CARAS
© 2023, Centre for Advanced Research in Agricultural Sciences
Research Journal of Agricultural Sciences
Volume 14; Issue 05 (Sep-Oct 2023); pp 1380–1385

Open Access
ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Full Length Research Article

Quantitative Analysis of Humic Acid using Spectrophotometric Method from Newly Formed Liquid Fertilizers Based on Panchagavya Formulation: A Novel Study

Rajkumar Mehra[1], Poonam Dehariya*[2], Meena Singh[3], Akanksha Kumari[4] and Ankita Jain[5]

[1-5] Mushroom Biology Laboratory, Department of Botany, Dr. Hari Singh Gour Vishwavidyalaya, Sagar - 470 003, Madhya Pradesh, India

Abstract

Humic acid plays a critical role in ecology. It offers several benefits to crop production due to its molecular makeup. This helps to loosen soil and improve soil fertility, boosting the holding capacity of water, reducing soil moisture loss, driving up seed germination, promoting soil microflora communities, and lowering the risk of diseases spread through contaminated soil. Several search articles and literature focused on the analysis of nutrients in Panchagavya. However, no study was found on isolating humic acid from Panchagavya. Therefore, we have paid attention to the analysis of humic acid from Panchagavya as well as novel formulations Capralac Extractum (CE) (goat-based) and Bubaluslac Extractum (BE) (buffalo based). These novel formulations are based on the Panchagavya formulation. We used the spectrophotometric method for qualitative analysis of humic acid at multiple wavelengths (365, 400, 565, and 665). Results show that CE-G has the highest amount of humic acid found at 565 and 665, a significant difference at the level of $p<0.01$. As we know, the content of humic acid in manure discussed is 1 to 3%, and peat or sapropel has 15 to 40%. Observation based on manure at the range of 565 to 665 nm and based on peat or sapropel at 400 nm range of wavelength the results are significant.

***Key words*:** Bubaluslac Extractum, Capralac extractum, Humic acid, Novel formulation, Panchagavya, Spectrophotometric method

Humic compounds are one of the most prevalent natural organic substances produced from microbial activity and the degradation of plant and animal wastes with a series of geochemistry processes [1-3]. Humic acid is important for ecology. They provide several advantages for plant production because of their molecular structure. They contribute to breaking up clay and enhancing soil fertility, slowing evaporation from soils, increasing seed germination rates, fostering the formation of microflora communities in soils, and reducing soil-borne diseases [3-4]. Most of the current research focuses on the isolation of humic acid from water, peat, sapropel, soil, and also manure [1], [5-7]. Studies have been conducted to investigate the influence of Panchagavya on plant growth and development [8-10]. Many research articles and literature focused on analyzing nutrients in panchagavya, but no study was found on isolating humic acid from Panchagavya [11-12]. Therefore, we focused on the panchagavya humic acid as well as novel formulations CE (goat based) and BE (buffalo based) which is also based on the Panchagavya formulation. This is the first time we prepared panchagavya-like formulations using two different domestic animals (goat and buffalo) and analyzed their properties for sustainable agriculture practices and forestry. The nomenclature of the Goat-based bio formulation is Capralac extractum which is based on the Latin word *Capraeae* means goat, *lac* word for milk-based component, and *extractum* is an extract. Similarly, Buffalo-based bioformulation is also called Bubaluslac extractum, in which *Bubalus* means buffalo (scientific name *Bubalus bubalis*). We know that nature gives special characteristics to each animal. Hazarika *et al*. [13] wrote an article about cow and goat urine and its beneficial role in disease control along with the urine of many other domestic animals and agriculture practices. Similarly, an investigation was done by Kapadiya *et al*. [14] on the properties of milk of cow buffalo and goat, concluding that buffalo milk has the highest amount of fat, protein lactose, and other minerals like Ca, Mg, and P. Researches also proved that the rumen of buffalo showed a large number of bacterial species and Shannon diversity as compared with that of the Holstein calf [15]. We also know that cow-dung manure and vermicompost are applied to the soil, increasing the organic matter content and improving water infiltration, retention, and cation exchange capacity [16]. This

Received: 17 Jul 2023; Revised accepted: 20 Sep 2023; Published online: 28 Sep 2023

Correspondence to: Poonam Dehariya, Mushroom Biology Laboratory, Department of Botany, Dr. Hari Singh Gour Vishwavidyalaya, Sagar - 470 003, Madhya Pradesh, India, Tel: +91 7247097160; E-mail: dpoonambot18@gmail.com

Citation: Mehra R, Dehariya P, Singh M, Kumari A, Jain A. 2023. Quantitative analysis of humic acid using spectrophotometric method from newly formed liquid fertilizers based on Panchagavya formulation: A novel study. *Res. Jr. Agril. Sci.* 14(5): 1380-1385.

