

# HUMIC ACID: SOURCES,EXTRACTION METHODS AND IT APPLICATION EFFECTS ON RICE CROP –A MINI REVIEW

## Abstract

*Oryza sativa* L., commonly known as rice, is a crop that is extensively cultivated worldwide and is recognized as the "global grain." In the current context, the pressing requirement for sustainable agricultural practices that ensure food production without compromising crop yield and producer income has become a significant objective. This is due to the escalating environmental pollution, augmented use of chemical fertilizers, and the gradual deterioration of cultivated soils. In order to mitigate the aforementioned environmental degradation, minimize the reliance on chemical fertilizers, reduce cultivation expenses, and achieve sustainable production, a viable and ecologically sound solution would involve the utilization of natural plant bio-stimulants (PBs). These PBs have the capacity to augment flowering, promote plant growth, facilitate fruit set, enhance crop productivity, and improve nutrient use efficiency (NUE). Additionally, they possess the ability to enhance tolerance against various abiotic stresses. Studies have demonstrated that bio-stimulants such as humic acid, which encompass an intricate blend of polysaccharides, micronutrients, and plant growth hormones, facilitate the growth of plants and enhance their ability to withstand abiotic stresses. Humic substances, being highly adaptable and indispensable constituents of the inherent soil-ecosystem, have endured for centuries. Foliar application of humic acid could increase osmotic regulators compared with other fertilizer resources, reducing the damage caused by drought stress to some functional traits of cultivars. Humic acid, in comparison with other nutritional systems at higher levels, lead to increase in chlorophyll levels. It also causes photosynthetic stability with increase in yield. Humic acid exerts a beneficial influence

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on the efficacy of enzymes and nutrients in plant metabolism. It improves the chemical, physical and biological characteristics of the soil. In this mini review, we detailed the humic acid sources, extraction methods and its benefits and effect of humic acid application combined with NPK fertilizer to rice.

**Key words:** Drought and saline stress, Humic acid, Rice, Yield

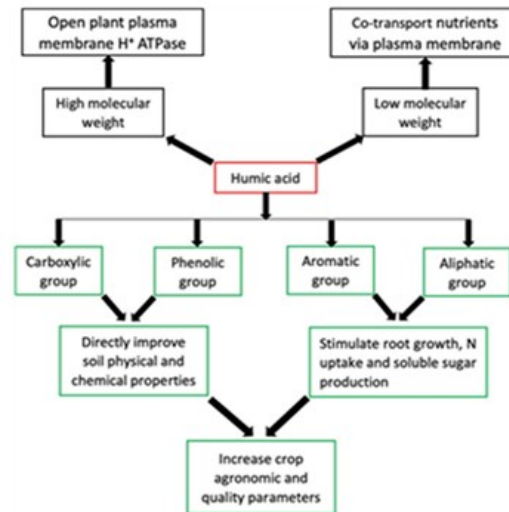
## I. INTRODUCTION

The enduring and multifaceted nature of humic substances make them fundamental components of the natural soil ecological community, having played a crucial role for ages (Mayhew, 2004). Humic acid stands as the hindmost culmination of the natural breakdown of flora and fauna. The genesis of humus arises from the combination of chemical and biological humification processes involving plant and animal matter, alongside the influential involvement of microbe. These substances are the most extensively dispersed organics of biosynthesis on the Earth's surface, exceeding the quantity of organic carbon found in all living organisms.

Humic substances offer a targeted and cost-effective alternative to traditional fertilization methods for replenishing depleted humus in soil. The integration of humic substances into the soil has demonstrated the ability to augment plant growth beyond the sole influence of nutrients. As they have profits in agricultural soils, particularly those with poor organic content, humic materials are widely utilized worldwide. Sources of Humic Acid include lignite, coal, organic materials, soils. The application of humic acid can have a positive impact on various soil properties, including soil structure, soil texture, pH, CEC, nutrient availability, soil carbon, nitrogen cycling and enzymes.

## II. HUMIC ACID

**1. Structure-Function Relationship of Humic Acids:** The characteristics of HA are correlated with their respective structures, which are contingent upon their sources. (Nardi et al., 2021).



