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Bioactive-Sulfur Coated Diammonium Phosphate Improves Nitrogen and Phosphorus Use Efficiency and Maize (*Zea mays* L.) Yield

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Abstract: Poor use efficiency of costly phosphatic fertilizer diammonium phosphate (DAP) is a very serious economic and environmental issue. Therefore, this research was designed to improve the effectiveness of DAP by its coating with bioactive-elemental sulfur (BES). First of all, BES was prepared by inoculating the elemental sulfur with sulfur-oxidizing bacteria (SOB). After that, BES and elemental sulfur coated DAP was prepared by coating different amounts (2.0, 4.0, 6.0 and 8.0 g) of BES & elemental sulfur respectively/100 g DAP. Finally, a field experiment on maize crop was conducted to evaluate the performance of coated DAP. Nine treatments (T_1=control, T_2=2% elemental sulfur coated DAP, T_3=2% BES coated DAP, T_4=4% elemental sulfur coated DAP, T_5=4% BES coated DAP, T_6=6% elemental sulfur coated DAP, T_7=6% BES coated DAP, T_8=8% elemental sulfur coated DAP and T_9=8% BES coated DAP) of coated DAP were evaluated in this experiment. Treatments were arranged according to randomized complete block design (RCBD) and each treatment was replicated thrice. Data regarding growth, physiology, yield and nutrition parameters were recorded. The collected data were analyzed by analysis of variance (ANOVA) using STATISTIX 8.1 software and means were compared through the least significant difference (LSD) test. Statistical analysis indicated that maximum improvement was noted with the application of T9 (8% BES coated DAP) which improved the plant height (39%), chlorophyll content (21%), 1000 grain weight (27%), kernel yield (79%), nitrogen concentration (56%) and phosphorus concentration (52%) as compared to control. It can be concluded that bioactive-sulfur coated DAP has the potential to enhance maize production under field conditions.

Keywords: Bioactive-sulfur, coated DAP, sulfur-oxidizing bacteria, fertilizer use efficiency.

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1. Introduction

Among the essential plant nutrients, phosphorous (P) is the 2nd most important macronutrient, required for profitable crop production. The optimum P concentration in soils ranges from 0.005 to 0.15 % and plants absorbed it as $H_2PO_4^-$ and HPO_4^{2-} (Wang et al., 2021) It is considered an essential constituent of hereditary materials called nucleic acids (DNA and RNA) and many other intermediary metabolites like adenosine phosphates and sugar phosphates which are fundamental part of metabolism in all life forms (Correll, 1998). P is critical in the early stage of plant growth. The critical P concentration in plants ranges

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from 0.1 to 0.5% depending on the plant species and growth stage. Application of P fertilizer induces a vigorous and deeper root system (Sharma, 2007). Moreover, P promotes early maturity in plants by decreasing the moisture content of grain and improving crop quality (Malakooti, 2000). Phosphatic fertilizer application has a significant impact on the yield and quality of crop by strengthening stem and straw and preventing lodging in cereal crops. Moreover, it enhances the immunity and resistance of plants against diseases (Ahmad et al., 2007).

Suboptimal P nutrition causes significant yield losses (10-15%) (Shenoy and Kalagudi, 2005). Deficiency of P is aggravated in alkaline and calcareous soils. Due to several limitations, P availability to plants is reduced under soil application. Moreover, P availability in acidic soil is decreased due to the adsorption of P by iron (Fe^{+3}) and aluminum (Al^{3+}). Likewise, a decrease in P availability in alkaline soils is attributed to its adsorption by Ca and Mg (Ali et al., 2014; Hashmi et al., 2017). Therefore, plants cannot use major components (>80%) of soil-applied P and the application efficiency of P fertilizer is very low (Mosali et al., 2006; Sharma et al., 2013). For profitable crop production, phosphatic fertilizers play a vital role, especially in soils with inherent phosphorus deficiency (Sharpley et al., 1994). Several phosphatic fertilizers are used to meet crop P requirements including diammonium phosphate (DAP), monoammonium phosphate (MAP), triple superphosphate (TSP) and single superphosphate (SSP). DAP is a widely used phosphatic fertilizer that contains 46% P_2O_5 and 18% N (Meyer et al., 2020).

