

Effect of Seaweed Extracts from Different Sources Combined with Urease and Nitrification Inhibitors

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Urease inhibitors (UIs) and nitrification inhibitors (NIs) still have limitations in increasing crop yield. Therefore, to improve the application effect of inhibitors, the combination of seaweed extracts (SE) from different sources and inhibitors was added to urea to provide a theoretical basis for the development of a new generation of efficient stabilized urea fertilizer with both biostimulant and inhibitor technologies. The combinations were tested in outdoor pots with no N- fertilizer (CK), application of urea alone (U) as control, and kelp polysaccharide (KP), margin polysaccharide (MP), N-(n-propyl) thiophosphoric triamide (NPPT), dicyandiamide (DCD), and combinations of SE with inhibitor were added to urea to make eight fertilizer prototypes. Compared with KP, MP showed better application effect, with significantly higher grain yield and nitrogen use efficiency (NUE) ($P < 0.05$). Compared with the addition of inhibitor alone, the combinations of NPPT with KP and MP, respectively, had opposite effects on urea-N transformation, meanwhile NPPT+KP had a positive effect. However, NPPT+MP significantly decreased yield, plant nitrogen uptake, and NUE ($P < 0.05$); DCD+MP decreased plant N uptake and NUE to some extent. Therefore, the addition of NPPT with KP and DCD with KP to urea significantly improved yield when planting maize in black soil.

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INTRODUCTION

Seaweeds are the most abundant bioresource in the ocean, containing a large number of nutrients that are lacking in terrestrial organisms (Wang *et al.* 2018). They have long been used in agriculture to augment plant productivity and food production (Craigie 2011). Recently, with the rapid development of biostimulant industry, biostimulants are increasingly known and applied, while seaweed extract (SE) is one of the most widely used biostimulants. The SE extracted by physical, chemical, and biotechnological methods are rich in active substances such as alginate, polysaccharides, betaine, and growth hormones (Battacharyya *et al.* 2015; Di Stasio *et al.* 2017). The SE have been shown to have significant effects in promoting seed germination (Sivasankari *et al.* 2006), root development and yield increase (Wang *et al.* 2018), and enhancing plant stress resistance (Goni *et al.* 2018). In agricultural production, the SE is mostly used in combination with

chemical fertilizers to reduce the negative effects of fertilizers on the environment (Chen *et al.* 2022).

Urea is one of the most widely applied commercial chemical nitrogen (N) fertilizers worldwide (Ray *et al.* 2021). Previous studies showed that urea applied to the soil is lost to the environment through various pathways (Zhu and Chen 2002), and less than 50% of the N is taken up by the crop (Sylvester-Bradley 1993). Meanwhile, large amounts of N released into the environment also cause various environmental risks, such as eutrophication of water bodies and intensification of the greenhouse effect (Lassaletta *et al.* 2014; Beeckman *et al.* 2018). However, as the population increases, there is still a global risk of insufficient food production (Tian *et al.* 2021); therefore, the application of N fertilizer in agriculture is necessary (Meng *et al.* 2020). To improve the N fertilizer use efficiency and reduce the environmental risks, researchers have been developing new N fertilizers.

Researchers have found that the addition of urease inhibitors (UIs) and nitrification inhibitors (NIs) to N fertilizers can effectively reduce the loss of N (Artola *et al.* 2011; Abalos *et al.* 2014), and is considered to have positive environmental benefits (Lam *et al.* 2018). Researchers define fertilizers with inhibitors as stabilized fertilizers. However, Silva *et al.* (2017) showed that the N that was not lost to the environment due to the addition of inhibitors was not fully absorbed by the plant. Singh *et al.* (2013) showed that the addition of UIs effectively increased the N uptake by herbage, but there was no significant difference on the dry matter. Similarly, Di *et al.* (2005) showed that the dicyandiamide (DCD) applied with 5 kg ha⁻¹ did not significantly affect the herbage N with respect to uptake and dry matter yield. Although the application of inhibitors has positive environmental benefits, it has a limited effect on crop yield increase. Therefore, to improve the effect of inhibitors and solve the problem of limited yield increase with application inhibitor alone, the authors combined SE and inhibitors into urea in an effort to achieve an efficient stabilized fertilizer to be applied in black soil.

Meanwhile, there are differences in the composition and effects of SE from different sources. It is not known whether the combination of SE from different sources with inhibitors can produce the same positive increase in yield. Therefore, the authors combined SE from different sources such as kelp polysaccharide (KP) and margin polysaccharide (MP)) with different types of inhibitors, and (N-(n-propyl) thiophosphoric triamide (NPPT) and dicyandiamide (DCD)) into urea to investigate the effect of the combinations on urea-N transformation and maize physiological and biological indicators. This study aimed to improve the application effect of traditional stabilized fertilizers and to provide a theoretical basis for the development of new efficient stabilized fertilizers.

