J. Appl. Res Plant Sci. Vol. 4(2), 545-552, 2023, https://doi.org/10.38211/joarps.2023.04.02.186

Research Article

OPEN ACCESS

Available on https://www.joarps.org
Journal of Applied Research in Plant Sciences
(JOARPS)
ISSN: 2708-3004 (Online), 2708-2997 (Print)

Response of wheat *(Triticum aestivum L.)* to combined application of organic compost along with plant growth promoting *Aspergillus* fungi

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Article Received 08-09-2022, Article Revised 10-01-2023, Article Accepted 26-02-2023.

Abstract

Bio-organic fertilizers can improve soil health and maintain microbial activity. The application of *Aspergillus* spp. can degrade organic matter, promote plant growth, and improve soil health by accelerating soil nutrients and biological activity. Previously, *Aspergillus* spp. has shown potential in phosphate solubilization and siderophore production. Still, a study gap remains, and focus has been placed on clarifying the impact of *Aspergillus* sp. AS2022 on soil microbial biomass and soil nutrient acceleration. For this purpose, a pot experiment was established with four different treatments: cow compost (CC), cow compost + AS2022 (CA), AS2022 (AS) strain only and control-only soil (CK). The amount of cow compost used was 180 mg N kg^{-1}. Results revealed that inoculation of AS2022 with cow compost stimulated nitrogen mineralization and enhanced available nitrogen and accelerated the soil enzyme activities, which proposed that it could contribute to wheat crop production through the initial and later phases of plant growth. Although a single application of AS2022 enhances plant growth compared to the control, it might be the release of secondary metabolites. However, our short-term findings showed that the combined application of beneficial fungal strain AS2022 along with cow compost was suitable culture for wheat crop production and improvement of soil quality through organic matter degradation and accelerating soil nutrients.

Keywords: *Aspergillus spp., cow compost, wheat crop, soil nutrients, soil microbial biomass*

