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HISTORY, CONSTRAINTS OF HEMP AND FUTURE PROSPECT AS A POTENTIAL LIVESTOCK FEED IN BANGLADESH

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ABSTRACT

This study explores the historical context and potential of hemp as livestock feed in Bangladesh. Hemp, or Cannabis sativa L., has a long history in the region, dating back to the 18th century when it was cultivated for various purposes, including medicinal and intoxicating uses. Hemp in Bangladesh serves various functions, primarily for medicinal purposes across different regions. Globally, the demand for hemp is rising, driven by its applications in fiber production, food, and medicine. Despite legal complexities, hemp has the potential to be a valuable livestock feed additive due to its nutritional qualities. Feeding hemp to ruminant animals can improve the fatty acid profile of their milk and meat. For non-ruminant animals like poultry, hemp seeds offer essential nutrients for growth and health. However, constraints exist, including legal issues and societal misconceptions regarding cannabis in Bangladesh. Changing these perceptions is crucial to realizing the potential of hemp in the country's agriculture and livestock industries.

1. INTRODUCTION

In this study, the term "hemp" refers to *Cannabis sativa* L. and any part of that plant. Hemp goes by various names in different countries. In English, "Hemp" is the most recognized term for cannabis, albeit with multiple meanings, encompassing its identity as a crop, a fiber, and a drug. Meanwhile, "Ganja" serves as the Hindi name for cannabis and is a common and familiar term in many parts of South Asia, including Bangladesh (Rahman *et al.*, 2022). However, in the realm of agriculture, certain crops hold the potential to revolutionize traditional farming practices, addressing the ever-growing challenges of feeding a global population. One such crop is hemp, a versatile and underutilized resource. Hemp serves as a versatile crop, offering a range of uses, including the production of food, fiber, and pharmaceuticals (Berzins *et al.*, 2014; Hill, 1972). The historical legacy of hemp production in Bangladesh has deep roots, dating back to the late 18th century under British colonial rule.

While the exact origins of cannabis cultivation remain shrouded in the mists of history, the earliest documented instances can be traced back to 1722 when the East India Company conducted experimental trials involving hemp as a fiber crop (Rahman *et al.*, 2022). In 1839, the plant's medicinal and intoxicating properties were discovered, driving its popularity as a drug (Mills, 2003). The cultivation of hemp, or "ganja" as it is known in Bangladesh, is believed to have commenced in the Jessore region in the early 18th century and later shifted to Naogaon District. However, its cultivation and production were banned in 1987 in Bangladesh due to international narcotics agreements. Hemp, often cloaked in a shroud of misconception and legal complexities, confronts a series of problems that have hindered its growth in Bangladesh. Legal restrictions surrounding its cultivation, stemming from international narcotics agreements, have cast a shadow over its potential utility. Additionally, prevailing negative societal perceptions and limited scientific evidence for its use in animals further exacerbate these challenges.

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These problems are the crux of a complex narrative that requires a holistic understanding to unlock the true potential of hemp. Recently, its global importance in addressing the increasing demand for both human and animal consumption, its nutritional qualities, and its potential impact on the performance of various animal species. The nutritional qualities of hemp and its effects on the performance of both ruminant and non-ruminant animals highlight the potential benefits it offers. In this study, we aim to unravel the historical background of hemp cultivation in Bangladesh, shedding light on its evolution and the factors contributing to its current status. We also delve into the myriad uses of hemp, from traditional medicine to livestock feed, seeking to understand its potential as a multifunctional crop. Besides, we explore the global importance of hemp production, assessing its relevance on the global stage and the demand it generates. Moreover, it will highlight the bioactivity of hemp and its role in enhancing animal health and the quality of products derived from these animals. This study endeavors to peel back the layers of history, problems, objectives, and importance surrounding hemp cultivation in Bangladesh. Through a comprehensive exploration of its past, present, and potential future, it shines a light on the transformative power of this versatile crop and its role in shaping the future of agriculture and food security.

2. MATERIALS AND METHODS

The information search was carried out with the following keywords: Ganja, hemp, Cannabis sativa, livestock, ruminant, non-ruminant animals, Bangladesh, global, socio-economic status, feeding and management, productivity, production, reproduction, bioactivity, milk and products quality and diseases. Databases such as Google, Google Scholar, Bangladesh Journal-Online (BJO), Research Gate, Scopus, and PubMed on the scientific literature published about hemp production, constraint, and feeding animals in Bangladesh and abroad were reviewed.

Historical background of hemp cultivation in Bangladesh

There is no exact information about when and how the cultivation of hemp started in Bangladesh. The earliest documented instance of hemp cultivation in Bengal can be dated back to 1722 (Ali, 2014; Khansaheb, 2007). During that period, the East India Company conducted experimental trials involving hemp cultivation as a fiber crop. In 1839, the plant's medicinal and intoxicating properties were discovered, propelling its popularity as a drug (Mills, 2003). However, cultivation of hemp is believed to have started in Jessore southern part of Bangladesh in the first decade of the eighteenth century (Ali, 2014). However, in 1873, a report on the cultivation of ganja in Bengal, along with insights into the consumer market for this commodity, was published in the Calcutta Gazette. Notably, the report highlighted that ganja was not cultivated in Jashore during that time because farmers were compelled to cultivate indigo instead (Ali, 2014; Hunter, 1876). Subsequently, the ganja cultivation area shifted to the Naogaon district in northwestern Bangladesh due to favorable environmental conditions (Uddin, 2006). Its cultivation was legally confined to the so-called "Ganja Mahal" in the Naogaon sub-district, comprising parts of three districts—Bogra, Dinajpur, and Rajshahi—before their merger in 1896 (Ali, 2014). Strict control over cultivation and production was entrusted to the Ganja Society, established in 1917, with its headquarters located in Naogaon within Ganja Mahal (Mills, 2003).

Uses of hemp in Bangladesh

An extensive body of literature comprising ethno medicinal plant survey reports has been disseminated in Bangladesh. This investigation discerned that *Cannabis sativa* L. (hemp) exhibited multifarious utilitarian applications across various districts and regions within the nation. Notably, the districts of Kushtia, and Chittagong stand out for their distinctive historical engagement with this plant, encompassing its utilization in sustenance and veterinary practices. Conversely, in other locales, *C. sativa* L. is primarily employed for therapeutic purposes, addressing a spectrum of ailments, including sleep disorders, neuropsychiatric and central nervous system maladies, infections, respiratory conditions, rheumatic afflictions, gastrointestinal disorders, gynecological concerns, cancer, and a myriad of other health-related issues, as delineated in Table 1. This table elucidated the vernacular nomenclature and constituent ingredients employed by local communities hailing from diverse regions within Bangladesh, along with the associated preparation techniques.

Global importance of hemp production

Cannabis sativa L., commonly called hemp, is vital in addressing the growing global demand for human and animal consumption. This demand stems from its diverse applications in fibre production, food, and medicine (Berzins *et al.*, 2014; Hill, 1972). Globally, the demand for hemp is rising, with regional variations; Asia leads with 45%, North America at 22%, and Europe at 19% (New Frontier data, 2021). Within plant taxonomy, the genus Cannabis encompasses three species: *C. sativa, C. indica, and C. ruderalis,* all classified under the solitary species *C. sativa*. While hemp has historically played a significant role in traditional medicine in Bangladesh, its use is declining due to legal complexities. Conversely, there is a notable increase in global consumption of Cannabis and its derivatives. Many countries have legalized cannabis-derived products for medical and recreational use (Pant, 2016). North America and Europe have passed laws permitting medical and recreational cannabis use. Hemp, defined as Cannabis sativa containing less than 0.3% THC on a dry weight basis, is recognized legally in the United States (US) and the European Union (EU). Despite the therapeutic potential of cannabinoids like CBD, limited scientific evidence exists for their use in animals, primarily focused on companion animals. The intricate interplay of legal, medical, and societal factors underscores hemp and Cannabis's multifaceted role in addressing the increasing global demand for human and animal consumption.

Nutritional quality of hemp

Hemp is more useful and applicable in the fields of agriculture, food, feeds, cosmetics and pharmaceuticals companies which have low environmental effects as well as rich source of bioactive components. Besides from other uses, it is also promising feed additive for livestock. Hemp seed is by the byproduct of this crop, and this is useful feed for animals. Despite the use of synthetic antibiotic growth promoter, hemp seed can be used as a livestock feed additive without any residual effects in animals. Hemp seeds are normally used as whole, hulled seed and dehulled seed (mainly kernel) as well as its processed products are also available for consumption. Hempseed is rich source of antioxidant, bioactive peptides, phenolic compounds and phytosterols (Irakli, 2019). Inclusion of hemp oil in livestock feed can be a good source of essential fatty acid (Cozma, 2015) as well as for fat and protein source, hemp cake can be used (Mierlita, 2019). Bodas (2010) stated that hemp seed oil and oil cake have high concentration of polyunsaturated fatty acids. Several terpenes were found in hemp seed oil and beta-caryophyllene was abundant (Leizer, 2000). 18-23% protein, 25-30% oil, 30-40% fiber and 6-7% moisture is present in hemp seed (Leonard, 2020). About 181 proteins are available in hemp seed and concentration edestin and globulin are 67%-75% which is the main storage protein as well as globular protein are also 25-37% (Aiello, 2016). The ratio of soluble and insoluble dietary fiber located in shell of hemp seed is 20:80 where the insoluble fiber of hemp seed contains 46% cellulose, 31% lignin, 22% hemicellulose (Maki, 2000). The core had the lowest concentration of phenolic chemicals, whereas the shells had the highest concentration. The two main phenolic components in hemp that are exhibiting a high antioxidant activity are lignan amides and hydroxy cinnamic acid (Fathordoobady, 2019). Tetrahydrocannabinol (THC) is present in hemp seeds in very small amounts less than 1% (Citti, 2018). In contrast, hemp seeds have a number of anti-nutritional components such phytic acid and trypsin inhibitors that prevent proper nutrition (Pojic, 2014).

Effects of hemp feeding on the performances of ruminant animals

Hemp, a versatile and fast-growing plant, has recently attracted interest for its possible use as ruminant feed for milk production. Ruminants have a unique stomach that allows them to absorb fibrous plant components, thus hemp could be a helpful supplement to their diets. Ruminants are animals that transfer less energy from feed into food due to losses caused by rumen fermentation processes. Ruminants, on the other hand, play a vital role in the bioeconomy by transforming food that people cannot eat (such as forages, crop residues, and agricultural byproducts) into high nutritional value food (Van Soest, 1982). As a result, alternative plants have been recently rediscovered and reintroduced on the agricultural surfaces for increasing livestock production. The hemp plant (*Cannabis sativa L.*) is undoubtedly one of the most cultivated plants throughout history in the world. The development of various feeding techniques to improve the chemical nutritional qualities of dairy milk and milk products, assuming that nutrition might influence milk composition in ruminants, has expanded in recent years. Hemp seeds are indeed a good source of both omega-3 (n-3) and omega-6 (n-6) fatty acids (FAs), particularly alpha-linolenic acid (ALA) for n-3 and linoleic acid (LA) for n-6.

These FAs are essential for human health and must be obtained through the diet because the human body cannot produce them on its own. It is true that the consumption of hemp seeds or hemp seed oil, which is rich in these FAs, can lead to an increase in the levels of these polyunsaturated FAs (PUFAs) in the diet. This, in turn, can potentially lead to an increase in their presence in milk and dairy products if consumed by animals. Feeding hemp seeds or hemp oil to livestock, such as cows, could potentially result in milk with a more favorable n-6/n-3 ratio because of the hemp's high n-3 content. This, in turn, could lead to dairy products with a more balanced FAs profile. It's worth noting that the extent to which this change occurs in animal products depends on various factors, including the animal's diet, genetics, and the duration of hemp seed consumption (Lopez *et al.*, 2016; Bennato *et al.*, 2020; Ianni *et al.*, 2019; Castro *et al.*, 2019; Cattani *et al.*, 2014; Gebreyowhans *et al.*, 2019). Karlsson *et al.* (2010) evaluated the effects of increasing the proportion of hempseed cake (HC) in the diet of dairy lactating cows on milk production and composition and found no effects in dry matter (DM) intake but significant linear increases in crude protein (CP), fat, and NDF intakes were observed with the increase of the proportion of HC in the diets. Mustafa *et al.* (1999) conducted a study regarding the DM and CP in situ degradability in two non-lactating rumen fistulated cows using four different protein sources: hemp, borage, canola, and heated canola meals.

The results of the study revealed that hemp meal was an excellent natural source of rumen undegradable protein (RUP), containing 774 g/kg of CP. This RUP content was found to be equivalent to that of heat-treated canola meal but higher than borage and canola meals. It is also recommended to maintain the diets as isoenergetic and isonitrogenous while adjusting the proportions of other ingredients. Additionally, to assess the potential nutraceutical benefits of hempseed meal, it would be valuable to determine the nutritional value of milk and its derivatives when hemp meal is incorporated into the diets." Ewes' milk naturally contains beneficial substances for human health, such as n-3 FAs and conjugated linoleic acid (CLA). Feeding hemp (both hemp seeds and hemp cake) to ewes increased milk production and fat content but reduced milk lactose. This hemp-based diet also elevated the levels of polyunsaturated fatty acids (particularly n-3 fatty acids) in ewes' milk and improved the n-6/n-3 fatty acid ratio. Moreover, the total content of CLA in the milk doubled in ewes that received hempseed and increased 2.4 times when hemp cake was included in their diet (Mierlita, 2018). The presence of alpha-tocopherol and antioxidant activity increased with hempseed inclusion in the diets, which helps reduce the risk of lipid oxidation in raw milk. In a study by Ianni *et al.* (2019), the impact of enriching the diet of dairy ewes with hempseed (constituting 5% of the dry matter) was evaluated. This dietary enrichment led to an increase in lactose concentration from 4.69% to 5.84%. However, there were no significant differences observed in milk fat, protein, casein, and urea. Additionally, no changes were detected in the total fat, protein, and ash content in the resulting cheeses. Notably, hempseed supplementation positively influenced the metabolic pathways related to energy production in lactating ewes, potentially enhancing their resistance to adverse climatic conditions such as low temperatures (Iannaccone *et al.*, 2019). Mustafa *et al.* (1998) noted that hemp meal (HM) serves as an excellent natural source of rumen undegradable protein (RUP) that is on par with heat-treated canola meal. When used as a protein source in diets with equivalent nitrogen levels (up to 200 g kg-1 of dry matter) compared to canola meal (CM), HM did not have adverse effects on feed intake or nutrient utilization in sheep.

In case goat, Cozma *et al.* (2015) evaluated the effect of a diet supplemented with hempseed oil in Carpathian goats during 31 days of experiment and no significant changes of milk yield were observed receiving the hempseed oil supplementation. Fat content increased significantly during the trial in milk produced by goats receiving hemp oil in comparison with the control group. In another study, evaluated the effects of the inclusion of 9.3% on DM of linseed or hempseed in diet for Alpine lactating goat and found milk yield was unaffected by the dietary treatment but linseed and hempseed supplementation significantly increased the milk fat content. Again, Cozma *et al.* (2015) found a significant increase of the PUFA concentrations (+45%) in milk produced by goats supplemented by hempseed oil, without an effect on n-3 fatty acids content. Overall, beneficial effects on human health can be obtained in goat milk with the inclusion of hempseed oil in the diets. The impact of utilizing hemp in various animal species and their findings are mentioned in Table 4.

Winders *et al.* (2022) carried out an experiment regarding the feeding of hempseed cake alters the bovine gut, respiratory and reproductive microbiota and found significant effects on the community structure and diversity of the ruminal, nasopharyngeal, vaginal, and uterine microbiota in beef heifers. In the rumen, hempseed cake-fed cattle showed increased microbial diversity and richness compared to the control group (fed corn dried distillers' grains with soluble). The ruminal microbiota composition was significantly affected by the inclusion of hempseed cake in the diet, with changes in the abundance of specific bacterial genera. The nasopharyngeal microbiota was also influenced by the diet, but to a lesser extent than sampling time. Hempseed cake did not significantly affect microbial richness and diversity in the nasopharynx. The vaginal microbiota of heifers fed hempseed cake showed reduced microbial richness compared to the control group. The uterus of hempseed cake-fed heifers had greater microbial diversity and richness compared to the control group. In another study, Ben Necib *et al.* (2022) investigates the effects of dietary substitution of whole hemp seeds in comparison with whole linseeds in a diet-induced obesity mouse model and demonstrates that whole hemp seed substitution does not affect weight gain, adiposity, or food intake, while linseed substitution does. Hemp seed substitution mitigates diet-induced obesity-associated increases in intestinal permeability and circulating PAI-1 levels. Both hemp seeds and linseeds are able to modify the expression of several endocannabinoidome genes. Potential beneficial metabolic effects of hemp seeds found, their role in improving intestinal barrier function, and their ability to decrease inflammation in mice under a high-fat, high-sucrose diet compared to linseeds. Endocannabinoids, particularly anandamide (AEA) and 2‐arachidonoylglycerol (2‐AG), are instrumental in regulating energy homeostasis and stress response. Van Ackern *et al.* (2021) contribute to the understanding of the endocannabinoid system (ECS) in dairy cows, which was previously not well-known in ruminants. It demonstrates that intraperitoneal administration of endocannabinoids (AEA and 2-AG) can attenuate stress-induced suppression of feed intake and increase short-term feed intake in cows. This research suggests that endocannabinoids have the potential to improve dairy health and productivity by modulating feed intake, energy metabolism, and stress response in cows. Parker *et al.* (2022) carried out an assessment of spent hemp biomass (SHB) as a potential ingredient in ruminant diet and mentioned that feeding SHB does not induce inflammation or affect the immune system, but it may have effects on metabolism, kidney function, and bone metabolism. It was found that feeding of SHB to lamb can have positive effects such as improved antioxidative status and better digestibility of the diet.

Constraints of hemp cultivation in Bangladesh

The legal cultivation of hemp, known as ganja, in Bangladesh, has a historical legacy dating back to the late 18th century under British colonial rule. This practice continued until 1987, when it ceased due to international narcotics agreements (Rahman *et al.,* 2022). The colonial British government introduced a licensing system in 1876 to regulate production. Before the introduced of licensing system there was no limitation on production and sales. Presently, hemp cultivation and sale are illegal in Bangladesh. Marijuana is classified as a B-Class narcotic per the Narcotics Control Act of 1990. However, Section 9 of the Act allows for the lawful manufacture, processing, import, export, supply, purchase, and sale of narcotics for approved medicinal use or scientific research with proper licensing and permits (Shakil *et al.*, 2021). In Bangladesh, there's a widespread misconception about hemp due to a lack of accurate information about its properties, resulting in a negative societal perception. However, there's a current shift where hemp is increasingly recognized as versatile. Historically, ganja, a variety of hemp, has been used for spiritual purposes in Bangladeshi society, particularly among Sufi Muslims (Haque, 1993; Malek, 1999).

3. CONCLUSION

Hemp, a versatile crop with a historical background in Bangladesh dating back to the late 18th century, offers the potential to revolutionize livestock feed practices in the country. While its historical use centered on narcotic purposes, the growing recognition of its versatile properties is reshaping its role in agriculture. Hemp boasts a range of nutritional benefits, including high-quality proteins, essential fatty acids, and bioactive components. This makes it an attractive candidate as a valuable animal feed additive. The rich protein content, beneficial fatty acids and antioxidant properties in hemp can enhance animal health and elevate the quality of animal-derived products. Introducing hemp into livestock feed can improve animal performance, potentially boosting meat and dairy production. By harnessing the untapped potential of hemp in livestock nutrition, Bangladesh can address food security challenges and contribute to more sustainable and nutritious agricultural practices. Achieving this transformation will necessitate legal and regulatory changes to facilitate its adoption in the country's agricultural sector.

In summary, the historical cultivation of hemp in Bangladesh has laid the groundwork for a new era where hemp has become a key component of livestock feed. Its nutritional richness, coupled with the ongoing shift in perception, promises to diversify agriculture and enhance the quality of animal feed, ultimately benefiting both animal and human health. Legal and regulatory adjustments will play a vital role in realizing this potential.

4. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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Table 1. Local name and ingredient used by the people of different locations in Bangladesh.

Locations	**Local name**	**Part(s) Used**	**Purposes**	**Formulations**	**Remarks**
Naogaon	Ganja	Seed	Food, oil	Fry ganja seed with rice (50/50) as a food, extracted oil from seed for pain	This study
		Leaf	Medicine	dry leaf powder mixed with mustard oil twice daily for fever and headache	
		Bud	Narcotic	Upper parts of flower spikes used as a drug	
		Stalk	fuel	stems using for cooking	
Rajshahi	Shider Gach	Seed	sex stimulant	The seeds are taken for sexual stimulation.	Nawaz *et al.*, 2009
		Leaf	Insomnia,	Leaves and seeds are dried, powdered and made into balls of about 1/16 kg each. One ball is taken daily for coughs, mucus, as a narcotic and to induce sleep.	
			narcotic		
			mucus, cough		
Kholabaria of Natore	Ganja	Leaf	Dandruff	Leaves make a good sunff for deterging the brain; juice removes dandruff.	Sultana, 2017
		Resin	Headache, asthma	The resin called charas is used prevents and cures headache, asthma	
Kushtia	Ganja	Seed	Birds & poultry feed	Take out the seed by hand	This study
		Bud	Narcotic	Cut the bud in small pieces put it inside the coconut bark ball	
Bandarban	Shiddir Ganja Bhang	Bark	Stomach and Urinal problem	Juice daily 3 times	This study
Chittagong	Ganja, Hemp	Stalk	Fiber	Stalks steeped in water 10-15 days; then beaten out on stone or block of wood in water.	O'Malley,1908
		Flower	Aninal food	Flowers used for cattle food and stalks making Sulphur matches	
Rangamati	Bhang, Siddi	leaf	Treatment of schizophrenia like psychotic episodes.	Leaves are used to make oil then message on the scalp till cure. If a patient is in severe condition, then the leaves were used to make vapor and the vapor is taken by the nose.	Ahmed and Azam,2014

Table 2. Chemical composition of hemp seed

Parameter of Analysis	Content (%)
Total Protein	21.00 ± 0.04
Total lipid	28.00 ± 1.35
Crude Fiber	12.00 ± 0.51
Ash	4.00 ± 0.08
Moisture	10.00 ± 0.33
Total carbohydrates	25.00 ± 1.39

El-Sohaimy, 2022

Essential amino acid and fatty acids are present as a balanced form in hemp seed oil (Callaway, 2004) also mentioned that this contains high level arginine and glutamic acid.

Table 3. Amino acid composition of hemp seed

Essential Amino Acid	Content, g/100 g	Non-Essential amino acids	Content, g/100 g
Isoleucine + Leucine	5.21	Arginine	15.52
Lysine	2.88	Histidine	3.2
Methionine + Cysteine	5.49	Proline	3.44
Phenylalanine + Tyrosine	9.63	Serine	4.05
Threonine	3.79	Alanine	3.85
Tryptophan	0.26	Glycine	3.7
Arginine	15.52	Glutamic acid + glutamine	3.91
Valine	4.53	Asparagine + aspartic acid	12.53
Total of essential amino acids	31.79		

El-Sohaimy, 2022

Table 4. The impact of utilizing hemp in various animal species

Species	Experimental duration	Type of hemp and Amount	Results	Reference
Swedish red dairy cows	5 weeks, 1 week (pre-experimental period)	Hemp seed cake: 14.3, 23.3, 31.8% (dry matter)	14.3% hemp seed cake: higher milk yield, 23.3 or 31.8% hemp seed cake: no benefits in milk performance	Karlsson *et al.* (2010)
Steers	166 days	Full-fat hemp seed: 9 or 14%	Significant increase in CLA a level, also trans and saturated fats in tissues, no effect on DMI, ADG, carcass traits	Gibb *et al.* (2005)
Male Holstein cattle	14 days	Industrial hemp (*Cannabis sativa L.*): 25g mixed in 200g of grain (Target daily dose of 5.5mg/kg cannabidiolic acid)	significant increase in lying behavior, significant decrease in cortisol level and prostaglandin E2(PGE2)	Kleinhenz *et al.* (2022)
Male Holstein calves	Single oral dose, 4 days	Industrial hemp: 35 g (target dose of 5.4 mg/kg cannabidiolic acid)	No significant changes in serum parameters	Kleinhenz *et al.* (2020)
Carpathian goats	31 days	Hempseed oil: 93 g/day	Higher milk fat content, increase in conjugated fatty acid and PUFAs, no effect on milk yield	Cozma *et al.* (2015)

Table 5: Health benefits of peptides derived from hempseed protein.

Bioactivity	**Main conclusions**	**References**
Antioxidation	Differences in DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, exhibiting the lowest IC50 value of around 2.3 mg/mL, along with Fe2+ chelation capacity demonstrated by the lowest IC50 values ranging from 1.6-1.7 mg/mL. Additionally, there is variance in reducing power, as indicated by the highest absorbance at 700 nm ranging from 0.31 to 0.35	Tang *et al.* (2009)
	The DPPH radical scavenging activity was notably less potent when compared to the fractionated peptides. The fractionation of HSP (heat-stable peptide) resulted in notable enhancements in ferric reducing power, DPPH, and hydroxyl radical scavenging activities. However, it led to a reduction in metal chelation capacity. Within the peptide fractions, those with longer chain lengths (ranging from 3-5 kDa and 5-10 kDa) exhibited superior metal chelation and ferric reducing power in comparison to the <1 kDa and 1-3 kDa fractions.	Girgih *et al.* (2011a)
Serum glucose regulation	HSP (heat-stable peptide) subjected to Alcalase treatment at a degree of hydrolysis measuring 27.24±0.88% displayed strong inhibition of alpha-glucosidase activity. This effect was attributed to the presence of two newly discovered alpha-glucosidase inhibitory peptides with the following sequences: Leu-Arg (with a molecular weight of 287.2 Da) and Pro-Leu-Met-Leu-Pro (with a molecular weight of 568.4 Da)	Ren *et al.* (2016)
Angiotensin I-converting enzyme inhibition	More pronounced inhibition in laboratory settings of the functions of angiotensin I-converting enzyme and renin, the primary enzymes responsible for the abnormal increase in blood pressure (hypertension).	Girgih *et al.* (2011b)
Antihypertension and renin inhibition	A 1% concentration of alcalase HPH proved to be the most efficient in lowering systolic blood pressure, leading to a reduction of -32.5±0.7 mmHg within 4 hours. On the other hand, the pepsin HPHs had a more sustained impact, resulting in a decrease of -23.0 ±1.4 mmHg even after 24 hours.	Malomo *et al.* (2015)
	Peptides consisting of a small number of amino acids (≤5), like Trp-Val-Tyr-Tyr (WVYY) and Pro-Ser-Leu-Pro-Ala (PSLPA), demonstrated the highest levels of antioxidant efficacy. Specifically, they exhibited a DPPH scavenging capacity of 67% and 58%, while their metal chelation activity reached 94% and 96%, respectively.	Girgih *et al.* (2014)
Acetylcholinesterase inhibition	Among the HPHs (hydrolyzed proteins), those treated with 1% pepsin exhibited the strongest inhibition of acetylcholinesterase, displaying an IC50 value of 6 µg/mL. In contrast, the IC50 values for the other HPHs ranged from 8 to 11.6 µg/mL.	Malomo & Aluko (2016)

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ZOONOTIC SPILLOVER AND THEIR RELATIVITY TO PANDEMICS

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ABSTRACT

The study aims to emphasize the relativity of pandemics and their zoonotic spillover. During recent decades the chance of pandemics occurring has increased many folds. So, the study thoroughly reviewed seven zoonotic spillovers from bats in history that led us to the common feature that close contact of bats with other species including humans is the major reason for pandemics. Due to anthropogenesis bats started moving into buildings for survival, reproduction, and swarming became the susceptible source of disease transmission. The study aims to suggest measures that could help to prevent bat-borne viral outbreaks in the future.

1. INTRODUCTION

The ongoing wave of Coronavirus disease-19 (COVID-19) pandemic around the globe caused by SARS-CoV-2 became a global public health concern (Jin *et al.*, 2020, Rothan & Byrareddy, 2020). It is obvious that bats are the convenient reservoir of the CoVs and probably the etiological agent of COVID-19 (Sabir *et al.*, 2016).

In the past century, Asia, Africa, and Arabian states were the major hotspot regions related to bat-born viral pandemics in human society (Calisher *et al.*, 2006, Wolfe *et al.*, 2005, Morse, 2004). We studied seven zoonotic spillovers that are Marburg virus (Towner *et al.*, 2009, Nyakarahuka *et al.*, 2016) , Ebola virus (Smith, 1978) Nipah virus (Paton *et al.*, 1999, Chua *et al.*, 2000), Hendra virus (Blum *et al.*, 2009), Severe Acute Respiratory Syndrome coronavirus in (Christian *et al.*, 2004) Middle East respiratory syndrome (Skariyachan *et al.*, 2019) and COVID-19 (Jin *et al.*, 2020).

