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Biostimulatory Evaluation of Vermicompost-Derived Humic Acid on Seed Germination and Seedling Growth of Raphanus sativus

A. Bhavani[1], M. Razia[2]

[1,2]Department of Biotechnology, Mother Teresa Women's University, Kodaikanal -624101, TamilNadu, India

Abstract

Vermicompost is a humified organic additive that is biologically active and can improve soil fertility and plant growth. Using Fourier Transform Infrared (FT-IR) spectroscopy, the current work sought to characterize vermicompost and its humic fractions and assess the impact of humic acid produced from vermicompost on radish (Raphanus sativus L.) seedling germination and early growth. Earthworms were used to break down animal feces to create vermicompost. Alkaline extraction and acid precipitation techniques were then used to obtain humic and fulvic acids. A variety of functional groups, including amines, alkanes, aromatic and aliphatic amines, alcohols, and alkenes, were found in vermicompost according to FT-IR analysis, showing a high concentration of carbon-rich organic compounds.The presence of organic molecules comprising nitrogen and oxygen was confirmed by the presence of hydroxyl and primary amine groups in fulvic acid fractions, while humic acid fractions showed distinctive amine and alkenyl functional groups. Using radish seeds exposed to varying doses and soaking times, the bioactivity of humic acid was evaluated using seed germination assays. Seeds treated with 0.5 ml humic acid had the maximum germination rate (60 ± 2.4%), while reduced germination rates were noted at higher doses, suggesting concentration-dependent effects. The germination percentage for the control treatment was 46 ± 0.4%. After seven days, a growth assessment of the seedlings revealed that the humic acid-treated seedlings had improved shoot and root development. The highest shoot and root lengths under the 0.5 ml treatment were 5.5 cm and 4.0 cm, respectively. The findings show that vermicompost is a useful source of humic compounds and has biologically significant functional groups. While greater quantities showed negative effects on early plant growth, moderate amounts of humic acid generated from vermicompost positively impacted seed germination and seedling vigor. These results imply that humic acid derived from vermicompost might be used as an environmentally benign biostimulant to enhance early seedling growth and seed germination in radish and possibly other crops.

Keywords: Vermicompost, Humic acid, Fulvic acid, FT-IR spectroscopy, Raphanus sativus, Biostimulant

1. Introduction

Interest in organic soil additives that can enhance crop yield, soil fertility, and environmental quality has increased due to the growing need for sustainable farming methods. Vermicompost, which is created when earthworms and related microbes biodegrade organic wastes, has become one of these effective bio-organic fertilizers. In addition to stabilizing organic residues, vermicomposting provides the substrate with humified organic compounds, beneficial microbial populations, plant-available nutrients, and enzymes. Among composting earthworms, E. fetida is extensively employed in vermicomposting owing to its rapid growth, prolific reproduction, and exceptional adaptation to organic waste-rich environments (Razia et al.,2026; Dominguez & Edwards, 2004). The main biologically active components of vermicompost are humic compounds, especially fulvic acid (FA) and humic acid (HA), which are essential for controlling plant development, soil aggregation, and nutrient cycling. According to recent research (Jakubus and Michalak-Oparowska, 2022; da Silva et al., 2022; Vyas et al., 2022), humic compounds extracted from vermicompost can improve nutrient uptake, boost microbial activity, and increase plant tolerance to environmental stresses. A diverse collection of intricate chemical compounds known as humic acid is created when organic matter is humified. Humic acid has garnered significant interest as a natural biostimulant in agricultural production systems due to its strong cation exchange capacity and physiological activity.

