



Effects of Novel Nitrogen Smart Fertilizer Formulations on Potato Production and Quality

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Abstract

Purpose Nitrogen (N) fertilization poses a series of concerns related to its impact on agroecosystems. This study evaluated a possible strategy to overcome those problems, investigating innovative urea smart fertilizers containing nitrification inhibitors (Methylene Blue – BM), urease inhibitor (Gallic Acid – GA), and their combinations (BMGA and GABM) for potato (*Solanum tuberosum* L.) fertilization. **Methods** A two-year study (2023–2024) was conducted on potato crop to compare the effects of four innovative urea smart fertilizers (GA, BM, GABM, BMGA) and a commercially available urea smart fertilizer (NBPT) applied at 100% and 75% of the recommended N dose (RND) on plant phenology, crop yield, crop biometry, tuber N content, nitrate (NO_3^-) leaching, and soil N contents. The unfertilized and business as usual fertilization, with urea provided at 100% of RND, were used as controls. **Results** A model based on cumulated growing degree days (GDD) and Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH) stage revealed that the smart fertilizers did not affect potato plant phenology. Soil analyses did not yield insightful results. Under BM–100, total and marketable yields, and plant height were the highest (40.3 ± 4.1 and $34.3 \pm 3.8 \text{ t ha}^{-1}$, $56.3 \pm 8.2 \text{ cm}$). Notably, smart urea at 75% dosage achieved yields comparable to commercial urea at 100% (on average $25.4 \pm 2.8 \text{ t ha}^{-1}$ of marketable yield) and produced tubers with 7% less Total Kjeldhal nitrogen (TKN) content. Conversely, the highest NO_3^- concentration in leaching was found under NBPT–100 ($169.2 \pm 18.2 \text{ mg L}^{-1}$) and BMGA–100 ($198.7 \pm 21.1 \text{ mg L}^{-1}$). **Conclusion** This pioneering study identifies BM as a promising nitrification inhibitor for enhancing potato yield, underscoring the utility of smart urea in reducing agricultural N inputs while maintaining productivity.

Keywords Potato yield · Potato phenology · Urease inhibitor · Nitrification inhibitor · Nitrogen fertilization

1 INTRODUCTION

Potato (*Solanum tuberosum* L.) is considered as the fourth most important food crop after rice, wheat, and maize (Zhang et al. 2017), and as the largest cropped vegetable in the world (Yusuf et al. 2024). According to the Food and Agriculture Organization of the United Nations, potato world production in 2022 was around 374 million tons of

fresh tubers from 21 million hectares (FAO, 2024). Optimal fertilization levels are crucial as they significantly influence tuber quality, one of the most critical factors in potato production. Its high fertilizer requirements reflect the role of N, phosphorus (P), and potassium (K) in increasing tuber weight and size while reducing non-marketable yields (Oliveira et al. 2021).

Among these nutrients, N stands out as particularly important for promoting vigorous crop growth, enhancing leaf development, and ultimately boosting both yield and tuber quality (Qiqige et al. 2017). N is commonly found as ammonium (NH_4^+) in various fertilizers. However, depending on soil conditions such as temperature, aeration, and drainage, soil bacteria rapidly convert NH_4^+ into NO_3^- through nitrification. Many crops, particularly cereals, absorb and utilize NO_3^- more efficiently than NH_4^+ . In contrast, some plants, like grasses and potatoes, respond equally well to both forms (Finch et al. 2014). The presence of NO_3^- in soil–water can

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pose significant environmental risks, as they can leach into waterways and contribute to eutrophication. According to the UN Environment Programme (2020), human activities have doubled the amount of N compounds present in soil, water, and air over the past century.

Given these factors, one of the key challenges in sustainable agriculture is promoting fertilizers that minimize environmental impact. These products are often referred to as “smart fertilizers.” While there is no universally accepted definition, they are generally characterized by their ability to release nutrients in a controlled or slow manner compared to conventional fertilizers (Raimondi et al. 2021). Smart fertilizers when applied, can address the specific nutrient needs of crops, at different growth stages (Calabi–Floody et al. 2018). Additionally, some definitions highlight their capacity to reduce environmental harm by minimizing nutrient losses and retaining more water post-application (Raimondi et al. 2021).

To make the transition towards more sustainable fertilization strategies, investment in the development of smart fertilizers is vital. Smart N fertilizers formulations have been developed and tested across various crops, including potatoes. These formulations aim for slower or controlled nutrient release and often incorporate inhibitors that target soil microbiome activities involved in the N cycle, thereby reducing nutrient losses. For instance, N-(n-butyl) thiophosphoric triamide (NBPT) is widely used to inhibit urease activity, while 3,4-dimethylpyrazole succinic (DMPSA) targets the nitrification process (Cantarella et al. 2018; Souza et al. 2021). Additionally, encapsulated fertilizers, such as polymer-coated urea (PCU) have been explored (Wilson et al. 2009). However, concerns persist regarding the environmental impact of synthetic polymers or inorganic inhibitor molecules in the soil and their toxicity (Guiney et al. 1998; Sarkar et al. 2021), as well as potential negative effects on different life forms (Mangas et al. 2016; Van Melis et al. 2022; Woodward et al. 2021).

Sustainable alternatives to synthetic polymers are innovative slow-release formulations using fertilizers coatings based on chitosan, a biopolymer obtainable as a seafood industry byproduct (Kondal et al. 2021). This recently formulated coating is offering potential solutions to environmental pollution caused by synthetic polymers in smart fertilizers. While, in alternative to synthetic urease and nitrification inhibitors, other organic molecules that can be biodegraded in the soil can be proposed. BM for instance, a heterocyclic aromatic compound already widely used in other fields of research, is known to inhibit the nitrifying bacteria and archaea (Collins et al. 2011; Sipos and Urakawa 2016). While GA, has been found to inhibit the urease enzyme (Haq et al. 2024; Demir et al. 2024). GA is a naturally occurring compound, and together with BM, they

are readily available on the market. Both molecules have low water solubility, are effective at low concentration, and are safe for humans. Low water solubility can help delay the early dissolution of urea in the soil solution, potentially improving N use efficiency (NUE) (Daneshfar et al. 2008; Khan et al. 2022). Effectiveness at relatively low concentrations ensures minimal reduction in N content within the fertilizer granules, if they have to be applied in smart fertilizer formulations (Saloni Kakkar 2019; Zada et al. 2024). Additionally, both organic inhibitors are not classified as carcinogenic or toxic to humans, nonetheless, GA is known to have neuroprotective properties (Tariq et al. 2021; Mef-tahi and Aboutaleb 2023). All these characteristics make GA and BM environmentally sustainable options for agricultural applications of N cycle inhibitors.