When we studied briefly these spillovers that resulted in the growth of the human population intensified the anthropogenic interference to wildlife (Plowright *et al.*, 2011). Wild animals are the potential hosts of viral diseases as in the case of Hendra, Nipah, and SARS-CoVs (Chua *et al.*, 2000, Philbey *et al.*, 1998, Murray *et al.*, 1995a). Similarly, the reason behind the EBOV and SARS-CoV outbreaks is the cultural traditions of eating wild animal meat or 'bush meat' in Africa and Asia (Dowell *et al.*, 1999). Businesses in live markets such as the desire for eating wild bats conceded the susceptible animals to come into contact with bats and let the transmission of SARS-CoV into the human's population (Webster, 2004, Guan *et al.*, 2003a, Lam *et al.*, 2003). On the contrary, in the case of MERS-CoV outbreak civets dromedary camels were identified as major intermediate hosts that were got infected elsewhere from the reservoir host (Zaki *et al.*, 2012).

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Moreover, the anthropogenic activities including deforestation and urban development with the increasing human population selecting synanthropic bat species, and so far the bats are benefiting from living close to humans, thereby increasing their numbers and the risk of disease transmission to humans (McFarlane *et al.*, 2012). The origin of the COVID-19 pandemic outbreak is still debatable but the very first cases of concentrated pneumonia were informed in Wuhan city of China and were linked with the wet animal market. Because of high human to human transmission, the COVID-19 rapidly spread to other countries of the world and global health emergency was announced by WHO (Jin *et al.*, 2020, Rothan and Byrareddy, 2020). The aim of our study is to curb pandemics in future. So that, we thoroughly reviewed seven zoonotic spillovers from bats in history that led us to common feature that is close contact of bats with other species including human is the major reason of pandemics. Due to anthropization bats started moving into building for survival, reproduction and swarming that became the susceptible source of disease transmission. Our suggestions could help to prevent bat-borne viral outbreaks in future if seriously implemented.

Zoonotic Spillover from bats

Spread of viruses via bats causing EIDS (Emerging infectious disease) is a major issue. A list of bats born dreadful pandemics/epidemics outbreak in human populations all over the world is given in Table 1 and Figure 1. The mode of transmissions of viral pandemics/epidemics to the human population from different reservoirs is represented in Figure 2. This figure also explained that bat close contact in outbreak areas becomes the source of disease transmission. Our suggestions could help to prevent bat-borne viral outbreaks in future if seriously implemented listed below.

Why bat research always our week area?

Americans were on the belief that bats are violent enough to attack human and pets even organization which worked for bat conservation used to avoid bats because of fear (Tuttle, 1979). Therefore, deep research is much needed in broad spectrum which should be able to cover all aspects of bats.

Minimize anthropogenic activities

Area of forests has been lessened and environmental diversity is nearer to human population due to anthropization. Unlike their natural habitat, these landscapes provided a large number of bats to make their habitat in this anthropized environment. Bats found there niches in human populated areas which are their alternative environment for their survival (Walsh *et al.*, 2017a, Afelt *et al.*, 2018).In the name of urbanization, large scale deforestation has been done that threatens and disturbing the natural habitats, bats started changing their habitat and moved to urban areas that made close contact with humans. If we do reforestation bats can be revived to their original habitat and it will also help the ecosystem, whereas the problem of the increasing population can be controlled by the social campaign, social awareness, education, and proper planning.

Detecting synanthropic bat species

Most of the species are living in man-made buildings in which bats are using different spaces of these buildings which are good element for hibernation and reproduction (Lesinski, 2006). Searching for bats in the buildings and its premises is hard task even for the chiropterologists therefore infrared cameras, endoscopes and ultrasonic detectors can be used to find bats but more inovative technological equipment can be used with the help of chiroptrologists to capture bats.

Chiropterological inventory survey

Roofs, wall cracks, attics can be examined and inspected with use of modern equipment's. It is necessary step that should be practiced during any inventory of bats in buildings and its premises (Janus & Lesinski, 2018). A survey must take place with modern techniques and equipment's with high probability detection rate in new buildings as well as existing buildings and its premises including all spaces, roofs, wall cracks and all possible places.

Create rehabilitees for bats

Killing and capturing of bats has become extensive and not limited in buildings. Major colonies of bats (*Myotis grisescens*) were burnt in caves (Tuttle, 1979) when health officials conjectured i that these species of Bats were outbreak cause rabies in foxes (Fredrickson & Thomas, 1965). This information makes bats very unsafe. Killing and capturing of bats has become extensive and not limited in buildings. Major colonies of bats (*Myotis grisescens*) were burnt in caves (Tuttle, 1979) when health officials conjectured that these species of Bats were outbreak cause rabies in foxes (Fredrickson & Thomas, 1965). Historically, there are always reasons and objects of fear and conflict that came across many societies possibly due to their dark and evasive behavior (Kingston, 2016). Capturing and killing bats or keep them away from buildings is not the solution. We need to make rehabilitation areas for bats in forests, mountains, areas far away from human population where these captured bats can revive, live, hibernate and swarm. By reviving bats and saving its population is necessary as bats play important role in our ecosystem.

Management Implications

Our study can support wildlife management based on bats conservation and its rehabilitation.

2. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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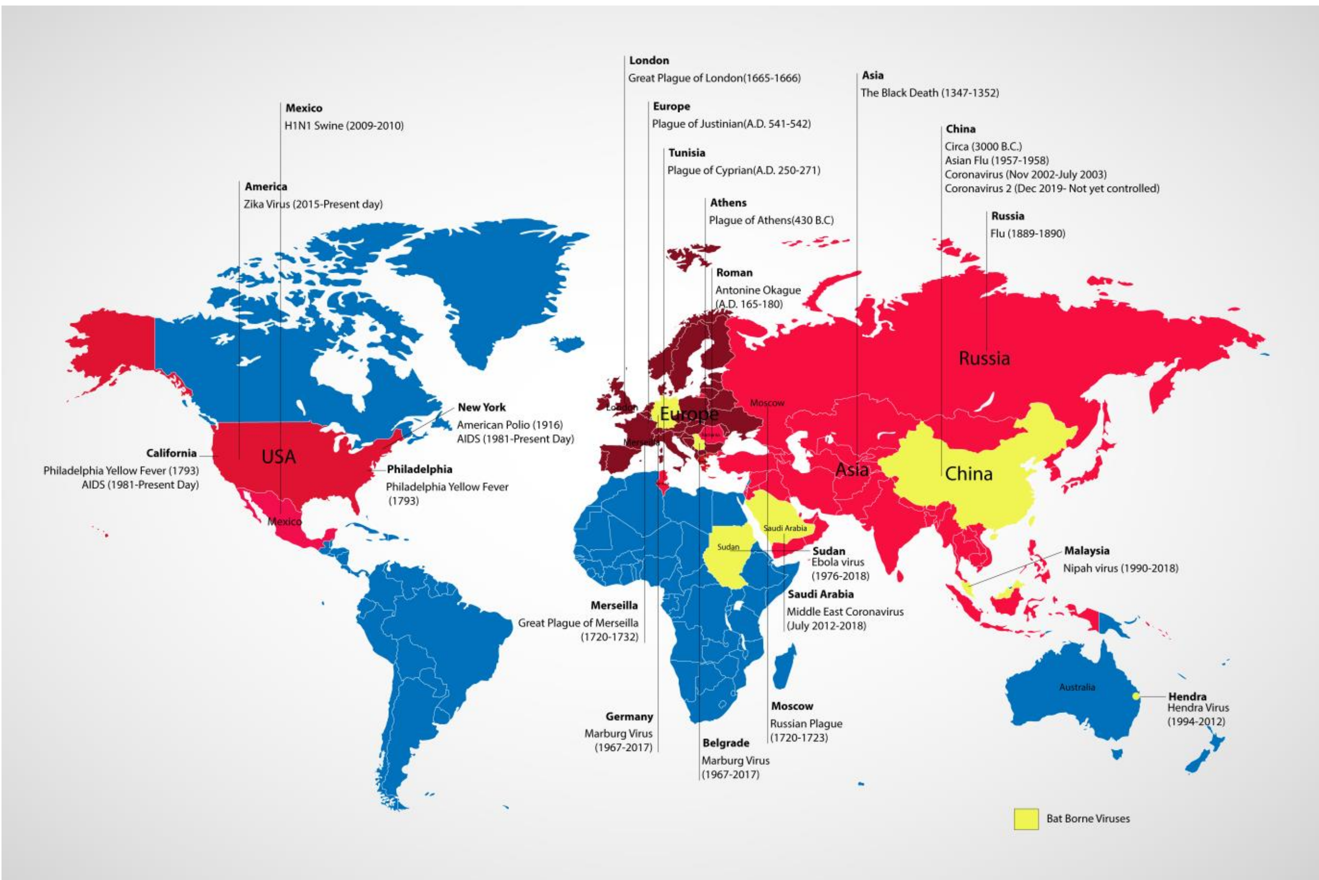

Figure 1: Visual map for the zoonotic spillover

Note: Yellow are representing the bat borne virus in maps

Table 1. A list of bat born dreadful pandemics / epidemics outbreak in human populations all over the world

Virus Name	Origin/ Geographical location	Epidemic potential/ Time period	Target Species /Zoonosis	Symptoms	Incubation Period (Days)	Targeted human's receptor	Causes	Mode of Transmission	Death rate	References
MVD	Marburg and Frankfurt, Germany and Belgrade, Yugoslavia (present-day Serbia)	1967-2017	Bat, Primates, Human	hemorrhagic fever headache chills muscle ache	2-21	TIM-1	Visitor, Experimental study on infected monkeys	Infected Research Areas, Eco-tourism	81%	(Nyakarahuka *et al.*, 2019, Lam *et al.*, 2003, LUBY and SANDERS, 1969, Amman *et al.*, 2012, Sissoko *et al.*, 2017, Timen *et al.*, 2009, Adjemian *et al.*, 2011, Bausch *et al.*, 2003, Beer *et al.*, 1999, WHO, 2018)
EBOV	Sudan	1976-2018	Bat Primates Pigs Human	high fever malaise fatigue and body aches	2-21	TIM-1	Lovers of game meat, Dealing with wild animals	Hunters, Poor medical management	41.7%	(Arunkumar *et al.*, 2019) (Leroy *et al.*, 2009) (McMullan *et al.*, 2019) (Malvy *et al.*, 2019) (Leligdowicz *et al.*, 2016) (WHO, 2020b)
NiV	Malaysia/ Singapore	1990-2018	Bat Pig Human	neurological and respiratory	6-14	EFNB2	Agricultural intensification, Globalized economy	Workers on pig farms, Sale of infected Pigs to another region Persistence of pathogen increased the transmission in pigs and to humans.	39%	(Arunkumar *et al.*, 2019) (Negrete *et al.*, 2005) (Bonaparte *et al.*, 2005) (Arunkumar *et al.*, 2019, Paton *et al.*, 1999, Chua *et al.*, 2000)
HeV	Hendra, Queensland, Australi	1994-2012	Bat Horses Human	influenza-like illness multiorgan failure and	21	EFNB2	Climate change, Habitat loss,	Bats movement in agricultural land Urbanization	57%	(Murray *et al.*, 1995b) (Symons, 2011) (Brearley *et al.*, 2013) (Jones *et al.*, 2013) (Giles *et al.*, 2018)

				progressive encephalitis			Habitat fragmentation			(Walsh *et al.*, 2017b) (Young *et al.*, 1996)
SARS-CoV-1 (SARS)	Guangdong, China	November 2002-July 2003	Bat Civet cat Human	fever malaise myalgia headache diarrhea shivering cough and breath shortness	2-7	ACE2	Hunting, Dealers of wild animals in markets International travel	Butchers, Wildlife animal husbandry, Infected Research Areas	10.9%	(Hui *et al.*, 2020b) (Kakodkar *et al.*, 2020) (Lu *et al.*, 2020) (Chan *et al.*, 2020) (Li *et al.*, 2005) (Webster, 2004) (Guan *et al.*, 2003b) (Lim *et al.*, 2004) (WHO, 2015)
MERS-CoV (MERS)	Saudi Arabia	July 2012-2018	Bat Camel Human	fever cough and shortness of breath common cold chills headache joint pain	5	DPP4	International travel, contact with camels	Consumption of camel meat or milk	35%	(Skariyachan *et al.*, 2019) (Kakodkar *et al.*, 2020) (Zaki *et al.*, 2012) (Wang *et al.*, 2013) (Perlman and Netland, 2009) (Saeed *et al.*, 2017) (Shehata *et al.*, 2016) (Kandeil *et al.*, 2019) (CDC, 2020)
SARS-CoV-2 (COVID-19)	Wuhan, China	December 2019- Not controlled yet	Bat Pangolin Human	fever cough and shortness of breath	2-14	ACE2	sale of bush meat in seafood market	Fecal-oral path, Droplet transmission, Conjunctiva Fomites	4.9%	(Jin *et al.*, 2020) (Borba *et al.*, 2020) (Hui *et al.*, 2020a) (Ong *et al.*, 2020) (Zhang *et al.*, 2020) (WHO, 2020a)

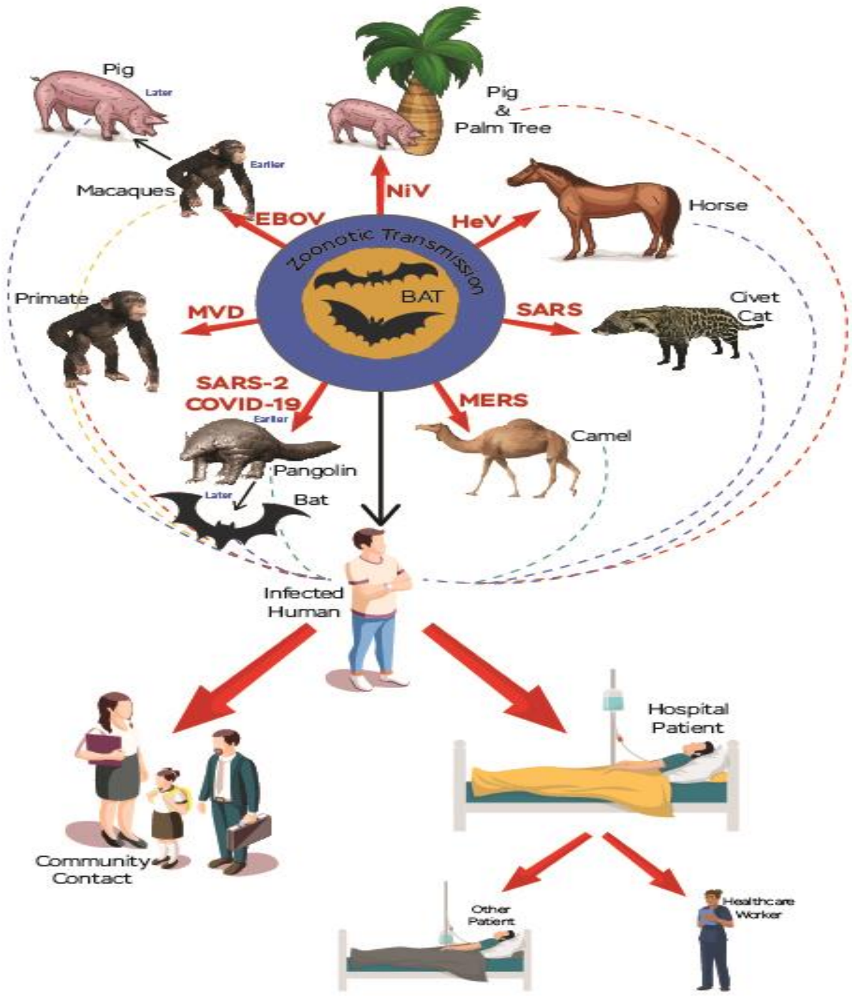

Figure 2: The mode of transmissions of viral pandemics/epidemics to the human population from different reservoirs is represented.

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THE ROLE OF FISH IN GLOBAL FOOD AND NUTRITION SECURITY: CURRENT ASPECTS AND FUTURE PROSPECTS

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ABSTRACT

This article describes the current aspects and future prospect of fish usage in consumer's meals to cover the deficiency of essential fatty acids, vitamins, and minerals, globally. Currently, most of the human population is facing health problems such as cancer, depression, early aging, heart, brain, and autoimmune diseases, etc. due to the lack of essential elements in the routine diet. Fish is blessed as an enriched source of Omega-3, Calcium, Iodine, IL-1, Lysine, Vitamin A, D3, and many more to combat above mentioned problems. Fish is not only a reliable source of income generation from the beginning of the World Era but also provides essential nutrients to the poor community. Traditionally, it is the source of earnings for more than 100 million people and supplies livelihood to 660-820 million people worldwide. It is estimated that fish production and consumption are going to increase from 178.5 to 204.4 million tons and 18.7 kg per capita to 22.5 kg, respectively, during 2018-2030. This review highlights the role of fish in global food and nutrition security by providing protein and essential nutrients to the community to attain the Sustainable Developmental Goals of the United Nations.

1. INTRODUCTION

The global human population will increase from the current 7.8 billion persons to about 9 billion in 2050 mainly due to a rapid population growth rate in developing countries (World Population Clock, 2020). This situation is raising hunger and affecting almost 821 million people, globally (FAO, 2019). Almost 14% of the humans in the world are protein and energy deficient (Béné *et al*., 2015). Therefore, the human population is facing severe problems of food security due to the sudden rise in hunger and malnutrition issues.

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Fish and its foodstuffs are beneficial for protein, fatty acids, and vitamins which play a vital part in the food security and economy of the globe. The Fish and its yield are also helpful in uplifting human livelihood mainly in developing countries (Chan *et al*., 2019). As regards, sixty million people are related to the inland fisheries which sustain almost 12% of the population of the world. In general, nearly 93-97 million people of the world are associated with culturing, capturing, processing, marketing, and retailing of fish (FAO-Food & Nations, 2014). Women are also connected in small-scale processing as well as retailing of fish in local level markets which need less skill and producing a sufficient income for their families (Huntington & Hasan, 2009). Almost 79% of fish were produced by developing countries in 2020.

More than 29,000 fish species are present globally. From which 1500 are recognized by humans. At present, fish production with a major share of aquaculture is rising @1.5% per year. It is not required the land for Fish farming. However, it can be kept in barren areas simply in water ponds which are beneficial for those countries which have less and land with low quality (Martin *et.al*., 2013). Furthermore, recently introduced bio-flock technology for fish farming requires less space and management than conventional farming. Concerning small-scale farmers, it is very important to keep the smaller native breeds besides the carp. It can be noticed that it does not put much trouble. However, the overall economic condition of farmers becomes superior with more revenue. The *Gudusiachapra* is kept with carps in India (Duarah & Mall, 2020). Tilapia spp, *Heterobranchusbodorsalis*, *Clariasgariepinus*, *Mugiespp*, *Chrysichthysnigrodigitatus*, *Heterotisniloticus*, *Ophiocephalusobscure*, *Cyprinuscarpio,* and *Megalospp* are the most common cultured species of fish (Adewuyi *et al*., 2010).

Fish is a cheaper protein source, vitamins, and other important micronutrients than beef or mutton mainly in developing countries (Garcia & Rosenberg, 2010). Fish consumption in a regular diet can accomplish the protein, vitamins, minerals, and some fatty acids deficiencies (Muir, 2013). Its meat is an instant supply of omega-3 unsaturated fatty acids. These FAs are very essential for a strong body (Calder, 2018). A raise in fish eating is a great accomplishment from the last half-century because per capita requirement is rising from 9.9 kg to 18.4 kg from 1960 to 2009, respectively. Fish can be consumed in different preserved forms which are dried, smoked, and powdered forms. These preserved forms of fish can be moved easily to remote areas (Huntington & Hasan, 2009). Fish farming and its products are very helpful which carry out the local market demands if fish farming is done on a small level. Large-scale farming could facilitate exports.

Many by-products are obtained from wild fish processing. These products are being utilized in the feed of livestock and aquaculture. Fish oil and fishmeal like by-products are an easily digestible source of constituents which are adopted in the feed of farmed fish (Sofia, 2018). United Nations (2018) has formed 17 Sustainable Development Goals in the Resolution to transform the world in 2030. From which, three goals such as no poverty, zero hunger; sustainable production, and consumption could be applied to fisheries and aquaculture. This sector can fulfill the nutritional requirement of human being and bring prosperity to the community (Duarah & Mall, 2020). Therefore, this article aimed to review the current aspects and future prospective of fish's role in global food security and nutrition.

Fish production

The current and future expected fish production in 2030 in different regions of the world is shown in Table 1. Fish production in 2018 has reached 179 million tons which is expected to attain 204 million tons in 2030. The share of aquaculture in overall fish production is 46% which is likely to increase 53% in 2030. Asia is the biggest producer of fish in the world that has a share of 19.2%. The number of people who are linked in fisheries and aquaculture can differ by diverse regions of the world. More than 59.5 million people were attached to fisheries and aquaculture in 2018 (Table. 2). Small-scale fisheries can give 90% of the resources in the marine fisheries zone (World Bank, 2012). The highest number of fishers and aquaculture workers are from the Asian region (85% of the world total) and next is Africa (9%). The employment rate is steadily increasing in Africa especially in aquaculture whereas; employment rate is declining in America and Europe.

Current scenario of fish industry

The seafood sector can provide protein and has a significant contribution in supplying food worldwide. In China, the increasing demand for seafood is providing the opportunity for aquaculture and global fishermen to improve their economic status (Batka et al., 2015). The largest growth of shrimp and tilapia aquaculture has been observed in Latin America, Southeast Asia, India, and the Caribbean. Shrimp and salmon aquaculture has significantly increased in the last two decades and marine biologists are also trying to begin the breeding of other species. Several marine fish (domestic) are carnivores and wildlife is needed to be used in their feed to fulfill their feeding requirements for effective breeding.

In the US, the best aquaculture business is the catfish industry and its growth and development lead the business of fish farms to complex and intensive management. The strategies for catfish marketing and agribusiness market are common (Engle, 2003). Fisheries are contributing to the marine system and food security by producing grazing fish. Their function is covered by the myth which is edible fish are not good for the use of humans. Furthermore, policymakers are currently working on the organization of these fishes so that their contribution should be enhanced to food security and economic development. To achieve a balance, consumers and industry play a vital role, which helps in maintaining the aquaculture and animal feed on one hand and help in poverty reduction and food security on other hand (Alder *et al*., 2008). Many research trials related to fisheries are in progress in the upper Mekong region. In this region, fisheries and fish are observed to determine the threats, and found major threat is hydrological changes followed by overfishing and extraterrestrial species introduction. Certain river systems said that the major threat is water pollution to migratory fish of Upper Mekong, but the low-lying areas of Upper Mekong's migratory fish are threatened by dam construction. For the migratory fish, Nanla and Buyuan River are considered as best breeding grounds on a priority basis (Kang *et al*., 2009).

The ecosystem and management practices have affected total fish production. The production of cichlid tilapia in Africa is seven times more than in Asia. There are many negative effects of environments on non-native fish which include increased resource competition, environmental degradation, land grazing, the spread of diseases, and mixing. Keeping in view these factors alone, seldom, it's sufficient to effectively quantify the general impact on the biodiversity which is presented (Gozlan *et al*., 2010). For a very long, the fishery has been a traditional part of families of coastal fishing and is a resource of income for over 100 million people internationally.

The marine ornamental fish industry is also flourishing nowadays. However, its sustainability is in danger due to the exploitation of coral reef areas due to different reasons. Furthermore, due to insensitive shipping and poorer husbandry practices, the over-harvesting of particular organisms and the increase in the level of mortality can badly affect the wild stocks, which warn a burning call to make biologically sustainable management practices for the marine ornamental creature (Madhu *et al*., 2009).

Fine aquaculture is civilized from high-intensity industrial production. It's a need for effective injections to cultivate the Fish at high density and also to prevent chronic and emerging types of diseases. Effective vaccination might have a constructive role in reducing the utilization of antibiotics. It was published that the beginning of vaccines in Norway with Atlantic salmon (Salarsalar) at the end of 80's and the start of 90's decreased the use of an antibiotic (Brudeseth *et al*., 2013). A very much active industry is Atlantic salmon aquaculture in coastal and coastal industries. For the past two decades, the fishery of Lake Victoria has been under increasing pressure. Fish production starts in the early 90s but now the catching of several species is declining. Furthermore, in the export market, the demand for dagaa (Rastrineobolaargentea) and Nile perch (Latesniloticus) fish of Lake Victoria is very high. Due to the dramatic commercial change, the present circumstances exist. It has taken place in Lake Victoria over the past 20 years (Abila, 2003).

Nutritive and health values of fish

Fish is a vital origin of protein, essential fatty acids, vitamins, and minerals. Fish meat is higher in nutrients that are needed to the body such nutrients are absent in the cereal-based diet (Aberoumand, 2012). Fish contain 12-25% protein with a higher biological value. It contains most of the essential amino acids that are very beneficial for human consumption (Vladau *et al*., 2008). Digestibility of fish is higher than the protein of plant sources in addition to this fish helps in the absorption of other sources of protein like maize; its proteins are partially absorbed due to the minute amount of lysine. All the essential amino acids are balanced in fish along with the high level of lysine that's the reason when fish is consumed with plant sources protein, the net intake of protein absorption is higher (Thilsted, 2012).

Eicosatetraenoic acid (EPA) and docosahexaenoic acid (DHA) are the two essential fatty acids that are present in many marines and freshwater fishes (Steffens, 1997). The risk of cardiovascular diseases is a decline by the consumption of fish having an abundant amount of these fatty acids. These essential fatty acids also act against depression, autoimmune diseases, and inflammation through the elevation of IL-1. Fish also protects our body against psoriasis, rheumatoid arthritis, crohn's disease, ulcerative colitis, and so on so forth (Simopoulos, 2002). A long time ago, fish was contemplated as 'brain food', due to the higher mental level of people that consume fish. Many researchers believe that fish helps in brain growth and reproduction. Fish play a vital role in other body functions as well.

One million premature deaths in a year due to deficiency of minerals, and vitamins highlight the need of creating awareness to focus on human nutrition improvement. Fish are an affluent source of iron, zinc, and vitamin A

(Thilsted, 2012). Vitamin A is an excellent antioxidant that helps in relieving stress and chronic diseases (Olson, 1996). Due to nutritive and health benefits, fish consumption has been significantly increased from the last decade. Fish contains DHA, a particular omega-3 fatty acid that stimulates the brain development of infants during the gestation period of their mother (Torpy *et al*., 2006). The demand for fish oil is very high and acquire from the head of tuna fish and used in school feeding meals (Thilsted *et al*., 2014).

In Bombay (India) fish is comparatively cheaper than meat, milk, or eggs. An ascertainment was carried out on 13 domestic species of fresh, salted, and dried fish for contributing to protein and minerals. It is observed that salted and dried fish provide more and good quality protein and minerals than fresh fish, at the same expense. The vitamin A contents of 15 varieties of domestic fish liver oils, estimated in Carr-Price blue units, were all higher than samples of the cod liver oil used in the domestic areas. The component of global seafood production is shellfish. It is the source of polyunsaturated fatty acids, essential amino acids, digestible proteins, bioactive peptides, carotenoids, astaxanthin, vitamins (vitamin B12), minerals (zinc, copper, selenium, potassium, sodium, inorganic phosphate, and iodine), and also other nutrients. However, the health of shellfish can be compromised by some facts like exposure to unwanted environments, false handling practices, unhealthy farming, and several other threats like parasites, pathogenic organisms, heavy metals, biotoxins, industrial and environmental pollutants, allergy-causing compounds, and process-related additives such as bisulfate and antibiotics. Mentioned threats can be prevented by proper precaution from harvesting to the consumption stage. Furthermore, all seafood items including shellfish are properly examined by native public health, international and governmental organizations (Venugopal & Gopakumar, 2017).

The economic potential of fish

Fish significantly pays its role through all forms of life; moreover, it covers earlier forms of life. Now a day fish serves as a significant source of food for people globally. The task of transferring or conveying fishes from their natural habitat to one's plate offers healthy life to humans. Mostly 660 to 820 million people deriving their incomes from the fisheries and aquaculture industry over the globe. In Hindustan, almost 10 million people survive upon fish to fulfill their livelihood of bread and butter (Gogoi, 2015). Currently, the role of aquaculture in total fish production is increasing and playing an important role in the GDP of various countries. Because of increasing in the number of the human race expansion and cessation in the growth of capturing fishes, it is direly needed to enhance the chain of supply from aquaculture to fulfill the livelihood (De Silva & Soto, 2009). Almost 70% of the total produced is analyzed earlier to the trade. Most of the needed ravage 20 to 80 percent is produced which is consumed as fish meal, fish silage, and fish sauce. Fish by-products are valuable sources and can be used as valuable crucial products such as gelatin, collagen, minerals, oils, amino acids, proteins, and so on and so forth.

Usually, duck droppings are used as a substitute for accessory feed which accounts for almost 60 percent of invest in fish culture (Abdel-Hakim *et al*., 2000). Marine fishing has brought revolutionary structural changes in the last few decades. In Albania, fisheries sector comes with regional values though on a national scale, it's not crucial as the Albanian Agriculture sector, however it provides revenues to government administration from license fees and other various taxes. Moreover, it's still ignored with having no supervision though it generates as such as other sectors of Albanian market (Flloko, 2005). Fishes are basic export of Iceland, and it provides almost 33% share in the total export of the country. A few numbers of people are involved as employ in fishing and fish processing. However, the economy is largely unprotected with any change in earnings from fish export (Kristinsson, 1987)
From the coastal shore site of Peru and Cerro Azul, The Fish and mammal bones light monitory quality just before Inca take over in 1470 A.D. with the help of pack llamas, dried and stored fishes were transported (Marcus *et al*., 1999). The definite strength of this sector is 80 to 85 billion dollars yearly. Moreover, with accessible base industry, there is an uncountable number of minor economical moves from boat building to international transport that is being supported by world fisheries. Thereafter these activities are rarely concentrated during evaluating economical crash. North-west Islands of Europe constitute a group called Shetland which financially totally depends on marine-based assets. The employment rate has significantly increased due to fish capturing, processing, packaging, and export activities (Coull, 1996).

