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Microgranular fertilizer and biostimulants as alternatives to diammonium phosphate fertilizer in maize production on marshland soils in northwest Germany

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Abstract

The eutrophication of groundwater through widespread diammonium phosphate (DAP) fertilization and excessive farm fertilizer is one of the major problems in European agriculture. Organomineral microgranular fertilizers that have a reduced phosphorus (P) content, alone or in combination with biostimulants, offer promising alternatives to DAP fertilization. We conducted a field experiment with maize (*Zea mays*) on a marshland soil site in order to compare the yield increase and the phosphorus balance of DAP and microgranular fertilizer variants. P content of the soil on the study site is 3.9 g P per 100 g soil. Treatments involved a combination of two fertilizers, namely DAP or a P-reduced microgranular slow-release organomineral fertilizer (Startec) and the biostimulants mycorrhiza, humic substances and soil bacteria, applied individually or along with two of the above biostimulants. Fertilizer variants were also tested individually without additional biostimulants. One in four plots was used as a control, treated only with biogas slurry, to identify site-specific spatial variability and to implement correction factors to process raw data using standardized methods. Startec performed as well as DAP in terms of both the yield and corn cob ratio, while the P excess was lower in plots treated with Startec (av. = 4.5 kg P_2O_5 ha^{-1}) compared to DAP (av. = 43.7 kg P_2O_5 ha^{-1}). The latter differences are of statistical significance. Individual biostimulants and a combination of multiple biostimulants rarely resulted in significantly higher yields, with the exception of some combinations with humic substances and mycorrhiza in individual years. The influence of the climatic conditions in each of the years was higher than the influence of the biostimulants. However, average increases in yield over three years would be economically beneficial for farmers in the case of the applied humic substances product and mycorrhiza. An adequate alternative to DAP was found in the form of a P-reduced microgranular fertilizer from Startec.

Keywords: microgranular fertilizer, diammonium phosphate, eutrophication, phosphorus balance, biostimulants

Introduction

Though the extent of existing phosphate rock reserves is a subject of heated debate in the literature, it is undisputed that these resources for conventional fertilizer production are finite (Edixhoven et al. 2014; Kisinyo and Opala 2020). Further ecological problems, such as the eutrophication of ground and surface water systems by agricultural phosphorus inputs (Torrent et al. 2007; Ulén et al. 2007), have led to policies in the European Union placing strict regulations on fertilizing-related nutrient management (91/676/EWG, 2000/60/EC). Thus, a more responsible usage of phosphorus fertilizer is necessary. Recently, new fertilizing systems, such as the application of microgranular fertilizer, also known as pop-up fertilizer, and biostimulants have been successfully tested (Balawejder et al. 2020; Olbrycht et al. 2020). In contrast to DAP and other fertilizers, which are applied as a fertilizer band at a distance of around 10 cm to the seed, microgranular fertilizer is ideally put in the soil together with the seed, at a distance of a few centimeters. The direct contact between the fertilizer and the seed both requires a lower amount of fertilizer and nutrients, especially phosphorus (P), to be used per plant and calls for the components of the fertilizer itself to have a lower salt index (Alley et al. 2010). Further, the dispersal of the granules, that are smaller than 2 mm in diameter, prevents long-term osmotic gradients. While microgranular slow-release fertilizer has started to be used more frequently in German agricultural practice, and thus begun to develop into a promising tool to counter the above ecological challenges, the application of biostimulants has not spread to the same extent. This is in contrast to the numerous studies at laboratory scale (germination assays), in greenhouses and successful field trials for different plant taxa (Mackowiak et al. 2001; Nardi et al. 2002; Cavaglieri et al. 2005; Jakobsen et al. 2005; Anjum et al. 2011; El-Hassanin et al. 2016; Eulenstein et al. 2016; Fan et al. 2018). However, the world market for biostimulants has been growing fast in the last decade (Calvo et al. 2014).

In the present study, field trials were carried out over three years in maize (*Zea mays*) cultivation to compare the effect of the standard fertilizer diammonium phosphate (DAP), which is rich in P, and a microgranular slow-release fertilizer with a lower P content (Startec) both individually and in combination with biostimulants, namely liquid humic substances extract, soil bacteria and mycorrhiza.

Materials and Methods

Area studied

The experiments were carried out as a field trial from 2018 to 2020 near Wanna in northwest Germany (53.729995, 8.810990). The region is classified as having a European Atlantic climate (Cfb) as defined by Köppen and Geiger (1930), characterized by mild winters and moderate summer temperatures. The average precipitation per year for Wanna is 735 mm and the average annual temperature is 9.9 °C. 45% of annual precipitation is during the maize crop season from April to September.

