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Review Article

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Bio Inspired Design Research Inspirations and Future of Medical Sciences: Mapping Review of Recent Developments

Ujjawal Shukla

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Abstract

The recent developments in the field of bio-inspired design have been inspiring greatly the direction of research in the modern times. To promote maximum efficiency in proposed solutions for modern problems, it is imperative that inspiration should be taken from natural sources, as nature has spent millions of years perfecting the organisms to deal with their various circumstances. This research area has resulted in ideal solutions to multiple problems that have the capability to be actually applied in multiple situations, hence the versatility provides for cost efficiency. Not to mention, the presence of the solution in nature allows for an easy proto type. The methodology involved in these researches is simple analyse a certain phenomenon in nature and then relate it with a problem in real life. The present study is outcome of rigorous review of literature with the help of scholarly reliable databases such as Web of Science and Google Scholar to find put the vast amount of literature. Major findings focus on the analogical aspects, hence concerned with the aspects of bio inspired design and bio computing. Microbial research has been a prime area as well to realize real life solutions of chemo-pathological problems. DNA and proteins can be better analysed for structural problems. The paper concludes with the idea that these are just some of the basic issues that can be solved through bio-inspired design where future belongs to more intricate researches.

Keywords: Bio-Inspired design; Nature; Microbes; Biotechnology; Bio-computing; India.

INTRODUCTION

Nature has frequently been the focus of designers and scientists, who get inspired by the aesthetic qualities and functional qualities respectively. The most important factor determining the pace and placement of this research should be avoidance of harmful by products that end up harming the environment immensely. Inter disciplinary brain storming along the lines of ecology and systems theory has shown that it is possible to develop alternate modes from biology. Uncountable instances of organisms utilizing various natural situations and adaptations to their advantages can be found and then taken inspiration from for the same. Researchers then would use modern technology to understand these activities and then apply the findings for creation of new and better products. Innovation forms the founding pillar of success for all companies. Innovation can be defined simply as an idea successfully conceived and produced for the company and customer. Therefore, a very basic stepping stone for success is the formation of new ideas that display hints for this

Author Affiliation: B. Tech Bioengineering Scholar, School of Bioengineering, Vellore Institute of Technology, Bhopal 466114, Madhya Pradesh, India.

Corresponding Author: Ujjawal Shukla, B. Tech Bioengineering Scholar, School of Bioengineering, Vellore Institute of Technology, Bhopal 466114, Madhya Pradesh, India.

E-mail: ujjawal.shukla2021@vitbhopal.ac.in

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innovation. Nature has provided in various aspects of life, some no table examples of viable innovations existing to deal with the various adversities faced, and all readily available to be utilized by humans in modified form. The application of knowledge gained from various natural sources in technological innovations is called Bio-Inspired design. In a prominent study, Stone et al.[1] (2014) brought out the idea that bio-inspired design (BID) has a purpose to "systematically mine biological knowledge to solve existing design problems". The present study has aimed to review the multifarious research directions though literature based on the search results from Google Scholar and other databases. The discussion in the study would move with the purpose to visualise the BID research direction in the present time through various sections framed in this article.

Conceptual Background

Bio inspired design cannot be directly utilized in the technical factions and demands proficiency in both biology and engineering for attaining mastery. But, contrary to this requirement, most professionals lack any special education in biology, hence hindering this field. Lest things chance, bio-inspired design "will remain the domain of a few innovators skilled and interested enough to decipher the primary biological literature".[2] A number of criteria and methods have been attempted to promote this biological knowledge among engineering students, such as C-K models and interdisciplinary teams.[3] Information that has been already modified can be viewed in digital databases and catalogues, however while these only show information that has been deemed worthy, more basic research is also readily accessible. However, someone skilled in biological knowledge is necessary to interpret the information. Bio-inspired design is highly related with the appropriate movement of analogies, whose formulation as well as the creation of new ideas to provide solutions requires an in-depth knowledge in biology as well as engineering[4], with the added of importance of gaining technical knowledge. A smoother transition into this field is dependent upon collaborations between engineers and biologists, to inspire a combination of their respective fields.

Modern Society and Bio Inspired Design

A single utility is not enough to justify the solutions of engineering problems arising in these modern times. However, the old, usual methods to derive solutions for these problems will simply not satisfy the purpose. Therefore a new design process has been discovered to be implemented for bio-inspired design.[5] The whole design process consists of various steps, that is:

1. Identification of biological solutions and their definition.
2. Major understanding and extraction of biological solution.
3. Coordination of extracted solutions and related principles.
4. Designing and reframing of biological solution along with the target/problem search.
5. Application as well as implementation of Biological solution.

One of the most modern methods of handling engineering problems is to utilize analogies from biological creatures, this has led to multiple innovations such as Velcro, and conceptual design of a bionic car.[6-8] For example: superhy drophobic coatings inspired from lotus leaves and their cleaning mechanisms,[9-12] body temperature regulating clothes inspired from pinecones[13-15] and water walking microbots replicating the basilisk lizard.[16,17] Soft materials have taken the lead spot in the race for bio-inspired designing, as they have high adaptation to environment, diversity of functions, etc. However, the advantage they provide is covered by their lack of accuracy and reliability. Reconfigurable design of these materials[5] however, can help in both, achieving accuracy and reliability as well as the adaptability and other functions.

Bio Inspired Design: Concept and Earlier Prominence

Bio-inspired designs have emerged in modern times to be utilized in multiple fields, such as shock resistance for advanced aero-spaces, flying robots and much more. They have also proven useful for organ/implant substitutions to provide for better and more accessible implants and bone reconstructions. The beginning of milleium observed the developments of flapping wing micro air vehicles[18], dynamic materials and robotics.[19,20] The research led into the function integration aspects, damage tolerance and patterns.[21-24] The last decade witnessed the intricate aspects such as design by analogy and characterisation and its links with problem solving tools.[25-27] The components such as BID and additive manufacturing, metal anode cycling have applied the structure function methods. Various studies[28-32] brought out the complexity of BID[33-35] in the simplest descriptions. Analogy analysing, power networks and solution findings have also been core to this field. Better materials for artificial joints and tissues have also

been increasingly discovered to aid the cause. Hence, the field is highly productive, both mean while and in the future.

Bio Inspired Design and Indian Contribution

Various researches to attempt people find their bio-inspiration and helping in their analysis for a variety of fields such as joint augmentations, asymmetric supra-molecular solutions, etc have also been made. In all and all, bio-inspired design has taking a turn for the future. The contribution of Indian researchers is reflecting a wide range. The bio inspired design of nanocages[36-39], molecular recognition among the bio-inspired design strategies with the application of molecular interactions, computer aids, is contemporary.

The facets such as visual representations and collaborations of engineers and biologists have done a tremendous job. BID is possible due to emerging interests from various subject fields.[40,15,41,42] The chemical incarnations and BID have gone hand-in-hand[43-45] and traversed to various research directions. The aspects such as bench marking of BID and providing conceptual tools for knowledge proliferation such as the sauri and architecture.[46-48] The increased usage of computer aids and basic engineering principles has paved way for previously unimagined research areas. The literature is reflecting the inspiration and sources in the recent times.[49,31,4] The design and analysis at the most intricate levels could become reality.[50,51] The recent studies have focused on interdisciplinary approaches of BID research.

Bio Inspired Design and VIT India

A brief overview of the research conducted on Bio-Inspired Design in VIT, India show cases interests in DNA cryptography, neural networks and operations algorithms. A lot of emphasis has also been placed on bio-computing for various factors such as applications in swarm problems[52] and information retrieval. Recent studies have presented a composite picture of varied aspects of BID researches.[53-56] The observations from the studies[57,58] presented the BID applications into the wireless sensor networks and information retrieval at the core of digital platforms. Bio inspired "glass fiber reinforced polymer sandwich" core and 3D printed "bio inspired sandwich beam" are few of the examples from the innovative research.[59,60] The upcoming times will watch the upsurge in the BID research in India.

Objectives and Methodology of the study

The present study is based on review of literature to provide a glimpse into Bio Inspired Design (BID) research. The background literature was searched through Google Scholar to build the conceptual background in the beginning of the article. To map the BID research since the long past and in recent times, the following objectives were framed to conduct the study:

1. To find out the recent trends in Bio Inspired Design research.
2. To observe and relate the interdisciplinary nature of Bio Inspired Design research.
3. To compare the highly cited BID research and its recent proliferation since the Web of Science data available.
4. To find out trends and contribution of India in Bio Inspired Design research.

METHODOLOGY

Mapping reviews: Mapping research and reviewing is "a systematic approach to under standing the map of a profession, theory, research question, or practice. The term mapping is also used for concept mapping, which shows how concepts are related in a visual way. Similarly, mapping studies can show how literature is disseminated through journals, books, websites, and other channels". Mapping reviews usually intend to provide an overview of "what is out there" on a particular topic "but does not focus on where literature may be found".[61] Mapping research is performed in various disciplines, including engineering, science, medical and biological issues, research policy, and more. These are done to support further research, and are aimed to "classify the primary research papers in that specific domain".[62] Mapping reviews provides assessment of research papers in a subcategory, and synthesize the included works, specifically in bio inspired design.[63,64] Mapping studies include vast areas and number of citations due to broader research objectives. Data was collected from Web of science Core Database in June 2022 with the following keywords/string:

1. Bio Inspired Design
2. Bio Inspired design in India
3. Bio Inspired design in India during 2020s
4. Bio Inspired Design and Bio-engineering
5. Bio Inspired Design in India and VIT

The repeat search was made on last week of June 2022 where no change in the results was observed. The repeated results and numbers were same; hence the data analysis was initiated.

The following steps were taken as mapping

study method practice:

1. *Determination of the topic:* The review of literature and background literature survey has reflected that no recent mapping review has been conducted in the research areas of Bio Inspired Design. The literature is vast so there has been a need to review the literature and observe the direction of research in the present study.
2. The present study has provided the background information about the specialty of Bio Inspired Design research so that the novice readers could also grasp the idea of this specific filed.
3. Identification of highly cited studies has been done by consulting and browsing the Web of Science Database.
4. In the next phase of the study, core literature was displayed in the spread sheets (Microsoft Excel). The inclusion and exclusion of information materials was already determined and it was limited to only research articles.
5. Calculated numbers and percentage were expressed in the tables to reflect the data and analyze the same for the topic being mapped.

Significance of the research mapping

Using "concept maps" and "visualizations for data presentation" is addressed with data analysis in study. Researcher has used visualizations (Appendix 1) to illustrate various BID concepts and complex connections, and reflecting the frame works of inter related research processes, and presents findings.

Statistical method and analysis:

The study has carried out count of differences, descriptive statistics, and linear trend analysis to depict the change over the time. The study has also presented the line and bar charts for the visualization of data. The MS-Excel software has been used for preparation of figures and visualizations.

