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Preface

We would like to present, with great pleasure, the inaugural volume-10, Issue-2, February 2024, of a scholarly journal, *International Journal of Environmental & Agriculture Research*. This journal is part of the AD Publications series *in the field of Environmental & Agriculture Research Development*, and is devoted to the gamut of Environmental & Agriculture issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

This journal was envisioned and founded to represent the growing needs of Environmental & Agriculture as an emerging and increasingly vital field, now widely recognized as an integral part of scientific and technical investigations. Its mission is to become a voice of the Environmental & Agriculture community, addressing researchers and practitioners in below areas.

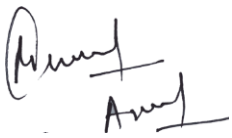
Environmental Research:

Environmental science and regulation, Ecotoxicology, Environmental health issues, Atmosphere and climate, Terrestric ecosystems, Aquatic ecosystems, Energy and environment, Marine research, Biodiversity, Pharmaceuticals in the environment, Genetically modified organisms, Biotechnology, Risk assessment, Environment society, Agricultural engineering, Animal science, Agronomy, including plant science, theoretical production ecology, horticulture, plant, breeding, plant fertilization, soil science and all field related to Environmental Research.

Agriculture Research:

Agriculture, Biological engineering, including genetic engineering, microbiology, Environmental impacts of agriculture, forestry, Food science, Husbandry, Irrigation and water management, Land use, Waste management and all fields related to Agriculture.

Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within that community who supported the idea of creating a new Research with *IJOEAR*. We are certain that this issue will be followed by many others, reporting new developments in the Environment and Agriculture Research Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IJOEAR* readers and will stimulate further research into the vibrant area of Environmental & Agriculture Research.



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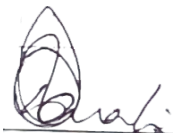

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Agricultural Sciences	
Soil Science	Plant Science
Animal Science	Agricultural Economics
Agricultural Chemistry	Basic biology concepts
Sustainable Natural Resource Utilisation	Management of the Environment
Agricultural Management Practices	Agricultural Technology
Natural Resources	Basic Horticulture
Food System	Irrigation and water management
Crop Production	
Cereals or Basic Grains: Oats, Wheat, Barley, Rye, Triticale, Corn, Sorghum, Millet, Quinoa and Amaranth	Oilseeds: Canola, Rapeseed, Flax, Sunflowers, Corn and Hempseed
Pulse Crops: Peas (all types), field beans, faba beans, lentils, soybeans, peanuts and chickpeas.	Hay and Silage (Forage crop) Production
Vegetable crops or Olericulture: Crops utilized fresh or whole (wholefood crop, no or limited processing, i.e., fresh cut salad); (Lettuce, Cabbage, Carrots, Potatoes, Tomatoes, Herbs, etc.)	Tree Fruit crops: apples, oranges, stone fruit (i.e., peaches, plums, cherries)
Tree Nut crops: Hazlenuts. walnuts, almonds, cashews, pecans	Berry crops: strawberries, blueberries, raspberries
Sugar crops: sugarcane. sugar beets, sorghum	Potatoes varieties and production.
Livestock Production	
Animal husbandry	Ranch
Camel	Yak
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Goats	Poultry
Bees	Dogs
Exotic species	Chicken Growth
Aquaculture	
Fish farm	Shrimp farm
Freshwater prawn farm	Integrated Multi-Trophic Aquaculture
Milk Production (Dairy)	
Dairy goat	Dairy cow
Dairy Sheep	Water Buffalo
Moose milk	Dairy product
Forest Products and Forest management	
Forestry/Silviculture	Agroforestry
Silvopasture	Christmas tree cultivation
Maple syrup	Forestry Growth
Mechanical	
General Farm Machinery	Tillage equipment
Harvesting equipment	Processing equipment
Hay & Silage/Forage equipment	Milking equipment
Hand tools & activities	Stock handling & control equipment
Agricultural buildings	Storage

Agricultural Input Products	
Crop Protection Chemicals	Feed supplements
Chemical based (inorganic) fertilizers	Organic fertilizers
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Environmental science and regulation	Ecotoxicology
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Production of Bayberry (*Myrica Nagi*) Wine using Baker Yeast

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***Abstract*—** *Bay berry have very less shelf life up to 3 days under ambient condition. Fortunately, presence of good amount of sugar makes desirable for substrate for wine production. Novelty of this work is to find the suitability of bayberries for wine production using yeast strain Saccharomyces cerevisiae. Juice was extracted from bayberries and analysed for TSS, titrable acidity, pH and alcoholic content and sensory evaluation, it was done at every 2 month intervals till 8th month's tenure. Further juice was adjusted by adding sugar syrup (5g) for initial 30, 60 and 90 days. Roasted wheat granules were added to ameliorate flavour. Sample was stored for the fermentation at 24± 2^0 C. Results have shown that pH was decreased as storage time progressed, initial pH was 3.18 and end pH was 2.40. Titrable acidity showed increased to certain point (2.74% - 3%) than slightly abated to 2.88%. TSS was reduced from 19.3^0 Brix to 17.1^0 Brix was recorded. Alcoholic content showed increase from 6.46% to 9.03%. The study have shown that acceptable and better wine can be produced from bayberry (myrica nagi) using saccharomyces cerevisiae.*

***Keywords*— *Wine, Bayberry, TSS, pH, Saccharomyces cerevices, Fermentation, Titrable acidity, alcoholic content*.**

I. INTRODUCTION

Bayberry also known as box berry and belongs to the family Myricaceae. It contain good amount of medicinal properties, phytochemicals such as polyphenols, carotenoids and vitamin C (Steinmetz and Potter et al, 1996). Of these phytochemicals, polyphenols are largely recognized as anti-inflammatory, antiviral, antimicrobial and antioxidant agents (Narayana et al, 2001).

It contains 80.60 percent moisture, TSS of about 19.50 % and 12.6 % total sugars. Also, 0.97 per cent protein (Parmar and Kaushal, 1982), Seal (2011) has reported crude fibre content as 7.53 ± 0.22 per cent in Myrica nagi fruits and also, very high amount of carbohydrates found was 76.33 ± 0.26 percent.

Wine is an alcoholic beverage produced from manifold fruit juices through fermentation action of microorganisms either spontaneous or seeding with a particular strain mainly of yeast species to adopt a particular quality of wine. Wine is resulted of anaerobic fermentation by yeast in which the sugars are converted to alcohol and carbon dioxide. (Zoeklein et al, 1995). It is most salient high value added products from fruits.

Commercially produced wines are commonly made from fermented grapes; current research would not using any chemicals or sugars despite different species of yeast to the crushed grapes. Yeast has the ability of changing fruit sugars into alcohol compounds. Wins can be produced from different types of fruits such as mango, pineapple, berries, grapes, Paw-Paw etc., wine name is given same as it is produced from particular fruit name or mixed fruits (Robinson, 2006).

Studies conducted in 2015 has showed that the global wine production has crossed 28 billion litres. Indian wine market has been flourishing from the past decade. The market size was about Rs. 3 billion in 2008 and by 2015 it had already reached Rs. 6 billion (Satish et al, 2018).

There merely less literatures are available for production of wine from bayberries. Therefore, the novelty of this experiment is to study was to optimize of the fermentation parameter also, effect of change in physicochemical parameters such as TSS, titrable acidity, alcoholic content, Sensory analysis such as aroma, taste and colour.

II. MATERIALS AND METHODS

2.1 Sample Collection:

Fruits were collected from farmer in Nainital hills of Uttarakhand in morning and directly brought to lab DRDO DIBER, Haldwani. Then fruits were washed with sterile water and air dried after that juice was extracted.

FIGURE 1: Bay berry fruit juice before clarification

FIGURE 2: Bay berry wine (2th month)

2.2 Methodology:

2.2.1 Experimental detail:

All experiments such as alcoholic content, TSS, titrable acidity, pH were recorded after every two months intervals up to a year. Sample was stored under 24±2^0 C during fermentation. Statistical analysis was done by following complete randomized design. TSS content of fruit was recorded by using digital refractometer H196801 and pH was measured by using pH mater titrable acidity was estimated following the method mentioned in the book Rangana, 1977. And alcoholic content of wine was analysed as per the FSSAI recommendation for alcoholic beverages.

2.2.2 Methodology:

Yeast strains was procured from Biotechnology lab, DIBER, DRDO, haldwani. Rehydration (Murli, Wine yeast) of yeast was done. Then 5% of sugar and yeast were added to the juice after transferring to conical flask after that pasteurisation (water bath) was done for 13-15 min at 60^0 C, Small amount of roasted broken wheat granules were also added to enhance the flavour. Aftermath, sample was placed at anaerobic condition (24±2^0 C). At the end for maturation sample was kept at 4^{0}C for 30 days tenure (Randall, 1987).

III. EXPERIMENTAL RESULTS

3.1 Total soluble solids:

A gradual decreasing trend of TSS was recorded at every 2 months intervals of time throughout the fermentation process till 8 months days. Initial TSS was 19.3^0 Brix and final TSS was 17.1^0 Brix. A gradual decrease trend in TSS was noted by many workers like Ezeronye, (2004) in their experiment with fruit wine.

3.2 Titrable acids:

It gives the total acids present in the wine also, it is the amount of citric acid (E. J. Ajit et al, 2018). Constant increasing in titrable acidity along with fermentation process was reported. Initial titrable acidity was 2.74 % and the final was found 2 .92%. Potty et al. (1978) also recorded increase in acidity in the wines, it is due to production of acids.

3.3 pH:

The pH decreased significantly in the wine, it is due to the increase in acidity (Patil et al., 1995). Initial pH was 3.18 subsequently it abated to 2.40 in 8th month.

3.3.1 Percent alcohol:

In the initial stage alcohol found 6.46%, it showed increase up to 9.13 in 4th. 6th and 8th months.

TABLE 1
MONTH WISE INCREASE IN ALCOHOL

Parameters	Days in month (2nd)	4th month	6th month	8th month
TSS	19.3	18.74	17.82	17.1
Titrable acidity	2.74	2.85	3.0	2.88
pH	3.18	2.9	2.52	2.40
Alcohol content	6.46	7.21	8.65	9.03

3.3.2 Sensory evaluation:

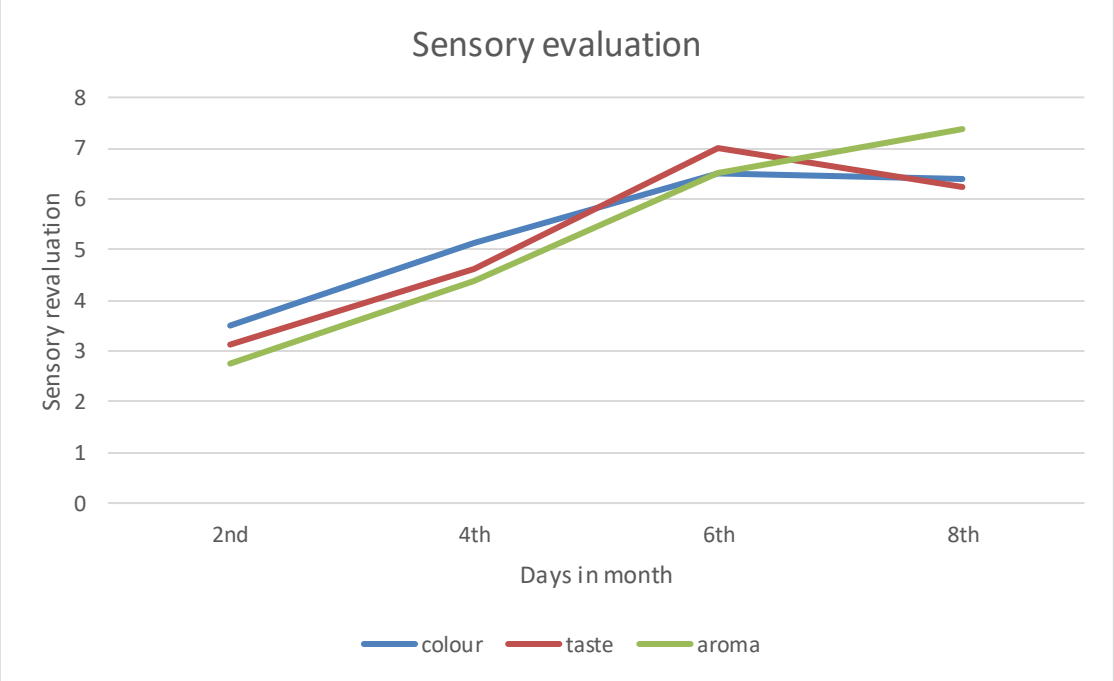

FIGURE 1: Sensory Evaluation Month wise

Sensory evaluation such colour, taste, aroma were done every after 2 months by semi trained members based on 7 hedonic scale points. This was followed up to 8th month duration. In beginning increase in colour and taste was seen over a period aftermath slight decline was seen. And in case of aroma evaluation, trainers reported that there was increasing in aroma throughout fermentation tenure.

IV. CONCLUSION

Bayberries contains good amount of sugar for production of wine. Our current research focused on production of good quality wine using yeast strain *Saccharomyces cerevisiae*. Ultimately 9.03% alcohol has produced in 8th month tenure. Also, broken wheat granules adds good acceptable flavour.

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The Forgotten Grains – Millets: A Review

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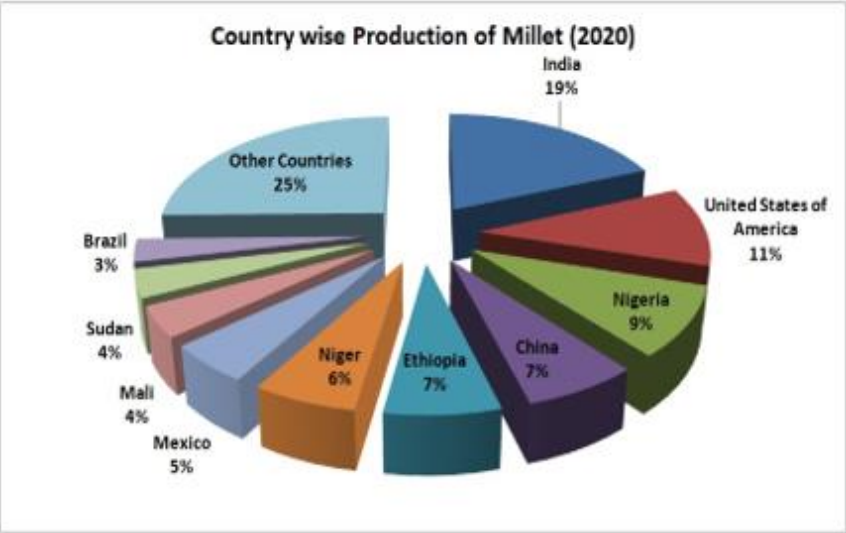
Abstract**—** *One of the earliest meals consumed by humans, millets may have been the first cereal grain employed in household cooking. Since ancient times, millets have been a primary source of nutrition for the inhabitants in semi-arid tropics in Asia and Africa, where other crops do not thrive. India and Asia have both been major millet consumers for centuries. Millet seeds are mashed and used to make the Indian flatbread roti. Despite all of these remarkable traits and capabilities of millet farming systems, the area dedicated to millet production has been declining over the past 50 years, and this decline accelerated during the green revolution. The little "grain" is free of gluten and rich in vitamins and minerals. Good quality protein, minerals, dietary fiber, phytochemicals, and vitamins are all abundant in millet grain, which is also extremely nutrient-dense. When compared to wheat and rice, millets have a lower glycemic index. When compared to wheat, the protein level of foxtail millet, proso millet, and pearl millet is greater. Kodo, tiny, foxtail, and barnyard millet have greater fiber contents. Modern as well as marginal farmers should be recommended to grow millets as it needs much less water and caring than its counterpart grains. Keeping this in mind a review of all the millets that can be grown in India has been included in this paper, pointing out millets from farmers perspective to include millets in his/her farming scheme as well from consumers perspective for its medical and health benefits.*

Keywords**— *Millets, Barnyard millet, Finger millet, Proso millet, Pearlmillet, Medical benefits.***

I. INTRODUCTION

1.1 Recognition of Millets on International level:

In 2023, the International Year of Millets was observed. The United Nations General Assembly declared 2023 the International Year of Millets in order to promote millet cultivation, consumption, and conservation. Millets are a type of grass with small seeds that is highly nutritious, drought resistant, and grows well in a variety of conditions. For thousands of years, they have been an important source of food for many communities all over the world. The International Year of Millets is predicted to have a number of positive effects, including enhanced food and nutrition security, higher income for smallholder farmers, and more sustainable use of natural resources. It is also intended to help achieve numerous SDGs, including SDG 1 (No Poverty), SDG 2 (Zero Hunger), SDG 3 (Good Health and Well-being), and SDG 13. (Climate Action). The International Year of Millets seeks to increase awareness of the importance of millets as a staple food, promote millet production and consumption, and encourage research and innovation in millet-based food systems.



Source: APEDA

1.2 Bring back Millets in farming again:

One of the first crops to be domesticated was millets. They were farmed in India for more than 5,000 years and were a staple meal in many ancient civilizations. From 3,300 to 1300 BCE, the Indus- Sarasvati civilisation ate millets. In 3,000 BC, the inhabitants of the Indus valley also ate them. They are also climate change resistant. They are an excellent alternative for dryland crops because of their innate endurance. More than rice output, millets accounted about 40% of all produced grains prior to the Green Revolution. With the effects of agriculture and the environment, millet output throughout time decreased from 40% of the grain production share to merely 10%. Wheat and rice are becoming common foods in India. During the last 60 years, our agricultural strategy has prioritised rice and wheat while ignoring millets, particularly small ones such as foxtail millet, tiny millet, browntop millet, and others. The availability of subsidised rice and wheat through the public distribution system and governmental nutrition programmes has also had a significant effect in shifting people's dietary choices from millets to rice in rainfed regions. Millets have vanished from our plates and fields as a result of systematic neglect. Millets are abundant in nutrients and include a lot of carbs, fibre, protein, and minerals including iron, magnesium, phosphorus, and zinc. They are also low in fat and have a low glycemic index, making them an excellent choice for diabetics.

II. TYPES OF MILLET

2.1 Sorghum Millet (Jowar) “Sorghum bicolor”:

Also known as great millet, broomcorn, guinea corn, durra, imphee, jowar, or milo.

2.1.1 Introduction:

Jowar, along with wheat, oats, maize and barley, is one of the world's top five grain products. Its origins are in Africa, which remains the biggest cultivator of this product. The produce has expanded to the south over time. Asia and the Americas are also included.

2.1.2 Expected medical and health benefits:

Sorghum has certain properties which makes it suitable for the patients suffered from chronic disorders, celiac disease, diabetes, obesity, celiac disease, oxidative stress and cancer. Sorghum's protein is not very easily digested. People with diabetes and obesity can benefit from sorghum as a food source due to its poor protein digestibility. Da Silva et al. (2011). Sorghum contains

a significant amount of dietary fiber (9.7–14.3 g), which is helpful for binding cholesterol, lengthening transit time, and delaying the absorption of carbs (Narasinga Rao, 2003). Sorghum lipid has the capacity to lower cholesterol. According to another research, sorghum grain includes nutrients that could be used as food additives or dietary supplements to lower cholesterol levels in adults (Carr et al., 2005). A good source of nutrients and minerals is sorghum. It typically resides in the sorghum grain's aleurone layer and germ. The main vitamins in sorghum include the vitamin B complex (pyridoxine, riboflavin, and thiamin) and a few fat-soluble vitamins (vitamins A, D, E, and K). (2012) MartinoI et al. For those with celiac disease, sorghum can be a good diet option because it is gluten-free thanks to gliadin-like peptides, according to Ciacci et al. Items made of sorghum could not reduce anti-transglutaminase antibodies after extended ingestion. In India, obesity is a growing problem that has been positively linked to many chronic diseases.

Coronary artery disease, stroke, insulin resistance, type 2 diabetes, hypertension, and metabolic condition are just a few of the well-known clinical co-morbidities associated with excessive body weight. According to experimental evidence (Alfieri et al., 1995), consuming foods high in dietary fiber helps to reduce the likelihood of becoming overweight. Sorghum is a rich source of dietary fiber and has unique physical properties including viscosity, the ability to hold onto water, and an upper limit on how much can be absorbed. These characteristics help to create the subsequent physiological conduct. It promotes fullness, aids in satisfying hunger, and has subsequent benefits. Sorghum lowers the chance of developing coronary heart disease. Sorghum has anti-carcinogenic chemicals that lessen the risk of oesophageal cancer. (1981, Van Rensburg). Antioxidant substances significantly contribute to halting the oxidation process and minimizing cellular damage. Antioxidant properties in sorghum can protect against reactive oxidative species. (Dayakar Rao and others, 2017).

Indian states suitable for cultivation: Maharashtra, Karnataka, MP, Tamil Nadu, Rajasthan, & Andhra Pradesh

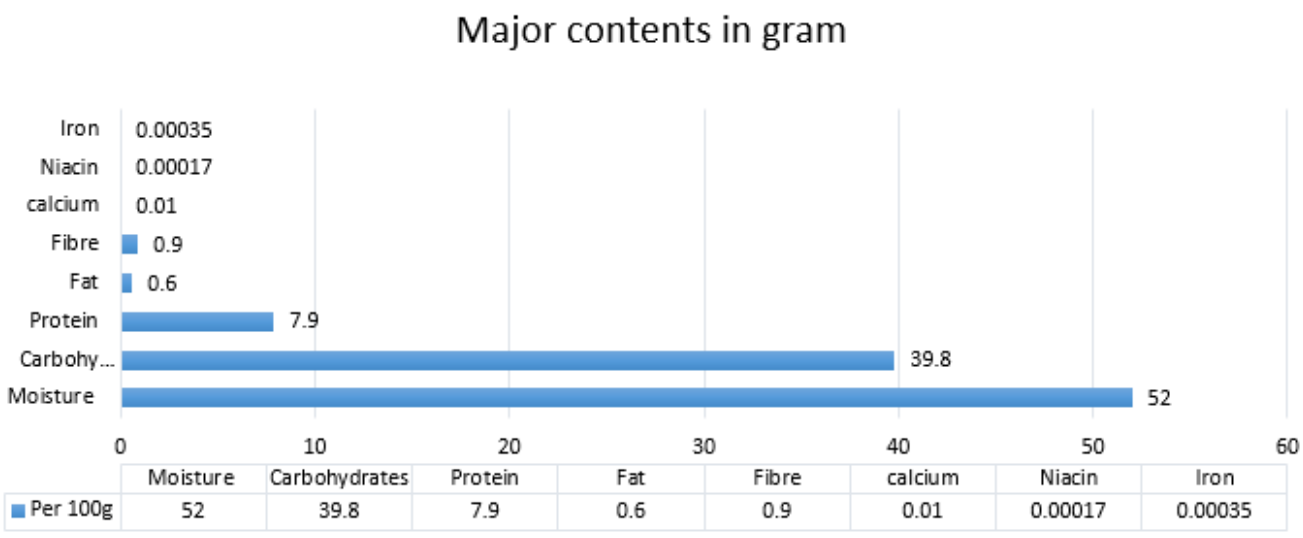
Cultivation months: Kharif season

Number of days: On average 115- 140 days

Total cost of production: On average 5557 rupees per hectare.

Cost per quintal in market sale: On average ₹3622.11./Quintal “variation between state markets”.

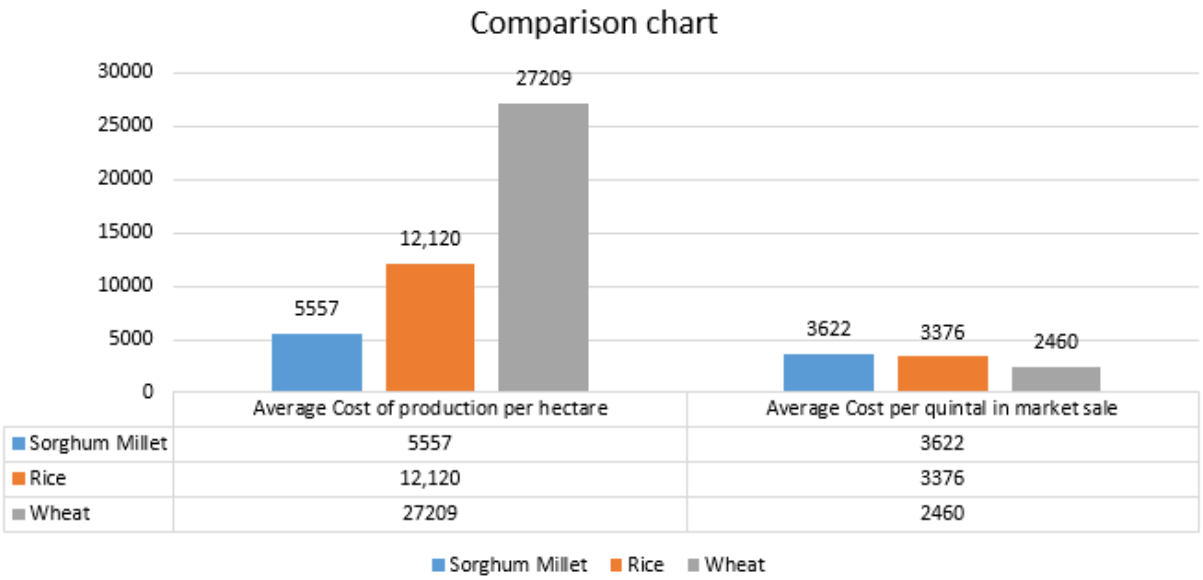
Major nutritional composition:



The nutritional composition of Sorghum bicolor grain includes energy (193 cal), moisture content (52 g), protein (7.1 g), fat (0.6 g), carbohydrates (39.8 g), fiber (0.9 g), calcium (10 mg), iron (3.5 mg), and niacin (1.7 mg). Phytochemical constituents include phenolics, polyflavonols, thiols, anthocyanins, tannins, 3-deoxyanthocyanidin, flavone, and flavanone (Dykes et al. 2009)

Expected Farmer income from one acre land: Net profit from 1 acre Sorghum farming,the farmer can get net profit of Rs.18, 995 – 20,000 by cultivating Sorghum in one acre land.

Comparison between rice and wheat on ground of cost and benefit:



2.2 Proso Millet (Chena / Barri) "Panicum miliaceum L."

Also known as common names, including proso millet, broomcorn millet, common millet, hog millet, Kashfi millet, red millet, and white millet.