EXPERIMENTAL

Experimental Site and Soils

An outdoor pot experiment was conducted at the national field observation and research station of Agroecosystems in Shenyang, Liaoning province (41°31'N, 123°24' E), in which Dongdan-6531 spring maize (*Zea mays* L., from May to October, 2020) was planted. The mean annual air temperature is 7 to 8 °C, and the mean annual precipitation is approximately 700 mm. The frost-free period is 147 to 164 days. The soil samples were collected from Nongan county (44°43'N, 125°18'E) in Jilin province of northeast China. The sampling site was planted with maize for a long period of time and was fertilized

regularly. The soil type is black soil with clay, silt, and sand of 37.3%, 52.2%, and 10.4%, respectively, and the texture structure is silt clay. Detailed physicochemical properties of the black soil are shown in Table 1.

Table 1. Physicochemical Properties of Black Soil (0 to 0.20 m Soil Layers)

pH	Organic matter (g/kg)	Total N (g/kg)	NH ₄ ⁺ -N (mg/kg)	NO ₃ ⁻ -N (mg/kg)	Total P (g/kg)	Available P (mg/kg)	Total K (g/kg)	Available K (mg/kg)
6.23	32.19	1.68	11.15	59.73	0.79	78.88	50.50	322.15

Experimental Design

The fine root debris and other debris from collected soil samples were removed and mixed thoroughly for use. Ten treatments were established with three replications each: (1) no N fertilizer (CK); (2) urea (U); (3) urea + KP (KP); (4) urea + MP (MP); (5) urea + NPPT (NPPT); (6) urea + DCD (DCD); (7) urea + NPPT + KP (NPPT+KP); (8) urea + NPPT + MP (NPPT+MP); (9) urea + DCD + KP (DCD+KP); (10) urea + DCD + MP (DCD+MP). Each pot contained 6 kg of air-dried soil. The fertilizers urea, triple superphosphate, and potassium chloride were applied at dosages of 0.7 g N, 0.12 g P₂O₅, and 0.15 g K₂O per kg soil, respectively. The application dosages of SE, N-butyl phosphorothioate triamine (NBPT), 3,4-dimethylpyrazolephosphate (DMPP), and 2-chloro-6-trimethylpyridine (CP) were 6%, 0.25%, 0.5%, and 0.25% (Xiao *et al.* 2022), respectively on the w/w basis of urea. The SE and inhibitors applied to each treatment were weighed respectively and mixed thoroughly with urea; then the prepared urea fertilizer was mixed with the soil, and the mixed soil was transferred into pots (diameter 26 cm, height 28 cm, cross-section was trapezoidal). After that, specimens were irrigated to achieve a moisture content reaching 60% field capacity. Five seeds were sown in each pot, and thinned to one plant per pot after germination and seedling establishment. During maize growth, the seeds were watered every day to ensure normal growth of maize and no water loss, and no topdressing during the growth period of maize.

Urea was supplied by the China National Pharmaceutical Group Corporation (Beijing), containing 46% N. Triple superphosphate was supplied by Yunnan Tianhua Group Co. (Kunming, China), containing 43% P₂O₅. Potassium chloride containing 60% K₂O was obtained from Russia (Uralkali, Perm Territory, Russia). Both KP and MP were supplied by a Chinese company (Shandong Qingdao Seawin Biotech Group, Qingdao, China), among which KP was extracted from brown algae, containing 7.18% kelp polysaccharide, 7.18% alginate, and 7.40% organic matter, at pH 6.5. The MP was extracted from green algae, containing 7.45% marshmallow polysaccharide, 7.21% alginate and 7.30% organic matter, pH 6.0. The UI, NPPT, and the NI, DCD were supplied by Macklin Biotechnology (Shanghai, China), with a purity of 99% and 99.5%.

Soil and Maize Sampling

Three replicates were set up for each treatment, and soil samples were collected at four growth stages (seedling, elongation, filling, and maturity, 38, 65, 102, and 135 days after planting, respectively) by the 5-point sampling method, and the soil samples were thoroughly mixed and passed through a 2-mm sieve for use. Leaf area and chlorophyll content were measured (YMJ-B and CCM-200 instruments, respectively) at silking stage.

At maturity, whole maize plants were collected, divided into kernels, stalks, and roots air-dried, and total biomass, grain yield, and root biomass were measured.

