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EFFICACY OF COW URINE AND PLANT LEAF AQUA-EXTRACTS AGAINST RAGI BLAST DISEASE

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Abstract

Ancient plants and plant based pathogens are the outcome of co-evolution. Ragi blast disease caused by *Magnaporthe grisea* is known to be the principal reason for its widespread ragi crop losses throughout southern Karnataka, India. The persistent use of chemical-based fungicides has contributed to the development of resistance in pathogens alongside environmental implications, highlighting the necessity for eco-friendly alternatives. Interest in cow urine (recognized for its microbicidal quality) as well as plant extracts from *Azadirachta indica*, *Ocimum tenuiflorum,* and *Datura metel*, due to their antifungal properties have gained importance as a mounting preference for an organic mode of disease management. The leaves of these plants were dried in the shade for seven days and ground into a fine powder, which was then used to prepare aqueous extracts at 1:20, 1:10, and 1:5 concentrations. The extracts were tested fresh and after 7 days of storage (at room temperature and under refrigeration). Each extract was combined with similarly treated cow urine in varying proportions and introduced into wells in Petri Plates (containing Sabouraud agar) inoculated with *Magnaporthe grisea* . Zones of inhibition were recorded after 96 hours. The highest zone of inhibition (28mm) was recorded at 60% tulsi extract and 40% cow urine (1:5 ratio). Tulsi leaf extract proved to be the most effective against M.grisea, followed by neem leaves. The results further confirm the efficiency of such traditional formulations as a cost-effective and sustainable alternative to chemical-based fungicides.

Keywords: Ragi blast disease, Magnaporthe grisea, Ocimum tenuiflorum, Datura metel, Azadirachta indica, cow urine, antifungal activity, organic disease management, aqueous leaf extract, zone of inhibition.

1. INTRODUCTION

Agriculture is the backbone of our Country and it is the most vital requirement which is responsible for nurturing the Entire country and the world. Rising awareness in sustainable agriculture encourages the shift towards eco-friendly alternatives. (Kumar et al., 2014). It is considered as a staple diet in several states of India, particularly Karnataka, Andhra Pradesh, Odisha, and Tamil Nadu, and is also widely consumed in parts of Africa. Globally, it is valued as one of the most nutritious cereals, rich in calcium, iron, dietary Fiber, and essential amino acids. Compared to the polished rice and refined wheat flour, Ragi is considered as one of highly nutritious crop because of its health-

promoting qualities such as managing the diabetes, finding solutions for malnutrition, and help in improving bone health (FAO, 2019). Finger millet is often referred to as a "climate-resilient crop" because of its ability to withstand drought, poor soils, and minimal input conditions. Despite being hardy and sustainable, Ragi production faces significant threats from various pests and diseases, among which blast disease is the most devastating. Globally among the 25 countries which contribute to the 4.5 million tonnes of Ragi, India produces more than 60% of the global production amounting to around 2.6 million tonnes (Sadhana Singh at al, 2023).

The Blast disease which is caused by the fungus Magnaporthe grisea is considered as one of the most destructive disease of Ragi. It is widespread in almost all finger millet-growing regions of the world and has the ability to cause yield losses ranging from 30–90% under severe conditions including both environmental as well as climatic conditions which can favour the growth of the fungi(ICAR, 2020). The disease appears in various forms which depends on the site of infection like leaf blast, node blast, neck blast, and finger blast. Leaf lesions initially appear as spindle-shaped spots with grayish centers and brown margins, which then expand rapidly under favorable conditions like temperature and humidity. Neck blast is the most damaging form, where infection at the panicle base weakens the stem that can cause lodging and poor grain filling which might affect the overall crop yield. The epidemiology of blast disease is influenced by high humidity, frequent rainfall, and moderate temperature, which are common in Ragi-growing regions during the cropping season. The disease is generally spread through the airborne spores which infects the plant and reduces it's yield as well as the grain quality. The current method of managing the blast disease is done using chemical fungicides such as tricyclazole, carbendazim, and mancozeb. The chemical -based fungicides are effective to some extent but, the long-term use of such fungicides can cause various problems like the development of resistant fungal strains, negative impact on the soil microflora, cause environmental pollution, and other health hazards for both farmers and consumers. And these chemical fungicides are less affordable by small scale farmers as they are not cheaper. These challenges highlights on the need for sustainable, eco-friendly, and farmer-friendly alternatives for ragi blast management.

Cows are considered as sacred in Indian culture, and their by-products have been utilized in agriculture from ancient times, as documented in Vrikshayurveda and in other traditional practices. Cow urine has been used as a growth promoter, pest repellent, and also as a soil fertility enhancer. The cow urine contains the chemical components like urea, uric acid, creatinine, phenolic compounds, salts, and volatile fatty acids which has antimicrobial properties. Studies have shown that cow urine can inhibit the growth of various bacterial and fungal pathogens (Singh et al., 2015). In management of plant diseases, the cow urine can act as both fungicidal agent as well as a plant growth stimulant.