Ukrainians have increased fish culture and production by storing the large sized fish seed (fingerlings) and by their use in feeding rice bran and oil cakes (especially groundnut oil cake). The total average share of feed expense was 7%, just which shows poor feed input use in fishponds (Radheyshyam *et al*., 2013). In meanwhile, it can be stated that 60 to 70 percent of their industry is in shade. The fish industry is playing an important role in food security and nutrition, it should be presented with a number of indications to represent or provide agricultural products. The basic theme of showing statically results on bio sources with aboriginal problems and emerging proposals is to give a rising humanitarian block of domestic activity (Radheyshyam *et al*., 2013).

Marine fishing has contributed a lot to uplifting the social and economic growth in various communities throughout the Islands and Highlands of Scotland. Carp fish is one of the primary species that has customary fishing in Israel. Fish in ponds are fertilized and fed with husky grains, oils, and lupins. To rescue health, we should work on agricultural science to develop new methods of farming for friendly environmental fish production. Fish farming with sustainability encloses new millennium, by using hydroponics with agriculture, we can gain a new modern technique such as aquaponics that can help in increase in sustainable agriculture and provide us sustainability to uplift economy with the efficiency of additional production (Blidariu & Grozea, 2011).

Water flow in Pakistan has thousands of aquatic lives; among them, fish has its economic value because of their demand and supply. Magnolia also has about 31 different species with subspecies, they play a pivotal and crucial role in their economy. Most of the landlocked countries are bestowed with lakes; among them, saline and freshwater come in from them they catch their annual fishing. Lack Hoysgol has a catching potential of 200 to 400 tons annually. Years ago, Lake Buyr had the highest fishing capacity but now it has just 130 tons so there is a dire need to increase the modern methodology of aqua-agriculture to increase fish utilization especially in Mongolian territories because their current utilization is very low (1kg per person per year).

Pakistan has grown up in recent years with the fish production capacity of 131,000 tons and two hundred twenty-six million USD value in 2011. Pakistan looks to utilize modern ways for increasing their ways in the export of seafood (Kartika,2014).

Export potential of fish and fish products

Global trading had greatly been affected by the exchange of fish and fish products. A chief part of the fish industry is occupied by tilapia, shrimp, salmon, and catfish (Anderson & Anderson, 2003). In global trading, 37% of total trade is accompanied by fish and fish by-products. This is the largest contribution to the food industry. While other major food items such as meat and milk have 9.8% and 6.7% input in the global market. According to a survey, about 77% of whole seafood produced throughout the globe is exported every year (Tveterås *et al*., 2012). The export of fish has fetched US$77 billion with annual growth of 7% in the last decade. The fish trade has not only expanded in numbers but also in species. Currently, 800 species of fish are traded in 197 countries (Disdier & Marette, 2010).

Developing states of the world make a major contribution to the food industry. Fifty percent of the total seafood exports were made by developing countries in the year 2010. In the same year, about 23% of total foodstuff was imported by these countries (Swartz *et al*., 2010). Future predictions about the fish industry indicate that this business will flourish at slower rates of 3.1% as in 2012 (FAO Outlook, 2013), and is expected to 1.8% in 2022 (Kobayashi *et al*., 2015). Internationally, supply and demand issues were resolved by introducing the gravity law. According to gravity law, “International market exchange is proportional to the outcome of commercial masses of trading countries and is inversely proportional to the terrestrial distances between them” (Stone & Jeon, 1999).

The gravity model was applied to measure the standards of food safety and tax-free exports to the United States of America and Europe (Disdier & Marette, 2010). In the 19th century, Japan had been the biggest exporter in the world, but in the 20th century, the country has imported seafood. China, India, Spain, the US, Canada, Norway, and Pakistan are the main fish trading countries of the world (Kazemi *et al*., 2017). The best strategic action plan of the food industry led China to make 14.1 billion dollars in export since 2002(Mallory, 2018). After China, Norway is the second prime exporter of seafood to the world. Seafood added 11% of the total economy of Norway in 2017 (Gautvedt, 2015). Vietnam has a 3260 km long coastline. This long-range makes the country strong for marine resources. The country has an Exclusive Economic Zone (EEZ) of 1 million km^2. The whole marine stock of the

Vietnam of seafood is 3.1 million metric tons (MT) with an exponential output of 1.4 million MT (Chanpiwat *et al*., 2016). In 2003 marine hunting of the country was about 1.6 million MT. Seafood provides one-tenth of total income drawn from shipping to other countries (Bartley, 2005). Pakistan had also contributed to international trading. Pakistan had exported fisheries foodstuffs of about 131000 tons of 226 million dollars' worth. Although there is great seafood production in advanced countries but developing countries transport about 50% of the seafood to these highly populated developed countries (Bailey *et al*., 2016). Seafood trading network empowers by stockholder'ˢ mutual interference (Zelbst *et al*., 2009). In the international trading of food items, seafood is considered a major component of interest. Developing countries are playing a major role by supplying 50% of seafood items to the world. High dense population and well-established individual economics in developed countries are the major factors of seafood consumption in those countries (Fox *et al*., 2018). The demand and supply chain of the seafood market is being used for future consumer limits, cost, and food security plans for the optimum productive strategies to ensure the food supply to the whole world (Shehata *et al*., 2019).

Future prospects of fish

Scientists and researchers are interested in the supply and demand problem of food items to fulfill the food security and nutritional requirements of the world. No one can deny the importance of fish in human food and therefore scientists are interested in determining its demand and supply. In this scenario, the assumptions are made based on income, population growth rate, and commercial rates of fish and its products. It is estimated that fish consumption will be increased from 18.7kg per capita to 22.5kg per capita for 2015-2030 years (Westlund, 2005).

Culturing of aquatic life is a suitable way to increase fish production without doing any harm to natural resources. According to FAO, aquaculture production is expected to reach 50% of total fish production by the year 2030 (Tidwell & Allan, 2001). Never exhausting mines; the Fishermen are acting as a fuel of the industry. These factors ensure that fishermen have a good impact on future policies regarding fish demand and supply. Effective policymaking in this sector will help in poverty elimination and fisheries industry empowerment (Béné, 2003). Fisheries landings peaked in 1989 at 89.7 million tons and have since fluctuated near this level, which indicates that the world is harvesting fisheries stock close to its maximum yield. Fisheries harvest covers near 69 million tons of human food, so in the years 2000, 2010, and 2025 demand for aquaculture should be near about 22-24, 35-37 and 52-55 million tons, respectively. However, this high demand cannot be achieved by aquaculture with a shortfall ranging from 1-3 million tons in the year 2000 and 9-13 million tons in 2025 (Chamberlain & Rosenthal, 1995).

Now a day, farming of seaweed is also becoming more popular and aquaculture production is also showing progressive growth manner. In both fisheries and aquaculture, the world's most important producer is China. Estimates show that by the year 2025 fish catching may increase about 1% of total fish production. Management will be the most focused factor of the fish stock. It is estimated that by 2025, 84% of total fish harvest will be consumed by human beings. Production of fish cultures has surpassed fishery production in 2014 and will provide 57% of total production by 2025. Growth will be fastest in developing countries (average 9.7%). Consumption will increase up to 8%. However, the trend may show an increase or decline in developed countries. Production from fish cultures will have a major share up to 31 to 41 percent by the end of 2020. The fisheries sector is showing an expansion in terms of production and consumption because of increased population and per capita income growth (Delgado *et al*., 2003). Therefore, an addition of 25 metric tons of fish is expected in the food supply chain.

According to the predicted trends for the future of fisheries by researchers, it is expected that the world will achieve its production target by 2050 by Rio pathways that are given as decentralized solutions, the global technology, and consumption change. The first aim is to focus on energy productiveness in specific areas, natural corridor making agriculture, and policies to make sure the distribution of food items is based on equality. Secondly, there is a special emphasis on the use of technical assistance in agriculture and fisheries to get production. And the third one is directed toward the area of consumption of food by human beings. It is reported that as the trade of fish products progresses the demand for fish is expected to increase. Fisheries are the world's fastest-growing industry. Christpho Bene and his team reported that in 2011, about 173 million tons of fish was harvested from different water ecosystems. They further stated that about 131 million tons of that harvest were used directly by human beings. But the rest of the fish harvest was not used or lost during or in the post-harvest stage. Fish consumption is reported to have increased three folds from 6kg to 18.8kg in 1950 to 2011, respectively in the last fifty years. Trends in the human population growth show that it will exceed 9 billion by 2050. To overcome the issue of food and nutritional security, fish and aquaculture stakeholders must shift their business to a corporate setup. In the current scenario of high population, aquaculture will be the best alternative protein source (Béné *et al*., 2015).

Increasing population and drastically changing climatic conditions harm this industry. These negative effects on industry caused an increase in the number of malnourished children on the planet from 8.5 to 10.13%. Keeping in view the population growth, it is estimated that there will be 76 to 84 million children mal nourished by 2050. Drought conditions are expected from the years 2030 to 2035.Due to global warming, an increase in temperature of 1-2% is expected till 2050 with drastic effects on water resources and crop production. Planned policies are required to cope with the harmful effects of expected environmental conditions (Nelson et al., 2010). Better economic conditions resulted in increased animal-based food consumption in the last five decades (Delgado, 2003). Statistical data of FAO indicates that in the past 54 years there is an overall increase in per capita meat and fish consumption from 23kg per capita to 42kg per capita (Sans & Combris, 2015). Animal origin food consumption is usually considered less flexible in developed countries, while it is much flexible in the case of underdeveloped or developing countries due to much flexible income (Muhammad *et al*., 2011). It is expected that consumption of animal origin products will slow down. Fish and animal meat are expected to be energized by developed countries of the world. In high-ranked economies like China and Brazil, the consumer market is considered to be at its maximum. However, many countries are showing a lower trend in the graph. This lower trend is attributed to the second nutrition transition (Rosegrant *et al*., 2013). An increase in meat and fish consumption in developing countries is expected as there are many gaps if compared with developed countries (Vranken *et al*., 2014).

Halal food certification and future of fish products

In the Quran Allah (S.W.T) said "Eat of that over which the name of Allah has been mentioned, if ye are believers in His revelations" (Quran VI: 119). In Islam consumption of only those aquatic animals is prohibited having poisonous, intoxication, or hazardous effects on health. According to the statement, fish is also halal (Latif, 2011). Fish need to grow on freshwater or saltwater as habitat and natural food for consumption (Naylor *et al*., 2000).

Due to poor education, water scarcity, and increased pollution farms are often contaminated by sewage water, pesticides used on crops (Qiu *et al*., 2017), and industrial wastes (Chanpiwat et al., 2016). This process is contaminating the natural quality of fish meat and its certification as halal is also questionable. Scientists and researchers think that consumption of contaminated fish for a long time is highly dangerous for the body (Virtanen *et al*., 2008). With the increase in population and advancement in technology, Muslim world is also expanding with a global impact. The Muslim population is to be expected 2.2 billion in 2030. Currently, there is 2.3 trillion US$ business of halal products (Wilson, 2014). Improved economic conditions are also increasing the demand for fish due to enhanced per capita income (Wilson, 2014) and recommendations by medical authorities in developed countries (Kris-Etherton et al., 2009). Fish by-products like fish oil and fish meal are being used in paint manufacturing, fertilizer, and animal feed (Abedin, 2018). The Halal research council has the main focus to give the consumer easy access to certified halal products. (Herpandi *et al*., 2011). With the increase in consumption of certified halal products in the next few years, there will be a decrease in consumption of non-food items. The credit of this decline goes to increased fish consumption by humans (Tacon *et al*., 2011).

2. CONCLUSION

The objective of this article was to highlight the importance of fish in the nutritional and food security of the world. Being the important source of protein, essential fatty acids, and minerals, the consumption of fish and its products has been increased. This could be useful to overcome malnutrition in children and women, especially in developing countries. Fish consumption per capita is expected to increase to 21.8kg/capita by the year 2025 with an 8% increased consumption rate but only a 1% increase in production rate. Therefore, modern production technologies should be implemented to cope with increasing demands.

There is a need to design a global food demand and supply chain to fill the gaps between the supply and demand of the essential nutrients. As our resources are squeezing (lands and freshwater resources), we have to pay attention to further strategies to overcome this crisis, especially the countries with more population and less area. Direct light exposure is not compulsory for fish farming. They can be farmed in the shade and deep water tanks. Global warming and water pollution are also having drastic effects on marine and freshwater life. An increase in global temperature causes the melting of glaciers, results in floods, wasting a high volume of freshwater lives including fishes. Better disease prevention and treatment strategies can increase both freshwater and farmed fish production. Breed improvement can also help in disease prevention and in production capacity. Policymakers of the world must take serious measures to minimize the effects of global warming and industrialization. National policies as special subsidies for investors can increase investment and stabilize the industry. Best plans, social awareness about the benefits of fish meat, well-designed strategies, and past experiences would take us toward a bright future in the fish industry.

3. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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Table 1: World Fisheries and aquaculture production: Current aspects and future prospects

Regions	Production			Of which aquaculture		
	2018 (1 000 tonnes)	**2030** (1 000 tonnes)	**Growth of 2030 vs 2018** (%)	**2018** (1 000 tonnes)	**2030** (1 000 tonnes)	**Growth of 2030 vs 2018** (%)
Asia	122 404	145 850	19.2	72 820	96 350	32.3
Africa	12 268	13 820	12.7	2 196	3 249	48.0
Europe	18 102	19 290	6.6	3 075	3 620	17.7
North America	6 536	6 981	6.8	660	838	27.1
Latin America and Caribbean	17 587	16 730	–4.9	3 140	4 170	32.8
Oceania	1 617	1 750	8.2	205	290	41.3
World	178 529	204 421	14.5	82 095	108 517	32.2
Developed countries	29 233	30 730	5.1	4 603	5 499	19.5
Developing countries	135 096	173 691	28.6	73 330	103 018	40.5

Source: FAO. 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome. https://doi.org/10.4060/ca9229en

Table 2: World employment for fishers and fish farmers (thousands) by region

Region	1995	2000	2005	2010	2015	2018
Fisheries and aquaculture						
Asia	31 632	40 434	44 716	49 427	49 969	50 385
Europe	476	783	658	648	453	402
Africa	2 812	3 348	3 925	4 483	5 067	5 407
Americas	2 072	2 239	2 254	2 898	3 193	2 843
Oceania	466	459	466	473	479	473
Total	**37 456**	**47 263**	**52 019**	**57 930**	**59 161**	**59 509**
Fisheries						
Asia	24 205	28 079	29 890	31 517	30 436	30 768
Europe	378	679	558	530	338	272

Africa	2 743	3 247	3 736	4 228	4 712	5 021
Americas	1 793	1 982	2 013	2 562	2 816	2 455
Oceania	460	451	458	467	469	460
Total	**29 579**	**34 439**	**36 655**	**39 305**	**38 771**	**38 976**
Aquaculture						
Asia	7 426	12 355	14 826	17 910	19 533	19 617
Europe	98	104	100	118	115	129
Africa	69	100	189	255	355	386
Americas	279	257	241	336	377	388
Oceania	6	8	8	6	10	12
Total	**7 878**	**12 825**	**15 364**	**18 625**	**20 390**	**20 533**

Source: FAO. 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome. https://doi.org/10.4060/ca9229en

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RECENT ADVANCES IN DAIRY ANIMAL BREEDING AND REPRODUCTION: A REVIEW

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ABSTRACT

This review has highlighted the transformative impact of recent technologies on dairy animal breeding and reproduction. Recent advances in the breeding and reproduction of dairy cows involve the integration of molecular genetics, cytogenetics, and reproductive biology into animal breeding practices. This integration of precision technologies has played a critical role in increasing the efficiency and productivity of breeding programs. The development of modern genetic technologies, such as genome mapping, marker-assisted selection, and transgenesis, has revolutionized the identification of superior genetic traits, contributing to accelerated genetic progress in dairy herds. Furthermore, advancements in reproductive technologies, including artificial insemination, embryo transfer, sperm sexing, and synchronization of estrus and ovulation in dairy cows, have supported the optimization of breeding strategies and facilitated improvements in economically important traits in livestock. Efforts have also been directed toward advancing the early detection of estrus stress in dairy cows using sensitive physiological indicators and sensor technologies, aiming to enhance decision-making in estrus management on dairy farms. In conclusion, recent improvements in the breeding and reproduction of dairy animals have demonstrated significant potential for enhancing reproductive efficiency, profitability, and the quality of milk and milk products. However, these technologies face limited applicability in developing countries due to challenges such as poor infrastructure, low costs, or a lack of human resources. Therefore, it is imperative to develop cost-effective technologies tailored to local and regional contexts, subsequently facilitating their broad dissemination within these regions.

1. INTRODUCTION

Global milk production is expected to rise by 1.6 percent per year, reaching 997 million tons in 2029 (FAO, 2021). In 2021, the total amount of milk produced by cows was 544 million metric tons (Shahbandeh, 2022); herd growth and yield growth contribute to the rise in milk production. Nonetheless, milk production in India, Pakistan, and several African countries that practice grazing-based animal husbandry has risen, mainly because of an increase in herd size rather than an increase in yield (Nimbalkar *et al.*, 2021). This plays a crucial role in mitigating food insecurity in various developing nations, serving as a primary source of both income and sustenance for a significant proportion of the rural population (FAO, 2011). Through the development of dairy farming, better balanced rural economies are possible, as well as reducing poverty, unemployment, and income inequality (FAO, 2009). Moreover, milk produced from small- and large-scale farms contributes to every nation's economy at the micro-level and, consequently, the global economy at the macro level (Nimbalkar *et al.*, 2021). However, this contribution depends on the factors that determine milk production, including feed availability and quality, health, management, and the genetic makeup of the animal, which fits it to a specific production system and the application of new technologies (Kumar, 2017).

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The development and application of these new technologies in breeding and biological techniques designed to increase reproduction and production efficiency through automated heat detection, embryo transfer, superovulation, and synchronization techniques have had a significant impact on the contribution of dairy farming to global economic growth (FAO, 2021). However, these novel farm innovations are suitable only for a particular area, physiological stage of animals, and economically viable options to enhance animal productivity. Therefore, low-cost and user-friendly dairy farming innovations suitable for all kinds of farms, maintained under rural conditions existing in different tropical countries are in need to enhance animal productivity and henceforth farmers' socio-economic welfare. This review aims to provide new insights into the most recent research and technologies for improving dairy animal breeding and reproduction.

Overview of development of global milk production

The global dairy industry is experiencing massive transformation whereas addressing a number of challenges, favorable long-term consumer trends, and otherwise emerging technologies, present a number of opportunities. According to FAOSTAT (2018), more than 270 million dairy cows were inhabiting the world including dual-purpose and 2600 kg/cow/year were the global average milk yield. However, the average milk yield /cow/ year in most of the developing countries was below 6000 kg/cow/year with 87% of the cattle population. On the other hand, 33 countries with about 13 % of the global livestock population produce about 40% of the world's milk production. Ensuring food security through increasing animal productivity is the primary focus of the dairy sector (Clay *et al*., 2020). This will be accomplished through continued selection strategies for milk yield, productivity, and functional traits (e.g. adaptation, welfare, resilience).

The differential selection process was undertaken around 10000 years ago starting from the early stage of cattle domestication and resulted in the development of nearly 1200 cattle breeds (FAO, 2015), with distinct characteristics such as milk yield, milk composition, environmental adaptation, coat color, body size, fertility, and overall resilience. Currently, only three breeds account for 95 percent of high-yielding dairy cows raised in the world's major dairy producing regions: Holstein (or Holstein-Friesian), Jersey, Brown Swiss, and their crosses. The global spread of these few breeds is primarily due to their higher levels of milk production and responsiveness to high-input production systems.

Generally, a continuously growing consumer demand for global milk and milk products, and technological advancement in dairy processing industries with advanced infrastructure to transport and store large amounts of dairy products were the main driven factors for the development of milk productivity which also compels dairy farmers to become more competitive. As a result, many industrialized or developed countries' overall milk yield has been raised, whereas in these countries, the overall number of dairy farms and cows has reduced, and larger herds are becoming less common (e.g., the United States and China; FAOSTAT, 2020).

Biotechnology Options for Improving Livestock Production

The traditional way of livestock productivity is no longer sustainable due to the dramatic growth of population growth and livestock product demand. A new insight that provides new opportunities to enhance livestock productivity in a way that alleviates poverty, improves food security and nutrition and promotes sustainable use of natural resources was mandatory. Therefore, scientists discover novel inventions to alleviate the above-said problems called biotechnology.

In recent years biotechnology is being used primarily for commercial and socioeconomic purposes to improve livestock production and productivity due to the fact that the livestock sector is a pillar of economic growth in developing countries. It creates an unpredicted horizon to improve the productivity of animals through increased growth, carcass quality and reproduction, improved nutrition, and feed utilization also, improved quality and safety of animal feed, improved health and welfare of animals, and reduced waste through more efficient utilization of resources (Roland, 2013).

Animal breeding and genetics

A significant and persistent genetic improvement has been made in livestock over the past several decades (Hill & Bunger 2004; Hill 2008). In recent years, the focus of animal breeding has rapidly shifted from short-term production goals to animal functionality, cost reduction, consumer perception, and product quality, resulting in overall animal production sustainability and long-term economic returns. Genetic selection for higher milk yield has been a key driver of dairy intensification, resulting in the development of highly specialized milk production systems with increasing herd size and a heavy reliance on cereals and protein sources (FAO, 2006).

Despite significant signs of productivity progress, the dairy industry's long-term success is dependent on the adoption of more sustainable breeding goals and management practices, particularly from an agroecological standpoint (Phocas *et al*., 2016). Current high-producing systems must be refined to place a greater emphasis on animal health and welfare, environmental efficiency, climatic adaptation, and increased preparedness for future challenges through the preservation of a diverse genetic pool. Several of these traits have recently been included in the breeding goals of some breeding programs, but there is still room for significant improvement.

Moreover, milk production and composition have been the primary selection goals in dairy cattle breeding programs for centuries (Miglior *et al*., 2017), and as a result, milk yield has increased dramatically. Economically, the success of selection for improved milk yield or feed efficiency in high-producing dairy cows is primarily attributable to reduced maintenance requirements as production levels increase (Brito *et al*., 2020a). The economic return from increased milk yield has been the primary driver of continued genetic selection for higher milk yield. In addition, increased milk yield is frequently regarded as a critical solution to the global challenges of ensuring food security and reducing greenhouse gas emissions, as dilution of maintenance results in both improved feed efficiency and lower methane emissions per kg of milk produced (Capper *et al*., 2009).

Furthermore, temperament (and other behavioral traits), physical and anatomical variables (e.g., coat color, body size), and milk production have been the key traits under artificial selection (primarily based on phenotypic performance) since domestication. The array of traits targeted for improvement has expanded significantly over the last five to six decades as a response to the dynamic requirements of dairy producers, consumers, and society in general, thanks to methodological advances in the areas of quantitative genetics, animal breeding, and phonemics. As multi-trait selection became the norm in dairy breeding programs, the development of selection indexes became critical in balancing each individual's genetic merit for each trait under selection, based on economic value or desired genetic gains (Byrne *et al*., 2016; Cole & Van Raden, 2018).

Animal breeding uses genetic marker technologies

Traditionally, animal breeding programs rely solely on phenotypes and pedigrees, whereas recent advances in molecular genetics and statistical methods for quantitative trait loci (QTL) mapping have made it possible to identify genetic factors that influence economically important traits. It is also important to identify genetic regions influencing phenotypic variation of complex traits through genetic interactions and environmental factors. These advancements have the potential to significantly increase the genetic improvement of livestock species through the use of MAS of specific loci, genomic sequence selection, gene introgression, and positional cloning (Aguet *et al*., 2023). The development and application of QTL mapping allow marker-assisted selection (MAS) (targeting specific loci for genetic improvement), genomic sequence selection (utilizing genomic information for trait enhancement), gene introgression (incorporating beneficial genes into breeding programs), and positional cloning (identifying and isolating genes of interest). The incorporation of detected QTL into genetic evaluation has the potential to improve selection accuracies, thereby expediting the genetic improvement of animal productivity (Jiang *et al*., 2010).

Marker-assisted selections (MAS) and gene-assisted selections (GAS)

The process of genetic improvement involves selecting outstanding individuals from a population to produce higher yields in future generations. Long-term genetic evaluations were used by dairy animal breeders to identify superior animals. The use of these animals improved phenotypic measures for milk production and milk components, especially in Holstein cattle. However, there are some drawbacks to choosing based on predicted breeding values. This method of selection has a limited ability to improve low-heritable traits without negatively affecting production. These traits are frequently associated with disease resistance, reproduction, productive life duration, and some conformational traits associated with fitness (Sonstegard *et al*., 2001).

Moreover, the evolution of intensive dairy systems has been fueled by a steady stream of innovations and technological breakthroughs, with conventional genetic selection playing a significant role in recent decades (Miglior *et al*., 2017). Animal breeding and genetics have been extensively conceptualized in artificial and standardized environments, where the linear equation: P (observed phenotype/performance) = G (additive genetic merit) + E (environmental effects) proved to be extremely efficient, particularly under controlled environmental conditions and high-input production systems.

In addition to genetic selection, the dairy industry has benefited from significant advances in nutritional practices, precision management, widespread adoption of reproductive technologies (e.g., artificial insemination, embryo transfer, sexed sperm), and precision health care and management. There is no doubt that many of these advancements have enhanced the effectiveness of genetic selection to increase productivity. Therefore, selecting a high-producing dairy cow is more than just the result of high genetic merit for key biological mechanisms and appropriate environmental factors; it also reflects complex positive feedback between these two components that occurred during the industrialization and intensification of dairy production.

Transgenes of Dairy animals

There are several applications for producing transgenic farm animals with exogenous DNA stably incorporated into their genome so that the 'transgene' is transmitted to offspring in a Mendelian fashion. In addition to the obvious scientific interest in studying genes and their regulation, transgenic animal technologies have been proposed to accelerate livestock improvement by introducing new genes or modifying the expression of endogenous genes that regulate economically important traits such as milk production traits (Wheeler, 2003). The ability to insert genes into livestock embryos, incorporate those genes, and ensure their stable transmission into the genome of the offspring will allow major genetic advances in animal agriculture to be realized. Genes that increase productivity (milk yield) or reduce costs (disease resistance) are most likely to be found in the species in question. If a gene is well characterized enough to be used in transgenesis, it will also be possible to genetically characterize individuals carrying the gene, making direct selection and propagation highly efficient. The majority of focus in dairy animals has been on genes that alter fat or protein synthesis in the mammary gland (Naqvi, 2007). This technology has been utilized to produce transgenic goats for the production of recombinant human protein therapeutics in their milk, achieved by introducing and stably integrating an engineered piece of DNA into the animal's genome, thereby directing the expression of the recombinant protein in the milk during the lactation period (Gavin *et al*., 2018).

Furthermore, research has focused on the development of a universal gene knock-in strategy for mammary gland-specific expression of recombinant proteins in dairy cattle, aiming to overcome the limitations of random transgene integration and subsequent epigenetic silencing (Lee *et al*., 2014). Additionally, transgenic goats have been engineered for the production of biosimilar antibodies in their milk, demonstrating the potential for using transgenic dairy animals as a production platform for biopharmaceuticals (Laible *et al.,* 2013). These applications highlight the diverse uses of transgenic technology in dairy animals, ranging from the modification of milk composition to the production of valuable recombinant proteins.

Progeny testing

The practical and best techniques, the performance of sires are evaluated based on their daughters' performance. AI services are delivered in a certain place for a particular breed in its native tract, and progeny produced in this way is evaluated for their performance. Progeny testing is practical and the best option for achieving genetic improvement in that breed (Vidya Nimbalkar *et al*., 2021). The effectiveness of progeny testing has been enhanced through the incorporation of genomic pre-selection (GS-PT), which allows for the selection of sires based on their estimated breeding values (EBV) and the performance of their progeny (Yamazaki *et al*., 2014).

The advantages of GS-PT over traditional progeny testing (PT) include increased annual genetic gain and more accurate selection of sires with higher heritability (Yamazaki *et al*., 2014). Some benefits of progeny testing in dairy animals include:

1. Identifying superior genetics: Progeny testing helps identify bulls with high genetic merit for various traits, such as milk production, quality, and health.
2. Genetic improvement: The evaluation of bulls through progeny testing has been a major source of genetic improvement in dairy animals.
3. Large-scale implementation: Progeny testing programs can be extended to farmers' herds, where large numbers of daughters per bull can be produced and recorded, increasing the accuracy of the selection process (Das *et al*., 2014).
4. Combining genomic pre-selection with progeny testing: The incorporation of genomic pre-selection into dairy cattle progeny testing has shown to increase annual genetic gain compared to traditional progeny testing (Yamazaki *et al*., 2014).

Overall, progeny testing in dairy animals is a valuable tool for evaluating and selecting superior genetics, leading to improvements in the performance and traits of dairy animals. The integration of genomic pre-selection and progeny testing has further enhanced the effectiveness of this method, allowing for more accurate and efficient selection of sires with high genetic merit.