communication deals with the work on humic acid due to its beneficial role in agriculture and plant development in nature. Humic acid was discovered to enhance cell growth and nutrient uptake by generating soluble ion complexes, enhancing cell energy as inducers of the plasma membrane, and also readily operating as root development regulators via cell auxin signalling [17]. Humic acid, conversely, can also increase the effectiveness of phosphate fertilizers by encouraging H^+ production in the rhizosphere and enhancing plant phosphate uptake [18]. Based on their molecular weight, and solubility, humic compounds can be divided into three classes: humic acid, fulvic acid, and humin. Fulvic acid has a moderate molecular weight range of 600 to 10000 Da and is soluble in water under all pH values. Humic acid is a high molecular weight fraction (ranging from approximately 10,000 to 100,000) soluble at higher alkaline media pH<2 but precipitates at acidic pH>2. But humin at any pH level is insoluble in water because its molecular weights range from approximately 100,000 to 10,000,000 [19-22]. UV spectroscopy is a widely used method for determining water samples and humic acid in water [23-24]. Due to its adaptability, simplicity, and viability, visible and near-infrared (VIS/NIR) spectroscopy is a quick, affordable, quantitative, and environmentally friendly method [25]. Therefore, without pretreatments, spectroscopy can adopt practices for delivering transient and record spectra for solid and liquid samples [26].

MATERIALS AND METHODS

Samples preparation

The present study was based on native domestic animals (cow, buffalo, and goat) of Madhya Pradesh (central India). For the preparation of formulation, three native domestic animals viz Malvi cow [27-28], Bhadawari buffalo [29-30], and Bundelkhandi goat [31-32] were used. Panchagavya was prepared as per the same general procedure and methods [33]. We have individually compared the plant nutrition of PG, CE, and BE. Each formulation of every animal is also based on the earthen pot (POT) and glass vessels (G) separately so that we may conclude whether earthen pot and glass vessels are preferable also. All the raw components of PG, CE, and BE were taken in the ratio 1:7:2:3:3, which stands for 1 kg ghee, 7 kg dung, 2 kg curd, and 3 lit. Urine and 3 lit milk of each animal for preparing formulation. As per the procedure of 25 days of fermentation of formulations, each formulation was put in three separate incubators at 32°C ± 2.

Methodology of extraction for humic acid

After 25 days of fermentation of all formulations, 2 g of all earthen pot and glass vessels formulations were dissolved in 100 ml of prepared alkaline solution (6.647 g sodium pyrophosphate loba chem. and 3.9 g sodium hydroxide EMPLURA 1.93502.0521 solution in 1000 ml distilled water) and shaken overnight at 200 rpm on a mechanical shaker. The next day samples were heated at 60 °C for 1 hour in the Yorco serological water bath in India, and the flasks were shaken the intervals every 20 minutes. Afterward, the samples were centrifuged in (REMI R24 centrifuge REMI ELEKTROTECHNIK LIMITED VASAI-401 208 INDIA), a centrifuge tube at 1800 rpm for 30 minutes, and then 100 µl Supernatant was carefully isolated from each sample a microwell plate, and absorbance was recorded through microplate reader (CYBER ELISA R01 salo terrace, Millbury MA01527, USA) at different 365, 400, 465, 665 nm ranges.

Standard solution

To prepare the standard humic acid (HA) stock solution, 1.05 g of 95% humic acid was dissolved in 1000 ml standard alkaline solution for 1 g/L. The HUMIGROW-95 CLSL India supplied humic acid. Several standard working solutions containing 0.1 to 1 g/L of HA were also produced in alkaline pyrophosphate solvent from this solution. The absorbance of humic compounds in the UV-VIS spectral range was measured in a sodium pyrophosphate alkali solvent mixture. Each standard working solution was placed into a spectrophotometric well plate, and the absorbance was recorded at 365, 400, 565, and 665 wavelengths for the preparation calibration series.

Statistical analysis

Data were exposed to paired comparison graphs, utilizing Origin pro-2021 software, to determine the impact of glass jar (G) and earthen pot (POT) on the humic acid content across all formulations. The calibration curve and SD (Standard Deviation) were determined using Microsoft® Excel® 2016 MSO (Version 2301 Build 16.0.16026.20002) 64-bit**.**

RESULTS AND DISCUSSION

Under the optimized experimental condition, the calibration graph established for the humic acid determination in its concentration range of 0.1 to 1 g/L is given in (Fig 1) at multiple wavelengths 365, 400, 565, and 665. It has a straight line with R^2, 0.9925, 0.9961, 0.9944, and 0.9907, respectively, with all wavelengths. The R^2 values accompanying our calibration curve evaluate how well our curve corresponds to the data we have produced. The more closely the values resemble 1.00, the more precisely our curve captures the detector response. R^2 values of 0.990 and higher are typically considered "good" results [34].