Soil and Plant Analyses

Soil pH was measured using a 1:2.5 soil to deionized water suspension (Cui *et al.* 2021). The organic matter content of the soil was determined by oxidation with potassium dichromate and followed by titration with ferrous ammonium sulfate (Schollenberger 1945). Total N content of soil was determined *via* dry combustion using an elemental analyzer (Vario Macro cube, Elementar, Hanau, Germany) (Yang *et al.* 2016). Soil total phosphorus (P) was digested by HClO₄ and available P was extracted with 0.5 mol/L NaHCO₃ solution, then both analyzed by the molybdenum blue method (Sommers and Nelson 1972; Zhao *et al.* 2004). Soil total potassium (K) was digested by HCl and available K was extracted with 1 mol/L NH₄OAc and determined by the flame photometric method (Gammon 1951; Zhao *et al.* 2004).

Soil Urea-N was extracted with KCl-PMA (2 mol/L KCl and 5 mg/L PMA), NH₄⁺-N and NO₃⁻-N were extracted with 2 mol/L KCl, and determined on a continuous flow analyzer (AA III, Seal, Norderstedt, Germany) (Mulvaney and Bremner 1979; Bracken *et al.* 2020).

All plant samples were dried at 65 °C until constant weight was achieved to calculate total biomass of maize, then ground and sieved through a 250-µm mesh for the analysis of total N in an elemental analyzer (Vario Macro cube, Elementar, Hanau, Germany).

Calculations and Statistical Analyses

Nitrification inhibition rate (%) was calculated using Eq. 1 (Xiao *et al.* 2022),

$$\text{Nitrification inhibition rate (\%)} = (a - b) / a \times 100 \quad (1)$$

where *a* denotes the NO₃⁻-N of soil applied urea only (mg kg⁻¹), *b* denotes the NO₃⁻-N of soil applied with biostimulants, UIs, and NIs.

The NUE values were calculated using Eq. 2 as follows (Cui *et al.* 2022),

$$\text{NUE} = (Y - YC) / NF \quad (2)$$

where *Y* represents plant N uptake with N fertilizer; *YC* is the plant N uptake with no fertilizer; and *NF* stands for the amount of N fertilizer applied.

Multiple comparisons were performed using the Duncan test, and significant differences were determined at *P* < 0.05. Statistical analyses were performed using Microsoft Excel 2010 (Redmond, WA, USA), IBM SPSS 21.0 (IBM Corp., Chicago, IL, USA), and R 4.2.1 (Revolution Analytics, Auckland, New Zealand). Graphs were prepared using R 4.2.1. The data in the tables denote the average value ± standard error.

RESULTS

The Contents of Urea-N in Soils

The Urea-N was not found in the soils of each treatment at the seedling stage, indicating that urea was fully hydrolyzed at this time. Thus the soil was not tested for Urea-N at the three subsequent maize growth periods.

The Contents of NH₄⁺-N in Soils

The soil NH₄⁺-N contents of each treatment tended to decrease with the growth of maize. At the seedling stage, the NH₄⁺-N contents of the added DCD treatments were above 48.16 mg kg⁻¹, which were significantly higher than other treatments; compared to the application of NPPT and DCD alone, the addition of SE significantly decreased the NH₄⁺-N contents, respectively ($P < 0.05$) (Table 2). At the filling stage, the NH₄⁺-N content of CK was higher than other treatments (Table 2); compared to DCD, the combination of DCD with SE significantly increased the NH₄⁺-N contents ($P < 0.05$). At the maturity stage, the NH₄⁺-N content of the DCD+MP was significantly higher than other treatments ($P < 0.05$), with 15.4 mg kg⁻¹, and the NH₄⁺-N contents of the other treatments tended to be the same (Table 2).

Table 2. Contents of NH₄⁺-N of Different Treatments in Black Soil (mg kg⁻¹)

Treatments	Seedling	Elongation	Filling	Maturity
CK	16.66 ± 1.24f	16.23 ± 0.91c	21.20 ± 1.03a	10.26 ± 1.04bc
U	29.61 ± 2.84d	18.39 ± 0.61abc	13.47 ± 0.39c	9.89 ± 0.66c
KP	32.55 ± 1.16d	17.08 ± 1.02bc	20.43 ± 1.08a	10.74 ± 0.35bc
MP	22.69 ± 1.56e	16.89 ± 0.41bc	16.78 ± 0.22b	9.74 ± 0.36c
NPPT	42.54 ± 2.14c	18.44 ± 1.98abc	11.28 ± 0.66d	10.90 ± 0.71bc
DCD	79.30 ± 2.58a	19.14 ± 1.28ab	8.27 ± 0.95e	10.31 ± 0.33bc
NPPT+KP	20.65 ± 2.86ef	17.82 ± 0.98bc	10.85 ± 0.74d	10.04 ± 0.77c
NPPT+MP	31.54 ± 0.98d	16.98 ± 1.41bc	11.19 ± 0.75d	10.09 ± 0.88c
DCD+KP	50.22 ± 4.55b	20.47 ± 0.77a	12.73 ± 0.48c	11.63 ± 0.85b
DCD+MP	48.16 ± 1.38b	18.96 ± 2.10ab	11.31 ± 1.00d	15.41 ± 0.94a