Data Analysis

Data was collected from Web of science database and has been arranged in various tables though Excel sheets. The data analysis is presented following under the sections given as:

a. Bio Inspired Design:

Upon using the keywords 'Bio Inspired Design' in Web of science core database, 10 highly cited articles as main results had been taken into consideration (Fig. 1). The researches have been arranged in the decreasing order of citations, with the top result peaking out at 2242 citations, mean while the bottom result still yielding an impressive 800+ results. Most citations show an increasing number from 2018 to 2021 this is the general trend. Bio Inspired Design in Web of Science Categories (Table 6) is reflected as data shed light on the keywords found for the topic 'Materials Science Multidisciplinary' topping the list with 2118 counts (Table 1) whereas 'instruments instrumentation' at

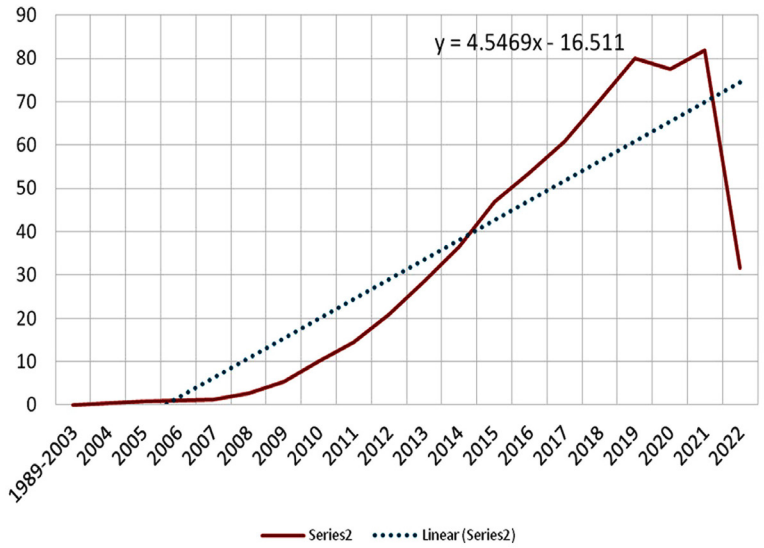

Fig. 1: displays the trend analysis of bio inspired design over the past 30 years 1989-2022. The results indicate a positive trend (Beta = 4.5469), which shows an average increase of 4.54 bio design concepts every year. However, an exception during the last two years is an indication of the impact of pandemic due to COVID-19.

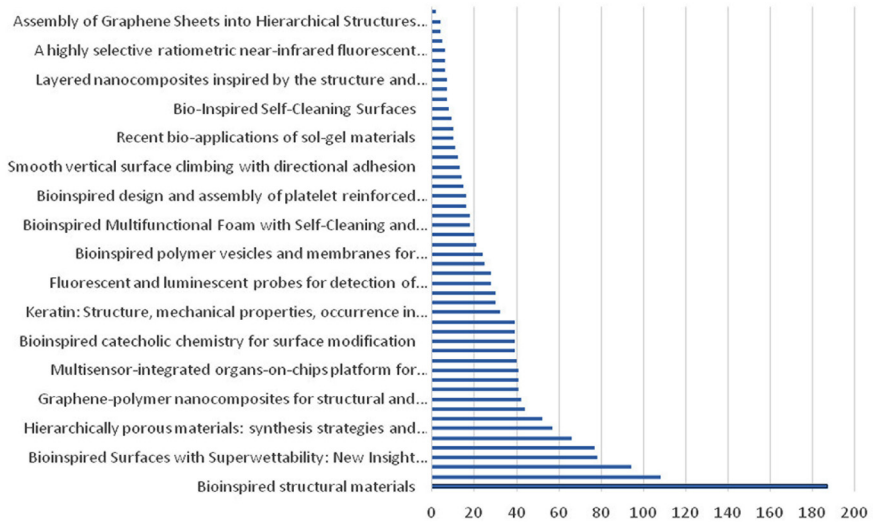

Fig. 2: presents the difference in number of subject relations over the past 30 years (1989-2003 to 2022) through web of science in the concept BID research designs (Table 1). The results show the maximum increase of 187 (count) in bio inspired structural materials, which was having no subject relations during 1989-2003.

Highly cited Publications from Web of Science Search Results

Table: 1 Bio Inspired Design Highly cited Publications from Web of Science Search Results

Citation	2018	2019	2020	2021	2022	Average Citation/ Year	Total Citation
Bioinspired structural materials Wegst, UGK; Bai, H; (...); Ritchie, RO Jan 2015 | Nature Materials 14 (1) , pp.23-36	321	376	396	438	187	280.25	2242
Biological materials: Structure and mechanical properties Meyers, MA; Chen, PY; (...); Seki, Y Jan 2008 | Progress In Materials Science 53 (1) , pp.1-206	151	153	148	137	39	104.47	1567
Soft robotics: a bioinspired evolution in robotics Kim, S; Laschi, C and Trimmer, B May 2013 | Trends In Biotechnology 31 (5), pp.23-30	161	194	203	152	66	109.8	1098
Bioinspired Surfaces with Superwettability: New Insight on Theory, Design, and Applications Wang, ST; Liu, KS; (...); Jiang, L Aug 26 2015 | Chemical Reviews 115 (16), pp.8230-8293	168	173	165	191	78	122.63	981
A molecular ruthenium catalyst with water-oxidation activity comparable to that of photosystem II Duan, LL; Bozoglian, F; (...); Sun, LC May 2012 | Nature Chemistry 4 (5) , pp.418-423	92	110	85	108	30	86.91	956
Bioinspired catecholic chemistry for surface modification Ye, Q; Zhou, F and Liu, WM 2011 | Chemical Society Reviews 40 (7) , pp.4244-4258	121	119	110	75	39	77.33	928
Photochemical conversion of solar energy Balzani, V; Credi, A and Venturi, M 2008 | Chemsuschem 1 (1-2) , pp.26-58	63	51	62	51	16	60.47	907
Bioinspired Design of a Superoleophobic and Low Adhesive Water/Solid Interface Liu, MJ; Wang, ST; (...); Jiang, LFeb 9 2009 | Advanced Materials 21 (6) , pp.665-+	102	114	107	114	40	63.57	890
Nanoconfinement controls stiffness, strength and mechanical toughness of beta-sheet crystals in silk Keten, S; Xu, ZP; (...); Buehler, MJ Apr 2010 | Nature Materials 9 (4) , pp.359-367	80	89	102	97	39	66.08	859
Bio-inspired, smart, multiscale interfacial materials Xia, F and Jiang, L Aug 4 2008 | Advanced Materials 20 (15) , pp.2842-2858	63	64	42	51	21	53.73	806

Table 6: Bio Inspired Design in Web of Science Categories

Web of Science Categories	Record Count	% of 6,049
Materials Science Multidisciplinary	2118	35.014
Chemistry Multidisciplinary	1649	27.261
Nano science Nanotechnology	1290	21.326
Chemistry Physical	1222	20.202
Physics Applied	853	14.102
Materials Science Biomaterials	566	9.357
Physics Condensed Matter	557	9.208
Multidisciplinary Sciences	410	6.778
Robotics	393	6.497
Engineering Biomedical	322	5.323
Polymer Science	310	5.125
Engineering Multidisciplinary	272	4.497
Engineering Electrical Electronic	246	4.067
Engineering Chemical	184	3.042
Engineering Mechanical	167	2.761
Computer Science Artificial Intelligence	164	2.711
Biochemistry Molecular Biology	162	2.678
Mechanics	154	2.546
Energy Fuels	138	2.281
Instruments Instrumentation	137	2.265

the bottom with 137 mentions.

b. Bio Inspired design in India

For the word string 'Bio Inspired design in India, a total of 10 highly cited studies and their citations have been taken and analysed (Table 2); anti-bacterial and copper surface researches have been less frequent, meanwhile the trend has been hot towards Functional Mimics of Glutathione.

Peroxidase: Bio inspired Synthetic Antioxidants and bio inspired nano structural surfaces. The trend again has been increasing instances of citations from 2018 to 2022. Bio Inspired design in India in Web of Science Categories is observed as 'Materials Science Multidisciplinary' has been the top search category (Table 2, 7) in the web of sciences, mean while robotics has been the bottom most category.

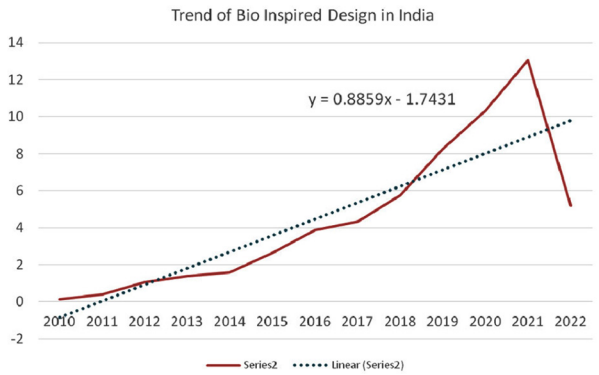

Fig. 3: displays the trend analysis of bio inspired design in India over the past 20 years 2010-2022. The results indicate a positive trend (Beta = 0.8859), which shows an average increase of 0.88 bio design in India concepts every year.

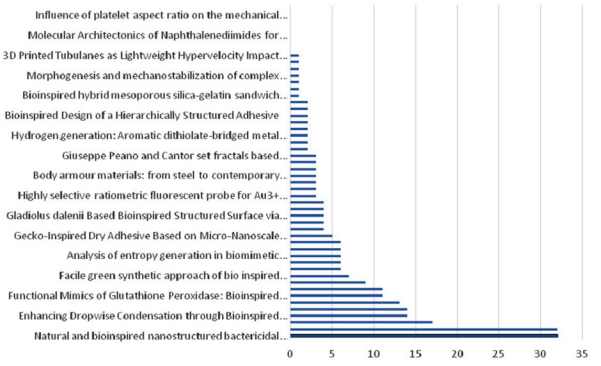

Fig. 4: presents the difference in number of subject relations over the past 30 years (1989 to 2022) through web of science in the concept BID research designs and India (Table 2). The results show the maximum increase of 32 (count) in Natural and bio inspired nano-structured bactericidal surfaces and applications of super hydrophobic nano-based coatings and surfaces, which was having no subject relations during 1989-2009.

