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Influence of Humic Acid on Growth and Yield of Mung Bean (*Vigna radiata* L.) under Different Irrigation Regimens

Fatemeh Lovimi*

PhD. Student, Department of Agro-Technology, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran.

RESEARCH ARTICLE

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ABSTRACT

BACKGROUND: Drought stress is one of the most important factors limiting the production of agricultural products. The use of natural fertilizers can lead to optimal water consumption in agriculture.

OBJECTIVES: In order to examine the impact of humic acid on yield of mung beans under different irrigation regimens, a study was conducted during the agricultural year 2014-15 in Ahvaz, south west of Iran.

METHODS: Current study was done by split-plot design according randomized complete blocks design (RCBD) with three replications. The investigated treatments included irrigation intervals at three levels (60, 100, and 140 mm evaporation from Class A pan) as the main factor and various concentrations of humic acid at three levels (0, 0.5, and 1 $g.lit^{-1}$) as the secondary factor.

RESULT: Results revealed that both irrigation and humic acid significantly affected seed yield, yield components and chlorophyll index. The highest seed yield, averaging 212 $gr.m^{-2}$, was achieved with the 100 mm irrigation treatment, while the lowest, averaging 102 $gr.m^{-2}$, was observed with the 140 mm irrigation treatment. Concerning the influence of humic acid, the treatment with 1 $g.lit^{-1}$ foliar application yielded the highest seed output (194 $g.m^{-2}$). The interaction between irrigation and humic acid had a noteworthy impact solely on seed yield and 100-seed weight. The most favorable seed yield and 100-seed weight were recorded in the treatment with 100 mm irrigation and 1 g.lit-1 foliar application of humic acid, while the least favorable results were associated with the 140 mm irrigation treatment without humic acid foliar application, resulting in a 29% decrease in yield compared to the treatment with 100 mm irrigation and 1 $g.lit^{-1}$ foliar application of humic acid. These findings underscore the significant role of humic acid in enhancing mung bean seed yield compared to scenarios without humic acid application.

CONCLUSION: In summary, the use of humic acid not only boosts Mung bean yield but also holds the potential to contribute significantly to sustainable agricultural objectives. Furthermore, its application can lead to a reduction in the reliance on chemical fertilizers, thereby mitigating environmental pollution, and the associated lower consumption contributes to cost-effectiveness.