Introduction

The major challenge to maintaining sustainable agriculture and meeting the needs of the world's expanding population is the rise in food demand and agricultural productivity. (Asghar & Kataoka, 2022b; Kabato, Ergudo, Mutum, Janda, & Molnár, 2022). Agricultural production relies on inorganic fertilizers, pesticides, and fungicides, leading to ecological problems such as declining soil health, loss of soil biodiversity, degradation, and poor water infiltration (Ju *et al.*, 2009; Zhu & Chen, 2002). Inorganic fertilizers and pesticides have more great nutrient contents than organic composts and organic-derived fertilizers and are readily available for the plant as per nutrient demand (M. Ahmed, Rauf, Mukhtar, & Saeed, 2017; Laird *et al.*, 2010). Ju *et al.* (2009) and Hou *et al.* (2010) documented that unnecessary utilization of inorganic fertilizers increases not only soil ecological pollution but also unintended ecological problems such as soil acidification, emission of greenhouse gases, and eutrophication. Furthermore, continuous application of inorganic fertilizers and conventional agriculture can distress the soil biogeochemical properties and results in damaging of soil biodiversity and imbalance of soil nutrients (Kim, Chung, & Malo, 2001; Majeed, Abbasi, Hameed, Imran, & Rahim, 2015). Therefore, alternative options are being considered to replace the inorganic fertilizers, aiming to improve agricultural production, reduce environmental problems, and maintain soil health. By using organic wastes and their byproducts to maintain soil health and boost agricultural productivity on a sustainable basis (Asghar, Mahmood, *et al.*, 2022). For sustainable agricultural production, applying organic wastes and their by-products can meet the nutritional demand of crops and sustain the soil biodiversity (S. Ahmed, Matiullah, Taqi, Ahmad, & Iqbal, 2022; Asghar & Kataoka, 2021). The organic-based amendments can be collected from municipal solid waste, dairy and poultry industries, and agricultural waste (Quilty & Cattle, 2011). Such as organic compost is a major stock for soil nitrogen and carbon with its 104 Pg N content in the compost, and it constitutes the major pool subjected to the N transformation in agriculture ecosystem (Ma *et al.*, 2022). Organic amendments reclaim and restore overworked farmland soils to maintain organic matter (OM) for soil fertility and agricultural production, particularly in the long-term, by releasing nutrients slowly (Asghar, Akça, *et al.*, 2022). By preserving soil biodiversity, adding organic matter, and ultimately promoting plant growth and development, the use of organic amendments in agricultural systems helps to sustain long-term soil quality (Mohanty *et al.*, 2011). Thus, applying organic amendments to manage microbial activity and soil fertility could improve soil quality and productivity. However, according to Tejada, Hernandez, and Garcia (2009), the utilization of organic amendments restores soil health and preserves the soil microbial community by supplying organic matter and long-term soil fertility through the delayed release of nutrients. In some ways, organic amendments are not being considered by farmers for suitable application because of slowly resealing of nutrients and not meeting the crop nutrient demands. Therefore, to find possible alternative strategies to ensure economic and environmental safety, increase crop yield, and maintain agroecosystems. Plant growth-promoting microbes (PGPM), including bacteria and fungi, are free-living soil microbes that rapidly colonize the plant roots/rhizosphere and increase the crop yield by promoting plant growth when inoculated (Asghar & Kataoka, 2021; Majeed *et al.*, 2015). PGPMs, such as rhizo/endophytic bacteria, and fungi, increase plant growth and root development by releasing a variety of secondary metabolites, producing volatile organic compounds, and enhancing nutrient availability (Hossain & Sultana, 2020; Naziya, Murali, & Amruthesh, 2019). Particularly, numerous plant growth-promoting fungi (PGPF) are recognized to produce auxin like indole-3-acetic acid (IAA) and siderophores and are additionally capable of improving the iron nutrition of plants by decomposing the organic matter and releasing the nutrients (Asghar & Kataoka, 2022a; Galeano *et al.*, 2021). However, many fungal species of the genera *Penicillium and Trichoderma* have been utilized based on bio-control agents, IAA, siderophores production, and P-solubilization (Bader, Salerno, Covacevich, & Consolo, 2020; Murali & Amruthesh, 2015). However, little attention has been paid to the impact of inoculation of *Aspergillus* spp. with cow composts on soil nutrient availability, plant growth promotion, and soil microbial biomass. In our previous report, we already demonstrated that *Aspergillus niger* could enhance phosphorus availability (Khan *et al.*), but still, many avenues remain and need to open in terms of enzymes activity, microbial biomass and nitrogen availability. In this regard, this study aimed to assess the growth promotion of wheat (*Triticum aestivum L.)* through inoculation of *Aspergillus niger* along with cow compost, and determine the possible mechanisms for plant growth and development.

Materials and Methods

Soil and compost collection: Soil (silty clay) was collected from the agricultural research institute of Gomal University, Dera Ismail Khan, KPK (31.8˚N, 70˚E). The soil was taken from the top layer, placed at room temperature, and removes the stones by passing 2mm sieve, roots, and surface litter, and then kept at 4 °C until it was used again. Further, the soil's basic chemical properties were measured and compiled in Table 1. However, cow manure was locally collected and turned into compost through the following methodology (Appelhof & Olszewski, 2017). After turning into compost, basic chemical properties were measured and shown in Table 1.

Table 1 Basic properties of soil and cow compost.

Properties	Soil	Cow Compost
pH (1:5)	7.95	--
Total N %	0.08	2.32
Total C %	1.24	38.35
C/N (%)	--	16.53
Total P %	--	4.16
Total K %	--	3.98
Available P mg kg^{-1}	161	--
Nitrate mg kg^{-1}	19.1	--
Phosphatase Enzymes ng g^{-1} soil/min	21.1	--
Glycosidase Enzymes ng g^{-1} soil/min	9.12	--

Fungal strain collection and preparation: *Aspergillus niger* strain (AS2022), hereafter known as AS2022, was collected from the Department of Soil Science, PMAS-Arid Agriculture University, Rawalpindi. The DNA was extracted from the isolated fungal strains using the ZR bacterial/fungal DNA Mini Prep Kit (Zymo Research Corp., Irvine, CA, USA). The universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′ TCCTCCGCTTATTGATATGC-3′) were used to amplify the ITS region. As earlier reported, the strain was isolated from the maize cultivated soil and with the ability of P solubilization (Khan *et al.*). The strain was carefully transported from Arid University to Gomal University by maintaining 25 ˚C. The strain was re-grown on new plates having PDA media and incubated for seven days at 25 ˚C. Later, the re-grown strain was cultured with 10 mL PDB broth and incubated for five days at 25 ˚C for better growth. Further, initial mass of spores were measured by dilution plating techniques to get and count the total colony forming units (CFUs) mL^{-1}, and method followed by (Zhang *et al.*, 2013).