**Figure 1:** Aromatic and Aliphatic functional groups of humic acid

- **Aromatic and Aliphatic Functional Groups of Humic Acid:** A recent study conducted by van Tol de Castro (2021) has demonstrated that the humic acid's aromatic and aliphatic functional groups are accountable for augmenting N uptake and soluble sugars, leading to a corresponding rise in rice yield. In contrast, an earlier discovery by Garcíá (2016) has validated that the aliphatic and aromatic functional groups of Humic substances stimulate root growth in rice seedlings.
- **Phenolic and Carboxylic Groups of Humic Acid:** The composition of humic acid encompasses a multitude of functional groups, among which the OH and COOH groups are the most prominent. The OH and COOH functional groups are primarily bound to the functions of humic acid, including enhancing soil chemical and physical properties as well as promoting plant growth.

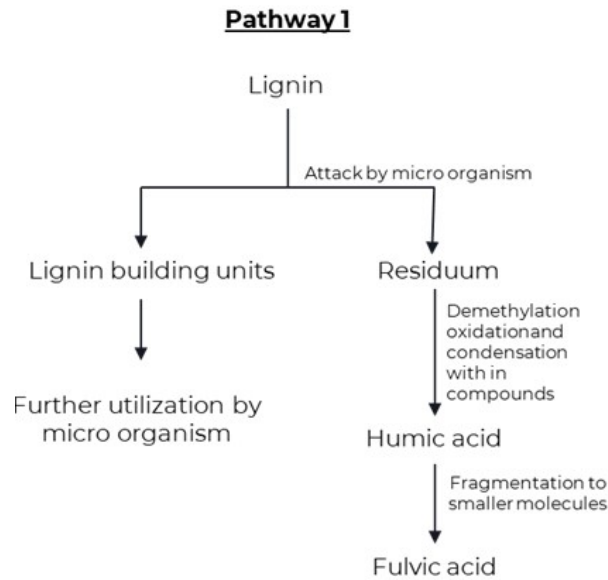
The disassociation of these functional groups leads to the creation of non-polar and polar terminations, which correspond to the hydrophobic and hydrophilic components. The hydrophilic end chiefly serves chelating functions, whereas the hydrophobic end is affiliated with repelling purposes.

- **Low molecular Weight Group and High Molecular Weight Group of Humic Acid:** Humic acids characterized by low molecular weight exhibit a higher concentration of phenolic and carboxylic groups compared to those with high molecular weight. The ability to chelate Humic acid has also been attributed to Low molecular weight HA, which is effective in modifying soil biochemical properties. The High molecular weight humic acid is potent in enhancing the soil physical conditions. Humic acid with high molecular weight has also been observed to elate plasma membrane H<sup>+</sup> ATPase, helping low molecular weight Humic acid in co-transporting minerals and execute different biological activities in plants.
- 2. Formation of Humic Substances:** Humification is a natural process whereby organic matter, undergoes transformation into humic substances through geo-microbiological mechanisms. The commencement of this procedure occurs upon the interaction of organic remains from flora and fauna with microorganisms present in the soil. Throughout the process of humification, microorganisms consume carbon compounds to fuel their metabolic activities, leaving behind undigested remnants that amass as humus. It is imperative to acknowledge that although diverse pathways are available for the production of humic substances in nature, their prevalence may differ across distinct environments. The initial stage of humus formation involves the ingestion of nutrients from organic residues by microorganisms. The aforementioned process ultimately culminates in the emergence of intricate chemical systems that exhibit greater stability compared to the original materials. Once the organic matter undergoes a specific level of decomposition, it can be referred to as humus, comprising a complex blend of organic compounds such as humic acid, fulvic acid, and humins.

Numerous theories have been postulated to elucidate the genesis of humic substances during the decomposition of animal and plant matter in soil. Nevertheless, irrespective of the particular pathway that is favored, all pathways are deemed feasible for

the natural synthesis of humic substances. However, the extent to which each pathway contributes may vary contingent upon the specific environmental conditions.

- **The Lignin Theory (pathway 1):** This theory posits that microorganisms do not fully utilize lignin, resulting in its residue becoming integrated into the soil humus. The conversion of lignin involves the removal of methoxy groups (-OCH<sub>3</sub>), leading to the formation of carboxyl groups (-COOH) and o-hydroxyphenols (C<sub>6</sub>H<sub>5</sub>OH). This remnants composes humic and fulvic acids.

