B-5204
[1-7]

RESEARCH ARTICLE Indian Journal of Animal Research

Elucidation of Biopotentiators in Organic based Formulation *Bijamrith* from Gir Cow (*Bos indicus*) Excrements and Urine using GC-MS

R. Sunitha[1], G. Gayathry[1], P. Maheshwari[2], K. Ganesan[1], M. Suganthy[1], S. Padmapriya[1], S. Shenbagavalli[1], A. Bharani[1]

10.18805/IJAR.B-5204

ABSTRACT

Background: Traditional organic formulation *bijamrith* prepared from fresh urine and dung of cow is an important element in natural farming and extensively applied in modern agriculture practices to improve the soil fertility, control pest and disease in plants. The scientific validation of the biologically active compounds in *bijamrith* is unexplored.

Methods: *Bijamrith* was prepared from excrements of Gir cow. Since, the volatile low molecular weight organic compounds from cow wastes could be derived from the solvent extract using GC-MS effectively, the compounds were separated by liquid-solvent extraction method using diethyl ether solvent and analyzed by GC-MS.

Result: GC-MS analysis revealed the elution of about 40 different compounds in *bijamrith* formulation. Among them, fatty acid namely 9-octadecenoic acid (Z)-, an ester compound 2,3-dihydroxypropyl occupied higher concentration with peak area of 13.96% at 29.29 minutes of retention time which were reported to possess antifungal, antibacterial and anti-inflammatory activity. The other biologically active compounds eluted were antioxidants, plant growth hormones, nematicidal agents for defense mechanism and improvement of plant health.

Key words: Bioactive compounds, GC-MS, Gir cow excrements, Organic *bijamrith*.

INTRODUCTION

Gir or Gyr is a milch cow and one of the prominent 'zebu breeds' that tolerates high temperature and highly suitable for tropical regions. It is the top most proven *desi* cow breed originated from mountains of Gir, Kathiawar, Gujarat, India, that produces 100% of easily digestible A2 protein of milk that plays a pioneer role in human dietetics, nutrition and is domesticated for breeding and improvement programme for its amenability in crossing with wide range of *desi* cow namely, Red Sindhi and Sahiwal of Indian breeds and also exotic breeds of Brazil and South America (Vitthalrao Khyade, 2021).

In natural farming, Gir cows play a very important and crucial role in soil health restoration and enhancement. Through the inherent feature of natural grazing habits, Gir cow very well contribute to nutrient recycling by organic matter spreading, improving soil texture, structure and fertility status. Incorporating Gir cow dung envisages a valuable resource potential of organic manures for soil essential nutrient enrichment, with increased yields in organic farming by minimizing the use of synthetic fertilizers (Adil Husnain, 2023).

Urine and dung are the two important biowastes excreted by cow (*Bos indicus*). These wastes are very cheaper and easily available to serve as bioenhancers/ biostimulant that possess the potentiality to increase yield of various crops. Organic formulations like *bijamrith*, *jeevamrith*/ *Gnanajeevamrith* derived from cow excrements and urine are advocated for soil and plant nutrition as a natural agriculture alternative that promotes lower production cost and can help to achieve high quality product and yield with lower or without the usage of chemical fertilizers and pesticides. Organic liquid formulations of *bijamrith* showed better shoot length, root length and seed vigour index in crops such as groundnut, soybean, moth bean and green gram. Crop diseases caused by bacteria, fungi and viruses was also controlled in early seed germination stage by formulations of *bijamrith* which acts as bio-enhancer in increasing seed viability and germination capability (Naikwade Pratap, 2019).

[1]Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.
[2]School of Agriculture and Animal Sciences, Gandhigram Rural Institute-(DTBU), Gandhigram, Dindigul-624 302, Tamil Nadu, India.