KEYWORDS: *Chlorophyll, Crop production, Organic matter, Pulse, Seed Yield.*

***Corresponding Author:** Fatemeh Lovimi ✉ fatemehloveimi58@gmail.com

1. BACKGROUND

Water stands as a pivotal element in agricultural productivity. The yield of agricultural crops is often curtailed by the various live and non living environmental stresses, resulting in a considerable disparity between actual and potential crop yields (Sadeghipour, 2009). Insufficient moisture levels throughout various growth stages lead to diminished water and the nutrient absorption, impeded nutrient transport within the plant, and ultimately culminate in reduced seed yield or overall crop output (Ebrahimzadeh, 2012). Water stress is considered to be one of the major problems in global field crop production which led to a decrease in growth and yield, especially in arid and semiarid regions where there is not enough rain (Robertson *et al*., 2004). Water deficit caused between 11 and more than 40% reduction of biomass across the forage crops due to a decline in leaf gas exchange and leaf area. In addition, the result showed that the Harvest index decreased as a result of irrigation withholding in different growth stages. Limited irrigation water availability poses the question as to when and how much to irrigate to achieve optimum production and water uses efficiency. It is quite sensitive to water stress when compared to a series of other crops (Al-Shareef *et al*., 2018). The research results of Eiraji *et al*. (2023) showed that the cut off irrigation had a significant effect on the number of pods per plant, hundred seed weight, seed yield, biological yield, protein percentage, relative water content and proline content of bean leaves. Utilizing natural fertilizers, including humic acid, devoid of detrimental environmental impacts, holds promise for augmenting crop yields. Humic acid, a natural polymer with H^+ sites affiliated with carboxylic and phenolic acid functional groups (cation exchange sites). Commercial humic acid is a rich source of many essential nutrients including 6-8% hydrogen, 46-42% oxygen, 44-58% carbon and 4-5% nitrogen, as well as many other nutrients which encourage plant growth. Humic acid when applied to field converted into readily available humic substances which directly or indirectly effect the plant growth (Büyükkeskin and Akinci, 2011). It was reported that humic substances promote the activity of plant growth stimulating rhizobacteria (PGPR) to induce the growth promoting hormones in rhizospheric zone and these hormones increase the efficiency of roots to transport water and nutrients from soil solution to plants (El-Hassanin *et al*., 2016). Humic acid also act as natural antioxidant. Its presence in plant tissues affects many biochemical processes by increasing nutrient uptake and maintaining levels of amino acids and certain vitamins (El-Bassiouny *et al*., 2014). It was also concluded that addition of humic acid may reduce the requirement of primary macronutrients (N, P and K) at optimal growth (Daur and Bakhashwain, 2013). Xi *et al*. (2010) also observe the stimulatory effect on the plant micro and macronutrients uptake due to humic acid application. Legumes are critical sources of protein-rich sustenance for both human and livestock consumption. About 22% of plant-based protein, 32% of fats, and 7% of essential carbohydrates for humans are derived from legumes. Legume seeds rank as the second most crucial plant-based protein source after seeds (Majnoun Hosseini, 2015). Utilizing natural fertilizers, including humic acid, devoid of detrimental environmental impacts, holds promise for augmenting crop yields (Albayrak and Kamas, 2014; Hyder *et al*., 2019). It stimulates both shoot and root growth, with a more pronounced effect on the root system, enhancing root volume and efficiency. Akhtar *et al*. (2017) reported that humic acid significantly increases root and shoot length and yield of mung bean by activating hormonal activity. The research results of *Zyada et al.* (2020) indicated a positive and significant effect of potassium consumption on the growth and yield of Cowpea. During the flowering stage of Chickpeas, Sadeghi Moghadam *et al*. (2013) noted that foliar application of humic acid had a significant impact on the number of pods per plant and seeds per pod. Researches results showed that the application of organic fertilizers such as humic acid increased the total chlorophyll, the number of pods per plant, the number of seeds per pod, the 100 seed weight and the seed yield of Cowpea plants (Faiyad *et al*., 2019; El-Beltagi, 2023). Khazraei (2013) observed an increase in the number of pods and seeds per pod in Red kidney beans with humic acid use. Sadeghipour (2009) found that the timing of irrigation significantly influenced the yield and yield components of mung beans. Non-irrigation during flowering reduced the number of pods per plant, seeds per pod, and seed yield, while limited irrigation during pod filling reduced 1000-seed weight. Haghparast *et al*. (2013), examining the alleviation of negative drought stress effects in Chickpeas through humic acid application, reported a significant interactive effect on the number of pods per plant and 100-seed weight. Additionally, the use of humic acid in foliar spray influenced the number of pods and seeds per pod. Shahbazi *et al*. (2015) studying wheat indicated that humic acid, applied during drought stress, improved root growth and development, enhancing access to water and nutrients, thus positively affecting characteristics such as increased photosynthetic organ volume. These factors directly contributed to a substantial enhancement in cultivated crop yield. By this, a better environment for roots in addition to the plant growth is provided (Davies *et al*., 2004). Humic acid improves the physical (Varanini *et al*., 1995), chemical and biological properties of soils (Mikkelsen, 2005). The role of humic acid is well known in controlling, soil-borne diseases and improving soil health and nutrient uptake by plants and mineral availability (Mauromicale *et al*., 2011). Humic acid-based fertilizers increase crop yield (Mohamed *et al*., 2009), stimulate plant enzymes/hormones and improve soil fertility (Sarir *et al*., 2005). Humic compounds can help to improve the soil structure by increasing the amount of pore space and enhancing the air exchange, water movement, water holding capacity and root growth. As a result, better drought resistance and reduction in water usage can be done (Khattak and Muhammad, 2006; Sharif *et al*., 2003). Besides water conservation, soil amendments have different, other benefits to quality of crop and soil (Peter *et al*., 2005; Piccolo *et al*., 2007).

2. OBJECTIVES

This experiment aims to investigate the impact of water stress at various growth stages on mung beans and the efficacy of foliar spray with humic acid in alleviating potential negative consequences of drought stress.

