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MICROGRANULES 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 the widespread Diammonium Phosphate (DAP) fertilization and excess of farm fertilizer is a major problem in the European agriculture. Organo-mineral fertilizer reduced in phosphorus (P) content, alone or in conducted a field experiment with maize (*Zea mays*) on a marshland soil site in order to compare the yield increase and the Phosphorus leachate of DAP and microgranule fertilizer variants. Treatments were realized as combination of two (organo-) mineral fertilizer, viz. DAP or a P-reduced microgranular depot-fertilizer (Startec) and the biostimulants mycorrhiza, humic substances and soil bacteria single or in parallel application with two of the mentioned biostimulants. Also (organo-) mineral fertilizer variants have been single tested without additional biostimulants. Every fourth parcel has been used as control, only treated with biogas slurry, to identify site-specific spatial variability and to implement correction factors to process raw data using standardized methods. Startec performed as good as DAP in both, yield and corn cob ratio, while P-balance is significantly better in parcels with Startec (av = 4,5 kg P2O5 / ha) compared to DAP (av = 43,7kg P2O5 / ha) resulting in small P-values of high statistical significance. Single and multiple combination of biostimulants rarely resulted in significant higher yields, with the exception of some combinations with humic substances and mycorrhiza in single years. The influence of the climatic conditions of the different years was higher compared to the influence of biostimulants. However, average increases in yield over three years would be economically beneficial for farmer in case of the applied humic substances product and mycorrhiza. An adequate alternative to DAP has been found in the P-reduced microgranulate fertilizer Startec.

Keywords: microgranule, diammonium phosphate, eutrophication, phosphorus balance, biostimulants

INTRODUCTION

Even if the extent of existing phosphate rock reserves is controversially discussed in literature, it is undisputed that these resources for conventional fertilizer production are finite (Edixhoven et al., 2014; Kisinyo and Opala, 2020). Further ecological problems as the eutrophication of ground and surface water systems by agricultural phosphorus inputs

(Torrent et al., 2007; Ulén et al., 2007) led politics in European Union to strict regulation of nutrient management and fertilizing systems (91/676/EWG, 2000/60/EC). Thus a more responsible usage of phosphorus fertilizer is necessary. Recently new fertilizing systems, as the application of microgranules, also known as pop-up fertilizer, and biostimulants, as alternative to wide spread Diammonium Phosphate (DAP) fertilization have been successfully tested (Lahde, 2016; Marilena and Aurel, 2021). In contrast to DAP and other fertilizer applied as fertilizer band with a certain distance of around 10 cm to the seed, ideally microgranules getting put together with the seed into soil or few centimeter distant to the seed. The direct contact of the fertilizer to the seed requires both a less amount of fertilizer and nutrients, especially phosphorus (P), used per plant and a lower salt index of the used components of the fertilizer itself (Alley et al., 2010). Further, the dispersal of the granules smaller than 2 mm in diameter prevents long term osmotic gradients. While microgranules as depot fertilizer become to be more frequently used in german agricultural practice, and thus develop into a promising tool to encounter the above mentioned ecological challenges, the application of biostimulants is not to the same extend spread. This stands in contrast to the numerous studies in laboratory scale (germination essays), 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 is growing fast since the last decade (Calvo et al., 2014).
Field trials are suitable to proof the concept of alternative fertilizing systems including the usage of biostimulants. In the present study field trials were carried out over three years to compare the effect of a standard fertilizer (DAP), rich in P, and a microgranular depot fertilizer with less P-content (Startec) in single or multiple combination with biostimulants, viz. liquid humic substances extract, soil bacteria and mycorrhiza in maize.

MATERIALS AND METHODS

Study area

Experiments were carried out as field trial with 22 parcels (50 x 6 m) each repeated 5 times during past three years (2018-2020) near Wanna in northwest Germany (53.729995, 8.810990). The region is classified as European atlantic climate (Cfb) according to Köppen and Geiger (1930) characterized by mild winter and moderate summer temperatures. The average precipitation per year for Wanna is 735 mm, average annual temperature is 9.9°C. 45% of annual precipitation is during maize crop season from April to September.
Hydromorphic loamy marshland soil, rich in humus, is present on the whole study site. 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 the small stream Emmelke.
The site has been used for maize cultivation over years and treated with following plant protectants Laudis, Spectrum Gold, Milagro Forte, Nagano (in 1, 2, 1, 0.5, and 0.3 litres per hectar). Regular tillage operations were ploughing and grubbing.