Corresponding Author: G. Gayathry, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.
Email: gayathryg@tnau.ac.in

How to cite this article: Sunitha, R., Gayathry, G., Maheshwari, P., Ganesan, K., Suganthy, M., Padmapriya, S., Shenbagavalli, S. and Bharani, A. (2023). Elucidation of Biopotentiators in Organic based Formulation *Bijamrith* from Gir Cow (*Bos indicus*) Excrements and Urine using GC-MS. Indian Journal of Animal Research.
DOI: 10.18805/IJAR.B-5204

Submitted: 22-07-2023 **Accepted:** 30-11-2023 **Online:** 14-12-2023

Urine from Gir cow had been proved to contain gold (Parmar, 2016) and various trace elements such as copper, calcium, zinc, iron (Ambika *et al.,* 2021). Urine distillates of Gir cow were found to improve hematological parameters and immuno-modulatory effects in tested rabbits (Joshi Ankita *et al.,* 2012). Gottimukkala *et al*. (2019) reported that urine of cow acts both as crop growth promoting bio-enhancer and also produces antibacterial substances in crops such as maize, wheat, fenugreek, basil and lemon grass. Cereal seeds treated with urine of cow showed higher growth characteristics and provided excellent capacity to build strength for seedlings and saplings at younger ages. Joseph *et al*. (2022) reported the bioactive compounds like sesquiterpenoids, neophytadiene, dimethyl phthalate, methyl ester, ambrin, oleic acids, octadecanoic acid, methyl isostearate in cow dung. Number of cow waste based bio-enhancers such as *Amrutpani*, *Bijamrut*, *Jivamrut, Panchagavya* and *Sanjivak* have been developed by growers in organic farming systems and were proved to increase yield in groundnut. Seed treatment with *bijamrith* and basal application of farm yard manure in soil along with foliar spray of *Jivamrut*, *Amrutpani, Sanjivak* and *Panchagavya* assured higher net returns and benefit cost ratio in groundnut crop (Mathukia *et al*., 2021).

Hence, the excrements of cow can rightly be called as biopotentiators, since liquid based organic formulations helps for biological control for plant diseases, improve soil fertility, productivity. The elucidation of most of the volatile composition of low molecular weight organic compounds from cow wastes could be harnessed and derived from the solvent extract using GC-MS. Hence, the scientific validation of the bioenhancers present in *bijamrith* prepared with Gir cow excrements was documented using GC-MS.

MATERIALS AND METHODS

Collection of urine and dung from Gir cow

Fresh urine was collected from heifers of Gir cow in a pre-sterilized vial. It was filtered through Whatman filter paper No. 1 to remove debris and other extraneous matters and used for preparation of *bijamrith*. The experiment was conducted in 2021-2022 at Nammazhvar Organic Farming Research Centre and Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

Preparation of organic based formulation *bijamrith*

Bijamrith was prepared from fresh dung and urine of Gir cow. About 5 kg of dung was taken in a muslin cloth and tied with a jute thread as a small bundle and hanged overnight (12 hours) in 20 liters of water. In another container 50 g of lime was dissolved in 1 litre of water and kept separately overnight. On the next day morning, cow dung was squeezed in water and added with a handful of field soil and to this 5 liters of cow urine and lime water (supernatant alone) was added and stirred well to prepare the formulation (Palekar, 2005).

Analysis of nutrient parameters and bioactive compounds in *bijamrith*

Bijamrith was analyzed for pH, Electrical Conductivity (EC), total Nitrogen, Phosphorous, Potassium and organic carbon content. The pH and EC of *bijamrith* was measured using a glass electrode and conductivity bridge instruments respectively (Falcon *et al*., 1987). Organic carbon content of *bijamrith* was estimated by the wet digestion method (Walkley and Black, 1934). About 0.5 ml of *bijamrith* was taken in a 500 ml conical flask and added with 10 ml of 1 N $K_2Cr_2O_7$ and 20 ml of conc. H_2SO_4. The contents were allowed to stand for 30 min. Then, distilled water (200 ml) orthophosphoric acid (10 ml) and diphenylamine (1ml) indicator were added and was titrated against 0.5 N $Fe.(NH_4)_2(SO_4)_2$ towards the end point of a bright green colour. The total nitrogen content was analysed by distillation method. *Bijamrith* was digested with diacid (H_2SO_4 and $HClO_4$ at 5:2) and volume was made up with distilled water. The extract was analyzed by Kjeldahl distillation method as per the procedures of Humphries (1956) and Biswas *et al*. (1977). Total phosphorus content was analyzed by vanadomolybdate colorimetric (ELICO-SL159) method and the triacid extract were analyzed for total potassium by flame photometer (Jackson, 1973).