Table 2: Bio Inspired design in India Highly cited Publications from Web of Science Search Results

Citation	2018	2019	2020	2021	2022	Average Citation/ Year	Total Citation
Functional Mimics of Glutathione Peroxidase: Bioinspired Synthetic Antioxidants Bhabak, KP and Mugesh, G Nov 2010 | Accounts Of Chemical Research 43 (11) , pp.1408-1419	37	46	31	35	11	29.31	381
Natural and bioinspired nanostructured bactericidal surfaces Tripathy, A; Sen, P; (...); Briscoe, WH Oct 2017 | Advances In Colloid And Interface Science 248 , pp.85-104	34	36	72	70	32	41.33	248
Enhancing Dropwise Condensation through Bioinspired Wettability Patterning Ghosh, A; Beaini, S; (...); Megaridis, CM Nov 4 2014 | Langmuir 30 (43) , pp.13103-13115	24	26	24	39	14	18.67	168
Recent progress in the preparation, properties and applications of superhydrophobic nano-based coatings and surfaces: A review Phuong, NT; Tran, HN; (...); Bajpai, AK Jul 2019 | Progress In Organic Coatings 132 , pp.235-256	0	11	39	78	32	40	160
An Adaptive Wearable Parallel Robot for the Treatment of Ankle Injuries Jamwal, PK; Xie, SQ; (...); Parsons, JG Feb 2014 | Ieee-Asme Transactions on Mechatronics 19 (1) , pp.64-75	21	17	18	14	4	14.89	134
Bioinorganic chemistry of molybdenum and tungsten enzymes: A structural-functional modeling approach Majumdar, A and Sarkar, S May 2011 | Coordination Chemistry Reviews 255 (9-10) , pp.1039-1054	8	12	6	6	0	9.92	119
Organic Solvent-Free Fabrication of Durable and Multifunctional Superhydrophobic Paper from Waterborne Fluorinated Cellulose Nanofiber Building Blocks Baidya, A; Ganayee, MA; (...); Pradeep, T Nov 2017 | ACS NANO 11 (11) , pp.11091-11099	14	31	26	31	13	19.17	115
DNA-Assisted beta-phase Nucleation and Alignment of Molecular Dipoles in PVDF Film: A Realization of Self-Poled Bioinspired Flexible Polymer Nanogenerator for Portable Electronic Devices Tamang, A; Ghosh, SK; (...); Mandal, D Aug 5 2015 | ACS Applied Materials & Interfaces 7 (30) , pp.16143-16147	11	28	18	14	4	13.38	107
Recent advances in engineering topography mediated antibacterial surfaces Hasan, J and Chatterjee, K 2015 | Nanoscale 7 (38) , pp.15568-15575	16	26	18	22	6	12.5	100
Bioinspired green synthesis of copper oxide nanoparticles from Syzygium alternifolium (Wt.) Walp: characterization and evaluation of its synergistic antimicrobial and anticancer activity Yugandhar, P; Vasavi, T; (...); Savithramma, N Oct 2017 | Applied Nanoscience 7 (7) , pp.417-427	3	16	18	22	14	12.17	73

Table 7: Bio-inspired design in india in web of science categories

Web of Science Categories	Record Count	% of 172
Materials Science Multidisciplinary	46	26.744
Chemistry Multidisciplinary	44	25.581
Nano science Nanotechnology	26	15.116
Chemistry Physical	23	13.372
Materials Science Biomaterials	14	8.14
Physics Applied	12	6.977

Table cont.....

Polymer Science	11	6.395
Biochemistry Molecular Biology	10	5.814
Energy Fuels	10	5.814
Engineering Biomedical	9	5.233
Engineering Electrical Electronic	9	5.233
Multidisciplinary Sciences	8	4.651
Chemistry Inorganic Nuclear	7	4.07
Engineering Mechanical	7	4.07
Biotechnology Applied Microbiology	6	3.488
Chemistry Organic	6	3.488
Engineering Chemical	6	3.488
Green Sustainable Science Technology	6	3.488
Chemistry Applied	5	2.907
Robotics	5	2.907

c. Bio Inspired design in India during 2020s

An over whelming amount of research has been conducted in India in 2020s upon Bio-Inspired Design. Stem cell and multi sensor integrated organs on chips have been the most cited researches, meanwhile researches regarding melanin and photodynamic microbial killings have received lesser citations (Fig. 5; Table 3). Bio Inspired design in India during 2020s in Web of science Categories as the Chemistry analytical has been the least common web of science categories, meanwhile multidisciplinary sciences (Table 3 & 8). have been the most popular categories, with over 25% of the total count.

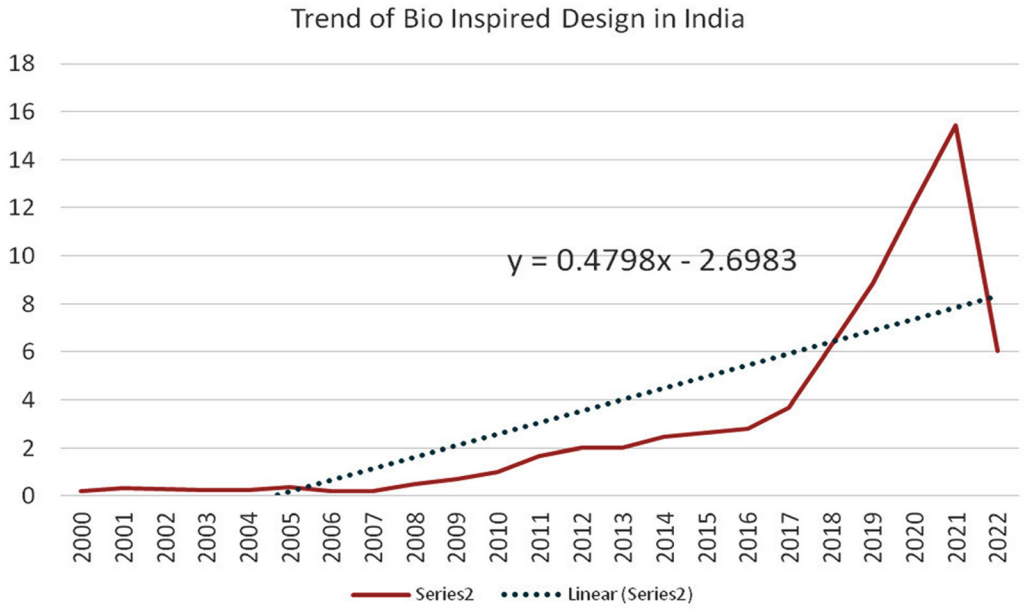

Fig. 5: displays the trend analysis of Bio Inspired design in India during 2020s over the past 20 years 2010-2022. The results indicate a positive trend (Beta = 0.4798), which shows an average increase of 0.47 bio design in India concepts every year.

Table 3: Bio Inspired design in India during 2020s Highly cited Publications from Web of Science Search Results

Citation	2018	2019	2020	2021	2022	Average Citation/ Year	Total Citation
Multisensor-integrated organs on-chips platform for automated and continual in situ monitoring of organoid behaviors Zhang, YS; Aleman, J; (...); Khademhosseini, A Mar 21 2017 | Proceedings of the National Academy of Sciences of The United States of America 114 (12) , pp.E2293-E2302	46	77	81	117	41	62	372
Bioinspired Materials for Controlling Stem Cell Fate Fisher, OZ; Khademhosseini, A; (...); Peppas, NA Mar 2010 | Accounts of Chemical Research 43 (3) , pp.419-428	16	19	15	14	2	17.92	233
Nano measurements with micro-devices: mechanical properties of hydrated collagen fibrils Eppell, SJ; Smith, BN; (...); Ballarini, R Feb 22 2006 | Journal of the Royal Society Interface 3 (6) , pp.117-121	17	14	11	6	5	10.65	181
Bioinspired polymeric materials: in-between proteins and plastics Barron, AE and Zuckermann, RN Dec 1999 | Current Opinion in Chemical Biology 3 (6) , pp.681-687	3	3	3	3	0	7.46	179
Bioinspired neuron-like electronics Yang, X; Zhou, T; (...); Lieber, CMMay 2019 | Nature Materials 18 (5), pp.510	0	24	44	64	16	37	148
Thermal gelation and tissue adhesion of biomimetic hydrogels Burke, SA; Ritter-Jones, M; (...); Messersmith, PB Dec 2007 | Biomedical Materials 2 (4) , pp.203-210	9	17	7	12	4	8.63	138
Direct-Ink-Write 3D Printing of Hydrogels into Biomimetic Soft Robots Cheng, Y; Chan, KH; (...); Ho, GW Nov 2019 | Acs Nano 13 (11) , pp.13176-13184	0	0	18	52	24	23.5	94
Bioinspired and Biomimetic Nanomedicines Chen, ZW; Wang, ZJ and Gu, Z May 2019 | Accounts Of Chemical Research 52 (5) , pp.1255-1264	0	8	39	32	15	23.5	94
Melanin and Melanin-Related Polymers as Materials with Biomedical and Biotechnological Applications-Cuttlefish Ink and Mussel Foot Proteins as Inspired Biomolecules Solano, F Jul 2017 | International Journal of Molecular Sciences 18 (7)	8	15	25	28	9	14.17	85
Targeting and photodynamic killing of a microbial pathogen using protein cage Architectures functionalized with a photosensitizer Suci, PA; Varpness, Z; (...); Young, M Nov 20 2007 | Langmuir 23 (24) , pp.12280-12286	3	3	4	3	1	5.25	84

Table: 8 Bio Inspired design in India during 2020s in Web of science Categories

Web of Science Categories	Record Count	% of 104
Multidisciplinary Sciences	27	25.962
Nano science Nanotechnology	25	24.038
Biotechnology Applied Microbiology	24	23.077
Materials Science Multidisciplinary	24	23.077
Chemistry Physical	21	20.192
Chemistry Multidisciplinary	20	19.231
Materials Science Biomaterials	18	17.308
Engineering Biomedical	17	16.346
Physics Applied	13	12.5
Physics Condensed Matter	10	9.615
Pharmacology Pharmacy	8	7.692
Robotics	5	4.808
Biochemistry Molecular Biology	4	3.846
Cell Biology	3	2.885
Engineering Multidisciplinary	3	2.885
Polymer Science	3	2.885
Biochemical Research Methods	2	1.923
Biophysics	2	1.923
Cell Tissue Engineering	2	1.923
Chemistry Analytical	2	1.923

d. Bio Inspired Design and Bioengineering

Bio-inspired design and Bioengineering has been a less popular field, with the peak citation only having 7 citations (Fig. 6; Table 4), where as the lowest cited research has 2 citations in total. Bio Inspired Design and Bioengineering in Web of Science Categories is evident from the table, materials science biomaterials have 50% of the total count, with the rest of the categories (Table 4,9) amounting to 25% of the categories.

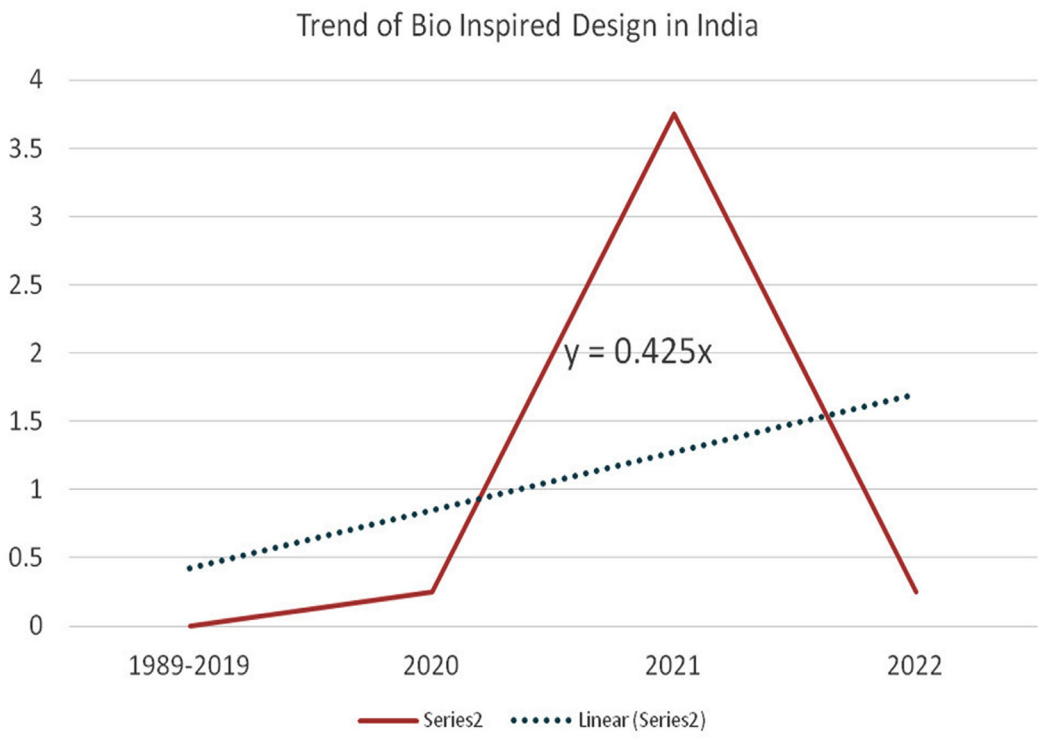

Fig. 6: displays the trend analysis of Bio Inspired design and Bioengineering over the past 30 years 2010-2022. The results indicate a positive trend (Beta = 0.425), which shows an average increase of 0.42 bio design and bioengineering during last three years.