According to earlier studies, humic compounds improve early plant establishment by influencing seed germination, cell division, membrane permeability, enzyme activity, and root elongation. Additionally, it has been demonstrated that vermicomposting processes improve biological activity and nutrient availability by increasing the concentration and quality of humic substances through earthworm-mediated transformation of organic materials (Wang et al., 2021; Zhou et al., 2022; da Silva et al., 2022). Therefore, extracting and characterizing humic and fulvic acids from vermicompost is a crucial method for comprehending their function in promoting plant growth and managing nutrients sustainably. Important markers of crop establishment and future productivity are seed germination and seedling vigor. Because of its rapid germination and development properties, radish is frequently used as a model vegetable crop for assessing seedling responses to bioactive chemicals. Through better nutrient mobilization and physiological regulation, humic acid administration has been shown to improve germination percentage, seedling vigor index, root architecture, and shoot development. Evaluation of germination measures like vigor index (VI), mean emergence time (MET), germination energy (GE), and germination percentage (GP) offers important information about the stimulatory effects of humic compounds during early plant growth. In order to assess the impact of humic acid on radish seed germination, seedling vigour, root length, and shoot growth under controlled laboratory circumstances, the current study was conducted preparation of vermicompost using Efetida and to extract and analysis humic and fulvic acids from vermicompost using FTIR analysis and study seed germination in Raphanus sativus.

2. Materials and Methods

Preparation of Vermicompost

Pre-decomposed animal feces must be placed in a trench that is 2 cm long, 1–1.5 m wide, and 0.9–1 m high. Apply a coating of cow dung first, leaving it for 20 to 30 days, depending on the environment. When a substance has partially decomposed. Earthworms start to consume the feed's upper surface as the substance breaks down. The material turns a deep black when it granulates. It indicates that the materials have been scraped down to the point where vermicompost worms are absent and are ready for collecting. Put all of the leftover materials in one pile and leave it there for two to three days. Worms may be easily separated by hand because they will sink to the bottom of the mound.

3. Humic Acid and Fulvic acid Extraction from Vermicompost

After weighing out 10g of air-dried organic material and adding 50 ml of 0.1 N NaOH to a 250 ml conical flask, the mixture was shaken for a whole day. To obtain the dark-colored supernatant solution, centrifugation was employed. To guarantee total humic acid extraction, the extraction process was carried out three times using 50 milliliters of extractant each time. To ensure complete removal of fine colloidal clays, supernatants were combined in a volumetric flask, and the resulting colored supernatant solution was centrifuged for 15 minutes at 15,000 rpm. The colored supernatant was gathered in a 250 ml beaker and the clay was thrown away. To precipitate humic acid, the pH electrode was dipped in the colored supernatant solution and 2 N HCl was added until the pH dropped to 2. After giving it a good shake, it was let to stand at room temperature for a full day. The coagulate (humic acid) and the supernatant (fulvic acid) were collected independently. A hot water bath was used to dry the gathered material (Stevenson, 1994).

4. Determination of Seed germination

A laboratory germination assay was used to assess seed germination and seedling vigor. For a whole day, uniform and healthy radish (Raphanus sativus) seeds were soaked in various humic acid solution concentrations; seeds soaked in distilled water were used as a reference. Following treatment, 25 seeds were kept under controlled laboratory settings (25 ± 2°C) in sterilized Petri dishes lined with two layers of Whatman No. 1 filter paper. Every therapy was carried out three times. Throughout the experiment, the filter papers were kept sufficiently moist by adding distilled water as needed. Seeds that showed radicle emergence of at least 2 mm were deemed germinated, and germination was monitored every day for seven days. The number of seeds that germinated was divided by the total number of seeds, then multiplied by 100 to determine the germination percentage. Root and shoot lengths were measured on the seventh day using 10 randomly chosen normal seedlings from each replicate.

5. Result and Discussion

FT – IR analysis of Vermicompost

To determine the primary functional groups and assess the vermicompost's chemical makeup, Fourier Transform Infrared (FT-IR) spectroscopy was utilized. Several distinctive absorption bands corresponding to various organic functional groups were visible in the FT-IR spectrum obtained in the 500–4000 cm^{-1} region. 3300–3500 cm^{-1} (N–H stretching of amines), 2800–3000 cm^{-1} (C–H stretching of alkanes), 1500–1650 cm^{-1} (N–H bending of primary amines), 1200–1300 cm^{-1} (C–N stretching of aromatic amines), 1000–1100 cm^{-1} (O–H stretching of alcohols), 1400–1500 cm^{-1} (C–H bending of alkanes), 900–1000 cm^{-1} (N–H vibrations of primary and secondary amines). These findings show that the vermicompost matrix contains oxygenated functional groups, aliphatic and aromatic carbon structures, and nitrogen-containing molecules. A high level of organic matter transformation during vermicomposting is suggested by the variety of functional groups found. While the creation of humified organic matter is indicated by the presence of aliphatic and aromatic carbon structures, the development of amine-related bands reveals microbial breakdown of proteinaceous materials and subsequent nitrogen stabilization (Lim et al., 2016). The spectrum's hydroxyl-containing chemicals could be linked to the alcohol and phenolic groups created during the breakdown process.