Elucidation of compounds in *bijamrith* by GC-MS profiling

A known quantity of *bijamrith* formulation was extracted using diethyl ether solvent for separation of compounds (liquid-solvent extraction method) and allowed for evaporation overnight.

Perkin Elmer Clarus SQ8C GC-MS model fitted with DB5 MS capillary standard non-polar column available at Department of Agricultural Microbiology, Agricultural College and Research Institute, Tamilnadu Agricultural University, Coimbatore, Tamil Nadu, India was used for elucidation of bioactive compounds from the solvent extractant of *bijamrith* (Sridharan *et al*., 2021).

The instrument was set with injector port temperature to 220°C, interface temperature at 250°C, source kept at 220°C. The oven temperature was set to, 75°C for 2 mins, 150°C @ 10°C/min, upto 250°C @ 10°C/min. Split ratio set as 1:12 and the injector used was splitless mode. The DB-5 MS capillary standard non-polar column with dimension 0.25 mm OD × 0.25 μm ID × 30 meters length procured from Agilent Co., USA was used for elution. Helium gas was used as the carrier gas at 1 ml/min. The MS was set to scan from 50 to 550 Da. The source was maintained at 220°C and $4.5e^{-6}$ motor vacuum pressure. The ionization energy was -70eV. The MS was also having inbuilt pre-filter which reduced the neutral particles. The data system has inbuilt libraries for searching and matching the spectrum. NIST MS Search 2.2v contains more than five lakh references. Interpretation of the mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST14). The spectrum of the compounds was

compared with the spectrum of the known components stored in the inbuilt library.

RESULTS AND DISCUSSION

Chemical characterization of *bijamrith* from Gir cow

The pH, EC, N, P, K and organic carbon content of *bijamrith* formulation were 7.08, 4.26 dSm^{-1}, 2.38%, 0.13%, 0.49% and 0.93% respectively.

GC-MS elucidation of bioactive compounds in *bijamrith* from Gir cow

The present findings of GC-MS elucidation of bioactive compounds in Gir cow urine and dung based bio-formulation *bijamrith* (Table 1, Fig 1) showed 40 different types of organic compounds, out of which 13 compounds were classified as antimicrobials and other compounds were found to be plant growth promoters, antioxidants/anti-inflammatory. The data

Table 1: GC-MS analysis of bioactive compounds in bijamrith from Gir cow.

Retention time (min)	Area (%)	Name of the compounds	Role/Activity (#)
6.03	0.69	Cyclohexanol, 3,3,5-trimethyl-	Plant hormone abcissic acid. (https://www.ncbi.nlm.nih.gov/pmc/articles/)
8.32	0.71	Phenol, 3-propyl-	Toxicant, pesticidal activity (https://www.ncbi.nlm.nih.gov/pmc/articles/)
11.45	3.82	2,5-cyclohexadien-1-one, 2,6-bis (1,1-dimethylethyl)-4-hydroxy-4-methyl-	Antimicrobial activity, Antioxidant. (https://www.ncbi.nlm.nih.gov/pmc/articles/)
11.57	0.77	2,5-Cyclohexadiene-1,4-dione, 2,6-bis (1,1-dimethylethyl)-	Antifungal, antimicrobial antioxidant property. (https://www.ncbi.nlm.nih.gov/pmc/articles/)
12.39	2.90	Butylated Hydroxytoluene	Antioxidant, anti-inflammatory activities. (https://www.ncbi.nlm.nih.gov/pmc/articles/)
13.39	0.66	Thiophene, 2,5-bis(2-methylpropyl)-	Polyacetylenic compound with strong bio cidal activity, suppress soil nematode. (https://www.ncbi.nlm.nih.gov/pmc/articles/)
13.91	1.64	1-Butanone, 1-(4-aminophenyl)-	Promote plant growth regulator, improve tol erance to abiotic stresses and induce plant de fensive responses. (https://www.ncbi.nlm.nih.gov/pmc/articles/)
15.55	3.25	Isopulegol	Component of essential and volatile oils ex tracted from natural sources. (https://www.ncbi.nlm.nih.gov/pmc/articles/)
15.81	0.52	4-(2,6,6-Trimethyl-cyclohex-1-enyl) -butan-2-ol	Antioxidant, cytotoxic, antimicrobial activity. (https://www.ncbi.nlm.nih.gov/pmc/articles/)
15.86	0.50	Octadecanoic acid, 4-hydroxy-, methyl ester	Antioxidant and anticancerous fatty acid molecule. (https://www.trc-canada.com/)
16.10	0.37	3,5-Di-t-butyl-4-methoxy-1, 4-dihydrobenzaldehyde	Allelopathic compound in rice with less radi cal scavenging activity (https://www.trc-canada.com/)
16.48	0.66	2,1-Benzisoxazole	Intermediate compound, anxiolytic agent, muscle relaxant and anticonvulsant. (https://www.trc-canada.com/)
17.71	0.44	10-Methyl-8-tetradecen-1-ol acetate	Antioxidant, anti-inflammatory, antimicrobial and anticancer property (https://www.trc-canada.com/)
20.66	0.35	7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione	Antimicrobial activity. (https://www.ncbi.nlm.nih.gov/pmc/articles/)
20.89	1.88	Di-n-octyl phthalate	Used as plasticizers in polymers (https://www.ncbi.nlm.nih.gov/pmc/articles/)
20.98	0.52	Methyl 9,12-epithio-9,11-octadecanoate	Synthetic intermediate and lubricity enhancer (https://www.trc-canada.com/)
20.14	1.62	Hexadecanoic acid, methyl ester	Anti-inflammatory activity (https://www.trc-canada.com/)