2.2.1 Introduction:

An annual grain crop called proso millet (Panicum miliaceum L.) was cultivated in semiarid China around 10,000 years ago. India, Nigeria, Niger, and China are the main growing countries for it. Despite being extremely nutritious and healthy, proso millet is utilized as bird feed and fodder throughout Europe and North America.

2.2.2 Expected medical and health benefits:

Compared to rice, wheat, and barley, Proso millet has a lower glycemic index (GI), making it a better choice of diet for those with type-2 diabetes and cardiovascular disease (CVD) goods made with 100% it had GI (%/g) values between 50 and 65, as opposed to 70 to 80 for goods made with refined maize and wheat Comparison between rice and wheat on ground of cost and benefit. Proso Millet protein (PMP) plays a crucial function in the metabolism of cholesterol because it can raise levels of adiponectin and high-density lipoprotein (HDL) cholesterol, particularly the isomer HDL2, without changing levels of LDL cholesterol. In addition to promoting lipid metabolism, adiponectin plays a critical role in enhancing insulin sensitivity The process of atherosclerosis, which causes heart attacks and strokes, is thought to start with damage to the blood vessel's inner walls. As a result, feeding PMP can actively lower blood sugar and insulin levels after eating a high-fat meal by increasing HDL and adiponectin levels. Additionally, because TNF and insulin sensitivity have a negative correlation, PMP also down regulates TNF .The only nutritional therapy available for a person with celiac disease is diet improvement and avoiding gluten. People who have this condition also have an allergy to gluten, a protein that is present in grains such wheat, rye, barley, and oats. Lecithin, which is abundant in PM and plays a significant function in the neurological health system by repairing and renewing myelin fiber and accelerating brain cell metabolism, is present in high concentrations. Niacin, folic acid, and vitamin B-complex are also present in large amounts in PM .Compared to main cereal grains, PM has a substantially greater mineral content. PM's high fiber and antioxidant content is also beneficial in preventing cancer and cardiovascular disease. Zhang et

al.'s (2014) study revealed that PM had antiproliferative characteristics against HepG2 human liver cancer cells and MDA human breast cancer cells, which were both originally identified as parts of the MD Anderson line of breast cancer cells.

Indian states suitable for cultivation: Tamil nadu, Andhra Pradesh, central and eastern Uttar Pradesh, western Bihar, North Eastern states.

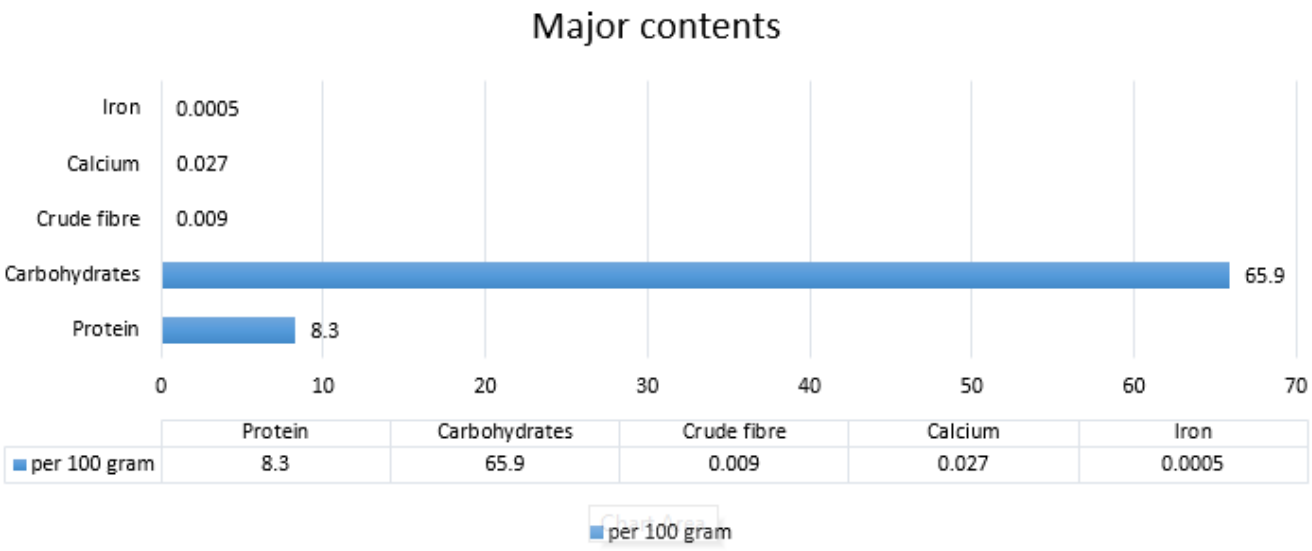
Cultivation months: The rainy season crop is sown in onset of monsoon preferable in July. September – October in Tamil nadu and Andhra Pradesh, Mid March-mid May in Bihar and Uttar Pradesh as irrigated catch crop.

Number of days: On average 60 to 100 days

Total cost of production: On average 5804 rupees per hectare.

Cost per quintal in market sale: On average 5500.00 INR/Quintal variation between state markets.

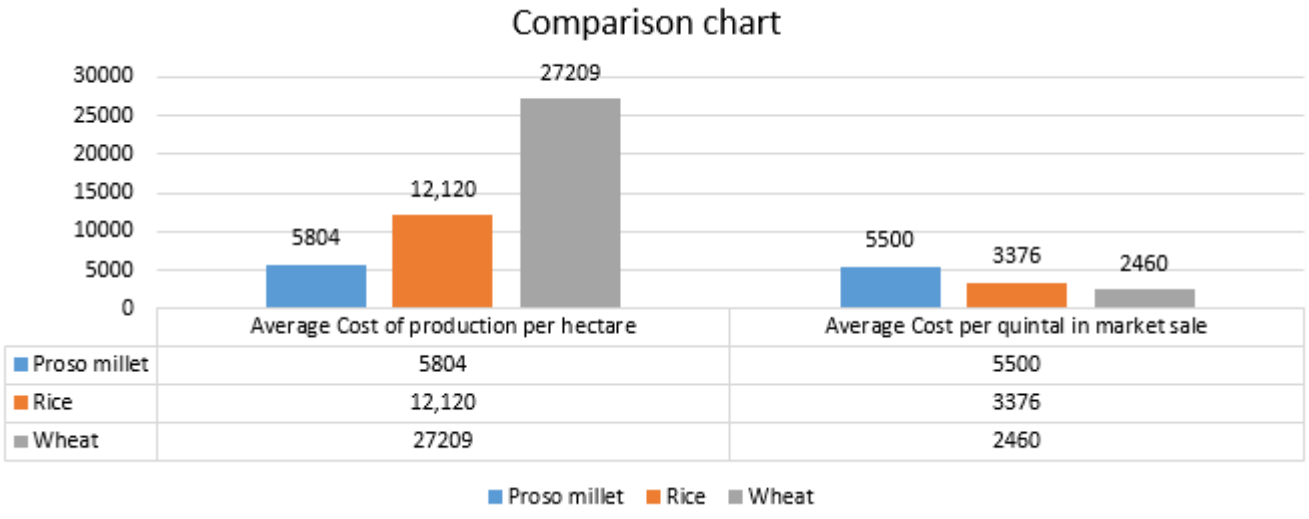
Major nutritional composition:



Niacin, B-complex vitamins, folic acid, PCa, Zn, Fe, essential amino acids (methionine and cysteine), starch, and phenolic compounds like antioxidants and beta-glucan are all abundant in proso millet grains.

Expected Farmer income from one acre land: Net profit from 1 acre Sorghum farming,the farmer can get net profit of Rs.43000-44800 per acre by cultivating proso in one acre land.

Comparison between rice and wheat on ground of cost and benefit:



2.3 Pearl Millet (Bajra) "Pennisetum glaucum (L.) R. Br.)":

This crop is also known as Pearl millet, candle millet, dark millet, bajra, indian millet, horse millet.

2.3.1 Introduction:

Pearl millet grain is considered as a staple food in Africa and India where it is used to make flour, bread, porridge and "couscous" (Ecoport et.al., 2009). As a feedstuff it is mainly grown to produce hay, silage, green-chop, pasture and standover feed grazed directly (FAO, 2009). but contain less lignin, more crude protein, have higher DM degradability and digestibility, and their overall quality does not drop as quickly as they mature, as occurs with normal mid-rib types (Hassanat et al.,2007).

2.3.2 Expected medical and health benefits:

Prevents Type 2 Diabetes, Pearl millet has a special composition that includes healthy carbs and dietary fiber that lower blood sugar levels. Slowly digesting starch: By regularly putting Pearl millet in your diet, you can reduce your chance of acquiring diabetes. Low glycemic index: When compared to foods with a high glycemic index, pearl millet raises blood sugar levels more gradually. Alternative without gluten: If you are gluten intolerant, try Bajra roti with methi, which is made from pearl millet. As one of the most significant Pearl millet Benefits, include Bajra, or Pearl Millet, in your diet might be helpful if you're trying to reduce weight. The protein in this millet aids in tissue repair and muscular growth. For vegetarians aiming to limit carbohydrates, it is a viable substitute due to its high protein content. Another advantage of pearl millet is that it lessens the risk of polycystic ovarian syndrome (PCOS). Women of all ages, including teens and those going through menopause, are susceptible to PCOS, a common hormonal condition. This disorder affects mood, causes tiredness, and unwelcome hair growth in addition to producing health problems.

Including Bajra (Pearl Millet) in the diet can be advantageous in addition to taking medicine, losing weight, and following strict dietary guidelines. Iron and fiber, both of which are abundant in pearl millet, aid in the reduction of visceral fat, especially in the abdominal area. In turn, this controls the menstrual cycle and shields against related lifestyle diseases. Magnesium and potassium, which are abundant in pearl millet and are essential for widening blood vessels and enhancing blood circulation, are present in high concentrations. This miracle millet can prevent artery blockages by lowering LDL cholesterol when consumed regularly. Furthermore, Omega-3 fatty acids and plant lignans found in Pearl Millet types contribute to heart health and general wellbeing. Bajra promotes healthy digestion and avoids constipation, which is crucial for your overall wellbeing. It's excellent for Celiacs as a gluten-free cereal. When consumed consistently, bajra's high insoluble fiber content may help ease constipation. By giving the stool more volume, this fiber encourages regular bowel motions and guards against digestive problems.

Indian states suitable for cultivation: Bajra in India and is mostly farmed in the states of Gujarat, Rajasthan, Maharashtra, Uttar Pradesh, and Haryana.

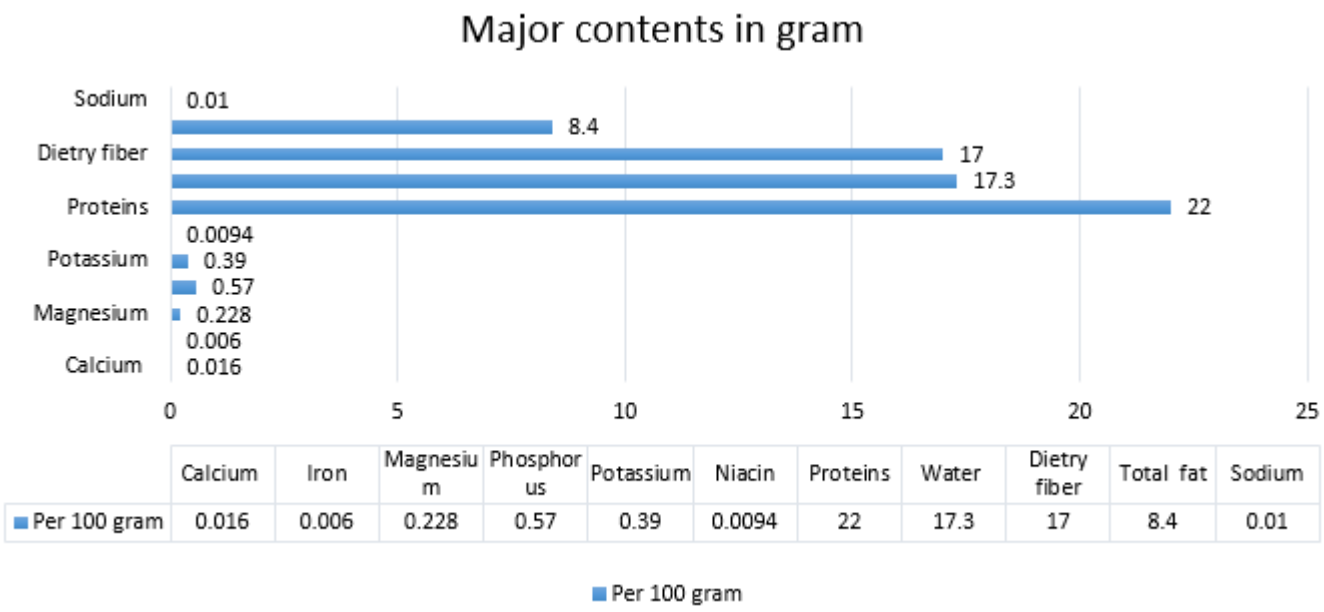
Cultivation months: In the north and center of the nation, kharif pearl millet should be sown during the first two weeks of July, just before the monsoon season begins. In Tamil Nadu, rabi season is appropriate during the first two weeks of October. Prior to the first monsoon rains, dry sowing is advised in the Maharashtra region of Marathwada. Summer pearl millet should be sown last week of January to 1st week of February to obtain higher production of summer pearl millet.

Number of days: On average 80 to 90 days.

Total cost of production: Approximately the cost of production per hectare is around Rs. 27020.70/ha.

Cost per quintal in market sale: On average ₹2200 /Quintal on average

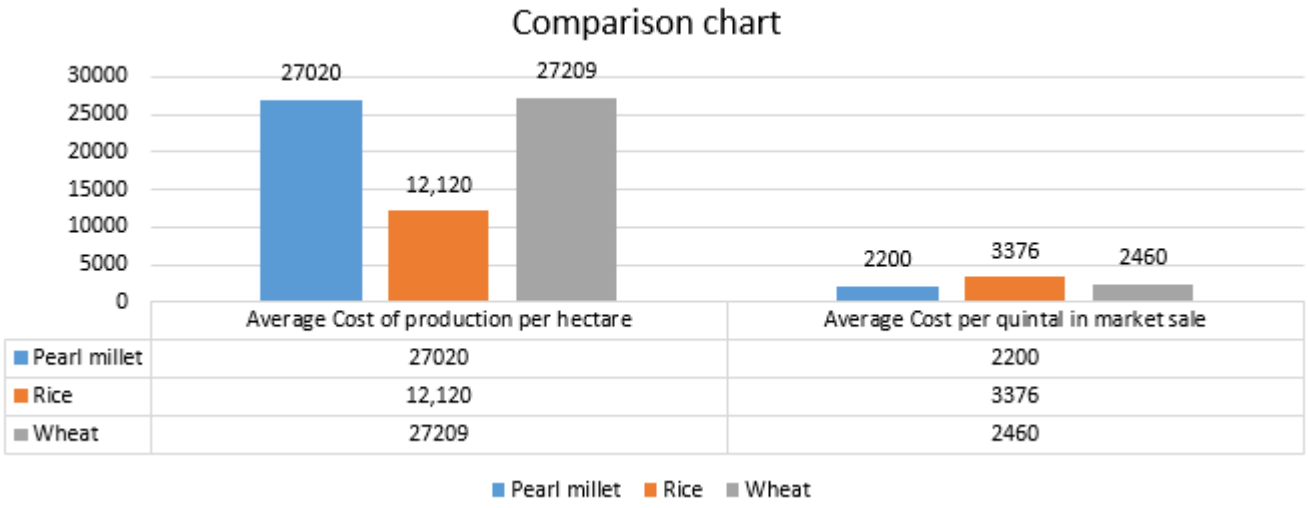
Major nutritional composition:



	Calcium	Iron	Magnesium	Phosphorus	Potassium	Niacin	Proteins	Water	Dietry fiber	Total fat	Sodium
Per 100 gram	0.016	0.006	0.228	0.57	0.39	0.0094	22	17.3	17	8.4	0.01

Expected Farmer income from one acre land: Net profit from 1 acre Sorghum farming under irrigated condition,the farmer can get net profit of Rs.50000-52000 per acre and in rainfed condition 31000 rupees per acre.

Comparison between rice and wheat on ground of cost and benefit:



	Average Cost of production per hectare	Average Cost per quintal in market sale
Pearl millet	27020	2200
Rice	12,120	3376
Wheat	27209	2460

2.4 Foxtail Millet (Kakum / Kangni) “Setaria italic”:

Second most widely grown millet in India is foxtail millet, also called as Kangni or Kakum in Hindi. Foxtail millet, also as Italian millet.

2.4.1 Introduction:

One of the first cereal grains to be domesticated, foxtail millet (Setaria italica (L.) P. Beauv.) is also the most economically significant species in the Setaria genus. However, it is also planted as a fodder plant. Foxtail millet is often grown for its grain (see the Foxtail millet grain datasheet). Setaria italica comes in a variety of cultivated and wild varieties and is interfertile. In temperate regions, annual weeds known as green foxtail millet are the wild forms. Height, habit, inflorescence form, quantity, and grain color vary among cultivars. The foxtail millet plant produces useful hay and silage.

2.4.2 Expected medical and health benefits:

According to an in vitro research conducted in 2003 by Chen et al., foxtail millets have a low glycemic index and may stimulate the pancreatic cells to create insulin, a hormone that controls blood sugar levels. These activities may lessen the blood sugar increase. Foxtail millets were found in 2003 by Choi et al. to improve the body cells' receptivity to insulin, a hormone that regulates blood sugar and has anti-diabetic properties. This suggests that eating foxtail millet may aid in controlling diabetes. The lower end of the digestive system's colon and rectum are both affected by colorectal cancer. According to published research, eating whole grains or cereals may help reduce the incidence of colorectal cancer. Foxtail millets were shown to have a new antifungal protein molecule in an experiment conducted by Wentao et al. in 2011. This protein molecule is known to have action against fungus like Botrytis cinerea and Alternaria alternative, which cause allergies and asthma. Foxtail millets may prevent the development of certain fungus, acting as an antifungal agent against them. This shows that foxtail millets may be able to treat fungus infections. A lack of nutritional intake is the primary cause of undernutrition. According to a meta-analysis by Seetha et al. from 2022, adding millets to the diet may assist with undernutrition since they include nutrients including methionine (an important amino acid), calcium, protein, zinc, and others. The results of this study lend credence to the usage of foxtail millets as an undernutrition remedy. Since foxtail millets are a great source of iron, which is necessary for the production of hemoglobin, increasing your iron intake may help you manage your anemia from iron deficiency. By lowering bile acid generation and bile cholesterol levels, foxtail millets' insoluble fiber content lowers the incidence of gallstones.

Indian states suitable for cultivation: Andhra Pradesh, Karnataka, Tamil Nadu, Rajasthan, Uttar Pradesh, Uttarakhand.

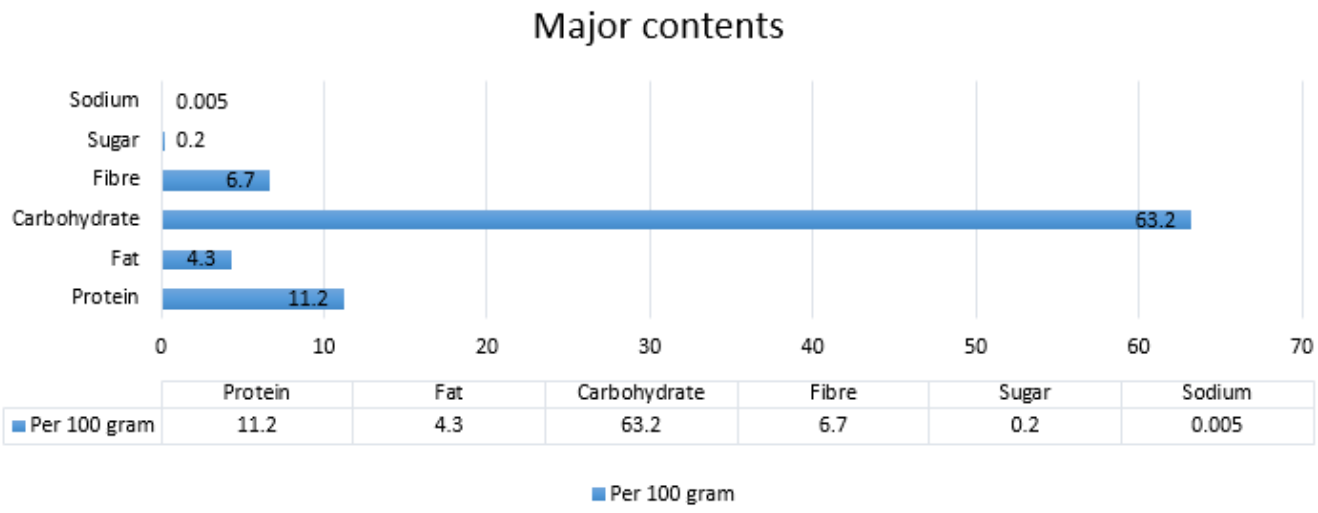
Cultivation months: August–September in Tamil Nadu., July-August in Karnataka. First fortnight of July in Andhra Pradesh, Second and third week of July in Maharashtra. In Tamil Nadu, Kharif irrigated crop is planted from the beginning of June to end of July and summer irrigated crop in January, Plains of Uttar Pradesh and Bihar, middle of June.

Number of days: On average 80 to 90 days.

Total cost of production: Approximately the cost of production per hectare around Rs.11607.2

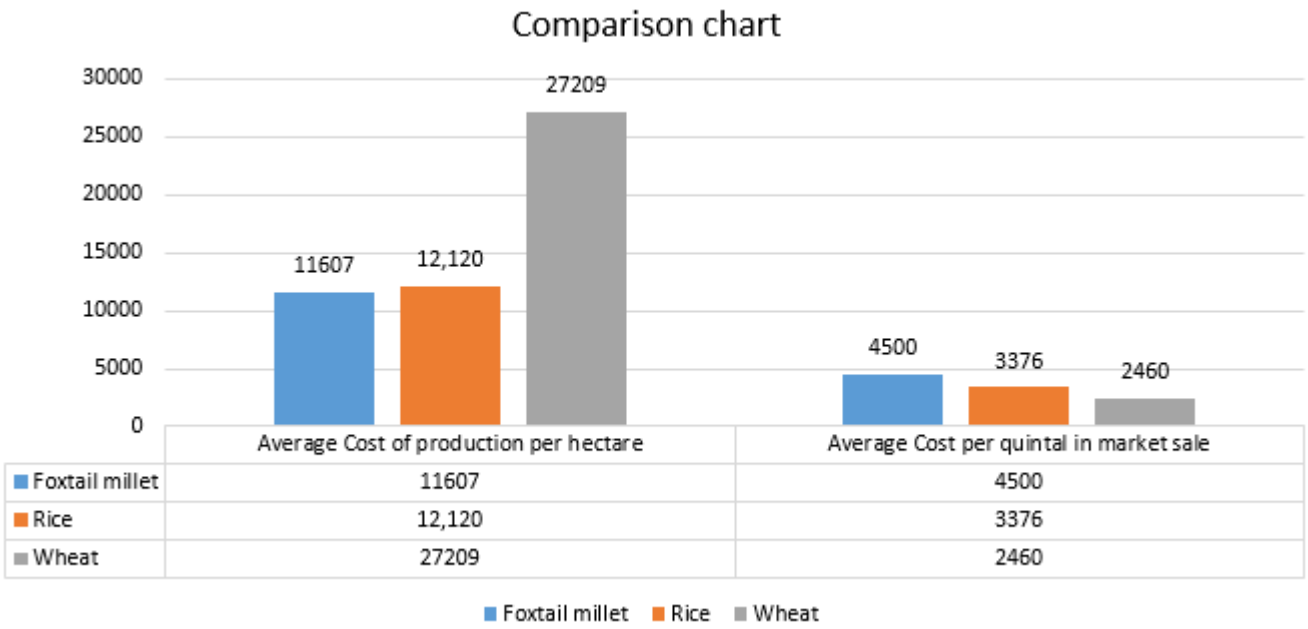
Cost per quintal in market sale: On average 4500 rupees per quintal.

Major nutritional composition:



Expected Farmer income from one acre land: Net profit from 1 acre foxtail millet farming is around 40000 rupees to 50000 rupees per acre.

Comparison between rice and wheat on ground of cost and benefit:



2.5 Finger Millet (Ragi) "Eleusine coracana"

Numerous names, including ragi (in Kannada, Telugu, and Hindi), Mandua/Mangal in Hindi, Kodra (in Himachal Pradesh), Mandia (in Oriya), Taidalu (in the Telangana area), Kezhvaragu in Tamil, etc., are also used to refer to finger millet in India.

2.5.1 Introduction:

The resilient crop finger millet thrives in arid highland regions of Asia and Africa. In times of drought and crop failure, its little, tough grain may be easily stored and is a dependable food supply.

The biggest yearly production on the continent is produced in Uganda, but finger millet is planted across the savannah and highlands of Eastern and Southern Africa.

2.5.2 Expected medical and health benefits:

Consuming finger millet, which is a very rich source of natural iron, aids in anemia recovery. Due to their high calcium and iron content, meals made from ragi are particularly well suited for older people and pregnant moms. For asthma, liver diseases, high blood pressure, and weak hearts, green ragi (finger millet) is advised. When a mother is nursing and her milk supply is low, green ragi is also advised. Regular consumption of finger millet may help stave off malnutrition, degenerative illnesses, and early aging. The phytochemicals in finger millet aid in slowing down the digestive process. This aids in the management of diabetes-related blood sugar levels. It has been discovered that a diet heavy in finger millet—which has more fiber than rice and wheat—helps diabetics. Additionally, the study discovered that a diet consisting mostly of whole finger millet has a

decreased glycemic response, or a capacity to raise blood sugar levels. This is because finger millet flour contains ingredients that reduce the digestion and absorption of carbohydrate. Regular consumption of finger millet promotes bone health, wards off conditions like osteoporosis, and may lower the chance of fracture. It is now well-established that phytates, polyphenols, and tannins can help millet foods' antioxidant activity, which is a crucial component in maintaining good health, slowing the aging process, and preventing metabolic illnesses FAO (1991).

Indian states suitable for cultivation: Ragi (finger millet) is mostly farmed and eaten in Karnataka in India, with smaller amounts also being produced and consumed in Andhra Pradesh, Tamil Nadu, Odisha, Maharashtra, Uttarakhand, and Goa.