Table 4: Bio Inspired Design and Bioengineering Highly cited Publications from Web of Science Search Results

Citation	2018	2019	2020	2021	2022	Average Citation/ Year	Total Citation
Cutting mechanics of wood by beetle larval mandibles Kundanati, L; Chahare, NR; (...); Gundiah, N Dec 2020 | Journal Of The Mechanical Behavior Of Biomedical Materials 112	0	0	1	5	1	2.33	7
Immobilization of Transglutaminase on multi-walled carbon nanotubes and its application as bioinspired hydrogel scaffolds Fatima, SW; Barua, S; (...); Khare, SK Nov 15 2020 | International Journal Of Biological Macromolecules 163 , pp.1747-1758	0	0	0	5	0	1.67	5
Evaluating the Impact of Tailored Water Wettability on Performance of CO2 Capture Rather, AM; Srikrishnarka, P; (...); Manna, U Nov 23 2020 | ACS Applied Energy MaterialS 3 (11) , pp.10541-10549	0	0	0	3	0	1	3
Bioinspired inorganic nanoparticles and vascular factor microenvironment directed neo-bone formation Kim, HD; Park, J; (...); Hwang, NS May 7 2020 | Biomaterials Science 8 (9) , pp.2627-2637	0	0	0	2	0	0.67	2

Table 9: Bio Inspired Design and Bioengineering in Web of Science Categories

Web of Science Categories	Record Count	% of 4
Materials Science Biomaterials	2	50
Biochemistry Molecular Biology	1	25
Chemistry Applied	1	25
Chemistry Physical	1	25
Energy Fuels	1	25
Engineering Biomedical	1	25
Materials Science Multidisciplinary	1	25
Polymer Science	1	25

e. Bio Inspired Design in India and VIT

An analysis for the 8 articles found for the topic 'Bio Inspired Design in India and VIT' show cases that bio inspired ionic liquid tagged cobalt salophen complex has been the most cited research, with a sky high 5.33 citations per year. Meanwhile 'Classification of Electroencephalogram Signal for Developing Brain Computer Interface Using Bio inspired Machine Learning Approach' (Table 5) has been the least cited research. Bio Inspired Design in India and VIT in Web of Science Categories as observed Bio Inspired Design in India and VIT when searched yielded 18 categories (Table 5,10,11) in the web of science database, with the top 4 categories spans across 2 record counts each and the remaining categories contributing 1 record each.

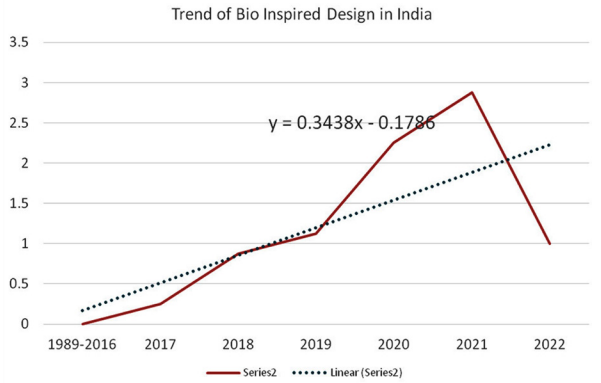

Fig. 7: displays the trend analysis of Bio Inspired design in India and VIT over the past 30 years 1989-2022. The results indicate a positive trend (Beta = 0.3438), which shows an average increase of 0.34 bio design in India and VIT contributions during last couple of years (2016-2022).

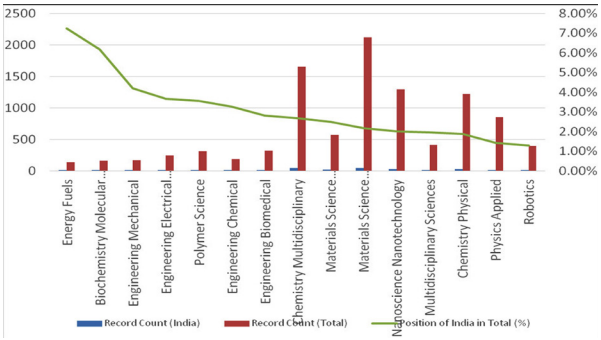

Fig. 8: presents the interdisciplinary nature and number of subject relations over the past 30 years (1989 to 2022) through web of science in the concept BID research designs and India. The results show the maximum increase of Chemistry Multidisciplinary (count=1649), Materials Science Multidisciplinary (count=2118), Nano science Nanotechnology (count=1290) and Chemistry Physical (count=1222) as conceptual relations and distributions of BID in modern research.

Table 5: Bio Inspired Design in India and VIT Highly cited Publications from Web of Science Search Results

Citation	2018	2019	2020	2021	2022	Average Citation/ Year	Total Citation
A bioinspired ionic liquid tagged cobalt-salophen complex for nonenzymatic detection of glucose Benjamin, M; Manoj, D; (...); Senthilkumar, S May 15 2017 | Biosensors & Bioelectronics 91 , pp.380-387	7	5	6	10	2	5.33	32
Thermal Co-reduction engineered silver nanoparticles induce oxidative cell damage in human colon cancer cells through inhibition of reduced glutathione and induction of mitochondria-involved apoptosis Dasgupta, N; Ranjan, S; (...); Ramalingam, C Nov 1 2018 | Chemico-Biological Interactions 295 , pp.109-118	0	4	9	8	2	4.6	23
Involvement of Bcl-2 Activation and G1 Cell Cycle Arrest in Colon Cancer Cells Induced by Titanium Dioxide Nanoparticles Synthesized by Microwave-Assisted Hybrid Approach Ranjan, S; Dasgupta, N; (...); Ramalingam, C Jul 15 2020 | Frontiers In Bioengineering And Biotechnology 8	0	0	1	4	2	2.33	7
Evidences of Biomimetic and Nonantibiotic Characteristics of the Zinc-Carboxymethyl Chitosan-Genipin Organometallic Complex and Its Biocompatibility Aspects Mishra, AH and Mishra, D Feb 2020 | Biomacrom-Olecules 21 (2) , pp.688-700	0	0	2	1	1	1.33	4
Investigations on Bending Characteristics of Soft Mesh Structure using Shape Memory Alloy Spring Towards Bio-Inspired Robotic Applications Muralidharan, M; Brolin, A; (...); Palani, IA Dec 2021 | May 2020 (Early Access) | Iranian Journal Of Science And Technology-Transactions Of Mechanical Engineering 45 (4) , pp.927-937	0	0	0	0	1	0.33	1
Investigation of free and forced vibration of GFRP corrugated bio-inspired sandwich beam with HSDT: Numerical and experimental study Gunasegeran, M and Sudhagar, PE May 2022 (Early Access) | Mechanics Of Advanced Materials And Structures	0	0	0	0	0	0	0
Free and forced vibration analysis of 3D printed bioinspired sandwich beam using HSDT: Numerical and experimental study Gunasegeran, M and Sudhagar, PE Jun 2022 | Apr 2022 (Early Access) | Polymer Composites 43 (6) , pp.3659-3677	0	0	0	0	0	0	0
Classification of Electroencephalogram Signal for Developing Brain-Computer Interface Using Bioinspired Machine Learning Approach Thilagaraj, M; Ramkumar, S; (...); Govindan, P Feb 25 2022 | Computational Intelligence and Neuroscience 2022	0	0	0	0	0	0	0

Table 10: Bio Inspired Design in India and VIT in Web of Science Categories

Web of Science Categories	Record Count	% of 8
Biochemistry Molecular Biology	2	25
Biotechnology Applied Microbiology	2	25
Materials Science Composites	2	25
Polymer Science	2	25
Biophysics	1	12.5
Chemistry Analytical	1	12.5
Chemistry Organic	1	12.5
Electrochemistry	1	12.5
Engineering Mechanical	1	12.5
Materials Science Characterization Testing	1	12.5
Materials Science Multidisciplinary	1	12.5
Mathematical Computational Biology	1	12.5
Mechanics	1	12.5
Multidisciplinary Sciences	1	12.5
Nano science Nanotechnology	1	12.5
Neurosciences	1	12.5
Pharmacology Pharmacy	1	12.5
Toxicology	1	12.5

Table 11: Web of Science Categories and India

Web of Science Categories	Record Count (India)	Record Count (Total)	Position of India in Total (%)
Energy Fuels	10	138	7.25%
Biochemistry Molecular Biology	10	162	6.17%
Engineering Mechanical	7	167	4.19%
Engineering Electrical Electronic	9	246	3.66%
Polymer Science	11	310	3.55%
Engineering Chemical	6	184	3.26%
Engineering Biomedical	9	322	2.80%
Chemistry Multidisciplinary	44	1649	2.67%
Materials Science Biomaterials	14	566	2.47%
Materials Science Multidisciplinary	46	2118	2.17%
Nanoscience Nanotechnology	26	1290	2.02%
Multidisciplinary Sciences	8	410	1.95%
Chemistry Physical	23	1222	1.88%
Physics Applied	12	853	1.41%
Robotics	5	393	1.27%
Chemistry Inorganic Nuclear	7	–	–
Biotechnology Applied Microbiology	6	–	–
Chemistry Organic	6	–	–
Green Sustainable Science Technology	6	–	–
Chemistry Applied	5	–	–

Findings

1. Bio-computing has been an emerging field and will continue to dominate the bio-inspired design research field.
2. Robotics has been taking huge structural inspiration from bacteria and other microbes for design.
3. DNA, protein and other nano-structural researches have yielded important results in real life applications.
4. Bio-inspired design has been an extremely hot topic in the recent times, both worldwide and in India, and will continue to grow.
5. Most solutions provided are versatile and can actually deal with multiple issues at once, hence providing cost efficiency.
6. Neural and neuro-degenerative disorders are also being treated efficiently and have a visible future for cure.

CONCLUSION

Bio-inspired design research is being conducted at an immense pace to ensure that the solutions to modern problems are efficient in all aspects and are ideal in all situations. In India, the research on Bio-Engineering has been focussed on computer aided bio-inspired designs, in addition to architectural designs concerned with molecular interactions. Research was also conducted on fields concerning the mingling of biological professionals with engineers. Due to technology improving at an immense rate, the scope of this field is only expected to grow manifold. The various fields they manage to cover will be instrumental in increasing the popularity of this research, despite the fact that this research needs expertise in both biology and advanced engineering.