Treatments: CK: no N fertilizer; U: urea; KP: urea + kelp polysaccharide; MP: urea + margin polysaccharide; NPPT: urea + N-(n-propyl) thiophosphoric triamide; DCD: urea + dicyandiamide; NPPT+KP: urea + N-(n-propyl) thiophosphoric triamide + kelp polysaccharide; NPPT+MP: urea + N-(n-propyl) thiophosphoric triamide + margin polysaccharide; DCD+KP: urea + dicyandiamide + kelp polysaccharide; DCD+MP: urea + dicyandiamide + margin polysaccharide. Different lowercase letters in the column mean a significant difference between treatments (Duncan test, $P < 0.05$).

Table 3. Contents of NO₃⁻-N of Different Treatments in Black Soil (mg kg⁻¹)

Treatments	Seedling	Elongation	Filling	Maturity
CK	31.41 ± 3.84h	2.16 ± 0.16g	2.26 ± 0.06e	3.77 ± 0.36bc
U	528.66 ± 21.06b	5.08 ± 0.33d	7.43 ± 0.43b	2.93 ± 0.21e
KP	505.41 ± 3.36c	6.48 ± 0.41c	6.88 ± 0.08bc	2.89 ± 0.09e
MP	442.99 ± 8.62d	3.97 ± 0.49ef	5.36 ± 0.52d	4.40 ± 0.49a
NPPT	435.67 ± 6.77e	4.55 ± 0.41de	8.13 ± 0.74ab	3.34 ± 0.39cde
DCD	261.53 ± 12.37g	2.49 ± 0.40g	7.67 ± 0.73b	4.22 ± 0.07ab
NPPT+KP	574.17 ± 5.28a	3.56 ± 0.30f	5.07 ± 0.72d	3.52 ± 0.37cd
NPPT+MP	409.76 ± 17.34e	4.97 ± 0.16d	5.83 ± 0.87cd	3.15 ± 0.32de
DCD+KP	259.09 ± 17.43g	14.75 ± 1.24a	9.16 ± 1.17a	3.71 ± 0.26bcd
DCD+MP	295.73 ± 14.10d	8.31 ± 0.61b	7.80 ± 0.58b	3.69 ± 0.14bcd

The Contents of NO_3^- -N in Soils

At the seedling stage, soil NO_3^- -N contents of applied N treatments were all significantly higher than of CK; compared to U, the addition of SE and inhibitors alone significantly decreased soil NO_3^- -N contents; to NPPT, the addition of KP significantly increased the NO_3^- -N content; to DCD, the addition of MP significantly increased the NO_3^- -N content ($P < 0.05$) (Table 3). At the elongation stage, the NO_3^- -N content of each treatment decreased rapidly, among which the NO_3^- -N contents of DCD+KP and DCD+MP were significantly higher than other treatments, and DCD+KP significantly higher than DCD+MP ($P < 0.05$). After the filling stage, the NO_3^- -N content of each treatment tended to be the same (Table 3).

Soil Nitrification Inhibition Rate of Different Treatments at the Seedling Stage

At the seedling stage, the DCD could effectively inhibit soil nitrification (Fig. 1), which was in line with the change of soil inorganic N (NH_4^+ -N and NO_3^- -N) (Tables 2 and 3), and the soil nitrification inhibition values of the added DCD treatments were all significantly higher than other treatments, with values above 44.06%, among which DCD+MP treatment significantly reduced the inhibition ($P < 0.05$) (Fig. 1). The SE inhibited nitrification to some extent, among which the inhibition of nitrification of MP was significantly higher than KP ($P < 0.05$) (Fig. 1). Compared to NPPT, NPPT+KP significantly reduced soil nitrification inhibition; however, NPPT+MP significantly increased the rate ($P < 0.05$) (Fig. 1).