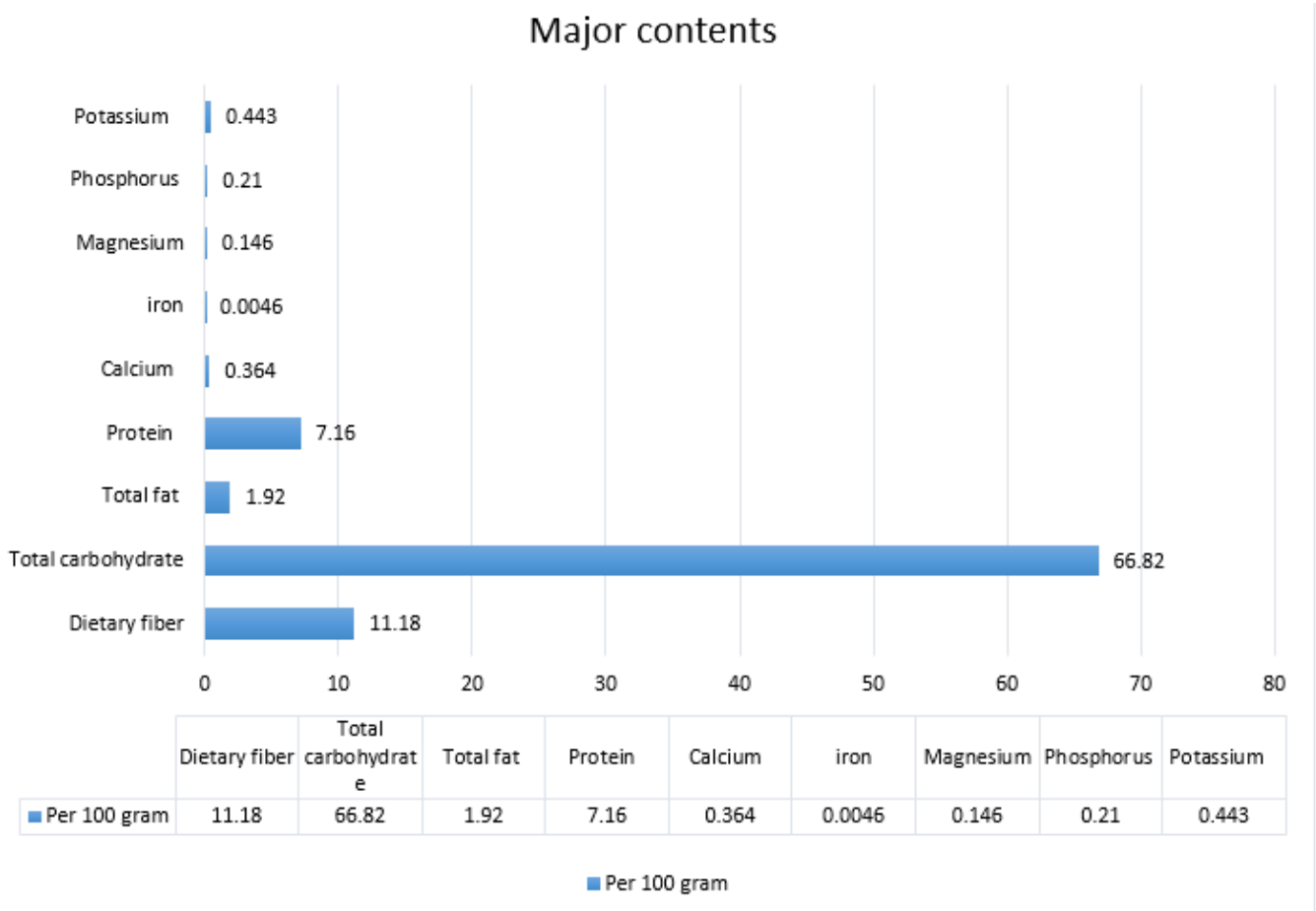
Cultivation months: In various regions of the nation, finger millet is farmed during all of the harvest seasons. The Kharif season is when more than 90% of the area is planted and cultivated.

Number of days: Depending on the tract and the type, the crop takes between 120 and 135 days to reach maturity.

Total cost of production: Ragi cultivation cost an average of Rs. 43,706 per hectare under rainfed conditions and Rs. 57,874 under irrigation conditions, respectively. Due to additional labor, FYM, fertilizer consumption, and irrigation costs, it was discovered that irrigated farming was more expensive than rainfed farming. Taking average cost of Rs. 36,900.

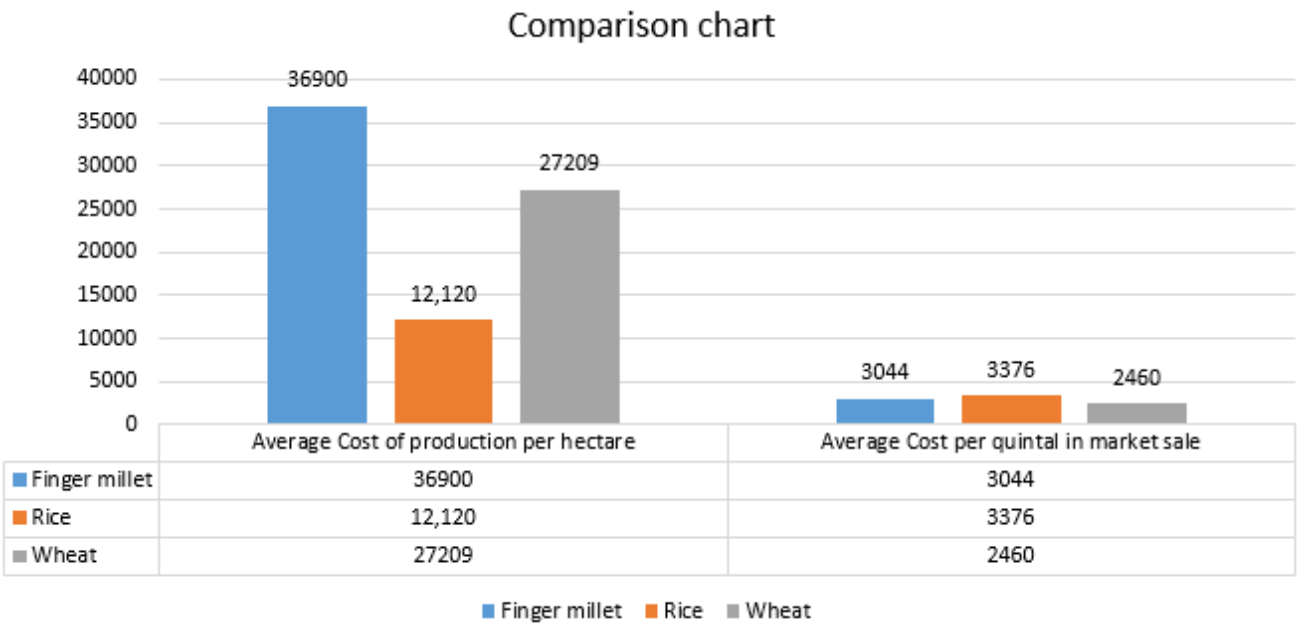
Cost per quintal in market sale: The average price of Ragi (Finger Millet), according to current market pricing, is Rs. 3044.45/Quintal.

Major nutritional composition:



Expected Farmer income from one acre land: Average net profit from 1 acre finger millet farming is around 30440 rupees per acre.

Comparison between rice and wheat on ground of cost and benefit:



	Average Cost of production per hectare	Average Cost per quintal in market sale
Finger millet	36900	3044
Rice	12,120	3376
Wheat	27209	2460

2.6 Browntop Millet (Korle) "Urochloa ramose":

Brown Top millet, also known as pala pul in Tamil, Kannada (Korale), and Telugu (Andakorra).

2.6.1 Introduction:

One of the rarest millet species is browntop millet, often known as signalgrass. It is indigenous to India and thrives in dryland areas. Browntop millet may be grown in low, often flooded locations and is heat and drought resistant. Its ability to tolerate shadows sets Korale apart from other crops. Even under tamarind trees, the crop that thrives in shade, grows well.

2.6.2 Expected medical and health benefits:

One of the greatest advantages of Brown top millet is this. Protein, fiber, and B vitamins including niacin, thiamin, and riboflavin are all included in brown top millet. Iron, phosphorus, and magnesium are also present. These substances benefit health. Finally, Brown Top Millet helps our body produce energy, red blood cells, and promotes immune system, cognitive, and physical health. This beneficial grain can help you manage diabetes by stabilizing blood sugar levels, lowering HbA1C, and improving insulin sensitivity. For better overall health, don't be afraid to incorporate this little powerhouse in your meals. Brown top millet is a fantastic option for anyone with Celiac disease or IBS because it is gluten-free. Bloating, cramping, and starch digestion are reduced. Brown top millet also relieves constipation and supports a healthy digestive tract by regulating bowel motions. Like any other millet, brown top millet is well known for enhancing heart health and reducing the risk of cardiovascular disease. Due to their high protein, fiber, and low carbohydrate content, these grains help to lower LDL cholesterol, prevent arterial clogs, and enhance heart function. Magnesium, calcium, and phosphorus are all vital nutrients for healthy bones and muscles, and brown top millet is an exceptional supplier of these nutrients.

Indian states suitable for cultivation: border territories between Karnataka and Andhra Pradesh, which include parts of the Karnataka districts of Tumkur, Chitradurga, and Chikkaballapura and the Andhra Pradesh district of Ananthpur.

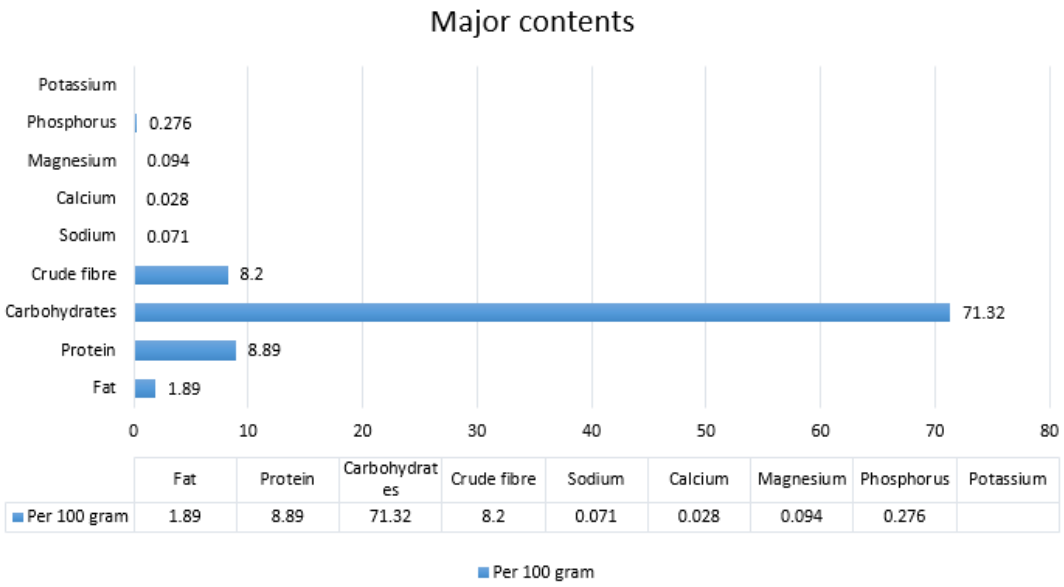
Cultivation months: Most places grow it from mid-April to mid-August, however later plantings will produce lesser yields. It may be grown either as a seasonal crop alone or in conjunction with other crops. Additionally, it is a great option when coupled with other millets. Redgram is really planted as part of a mixed crop, with 12 rows of browntop millet.

Number of days: On average needs 75 to 80days to mature.

Total cost of production: On average Browntop millet cost around 3334 rupees per hectare for cultivation in India.

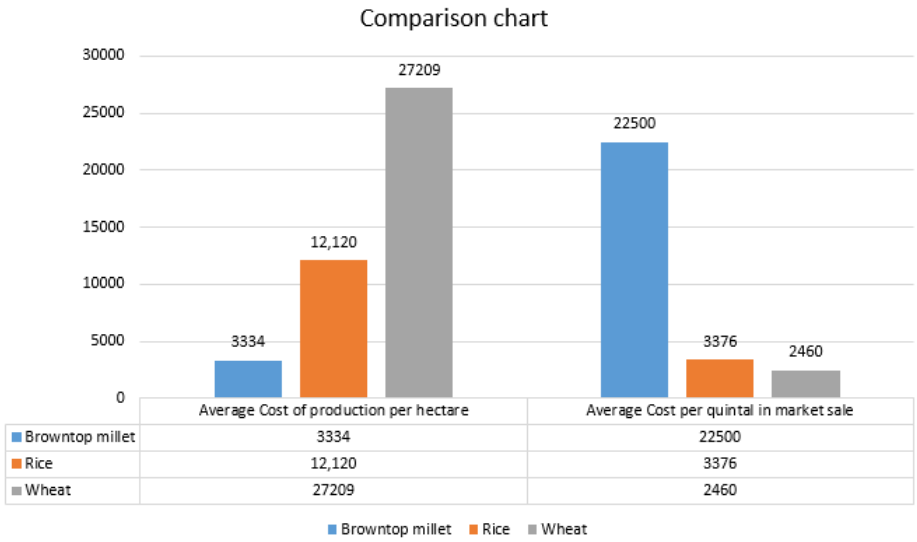
Cost per quintal in market sale: The average price of Browntop millet according to current market pricing, is Rs. 22500 per quintal.

Major nutritional composition:



Expected Farmer income from one acre land: Approximate net profit from well irrigated 1 acre browntop millet farming is around 130000 -140000 rupees per acre.

Comparison between rice and wheat on ground of cost and benefit:



2.7 Barnyard Millet (Sanwa) "Echinochloa esculenta":

Also Commonly named as Sanwa (Hindi), oodalu (Kannada), Kavadapullu (Malayalam), Kuthiravali (Tamil), Udalu (Telugu), Kira (Oriya).

2.7.1 Introduction:

A less significant grain crop is barnyard millet. It can survive water logging situations in addition to being particularly drought resistant. Typically, it is farmed as a rainfed crop. Like rice, kudiraivali grains are eaten. They are also employed in the creation of kheer, or rice pudding. Protein has a 40% digestibility rate.

2.7.2 Expected medical and health benefits:

Barnyard millet contains a high level of amylase retrogradation, which promotes the development of more resistant starches in increasing concentrations. Therefore, it could be suggested to those who have diabetes and cardiovascular disease. Due to its magnesium content, it lowers blood pressure. Increased dietary fiber helps to raise good cholesterol and reduce bad cholesterol. Barnyard millet is a great food to treat anemia because of its high iron level. Barnyard millet provides the necessary elements for a healthy metabolism when consumed in moderation. Barnyard millet can aid with symptoms like these, especially in anaemia situations when the body feels perpetually low on energy and exhausted. Barnyard millet is an excellent source of antioxidants that are beneficial for preserving skin health since it is packed with phenols and flavonoids. Additionally, the inclusion of iron and zinc aids in the promotion of robust hair growth. Barnyard millet prevents constipation, bloating, stomach pains, and acid reflux while promoting regular bowel motions.

Indian states suitable for cultivation: In Malaysia, the East Indies, China, Japan, and India, barnyard millet is cultivated. When the rice harvest fails, it is rumored that it is produced as a replacement crop in China and Japan. Africa and the United States of America are somewhat affected by it as well. Madhya Pradesh, Uttar Pradesh, Tamil Nadu, Andhra Pradesh, Karnataka, Maharashtra, and Bihar are among the Indian states where it is cultivated.

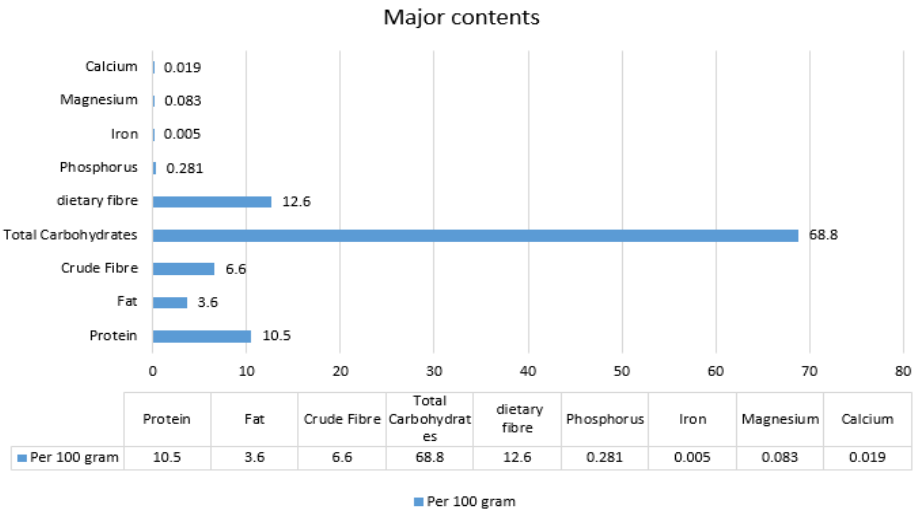
Cultivation months: A crop with a brief growing season, barnyard millet may be produced all year round. Early October to November is the ideal time to grow barnyard millet. The ideal soil temperature is roughly 18°C. Early May or April is when the rainfed early crop is seeded. The primary crop for the Kharif season is seeded in June or May.

Number of days: Millets grow quickly from planted seeds to mature, harvest-ready plants, taking just around 65 days.

Total cost of production: Barnyard millet cost around 13,585 rupees per hectare for cultivation in India.

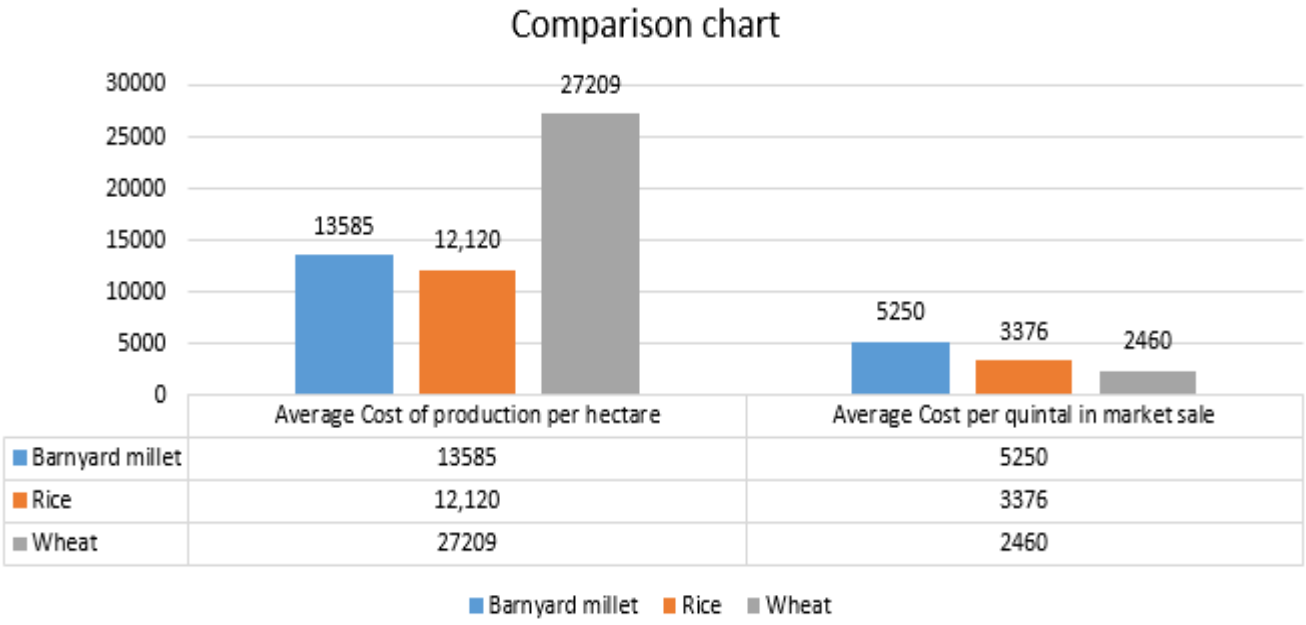
Cost per quintal in market sale: The average price of Barnyard millet according to current market pricing, is Rs. 5250 per quintal.

Major nutritional composition:



Expected Farmer income from one acre land: Approximate net profit from well farmed 1 acre farming is around 125000 rupees per acre.

Comparison between rice and wheat on ground of cost and benefit:



	Average Cost of production per hectare	Average Cost per quintal in market sale
Barnyard millet	13585	5250
Rice	12,120	3376
Wheat	27209	2460

2.8 Little Millet (Moraiyo) "Panicum sumatrense":

The regional names include Kutki in Hindi, Chama in Malayalam, Same in Kannada, Samai in Tamil, and Sama in Telugu.

2.8.1 Introduction:

Panicum sumatrense, sometimes known as little millet, is a cereal that grows quickly and only lasts a brief time and is resistant to both drought and water logging. It is a significant crop raised for food and for livestock.

2.8.2 Expected medical and health benefits:

The glycemic index of little millet is low. Its low GI causes a gradual increase of blood sugar. This makes it a fantastic option for managing blood sugar in diabetics. Foods with low GI levels provide continuous energy and help minimize blood sugar increases by being digested and absorbed more slowly. Little millet is a good option for preserving stable blood sugar levels because of its low GI. Little millet's fiber aids in maintaining regular bowel motions and guards against constipation. By adding weight and facilitating the easy transit of waste through the digestive tract, it also promotes a healthy gut. A small amount of millet in your diet can help your digestion work properly and lower your risk of gastrointestinal diseases. Little millet can be safely included in gluten-free diets. Gluten-intolerant persons may now enjoy a wider variety of dishes thanks to this grain, which provides a healthy alternative to wheat and other grains containing gluten. Little millet is inherently gluten-free, in contrast to wheat, barley, and rye. When someone has a problem connected to gluten, it might cause negative effects. If you want to eliminate gluten in your diet, Little Millet is a safe and wholesome alternative.

Indian states suitable for cultivation: Karnataka, Tamil Nadu, Odisha, Madhya Pradesh, Chattisgarh, Jharkhand, Andhra Pradesh, Uttarakhand, Maharashtra, and Gujarat are the Indian states that cultivate small millet.

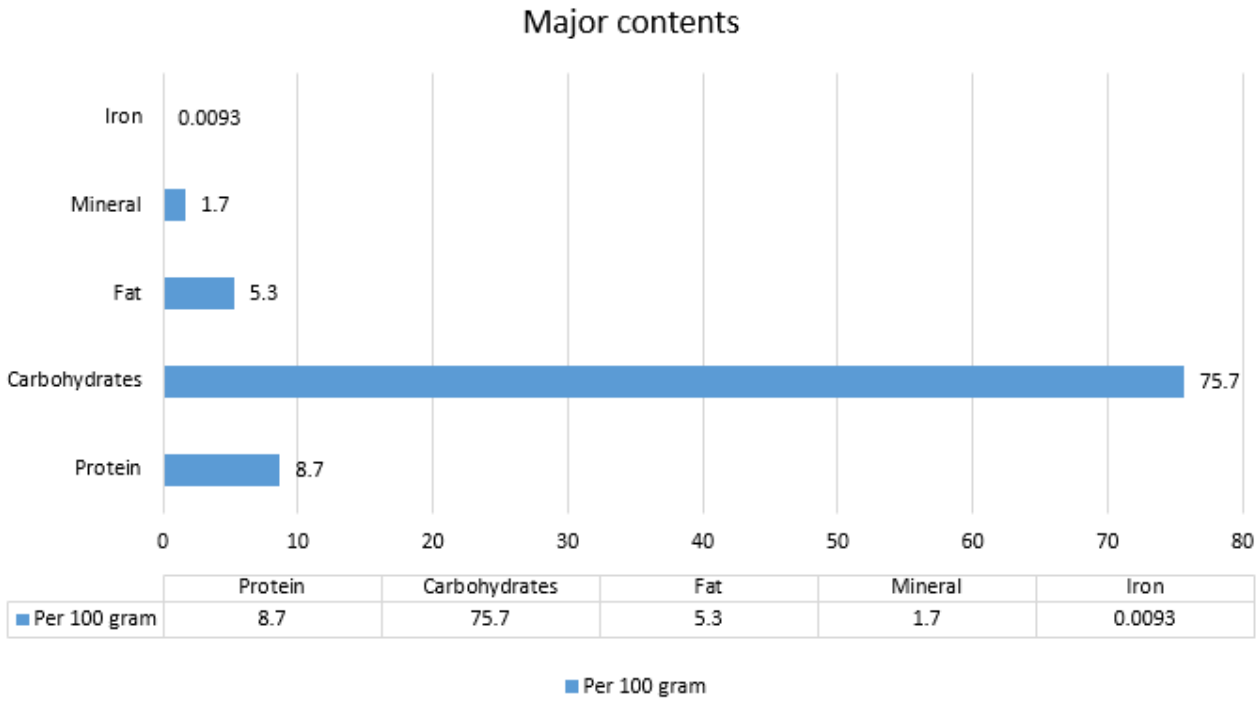
Cultivation months: Little millet is mostly grown in the kharif season. While the crop is planted in June in Tamil Nadu and the middle of June in Odisha, Madhya Pradesh and Karnataka, the seeding season is from the end of June to the first week of July.

Number of days: After seeding, the crop is ready for harvest 65 to 75 days later.

Total cost of production: Around 15,031 rupees per acre.

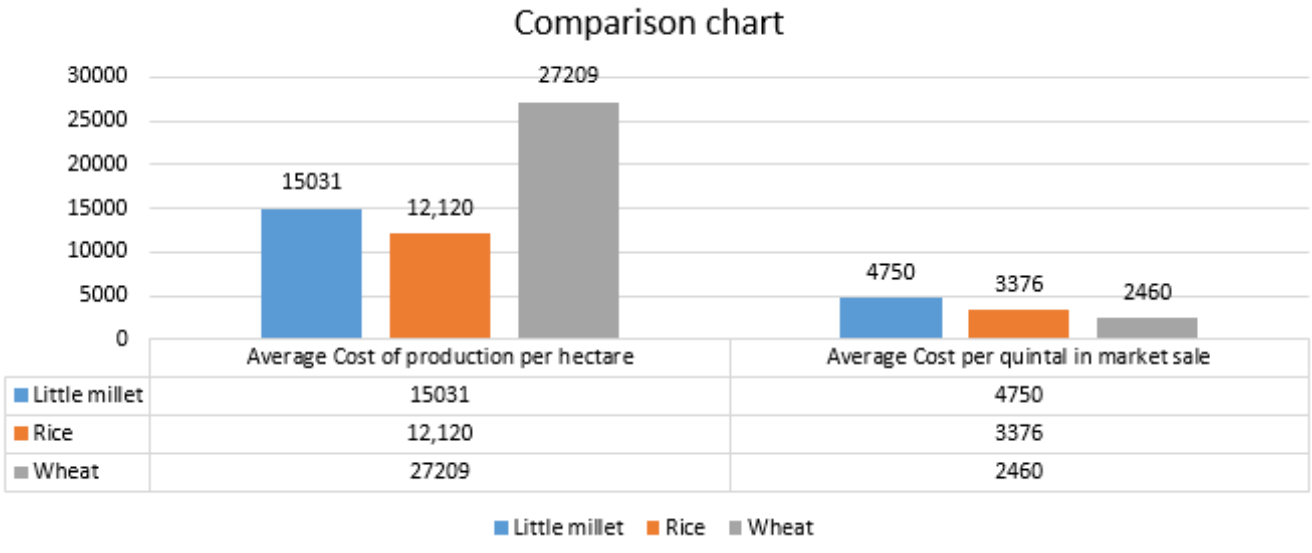
Cost per quintal in market sale: 4750 rupees per quintal.