Table 1: Continue....

Table 1: Continue....

21.20	0.45	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11, 11,13,13,15,15-hexadecamethyl-	Enhances plant growth and development (https://www.trc-canada.com/)
21.56	1.30	Docosanoic acid, methyl ester	Antioxidant property
21.62	0.37	Glycyl-L-histidyl-L-lysine acetate	Stimulates hepatic erythropoietic factor production and used in cosmetic industry (https://www.trc-canada.com/)
22.70	0.33	17-(1,5-Dimethylhexyl)-10,13-dimethyl-3-styryl hexa decahydrocyclopenta[a] phenanthren-2-one23.92	Fungal bio-diversity and role in soil health (https://www.ncbi.nlm.nih.gov/pmc/articles/)
0.54		Cyclooctasiloxane, hexadecamethyl-	Antimicrobial activity, Secondary metabolite (https://www.ncbi.nlm.nih.gov/pmc/articles/)
24.29	1.85	9,12-Octadecadienoic acid, methyl ester	Synthetic intermediate, biodiesel component used as lubricant (https://www.ncbi.nlm.nih.gov/pmc/articles/)
24.42	2.91	13-Octadecenoic acid, methyl ester	Monosaturated fatty acid used in agrochemi cal, pharmaceutical and dyestuff (https://www.trc-canada.com/)
24.92	0.77	Heptadecanoic acid, 16-methyl-,methyl ester	Inhibits plant pathogens and trigger plant immunity (https://www.trc-canada.com/)
25.82	0.42	1H-Cyclopropa[3,4]benz[1,2-e]azulene-4a,5,7b,9,9a(1aH)-pentol, 3-[(acetyloxy)methyl]-1b,4,5,7a,8,9-hexahydro-1,1,6,8-tetramethyl-, 9, 9a-diacetate, [1aR-(1aa,1ba,4aa,5a,7aa, 7ba,8a,9a,9aa)]-	Antimicrobial, antifungal, antioxidant, antitumor (https://www.trc-canada.com/)
26.46	0.91	Cyclononasiloxane, octadecamethyl-	Phytochemical, cytotoxic, antimicrobial and antibacterial activity (https://www.trc-canada.com/)
27.20	0.38	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	Antioxidant, anti-inflammatory, antimicrobial property, bioactive compound (https://www.ncbi.nlm.nih.gov/pmc/articles/)
27.38	0.35	4-Piperidineacetic acid, 1-acetyl-5-ethyl-2-[3-(2-hydroxyethyl)-1Hindol-2-yl]-à-methyl-, methyl ester	Antioxidant with free radical scavenging activity (https://www.ncbi.nlm.nih.gov/pmc/articles/)
27.51	3.30	Cyclononasiloxane, octadecamethyl-	Phytochemical, cytotoxic and antimicrobial activity (https://www.ncbi.nlm.nih.gov/pmc/articles/)
27.94	0.94	Ethyl iso-allocholate	Inhibitor for dihydropteroate synthase (https://www.trc-canada.com/)
28.04	0.75	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis-	Antioxidant property (https://www.trc-canada.com/)
28.43	0.45	Octadecanoic acid, 9,10-dichloro-, methyl ester	Antibacterial agent, an anti-inflammatory agent, a human metabolite, volatile oil component, plant metabolite and an algal metabolite (https://www.ncbi.nlm.nih.gov/pmc/articles/)
28.66	1.05	Cyclononasiloxane, octadecamethyl-	Antibacterial specifically against Bacillus cereus (https://www.ncbi.nlm.nih.gov/pmc/articles/)
28.85	0.71	Glycodeoxycholic acid	Regulate the metabolism in plants (https://www.trc-canada.com/)
29.15	5.29	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Antibacterial agent, anti-inflammatory agent, human metabolite, volatile oil component, algal and plant metabolite (https://www.trc-canada.com/)