Plant-based extracts are one considered as one of the rich sources of secondary metabolites like alkaloids, flavonoids, tannins, phenols, terpenoids, and essential oils, which possess antimicrobial activity. Botanicals like neem (Azadirachta indica), tulsi (Ocimum sanctum) and Datura (Datura metel) have demonstrated wide range of antifungal properties against crop pathogens. The advantage of aqueous extracts is that they are biodegradable, non-toxic to humans and animals, and they are often readily available in rural regions. Various reports confirm that these plant extracts are potent in inhibiting the germination of spores, reduce the mycelial growth, and delaying the disease progression in field crops (Kumar et al., 2018). The active compounds in neem, such as azadirachtin, nimbin, and salannin, are best known to exhibit the effects like antimicrobial and antifungal effects. The active compounds in tulsi like eugenol, ursolic acid, and flavonoids play an important role in inhibiting the growth of various fungal pathogens. The Datura plant contains several compounds like alkaloids, flavonoids, and terpenoids that are toxic to certain fungi. However, further research is needed to fully understand its safety, efficacy, and appropriate application methods. (Asma Rinez et al, 2013) (G. Krishna Kishore, 2005). Both cow urine and the effect of plant leaf extracts have been studied separately but their combined application could provide a synergistic effect on preventing the fungal growth and enhance the crop yield. Cow urine can also act as a solvent that enhances the activity of bioactive compounds obtained from plants. Additionally, its nitrogen content can improve the shelf-life and potency of extracts when it is applied in the field. Previous studies on other crops suggest that such combinations will not only control the diseases effectively but can also increase the plant growth and yield. However, very little is known about their combined use against Ragi blast disease which leaves a significant gap in current research.

2. REVIEW OF LITERATURE

History of fungicides

Fungal infections have accompanied plants (through evolution) since the dawn of time. From the 16th century, scientists began experimenting with plant-based fungal infections and factors that may inhibit their growth. In the year 1755, Tillet determined that seedborne fungi such as *Tilletia tritici* and *Tilletia laevis* were the causative agents of wheat bunt and could be controlled by the treatment of seed with lime or a combination of lime and salt. In 1807, Bénédict Prévost came across a chemical that was effective in controlling plant diseases, especially smuts and bunts in cereal crops. Through his experiments, he determined that farmers could prevent bunts in wheat crops by dousing

the wheat kernels in a weak copper sulphate solution. Such copper-based seed treatments were prevalent in many countries, especially France, till the end of the 20th century. Berkeley and Anton de Bary conducted their research on the Late blight fungus among potatoes in the 1950s. Despite their discoveries, the significance of their work wasn't recognized until the work of Pasteur and Koch on Human Pathogens in the 1860s, which led to the discovery of germ theory. (Bailey, S.L. et al., 2024)

During the year 1882, Millardet observed that the grape wines, when sprayed with a bluish-white mixture of copper sulphate and lime, were protected from pilferers and were able to retain their leaves throughout the season. A couple of decades later, the fungi were determined as the causal agent for several plant-based diseases and were regarded as easily noticeable symptoms among infected plants. In the year 1913, during a meeting at Atlanta, L.R.Jones began to contemplate whether it would be a good idea to classify plant pathology as an individual discipline apart from botany. (Baibakova, E.V. et al., 2019)

Since the Millardet concoction was effective against only a handful of diseases, several researchers began to explore certain organic compounds, which, when mixed with metal, could be effective in controlling follicular diseases. This led to the discovery of dithiocarbamate fungicides by Tisdale and Williams in 1934, which proved to be a turning point in the field of fungicides and was later commercialized as an effective form of seed treatment. This technology further advanced with the discovery of ethylenebis by Nabam, Zineb and Maneb and the development of Mancozeb by Rohm and Haas in 1961. Due to their huge spectrum, these fungicides aided farmers in controlling devastating diseases such as Potato Blight and Leaf Spots, caused due to fungal attack and provided a huge benefit to fruit and vegetable cultivators as well due to their low toxic level. Despite its success, dithiocarbamate fungicide failed to cater to several other plant diseases, such as powdery mildew and rusts and required application before the occurrence of infection in plants, requiring full coverage. (Baibakova, E.V. et al., 2019)

Benomyl was the first fungicide to have a spectrum broader than that of dithiocarbamate, ie, it had a wider range of action in the elimination of various fungi such as *Botrytis*, *penicillium* and *Rhizoctonia solani*. Launched by Dupont in 1970, Benomyl was cost-effective, extremely curative and even ensured plant and mammalian safety. Using this fungicide, farmers were able to treat existing infections and not worry about frequent application or about perfect coverage. Despite its popularity, frequent application of this fungicide over a long period of time gave rise to fungicide-resistant fungal populations. (Bailey, S.L. et al., 2024)

In the year 1977, Ciba-Geigy launched a phenylamide-based fungicide, Metalaxyl, which was a great success due to its high potency, excellent curative power and protectant activity, excellent coverage, flexible administration methods and protection against new fungal growth. But frequent administration or overuse resulted in resistant fungi. Therefore, Ciba-Geigy developed fungicide prepacks containing Metalaxyl, along with certain protectant fungicides like Mancozeb, increasing the shelf life of the product. (Bailey, S.L. et al., 2024)

By 1996, many fungicides were commercialized as they simulated the plant's natural defenses to protect it from fungicides. Besides simulating the plant defense system, these fungicides could be administered before the occurrence of infection, as a form of protectant. (Baibakova, E.V. et al., 2019)

Agricultural use of fungicides

Playing a vital role in modern agriculture, fungicides help protect crops from various kinds of fungal attack and also enhance crop production, thereby increasing profit margins. Their use is currently essential due to the sudden dietary shift towards fruits and vegetables that are even more susceptible to such attacks. (Ragsdale, N. N. (Ed.), 1991).