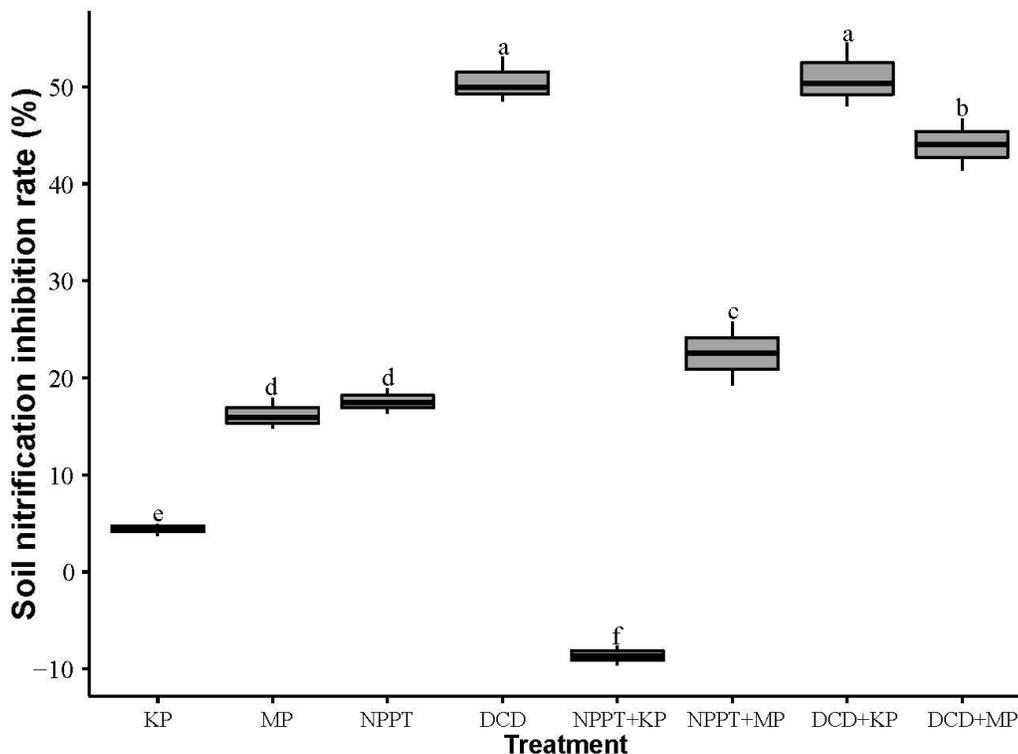


Fig. 1. Soil Nitrification Inhibition Rate (%). Error bars represented standard deviations ($n = 3$). Different letters indicate significant differences between different treatments at $P < 0.05$ by Duncan test.

Maize Plant Physiological and Biological Indicators

Compared to U, the addition of SE from different sources and inhibitors significantly increased chlorophyll contents ($P < 0.05$), among which NPPT+MP was most beneficial to crop growth and photosynthesis with the chlorophyll content, leaf area, height, and stalk thickness of 70.44, 674.61 cm², 263.67 cm, and 23.25 mm, respectively (Table 4). To MP, KP had a better application effect, and the leaf area and stalk thickness of KP were significantly higher than MP ($P < 0.05$) (Table 4). To NPPT, NPPT+KP significantly increased chlorophyll content 14.55% ($P < 0.05$); to DCD, DCD+MP significantly decreased height 10.55% ($P < 0.05$) (Table 4).

Table 4. Maize Plant Physiological at Silking Stage and Biological Indicators at Maturity Stage

Treatment	Chlorophyll	Leaf Area (cm ²)	Height (cm)	Stalk Thickness (mm)
CK	25.88 ± 2.88f	352.49 ± 9.12g	243.33 ± 13.50c	17.28 ± 1.57c
U	35.81 ± 2.53e	518.95 ± 31.34f	260.33 ± 3.79abc	22.03 ± 0.35ab
KP	60.42 ± 5.32cd	688.67 ± 64.57a	271.00 ± 5.29ab	24.33 ± 2.95a
MP	62.81 ± 5.59bc	626.69 ± 24.15bcd	255.67 ± 15.01abc	20.91 ± 0.90b
NPPT	63.86 ± 3.75bc	648.01 ± 45.40abc	265.33 ± 9.71abc	22.30 ± 0.34ab
DCD	55.88 ± 2.25cd	579.86 ± 15.86de	275.00 ± 10.58a	22.99 ± 0.91ab
NPPT+KP	73.14 ± 2.59a	614.03 ± 8.68cd	258.67 ± 14.64abc	21.55 ± 0.64ab
NPPT+MP	70.44 ± 5.20ab	674.61 ± 10.18ab	263.67 ± 24.34abc	23.25 ± 1.89ab
DCD+KP	63.23 ± 7.10bc	546.83 ± 24.57ef	265.67 ± 4.04abc	21.91 ± 1.60ab
DCD+MP	54.44 ± 3.73d	547.40 ± 41.04ef	246.00 ± 17.35bc	21.86 ± 1.71ab

Treatments: CK: no N fertilizer; U: urea; KP: urea + kelp polysaccharide; MP: urea + margin polysaccharide; NPPT: urea + N-(n-propyl) thiophosphoric triamide; DCD: urea + dicyandiamide; NPPT+KP: urea + N-(n-propyl) thiophosphoric triamide + kelp polysaccharide; NPPT+MP: urea + N-(n-propyl) thiophosphoric triamide + margin polysaccharide; DCD+KP: urea + dicyandiamide + kelp polysaccharide; DCD+MP: urea + dicyandiamide + margin polysaccharide. Different lowercase letters in the column mean a significant difference between treatments (Duncan test, $P < 0.05$).