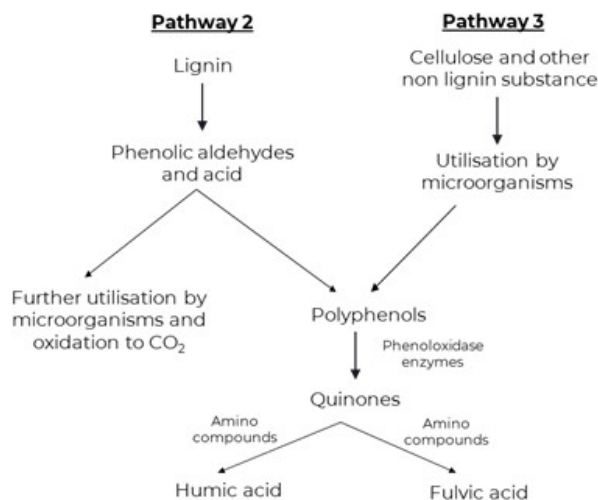


**Figure 2: Lignin theory**

The lignin theory of humic acid formation was supported by subsequent evidence cited by Waksman (1932), which included the following observations:

- The majority of fungi and bacteria struggle to decompose both lignin and humic acid.
  - Both lignin and humic acid exhibit partial solubility in alcohol and pyridine.
  - Both lignin and humic acid exhibit solubility in alkali solutions and can be precipitated by the addition of acids.
  - Both lignin and humic acid possess acidic properties.
  - The warming of lignins with aqueous alkali results in their conversion into methoxyl-containing humic acids.
  - Humic acids share similarities with oxidized lignins.
- **The Polyphenol Theory (Pathway 2 and 3):** Pathways 2 and 3 exhibit a remarkable degree of similarity, with the exception that polyphenols in pathway 2 are synthesized by microorganisms utilizing non-lignin carbon (C) sources such as cellulose. The polyphenols undergo enzymatic oxidation, resulting in the formation of quinones. These quinones are subsequently converted into humic substances. In pathway 3, it is

postulated that the primary building blocks of humic and fulvic acids are quinones which is originated from lignin, in conjunction with compounds produced by microorganisms. The starting materials for pathway 3 are cellulose and other non-lignin substances.



**Figure 3:** Polyphenol theory

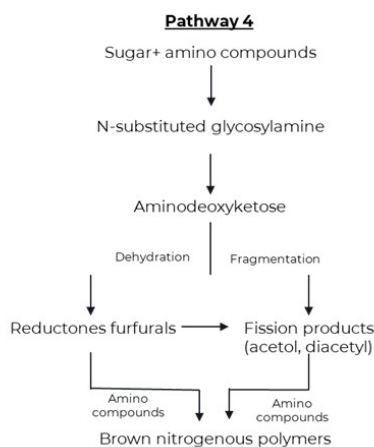
In this pathway, lignin continues to perform a decisive role in the synthesis of humus, albeit in a distinct manner from the traditional understanding of lignin. When lignin (the starting material for pathway 2) is attacked by microbes, phenols, aldehydes, and acids are released and go through enzymatic modification into quinones. These quinones then polymerize, with or without the existence of amino compounds, to form micromolecules that resemble humic substances (Stevenson, 1982). Amid the deterioration of plant residues, lignin becomes detached from cellulose and undergoes oxidative splitting, resulting in the genesis of primary structural units that are by-products of phenyl propane.

The side chains of these lignin units are subsequently oxidized, leading to demethylation. Polyphenols ensuing from this process are then transformed into quinones by polyphenoloxidase enzymes. These quinones, originating from lignin and potentially other sources, react with nitrogen-containing compounds to produce dark-colored polymer humic materials.

- **Sugar Amine Condensation (Pathway 4):** In accord with to pathway 4, reducing sugars and amino acids, which are derivatives of microbial metabolism, go through nonenzymatic polymerization to form brown nitrogenous polymers similar to those formed at moderate temperatures, while dehydrating specific food items. Dire and recurrent changes in the soil environment along with the mixing of reactants and mineral substances with catalytic properties can also aid condensation.

An intriguing facet of this theory pertains to the copious production of reactants, such as sugars and amino acids, by the actions of microorganisms. The

process of sugar and amine condensation associates the addition of the amine to the aldehyde group of the sugar, resulting in the formation of n-substituted glycosylamine. Subsequently, this glycosylamine undergoes oxidation to yield n-substituted-1-amino-deoxy-2-ketose. This compound goes through fragmentation and dehydration processes. Fragmentation leads to the production of 3-carbon chain aldehydes and ketones like acetol and diacetyl, whilst dehydration results in the genesis of reductones and hydroxymethyl furfurals. In the presence of amino compounds, the aforementioned compounds undergo facile polymerization to yield brown-colored products, specifically humic and fulvic acids (Stevenson, 1982).