Apart from yield protection, fungicides also help safeguard plants against mycotoxins and phytoalexins, which lead to severe health crisis in humans. Mycotoxin is responsible for fatal outbreaks, blood disorders and even cancer, whereas phytoalexins are known to cause reproductive issues and toxic syndromes that can lead to organ failure. (Ragsdale, N. N. (Ed.), 1991).

Case studies, conducted to date, highlight the economic and public health importance of fungicides. Such treatments have been known to have increased yields among wheat, soybeans, asparagus, almonds, and grapes, thereby preventing losses by about 70%. Without the use of such fungicides, fungal diseases such as soybean sudden-death syndrome, wheat rusts, and fruit rots would contribute to crop losses at a devastating level, impairing food supply chains, and increasing toxin-related health risks. (Ragsdale, N. N. (Ed.), 1991).

The risks caused due to uncontrolled fungal toxins are often greater than those from carefully managed fungicide use. Thus, they serve as crop protectants as well as public health safeguards. Responsible regulation must therefore balance minimal fungicide risks against the far greater dangers posed by fungal toxins and plant defense chemicals. (Ragsdale, N. N. (Ed.), 1991).

Harmful effects of fungicides

Despite their valuable role in the management of plant diseases, fungicides are known to pose several harmful effects on soil micro-organisms, human health and also the health of other organisms. The application of certain fungicides (difenoconazole) has proved to decrease the activity of soil-based microbes, thereby reducing the activity of enzymes

in the soil, as determined by the respiratory quotient. Several other fungicides are also known to reduce saprophyte populations in the phyllosphere region in crops, which poses a minimal threat to certain pathogens, increasing the rate of infection among crops. Furthermore, these fungicides couldn't be applied just before harvest. Many systemic fungicides have been found to minimize competition for nutrients by common soil fungi, such as Penicillium and Trichoderma species, leading to an increase in pathogens that are insensitive to the compounds. Due to the difference in sensitivity of pathogens, it can result in a condition known as disease trading, caused due to a change in the main disease-causing pathogen. (Goswami, S.K. et al, 2018)

Use of fungicides in agriculture has been reported to cause ecological imbalance, causing an increase in health hazards among people who have been exposed to the toxin. Organic sulphur fungicides have proved to be toxic to humans, causing inflammation of the eyes, upper respiratory tract and skin allergies, when consumed orally, whose sensitivity increases upon consumption of alcohol. Certain agro-chemicals have also been reported to be hazardous, affecting the human endocrine system and immune system, which in certain cases lead to cancer. EBDC fungicides decompose to yield ethylenethiourea when exposed to high temperature and oxidative conditions, increasing the risk of thyroid cancer along with other mutagenic and embryotoxic defects. The National Research Council (NRC) has reported that these chemicals pose the risk of cancer among children who consume residue-laden food. The Food and Drug Administration (FDA) has found that less than 1% of processed food products contain higher levels of toxicants. (Goswami, S.K. et al, 2018)

The Government of India and Denmark have imposed a ban on certain fungicides like Captafol, due to their hazardous nature, causing skin allergies among people exposed to the chemical. Mercury-based fungicides (Organic or Inorganic), used for controlling seedborne diseases, are found to change the soil, thereby releasing mercury. According to WHO (World Health Organization), such fungicides have been deemed to high hazardous and cause irritation of the skin and mucus membrane, dermatitis, other allergies and also risk permanent damage to the nervous system. Such compounds have currently been banned by several countries due to the possibility of bio-magnification, resulting in mass poisoning. (Goswami, S.K. et al, 2018)

The usage of fungicides is also responsible for causing air pollution, for eg, the powdered sulphur is used against powdery mildew on grapes, which is also responsible for colouration on grapes. This also leads to the oral consumption of these grapes, causing oral toxicity. During spraying, many fungicides can enter the human body through the skin and respiratory tract (inhalation) as well. Prolonged exposure to fungicides leads to the development of neuronal disorders, as well as degenerative diseases, especially during fetal growth and has been proven to be extremely carcinogenic to most living organisms. (Goswami, S.K. et al, 2018)

Importance of ragi

The traditional crop Jharkhand, Maharashtra and most of South India (Karnataka, Tamil Nadu, Andhra Pradesh, Telangana), finger millet (*Eleusine coracana*) or ragi or nachani or rajika or markataka is one of the oldest crop known to be cultivated in India and gets its name from ancient Indian Sanskrit literature, meaning "Dancing Grain". Ranked as the 4th most important crop in India, the grains of ragi are classified into 3 types—white, light brown and brown. White grains are more prominent in the baking industry, while light brown grains are preferred for making porridges. Brown grains are specifically utilized for brewing a type of traditional opaque beer, native to Southern Africa. (Singh.S et al, 2023)