Considering the abovementioned facts, development of innovative approaches is necessary to improve the application efficiency of P fertilizers (Yu et al., 2021). Various strategies are being used in this regard, including band placement (Freiling et al., 2021), intercropping (Tang et al., 2021), use of microorganisms (Sarkar et al., 2021), and fertilizer coating (Lawrencia et al., 2021) are widely used to enhance fertilizer application efficiency. Fertilizer coating has been documented as a very promising approach in this regard (Azeem et al., 2014; Lawrencia et al., 2021). Coated fertilizers have an additional layer of the barrier which prevents the direct contact of fertilizer with the soil. Fertilizer coating has minimized the nutrient dissolution rate (Nunes et al., 2021). Diverse materials are being used for coating fertilizers, including biodegradable organic coating, polymers (Nunes et al., 2021; Naik et al., 2017), elemental sulfur (Haseeb-Ur Rehman et al., 2021) starch (Zhai et al., 2011), and waxes (El Assimi et al., 2020).

Polymer coating of phosphatic fertilizers increases the fertilizer application use efficiency by preventing its fixation (Nunes et al., 2021; Perveen et al., 2021), however, the expensive nature of polymer coating restricted its application. Contrarily, sulfur coating is a cheaper and easy process, therefore preferred for coating fertilizers. Furthermore, sulfur is a secondary nutrient and fungicide, therefore providing an additive nutrient advantage. Sulfur reduces soil pH, increases nutrient availability, especially P in the soil (Ayşen et al., 2019). Due to these advantages, sulfur coating is gaining interest to decrease nutrient deficiency in alkaline and calcareous soils (Lawrencia et al., 2021).

Sulfur oxidation to transform sulfuric acid (H_2SO_4) decreases the soil pH of calcareous soils and enhances nutrient availability (Griffith et al., 2015; Rahmani et al., 2018). Elemental sulfur is a standard acidulant for decreasing the soil pH (Kulczycki, 2021; Karimizarchi et al., 2018). Oxidation of coated elemental sulfur solubilizes the fixed P and increases its availability (Mohamed et al., 2014 Pourbabaee et al., 2020). Generally, soil has sulfur-oxidizing bacteria, however, their population is inadequate to attain satisfactory solubilization of fixed phosphorous. Therefore incorporation of sulfur-oxidizing bacteria in soil is required to achieve satisfactory results (Stamford et al., 2015). Consequently, we hypothesized that increasing the population of SOB in the vicinity of DAP granules by coating with elemental sulfur inoculated with SOB can help to increase phosphorous availability and phosphorus use efficiency more efficiently. No study has been found in the existing literature regarding this aspect. Therefore, this project was designed to prepare bioactive-elemental sulfur (BES) coated DAP fertilizer and its evaluation for improving maize production.

2. Materials and Methods

A field trial was conducted at the research farm of MNS- University of Agriculture Multan, Pakistan. Before experiment, soil was analyzed for different physicochemical properties (Table 1).

Table 1: Soil physico-chemical propertied of experimental soil

Parameters	Units	Values
Texture	-	Sandy loam
pH	-	8.3
EC	$dS\ m^{-1}$	4
Organic matter	%	0.7
Total N	%	0.051
Available P	$mg\ kg^{-1}$ soil	7.2
Extractable K	$mg\ kg^{-1}$ soil	84

2.1. Collection of sulfur-oxidizing bacteria and preparation of broth culture

Sulfur-oxidizing bacteria were obtained from the Department of Soil and Environmental Sciences, MNS University of Agriculture, Multan, Pakistan, and broth culture was prepared by using Starkey media. The composition of 1L Starkey media was 3g KH_2PO_4, 0.2g $MgSO_4$. $7H_2O$, 0.2 g $CaCl_2.2H_2O$, 0.5g $(NH_4)_2$ SO_4 and traces of $FeSO_4$ (Starkey, 1935). Then pH of the media was adjusted to 8 by 1N NaOH and H_2SO_4 and media was sterilized, after sterilization, media was cooled to around 40°C. Finally, media was inoculated with liquid culture of SOB and incubated at 30°C for seven days in an orbital shaker incubator at 120 rpm. The change in color of broth media (from purple to yellow) indicated the growth of SOB. Then bio-activation of elemental sulfur with SOB was done by adding 10 ml broth culture of SOB per 100g ES. The mixture was stirred with spatula for even mixing of broth and ES.