In view of the above reported considerations, the objective of this study was to evaluate the agronomic performance and environmental impact of urea granules coated with either GA, BM, or their combinations (BMGA and GABM). These coated urea granules are designed with an onion-like structure containing one or both inhibitors depending on the treatment. The study was based on the premise that the use of GA, BM, or their combinations as coating agents for urea would reduce NO_3^- leaching compared to uncoated urea, while maintaining or improving crop phenology, biometric parameters, yield and tuber quality. Accordingly, the main objectives were to assess the agronomic response of the crop as well as to quantify the environmental impact of these smart fertilizers, particularly regarding the quality of water percolating through the soil profile.

2 Materials and Methods

2.1 Field Site Description and Management

The field experiment was set up in the University of Padova experimental farm “L. Toniolo”, located in Legnaro, Italy (45°21' N; 11°56' E). The experiment was repeated in two different years in the same location. The area is characterized by a humid subtropical climate (Cfa in Köppen–Geiger classification). Weather data (air and soil temperature, precipitation) were collected from the meteorological station of the Veneto Regional Agency for Environmental Prevention and Protection (ARPAV), located within the farm about 500 m far from the experimental site. GDD ($^{\circ}\text{C day}^{-1}$) were calculated as the difference between the daily average temperature (T_a) and the lower base temperature (T_b), following the formula given in Pathak and Stoddard (2018). T_b was set to 6 $^{\circ}\text{C}$ while T_{cutoff} equal to 29 $^{\circ}\text{C}$ (Weixing & Tibbitts, 1995; Pantelić et al., 2018). The experimental site soil is classified as loam according to USDA Soil Textural

Triangle (Ditzler et al. 2017). The basic soil parameters are listed in Table 1.

Potato (*Solanum tuberosum* L.) cv Desiree (HZPC, The Netherlands) was hand-sowed in ridges on March 20, 2023, and March 21, 2024, in 4 m² plots at a density of 7 plants m⁻². The experiment was arranged in a Randomized Complete Block Design (RCBD) with twelve fertilization treatments, each replicated once across three blocks, resulting in a total of 36 plots. The twelve treatments included standard commercial urea 46% (UREA), which was applied as the positive control 170 kg N ha⁻¹ (RND 100%), and a non-fertilized treatment (NO FERT) as a negative control RND 0%. Additionally, five smart urea formulations were tested, comprising NBPT-enhanced urea (commercially available as AMIFERT 46, Panfertil, Italy) and four innovative formulations provided by the CASALE Group. The CASALE formulations were urea granules coated with an external inhibitor layer and included a nitrification inhibitor BM, a urease inhibitor GA, and combined treatments containing both inhibitors (GABM and BMGA). For the combined formulations, the inhibitor listed first (GABM: GA; BMGA: BM) was applied as the external layer, while the second inhibitor was applied as an internal layer. Combined inhibitors were formulated with the same concentration of both BM and GA formulation, exhibiting a double inhibitory influence on the soil microbiome.

The innovative granulation process was carried out using a specialized granulation apparatus, in which a urea melt containing one or more inhibitors is sprayed onto a bed of solid urea particles. This layering technique is advantageous, as it enables nutrient release that is better synchronized with crop uptake. The resulting urea granules are ideally around 3 mm in diameter (Marrone et al. 2024).

All smart fertilizer treatments were applied at two doses: 100% and 75% of RND. The 100% RND was based on local fertilization practice, developed in accordance with scientific literature (Bi et al. 2020; Marsh 2019; Setu et al. 2018), and consisted of 170 kg N ha⁻¹, 80 kg P₂O₅ ha⁻¹, and 200 kg K₂O ha⁻¹. The 75% RND received 124.5 kg N ha⁻¹ whereas the P and K were supplied as the 100% RND treatment. The NO FERT treatment received no fertilization. Smart fertilizers were distributed in a single application, by hand, onto

the soil surface before sowing, whereas standard commercial urea was split into two applications: half at pre-sowing and the remaining distributed manually on the ridge and incorporated mechanically during hilling (June 12, 2023 and June 5, 2024).

The plots were managed under rainfed conditions with emergency irrigation provided when wilting symptoms were occurring. In June 2023 the crop experienced water stress conditions, hence on the 21 st of June the crop was irrigated. As well in June 2023, the Colorado potato beetle (*Leptinotarsa decemlineata*), first emerged as a significant pest. Thus, on June 7 AMPLIGO (Syngenta, Italy) was applied at the dose of 0.5 mL L⁻¹, while on June 23, EPIK SL (Sipcam, Italy) was applied at the dose of 1.5 mL L⁻¹. In 2024, an overwintering beetle population attacked early-stage potato plants in March, requiring two applications of AMPLIGO in April, which successfully eradicated the pest. Downy mildew (*Phytophthora infestans*) posed another serious threat, necessitating the application of copper sulfate, formulated as Kauritil® Ultra WG (BASF, Italy), as a protectant fungicide. It was applied at 0.8 kg ha⁻¹ on June 7, 2023, and April 20, 2024. In 2024, the systemic fungicide LIETO SC (Sipcam, Italy) was also applied on June 20 at the dose of 4.5 mL L⁻¹, ensuring that no symptoms of downy mildew appeared throughout the season.

2.2 Measurements and Sampling

2.2.1 Crop Phenology, Plant height, Yield and Yield Components

Data about crop phenology (Meier, 2001) was collected every two weeks on 10 inner plants of each of the 36 plots. From the 10 BBCH readings a mode was used to describe the overall progress in the phenological stage for the plot. The sampling started on the 5th week after sowing and was repeated once every two weeks until the 15th week after sowing. Plant height (cm) was recorded as well. Crop harvesting occurred on 20–21st July 2023 and 22–23rd July 2024. All tubers were harvested to record data about Total Tuber Yield (TTY – t ha⁻¹), which is the production of all tuber classes, and it is expressed as fresh tuber weight. Marketable Tuber Yield (MTY – t ha⁻¹), which is the production of tubers with size class larger than 4 cm, also considered as fresh tuber weight. Small Tubers Percentage (STP – %), which was calculated as the ratio between the Small Tuber Yield (STY – size class < 4 cm) and the TTY. From the marketable tubers, for each plot, a 0.5 kg subsample was oven-dried at 65 °C to constant weight to determine its Dry Matter Content (DMC – %). Then the dried samples were ground and analyzed to determine TKN – % content by the AOAC method (FOSS analytical 2000).