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Aflatoxicosis; Diagnosis and Treatment in Livestock

Deep Narayan Singh[1], Mamta[2], Ajay Kumar[3], Rajneesh Sirohi[4]

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Abstract

Aflatoxicosis is a fungal borne toxicosis that may affect almost all species of livestock in India as well as abroad. Aflatoxins are major class of mycotoxins produced primarily by Aspergillus flavus i.e. most common grain mold fungi. The fungus grows on carbohydrate rich feeds such as peanuts, cottonseed, corn, sorghum and cereal grains when they are stored in hot conditions without adequate drying and aeration. Aflatoxin M1 found in milk of dairy cattle is the metabolite of Aflatoxin B1 that occurs in feed materials. Aflatoxicosis is a problem in livestock, most notably swine and cattle. Gross lesions include hepatic enlargement, congestion, yellow discoloration, and friability; petechiae or more generalized hemorrhage; and edema and ecchymotic or petechial hemorrhages of the gall bladder. Cattle are more resistant than pigs, but the typical lesions of aflatoxicosis, as described above, can be found following exposure. Fibrosis and bile duct proliferation may be extensive and found together with fibrotic veno-occlusion of the central veins. Sheep are resistant to aflatoxin.

Keywords: Aflatoxin; Corn; Fungus; FDA.

INTRODUCTION

Many different fungi may grow as molds on stored grains. Fusarium and Aspergillus fungi are among the most common grain molds. Not all fungi produce toxins, but Aspergillus flavus, which produces aflatoxin, is among the most common grain mold fungi.

The development of aflatoxins depends on the infestation and growth of the Aspergillus mold in grain. High temperatures and high humidity favor the infection of corn kernels through the silks by the Aspergillus fungi. High humidity prone areas are more susceptible for aflatoxin poisoning. Below normal soil moisture (drought stress) has also been found to increase the number of Aspergillus spores in the air. Therefore, when drought stress occurs during pollination, the increased inoculums load (spores in the air) greatly increases the chances of infection. Further more, drought stress, nitrogen stress, and other stresses that affect plant growth during pollination can increase the level of aflatoxins produced by Aspergillus fungi.

Aflatoxins are poisonous, carcinogenic by products produced during the growth of several species of the mold fungus Aspergillus flavus. These byproducts are produced as the fungi grow

Author Affiliation: [1]Associate Professor, Department of Livestock Farm Complex (LFC)/Livestock Production Management (LPM), Bihar Veterinary College, Bihar Animal Sciences University, Patna 800014, India, [2-4]Assistant Professor, Department of LPM, College of Veterinary Science & Animal Husbandry, U.P. Pandit Deen Dayal Upadhayaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan, Mathura 281001, Uttar Pradesh, India.

Corresponding Author: Deep Narayan Singh, Associate Professor, Department of Livestock Farm Complex (LFC)/ Livestock Production Management (LPM), Bihar Veterinary College, Bihar Animal Sciences University, Patna 800014, India.

E-mail: drdeep25@gmail.com

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in feed grains, processed feed, and food products. Aflatoxins are primarily a problem in corn but can also occur in other grain crops. Aflatoxins are highly toxic to livestock, poultry, and people. Consumption of low concentrations by animals sensitive to aflatoxins can lead to death in 72 hours. In general, at nonfatal levels, the health and productivity of animals fed contaminated feed are seriously impaired. As a result, the Food and Drug Administration (FDA) has set an action level for aflatoxins in corn at 20 parts per billion (ppb). Corn containing aflatoxin levels of 20 ppb or more cannot be sold in interstate commerce, and, in general, should not be fed to young poultry, swine, and livestock, or to lactating animals, and must not be milled for human consumption.

Several other factors play a role in the development of Aspergillus mold and aflatoxin production. Because drought stress plays such an important role, practices that reduce drought stress in plants should reduce the levels of infection and aflatoxin production. Irrigation has been shown to be very effective in reducing Aspergillus infection and aflatoxin development, even if done only during pollination. Tillage practices have not been as effective and have only been demonstrated to reduce aflatoxin by sub soiling in areas with hardpans. Occasionally during droughty periods, hybrids of differing maturities or those planted early will pollinate during periods when drought stress is less often observed in South Dakota. Escaping drought with planting dates and hybrid maturity may differ from one year to the next. Time of harvest has also been shown to be important in influencing the occurrence and levels of aflatoxin because Aspergillus does not compete well with other molds when corn is above the 20 percent moisture content. Harvesting corn when moisture content is above 20 percent followed by rapid drying to at least a moisture content of 14 percent within 24 to 48 hours of harvest keeps further Aspergillus growth and toxin production at a minimum.

Aflatoxicosis and Livestock:

Aflatoxicosis is a disease caused by the consumption of aflatoxins. Aflatoxins are secondary mold metabolites produced by some strains of Aspergillus flavus and other related species of Aspergillus fungi. The four most common aflatoxins are B1, B2, G1, G2 and M. Contaminated grains and grain by products are the most common sources of aflatoxin. Corn silage may also be a source of aflatoxins, because the ensiling process does not destroy toxins already present in silage. Aflatoxins are metabolized in ruminants by the liver and are excreted in the bile. Aflatoxin B1 is the most potent mycotoxin (toxic substance produced by a mold) to affect cattle. B1 increases the apparent protein requirement of cattle and is a potent carcinogen (cancer causing agent). When significant quantities of B1 are consumed, the metabolite M1 appears in milk within 12 hours. Research suggests M1 is not as carcinogenic or mutagenic as B1, but it does appear to be as toxic as its parent compound.

Aspergillus thrives at high grain moisture levels (22% to 26%) and temperatures of 82 °F to 90°F. Drought stressed corn is especially susceptible to aflatoxin contamination.

The swine are also affected by Aflatoxicosis. The effects of feeding aflatoxin contaminated corn depends on the age of the pig and the concentration of the toxin in the feed. Low levels (20 to 200 ppb) can affect pig performance through reduced feed in take and suppression of the immune system. High levels (1,000 to 5,000 ppm) can result in death. A metabolite of aflatoxin (aflatoxin M) can be found in milk from sows fed diets with aflatoxin. Lactation diets with 500 to 750 ppm aflatoxin can result in mortality and reduced growth of nursing pigs. There are no maximum a safe levels of aflatoxin in swine diets, as effects can be found with low concentrations (20 ppb). There are published maximum a tolerable@ levels in complete diets for some classes of swine: pigs less than 50 pounds - 20ppb; finishing pigs - 200ppb; breeding swine - 100ppb. If the fungus is present and viable, aflatoxin levels can increase during grain and feed storage. One severe case of aflatoxicosis (850ppb aflatoxin in the finished feed) resulted from feeding corn with less than 20 ppb aflatoxin, but the feed was stored in large, outside feeders for seven to 14 days. There is a risk of aflatoxin toxicity in feed from low levels of aflatoxin in corn. The first step in addressing aflatoxin in swine on the farm is prevention. The potential for aflatoxin is reduced by drying (less than 15% moisture), removal of foreign material and cracked kernels, and routine aeration of stored corn. Suspect corn should be analyzed for aflatoxin B1 and aflatoxin B2, through a commercial lab or the veterinary diagnostic lab. If aflatoxin is present, the concentration will determine the next step. The simplest method of dealing with aflatoxin contaminated corn for on-farm use is blending.

Contaminated corn can be mixed with a clean corn to reduce aflatoxin to an acceptable level. For example, mixing 500 pounds of corn containing 400ppb aflatoxin with 1500 pounds of aflatoxin free corn reduces the aflatoxin level to 100ppb. If the contaminated corn contains viable fungus, the

aflatoxin levels will increase with high moisture and warm temperatures. Fungal inhibitors such as propionic acid can reduce or prevent fungal growth. These inhibitors can be expensive to use and difficult to apply properly. They will have no effect on the aflatoxin already present in the corn. The commercial feed industry often uses compounds called aluminosilicates to improve the pelleting and flow properties of feed. In the early 1980s, research showed some of these compounds reduced the negative effects of feeding aflatoxin contaminated corn. Mixing 10 pounds per ton sodium bentonite in feed using corn with 750ppb aflatoxin produced growth comparable to aflatoxin free feed. Similar benefits were found using a hydrated sodium calcium aluminosilicate. These compounds partially bind aflatoxin in the digestive tract and reduce their absorption. Aflatoxin is a problem that can often be controlled through proper grain handling. During drought conditions, however, it is a constant possibility. Suspect corn should be tested, and appropriate action taken based on the level of aflatoxin and the age and weight of the pigs to be fed. For more information, contact your local county Extension office or veterinarian, or the Alabama Department of Agriculture and Industries feed control office.

Development of Aflatoxin

Mature corn that remains in the field or corn that is stored without adequate drying can be subject to Aspergillus growth and aflatoxin production. Temperatures between 80°F and 100°F and relative humidity of 85 percent (corresponding to 18 percent grain moisture) are optimum for growth of Aspergillus. Growth of the fungus is poor below 55°F, but if the grain is moist enough, toxins can still be produced. However, simply reducing the moisture content to as low as 12 percent does not kill the fungus and does not reduce the levels of toxins that have already been produced. If moisture levels rise again above 12 percent anytime during storage, and temperatures are high enough, then mold growth and toxin production will resume. It is important to note that conditions favoring the growth of Aspergillus also favor the growth of other fungi that can have harmful effects on humans if they are inhaled or ingested while working in grain handling facilities. Always wear a dust respirator when working in grain or feed storage and handling areas.

Preventing Aflatoxin Contamination

Resistance to aflatoxin accumulation in corn kernels has been recently identified. Hybrids resistant to aflatoxin and other fungal toxins should become available in the near future.

Detecting Aflatoxin Contamination:

Once aflatoxin is produced, it is stable. Heat, cold and light do not affect it. It is also colorless, odorless and tasteless, and because of the low concentrations involved and the uneven distribution in grain bins, aflatoxins are difficult to detect. In the past, elevator operators and buyers used the black light test, but this test simply detects compounds that fluoresce (aflatoxins and others) and should only be used to select samples that require further testing. Similarly, mini-column tests are no longer recommended, as they were

Prone to give false positive results if used improperly.

However, aflatoxin contaminated feed can be tolerated by some livestock, particularly older animals. Obviously, the higher the level of contamination, the greater the risk in feeding contaminated corn to animals. Further more, continued proper storage is essential so that aflatoxin levels do not continue to increase in the corn or feed before use. Detoxification of feed continues to be an elusive goal. However, certain feed additives have been successfully used to inhibit mold growth and to reduce the incidence of aflatoxicosis in animals. Organic acids such as propionic, sorbic, and benzoic acids as well as their salts such as calcium propionate and potassium sorbate, and copper sulfate can be used to inhibit mold growth in feed. Mineral clays such as zeolite and bentonite as well as hydrated sodium calcium alumino-silicate (HSCAS) can bind to aflatoxin, protecting animals from absorbing the toxin that may be in the feed. These products, according to FDA rules, cannot as yet be labeled as mycotoxin binders, and are sold as anti-caking and free flow feed additives. There are no clear cut safe levels for different animal species regarding their resistance or tolerance to aflatoxins. The following section on aflatoxicosis and ruminants and the general guidelines for dealing with aflatoxin contaminated feed may assist you in deciding.

Sampling for Aflatoxins

Regardless of the test procedure used, the single most important factor for reliable and accurate testing of grain for aflatoxins is obtaining a representative sample. The ideal sample size should be at least 10 pounds of corn. The sample should consist of several smaller subsamples (10 or more 1 pound samples) that have been taken from different spots and then mixed together. Handle each bin or truck separately, and take a 10 pound

sample from each source. Place samples in a cloth or paper container that allows air exchange. Air tight containers or plastic bags allow condensation, which raises the moisture content, resulting in the possibility of continued growth and toxin production of the fungus. Send or take samples to a testing lab as quickly as possible.