According to Aira et al. (2016), these functional groups have a major impact on soil fertility increase, cation exchange capacity, and nutrient retention. Vermicomposting successfully transforms organic waste into a stable organic amendment rich in bioactive molecules, as evidenced by the quantity of carbon-rich compounds found in the current study. Amines, alkanes, alcohols, and aromatic chemicals have been linked to humification and stabilization processes in mature vermicomposts made from agricultural and animal wastes, according to similar FT-IR profiles (Jakubus and Michalak-Oparowska, 2022). Many people believe that the development of these functional groupings is a sign of enhanced agronomic quality and compost maturity. Additionally, humic compounds and complex organic molecules that improve soil structure, nutrient availability, and microbial activity are produced during earthworm-mediated decomposition (da Silva et al., 2022).

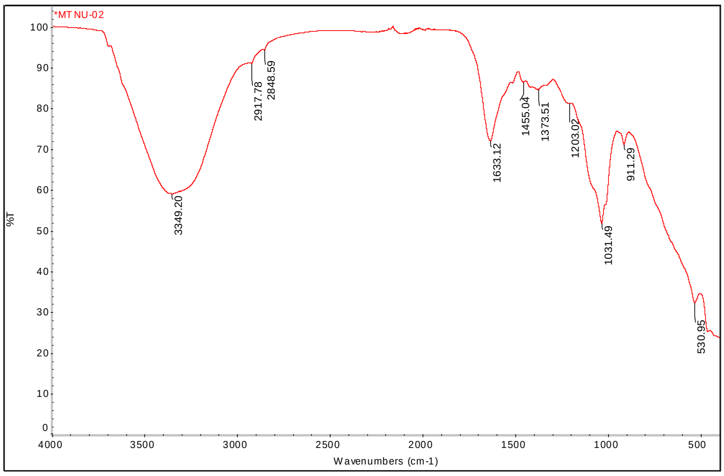

Figure1: FT – IR analysis of Vermicompost

Table 1: FT – IR analysis of Vermicompost

S. No	Characteristic Absorption (Cm^{-1})	Functional Group	Types of Vibration
1	3300- 3500	N – H	Amine
2	2800- 3000	C – H	Alkane
3	1500- 1650	N- H	Primary amines
4	1000- 1100	C- N	Aliphatic amines
5	1200 – 1300	C – N	Aromatic amines
6	1300 – 1400	O – H	Alcohol
7	1400 – 1500	-C – H	Alkane

8	900 – 1000	N- H	Primary secondary amines
9	500 – 600	=C – H	Alkenes

6. FT- IR analysis of Humic acid

The extracted humic acid showed distinctive absorption bands at 3000–3350 cm^{-1} and 1500–1650 cm^{-1}, which corresponded to the C=C stretching vibrations of alkenyl compounds and the N–H stretching vibrations of amine groups, respectively, according to FT-IR analysis. These functional groups show that the humic acid fraction contains complex carbonaceous structures and organic molecules that contain nitrogen. The presence of both amine and alkenyl groups indicates that stable organic molecules created during the humification process are present in the extracted humic acid. While alkenyl structures indicate aromatic and unsaturated carbon components typical of mature humic substances, amine groups are linked to the inclusion of nitrogenous compounds obtained from microbial degradation and transformation of organic matter. Humic acids extracted from vermicompost and composted organic residues have been shown to have similar FT-IR absorption bands, indicating the gradual stabilization and humification of organic matter (Sharma et al., 2021). The cation exchange capacity and nutrient-holding potential of humic substances are improved by the presence of carbon and nitrogen-containing functional groups, which promotes soil fertility and plant growth (da Silva et al., 2022). Additionally, it has been demonstrated that humic acids high in these functional groups promote rhizosphere microbial activity, root growth, and nutrient uptake (Canellas et al., 2023). Thus, the synthesis of chemically stable and physiologically active humic compounds with potential agricultural applications is confirmed by the FT-IR profile obtained in this work.