**Figure 4:** Sugar amine condensation theory

- 3. Sources of Humic Acid:** Humic substances can also derive from organic matter such as roots, litter, deceased organisms, and excretions of living things found in both terrestrial and aquatic environments. Historically, scholars have been segregating humic materials from soils and water. These humic substances have been regenerated within the soil through agricultural practices including crop rotation, cultivation of legumes, incorporation of green manure, and treatment by compost.

**Table 1:** Different sources of humic substances with different concentrations

Natural Source	% Humic acid / Fulvic acid
Leonardite	25 to 90
Compost	5 to 25
Peat	5 to 20
Lignite	5 to 15
Manure	1 to 3
Soft coal	2 to 5
Hard coal	0 to 1

#### 4. Extraction of Humic Acid

- **Extraction of Humic acid from lignite by fractionation procedure by Stevenson (1982)**

The Lignite is pulverized and subsequently filtered through a sieve with a mesh size of 0.25mm.



The lignite powder that has been passed through a sieve is dissolved in a solution of sodium hydroxide with a concentration of 0.5N. The ratio of lignite to sodium hydroxide is 1:10.



The dark brown solution is subjected to filtration using Whatman No.1 filter paper.



The filtrate is gathered, and subsequently, the pH of the solution is modified to 1.0 using concentrated hydrochloric acid (HCl). Following this adjustment, the precipitate (HA) is permitted to settle.



The liquid portion, known as the supernatant (FA), is extracted through siphoning, while the mixture is subjected to filtration.



The precipitate undergoes purification through redissolution in a solution of 0.5N NaOH, followed by reprecipitation using concentrated HCl. This process is repeated a total of five times.



Subsequently, the humic acid is subjected to a thorough rinsing process using distilled water until all traces of chloride are eliminated. Following this, the humic acid is dried and subsequently pulverized into a finely textured powder.



- **Extraction of Humic Acid from Lignite by KOH-Hydrothermal Method**

The present study involves the treatment of a 20 g sample of lignite powder with potassium hydroxide (KOH) and distilled water while maintaining its air-dried state.



Subsequently, the amalgamation is introduced into an oven set at a temperature range of 130 to 190 degrees Celsius, and allowed to cool naturally until it reaches room temperature.



The supernatant is isolated from the residues through the process of centrifugation. Then, the residue is subjected to multiple washes with distilled water until it approaches a state of near-neutral pH.



The remaining substance is subjected to filtration and subsequently subjected to desiccation in a vacuum oven at a temperature of 105 °C.



The supernatant is acidified to a pH value below 2 using hydrochloric acid (HCl), subsequently subjected to centrifugation for further separation, and finally dried in a vacuum oven at a temperature of 60 °C.



The dried material acquired corresponds to Humic Acid.

- **Extraction of Humic Acid from Lignite by Ion Exchange Method**

The extraction of humic acid from lignite involves the combination of two methods: nitric acid peroxidation and alkali solution and acid eduction.



Lignite undergoes a process of crushing and sieving, employing 80 mesh sieves.



The lignite sample, weighing 10 grams, is subjected to centrifugation for a duration of one hour in the presence of 0.5M NaOH.

↓

The solution obtained is subjected to filtration using a Whatman filter paper.

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In order to obtain a solid form of humic acid, the filtrate is subjected to treatment with resin cation.

## 5. Benefits of Humic Acid

### • Physical Benefits

- Enhanced soil structure: Mitigates excessive water and nutrient depletion in sandy soils, thereby transforming them into fertile soils through decomposition.
- Mitigates soil cracking, surface water runoff, and soil erosion by harnessing the cohesive properties of colloids.
- Facilitates soil loosening and disintegration, thereby improving soil aeration and workability. Enhances water retention capacity, thereby aiding in drought resistance.
- Deepens the soil's hue, thereby facilitating absorption of solar energy.