3. MATERIALS AND METHODS

3.1. *Field and Treatments Information*

This study was conducted during the agricultural year 2014-15 at Shahid Salemi Farm in Ahvaz, situated at 36 degrees and 24 minutes north latitude, 45 degrees and 9 minutes east longitude, with an elevation of 20 meters above sea level. The soil at the research site had a loamy clay texture, a pH of 7.5, and an electrical conductivity of 1.3 dS/m. The experiment was arranged using a split-plot design within randomized complete blocks design (RCBD) with three replications. The main factors studied included three irrigation intervals (60, 100, and 140 mm evaporation from Class A pan), and the secondary factor involved various levels of humic acid (0, 0.5, and 1 $g.lit^{-1}$) applied as foliar spray at two growth stages (four-leaf and flowering). Soil tillage and fertilization operations (50 kg pure nitrogen from urea, 80 kg pure phosphorus from ammonium phosphate, 80 kg pure potassium from potassium sulfate) were carried out.

3.2. *Farm Management*

Following fertilization, the field soil was mixed using a light disk, and furrows were created at 50 cm intervals using a furrow opener. Planting was done manually on the 1st of July, 2014, using seeds of the Gohar variety. The experiment consisted of 27 plots, each containing six rows of crops, each 5 meters long, with a spacing of 10 cm between plants on the row. The main plot spacing was 1.5 meters, and the sub-plot spacing was 1 meter. The first irrigation was performed one day after planting, and subsequent irrigations were based on the treatments according to Class A pan evaporation.

3.3. *Measured Traits*

To measure yield and its components, the side rows and half a meter from the beginning and end of each plot were removed as margins. The final harvest took place on the 1st of November, 2014, in an area equivalent to 2 m^2 from rows 3, 4, and 5. For measuring the number of pods per plant, 10 samples were randomly selected from the harvested samples. The 100-seed weight was determined by selecting five samples randomly from the harvested seeds of each plot. The weight was measured using a digital scale with an accuracy of 0.1 g. In addition, by measuring the weight of samples taken from each plot, the seed yield was estimated. To measure the chlorophyll index during the flowering stage, three leaf parts from 10 selected plants were chosen using a SPAD-502 chlorophyll meter. The relative leaf water content was calculated using the formula (Alizadeh, 2002):

Equ.1. Relative water content (RWC%) =[(Leaf fresh weight (g) - Leaf dry weight (g))/ ((Leaf saturated weight (g)- Leaf dry weight (g)] ×100

3.4. Statistical Analysis

Analysis of variance and mean comparisons were done via SAS (Ver.8) software and Duncan multiple range test at 5% probability level.

4. RESULT AND DISCUSSION

4.1. *Number of Pod per Plant*

The results from the analysis of variance underscore the significant impact of humic acid and irrigation intervals on the number of pods per plant (Table 1). The highest number of pod per plant is linked to the 100-millimeter evaporation from the Class A pan treatment, registering an average of 12.3, while the lowest pod per plant is attributed to the 140 millimeter evaporation treatment, averaging 9.18 (Table 2). The reduction in pod count in the 140 millimeter evaporation treatment is likely due to a decrease in the number of flowers or the shedding of flowers and pods. Plants exposed to stress during the flowering stage often exhibit a diminished number of pods and seeds due to a shortened flowering period and sterility of certain flowers, aligning with the previous studies indicating a decrease in pod formation and an increase in pod shedding under post-flowering drought stress (Mathur *et al*., 2007). A comparison of different levels of humic acid on the number of pods per plant (Table 2) reveals that the highest pod per plant is observed in the treatment with 1 g of humic acid per liter, averaging 11.71, while the lowest pod per plant is associated with the treatment without humic acid, with an average of 10.3. The upward trend in the number of pods per plant, influenced by increasing humic acid, strongly indicates the positive impact of this treatment on this trait.

Table 1. Result analysis of variance of measured traits

S.O.V	df	No. pods per plant	No. seeds per pod	100 seed Weight	Seed yield	Chlorophyll Index	Relative leaf water content
Replication	2	0.597	0.03028	1.0544	1015.2	11.75	0.43
Irrigation intervals (a)	2	24.23**	3.19**	10.34**	26993.7**	83.49*	93.15*
Error I	4	2.1859	0.09111	0.4621	391.7	10.12	38.95
Humic acid (b)	2	4.482**	2.33**	10.12**	15559.5**	49.49*	58.22*
a × b	4	0.119^{ns}	0.075ns	1.86**	1848.7**	5.06ns	11.33ns
Error II	12	0.1296	0.086	0.2727	144.1	13.24	18.57
CV (%)		3.26	2.29	7.56	10.9	6.4	5.07

$^{ns,\ *\ and\ **}$: no significant, significant at 5% and 1% of probability level, respectively.