Ragi is deemed to be highly nutritious as it is composed of carbohydrates, dietary fiber, starch, proteins and minerals as well as crude fiber. Despite being referred to as a poor man's crop, ragi has been acknowledged to be a future smart food, due to its rich nutritional value, resistance to climate change and resilience to water scarcity. These grains can be stored for many years without insect or pest infestation. (Dhanushkodi.V, 2023)

Global distribution of ragi blast disease

Ragi or finger millet is commonly grown in countries across Asia and Africa, with the top producers being India, Nepal, Uganda and China. Considering the global production of ragi, India produces around 60% of the world's ragi with a yearly harvest of 2.5 million tonnes out of the global production of 4.5 million tonnes. (Singh.S et al, 2023)

In India, ragi is majorly grown in the hilly regions of Uttar Pradesh, Himachal Pradesh, Karnataka, Tamil Nadu, Andhra Pradesh, Orissa, Bihar, Gujarat and Maharashtra. This crop is known to thrive in regions with very high rainfall (600mm to 1200mm), acidic soil and acquires maturity within 100 to 120 days. (Dhanushkodi.V, 2023)

Causal organism of ragi blast disease

Despite being exceptionally varied, *Magnaporthe grisea*, also known as Ragi Blast, is host-specific and known to infect ragi plantations, which spreads via numerous air-borne asexual spores (conidia), which enter the cells of its ideal host, i.e., ragi plant, through the stomata. Different species of this pathogen have different hosts among cereal crops (eg, rice, wheat) and germinate under highly humid conditions (more than 90%) at temperatures (25˚C to 28˚C).

The occurrence of frequent rains accompanied by cloudy skies further assists spore germination of *M.grisea* within its host due to the extremely humid conditions and the presence of water droplets, thus boosting its germination. (Vikaspedia, 2024) (Puri.S and Kumar.J, 2018)

Disease symptoms and cycle

Magnaporthe grisea is known to infect crops at different stages, resulting in the occurrence of leaf blast, neck blast, finger blast, etc. It usually appears on the plant as circular lesions, which are pointed at either end. The spots are greyish in colour at the center, and brownish in colour at the border. Under certain specific conditions, several of such spindle-shaped spots coalesce together, causing the entire leaf to dry. In other cases, this pathogen develops specs on the leaves and neck, turning it brownish black in colour and causing the peduncle to break. (Vikaspedia, 2024)

The life cycle of a pathogen can be observed by the varied change in colour from creamy white to yellow-green to black. The black colour indicates that the spores are ready for germination, while the freshly germinated spores are white in colour, with the intermediate ranging in colours from yellow-green to orange. (Vikaspedia, 2024)

Morphological management against magnaporthe grisea

The greyish mycelium of the fungus, taken from the infection point on the rachis of the several independent spikes, was observed. The incubation of several infected spikes or leaves in a creamy layered moist blotter at room temperature with 12 12-hour light and dark cycle for 5 days led to the formation of pyriform conidia. The typical pyriform (pear-shaped) and septate hyaline conidia, observed in the infected site after 5 days, were similar to those of *Magnaporthe oryzae*. (Meshuk.A.A, 2021)

The fungi showed higher mean mycelial growth rate on Oat Meal Agar (15.43mm per day) media, followed by Potato Dextrose Agar (14.34mm per day). The blast fungus is caused by the haploid filamentous and ascomycetous fungus, *Magnaporthe oryzae*. (Meshuk.A.A, 2021)

Magnaporthe grisea is a fungus that also belongs to the genus *Magnaporthe*, due to which it shares most of its morphological characteristics with *Magnaporthe oryzae*.

Use of neem leaf extract as a biofungicide

Due to its wide range of medicinal properties, the neem tree has served as the medium of attraction to several researchers and scholars worldwide. According to traditional Indian documents (Charaka Samhita and Sushruta Samhita) neem tree has been referred to as “Sarva roga nivarini”, meaning “the curer of all ailments”. (Shrivastava.D.K and Swarnkar.K, 2014)

The constituents of neem leaf are known to exhibit immunomodulatory, anti-inflammatory, anti-hyperglycemic, anti-ulcer, anti-malarial, anti-fungal, anti-bacterial, anti-oxidant, anti-mutagenic and anti-carcinogenic properties. (Mahmoud.D.A et al, 2011)

When tested against *Aspergillus flavus, Alternaria solani* and *Cladosporium*, neem was shown to possess inhibitory properties against those fungi. Compared to the synthetic fungicides, which are responsible for various side effects, Azadirachta leaves possess excellent antifungal activity and have been found to be extremely useful in primary health care. The neem leaf extracts were proven to be useful against several harmful fungi by inhibiting their growth. (Shrivastava.D.K and Swarnkar.K, 2014)

Use of tulsi leaf extract as a biofungicide

A very well-known, sacred plant of the Hindus, Tulsi (*Ocimum sanctum*) or the sacred basil, is used against many conditions such as fever, common cold, sore throat and respiratory disorders. This plant, as a whole, has demonstrated to have antibacterial, antiperiodic, hypotensive and expectorant properties. (Sharma.S et al, 2019) (Prajapati.M, 2021)