Table 1: Continue....

Table 1: Continue....

29.29	13.96	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	Antibacterial, anti-inflammatory, volatile oil component, human, plant and an algal metabolite (https://www.trc-canada.com/)
29.48	4.66	Z,Z-3,15-Octadecadien-1-ol acetate	Sex pheromone (https://www.ncbi.nlm.nih.gov/pmc/articles/)
29.60	2.67	Trilinolein	Antioxidant activity and myocardial protective agent (https://www.ncbi.nlm.nih.gov/pmc/articles/)
29.78	1.74	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 2,4a,9,9atetrakis (acetyloxy)-3, [(acetyloxy) methyl]-1,1a,1b,2,3,4,4a,7a,7b,8,9, 9a-dodecahydro-2,7b-dihydroxy-1,1,6,8-tetramethyl-, [1aR-(1aa,1ba, 2a,3a,4aa,7aa, 7ba,8a,9a,9aa)]	Intermediate compound, kinase inhibitor and anti-cancer agent. (https://www.trc-canada.com/)

(#) Source: https://www.ncbi.nlm.nih.gov/pmc/articles/ and https://www.trc-canada.com/.

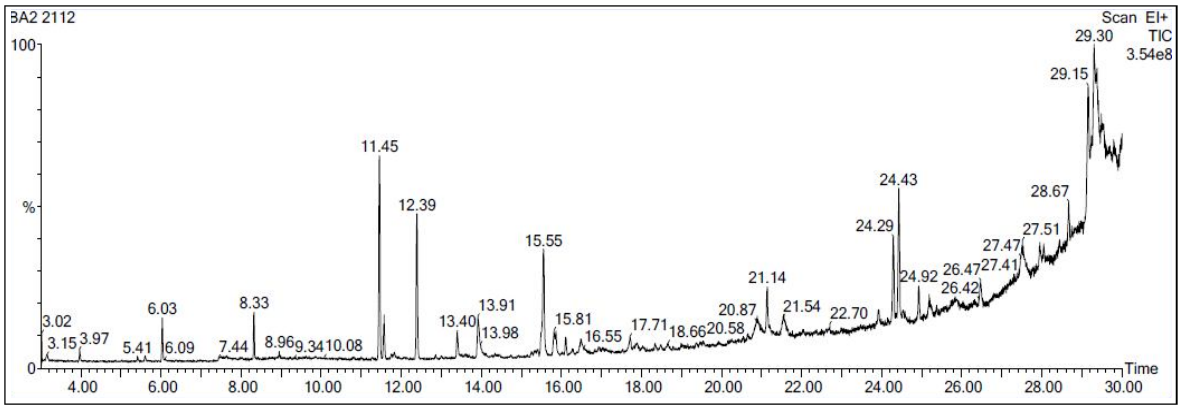

Fig 1: GC-MS elucidation of bioactive compounds in *bijamrith* from Gir cow excrements.

on application/activity of the compound eluted were retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/ and https://www.trc-canada.com/.