Table 1 Soil main physical and chemical characteristics

Parameter	Value
Sand (%)	33.0±1.8
Silt (%)	46.7±1.6
Clay (%)	20.3±0.3
pH	8.10±0.1
ECe (dS m ⁻¹)	0.30±0.1
Total carbonate (%)	18.7±1.4
Soluble carbonate (%)	4.00±0.4
Organic carbon (%)	0.70±0.1

2.2.2 Soil Sampling

Soil samples were collected at two key points: before the initial fertilization and after harvesting. In 2023, at the start of the experiment, three random soil samples were taken across the experimental field at two depths: 0–20 cm and 20–40 cm. At the end of the growing season, as the field had received different treatments, one soil sample per plot was collected per each depth. In 2024, soil sampling was conducted for all plots both before sowing and after harvesting. The analysis focused on evaluating various N forms, including TKN (FOSS analytical 2000), nitrite (NO_2^-), NO_3^- , and NH_4^+ , through ICS–900 Ion Chromatography system (Dionex Corporation).

2.2.3 Percolation Water

Over the 36 plots under investigation, 24 plots were equipped with a system able to catch percolated water and thus making possible the analysis on the quality of water, particularly in terms of NO_3^- content (mg L^{-1}). The system for conducting this activity consisted of porous ceramic suction cups (SK20 UMS, USA), which were installed 60 cm deep in the soil (Curley and McDonnell 2010). To maintain the suction of the porous cups at 0.2 bar, a manually electric vacuum pump was used (Vacuporter, USA). After each heavy rainfall, when water percolation in soil occurred, a water sample from the subsoil was collected for a total of 3 samples in 2023 and 9 samples in 2024 per plot; these samples were analyzed for the NO_3^- concentration assessment, through ICS–900 Ion Chromatography system (Dionex Corporation).

2.2.4 Data Analyses

All statistical analyses were conducted using R Studio (version 2024.09). A significance level of $\alpha=0.05$ was applied throughout. Differences among fertilization treatments were assessed using Analysis of Variance (ANOVA), followed by Sidak-adjusted post-hoc tests for multiple comparisons.

Response variables such as TTY, MTY, and plant height were analyzed using generalized linear mixed-effects models (GLMMs) with a gamma distribution, implemented via the *glmer* function from the *lme4* package (Ruíz et al. 2023). Variables characterized by a beta distribution, including STY and DMC, were modeled using the *glmmTMB* function (*glmmTMB* package; Alves et al. 2024). All models included treatment and year as fixed effects and blocks as random effects.

For NO_3^- concentrations in leached water, a GLMM with a gamma distribution was fitted (*glmer*), incorporating treatment, Days After Sowing (DAS), and year as fixed effects,

and blocks as random effects. Similarly, gamma-distributed GLMM was used to model tuber TKN content, with formulation type, N dose, and year as fixed effects, and blocks as a random effect.

To assess the influence of fertilization on potato phenology, BBCH development stages were compared across treatments and years using a linear mixed-effects model (*lmer*), with blocks as random effects and fixed effects for treatment and year (Pagliari et al. 2022). Additionally, a Gompertz model was fitted to BBCH data in relation to GDD, as this model has been validated in similar studies (Schieler et al. 2023), and used to estimate GDD thresholds corresponding to main phenological stages for each year.

Soil N dynamics (TKN, NO_2^- , NO_3^- , NH_4^+) from sowing to harvest were analyzed using linear mixed-effects models (*lmer*), where N form differences were calculated as $\Delta\text{N} = \text{N}_{\text{sowing}} - \text{N}_{\text{harvest}}$. Fixed effects included fertilizer type, N dose, year, and sampling depth, with blocks as random effects. Interactions were tested in all these models to explore treatment dependencies.

Finally, plant height data across DAS was modeled using a nonlinear logistic growth function via the *nlme* function from the *lme4* package (De Boeck et al. 2011). A nonlinear mixed-effects model was used, treating treatment as a fixed effect and specifying a nested random intercept structure for year and block ($\sim 1 \mid \text{Year/Block}$) to account for repeated measures and experimental hierarchy (Gregorczyk and Gregorczyk 1991). Plant heights were then compared by the model estimated asymptote for each treatment. The logistic function asymptote provides an estimation of the average maximum plant height reached during the cropping cycle.

3 Results

3.1 Meteorological Conditions

Air temperature and rainfall observed in the two experimental periods have been compared with the long-term observation (1994–2022) (Supplementary Fig. 1). In 2023, the total rainfall during the cropping season was lower (–25.2%) than in 2024 (413 mm). In both years, May has been the month with the highest rainfall and it exceeded more than double the long-term average rainfall. While in March 2023 the monthly rainfall was about half of the long-term average, in March 2024 the monthly rainfall exceeded significantly the long-term average. In 2023 the driest month was June while in 2024 it was July, both cases significantly below the long-term average. The average daily temperature measured during the cropping season was slightly higher in 2024 (19.6 °C) compared to 2023 (18.5 °C), and in both years higher than the long-term average (17.2 °C). In both years,

Table 2 Results of the BBCH analyses comparing different treatments and years for the six different weeks of monitoring

Year	Week					
	5th	7th	9th	11th	13th	15th
2023	121	135	505	700	901	903
2024	104	106	501	609	900	903
Year	$p < 0.001$	$p < 0.001$	n.s.	$p < 0.001$	n.s.	n.s.
Treatment	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 3 Cumulated growing degree days (GDD) ($^{\circ}\text{C}\cdot\text{day}^{-1}$) $\pm$ SE necessary to reach each principal growth stage of the potato BBCH scale in 2023 and 2024, retrieved from the fitted Gompertz model

