary and monitoring was carried out for pests and diseases until maturation.

Basal fertilizer was supplied when the sprouts first appeared, and was then covered with soil. Three different N fertilizer rates were used for the basal application: 0 (no N fertilizer, T_1), 50 kg N ha^{-1}, 62.5 kg P_2O_5 ha^{-1}; and 0 kg K_2O ha^{-1} (rates used by local farmers, T_2), and 19 kg N ha^{-1}; 19 kg P_2O_5 ha^{-1}; and 9.5 kg K_2O ha^{-1} (rate based on soil and leaf analyses, T_3). A dressing application was applied at 2 months after the basal application. Chemical fertilizer (143.75 kg N ha^{-1}; 0 kg P_2O_5 ha^{-1}; and 75 kg K_2O ha^{-1}) was added based on the rates used by farmers (T_2) and at 20.125 kg N ha^{-1}; 0 kg P_2O_5 ha^{-1}; and 30 kg K_2O ha^{-1} based on the soil analysis (T_3-T_{10}). The inhibitor treatments included added inhibitors associated with chemical fertilizer at the soil analysis rate. Synthetic and natural NIs were added at basal application. DMPP at 5% of the N fertilizer amount was added in the T_4-T_6 treatments. Ground neem seed at 20% of the N fertilizer amount was added in the T_7-T_9 treatments and praxelis at 200% of the N fertilizer amount was added in the T_{10} treatment. Various UIs and NIs were added at dressing application. NBPT and DMPP were added in the T_4 and T_5 treatments at the rate of 5% of the N fertilizer amount. NBPT and DMPP were added in the T_6 treatment at the rate of 2.5% of the N fertilizer amount. The T_7, T_8, and T_{10} treatments included garlic, neem, and praxelis at rates of 10%, 20% and 200%, respectively, of the N fertilizer amount. Garlic and neem were added in the T_9 treatment at the rates of 5% and 10% of the N fertilizer amount, respectively (Table 1).

2.2 Field measurement

After fertilization, monthly measurements of the sugarcane height, stalk diameter and length and width of sugarcane leaves were recorded, followed by bimonthly observations until maturity. The sugarcane height was randomly measured in cm from the middle 2 rows with the assistance of a measuring tape from the surface of the soil to the top visible dewlap (TVD). Stalk diameter was measured using a set of vernier calipers at the first internode

Table 1. List of treatment details, fertilizers ($N-P_2O_5-K_2O$), and inhibitors applied

Treatment	1st Application		2nd Application	
	Fertilizer rate ($kgN-P_2O_5-K_2O$ ha^{-1})	Inhibitor rate (% of N fertilizer)	Fertilizer rate ($kgN-P_2O_5-K_2O$ ha^{-1})	Inhibitor rate (%N fertilizer)
T_1	-	-	-	-
T_2	50 - 62.5 - 0	-	143.75 - 0 - 75	-
T_3	19 - 19 - 9.5	-	20.125 - 0 - 30	-
T_4	19 - 19 - 9.5	DMPP 5%	20.125 - 0 - 30	NBPT 5%
T_5	19 - 19 - 9.5	DMPP 5%	20.125 - 0 - 30	DMPPP 5%
T_6	19 - 19 - 9.5	DMPP 5%	20.125 - 0 - 30	NBPT 2.5%+DMPP 2.5%
T_7	19 - 19 - 9.5	Neem 20%	20.125 - 0 - 30	Garlic 10%
T_8	19 - 19 - 9.5	Neem 20%	20.125 - 0 - 30	Neem 20%
T_9	19 - 19 - 9.5	Neem 20%	20.125 - 0 - 30	Garlic 5%+Neem 10%
T_{10}	19 - 19 - 9.5	Praxelis 200%	20.125 - 0 - 30	Praxelis 200%

Description: control (T_1), farmer (T_2), site-specific nutrient management (SSNM) (T_3), and SSNM with inhibitors divided into DMPP/NBPT (T_4), DMPP/DMPP (T_5), DMPP/NBPT + DMPP (T_6), neem/garlic (T_7), neem/neem (T_8), neem/garlic + neem (T_9) and praxelis/praxelis (T_{10})

above the soil. Length and width of sugarcane leaves were measured using a measuring tape, and leaf area was calculated based on equation 1:

$$\text{Leaf area} = \text{leaf length} \times \text{leaf width} \times 0.75 \quad (1)$$