Molecular marker-assisted introgression (MAI)

Molecular marker-assisted introgression (MAI) is a breeding technique that utilizes molecular markers to identify and select desirable traits in dairy animals. This technique involves the identification of molecular markers linked to specific traits of interest, such as milk production or disease resistance, and the use of these markers to select animals with the desired traits for breeding (Hariyono *et al*., 2022). Those markers are used to help livestock breeders choose individuals who express the introgressed gene. In conventional breeding, a series of backcrosses to the recipient parent is usually performed. However, the use of molecular markers shortens the time and cost for used backcrossing cycles required to select and identify the desired individual. It is also increasing the efficiency of breeding programs to improve livestock traits such as growth, meat quality, wool quality, milk production and quality, and disease resistance (OMIA, nd).

Additionally, MAI has been used to introgress desirable traits from wild or exotic breeds into domesticated dairy animals, resulting in improved performance and adaptation to changing environmental conditions for example, the use of molecular markers has been shown to be effective in selecting thermo-tolerant animals in dairy cattle breeding, allowing for the selection of superior thermo-tolerant animals through marker-assisted selection (MAS) (Hariyono *et al*., 2022).

In general, the use of molecular marker-assisted introgression in dairy animal breeding has shown promise in improving the efficiency and effectiveness of breeding programs, leading to improved performance and adaptation to changing environmental conditions.

Screening for undesirable genes

Screening for undesirable genes in dairy animals is essential for maintaining the health and performance of the herd. Some examples of undesirable genes in dairy animals include those associated with genetic disorders, such as Deficiency of Uridine Monophosphate Synthase (DUMPS), Factor XI deficiency (FXI), and Complex Vertebral Malformation (CVM) (Ghanem & Nishibori, 2008). These genetic disorders can lead to poor production and reproduction performance, structural unsoundness, and other health issues in dairy animals (Magotra *et al*., 2020). Molecular markers can be used to trace and document genetic diseases and physical defects in livestock animals. The genetic changes and DNA mutations that manifest in protein structure and function are easily traced as the cause and origin of these problems (Womack, 2005).

To screen for undesirable genes in dairy animals, the following methods and techniques can be employed:

- **Genetic testing**: Advanced genetic testing technologies, such as polymerase chain reaction (PCR) and DNA sequencing, can be used to detect the presence of specific mutations or genetic markers associated with undesirable traits.
- **Phenotypic assessment:** Visual observation and evaluation of the animal's physical appearance, behavior, and performance can help identify signs of genetic disorders or other undesirable traits.
- **Pedigree analysis:** Analyzing the pedigree of animals can help identify potential carriers of undesirable genes, as well as plan for future breeding strategies to minimize the risk of passing on these traits to offspring.
- **Progeny testing:** As mentioned earlier, progeny testing can be used to evaluate the genetic potential of animals, particularly bulls, for their performance and traits. By evaluating the offspring's performance, the bull's genetic merit can be assessed, and the presence of undesirable genes can be identified (Magotra *et al*., 2020).

Then after, animals carrying defective genes are easily identified using DNA testing and are culled from the livestock breeding program.

Generally, intensive selection for production traits, combined with the intensification of dairy cattle production systems, has resulted in animals that are more susceptible to behavioral, physiological, and immunological disorders. The discovery of precise technologies to measure novel traits like resilience, welfare, and environmental efficiency used across multiple production systems has a significant impact.

Advancement in dairy animal Reproduction

The goals of using reproductive biotechnologies in livestock are to increase output, improve reproductive efficiency, and genetic improvement rates. Many options for managing the reproduction of small and large ruminants have become available over the years (Said *et al*., 2020). The main technologies that are widely used are artificial insemination (AI) and sperm preservation. Assessing sperm fertilization capacity, sexing sperm, synchronization and fixed-time insemination, superovulation, embryo transfer (ET), and in vitro embryo production (IVEP) are other techniques that can improve reproductive and pregnancy rates. Molecular DNA markers can also be used to improve genetics through marker-assisted selection (MAS), as well as to characterize and conserve an animal's genetic resources.

Estrus synchronization

Estrus synchronization is a technique by which most of the females in a population or a herd can be brought into estrus at a predetermined time. Recent advancements in estrus synchronization in dairy animals have improved the efficiency and effectiveness of breeding programs. These programs have become standard components in the current breeding management of cows in most dairy industries, and many are based on protocols that allow timed inseminations (TAI) to overcome the practical difficulties associated with estrus detection (Channo *et al*., 2021; Macmillan, 2010). These difficulties are exacerbated in modern herds of high-producing cows due to increasing herd size, individual animal monitoring difficulties, and often subjective observations (Macmillan, 2010).

The major limitation of estrus-synchronization programs is their inability to induce potentially fertile estrus and ovulation in non-cycling cattle (i.e., prepubertal heifers and anoestrous suckling cattle). However, the most recently developed programs include protocols for resynchronization following first or subsequent inseminations, which may involve selected forms of hormonal intervention during the diestrus and pro-estrous periods following TAI or following pregnancy diagnosis by ultrasound from 28 days after TAI (Macmillan, 2010). PGF2α, GnRH, and Progestin are the most important Hormones for estrus synchronization. Heifers respond extremely well to the MGA/PGF2α system (Amare & Ayalew 2021).

Overall, recent advancements in estrus synchronization have improved the accuracy and efficiency of breeding programs, leading to improved reproductive efficiency and genetic selection in dairy animals. It also provides significant economic benefits for dairy farmers and breeders through reduced labor costs, improved pregnancy rates, cost savings on feed and housing, and increased profitability.

Artificial insemination (AI)

Artificial insemination is the most widely used biotechnology in livestock, particularly in cattle production to deposit a proven sire's stored sperm directly into a cow's uterus (Vidya Nimbalkar *et al*., 2021). It is still considered to be one of the most important assisted reproductive technologies (Jacquelyn & Laura, 2008). The technique is used to improve desired characteristics quickly through intensive genetic selection. This innovative technique has achieved benefits such as facilitating the use of superior quality semen without the expense and risk of sire ownership; and lowering the risk of introducing venereal diseases into the herd (Vidya Nimbalkar *et al*., 2021). It also eliminates the need to keep a bull for natural service and aids in the utilization of excellent germplasm up to the fullest extent. Because AI is simple, inexpensive, the quickest, and most effective method of breeding, developing countries such as India could become the world's top milk-producing country. Moreover, about 90% of genetic improvement in a commercial herd is dependent on AI. Furthermore, the sperm from the superior sire can be preserved for a long period by the technique of sperm cryopreservation in liquid nitrogen temperatures at -196°C. However, the viability of sperm is critical to the success of AI (Jindal & Sharma 2010).

Embryo transfer and multiple ovulations (MOET)

Embryo transfer technology (ETT) is one of the most recent tools available for faster livestock improvement worldwide, particularly for exploiting the genetic potential of high-quality females and males at the same time (Said *et al*., 2020). It is the process of implanting embryos in the uterus of a female (cow) to establish a pregnancy. The process involves three steps: superovulation with follicle-stimulating hormones, embryo collection surgically or nonsurgical, and embryo transfer. Prior to the development of this technology, a superior/high milk-producing cow could only produce a limited number of offspring in her lifetime. Higher technology costs and a low conception rate may be factors limiting its implementation (Vidya Nimbalkar *et al*., 2021). The benefits of embryo transfer include breed preservation and conservation, disease-free herd creation, economical livestock transport, and rapid multiplication of elite female breeding stock.

Furthermore, various hormone protocols are being used to achieve group calving or desired calving in a year. MOET has the potential for genetic improvement by increasing the selection intensity on the female by increasing the number of calves, either male or female, from genetically superior donors. The application of MOET can also lead to increased reproductive capability and ability of precious or valuable animals, as well as an increase in the percentage of genetic improvements in the herd (Faizah *et al*., 2018). It can also be used to screen bulls for inherited defects.

Embryo splitting

Embryo splitting is the artificial microsurgical splitting of an embryo which results in the formation of twins or multiples. It is used to increase the rate of genetic improvement in dairy cattle through embryo transfer and splitting. The genetically identical embryos can continue to develop after separation. Before embryos are transferred to a surrogate female, the morale or blastocyst stages can be cut into two equal halves using an inverted microscope connected to a micromanipulator and a microsurgical knife. This method can produce genetically identical animals. This procedure appears to mimic the natural process of producing monozygotic twins (Said *et al*., 2020). Embryo splitting is used to increase the rate of genetic improvement in dairy cattle through embryo transfer and splitting.

In general, the use of embryo splitting, and transfer has been integrated into dairy cattle improvement programs, offering advantages such as increased genetic gains and greater control over recording, breeding, and selection, leading to a larger proportion of the possible genetic gains being realized in practice. Additionally, embryo manipulation and gene transfer technologies have had a major impact on genetic strategies in animal production, allowing for advancements such as embryo splitting producing monozygotic twins, in vitro fertilization, and chimera production of tetra parental animals. The importance of embryo splitting in dairy animal breeding lies in its potential to increase the rates of genetic improvement, enhance control over genetic strategies, and facilitate the production of transgenic animals for the purpose of generating valuable proteins in the milk of dairy animals.

Sperm sexing

In dairy farming farmer's desire is to get female calves, sperm sexing is a guarantee for them. The 'Y' chromosomes in sperm cells are removed through the sorting process; sexed semen predominant with 'X' chromosomes can ensure the birth of a female calf. The application of this technology is popular among dairy farmers are a reduction in economic burden and the production of a greater number of female calves as future productive cattle. There have been advancements in the development of cost-effective indigenous sperm sexing techniques, such as microfluidics and bio electromechanical systems (BEMS), which may hold the key to the development of a portable device for semen sexing with minimal tampering of the sperm structure (Rai, 2018) (Figur 1). Although this technology has been used on a variety of species, the vast majority of pregnancies have been in cattle, almost entirely as a result of sexed sperm that has been frozen (Seidel, 2007).

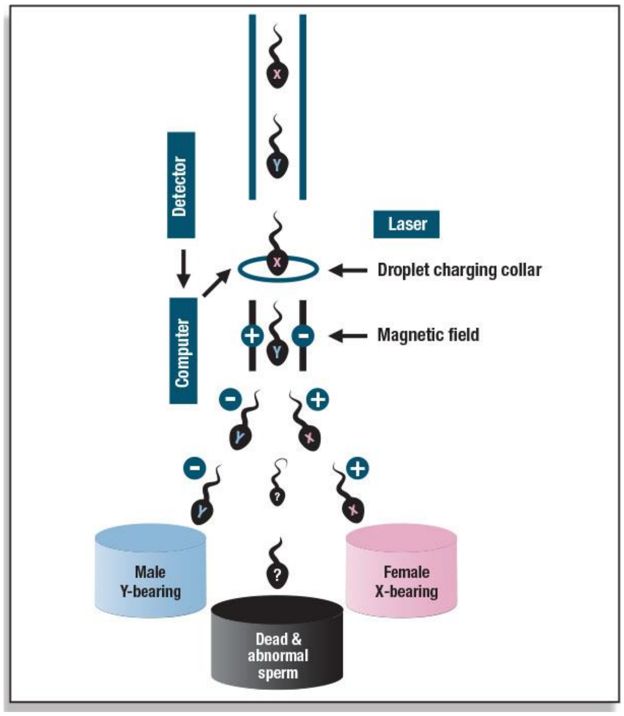
The use of sperm sexing in dairy animal breeding offers several advantages this includes.

1. Improved productivity: By producing more female dairy animals, sperm sexing can help increase the productivity of dairy herds, leading to higher milk yields and better economic returns for dairy farmers.
2. Cost-effective approach: Sperm sexing is a cost-effective approach for achieving sex pre-selection, as it can lead to more female dairy animals, which are more valuable for dairy farmers, while male dairy crossbred animals are more valuable for beef production.
3. Genetic improvement: Sperm sexing can help improve the genetic makeup of dairy herds by focusing on the production of female dairy animals, which are more valuable for dairy farmers.
4. Prevention of genetic disorders: Pre-implantation sexing of embryos can help diagnose genetic disorders at the prenatal stage, benefiting both the management, production, and breeding programs of livestock and the diagnosis of genetic disorders.

Although the evolution of sexed semen in dairy animal breeding has been marked by significant technological advancements and a growing recognition of its economic and practical applications, Vidya Nimbalkar *et al.* (2021) states that the higher cost of semen, combined with the low conception rate, are important factors to consider before using it, especially in heifers or primiparous animals. In addition, the technique does not involve any genetic modification or manipulation, and therefore, it does not have a significant impact on genetic diversity.

In general, it enables the production of more female dairy animals, leading to increased productivity and better economic returns for dairy farmers and breeders.

Figure 1 Semen sorting process



Embryonic stem cells

Embryonic stem cells (ESCs) are stem cells derived from the donor mother animal's undifferentiated inner cell mass of an embryo. Stem cells are pluripotent cells that can self-replicate and develop into specialized cells. It can be found at various stages of fetal development and in a variety of adult tissues. In the laboratory, stem cells are manipulated to accept new genes, which can then change their behavior. This procedure entails removing the donor mother's ovaries and dosing her with progesterone, which alters the hormone environment and allows the embryos to remain free in the uterus. The embryos are harvested after 4–6 days of intrauterine culture and grown in vitro until the inner cell mass forms egg cylinder-like structures (Said *et al.*, 2020). Moreover, the use of embryonic stem cells allows for the direct manipulation of the germline, making it possible to add new milk-protein genes to dairy animals and remove or replace endogenous genes (John & Clark, 2005).

Recent advancements in transgenic technology have made it possible to produce transgenic large domestic animals, such as sheep and cattle, through nuclear transfer, which opens up a means of ruminant transgenic production with an efficiency that entitles us to consider it a serious alternative to microinjection. Additionally, the isolation and culture of spermatogonial stem cells (SSCs) from cryopreserved dairy goat testicular tissues have been explored as an efficient procedure for cryopreserving dairy goat testis tissue, offering an efficient way to preserve SSCs for infertility and rare animals and eminent livestock (Ze-hua, 2011). The use of embryonic stem cells in dairy animal breeding has shown promise in enhancing milk protein properties and improving genetic selection, leading to improved productivity and economic returns for dairy farmers and breeders.

In general, improvements in reproductive performance had the largest influence on revenues followed by energy efficiency and then by disease reduction. The adoption of reproductive technologies has a great impact on the incidence of ketosis, milk fever, conception rates at first service, metritis on days open, unrealized milk, veterinary costs, labor, and discarded milk.

Application of biotech in Ethiopia

Ethiopia has an abundance of fauna and flora genetic diversity, but its biotechnology facilities lack precise information about their capabilities, capacities, and associated technical and administrative gaps. In most cases, biotech equipment fails to operate due to either a lack of skilled technicians or an inability to maintain it. In light of the foregoing, the Ethiopian government has designated biotechnology as a critical science that requires special attention and support to sustain the country's rapid economic growth. Biotechnology is used as critical support for GT-I and II, to drive economic and social development and achieve middle-income status by 2025 (Abu & Rachel, 2018).

Moreover, information from different literature shows that seven institutions with a various developmental stage, primarily tissue culture, but also bio-fertilizers, molecular markers, embryo transfer, immunology, vaccine, diagnostic kit development, and epidemiology. Ten centers have laboratories that range from basic to well-equipped, and a few more are in the process of being built. In general, the future success of biotechnological research and development in Ethiopia is dependent on the government's attention to capacity building and the level of collaboration among institutions (Dereje, 2011). To exploit the indigenous livestock's genetic potential the Ethiopian government planned and implement the following applied research.

- Detection of selected signatures for trypano-tolerance traits/genes in Ethiopia cattle.
- Genome-wide association studies for egg production, egg quality, and natural antibody (NAB) traits in indigenous chicken in Ethiopia.
- Harnessing fecundity and muscle growth gene to improve the productivity of indigenous sheep in Ethiopia using a genomic approach.
- Other related animal biotechnology research includes the development of vaccines and diagnostic kits (Abu & Rachel, 2018).

2. CONCLUSION

The demand for milk and products dramatically increased as a result of rapid population growth and income growth, however, the resources are limited, and building sustainable food production is a global challenge. The application of biotechnology in the dairy sector plays a significant role in solving problems in the areas of breeding, reproduction, management, diseases and milking to meet the growing demand for milk and products. The use of genomic selection techniques has proven to be a major breakthrough, enabling accurate prediction of genetic performance at early life stages. This not only accelerates the pace of genetic improvement, but also minimizes the generation interval, accelerating the overall progress of dairy herds. Moreover, advanced reproductive technologies such as sexed semen and in vitro fertilization have given dairy farmers greater control over breeding, thereby optimizing the production of desirable traits. It has been proven that the integration of these cutting-edge technologies in breeding and reproduction has led to significant improvements in the precision and efficiency of breeding programs, which in turn have led to sustainable and economically viable strategies.

Even though recent advances in dairy cow breeding and reproduction have introduced a new era in dairy farming, enabling higher productivity and genetic advantages for dairy farmers, these advanced technologies have been primarily used in developed countries such as the United States, Brazil, Europe, and others due to a lack of infrastructure, technical and educational capacity, and economic factors. Therefore, there is a need to develop local and region-specific technologies and disseminate them in similar socio-geographical regions to build sustainable dairy sectors in these nations. In addition, the following issues should be addressed to improve the dairy sector in developing countries.

1. Government intervention provided a pillow for rather soft budgeting of farms with dairy production,
2. The mobilization of research, knowledge transfer and innovation became one of the priority areas for government organizations, research centers, NGOs,

and Universities in developing countries like Ethiopia.

3. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

4. DEDICATION

This paper is dedicated to the late researcher and advisor, Dr. Moges Derege.

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A ZOONOTIC TREMATODE INFECTION IN AN IMPORTED GOLDFISH, *CARASSIUS AURATUS*

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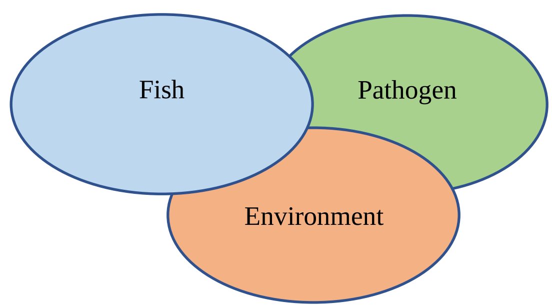
Centrocestus formosans, Dactylogyrus, ichthyobodo necatrix, pathogen, FAO,

ABSTRACT

The study was conducted for evaluation of parasite in goldfish and rainbow shark. Fifty fish samples goldfish (n= 40) were examined. Different parasites were recorded in goldfish such as *Dactylogyrus* sp, *Centrocestus formosans,* and unidentified metacerceria, *Gyrodactylus, ichthyobodo necatrix and* tetrahymena. In 38 fishes out of forty in goldfish. A total of 7,407 parasites were recorded. Most of the parasites were at the tips and middle part of the gills. The infection level having prevalence in goldfish was *Centrocestus formosans 87.5%, Dactylogyrous* sp. was 95%, gyrodactylus was 01%, *Ichthyobodo necatrix* also called costia was 05%, tetrahymena 2.5%. Experiments were conducted in June to August 2018, and prevalence of parasites was compared with Annahita (2017) that primarily focus on prevalence of *Centrocestus formosans.*

1. INTRODUCTION

Fish is a viral source of food for people. It Contain high quality protein. According to Food and agriculture organization (FAO) of the United Nation (1997), it provides ~16% of animal protein consumed by world's population. The FAO estimates that one billion people worldwide rely on fish as a primary animal protein source (FAO, 2000). Fish has high-quality protein and low fat. Animals kept in aquariums that include fish species, mollusks such as snails, clams, invertebrates for example corals live rocks etc. are called ornamental fish (Livengood & Chapman, 2009). The most popular ornamental fish species**.** Goldfish is a freshwater ornamental fish in family Cyprinidae of order Cypriniformes.it is the most common fish kept in aquarium. Disease is a result of the complex interaction between the host, the pathogen and the environment (Snieszko, 1974). Ornamental fishes have extensive connection with different pathogenic and nonpathogenic environment (Snieszko, 1974)



microorganism resulting a huge economic loss (Aly *et al.*, 2008). But among all the factors parasites are most impactful issue with major sign of disruption in weight loss, reproduction capacity, blindness, abnormal behavior, epithelial lesions, deformation of gills that result in economic loss in aquaculture industry (Jaberi *et al.*, 2016).

There are serval kind of fish diseases including.

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Viral Diseases, Bacterial Diseases, Fungal Diseases Parasitic Diseases

The occurrence of parasites on ornamental fishes and their transport to other countries has been reported worldwide; in Germany (Moravec *et al.,* 1994), Australia (Evans & Lester, 2001), Korea (Kim *et al.,* 2002), Sri Lanka (Thilakaratne *et al.,* 2003), Norway (Levsen *et al.,* 2003), in Brazil (Pizza *et al.,* 2005; Tavares-Dias *et al.,* 2010) and Pakistan (Iqbal *et al.,* 2014). Parasites on freshwater fishes are primarily protozoans, myxozoans, helminthes and crustacean. Several species of parasites affect marine and freshwater species of fish. These agents include protozoa (microscopic one-celled organisms), trematodes (flukes/monogenean/digenean parasites), cestodes (tapeworms), nematodes (roundworms), acanthocephalans (thorny-headed worms), and crustaceans (a large class of creatures with hard outer shells, such as lobster, crab, shrimp, woodlice, water fleas, barnacles, etc.). A number of those parasites that affect are the following.

A) Argulosis
B) Lerneacyprincea
C) Ichtyopthiariasis:
D)Trichodiniasis
F) Costia
G) Centrocestiasis:
H) Acanthocephalan infection:
I) Helminthiasis:
J) Gyrodactylosis:
K) Dactylogyrosis:

2. MATERIALS AND METHODS

The present study was conducted in the zoological lab department of zoology, university of Punjab. Fifty imported ornamental fish goldfish (n=40) were bought from three different ornamental fish shops located in Lahore (Pakistan). Morphometric measurements such as body depth (B. D), total length (T. L), Standard length (S. L), focal length (F. L) and body weight (B.W) of experimental fish specimens were calculated. Sterilized magnifying glass was used to scan their body thoroughly to identify any abnormalities or infection. Gills, fins and skin seemed to be infested by parasites. Wet mount was prepared to determine infection level organs and pathological observations were recorded. Each body part of ornamental fish was observed carefully with magnifying glass Dorsal fin, caudal fin, anal fin, pectoral fin, pelvic fin, gills scales, skin mucus and eyes from each fish were examined. By examining lesions, wounds or any other clinical symptom, parasitic infection was carefully calculated. Parasites that can be observed with naked eye such as Argulus species were recorded and their position on mount and number was noted. Skin mucus was taken to make smear for observation under light microscope. Fins were removed from the body and placed on slides to examine any parasitic infection under a light microscope. Gill flaps from both sides of fish samples were separated and placed on glass slides to observe presence of pathological infection. At different magnifications (4X, 10X, 40X), Photographs of parasitic infection were captured using digital camera. (Hossain *et al.*, 2007) protocol was followed for histopathological study of gills. Statistical analysis was followed by (Margolis *et al.* 1982). Mean intensity is calculated by formula.

Number of parasites counted ÷Total number of fish host infected.

Abundance of parasites is calculated by the following.

Number of parasites counted ÷Total number of fish host examined.

3. RESULTS AND DISCUSSION

The study was conducted to parasitic evaluation for parasites in goldfish and rainbow sharks. Fifty fish samples goldfish (n= 40) were examined. Different parasites were recorded in goldfish such as *Dactylogyrus* sp, *Centrocestus formosans,* and unidentified metacerceria, *Gyrodactylus, ichthyobodo necatrix and* tetrahymena. In 38 fishes ut of forty in goldfish. A total of 7,407 parasites were recorded. Most of the parasites were at the tips and middle part of the gills. Infection level having prevalence in goldfish was *Centrocestus formosans 87.5%*, dactylogyrous sp. was 95%, gyrodactylus was 01%, ichthyobodo necatrix also called costia was 05%, tetrahymena 2.5%,

Experiments were conducted in June 2018 to August 2018. And prevalence of parasites was compared with (Annahita 2017) that primarily focus on prevalence of Centrocestus formosans. Parasites in host fish population is set made of parasite species in that environment. (Marcogliese, 2016) and influenced by climate, external factors, larval stages, food structure and the presence of it in the environment.

4. CONCLUSION

The study shows high parasitic diversity in goldfish. And due to this fish morality increases. There should be strict check for importation of infected fish into Pakistan. Otherwise, infected exotic fishes may cause infection to native species.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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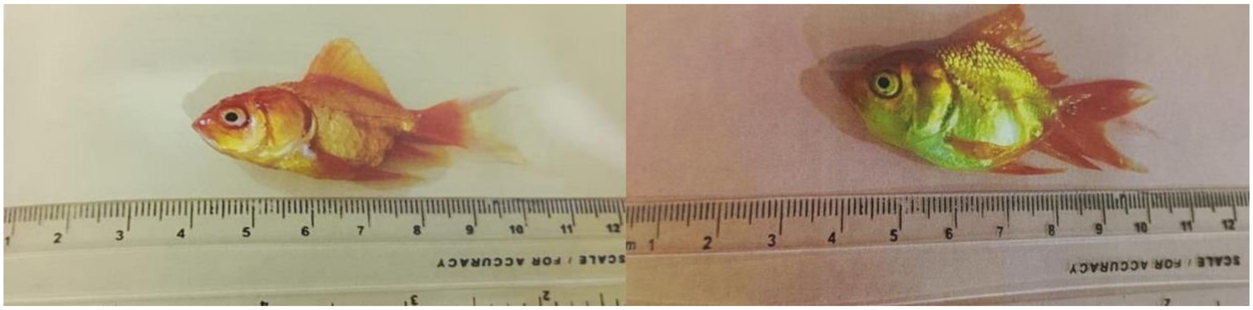
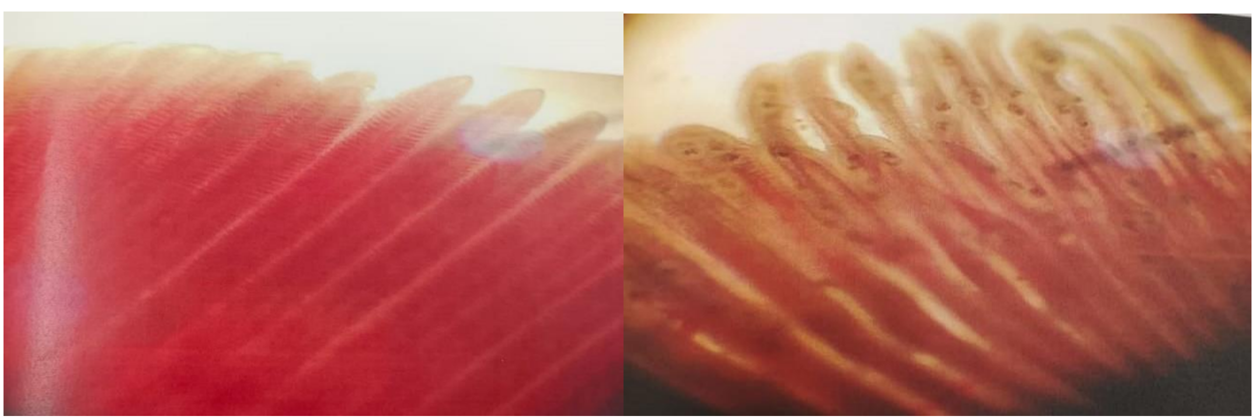


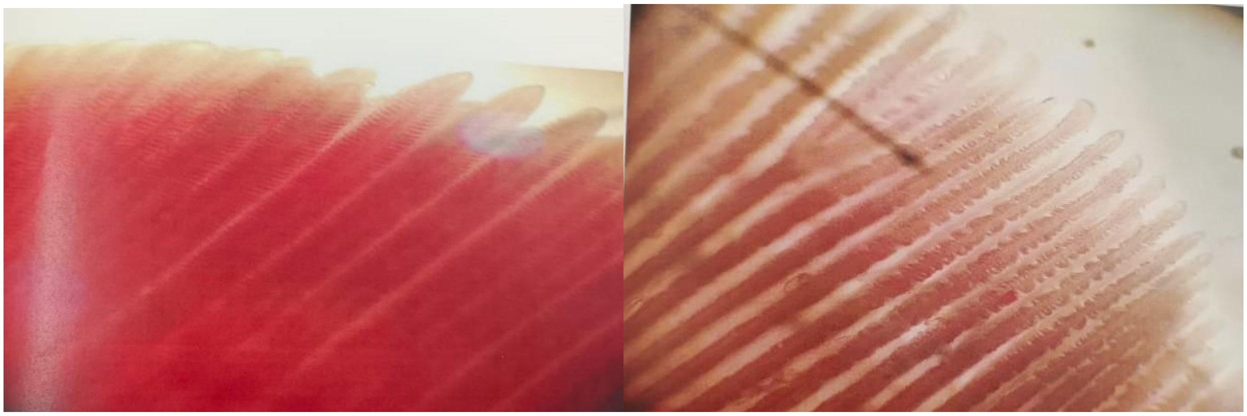
(A) **(B)**

(A) Goldfish, *Carassius auratus* with healthy fins **(B)** Goldfish, *Carassiu auratus* with eroded fins.

(A) **(B)**



(A) **(B)**



(A) **(B)**

Table 4.3. Parasitic fauna observed in goldfish, *Carassius auratus*.