Pre-germination seeds preparation: Wheat (*Triticum aestivum L.*) inqlab-91 seeds were obtained from the local market, washed, and placed on wet tissue paper plates for 100% germination. For the enhancement of seed germination, plates were later kept in a growth chamber during the day and night cycle.

Experimental setup: To elucidate the effects of the combined application of cow compost along AS2022 on the plant growth promotion of wheat, for this purpose a pot experiment was established. Pots were filled with 4 kg soil by adding cow compost with (180 mg N kg^{-1} of soil**)** based on the proportion of the total N used (the N percentage is displayed in Table 1). Four treatments with five replications were organized as follows: cow compost (CC), cow compost + AS2022 (CA), AS2022 (AS) strain only and control-only soil (CK). Pre-germinated seeds (20 seeds per/pots) were transferred to the each experimental pot. Moisture content of soil was adjusted at 65% of soil water holding capacity, and water was maintained through daily basis monitoring. Experimental pots were placed in a growth chamber, and plants were grown for until maturity. After six weeks of plant growth, above and below-ground parts of plants were collected, including the plant height, the number of grains and spike length. At the same time, soil samples were also collected for analysis of available nitrogen (NH_4^+-N and NO_3^-‒N), microbial biomass (fungal and bacterial CFUs), and soil enzyme activities (β- glycosidase, phosphates, cellulase and inervtase).

Data analysis: Available nitrogen, β- glycosidase, and phosphates enzyme activities were measured based on (Asghar & Kataoka, 2021). Microbial biomass (fungal and bacterial CFUs) was measured by following the series dilution plating techniques (10^{-2}, 10^{-3}, and 10^{-4}) through following (Martin, 1950). However, cellulase and inervtase were measured as follows (Zhao, Zhang, Li, Gao, & Zhang, 2020). Tukey's HSD test was directed in Statistix 8.0 (Analytical Software, FL, USA) for statistical analysis.

Results

Response of wheat: A pot experiment was established to elucidate the effects of different treatments. The plant height, number of grains, and spike length were subjected to monitor the function and effects of compost's combined and alone application. Regarding the plant height, the number of grains and spike length was significantly affected by CA (Tukey's $P < 0.05$) treatment as compared to CC, AS, and control, respectively (Fig.1a, b, c). It was noted that a single application of AS2022 also enhanced the plant growth in terms of number of grains, plant height, and spike length compared to the control.

Post-harvest soil status: After plant harvesting, the soil was collected to monitor the effects of different treatments and understand the better function and mechanisms. The soil nutrients parameters were subjected, including available nitrogen, enzyme activities, and microbial biomass in fungal and bacterial CFUs. In this regard, soil-available nitrogen and enzyme activities were significantly (Tukey's $P < 0.05$) affected by CA treatments as compared to the rest of the treatments, respectively (Fig.2, 3a, b, c, d). The CA treatment changes the soil available nitrogen and enzyme activities; it might be the reason to enhance the wheat crop compared to the rest of the treatments. In terms of microbial biomass, soil fungal and bacterial CFUs were also significantly (Tukey's $P < 0.05$) affected by CA treatment (Table 2). Furthermore, compared to the compost application and AS2022 was not significantly different, and both were able to enhance the soil enzyme activities and change the soil microbial biomass.