2.2. Coating of DAP with bioactive-sulfur

Coating of DAP with bio-activated sulfur was done in seed rotating drum. Eight types of coated DAP were prepared with varying amount of elemental sulfur (2.0%, 4.0%, 6.0% and 8.0%) and BES (2.0%, 4.0%, 6.0% and 8.0%). First of all, a specific amount of DAP was coated with sticky solution (35%) of gum acacia. Then different amount of elemental sulfur and BES (2.0-8.0) was added and coated by rotating the drum for 15 minutes. Finally, coated DAP was shifted to plastic trays for drying overnight at room temperature.

2.3. Field experiment

After preparation of bioactive sulfur-coated fertilizer, a field study was directed on maize crop to evaluate the potential of BES coated DAP. Seeds of maize cultivar NK 8441 were done on 18th February 2019 and harvested at full maturity stage. Land preparation was done with deep plowing by the chisel plow. Fertilizers were broadcasted at following local recommendations. Nitrogen was applied @ 100 kg acre-^{1} in four splits. While phosphorus and potassium application was done @ 60 kg P_2O_5 and 40 kg K_2O acre^{-1} at the time sowing. A field experiment was laid according to RCBD and each treatment was replicated thrice. Nine treatments (T_1=control, T_2=2% elemental sulfur coated DAP, T_3=2% BES coated DAP, T_4=4% elemental sulfur coated DAP, T_5=4% BES coated DAP, T_6=6% elemental sulfur coated DAP, T_7=6% BES coated DAP, T_8=8% elemental sulfur coated DAP and T_9=8% BES coated DAP) of coated DAP were evaluated in this experiment. 75cm and 20 cm distance was maintained row to row and plant to plant respectively. Data regarding growth yield and nutrition was noted at different stages during the crop growth. Plant height of maize plants was measured at harvesting using a meter rod. Chlorophyll contents were determined by SPAD meter. Nitrogen and P content in grain and straw were determined by the methods described in the Soil and Plant Analysis Laboratory Manual. Nitrogen and P use efficiency (kg grain kg^{-1} N or P applied) was calculated (Ortiz-Monasterio et al., 2001) P uptake in grain was calculated by using following formula

P uptake in grain = % P in grain /100 × dry grain weight (g)

Analysis of variance (ANOVA) and STATISTIX 8.1 software were used to analyse the obtained data, and mean was compared through least significant difference (LSD) test.

3. Results

3.1. Growth and yield parameters

Bioactive sulfur-coated DAP significantly improved the plant height, chlorophyll contents, kernel weight per cob, number of grain per cob, 1000 grain weight, kernel yield as compared to uncoated DAP (Table 2). Maximum plant height (221 cm) was observed in T_9 (8% BES coated DAP) which was 39% higher than T_1 (158 cm). Similarly, chlorophyll contents and leaf area were increased by 21% and 81% respectively by T_9 as compared to control. In case of yield parameters T_9 produced a higher yield as compared to control. The numbers of grain per cob were 288 in control which increased to 530 in T_9. In case of 1000 grain weight highest weight (386 g) was observed in T_9 and T_7 which was 27% higher than T_1 (303 g). However, maximum kernel yield (kg m^{-2}) of maize (2.17 kg) was observed in T_9 which 79% higher was as compared to control (1.21 kg).

3.2. Nutritional parameters

The use of bioactive sulfur coated DAP enhanced the N, P, K, S, Zn and Fe content in grain, NP uptake in grain and NP use efficiency of maize as compared to control demonstrated in Table 3. Nitrogen content in the grain of control was only 0.64% which improved to 1.01% by T_9. And highest P concentration (0.90%) was observed in T_9 which is 52% higher than T_1 (0.59%). While maximum grain k concentration (0.88%) was observed in T_9 (8% BES coated DAP) which is 62% higher than T_1 (0. 54%).