Prior to harvest, the number of millable canes was counted from a 21 m^2 (the middle 2 rows) evaluation area and converted to a hectare basis. Total canes from the middle 2 rows were weighed and stalk weight per stalk was calculated. The lengths of 10 randomly selected canes from the middle 2 rows were measured from the base (ground level) to the top node and then averaged and then measured for the internode length. Then 10 subsamples of millable canes were collected and measured for the juice sucrose (%pol), total soluble solids content (%brix), and fiber content using a polarimeter, refractometer, and total solids determination in the sugarcane, respectively. The commercial cane sugar (CCS) from each treatment was calculated based on equation 2:

$$CCS = [3P(1-(F+5/100))]/2 - [B(1-(F+3/100))]/2 \quad (2)$$

where P is the pol % of the juice, B is the brix % of the juice, and F is the fiber % of the juice.

Three randomly selected canes from the middle 2 rows were dried at 60-65ºC and weighed to determine the aboveground biomass. Total N was determined using the Kjeldahl method (Greenberg et al., 1992). Powdered cane, leaf, and straw were digested with concentrated H_2SO_4 in the presence of a K_2SO_4 and $CuSO_4$ catalyst mixture. Then, the N in the digest was collected using distillation with NaOH; afterward, the distillate captured in the H_3BO_3 indicator solution was titrated using standard HCl.

2.3 Statistical analysis

The data collected were analyzed to evaluate the effects of fertilizer rate, UI, and NI applications, and the number of applications on sugarcane height and stalk diameter. The statistical model used was a repeated measure. Multiple comparisons were tested using Duncan's multiple range test with $P < 0.05$ as the significance level. Factor analysis, using the principal component method, was performed to determine the pattern of treatments (N rates and inhibitors).

3. Results and Discussion

3.1 Sugarcane growth and certain morphological traits

Sugarcane height was significantly affected by treatment, time, and their interaction (Table 2). Sugarcane growth responded to the amount of fertilizer applied. Compared to the soil analysis rate (T_3) and no N fertilizer (T_1), the common farmer rate (T_2) had the highest sugarcane growth attributes, with sugarcane growth improving as the N rate applied was raised. Increasing the N rate applied enhanced the sugarcane height, in the sequence $T_2 > T_3 > T_1$ in this study. After 4 months of planting, the sugarcane entered a stalk elongation phase. The sugarcane started elongating and increasing in stalk diameter, allowing the plant to grow quickly. This phase continued until about 7-8 months after planting. When the sugarcane entered a maturity and ripening phase, the growth rate slowed down significantly compared to the previous phase. The sugarcane started to accumulate sugar in the stalks. Therefore, the stalk heights at 10 months after planting showed no significant differences in all treatments. However, at maturity, T_2 and T_3 had higher stalk height (244.1 and 233.6 cm, respectively) than T_1 (216.2 cm). This observation was consistent with another published study where adding N fertilizer significantly promoted stalk height, stalk diameter, and millable stalk ha^{-1} distribution for sugar cane and consequently for sugar productivity (Zeng et al., 2020). N is a functional component of amino acids and chlorophyll, which affect photosynthesis and the assimilation of minerals that can affect plant growth and development (Wu et al., 2019). In the current study, the effects of applied N fertilizer along with UIs, NIs, and UINIs resulted in a similar or greater stalk height than for T_2, and a much taller stalk (up to 11%) than for only the N fertilizer treatment (T_3). The results confirmed that the application of inhibitors with N fertilizer significantly promoted photosynthesis, which improved the stalk height. In addition, it appeared that the synthetic inhibitors had a greater effect on plant height than BNIs. T_5 and T_6 achieved significantly higher stalk heights at maturity when compared to BNIs (T_7 and T_{10}) and the only treatments (T_2 and T_3).

The stalk diameter was lower in the T_3 than in the T_2 treatment and seemed slightly higher in the inhibitor treatments (Table 3). This was consistent with a study using physic nut (*Jatropha curcas* L.), where the stem diameter increased due to an increase in the N rate application (Montenegro et al., 2019). However, this result for stalk diameter was different from stalk height, for which the potential of the various inhibitors was rarely significant during the sugarcane growth period. The application of inhibitors promoted the stalk diameter by up to 1.6-7.4%, with the synthetic inhibitors tending to have a slightly smaller effect than natural inhibitors.