Phenological stage	BBCH	GDD 2023	GDD 2024
Leaf development	100	213 $\pm$ 4	331 $\pm$ 4
Side shoots formation	200	309 $\pm$ 3	435 $\pm$ 3
Main stem elongation	300	388 $\pm$ 2	521 $\pm$ 3
Tuber formation	400	464 $\pm$ 2	601 $\pm$ 3
Inflorescence emergence	500	544 $\pm$ 2	685 $\pm$ 3
Flowering	600	636 $\pm$ 3	777 $\pm$ 4
Fruit development	700	752 $\pm$ 4	889 $\pm$ 5
Fruit ripening	800	929 $\pm$ 6	1040 $\pm$ 6
Senescence	900	1464 $\pm$ 56	1313 $\pm$ 15

Table 4 Results of the logit model trained over treatment, year and blocks in terms of asymptotic maximum height (AMH). AMH values with no letter in common differ significantly at $p \leq 0.05$ (Sidak HSD test). AMH values are presented as mean $\pm$ SE (standard error). The treatments represent different coating materials for Urea granules, together with controls. BM (Blue methylene), GA (Gallic Acid), BMGA (GA inner and BM outer layer), GABM (BM inner and GA outer layer). Treatments with -100 or -75 are indicating the percentage of recommended N dose, NO FERT is 0% N

Treatments (T)	AMH (cm)	
BM-75	57.0 $\pm$ 8.2	a
BM-100	56.3 $\pm$ 8.2	a
NBPT-100	55.4 $\pm$ 8.2	ab
BMGA-75	54.9 $\pm$ 8.2	abc
BMGA-100	54.7 $\pm$ 8.2	abc
GA-100	54.5 $\pm$ 8.2	abc
GABM-100	52.6 $\pm$ 8.2	bcd
GABM-75	52.2 $\pm$ 8.2	cd
GA-75	50.0 $\pm$ 8.2	de
NBPT-75	47.7 $\pm$ 8.2	e
UREA-100	42.1 $\pm$ 8.2	f
NO FERT-0	41.7 $\pm$ 8.2	f

average daily temperature never reached a value lower than T_b (6 $^{\circ}\text{C}$) whereas the average daily temperature exceed the T_{Cutoff} (29 $^{\circ}\text{C}$) twice in 2023 and thrice in 2024.

3.2 Crop phenology, height, Tuber Yield, and Yield Components

The cultivation year significantly ($p < 0.001$) influenced the phenological stage at the 5th, 7th, and 11th week after sowing whereas no effect ($p > 0.05$) was observed 9th, 13th, and 15th week after sowing. Instead, the effect of fertilization

Table 5 Summary of the ANOVA outcomes from the models built on yield and yield components data. From left to right: marketable (MTY) and total potato tuber production (TTY), tubers smaller than 4 cm (STP), and tuber dry matter content (DMC). n.s. not significant

	<i>p</i> -value			
Source of Variation	MTY	TTY	STP	DMC
Treatment (T)	0.0008	0.005	< 0.0001	n.s.
Year (Y)	< 0.0001	< 0.0001	< 0.0001	< 0.0001
T x Y	n.s.	n.s.	n.s.	n.s.

treatment on the model was never found to be significant ($p > 0.05$) (Table 2). The relationship between BBCH stages and GDD cumulated values was evaluated with a Gompertz model (Table 3). In 2023 the crop accumulated less GDD to move to the next phenological stage, compared to 2024. However, in the last part of phenological development, more cumulated GDD were required in 2023 than 2024 to reach senescence (BBCH stages 900).

Plants height dynamics, modeled through a logistic non-linear mixed-effects model, showed clear differences in the asymptotic maximum height (AMH) among fertilization treatments ($p < 0.05$). In Table 4 the estimated asymptote for each treatment is reported. The significantly ($p \leq 0.05$) lowest AMH was estimated under NO FERT and UREA-100 treatments (on average 41.9 cm) whereas the significantly ($p \leq 0.05$) highest under BM-75 and BM-100 treatments (on average 56.7 cm) (Table 4).

Tubers' yield, STP and DMC were significantly influenced by the cultivation year with better performance in 2023 than 2024, whereas the treatment only significantly affected the yield (Table 5). The interaction treatment x year was never found to be significant. Focusing on the treatments (Table 6), the averages and standard errors across the two experimental years for tuber production and quality parameters were $25.9 \pm 2.9 \text{ t ha}^{-1}$ for MTY, $32.0 \pm 3.2 \text{ t ha}^{-1}$ for TTY, $19.3 \pm 1.8\%$ for STP and $19.3 \pm 0.6\%$ for DMC. Both MTY and TTY significantly increased under BM-100 compared to NO FERT and produced 77% more marketable yield than under UREA-100. Moreover, MTY was significantly greater under BMGA-100 than under control, while STP was significantly lower under BMGA-100 than under UREA-100. Notably, yield did not significantly differ in smart fertilizer formulation applied at 75% of the RND compared to UREA-100%. STP was found to be higher

Table 6 Summary of the yield and yield components results averaged over the ANOVA factors. From left to right: marketable (MTY) and total potato tuber production (TTY), tubers smaller than 4 cm (STP), and tuber dry matter content (DMC). Values are presented as mean $\pm$ SE (standard error). Column values with no letter in common differ significantly at $P \leq 0.05$ (Sidak HSD test). The treatments represent different coating materials for Urea granules, together with controls. BM (Blue methylene), GA (Gallic Acid), BMGA (GA inner and BM outer layer), GABM (BM inner and GA outer layer). Treatments with –100 or –75 are indicating the percentage of recommended N dose, NO FERT is 0% N