Table 2: Effect of bioactive-elemental sulfur coated DAP on growth and yield parameters of maize

Treatments	Plant height (cm)	Chlorophyll contents	Kernel weight cob^{-1} (g)	Grains cob^{-1} (g)	1000-grain weight (g)	Kernel yield (kg m^{-2})
T_1 (control)	158^{e} (0)	44^{c} (0)	115^{c} (0)	288^{f} (0)	303^{e} (0)	1.21^{d} (0)
T_2	164de(4)	45.4^{bc} (4)	116^{c} (1)	307^{ef} (6)	322de (6)	1.16^{d} (-4)
T_3	164de (4)	49.5^{ab} (14)	150^{bc} (30)	362de (25)	335^{cd} (10)	1.50^{cd}(23)
T_4	176^{cd}(11)	49.8^{ab} (14)	118^{c} (3)	372^{d} (29)	343^{bcd} (13)	1.18^{d} (-2)
T_5	175^{cd} (10)	50.5^{ab}(16)	180^{ab} (56)	414^{cd}(43)	349^{bc} (15)	1.8^{bc} (48)
T_6	180^{bc} (14)	51.4^{a} (18)	187^{ab} (62)	449^{bc} (55)	346^{bc}(14)	1.87^{ab}(54)
T_7	188^{bc} (19)	51.4^{a} (18)	188^{a} (63)	506^{ab} (75)	386^{a}(27)	1.88^{ab}(55)
T_8	194^{b} (22)	52^{a} (19)	203^{a} (76)	522^{a} (80)	358^{b}(18)	2.03^{ab}(67)
T_9	221^{a} (39)	52.7^{a} (21)	217^{a} (88)	530^{a} (84)	386^{a}(27)	2.17^{a} (79)

Data are shown as mean of three values. The means with the same letter(s) are not statistically different according to least significant difference (LSD) test. Values shown in parenthesis represent the % increase from control. T_2, T_4, T_6 and T_8 represent coated DAP with 2, 4, 6 and 8% elemental sulfur respectively. T_3, T_5, T_7 and T_9 represent coated DAP with 2, 4, 6 and 8% bioactive elemental sulfur respectively.

Similarly, S and Zn contents were 0.12% and 0.80% in control and increased to 0.20% and 0.85% respectively by T_9, while Fe contents were recorded 0.11% in control and enhanced by 0.16 by T_8. NUE and PUE was also affected by BES coated DAP. The highest NUE and PUE were observed in T9 which was increased 79% and 78% respectively as compared to control. N and P uptake were increased from 77 and 72 kg ha-^{1} to 218 and 195 kg ha-^{1} respectively.

4. Discussion

The present study was aimed to assess the efficiency of BES coated DAP to improve maize growth, yield and nutrient use efficiency. The results revealed that BES coated DAP performed better in enhancing the growth, yield and nutrient use efficiency of maize as compared to uncoated DAP. Obtained results highlighted enhanced growth parameters such as plant height of maize under field conditions by using BES and elemental sulfur-coated DAP. Improvement of growth parameter can be attributed to increased root growth by the P availability, and increased nutrient uptake from rhizosphere which helped in enhancing the growth parameter. These results are in agreement with an earlier report that plant height, kernel weight per cob of maize increased with the application of elemental sulfur in combination with P sources (Khan et al., 2005). Findings of Tarabily et al., (2006) also supported our results. They concluded that optimum maize growth was obtained when strains of sulfur-oxidizing bacteria strains were applied with elemental sulfur, as compared to control by significantly reducing the soil pH and resulting in increased P availability.

Table 3: Effect of BES coated DAP on nutrients parameters of maize

Treatments	Grain N (%)	Grain P (%)	Grain K (%)	Grain S (%)	Grain Fe (%)	Grain Zn (%)
T_1 (control)	0.64^{f} (0)	0.59^{c} (0)	0.54^{e} (0)	0.80 ^{c} (0)	0.11 d(0)	0.12 e(0)
T_2	0.78^{cde} (21)	0.74^{b} (25)	0.51^{de} (-5)	0.81^{c} (1.2)	0.10d (-9)	0.13e(8)
T_3	0.68^{ef} (6.25)	0.76 ^{b}(28)	0.48^{e} (-11)	0.81^{c} (1.2)	0.12 cd(9)	0.16cd (33)
T_4	0.73^{def} (14)	0.75^{b}(27)	0.55^{cde} (1.8)	0.814^{c} (1.2)	0.14 abc(27)	0.14cde(16)
T_5	0.81^{cd} (26)	0.80^{b}(35)	0.60^{bcde} (11)	0.82^{b} (2.5)	0.12 cd(9)	0.15cd (25)
T_6	0.86^{bc}(34)	0.81^{b}(37)	0.64^{bc} (18)	0.83^{a} (3.7)	0.13 bcd(18)	0.13de (8)
T_7	0.94^{ab}(48)	0.76^{b} (28)	0.62^{bcd}(14.8)	0.83^{a}(3.7)	0.15abc (36)	0.16bc(33)
T_8	0.95^{ab}(48)	0.83^{ab}(40)	0.71^{b}(23)	0.84^{a} (5)	0.16a (45)	0.18 ab(50)
T_9	1.01^{a}(56)	0.90 ^{a}(52)	0.88^{a}(62)	0.85^{a} (6.2)	0.15 ab(36)	0.20a (66)