The study site contains hydromorphic loamy marshland soil, rich in humus and contains 3.9 g P and 5.2 g K per 100 g soil. Ground water levels are 40–60 cm below the surface, with insignificant changes over the year due to the presence of drainage channels communicating directly with the regulated system of a small stream called the Emmelke. The site has been used for maize cultivation for years and treated with the plant protection products Laudis, Spectrum Gold, Milagro Forte and

Nagano (with 1, 2, 1, 0.5 and 0.3 liters per hectare). Regular tillage operations involve plowing and land clearing.

Experimental setup

The maize cultivar Amaroc S230 was sown with a density of 8.5 seeds per square meter using the AMAZONE single corn seeder system (EDX 6000-2C precision air seeder). Eleven different fertilizer combinations were repeated five times and regularly separeated by five control parcels on plots each measuring 50 m x 6 m.

DAP fertilizer was applied in a band 12 cm below the soil surface and Startec microgranular fertilizer (De Ceuster Meststoffen NV (DCM) Bannerlaan 79, 2280 Grobbendonk, Belgium) was applied a few centimeters beneath the corn, respectively. DAP was applied in amount of 100 kg ha^{-1}. The latter contains 18% total N, all in the form of NH_4-N, and 46% P_2O_5. Startec can be classified as a microgranular organomineral fertilizer, of which 80% (of the original substance) is made up of the organic industrial by-products oil cake and bone meal and the mineral components ammonium phosphate, ammonium sulfate, EDTA-chelated Fe, Mn, Zn, zinc sulfate and zinc oxide. The nutrient composition of Startec is 7.5% N, 22% P_2O_5, 4% K_2O, 10% S, 0.5% Fe and Mn respectively, plus 1.5% Zn. In the present study, Startec was applied at a rate of 25 kg ha^{-1}. The study site was treated with biogas slurry (30 m³ ha^{-1}) containing 4.3 kg of total N, 1.3 kg of P_2O_5 and 5.2 kg of K_2O per m³. The nutrient types and input rates are given in Table 1.

Table 1. Application rates and nutrient inputs per hectare of used fertilizer

Fertilizer (type[1])	Application rate per hectare in kg	Nutrient type and input rate per hectare						
		N	P_2O_5	K_2O	SO_3	Fe	Mn	Zn
Diammonium phosphate (mineral fertilizer)	100	18	46	-	-	-	-	-
Startec (organomineral microgranular fertilizer)	25	1.75	5.5	1	2.5	0.125[2]	0.125[2]	0.375[2, 3]
Pre-treatment of the soil with biogas slurry	30000	129	39	156	-	-	-	-

[1] For seed band application; [2] EDTA-chelated; [3] EDTA-chelated and as oxide; - Absent or no data available.

Mycorrhiza, grown on expanded clay, and soil bacteria, sprayed on natural zeolite (clinoptilolite) as a carrier material, were pulverized and poured into separate chambers of the AMAZONE precision seeder for exact, plot-specific application along with a fertilizer treatment. Humic substances were sprayed directly on the soil after treatment with biogas slurry. This form of application was used for organizational reasons and differs from the manufacturer’s instructions. The manufacturer of the humic product (GeoFert Germany GmbH, Koppelbergstraße 4, 17166 Tetrow, Germany) recommends mixing the humic substances directly into the slurry to reduce the technical effort in agricultural practice.

Treatments were realized as a combination of two mineral fertilizers, namely DAP or the P-reduced microgranular slow-release fertilizer from Startec and the biostimulants mycorrhiza (abbreviated as M), humic substances (abbreviated as HS) (GeoOrganic®, GeoFert Germany GmbH) and the soil bacteria *Bacillus subtilis* (abbreviated as Bac) (BactoFert®, GeoFert Germany GmbH) either alone or together with one or two of the above biostimulants. Mineral and organomineral microgranular fertilizer variants were also tested alone, without any additional biostimulant. One in four plots was used as a control, treated only with biogas slurry, to identify site-specific spatial variabilities and to implement correction factors in data analysis. Manual harvesting was performed by randomly removing 20 plants per plot. The cobs and the remaining plants were weighed and shredded separately using a garden shredder (AL-KO Master 32-40). For each of the five repetitions of a variant, the shredded cob and corn material was used to prepare samples for the measurement of dry matter, and afterwards pulverized for NIRS analyses using a FOSS 5000-M NIRS spectrometer (FOSS NIRSystems). The P and K contents were measured after aqua regia dissolution via ICP-OES. The latter data were used to calculate the year-specific removal of N, P_2O_5 and K_2O by harvesting.