Major nutritional composition:



Expected Farmer income from one acre land: Approximate net profit from well farmed 1 acre farming is around 20250 rupees per acre.

Comparison between rice and wheat on ground of cost and benefit:



2.9 Buckwheat Millet (Kuttu) "Fagopyrum esculentum":

Also known as kuttu in Indian states.

2.9.1 Introduction:

Buckwheat is a member of the food category known as pseudocereals. Pseudocereals are seeds that grow on grasses yet are used as cereal grains. The pseudocereals quinoa and amaranth are also popular. Buckwheat is gluten-free because, in spite of its name, it is not linked to wheat. It may be converted into groats, flour, and noodles or used in buckwheat tea.

2.9.2 Expected medical and health benefits:

Niacin and fiber are two elements found in whole grain diets that are crucial for heart health. Buckwheat has a lot of fiber. A form of plant-based carbohydrate known as dietary fiber is one that the body cannot digest. Fiber aids in the proper digestion of food by the intestines and facilitates the passage of food through the digestive system. Additionally, it could promote weight loss and shield against cardiovascular disease. Foods that prolong satiety can stave off hunger for longer periods of time and may lower the daily caloric intake of a person. Buckwheat has a lot of protein. High-protein meals are crucial for weight management, according to research, since they increase satiety while containing less calories than other food categories. FAO (2009). A healthy diet that includes buckwheat may increase satiety and aid with weight control. A source of complex carbohydrates is buckwheat. People who consume this type of carbohydrate can better control their blood sugar levels. Complex carbs are digested by the body more slowly than simple carbohydrates. As a result, digestion is slowed and blood sugar levels are kept steady for longer. An illustration of a simple carbohydrate is white bread.

Indian states suitable for cultivation: In India, Buckwheat is grown majorly in hill areas of Jammu and Kashmir ,Uttarakhand, Himachal Pradesh , Chattisgarh ,Uttar Pradesh , West Bengal , Upper Assam region, Sikkim , Meghalaya, Manipur, Arunachal Pradesh , Nilgiris and Palani hills of Tamil Nadu, and Kerala

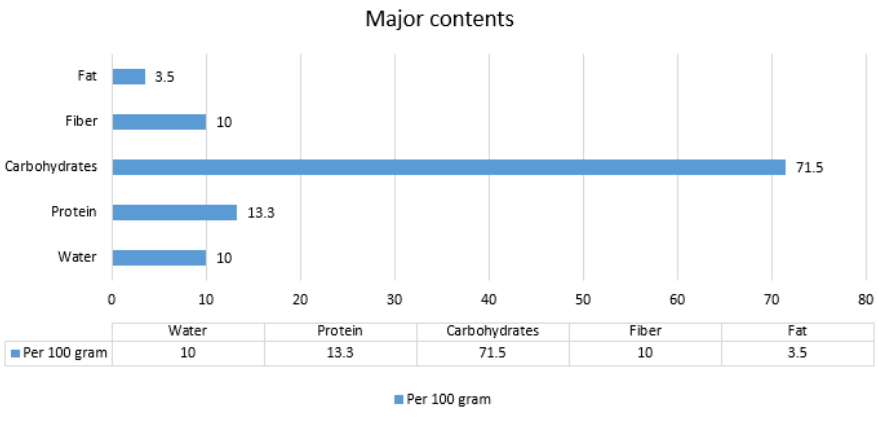
Cultivation months: Buckwheat seeds should be sown in the months of October and November. It can also be grown from February to October in controlled conditions.

Number of days: It takes about on average 80 to 90 days to mature.

Total cost of production: On average the cost of production per hectare is Rs.3,087

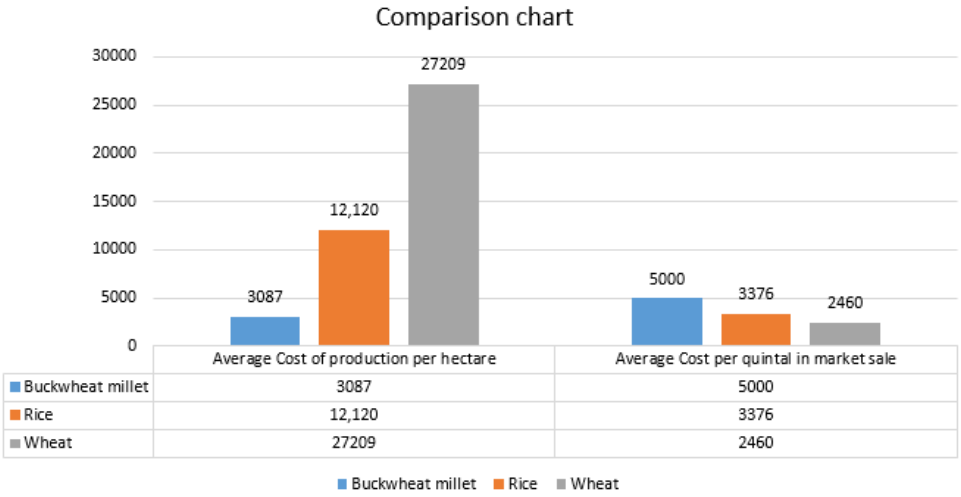
Cost per quintal in market sale: On average 5000 rupees per quintal.

Major nutritional composition:



Expected Farmer income from one acre land: Approximate net profit from well farmed 1 acre farming is around 20250 rupees per acre.

Comparison between rice and wheat on ground of cost and benefit:



2.10 Amaranth Millet (Rajgira) "Amaranthus caudatus L":

It is known as rajgira (king of seeds) in Gujarati, ramdana (seed provided by god) in Bihar, Odisha, and Uttar Pradesh, chuka in Bengal, kalaghesa, chumera, and ganhar in Central India, and bathu in Himachal Pradesh, among other names in Indian languages.

2.10.1 Introduction:

High concentration of micronutrients and higher concentration of higher-quality protein, GRAIN amaranth, an edible pseudocereal, is currently a crop of attention. The C4 pathway in grain amaranth provides the physiological benefit of a high rate of photosynthesis.

2.10.2 Expected medical health benefits:

For thickening soups, stews, sauces, and other meals, amaranth flour provides a gluten-free substitute for wheat flour. Additionally, it may be cooked using gluten-free flours and gums. decreases cholesterol levels Both LDL and triglyceride cholesterol levels are reduced by the oils and phytosterols. Amaranth contains peptides and oils that have anti-inflammatory effects that can aid with pain relief and swelling reduction. Blood pressure can be lowered using amaranth. This seed is generally a healthy meal for the heart since it reduces blood pressure, inflammation, and cholesterol. Compared to the protein in other seeds and grains, the protein in amaranth is simpler to digest. Its digestion has been contrasted with milk protein's. A food with a lot of fiber is amaranth. This increases its filling capacity, aids in digestion, lowers blood pressure and cholesterol, and delays the absorption of sugar so the body can continue to produce energy. Lack of fiber may cause constipation, flatulence, or even increased fat storage. It's a good idea to include dietary fiber-rich foods like amaranth.

Indian states suitable for cultivation: This crop is grown as a minor crop in the Asia-Pacific areas, which include Israel, India, China, Manchuria, Nepal, Bhutan, Afghanistan, Indonesia, Japan, Thailand, and Nepal.There was grain amaranth in India. largely in hilly areas, although as of the late 1990s, its cultivation accelerated in Central and areas of the western Plateau. However, According to estimates, the crop is grown. in an area of 40–50 thousand acres India.

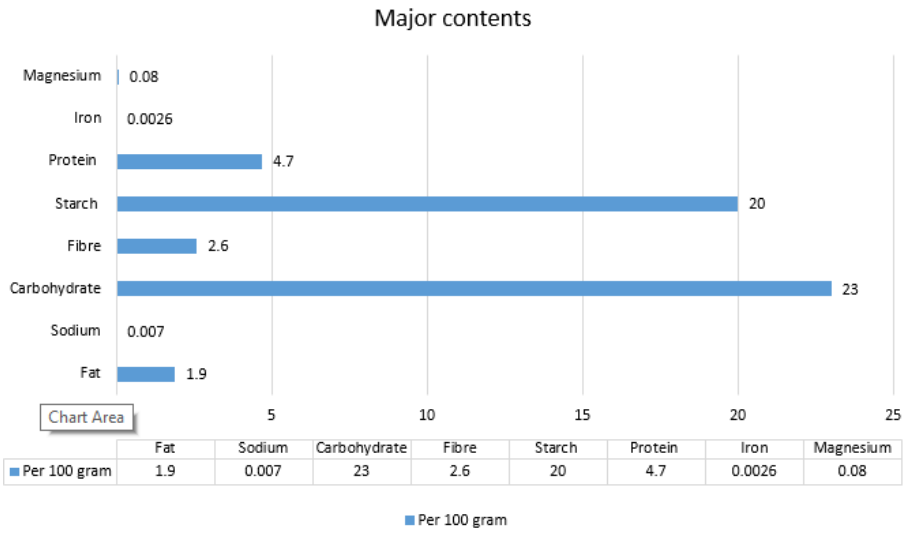
Cultivation months: In hills, the crop is generally sown in the months of May–June soon after onset of monsoon. However, in plains it can be sown either in rabi (winter) or kharif (summer) season. But, generally it is cultivated in rabi season and is sown in months of October–November.

Number of days: It takes about 120 to 130 days to mature.

Total cost of production: The cost of cultivation per acre around 24700 rupees.

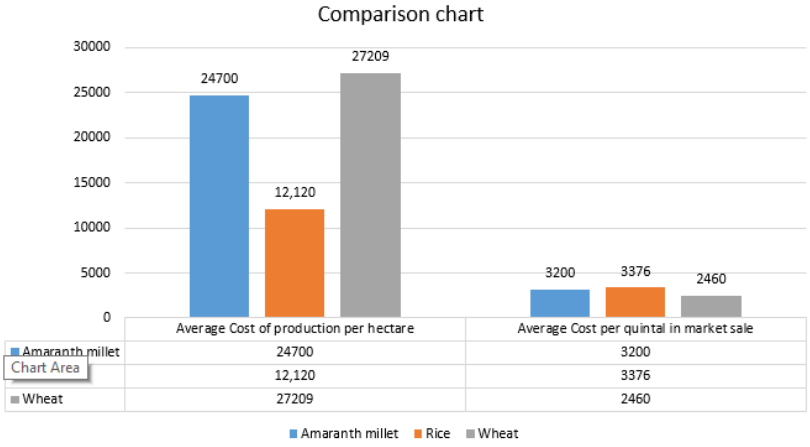
Cost per quintal in market sale: On average 3200 rupees per quintal in market mandi.

Major nutritional composition:



Expected Farmer income from one acre land: Approximate net profit from well farmed 1 acre farming is around 28,800 rupees per acre.

Comparison between rice and wheat on ground of cost and benefit -



2.11 Kodo Millet "Paspalum scrobiculatum":

Locally known as rice grass, ditch millet, cow grass in English, araka in Telugu and kodra in Marath.

2.11.1 Introduction:

It is well renowned for having the strongest drought resilience of all the millets that are available, and because it yields a high amount of grain quickly, it has significant economic significance. Kodo millets are produced in the largest quantity in the world, hence it is important economically to grow them. Kodo millets come in a variety of kinds, including Indira kodo, Jawahar kodo, TNAU, etc., and are grown during the kharif season (the monsoon season). Kodo millets are transformed into premium dishes and beverages. In addition to its economic and gastronomic advantages, kodo millets have a host of health advantages FAO (2002).

2.11.2 Expected medical and health benefits:

Because of its greater amounts of fiber, lower levels of uric acid production, and lower levels of potassium, kodo millet is beneficial for renal problems. The flavonoids and antioxidants in Kodo millet aid in blood purification and reduce the risk of kidney and gallbladder stone development. Kodo millet has phytochemicals such phytic acid, which reduces cholesterol, and phytate, which has been associated with Kodo millets contain polyphenols and little sugar. Both of these assist in lowering blood sugar levels. Kodo millets are said to aid in the management of type 2 diabetes, according to a study done in 2022 by Han et al. Additionally, a study discovered that Kodo-dependent foods, such as Kodo idly and upma, have a 60% lower glycemic index, making them a perfect replacement for cooking diabetic-friendly meals. a decreased risk of cancer. Heart attacks, atherosclerosis, and other chronic illnesses can be avoided by eating Kodo millet since it contains potassium, magnesium, and prebiotic fiber. Kodo millets have a greater polyphenol content, which reduces the risk of obesity, according to research. Additionally, it aids in lowering inflammation, adipose tissue fat synthesis, and weight gain. Antioxidants and polyphenols found in Kodo millets aid in the fight against free radicals and have cell-repair properties. A traditional method of treating cuts and wounds is to apply Kodo millet flour paste to them.

Indian states suitable for cultivation: As a food grain, it is grown throughout India, from the southern states of Kerala and Tamil Nadu to the northern states of Rajasthan and Uttar Pradesh, and the eastern state of West Bengal.

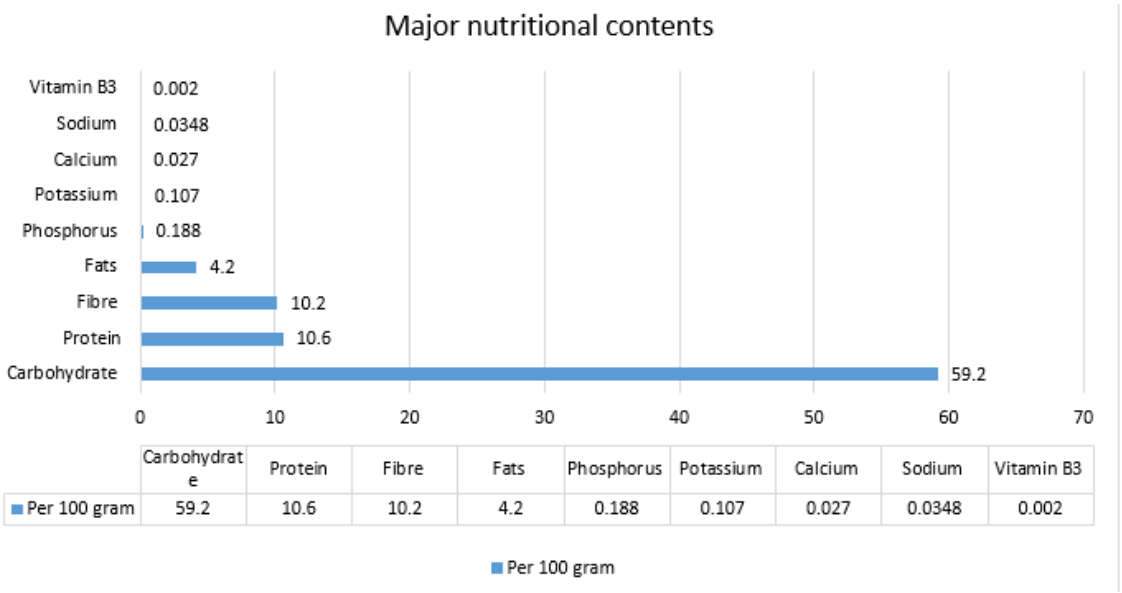
Cultivation months: Kodo millet is sown in early June and is a "kharif" or monsoon crop.

Number of days: Its growth period, which ranges from 120 to 180 days, is rather brief.

Total cost of production: Minimum 24700 rupees is cost of production for a hectare.

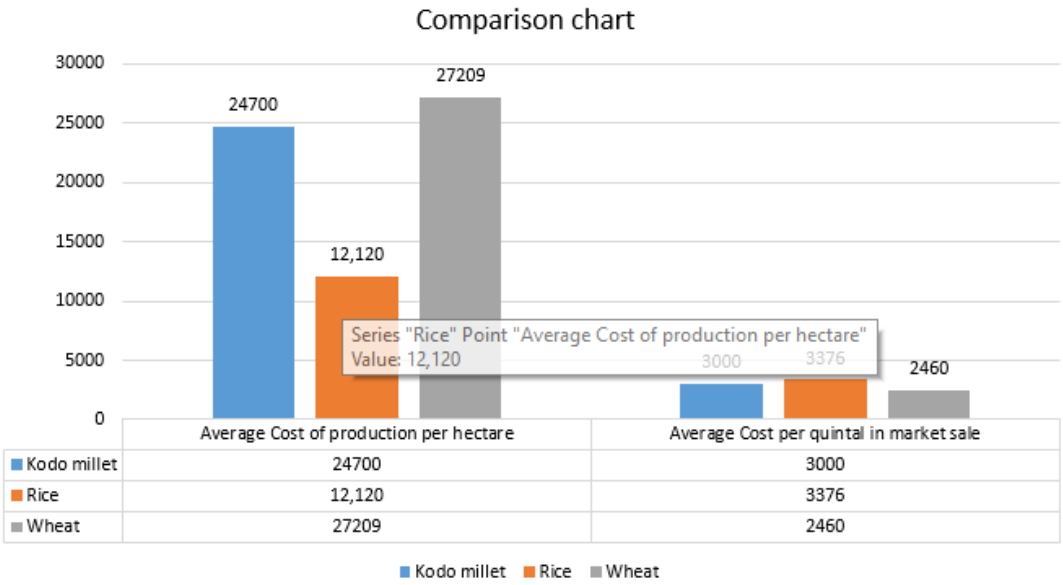
Cost per quintal in market sale: In market a farmer can get 3000 rupees per quintal.

Major nutritional composition:



Expected Farmer income from one acre land: Approximate net profit from well farmed 1 acre farming is around 27,000 – 28,000 rupees per acre.

Comparison between rice and wheat on ground of cost and benefit:



III. CURRENT SITUATION OF MILLETS IN INDIA:

One of the top five nations in the world for millets exports is India. India exported millets for $75.46 million in 2022–2023. Around 173 lakh tonnes of millets are produced in India, which accounts for 20% of world output and 80% of that in Asia. Top manufacturer of Barnyard is India (99.9%). Finger (53.3%), Kodo (100%),(100%) Little millet,(44.5%) Pearl millet. In India, millet cultivation produced an average of 1208 kg per hectare in 2021–2022. Despite India's millet farming area steadily declining since 1971–72, the production of millets rose by 7% from 1966 to 2022.

IV. FEW STARTUPS BUSINESS ON MILLETS

In India, millet production in 2021–2022 averaged 1208 kg per acre. Despite India's millet agricultural area continuously dropping since 1971–1972, millets were produced in greater quantities from 1966 to 2022, increasing by 7%. The government is providing funding for initiatives such as market and value chain development, research and development, raising consumer awareness for increased consumption, and supporting sustainable production. In 212 districts throughout 14 States, the National Food Security Mission's (NFMS) nutritious cereal component for millets is being implemented. The ministry is collaborating with other central ministries, the state governments, and other stakeholder groups to promote millet production and consumption. The Public Distribution System must now reorient its distribution programs away from providing only basic calories and toward offering a more varied food basket that includes millets in order to enhance the nutritional health of young children and women who are of childbearing age. In April 2018, the Indian government declared millet to be a wholesome cereal. The Poshan Mission program has also incorporated millet.

V. CONCLUSION

With the increasing population every year equivalently increasing pressure for higher production leads to higher consumption of water, fertilizers, pesticides etc. Rice, wheat, maize, sugarcane especially consume much water and time for giving relevant production, to counter this in present time, millets gives much higher counting benefits than any of the farming cereal. Before the rise of wheat and rice in late 1960's during green revolution, millets were playing the part of India's major cereal. Afterwards high yielding rice and wheat took over the area from millets but now after five decades it is realized that need of millets is not only for saving cultivation price but also to fulfil nutritional need.

CONFLICTS OF INTEREST

There is no conflicts of interest of any type in drafting this review paper.

AUTHOR'S CONTRIBUTION

Both author contributed equally in conceived and design the analysis, collected the data, performed the analysis and wrote the full length paper.

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Organic Farming: A Review on Viable Path to Safe and Healthy Food Production

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Abstract**—** *The Green Revolution, though successful in meeting rising food demands, has incurred significant environmental costs. Organic farming emerges as a holistic alternative, promoting agroecosystem health and safe food production. This paper reviews the principles and effectiveness of organic farming practices, focusing on liquid organic formulations like Panchagavya, Beejamruth, and Jeevamruth. These formulations, derived from natural sources, stimulate plant growth and immunity, addressing concerns about soil health and water contamination. Despite increasing adoption by farmers, scientific validation of these inputs is lacking. This paper highlights the importance of on-farm experimentation for wider acceptance. India, with its historical reliance on cattle-based agriculture, is poised for a transition to organic farming. Organic inputs, such as vermiwash and Panchagavya, enhance soil properties and crop productivity, offering a sustainable and eco-friendly agricultural approach. As organic farming gains momentum globally, these alternatives play a crucial role in promoting soil health, reducing chemical dependency, and ensuring food safety. Embracing organic practices aligns with the growing global shift towards environmentally conscious agriculture, promising a more sustainable future for food production.*

Keywords— Organic farming, Green Revolution, Liquid organic formulations, Panchagavya, Beejamruth, Jeevamruth, Sustainable agriculture, Agroecosystem health.

I. INTRODUCTION

The Green Revolution, while successful in meeting the escalating demand for food and fiber, has come at a considerable environmental cost, marked by the loss of natural ecosystems, groundwater depletion, food pollution, and environmental degradation. As the nation grapples with the challenge of providing safe food for its growing population, organic farming emerges as a holistic alternative to conventional food production. Organic agriculture, a comprehensive production management system, promotes the health of agro ecosystems and ensures the production of safe food for human consumption. Organic farming diverges from conventional practices by avoiding extensive use of synthetic fertilizers, growth regulators, and livestock feed additives. Instead, it relies on sustainable methods such as green manures, crop rotations, crop residues, and animal manures. Liquid organic formulations like Panchagavya, Beejamruth, Jeevamruth, and Amritpani, prepared with locally available materials, play a crucial role in supporting plant growth. These formulations, fermented products rich in beneficial microflora, stimulate vegetative growth and contribute to better yields. The modern organic movement, significantly different from its origins, is gaining recognition commercially, socially, and environmentally. Liquid formulations like Panchagavya, Beejamruth, and Jeevamruth, derived from agricultural by-products, are found to be excellent growth enhancers. The growing awareness of safe and healthy food, coupled with concerns about soil health and water contamination, has led to increased demand for organic farming practices. With farmers realizing the benefits of organic farming, the use of organic liquid manures such as Panchagavya, Beejamruth, and Jeevamruth is on the rise. While these inputs are proving successful in promoting plant growth and immunity, scientific validation is lacking. The need for alternative technologies that ensure safe and healthy food while being environmentally friendly underscores the significance of organic farming practices. Scientific validation of these organic inputs through on-farm experimentation becomes essential for wider acceptance and adoption in mainstream agriculture. Organic farming has witnessed rapid development in recent years, and India, with its lower per capita and per hectare consumption of chemical fertilizers compared to global standards, stands poised for a transition toward organic

agriculture. The historical practice of cattle-based agriculture, where cows were revered for their contributions to farming through milk, dung, and urine, lays the foundation for the integration of organic principles in Indian agriculture. Despite the success of the Green Revolution in the late 1960s, reports of stagnating or declining crop production levels, over-exploitation of natural resources, and excessive chemicalization of agriculture have raised concerns about the sustainability of conventional farming practices. In this context, organic farming emerges as a viable alternative, emphasizing a holistic production management system that promotes and enhances the health of agro ecosystems. Organic farming eschews synthetic fertilizers, pesticides, growth regulators, and livestock feed additives, relying instead on natural practices such as crop rotation, crop residues, animal manures, green manures, and biofertilizers. The use of cow-based organic manures like Panchagavya, Jeevamruth, and Beejamruth is gaining popularity due to their rich nutritional content and role in promoting plant growth.

II. ORGANIC FARMING

Organic farming is developing very rapidly in recent years. Indian agriculture has a better chance to convert itself as organic agriculture because, the per capita and per ha consumption of chemical fertilizer in the country is much lower than the global standards. In olden days, cattle based agriculture was widely practiced. Cow is greatly respected and worshipped. The cow is an inseparable part of the farming community. We directly benefit from cow in terms of milk, dung and urine.

India's food production is a success story following the green revolution in late 1960's. However, there have been reports of either stagnating or declining levels of crop production. Over exploitation of natural resources and excessive chemicalisation of agriculture have led to poor sustainability of farm production. So these technologies are not environmentally sound and sustainable. We have to think of several alternatives for sustaining our food production without sacrificing the environment and ecology. One of the alternatives is organic farming. In this context, the present investigation was taken up to evaluate organic inputs (Jeevamruth and Beejamruth) and to test their efficacy on greens. The literature relevant to the study are reviewed here under.

2.1 Concept of organic farming:

Organic farming is a production system which avoids or largely excludes the use of synthetically compounded fertilizers, pesticides, growth regulators and livestock feed additives. To the maximum extent possible organic farming system rely upon crop rotation, crop residues, animal manures, legumes, green manures, off farm organic wastes, mechanical cultivars, mineral bearing rocks and bio fertilizers to maintain soil productivity as well as tilth and as plant nutrients and biological means to control insects, weeds and other pests. Chakraborty (1998) stated that organic farming means farming in the spirit of organic relationship. It places farming on integral relationship between soil, water and plants; between soil microbes and waste products; between agriculture and forestry; among soil, water and atmosphere. Organic farming is known by various other names also: alternative agriculture, low external input sustainable agriculture (LEISA), ecological agriculture and regenerative agriculture.

Organic farming is both a philosophy and a system of agriculture. The objects of environments, social and economic sustainability lie at the heart of organic farming and are among the major factors determining the acceptability or otherwise of specific production practices (Stockdale *et al*., 2001).