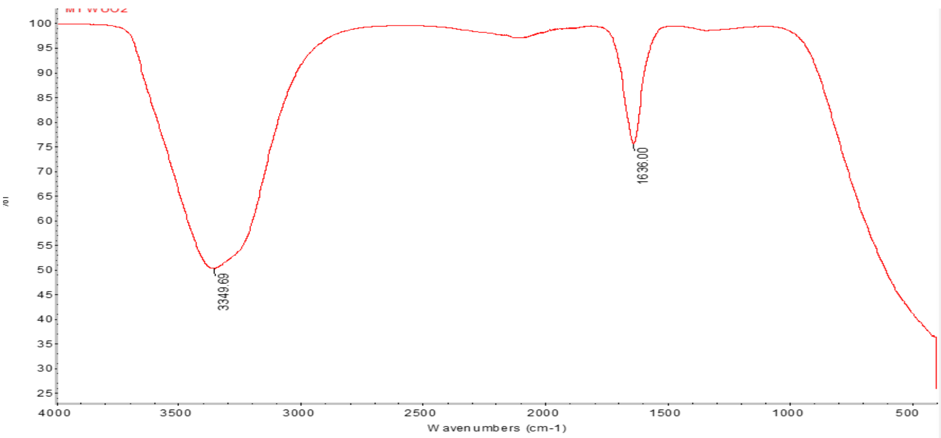

Figure 1: FT- IR analysis of Humic acid

Table 1 : FT- IR analysis of Humic acid

S.No	Characteristic absorption (Cm^{-1})	Functional Group	Types of Vibration

1	3000 – 3350	N - H	Amine
2	1500 – 1650	C=C	Alkenyl

7. FT-IR analysis of Fulvic acid

FT-IR analysis of the extracted fulvic acid revealed characteristic absorption bands at 3000–3350 cm^{-1} and 1500–1640 cm^{-1}, corresponding to O–H stretching vibrations of alcohol groups and N–H bending vibrations of primary amines, respectively. The presence of these functional groups indicates that the fulvic acid fraction contains oxygenated and nitrogen-containing organic compounds. These results suggest that the vermicompost-derived fulvic acid possesses a chemically active structure enriched with polar functional groups that contribute to its high solubility and biological activity.

The prominent O–H absorption band observed in the fulvic acid spectrum is characteristic of hydroxyl-containing compounds, including alcohols and phenolic structures commonly found in humified organic matter. Such oxygen-rich functional groups are known to enhance the chelation of micronutrients and facilitate nutrient transport in soil–plant systems (Canellas et al., 2023). The presence of primary amine groups indicates the incorporation of nitrogenous compounds generated through microbial decomposition and transformation of organic residues during vermicomposting. Similar FT-IR profiles have been reported for fulvic acids extracted from composts and vermicomposts, where hydroxyl and amino functional groups were associated with increased biological activity and nutrient availability (Zhou et al., 2022). Fulvic acids are recognized as the most chemically reactive fraction of humic substances due to their lower molecular weight and greater abundance of oxygen-containing functional groups. These compounds play a significant role in improving nutrient uptake, stimulating root growth, and enhancing microbial activity in the rhizosphere (da Silva et al., 2022). The occurrence of hydroxyl and amino groups in the present study indicates an advanced degree of organic matter humification and the formation of stable bioactive compounds. Similar findings were reported by Jakubus and Michalak-Oparowska (2022), who observed that fulvic acids rich in oxygenated functional groups contribute substantially to the agronomic quality of vermicompost. Therefore, the FT-IR results confirm that the extracted fulvic acid contains biologically important functional groups capable of supporting plant growth and soil fertility through improved nutrient mobilization and biochemical interactions within the soil ecosystem.