	MTY	TTY	STP	DMC
Treatments (T)	t ha ⁻¹	t ha ⁻¹	%	%
BM – 100	34.3 a $\pm$ 3.8	40.3 a $\pm$ 4.1	15.4 c $\pm$ 1.7	19.1 $\pm$ 0.6
BMGA – 100	30.9 ab $\pm$ 3.5	37.1 ab $\pm$ 3.8	16.1 bc $\pm$ 1.8	19.2 $\pm$ 0.6
GA – 100	28.5 abc $\pm$ 3.2	35.5 ab $\pm$ 3.6	19.6 abc $\pm$ 1.9	19.1 $\pm$ 0.6
GABM – 75	28.3 abc $\pm$ 3.2	34.3 ab $\pm$ 3.5	17.5 bc $\pm$ 1.8	19.4 $\pm$ 0.6
GABM – 100	27.7 abc $\pm$ 3.1	33.7 ab $\pm$ 3.4	17.8 bc $\pm$ 1.8	19.1 $\pm$ 0.6
NBPT – 100	27 abc $\pm$ 3	32.5 ab $\pm$ 3.3	17.1 bc $\pm$ 1.8	20.0 $\pm$ 0.7
BM – 75	26.2 abc $\pm$ 2.9	31.9 ab $\pm$ 3.2	17.7 bc $\pm$ 1.8	20.1 $\pm$ 0.7
BMGA – 75	25.9 abc $\pm$ 2.9	31.6 ab $\pm$ 3.2	18.6 abc $\pm$ 1.9	18.6 $\pm$ 0.6
NBPT – 75	24.9 abc $\pm$ 2.8	31.0 ab $\pm$ 3.1	19.9 abc $\pm$ 1.9	19.9 $\pm$ 0.7
GA – 75	21.2 abc $\pm$ 2.4	25.9 ab $\pm$ 2.6	19.1 abc $\pm$ 1.9	19.7 $\pm$ 0.7
UREA – 100	19.3 bc $\pm$ 2.2	26.3 ab $\pm$ 2.7	28.3 a $\pm$ 2.2	19.4 $\pm$ 0.6
NO FERT	17.8 c $\pm$ 2	23.8 b $\pm$ 2.4	25.2 ab $\pm$ 2.1	18.6 $\pm$ 0.6

under UREA–100 (28.3%) than under BM–100 (15.4%). No

differences in tuber DMC were observed across treatments.

3.3 NO₃⁻ Leaching Through Water Percolation

NO₃⁻ content in percolated water significantly depended on treatment and year ($p < 0.001$). The effect of DAS factor on the model, did not significantly affect the NO₃⁻ content in percolated water ($p = 0.09$), no interaction between factors was found significant. A significant lower (on average – 41%) NO₃⁻ concentration was found under NO FERT than under BMGA–100 (fitted average 199 mg L⁻¹) and NBPT–100 (fitted average 169 mg L⁻¹) whereas no significant difference we found among the other treatments (Figure 1).

3.4 Concentration of N Forms in the Soil

The results of soil N form analyses, averaged per treatment, are summarized in Table 7. Among the four N forms evaluated, NO₃⁻ was the second highest in concentrations only after TKN. However, NO₃⁻ concentrations demonstrated high standard deviations and a general decreasing trend over the crop cycle. Meanwhile, TKN values appeared to be the most stable compared to other N values. The year significantly ($p < 0.05$) affected soil Δ TKN, Δ NO₂⁻, Δ NO₃⁻, and Δ NH₄⁺ between potato sowing and harvesting. Soil depth had a significant influence only for Δ NH₄⁺ and Δ NO₂⁻. On average, across the two years, between potato sowing and harvesting, Δ NH₄⁺ values were negative in the topsoil and positive in the subsoil, measuring –0.52 mg kg⁻¹ for the 0–20 cm soil layer and +0.27 mg kg⁻¹ for the 20–40 cm soil layer, respectively. Fertilizer type significantly influenced Δ NO₃⁻ ($p < 0.001$) in 2023 for the topsoil layer. On the average of the two considered soil profiles, the estimated mean

Fig. 1 Bar chart illustrates model-predicted average nitrate concentrations (mg L⁻¹) in leachate across multiple sampling dates. Treatments labeled with different letters are significantly different (Sidak post-hoc test; α : 0.05). Error bars show model predicted standard error. The treatments represent different coating materials for urea granules, together with controls. BM (Blue methylene), GA (Gallic Acid), BMGA (GA inner and BM outer layer), GABM (BM inner and GA outer layer). Treatments with –100 or –75 are indicating the percentage of recommended N dose, NO FERT is 0% N

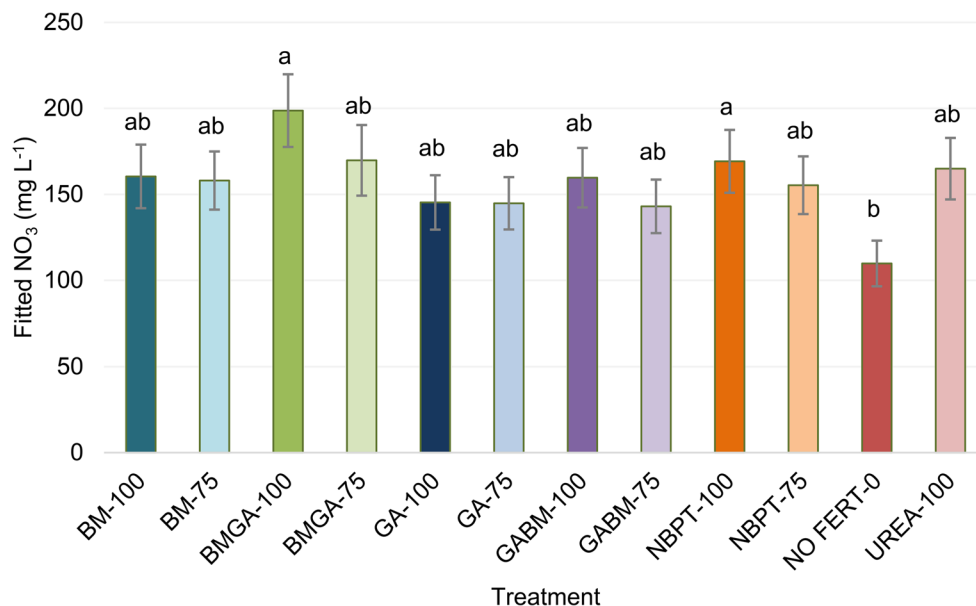
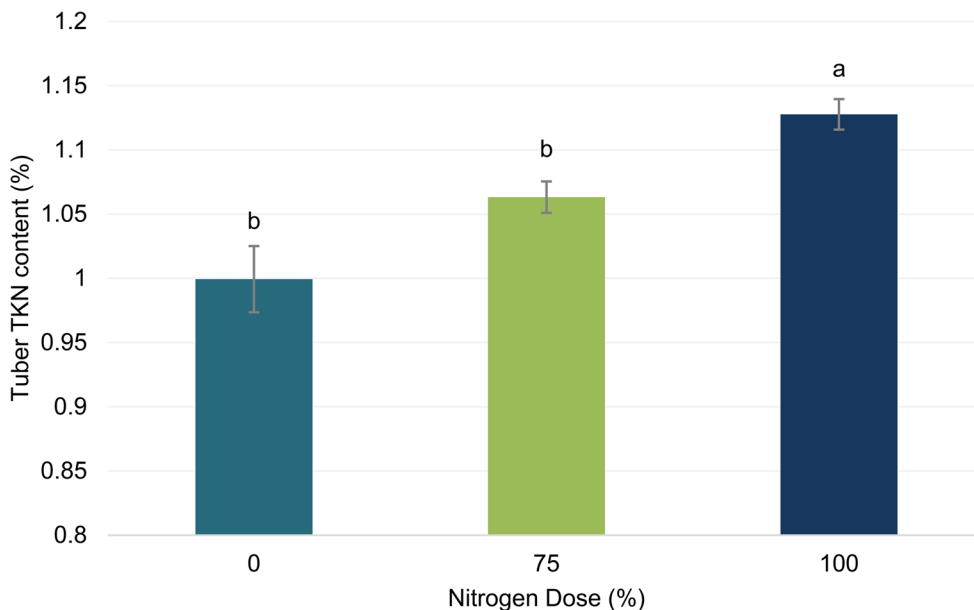