The effects of different wavelengths on the sample's absorbance are shown in (Table 1). The lowest absorbance was found at 365 nm, and the highest absorbance was found at 400 nm. All range from 365 to 665 nm in the visible range were is, 365 nm range is nearer to UV and 665 far from UV but nearer to IR and both show the lowest absorbance in samples. Absorption of humic acid increases uniformly with the decreasing wavelength [35].

Table 1 Effect on the absorbance of PG, CE, BE at 365, 400, 565, and 665 nm wavelength in 2% concentration of samples. Results were taken in triplicates

Wavelength nm	PG-POT	PG-G	CE-POT	CE-G	BE-POT	BE-G
365	0.3440	0.409	0.339	0.503	0.761	0.676
400	2.849	3.104	2.956	3.189	2.888	3.33
565	1.527	1.721	2.3	2.828	2.143	2.365
665	1.259	1.527	2.207	2.383	1.562	2.182

PG-POT (Panchagavya- Earthen Pot), PG-G (Panchagavya- Glass Vessel), CE-POT (Capralac Extractum- Earthen Pot), CE-G (Capralac Extractum-Glass Vessel), BE-POT (Bubaluslac Extractum-Earthen Pot), BE-G (Glass Vessel)

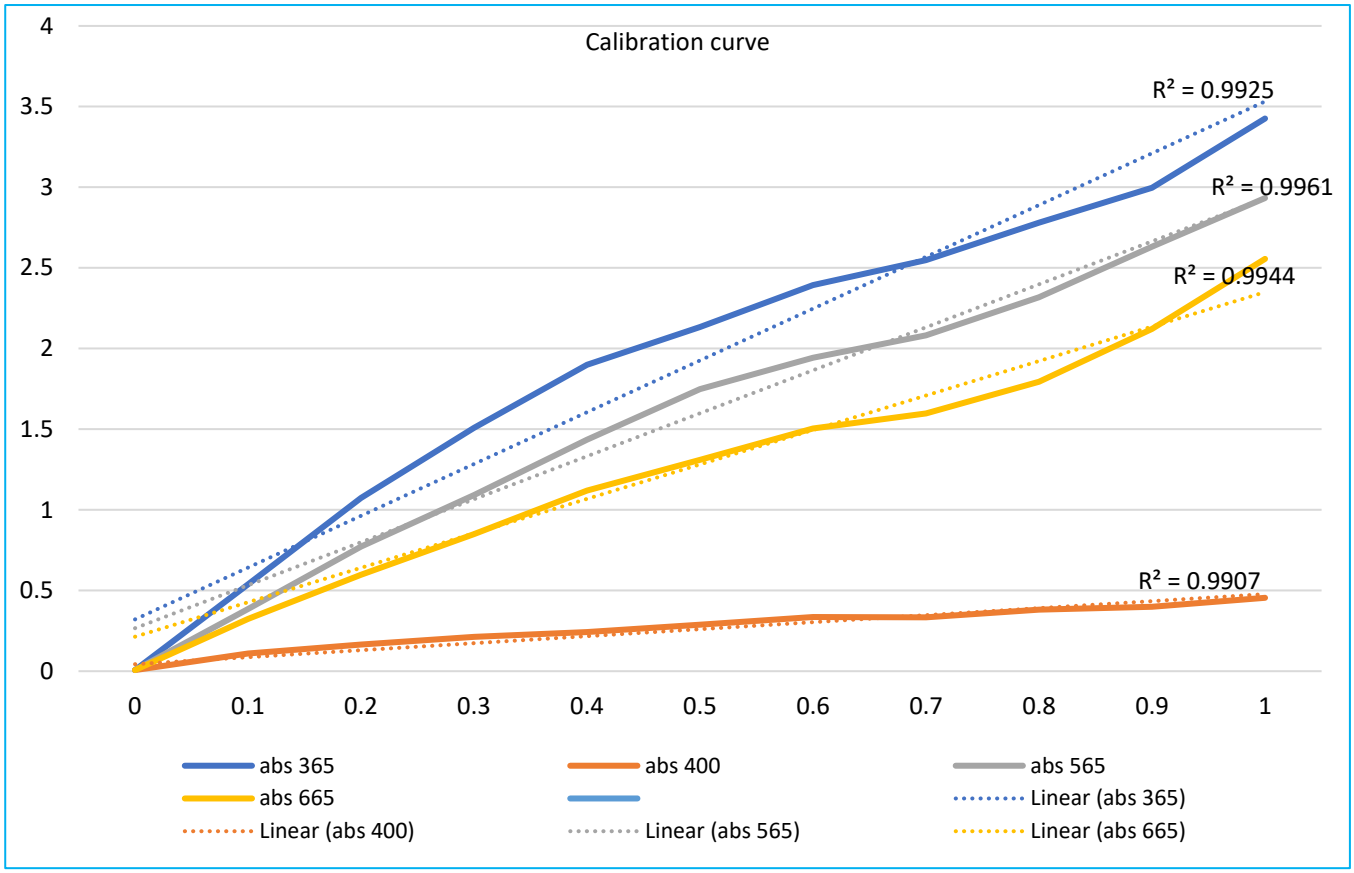

Fig 1 Calibration graph of standards containing 0.1 to 1g/L humic acid at 365, 400, 565, and 665 nm wavelength with R^2 value

Table 2 Average quantitative measurement of humic acid in mg/L at 365, 400, 565, and 665 nm wavelength in 2% concentration of samples with ±SD (Standard Deviation). Results are in the average of triplicate

Wavelength nm	PG-POT	PG-G	CE-POT	CE-G	BE-POT	BE-G
365	11.464±0.34	31.7±0.46	9.19±0.63	59.31±0.77	137.55±2.32	113.33±0.34
400	6695.3±18.17	7246.6±153	6974.3±9.6	7523.3±12.34	6780.3±27.53	7828.3±51.15
565	482.3±8.6	581.3±19	762±42	974±16.7	716.3±10.1	793.3±5.7
665	502±1	617.33±1.52	904.3±1.52	982.6±1.15	630.6±2.5	897±1.5