Experimental setup

The maize cultivar Amaroc S230 has been sown with a density of 8.5 seeds per square meter using AMAZONE single corn seeder system (EDX 6000-2C precision air seeder). DAP fertilizer has been applied as band 12 cm below the soil surface and Startec (De Ceuster Meststoffen NV (DCM) Bannerlaan 79, 2280 Grobbendonk, Belgium) microgranules few centimetres underneath the corn respectively. 100 kg/ha of DAP has been applied. The latter contains 18% total N, all in form of NH_4-N, and 46% P_2O_5. Startec can be classified as organomineral fertilizer consisting by 80% (of the original substance) of the organic industrial by-products oil cake and bone meal and mineral components Ammonium phosphate, Ammonium sulphate, EDTA-chelated Fe, Mn, Zn, Zinc sulphate and Zinc oxide. Nutrient composition of Startec is 7.5% N, 22% P_2O_5, 4% K_2O, 10% S, 0.5% Fe and Mn respectively and 1.5% Zn. The application rate of Startec in the present study was 25 kg/ha. Mycorrhiza, grown on expanded clay, and soil bacteria, sprayed on natural zeolite (clinoptilolite) as carrier material were powdered and filled into separate chambers of AMAZONE precision seeder for exact, parcel-specific application in the same go as fertilizer treatment. The study site has been treated with biogas slurry (30 m^3/ha) containing 4.3 kg total N, 1.3 kg P_2O_5, 5.2 kg K_2O per m^3. Humic substances were sprayed directly on the soil after treatment with biogas slurry. This form of application has been used due to organizational reasons and differs from manufacturer's prescription. Manufacturer of the humic product (GeoFert Germany GmbH) prescribed to mix the humic substances directly into the slurry to reduce technical effort in agricultural practice. Treatments were realized as combination of two mineral fertilizer, viz. DAP or the P-reduced microgranular depot-fertilizer Startec and the biostimulants mycorrhiza (abbreviated as M), humic substances (GeoOrganic®, GeoFert Germany GmbH) (abbreviated as HS) and soil bacteria (BactoFert®, GeoFert Germany GmbH) (abbreviated as Bac) single or in parallel application with up to two of the mentioned biostimulants. Also mineral fertilizer variants have been single tested without additional biostimulant. Every fourth parcel has been used as control, only treated with biogas slurry, to identify site-specific spatial variability and to implement correction factors in data analysis. Hand-harvest was performed by removing 20 plants involuntarily per parcel. Cob and the remaining plant were weighed and shredded separately using a garden shredder (AL-KO Master 32-40). Shredded material of cob and corn respectively for each of the five repetitions of a variant was used to prepare samples for measurement of dry matter and afterwards pulverized for NIRS-analyses using a FOSS NIRS-spectrometer 5000-M (FOSSNIRSystems). The latter data were used to calculate the year-specific removal of N, P_2O_5 and K_2O by harvest.

Statistical analysis

Control parcels without additional fertilization have been used to detect soil spatial variability on the study site. Differences in control parcels have been used to implement correction factors as described in Thomas (2006) and Dospechov (1979). To ensure normal distribution, yield was transformed via an exponential function. Differences between fertilizer variants were tested using students 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 by using the package ggplot2 (Wickham, 2009).