Table 7 Soil N forms total Kjeldahl nitrogen (TKN), NO_2^- , NO_3^- and NH_4^+ at the beginning and the end of the two experimental growing seasons. For each plot a sample was taken before the pre-sowing fertilization and before the tubers harvest. Values are presented as mean $\pm$ standard deviation

			TKN	NO_2^-	NO_3^-	NH_4^+
			%	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹
2023						
Sowing	0–20 cm		0.107 $\pm$ 0.015	0.033 $\pm$ 0.059	64.93 $\pm$ 8.678	0.968 $\pm$ 0.212
	20–40 cm		0.103 $\pm$ 0.071	0.200 $\pm$ 0.136	47.76 $\pm$ 6.780	1.600 $\pm$ 0.404
Harvesting	0–20 cm		0.109 $\pm$ 0.005	0.288 $\pm$ 0.100	26.48 $\pm$ 16.46	1.913 $\pm$ 0.308
	20–40 cm		0.102 $\pm$ 0.016	0.285 $\pm$ 0.129	18.69 $\pm$ 7.770	1.611 $\pm$ 0.597
2024						
Sowing	0–20 cm		0.105 $\pm$ 0.006	1.171 $\pm$ 0.435	18.58 $\pm$ 3.442	2.868 $\pm$ 2.128
	20–40 cm		0.104 $\pm$ 0.005	1.397 $\pm$ 0.549	22.73 $\pm$ 4.359	2.683 $\pm$ 0.590
Harvesting	0–20 cm		0.185 $\pm$ 0.011	0.023 $\pm$ 0.091	9.951 $\pm$ 4.691	2.108 $\pm$ 0.979
	20–40 cm		0.186 $\pm$ 0.009	0.060 $\pm$ 0.159	8.057 $\pm$ 3.197	1.676 $\pm$ 0.686

Fig. 2 Bar chart showing the model-predicted Total Kjeldahl Nitrogen content (TKN%) of potato tuber dry matter samples across the three N doses. Different letters denote statistically significant difference (Sidak post-hoc test; α : 0.05). Error bars show model predicted standard error. The treatments represent different coating materials for urea granules, together with controls. BM (Blue methylene), GA (Gallic Acid), BMGA (GA inner and BM outer layer), GABM (BM inner and GA outer layer). Treatments with –100 or –75 are indicating the percentage of recommended N dose, NO FERT is 0% N



ΔNO_3^- between potato sowing and harvesting did change significantly according to treatments: (i) the ΔNO_3^- was significantly lower for UREA–100 than for the other treatments, with an estimated mean of -8.2 ± 4.9 mg kg⁻¹; and (ii) for NO FERT and smart fertilizer treatments the ΔNO_3^- ranged from $+33.5$ to $+44.9$ mg kg⁻¹.

3.5 Tuber N Content

Both year and RND affected tuber TKN content ($p < 0.001$), while the formulation of the fertilizer did not. TKN concentrations were on average 1.00%, 1.06%, and 1.13% for NO FERT, 75% RND, and 100% RND, respectively (Figure 2).

4 DISCUSSION

Because meteorological variability critically affects nitrogen transformation pathways and the performance of urea-based fertilizers, the discussion first addresses the climatic conditions prevailing during the two experimental years.

Recorded temperatures exceeded the 20-year average, consistent with the broader impacts of global warming observed in many regions (Cnossen et al. 2022). Climate change experts predict that future rainfall patterns will become shorter and more intense, increasing the risk of water percolation and NO_3^- leaching (Westra et al. 2014). As a result, traditional urea formulations are expected to become increasingly prone to environmental losses, emphasizing the need for controlled-release alternatives. Notably, in May of both years, average rainfall approximately doubled the long-term trend.

The observed climatic differences between years may have contributed to variations in plant growth and developmental rates, affecting height, yield potential, and phenological advancement. Mean annual temperatures in 2024 were higher than in 2023, leading to a proportional increase in GDD accumulation (Del Pozo et al. 2019). In 2024, due to higher temperatures compared to 2023, each potato plant accumulated more GDD to reach a given BBCH stage. This can be justified by considering that high temperatures can sometimes inhibit plant development, temporarily slowing

growth. As a result, more heat accumulation (GDD) was required in 2024 for the plants to progress to the same phenological stage. A similar trend has been reported in tomato phenology: later-season plantings in warmer conditions accumulated more GDD to reach each phenological stage compared to earlier-season plantings in the same year (Pathak and Stoddard 2018).

The largest difference in GDD accumulation between the two years was observed at the senescing stage, with a gap of 151 GDD. This variation of GDD to reach the same BBCH stage is consistent with findings from another study conducted in the Veneto region on maize crops over a three-year period (Berti et al. 2019). In general, it is known that the Gompertz model varies depending on the environmental conditions from which the data are collected (Barker et al. 2010).

The effect of different urea formulations and application doses resulted in not influencing the timing of the phenological development of the potato. This result confirms a study conducted in Russia, where the use of traditional urea was compared with urea added with NBPT on potato (Vizirskaya et al. 2021).