Effects of Aflatoxins on Animals

Effects of aflatoxin consumption are similar in all animals; the animal's susceptibility to aflatoxin, however, varies by species, age, and individual variation. In acute clinical aflatoxicosis, signs of acute hepatic injury are seen as coagulopathy, increased capillary fragility, hemorrhage, and prolonged clotting times. Blood pigments may appear in the urine and mucous membranes are icteric. The liver shows gross changes caused by centrilobular congestion and hemorrhage and fatty changes of surviving hepatocytes. Death of the animal may occur within hours or a few days. In chronic aflatoxin poisoning, most of the effects are still referable to hepatic injury, but on a milder scale. The most sensitive clinical sign of chronic aflatoxicosis is reduced rate of growth of young animals. Other signs include prolonged clotting time, in- creases in serum glutamic oxalacetic trans aminase, ornithine carbamyl transferase, and cholic acid levels. Hepatic pathology includes a yellow to brassy color, enlarged gall bladder, dilute bile, histologic signs of fatty changes in the hepato cytes, and bile duct proliferation. Frequently the signs of chronic aflatoxins are so protean that the condition goes undiagnosed for long periods. Chronic aflatoxin poisoning, however, is the man ner in which animals are most frequently affected and. The economic consequences are often considerable. Both acute and chronic aflatoxin poisoning can impair immune responses and native defense mechanisms. In addition to the liver, the thymus is also a target organ for aflatoxin; thymic involution results with loss of cortical thymocytes. It is primarily the cellmediated immune responses that are affected by aflatoxin; prominent among these are diminished responses in delayed cutaneous hypersensitivity, graft vs. Host reaction, leukocyte migration, and lymphoblastogenesis. Aflatoxin also reduces phagocytic activity in a dose related manner; this is important not only to the phagocytic clearance of invading organisms but also to presentation of antigens to other components of the immune system. Some humoral components are diminished by aflatoxin, including complement C4), interferon, IgG, and IgA, but not IgM. Usually only dramatically high levels of aflatoxin will affect antibody titers and gut associated lymph tissue or the bursa or Fabricius in Immunity acquired through vaccination procedures is impaired in fowl cholera and porcine erysipelas, but not New castle disease in chickens. Similarly, susceptibility to some infectious agents (e.g., Salmonella, Candida, Treponema, Eimeria, and infectious bursal disease virus) is increased, but not all infectious processes seem to be affected (Pier and McLoughlin, 1985). of considerable potential economic consequence is the fact that aflatoxin can suppress the immune system of young animals by in utero transfer across the placenta of the pregnant dam (Pier et al., 1985). In these cases the affected newborn animals lack resistance to infection and cannot respond well to vaccines. These are reactions of considerable consequence in colonized animals in which we rely on elective vaccination procedures in disease prevention. Several of the major mycotoxins exert their effects through different organ systems and different biological pathways. Aflatoxin, ochratoxin, and T-2 toxin all interfere with protein formation, but each does so in a different manner; aflatoxin binds to both RNA and DNA and blocks transcription. T-2 toxin blocks initiation of translation, and ochratoxin blocks phenylalaninet RNA synthetase, and thus blocks translation. Each toxin causes different effects on globulin formation; aflatoxin reduces IgG and IgA but not IgM and usually does not reduce antibody titers, ochratoxin reduces IgG and IgM and regularly reduces antibody responses, T-2 reduces IgM and IgA but usually not IgG and often reduces antibody response. Both aflatoxin and T-2 effect reduced complement activity, but in different ways; aflatoxin reduces C4 activity, whereas T-2 reduces C 3 activities. Thus, one might expect that when mixtures of mycotoxins are encountered in feed mixtures that some interaction of these toxins might be apparent. Experiments combining aflatoxin and T-2 toxin show a synergistic effect on lethality, but only additive suppression was seen on weight gain and selected immunologic traits (Pier et al., 1988). Another mycotoxin mixture (aflatoxin and cyclopiazonic acid) was studied in guinea pigs (Pier et al., 1989). This was an interesting mixture because these two toxins appear together in nature; some strains of A. flavus are capable of producing both toxins in corn and other substrates. Synergistic interaction was seen on lethality, weight gain, and histopathologic changes in the liver. However, an interesting observation was that cyclopiazonic acid over came the immunosuppressive effects of aflatoxin on cell poultry. mediated immune responses. From these and other studies it is

apparent that the immune system is a sensitive register for the effects of various mycotoxins and that mixtures of mycotoxins can profoundly affect the animal organism.

Implications

The economic consequences of aflatoxicosis on young growing animals are substantial and varied. Aflatoxins cause clinical illness and death when consumed in high quantity; at lesser levels they reduce the growth rate and feed efficiency of young animals and they reduce the animals' ability to cope with infections. Undoubtedly we will recognize yet other effects of the aflatoxins on animal and human health as investigations continue. Because of their exceptional biological activities and their propensity to peak in essential field crops during problem years, aflatoxins promise to be a continuing problem in animal production.

Symptoms of Aflatoxicosis

Beef and dairy cattle are more susceptible to aflatoxicosis than sheep or horses, although other mycotoxicoses occur in these species, such as facial eczema in sheep and leukoence phalornalacia in horses. Young animals of all species are more susceptible than mature animals to the effects of aflatoxin. Pregnant and growing animals are less susceptible than young animals, but more susceptible than mature animals. Feed refusal, reduced growth rate and decreased feed efficiency are the predominant signs of chronic aflatoxin poisoning. In addition, listlessness, weight loss, rough hair coat and mild diarrhea may occur. Anemia along with bruises and subcutaneous hemorrhage are also symptoms of aflatoxicosis. The disease may also impair reproductive efficiency, including abnormal estrous cycles (too short and too long) and abortions. Other symptoms include impaired immune system response, increased susceptibility to disease, and rectal prolapse.

Clinical Pathology

Clinical laboratory findings vary with the animal species, level of aflatoxin in the ration, and the duration of feeding. There are no consistent diagnostic changes in haematocrit, hemoglobin, and differential cell counts in animals fed aflatoxin. Leukocytosis may occur in animals with secondary bacterial infections. Serum bilirubin levels may be elevated and typically serum protein levels are decreased. Lesions observed at necropsy related to either acute or chronic liver disease are dependent upon the level of aflatoxin and the duration of feeding. A majority of acute liver damage observed has been the result of experimentally high doses, while chronic liver damage is a more common field observation. The liver is usually pale tan, yellow or orange. Hepatic fibrosis and edema of the gallbladder may also be observed.

Diagnosis

The diagnosis of aflatoxicosis is often difficult because of the variation in clinical signs, gross pathological conditions and the presence of infectious diseases due to the suppression of the immune system. On the farm, more than one mold or toxin may be present in the contaminated feed, which often makes definitive diagnosis of aflatoxicosis difficult. The prognosis of aflatoxicosis depends upon the severity of liver damage. Once overt symptoms are noticed the prognosis is poor. Treatment should be directed at the severely affected animals in the herd and furt her poisoning prevented.

Serological tests for diagnosis of Aflatoxins:

Serological tests are now considered to be more reliable and their accuracy has been validated by comparison to more costly and time consuming analytical procedures. Serological test kits using such methods as ELISA do not require specialized labs, equipment, or training and when conducted according to manufacturer's instructions can give accurate results for the presence (qualification) and amount (quantification) of Aflatoxins in grain samples.

Determination of Aflatoxins in laboratory

Modified Romer's method:

Method: The aflatoxin is extracted with acetone, treated with Cupric carbonate and ferric gel to eliminate fluorescent material other than aflatoxin, washed with acid and alkali and extracted with chloroform, dried, rediluted with chloroform and spotted in an activated TLC plate with standards and ascertained the concentration by visual comparison method in a UV viewing cabinet.

Preparation and Requirement of reagent

1. 0.2 M NaOH: Dissolve 8 gm of NaOH in 1000 ml solution.
2. 0.41 M FeCl3: Dissolve 17 gm of FeCl3 to make 1 lit of solution with distilled Water.
3. 0.03% H2SO4: 0.3 ml of conc. H2SO4 in 1 lit of distilled water
4. 4.0.02 M KOH and 1% KCl: Dissolve 1.222 gm of KOH and 10 gm of KCl in 1 lit standard flask with distilled water.

Preparation of Activated TLC plate:

To prepare two plates (10cmx10 cm or 10cmx5cm)

of 0.2mm thickness dissolve 16 gm of silica gel (G) in 35 ml of distilled water, apply on the plate using applicators allow it to natural dry. Then keep the plate at 105°C for 1hour and cool. Draw lines with 1 cm space such that standards are at the middle of the plate and four sample spots can be applied on either side of the standard.

Procedure:

1. Take 10 gm of sample. Add 40 ml of distill water. Beat it in the mixie for 2 minutes.
2. Add 60 ml of acetone and again it for the 2 minutes. Contents may slightly be heated up. High temperature should be avoided.
3. Filter the contents. Take 30 ml of filtrate and add approximately 0.6 gm of cupric carbonate in beaker (A).
4. In another beaker (B), take 34 ml of 0.2 M NaOH + 6 ml of FeCl3 (0.41 M) and swirl the contents.
5. Add the contents in the beaker (B) to beaker (A) and again mix it slowly by swirling movements.
6. Filter the contents through Whatman No. 1 Filter paper.
7. Take 40 ml of filtrate in a 25 ml separating funnel.
8. Add 40 ml of (0.03%) H2SO4 and 10 ml of chloroform. Mix it slowly.
9. Collect the chloroform layer in a 100 ml beaker, add 10 ml of Chloroform, mix thoroughly, allow to settle and collect the chloroform in the same 100 ml of beaker.
10. In a second separating funnel, take40ml of 0.02 M KOH and 1% KCl mixture.
11. To this, add the collected 20 ml of chloroform extract. mix it slowly and collect the layer through anhydrous Sodium sulphate bed drop by drop to remove ant traces of moisture.
12. Keep the chloroform extract in an oven at 50 C till it becomes dry.
13. The dry afflatoxins film is rediluted with0.2 ml Chloroform and spot on the TLC plate taking exactly 5 micro liter, 10 micro liter, 20 micro liter and 40 micro liter besides the standard spots of 5 micro liter and 10 micro liter.

Preparation of Aflatoxins Standard

Carefully prepared the given Aflatoxin in a suitable standard flask with Benzene: acetonitile (98+2) mixture to give a concentration of 10 micro gram per ml. Standardize the concentration of stock solution using spectrophotometer. From this stock solution, prepare Aflatoxin solution in benzene: acetonitrile containing 4 micro gram per ml in a suitable standard flask which is the working standard. After spotting the standards and sample, develop the spots in an unsaturated developing time containing chloroform: acetone: water in the ratio 88:12:1. after developing 3/4th of the plate, the plate is carefully remove from the tank, dried well and viewed in UV cabinet viewer using long wavelength (364 nm)

Calculation:

$$\text{Aflatoxin content in ppb} = \frac{S \times C \times d \times 100}{T \times 1.714}$$

Where,

S = Standard which compare with the sample in the fluorescent intensity.

C= Concentration of standard.

d= dilution factor.

T= sample which compares standard in fluorescent intensity.

1.714= effective weight.