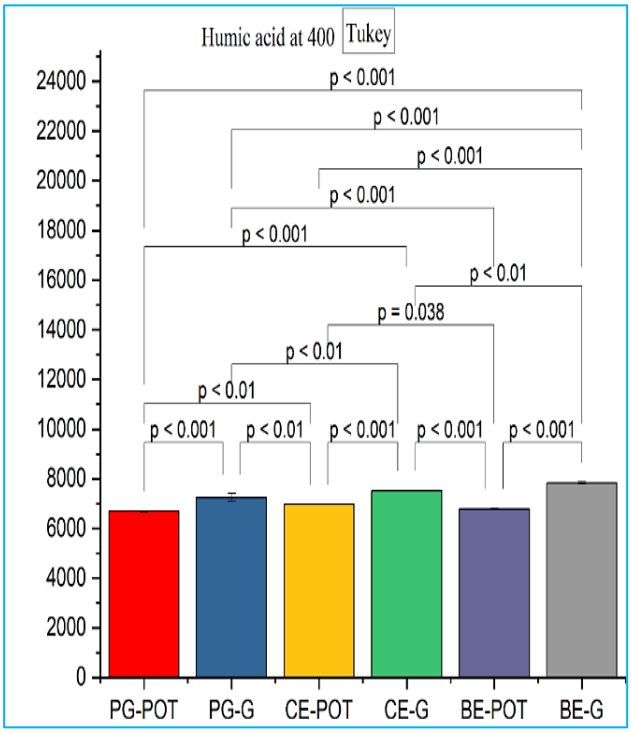

Fig 2.1

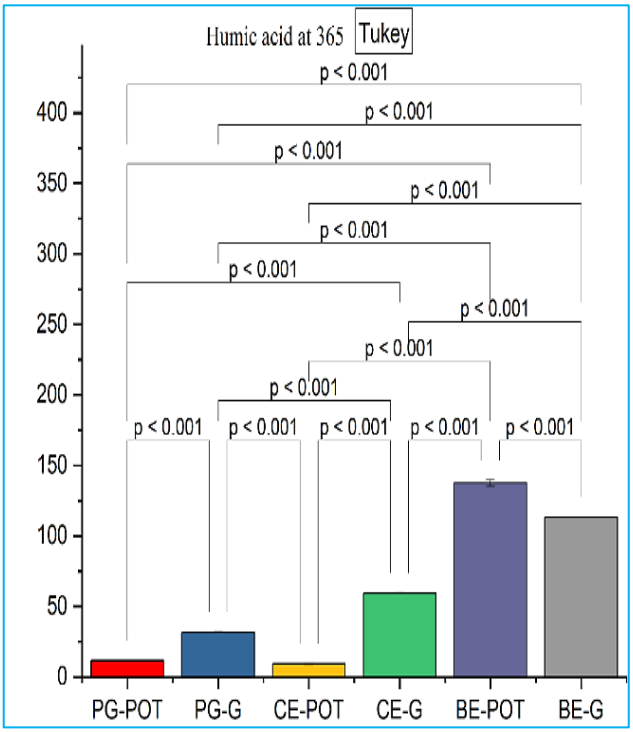

Fig 2.2

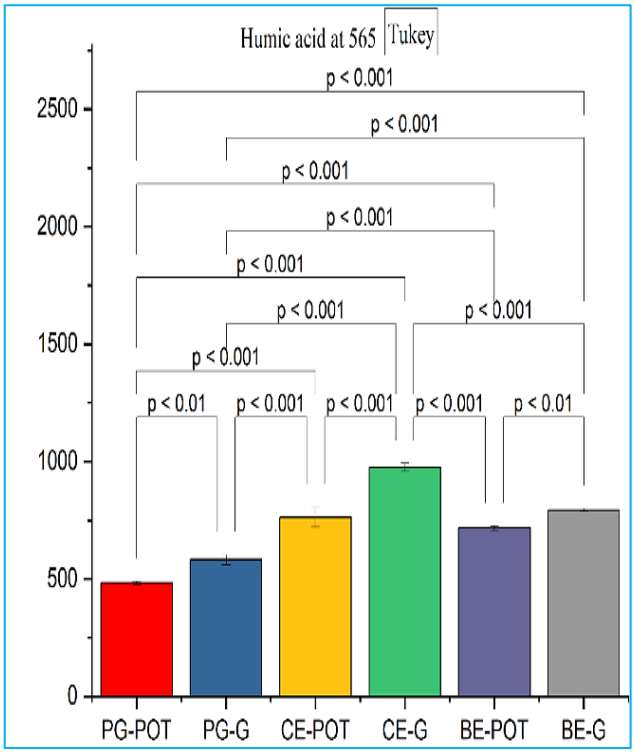

Fig 2.3

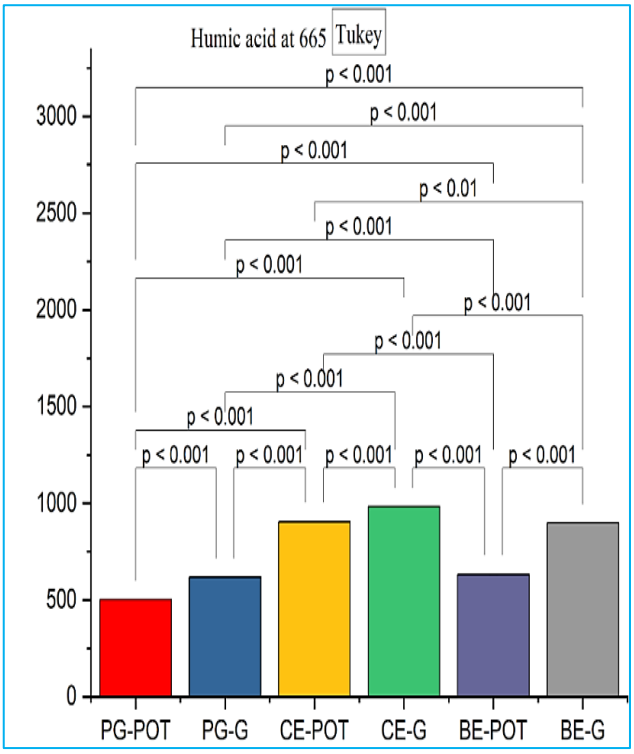

Fig 2.4

Fig 2 The comparative concentration of humic acid among the formulations at 365, 400, 565, and 665 nm with p values using paired comparison graph