Table 2. Stalk height during experimental period following treatment with N fertilizer with UIs, NIs, and UINIs compared to treatment with only fertilizer under field conditions

Treatment	Stalk height (cm)						
	3 months	4 months	5 months	6 months	8 months	10 months	12 months
T_1	28.5	61.0	82.8^{e}	111.7^{f}	174.0^{f}	208.3	216.2^{e}
T_2	22.4	71.1	114.6^{ab}	140.2^{a}	228.2^{a}	227.0	244.1^{bcd}
T_3	30.2	68.3	98.8^{d}	116.6^{ef}	186.3^{e}	218.3	233.6^{d}
T_4	27.0	68.5	118.6^{a}	131.2^{bc}	208.6^{bc}	234.3	251.4^{ab}
T_5	26.2	61.1	113.0^{ab}	136.1^{ab}	212.0^{b}	219.3	259.9^{a}
T_6	28.4	61.0	109.2^{abc}	135.2^{ab}	213.6^{b}	234.9	257.4^{a}
T_7	26.1	73.5	107.2^{bcd}	124.2^{cde}	200.0^{cd}	238.7	244.2^{bcd}
T_8	24.8	61.3	99.5^{cd}	121.3^{de}	198.5^{cd}	216.9	245.2^{bcd}
T_9	31.5	66.1	109.2^{abc}	128.4^{bcd}	194.0^{de}	224.4	246.0^{bc}
T_{10}	24.6	63.4	102.6^{cd}	122.0^{de}	204.8^{bc}	226.4	237.2^{cd}
F-test	NS	NS	**	**	**	NS	**

Description: Control (T_1), farmer (T_2), SSNM (T_3), and SSNM with inhibitors divided into DMPP/NBPT (T_4), DMPP/DMPP (T_5), DMPP/NBPT + DMPP (T_6), neem/garlic (T_7), neem/neem (T_8), neem/garlic + neem (T_9) and praxelis/praxelis (T_{10}). Different lowercase letters indicate significant differences ($P < 0.05$) according to Duncan's multiple range test. NS = not significantly different at $P < 0.05$ * and ** indicated the significance at $P < 0.05$ and 0.0001, respectively.

Table 3. Stalk diameter during experimental period following treatment with N fertilizer with UIs, NIs, and UINIs compared to treatment with only fertilizer under field conditions

Treatment	Stalk diameter (cm)						
	3 months	4 months	5 months	6 months	8 months	10 months	12 months
T_1	1.92^{ab}	1.98	2.23	2.38	2.76^{ab}	2.82^{c}	3.13
T_2	1.91^{ab}	2.03	2.30	2.59	2.79^{a}	3.15^{a}	3.21
T_3	1.87^{ab}	2.02	2.28	2.59	2.69^{c}	3.02^{b}	3.10
T_4	1.95^{a}	1.99	2.25	2.66	2.75^{abc}	3.10^{ab}	3.29
T_5	1.82^{abc}	2.03	2.25	2.39	2.71^{bc}	3.06^{ab}	3.20
T_6	1.81^{abc}	1.97	2.22	2.53	2.70^{bc}	3.09^{ab}	3.15
T_7	1.83^{abc}	2.00	2.26	2.49	2.69^{c}	3.11^{ab}	3.25
T_8	1.78^{bc}	2.02	2.27	2.53	2.73^{abc}	3.07^{ab}	3.31
T_9	1.71^{c}	2.00	2.25	2.52	2.70^{bc}	3.14^{a}	3.33
T_{10}	1.84^{abc}	2.00	2.25	2.63	2.71^{bc}	3.06^{ab}	3.23
F-test	*	NS	NS	NS	*	**	NS

Description: control (T_1), farmer (T_2), SSNM (T_3), and SSNM with inhibitors divided into DMPP/NBPT (T_4), DMPP/DMPP (T_5), DMPP/NBPT + DMPP (T_6), neem/garlic (T_7), neem/neem (T_8), neem/garlic + neem (T_9) and praxelis/praxelis (T_{10}). Different lowercase letters indicate significant differences ($P < 0.05$) according to Duncan's multiple range test. NS = not significantly different at $P < 0.05$. * and ** indicated the significance at $P < 0.05$ and 0.0001, respectively.