Organic farming is focused on the whole farm system and its interaction with climate, environment, social and economic conditions, rather than considering the farm as comprising of individual enterprises. Organic farming represents the restructuring of whole farm system, rather than the adoption of current practice to reduce environmental impact (Chaudhry, 2002). The objectives of organic agriculture being concisely expressed in the standard document of the International Federation of Organic Agriculture Movement (IFOAM) are,

- To produce food of high quality in sufficient quantity
- To work within natural systems and cycles
- To encourage and enhance biological cycles within the farming system, involving micro-organisms, soil flora and fauna, plants, and animals.
- To maintain and increase the soil fertility for long term.
- To maintain the genetic biodiversity of the production system and its surroundings.
- To promote the sustainable use of natural resources.
- To give all livestock conditions of life with due considerations for the basic aspects of their inmate behaviour.
- To consider the wider social and ecological impact of the farming system.

2.2 Organic manures for crop production:

Klute and Jacob (1949) determined that continued use of manure resulted in an increase in organic matter in the soil, which in turn increased cation exchange capacity, available water and decreased the bulk density of soil. All of these soil characteristics are favourable to crop production and soil health restoration.

The Rothamsted experiment results revealed the superiority of organic manures over NPK mineral fertilizers. In Samundham, the long range trials on a sandy clay soil for sixty years, the yields were more or less similar with FYM and mineral fertilizer. In the field experiments also, the wheat yields over a period of thirteen years with organic manure were similar to that with suitable combination of mineral fertilizers (Cooke, 1970).

It is obvious that organic manures have both direct and indirect effects on crop productivity and environment. The uptake of humic substances or its decomposition products results in better growth, yield and efficient metabolism (Mathur and Gaut, 1977).

2.3 Organic nutrient sources:

Organic manures in agriculture add much needed organic matter and minerals to the soil. The important manures used in organic farming are farmyard manure, vermicompost, green manures and liquid organic manures like Panchagavya, Jeevamruth and Beejamruth. The beneficial effects of organic manures in agricultural production and soil fertility are known for many decades, but they are inadequate in nutrient supply and low in nutrient concentrations. The total nutrients recycled from organic matter decomposition are much less than the amount of nutrients utilized by the crop plants. This necessitates the enrichment of manures with beneficial microbial inoculants like free living nitrogen fixers or phosphate solubilizers, to improve the nutritional status of the manures. The enrichment manures with rock phosphate and beneficial microbial cultures result not only in improvement of nutritive value but also in higher growth and yield of crops. The microbial enrichment of organic manures will further contribute to the enhancement of P solubilisation and N fixation. Organic manures are available in the form of green and dry plant residues, fresh animal wastes, decomposed materials of plants and animal origin and biologically, active preparations (Palaniappan *et al*., 1995). Manures and biologically active preparations of animal and plant origin were most commonly used by those farmers who aimed for sustainable production in Tamil Nadu (Somasundaram, 2002).

2.4 Vermicompost:

Vermicompost is blackish brown humus like coarse, granular material which is loose, fine, soft to touch light in weight and free from any foul smell having electrically charged particles meant for improved adsorption of plant nutrients in the soil (Neena and Battish, 2005). Marinari *et al*., (2000) also observed that vermicompost had pH of 7.7, 2.0% of N, 34.2% of total carbon, 813.0 μg N g^{-1} of NO_3-N, 133.7 μg N g^{-1} of NH_4-N, and 47.0 μg P g^{-1} of available P. Vermicompost being a rich source of macro and micro nutrients, vitamins, plant growth regulators and beneficial micro flora, appeared to be the best organic source in maintaining soil fertility on sustainable basis towards an eco-friendly environment. Vermicompost besides being a rich source of micronutrients also acts as chelating agent and regulates the availability of metallic micronutrients to the plants and increases the plant growth and yield by providing nutrients in the available form. Vermicompost contains growth promoting substances like auxins and cytokinins.

2.5 Liquid Formulation:

To overcome the problems faced with solid carrier based formulations, there is need to develop new inoculant formulations which would ensure longer survival, no contamination, ease of applicability. In recent years, many of the formulations of the liquid based inoculants are introduced which have been shown to tolerate adverse environmental conditions in a better way and are free from other problems that are encountered with solid carrier based preparations (Hynes *et al*., 2001). Liquid bioinoculants are special formulations containing not only the desired microorganisms and their nutrients, but also, special cell protectants or substances that encourage the longer shelf life and tolerance to adverse conditions (Vora *et al*., 2008). Characteristics of a good liquid inoculant include non-toxicity, low cost, readily available uniform materials, adaptable to normal cell culture conditions, amenable to nutrient supplements, rapid release of microorganisms in the soil, support their growth and survival and are easily manageable in the mixing and packaging operation (Smith, 1992).

2.6 Panchagavya:

Panchagavya is a bio stimulant consisting of a combination of five products obtained from cow, which includes cow dung, cow urine, cow milk, curd and ghee. The term Panchagavya represents 'Pancha'-five, 'Gavya'-produce from cow. It acts as an immuno stimulant that promotes growth, increases the overall yield and also renders resistance to diseases and pests. The

materials required for preparation of Panchagavya (Boomiraj, 2003) are cow dung-5 kg, cow urine-3 litre, cow milk-2 litres, curd -1 litre, ghee-1 litre, sugarcane juice-3 litres, tender coconut water-3 litre, Ripe banana (cv. Poovan)-12 numbers and coconut toddy-2 litres. Fresh cow dung (5kg) and one litre of ghee were mixed well and retained in a plastic bucket for three days under shade.

It was stirred well twice a day, then on fourth day, the remaining ingredients were added to the mixture. The slurry was mixed well three (or) more times a day up to 15th day. Thus panchagavya was ready for use in a period of 15 days which was later diluted to 3 per cent and then sprayed. Other than cow's products, the added materials like riped banana, act as fermenting agents and preservators to panchagavya (Natarajan, 2002).

2.7 Jeevamruth:

Jeevamruth is a bio stimulant consisting of a combination of five products, which includes cow dung, cow urine, pulse flour, jaggery, handful of garden soil and some amount of water. The term 'Jeevamruth' represents 'Jeev'-life, 'amruth'- a valuable food. It acts as an immune-stimulant that promotes growth, increases the overall yield and also renders resistance to diseases and pests. Palekar (2006) has given the method to prepare Jeevamruth with the following ingredients,

No.	Ingredients	Quantity
1	Cow dung	10 kg
2	Cow urine	10 litres
3	Pulse flour	2 kg
4	Jaggery	2 kg
5	Soil	100 grams
6	Water	200 litres

2.8 Beejamruth

Beejamruth is used for the seed treatment and the name represents 'Beej'-seed, 'amruth' – a valuable food. Beejamruth is very useful for seed pre-treatment as it increases the germination ability of the seeds. The materials for preparing Beejamruth (Palekar, 2006) are as follows,

No.	Ingredients	Quantity
1.	Cow dung	10 kg
2.	Cow urine	10 litres
3.	Lime	200 grams
4.	Soil	100 grams
5.	Water	100 litres

2.9 Vermiwash:

Earthworm produced plant growth substances in alimentary canal and excreted it along with earthworm cast. Vermiwash, liquid organic manure is an aqueous extract of a column of fresh vermicompost and surface washing of earthworms which contain beneficial microorganisms and water soluble fractions. The nutrients present in vermiwash are in water soluble form and intermediate requirement of a number of components can be met from a single source. The vermiwash, used as the ingredient of the spray preparation, act as a nutrient medium for the crops (Dutt, 1996).

Vermiwash is a collection of excretory and secretory products of earthworm along with other micronutrients. The fresh vermiwash houses a large number of beneficial microorganisms, which helps in plant growth and protects it from a number of infestations. Vermiwash contains sugars, amino acids and phenols along with plant growth promoting hormones such as indole acetic acid and humic acid (Gulsar *et al*., 2006).

2.10 Effect of organic inputs on soil biological properties:

The various biochemical process associated with nutrient recycling are mediated by soil enzyme which are derived from microorganisms, plant roots and soil enzymes (Tabatabai, 1982). Yin-Powang and Chenchingchao (1995) reported that chicken manure used in organic farming treatment enhanced the bacterial and fungal population. A field experiment conducted in Vellayani, Thiruvanathapuram, Kerala, India during 1995– 97 on *Momordica charantia* cv. Preethi revealed that poultry manure application increased the bacterial population in soil than chemical fertilizers (Rajasree and Pillai, 2002).

2.11 Soil microorganisms:

Compost carries with it a very large population of Actinomycetes, fungi and bacteria and by their incorporation into soil, not only millions of microorganisms are added but those already present in the soils are stimulated by the fresh supply of humic materials (Gaur, 1982). Ammonification, nitrification and N fixation are increased due to improved microbiological activity. Compost also stimulates the mycorrhizae, which live in symbiotic association with the roots of plants and trees and play an important role in transferring certain nutrients from soil to plant (Gaur, 1982). The soil microbes are sole agents responsible for all of the biological transformations in the soil. These are carried out through a variety of biochemical reactions carried out or catalysed by group of enzymes (De and De, 1988).

2.12 Soil enzymes:

Soil enzymes activity is considered as an index of microbial activity in the soil. Therefore, any management practices that influences the microbial population of the soil would be expected to produce changes in the soil enzyme activity and level of enzyme activity can be used as an indicator of soil fertility (Burns, 1982). The measurement of dehydrogenase activity in soil gives correlating information on biological activities of microbial populations in soil (Casida *et al*., 1964). Organic manures stimulate soil phosphatase activity (Golian, 1968). Addition of organic matter increased activities of urease, catalase, dehydrogenase and amylase in soil (Garcia *et al*., 1993).

2.13 Effect of organic inputs on crops:

All crops respond to organic manuring and the extent of response depends on several factors such as degree of humification, maturity of the compost, its C/N ratio, the time and method of its application and on soil type, agro climatic conditions and moisture regime of soil during the growth of the crop (Gaur, 1982). From various pot culture and field experiments, it is evident that increased yield and nutrient uptake were related mainly to the improved physical condition or to the nutrient contents of the organic manure or wastes (Narwal *et al*., 1993; Kapur, 1995).

2.14 Field Crops

Dwivedi *et al*., (1993) reported that the application of nitrogen through compost accelerated the metabolic activities, which enhanced the synthesis of aminoacids, protein and carbohydrate resulting in higher assimilation of these contents in black gram and wheat. Subbaraj and Ramaswami (1995) studied the effect of organic amendments on oil yield of groundnut and recorded the highest oil content in composted coir pith treatment, which ranged from 34.7 to 47.7 %. Thilagavathi and Mathan (1995) reported an increase in panicle length, grain per panicle, root length, density and grain yield of coir pith amended soils. Amanullah (1997) reported that the growth parameters and yield of cassava increased due to application of organic manures especially composted poultry manure either alone or with FYM. Math and Trivedi (2000) reported an increased wheat yield and grain yield in organic amended soils over control.

2.15 Horticultural crops:

The results of more than thirty experiments with potato showed that there has been an increase in yield with application of organic manure varying from 4 to 30% over control (Gaur, 1982). The yield increase was also reported in Chillies, Fenugreek, Onion, Sweet potato and Tomato. Organic waste materials can be used as a medium for growing cucumbers in glass house condition with encouraging results (Tzvetkove and Vargov, 1991). According to Kostov *et al*., (1995), the production of fruits on the compost applied field, started 10 to 12 days earlier and compost treatments showed a significantly higher yield. Compost application has an added advantage of being suppressive to numerous plant diseases (Shyng, 1994) and weed population (Son, 1995).

2.16 Panchagavya:

2.16.1 Properties of Panchagavya

Cow's urine (Gomoothra) is rich in urea and acts both as a nutrient as well as hormone. Urine also contained uric acid and hippuric acid in large quantities along with other minerals like NaCl, sulphates of Ca and Mg and potassium hippurate.

Cowdung is rich in bacteria, fungi and other microbial organisms. Cowdung has 82% of water and solid matter of 18% (Singh, 1996). Cow's milk is a good medium for saprophytic bacteria and acts as virus inhibitor. Milk contains protein, fat, carbohydrate, amino acid, calcium, hydrogen, lactic acid and *Lactobacillus bacterium*. Many microorganisms could ferment either five or six carbon sugars, but the *Lactobacillus bacterium* could ferment both (Linda Mc Graw, 1999).

2.16.2 Effect of Panchagavya on growth, yield and quality of crops:

Panchagavya sprayed on chillies crop produced dark green color in leaves and new growth within 10 days (Subhashini Sridhar *et al*., 2001). Vivekanandan (1999a) reported that the panchagavya spray on 25 DAS and 40 DAS had advanced the paddy harvest by ten days. Pod yield of moringa was increased by the treatment combinations of poultry manure + neem cake + panchagavya along with increased dose of fertilizer (Beaulah *et al*., 2002a). The key feature of panchagavya was its efficacy to restore the yield level of all crops during the transitory period of switching over to organic farming from the very first year (Natarajan, 2002).

2.17 Microbiological aspects:

Effective microorganism like *Lactobacillus, Saccharomyces, Streptomyces, Aspergillus* found in panchagavya improved the soil quality and growth and yield of sweet corn, which was equal to or higher than from chemical fertilizer (Xu and Xu, 2000). In Panchagavya, proven bio fertilizers such as Azospirillium (1010), Azotobacter(109), Phosphobacterium (107) and *Pseudomonas* (106) were found besides *Lactobacillus* (Solaiappan, 2002). The crude extract of *Pseudomonas* was found to enhance the growth of garden pea seeds as compared to control (incubated with distilled water), since it contained IAA and GA3 (Mahalingam and Sheela, 2003).

2.18 Effect on pest and diseases:

Ramachandra Reddy and Baskara Padmodaya (1995) reported that the Panchagavya spray controlled the wilt of banana. Soil drenched with 10% successfully controlled the wilt of tomato (Mishra, 2002) and it was found to be superior to carbendazim in reducing the plant disease index and increasing the vigour of the plant and fruit yield of tomato. Panchagavya was found to activate soil microorganisms and to protect plants from diseases (Upendra Shendy *et al*., 2000). Panchagavya spray with Agniastra (fumigation in the field) recorded the least population of cutworms and highest yield of potato (Selvaraj, 2003). Contrary to the above, on the fourth day after spraying panchagavya, there was an aphid attack on the crops. Flowers started withering due to sucking of sap (Jayashankar *et al*., 2002).

2.19 Characterization of organic inputs in soil:

2.20 Panchagavya:

The chemical characteristics of Panchagavya reveal that they possess almost all the major nutrients, micronutrients and hormones (IAA and GA) required for crop growth. Predominance of microorganisms like yeast and Lactobacillus in Panchagavya is due to the combined effect of low pH, milk products and addition of jaggery as substrate for their growth. The low pH of the medium is due to the production of organic acids by the fermentative microbes as evidenced by the population dynamics and organic acids determination in GC analysis. Lactobacillus produces various beneficial metabolites such as organic acids, hydrogen peroxide and antibiotics which are effective against other pathogenic microorganisms besides its growth promotion effect on animals and human beings as probiotics. The mixture of 1 part of Panchagavya with 50 parts of water has some notable physico-chemical characteristics. The pH range of mixture is around 4.60 and the EC is around 0.54 mS cm^{-1}. The IAA is around 12.1 ppm and the GA content is around 5.2 ppm. The observed values of N, P & K are 6650 ppm, 4310 ppm and 5200 ppm respectively.

2.21 Jeevamruth:

The filtered extract from Jeevamruth is used as soil application and is believed to enrich soil microbiologically through in-numerous beneficial microorganisms. Jeevamruth is reported to contain very large population of nitrogen fixers, phosphate solubilizes and siderophore producers. Microbial fermentation during preparation is also believed to develop some growth promoting hormones which help in better germination and seedling emergence (Sanjeev kumar *et al*, 2012).

2.22 Beejamruth:

The application of Beejamruth in soil application results in rapid proliferation of microbes. This adds up to the beneficial effect of the application of Beejamruth. These microorganisms produced IAA and GA which resulted in the improvement of seed germination and seedling length and seed vigour in chilli (Nemagoudar *et al*., 2012).

III. CONCLUSION:

In conclusion, the utilization of organic inputs, exemplified by vermiwash and Panchagavya, represents a crucial paradigm shift towards sustainable and eco-friendly agricultural practices. These organic alternatives not only enhance soil biological properties and stimulate microbial activity but also significantly contribute to the growth, yield, and quality improvement of crops. The positive impact extends across various aspects of agriculture. Vermiwash, with its rich nutrient content and beneficial microorganisms, emerges as a potent liquid organic manure, fostering plant growth and fortifying crops against infestations. Meanwhile, the holistic composition of Panchagavya, combining cow-derived elements, demonstrates diverse properties that advance crop maturity, induce vibrant growth, and fortify plants against diseases. The integration of organic inputs into farming practices is not only environmentally responsible but also economically viable. As organic farming gains momentum, these alternatives play a pivotal role in promoting soil health, reducing dependence on synthetic chemicals, and fostering a more sustainable agricultural ecosystem. By embracing these organic practices, farmers can contribute to mitigating environmental degradation, ensuring food safety, and promoting long-term soil fertility. As the world faces escalating challenges in agriculture, the adoption of organic inputs stands as a beacon for a resilient and sustainable future in farming. This shift aligns with the growing global consciousness towards environmentally conscious and socially responsible agricultural practices, paving the way for a healthier planet and more sustainable food production systems.

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Standardization of *In Situ* Hybridization Techniques and Electron Microscopy for the Diagnosis of Aquatic Organism Diseases

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Abstract— *Numerous etiological agents cause acute, chronic, granulomatous, systemic, or focal diseases in animals. In aquaculture, especially in fish, reptiles, amphibians, and crustaceans, pathogenic microorganisms have led to significant production losses due to the mortality of infected animals or the deteriorating condition of diseased ones, rendering commercialization unfeasible and resulting in economic losses. Furthermore, some of these pathogens affecting aquatic animals are zoonotic, impacting public health. Therefore, with the aim of aiding in the rapid and efficient diagnosis of diseases in aquatic organisms, the standardization of diagnostic techniques began in 2009 at the Interinstitutional Laboratory for Aquaculture Health - LISA (Instituto Biológico/São Paulo/Brazil). These techniques include the in situ hybridization (ISH) under light or photonic microscopy and negative staining (rapid preparation) immunoelectron microscopy (IEM), immunocytochemistry with colloidal gold particles (IMCG), and embedding of fragments in resin for transmission electron microscopy. Adaptations to the initially developed protocols for mammals were made, such as the removal of melanin from melanomacrophages. Due to the high presence of melanomacrophages with brownish melanin granules in the organs of ectodermic animals (frogs and fish), it was necessary to remove this melanin to facilitate the visualization of the diaminobenzidine (DAB) chromogen without interfering with the in situ hybridization technique, in which specific nucleotide sequences were identified in histological sections. This modification prevented false-positive results. These standardized techniques aided in the accurate and efficient diagnosis of pathologies such as White Spot Disease in shrimp, the occurrence of Mycobacterium spp. and Francisella spp. in fish, and chytridiomycosis in frogs.*

Keywords— In situ hybridization, Electron microscopy, diseases, aquaculture.

I. INTRODUCTION

1.1 *In Situ* Hybridization:

This technique was first described in 1969 (Gal, 1969), but it only garnered the interest of the medical community in the 1980s. The appearance of cells and their architectural arrangement within a morphologically complex tissue represents only a fraction of the information within a histological section. These tissues contain all cellular proteins and express genes that will determine the biological behavior of the cell, and even provide clues about the origin and pathogenesis of diseases and their varying degrees (Murakami, 2001).

From this perspective, Jin & Lloyd (2001) assert that DNA or RNA analysis techniques will become increasingly commonplace, shedding light on unresolved questions. The undeniable benefits arising from the integration of this more refined technology with conventional Pathology are contributing to a remarkable increase in our knowledge of certain diseases.

The primary allure of the *in situ* hybridization reaction lies in its ability to precisely locate a specific gene or its transcripts within paraffin-embedded or frozen tissue. While PCR can detect mRNA or DNA in tissue extracts, it does not allow us to observe the distribution of transcripts or DNA within a specific population of cells or in areas of adult or developing tissue (Young, 1989).

1.2 Nucleic Acid Probes:

In *in situ* hybridization, probes are utilized to locate specific nucleic acid sequences at a subcellular level. Biotin is commonly employed in probes for non-radioactive detection. Biotin can be visualized through numerous methods, utilizing either avidin or streptavidin, both of which exhibit high affinity for this amino acid. Standard biotin detection employs enzymatic conjugates of streptavidin, producing a precipitation product that signals chromogenic enzyme substrates. Following the initial binding of biotinylated probes with streptavidin-peroxidase, peroxidase catalyzes the oxidation of biotinyl-tyramide, and this reaction deposits a significant amount of biotin at the hybridization site. This free biotin captures more streptavidin-peroxidase, amplifying the cycle further until the reaction reaches saturation. This signal is ultimately revealed using the chromogenic indicator diaminobenzidine (DAB), which is oxidized by peroxidase, yielding a dark brown precipitate at the hybridization site (Braissant and Wahli, 1998).

A probe is a known segment of DNA or RNA obtained through molecular cloning or chemical synthesis, which is complementary to a target sequence of interest and contains a label that enables selective visualization. DNA probes function similarly to antibodies used in immunocytochemistry in that they bind to a target and carry a signal. However, DNA hybridization probes offer certain advantages over immunodiagnostics because DNA is much more stable and easily preserved than most proteins. RNA is readily degraded by ribonucleases. The optimal probe size is around 200-500 base pairs for improved tissue penetration (although larger probes can be designed). The optimal hybridization temperature typically ranges from 15 to 25°C below the melting temperature (Warford and Lauder, 1991).

1.3 Application:

This technique can primarily be used to detect RNA or DNA from microorganisms and differentiate productive from non-productive infections. It provides morphological information and allows for the observation of gene expression (mRNA), especially when protein expression is low or when it is rapidly exported from the cell, making it challenging to detect through Immunohistochemistry. Additionally, it also verifies the possibility of post-transcriptional control mechanisms (for example, the hybridization of viral mRNA in the liver has helped in understanding the complexity of hepatitis B infection) (McNicol and Fraquharson, 1997).

1.4 Electron Microscopy Negative Staining Technique:

The negative staining technique involves a quick and easy preparation, providing results within minutes, making it the most productive approach in electron microscopy in terms of the number of samples. Particles from a suspension are adsorbed onto the surface of a specimen support, stabilized, and typically contrasted with drops of heavy metals. With this approach, particles can be visualized down to subnanometer sizes and categorized based on their morphology. The original term "negative staining" was introduced by Brenner and Horne in 1959.

Due to its ease of use and comparatively high yield, negative staining is often employed for quality assurance, such as testing virus cultures. Various types of samples can be easily transferred into a suspension without disrupting the structure of viral particles. Efficiency in terms of preparation speed is a critical factor in transmission electron microscopy diagnostics, making it a frontline method in this field (Curry et al., 2006). Furthermore, the open view of electron microscopy provides direct information about all nanoparticles present in a sample. Virus particles are identified based on morphological parameters such as size, shape, surface structure, and peculiarities. Considering that the morphology of a virus is relatively stable throughout evolution, diagnosis is easily achieved even if nucleic acids have undergone significant mutation, making identification through other methods more challenging. Therefore, diagnostic electron microscopy is valuable for identifying viruses in emerging infectious diseases or suspected bioterrorism cases. In veterinary medicine, diagnostic electron microscopy plays an even more crucial role because other diagnostic tools are often unavailable (Laue, 2010).

To combine structural information with molecular data, negative staining can be paired with immunolabeling. This immunonegative staining can enhance specificity in diagnostic electron microscopy or provide insights into the molecular topology of viruses (Biel and Gelderblom, 1999; Biel and Madeley, 2001).

1.5 Electron Immunomicroscopy:

Electron immunomicroscopy (IEM) is employed when the number of viral particles in a sample is very low, when virions are pleomorphic and difficult to identify due to the absence of typical viral morphology such as defined symmetry, shape, spikes, particle size, or capsomer number and arrangement or when samples are very electron-dense, and the aggregates generated by the technique can aid in identification (Lavazza et al., 2015). It allows for virus identification through specific antigen-antibody reactions, relying on the morphological characteristics. It is also used to serotype morphologically similar (but antigenically distinct) particles (Katz Kohn, 1984; Fields et al., 1996).

Various variations of the method, such as immune agglutination or direct immunoelectron microscopy (DIEM) (Anderson et al., 1973) or immune aggregation electron microscopy (IAEM) (Lavazza et al., 2015), solid-phase immunoelectron microscopy (SPIEM) (Derrick, 1973) have been employed. Hyperimmune sera, monoclonal antibodies, or convalescent sera can be used in performing the technique (Hazelton et al., 2003; Lavazza et al., 2015). SPIEM has been used to detect most viruses causing gastroenteritis, such as bovine rotavirus, swine rotavirus, equine rotavirus, canine parvovirus, and bovine viral diarrhea virus (BVDV).

IAEM has been used to detect porcine rotavirus (PoRV), porcine torovirus (PoToV), and porcine epidemic diarrhea virus (PEDV) in pigs with enteritis using convalescent serum (Lavazza et al., 2015).

1.6 Immunogold Colloidal Particle Labeling in Negative Staining Technique :

In this technique, the antigen-antibody reaction is enhanced by labeling the antigen with colloidal gold particles associated with protein A, using specific antibodies for the type and genus. This method also allows for the detection and identification of virus-induced antigen structures and their location in infected cells, serotyping viral strains (Kjeldsberg, 1986), and determining antigenic variants in isolated strains (Patterson & Oxford, 1986).

This technique has been used to label porcine epidemic gastroenteritis virus (TGEV) particles in feces and small intestine fragments of infected pigs (Martins et al., 2013), type A rotavirus and coronavirus in fecal samples from diarrheic calves and winter dysentery in cattle (Kooijman et al., 2016), the simultaneous presence of coronavirus and rotavirus in the feces of calves with diarrhea (Catroxo et al., 2007), and BVDV in the feces of cattle with diarrhea (Catroxo et al., 2007).