Previous studies have reported that humic acid, through its positive physiological effects such as increased metabolism within cells and higher chlorophyll content in leaves, enhances leaf longevity and ultimately increases plant yield and yield components (Nardi *et al*., 2002; Ayman *et al*., 2009).

4.2. *Number of Seeds per Pod*

The results indicated that the number of seeds per pod was significantly influenced by humic acid and irrigation intervals (Table 1). A comparison of the average effect of irrigation intervals on the number of seeds per pod showed the highest number of seeds per pod in the treatment with 100 millimeters evaporation from the pan, with an average of 10.5, and the lowest number of seeds per pod in the treatment with 140 millimeters evaporation from the pan, with an average of 9.4 (Table 2). In the treatment with 100 millimeters evaporation from the pan, the plant was able to allocate the highest number of pods and the highest number of seeds per pod to itself by appropriately utilizing all environmental conditions and sufficient development of the vegetative organs and adequate production of photosynthetic materials. Sadeghipour (2009) stated in an experiment on the effect of limited irrigation on mung bean that the effect of water scarcity on its yield and components was significant. The treatment without irrigation in the flowering stage reduced the number of pods per plant, the number of seeds per pod, and seed yield, which was consistent with the results of this study. Comparisons of the average effect of different levels of humic acid also showed that the highest number of seeds per pod was related to the treatment with 1 g.lit^{-1}, with an average of 10.47, and the lowest number of seeds per pod was related to the treatment without humic acid, with an average of 9.51 (Table 2).

Table 2. Mean comparison effect of different level of irrigation intervals and humic acid on studied traits

Treatments	No. pods per plant	No. seeds per pod	Chlorophyll index	Relative leaf water content (RWC)
Irrigation intervals				
60 mm evaporation from class A evaporation pan	11.48b	10.33a	55.32b	85.13b
100 mm evaporation from class A evaporation pan	12.36a	10.53a	58.63a	88.6a
140 mm evaporation from class A evaporation pan	9.18c	9.41b	54.77b	80.8c
Humic acid				
Zero or control	10.3b	9.51b	54.14b	83.4b
0.5 g.lit^{-1}	11.03a	10.28a	57.17a	85.2a
1 g.lit^{-1}	11.71a	10.47a	57.41a	85.9a

***Means with similar letters in each column are not significantly differentt by Duncan's test at 5% probability level.

It seems that foliar application with humic acid compounds influenced the number of seeds per pod. In this study, foliar spraying with humic acid affected the number of seeds per pod and increased this trait, which was consistent with the results of Haghparast *et al*. (2013). In an investigation conducted by Kaya *et al*. (2014) on Chickpeas, it was found that foliar spraying of humic acid significantly increased yield and yield components in the plant during the 3 to 6 leaf stage. Additionally, the humic acid treatment led to an increase in the number of seeds per pod and the number of pods per plant.

4.3. *100-Seed Weight*

The results indicate a substantial influence of humic acid, irrigation intervals and their interplay on the 100 seed weight (Table 1). Exploring the interaction between humic acid and irrigation intervals, the most significant weight (72.9 g) is linked to the treatment involving 100 millimeters evaporation from the Class A pan coupled with foliar application of 1 $g.lit^{-1}$ humic acid. Conversely, the lowest weight (25.5 g) is associated with the treatment of the 140 millimeters evaporation from the Class A pan without foliar application of humic acid (Table 3). Ayman *et al*. (2009) previously reported an augmentation in Chickpea's weight of 100 seeds weight due to the synergistic impact of natural materials in foliar spraying with humic acid under both normal and drought stress conditions. In this study, withholding irrigation during the flowering stage led to reduced pod per plant, seeds per pod, and overall seed yield. On the other hand, limited irrigation during the pod-filling stage resulted in diminished weight of 1000 seed weight. Notably, traits such as the 1000 seed weight in mung bean exhibited lower susceptibility to drought stress (Zabet *et al*., 2003). Analyzing various concentrations of humic acid on the 100 seed weight (Table 3) reveals that the treatment with 1 $g.lit^{-1}$ of humic acid yielded the highest weight (averaging 77.1g). In contrast, the treatment without humic acid exhibited the lowest weight (averaging 75.5g). The observed increase in seed weight in the 1 $g.lit^{-1}$ humic acid treatment could be attributed to enhanced development of antheridium cells, amyloplasts, and photosynthetic materials, possibly influenced by the growth-promoting effects of hormones. The findings suggest that foliar application of humic acid contributes to elevated plant growth, increased pod per plant, higher pod weight, augmented protein content, and enhanced chlorophyll levels in the plant through improved nutrient absorption. These results align with Ayman *et al*. (2009) findings in Chickpeas, underscoring the positive impacts of foliar spraying with humic acid on various growth and yield-related parameters. Further exploration of the molecular and the physiological mechanisms underlying these responses holds promise for refining crop management practices.