Nonetheless, the findings demonstrated that synthetic inhibitors produced greater stalk length and smaller stem diameter compared to inhibitors from plants as shown at 12 months after planting. These results may have been caused by different active compounds that had the potential to influence the sugarcane plant's morphological traits and growth. This could be explained by the inhibitory effect of synthetic inhibitors being effective shortly after application, which tended to increase the plant height, but this effect was not long-lived (Upadhyay et al., 2011). On the other hand, the BNIs had a more stable inhibitory effect than synthetic inhibitors and so could inhibit soil NH_4^+-N transformation for a longer period (Subbarao et al., 2013). This might have been expressed as the larger diameter in the sugarcane. In addition, the results showed that T_{10} had a similar trend in stalk length and diameter to the other natural inhibitors.

The leaf area in sugarcane was affected by different levels of N fertilizer, with the T_2 and T_3 treatments (499.9-514.9 cm^2) having higher leaf areas than the T_1 treatment (Table 4). This result was in agreement with another study (Idris et al., 2021), where the application of N fertilizer tended to improve the leaf area. In general, N is an important nutrient for plants, being an important component of many biochemical compounds in plants. N helps plants to produce more photosynthetic products by boosting chloroplast production and function when taken up by the plants, resulting in increases in the photosynthetic rate and leaf area (Omondi et al., 2019). However, excess N fertilizer could potentially decrease growth, leaf area, and productivity because of decreases in photosynthetic enzymes and thylakoid N; therefore, reducing the photosynthetic process (Mu & Chen, 2021).

Table 4. Leaf width, leaf length and leaf area at maturation following treatment with N fertilizer with UIs, NIs, and UINIs compared to treatment with only fertilizer under field conditions

Treatment	Leaf Width (cm)	Leaf Length (cm)	Leaf Area (cm^2)
T_1	5.3	122.8^{e}	488.7^{c}
T_2	5.1	130.2^{cd}	500.0^{c}
T_3	5.3	128.5^{de}	514.9^{bc}
T_4	5.3	129.3^{d}	514.7^{bc}
T_5	5.4	129.7^{cd}	519.1^{bc}
T_6	5.8	130.1^{cd}	563.6^{ab}
T_7	5.6	134.8^{cd}	563.0^{ab}
T_8	5.6	136.2^{bc}	568.3^{ab}
T_9	5.4	145.6^{a}	592.7^{a}
T_{10}	5.6	141.7^{ab}	594.7^{a}
F-test	NS	**	*

Description: control (T_1), farmer (T_2), SSNM (T_3), and SSNM with inhibitors divided into DMPP/NBPT (T_4), DMPP/DMPP (T_5), DMPP/NBPT + DMPP (T_6), neem/garlic (T_7), neem/neem (T_8), neem/garlic + neem (T_9) and praxelis/praxelis (T_{10}). Different lowercase letters indicate significant differences ($P < 0.05$) according to Duncan's multiple range test. NS = not significantly different at $P < 0.05$. * and ** indicated the significance at $P < 0.05$ and 0.0001, respectively.

The efficacy of the inhibitors used in this study in terms of leaf area promotion are shown in Table 4. Due to the differences in leaf length and width, synthetic inhibitors appeared to have led to smaller leaf areas than the BNIs. Compared to T_3, the T_{10} and T_9 treatments (594.7 and 592.7 cm^2, respectively) had the significantly highest leaf areas, indicating that the leaf area increased due to the addition of inhibitors. Furthermore, the study by Ni et al. (2018) reported that the use of UIs could delay urea hydrolysis, resulting in the preservation of N in the soil-plant system, and enhancing the amount of N in the soil. However, the NIs acted to retard NH_4^+-N oxidation; consequently, most of the mineral N in form of NH_4^+-N and NO_3^--N was prolonged and taken up by plants, providing N in the plant. The efficiency of inhibitors varies essentially depending on the type of active sites present (Peixoto & Petersen, 2023) and modes of action of the compounds (Subbarao et al., 2012). Although the BNIs produced a shorter sugarcane stalk, they had the capacity and traits to slow down the nitrification process over time after N fertilization and seemed to produce a larger leaf area than synthetic inhibitors.