Maize Plant Physiological and Biological Indicators

Compared to U, the addition of SE and inhibitors significantly increased maize yield, plant N uptake, and NUE ($P < 0.05$) (Table 5). The MP had the best application with the total biomass, grain yield, plant N uptake, and NUE of 524.45 g, 247.48 g, 5.08 g, and 73.20%, respectively (Table 5). Compared to NPPT, the NPPT+KP significantly increased total biomass, grain yield, and root biomass 16.22%, 25.27%, and 26.13%, respectively, and plant N uptake and NUE were also increased. However, NPPT+MP significantly decreased total biomass, grain yield, plant N uptake, and NUE by 16.44%, 22.71%, 11.93%, and 22.76%, respectively, and the combination of NPPT with SE could significantly increase root biomass ($P < 0.05$) (Table 5). Compared to DCD, the DCD+KP significantly increased total biomass and grain yield 7.49% and 24.50%, respectively, and root biomass was significantly decreased 24.19%. The DCD+MP treatment significantly increased grain yield and root biomass 13.17% and 17.24%, respectively ($P < 0.05$) (Table 5).

Table 5. Maize Plant Physiological at Silking Stage and Biological Indicators at Maturity Stage

Treatment	Total Biomass (g)	Grain Yield (g)	Root Biomass (g)	Plant N Uptake (g)	NUE (%)
CK	200.38 ± 3.12f	91.76 ± 2.54f	12.45 ± 2.28e	2.01 ± 0.05f	–
U	255.53 ± 3.78e	115.30 ± 1.56e	22.55 ± 1.05bc	2.67 ± 0.06e	15.76 ± 1.41e
KP	514.72 ± 19.83a	221.22 ± 14.65b	25.24 ± 2.17a	4.77 ± 0.32ab	65.79 ± 7.66ab
MP	524.45 ± 12.09a	247.48 ± 11.83a	22.09 ± 1.37c	5.08 ± 0.08a	73.20 ± 1.85a
NPPT	434.46 ± 11.42c	198.25 ± 12.63bc	16.45 ± 0.39d	4.22 ± 0.08c	52.67 ± 1.95c
DCD	446.24 ± 6.98c	177.27 ± 16.22c	21.25 ± 1.67c	4.43 ± 0.06bc	57.61 ± 1.38bc
NPPT+KP	504.91 ± 29.60ab	248.35 ± 18.35a	20.75 ± 1.01c	4.57 ± 0.33bc	61.04 ± 7.80bc
NPPT+MP	363.04 ± 14.63d	153.22 ± 3.76d	20.63 ± 1.18c	3.72 ± 0.18d	40.68 ± 4.32d
DCD+KP	479.68 ± 15.96b	220.69 ± 18.05b	16.11 ± 1.62d	4.73 ± 0.32b	64.74 ± 7.55ab
DCD+MP	449.05 ± 10.82c	200.62 ± 14.02b	24.91 ± 0.53ab	4.23 ± 0.05c	52.92 ± 1.29c

Treatments: CK: no N fertilizer; U: urea; KP: urea + kelp polysaccharide; MP: urea + margin polysaccharide; NPPT: urea + N-(n-propyl) thiophosphoric triamide; DCD: urea + dicyandiamide; NPPT+KP: urea + N-(n-propyl) thiophosphoric triamide + kelp polysaccharide; NPPT+MP: urea + N-(n-propyl) thiophosphoric triamide + margin polysaccharide; DCD+KP: urea + dicyandiamide + kelp polysaccharide; DCD+MP: urea + dicyandiamide + margin polysaccharide. Different lowercase letters in the column mean a significant difference between treatments (Duncan test, P < 0.05).