Data are shown as mean of three values. The means with same letter(s) are not statistically different according to least significant difference (LSD) test. Values shown in parenthesis represent the % increase from control. T_2, T_4, T_6 and T_8 represent coated DAP with 2, 4, 6 and 8% elemental sulfur respectively. T_3, T_5, T_7 and T_9 represent coated DAP with 2, 4, 6 and 8% bioactive elemental sulfur respectively.

Improvement in yield parameters such as kernel weight per cob, number of maize kernel per cob, 1000 kernel weight and kernel yield also cleared from our findings by using the BES coated DAP. Findings of Salimpour et al., (2010) are in line with presented results, as they concluded that application of rock phosphate with ES, *Thiobacillus* sp. and organic matter resulted in the highest amount of yield and stover in canola which was increased by 38% to 70% respectively as compared to control. Concluding remarks of Motior et al., (2011) research in field also agreed our results they revealed that yield of cucumber was considerably improved with BES and SOB application. Our outcomes are also in line with the results of Shivay et al., (2016). They recorded that grain yield of wheat was increased from 9.85 to 11.21 % by using sulfur-coated fertilizer. Our results were also supported by Pooniya et al., (2017) they noted that maize yield was increased 12.8% by sulfur coated urea fertilizer as compared to uncoated fertilizer.

The nutritional component of maize grains also increased in our research by using the BES and elemental sulfur-coated DAP. This enhancement may occur due to more availability of P by reducing the soil pH by solubilization of fixed P with the application of bioactive sulfur. It is also clear from the concluding remarks of Salimpour et al., (2012). They testified that elemental sulfur reduced the soil pH when inoculated with SOB and increased the plant nutrients availability such as P. Results of Arian et al., (2010) also sustained our outcomes. They concluded that P solubility was increased 2.4 times by the application of elemental sulfur inoculated with strains of sulfur-oxidizing bacteria, compare to control. Garcia et al., (1997) also reported that P availability by plants was increased with coated DAP and SOP as compared to uncoated DAP and SOP. Findings of Noor et al., (2017) also declared that N and P contents were also increased in wheat grains and straws of coated fertilizer as compared to uncoated fertilizer.

5. Conclusion

The present research was designed to evaluate the effect of BES and elemental sulfur-coated DAP on the efficiency of P and maize production under field conditions. The results indicated that BES and ES-coated DAP significantly increased maize output under field conditions. Statistical analysis indicated that maximum improvement was noted with the application of T9 (8% BES coated DAP). The BES and elemental sulfur-coated DAP treatments were shown to be superior to the control (non-coated DAP) in terms of their influence on all parameters, depending on the percentage increase. The comparison of elemental sulfur (T_2, T_4, T_6, and T_8) and BES (T_3, T_5, T_7, and T_9) coated treatments based on % increase revealed that BES coated treatments performed better than elemental sulfur coated treatments. A comparison among elemental sulfur-coated DAP treatments showed that T8 (8% sulfur coated DAP) performed better than other sulfur-coated treatments. T9 (8% BES coated DAP) showed more influence on all the parameters as compared to other BES coated treatments. Therefore, this study concluded that bioactive-sulfur coated DAP has the potential to enhance maize production under field conditions.

Competing Interest Statement: The authors declare no conflict of interest.

List of Abbreviations: DAP, diammonium phosphate; BES, bioactive elemental sulfur; RCBD, randomized complete block design; ANOVA, analysis of variance.

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