RESULTS AND DISCUSSION

P is an essential and often yield limiting nutrient for plants in general and in crop production in particular (Sharpley, 1997). The low solubility of P in water forces the usage of mineral fertilizer (as DAP) containing P-compounds, which solves in watered soil matrix into highly plant available form. Thus the major source of P in commercial crop fertilization is usually not applied by using farm fertilizer like slurry. However, P is also in organic form in Startec. Organic P in Startec is predominantly in bone meal as Hydroxyapatite ($Ca_5(PO_4)_3(OH)$), typically present in bone structures (Kattimani et al., 2016). Within the framework of experimental setup design it was supposed that the organic bounded P and other organically bound nutrients in Startec will be transformed in plant available form better, if biostimulants are used. Contrary to the hypothesis of a higher benefit of the mutual application of biostimulants and Startec the effect on DAP-fertilized plants has been higher. After data sampling of the three years study, average dry matter yield of all DAP combinations with biostimulants has been 14.8 % higher than DAP without any biostimulant (Figure 1). In comparison the effect of biostimulants on Startec has been smaller and overall insignificant resulting in 4% higher yield compared to Startec without biostimulants. A possible explanation may be the positive effect of Startec's organic compounds as oil cake and bone meal on microbial activity. Oil cakes are used to rise microbial activity in bioremediation of soils (Govarthanan et al., 2015) and further ranges of biotechnological application (Ramachandran et al., 2007). Also bone meal is known to rise mineralization dynamics and thus extractable macronutrients in soils (Mondini et al., 2008) and act as biostimulant for bacteria (Liu et al., 2019). In other words, the supposed positive effect of biostimulants on microbial activity may already be contributed by the organic compounds in Startec acting in direct periphery of the roots of young maize plants. The concept of Startec's mode of action on root growth is promotion through fine microgranule dispersal of organic nutrients, which are in that form mineralizable within the vegetation period, and direct attraction of root growth into the soil-microgranule matrix by mineral NH_4-N. On the other hand the DAP fertilizer band, which is more distant to the seed within the soil, also attracts root growth by ammonia but may not be able to support microbial activity to the same extend as Startec's organic, mineralizable pool for macro- and micronutrients. However, adding humic substances or/and mycorrhiza can support beneficial plant-microorganism interactions. Another hypothesis for the lower effect of the used biostimulants on yields gained with Startec is that the soil P was sufficient and not the limiting factor. Thus lower mineral P-inputs will not result in lower yields. It is noteworthy that in case of Bac effects have been neutral on dry matter yield of DAP and negative, albeit non-significant, on Startec (-10.3%) (data not shown). Further no effect has been found in combination between mycorrhiza plus HS and Bac plus mycorrhiza on yield with Startec alone (data not shown) while these combinations resulted in higher average yields over three years in DAP (HS plus mycorrhiza: +11.7%; Bac plus mycorrhiza +32.1%). Greatest positive differences in

average yield over the three years were found in Bac plus mycorrhiza (+32.1%) and application of HS (+15.2%) for DAP and HS (+13.75) and mycorrhiza (+14.9%) on Startec. Statistically significant differences were only present in case of the effect of HS plus mycorrhiza on DAP in 2018 (P = 0.0127), the effect of Bac plus mycorrhiza on DAP in 2019 (P = 0.0041) and HS on Startec's yield in 2018 (P = 0.0087).