### • Chemical Benefits

- Enhances the soil's buffering properties.
- Functions as a natural chelator for metal ions in alkaline conditions, facilitating their absorption through the roots.
- Exhibits remarkably high cation-exchange capacities.
- This process enables the transformation of essential nutrient elements, including nitrogen, phosphorus, potassium, iron, zinc, and other trace elements, into readily available forms that can be utilized by plants.
- Enhances the uptake of nitrogen by plants.
- Mitigates the interaction between phosphorus and calcium, iron, magnesium, and aluminum, releasing it in a form that is beneficial and accessible to plants.
- Releases carbon dioxide from soil calcium carbonate, enabling its utilization in photosynthesis.
- Diminishes the presence of toxic substances in soils.

### • Biological Benefits

- Functions as an organic catalyst in numerous biological processes.
- Enhances the growth and proliferation of suitable microorganisms in soil.
- Augments the plant's inherent resistance against diseases and pests.
- Stimulates root growth, particularly in a vertical direction, and facilitates improved absorption of nutrients.

- Enhances root respiration and promotes the formation of roots.
- The process of photosynthesis in plants is facilitated by the presence of certain compounds that support the development of crucial components such as chlorophyll, sugars, and amino acids.
- Promotes plant growth (resulting in higher biomass production) by expediting cell division, accelerating root system development, and increasing the yield of dry matter.
- Enhances the quality of yields, improving their physical appearance and nutritional value.

**6. Effect of Humic Acid on Growth and Yield of Rice:** The research conducted by Karenavar et al. (2022) investigated the effects of a humic-acid based bio-stimulant on the growth, yield, and yield attributing characters of kharif rice (*oryza sativa* L.). The results showed that soil application of the bio-stimulant at a rate of 20 kg ha<sup>-1</sup>, applied at 2-3 weeks after transplanting and at panicle initiation of rice, resulted in higher crop growth parameters such as plant height, number of leaves per hill, tillers per hill, and dry weight. Additionally, it also led to higher yield parameters including panicle length, number of panicles per hill, number of grains per panicle, grain yield, and straw yield. The rice grain yield was found to be increased by 30.5% compared to the control treatment.

Mitkar et al. (2022) also observed positive effects of bio-stimulants on the growth, yield, and yield attributing characters of kharif rice. They found substantially higher values of growth parameters at 60 days after transplanting (DAT), 90 DAT, and at harvest with the application of humic acid at a rate of 0.5%.

Armeylee Joneer and Lum Mok Sam (2022) demonstrated that the application of humic acid at a concentration of 2 g/mL, as well as half of the recommended NPK, happened in superior vegetative growth and yield of Tadong upland rice. Moreover, it was discovered that the implementation of this approach resulted in a decrease in the utilization of chemical fertilizers during the cultivation of rice.

In a study by Kalyanasundaram et al. (2021) on yield maximization of direct sown rice under water constraint situations, it was concluded that the use of tensiometer-based irrigation with soil application of humic granules at a rate of 2.5 kg ha<sup>-1</sup> can be a practicable method for accentuating grain yield and conserving water in the north eastern region of Tamil Nadu. This approach promotes water use efficiency in direct rice cultivation areas.

The acid humic plays a positive role in enhancing the effectiveness of enzymes and nutrients in plant metabolism, succeeding in a higher formation of carbohydrates for most plants. The application of humic acid has been found to result in an increase in plant yield. This effect is attributed to the significance of humic acid on various metabolic processes of the plant, including respiration and photosynthesis, as well as its antioxidant properties that aid in the preservation of chlorophyll content. (Ragheb hadi AL-bourky et al., 2021)

The application of humic acid 12% at a rate of 12.5 liters per hectare (T5) resulted in improved growth attributes. Based on the results of the experiment, it can be inferred that the application of humic acid at a concentration of 12% and a rate of 12.5 liters per hectare is a superior agronomic practice that is both economically sustainable and ecologically viable for rice cultivation. (Kumaravel et al., 2022)

The natural buffering group of humic acid, consisting of the amino and carboxylic groups, plays a crucial role in ion exchange in soil agriculture. The composition of humic acid includes carbon, oxygen, aluminum, silicon, and potassium chloride. Humic substances are commonly used to condition soils, either by direct application as soil fertilizer. This research focuses on the short-term impact of humic acid application on rice growth. The use of humic acid and leonardite proved to be beneficial for the growth of rice leaves and roots compared to the control group. (Buntita jomhataikool et al., 2019)

The study conducted by Wanti Mindari et al. (2018) investigated the impact of various sources and doses of humic acid on the chemical and physical properties of saline soil, as well as the growth and yield of rice. The findings revealed that peat humic acid exhibited superior effects compared to other substances, resulting in increased plant biomass, plant root weight, tiller number, and chlorophyll content. Moreover, the application of humic acid effectively enhanced rice yield by 10-20% through the adjustment of soil pH, nutrient availability, and soil salinity.