Parasites	No. of fish examined	No. of fish infected	No. of Parasites	Prevalence (%)	Abundance	Mean intensity	Location
Centrocestus formosans	40	35	3102	87.5	77.55	88.62	Gills
Dactylogyrus sp.	40	38	4305	95	107.6	113.2	Gills
unidentified metacercariae	40	02	02	05	0.05	01	Gills
Gyrodactylus sp.	40	04	05	01	0.125	1.25	Fins
ichthyobodo necatrix	40	02	04	05	0.1	02	Gills
tetrahymena	40	01	03	2.5	0.075	03	Gills
Argulus	40	02	02	05	0.05	01	Skin

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EFFECT OF DIFFERENT SALINITY LEVELS ON GROWTH PERFORMANCE, HEMATOLOGICAL PARAMETERS AND PROXIMATE COMPOSITION OF *CYPRINUS CARPIO*

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Key words:
Cyprinus carpio, Salinity levels Growth performance, Hematological parameters, Proximate composition.

ABSTRACT

The current study was conducted to evaluate the effect of different salinity levels on the growth performance, proximate composition, and hematological parameters of Common carp (*Cyprinus carpio).* The experiment was designed under Laboratory conditions with twice replica as five treatments: T0: 0-ppt, (control); T1: 2-ppt, T2: 4-ppt, T3: 6-ppt and T4: 8-ppt and fish were randomly stocked (5 fish /aquarium). The *C. carpio* were fed 5% (commercial diet CP-30%) of their body weight and water was replaced regularly after every alternate day. The present study describes the significant changes ($p<0.05$) in growth parameters (viz; final weight, weight gain, feed intake and feed conversion ratio) with poor feeding behavior and stress were observed with further increase in salinity. Furthermore, body composition (Protein, fat, moisture, and ash contents) also showed significant changes ($p<0.05$). The crude protein and moisture contents were significantly decreased while crude fat and ash contents were increased with the increase of salinity respectively. The results of hematological parameters also decrease with the increase of salinity and found significant changes ($p<0.05$). The results of this study indicate that *C. carpio* exhibits a great degree of adaptability and resistance to salinity stress. This study serves as a basis for developing strategies to optimize the rearing conditions and welfare of common carp in different salinity regimes. Further research is warranted to elucidate the molecular and cellular mechanisms involved in the observed responses, enabling more precise management practices for the sustainable cultivation of *C. carpio*.

1. INTRODUCTION

Salinity is an abiotic factor that affects body composition, growth performance and hematological parameters of aquatic organisms (Moffett *et al.*, 2023). It is considered as an important factor that impact on osmosis, physiological functions, hormones, enzymes, immune metabolism and survival of fish (Wang & Zhu, 2002; Akhtar *et al.*, 2010).

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High salinity causes mortality, low reproductive rates and in some freshwater species reproductive failure is also reported (Hintz & Relyea, 2019). In the worldwide, natural waters are exposing to continuous rise in salinity levels due to human activities such as the use of salt or desalination of plants and uneven rainfall (Al-Faiz *et al.*, 2009; Fazio *et al.*, 2013; Hintz & Relyea, 2019). Therefore, climatic conditions of Pakistan do not sustain and changed into underground brackish and saline water (Jarwar, 2014; Khan *et al.*, 2016).

Aquaculture plays an important role in solving worldwide crises such as salinity, famine and malnutrition by the production of fish in intensive and non-intensive culture systems. In aquaculture practices some organisms have evolved to fully adapt to tolerate a wide range of high salinity levels, such as the common carp (*Cyprinus carpio*) (Triantaphyllopoulos *et al.*, 2020). *C. carpio* belongs to Family Cyprinidae considered as potential candidate live in freshwater habitat, although it can tolerate to some salinity and has been testified to improves growth and survival, but higher levels of salinity seem to be injurious (Barus *et al.*, 2001; Mangat & Hundal, 2014). It is known as a potential candidate for profitable aquaculture worldwide (Eurasia) due to its high adaptive capabilities (Parkos & Wahl, 2014; Tessema *et al.*, 2020). The common carp is stenohaline fish that can tolerate various environmental conditions such as extreme salinity and resistance to pathogens. It can adjust the hematological characteristics, allied with immune response display physiological status which effects on growth and physical appearance (Akinrotimi *et al.*, 2012). In aquaculture, common carp is a familiar fish due to its fabulous taste and its excellent market demand (Nedoluzhko *et al.*, 2021).

Salinity also can impact on hematological parameters which are assessed as indicators, play vital roles in maintaining physiological functions and overall health of organisms (Kim & Kang, 2016; Ahmed *et al.*, 2020). The alterations in blood parameters are thought to be adaptive responses to changes in the osmotic balance caused by variations in salinity. The increase in salinity levels inspiring to decrease in red blood cell counts, suppress immune responses, leading to decrease in the number of white blood cells and a compromised immune system (Soegianto *et al.*, 2017; Ramesh *et al.*, 2018). Such immunosuppression can render the fish more susceptible to various diseases and infections in brackish or seawater environments (Jackson *et al.*, 2020).

The aim of this scientific study is to investigate the impact of varying salinity levels on growth performance, hematological parameters and proximate composition of *Cyprinus carpio* to provide valuable insights into the species' adaptability and optimize their husbandry in diverse aquatic environments.

2. MATERIALS AND METHODS

The Common carp (*Cyprinus carpio*) were collected from pond of fish hatchery, Saline Water Aquaculture Research Centre (SWARC) Muzaffargarh and transported to Laboratory. Fish were acclimatized for 7 days in freshwater glass aquaria before the experiment began. The experiment was designed into five treatments (T1: 2-ppt, T2: 4-ppt, T3: 6-ppt and T4: 8-ppt) respectively and control group (T0: 0-ppt). Each treatment had two replicas and 5 fish/aquarium were randomly stocked in each treatment. Fish were fed 5% of their body weight with Commercial diet CP-30% and water was exchanged regularly after every alternate day. The aquariums were properly equipped, and continuous aeration was provided to maintain dissolved oxygen. All of the physicochemical parameters were maintained by using Apera 8500 EC meter, Apera 8500 pH meter and P-512 dissolved oxygen meter on daily basis. The salinity of the treatments were gradually maintained by increases of salt and tested by Salinity meter on daily basis. Detritus and uneaten feed were removed on daily basis by siphoning out.

Growth performance

Growth parameters viz; Final weight, weight gain, feed intake, growth rate and FCR were recorded by following formulas:

Weight gain = Final weight- Initial weight
FCR= feed given (g)/ Weight gain (g)
Growth Rate (%); WG (g)/ WI (g) X 100
Feed Intake; FCR × Weight gain (g)

The following measurements were taken (Owais *et al*., 2023)

Proximate composition

The proximate inspection of desiccated fish meat was supported by procedures of Association of Analytical Chemist (AOAC, 1984). Samples were weighted at 105°C to determine moisture. Protein content was determined by measuring nitrogen (N×6.25) using the Kjeldahl method. Fat was confirmed by ether extraction using Soxhlet method. Crude ash was calculated following combustion at 550 °C for 6h (Owais *et al*., 2023).

Blood sampling

The blood samples of experimental and control group fish were taken at the end of the experiment. It was collected by using a 5 ml disposal syringe. From each fish 1 ml of blood was collected into blood count test tubes that contain anticoagulant EDTA solution. The hematological parameters (Hemoglobin: Hb, Platelet count: PLT, Red blood cell: RBC, mean corpuscular volume: MCV, Hematocrit: HCT and Mean corpuscular hemoglobin: MCH) were tested by using a fully automated blood cell counter machine.

Statistical Analysis

SPPS (ver. 22, USA) was used for statistical analysis of the data. Data were subjected to one-way ANOVA and Duncan's multiple range tests to determine the significant differences between the means.

3. RESULTS AND DISCUSSION

The results of growth performance revealed that decrease in Final weight (g), Weight gain (g), Feed intake (g), Growth rate (%) while feed conversion ratio increased when salt concentrations gradually rose from 0-ppt to 8-ppt. The statistical analysis shows significant differences ($P \geq 0.05$) in treatments. Results of growth parameters show weight gain (g); T0: 0-ppt (11.28±0.28), T1: 2-ppt (10.34±0.38), T2: 4-ppt (9.02±0.17), T3: 6-ppt (8.52±0.27) and T4: 8-ppt (7.64±0.26) respectively. Feed intake (g); T0: 0-ppt (14.58±0.37), T1: 2-ppt (14.47±0.42), T2: 4-ppt (12.95±0.12), T3: 6-ppt (12.26±0.36) and T4: 8-ppt (12.38±0.51) respectively. Growth Rate (%); T0: 0-ppt (95.72±0.93), T1: 2-ppt (87.39±1.75), T2: 4-ppt (81.52±2.95), T3: 6-ppt (73.54±2.51) and T4: 8-ppt (69.73±3.33) respectively. Feed conversion ratio; T0: 0-ppt (1.27±0.01), T1: 2-ppt (1.39±0.01), T2: 4-ppt (1.40±0.03), T3: 6-ppt (1.41±0.02) and T4: 8-ppt (1.65±0.05) respectively (Table 1, Figure 1).

Results of hematological studies revealed that decrease in blood counts like red blood cells (RBC), hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), hematocrit (HCT) and platelet count (PLT) when the salt concentrations gradually rose to 0-ppt to 8-ppt. The statistical analysis showed significant differences ($P \geq 0.05$) in treatments. The RBC $10^6 \times mm3$ counts as in treatments; T0: 0-ppt (0.42±0.09), T1: 2-ppt (0.38±0.05), T2: 4-ppt (0.33±0.06), T3: 6-ppt (0.29±0.06) and T4: 8-ppt (0.23±0.01) respectively. Hb g/dl**;** T0: 0-ppt (3.33±0.07), T1: 2-ppt (3.28±0.07), T2: 4-ppt (2.62±0.21), T3: 6-ppt (2.22±0.01) and T4: 8-ppt (2.17±0.01) respectively. MCH pg; T0: 0-ppt (78.70±0.45), T1: 2-ppt (75.86±0.39), T2: 4-ppt (73.68±0.19), T3: 6-ppt (70.62±0.33) and T4: 8-ppt (65.00±0.59) respectively. MCV μm3**;** T0: 0-ppt (76.68±0.43), T1: 2-ppt (73.30±0.48), T2: 4-ppt (69.24±0.53), T3: 6-ppt (65.06±0.39) and T4: 8-ppt (59.14±1.34) respectively. HCT %; T0: 0-ppt (3.34±0.03), T1: 2-ppt (3.25±0.06), T2: 4-ppt (2.83±0.02), T3: 6-ppt (2.68±0.02) and T4: 8-ppt (2.35±0.01) respectively. PLT 10^9/l; T0: 0-ppt (717.40±1.02), T1: 2-ppt (710.60±1.12), T2: 4-ppt (670.00±7.07), T3: 6-ppt (654.20±1.52) and T4: 8-ppt (562.00±2.67) respectively (Table 2, Figure 2).

The results of proximate composition revealed that decrease in Crude Protein and Moisture with the increase of salinity while Crude fat and Ash content increase with the increase of salinity. The significant differences are found in the treatments. The values of Crude Protein %; T0: 0-ppt (17.44±0.16), T1: 2-ppt (16.48±0.08), T2: 4-ppt (15.42±0.15), T3: 6-ppt (14.24±0.10) and T4: 8-ppt (13.64±0.12) respectively. Crude fat %; T0: 0-ppt (9.38±0.08), T1: 2-ppt (11.44±0.23), T2: 4-ppt (13.44±0.16), T3: 6-ppt (14.32±0.11) and T4: 8-ppt (15.28±0.08) respectively. Moisture %; T0: 0-ppt (80.44±0.14), T1: 2-ppt (77.74±.049), T2: 4-ppt (73.92±0.54), T3: 6-ppt (71.18±0.46) and T4: 8-ppt (65.16±0.98) respectively. Ash %; T0: 0-ppt (1.46±0.02), T1: 2-ppt (1.74±0.05), T2: 4-ppt (2.68±0.08), T3:6-ppt (2.86±0.09) and T4: 8-ppt (3.60±0.07) respectively (Table 3, Figure 3).

The present study was conducted to the Effect of different salinity levels on growth performance, Hematological parameters and proximate composition of Cyprinus carpio. The findings of the study revealed that no mortality on different salinity regime between 0 to 8-ppt. The growth parameters revealed that decrease in Final weight (g), Weight gain (g), Feed intake (g), Growth rate (%) while feed conversion ratio increased with the increased of salinity. Mylonas *et al.*, (2009) revealed that regime in salinity 0-ppt to 8-ppt affects the growth rate, feed conversion and feed intake. However, fish was perfectly able to normalize body functioning such as osmoregulatory process and metabolic rate. The high level of salinity increases metabolic rate because of new osmotic conditions leads to control necessary ions through osmoregulation and to maintain the internal stability (homeostasis) which needs high energy cause reduction in growth rate and effects the feed conversion ratio and feed intake. Abo- Hegab & Hanke, (1982) describe changes in feed conversion rate of common carp with the increase of salinity upto15-ppt. DeBoeck *et al.* (2000) studied common carp (*Cyprinus carpio*) at 10-ppt salinity and found negatively impact on feed conversion rate, weight gain and feed intake while Laiz-Carrión *et al.* (2005) describes the effect of salinity on fish reported poor growth performance depends on species and duration of exposure. Barman *et al.* (2005) entitled that decline in growth parameters viz; feed conversion and feed intake of grey mullet (*Mugil cephalus*) on high salinity. Luz *et al.*, (2008) studied the goldfish *Carassius auratus* and reported low feed conversion rate at 8-ppt. Furthermore, Xia *et al.*, (2010) studied grass carp *Ctenopharyngodon Idella* at different salinity levels and reported low feed conversion rate at 10-ppt.

The current study results exhibited a significant effect of various salinity levels on hematological parameters decrease in haemoglobin content, RBCs and haematocrit in treated groups. These findings are in similar agreement with another researcher who found a significant effect of salinity on RBCs, HCT and Hb of different fish species and high salinity levels with the osmoregulatory dysfunction (Fazio *et al.*, 2013; Soltanian *et al.* 2016). McCormick, (2001) explained that blood parameters play a significant role in health status of fish and reported salinity influence on physiological changes which leads to decrease in blood parameters. *Cyprinus carpio* hematological parameters are found significantly decreases in blood parameters at high salinity.

Luz *et al.* (2008) explained that salinity is used as stress indicators for hematological and physiological parameters of the fish and reported that increase in salinity level affects the ion exchange mechanism. Al-Hilali & Al-Khshali, (2016) examined the impact of high salinity on the blood parameters of common carp and reported that salinity levels did influence certain hematological parameters. Elarabany *et al.* (2017) reported significant changes in hematological parameters of Nile tilapia (*O. niloticus*) at higher salinities levels. Mubarik *et al.* (2019) examined hematological parameters of *Cyprinus carpio* under different critical and chronic salinity regimes. It found significant effects on various blood parameters, including hemoglobin concentration. Murmu *et al.*, (2020) explained the effect of salinities on hematological parameters on Rohu fingerlings and explained the decreased in RBC counts at 6-ppt. Similarly, MCH and haemoglobin content are also drastically reduced in the fishes treated with high salinity. Salati *et al.* (2021) reported the impact of salinity levels in *C. carpio* and demonstrate variations in hematological parameters.

The proximate composition such as moisture, protein, lipid, and ash of common carp are affected by salinity. The result revealed significant differences among all of the treatments. The protein and moisture contents decreased with increase of salinity while minimum protein and moisture content were found at 8-ppt. Fat and ash contents increased with increase of salinity, maximum contents were found at 8-ppt respectively. Barman, (2012) studied milk fish proximate composition which shows significant reduction in level of moisture with increase in salinity from 0 to 15-ppt. Ljubojević *et al.* (2015) reported decreasing in protein content and the increasing in lipid contents in the *Lutjanus guttatus* and carp. Daudpota *et al.* (2016) studied body composition of *C. carpio* at higher salinities and reported changes in the protein, moisture, lipid, or ash contents because osmoregulation expenditure is required high energy contents. Rahim *et al.* (2017) studied the body composition of the common carp at higher salinity and found significantly higher fat content because fish store more energy to cope with the osmotic stress of salinity. Mandal *et al.* (2020) studied the proximate composition of *Pangasius* at different salinity levels, found protein and moisture contents decreased with the increase of salinity while Fat and ash contents increased with the increase of salinity.

4. CONCLUSION

This study summarized that growth performance, hematological parameters and proximate composition of common carp (*Cyprinus carpio*) are influenced by different salinity levels. *C. carpio* exhibits a great degree of adaptability and resistance to salinity stress, which can impact its overall health and physiological responses. These findings contribute to a profound understanding about ecological significance of salinity in aquaculture and natural aquatic environments. This study may serve as a basis for developing strategies to optimize the rearing conditions and welfare of common carp. Further research is warranted to elucidate the molecular and cellular mechanisms involved in the observed responses, enabling more precise management practices for the sustainable cultivation of this economically important fish. Overall, this scientific investigation sheds light on the intricate relationship between salinity levels, growth and physiological well-being of aquaculture industry and contributes to understanding of aquatic ecosystems.

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6. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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Table 1. Showing growth performance (Mean ± SEM) of *Cyprinus carpio* at different salinity levels (0-ppt, 2-ppt, 4-ppt, 6-ppt and 8-ppt and) during the 60-days experiment.

Treatments	**Weight gain (g)**	**Feed intake (g)**	**Growth Rate (%)**	**FCR**
0-ppt	11.28±0.28d	14.58±0.37b	95.72±0.93a	1.27±0.01a
2-ppt	10.34±0.38c	14.47±0.42b	87.39±1.75b	1.39±0.01b
4-ppt	9.02±0.17b	12.95±0.12a	81.52±2.95b	1.40±0.03b
6-ppt	8.52±0.27b	12.26±0.36a	73.54±2.51c	1.41±0.02b
8-ppt	7.64±0.26a	12.38±0.51a	69.73±3.33c	1.57±0.05c

Means of the same raw with different letters are significantly different ($p<0.05$). Data was presented as (mean ± standard error).

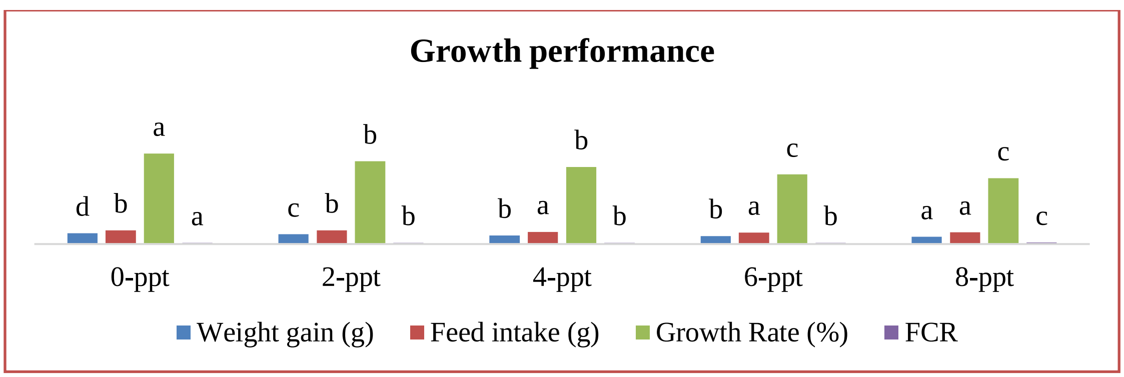

Figure 1. Clustered column bar graph showing growth performance of Cyprinus carpio at different salinity levels (0-ppt, 2-ppt, 4-ppt, 6-ppt and 8-ppt) during the 60-days experiment.

Table 2. Showing growth concentrations of hematological parameters (Mean ± SEM) of Cyprinus carpio at different salinity levels (0-ppt, 2-ppt, 4-ppt, 6-ppt and 8-ppt and) during the 60-days experiment.

Groups	**RBC10^6×mm3**	**Hb g/dl**	**MCH pg**	**MCV µm3**	**HCT %**	**PLT 10^9/l**
0-ppt	0.42±0.09e	3.33±0.07a	78.70±0.45e	76.68±0.43e	3.34±0.03d	717.40±1.02a
2-ppt	0.38±0.05d	3.28±0.07a	75.86±0.39d	73.30±0.48d	3.25±0.06d	710.60±1.12a
4-ppt	0.33±0.06c	2.62±0.21b	73.68±0.19c	69.24±0.53c	2.83±0.02c	670.00±7.07b
6-ppt	0.29±0.06b	2.22±0.01c	70.62±0.33b	65.06±0.39b	2.68±0.02b	654.20±1.52b
8-ppt	0.23±0.01a	2.17±0.01c	65.00±0.59a	59.14±1.34a	2.35±0.01a	562.00±2.67c

Means of the same raw with different letters are significantly different ($p<0.05$). Data was presented as (mean ± standard error).

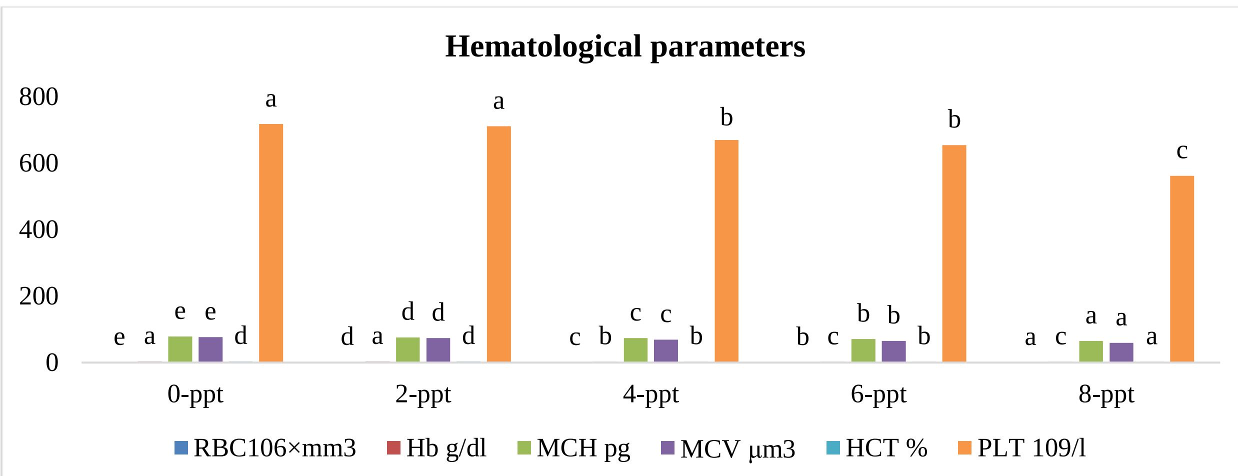

Figure 2. Clustered column bar graph showing hematological parameters of Cyprinus carpio at different salinity levels (0-ppt, 2-ppt, 4-ppt, 6-ppt and 8-ppt) during the 60-days experiment.

Table 3. Showing proximate composition (Mean ± SEM) of Cyprinus carpio at different salinity levels (0-ppt, 2-ppt, 4-ppt, 6-ppt and 8-ppt and) during the 60-days experiment.

Parameters	**Treatments**				
	0-ppt	**2-ppt**	**4-ppt**	**6-ppt**	**8-ppt**
Crude Protein %	17.44±0.16e	16.48±0.08d	15.42±0.15c	14.24±0.10b	13.64±0.12a
Crude fat %	9.38±0.08a	11.44±0.23b	13.44±0.16c	14.32±0.11d	15.28±0.08e
Moisture %	80.44±0.14e	77.74±.049d	73.92±0.54b	71.18±0.46b	65.16±0.98a
Ash %	1.46±0.02a	1.74±0.05b	2.68±0.08a	2.86±0.09c	3.60±0.07d

Means of the same raw with different letters are significantly different ($p<0.05$). Data was presented as (mean ± standard error).

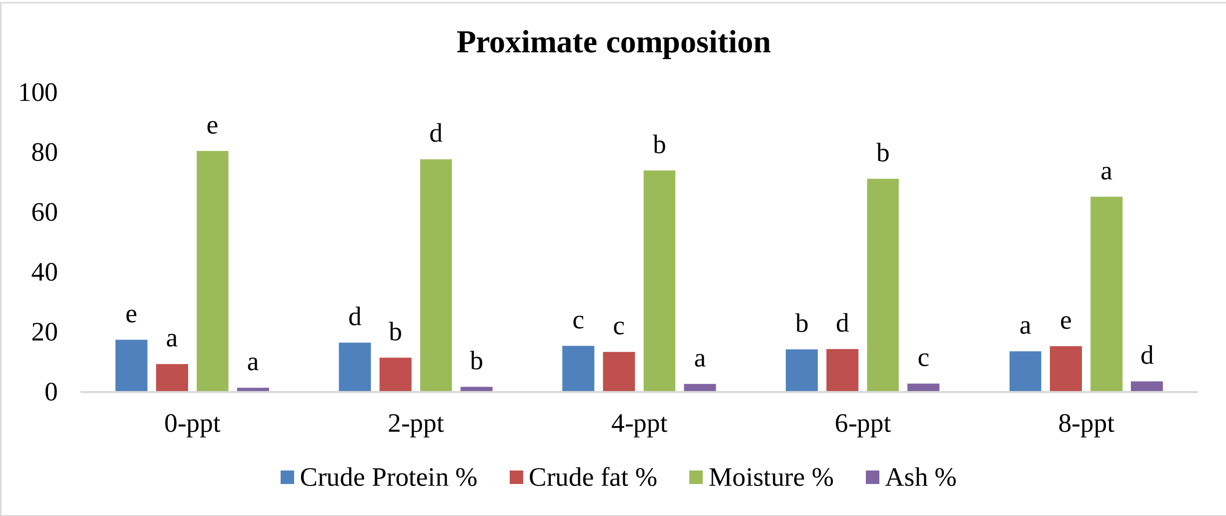

Figure 3. Clustered column bar graph showing proximate composition of *Cyprinus carpio* at different salinity levels (0-ppt, 2-ppt, 4-ppt, 6-ppt and 8-ppt) during the 60-days experiment.

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COMPARATIVE EFFICACY OF BOTANICAL EXTRACTS ALONG WITH THEIR BIOSYNTHESIZED ZINC OXIDE NANOPARTICLES AGAINST HOUSE FLY *MUSCA DOMESTICA* L. (DIPTERA: MUSCIDAE)

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Key words:
Musca domestica, Botanicals, Zinc oxide nanoparticles, Azadirachta indica, Citrus limon and Mortality.

ABSTRACT

The current study was conducted in 2023 at the Department of Entomology, University of Agriculture, Faisalabad, in a Completely Randomized Design with three replications. The objective of this research was to determine the efficiency of botanical extracts and their green synthesized zinc oxide nanoparticles against *Musca domestica*. Results revealed that at 50% concentration, *Azadirachta indica* extracts exhibited the highest mortality on larvae (51%), pupae (49%) and adults (53%), respectively, while at same concentration *Citrus limon* extracts exhibited the highest mortality on larvae (41%), pupae (45%) and adults (44%), respectively. Similarly, at 400ppm, ZnO Nps of *A. indica* showed 64, 57 and 67% mortality on larvae, pupae, and adults, respectively. While ZnO Nps of *C. limon* showed mortality on larvae (50%), pupae (54%) and adults (55%), respectively. The findings showed that *A. indica* extract at 50% concentration was more toxic to *M. domestica* as compared to *C. limon* extract. Additionally, *A. indica* ZnO Nps at 400ppm concentration were highly toxic to *M. domestica* as compared to *C. limon* ZnO Nps extract.

1. INTRODUCTION

The House fly, *Musca domestica* L. (Diptera: Muscidae) is a global health pest that poses a significant threat to both humans and animals by vectoring a variety of contagious diseases (Baker *et al.,* 2020). The house fly spread some of its microbial content whenever it lands on human drinks or food by depositing it in saliva or feces (Park *et al.,* 2019). Flies pick up pathogens via sewage, waste and other unsanitary sources (Abbas *et al.,* 2013). The house fly has a tendency for dispersing illnesses investigation has also revealed that house flies contain a diverse range of pathogens and viruses (Bahrndorff *et al.,* 2017).

More than 100 pathogens that are dangerous to humans can be carried with house flies, including bacteria like *Yersinia pseudotuberculosis*, *Vibrio cholerae*, *Helicobactor pylor*, *Campylobacter jejuni*, *Salmonella enteritidis* and *Escherichia coli* (Khamesipour *et al.,* 2018). It was discovered that house flies are important avian flu virus transmitters. Which poses a threat to humans, poultry and the global livestock industry (Wanaratana *et al.,* 2013). According to reports, the seasonal abundance of house flies in developing countries like Pakistan is a contributing factor to the spread of digestive disorders like diarrhea in urban and rural regions (Khan *et al.,* 2013).

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Insecticides play a significant role in suppressing this destructive pest. Many chemicals are used to control house flies and that is considered as rapid control of pest population (Walsh *et al.,* 2022). Unknowingly implemented of insecticides at greater quantities in lawns, gardens and houses that seriously jeopardizes the quality of the environment, living things and food. In mammals by inhalation, insecticides can cause oxidative stress, liver toxicity, kidney damage, hepatotoxicity, teratogenicity, carcinogenicity, mutagenicity, as well as neurodegeneration (Muhoro & Farkas, 2021; South *et al.,* 2020). Pesticide usage is less successful due to several variables, including insecticide resistance, rising insecticide prices and insecticide toxicity for other beneficial insects (Scott, 2017).