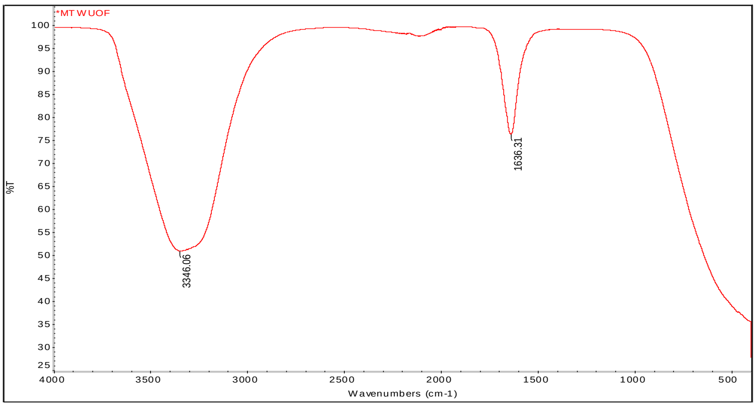

Fig 2: FT-IR analysis of Fulvic acid

Table 2: FT-IR analysis of Fulvic acid

S. No	Characteristic absorption (Cm^{-1})	Functional Group	Types of Vibration
1	3000 – 3350	O - H	Alcohol
2	1500 – 1640	N - H	Primary amines

8. Seed treated with Humic acid

Table 3 showed how humic acid affected radish (R. sativus) seed germination and early seedling growth. When compared to the water-treated control, the germination % was considerably impacted by the humic acid treatment. Seeds treated with the ideal concentration of humic acid had the highest germination percentage (60 ± 2.4%) among the treatments, suggesting a favorable impact on seed viability and germination. Another humic acid treatment similarly showed a moderate increase in germination (50 ± 2.4%), while the control had 46 ± 0.4% germination. On the other hand, one of the higher amounts produced a reduced germination percentage (36 ± 0.4%), indicating that too much humic acid may have a negative impact on seed germination. These results showed that radish seeds responded to humic acid in a concentration-dependent manner, with lower or moderate doses encouraging germination more successfully than higher concentrations. Humic acid's capacity to increase water absorption, activate hydrolytic enzymes involved in reserve mobilization, and improve membrane permeability during the early stages of seed germination may be responsible for its stimulatory effect on germination. Additionally, humic compounds have been shown to have hormone-like properties, especially those similar to auxins, which can promote cell division and elongation and enhance seedling establishment. The current study's increased germination was in line with other findings showing that humic acid functions as a biostimulant by enhancing nutrient availability and metabolic activity in seeds that are germinating.

The growth metrics assessed after seven days provided additional evidence of humic acid's advantageous function in seedling development. Compared to the control, seedlings grown from humic acid-treated seeds typically showed more root and shoot growth, improving seedling vigor. Better nutrition and water absorption may be made possible by the improved root development, which would encourage shoot growth and overall seedling performance. However, the decrease in germination seen at higher doses raises the possibility that too much humic acid could affect physiological processes or cause osmotic stress, which would impair seed performance. Therefore, using humic acid at the right doses may be a useful tactic for enhancing radish germination and early seedling growth. The current study's findings support those of Canellas and Olivares (2014), who found that humic chemicals promote plant development via stimulating physiological and biochemical processes. Calvo et al. (2014) have published similar findings, showing that humic-based biostimulants enhance crop yield, nutrient uptake, root architecture, and seed germination. Thus, humic acid could be a sustainable growth-promoting additive for radish farming.

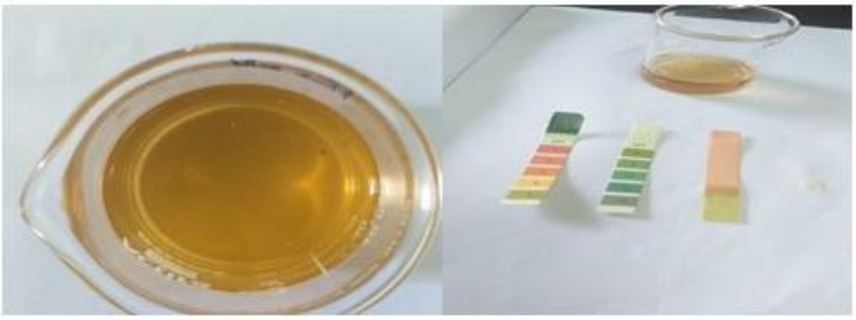

Fig 3: Humic acid from Vermicompost

Fig 6: a)Control seed b)Control germination c) & d) 0.5-1 ml concentration of humic acid for 5 minutes and 10 minutes.