The present research findings of *bijamrith* prepared from urine and dung of Gir cow elucidated the occurrence of entirely different types of compounds such as cyclohexanol and tetradecenol. Similar type of studies on the ethyl acetate extract fractions of urine from Sahiwal cow at different physiological stages such as heifer, lactating, pregnancy period showed the presence of 511 sequences of peptides and aqueous extract showed about 46 antimicrobial compounds (Kumar *et al*., 2023). Bioactive fractions of Badri cow urine, exhibited antibacterial activity against human pathogenic bacteria, GC-MS elucidation showed the presence of 12 important compounds and the prime molecules were heneicosanol, heptadecanol, nonadecanol, hexanol, pentadecanal, tetra siloxane, pyrazole, cycl octasiloxane and FTIR analysis further confirmed the presence of alcohol, amide, alkene, alkyl halide functional groups (Nautiyal and Dubey, 2021).

Bijamrith formulation prepared from Gir cow urine and dung showed the presence of cyclohexanol, 3,3,5-trimethyl-, compounds exhibiting properties of abcissic acid. Similarly, Radha and Rao (2014) demonstrated that bio-dynamic formulations from cow dung and urine such as cow pat pit and *panchagavya* attributed to plant growth promoting properties with the production of auxins, IAA, GA, phosphorus solubilizing ability and control of various soil and root borne pathogens in maize. Combined application of cow dung and urine formulations reduced blight disease incidence in a plant called Ribwort (*Plantago lanceolata*).

In the present elucidation of compounds in *bijamrith,* sex pheromone compounds namely Z,Z-3,15-octadecadien-1-ol acetate has been identified. Lestari and Andi Andrian (2017) illustrated that plant growth parameters such as plant height, leaf length and leaf width of sorghum were increased by application of 100cc/l of cow urine. The plant growth promoting compounds in the urine might have contributed to improved plant growth parameters. Urine of Punganur cow at various physiological stages showed that the hormones such as growth hormones, estrogen in heifers, cortisol in milch cows, thyroxine in dry cow were released depending upon the feed intake (Chitteni *et al*., 2022).

The GC-MS results of the current study showed the occurrence of fungicidal compounds such as heptadecanoic acid, 16-methyl-, ester compounds. It was found that 15%

concentration of cow urine showed inhibition of plant pathogenic fungi such as *Fusarium*, *Rhizoctonia*, *Sclerotium* and served as a potential biopesticide (Jandaik *et al*., 2015). In the present GC-MS findings of Gir cow based *bijamrith*, the same type of antimicrobial compound has been elucidated. The secondary metabolites of the *Colletotrichum siamense* analyzed from the GC-MS analysis revealed the presence of compounds namely methyl 9,12-epithio-9,11-octadecanoate that were reported to have antibacterial, antifungal and antiviral activities (Vaijayanthi *et al*., 2023).

CONCLUSION

The Gir cow based organic formulation namely *bijamrith* prepared using dung and urine elucidated 40 different types of plant growth promoting, antibacterial and antifungal compounds. Amongst all, 9-octadecenoic acid (Z)-, ester compound namely 2,3-dihydroxypropyl were the major biologically active molecules identified in *bijamrith* formulation. The present study on scientific validation of *bijamrith* from Gir cow opens a broader avenue for the application of *bijamrith* in seed coating, pelleting or seed priming for the development of designer seeds in near future.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Director of Research, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India for granting core research grant for scientific evaluation of various organic based formulations in a successful manner.

Conflict of interest

The authors declare no conflict of interest and all the authors contributed equally.

REFERENCES

Adil, H. (2023). The role of Gir cow in organic farming and its economic considerations. INSC Digital Magazine. August 2, 2023 (URL: https://theinscribermag.com/the-role-of-gir-cow-in-organic-farming-and its economic considerations).

Ambika, S., Rajesh, N., Ashish, K., Arvind, T., Atul, P., Debashis, R. (2021). Trace elements detection in cow urine by Atomic Absorption Spectrophotometer. Chemical Science Review and Letters. 10(38): 195-197.

Biswas, T.D., Jain, B.L., Maldal, S.C. (1977). Cumulative effect of different levels of manures on the physical properties of soil. Journal of the Indian Society of Soil Science. 19(1): 31-37.

Chitteni, P., Padmaja, K., Prasad, P.E., Kumari, B.P., Ravi, A. (2022). Chemo profiling of Punganur cow urine at different physiological stages. Indian Journal of Animal Research. 12(2): 229-235.