1.7 Resin Fragment Inclusion Technique:

The resin embedding technique, followed by ultrathin sectioning, is especially important for revealing fine details of the ultrastructure of all types of cells and tissues (Martins et al., 2013). In an infectious process, it allows the observation of infection pathogenesis and the identification of the agent (Fields et al., 1996). Ultrathin sections have the advantage of allowing the observation of virus-cell interaction, revealing the site of viral replication and maturation within host cells, which is pertinent information for the identification of unknown viruses (Fong, 1989). The overall ultrastructural details not only determine infection but also the course of disease in populations (Catroxo & Martins, 2015). The resin embedding technique has allowed the study of various ultrastructural aspects of the intracellular behavior of TGEV in intestinal fragments of infected pigs (Martins et al., 2013) and parvovirus in intestinal fragments of neonatal dogs with diarrhea (Catroxo & Martins, 2015). This technique also enables the study of the effectiveness of vaccines based on non-infectious virus-like particles (RVLPs) produced *in situ* (Meier et al., 2017).

In this work, we aimed to review the standardizations and protocols of *in situ* hybridization techniques in optical or photonic microscopy and immunoelectron microscopy (IEM), immunocytochemistry with immunogold colloid particle labeling (IMCG), and resin fragment embedding in transmission electron microscopy to enhance the efficiency of diagnosing diseases in aquatic organisms.

II. MATERIAL AND METHODS

The material and methods follow a chronological order as the techniques were developed and adapted. The first disease to be studied was White Spot Syndrome Virus in 2009, with the diagnostic techniques of *In Situ* Hybridization and Electron Microscopy being described sequentially.

White Spot Syndrome Virus (WSSV) Hipólito et al. (2012) published an article on the use of ISH and EM in the diagnosis of White Spot Syndrome Virus in shrimp.

Shrimp farming stands out as one of the most successful and economically attractive agro-industrial segments. This rapid advancement of marine shrimp farming is associated with, among other factors, the introduction of the exotic species known as Pacific white shrimp (*Litopenaeus vannamei*).

However, risk analyses of cultivated shrimp highlight that diseases caused by viruses are a highly limiting factor in the industry. Monitoring the health status of cultivated shrimp is important not only from a bioecological perspective but also from an economic-financial standpoint.

White Spot Disease, or White Spot Syndrome (WSS), is caused by the virus of the same name (WSSV), belonging to the Whispovirus genus. This virus has a wide range of hosts, has been detected in all growth phases, and can be transmitted horizontally or vertically. At the end of 2004, the first cases of this disease in Brazil were reported in the southern region, causing alarm in the shrimp farming sector due to its severity, leading to 100% mortality on some farms. Due to limited knowledge of this disease, its occurrence disrupted the entire production sector (aquaculture and fisheries), leading to restrictions on the movement and trade of any aquatic species.

For the detection of WSSV, techniques such as *in situ* hybridization and Nested-PCR are used. The main objective of this project was to detect the presence of White Spot Virus in shrimp destined for the State of São Paulo using methods recommended by the World Organization for Animal Health (WOAH). The project aimed to implement protocols for histopathology, *in situ* hybridization, Nested-PCR, and Transmission Electron Microscopy and train technicians from the Department of Agriculture in these techniques. To achieve this, samples of WSSV from clinically affected shrimp (*L. vannamei*) from farms in the State of Santa Catarina were collected. The target organs for diagnosis were the gills, digestive tract, pleopods, and hemolymph. Through training and the implementation of these techniques in Department of Agriculture laboratories, the goal was to fill a gap in the detection of diseases in aquatic organisms, especially viral diseases that are subject to mandatory reporting, which is currently observed in the State of São Paulo.

2.1 *In Situ* Hybridization Technique:

For in situ hybridization, a commercial kit for *In Situ* Hybridization (ISH) was used, along with biotinylated probes in the diagnosis of WSSV. The biotinylated probe was constructed in the same sequence as the PCR, with sequences 146F1 (5'-ACT-ACT-AAC-TTC-AGC-CTA-TCTAG-3') and 146R1 (5'-TAA-TGC-GGG-TGT-AAT-GTT-CTT-ACG-A-3'). (FIG 1, 2, and 3).

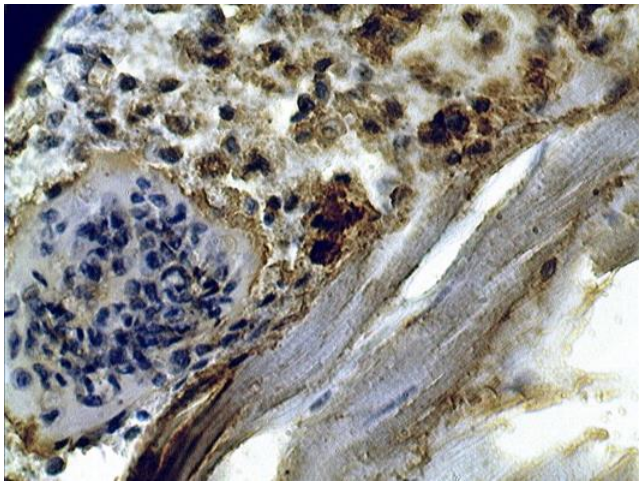


FIGURE 1: Photomicrograph in brown, positive result (white arrow), negative result (black arrow) Heart and hemocytes x 630.

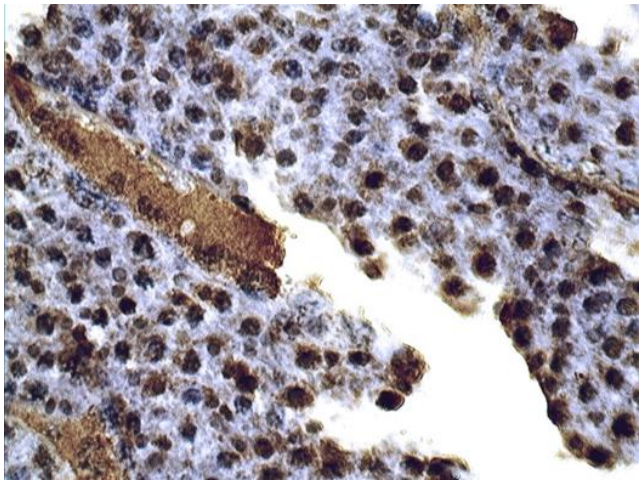


FIGURE 2: Photomicrograph in brown, positive result (white arrow), negative result (black arrow) Hepatopancreas x 630.

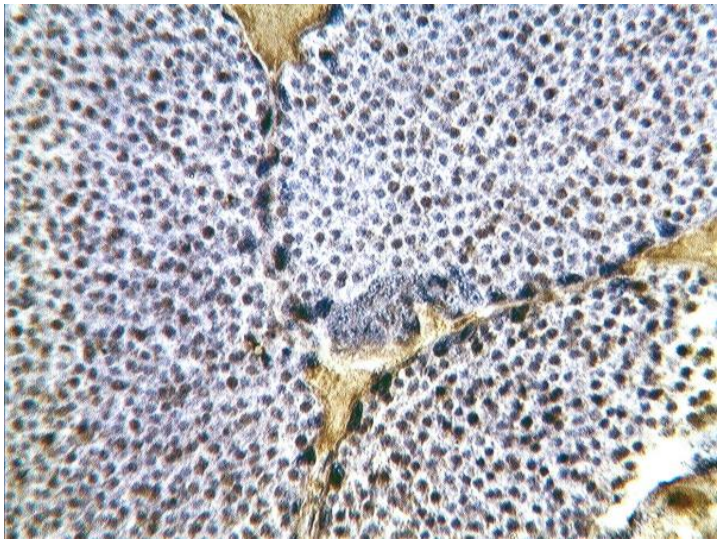


FIGURE 3: Photomicrograph in brown, positive result (white arrow), negative result (black arrow) Testicle x 400.

2.2 Transmission Electron Microscopy:

Another one of these additional tests involved the utilization of transmission electron microscopy. In this instance, the employed technique encompassed transmission electron microscopy in conjunction with immunoelectron microscopy (IEM) and immunocytochemistry, such as immunolabeling with colloidal gold particles (IMCG). The samples, fragments of hepatopancreas and pleopods, originated from marine shrimp, L. vannamei, cultivated intensively within Brazilian shrimp farms. In IEM, the fragments were processed through grids brought into contact with specific monoclonal antibodies. Following washes with PBS drops, the grids were incubated in viral suspension drops. Subsequently, the grids were contrasted using 2% ammonium molybdate at pH 5.0. In IMCG, for negative staining, screens were exposed to viral suspension and specific monoclonal antibodies. After successive PBS drop washes, the screens were incubated with protein A in conjunction with 10 nm colloidal gold particles. The grids were then contrasted with 2% ammonium molybdate at pH 5.0. We employed a Philips EM 208 transmission electron microscope. The results revealed a positive reaction for both techniques. In transmission electron microscopy for IEM, the presence of virus-antibody aggregates was observed. In IMCG, the antigen-antibody reaction was enhanced by the colloidal gold particles. Viral particles displayed oval to bacilliform or ellipsoid shapes, with measurements ranging from 230-290 nm in length and 80-160 nm in diameter, exhibiting projections within the nucleus. It is noteworthy that this is the first study employing these techniques in samples of marine shrimp, *L. vannamei*, in Brazil and it has demonstrated its viability as a complementary tool for diagnosing the presence of the virus (Figures 4, 5, 6, 7).

2.3 Negative Staining, Immunoelectron Microscopy, and Immunolabeling with Colloidal Gold Particle Techniques:

For negative staining, fragments of hepatopancreas and carapace were suspended in 0.1M phosphate buffer at pH 7.0. These suspensions were placed on metal grids, previously coated with collodion film and stabilized with carbon. Subsequently, the screens were drained with filter paper and negatively contrasted with 2% ammonium molybdate at pH 5.0.

In the immunoelectron microscopy (IEM) technique, copper grids, prepared in the same manner, were incubated for 15 minutes in 40 µL of viral suspension (antigen), sensitized with primary polyclonal anti-WSSV antibody against VP 664 protein (Abcam®), diluted at 1:200, and washed with 40 drops of PBS buffer. They were then incubated for an additional 10 minutes with WSSV viral suspension, washed successively with distilled water, and negatively contrasted with ammonium molybdate under the same conditions (Hayat and Miller 1990).

For the immunolabeling with colloidal gold particle technique (IMOC), copper grids, also previously prepared, were incubated for 30 minutes in 40 µL drops of viral suspension, sensitized with the same antibody diluted at 1:80, and washed with PBS buffer. Subsequently, they were incubated for 30 minutes with secondary antibody (protein A conjugated with 10nm diameter

colloidal gold particles - Electron Microscopy Sciences®) diluted at 1:20 in 0.5% PBS and also negatively stained using ammonium molybdate (Knutton 1995).

The samples were examined via transmission electron microscopy using a Philips EM 208 microscope. As a control for the technique, every 10 incubated samples, one was treated as described previously, replacing the antibody with distilled water.

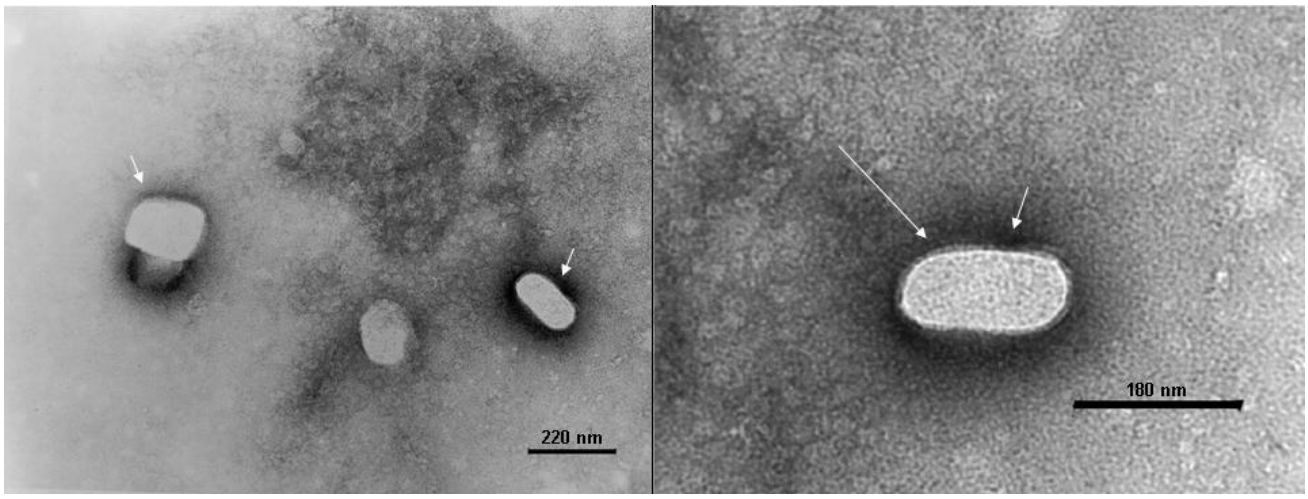


FIGURE 4: Electrophotomicrograph of WSSV viral suspension from L. vannamei hepatopancreas. Particles with oval and bacilliform shapes (large arrow) and an outer envelope (small arrow).

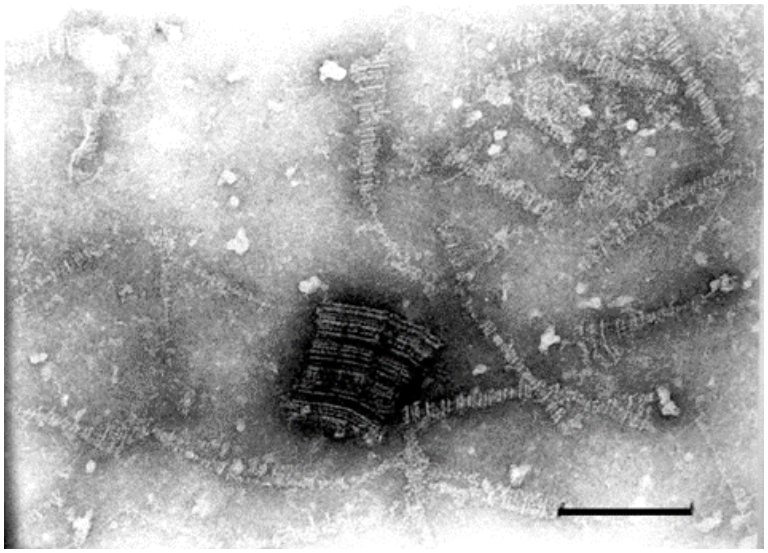


FIGURE 5: Electrophotomicrograph of WSSV viral suspension from L. vannamei carapace, showing WSSV nucleocapsids with streaks. Negative staining technique.

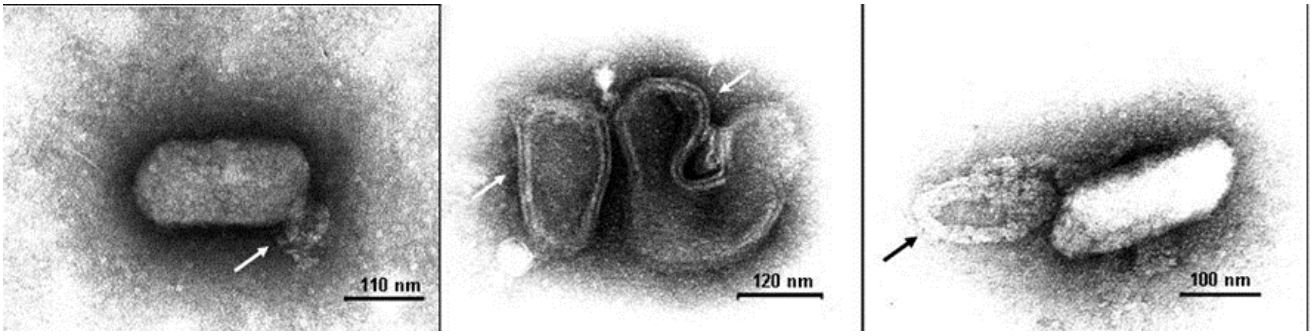


FIGURE 6: Negative staining of WSSV viral suspension showing ring -like particles attached to the virion (arrows).

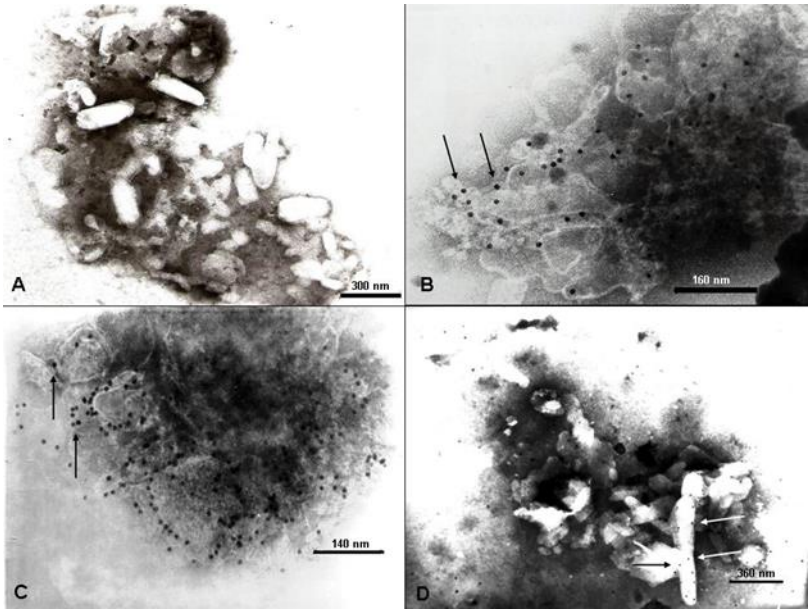


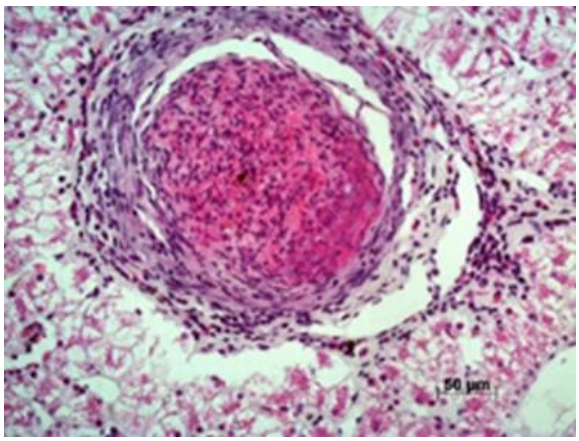
FIGURE 7: Immunoelectron microscopy and immunolabeling with colloidal gold particles. A - WSSV particles aggregated to the immune complex. B, C, D - Strong antigen-antibody interaction evidenced by gold particles on the virion (Arrows).

2.4 Chytridiomycosis (*Batrachochytrium dendrobatidis*):

Martins et al. (2020) described the melanomacrophage bleaching technique, which was proposed due to the significant presence of melanomacrophages in the organs of ectodermal animals such as amphibians and fish. These animals exhibit brownish melanin granules in various tissues, and to facilitate the observation of organ fragments under direct light microscopy during the use of biotinylated probes, it was decided to remove this pigment.

This bleaching facilitated the visualization of the DAB (diaminobenzidine) chromogen without interfering with the in situ hybridization technique, in which specific nucleotide sequences were identified in histological sections, and it prevented false-positive results (Figures 8 and 9). The bleaching process is gradual oxidation by hydrogen peroxide (KORYTWSKI & Sarna, 1990).

Fragments of both healthy and diseased animals were fixed in 10% formalin, dehydrated in increasing alcohol grades, cleared in xylene, and embedded in paraffin. Histological sections of 4.5 microns in thickness were affixed to silanized slides. These slides used for bleaching were immersed in 10% hydrogen peroxide (H2O2) in 0.2 mol/L Tris-HCl buffer at pH 7.4 for 24 hours at room temperature. During this process, the material was kept in the dark. Following this procedure, the normal staining protocol for IHC and ISH was followed.



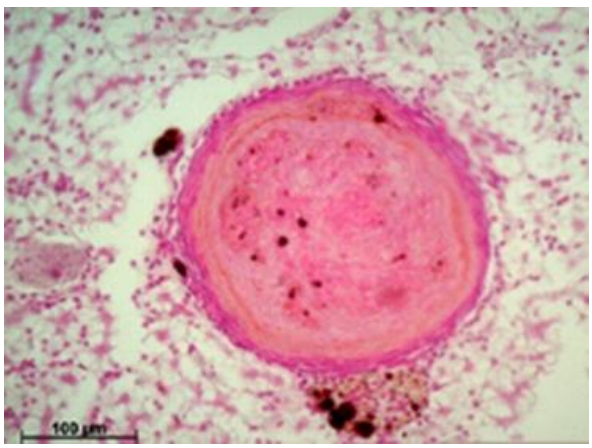


FIGURE 8 AND 9: Photomicrographs of granulomas in tilapia organs with bleaching treatment (left) and without treatment, thus containing melanin in the granuloma and melanomacrophages (right).

2.5 *In Situ* Hybridization Technique:

Schloegel et al. (2010) detected the non-hyphal fungus *Batrachochytrium dendrobatidis* in captive frogs in Brazil, affecting amphibians (Urodela and Anura) at all stages of their life, developing exclusively in the outer keratinized layer of the epidermis. Chytridiomycosis is an emerging disease and considered the primary cause of the global decline of these animals.

Subsequently, in line with this study, Hipolito et al. (2013) employed *in situ* hybridization and resin embedding techniques in electron microscopy as diagnostic tools to analyze keratinized tissues of larval forms of *L. catesbeianus* suspected of chytridiomycosis. The material utilized comprised parts of the tadpole mouth with partial discoloration of typically dark-colored denticle structures. *In Situ* Hybridization (ISH) is a molecular biology technique that detects the presence of nucleic acids in lesions using specific probes, which are complementary nucleotide sequences marked with biotin, binding to the fungus DNA in the infected tissue. The presence of bound biotin is amplified by the streptavidin-peroxidase enzyme complex and the chromogenic indicator DAB (diaminobenzidine). The "Gen-Point® DAKO Amplification System" kit and DAKO® S2450 hybridizer were employed. Hybridization was performed on slides containing sections of tadpole mouth tissues from bullfrog tadpoles. The biotinylated probe used consisted of Primer 1 (Forward Primer): ITS1-3 Bd: 29 bases [Biotin-5'-CCT-TGA-TAT-AAT-ACA-GTG-TGC-CAT-ATG-TC-3'] and Primer 2 (Reverse Primer): 5.8S Bd: 22 bases [Biotin-5'-AGC-CAA-GAG-ATC-CGT-TGT-CAA-A-3']. A positive result for hybridization is indicated by a dark brown precipitate, resembling structures akin to zoosporangia in this coloration (Figure 10).

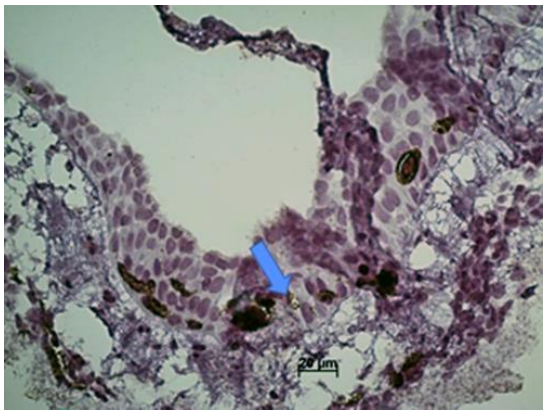


FIGURE 10: Photomicrograph of histological section of the mouth of a bullfrog tadpole, Lithobates catesbeianus, using the *In Situ* Hybridization (ISH) technique, demonstrating a vacuolar structure resembling sporangia, containing spores, in brown coloration (arrow).

2.6 Transmission Electron Microscopy:

2.6.1 Resin Embedding Technique Followed by Positive Contrast of Ultra-Thin Sections:

Samples of tadpole mouth fragments were processed following standard resin embedding procedures based on Gonzalez-Santander's methods (1969). When employing this technique, freshly collected samples are fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer at pH 7.0, post-fixed in 2% osmium tetroxide in 0.2M phosphate buffer at pH 7.0, stained with 0.5% uranyl acetate, dehydrated in an increasing acetone series (50 to 100%), and embedded in Spurr resin. After ultrasectioning of the blocks, the ultrathin sections obtained are positively stained through a sequential treatment with uranyl acetate (Watson, 1958) and lead citrate (Reinolds, 1963) before being observed under the Philips EM 208 transmission electron microscope (Figure 11).

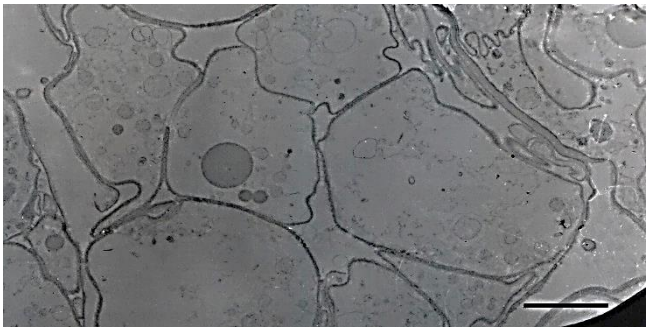


FIGURE 11: Transmission electron micrograph of ultrathin section of the mouth of a tadpole infected with the fungus *Lithobates catesbeianus*.

Martins et al. conducted an analysis in 2021 on various diagnostic techniques for *Mycobacterium* spp. and *Francisella* spp. in captive or free-ranging fish. This study focused on granulomatous diseases caused by these bacteria, which have significant implications for Animal Health, Public Health, and the Agribusiness sector. In this context, the occurrence of circulating samples of these bacteria was determined using fragments of organs obtained from passive and active collections in the State of São Paulo, employing the following techniques: *In Situ* Hybridization (ISH), Immunohistochemistry (IHC), Optical Microscopy (OM) (H&E and Ziehl-Neelsen or Fite-Faraco staining), and Transmission Electron Microscopy (TEM) with negative staining.

Mycobacterium spp. is a pathogen capable of causing severe and costly diseases in various invertebrates and vertebrates, including humans (tuberculosis, leprosy, Buruli ulcer), livestock (bovine tuberculosis), and ectothermic animals (reptiles, amphibians, and fish). In recent years, there has been increased interest in aquaculture due to a decline in fishing activities, which has favored the development of diseases such as mycobacterioses (Whipps, Watral, Kent, 2003).

More recently, the bacterium belonging to the *Francisella* spp. genus, highly virulent for numerous animal species, has been found in marine and freshwater fish, amphibians, reptiles, and even mollusks. It has been associated with massive mortalities of tilapia in commercial farms in Taiwan, Hawaii, and Costa Rica (Mauel et al., 2007; Soto et al., 2009), leading to declines in production ranging from 5% to 80%, with an average of 50%. In 2005, this bacterial disease, initially mistaken for a disease caused by bacteria of the Piscirickettsia genus (commonly responsible for septicemia in salmonids), decimated the tilapia stocks of one of the major producers and exporters of fresh fillets to the United States, Aqua Corporation, in Costa Rica.