The average stalk weight in T_3 had a greater effect from the application of N fertilizer than from T_2 and T_1 (Table 5). The highest stalk weight was in T_{10} followed by T_4, T_6, and T_5 (1.95, 1.94, 1.94 and 1.88 kg $stalk^{-1}$, respectively). In general, adding N fertilizer provided mineral N availability for plants to uptake, resulting in larger stalks, which is related to the transformation of mineral N from root to the stalk. Furthermore, adding UIs, NIs, and UINIs affected the stalk length in sugarcane because the UIs slowed down the hydrolysis of urea and the NIs delayed the NH_4^+-to-NO_3^--N transformation. As a result, mineral N in the form of NH_4^+-N was present in the soil for a longer period, enhancing N uptake by the plants. Similarly, Tawfic et al. (2008) reported that the stalk weight had direct correlation with stalk length, stalk diameter, number of internodes and number of tillers. Table 5 shows that adding N fertilizer improved the number of millable canes. Compared to T_3, a greater number of millable canes were found in inhibitor treatments except for T_6 and T_8, which gave a lower number of millable canes. There was large variation in the millable canes per planted area, which was less pronounced. However, in this study, the application of inhibitors along with a reduced N rate tended to have a greater capacity to improve the number of millable canes per planted area, leading to an increase in sugarcane production.

The Department of Agriculture (DOA) in Thailand conducted the investigations on some characteristics of sugarcane Khon Kaen 3 variety in 35 experimental plots. The DOA reported that Khon Kaen 3 had erect plant canes, medium stems and curved internodes, average heights of 278 cm, stalk diameters of 2.73 cm, 64,694 stalk ha^{-1} and 14.6% of CCS value in the first year crop (Ponragdee et al., 2011). Compared to the DOA findings, the results in this study seemed to have greater stalk diameter and number of millable cane while cane heights and CCS values were lower. The difference in this study and the DOA study might be due to variation in the site and climate.

3.2 Sugarcane quality parameters

The values of fiber, brix, pol, purity, and %CCS of sugarcane were non-significantly affected by decreasing N application (Table 6). While %CCS increased with increasing N rates, T_2 produced a higher value than the T_3 and T_1 treatments, with increases of 0.68 and 1.81%, respectively. This result was in contrast with Asokan et al. (2005), who reported that there was no significant increase effect of N application on %CCS. An excessive N fertilizer rate had adverse effect on sucrose in the culm, which referred to the % pol, the quantity of sugar in the sugarcane, and probably contributed to a lower CCS (Kingston,

Table 5. Internode length, number of millable cane and stalk weight at maturation following treatment with N fertilizer with UIs, NIs, and UINIs compared to treatment with only fertilizer under field conditions

Treatment	Internode Length (cm)	Number of Millable Cane (stalk ha^{-1})	Stalk Weight (kg stalk^{-1})
T_1	10.7	63,928	1.48
T_2	10.9	71,250	1.91
T_3	10.4	69,583	1.84
T_4	10.9	73,333	1.94
T_5	10.2	74,583	1.88
T_6	10.3	67,083	1.94
T_7	10.6	77,916	1.76
T_8	11.1	69,166	1.77
T_9	10.2	75,000	1.84
T_{10}	9.9	71,250	1.95
F-test	NS	NS	NS

Description: control (T_1), farmer (T_2), SSNM (T_3), and SSNM with inhibitors divided into DMPP/NBPT (T_4), DMPP/DMPP (T_5), DMPP/NBPT + DMPP (T_6), neem/garlic (T_7), neem/neem (T_8), neem/garlic + neem (T_9) and praxelis/praxelis (T_{10}). NS = not significantly different at $P < 0.05$.

Table 6. Sugarcane quality parameters following treatment with N fertilizer with UIs, NIs, and UINIs compared to treatment with only fertilizer under field conditions

Treatment	Fiber (%)	Brix (%)	Pol (%)	Purity (%)	CCS (%)
T_1	11.32	19.06	15.74	82.86	11.59
T_2	11.71	19.36	16.05	82.83	11.80
T_3	11.83	19.65	16.10	81.86	11.72
T_4	11.91	19.83	17.06	85.96	12.83
T_5	11.80	19.80	17.03	85.86	12.82
T_6	12.44	20.37	17.30	84.89	12.81
T_7	11.74	19.73	16.70	84.62	12.44
T_8	11.49	20.55	17.64	85.69	13.31
T_9	11.75	19.91	16.69	83.82	12.36
T_{10}	12.54	19.82	17.00	85.73	12.65
F-test	NS	NS	NS	NS	NS

Description: control (T_1), farmer (T_2), SSNM (T_3), and SSNM with inhibitors divided into DMPP/NBPT (T_4), DMPP/DMPP (T_5), DMPP/NBPT + DMPP (T_6), neem/garlic (T_7), neem/neem (T_8), neem/garlic + neem (T_9) and praxelis/praxelis (T_{10}). NS = not significantly different at $P < 0.05$.