When the fungicidal effect of Tulsi leaves was evaluated against 10 fungal pathogens (*Rhizoctonia solani, R. bataticola, Phoma sorghina, Colletotrichum gloeosporioides, Fusarium pallidoroseum, F. oxysporum (ciceri), Sclerotium rolfsii, Sclerotinia sclerotiorum, Alternaria solani* and *A.alternata*), it was found to inhibit 75% of overall fungal growth. It was also demonstrated that the aqua extract of Tulsi leaves was far superior in their fungicidal properties than ethanol extracts. (Sharma.S et al, 2019) (Kaur.P et al, 2024)

Use of datura leaf extract as a biofungicide

Belonging to the Solanaceous species. Datura metel is a sub-glabrous shrubby herb that exists throughout the world. This plant has not been the subject of most biological and chemical research, due to its poisonous nature. Datura was demonstrated to showcase antifungal properties against various plant pathogens. The potential of aqua extracts of Datura to inhibit the growth of pathogenic antagonistic fungi differed on the basis of the origin of the extract, its concentration and the pathogen as well. Some fungi, such as *F.oxysporum.f.sp.melonis* were extremely sensitive to Datura extracts that other fungi (even from the same genus). An increase in the degree of inhibition was observed with

an increase in extract concentrations, but when Datura leaf aqueous extracts were exposed to *F.oxysporum.f.tuberosi*, *Lycopessici* proved to be less sensitive than *tuberosi* with the highest inhibition (radial growth) at 32% (Rinez.A et al, 2013)

Due to its antifungal property, Datura metel inhibited the spread or germination of urediniospores of P.arachidis under laboratory conditions. When applied as a prophylactic spray (in combination with Datura metel and L.inermis), it reduced LLS lesions and rust pustules by 65%-74%. It was later found that during field studies that 1 spray of chlorothalonil at 45 DAS (Days After Sowing), when combined with 3 sprays of Datura metel at an interval of 15 days each, was effective in reducing the severity of LLS and rust. Integrated applications of Datura metel extract and Chlorothalonil may reduce the need for fungicides in groundnut cultivation. (Kishore.G.K and Panda.S, 2005)

Fungicidal properties of cow urine

In ancient Ayurveda, cow urine is highly revered due to its pharmacological importance. One of the ingredients of "Panchagavya|", cow urine, is capable of treating many diseases and is the best remedy for fungal and bacterial diseases, in animals as well as in plants. It is also known to contain many beneficial elements that contribute to its antimicrobial activity. Cow urine is composed of 95% water, 2.5% urea and 2.5% a mixture of salts, hormones, enzymes and minerals and is very useful in the form of bio-fertilizer and bio-pesticide. (Jandaik.S et al, 2015) especially against insect pests, infecting Bt cotton (Patel.C.C et al, 2019)

The laboratory analysis of cow urine revealed that it was made up of nitrogen, sulphur, phosphate, sodium, manganese, iron, silicon, chlorine, and magnesium. Malic, citric, titric, succinic, carbonic oxide, calcium salts, vitamins (A, B, C, D & E), lactose, certain enzymes, creatinine, hormones and gold acids. Cow urine possesses antimicrobial properties due to the presence of certain volatile and non-volatile components such as urea, creatinine, aurum, hydroxide, carbolic acid, phenols, calcium and manganese. When tested against 6 fungal species (*Aspergillus, Rhizopus spp, Mucos sp, Penicillium sp, Alternasia sp, Macrophomina sp*), Cow urine proved to be effective in the inhibition of fungal growth (Ghosh.T, Biswas.M.K , 2018)(Kgakudi.B.K and Mantswe.M, 2020)

In ancient scriptures, cow urine has been described as an "elixir of life". It is nature-friendly and is the safest method of treatment. Cow urine is also an excellent form of fertilizer as it increases the nitrogen level along with other minerals in the soil, leading to better plant growth. (Mohanty.I et al, 2014)

Cow urine can prove to be an excellent source to increase the fertility of the soil, crop productivity and crop quality. In recent years, cow urine has slowly become an alternative form of fertilizer. When combined with manures and fertilizers, frequent usage of cow urine can pave the way for more sustainable agriculture. (Pradhan.S.S et al, 2018)

Synergistic effect of neem and cow urine

Antimicrobial resistance poses a major global challenge, prompting the search for alternative therapies. This study investigated the antimicrobial properties of neem tree (*Azadirachta indica*) extracts—derived from leaves, seeds, and bark—both alone and in combination with cow's urine. Samples were collected, processed, and subjected to standard phytochemical and antimicrobial tests.

The extracts contained alkaloids, tannins, saponins, polyphenols, flavonoids, anthraquinones, terpenoids, reducing sugars, and steroids, all known for bioactive potential. Antimicrobial assays were performed against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Aspergillus niger, Saccharomyces cerevisiae,* and *Candida albicans*.

Neem extracts in the absence of cow's urine demonstrated inhibitory zones ranging from 7–25 mm, while extracts mixed with cow's urine showed slightly higher inhibition (9–27 mm). Minimum inhibitory concentration (MIC) tests, which were conducted in both bacterial and fungal strains, cow urine–neem extract mixtures generally produced stronger effects. (Vats.S and Miglani.K, 2011)

Despite this, enhanced antimicrobial activity with urine supplementation was observed in several pathogens, likely due to the synergistic effect of bioactive compounds in both neem and cow's urine. The study concludes that neem extracts possess broad antimicrobial potential and, when combined with cow urine, may offer an improved natural remedy. Large-scale testing is recommended for validation.