Statistical analysis

Control plots without additional fertilization were used to detect soil spatial variability on the study site. Differences in control plots were used to implement correction factors as described in Thomas (2006) and Dospechov (1979). To ensure that the distribution was normal, the yield was transformed using an exponential function. Differences between the fertilizer variants were tested using Student's t-test. All statistical analyses were performed in R (R Core Team 2014). For data selection, the package dplyr (Wickham et al. 2018) was used. Visualization in R was conducted using the package ggplot2 (Wickham 2009).

Results

The high range of fluctuation in the repetitions of certain fertilizer variants mainly resulted in statistically insignificant differences in yield. The average dry matter yields of DAP in combination with all the individually and jointly applied biostimulants that were tested were 14.8% higher than the dry matter yield of plots only fertilized with DAP. Biostimulants had a negligible effect on Startec. Statistically significant differences were found in the case of the effect of HS plus M on DAP in 2018 ($P = 0.0127$), the effect of Bac plus M on DAP in 2019 ($P = 0.0041$) and the effect of HS on Startec's yield in 2018 ($P = 0.0087$). Differences in the phosphorus balance between all the Startec variants compared to all the DAP variants were of the highest statistical significance across the entire study time and in the individual years ($P < 0.0001$). The average P excess of plots fertilized with Startec was 4.5 kg per hectare and year. DAP fertilization resulted in a P excess of 43.7 kg per hectare and year.

Discussion

In the present study, the amounts of phosphorus per unit of area vary over the two different fertilizing systems using DAP and Startec (Table 1). The reduction of macroelements, especially phosphorus, is known to play a yield-limiting role in plant growth (Sharpley 1997). The low solubility of P in water makes it necessary to use mineral fertilizer (such as DAP) containing P compounds, which dissolve in an irrigated soil matrix in a highly plant-available form. Thus, mineral fertilizer is usually an important source of P in commercial crop fertilization. However, P is also found in organic form in Startec fertilizer. The organic P in Startec is predominantly present in bone meal as hydroxyapatite ($Ca_5(PO_4)_3(OH)$), which is typically present in bone structures (Kattimani et al. 2016). When designing the experimental setup, it was expected that the organically bound P and other organically bound nutrients in Startec would be converted into a plant-available form better if biostimulants were used. Contrary to the hypothesis that the joint application of biostimulants and Startec would have a higher benefit, the effect on DAP-fertilized plants was higher. The average dry matter yield during the three-year study of all DAP combinations with biostimulants was 14.8% higher than DAP without any biostimulant. In comparison, biostimulants had less effect on Startec; the impact was insignificant overall, resulting in a 4% higher yield compared to Startec without biostimulants. One possible explanation may be the positive effect that Startec’s organic compounds such as oil cake and bone meal have on microbial activity. Oil cakes are used to increase microbial activity during soil bioremediation (Govarthanan et al. 2015) and other types of biotechnological application (Ramachandran et al. 2007). Bone meal is also known to increase the mineralization dynamics and thus the amount of extractable macronutrients in soils (Mondini et al. 2008) and to act as a biostimulant for bacteria (Liu et al. 2019). In other words, the supposed positive effect of biostimulants on microbial activity may already be affected by the organic compounds in Startec acting in the direct periphery of the roots of young maize plants. The concept behind Startec’s mode of action on root growth is that it is promoted by the fine microgranular dispersal of organic nutrients. In that form, they can be mineralized during the growing season. It also promotes direct root growth into the soil’s microgranular matrix by mineral NH_4-N. On the other hand, the DAP fertilizer band, which is more distant from the seed within the soil, also attracts root growth by providing ammonia, but may not be able to support microbial activity to the same extent as Startec’s organic mineralizable pool of macro- and micronutrients. However, adding humic substances (HS) and/or mycorrhiza (M) can support beneficial plant–microorganism interactions (Artursson 2005; Canellas et al. 2011; Olivares et al. 2017; Cozzolino et al. 2021; Rozmoš et al. 2021). Another hypothesis to explain why the biostimulants used have a lower effect on yields gained with Startec is that the soil P is sufficient, and not a limiting factor. Thus, lower mineral P inputs will not result in lower yields. It is noteworthy that when soil bacteria (Bac) were applied, the effects were neutral on the dry matter yield of DAP and insignificantly negative on Startec (–10.3%) (data are not presented). Further, with a combination of mycorrhiza plus humic substances (HS_M) and soil bacteria plus mycorrhiza (Bac_M), no effect was found on the yield with Startec (data are not presented), while these combinations resulted in higher average yields over three years in the case of DAP (HS_M +11.7%; Bac_M +32.1%). The highest positive impacts on the average yield gained with Startec over the three years were found when HS (+13.75%) and M (+14.9%) were applied. Statistically significant differences were only present in the case of the effect of HS_M on DAP in 2018 ($P = 0.0127$), the effect of Bac_M on DAP in 2019 ($P = 0.0041$) and the effect of HS on Startec's yield in 2018 ($P = 0.0087$) (Figure 1).