2013). Table 6 reveals that the application of the optimum N rate seemed to be associated with inhibitor effects on the CCS value, but differences were not significant. These results suggested that the application of N fertilizer based on the soil analysis combined with either UIs or NIs had the potential to increase soil N availability for plant uptake and CCS value. A similar observation was mentioned by Ruser & Schulz (2015) and Folina et al. (2021). Reducing the N fertilizer had little effect on the CCS because the soil already had sufficient nutrients, therefore, there was no need to add large amounts of nutrients. In addition, adding an appropriate amount of nutrient needed to be combined with the inhibitors to improve the efficiency of N use by plants by delaying urea hydrolysis and oxidation conversion of NH_4^+-N (Klimczyk et al., 2021). Similarly, Muller et al. (2022) reported that the use of NIs with a reduced N rate significantly decreased N fertilizer losses by up to 98% and also maintained the yield.

As mentioned earlier concerning sugarcane development and specific morphological attributes, there were no appreciable differences in the amount of millable cane, stalk weights and CCS of each treatment when inhibitors were applied with N fertilizer. Nevertheless, the application of an appropriate N fertilizer rate associated with UIs, NIs and UINIs could minimize fertilizer costs by up to 61 to 69% compared to the fertilizers commonly applied by farmers (T_2), while yields were not decreased (data not shown). Nonetheless, the findings confirmed that the farmer could minimize costs while safeguarding crop nutrient uptake.

3.3 Aboveground biomass and N contents

The aboveground biomass was highest in the T_5 (62.8 t ha^{-1}) and significantly different from the other treatments (Figure 1). The other inhibitors produced more dry matter than the only fertilizer treatment (Figure 1), indicating that the application of UIs and NIs promoted the cane dry matter. In this study, the increases in stalk height and diameter increased the dry matter at maturation, which confirmed that reducing the N rate applied in association with inhibitors had an effect on crop growth and nutrient uptake by the plants and promoted a higher crop yield and yield quality. Liu et al. (2013) reported that DMPP application significantly increased wheat yields and aboveground biomass.

This study showed that reducing N fertilizer rate reduced the N content in sugarcane in the T_3 treatment by up to 13.91% less than for the T_2 treatment (Table 7). However, the results also showed that the N content increased when inhibitors were added together with a reduced N fertilizer rate, with an increase by 4.78-22.17% compared to T_3. The inhibitor treatments produced significantly greater N contents in the cane, except for T_9, compared to the T_3 treatment. The highest N content in sugarcane of 2.81 g kg plant^{-1} was obtained in the T_4 treatment followed by 2.79 and 2.57 g kg plant^{-1} in the T_6 and T_5 treatments, respectively. These results agreed with another study where the application of UIs and NIs was one factor in increasing the aboveground biomass and subsequent N uptake in plants (Wang et al., 2021). These findings demonstrated that the addition of inhibitors in the form of UIs and NIs improved the total aboveground biomass and N content in clayey soil. Using synthetic UIs and NIs seemed to perform much better than the plant-sourced inhibitors. Thus, it could be concluded that both synthetic substances (DMPP and NBPT) and active compounds in natural inhibitors, such as thiosulfinate, 3,4-dimethylpyrazole, tetranortriterpenoids, phenylpropanoids, and flavonoids, greatly inhibited the urea transformation and nitrification processes. This inhibition led to an increase in plant N content, indicating a reduction in fertilizer N losses.

3.4 Principal component analysis

The principal component analysis (PCA) between sugarcane morphological traits and yields was performed. The result explained 56% of the total variance (Factor 1: 38% and Factor 2: 18%) (Figure 2).