Table 3. Mean comparison interaction effects of different levels of irrigation and humic acid on 100 Seed weight, seed yield

Irrigation interval	Humic acid ($g.lit^{-1}$)	100 Seed weight (g)	Seed yield ($g.m^{-2}$)
60mm evaporation from class A evaporation pan	Zero or control	5.737d*	119.825de
	0.5	7.693bc	183.918c
	1	7.057bc	187.045c
100mm evaporation from class A evaporation pan	Zero or control	6.093cd	141.349d
	0.5	8.247ab	219.737bc
	1	9.72a	275.17a
140mm evaporation from class A evaporation pan	Zero or control	5.293d	79.817e
	0.5	5.983cd	106.341de
	1	6.363cd	122.198de

*Means with similar letters in each column are not significantly differentt by Duncan's test at 5% probability level.

4.4. *Seed yield*

The results from the variance analysis underscore a substantial influence of humic acid and irrigation intervals on seed yield, with a notable interplay between humic acid and irrigation intervals (Table 1). A closer examination of the interactive effect reveals that the most robust seed yield, amounting to 172.275 $g.m^{-2}$, is linked to the treatment involving 100 millimeters of evaporation from the Class A pan coupled with a foliar application of 1 $g.lit^{-1}$ of humic acid (Table 3). Conversely, the reduction in the irrigation interval in the treatment with 60 millimeters of evaporation, due to the imposition of waterlogging stress and suboptimal utilization of environmental resources, resulted in a more pronounced decline in seed yield compared to the treatment with 100 millimeters of evaporation, aligning with the findings of Tohidi (2015). Thomas *et al*. (2003) reported that the drought stress, the influencing physiological processes in plants, leads to a decrease in stomatal conductance, current photosynthesis and the plant's capacity to use available resources, consequently diminishing seed yield in Mung beans. Haghparast *et al*. (2013) observed a 13% reduction in seed yield in various Chickpea varieties under drought stress. Nardi *et al*. (2002) highlighted that humic acid ensures the sustained activity of photosynthetic tissues, enhancing plant yield by positively affecting the metabolism of plant cells and increasing chlorophyll concentration in leaves, thereby elevating overall plant yield. Sharif *et al*. (2002) reported an augmentation in maize seed yield due to the positive impact of humic acid on improving photosynthesis and enhancing nutrient uptake in plants.