Treatment

Aflatoxicosis is typically a herd rather than an individual cow problem. If aflatoxicosis is suspected, the ration should be analyzed immediately. If aflatoxins are present, the source should be eliminated immediately. Levels of protein in the ration and vitamins A, D, E, K and B should be increased as the toxin binds vitamins and affects protein synthesis. Good management practices to alleviate stress are essential to reduce the risk of secondary infections. Secondary infections must receive immediate attention and treatment.

Aflatoxicosis can only be prevented by feeding rations free of aflatoxin. Preventing aflatoxin contamination is outlined on the preceding page, but since preventing contamination is not always possible, here are a *few keys facts to remember when dealing with contaminated feeds in animal rations:*

- The recommended feeding level is 0 parts per billion (ppb).
- The level of aflatoxin an animal can tolerate will depend upon the age and sex of the animal, its health status, and overall management level of the farm.
- To avoid contamination of milk, lactating dairy cattle should not receive more than 20 ppb in the total ration.
- Calves should not receive milk from cows fed

in excess of 20 ppb, because they can ingest aflatoxin from the milk.

- Beef cattle can tolerate slightly higher levels of aflatoxin, but yearlings and mature cows should not receive more than 400 ppb in the total ration. Weanlings should not receive more than 100 ppb in their total daily ration.
- Poultry and swine are more sensitive to aflatoxin contamination.

Under no circumstances should these livestock species be fed more than 20 ppb aflatoxin in their daily rations. The above are only guidelines. This does not suggest that feeding at these levels or below will reduce or eliminate the potential for aflatoxicosis. There are no clear cut safe feeding levels. Safe levels vary with each individual animal. Remember that ingestion of aflatoxins at levels even lower than those listed in the guidelines may cause some undesirable side effects and depends on such factors as age, sex, and general health of the animals. To feed at a level other than 0 ppb is a risk assumed by the person making the decision to do so. In all cases, monitor animal health closely and discontinue the use of contaminated feed immediately if undesirable effects are noticed.

CONCLUSION

Aflatoxins are highly toxic to livestock, poultry, and people. Even when fed at nonfatal levels, aflatoxin can seriously impair animal health and productivity. For lactating dairy cattle, do not exceed 20 ppb aflatoxin in rations to avoid exceeding the Food and Drug Administration level of 0.5 ppb in milk. Aflatoxin is just one of many mycotoxins that can adversely affect animal health and productivity.

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Case Report

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Traditional Practices Impact on Positive Environment in Selected Villages of Fatehgarh Sahib District, Punjab: A Field Visit Report

P. Chitra

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Abstract

Cattle comprises the top most domestic animal in India. Cow is connected with all beings for the use of milk in crucial time of their life. Cow milk products and by products of cow dung and cow urine also have more therapeutic benefits practiced in centuries in India. The cow is treated as holy animal and Hindu religion worshipped as Goddess. The author chosen to visit North Region of Punjab, Fategarh Sahib district, selected two villages to observe the traditional practices of cow dung and manure preparation for the villagers day today life. The field visit the author interviewed the Sarpanches (Panchayat President's) and gathered the information socio economic demographic variables and health of villagers and communicable diseases data of the people. It is reported that there is no incidence of specific and periodic diseases occurred for past years. The author observed the practice of cow dung and manure preparation for long time it enhances the ecological environment and thus prevent the diseases and promote the health of people and positive atmosphere of community climate.

Keywords: Traditional Practices; Environment Health; Cow Dung; Manure; Guhara.

INTRODUCTION

Cattle comprises the top most domestic animal in India. The cow milk is very useful for human life from infant to end life. Other animals off springs for life surviving when mother dead or not available to feed it only depends on cow milk. The cow role not possible to substitute of any other animals for feed of any living beings in this World. For motherof her kindness to the humanity she is treated Goddess in Hindu religion. Cattle by products of cow dung and urine will be used for therapeutic and hygienic purposes for centuries. Many studies says the cow dung is natural remedy of purified environment and it exposure controls the adverse effects of pollutions.

Cow dung is also called as cow pats, cow pies, or cow manure, is the waste product (faeces) of bovine animal species. A deposit of cow dung is referred to in American English as a 'Cow pie' or less commonly 'cow chip' (when dried) and in British English as a Cowpat.[1]

Cow dung is the undigested residue of plant matter which has passed through the animal's gut. The faecal matter is rich in Minerals. Cow dung provides food for a wide range of animal and fungus species, which break it down and recycle it into the food chain and into the soil. Cow dung

Author Affiliation: Director of Research & Development, Desh Bhagat University, Fatehgarh Sahib, Punjab 147301, India.

Corresponding Author: P. Chitra, Director of Research & Development, Desh Bhagat University, Fatehgarh Sahib, Punjab 147 01, India.

E-mail: prof.pchitra@gmail.com

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is also used in Hindu religious fire Yajna as an important ingredient.[2] Cow dung is also used in the making of Panchgavya for use in Hindu rituals.[3]

Cow dung is utilized for flower and plant pots. It is plastic free, biodegradable and eco-friendly. Cow dung pots dissolves naturally and becomes excellent manure for the plant from 20 July of 2020, state Government of Chhattisgarh India started buying cow dung under "Godhan NyayYojna" Scheme. Cow dung procured under this scheme will be utilized for the production of vermi compost fertilizer.[4]

Cattles population

India cattle inventory amounted over 305 million in 2021. India had the highest cattle population followed by Brazil, China and USA.[5] The 19th live stock censuses 2012 reported that there are around 300 million bovines in India, which is the 18% of the bovine population existing in the world. The Hindu Vedas mention cow as a holy animal and must be worshiped. Cows are very essential animal resources in India. Cows are vast useful in dairy industry and agriculture.[6] India has 190.9 and 108.7 million cows and buffaloes respectively.[7] From time immemorial, cows have been a part of civilized life[8,9] especially in India.

Characteristics of cow dung

Fundamentally, the dung of the cow is the throwouts or discards containing the undigested herbivorous materials which are processed in their guts by the symbiotic bacteria which resides in the stomach compartment, rumen of the cow. These rejected matters or the faecal matter are very is rich in the mineral content (from the human point of views). While cow dung is just a waste by product of the cattle, Ayurveda describes the cow dung (gomeya) as not mere a rejected material but as a best natural purifier (which purifies all the waste products of nature).[10]

Cow dung contains around twenty four minerals like potassium, nitrogen, fewer quantities of sulphur, calcium, magnesium, manganese, cobalt etc. Also, it consists of crude fibres like cellulose with lignin hemicelluloses and crude proteins. The overall composition of cow dung is the undigested fibre, sloughed of intestinal epithelium, fewer excreted products which are derived from mucus, bacteria present in the intestine and pigments of bile. The green colouration of the cow dung is due to the presence of the bile pigment called biliverdin. The emulgent property of the cow dung is also due to the salts of bile (confers hydrophilic coat to the hydrophobic droplets).[11]

The microbial population of the cow dung consists of plentiful counts of bacilli (lactobacilli) and cocci. Few revealed and unknown and/ oranonymous fungi and yeasts are also present. As a whole, more than sixty bacterial species, hundred species of protozoa and yeasts are present in the cow dung.[12,13]

Table 1. Composition of cow dung

S.No.	Composition	Percentage
1.	Water	80%
2.	Nitrogen	1.74%
3.	Phosphate	1.7%
4.	Potash	0.6%
5.	Calcium	0.37%
6.	Magnesium	0.53ppm
7.	Ferrous	1400 ppm
8.	Zinc	90 ppm
9.	Manganese	210 ppm
10.	Copper	7.1 ppm
11.	Boron	5.0 ppm
12.	Bacteria	60 species
13.	Protozoans and yeasts	100 species

*ppm- parts per million

Macdonell AA, Keith AB 2007

The cow dung obtained from a matured cow is approximately 5.4 tonnes per annum. It varies from the cow dung obtained from the calf. As a result, the dry cow dung would be nearly 1620 kg per annum (54. x 0.30).[14] Hence, cow dung is considered as a "gold mine" because of the broad applications in department of resource of energy, department of agriculture, protection of environment department and the therapeutics.[15]

Environment Protection of Cow dung

Cow dung is considered as an important substance in the preservation of the environment. The atmospheric temperature is balanced when the cow dung is burnt. Cow dung controls the radio active rays and also the heat impact of the environment. The dwellers are protected by coating the walls and cleaning the floors of the house with the mixture of dung and water. The Bhopal gas leak in 1984 killed around 20,000 people, but the people who lived in the cow dung coated walls were less affected. Till today, cow dung is used to shield the radiation in the atomic power centres located in India and Russia. This also prevents the harmful radiations from sun and shields the earth. Overall,

cow dung prevents the environmental pollution by keeping it away from pollutants and do not allow any harmful effects of radiation.[16] Reduction of acid content in water is processed to reduce the percentage of acid by the treatment of water with the dung. This procedure can be followed for cleansing the pond and also to neutralize the acid present in the pond.

Cow dung possesses disease preventing properties. It destroys micro organisms which cause diseases, fermentation and put refaction. Since olden days to till date (in some villages through out India), there exists the habit of cleaning and moping the floors, especially the clay floorings using the mixture of water and cow dung. Slowly the above practice has vanished due to the transformation of the floorings from clay to mosaic, marble, granite, etc., but in some places still they follow this practice. People who still wanted to practice this traditional system, in spite of their flooring not made in clay, they sprinkle the mixture containing water at the entry of the door in their house.[17,18]

There is a ritual named "*Gobarlep*" performed by Hindus. The meaning of "*Gobarlep*" is nothing but spreading of a thin film of the mixture of cow dung and water of the floors and walls. The films of the mixture of cow dung mainly consist of cellulose in an undigested form. When the moisture in the mixture gets evaporated due to natural sunlight drying, the alkaline content comes up forming a binding coat. The viral particles are mostly acidic in nature and hence when these particles fall on the ground containing a thin film of cow dung, they feel an ionic blow and eventually their virulent effect gets reduced. The neutral viral particles fall on the ground and get attached on to the film. Hence, there will not be any active microbes present on the ground when there is a coat of cow dung.[19]

Observational visit to Khumna &Khanian villages.

The author visited to the villages of Khumna and Khanian of Fatehgarh Sahib district, Punjab to observe the environmental health and any hazards exposure of village people.

Firstly met Sarpanches (Head) of villages in prior appointment and arranged in person interview with specific day. In each village a day is fixed and arranged village tour to visit the village. With their permission photographs taken for study purpose. In interview gathered the information about village population, demographic and economic variables, domestic animals, traditional practices of cow dung and manure preparation, facilities available at village, health status of the villagers and any specific diseases for past and present due environmental pollutions of water , soil and air.

Surprisingly, the author found that the villagers most of them rearing cattle for their own use of milk, cow dung and farming work purposes in their houses or farms.

The villagers for traditional and cultural practices is existing in system is naturally occurring phenomenon ecologically good for health.

The both villages the similarities are observed in cattle care, cow dung cake preparation and storation house named Guhara and manure preparation process for cultivation.

Khumna is a village located in Amloh Tehsil of Fatehgarh Sahib district, Punjab with total 327 families residing. The village population is 1558 of which 839 are males while 719 are females as per population census 2011. The village population of children with age 0-6 is 165, literacy rate of was 71.79%.The male literacy is at 75.17% while female literacy rate is 67.77%. This village most of the %.This villagemost of the population are from schedule caste constitutes 74.07 % of total population. Children the age group of 5-18 years around 500 .Out of 625 workers engaged in employment work, 20 were cultivators (owner or co-owner) while 7 were agricultural laborers.