In (Fig 1), we observed that, at 400 nm, absorption was less than wavelengths 365, 565, and 665 nm in the case of the standard humic acid solution. But in the case of samples, (Table 1), shows the opposite. For analysis of humic acid and fulvic acid generally 465/665 nm wavelength was used [36-38]. In some literature, 350 and 450 nm were also used [6], [39]. In some pieces of literature highest absorbance λ wavelength or isosbestic point was also selected for wavelength selection for quantitative analysis [40-41]. The use of multiple wavelengths can sometimes add to the accuracy of the method [41]. Kumada [35] also explains the "P" type of humic acid which was first found in podzol and later also found in B and C horizons of soil, red, and yellow soil, volcanic ash, and brown forest soils, and shows three distinct absorption bands at 615, 570, and 450 nm. Wavelengths of 465 and 665 nm were also used by Fuentes *et al*. [42] for humic acid determination. If we consider the prepared standard's highest absorption wavelength, we select 365 nm, but samples have the lowest absorbance at this range. At 400 nm wavelength-prepared standards have lower absorption, but the sample has the highest absorption. Natural compost and peat contain 5 to 20% of humic acid, the same as 1 to 3% in manure [43]. Commercial organic fertilizers usually contain between 15 to 85 % humic acid extracted from lignite, leonardite, or peat [42]. The solution of 2000 ppm humic acid is effective for plant growth and development, according to [44]. Li *et al*. [45] also worked on humic acid recovery from sludge and found that alkaline pretreatment, ultrafiltration separation, and subsequent anaerobic digestion significantly improved the humic acid quantity. This completely proved that accuracy depends on advanced methodology and procedure. During this research, we used the spectrophotometric methodology for humic analysis. It may not give accurate results but we can assume a value approximately relative. This is the very first time we are analyzing humic acid from a very novel formulation CE (*Capralac extractum*) and BE (*Bubaluslac extractum*) along with Panchagavya formulation using multiple wavelengths through UV vis spectrophotometer. Fuentes et al. [42] used the organic carbon oxidation method. Karpukhina *et al*. [46] used FTIR, ATR (Attenuated Total Reflectance), and total carbon analyzer for humic acid and ICP-AES for the determination of silicon and aluminum contents were used in the research. Peat is an incompletely decomposed organic material form of soil and sapropel is long time decomposed organic inorganic material under anaerobic condition that accumulate in the bottom of water bodies, both contain 15 to 40% humic acid [38], [47]. Deryagina and Konyukhova [6] also worked on sapropel humic acid and found 2.7 to 3.6 g/l humic acid from different lake sapropels. If we convert the value in mg to percentage in 100% concentration of samples, then we found minimum to maximum humic acid at 365 nm, 0.045% to 0.68% in PG-POT and BE-POT respectively; at 400 nm, 33.4% to 39.14% in PG-POT and BE-G respectively; at 565 nm, 2.41% to 4.87% in PG-POT and CE-G respectively; and at 665nm, 2.51% to 4.91% in PG-POT to CE-G respectively. As discussed earlier, humic acid content in manure is 1 to 3%, and peat or sapropel have 15 to 40%. If we match these values based on manure, then our results are perfectly good at the 565 to 665 nm range. But if we check out results on the base of peat or sapropel then results are also matched at 400 nm wavelength range. (Fig 2) shows significant differences among all earthen pot and glass vessel formulations. (Fig 2.1), at 365 nm wavelength humic acid concentration shows the highest amount in all glass vessel formulations with the difference $p < 0.001$ and the largest value found in BE-POT. (Fig 2.2) At 400 nm wavelength, the highest concentration of humic acid was found in BE-G, as in (Table 2), 7828 mg/L, and the second highest value was observed in CE-G (7523 mg/l). But both BE-POT and CE-G have significant differences at $p<0.01$. We also found the highest concentration of humic acid in CE-G at 565 and 665 wavelengths, which we can observe in Table 2. In CE-G, 974 and 982 mg/l humic acid was found at 565 and 665 nm, respectively. In (Fig 2), (2.1, 2.2, 2.3, and 2.4) have one similarity related to humic acid concentration. It is that all-glass vessels (G) values (PG-G, CE-G, and BE-G) have high

amounts of humic acid concentration with a significant difference compared to other earthen pot (POT) values. It may be because of no water percolation in a glass vessel compared to an earthen pot.

CONCLUSION

This research does not criticize any animals. As far as we know, each animal has a unique set of attributes from nature. The study concludes that the highest amount of humic acid found in CE-G at 565 and 664 nm (4.87 to 4.91 mg/l in 100% in the concentration of formulation) and matches with earlier research concluding that manure contains 1 to 3 % of humic acid. But along with these, other formulations include lower limits of humic acid but remain in manure humic acid concentration limits. A concentration of 2000 ppm of humic acid is good for plants and crops, which is CE-G also contained at 565, and 665 nm in a 5% concentration of samples. Based on results, research also concludes that Glass vessels are preferable to earthen pots for high content of humic acid in formulation.

Acknowledgments

The authors would like to express their gratitude to Prof. A.N Rai for his consistent support of my philosophical viewpoints and for continuously offering suggestions for the improvement of my study and research work through highly insightful discussions. The authors are also thankful to the Head, of the Department of Botany, Dr. Hari Singh Gour University Sagar M. P., for providing laboratory facilities. This work was financially supported by UGC, Govt. of India to senior author (R.M.).