Plant height was greater in plots fertilized with BM-100 compared to UREA-100, aligning with the higher tuber yield observed and indicating more vigorous vegetation growth. In this experiment, the model predicted an average plant height for UREA treatments of 33.1 cm at 67 DAS, increasing to 39.5 cm by DAS 85, averaged across years. Similarly, in an experiment conducted in Nepal with the cv. Desiree, plant heights were comparable with our observations (33.6 cm and 40.8 cm at 67 and 85 DAS, respectively) (Gainju et al. 2019).

Between 1999 and 2003, marketable tuber production in Italy ranged from 22 to 26 t ha⁻¹ (Pedersen et al. 2005). The average yield across all treatments in this study (25.9 t ha⁻¹) is on the higher end within this historical range. However, the positive control, UREA, yielded less than the long-term average (19.2 t ha⁻¹). This lower yield may be attributed to the use of manual cultivation techniques, as automated farming methods are known to generally increase yield (Stockem et al. 2023).

The higher production in 2023 could be partially attributed to more favorable environmental conditions compared to 2024. However, it is worth noting that the heavy Colorado beetle infestation in the second year likely had a negative impact on yields. Year-to-year variability in yield is not uncommon in field experiments, as environmental factors and pest pressures can vary significantly. Similar variability has been reported in studies involving Slow-Release Fertilizers on rainfed potato cropping systems, where the use of urease and nitrification inhibitors resulted in significant differences in tuber yields across different years (Souza et

al. 2019; Zebarth et al. 2012). The lower tuber yield in 2024 likely influenced other yield parameters, such as the proportion of small tubers and the DMC. Lower tuber yields are often correlated with smaller tuber sizes, as demonstrated in a previous study (Khayatnezhad et al. 2011). Additionally, smaller tubers are typically associated with a higher DMC (Ifenkwe et al. 1974). This relationship may be due to reduced competition for resources within the tubers, allowing for a more concentrated accumulation of DMC.

The highest tuber yield in this experiment was observed under BM-100. In a recent meta-analysis on N enhanced efficiency fertilizers done in potato cropping, smart fertilizers formulated with nitrification inhibitor showed an average increase of yield of 3.1%, and not always significantly higher than the business-as-usual practice (Pan et al. 2023). This puts the novel proposed nitrification inhibitor BM ahead of most other existing molecules. Additionally, BM is known for its antioxidant properties, acting as a reactive oxygen species scavenger in plant tissues (Honda and Oda 1960). This antioxidant mechanism may contribute to alleviating environmental stressors, potentially improving crop yield. Higher MTY and a reduced proportion of small tubers were observed under BM-100 and BMGA-100 treatments compared to NO FERT, leading to increased marketability. Similar tuber yields were found under GA and NBPT, both urease inhibitors, without significant differences with UREA-100. On average, the yield gain associated with NBPT usage is approximately 6.0% across different crops (Cantarella et al. 2018). However, in this study, no significant yield difference was found between the NBPT-100 and UREA-100 treatments. Similar mixed results have been observed in other studies. For instance, in an experiment conducted in Czech Republic with three different N dosages, tuber yields were similar or even lower under NBPT-treated urea compared to urea-only, except at the lower dosage (54 kg N ha⁻¹), where a slight yield advantage was observed. (Lošák et al. 2016). Conversely, at a higher dosage (200 kg N ha⁻¹), potato yields were improved under NBPT-treated urea compared to urea-only in Pakistan (Khan et al. 2014), while in Russia, marketable yield increased modestly by 4.98% with NBPT application compared to urea-only (Vizirskaya et al. 2021).

Smart fertilizers applied at a reduced dosage (75% of RND) promoted the same yield as urea at the full dose. This finding is particularly significant because it demonstrates that using inhibitors enables a reduction in the total fertilizer applied while maintaining comparable yields. Such formulations can reduce the environmental impact and the frequency of fertilizer application without compromising productivity.

Similar results have been observed in controlled experiments. For instance, in a greenhouse study, yield was higher

with sulfur-coated urea applied at 66% of the RND compared to the urea-only treatment (Zareabyaneh and Bayatvarkeshi 2015). Similarly, a field study conducted in the USA and China found that a single application of PCU produced potato tuber yield and quality comparable to those achieved with urea-only. While PCU was applied only once, urea was applied once before sowing and then through five subsequent fertigation interventions (Xue et al. 2024).

The same study also highlighted that both the total N rate and the application frequency were reduced by PCU, which helped offset its higher cost compared to standard fertilizers (Xue et al. 2024). Improvements in NUE, reductions in environmental risks, and decreases in labor and operational costs associated with multiple fertilizer applications reinforce the potential of smart fertilizer formulations. In this experiment, no significant differences in tuber DMC were observed among the treatments. However, other studies have reported that N fertilization levels can influence DMC in potatoes. For instance, Simson et al. (2016), noted that higher N levels were associated with reduced DMC in tubers. The lack of differences in the presented study might be attributed to the relatively small variations in N management practices among the treatments. These differences may not have been substantial enough to affect DMC accumulation.

Yield, quality parameters, and environmental impact did not improve under the combined BMGA and GABM treatments compared to the single-inhibitor formulations (GA and BM). Despite containing a higher dose and different mechanism of inhibitors, combined formulations did not demonstrate a higher performance compared to single inhibitor formulations. This result is opposite the expectation that the combined formulations would surpass treatments with GA or BM alone. However, it is possible that the interaction between the two inhibitors resulted in an antagonistic effect rather than synergy. This finding aligns with an experiment conducted in Minnesota, where the results obtained from a mixture of urease and nitrification inhibitors were not significantly better than those from urea-only treatments (Souza et al. 2019). A constant supply of bioavailable N to the potato plant should be ensured through the use of urease and nitrification inhibitors, without leading to excess N fertilization. An over availability of N is indeed not beneficial as the plant invests too much in vegetation and less in tuber production impacting negatively yield (Ruža et al. 2013). Nevertheless, when nitrogen uptake is not synchronized with plant demand, the surplus nitrogen remains susceptible to loss through leaching processes. The concentration of NO_3^- leached did not differ significantly between any of the smart N formulations and the control UREA treatment. However, the highest NO_3^- concentration compared to NO FERT was found under BMGA-100 and NBPT-100.