The village have open drainage system and few places obstructed the drainage, mosquitoes breeding may be for long times happened. People are managing with mosquitoes repellents with cowdung smoke at evening in their house premises to control and prevent health issues. The waste disposable is road side dumping or anywhere throwing is the real practice there is no facilities to follow waste management of using different dust bins for disposable and regular collecting waste and in different points. No sanitary workers and other Government benefits not reached to this village. This village have Primary school and Primary Health Centre for their use. In this village cattle are living around 300.

Khanian is a large village located in Amloh Tehsil of Fatehgarh Sahib district, Punjab with total 446 families residing. The village population is 2046 of which 1056 are males while 990 are females as per population census 2011.The children with age 0-6 is 215 which makes up 10.51 % of total population of village. Average sex ratio of village is 938 Child sex ratio for as per census is 870, The village has higher literacy rate in 2011, The male

literacy stands at 86.72 % while female literacy rate was 77.30 %.Schedule Caste (SC) constitutes 28.05 % of total population village.

In Khanian village out of total population, 629 were engaged in work activities. 93.64 % of workers describe their work as main work (employment or earning more than 6 months) while 6.36 % were involved in marginal activity providing livelihood for less than 6 months. Of 629 workers engaged in main work, 124 were cultivators (owner or co-owner) while 92 were agricultural laborers. Schedule Caste (SC) constitutes 28.05 % of total population in village.

The village have good administrative system of by own villagers. They have farmers cooperative society for the benefits of farmers. The village around 900 acres are cultivating by villagers. It is rich village .The Government higher secondary school available for boys and girls. Veterinary hospital facility available for their domestic animals. In this village cattle around 600 numbers.They also doing same way of cow dung and manure preparation

The village Khumna – Photographs of Cow dung

systematically in regular work of their part of life.

The village practiced open drainage system and maintained somewhat cleanliness. Sanitary worker arranged by their own to disposable of waste in regular basis. When the observation the road side dumping is commonly seen and plastics found everywhere in village. It may affect the cultivating land.

As per constitution of India and PanchyatRaaj Act, Khumna and Khanian villages are administrated by Sarpanch (head of village) who is elected as representative of village.

Cow dung Cake Preparation of the Villages

Cow dung is collected in daily basis. Stored in available space of the home or near by place. Then

The Village Khanian - Photographs of Cow dung & Manure Pits

it is taken as big ball size and made it flat and round shape for easy dry.

The first day it kept in the upper part exposed to sunlight next day it is taken lower part to be dried in sunlight, later it is easy to dry with 2 cow dung cakes together support each other. Depends on sunlight it will be dried and beautifully arranged in House of cow dung named in Punjabi as Guhara. It is like dome shape of arrangement. Broad in bottom and layers by layers it reches cone shape in top. Whenever it is required fire, smoke it is available for whole year. Also cow dung is used for Manure of the land for cultivation.

Manure Preparation

The cow dung is dumped into the 'manure pits

Picture of Guhara

'and covered with earth after each day dumping. Two such pits will be needed, when one is closed , the other will be use in 5 to 6 months time , the cow dung is converted into manure which can be returned to the field.

The organic manure is ready after 6 months to 1 year for the cultivation. Few of the farmers doing just cow dung dumping for long time in pit and finally closing with earth , not using earth in between time in summer seasoning .

CONCLUSION

Cow dung prepared by the all regions of India. Some difference may be seen in design and thickness. In Punjab state it is broad and very thick round in big size where as in Tamilnadu it is thin and round in size and which is dried in floors of land

and walls of the mud houses and stored for seasons usage. Where the cattles are more there is naturally ecological positivism can happened and also prevention of many diseases around the premises. For using evening smoke at in front of houses it totally runaway from the area. Land filling manure is useful in organic production of vegetables paddy and wheat to consume for healthy and long life of humans and animals.

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Case Report

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Successful Management of Vaginal Hyperplasia in a Non-Descript Bitch

Kabir Alam[1], Daund Sushant S.[2], Rajesh Kumar[3]

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Abstract

A 3 year old non-descript bitch was presented to our teaching veterinary clinical complex with the history of protrusion of red coloured mass through the vulva. Clinical examination revealed a pear shaped pendulous mass was hanging from the vulva. The dog was found to be systematically healthy. Based on the clinical and physical examination, the case was identified as a type-3 vaginal hyperplasia. The treatment involved a medicinal management with the Gn RH and hCG but due to recurrence of the condition, the amputation of the protruded mass along with the OHE was carried out. The bitch was recovered uneventfully without any recurrence of the condition.

Keywords: Bitch; Hormone; Management; Vaginal Hyperplasia.

INTRODUCTION

In canines, hyperplasia of the vaginal mucous membrane is an unusual reproductive disorder. It is most commonly seen in young bitches during follicular phase of the estrous cycle. The brachiocephalic breeds are more prone to vaginal hyperplasia (Post et al, 1991), with little incidence in case of non-descript breeds (Anila et al, 2020). It is characterised by the swollen and oedematous vaginal mucosa, cranial to the urethral orifice with protrusion through opening of the vulva, giving a dome-shaped or dough nut-shaped appearance of the protruded mass (Post et al, 1991). It is believed to be due to the increased concentrations of serum estradiol (Concannon, 2011). Early veterinarian assistance is needed to avoid trauma, inflammation and self-mutilation to the protruded mucosa (Wykes, 1986). In this case report we described the successful management of stage 3 vaginal hyperplasia in a bitch.

Author Affiliation: [1]PhD Student, [2]PG Student, [3]Assistant Professor, Department of Veterinary Gynecology and Obstetrics, College of Veterinary Science and Animal Husbandry Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya 224229, Uttar Pradesh, India.

Corresponding Author: Rajesh Kumar, Assistant Professor Department of Veterinary Gynecology and Obstetrics, College of Veterinary Science and Animal Husbandry Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya 224229, Uttar Pradesh, India.

E-mail: drrajesh25@gmail.com

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Case history and observations

A 3 year old non-descript bitch was presented to our veterinary clinical complex (VCC), ANDUAT, Kumarganj, Ayodhya (UP) with the complaint of protrusion of red coloured mass through the opening of vulva since 9 days. The case was previously treated by a local veterinarian with manual reposition of the protruded mass but the recurrence again occurred. Clinical examination revealed a red, semi-soft large pedunculated mass protruding from the vulva (Fig. 1). The animal had normal appetite, urination and defaecation and was in proestrus just before the initiation of this condition. The diagnosis of this condition is done after the clinical and physical examination and differentiating it with vaginal or vulvar tumor, as stage 3 vaginal hyperplasia.

Fig. 1: Vaginal hyperplasia in bitch

Treatment

Medical treatment

Firstly, we cleaned the protruding mass and applied a lubricating jelly to protect it from drying and advised to place a elizabeth collar to avoid self-mutilation. Gonadotropin releasing hormone (GnRH) 5µg twice a day intramuscularly was given for 7 days. The result of this treatment was not found to be satisfactory so we given human chorionic gonadotropin (hCG) 250 IU once a day for 4 days. After treating with hCG, the mass was reduced gradually upto 70 percent within 9 days of treatment but again the recurrence of the condition was there so after consulting with the owner we decided for the surgical management through ovariohysterectomy as the bitch is not being used for future breeding purposes.

Surgical management

The surgery was performed under general anaesthesia. The atropine sulphate @0.04 mg/ kg body weight was given as preanaesthetic. The anaesthesia was induced and maintained with xy lazine @ 1mg/kg body weight intravenously and ketamine @5mg/kg body weight in travenously. The animal was placed in lateral recumbency, and the protruded mass was cleaned with normal saline. To avoid damage during sutures or surgery, the external urethral orifice was identified and catheterized. The lobulated, edematous mass was ligated with absorbable sutures (Petcryl, no1, Futura Surgicare Pvt. Ltd.), taking care not to encircle the urethra. The prolapsed mass was amputated after it had been properly ligated. The urinary catheter was removed after the completion of surgery. The OHE was also performed to avoid the recurrence of the vaginal hyperplasia. Post-operatively the animal was given inj.cefoperazone @20mh/kg b.wt. BID for 7 day, inj. Meloxicam @0.2mg/kg b.wt. IM OID for 5 days, inj. Vitamin B complex @2 ml IV BID for 7 days and tab. Tissue aid @1 tab BID orally for 5 days. The surgical wound was antiseptically dressed with Betadine solution until complete healing occurred, and the animal was successfully recovered without reccurence of the condition.

DISCUSSION

Vaginal hyperplasia is a benign, proliferative disorder of the vaginal mucosa characterised by excessive growth of the vaginal epithelium mainly due to the exaggerated response of the vaginal mucosa to estrogen during the proestual-estral phase of the cycle leading to protrusion through the vulvar lips (Bucci, 2022). Other factors such as age, breed and genetic predisposition may also play a role in the pathogenesis of this condition (Johnston, 1990). Based on the degree of tissue involved, the vaginal hyperplasia is divided into 3 stages (Manothaiudom, 1991). In this case, both the medicinal and surgical approaches were carried out. The GnRH and hCG exerts there effect by releasing LH and cause a subsequent rise in serum concentration of progesterone but due to reccurence of the vaginal hyperplasia, amputation of the prolapsed mass along with the OHE is found to be the best method to treat the case of vaginal hyperplasia in bitch.

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Standard abbreviations should be used and be spelt out when first used in the text. Abbreviations should not be used in the title or abstract.

Checklist

- Manuscript Title
- Covering letter: Signed by all contributors
- Previous publication/ presentations mentioned, Source of funding mentioned
- Conflicts of interest disclosed

Authors

- Middle name initials provided.
- Author for correspondence, with e-mail address provided.
- Number of contributors restricted as per the instructions.
- Identity not revealed in paper except title page (e.g.name of the institute in Methods, citing previous study as 'our study')

Presentation and Format

- Double spacing
- Margins 2.5 cm from all four sides
- Title page contains all the desired information. Running title provided (not more than 50 characters)
- Abstract page contains the full title of the manuscript
- Abstract provided: Structured abstract provided for an original article.
- Key words provided (three or more)
- Introduction of 75-100 words
- Headings in title case (not ALL CAPITALS). References cited in square brackets
- References according to the journal's instructions

Language and grammar

- Uniformly American English
- Abbreviations spelt out in full for the first time. Numerals from 1 to l0 spelt out
- Numerals at the beginning of the sentence spelt out

Tables and figures

- No repetition of data in tables and graphs and in text.
- Actual numbers from which graphs drawn, provided.
- Figures necessary and of good quality (color)
- Table and figure numbers in Arabic letters (not Roman).
- Labels pasted on back of the photographs (no names written)
- Figure legends provided (not more than 40 words)
- Patients' privacy maintained, (if not permission taken)
- Credit note for borrowed figures/tables provided
- Manuscript provided on a CDROM (with double spacing)

Submitting the Manuscript

- Is the journal editor's contact information current?
- Is the cover letter included with the manuscript? Does the letter:

1. Include the author's postal address, e-mail address, telephone number, and fax number for future correspondence?
2. State that the manuscript is original, not previously published, and not under concurrent consideration elsewhere?
3. Inform the journal editor of the existence of any similar published manuscripts written by the author?
4. Mention any supplemental material you are submitting for the online version of your article. Contributors' Form (to be modified as applicable and one signed copy attached with the manuscript)