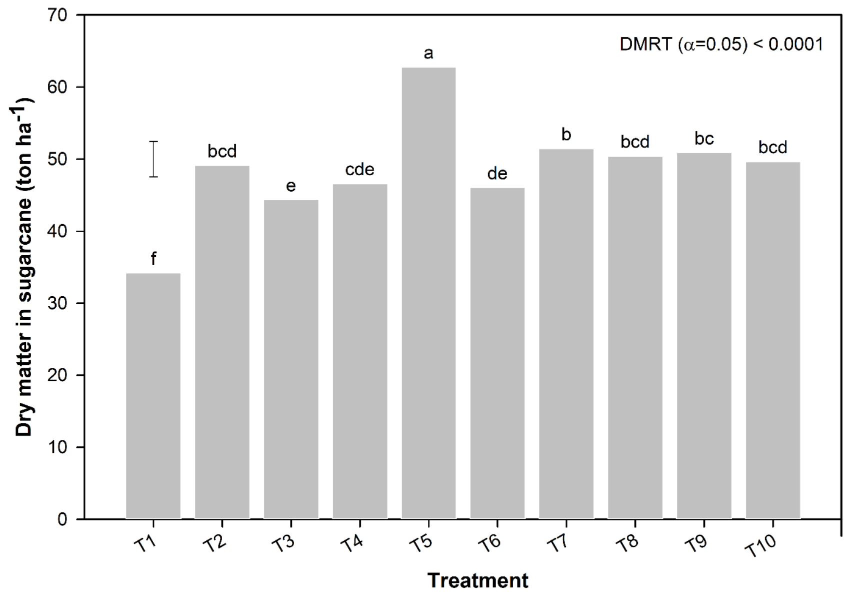

Figure 1. Dry matter in sugarcane following treatment with N fertilizer (with UIs, NIs, and UINIs) compared to treatment with only fertilizer under field conditions. Different lowercase letters above bars indicate significant differences (P<0.05) according to Duncan's multiple range test. Control (T_1), farmer (T_2), SSNM (T_3), and SSNM with inhibitors divided into DMPP/NBPT (T_4), DMPP/DMPP (T_5), DMPP/NBPT + DMPP (T_6), neem/garlic (T_7), neem/neem (T_8), neem/garlic + neem (T_9) and praxelis/praxelis (T_{10}).

Table 7. N content in canes following treatment with N fertilizer (with UIs, NIs, and UINIs) compared to treatment with fertilizer only under field conditions

Treatment	N Content in Plants (g kg^{-1})	Compared to T_2 (%)	Compared to T_3 (%)
T_1	1.41 d		
T_2	2.30 bc		
T_3	1.98 c	-13.91	
T_4	2.81 a	22.17	41.92
T_5	2.57 ab	11.74	29.80
T_6	2.79 a	21.30	40.91
T_7	2.49 ab	8.26	25.76
T_8	2.49 ab	8.26	25.76
T_9	2.41 abc	4.78	21.72
T_{10}	2.55 ab	10.87	28.79
F-test	**		

Description: control (T_1), farmer (T_2), SSNM (T_3), and SSNM with inhibitors divided into DMPP/NBPT (T_4), DMPP/DMPP (T_5), DMPP/NBPT + DMPP (T_6), neem/garlic (T_7), neem/neem (T_8), neem/garlic + neem (T_9) and praxelis/praxelis (T_{10}). Different lowercase letters indicate significant differences (P < 0.05) according to Duncan's multiple range test. ** indicates the significance at P < 0.0001.