Traditional approaches of managing insect pests may be replaced with botanical pesticides, which are abundant in bioactive chemicals (Gusmao *et al.,* 2013). They are effective against specific target species, are non-toxic to mammals and humans, and useful in integrated pest management (Ngegba *et al.,* 2022). It has been demonstrated that the chemical components of biopesticides have insecticidal and repellant properties (Nwanade *et al.,* 2020). Natural plant-based insecticides are a promising alternative to synthetic chemical pesticides for the management of insect pests (Ahmed *et al.,* 2021). The neem tree, *Azadirachta indica*, contains a variety of chemical materials that can be used as insecticides and has the potential to control insect pests (Boeke *et al.,* 2004). Azadirachtin is very well known for its capacity to suppress phytogenic pest growth. It prevents pests of various orders from feeding and growing (Pavela, 2007). Because of their several mechanisms of action on insects, neem compounds have little toxicity to birds, fishes and humans and are less likely to cause resistance. Neem has been used in a variety of mosquito control efforts due to its effectiveness in controlling agricultural pests, environment protection and public acceptance of its products (Acharya *et al.,* 2017). Lemon, also known as *Citrus limon*, has been investigated for its potential as a biopesticide because of its inherent ability to deter or suppress pests (Morya *et al.,* 2010). Numerous substances found in lemons have been demonstrated to have insecticidal and repellant effects. Citronellal, citronellol and limonoids are some of the active substances present in lemons that contribute to their biopesticide activities (Aslam *et al.,* 2011). These substances are helpful in pest management methods since they may deter and even kill some pests (Mossa, 2016).

Due to their antibacterial and pesticide properties, nanoparticles have received a lot of interest nowadays (Kalpana *et al.,* 2018). Additionally, compared to their synthetic equivalents, these environmentally friendly nanoparticles are said to be more effective pesticides, cheaper, biodegradable and safer for both humans and the environment (Murugan *et al.,* 2016). For the creation of nanoparticles, a variety of botanicals with different reductive groups can serve as reducing and protecting agents. Harmful chemicals are not used in the synthesis process, using botanicals to create nanoparticles has many advantages (Borase *et al.,* 2014). On the *M. domestica*, green synthesized nanoparticle has been found to exhibit bio insecticidal properties (Al-Nasser *et al.,* 2022). The experiments showed that ZnO Nps have an exceptionally powerful antimicrobial action at incredibly low gram-negative and gram-positive bacterial concentrations, suggesting a stronger antibacterial than chemically produced ZnO Nps (El-Ghwas *et al.,* 2022). The objective of this research is to investigate the comparative efficacy of botanical extracts *A. indica* and *C. limon*, along with their biosynthesized zinc oxide nanoparticles against larvae, pupae and adults of *M. domestica*.

2. MATERIALS AND METHODS

Study area

This study was conducted in the Department of Entomology at the University of Agriculture in Faisalabad.

Collection of house fly

Adult houseflies were collected by using the sweep net from garbage, livestock farms and chicken shops at various locations in Faisalabad, Punjab, Pakistan.

Laboratory rearing

The collected house flies were released in plastic cages having size 40 cm × 45 cm × 45 cm. A cotton wick moistened in the water along with powdered milk and sugar mixed (1:1 w/v) was served as food source in a separate petri dish for adults. Larvae was reared on artificial diet that contains powdered milk, sugar, yeast, grass meal and wheat bran in the following proportions: 0.3:0.3:1:2:4 w/v, respectively (Francuski *et al.,* 2020). House flies were kept under controlled laboratory environment of 26±28°C, R.H 60–70%, and a photoperiod of (14:10 LD). To determine the exact age of adults, emerged adults from pupae were transferred to separate containers.

Collection of leaves and peel

Neem leaves that were fully developed and fresh as well as lemon peel were taken from the University of Agriculture, Faisalabad botanical garden.

Preparation of extract

Fresh neem leaves and lemon peel were washed, dried in the shade for eight days and then chopped. The chopped materials were ground to a fine powder using a grinder and sieved through a 40-mesh size. Then 5g of each powder was taken, mixed in 500ml of distilled water and shaken on a shaker. The mixture was then heated at 50°C for 1 hour at 40 rpm in a rotary evaporator, separately for both samples. The resulting solutions were purified using Whatman no. 1 filter paper to obtain pure liquid extracts, which were collected in a bottom flask and stored at 4°C.

Biosynthesis of ZnO nanoparticles

A magnetic stirrer was used to heat 100ml of prepared extracts and 0.81g zinc oxide to make a 10mM solution in 1000ml of distilled water at room temperature. It was covered with aluminum foil to prevent hydrolysis when exposed to light. The change in color indicated the formation of ZnO nanoparticles. The solution was kept in incubator at 28°C. Then a chemical reaction occurred after the couple of days and then nanoparticles were formed in liquid state.

UV spectrophotometry of ZnO NPs

UV spectrophotometry was used to clarify nanoparticles.

Larvicidal bioassay

The relative efficacy of plant extracts and ZnO Nps against *M. domestica* larvae was evaluated using the larvae dipping method. Different concentration of ZnO Nps (400, 300, 200 and 100 ppm) and botanical extract (50, 25, 12.50 and 6.25%) was made from stock solution. Twenty larvae of the third instar were carefully immersed for 30 seconds within every dosage before being returned to the rearing medium. For the control, larvae were immersed in distilled water. The experiment consists of three replications. After (24, 48, 72 h) of exposure, susceptible larvae were counted by being touched with a soft camel hairbrush.

Pupicidal bioassay

The topical application method was used to evaluate the pupicidal activities of botanical extracts and ZnO Nanoparticles. Each concentration of an individual botanical extracts and ZnO Nanoparticles was administered to twenty pupae that are one day old with the help of micropipette. After 7 days of exposure, un-emerged adults from were counted. The experiment was replicated three times.

Adulticidal bioassay

Twenty 3-4 days old *M. domestica* were given each dosage of botanical extract and ZnO Nps on the thoracic notum for adulticidal toxicity studies. Only distilled water was used to treat *M. domestica* in the control group. Following treatment, *M. domestica* was moved to enclosures for rearing and given dry sugar, milk powder and cotton swabs wet in water. The experiment consists of three replications. The number of dead estimates were made (24, 48, 72 h) after the exposure.

Statistical analysis

All collected results were subjected to statistical analysis. The data collected were analyzed using a one-way analysis of variance (ANOVA) and means were compared using Tukey's HSD test.

3. RESULTS AND DISCUSSION

Mortality is caused by botanicals and biosynthesized zinc oxide nanoparticles on the larval stage of Musca domestica.

All tested botanicals and biosynthesized zinc oxide nanoparticles caused significant mortality of house fly larvae after 72 hours of application as shown in Table 1 and 2. The *Azadirachta indica* mediated ZnO Nps was statistically highly effective with mortality of 30.67% followed by *Citrus limon* mediated ZnO Nps, *A. indica* and *C. Limon* with mortality of 27.33, 24.67 and 20.00% respectively, after 24 hours of application. The *A. indica* mediated ZnO Nps was again significantly more efficient with mortality of 50% than the *C. limon* mediated ZnO Nps, *A. indica* and *C. Limon* was 40.33, 37.00 and 33.67% respectively, after 48 hours of application. However, after 72 hours application, *A. indica* mediated ZnO Nps caused the highest mortality 63.67% followed by *C. limon* mediated ZnO Nps, *A. indica* and *C. Limon* with mortality of 50.00, 46.33 and 40.67% respectively which were also significantly different from each other. The results demonstrated that *A. indica* extract was more toxic to *M. domestica* larvae as compared to *C. limon* extract at a 50% concentration. In contrast to *C. limon* ZnO Nps extract, *A. indica* ZnO Nps at 400 ppm concentration was highly lethal to *M. domestica* larvae.

Mortality is caused by botanicals and biosynthesized zinc oxide nanoparticles on the pupal stage of Musca domestica.

All tested botanicals and biosynthesized zinc oxide nanoparticles caused significant mortality of house fly pupae after 7 days of application as shown in Table 3 and 4. The *Azadirachta indica* mediated ZnO Nps was statistically highly toxic with mortality of 56.33% followed by *Citrus limon* mediated ZnO Nps, *A. indica* and *C. Limon* with mortality of 53.33, 48.33 and 45.00% respectively, after 7 days of treatment exposure. The results showed that *A. indica* extract was more toxic to *M. domestica* pupae as compared to *C. limon* extract at a 50% concentration. In contrast to *C. limon* ZnO Nps extract, *A. indica* ZnO Nps at 400 ppm concentration was highly lethal to *M. domestica* pupae.

Mortality caused by botanicals and biosynthesized zinc oxide nanoparticles on the adult stage of Musca domestica.

All tested botanicals and biosynthesized zinc oxide nanoparticles caused significant mortality of house fly adult stage after 72 hours of application as shown in Table 5 and 6. The *Azadirachta indica* mediated ZnO Nps was statistically highly effective with mortality of 40.00% followed by *Citrus limon* mediated ZnO Nps, *A. indica* and *C. Limon* with mortality of 35.67, 32.33 and 26.33% respectively, after 24 hours of application. The *A. indica* mediated ZnO Nps was again significantly more efficient with mortality of 52.33% than the *C. limon* mediated ZnO Nps, *A. indica* and *C. Limon* was 43.00, 40.67 and 32.33% respectively, after 48 hours of application. However, after 72 hours application, *A. indica* mediated ZnO Nps caused the highest mortality 66.67% followed by *C. limon* mediated ZnO Nps, *A. indica* and *C. Limon* with mortality of 55.00, 52.67 and 43.33% respectively which were also significantly different from each other. The botanicals and biosynthesized zinc oxide nanoparticles after 72 hours of treatments application showed gradual reduction in percentage mortality of the house fly larvae as compared to 24 and 48 hours after treatment exposure. The findings showed that *A. indica* extract at 50% concentration was more toxic to *M. domestica* adults as compared to *C. limon* extract. Additionally, *A. indica* ZnO Nps at 400ppm concentration were highly toxic to *M. domestica* adult stage as compared to *C. limon* ZnO Nps extract.

The current findings are in line with the findings of Aisvarya *et al*., (2023) reported that silica nanoparticles showed 73% mortality of *Sitophilus oryzae* at the dose of 9 mg after 4 days. Similar observation was also recorded by Al-Azzazy and Ghani (2023) determined the effect of copper nanoparticles against *Phyllocoptruta oleivora*. Results showed that 86% mortality of *P. oleivora* at the dose of 320 ppm after one week. Our results were similar to Ayinde *et al*., (2020) investigated the larvicidal activity of *Azadirachta indica* seed oil on *Anopheles gambiae.* A larval mortality rate of 100.0% was observed in *Anopheles gambiae* within a three-day period at a concentration of 500 ppm. Our results were similar to Gogate *et al*., (2018) showed that biosynthesized ZnO Nps revealed 83% toxicity in *Corcyra cephalonica*. Our findings were similar to Ghidan *et al*., (2017), who stated that ZnO nanoparticles, facilitated by *Punica granatum*, resulted in a mortality rate of 75.5% for the first and second nymphal instar of *Myzus persicae* after 24 hours at a concentration of 8000 µg/ml. Our results were consistent with the findings of Buhroo *et al*., (2017), who observed that ZnO nanoparticles induced 100% mortality in 4th instar larvae of *Mythimna separate* at a concentration of 500 ppm under laboratory conditions. Additionally, concentrations of 400 ppm, 300 ppm, and 200 ppm resulted in mortality rates of 93%, 83%, and 46% respectively for the 4^{th} instar larvae of *M. separate*.

4. CONCLUSION

These findings highlight the high efficacy of zinc oxide nanoparticles in managing the population of *Musca domestica* while posing no risks to human health and the environment, making them a promising tool for integrated pest management.

5. ACKNOWLEDGEMENTS

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6. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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Table 1. Mean percent mortality (M±SE) of *Musca domestica* larvae at 24, 48 and 72 hours after the application of botanicals in a laboratory test.

Sr. No.	Treatments	Concentration (%)	24 h	48 h	72 h
1	*Azadirachta indica*	6.25	1.23 ± 1.05^{b}	10.00 ± 3.33^{b}	16.67 ± 3.33^{c}
		12.50	6.67 ± 2.05^{ab}	13.33 ± 2.28^{bc}	20.00 ± 2.33^{c}
		25	16.67 ± 2.05^{ab}	26.67 ± 3.32^{ab}	40.00 ± 2.45^{b}
		50	24.67 ± 3.33^{a}	37.00 ± 2.46^{a}	50.67 ± 3.33^{a}
2	*Citrus limon*	6.25	6.67 ± 1.20^{ab}	13.33 ± 1.12^{c}	16.67 ± 3.33^{bc}
		12.50	10.00 ± 2.50^{ab}	13.33 ± 2.85^{ab}	20.00 ± 2.85^{bc}
		25	13.33 ± 3.33^{ab}	16.67 ± 4.08^{ab}	30.00 ± 3.33^{bc}
		50	20.00 ± 4.08^{a}	33.67 ± 3.33^{a}	40.67 ± 3.04^{a}

Means within a column not sharing a common letter are significantly different at $p < 0.05$ using Tukey's test.
M±SE: Mean ± Standard error

Table 2. Mean percent mortality (M±SE) of *Musca domestica* larvae at 24, 48 and 72 hours after the application of biosynthesized ZnO Nps in a laboratory test.

Sr. No.	Treatments	Concentration (ppm)	24 h	48 h	72 h
1	*Azadirachta indica* ZnO Nps	100	6.67 ± 3.04^{b}	13.33 ± 2.20^{cd}	16.67 ± 3.33^{cd}
		200	13.33 ± 2.08^{ab}	20.00 ± 3.33^{bc}	36.67 ± 2.45^{ab}
		300	20.00 ± 1.23^{ab}	30.00 ± 2.78^{b}	40.00 ± 2.45^{ab}
		400	30.67 ± 3.33^{a}	50.00 ± 3.33^{a}	63.67 ± 3.33^{a}
2		100	6.67 ± 2.76^{b}	16.67 ± 1.45^{ab}	20.00 ± 3.12^{bc}

	Citrus limon ZnO Nps	200	10.00±1.46^{ab}	20.00±2.38^{ab}	30.33±3.33^{ab}
		300	16.67±2.23^{ab}	23.33±3.33^{ab}	38.33±2.45^{ab}
		400	27.33±3.33^{a}	40.33±3.48^{a}	50.00±3.33^{a}

Means within a column not sharing a common letter are significantly different at $p < 0.05$ using Tukey's test.
M±SE: Mean ± Standard error
ZnO Nps: Zinc oxide nanoparticles
Ppm: Part per million

Table 3. Mean percent mortality (M±SE) of *Musca domestica* pupae after 7 days of application of botanicals in a laboratory test.

Sr. No.	Treatments	Concentration (%)	7 days
1	*Azadirachta indica*	6.25	14.33±3.33^{b}
		12.50	23.00±2.33^{b}
		25	36.00±2.58^{ab}
		50	48.33±3.33^{a}
2	*Citrus limon*	6.25	13.33±3.33^{bc}
		12.50	20.67±1.33^{ab}
		25	32.33±1.58^{ab}
		50	45.00±4.38^{a}

Means within a column not sharing a common letter are significantly different at $p < 0.05$ using Tukey's test.
M±SE: Mean ± Standard error

Table 3. Mean percent mortality (M±SE)) of *Musca domestica* pupae after 7 days of application of botanicals in a laboratory test.

Sr. No.	Treatments	Concentration (ppm)	7 days
1	*Azadirachta indica* ZnO Nps	100	19.33±1.33^{c}
		200	25.33±2.33^{bc}
		300	40.67±2.58^{b}
		400	56.33±3.33^{a}
2	*Citrus limon* ZnO Nps	100	16.67±3.33^{a}
		200	22..00±3.21^{bc}
		300	36.33±1.49^{b}
		400	53.33±3.33^{a}

Means within a column not sharing a common letter are significantly different at $p < 0.05$ using Tukey's test.
M±SE: Mean ± Standard error
ZnO Nps: Zinc oxide nanoparticles
Ppm: Part per million

Table 5. Mean percent mortality (M±SE) of *Musca domestica* adults at 24, 48 and 72 hours after the application of botanicals in a laboratory test.

Sr. No.	Treatments	Concentration (%)	24 h	48 h	72 h
1	*Azadirachta indica*	6.25	13.33±3.04^{bc}	16.67±3.33^{b}	20.00±2.33^{bc}
		12.50	18.67±3.46^{b}	22.00±3.12^{b}	28.33±3.33^{b}
		25	23.33±0.33^{ab}	30.33±4.32^{ab}	36.67±2.45^{ab}
		50	32.33±3.33^{a}	40.67±3.33^{a}	52.67±4.43^{a}
2	*Citrus limon*	6.25	10.00±0.21^{c}	13.67±1.12^{c}	20.00±2.50^{bc}
		12.50	13.33±3.33^{bc}	19.00±2.61^{ab}	25.35±3.33^{abc}
		25	20.00±3.58^{ab}	23.33±3.23^{ab}	33.33±4.00^{ab}
		50	26.33±3.67^{a}	32.33±3.67^{a}	43.33±3.04^{a}

Means within a column not sharing a common letter are significantly different at $p < 0.05$ using Tukey's test.
M±SE: Mean ± Standard error

Table 6. Mean percent mortality (M±SE) of *Musca domestica* adults at 24, 48 and 72 hours after the application of biosynthesized ZnO Nps in a laboratory test.

Sr. No.	Treatments	Concentration (ppm)	24 h	48 h	72 h
1	*Azadirachta indica* ZnO Nps	100	16.37±0.67^{b}	23.33±1.02^{d}	26.67±3.04^{c}
		200	20.00±1.06^{bc}	26.67±1.56^{b}	38.33±4.32^{bc}
		300	29.67±2.23^{ab}	36.67±2.78^{ab}	50.00±2.45^{ab}
		400	40.00±3.33^{a}	52.33±3.33^{a}	66.67±3.33^{a}
2	*Citrus limon* ZnO Nps	100	14.33±3.04^{ab}	18.67±1.70^{bc}	23.33±4.08^{bc}
		200	21.33±2.46^{ab}	25.33±2.38^{abc}	32.67±3.33^{bc}
		300	27.67±3.23^{a}	33.67±3.12^{ab}	42.00±2.45^{ab}
		400	35.67±4.13^{a}	43.00±4.02^{a}	55.00±3.33^{a}

Means within a column not sharing a common letter are significantly different at $p < 0.05$ using Tukey's test.
M±SE: Mean ± Standard error
ZnO Nps: Zinc oxide nanoparticles
Ppm: Part per million

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EVALUATION OF INSECTICIDES AND BOTANICAL OILS AGAINST COTTON WHITEFLY *BEMISIA TABACI* (HEMIPTERA; ALEYRODIDAE)

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Key words:
Efficacy, pesticides, cotton whitefly, pest management, imidacloprid, neem oil

ABSTRACT

The research study was conducted to compare the efficacy of insecticides viz. imidacloprid, acetamiprid, lambda-cyhalothrin and botanical oils viz. neem oil, castor oil and linseed oil against *Bemisia tabaci* (Gennadius, 1889) under laboratory conditions. By following Completely Randomized Design, bioassay was performed by leaf dip method with three replications and mortality data were collected after 24, 48, 72 and 96 hours of treatment. The study revealed that both botanical and synthetic pesticides had significant effects on whitefly nymphal mortality. However, the most effective pesticides for whitefly up to 96 hours were imidacloprid and neem oil, while lambda cyhalothrin and linseed oil remained least effective, and the others showed 50-60% mortality throughout the experiment.

1. INTRODUCTION

Cotton whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) is a destructive pest and virus vector, worldwide that infests food and fiber crops, including cotton, leguminous plant, vegetables, and ornamental plants (Horowitz *et al.,* 2020). Whitefly is one of the most important sucking insect pest of cotton in the Middle East, Europe, North America, and Central America (Li *et al.,* 2021) and in Pakistan (Khalil *et al.,* 2017). It sucks the cell sap of plants and excrete honeydews on leaves, which promotes the growth of sooty mould and limit photosynthesis, hence decrease crop quality and quantity (Jones, 2003). It causes severe economic damage directly by sucking plant sap and indirectly by transmitting 111 plant viruses especially begomoviruses on about 900 plant species (Polston *et al.,* 2014). These are highly important pests for cotton throughout the seedling and vegetative stages because they suck the plant sap, weaken it, and cause wilting and leaf loss in cases of severe infestation (Abro *et al.,* 2004).

Since the 1970s, when whitefly outbreaks started to become more common, pesticides have been used in Pakistan to control whitefly in commercial cotton plantations (Kumar *et al.,* 2022), resulting in whitefly feeding damage and illnesses brought on by the cotton leaf curl virus complex (Parola-Contreras *et al.,* 2022). It tends to acquire resistance to many types of insecticides, including carbamates, organophosphates, pyrethroids, and several chemicals recently introduced for use in Pakistan, in cotton-vegetable cropping systems. which resulted in failure of whitefly control and lead to significant damage to the cotton crop (Ahmad & Khan, 2017; Shah *et al.,* 2021). This situation has led to a search for other efficient control methods, such as chemicals of plant origin, or compatible pest control strategies (Kumar *et al.,* 2020). The majority of these botanical pesticides are non-toxic to both humans and the environment even though it works well against a wide range of insect species (Patel *et al.,* 2022). Botanical pesticides which are rich sources of bioactive compounds, could be a substitute for traditional methods of controlling whiteflies (Gusmao *et al.,* 2013).

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They are target specific and non-toxic to mammals and humans and are potentially suitable for use in integrated pest management (Tare *et al.*, 2004). In modern agriculture and an increasingly regulated world, natural botanical pesticides can be feasible in pest management practices and an effective alternative to synthetic pesticides to protect crops (Isman, 2006). Several plant-based materials have been considered for use as insecticides, antifeedants or repellents, which include terpenes, flavonoids, alkaloids, phenols, and other related compounds (Adeyemi, 2010).

Thus, there is a safe and alternative strategy for the control of whiteflies in the agricultural field is needed. Hence, the present study was focused to evaluate the efficacy of insecticides and botanical oils to combat the whitefly infestation in cotton in Pakistan.

2. MATERIALS AND METHODS

Collection and rearing of whiteflies

Whiteflies (*Bemisia tabaci* L.) were collected from pesticide-free cotton fields at the University of Agriculture, Faisalabad, using an aspirator. Subsequently, these whiteflies were reared on brinjal plants in a cage under semi-natural conditions. Brinjal leaves having whitefly nymphs were collected for bioassay.

Leaf discs

Brinjal plants were grown in pots and kept in semi-natural conditions that were free from any pesticide exposure. Middle-aged brinjal leaves, approximately three months old, were selected. The leaves were carefully trimmed to obtain circular leaf discs, each measuring 1.7 cm in diameter. The use of a cork borer ensured precision and uniformity in obtaining leaf samples (Kongchuensin & Takafuji, 2006).

Botanical pesticides

The common botanical pesticides which are used to control the sucking pest complex were selected. Different botanicals i.e., Neem (*Azadirachta indica*) seed oil, Castor (*Ricinus communi*) oil and Linseed (*Glycine max*) oil were purchased from the local market.

Synthetic pesticides

The insecticides used in the experiments are (imidacloprid, acetamiprid and lambda cyhalothrin). These pesticides were purchased from the local market.

Bioassay

An experiment was performed to screen the effectiveness of selected synthetic pesticides and botanical oils against whitefly nymphs on brinjal plants. The pesticides used were imidacloprid, acetamiprid, lambda-cyhalothrin, neem oil, castor oil and linseed oil with five concentrations (20%, 10%, 5%, 2.5% and 1.25%) tested for each. Water was used as the control treatment. Brinjal plants were grown under semi-natural conditions and infested with adult whiteflies. After one month, leaves with 3rd instar nymphs were selected and washed to remove debris. The selected pesticides were diluted and infested leaves were dipped in the solutions for 10-15 seconds. After air-drying, the treated leaves were placed on agar in petri plates. The effects on whitefly nymphs were observed under a microscope and mortality data was recorded.

Experimental conditions

The experimentation was carried out in a growth chamber under constant conditions, maintaining a temperature of 26±2°C and a relative humidity of 70±5%.

Data collection

Data was collected after 24, 48, 72 and 96 hours to assess the impact of insecticides and botanicals.

Data analysis

Minitab software was used for the Analysis of Variance (ANOVA) and comparison of means of significant treatments. Means were compared using the Tukey HSD test at α= 5%. (Najafpoor *et al.*, 2018).

3. RESULTS AND DISCUSSION

Efficacy of insecticides against whitefly

All tested insecticides caused significant mortality of whitefly after 96 hours of application (Table 1). Imidacloprid was statistically highly effective with mortality of 46.67% followed by acetamiprid and lambda cyhalothrin with mortality of 40.67% and 23.33% respectively, 24 hours of application. Statistically, whitefly mortality percentage caused by imidacloprid and acetamiprid at par while lambda cyhalothrin was least effective to control whitefly. The imidacloprid was again significantly more efficient with mortality of 50% than the acetamiprid and lambda cyhalothrin 43.33% and 28.67% respectively, 48 hours of application. After 72 hours of exposure, maximum whitefly mortality was 57% followed by acetamiprid 53.33% and lambda cyhalothrin 36% which were significantly different from each other respectively. However, after 96 hours application, imidacloprid caused the highest mortality 76.67% followed by acetamiprid and lambda cyhalothrin with mortality of 68.08% and 60% respectively which were also significantly different from each other, respectively. The insecticides 96 hours after treatments application showed gradual increase in percent mortality of the whitefly as compared to 24, 48 and 72 hours after treatment exposure.

Efficacy of botanical oils against whitefly

Table 2 showed significant differences among all the botanical oils. Neem oil was statistically highly effective with mortality of 30% followed by castor oil and linseed oil with mortality of 28.35% and 26.86% respectively, 24 hours of application. The neem oil was again significantly more efficient with mortality of 37.33% than the castor oil and linseed oil 34.73% and 31.12% respectively, 48 hours of application. After 72 hours of exposure, maximum whitefly mortality was 50% followed by castor oil 48.67% and linseed oil 45.67% which were significantly different from each other respectively.

However, after 96 hours application, neem oil caused the highest mortality 60.63% followed by castor oil and linseed oil with mortality of 53.33% and 50.60% respectively which were also significantly different from each other, respectively. The botanical oils 96 hours after treatments application showed gradual increase in percent mortality of the whitefly as compared to 24, 48 and 72 hours after treatment exposure. Amusan *et al*. (2013), Hossain *et al*. (2015), Mamun *et al*. (2015), Padmavathi *et al*. (2017) and Hussain (2022) who observed significant mortality of against sucking and chewing pests with application of neem oil. The finding of the current study agreed the results of Mohan and Katiyar (2000) who stated that imidacloprid was the most effective in controlling the whitefly population and its continuous use resulted in increased whitefly population due to development of resistance in this pest against imidacloprid.

4. CONCLUSION

Imidacloprid demonstrated effective control of whitefly as a synthetic insecticide, while neem oil showed promising results among botanicals. Among insecticides lambda cyhalothrin while among botanicals linseed oil demonstrated the lowest level of efficacy. In conclusion, botanical pesticide (neem oil) can be an efficient alternative to chemical pesticides for managing whitefly in agriculture, especially in situations where chemical resistance is a concern.

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6. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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Table 1. Mean percent mortality (M±SE) of *Bemisia tabaci* after 24, 48 72- and 96-hours application of insecticides in a laboratory test.

Treatments	Concentrations (%)	Percent Mortality (M±SE)			
		24 hours	48 hours	72 hours	96 hours
Imidacloprid	1.25	3.33±3.04^{b}	6.67±3.67^{c}	16.67±2.50^{bc}	23.33±1.25^{bc}
	2.5	6.67±3.33^{b}	15.00±3.58^{bc}	23.67±3.33^{bc}	33.33±2.23^{bc}
	5	16.67±0.33^{b}	20.00±3.33^{bc}	26.67±4.00^{b}	40.00±3.33^{b}
	10	20.00±3.46^{b}	26.67±0.21^{b}	36.67±4.33^{b}	50.00±3.67^{b}
	20	46.67±3.33^{a}	50.00±2.23^{a}	57.00±4.00^{a}	76.67±4.67^{a}
Acetamiprid	1.25	3.33±2.65^{bc}	6.67±2.32^{c}	13.33±2.40^{bc}	23.33±1.99^{bc}
	2.5	10.33±2.18^{bc}	16.67±2.65^{bc}	23.33±2.90^{bc}	30.00±2.45^{abc}
	5	13.33±3.33^{bc}	20.00±3.05^{bc}	30.00±3.25^{bc}	40.00±2.88^{abc}
	10	16.67±3.64^{b}	30.00±3.88^{ab}	33.33±3.84^{ab}	46.67±3.05^{ab}
	20	40.67±4.12^{a}	43.33±4.09^{a}	53.33±4.00^{a}	68.08±3.55^{a}
Lambda-cyhalothrin	1.25	3.33±0.19^{c}	6.67±2.87^{b}	13.33±3.33^{c}	20.00±2.13^{d}
	2.5	7.67±1.20^{bc}	11.16±2.98^{ab}	16.67±3.65^{bc}	27.67±2.18^{cd}
	5	12.33±1.34^{ab}	19.00±3.83^{ab}	26.67±2.56^{ab}	34.78±2.98^{bc}
	10	14.67±2.98^{ab}	22.67±3.33^{a}	33.33±3.33^{ab}	40.71±3.11^{b}
	20	23.33±3.33^{a}	28.67±4.32^{a}	36.00±4.19^{a}	60.00±3.33^{a}

Means in a column sharing same letter (s) are significant at 5% probability level using Tukey's test.
(M±SE)= Mean±Standard Error

Table 2. Mean percent mortality (M±SE) of *Bemisia tabaci* after 24, 48 72- and 96-hours application of botanical oils in a laboratory test.