Falcon, M.A., Corominas, E., Perez M.L., Perestelo, F. (1987). Aerobic bacterial populations and environmental factors involved in the composting of agricultural and forest wastes of the Coronary islands. Biological wastes. 20: 89-99.

Gottimukkala, K.S.V., Mishra, B., Joshi, S., Reddy, M.K. (2019). Cow urine: Plant growth enhancer and antimicrobial agent. Journal of Horticulture and Plant Research. 8: 31.

https://www.ncbi.nlm.nih.gov/pmc/articles/.

https://www.trc-canada.com/.

Humphries, E.C. (1956). Mineral Components and Ash Analysis. In: Modern Method of Plant Analysis, Springer -Verlar, Berlin. Pp: 468-502.

Jackson, M.L. (1973). Soil Chemical Analysis. New Delhi: Prentice Hall of India (Pvt.). Ltd.

Jandaik, S., Thakur, P., Kumar, V. (2015). Efficacy of cow urine as plant growth enhancer and antifungal agent. Advances in Agriculture. 62038: 1-7.

Joseph, R., Saminathan, K., Deepthi, M.P., Kathireswari, P. (2022). Comparative analysis on bioactive compounds presents in dung material of *Bos taurus* and *Bos indicus*. Materials Today: Proceedings. 48: 181-185.

Joshi, A., Bankoti, K., Bisht, T., Chauhan, R.S. (2012). Immunomodulatory effect of Gir cow urine in rabbits. Journal of Immunology and Immunopathology. 14(1): 57-61.

Kumar, R., Kaushik, J.K., Mohanty, A.K., Kumar, S. (2023). Identification of bioactive components behind the antimicrobial activity of cow urine by peptide and metabolite profiling. Animal Bioscience. 00(1): 1-13.

Lestari, S.U. and Andrian, A. (2017). Effects of urine cow dosage on growth and production of sorghum plant (*Sorghum bicolor* L.) on peat land. IOP Conference Series: Earth and Environmental Science. 97(1): 012052. IOP Publishing.

Mathukia, R.K., Chhodavadia, S.K., Vekaria, L.C., Vasava, M.S. (2021). Organic cultivation of summer groundnut using cow-based bio-enhancers and botanicals. Legume Research. 46(10): 1351-1355.

Naikwade, P. (2019). Effect of Bijamrita and other organic liquid treatments on seed germination and seedling growth of legume crops. Online International Interdisciplinary Research Journal. 9(3): 59-68

Nautiyal, V. and Dubey, R.C. (2021). FTIR and GC-MS analyses of potential bioactive compounds of cow urine and its antibacterial activity. Saudi Journal of Biological Sciences. 28(4): 2432-2437.

Palekar, S. (2005).The Philosophy of Spiritual Farming I. 2nd ed. Amravati: Zero Budget Natural Farming Research, Development and Extension Movement, Amravati, Maharashtra, India.

Parmar, V. (2016). Holy cow! Junagadh Agricultural University scientists find gold in Gir cow urine.Retrievedfrom:https://timesofindia.indiatimes.com/city/rajkot/holy-cow-junagadh-agricultural-university-scientists-find-goldin-gir-cow-urine/article show/52948435.cms.

Radha, T.K. and Rao, D.L.N. (2014). Plant growth promoting bacteria from cow dung based biodynamic preparations. Indian Journal of Microbiology. 54: 413-418.

Sridharan, A.P., Sugitha, T., Karthikeyan, G., Nakkeeran, S., Sivakumar, U. (2021). Metabolites of *Trichoderma longibrachiatum* EF5 inhibits soil borne pathogen, *Macrophomina phaseolina* by triggering amino sugar metabolism. Microbial Pathogenesis. 150: 104714.

Vaijayanthi, S., Ranjani, S., Hemalatha, S. (2023). Comparison of myco nanoparticles and myco nano emulsion incorporated biomaterials in controlling multidrug-resistant bacterial pathogens. Journal of Environmental Chemical Engineering. 11(3): 109672.

Vitthalrao Khyade, B. (2021). Gir cow milk: The wealth for human health. Acta Scientific Veterinary Sciences. 3(7): 9-12.

Walkley, A. and Black, T.A. (1934). An experimentation of vegetative method for determining soil organic matter and proposed modification of the chromic acid titration method. Soil Science. 37: 38-39.