4.5. *Chlorophyll Content*

The results derived from the variance analysis indicate a significant influence of humic acid and irrigation intervals on the Chlorophyll Content (Table 1). Delving into a comparison of mean irrigation intervals unveils that the treatment involving 100 millimeters evaporation from the Class A pan boasts the highest chlorophyll index, registering an average of 6.58%. On the flip side, the treatment subjected to 140 millimeters evaporation from the Class A pan exhibits the lowest chlorophyll index, standing at an average of 5.54% (Table 2). The maximum of the chlorophyll index during the growth season is attributed to the treatment involving 100 millimeters evaporation from the Class A pan, owing to optimal provision of water, nutrients, and the absence of pathogenic influences. Leaves with heightened chlorophyll indices tend to prolong their exposure to radiation, positively impacting their photosynthetic efficiency (Lindquist *et al*., 2005). Conversely, the nadir of the chlorophyll index is observed in irrigation intervals of 60 and 140 millimeters evaporation from the Class A pan. This is attributed to the fact that drought stress induces a reduction in leaf surface area(LAI) and the rate of photosynthesis, primarily due to biochemical constraints such as diminished photosynthetic pigments, notably chlorophylls (Earl and Davis, 2003). Considering the decrement in plant water potential under drought stress, the plant responds by modulating relative water content through partial stomatal closure during the daytime. The concomitant stomatal closure leads to an escalation in oxidative stress intensity, chlorophyll degradation, and a reduction in leaf chlorophyll content. This deduction is consistent with findings reported by other researchers (Brevedan and Egli, 2003). An examination of mean levels across different humic acid treatments reveals that treatments with 0.5 and 1 $g.lit^{-1}$ of humic acid do not exhibit a significant difference but both show a marked distinction compared to the control treatment. The apogee of the chlorophyll index is associated with the treatment involving 1 $g.lit^{-1}$ of humic acid, registering an average of 4.57%. Conversely, the nadir of the chlorophyll index is linked to the treatment without humic acid, reflecting an average of 4.54% (Table 2). This aligns with Khazraei (2013) findings in a study on the growth characteristics of Red kidney beans, where the use of humic acid resulted in an increased leaf chlorophyll index compared to the control treatment. Ayman *et al*. (2009), in their investigation of fava beans, reported that foliar application of humic acid enhanced plant growth and leaf chlorophyll content by augmenting nutrient absorption.

4.6. *Relative Leaf Water Content* (RWC)

The results of the analysis of variance for the data indicated that the relative leaf water content was significantly affected by humic acid and irrigation intervals (Table 1). A comparison of the average effect of irrigation intervals showed that the highest percentage of relative leaf water was associated with the treatment of 100 millimeters evaporation from the pan, with an average of 88.6%, and the lowest percentage of relative leaf water was related to the treatment of 140 millimeters evaporation from the pan, with an average of 80.8% (Table 2). The low relative leaf water content in the 140 millimeters evaporation from the pan treatment can be argued to have a close relationship with plant water potential. Water scarcity stress leads to stomatal closure and reduced leaf development due to the consumption of photosynthetic materials in regulating the plant's water status. Additionally, due to the reduction of carbon dioxide available to the plant, the rate of photosynthesis decreases. O'Neill *et al*. (2006) stated that severe water scarcity stress increases leaf temperature, resulting in wilting, curling, and premature aging of corn leaves. This also leads to a reduction in the absorption of active photosynthetic radiation and a decrease in dry matter production. As water scarcity stress increases, osmolytes accumulate in the plant, consuming a considerable amount of energy. Consequently, the energy that could be used for the growth and development of leaves is diverted to reduce osmotic potential, resulting in a decrease in the leaf surface area index. Comparison of the average levels of different humic acid treatments on the percentage of relative leaf moisture showed (Table 2) that the highest percentage of relative leaf moisture was associated with the treatment of 1 gram per liter of humic acid, with an average of 85.9, and the lowest percentage of relative leaf water was related to the treatment without humic acid, with an average of 83.4. Resaei *et al*. (2012) reported that the application of humic acid had a significant effect on the chlorophyll a, b, and total leaf water content in comparison to its non-application in chickpea varieties. In general, it can be concluded that the application of humic acid in improving some physiological traits related to drought stress can be beneficial, which is consistent with the findings of this study.

5. CONCLUSION

The utilization of humic acid demonstrates a positive influence on Mung bean yield and certain agronomic characteristics associated with seed yield. These impacts are likely attributed to the physiological effects of these compounds. Notably, the interplay between irrigation intervals and humic acid significantly affected seed yield and the 100 seed weight. The most noteworthy results, in terms of both seed yield and weight of 100 seed weight, were observed in the treatment involving 100 millimeters of pan evaporation and a foliar application of 1 $g.lit^{-1}$ of humic acid. The application of 0.5 and 1 $g.lit^{-1}$ of humic acid resulted in a higher number of pod per plant and increased 100 seed weight, indicating enhanced economic efficiency compared to alternative treatments. In summary, the use of humic acid not only boosts Mung bean yield but also holds the potential to contribute significantly to sustainable agricultural objectives. Furthermore, its application can lead to a reduction in the reliance on chemical fertilizers, thereby mitigating environmental pollution, and the associated lower consumption contributes to cost-effectiveness.

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FOOTNOTES

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