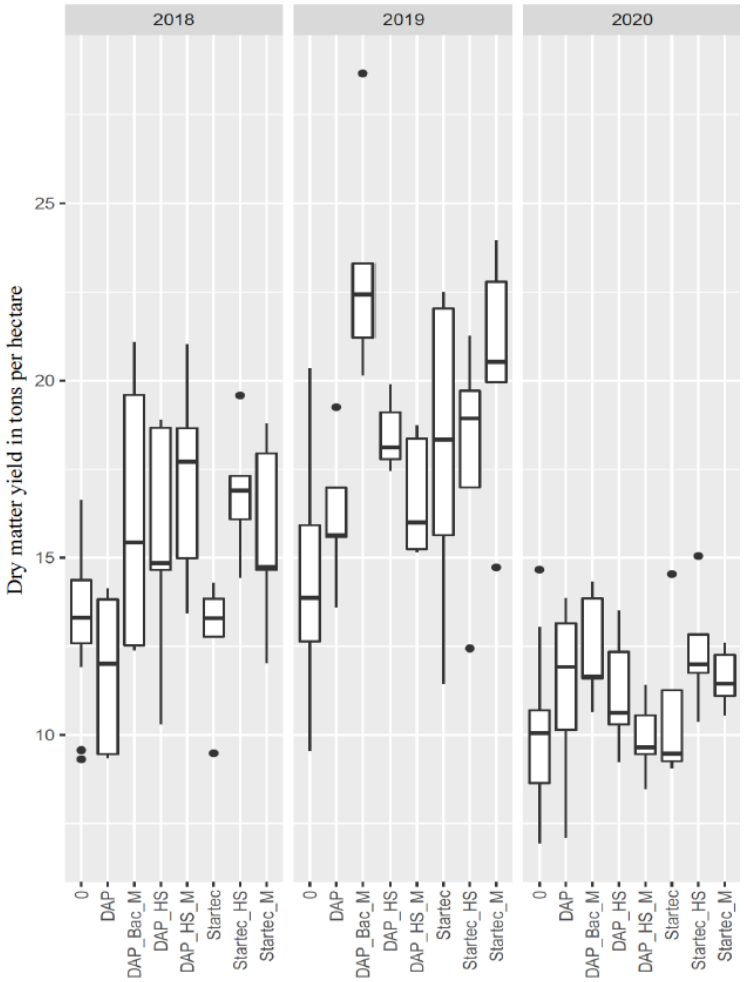

Figure 1. Dry matter yield per hectare gained with fertilizer variants DAP and Startec single or in combination with biostimulants soil bacteria (BAC), mycorrhiza (M) and humic substances (HS) in 2018, 2019 and 2020, 0 shows yield of control parcels. Variants not shown: Startec_Bac, DAP_Bac, Startec_HS_M, Startec_Bac_M

Discontinuous impacts of biostimulants over the years were caused by higher influence of climatic conditions. In other words, the influence of the year was higher than the influence of the biostimulant, which was verified via modelling in R-software. The study site has been chosen to minimize fluctuation in soil water and temperatures over the three cropping

periods. However, compared to the other years 2018 has been dry in spring, which may provoke osmotic stress in DAP-fertilized plants without biostimulants and accordingly a lower yield. Mycorrhiza and humic substances alleviation of osmotic stress (Ruiz-Lozano, 2003; Anjum et al., 2011; Aydin et al., 2012; Santander et al., 2017) may play a role in better performance of DAP-biostimulant combination compared to DAP control application in 2018. The locations of the parcels were not precisely the same each year. An occurring shift may prevent control parcels from gradual decreasing P-contents in soil over the years. To avoid shifts of the parcels on the study site and for higher statistic validity future studies should be realized in fully randomized experimental setup. While the average dry matter yield gained with Startec applied without biostimulants is slightly, viz. 4.8%, higher than DAP (without biostimulants), the phosphorus balance of all Startec variants over the three years study is close to be neutral (4.5 kg excess per hectare and year) compared to DAP phosphorus excess of 43.7 kg/ha over all DAP-variants (Figure 2).

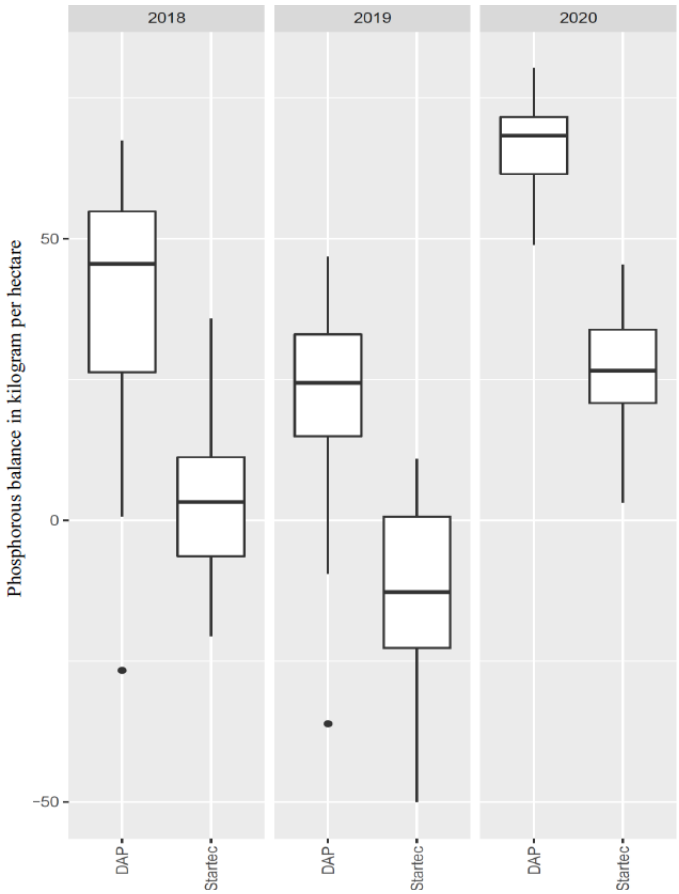

Figure 2. Phosphorus balance of all variants with and without biostimulants gained with DAP and Startec in 2018, 2019 and 2020.