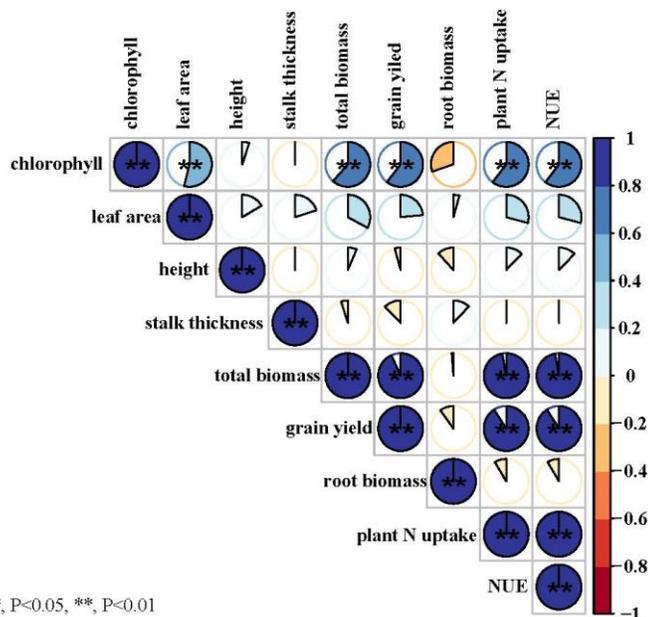


Fig. 2. Pearson correlation analysis among physiological and biological indicators, plant N uptake, and NUE

Pearson Correlation Analysis among Physiological and Biological Indicators, Plant N Uptake, and NUE

There were significant positive correlations between chlorophyll content and leaf area, total biomass, grain yield, plant N uptake, and NUE ($P < 0.01$) (Fig. 2), indicating that the increase in chlorophyll content was positive for the increase in yield, plant N uptake, and NUE, while no significant correlation existed between leaf area, plant height, stem thickness, yield, root biomass, plant N uptake, and NUE ($P < 0.05$) (Fig. 2), indicating that those indexes were not determining factors affecting maize yield, plant N uptake, and NUE.

DISCUSSION

Effect of SE from Different Sources and Inhibitors on Urea-N Transform

The soil treatment using DCD significantly increased the soil $\text{NH}_4^+\text{-N}$ contents (Table 2) at the seedling stage ($P < 0.05$), similarly to Ibarra *et al.* (2021). The SE used in this experiment all contained polysaccharides, while Jagtap *et al.* (2021) showed that glycoside hydrolases and polysaccharide hydrolases present in soil could hydrolyze or cleave algal polysaccharides into algal oligosaccharides (AOS). They also showed that the negatively charged functional groups contained in AOS could bind to the $\text{NH}_4^+\text{-N}$ in the soil, and then give the soil $\text{NH}_4^+\text{-N}$ some abiotic protection, so both SE could inhibit nitrification to some extent. Wang *et al.* (2016) showed that SE could increase soil urease activity, accelerating the hydrolysis rate of urea. After a short time, a large amount of $\text{NH}_4^+\text{-N}$ is released into the soil, and then it increased ammonia volatilization loss; therefore compared to normal urea, the addition of SE alone decreased the inorganic N contents, which also decreased the nitrification substrate concentration and inhibited nitrification. As a new type of urease inhibitor, NPPT has a similar basic structure and functional groups with N-butyl phosphorothioate triamine (NBPT) (Krol *et al.* 2020). The NPPT can effectively delay the transformation of Urea-N to $\text{NH}_4^+\text{-N}$ (Zhou *et al.* 2019), and thus it can inhibit nitrification to some extent. The addition of SE from different sources had the opposite effect on Urea-N transformation, which was caused by the difference in composition between KP and MP. Hashem *et al.* (2019) showed that there were obvious differences in the composition of SE from different sources, with brown algae extracts containing higher concentrations of phenolics and green algae extracts containing more proline, indole acetic acid (IAA), cytokinins, and carbohydrates.

Engel *et al.* (2015) showed that the degradation of NBPT in soil is mainly affected by the activity of microorganisms and the half-life in unsterilized soil is only 0.07 to 3.43 days, while NPPT have similar structure, effect, and effective time with NBPT (Zhou *et al.* 2016). Thus, the degradation rate of NPPT in soil is also affected by soil microorganisms. The high concentration of amino acids, growth hormones, and carbohydrates in MP could increase microbial community diversity (Bais *et al.* 2006). Meanwhile, MP contained a high concentration of plant growth hormone, which increased the activity of root, and more carbon C could be incorporated into soil through the root system (Kuzyakov and Domanski 2000). The metabolites produced by root also benefit the growth of microorganisms (Meier *et al.* 2017) and thus NPPT+MP caused the NPPT to be under a stronger microbial degradation, shortened its effective action time, increased the ammonia volatilization loss, and significantly decreased the inorganic N contents (Tables 2 and 3), which was not beneficial to the effect of NPPT. Zhao *et al.* (2022) showed that seaweed phenolics have