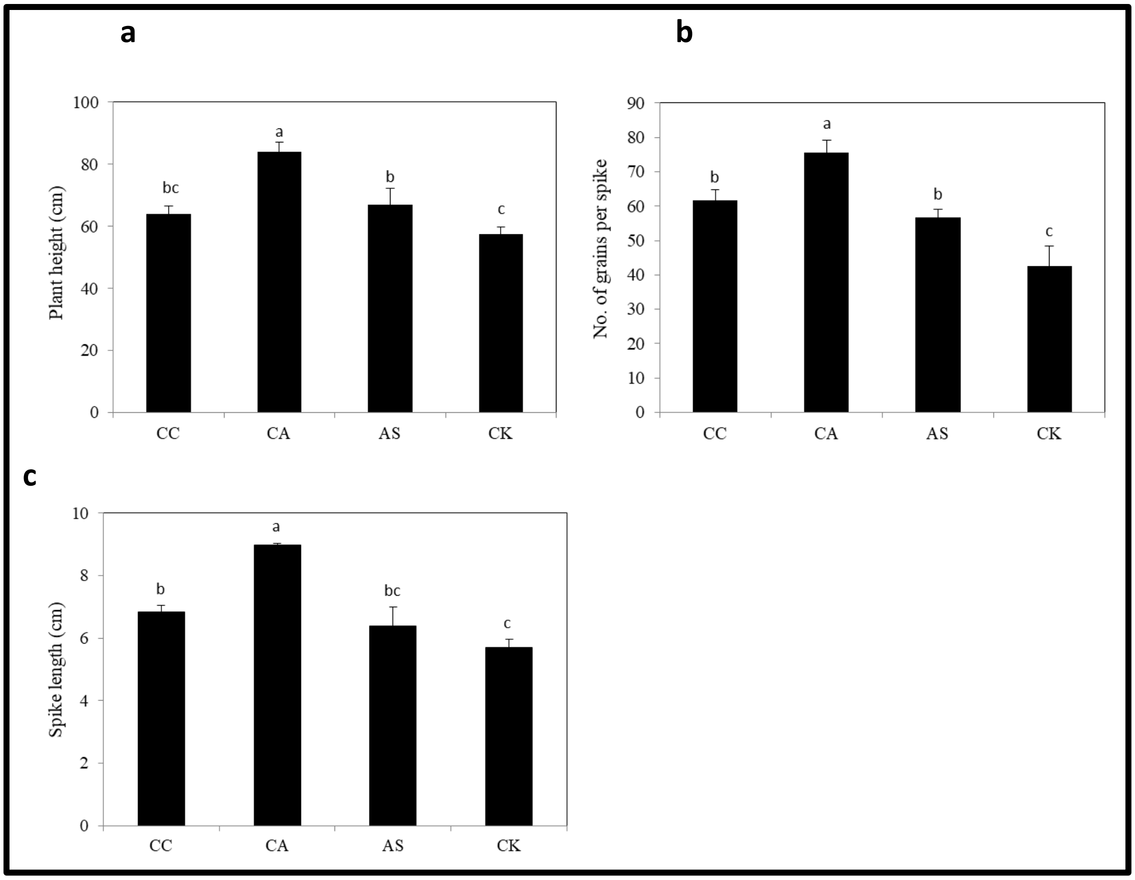

Fig. 1 Growth of wheat in a pot experiment with various treatments (cow compost (CC), cow compost + AS2022 (CA), AS2022 (AS) strain only and control-only soil (CK). The bars shown the standard deviation among replicates (n=5), and different letters indicates the statistical significance between treatments using Tukey's test (P <0.05). While, (Fig a) indicating the plant height, (b) the number of grains and (c) spike length.

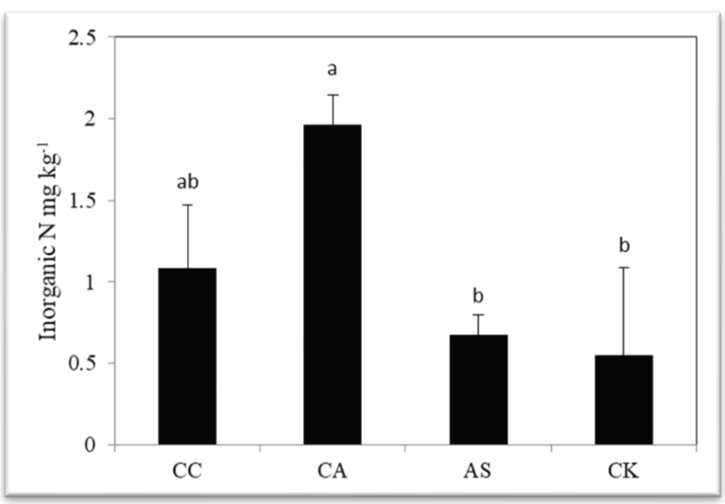

Fig. 2 The amount of inorganic nitrogen with various treatments (cow compost (CC), cow compost + AS2022 (CA), AS2022 (AS) strain only and control-only soil (CK). The bars shown the standard deviation among replicates (n=5), and different letters indicates the statistical significance between treatments using Tukey's test (P <0.05).