India has a long tradition of using natural resources for health, with plants and animals considered medicinally valuable. *Azadirachta indica* (Neem), known as the "village pharmacy," possesses broad therapeutic effects including antibacterial, antifungal, anti-inflammatory, and immunomodulatory activity due to compounds such as nimbin, nimbidin, and gedunin. Similarly, cow urine (Go-muttra) has been described in Ayurveda as a therapeutic agent for various diseases including cancer, asthma, renal and skin disorders, and is believed to have antimicrobial and immune-stimulant properties. Neem extract was prepared from fresh leaves, while cow urine was photo-activated and centrifuged to remove contaminants. Antimicrobial assays using agar diffusion and broth dilution methods revealed that Neem extract alone inhibited *Candida albicans, C. glabrata, Escherichia coli, Pseudomonas aeruginosa*, and *Streptomyces aureofaciens*, while cow urine alone showed strong activity *against E. coli, C. albicans,* and *S.*

aureofaciens, but when used together, Neem and cow urine produced synergistic effects against *C. tropicalis, C. glabrata, P. aeruginosa*, and *S. aureofaciens*. (Waitaka.P et al, 2016)

Synergistic effect of tulsi and cow urine

A widely revered medicinal plant in Ayurveda, Tulsi (*Ocimum sanctum*) has been considered to be of a high value due to its antioxidant, antimicrobial, and immunomodulatory properties. The phytochemicals present , including eugenol, ursolic acid, flavonoids, and tannins, contribute to the strong radical scavenging and antibacterial effects.. The methanol extract of tulsi demonstrated the highest antioxidant potential, with 96.35% DPPH radical scavenging activity, nearly equivalent to ascorbic acid, indicating its efficiency as a natural antioxidant. In antibacterial assays, tulsi extracts displayed inhibitory effects *against Escherichia coli, Proteus vulgaris*, and *Staphylococcus aureus*, though activity varied depending on the solvent used.

In Ayurveda cow urine is referred to as "Amrita" (life-giving), for its immunostimulant and antimicrobial properties. Cow urine produced the highest zone of inhibiton when combined with tulsi extract suggesting its efficiency in solubilizing bioactive compounds.

Tulsi extracts prepared with cow urine showed enhanced antibacterial effects compared to individual use. These results confirmed that the combination of cow urine and tulsi leaf extract offers synergistic antimicrobial and antioxidant activity, supporting their potential in developing natural alternatives to synthetic drugs.

3. METHODOLOGY

A) Preparation of Plant Leaf Extracts:

1. Leaves of Neem (Azadirachta indica), Tulsi (Ocimum tenuiflorum) and White Flower Datura (Datura metel) is shade dried for 7 days and powdered individually.
2. Plant leaf powders are mixed with distilled water in the ratio 1:10, i.e., 10g of extract in 100ml of water.
3. The beakers are covered with aluminium foil and incubated for 30 minutes.
4. The concoctions are filtered into conical flasks, labelled as 1:10 neem leaf extract, tulsi leaf extract and Datura leaf extract, using Whatman No.1 filter paper.
5. The resultant extracts are divided into 2 parts each, where one set of extracts is placed in the refrigerator while the second set of extracts are placed at room temperature for 7 days.
6. The above steps 1 to 4 are followed on day 7 to prepare fresh extracts.
7. The above steps 1 to 6 are repeated to extract concentrations 1:5 and 1:20.

B) Preparation of Sabouraud Agar Media:

1. Add 40g of Dextrose, 15g of Agar and 10g of Peptone in 1 litre of distilled water and mix well using a glass rod.
2. Pour the mixture into a clean, dry conical flask and apply a cotton plug to its mouth.
3. Autoclave the media to avoid contamination.

C) Preparation of Urine – Plant Leaf Extract Mixtures:

1. Take 11 clean and dry test tubes and label them 1 to 11, with test tube no.1 containing cow urine and test tube no.11 containing only extract (Neem, Tulsi or Datura).
2. Different aliquots of neem leaf extract (0.1ml, 0.2ml, 0.3ml, 0.4ml, 0.5ml, 0.6 ml, 0.7ml, 0.8ml & 0.9ml) are added into the test tubes labelled 2 to 10.
3. The volume is made up to 1ml using urine
4. The test tubes are shaken well to mix the contents.

5. The above steps 1 to 4 are repeated with Tulsi leaf extract and Datura leaf extract.
6. The above steps 1 to 5 are repeated with Room Temperature Extract and Refrigerated Extract.
7. The above steps 1 to 6 are repeated with the extract concentration 1:5 and 1:20

D) Performing the Experiment:

1. Autoclave 55 Petri plates and pour the Sabouraud Agar Media into all the plates. Let it cool
2. Inoculate the media with the fungi "Magnaporthe grisea" in the laminar air flow.
3. Incubate the Petri plates for 48 hours.
4. Punch 5 holes, labelled as 1, 2, 3, 4 and 5 in the previously incubated Petri plates containing the fungi "Magnaporthe grisea".
5. 1 Petri plate will contain 100% Cow Urine. The balance 54 Petri plates are divided into 9 sets. Each set contains 6 Petri plates. The Petri plates in each set are divided as follows:
   i) Petri plate 01 and Petri plate 02 contains Cow Urine-Tulsi Leaf Extract.
   ii) Petri plate 03 and Petri plate 04 contains Cow Urine-Neem Leaf Extract.
   iii) Petri plate 05 and Petri plate 06 contains Cow Urine-Datura Leaf Extract.
6. Take one drop each from the test tube 2 to test tube 11, containing different aliquots and put them into each of the holes of Petri plate 01 and Petri plate 02 (total 10 holes) containing Cow Urine-Tulsi leaf extract, respectively.
7. Repeat the above step for Petri plate 03 and Petri plate 04 containing Cow Urine- Neem leaf extract and Petri plate 05 and Petri plate 06 containing Cow Urine-Datura leaf extract.
8. Repeat steps 6 and 7 for refrigerated extract and room temperature extract.
9. Repeat steps 6. To 9. for refrigerated urine and room temperature urine.
10. Repeat the above steps 6 to 10. for extract concentration 1:5 and 1:20.
11. Tabulate the results obtained and repeat the experiment two more times to obtain three sets of data.
12. Calculate the mean of each set of readings. Draw a graph for the mean obtained to determine statistical analysis.

4. RESULTS AND INTERPRETATION

The results obtained from the above-mentioned experimental setup using cow urine and the aqua-extracts of neem, tulsi and Datura leaves provided different ranges of effectiveness of the bio-fungicide. On an average, Cow urine, under room temperature condition proved to be the most effective as a bio fungicide, closely followed by refrigerated cow urine.the highest zones of inhibition, for each extract, was demonstrated as follows:

- Tulsi leaf extract (at concentration 1:5) , under refrigerated condition, when combined with fresh urine yielded a diameter of 28mm as the zone of inhibition
- Neem leaf extract (at concentration 1:10) , under fresh condition, when combined with room temperature urine yielded a diameter of 20mm as the zone of inhibition
- Datura leaf extract (at concentration 1:10) , under room temperature condition, when combined with refrigerated urine yielded a diameter of 16mm as the zone of inhibition

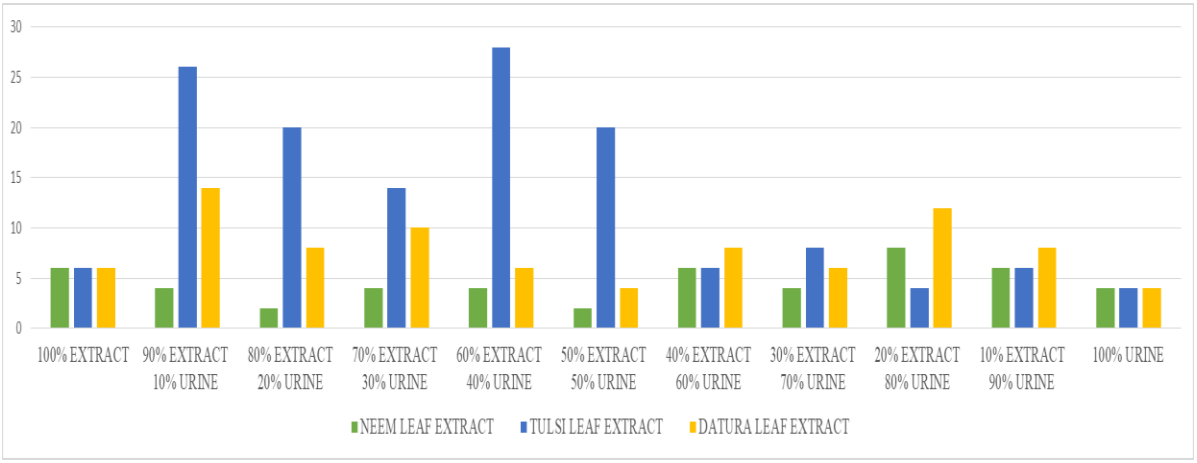


Table 1 depicting the diameter of zone of inhibition using a combination of refrigerated extract and fresh cow urine

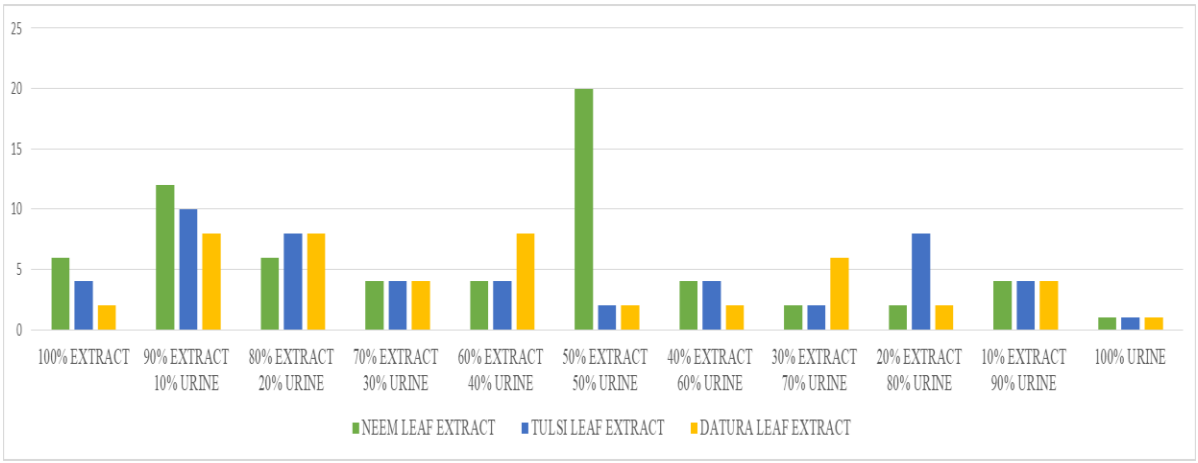


Table 2 depicting the diameter of zone of inhibition using a combination of fresh extract and room temperature cow urine