The difference in phosphorus balance between all Startec variants compared to all DAP variants were of highest statistical signifincance ($P < 0.0001$). Thus microgranular P-depot fertilizer Startec turned out to be an adequate alternative to DAP-fertilization in maize on fertile, well-watered marshland soils. No differences were present in corn cob ratio over all variants. For regions with high densities of livestock units and biogas plants the export of slurry and manure is resource consuming and puts farmer under financial pressure. By using alternative fertilizer with lower P-content more organic P from regional farm fertilizer can be used and inefficient export in regions with lower densities of livestock units can be avoided. Potential modes of action on P availability for plants of each used biostimulant in the study were hypothetical to present state of knowledge. However, in literature it is often stated that solubilization of soil P into plant available form is driven by both plant-soil interaction and microorganism-soil interaction. The latter types of interaction are not independent but rather characterized by interrelated processes within the rhizosphere. Microorganisms solubilize soil P for example by releasing small, two to six C-atom, organic anions (Khan et al., 2007) and incorporate it in labile structures as membranes and metabolism related molecules (Achat et al., 2010). Due to the short lifespan of dominant soil bacteria the microbial P has habitat-specific turnover rates, which are shorter than a vegetation period (Oberson et al., 2001; Bonkowski, 2004). In other words, organic and inorganic bound soil P can be transferred in plant available form trough incorporation into soil microorganisms and mineralization of the latter. The use of soil bacteria, as performed in the experiment by using BactoFert® (*Bacillus subtilis*), (GeoFert Germany GmbH) or the application of leonardite derived humic substances (GeoOrganic®, GeoFert Germany GmbH), and thus the increase of microbial activity (Lovley et al., 1996; Field et al. 2000), has the potential to support the above described process of soil-P turnover by microorganisms. Further the growth of roots can be increased by humic substances (Adani et al., 1998; Nardi et al., 2000), soil bacteria (Araújo et al., 2005) and mycorrhiza (Vessey and Heisinger, 2001) by raising the effective root surface for P-acquisition. In case of mycorrhizal effect on plant P uptake Vessey and Heisinger (2001) point out that the effect can be traced back to the increase of effective root surface and is thus indirect. However, effects of microorganisms may be also negative because of direct competition between plants and microorganisms for orthophosphate (Oehl et al., 2001; Bühnemann et al., 2007; Ehlers et al., 2010). Especially for mycorrhizal fungi it is known that positive effects on plants are limited in soils with high biological activity before the treatment (Eulenstein, 2016) or even negative on well-watered sites (Lahde, 2016). Also in terms of microbial P turnover the potential competition between plants and mycorrhiza may be higher due to a longer lifespan of fungi compared to bacteria, which lacks robust chitin structures of fungi and higher symbiosis related resistance to environmental fluctuation (Kassim et al., 1981; Simpson et al., 2004). In general positive effects of biostimulants predominate in literature and in the present study as well.

CONCLUSION

The used microgranular fertilizer Startec performs as good as DAP in yield and can be considered to be an adequate alternative to DAP-fertilization in maize cultivation on fertile

marshland soils. Phosphorus balance of Startec variants were around nine times lower than all DAP-variants ((P < 0.0001). The impact of biostimulants has been discontinuous in general, comprising years with a significant positive and without remarkable impact. The influence of the climatic conditions of the years has been higher than the influence of the biostimulants. In average the effects of humic substances and mycorrhiza where economically beneficial if established into agricultural practice. Influence of biostimulants on less fertile or less watered soils is supposed to be higher. Thus, further studies has to be realized comprising parallel trials on different soil types, including soils with low soil-P content, during the same year and additional microbiome monitoring to prove the above mentioned hypothesis of the biostimulants mode of action.

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