2.7 *In Situ* Hybridization Technique For *in situ* hybridization, we utilized the "Gen-Point® DAKO Amplification System" kit and DAKO® S2450 hybridizer, along with the following probes:

2.7.1 *Francisella spp.* (Hsieh et al., 2007). Individual primer sequences:

- FLB16S180f: 5'-GCG-GATTAA-AGG-TGG-CCT-TTG-C-3' (forward primer)
- FLB16S465r: 5'-CCT-GCA-AGC-TAT-TAA-CTC-ACAGG-3' (reverse primer)

2.7.2 *Mycobacterium spp.* (Talaat et al., 1997). Primers specifically amplified:

- 924-bp fragments, T-39 (5′-GCGAACGGGTGAGTAACACG-3′) and T-13 (5′-TGCACACAGGCCACAAGGGA-3′). Or Partially degenerate primers (Zerihun et al., 2011):
- Myco-rtf (5¢-GGTGGACRTCATCYTGAACA-3¢) and Myco-rtr (5¢-TCCARRATCTGGCCGATGT-3¢).
- Amplifying a 63bp fragment and containing the TaqMan probe: Myco-rtpr (5¢-CACGGTGTGTGCCGCGTCGTATG-3¢) (Figures 13, 14, 15).

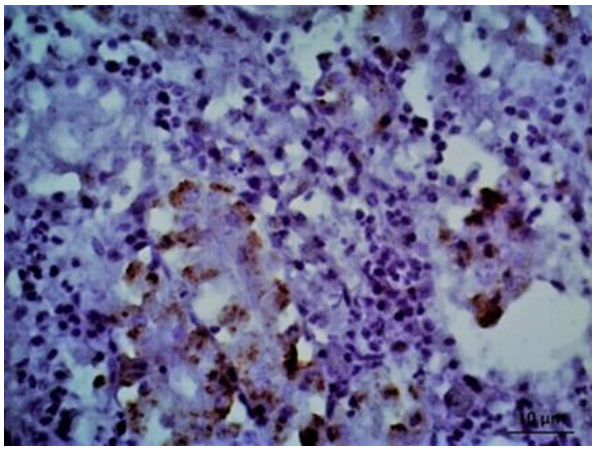


FIGURE 13: Photomicrograph of kidney and liver positive for Mycobacterium spp. using the *In Situ* Hybridization (ISH) technique at 400x magnification

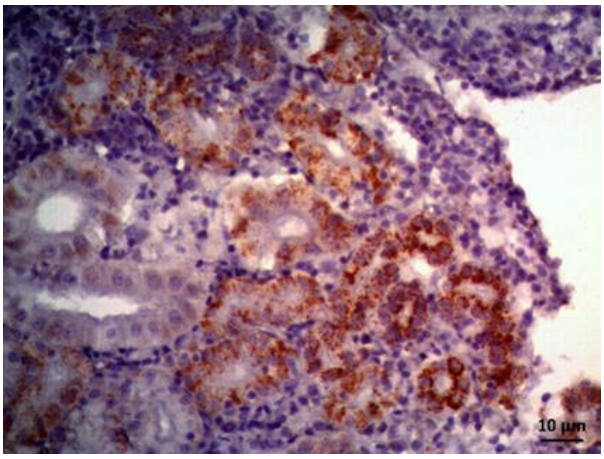


FIGURE 14: Photomicrograph of a kidney positive for Francisella spp. using the *In Situ* hybridization technique at 400x magnification.

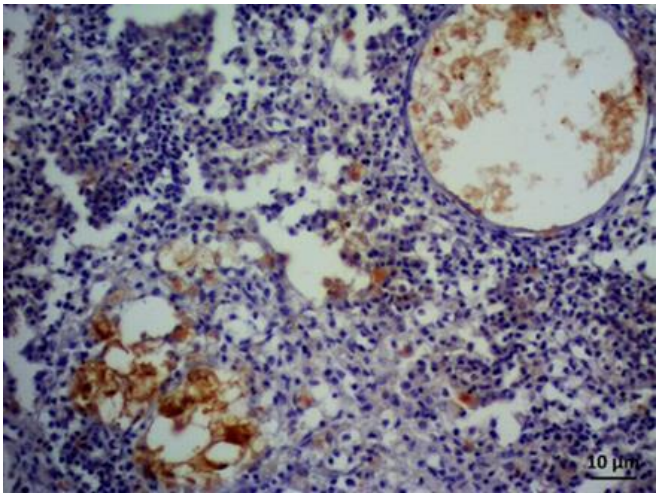


FIGURE 15: Photomicrograph of spleen positive for Francisella spp. using the *In Situ* Hybridization (ISH) technique at 400x magnification.

2.8 Negative Staining Technique

Fragments of fish organs were suspended in 0.1M phosphate buffer at pH 7.0. Drops of the obtained suspensions were placed in contact with copper mesh grids, previously coated with collodion film and stabilized with carbon. After draining with filter paper, the grids were negatively stained with 2% ammonium molybdate at pH 5.0 and examined under a Philips EM 208 transmission electron microscope (Figures 16, 17).

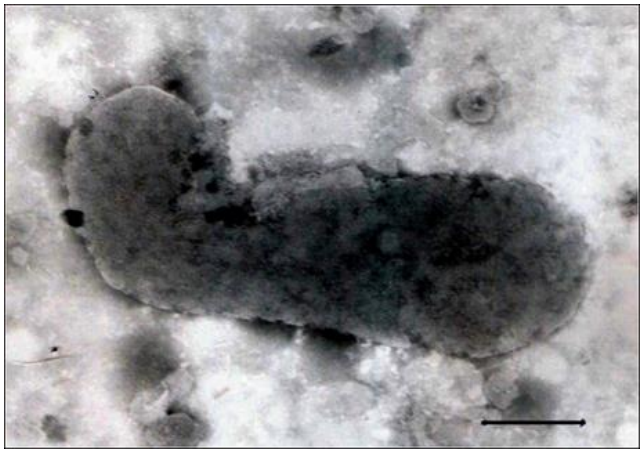


FIGURE 16: Electron micrograph of *Mycobacterium* spp., contrasted with ammonium molybdate using the negative staining technique. Scale bar: 100 nm.

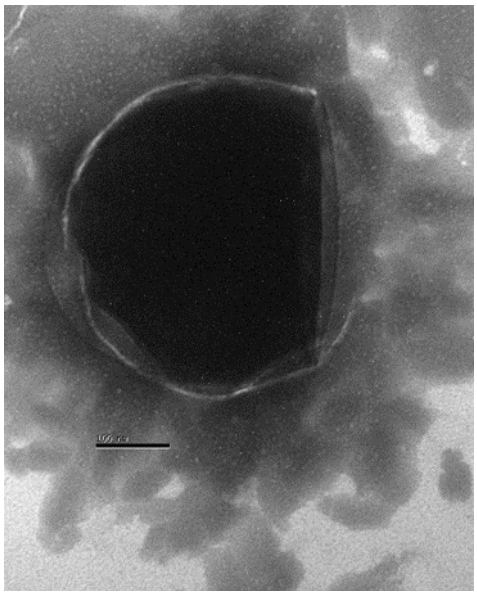


FIGURE 17: Electron micrograph of *Francisella* spp., contrasted with ammonium molybdate using the negative staining technique. Scale bar: 100 nm.

III. CONCLUSION

Considering that several etiological agents cause acute, chronic, granulomatous, systemic or focal diseases in aquaculture animals (fish, reptiles, amphibians and crustaceans), the standardization of histopathology and transmission electron microscopy techniques was essential to assist in the rapid and efficient diagnosis of such microorganisms, avoiding the mortality of infected animals, the unfeasibility of commercialization and economic losses to farms.

ACKNOWLEDGMENTS

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Albanian Agricultural Producers: Challenges for Increasing Production Capacity and the Effects of Marketing in the Development of the Local Market

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Abstract— *Referring to agricultural potentials and resources in Albania, the development of production capacities is below the level of the demands and needs of consumers with local products. Substitution of imports of agricultural products with products of local companies and farms is a very important factor for economic growth, increasing the competitiveness of agricultural economies and opening new jobs. To achieve this, companies and agricultural farms in Albania must apply clearer marketing schemes to identify their potentials and opportunities, through efficient management, based on development plans for each product or agricultural sector. The purpose of this study is the analysis of the competitiveness and performance growth in the market, for the agricultural products taken in the study, as well as the impact of the marketing application, based mainly on the market study. The methodology used in this research include multivariable method along with factor analysis, regression techniques and correlations. The results show that agricultural production companies and farms have started to apply marketing in different forms, both in social and traditional media, creating a better competitive position in the market. This had a positive effect on increasing the presence of local products and the substitution of imported products, increasing the credibility of consumers for products produced in Albania, as well as creating a market with a variety of local products*.

Keywords— Marketing, Agricultural Products, Competitive Advantages, Substitution, Import.

I. INTRODUCTION

The economy of Albania has sufficient human resources for market research and the development of new agricultural products, which are in demand in the European Union (EU) markets. Due to the fact that they are of a high ecological level, they do not harm the environment and have more favorable prices than the products of developed EU countries (Bajramović, et al., 2016). The climate that prevails in Albania and the type of agricultural lands favor many new agricultural products and crops that have higher profits than other products that have been cultivated before. The development and growth of existing products is one of the main components in a small or medium-sized company. A progressive company must deal in advance with planning and expanding the variety of products and cultures where it operates in order to be competitive in the market. In order to achieve this, the companies today must apply the most effective marketing, in which all factors in the marketing process will be taken into account and only then will the marketing strategies be determined which would facilitate the movement of products from the manufacturer to the end consumer. Marketing in agriculture must be customer-oriented, mainly in identifying consumer needs and meeting these needs as best as possible, but bearing in mind that to always offer the agricultural producer (farmer), the transporter, the trader and the processor a certain rate of return.

Referring to the considerable agricultural resources until the last ten years, Albania has not moved in the right steps and has not met the demands and needs of consumers with its own products, being forced to meet these demands with imported products. The data of the previous years show positive changes, which have resulted in a decrease in the import of agricultural products and a small increase in export (Hoxha, 2022). This also means the replacement of agricultural imports with domestic production. These positive trends in the agribusiness economy come as a result of the awareness of farmers producing better

quality products that are more in demand in the market (Republic of Albania, Council of Ministers, 2022). This awareness of farmers also comes thanks to the support of various international organizations that support agriculture and undoubtedly thanks to the support of the Government of the Republic of Albania which, through the Ministry of Agriculture, has started supporting farmers through various grants and subsidies in order to increase of the productive potential of agricultural products, as well as increasing their competitive position in the market (Ministry of Agriculture and Rural Development, 2019).

The replacement of imports of agricultural products with local production is a very important factor for economic growth, increasing the competitiveness of agricultural economies in Albania and opening new jobs (Republic of Albania, Council of Ministers, 2016). To achieve this, companies and agricultural farms in Albania should, through the application of consumer-oriented marketing, identify the needs in the market and meet them as best as possible. This will only be achieved through a preliminary market study to analyze all external factors and competition within the industry, to be as competitive as possible in the market and to best meet consumer needs.

TABLE 1
FLOW OF GOODS IN FOREIGN TRADE 2018-2023(Q3)-THE PARTICIPATION OF AGRI-FOOD PRODUCTS SECTOR.

Million ALL

Year	Export/ FOB	Import/CIF	Participation in export of agri-food products	Participation in import of agri-food products	The Percentage of Coverage
1	2	3	6	7	
2019	298,792	649,118	37,818	127,695	46.0
2020	271,955	605,262	40,861	123,558	44.9
2021	368,769	800,718	46,646	144,937	46.1
2022	486,784	950,381	54,603	166,246	51.2
Q3-2023	371,123	723,356			49.0

Source of Information: INSTAT, 2023

This negative trade balance of agricultural products requires changing the operating environment in order to improve the competitive position in the international market, through effective policies, to increase internal capacities that would contribute to the replacement of imports with local production as well as increasing exports (Xhepa and Liperi, 2021). In this effort, the replacement of agricultural imports with domestic products remains one of the main challenges for policy-makers and other actors of economic development, to design and implement sound policies that reduce the trade deficit.

The uncertain market of agricultural products and the import of these products from abroad is the biggest concern that the producers of agricultural products fear. Small agricultural producers are faced with high production costs, and as a result, they command high prices in the market.

These two factors prevent these businesses from competing in the market equally with large agricultural producers and imported products. For this reason, the small producer is forced to cooperate with large producers in order to reduce the cost of production and secure the market, because agricultural products have a short shelf life. As a result of this uncertainty from unstable prices of small producers, influenced by the competition of large producers or imports, caused this to be reflected in the following years. Multi-year investment in product marketing leads to product differentiation and the establishment of its recognition and reputation among consumers.

II. METHODOLOGY

The purpose of this paper is the analysis of the existing state of competitiveness of agricultural products and the possibility of substitution of imported products, as well as the impact of the application of marketing. Market study and awareness of agricultural producers to increase their performance in the market as well as the proposal of a marketing model for the Albanian

agricultural producer, to reduce the cost, promote the production of new agricultural products and increase competitiveness in the market.

The main objectives in fulfilling the purpose of the work are:

- Analysis of marketing implementation and preparation of research methodology, according to the requirements arising from the questionnaires and basic hypotheses.
- Preparation and distribution of questionnaires for agricultural producers, as well as collection, processing and presentation of data for discussion.
- Drawing conclusions and giving recommendations to agricultural producers and proposing a marketing model to be followed by Albanian agricultural producers.

In order to carry out this work and to achieve the aim and objectives of the research, a basic hypothesis has been put forward: ***The application of marketing to domestic products enables the substitution of imported products***. During the handling of the papers, some research questions will be answered as follows:

- What is the state of implementation of marketing and how oriented are agricultural businesses in the application of marketing?
- What is the competitive position of local producers in front of agricultural products that are imported from abroad?

The expected contribution will be the proposed model for marketing to agricultural companies in Albania, while the research in this article can be considered exploratory or confirmatory. The results achieved help in decision-making, suggesting a specific orientation of actions.

The quantitative method of data collection includes the analysis of primary data collected through questionnaires. The sample size is not less than 30 and not more than 50, being considered adequate to carry out this work. The applied methodology of this paper is in function of its purpose and is based on these research elements:

Sources of data is a combination of secondary and primary data. The main research is supported by the data collected through questionnaires distributed to the interviewed businesses, while the secondary data is the result of the information provided through the literature.

During the work, the quantitative method of data collection was used, mainly the questionnaire. This method has helped to compare the different variables used to test the hypotheses presented.

Data processing is based on the use of various statistical methods such as: SPSS Excel, descriptive analysis, cross tables, methods of analysis, representation and correlation between phenomena.

30 different businesses dealing with the production or processing of agricultural products were surveyed and that: 5 producers/processors of milk, 5 producers/processors of non-alcoholic beverages from trees, 5 producers of vegetables, 5 producers of alcoholic beverages, 5 meat processors, 2 processors of flour products and 2 processors of oil products.

Research methods in this study include: multivariable method along with factor analysis, regression techniques, correlation and forecasting techniques.

III. STUDY RESULTS ANALYSIS

The results of the research come mainly from the answers to the general questions, to continue with the part of the questions related to the marketing activities and ending with the part of the validation of the hypotheses according to the Pearson Correlation model. To analyze this paper, in the study part, 30 managers of 30 companies from different agricultural sectors were interviewed and only in those companies/farms. that have more than 10 employees.

30 different businesses dealing with the production or processing of agricultural products were surveyed and that: 5 producers/processors of milk, 5 producers/processors of non-alcoholic beverages from trees, 5 producers from vegetables, 5 producers of alcoholic beverages, 5 meat processors, 3 processors of flour products and 2 processors of oil products.

TABLE 2
WHAT ARE THE FORMS OF MARKETING THAT YOUR COMPANY MOSTLY IMPLEMENTS?

		Frequency	Percent	Valid Percent	Total Percent
Valid	• Internet Marketing	15	25	25	25
	• Television and radio marketing	4	6.7	6.7	31.7
	• Catalog marketing	2	3.3	3.3	35
	• Internet Marketing + Television and Radio Marketing	6	10	10	45
	• Internet Marketing + Television and Radio Marketing + Catalog Marketing	19	31.7	31.7	76.7
	• Internet and catalog marketing	13	21.7	21.7	98.3
	• Internet Marketing + Fairs	1	1.7	1.7	100
	• Total	60	100	100	

The most frequent forms of marketing used by the interviewed businesses are:

- The combination of Internet Marketing + Television and Radio Marketing + Catalog Marketing with 31.7%,
- The combination of Internet Marketing with 25%.
- The combination of Internet Marketing and catalogs with 21.7%.

Competition in the market is considered strong by all sectors, because over 80% of company managers from each sector have declared that they face strong competition.

TABLE 3
DO YOU DO BUSINESS PROMOTION TO PROVIDE INFORMATION ABOUT THE SCOPE OF THE COMPANY?

		Frequency	Percent	Valid Percent	Total Percent
Valid	Yes	38	63.3	63.3	63.3
	Sometimes	22	36.7	36.7	100
	Total	60	100	100	

A significant number of businesses, even 63.3% of them do promotion to provide information about the business, while 36.7% of them only sometimes provide information about the scope of the enterprise.

TABLE 4
IF YES, FOR DESIGNING THE MESSAGE AND ADVERTISING OF YOUR PRODUCTS, YOU ENGAGE

		Frequency	Percent	Valid Percent	Total Percent
Valid	The marketing department within the enterprise	12	20	20	20
	Enterprise staff	19	31.7	31.7	51.7
	Firms specializing in advertising	12	20	20	71.7
	Enterprise staff + Specialized firms	2	3.3	3.3	75
	Marketing department + Specialized firms	15	25	25	100
	Total:	60	100	100	

In the design of the advertising message, 31.7% of the enterprises use the marketing staff, with 20%, respectively, using the marketing department within the enterprise and specialized firms for advertising, 25% of them use the combination: marketing department + specialized firms, while 3.3% of them use the combination: Company staff + specialized advertising firms.

IV. RESULTS FROM CROSSTABS

In this part, the results from processing with SPSS are presented, mainly through the model of cross tables. This model is used to observe the correlation between the independent variables x and the dependent variables y, and also the cross tables where the results confirming the correlation or rejecting it are presented through the "Pearson Correlation" with the two-sided significance test (sig 2 – tailed).

TABLE 5
CORRELATION - PRICING - COMPETITIVE PRESSURE

CORRELATES

		Do you price your products on a geographic basis (rural and urban areas)	**Do you consider that your competition is increasing the pressure to lower product prices?**
Do you price your products on a geographic basis (rural and urban areas)	Pearson Correlation	1	0.131
	Dig(2-Tailed)		0.317
	N	60	60
Do you consider that your competition is increasing the pressure to lower product prices?	Pearson Correlation	0.131	1
	Dig (2-Tailed)	0.317	
	N	60	60

In table 5, the variable: Do you set product prices on a geographic basis (rural and urban regions) is compared with the other variable: Do you consider that your competition is increasing the pressure to reduce product prices?. From the calculations, it can be observed that a Pearson Correlation coefficient revealed that there is no positive relationship between the two variables, r = .131, n = 60, p = 0.317. In general, there is no positive relationship between the two variables, because even during the interviews it was observed that only a small number of enterprises make price "differentiation" according to geographical areas and this indirectly does not even affect any pressure from the competition to do such a thing nor to reduce the cost.

Verification of hypotheses

In the tables below, the validation of the hypotheses is presented.

H 1: The genuine competition of enterprises in assortments of agricultural products has a negative correlation with the level of consumer involvement in the process of replacing these products.

TABLE 6
HYPOTHESIS 1

CORRELATES

		Does your product range in the market face genuine competition?	**How easy is it for the customer to find other products as substitutes for your products in the market?**
Does your product range in the market face genuine competition?	Pearson Correlation	1	0.19
	Dig(2-Tailed)		0.147
	N	60	60
How easy is it for the customer to find other products as substitutes for your products in the market?	Pearson Correlation	0.19	1
	Dig(2-Tailed)	0.147	
	N	60	60

Table 6 presents the results of the third hypothesis which was tested as correct. The first hypothesis is based on the assumption that the assortment of products in the market when faced with real competition is not strongly related to the level of consumer involvement in the process of replacing these products.

A Pearson correlation coefficient sheds light on the negative correlation between the two variables, r = 0.190, n = 60, p = 0.147.

In this regard, we can conclude that these two variables do not correlate with each other. This proves anyway that the competition in this sector is real and when there is real competition the consumer is not interested in replacing the products of the company he likes with the same products of other companies because of the price or because of any variable other.

H 2: The degree of substitution of agricultural products due to their country of origin has a negative correlation with the degree of the risk of substitution of these same products due to production with advanced technology.

TABLE 7
HYPOTHESIS 2

CORRELATES

		Do you foresee your customer substituting your products because of the country of origin?	**Is there a risk that the consumer will replace your products with those of competitors due to advanced technology production?**
Do you foresee your customer substituting your products because of the country of origin?	Pearson Correlation	1	0.11
	Dig(2-Tailed)		0.934
	N	60	60
Is there a risk that the consumer will replace your products with those of competitors due to advanced technology production?	Pearson Correlation	0.011	1
	Dig(2-Tailed)	0.934	
	N	60	60

Table 7 presents the results of the second hypothesis which was tested as correct. The second hypothesis was based on the assumption that: The degree of substitution of agricultural products due to their country of origin has a negative correlation with the degree of risk of substitution of these same products due to production with advanced technology.

A Pearson correlation coefficient sheds light on the negative correlation between the two variables, r = 0.011, n = 60, p = 0.934.

In this regard, we can conclude that these two variables do not correlate with each other and that the correlation is negative. This shows that the consumer is not interested in replacing local products with products from abroad because of the advanced technology used by foreign competition and that in percentage 46.7% of managers think that the Kosovar consumer does not change or replace the product because of technology of advanced for producers from outside Albania.

V. CONCLUSIONS AND RECOMMENDATIONS

Based on the literature used, on the purpose and objectives presented, on the methodology used in the paper, as well as on the findings from the study, we come to three types of conclusions: on the theoretical level, on the methodological level and on the practical level.

5.1 Conclusions on the theoretical plane:

- Marketing as a new science developed from the end of the 30's is of exceptional importance for companies that are oriented towards the market, respectively from the end consumer in order to identify his needs and desires and to meet them in time, quantity and in the right place (Kotler, Kartajaya, and Setiawan, 2021).
- The marketing strategy requires several steps or stages before it is implemented in practice, this is also realized through strategic alternatives.

- Creating consumer trust through the quality of certain products or services, today everywhere in the world is a priority of consumer-oriented companies and the same should happen in Albania.
- The policy of physical distribution of products means finding the easiest and fastest way/channel to penetrate to the final consumer, considering the fact that the company that distributes the goods close to the consumer gains a competitive advantage in the market (Dieppe, 2020).
- The Albanian economy after the 2000s, has been an economy oriented mainly on trade and without a focus on production, to replace the high level of imports, especially in agriculture, but in the last 5 years a better progress can be observed in this sector.
- Maintaining consistency in production, especially in agricultural products, is more than necessary to satisfy the consumer and maintain continuity in the market.

5.2 Methodological conclusions:

- To recognize the implementation of marketing activities and strategies in agricultural production companies in Albania, it was necessary to use surveys and analyze them. For this purpose, those companies that employ more than 10 workers were selected as a sample, while the interviews were conducted mainly with people who are owners of companies/farms or at the managerial level.
- The questionnaire is the research instrument used to collect data from companies and is built from various questions related mainly to questions about marketing strategy activities but also other questions about competitiveness and competitive advantage in the market, through which they are achieved conclusions of interest, to have a clear overview of how they are being implemented in practice and how much importance is given to marketing in manufacturing companies.
- The study contains a comparative look between the same and different businesses within a sector.

5.3 Conclusions on the practical level:

- Customer care and trust means repeat purchases from existing customers and increase in the number of new customers, thus leading to increased company profits. This has been proven in those companies that were part of this study, as well as by the proven hypothesis related to these variables.
- The Albanian consumer, according to the answers given by the interviewees, does not prefer to replace local products if the variety of products offered in the market matches their requirements.
- The Albanian consumer also does not prefer to replace local products, due to advanced technology from competitors targeting the local market.
- Setting the right objectives and measuring managerial performance has influenced the increase in sales from agricultural companies in Albania.
- Most of the respondents assess the risk from import substitute products and about 56% of them think that there is a risk for their businesses from these products, while 30% do not have that opinion.

5.4 Recommendations

- Development through acquisitions is an alternative that gives the opportunity to enter new markets/products. In some cases, products and markets change rapidly, and this is presented as the only way to enter these markets, while internal development is considered a very slow path. Companies in the food sector in Albania have so far hesitated to act with such a strategic alternative. Therefore, it is recommended that they take the risk to enter this strategic alternative in order to enrich their markets/products and create a competitive advantage in the market.
- The modification of current products and the entry into production of new products, similar to the products that dominate the domestic market, is a recommendation that is made especially to those companies that have reached the stage of maturity with their current products and for which consumers are looking for innovation.
- Maintain stability in the production of their products, paying special attention to the quality of the products in order to convince the consumer that they are meeting their requirements and expectations.

5.5 Recommendations for producers/processors

- The agricultural production enterprises part of this study has to some extent combined well the elements of mixed marketing, in particular promotion and distribution channels, but they have not performed well in terms of cost reduction and variety in products.
- Another shortcoming that has been observed in these businesses is the use of insufficient knowledge related to marketing strategies.
- Efficiency in production and reduction of costs, using several strategies that affect the increase of competitiveness in the market and the increase of consumer confidence, ultimately lead to the replacement of imports with domestic products.

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A Review on Impacts of Violence Against Women (VAW) on Socio Economic Development Sector

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***Abstract*—** *Violence against women (VAW) is any act of physical, emotional, psychological and sexual abuse which occurs either in home or community against women such as domestic violence, rape, sexual assault, honor killings, female genital mutilation (FGM), forced marriage, forced prostitution, trafficking of women and so on. This study is based on the secondary sources of data to assess the socio economic impacts of violence against of women in national and global scenario. Violence against women (VAW) was found affecting socio economic sector such as agriculture, manufacturing and service sector. Therefore, government should formulate and effectively implement strict laws and procedures to deter crime, safeguard underprivileged groups and punish perpetrators.*

Keywords*— *Socio economic, secondary sources, women, VAW.