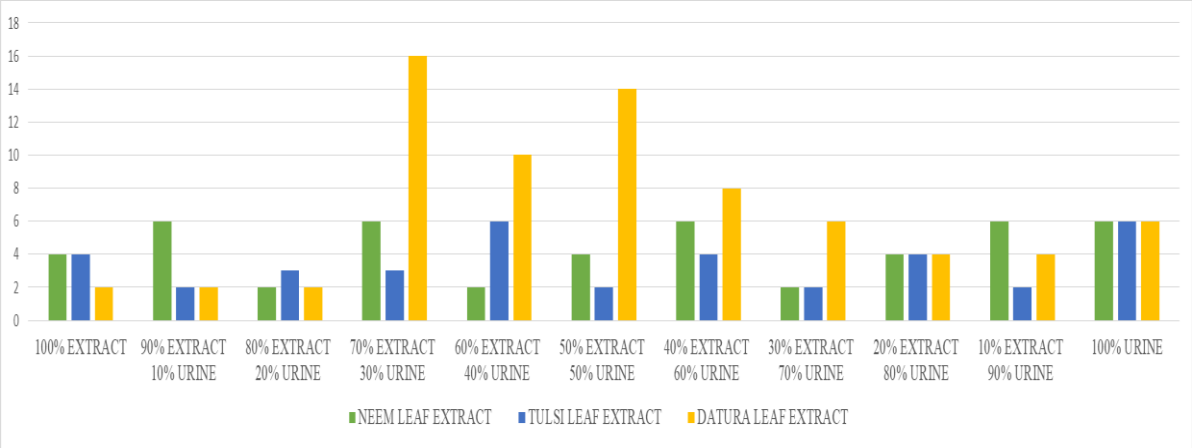


Table 3 depicting the diameter of zone of inhibition using a combination of room temperature extract and refrigerated cow urine

Similarly lower zone of inhibiton was observed at concentration 1:20 due to the extracts being diluted. This shows that extracts play a vital role in enhancing the microbicidal properties of cow urine

5. CONCLUSION

Finger millet (Eleusine coracana), popularly known as ragi, is a highly nutritious and climate-resilient crop that plays an important role in food security, especially for small-scale farmers in semi-arid regions. However, its productivity is severely affected by blast disease caused by Magnaporthe oryzae. This fungus infects ragi by forming appressoria—specialized infection structures that exert high turgor pressure to penetrate the leaf cuticle. The pathogen survives in slightly acidic to neutral pH and moist environments, relying on enzymes such as cutinases and proteases for host colonization (Ghosh T. et al., 2018; Sharma R. et al., 2015).

The present study demonstrated that bio fungicides prepared from cow urine and aqueous plant extracts are effective in reducing fungal growth under in vivo conditions. Refrigerated cow urine, by preserving urea and volatile antifungal compounds, releases ammonia upon application, which alkalinizes the leaf surface and disrupts the fungal enzyme activity as well as destabilises protein. This creates an unfavorable environment for appressoria formation and subsequent infection (Sharma R. et al., 2015).

The Integration of such biofungicides into organic farming is highly significant. Unlike synthetic fungicides, these natural formulations are eco-friendly, biodegradable, and safe for both consumers and the environment. More importantly, they reduce input costs for small-scale farmers, who often struggle with the financial burden of chemical fungicides. By improving ragi yield through sustainable disease management, biofungicides not only ensure food security but also empower marginal farmers economically.

In conclusion, the efficacy of cow urine and aqueous plant extracts against ragi blast highlights their potential as eco-friendly biofungicides. Their role in organic farming supports the broader goals of sustainable agriculture by maintaining soil health, reducing chemical dependency, and promoting affordable crop protection strategies for resource-limited farmers. Thus, such practices contribute both to environmental conservation and the socio-economic upliftment of farming communities (Ghosh T. et al., 2018; Sharma R. et al., 2015).

6. STATEMENTS & DECLARATIONS

AI Statement: The authors declare that they have not used generative artificial intelligence, specifically ChatGPT, in the writing of this manuscript and/or in the creation of images, graphics, tables, or their corresponding captions.

Authorship Contribution: Shrinidhi Sriram, Lavanya.J.M: Carrying out the data collection, data curation, and writing the original manuscript. Devika.S: Reviewing the draft and supervision.

Ethical Standards: All the ethical research standards were followed while writing this conceptual paper.

Conflict of Interest: The authors state that they do not have any conflict of interest.

Informed Consent / Ethical Compliance: As this is a conceptual paper, no consent is required.
Human or animal involvement in the article: None
Data Availability: All data included in this research article will be provided on request.

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