LITERATURE CITED

1. Wang X, Li BQ, Zhai HL, Xiong MY, Liu Y. 2016. An efficient approach to the quantitative analysis of humic acid in water. *Food Chemistry* 190: 1033-1039.
2. Maccarthy P. 2001. Soil organic matter as function of nitrogen fertilization in crop successions. *Soil Science* 166: 738-751.
3. Li Y, Fang F, Wei J, Wu X, Cui R, Li G, Tan D. 2019. Humic acid fertilizer improved soil properties and soil microbial diversity of continuous cropping peanut: A three-year experiment. *Scientific Reports* 9(1): 1-9.
4. Javanshah A, Saidi A. 2016. Determination of humic acid by spectrophotometric analysis in the soils. *Int. Jr. Adv. Biotechnology Research* 7: 19-23.
5. Deryagina MS, Konyukhova OM. 2023. Determination of humic acid content in sapropel. *In*: *BIO Web of Conferences* (Vol. 57, p. 06002). EDP Sciences.
6. Jindo K, Sonoki T, Matsumoto K, Canellas L, Roig A, Sanchez-Monedero MA. 2016. Influence of biochar addition on the humic substances of composting manures. *Waste Management* 49: 545-552.
7. Khan MS, Akther T, Hemalatha S. 2017. Impact of Panchagavya on *Oryza sativa* L. grown under saline stress. *Journal of Plant Growth Regulation* 36: 702-713.
8. Kumar RS, Ganesh P, Tharmaraj K, Saranraj P. 2011. Growth and development of blackgram (*Vigna mungo*) under foliar application of Panchagavya as organic source of nutrient. *Current Botany* 2(3): 9-11.
9. Kumar P, Khan N, Singh PD, Singh A. 2018. Yogesh, Vikas Yadav. Panchagavya: A low-cost organic input in organic farming-a review. *International Journal of Chemical Studies* 6(6): 575-577.
10. Chakraborty B, Sarkar I. 2019. Quality analysis and characterization of Panchagavya, Jeevumrutha and Sasyamrutha. *Int. Jr. Curr. Microbiol. App. Science* 8(5): 2018-2026.
11. Ali MN, Ghatak S, Ragul T. 2011. Biochemical analysis of Panchagavya and Sanjibani and their effect in crop yield and soil health. *Jr. Crop Weed* 7(2): 84-86.
12. Hazarika S, Das S, Sarma H, Sharma HK. 2018. Application of cow and goat urine in traditional systems of medicines: A brief review. *International Journal of Pharmaceutical and Biological Archive* 9(4): 197-203
13. Kapadiya DB, Prajapati DB, Jain AK, Mehta BM, Darji VB, Aparnathi KD. 2016. Comparison of Surti goat milk with cow and buffalo milk for gross composition, nitrogen distribution, and selected minerals content. *Veterinary World* 9(7): 710.
14. Wang Q, Gao X, Yang Y, Zou C, Yang Y, Lin B. 2020. A comparative study on rumen ecology of water buffalo and cattle calves under similar feeding regime. *Veterinary Medicine and Science* 6(4): 746-754.
15. Mandal KK, Rajak A, Debnath SW, Hasan MA. 2013. Integrated nutrient management in aonla cv A-7in the red laterite region of West Bengal. *Journal of Crop and Weed* 9: 121-123.
16. Canellas LP, Piccolo A, Dobbss LB, Spaccini R, Olivares FL, Zandonadi DB, Façanha AR. 2010. Chemical composition and bioactivity properties of size-fractions separated from a vermicompost humic acid. *Chemosphere* 78(4): 457-466.
17. Jing J, Zhang S, Yuan L, Li Y, Lin Z, Xiong Q, Zhao B. 2020. Combining humic acid with phosphate fertilizer affects humic acid structure and its stimulating efficacy on the growth and nutrient uptake of maize seedlings. *Scientific Reports* 10(1): 17502.
18. IHSS. 1985. International Humic Substance Society.
19. Pettit RE. 2004. Organic matter, humus, humate, humic acid, fulvic acid and humin: their importance in soil fertility and plant health. *CTI Research* 10: 1-7.
20. Sharma A, Anthal R. 2022. Fulvic acid isolation and characterization from water of a Ramsar Lake Mansar, J&K, India. *Applied Water Science* 12: 1-7.
21. Gan D, Kotob SI, Walia DS. 2007. Evaluation of a spectrophotometric method for practical and cost-effective quantification of fulvic acid. *Annals of Environmental Science* 1: 11-15.
22. Singh P, Singh MK, Beg YR, Nishad GR. 2019. A review on spectroscopic methods for determination of nitrite and nitrate in environmental samples. *Talanta* 191: 364-381.
23. Sheng GP, Zhang ML, Yu HQ. 2007. A rapid quantitative method for humic substances determination in natural waters. *Analytica Chimica Acta* 592(2): 162-167.
24. Peng X, Shi T, Song A, Chen Y, Gao W. 2014. Estimating soil organic carbon using VIS/NIR spectroscopy with SVMR and SPA methods. *Remote Sensing* 6(4): 2699-2717.