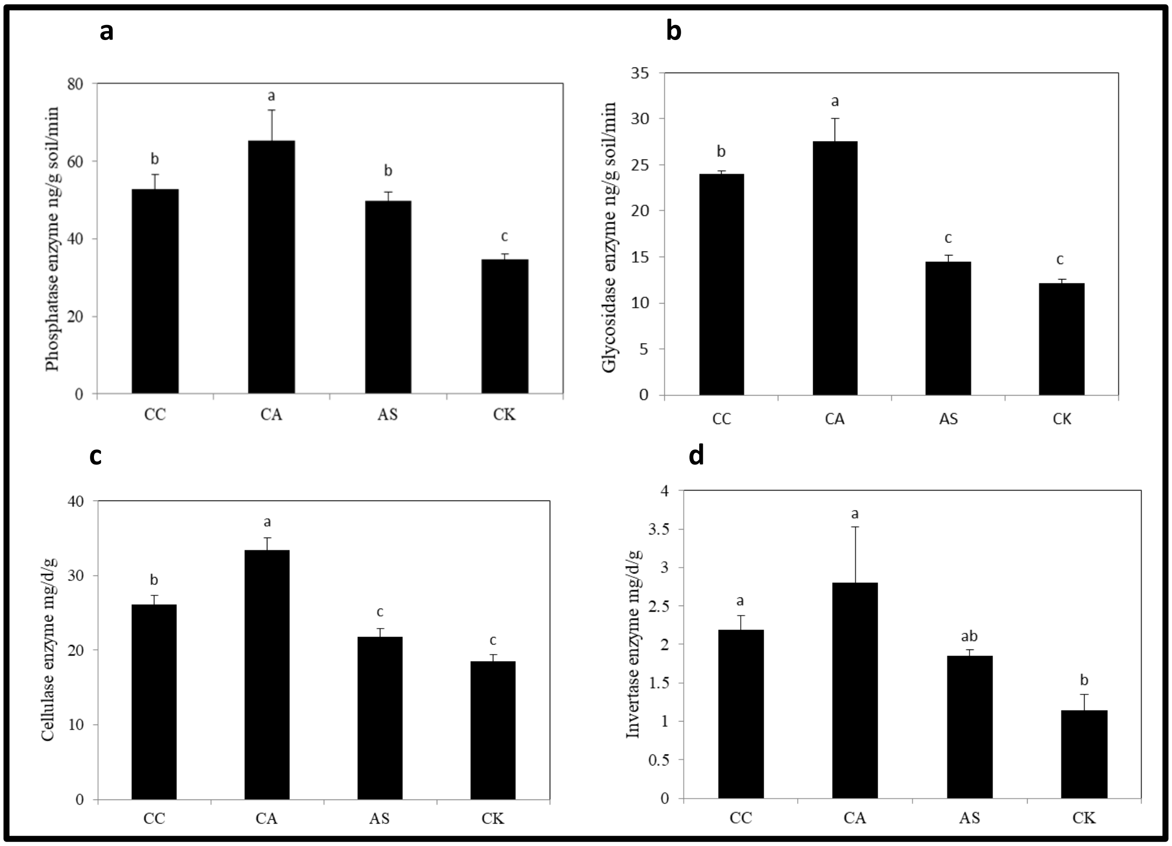

Fig. 3 The dynamic of enzyme activities in soil with various treatments (cow compost (CC), cow compost + AS2022 (CA), AS2022 (AS) strain only and control-only soil (CK). The bars shown the standard deviation among replicates (n = 5), and different letters indicates the statistical significance between treatments using Tukey's test (P <0.05). While (a) indicating the phosphatase enzyme (b), β-glycosidase enzyme (c), cellulase enzyme and (d) invertase enzyme.

Table 2 Microbial (fungal and bacterial) CFUs recovered from the each treatment of experimental soil.

Treatments	**CFU fungi g of soil (10^4)**	**CFU bacteria g of soil (10^6)**
CC	22±1 ab	39±5 b
CA	30±5 a	84±4 a
AS	25±4 ab	48±5 ab
CK	17±1 b	18±1 c

Note: CFUs (colony-forming units), values are shown as means ± SD (n=3). Different letter in the same row indicates significant differences using Tukey's test (P < 0.05).