Treatments	Concentrations (%)	Percent Mortality (M±SE)			
		24 hours	48 hours	72 hours	96 hours
Neem oil	1.25	10.00±3.33^{ab}	20.00±2.56^{bc}	23.33±1.56^{bc}	30.00±1.48^{b}
	2.5	16.67±0.54^{ab}	21.25±3.04^{bc}	26.67±3.33^{bc}	36.87±3.3^{b}
	5	23.33±1.46^{a}	25.64±3.33^{ab}	36.53±2.45ab	45.00±3.46^{ab}
	10	26.00±2.15^{a}	30.25±2.33^{ab}	40.37±1.25^{ab}	50.00±1.34^{ab}
	20	30.00±3.33^{a}	37.33±3.46^{a}	50.00±3.11^{a}	60.63±4.33^{a}
Castor oil	1.25	6.67±1.48^{cd}	13.23±1.08^{cd}	20.00±2.48^{cd}	26.23±1.70^{b}
	2.5	16.47±2.31^{bc}	20.00±4.10^{bcd}	26.67±1.3^{bc}	36.73±3.33^{ab}
	5	20.00±3.33^{b}	26.67±1.45^{abc}	33.63±2.46^{abc}	42.00±1.46^{ab}
	10	26.45±1.34^{ab}	31.33±3.33^{ab}	43.53±1.34^{ab}	47.36±3.34^{a}
	20	28.35±2.33^{a}	34.73±2.13^{a}	48.67±3.33^{a}	53.33±3.33^{a}
Linseed oil	1.25	6.67±1.20^{bc}	10.00±1.82^{bc}	16.43±3.33^{bc}	28.53±2.10^{b}
	2.5	13.33±1.85^{abc}	20.00±3.25^{abc}	25.13±2.43^{abc}	36.68±2.11^{ab}
	5	20.00±2.39^{ab}	23.67±3.33^{ab}	28.47±1.48^{ab}	40.82±3.33^{ab}
	10	23.33±3.31^{a}	26.34±2.67^{ab}	34.00±4.08^{ab}	46.48.00±4.11^{a}
	20	26.86±4.38^{a}	31.12±2.96^{a}	45.67±3.33^{a}	50.60±2.91^{a}

Means in a column sharing same letter (s) are significant at 5% probability level using Tukey's test.
(M±SE)= Mean±Standard Error

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FAUNISTICS OF PIERID BUTTERFLIES (LEPIDOPTERA: PIERIDAE) WITH SOME NEW RECORDS FROM SWAT, PAKISTAN

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ABSTRACT

This study was conducted to explore the Pierid butterfly fauna of Swat over a one-year period from March 2021 to April 2022. A total of 100 specimens of butterflies were collected from different localities in Swat. Among the 100 specimens collected, 15 species spanning 10 genera categorized them into two subfamilies: Pierinae and Coliadinae. Within the identified species, nine belonged to the subfamily Pierinae, while the subfamily Coliadinae comprised six species. One species, *Eurema brigitta* (Stoll, 1780), is reported for the first time from Khyber Pakhtunkhwa, while five species—*Pieris rapae* (Linnaeus 1758), *Ixias pyrene* (Linnaeus 1764), *Euchloe daphalis* (Moore 1865), *Aporia nabellicahesba* (Boisduval, 1836), *Colias eratae* (Esper 1805) are reported for the first time from Swat.

1. INTRODUCTION

A healthy environment is essential for a healthy biological community. More than half of the world's species are insects, which also serve as pollinators, seed dispersers, biocontrol agents, and ecological indicators (Mengal *et al.*, 2019). Lepidoptera is regarded as the most varied group of insects. (Gay *et al.*,1992). It includes butterflies and moths (Shields, 1989). Butterflies account for one-tenth of all known Lepidoptera-species (Carter, 1992). About 19,238 species of butterflies have been reported around the world (Heppner, 1998). Butterflies are the most well-known insect to humans, due to their enormous size and dazzling vibrant colours (Bibi *et al.*, 2021). As they fly from one colour and sun-loving lifestyle. We are amused by their plant to another, butterflies aid in pollination. The majority of butterfly species are seasonal and prefer a specific environment. (Kunte,1997).

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They are also the best indications of undesired activity and disruptions in the surroundings. (Kocher & Williams, 2000). The family Pieredae consists of medium-to-small sized butterflies, mostly white and yellow in colours. They are characterized by the absence of cleaning spur on fore tuba and bifid claws, hindwings are without pre-costal veins (Roberts, 2001). They are also generally known to be species of open areas. Members of the family Pieridae are distributed throughout the globe, except Polar regions (Khan *et al.*, 2004). They act as pollinator, but some are serious pests of many crops and fruits in their larval stages. Larva of *Pieris brassicae, Pontia daplidice* and *Anaphaeis aurota* are the pest of many plant families like *Cruciferae*, Leguminosae and Rhamnaceae. A large number of cabbage crop yield losses are due to the pests of *Pieris rapae* (Shah & Rafi, 2016). *Pieredae* is second largest family of butterflies with about 76 genera containing approximately 1100 species (Evans, 1903).

The Pieridae are generally divided in four subfamilies: Dismorphiinae, Pierinae, Coliadinae, Pseudopontiinae (Braby, 2005). Only two subfamilies PierIne and Coliadene represent their taxa in Pakistan (Shah & Rafi, 2016).

Many researchers worked on the Pieredae butterfly's fauna of Pakistan. Malik (1970) reported 21 species while Iqbal (1978) reported 12 species of Pieridae from Islamabad. Hasan (1994) documented 13 species of Pieredae from Murree and Islamabad. Shah & Rafi (2016) reported 10 piered species from district Kohat. A total of 320 species of butterflies have been reported from Pakistan, including 56 species of Pieridae butterflies (Roberts, 2001). Naz *et al.* (2001) recorded 10 species of Pierid from district Buner. During a taxonomic study five species of piered were recorded by Abbas *et al.* (2002) from Sakrdu region. Similarly, in Azad Kashmir seven pierid species were documented by Khan *et al.* (2007). Perveen & Ahmad (2012) reported 11 species of pierid butterfly from Kohat while from District Mansehra five species of Pierid butterfly were recorded by Perveen & Fazal (2013).

The butterfly fauna from Poonch Azad Kashmir was recorded by Khan *et al.* (2014), yielding 13 species of Pierid butterfly. From District Chitral, five species of Pierid butterfly have been reported (Afshan *et al.*, 2015). In District Dir Lower, of the total 24 species of butterflies only five belonged to the family Pieridae (Khan, 2016). Recently, Mengal *et al.* (2017) conducted a study on butterfly fauna of Quetta and found seven species of pierid butterflies. From District Battagram of the total eight species, 50 % were from family Pieredae (Bibi *et al.*, 2021). Most recently, out of the total 23 species of butterflies from district Haripur seven belonged to the family Pieridae (Khalid *et al.*, 2022) while Taj *et al.* (2022) reported eight species of Pierid butterfly from District Abbottabad. Tshikolovets & Pagès (2016) reported six families of butterflies comprising of approximate 436 species of butterflies in Pakistan. Present study is an approach to record diversity and distribution of Pierid butterflies in various habitats of District Swat prepare a Checklist for future study.

2. MATERIALS AND METHODS

Study Area

Swat is a popular tourist destination in Khyber Pakhtunkhwa. Swat district covers a total size of 5,337 square kilometers (2,061 square miles). Swat is bounded administratively by Chitral, Upper Dir, and Lower Dir to the west, Gilgit-Baltistan to the north, and Kohistan, Buner, and Shangla to the east and southeast, respectively. The district is divided into seven administrative Tehsils: Babozai, Kabal, Barikot, Matta, Charbagh, and Khwazakhela, as well as Bahrain. Swat has an average elevation of 980 meters (3,220 feet) (Mohiuddin, 2007)

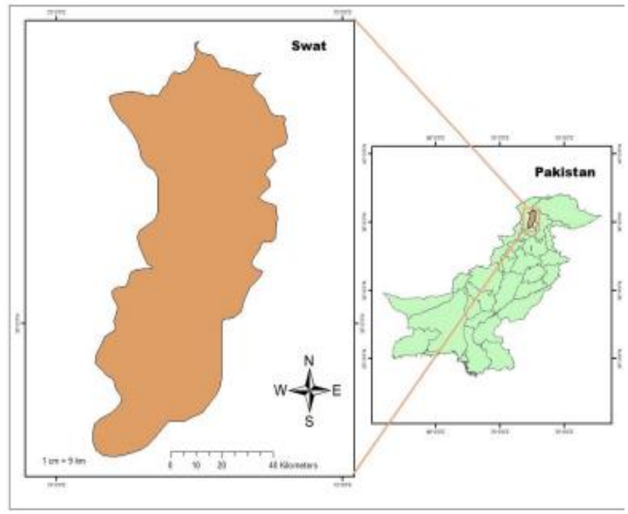

Fig 1 Map of study area

Collection

Butterflies were collected from the seven Tehsils of district Swat: Babozai, Barikot, Kabal, Bahrain, Khwazakhela, Charbagh, and Matta, throughout a one-year period from March 2021 to April 2022. Butterfly nets were used to collect butterflies, and photographs were shot in the field. The specimens were killed by pushing on the thorax. However, sensitive species were stored in the refrigerator overnight. On a thermophile setting board, dead specimens were stretched for Pinning. Butterflies were then labelled with the following information: common name, scientific name, date of collection, location of collection, and collector. After labelling, the butterflies were moved to rectangular wooden and plastic boxes with naphthalene balls in one side to prevent pests from entering.

Photography:

All photographs were taken using a digital camera model "Nikon D7200" equipped with a 70-300mm AFP or lens wide zoom optical moveable lens, and close-up images were made with an Oppo A16.

Identification:

The identification of butterflies in this study was done with the help of available literatures (Inyatullah *et al.*, 2002; Khan *et al.*, 2004; Khan *et al.*, 2014; Mengal *et al.*, 2017; Roberts, 2001; Sabir *et al.*, 2000; Tshikolovets & Pagès, 2016; Yu-Feng *et al.*, 2020).

3. RESULTS AND DISCUSSION

In the current survey 100 specimen of Family Pieridae were collected from the seven Tehsil of District Swat; Babozai, Barikot, Bahrain, Kabal, Matta, Khwazakhela and Charbagh for one-year period from March 2021 to April 2022. Collected specimens were identified into two sub-families, 10 genera and 15 species (Table 1).

Sub-family Pierinae comprised of nine species *Pieris canidia* (Linnaeus 1768), *Pieris rapae* (Linnaeus 1758), *Pieris brassicae* (Linnaeus 1758), *Belenois aurota* (Linnaeus 1758), *Pontia daplidice* (Linnaeus 1758), *Ixias pyrene* (Linnaeus 1764), *Euchloe daphalis* (Moore 1865), *Aporia nabellicahesba* (Boisduval,1836) while subfamily Coliadinae composed of six species Colias fieldii (Menetries 1855), *Colias eratae* (Esper 1805), *Eurema hecabe* (Linnaeus 1758), *Eurema brigitta* (Stoll ,178), *Gonepteryx nepalensis* (Doubleday, 1847), *Catopsilia pomona* (Fabricius, 1775), *Catopsilia pyranthe*. One species *Eurema brigitta* (Stoll ,178) is first time reported from Khyber Pakhtunkhwa while 5 species *Pieris rapae* (Linnaeus 1758), *Ixias pyrene* (Linnaeus 1764), *Euchloe daphalis* (Moore 1865), *Aporia nabellicahesba* (Boisduval,1836), *Colias eratae* (Esper 1805) are reported first time from Swat. *Pieris canidia* is the most abundant and *Aporia nabellicahesba* is comparatively rare. Highest number of collections was made in Tehsil Babozai.

Phylum Arthropoda
Class Insecta
Order Lepidoptera
Family Pieridae
Sub-family Pierinae

1. *Pieris canidia* (Linnaeus 1768)
Indian Cabbage White

Material examined: Pakistan: Khyber Pakhtunkhwa: Swat, Mingora, 24.ix.2021, 0.vii.2021; leg. Abdur Rehman. ; leg. Usama 12.vii.2021; leg. Usama, Matta, Balasoor, 9. x.2021; leg .A. Rehman

Distribution in Pakistan: Gilgit Baltistan , Gilgit by (Hasan, 1997); Azad Kashmir, Poonch (Khan *et al.*, 2014); Punjab from Lahore (Ahsan & Iqbal, 1975; Puri, 1931) , Attock (Rahman *et al.*, 2011), Murree Rawalpindi & Islamabad (Hasan, 1994; Iqbal, 1978) , Potohwar (Rafi, 2016); Khyber Pakhtunkhwa, Abbottabad (Taj *et al.*, 2022), Peshawar by Malik (1970), Buner (Naz *et al.*, 2001), Chitral (Afshan *et al.*, 2015), Shabqadar (Ullah *et al.*, 2017), Tangi (Yu-Feng *et al.*, 2020), Koaz Behram (Haroon *et al.*, 2014), Swat (Tshikolovets & Pagès, 2016).

2. *Pieris rapae* (Linnaeus 1758)
Small Cabbage White

Material Examined: Pakistan: Khyber Pakhtunkhwa; Swat**,** Babozai, Mingora,22.ix.2021, 0.vii.2021; leg. Usama. Jahanabad 23.vii.2021, Matta, Balasoor,10. x.2021, Khwazakhela, Chamtalai, 4.III.2022; leg.A. Rehman

Distribution in Pakistan: Azad Kashmir, Dhirkot (Abbasi *et al.*, 2019) ; Gilgit Baltistan Skardu (Abbas *et al.*, 2002) ; Punjab, Muree (Malik 1970) ,Potohwar (Shah & Rafi, 2016); Khyber Pakhtunkhwa Kohat (Perveen & Ahmad, 2012),Karak (Usman *et al.*, 2017);Haripur (Khalid *et al.*, 2022) ; Sindh (Mal *et al.*, 2014) ; Baluchistan, Takhti Suliman (Roberts, 2001) ,Quetta (Malik 1970; (Mengal *et al.*, 2017), Ziarat (Tshikolovets & Pagès, 2016).
Remarks; New record for Swat

3. *Pieris brassicae (*Linnaeus 1758)
Large Cabbage White

Material Examined: Pakistan: Khyber Pakhtunkhwa; Swat**,** Babozai, Mingora, Ingaradherai, 5.v.2021, 10.vii.2021; leg. Atif. Manglawar,10.vi.2021, Kabal, Hazara,14.ix.2021, Khwazakhela, Topsin,25.ii.2022; leg. A. Rehman

Distribution in Pakistan ; Azad Kashmir from district Muzaffarabad & Azad Kashmir by (M. Khan *et al.*, 2004) similary (Khan *et al.*, 2014) from Poonch Malik (1970) from Rawalpindi Gilgit Baltistan Skardu (Abbas *et al.*, 2002) Punjab , Lahore (Ahsan & Iqbal 1975), Potohwar (Rafi, 2016); Khyber Pakhtunkhwa, Abbottabad (Taj *et al.*, 2022), Buner (Naz *et al.*, 2001), Malakand & lower Swat (Inyatullah *et al.*, 2002; Roberts, 2001) , Dir Lower (M. I. Khan, 2016), Dir Upper (Attaullah *et al.*, 2018), Karak (Usman *et al.*, 2017) , Haripur (Khalid *et al.*, 2022) ; Sindh (Mal *et al.*, 2014), Swat (Malamjabba, Kalam & Miandam) (Tshikolovets & Pagès, 2016); Baluchistan , Quetta (Mengal *et al.*, 2017).

4. *Belenois aurota* (Fabricius 1758)
Pioneer White

Material Examined: Pakistan: Khyber Pakhtunkhwa; Swat, Babozai, Mingora, Marghozar, 20.iii.2021, 25.vii.2021, Barikot, Amlookdara, 21. viii.2021, Charbagh;leg.Usama. Badar,9.ix.2021, Matta,Beha, 13.x.2021,Kabal,Hazara,17.ix.2021;leg.A.Rehman

Distribution in Pakistan: Azad Kashmir Poonch (Khan *et al.*, 2014) ; Gilgit Baltistan ; Khyber Pakhtunkhwa ,Swat (Roberts, 2001) ,Kohat (Perveen & Ahmad, 2012) , Dir lower (Khan, 2016), Tangi Charsadda (Yu-Feng *et al.*, 2020) ; Baluchistan , Quetta (Mengal *et al.*, 2017; Roberts, 2001).

5. *Pontia daplidice* (Linnaeus 1758)
Bath White

Material Examined: Pakistan: Khyber Pakhtunkhwa; Swat**,** Babozai, Mingora,28.iv.2021, Marghozar 10.vii.2021, Barikot, Amlookdara, 21.viii.2021; leg.Azan .Charbagh ,Badar, 9.ix.2021, Matta Beha,13.x.2021, Bahrain,Utrur,8.viii.2022,Kabal,Hazara,28.VII.2021, Khwazakhela,Behar,17.vi.2021;leg.A.Rehman

Distribution in Pakistan : Hassan (1997) reported it from Rawalpindi Islamabad and Murree hills ;Azad Kashmir , Poonch (Khan *et al.*, 2007) , Dhirkot (Abbasi *et al.*, 2019) ; Gilgit Baltistan ,In Gilgit by Hasan (1997), Skardu (Abbas *et al.*, 2002) ; Khyber Pakhtunkhwa, Buner (Naz *et al.*, 2001) Chitral, Swat (Roberts, 2001) , Shabqadar (Ullah *et al.*, 2017), Dir Upper Doag Dara (Attaullah *et al.*, 2018), Manshera (Perveen & Fazal, 2013), Battagram (Bibi *et al.*, 2021); Punjab , Potohwar (Shah & Rafi, 2016).

6. *Ixias pyrene* (Linnaeus 1764)
Yellow Orange Tip

Material Examined: Pakistan: Khyber Pakhtunkhwa; Swat**,** Babozai, Mingora, Saidu Sharif,10.ix.2021, Marghozar, 10.vii.2021; leg. Usama. Barikot, Amlookdara, 21.viii.2021, Charbagh Badar; 9.v.2021; leg.Yaseen. Kabal, Hazara, 17.iii.2021; leg. A.Rehman.

Distribution in Pakistan; Azad Kashmir; Gilgit Baltistan; Punjab, Lahore Ahsan & Iqbal (1975) Taxila (Roberts, 2001); Khyber Pakhtunkhwa, Buner (Naz *et al.*, 2001), Abbottabad, Hevellian (Roberts, 2001); Sindh (Mal *et al.*, 2014)
Remarks: New record to Swat

7. *Euchloe daphalis* (Moore 1865),
Pearl White

Material Examined: Pakistan: Khyber Pakhtunkhwa; Swat, Babozai, Marghozar,3.iii.2021, Charbagh ,Badar, 19.ii.2022; leg.A.Rehman
Distribution in Pakistan; It has been reported in Chiltan, karakoram Baluchistan, Torghar, Saiful Maluk, Chitral (Roberts, 2001), Gilgit, Chitral, Astore (Tshikolovets & Pagès, 2016).
Remarks: New record to Swat

8. *Aporia nabellicahesba* (Boisduval,1836)
The Dusky Blackvein

Material Examined: Pakistan: Khyber Pakhtunkhwa; Swat, Kalam, 25.vii.2021. leg.A.Rehman
Distribution in Pakistan: Kaghan Valley, Shograh, Chitral (Roberts, 2001; Tshikolovets & Pagès, 2016).
Remarks: New record to Swat

Subfamily Coliadinae
9. *Colias fieldii* (Menetries 1855),
Dark Clouded Yellow

Material Examined: Pakistan: Khyber Pakhtunkhwa; Swat**,** Babozai, Mingora, 22.iii.2021, 29.vii.2021; leg. Taimur. Barikot, Amlookdara, 21.v.2021, Charbagh, Badar, 9.ix.2021, Matta, Beha, 13.v.2021, Bahrain,Mankiyal,15.iii.2022,Kabal, Hazara,17.ix.2021; leg.A.Rehman

Distribution in Pakistan: Azad Kashmir, kotli, Mirpur & Bhimber (Khan *et al.*, 2007), Poonch (Khan *et al.*, 2014) ; Gilgit Baltistan Skardu (Abbas *et al.*, 2002) ; Punjab ,Lahore (Ahsan & Iqbal, 1975), Murree (Roberts, 2001) ; Khyber Pakhtunkhwa ,Buner (Naz *et al.*, 2001) ,Hazara (Roberts, 2001) Koaz Behram (Haroon *et al.*, 2014), Dir Lower (Khan, 2016),Swat (Tshikolovets & Pagès, 2016), Tangi Charsadda (Yu-Feng *et al.*, 2020) ; Baluchistan, Suleiman range (Roberts, 2001).

10. *Colias erate* (Esper 1805),
Pale Clouded Yellow

Material Examined: Pakistan: Khyber Pakhtunkhwa; Swat, Babozai, Mingora,17.vi.2021, Takhtab 10. v.2021; leg. Usama Badar, 9.ix.2021, Kabal, Kanju, 5.iii.2022, Khwazakhela, Behar, 13.x.2021. leg.A. Rehman

Distribution in Pakistan: Azad Kashmir (kotli, Mirpur and Bhimber) (Khan *et al.*, 2007) ,Poonch (Khan *et al.*, 2014) ; Gilgit Baltistan Skardu (Abbas *et al.*, 2002) ; Punjab ; Khyber Pakhtunkhwa ;Buner (Naz *et al.*, 2001), Takht-i-nasrat (Roberts, 2001) Tangi Charsadda (Yu-Feng *et al.*, 2020) , Abbottabad (Taj *et al.*, 2022), Malakand (Inyatullah *et al.*, 2002) Dir Upper (Attaullah *et al.*, 2018) ; Dir Lower, Chitral (Tshikolovets & Pagès, 2016); Sindh (Malik, 1970 ; Mal *et al.*, 2014).
Remarks: New record to Swat

11. *Eurema hecabe* (Linnaeus 1758)
Common Grass Yellow

Material Examined: Pakistan: Khyber Pakhtunkhwa; Swat**,** Babozai, Mingora,25.IV.2021, Marghozar, 10.vii.2021; leg. Usama. Badar, 9.viii.2021, Matta,Beha,13.x.2021,Bahrain, Mankiyal,15.iii.2022, Kabal, Hazara,17.ix.2021, Khwazakhela, Behar, 9.vi.2021.leg.A.Rehman

Distribution in Pakistan; Azad Kashmir Poonch (Khan *et al.*, 2014) Gilgit Baltistan Punjab Khyber Pakhtunkhwa , Buner (Naz *et al.*, 2001) , Lower Swat ,Charbagh (Inyatullah *et al.*, 2002), Kohat (Perveen & Ahmad, 2012) , Manshera (Perveen & Fazal, 2013) , Koaz Behram (Haroon et al., 2014) Chitral (Afshan *et al.*, 2015) ,Dir Lower (M. I. Khan, 2016) , Karak (Usman *et al.*, 2017) Tangi Charsadda (Yu-Feng *et al.*, 2020) Abbottabad (Taj *et al.*, 2022) ; Sindh (Mal *et al.*, 2014) ; Baluchistan ,Quetta (Mengal *et al.*, 2017).

12. *Eurema brigitta* (Stoll ,178)
Small Grass Yellow,

Material Examined: Pakistan: Khyber Pakhtunkhwa; Swat**,** Babozai, Mingora, 25.iii.2021; leg. Usama Badar, 9.iv.2021, Kabal, Hazara, 17.vii.2021. leg. Waqas

Distribution in Pakistan; Punjab, Lahore (Ahsan & Iqbal, 1975; Roberts, 2001); Islamabad, Margalla Hills, Poonch (Tshikolovets & Pagès, 2016).
Remarks: This species is documented for the first time from Khyber Pakhtunkhwa.

13. *Gonepteryx nepalensis* (Doubleday, 1847)
Himalayan Brimestone

Material Examined: Pakistan: Khyber Pakhtunkhwa; Swat**,** Babozai, Marghozar, 5.iii.2021, Islampur, 10.vii.2021;leg.Usama.Malamjabba,7.x.2021,Matta,JarogoValley,19.ix.2021,Khwazakhela,Behar,2.iv.2022. leg. Taimur

Distribution in Pakistan: Azad Kashmir, Dhirkot (Abbasi et al., 2019). Gilgit Baltistan ; Punjab ; Khyber Pakhtunkhwa, Buner (Naz *et al.*, 2001), Marghuzar (Swat) (Inyatullah *et al.*, 2002) ; Sindh Baluchistan

14. *Catopsilia Pomona* (Fabricius, 1775)
Common Emigrant

Material Examined: Pakistan: Khyber Pakhtunkhwa; Swat**,** Babozai, Mingora,5.viii.2021, Odigram,10.ix.2021, 21.viii.2021, Charbagh ,Malamjabba, 28.vii2021.Leg.A.Rehman

Distribution in Pakistan: Azad Kashmir ; Gilgit Baltistan ; Punjab , Lahore by Ahsan & Iqbal (1975),Taxila ,Muree Hills & Bahawalpur (Tshikolovets & Pagès, 2016) ; Khyber Pakhtunkhwa, Swat ,Lower Chitral (Roberts, 2001) , Kohat (Perveen & Ahmad, 2012) ,Koaz Behram (Haroon et al., 2014) Shabqadar (Ullah et al., 2017), Karak (Usman et al., 2017), Tangi Charsadda (Yu-Feng et al., 2020) ; Sindh, Karachi (Malik 1970;Mal et al., 2014) ; Baluchistan , Quetta (Mengal et al., 2017; Roberts, 2001)

15. *Catopselia pyranthe* Linnaeus
Mottled Emigrant

Material Examined: Pakistan: Khyber Pakhtunkhwa; Swat**,** Babozai, Mingora, 10.iii.2021, Saidu sharif,10.vi.2021; leg. Usama, Kabal, Hazara, 2.viii.2021. leg. A. Rehman.

Distribution in Pakistan: Azad Kashmir, Poonch (Khan *et al.*, 2014); Islamabad , Muree Hills (Hasan 1994) Gilgit Baltistan ; Punjab, Multan , Bahawalpur, Taxila (Tshikolovets & Pagès, 2016) ; Khyber Pakhtunkhwa Kabal (Inyatullah *et al.*, 2002), Swat (Roberts, 2001) Koaz Behram(H. Khan & Perveen, 2015), Shabqadar (Ullah *et al.*, 2017) , Tangi Charsadda (Yu-Feng *et al.*, 2020); Sindh (Mal *et al.*, 2014).Baluchistan (Roberts, 2001).

4. CONCLUSION

It is stated that the research area's high level of vegetation contributes to the diversity of the butterfly fauna. To fully understand the butterflies of the Swat Valley, more in-depth research is advised.