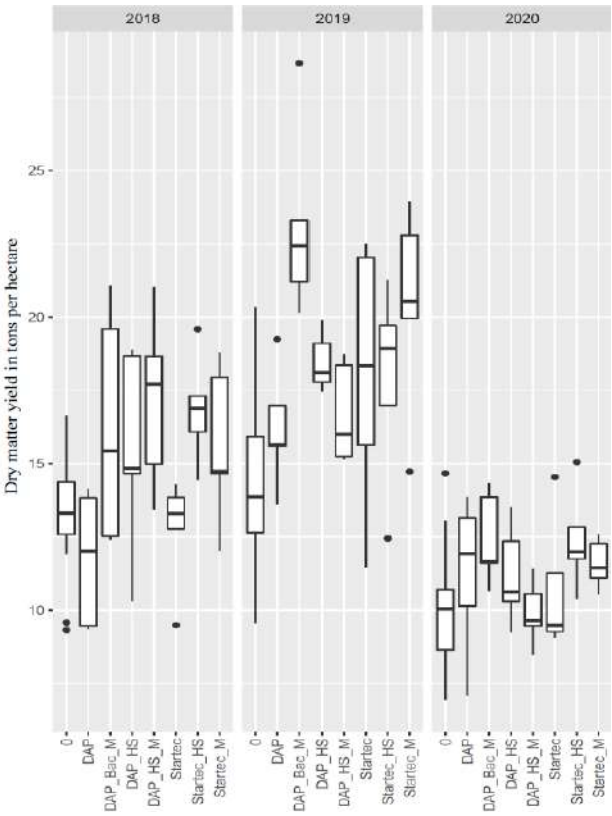

Figure 1. Dry matter yield per hectare gained with the DAP and Startec fertilizer variants individually or in combination with the biostimulants soil bacteria (Bac), mycorrhiza (M) and humic substances (HS) in 2018, 2019 and 2020; 0 represents the yield of the control plots.

Variants not shown: Startec_Bac, DAP_Bac, Startec_HS_M, Startec_Bac_M

The absence of statistically significant differences in 2020 can be traced back to the high precipitation spread equally over that year. Thus, while the biostimulants used during the comparatively dry springs of 2018 and 2019 are thought to have alleviated osmotic stress during the early development of the maize plants, this may not be relevant in 2020. The discontinuous impacts of biostimulants over the years were caused by the higher influence of climatic conditions. In other words, the influence of the year was higher than the influence of the biostimulants. The study site was chosen to minimize fluctuations in soil water and temperatures over the three cropping periods. However, compared to the other years, 2018 and to a certain extent also 2019 were dry in spring, which may lead to osmotic stress in DAP-fertilized plants without biostimulants and accordingly to a lower yield. The alleviation of osmotic stress by mycorrhiza and humic substances (Ruiz-Lozano 2003; Anjum et al. 2011; Aydin et al. 2012; Santander et al. 2017) may play a role in the better performance of the DAP–biostimulant combination compared to the DAP control application in 2018 and 2019. The increase in plants' resistance to drought through mycorrhizal symbiosis depends on the long-term water supply. If a lack of water limits their carbon uptake, the nutrition of mycorrhizal fungi may decrease shoot growth (Tinker et al. 1994; Ruiz-Lozano et al. 1995; Aikio and Ruotsalainen 2002). The locations of the plots were not precisely the same each year. If a shift occurs, this can prevent soil P contents in the control plots from gradually decreasing over the years. To avoid shifts in the plots on the study site and for higher statistical validity, future studies should be realized with a fully randomized experimental setup. While the average dry matter yield gained with Startec applied without biostimulants is slightly higher than DAP (without biostimulants), at 4.8%, the phosphorus balance of all Startec variants over the three years study is close to neutral (4.5 kg excess per hectare and year) compared to DAP phosphorus excess of 43.7 kg ha^{-1} over all DAP variants (Figure 2).