strong antibacterial ability, which could show toxicity to some soil microorganisms (Geng *et al.* 2017). The authors concluded that this could delay the degradation of NPPT in the soil, further reducing the ammonia volatilization loss, and significantly increasing the inorganic N contents (Tables 2 and 3) ($P < 0.05$). Qiao *et al.* (2015) showed that the addition of NIs increased ammonia volatilization loss by approximately 20%, while SE inhibited nitrification to some extent, resulting in high concentrations of NH_4^+ -N maintained in the soil for a longer time. Thus, the combination of DCD with SE further increased ammonia volatilization loss, and then significantly decreased soil NH_4^+ -N contents (Table 2). Schwarzer *et al.* (1998) showed that the degradation process of DCD in soil includes not only chemical degradation, but soil microorganisms also showed degradation behavior for DCD. The above discussion concluded that amino acids and carbohydrates contained in MP can improve the activity of microorganisms and shorten its effective action time. Thus, DCD+MP significantly increased NO_3^- -N content (Table 3) ($P < 0.05$) and reduced the soil nitrification inhibition rate (Fig. 1). Previous study showed that DCD could effectively inhibit nitrification over 35 days (Barth *et al.* 2020). Although KP could inhibit the activity of microorganisms and delay the degradation of DCD in the soil, the addition of DCD alone could still inhibit nitrification at the seedling stage (38 days after planting), thus DCD+KP did not significantly affect soil nitrification inhibition rate (Fig. 1) ($P < 0.05$).

Effects of SE from Different Sources and Inhibitors on Physiological and Biological Indicators, Yield, Plant N Uptake, and NUE

Compared to the normal urea, the effects of added SE and inhibitors on plant photosynthesis, yield, plant N uptake, and NUE were similar to previous studies (Wallace *et al.* 2020; Del Buono 2021). Compared with KP, MP had a better application effect because of the higher concentration of plant growth hormones, in accordance with Hashem *et al.* (2019). The addition of SE from different sources showed an opposite effect on yield, plant N uptake, and NUE, in line with the changes in soil inorganic N contents (Tables 2 and 3). Because KP could delay the degradation of NPPT and further reduce ammonia volatilization losses, NPPT+KP significantly increased maize yield, plant N uptake, and NUE (Table 5) ($P < 0.05$), and NPPT+MP was not beneficial to maize yield and NUE. Brown algal polyphenols contained in KP enhance plant stress resistance (El-Katony *et al.* 2020), and avoid the accelerated rate of chlorophyll degradation in leaves when subjected to drought stress (Nyarobi *et al.* 2022). Thus, NPPT+KP significantly increased chlorophyll content (Table 4), while the chlorophyll content was the determining index of yield increase (Fig. 2), which is in accordance with the previous study (Orzech *et al.* 2022), and thus significantly increased yield. The NPPT+MP treatment accelerated the rate of NPPT degradation in the soil, increased the ammonia volatilization loss, then caused a significant decrease in yield, plant N uptake, and NUE (Table 5) ($P < 0.05$). The DCD+KP significantly increased total biomass and grain yield (Table 5) ($P < 0.05$), mainly because KP could delay the microbial degradation of DCD. Nitrous oxide (N_2O) emission is the main pathway of N loss in upland fields (Ju *et al.* 2009), while DCD+MP significantly increased soil NO_3^- -N content (Table 3) ($P < 0.05$), which resulted in an increase in N_2O emission and N loss (Deiglmayr *et al.* 2006), and caused a decrease in NUE (Table 5). Meanwhile, the combination of DCD with SE significantly increased the grain yield. The authors concluded that the combination of DCD and SE could affect the transfer and distribution of photosynthetic products. However, there was no report on this phenomenon, and the authors will investigate this phenomenon in the subsequent work.

CONCLUSIONS

1. The addition of seaweed extract (SE) from different sources to urea significantly increased chlorophyll contents, leaf area, grain yield, plant N uptake, and nitrogen use efficiency (NUE). While SE could inhibit soil nitrification to some extent, MP has a better application effect.
2. The N-(n-propyl) thiophosphoric triamide kelp polysaccharide (NPPT+KP) treatment significantly increased chlorophyll content and yield; however, the corresponding treatment with margin polysaccharide (NPPT+MP) significantly decreased the yield, plant N uptake, and NUE. The combination of dicyandiamide (DCD) with SE significantly increased grain yield, which was positive for economic benefits. The DCD+KP produced positive results; the plant N uptake and NUE were increased. But DCD+MP reduced the soil nitrification inhibition rate, plant N uptake, and NUE.
3. Application of urea with the combination of KP with NPPT and DCD, respectively, was beneficial for maize yield and NUE when planting maize in black soil.

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