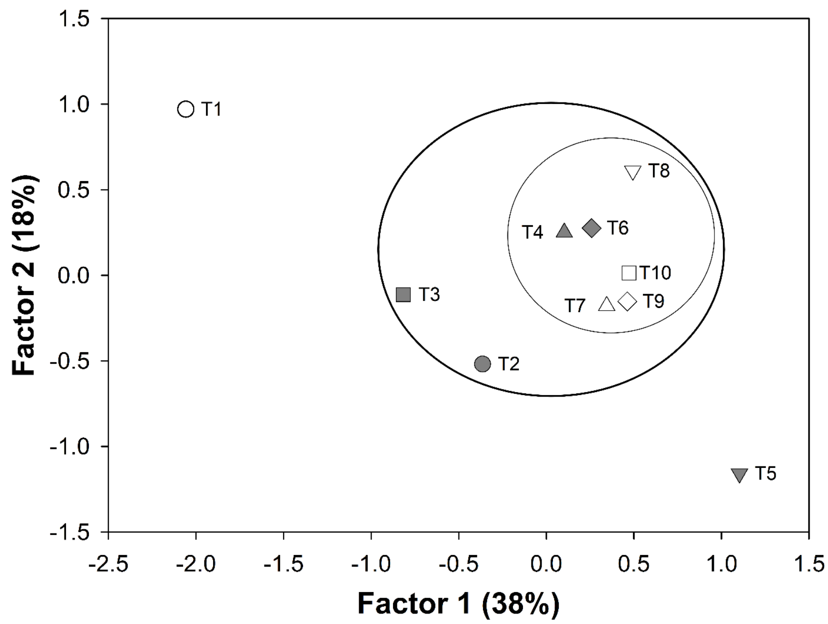

Figure 2. Principal component analysis of sugarcane morphological traits and quality in this study for growth and morphological traits, aboveground biomass, %CCS and N content in all treatments (T1-T10). Control (T_1), farmer (T_2), SSNM (T_3), and SSNM with inhibitors divided into DMPP/NBPT (T_4), DMPP/DMPP (T_5), DMPP/NBPT + DMPP (T_6), neem/garlic (T_7), neem/neem (T_8), neem/garlic + neem (T_9) and praxelis/praxelis (T_{10})

The results revealed that all treatments consisted of four main groups: Group 1 (T_1), Group 2 (T_2 and T_3), Group 3 (T_4 and T_6-T_{10}) and Group 4 (T_5). In the first component, Group 1 had a negative loading that was completely opposite to Groups 2-4. This could be effectively explained by sugarcane growth and morphological traits. Nonetheless, the second component also showed a close loading for Groups 2 and 3, with separated loadings for Group 1 and Group 4. The second component was well explained by sugarcane yield components.

The PCA in Figure 2 provides information about the relationships among treatments. T_4 and T_6 (synthetic inhibitors) were directly correlated with T_7-T_{10} (inhibitor from plants) and related with T_5. However, there was an inverse correlation between T_2 and T_3 (only fertilizer) and T_1 (no N fertilizer). These observations were consistent with the findings on sugarcane growth, morphological traits, and N content in the canes, with all inhibitors having a similar relationship among factors. In addition, T_5 produced the greatest stalk length and this had a direct effect on the aboveground biomass; thus, there was a separate high loading for T_5.

4. Conclusions

The results from this study inferred that the N application rate had an effect on sugarcane growth, leading to a greater yield in terms of aboveground biomass and N content. Nevertheless, the optimum N rate application associated with inhibitors (UIs and NIs) could help to maintain the effect of adding N fertilizer, provide a longer period of supply of the available N in the soil that also presented in the aboveground biomass and N content in the canes. Subsequently, plants could uptake suitable amounts of N to satisfy the crop requirements. Furthermore, these findings highlighted that the synthetic inhibitors and the inhibitors from plants had different effects on morphological traits of sugarcane, with the synthetic inhibitors producing a long stalk length with a smaller stalk diameter than the BNIs that was correlated to the yield in terms of the amount of N in the canes. Therefore, it was necessary to apply the proper N fertilizer rate with inhibitors. In this study, the T_5 treatment seemed to achieve the best sugarcane growth attributes and a significantly greater dry matter content compared to the other inhibitor treatments; however, regarding the qualitative effects (%CCS and N content in the cane), all inhibitors used in this study had a potential similar to the T_5 treatment. Although the praxelis produced a lower stalk length and diameter, it had the highest leaf area, which was driven by the N content in the plant and therefore enhanced the N uptake by the plants. Furthermore, based on the results of this study, further experiments need to be conducted with praxelis that include a consideration of side effects and greater clarification of the active components.

5. Acknowledgements

The Thailand Research Fund provided a scholarship through the Royal Golden Jubilee Ph.D. program [Grant No. PHD/0154/2559] to Miss Phatchariya Welutung and Assoc. Prof. Dr. Patthra Pengthamkeerati. The Lop Buri Research Station, Faculty of Agriculture, Kasetsart University, Lop Buri province, Thailand provided the research area.

6. Conflicts of Interest

The authors declare that we have no conflict of interest.

ORCID

Phatchariya Welutung https://orcid.org/0009-0001-3351-5719

Patthra Pengthamkeerati https://orcid.org/0000-0003-2155-6414

Boonlue Kachenchart https://orcid.org/0000-0002-5841-3030

Saowanuch Tawonpruek https://orcid.org/0000-0001-7229-4640

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