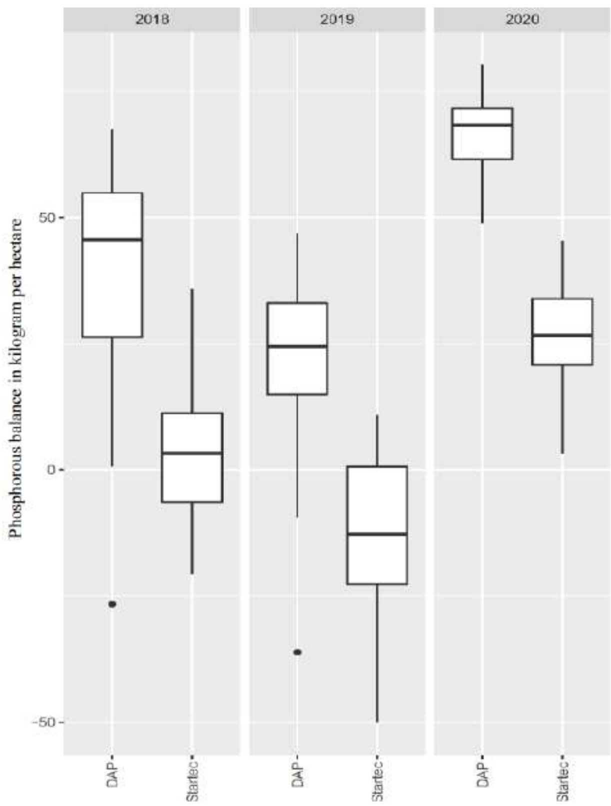

Figure 2. Phosphorus balance (P_2O_5) of all variants with and without biostimulants gained with DAP and Startec in 2018, 2019 and 2020.

Differences in phosphorus balance between all the Startec variants compared to all the DAP variants were of the highest statistical significance across the entire study period and in the individual years ($P < 0.0001$). Thus the Startec organomineral microgranular slow-release P fertilizer turned out to be an adequate alternative to DAP fertilization in maize on fertile, well-watered marshland soils. No differences were present in the corn cob ratio over all variants. For regions with high densities of livestock units and biogas plants, exporting slurry and manure is resource-consuming and puts farmers under financial pressure. By using alternative fertilizers with a lower P content, more organic P from regional farm fertilizers can be used, and inefficient exports to regions with lower densities of livestock units can be avoided. Potential modes of action affecting P availability for plants of each biostimulant used in the study were hypothetical, based on the present state of knowledge. However, it is known that the conversion of soil P into a plant-available form is driven by both plant–soil interaction and microorganism–soil interaction. The latter types of interaction are not independent, but instead characterized by interrelated processes within the rhizosphere. Microorganisms solubilize soil P, for example, by releasing small organic anions with two to six C atoms (Khan et al. 2007) and incorporating them into unstable structures as membranes and metabolism-related molecules (Achat et al. 2010). Due to the short lifespan of dominant soil bacteria, microbial P has habitat-specific turnover rates which are shorter than one growing season (Oberson et al. 2001; Bonkowski 2004). In other words, organic and inorganic bound soil P can be transferred in plant-available form through its incorporation into soil microorganisms and the mineralization of the latter. The use of soil bacteria, as performed in the experiment by using Bac or the application of leonardite-derived humic substances (HS), and thus increasing microbial activity (Lovley et al. 1996; Field et al. 2000), has the potential to support the above process of soil P conversion by microorganisms. Further, the nutrient uptake and root growth can be increased by humic substances (Adani et al. 1998; Nardi et al. 2000), soil bacteria (Araújo et al. 2005; Hansen et al. 2020; Rozmoš et al. 2021) and mycorrhiza (Vessey and Heisinger 2001; Bashan et al. 2014; Cozzolino et al. 2021) by raising the effective root surface for P uptake and other modes of action. In the case of the mycorrhizal effect on plant P uptake, Vessey and Heisinger (2001) point out that the effect can be traced back to the increase in the effective root surface and is thus indirect. However, microorganisms can also have a negative effect because of direct competition between plants and microorganisms for orthophosphate (Oehl et al. 2001; Bünemann et al. 2007; Ehlers et al. 2010). In the case of mycorrhizal fungi in particular, it is known that positive effects on plants are limited in soils with high biological activity before the treatment (Eulenstein et al. 2016) and the effect can even be negative on well-watered sites (Lahde 2016). Mycorrhiza applications can also have adverse effects if an unsuitable soil microbiome is present (Bowen and Theodorou 1979; Garbaye et al. 1994; Founoune et al. 2002; Frey-Klett 2007). Moreover, in terms of the microbial P turnover, the potential competition between plants and mycorrhiza may be higher due to the longer lifespan of fungi compared to bacteria, which lack the robust chitin structures present in fungi, and fungi's higher symbiosis-related resistance to environmental fluctuation (Kassim et al. 1981; Simpson et al. 2004). In general, the positive effects of biostimulants predominate both in the literature and in the present study.