Table 5: Germination Percentage of Radish Seeds Treated with Different Concentrations of Humic Acid

S.No	Time (Minutes)	Treatment (Humic acid)	Germination Percentage (%)
1	5	0.5 ml	40±0.8
2	10	1.0ml	50±2.4
3	5	0.5 ml	60±2.4
4	10	1.0ml	36±0.4
5	10	Control	46±0.4

9. Conclusion

Vermicompost is a rich source of humic compounds with a variety of bioactive functional groups, as this study showed. Vermicompost, humic acid, and fulvic acid fractions were found to have carbon-, nitrogen-, and oxygen-containing chemicals, according to FT-IR analysis. Vermicompost-derived humic acid improved radish seedling germination percentage, root growth, and shoot development at optimal concentrations, but greater concentrations indicated negative effects, according to seed germination tests. According to these results, humic acid isolated from vermicompost may be used as an environmentally benign biostimulant to enhance early plant development and seed germination in sustainable agricultural systems.

References

1. Abdul-Baki, A.A. and Anderson, J.D., 1973. Vigor determination in soybean seed by multiple criteria. Crop Science, 13(6), pp.630–633.
2. Aira, M., Gómez-Brandón, M., Lazcano, C. and Domínguez, J., 2016. Earthworm-mediated decomposition and humification of organic wastes. Waste Management, 48, pp.206–215.
3. Calvo, P., Nelson, L. and Kloepper, J.W., 2014. Agricultural uses of plant biostimulants. Plant and Soil, 383(1–2), pp.3–41.
4. Canellas, L.P. and Olivares, F.L., 2014. Physiological responses to humic substances as plant growth promoters. Chemical and Biological Technologies in Agriculture, 1, p.3.
5. Canellas, L.P., Olivares, F.L. and Aguiar, N.O., 2023. Humic and fulvic acids as plant biostimulants: mechanisms and agricultural applications. Agronomy, 13(5), p.1254.
6. da Silva, M.S.R.A., et al., 2022. Effect of the application of vermicompost and millicompost humic acids on the soybean microbiome under water restriction conditions. Frontiers in Microbiology, 13, p.1000222.
7. Domínguez, J., 2004. State-of-the-art and new perspectives on vermicomposting research. In: C.A. Edwards, ed. Earthworm Ecology. 2nd ed. Boca Raton, FL: CRC Press, pp.401–424.
8. Jakubus, M. and Michalak-Oparowska, W., 2022. Valorization of quality of vermicomposts and composts using various parameters. Agriculture, 12(2), p.293.
9. Lim, S.L., Lee, L.H. and Wu, T.Y., 2016. Sustainability of using composting and vermicomposting technologies for organic solid waste biotransformation. Journal of Cleaner Production, 111, pp.262–278.
10. Razia, M., Lavanya, M. and Merlyn Sujatha, R., 2026. Molecular identification and functional applications of native earthworm species in Kodaikanal Hills. International Journal of Advanced Research in Biological Sciences, 13(4), pp.47–56.
11. Reed, R.C., Bradford, K.J. and Khanday, I., 2022. Seed germination and vigor: ensuring crop sustainability in a changing climate. Heredity, 128, pp.450–459.
12. Sharma, A., Dhiman, A. and Kumar, R., 2021. Characterization of humic substances derived from organic waste composts using spectroscopic techniques. Environmental Technology & Innovation, 24, p.101860.
13. Vyas, P., Sharma, S. and Gupta, J., 2022. Vermicomposting with microbial amendment: implications for bioremediation of industrial and agricultural waste. Biotechnology & Biotechnological Equipment, 36(1), pp.203–215.
14. Wang, F., Yao, W., Zhang, W., et al., 2021. Humic acid characterization and heavy metal behaviour during vermicomposting of pig manure amended with 13C-labelled rice straw. Waste Management & Research, 40(6), pp.803–814.
15. Zhou, X., Li, J., Zhang, J., et al., 2022. Bioaugmentation mechanism on humic acid formation during composting of food waste. Science of the Total Environment, 830, p.154783.