I. INTRODUCTION

Violence against women (VAW) is one of the type of gender-based violence that encompasses harm and abuse to women on a physical, sexual, psychological and economic level. Domestic violence against women can take the form of intimate partner violence (IPV), rape, sexual assault, human trafficking, stalking and cyberbullying. According to Krantz and Garcia (2005) VAW is a serious violation of women's human rights. The nature of violence against women differs from one nation to another (Cousineau and Rondeau, 2004). De Judicibus and McCabe (2001) claimed that the traditional gender-role attitudes are associated with greater flourishing environment for violence against women. Gender-based violence is widespread in Nepal and is a public health problem. In the past ten years, women's entrepreneurship has been recognized as a significant untapped source of economic growth (Georgeta, 2012). Violence against women (VAW) is a widespread global issue, with more than one-third of women worldwide having experienced VAW (World Health Organization, 2013). The physical and mental health of women is negatively affected by violence against them for a very long time. It can result in substance misuse, bodily harm, post-traumatic stress disorder, depression, anxiety, and even death. It also has a financial cost in terms of medical expenses, lost wages from the victims' inability to work, and an impact on workplace efficiency. Finally, violence against women inhibits efforts to reduce poverty and advance human rights, as well as efforts to achieve gender equality.

According to a study conducted by Puri (2023) in Nawalparasi district of Nepal had found that along with the enforcement of laws against intimate partner violence (IPV) their findings indicate that special attention should be paid to women during a crisis time, such as the current COVID-19 pandemic, particularly those who experience other household stressors. Gender-based violence is becoming more prevalent in Nepal. Nepal is experiencing a significant social issue with violence against women. Stakeholders must work together to increase awareness, strengthen legal enforcement, and offer required resources to survivors in order to address gender-based violence. A gender-sensitive public health response to violence against women has also begun to be promoted by the government, as has the Safe Cities Campaign.

The root causes of Gender Based violence (GBV) are "gender inequality, abuse of power, and harmful norms." Governments must take action to guarantee that all laws and policies are in line with the recognized international commitments of the state in order to control violence against women. Teaching and training to police, prosecutors and other law enforcement professionals is necessary to combat GBV. Women who face violence must also receive proper support services. Governments must develop initiatives that give women access to economic and educational resources. Consent-based education should be taught in schools so that kids can learn about respect, healthy relationships, and gender-based violence (UNHCR, 2020).

Violence against women is a global public health problem and a human rights issue with devastating consequences for the physical and mental health of women, their families, and society at large. World Health Organization, (2013) defines violence against women as any act of gender-based violence that results in, or is likely to result in, physical, sexual, or mental harm or suffering to women, including threats of such acts, coercion or arbitrary deprivation of liberty whether occurring in public or in private life. Campbell and Soeken (1999) had found that 40%–52% of women residing in USA and Mexico were found experiencing physical violence by an intimate partner. Intimate partner violence refers to the abuse taking place usually between husband and wife. World Health Organization, (2002) had divided violence into three broad categories viz. self-directed violence, interpersonal violence and collective violence. In many parts of the world including Nepal there is still social preference of boys over girls. According to Miller (2001) the social preference of boys over girls leads to sex selective abortions of females by identifying the sex of fetus through ultra sound technique. Women and girls were found victimized in the name of dowry. Dowry is the payment to be made to the groom's family to marry daughter. Dowry has been the one of the major reasons for the disputes in families where the groom's family demanding more than the bride's family leading to dowry related of deaths of women and girls in certain parts of India and other South Asian countries (Gangrade, 1995). According to Rabbani et al. (2008), a number of factors play a role in the occurrence of gender-based violence (GBV) in Pakistan. Among the contributing causes include poverty, a lack of education, a lack of support for and protections for GBV victims, a lack of women's empowerment and a lack of women's representation in government.

Women engage in decision-making processes in both public and private enterprises, according to the results of a study carried out in Malaysia by Ismail (2023), but multicultural education is still in its infancy. When someone is harmed due to their gender identity or expression, it is referred to as gender-based violence. It can take many different forms, such as cyberbullying as well as physical, sexual, and psychological abuse. The United Nations has identified gender-based violence against women as a global health and development issue, and numerous pieces of legislation, public awareness campaigns, and action programs aimed at reducing gender-based violence have been put into place globally (Russo, 2006). It can afflict persons of any gender identity or expression, although it is more common among women and lesbian, gay, bisexual, transgender, or queer (LGBTQ) people. Heise (2002) cites several instances of gender-based violence, including dowry-related murder, marital rape and selective malnourishment of female children, forced prostitution, female genital mutilation, and mistreatment of female children. The government of Nepal has put in place a range of legislative, regulatory, and programmatic actions to protect women and advance gender equality in order to combat gender-based violence.

II. METHODOLOGY

Secondary sources of data serve as the foundation for this study. Various reports and studies that were published by governmental and non-governmental organizations were reviewed and analyzed qualitatively to assess the impacts of violence against women on socio economic development sector.

III. DISCUSSIONS

VAW has a profoundly negative effect on socioeconomic development of any country including Nepal. Women who experience this type of violence may have difficulty getting an education and finding employment, leading to poverty and poor health outcomes. Additionally, this violence can lead to gender-based discrimination in the workplace, as well as psychological trauma and physical injury. This in turn limits the ability of women to fully participate in the socio-economic development of their country, making it difficult for a nation to achieve its development goals.

In addition to a human rights issue violence against women has devastating impacts on the physical and mental health of women, their families, and society at large. Generally, VAW is encountered high during the time of disasters and clashes between or within the country. In this context. Bradely et. al (2023) suggests that VAW should be integrated into all emergency responses when a climatic disaster occurs. The perpetration of Violence against women can be identified in patriarchal privilege, poverty, childhood violence and negligence of substance abuse and mental health concerns (Gibbs et al., 2020). The VAW is generally three type's i.e self-directed, interpersonal and collective (Figure 1). The self-directed violence is caused by suicidal behavior and self-abuse while the interpersonal violence is caused by family or partner or community. The members of family subjected to VAW may be children, young girls or adolescents and women of reproductive age. The collective violence is caused by social, political and economic situations of the country.

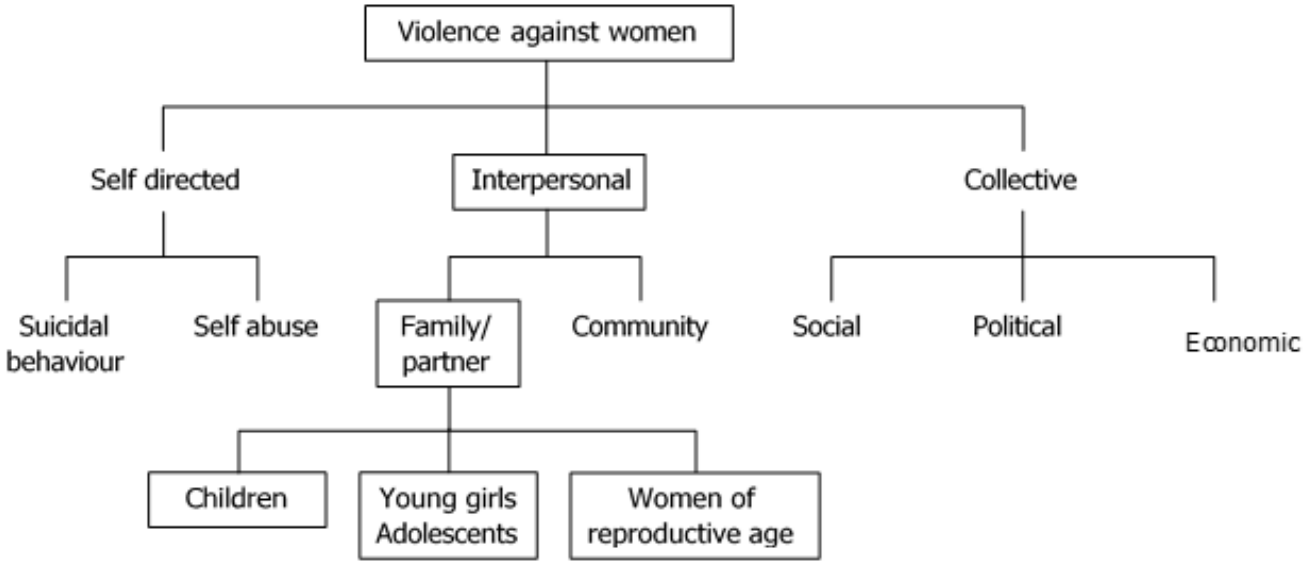

FIGURE 1: Types of violence against women
Source: Krantz and Garcia (2005)

3.1 The major impacts of VAW on agriculture sector

Violence against women can have a devastating effect on the agricultural sector of a country. **For example,** when women experience violence, they are more likely to suffer from poor physical and mental health, which can lead to decreased production and productivity of agriculture sector eventually leading to lowered income for female farmers.

According to Agriculture and Livestock Diary (2023), majority (72.8 %) of female are engaged in agriculture and the violence against women can often lead to isolation, which contributes to a lack of access to information, tools, and resources, making it difficult for them to keep up with changes in this sector and take advantage of agricultural opportunities. The relationship between VAW and women farmers is closely interrelated with each other because women farmers are highly vulnerable to the various forms of gender-based violence. In many communities, women farmers are targeted due to their high status in the community, making them more likely to experience physical, psychological, and sexual abuse. Furthermore, they often lack access to resources such as land, water, and knowledge that could enable them to succeed with their farming endeavors. This can further increase their vulnerability to violence and limit their ability to improve their livelihoods.

3.2 The major impacts of VAW in primary production sector, manufacturing sector and service sector

In the **primary production sector**, violence against women has been found to limit women's access to land and farming resources. **For example**, when women are victimized by violence they may lose their access to agricultural land or other key resources, thus decreasing their ability to make a living.

According to study by World Health Organization, (2021) one in four young women were found experiencing intimate partner violence by the time they rich mid-twenties. However, only 14% of countries were found recognizing their risks and include specific services for them in national policies.

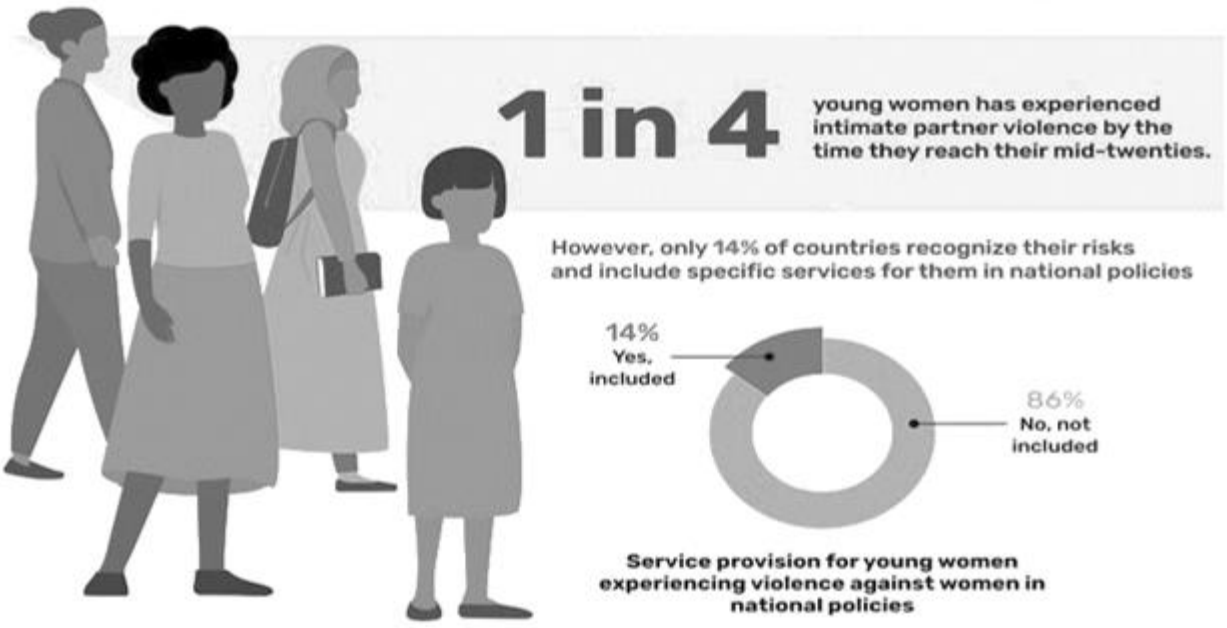

FIGURE 2: Intimate partner violence against women

Source: (World Health Organization,2021)

In the **manufacturing sector**, violence against women can lead to a decrease in women's participation and employment within industrial settings. **For example,** when women experience violence, they were more likely to skip work, thus leading to reduced wages and fewer opportunities for advancement.

In the **service sector**, violence against women can have implications for women's ability to access high-level professional positions. **For example**, when women experience violence were more likely to experience lower job satisfaction and lower levels of job authority than their male counterparts. This could diminish women's career prospects and limit their ability to advance in their fields. In the same context the study of Hossain (2021) in Bangladesh women engaged in garment industry were found exposed to different discrimination and structural violence. It clearly reflects that women are highly vulnerable to violence in their work place as well. According to Medica Mondiale (2023) the violence against women has many faces such as sexism in society, gender stereotypes, discriminatory laws, a lack of political participation, healthcare services do not cover needs of women, unpaid care work, direct physical, psychological and sexualized violence (Figure 3).

FIGURE 3: The violence against women has many faces

Source: (Medica Mondiale, 2023)

IV. CONCLUSIONS

VAW is a worldwide issue that needs to be addressed by comprehensive and focused interventions with the active involvement of all concerned stakeholders. In this study, VAW was found having significant effects on socio economic development of the country. Therefore, government should take careful consideration in formulation and effective implementation of policies against VAW, strengthening the community involvement and public education campaigns about gender equality, increasing access to justice and legal aid services such as legal education and counseling to survivors of gender-based violence and creation of efficient monitoring and reporting systems to gather information and reduce the violence against women and girls.

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Effect of Abiotic Stress and it's Mitigation Strategies in Wheat

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***Abstract*—** *India's staple grain is wheat, which is cultivated in the majority of the nation in the winter. The earlier investigations and studies on the impact of abiotic stress on wheat are covered in this succinct analytical piece. Numerous alterations in plant metabolism are brought about by various abiotic stressors and many of these modifications overlap with one another. Low yields are the result of crop growth being hindered by metabolic changes brought on by stress. Abiotic stressors have also been found to be a significant role in yield loss, productivity decline and net profit shrinkage in long-term studies carried out by different researchers in different locations. As a result abiotic stress such as Heat, drought, salinity, water logging and Heavy metal most be effectively mitigate through management practices such as stress resistance cultivars, irrigation scheduling, tillage and planting choices, residue management, sowing time and integrated nutrient management to preserve natural resources while minimizing the negative effects and ensuring long term wheat output.*

***Keywords*— *Wheat, Abiotic stresses, Heat stress, Salinity, Waterlogging, Drought, Mitigation Strategies*.**

I. INTRODUCTION

Wheat (*Triticum aestivum* L.) is a cereal crop grown on large area worldwide in a number of agro ecologies. Due to its economic and social benefits, it is widely grown in Asia, particularly in China, India and Nepal. Wheat production in India has progressively scaled new heights over the years with phenomenal increase in area, production and productivity. Globally, wheat is grown on 221.85 million hectares area with annual production around 785.12 million MT [1]. Wheat is called as the "King of cereals" because of its large cultivation in area, economic importance and potential for high productivity. Wheat is used to prepare pasta, pastry, semolina, chapatti and cookies among other items. It is a major staple food that accounts for approximately 35% of all food eaten by the world's population. In India, wheat is cultivated across five agro-climatic zones covering an area of about 29.8 million hectares with annual production of 110.59 million MT [1] and holds second position in wheat production worldwide. To provide food security for its growing population, the country has to produce 140 MT wheat by 2050 with productivity of 4.7 MT ha^{-1} [2].

Despite the fact that wheat has the highest total harvested area (38.8%) among cereals (including rice and maize), its total productivity is the lowest. Various factors such as biotic and abiotic stresses are responsible for the majority of wheat production losses. Biotic stress in wheat contains a number of pathogenic fungi and viruses that causes root diseases. Abiotic stresses, which include low or high temperature, inadequate or excessive water, high salinity, heavy metals and ultra-violet radiation, are all detrimental to wheat posing a significant threat to agriculture and the environment and responsible for substantial crop yield loss. Abiotic stress decreases productivity by 50% in most agriculturally valuable plants, including wheat [3]. Even though over 95% of India's wheat crop is irrigated, large portions of the country experience water shortages as a result of limitations on the amount of water available, especially during crucial periods of plant growth. Since the remaining wheat is dependent on rainfall, it is particularly susceptible to water stress, which lowers output and productivity. Although 21 to 24 degrees Celsius are thought to be the most ideal temperature range for wheat plant growth and development, the actual temperature range may differ based on the local agro climatic conditions. The whole wheat-cropped area in India experiences heat. While the central and peninsular parts experience heat stress all through the crop season, significant parts of north-western and north-eastern plains experience terminal heat. The trend by farmers in north-western and central India towards early sowing

of wheat to take advantage of residual moisture is demanding development of wheat genotypes for both early and terminal heat tolerance.

II. WHAT IS ABIOTIC STRESS?

- The negative impact of non-living factors on living organisms in a specific environment called Abiotic stress.
- In the recent past years, global warming and climate change have drastically affected the agricultural crop productivity grown in tropical and subtropical areas by appearing of several abiotic stresses.

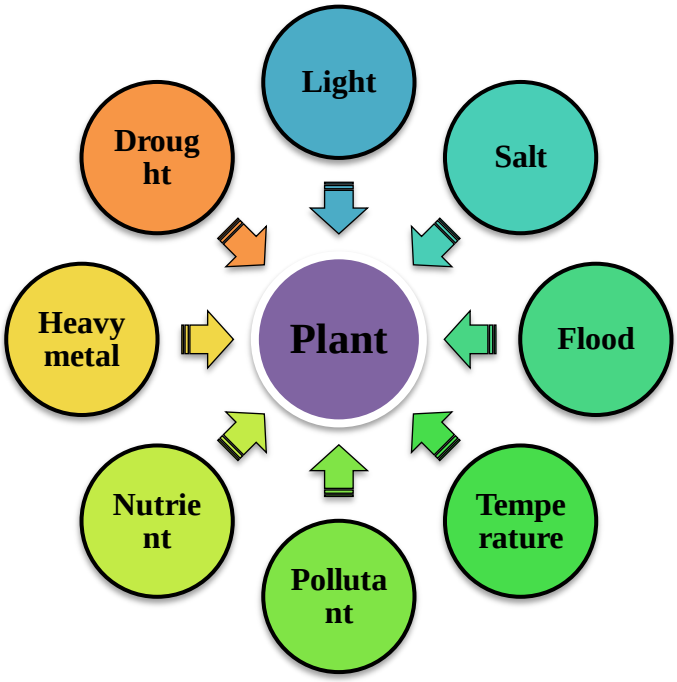

FIGURE 1: Various type of Abiotic stresses

2.1 High Temperature/ Heat Stress

Temperature regulates the growth and developmental processes of plants. Plant faces generally two types of stress with reference to temperature; high and low temperature. High temperature in plants adversely affects the normal metabolic processes which can induce premature leaf senescence, reduce the rate of photosynthesis and biomass production in plants.

Wheat is especially susceptible to heat stress during certain physiological growth stages [4]. Heat stress caused by high temperature is characterized as a rise in air temperature above a threshold level for a specific period of time that is sufficient to cause injury or irreversible damage to crop plants in general. For every 1 degree Celsius increase in temperature, there is a yield loss about 4.1 % to 6% [5]. Heat stress causes pollen sterility, Decreases carbon dioxide assimilation and improves photorespiration in wheat, according to previous studies [6]. According to [7], high temperatures damage the photosynthesis process, which has a negative effect on wheat growth and yield [8].

2.1.1 Mitigation strategies:

- **Selecting optimum time of planting:**

It helps in avoiding high temperature stress during anthesis and grain filling

- **Improving soil Quality**

Enhancing soil quality and structure to improve water retention and Nutrient Availability, thereby helping plants better cope with heat stress.

- **Implementing shade structure**

Erecting temporary shade structure or planting cover crops to provide shade and reduce direct exposure of wheat plants to heat stress.

- **Use of mulch/Zero tillage-**

It helps to protect seedlings from temperature stress and conserve moisture.

- **Selection of heat/high temperature tolerant variety**

GW 513, DBW 187, DBW 327, HI 8823 etc.

- **Use of sprinkler/drip irrigation-**

It helps in maintaining adequate soil moisture thereby reduces canopy/soil temperature.

- **Adoption of Precision Agriculture technologies**

Utilizing advanced technologies such as remote sensing and precision irrigation to monitor and manage wheat crops more effectively, including during periods of heat stress.

2.2 Drought Stress

Drought is one of the most significant abiotic stresses which have adverse effect on growth and development of plants. The productivity in wheat is most affected by water deficits. Water scarcity is a global problem that has major constrain in wheat production. The effect of drought on wheat becomes more conspicuous when the south-west monsoon fails to precipitate sufficient soil moisture essential for early establishment of the crop. Drought stress occurs when the available water in the soil is low to some critical levels for the plants. Drought causes huge damage to the physiological machinery of the plants resulting in degradation of morphological topology, anatomical structure and biochemical activity i.e. enzyme activity, protein content, sugar content, etc. It may be the most serious problem that diminishing wheat productivity by affecting various perspectives such as water relations, membrane integrity, pigment content. Crop yield is reduced mostly when water stress occurs at heading time, but its effect on yield is highest when it occurs after anthesis.

2.2.1 Mitigation Strategies

Drought-Tolerant Varieties: Developing and cultivating wheat varieties that exhibit enhanced tolerance to drought conditions through conventional breeding or biotechnology.

Optimized Irrigation Management: Implementing efficient irrigation practices such as drip irrigation, soil moisture sensors and scheduling irrigation based on crop water requirements to conserve water and minimize drought stress.

Conservation Tillage: Adopting conservation tillage practices such as no-till or reduced tillage to conserve soil moisture, improve soil structure and reduce evaporation, thereby mitigating drought stress.

Mulching: Applying organic or synthetic mulches to the soil surface to reduce water evaporation, maintain soil moisture levels and suppress weed growth, thus helping to alleviate drought stress on wheat crops.

Soil Amendments: Incorporating organic matter, such as compost or manure, into the soil to improve its water-holding capacity, nutrient availability and overall resilience to drought conditions.

Regulated Deficit Irrigation (RDI): Applying water strategically during critical growth stages to optimize water use efficiency and minimize the impact of drought stress on wheat yield and quality.

Seed Priming: Priming of seed enables the faster and better germination in plants under stressful conditions.

2.3 Salinity Stress

The salinity of arable land is considered as one of the most important abiotic stressors that restricted wheat production as well as its quality and security. It is one of the most destructive abiotic pressures, wreaking havoc on plant morphological, physiological and biochemical characteristics such as photosynthesis, nutrient absorption and yield. Higher salinity decreases germination and increases the concentration of Na^+ and Cl^- ions, disrupting wheat plants normal metabolic processes. Salt stress affects plants in a number of ways including osmotic effects, ion toxicity and nutritional disorders. Salinity also negatively affects wheat phenological developments such as leaf number, leaf expansion rate and root/shoot ratio and biomass. The saline environment disturbs plant water relations including relative water content, leaf water potential, water uptake, transpiration rate, water retention and water use efficiency.

2.3.1 Management of Salinity Stress:

- Application of gypsum @ 50% Gypsum Requirement (GR).

- Incorporation of Dhaincha (6.25 t/ha) in soil before planting.
- Foliar spray of 0.5 ppm brassinolode for increasing photosynthetic activity.
- Foliar spray of 2% DAP + 1% KCl (MOP) during critical stages.
- Spray of 100 ppm salicylic acid.
- Foliar application of ascorbic acid alone increased number of leaves and leaf area, while in combination with zinc sulfate increased the plant height and total plant biomass.

2.4 Waterlogging Stress

Wheat (*Triticum aestivum* L.) Is one of the most intolerant crops to soil waterlogging and the plants that are not tolerant to waterlogging may suffer from Fe or Mn toxicity. Wheat is very sensitive to waterlogging at sowing time and during seedling, flowering and grain-filling periods; waterlogging for 30 days during these periods reduced grain yield by 50-70% due to poor seed set and fewer spikes per unit area. In waterlogged soil, diffusion of gases through soil pores is so strongly inhibited by their water content that it fails to match the needs of growing roots. A slowing of oxygen influx is the principal cause of injury to roots and the shoots they support. Oxygen deficiency caused by waterlogging reduces shoot and root growth of plants, as well as yield. Waterlogging affects physiological processes of plants, such as absorption of water, root and shoot hormone relations and decreases the uptake and transport of ions through roots, causing nutrient deficiencies.

2.4.1 Management of Waterlogging Stress

- Surface drainage
- Raised bed system
- Early sowing and vigorous crop
- Nutrient application in particular
- Use of Plant growth regulator
- Use of Tolerant species and varieties
- Use of anti-ethylene agents
- Strategic deep tillage and subsoil manuring
- Controlled traffic farming

2.5 Heavy Metal Stress:

2.5.1 Major source of heavy metal release:

Natural: Rocks, Volcanic eruption, dust particle, aerosols, sea spray

Agricultural: inorganic fertilizer, pesticides, wastewater, fungicides, sea sludge, fly ash

Industrial: thermal power, industrial waste, Mining industries, rifaenaries, chemical industries

Domestic waste: organic waste, inorganic waste, biomass burning, e-waste,used filters, used batteries,

2.5.2 Management of Heavy Metal

- **Phytoremediation:** It is the best effective way for removal of heavy metals from contaminated soil with low and moderate heavy metal concentrations. It is eco-friendly and low cost way.
- **Phytoextraction:** The use of plants to remove heavy metals from soil
- Bioremediation of heavy metal toxicity:
    - Exclusion
    - Active transport of metals away from the cell
    - Intracellular and extracellular sequestration

- Enzymatic degradation of toxic metals to lesser toxic forms
- Reduction in metal sensitivity of cellular targets

- Soil washing
- Landfilling and leaching
- Soil immobilization

III. CONCLUSION

Abiotic stresses like high temperature (20%), low temperature (7%), salinity (10%), drought (9%) and other form of stresses (4%) are adversely affect wheat crop by physiologically, morphologically, biochemically that result in poor yield which ultimately affects the farmer's socio-economic condition. Hence, there is further scope to increase the productivity of wheat through adaptation of suitable agronomic management practices like adjusting the time of sowing, use of mulches, antitranspirants and reclamation of soil through appropriate soil conditioners with respect to edaphic and climatic conditions of the area.

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