- 7. Effect of Humic Acid on Growth of Rice Under Drought and Saline Stress Condition:** In a separate study by Jaber Mehdiniya Afra et al. (2022), it was demonstrated that foliar application of humic acid led to higher levels of osmotic regulators when compared to other fertilizer resources. This, in turn, mitigated the detrimental effects of drought stress on certain functional traits of cultivars. Additionally, humic acid exhibited a greater capacity to increase chlorophyll levels and promote photosynthetic stability, resulting in improved yield.

Amira M. Okasha et al. (2019) found that the most effective treatment for enhancing rice grain quality, growth, and yields was the foliar spray of a mixture containing humic acid and K<sup>+</sup> during the panicle initiation and mid-booting stages. This combination proved to be highly beneficial in developing rice quality and productivity, particularly under salt stress conditions, for the Giza 179 cultivar.

Furthermore, the application of gypsum, farmyard manure, and humic acid, as observed in the study conducted by M. Shaaban et al. (2013), contributed to the improvement of soil properties and the leaching of excessive ions to deeper layers. Consequently, the concentration of salts in the upper layers decreased, creating a favourable environment for plant growth and resulting in a significant increase in rice grain production.

- 8. Effect of Humic Acid on Nutrient Uptake of Rice:** Humic substances, specifically humic acid, possess the ability to attract positively charged ions. They form chelates with micronutrients and gradually liberate them as needed by plants. Additionally, these substances act as chelating agents, thereby preventing the creation of fixation, oxidation, precipitation and leaching of micronutrients in the soil. Humic substances, including

humic and fulvic acids, exhibit auxin activity and exert hormonal effects on catalytic activity, cell permeability, and nutrient uptake, ultimately leading to an increase in dry matter yield. This information was derived from a study conducted by M Eshwar et al. in 2017.

In a separate study conducted by Arnab Kundu et al. in 2020, the differential responses of rice (*Oryza sativa* L.) to foliar fertilization of organic potassium salts were examined. Among the various applications of potassium salts, potassium humate demonstrated the most favourable results. It significantly increased the nutrient uptake, root biomass and number of leaves. This study provided confirmation of the growth-promoting properties of organic potassium salts, as they improved the yield and nutrient uptake of submerged rice.

Furthermore, the application of humic acid or compost extract through spraying resulted in significant increases in various yield parameters and components. It also enhanced the nitrogen, phosphorus, and potassium content of grains and straw compared to the absence of such organic compounds. This finding was reported by El-Gohary et al. in 2010.

- 9. Effect of Humic Acid on Nutrient Availability:** The experimental results show that peat humic acid can increase the plant biomass, plant root weight, tiller number and chlorophyll content better than other humic acids. Humic acid efficiently can improve rice yields 10–20% supported by the suitability of soil pH, nutrient availability and soil salinity. (Wanti Mindari et al., 2019)

From the experiment on Effect of Lime, Humic Acid and Moisture Regime on the Availability of Zinc in Alfisol, Sushanta Kumar Naik and Dilip Kumar Das (2007) concluded that the application of humic acid has a positive impact on the availability of zinc under saturated conditions.

### III. SUMMARY AND CONCLUSIONS

The utilization of humic acid possesses the capacity to considerably influence the agronomic efficacy of crops and diverse parameters associated with soil quality. The impression of humic acid on crop behaviour is influenced by several factors, including its solubility, chemical composition, the specific soil & crop under treatment and application rate. Based on the aforesaid findings, it is noticeable that humic acid possesses a superior crop response, augmented yield, and enhanced yield attributing characteristics. Additionally, there is a notable enhancement in nutrient uptake and availability. In recent times, there is a growing trend in the utilization of organic compounds as fertilizers or soil amendments. This interest can be regarded as the desire to reduce the reliance on chemical fertilizers, concerns regarding the possible pollution caused by chemicals in soil, and the need for energy conservation. Thereupon, humic material develops as a valuable organic resource that has the potential to address some of these requirements.

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