Discussion

In this study we assessed the probability of wheat growth promotion with plant growth-promoting *Aspergillus* sp. strain AS2022 inoculation. As per our knowledge, this study was conducted for the very first time under controlled climate conditions to assess the effect of combined and alone application of fungal strains along with cow compost. It was noted that CA had greater biomass in terms of plant height, the number of grains, and spike length in the short-term pot experiment.

According to the parameters evaluated, the combined application of beneficial fungal strain AS2022 and compost promoted wheat crop compared to the alone application. *Aspergillus* sp. enhanced plant growth promotion by providing nutrient availability from the rhizosphere and their assimilation (Asghar & Kataoka, 2022a). Regarding the plant parameters, higher values were recorded for the combined application of AS2022 along compost, including plant weight, number of seed and spike height. Hu and Chabbi (2021) reported that when root growth is increased, plant enhances their efficiency and absorbs more nutrients and water. The ability shown for AS2022 is significant for plant growth promotion. Galeano *et al.* (2021) reported that *Aspergillus* sp. can P solubilization and siderophore production during quantitative and qualitative analysis. In agriculture, the availability of P is enhanced by siderophores, as they solubilize iron phosphate and chelate heavy metals, boosting nutrient uptake for plant growth promotion (Gontia-Mishra, Sapre, Sharma, & Tiwari, 2016). Furthermore, Asghar and Kataoka (2022a) and Mahmood, Iguchi, and Kataoka (2019) also reported similar results and documented that *Aspergillus* sp. can enhance plant growth promotion by changing the soil nutrient status.

Interestingly, it was noted that the combined application of strain AS2022 with cow compost accelerated the available nitrogen and extracellular enzyme activities (Fig.2, 3), compared to the single application of compost; it could directly or indirectly enhance the wheat crop and change the soil nutrient status. Schneider *et al.* (2010) reported that *Aspergillus* sp. can degrade organic matter and mineralize the nutrient in the rhizosphere, increase the accessibility for plant uptake, and also capable to solubilize nutrients like P and Fe. While Mahmood *et al.* (2019) reported that *Aspergillus* sp. can degrade the organic matter and release the nitrate and available phosphate. Thus, it was proposed that inoculation of AS2022 with compost resulted in wheat crop promotion through enhancing nutrients availability and increased soil enzyme activities, and improved the soil quality and health. Therefore, our results showed that combined application of beneficial fungal strain along compost is suitable and ecological friendly approach to enhance plant growth promotion through contributing organic matter degradation.

Soil microorganisms, including fungi and bacteria, can enhance plant growth by solubilizing nutrients, fixing atmospheric nitrogen and accelerating nutrient absorption (Harrison, 2003). Ideally, it was noted that soil bacterial and fungal CFUs were also significantly affected by the combined application of strain AS2022 along compost (Table 2). The change of microbial biomass, which could be subsidized to the degradation of organic matter and plant nutrient distribution from the rhizosphere to plant (Asghar & Kataoka, 2022a). Chuang, Kuo, Chao, and Chao (2007) stated that inoculation of beneficial fungi *Aspergillus* sp. change the soil fungal biomass, directly or indirectly promoting plant growth. The results predicted that soil microbial biomass might be responsible for the acceleration of soil enzyme activities and the availability of inorganic nitrogen. However, our short-term findings showed that the combined application of beneficial fungal strain AS2022 along with cow compost was suitable culture for wheat crop production and improvement of soil quality through organic matter degradation and accelerating soil nutrients.

Conclusions and Recommendations

In conclusion, we evaluated that inoculation of strain AS2022 along with cow compost was suitable for the acceleration of soil nutrients and wheat crop. The combined application of AS2022 and cow compost enhanced soil biological activities measured by soil microbial biomass and enzyme activities. Although, adding organic compost with AS2022 changed the soil nutrient status, such as inorganic nitrogen, which might enhance the wheat crop production. Inclusively, results suggested that combining organic materials with beneficial microbial strains can enhance soil nutrient availability and plant growth and development. In the future, functional bio-organic materials should be promoted for sustainable crop production and maintaining soil health, which might be affected by the heavy use of unnecessary synthetic fertilizers.

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