It was expected that NBPT-100 would reduce NO_3^- leaching compared to UREA, at least partially, but it didn't happen. However, NO_3^- leaching did not increase with the newest formulations, BM-100 and GA-100, applied at 100% of RND, compared NO FERT. The same trend was observed under UREA-100. The combinations of urease and nitrification inhibitors (GABM and BMGA), did not significantly reduce NO_3^- leaching compared to UREA, possibly due to an antagonistic interaction between these two types of inhibitors. NO_3^- leaching from BMGA was significantly higher than expected. However, other studies have reported that the use of both urease and nitrification inhibitors (e.g., NBPT+DMPSA) together can reduce NO_3^- leaching by up to 25% compared to urea-only treatments (Souza et al. 2021).

While in this experiment the NO_3^- content in the leached water was not significantly influenced by the NBPT treatment compared to traditional urea, studies in Minnesota have shown that urea treated with NBPT reduced NO_3^- leaching by 21% across growing seasons compared to urea-only treatments (Souza et al. 2021). In another study from Minnesota, a Polymer Coated Urea (PCU) formulation was compared to soluble N fertigation at a N dose of 270 kg N ha^{-1} . NO_3^- leaching per hectare was 37.8% lower with the PCU formulation than with the soluble N treatment. (Wilson et al. 2010). However, no yield differences were observed between treatments in the same experiment (Wilson et al. 2009). These examples demonstrate that controlled-release urea formulations do not always lead to a reduction in N pollution.

In general, subsoil water studies are difficult to interpret due to the influence of many environmental effects on results. In a potato and barley rotation experiment on a *Luvisol*, no differences in NO_3^- concentration in subsoil waters were observed between a nitrification inhibitor treatment and conventional ammonium nitrate fertilizer (Tripolskaja et al. 2017). Similarly, in another study involving PCU formulations, the variation in groundwater NO_3^- concentrations among plots was too large to detect significant differences among fertilization treatments (Bero et al. 2014). These findings underscore that NO_3^- leaching assessments can yield inconclusive results due to high variability and the challenge of accounting for covariates.

Complementary insights can be gained by examining the concentration of nitrogen forms in the soil, which reflect the balance between mineralization, uptake, and potential leaching losses. All soil samples contained less than 1% of total N, classifying these plots as N-poor. The low to medium concentrations of NO_2^- and NH_4^+ suggest that the nitrification process functioned as expected (Morris 2015; Haberle et al. 2018). The higher concentration of NO_3^- could be attributed to organic matter mineralization, which

releases N into the soil (Haberle et al. 2018). Generally, the low TKN values at the beginning of the crop cycle indicate that N fertility was insufficient at the start, necessitating fertilization. The similarly low TKN values at the end of the cycle suggest that most of the N was effectively absorbed by the plants, with minimal residual N left in the soil (Morris 2015).

In 2023, NO_3^- concentration in the topsoil after harvesting was higher under UREA-100 than under any of the other treatments. Except for ΔNO_3^- , none of the other N forms in the soil were significantly influenced by the treatment. Early nutrient uptake likely occurred in potato plants fertilized with urea-only, suggesting that the available nutrients were utilized early in the growth cycle. However, as N from urea was depleted, plants appear to have relied more heavily on NO_3^- reserves in the soil, leaving it comparatively NO_3^- -poor (Chen et al. 2024). In contrast, in a pot experiment using a nano-chitosan coating for slow-release urea, soil NH_4^+ and NO_3^- levels were reduced by 10.69% and 4.55%, respectively, compared to urea-only treatments (Kondal et al. 2021). The differences between field and pot experiments likely stem from the greater variability in open-field conditions, which can obscure nutrient cycling dynamics and complicate comparisons of N transformations among treatments.

Regarding soil depth, significant differences in N concentrations between the topsoil (0–20 cm) and subsoil (20–40 cm) were observed only for NH_4^+ and NO_3^- . During the growing cycle, the potato plant's root system likely accessed N relatively uniformly across both soil layers, leading to similar nutrient uptake. However, the topsoil showed a reduction in NH_4^+ from sowing to harvest, while NH_4^+ concentrations in the subsoil appeared higher in same period. This does not indicate a downward movement of NH_4^+ into the subsoil but rather reflects the depletion of NH_4^+ from the topsoil, leaving the relative concentration in the subsoil higher by comparison (Ghosh et al. 2019). This pattern may also reflect limited NH_4^+ availability at the surface due to volatilization or plant uptake dynamics.

The distribution of nitrogen forms in the soil ultimately determines the amount of N available for plant uptake and accumulation in tubers. In this experiment, an increase in TKN content in tubers was observed with higher N fertilization dose, a trend also confirmed by Zotarelli et al. (2014). Reducing the fertilizer application to 75% of the standard dose not only lowered fertilizer uses and environmental pollution but also resulted in tubers with reduced TKN content.

Although the type of fertilizer formulation did not significantly influence the TKN content of tubers in this experiment, similar studies have shown that smart fertilizers can reduce harmful N forms in tubers. For instance, sulfur-coated urea resulted in lower NO_3^- and NO_2^- concentrations

compared to ammonium nitrate fertilization (Ezzat and Abd El-Hameed 2010). These findings suggest that while fertilizer type may not always affect TKN under certain conditions, smart formulations like sulfur-coated urea could provide additional benefits by mitigating N accumulation in tubers, particularly in scenarios with different environmental or agronomic conditions.

5 CONCLUSIONS

This study demonstrated that novel smart N fertilizer formulations applied at 75% of recommended nitrogen dose can support a marketable tuber yield in a single application ($p < 0.05$) like that obtained applying urea at 100% of recommended nitrogen dose, split in two applications.

Although nitrogen fertilization influenced plant height, no significant differences in phenological development were observed among treatments. In terms of environmental outcomes, nitrate (NO_3^-) leaching showed high variability, likely due to fluctuating field conditions. Nevertheless, a significant trend emerged: plant fertilized with commercially available urease-inhibitor urea (NBPT-100) had higher NO_3^- concentrations in subsoil water ($169.2 \pm 18.3 \text{ mg L}^{-1}$) compared to unfertilized controls ($109.8 \pm 13.3 \text{ mg L}^{-1}$).

Further research is necessary to better understand the environmental and ecological impacts of novel urea-inhibitor formulations, particularly their interactions with the soil microbiome. Future studies conducted under controlled conditions are also recommended to minimize environmental variability and more accurately assess NO_3^- leaching dynamics.

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