Conclusion

The microgranular fertilizer Startec performs as well as DAP in terms of yield and can be considered an adequate alternative to DAP fertilization in maize cultivation on fertile marshland soils. The phosphorus balances of Startec variants were around nine times lower than all DAP variants ($P < 0.0001$). The impact of biostimulants was discontinuous in general: in some years there was a significant positive effect and in others there was no notable impact. The influence of the climatic conditions in each of the years was higher than the influence of the biostimulants. On average, the effects of humic substances and mycorrhiza were economically beneficial if established in agricultural practice under comparable conditions to those at the study site. The influence of humic substances on less fertile or less watered soils is believed to be higher. Further studies must be carried out comprising parallel trials on different soil types, including soils with a low P content, in the same year and with additional microbiome monitoring to prove the above hypothesis regarding the biostimulants' mode of action and possible limitations in agricultural practice.

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Mikrogranularno đubrivo i biostimulansi kao alternative diamonijum fosfatnom đubrivu za proizvodnju kukuruzana močvarnim zemljištima u severozapadnoj Nemačkoj

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Izvod

Eutrofikacija podzemnih voda usled široke rasprostranjenosti đubrenja diamonijum fosfatom (DAP) i prekomernih farmskih đubriva je jedan od najvećih problema u evropskoj poljoprivredi. Organomineralna mikrogranularna đubriva koja smanjuju sadržaj fosfora (P), samostalno ili u kombinaciji sa biostimulansima, nude obećavajuće alternative DAP đubrenju. Sproveden je poljski eksperiment sa kukuruzom (*Zea mays*) na močvarnom zemljištu kako bi se upredili povećanje prinosa i ravnoteža fosfora u varijantama sa DAP i mikroglanuralnim đubrivom. Sadržaj fosfora u zemljištu oglednog polja je 3.9 g P na 100 g zemljišta. Tretmani su uključivali kombinaciju dva đubriva, DAP ili P-redukovano mikrogranularno organomineralno đubrivo sa sporim oslobađanjem (Startec), i biosimulansa mikoriza, humične materije i zemljišne bakterije, primenjenih pojedinačno ili zajedno sa dve od gore navedenih biostmulanasa. Varijante sa đubrivom su takođe testirane pojedinačno bez dodavanja biostimulanasa. Jedna od četiri parcela je korišćena kao kontrola, i tertirana je samo sa muljem biogasa, za identifikuju prostorne varijabilnosti specifične za lokaciju i za implementaciju factora korekcije za obradu sirovih podataka korišćenjem standarizvanih metoda. Startec se pokazao dobar kao i DAP u pogledu prinosa i odnosa kukuruza i klipa, dok je višak P bio manji na parcelama tretiranim Startec-om (pros.= 4.5 kg P_2O_5 ha^{-1}) u poređenju sa DAP (pros. = 43.7 kg P_2O_5 ha^{-1}). Poslednje razlike su od statističkog značaja. Pojedinačni biostimulansi i kombinacija više biostimulanasa retko su davale značajno veće prinose, sa izuzetkom nekih kombinacija sa humičkim materijama i mikorizom u pojedinim godinama. Uticaji klimatskih uslova u savakoj od godina je bio veči od uticaja biostimulanasa. Međutim, prosečno povećanje prinosa tokom tri godina bilo bi ekonomski korisno za poljoprivrednike u slučaju primenjenog proizvoda od humičkih materija i mikorize. Pronađena je adekvatna alternativa za DAP u obliku mikroglanuralnog đubriva sa redukovanim P iz Startec-a.

Ključne reči: microgranularno đubrivo, diamonijum fosfat, eutrofikacija, ravnoteža fosfora, biostimulansi

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