25. Blanco M, Villarroya INIR. 2002. NIR spectroscopy: a rapid-response analytical tool. *TrAC Trends in Analytical Chemistry* 21(4): 240-250.
26. Raja MMM, Manne R, Devarajan A. 2021. Benefits of cow dung-a human ignored gift. *Journal of Natural Remedies* 21(3): 189-202.
27. Wikipedia contributors. (2023, February 10). Indigenous cattle breeds of India. *In*: *Wikipedia, The Free Encyclopedia*.
28. Singh S, Kushwaha BP, Maity SB, Singh KK, Das N. 2015. Effect of dietary protein on intake, nutrients utilization, nitrogen balance, blood metabolites, growth and puberty in growing Bhadawari buffalo (Bubalus bubalis) heifers. *Tropical Animal Health and Production* 47: 213-220.
29. Pundir RK, Vij PK, Singh RV, Nivsarkar AE. 1996. Bhadawari buffaloes in India. *Animal Genetic Resources/Resources Génétiques Animales / Recursos Genéticos Animales* 17: 101-113.
30. Verma NK. 2013. Status and characterization of goat genetic resources of India. *Sustainable Utilization of Indigenous Animal Genetic Resources of India* 78.
31. Verma NK, Mishra P. 2014. Goat genetic resources of India Bundelkhandi goat – An important germplasm of Bundelkhand region ICAR- National Bureau of Animal Genetic Resources Karnal- 132 001 (Haryana), India monograph # 84/2014 ISBN: 978-93-83537-19-8.
32. Gunwantrao BB, Patil CR. 2015. Bacterial succession during Panchagavya making as revealed by DGGE analysis. *Journal of Pure and Applied Microbiology* 9(4): 3005-3014.
33. Amanda R. 2016 *The Practical Chemist by Calibration*. Part II – Evaluating Your Curves. (https://cannabisindustryjournal.com/category/the practicachemist/).
34. Kumada K. 1965. Studies on the colour of humic acids: Part 1 on the concepts of humic substances and humification. *Soil Science and Plant Nutrition* 11(4): 11-16.
35. Eshwar M, Srilatha M, Rekha KB, Sharma SHK. 2017. Characterization of humic substances by functional groups and spectroscopic methods. *International Journal of Current Microbiology and Applied Sciences* 6(10): 1768-1774.
36. Faithfull NT. 2002. *Methods in Agricultural Chemical Analysis: A Practical Handbook*. Cabi.
37. Jarukas L, Ivanauskas L, Kasparaviciene G, Baranauskaite J, Marksa M, Bernatoniene J. 2021. Determination of organic compounds, fulvic acid, humic acid, and humin in peat and sapropel alkaline extracts. *Molecules* 26(10): 2995.
38. Shamia IS, Halabi MN, El-Ashgar NM. 2017. Humic acid determination in some compost and fertilizer samples. *IUG Journal of Natural Studies* 25(2): 42-50.
39. Kamal AH, El-Malla SF, Hammad SF. 2016. A review on UV spectrophotometric methods for simultaneous multicomponent analysis. *Eur. Jr. Pharm. Med. Research* 3(2): 348-360.
40. Morris R. 2015. Spectrophotometry. *Current Protoc. Essential Lab. Tech.* 11: 2.1.1-2.1.30. doi: 10.1002/9780470089941.et0201s11.
41. Fuentes M, Baigorri R, González-Gaitano G, García-Mina JM. 2018. New methodology to assess the quantity and quality of humic substances in organic materials and commercial products for agriculture. *Journal of Soils and Sediments* 18: 1389-1399.
42. Mayhew L. 2004. Humic substances in biological agriculture. *Rev. Acres* 34(1/2): 80-88.
43. Dunoyer AAT, Castillo FC, Camargo JM. 2022. Effect of humic acid on the growth of seedling tomato (*Solanum lycopersicum*) and melon Cucumis melo. *Revista Ambiente and Água* 17(4): e2808.
44. Li H, Li Y, Jin Y, Zou S, Li C. 2014. Recovery of sludge humic acids with alkaline pretreatment and its impact on subsequent anaerobic digestion. *Journal of Chemical Technology and Biotechnology* 89(5): 707-713.
45. Karpukhina E, Mikheev I, Perminova I, Volkov D, Proskurnin M. 2019. Rapid quantification of humic components in concentrated humate fertilizer solutions by FTIR spectroscopy. *Journal of Soils and Sediments* 19: 2729-2739.
46. Blecic A, Railic B, Dubljevic R, Mitrovic D, Spalevic V. 2014. Application of sapropel in agricultural production. *Poljoprivreda i Sumarstvo* 60(2): 243.