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6. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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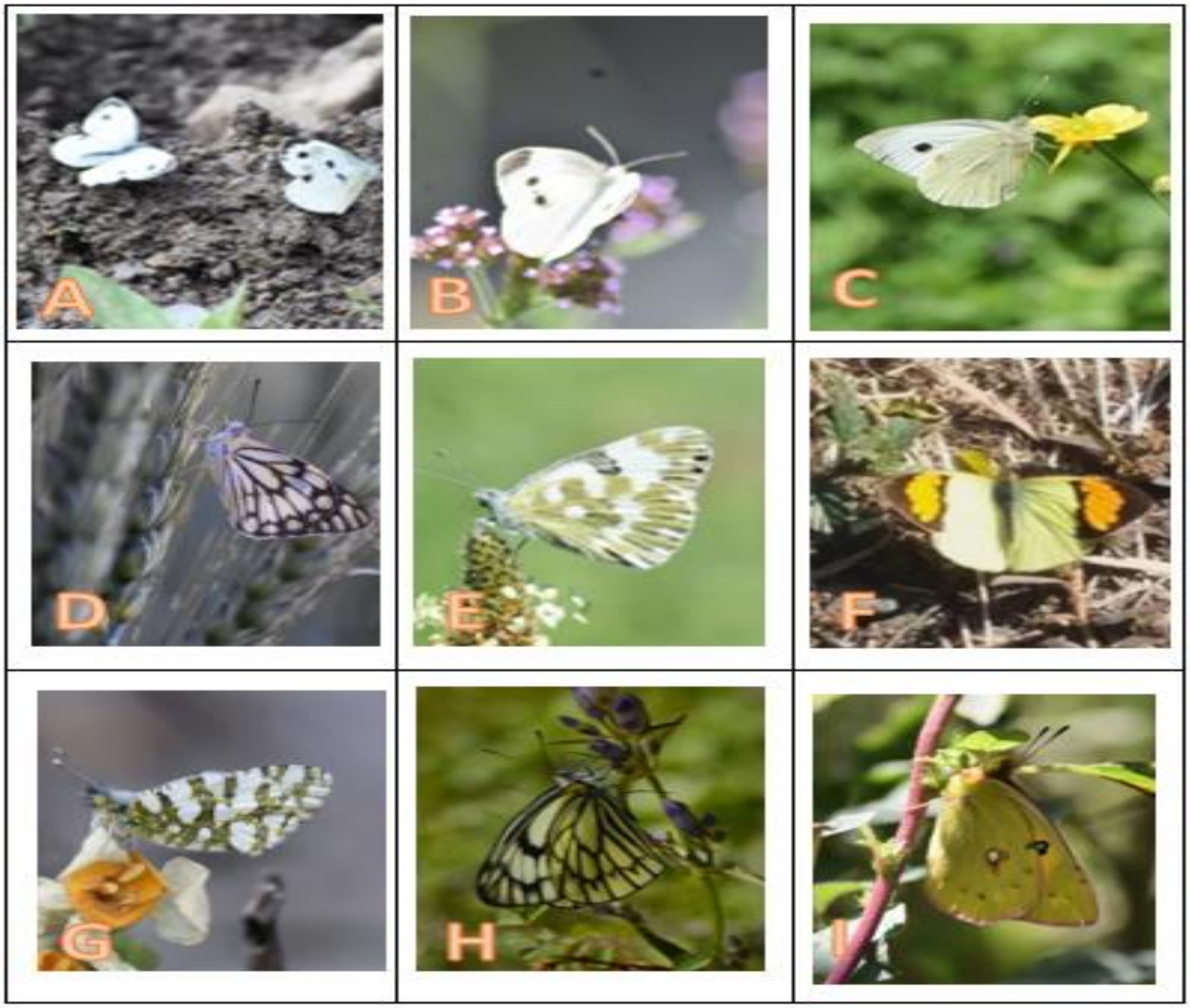

Figure 1 A, *Pieris canidia* (Linnaeus 1768); B, *Pieris rapae* (Linnaeus 1758) ; C, *Pieris brassicae* (Linnaeus 1758) ; D, *Belenois aurota* (Linnaeus 1758) ; E, *Pontia daplidice* (Linnaeus 1758) ;F, Ixias pyrene (Linnaeus 1764) ; G *Euchloe daphalis* (Moore 1865) ; H, *Aporia nabellicahesba* ; I, *Colias fieldi*

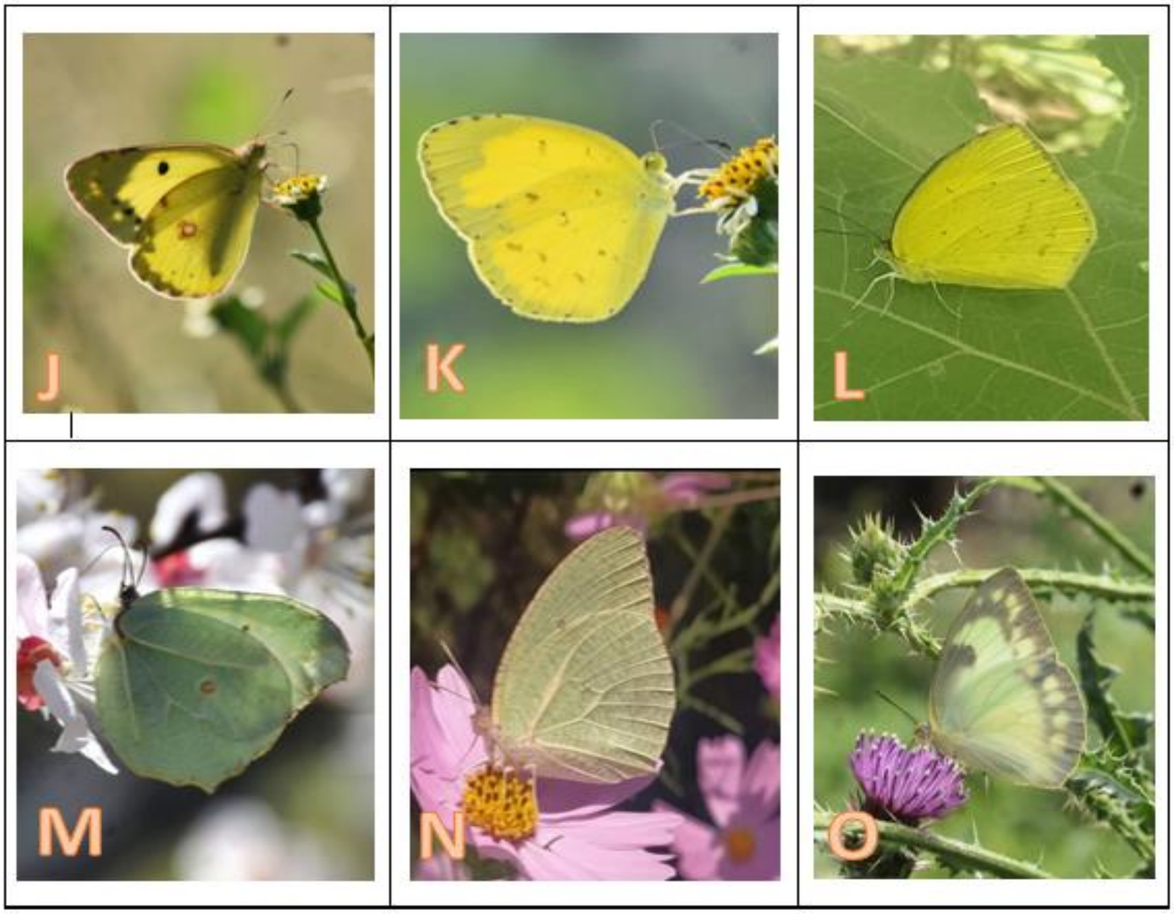

Figure 2, J, *Colias eratae* (Esper 1805); K *Eurema hecabe* (Linnaeus 1758); L, *Eurema brigitta* (Stoll ,1778) ; M, *Gonepteryx nepalensis* (Doubleday, 1847) ; N, *Catopsilia pyranthe* ; O, *Catopsilia pomona* (Fabricius, 1775),

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POPULATION TREND OF CANOLA APHID, *LIPAPHIS ERYSIMI* (KALT) (HOMOPTERA: APHIDIDAE) UNDER FIELD CONDITION

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ABSTRACT

The field experiment on the population trend of *Lipaphis erysimi* (Kalt.) was conducted at New Developmental Farm, The University of Agriculture, Peshawar, from 2021 to 2022. The results revealed that aphids appeared at rates of 1.00 and 0.75 per leaf during January 8-14, reaching their peak at 10.10 and 11.35 per leaf during March 12-18. Subsequently, they disappeared from the field after April 9-15 in both years (2021 and 2022). Therefore, as L. erysimi reaches its peak population during SMW 11 (March 12-18), plants should be closely observed during this period, and control methods with rapid curative action should be applied if needed.

1. INTRODUCTION

Canola, *Brassica napus* L. is one of the key oil seed crops. It is an herbaceous, annual crop, which belongs to family *Brassicaceae*. Primary it is cultivated for seeds and edible oil (Possent *et al*., 2017). In Pakistan, it has been grown in all the regions over an area of 26.02 thousand hec with annually production of 102.0 thousand tones contributing up to 17 percent of the local edible oil production. The major cultivating areas of Rapeseed and Mustard include Rawalpindi, Attock, Jhelum, Faisalabad, Chakwal, Multan, Bahawalpur, Bahawalnagar, Muzaffargarh, Rahim Yar Khan (AARI, 2019). In Pakistan, the low production of canola crop is due to many factors including a widespread series of biotic and abiotic stresses. Abiotic stresses include flooding, drought, cold, heat, extreme light intensity and salinity. In many crops, the most susceptible phase is reproductive stages has been studied towards temperature stress. Biotic stresses include a wide range of insects and pathogens (Buchanan *et al*., 2000). In canola crop aphids can reduce yields up to approximately 97 percent.

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These losses in yield are affected by insect pests, like, the phloem-feeding aphids, which parasitize the crop by deploying their defensive mechanism (Giordanengo *et al.* 2010; Jaouannet *et al.* 2014; Kumar, 2019). The nymph and adult are the major stages of aphid life cycle which damage plants by directly sucking cell sap from different portions. The pest raises parthenogenetically, and females can provide birth between 25 to 135 nymphs, which increase rapidly and reproduced in 6 to 9 days. Managing approaches for insects largely depend upon chemical insecticides (Karami *et al*., 2018). Presently, the easiest and most effective approach towards this pest is use of systemic insecticides such as neonicotinoids (El-Wakeil *et al*., 2013; Stapel *et al*., 2000). Though, this type of controlling pest is ecologically unmaintainable (Zhang *et al*., 2014b). Application of insecticides and natural enemies use have limited achievement in the management of insect pests. Though, insecticides create a negative effect on bio-control agents (Capinera, 2008), while resistance has developed in aphids against mostly synthetic insecticides (Mottaghinia *et al*., 2011). Therefore, HPR has benefit in managing the population of pests with eco-friendly method (Smith, 2005).

Many efforts have been taken in Pakistan, to increase the quality, productivity and resistance in rapeseed canola varieties. In order to these, fresh varieties have been evaluated and tested in multiple regions of Pakistan. New varieties are better in oil contents, quality and strength (Swati, 2005). Thus, keeping in view, the significance of the crop and aphid this study is aimed to population trend of canola aphid, *lipaphis erysimi* (kalt) (homoptera: aphididae) on selected canola variety (Abaseen) under field condition.

2. MATERIALS AND METHODS

The field experiment (Population trend of *L. erysimi* Kalt.) was conducted in New Developmental Farm, The University of Agriculture, Peshawar.

Population trend of L. erysimi (Kalt)

To study the population trend of *L. erysimi*, a susceptible variety (Abaseen) was sown in sub plots with four replications. The number of aphids were recorded on 3 leaves from top, middle and lower part of four randomly selected plants from each replication and evading the boarder rows of every plot. The number of aphids was counted through a magnifying glass and data were recorded on weekly basis (Ahmad *et al*., 2013).

Statistical Analysis

The data were analyzed by using analysis of variance (ANOVA) with help of Statistix 8.1. The significant data were separated by calculating least significant difference (LSD) at 5% level of significance (Steel & Torrie, 1997).

3. RESULTS AND DISCUSSION

Population trend of L. erysimi

Data was recorded on a weekly basis to determine the population trend of aphids on susceptible variety Abaseen during 2021 and 2022 (Table No. 1 and Figure No. 1). The pest appears in the field during Standard Meteorological Week 2 (SMW 2) (January 8-14) in 2021 (1.00 aphids' leaf^{-1}) and 2022 (0.75 aphids' leaf^{-1}). The pest continues to increase its population gradually till its peak was recorded in SMW 11 (March 12-18) during both years (8.80 aphids' leaf^{-1} in 2021 and 10.35 aphids' leaf^{-1} in 2022). After that, the pest population started to decline gradually till SMW 15 (April 9-15) (0.32 aphids' leaf^{-1} in 2021 and 0.47 aphids' leaf^{-1} in 2022). From SMW 15 onwards the pest disappeared from the field. Canola aphids *L. erysimi* is one of the economically important pests of canola crops and cause significant yield losses every year. This research was performed to find the population trend of the pest in this region. The population trend was observed on variety 'Abaseen' in 2021 and 2022. Where, aphids appear in the field during Standard Meteorological Week 2 (January 08-14) then the pest reaches to its peak population during SMW 11 (Mar 12-18) after that the insect population declined gradually and disappears from the field after SMW 15 (April 09-15) during both years.

Similar, result was recorded by Aslam *et al.,* (2005) and Saljoqi *et al.,* (2011). Furthermore, Jitendra *et al.,* (2000) has also observed a parallel population density of mustard aphid on Brassica germplasm. Khan *et al.,* (2015) recorded that at young stage, crop have fleshy leaves which provide medium for higher infestation of aphids in comparison with later stages, then plant tissues are rough and harden. Preceding works have verified different theories and conclusions concerning the reasons that may be responsible for the population trend of aphids in vivo conditions such as physiological age of plant (Smith, 2005) soil fertility, growth pattern (Painter, 1951), natural enemies and environmental stresses Aheer *et al.,* (2007).Thus it is concluded that no single factor might be responsible for the aphids disappearing from field, which is a complex phenomenon and may be convoyed by multiple factors.

4. CONCLUSION

The current research work concluded that the Peak population of *L. erysimi* was recorded in SMW 11 (March 12-18) during both years (2021 and 2022) while the pest disappears from the field till SMW 15 (April 9-15). Thus, As the *L. erysimi* reaches to its peak population during SMW 11 (March 12-18) thus, the plants should be observed closely during this period and control methods having rapid curative action should be applied if needed.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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Table 1. Mean population trend of *L. erysimi* on a susceptible variety (Abaseen) during 2021 -2022 under field condition.

Months and Dates	Standard Meteorological Weeks	Population Density 2021	Population Density 2022
Jan (08-14)	SMW 02	01.00 ± 0.40 i	00.75 ± 0.47 i
Jan (15-21)	SMW 03	02.00 ± 0.70 h	03.50 ± 0.64 g
Jan (22-28)	SMW 04	03.30 ± 0.23 g	04.75 ± 0.62 f
Jan-Feb (29-04)	SMW 05	05.00 ± 0.40 f	06.05 ± 0.62 e
Feb (05-11)	SMW 06	06.20 ± 0.40 e	07.05 ± 0.62 d
Feb (12-18)	SMW 07	07.00 ± 0.40 de	07.75 ± 0.62 d
Feb (19-25)	SMW 08	07.50 ± 0.40 cd	08.65 ± 0.62 c
Feb-Mar (26-04)	SMW 09	08.10 ± 0.40 bc	09.35 ± 0.62 c
Mar (05-11)	SMW 10	08.80 ± 0.40 b	10.35 ± 0.62 b
Mar (12-18)	SMW 11	10.10 ± 0.40 a	11.35 ± 0.62 a
Mar (19-25)	SMW 12	08.10 ± 0.40 bc	09.25 ± 0.62 c
Mar-Apr (26-01)	SMW 13	04.10 ± 0.40 g	05.05 ± 0.62 f
Apr (02-08)	SMW 14	02.10 ± 0.40 h	02.05 ± 0.62 h
April (09-15)	SMW 15	00.32 ± 0.25 i	00.47 ± 0.28 i
LSD (0.05)		1.195	1.719

Mean followed in column by same alphabets are insignificant at 5% level of probability.

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MELISSOPALYNOLOGY CHARACTERIZATION OF PAKISTANI HONEY

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SQ & MS conceived the idea; BA, ZH, SK honey samples collection and data analysis; HU, UN & S conducted analysis for pollen; KK, ZA & M identification of pollens.

Key words:
Pakistani honey, Pollens, *A. mellifera*, *Ziziphus* spp.

ABSTRACT

The aim of the study was to add latest scientific information regarding bee flora through pollen analysis available for honeybees in different districts of Punjab province, Pakistan. A total of 50 honey samples, natural (n=32) and branded (n=18) were collected and examined as per the method recommended by International Honey Commission. Natural honey samples were collected from beekeepers. Pollens of families *Poacea* (26.5%), *Azadirachta* spp. (22%), *Citrus* spp. (17.45%), *Pisum* spp. (16.41%), *Ziziphus* spp. (13.99%), *Prosopis* spp. (13.13%), *Brassica* spp. (8.57%), Malvacea (8.08%), *Syzygium* spp. (7.29%), *Cassia* spp. (6.2%), *Acacia* spp. (5.17%) and *Eucalyptus* spp. (4.35%) were common in both branded and fresh honeys. Whereas *Morus* spp. (8.5%), *Moringa* spp. (4.46%), *Psidium* spp. (4.23%), *Bombax* spp. (1.9%), *Mangifera* spp. (1.9%) were found in fresh honeys only. Similarly, four different types of Pollens (*Melilotus* spp. (8.6%), *Alfa* spp. (6.4%), *Benincasa* spp. (6.4%) and *Halianthus* spp. (4.3%)) were detected exclusively in various branded honeys Muqeet (n=4), Sary (n=2), Swat honey, Marhaba (n=3), Youngs (Beehive), Ubqari, Salman (Pak honey) Al-Shifa, Ponam, Langanase and Aftab Qarshi. There was a correlation (r=0.24) between pollens of same taxa and families in branded and fresh honeys. High quality pictures were taken by camera fitted on light microscope.

1. INTRODUCTION

Pollen grains are fine powdery material formed by the anthers of seed plants. During nectar collection from flowers, bees visited number of flowers and get some quantity of pollen with them. Pollen either adheres to the "hairy" legs and body of bees while crawling over flowers or removed from an anther by them using tongue and mandibles. The bees' combs pollen from her body, head, and forward appendages, collected and mixed with salivary glands secretion or nectar before placing in specific baskets known as "corbicula" that is located at their tibia (hind legs), and finally transferred to the beehive (Von Der Ohe *et al.*2004; Paray *et al.*, 2020). As pollen s are packed into the comb, they are supplemented with phytocidal acid to prevent bacterial growth and delay pollen germination. To prevent anaerobic metabolism and fermentation other enzymes produced by worker bees are also added for enhancing longevity of the stored pollen (Sajwani, *et al.*, 2014) . The pollen comb is referred to as "bee bread" when it is completely processed for storage, (Pospiech *et al.*, 2019 & 2021). After the nectar has converted into honey some of the pollen

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remains in honey and served as blueprint of botanical origin of the honey. Airborne pollen is also a source of pollen in honey. When ripened honey is being removed from a hive by beekeeper sometimes airborne pollen is deposited into it (Jones & Bryant, 2014).

2. MATERIALS AND METHODS

Sample collection

A total of 50 samples (fresh and branded) were collected from the different districts of Punjab, Pakistan, including: Jhang, Faisalabad, Kahnewal, Gujranwala, Bahwalpur, Toba tek singh and Muzaffargarh district. Branded honeys were collected from the local markets of Punjab, Pakistan. Honey brands were including: Marahaba, Salman Pak honey, Al-Shifa, Ubqari, Muqeet, Sary, Langanase, Youngs honey, Ponam, Swat honey and Aftab Qarshi. All honeys were subjected to the pollen analysis.

Method

Melissopalynological studies in present research was carried out according to the method that was recommended by International Commission for Bee Botany of IUBS and (Louveaux *et al.*, 1978; Ullah *et al.*, 2019).

Procedure

Five (5) g of honey was weighed out and dissolved in 10ml hot (not above 40°C) water (distilled water or clean tap water) in a beaker. This honey solution was transferred to the falcon tubes of 15ml capacity. Honey solution was centrifuged for 10 min (2500 r/min) and the supernatant liquid was decanted with the help of pipette. Honey sediment was distributed again with 10 ml of distilled water to eliminate sugars and centrifuged for 5 min. This step was repeated until a clear supernatant appeared; the honeys rich in colloidal matter were initially centrifuged with distilled water thrice. Then the pellets were centrifuged and washed with dilute Sulphuric acid followed by potassium hydroxide (5 g H_2SO_4 or 100 KOH to 1 liter of water). The sediment was washed with distilled water twice to remove remaining chemicals.

Preparation of slides

After washing the honey sediment was put over a slide and spread over it with the help of a micropipette of 10-100 µl capacity. After drying, the sediment was mounted with stained glycerin gelatin. For the preparation of glycerin jelly 0.48g of gelatin was dissolved with 25.5 ml of distilled water, 30ml pure glycerin was added to the mixture to prevent excess dehydration, followed by 0.6ml of phenol to prevent microbial decomposition. Safranin stain was added to this mixture to make the pollens more visible. About 2-3 drops of this glycerin-gelatin mixture were placed in the center of slide and a cover slip was placed over the slide in a way to prevent air bubble formation.

Performance of microscopic analysis

The determination of identification and counting of pollen grains in honey is a base to determine the geographical origin. Identification was made by comparing pollen grains with the reference to the literature. Microscopic examination was carried out at 40X and 100X magnification. An imaging software named "TCapture" was used to capture the micrographs from microscope.

Pollen counting

For pollen counting, heamocytometer was used. About 10 µl of the sediment was transferred to the haemocytometer, and it was allowed to charge. After these pollens were counted in the squares at 10X magnification of microscope. And for the identification of pollens magnification was increased to 40X. Formula of cell counting was applied to the calculated No. of pollens to calculate the No. of pollens per µl of the sample.

The formula is:

Particles per µl volume= $\frac{(counted\ particles)}{(counted\ surface \times Chamber\ depth)}$

To calculate the No. of pollens per ml of the sample following formula was applied:

Particles per ml volume= $\frac{(counted\ particles)}{(No.of\ squares\ counted)} \times 10000$

3. RESULTS AND DISCUSSION

Poacea

In branded honeys average pollens of Poacea was higher (15.28%) than fresh honeys that showed 11.17% of this pollen.

***Azadirachta* spp.**

Average %age of *Azadirachta* spp. pollen was greater 12.23% in fresh honeys and a lower value of 9.84% was there in branded honeys.

***Citrus* spp.**

Fresh honeys presented a higher average percentage (12.11%) of this pollen than that of branded honeys having lower (5.35%) percentage of this pollen.

***Pisum* spp.**

Pisum spp. had pollens in higher percentage (12.11%) in fresh samples as compared to the branded honeys those consisted of a lower percentage (4.28%) of this pollen type.

***Ziziphus* spp.**

Slightly greater (8.46%) average percentage of this spp. was calculated in fresh honeys as compared to branded honeys which showed 7.84% pollen of this taxa.

***Prosopis* spp.**

An average of 7.13% of *Prosopis* spp. pollens were counted in branded honeys and fresh honeys were having lower (3.95%) percentage than honey brands.

***Brassica* spp.**

An average percentage of the *Brassica* spp. pollens was recorded as 5.7% of branded honeys that was higher than that of fresh honeys which showed 2.8% of this pollen.

Malvacea

Pollens belonging to family Malvacea in branded honeys were in higher (4.28%) average percentage as compared to the pollens in fresh honeys those were having 3.76% of total pollens.

***Syzygium* spp.**

Pollens of this taxa showed a moderately higher percentage (4.28%) in branded samples and lower (3.01%) in fresh honeys.

***Cassia* spp.**

Cassia spp. showed a higher (4.28%) percentage in branded honeys as compared to fresh honeys those contained 1.88% of this pollen.

***Acacia* spp.**

Acacia pollens were having a higher (3.17%) average percentage in fresh honeys and branded honeys were consisting of 2.03% pollens of this taxa.

***Eucalyptus* spp.**

Fresh honeys contained greater (4.3%) average percentage of this pollen than that of branded honeys those were having 3.56% of this pollen.

Melilotus spp., *Alfa* spp., *Benincasa* spp. and *Halianthus* spp. were recorded only in branded honeys having 8.56%, 6.4%, 6.4%, and 4.28%, respectively. *Morus* spp., *Moringa* spp., *Psidium* spp., *Bombax* spp. and *Mangifera* spp. were found only in fresh honey samples having a percentage of 8.46%, 4.46%, 4.23%, 1.88% and 1.88%, respectively.

Pollen analysis provides very valuable information and knowledge about the botanical and ecological sources of honey. It presents an idea about the vegetation of the specific area from where the honey is collected. Bahadur *et al*., (2019); Gul *et al*., (2021) conducted Palynological characteristics of selected.

Lamioideae taxa and its taxonomic significance. Greater or lesser frequencies of *Citrus* spp. (14.41%), *Eucalyptus* spp. (9%), *Psidium* spp. (15%) and Poacea pollens (<3%) were reported by Sahney *et al.* (2018) in the honeys from Bankura and Paschim Medinipur districts of West Bengal. Mangi *et al.* (2018) studied the shapes, size and number of pollens in honey such as *Ziziphus* spp., *Azdirachta* spp., *Psidium* spp., *Brassica* spp. and *Acacia* spp. in natural honeys of district Dadu, Sindh Pakistan.

Adekanmbi and Alebiosu (2018) identified pollens of native flora from Nigeria only. Pollens of *Syzigium* spp. (3.01%) and (4.28%), *Brassica* spp. (2.87%) and (5.7%), *Eucalyptus* spp. (4.35%) and (3.56%), *Prosopis* spp. (4%) and (7.13%), *Acacia* spp. (3.17%) and (2%), *Mangifera* spp. (1.9%), *Bombax* spp. (1.9%) and *Moringa* spp. (4.46%) in fresh and branded honeys, respectively were found in the study of honey pollens of Punjab, Pakistan closer to the findings of Chauhan *et al.* (2017) in his work with Indian honeys, frequencies of *Syzigium* spp. pollens (3-15%) in the New Hyderabad (Lucknow) honeys, *Bombax* spp. pollens (<3%) in the honey of Jhansi, pollens of

Brassica spp. (<3%) in the New Hyderabad (Lucknow), Ashakhera and Jhansi honeys, *Eucalyptus* spp. pollens (3-15%) in the honeys of Girar, Bahraich, Ashakhera, New Hyderabad (Lucknow) and Malihabadand districts, pollens of *Prosopis* spp. (3-15%) in the honeys of Ashakhera district, *Bombax* spp. pollens (<3%) in the Jhansi and Trilokpur honeys, pollens of *Acacia* spp. (3-15%) in the Trilokpur and Mallawan district's honeys, *Mangifera* spp. pollens (<3%) in the honeys of Jahnsi, *Moringa* spp. pollens (3-15%) in the honeys of New Hyderabad (Lucknow) made this study closer to the current finding.

Pollen types in the current study were having following frequencies: *Syzygium* spp. 3.01% and 4.28%, *Brassica* spp. 2.87% and 5.7%, *Ziziphus* spp. 8.46% and 5.53%, *Cassia* spp. 1.9% and 4.3%, *Acacia* spp. 3.17% and 2% in fresh and branded honeys, respectively. These frequencies were closer to the (*Syzygium* spp. 2.26% in Kakragaon 3.78% in Chapaguri, 3.64% in Bidyapur district 3.18% in Chatibargaon, 3.78% in Chapaguri and 4.60% in Sidli, *Ziziphus* spp. 4.78% in Bidyapur, 6.57% in Basugaon, 6.78% in Bidyapur, 4.50% in Chapaguri and 10.43% in Dalogaon *Cassia* spp. 1.78% in Chalekati and 4.50% in Sidli, *Acacia* spp. 1.87% in Basugaon and 1.67% in Chapaguri and 1.32% in Sidli honeys) frequencies of pollens calculated by Tripathi *et al.* (2017) in the honeys collected from different regions of northeast India (Bongaigaon district of Assam).

4. CONCLUSION

This study contributes valuable insights into the diverse bee flora present in Punjab, Pakistan, as evidenced by the pollen analysis of honey samples from different regions. The distinct pollen profiles in natural and branded honeys highlight the potential regional variations in floral sources, providing a foundation for future research and quality monitoring in the apicultural industry.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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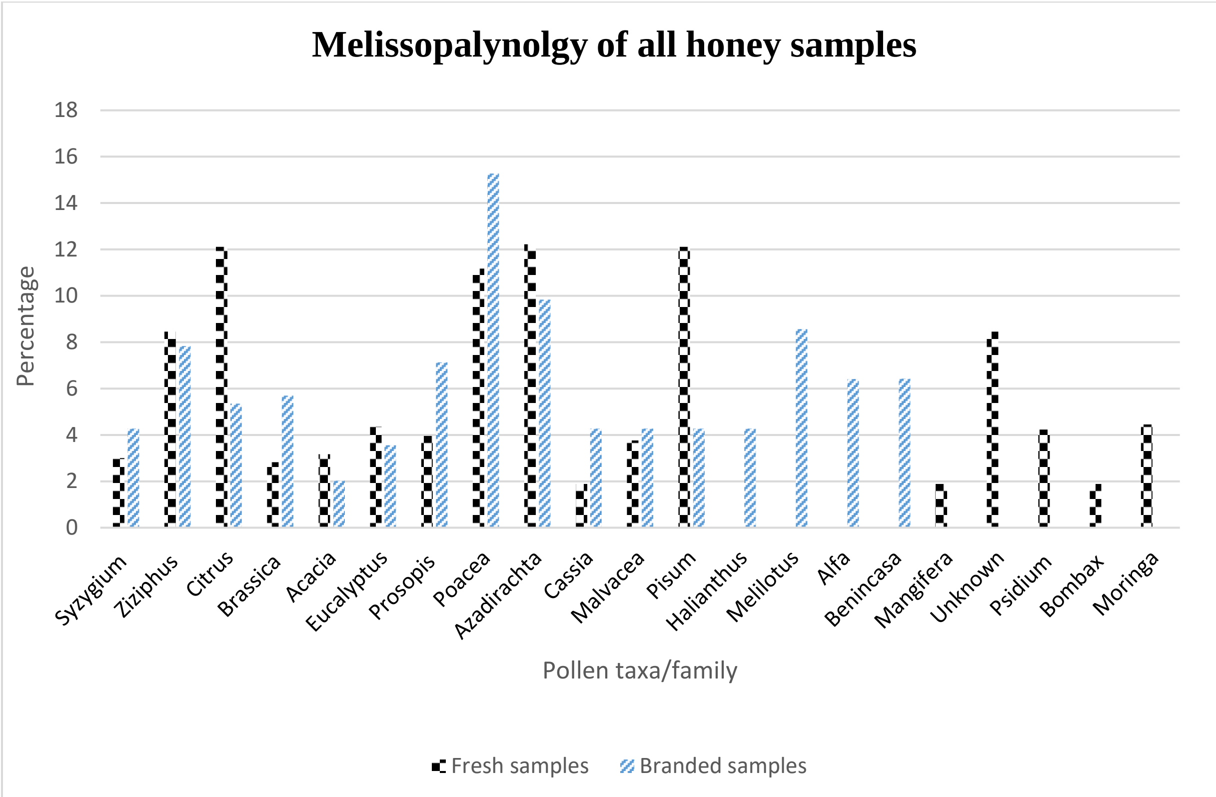
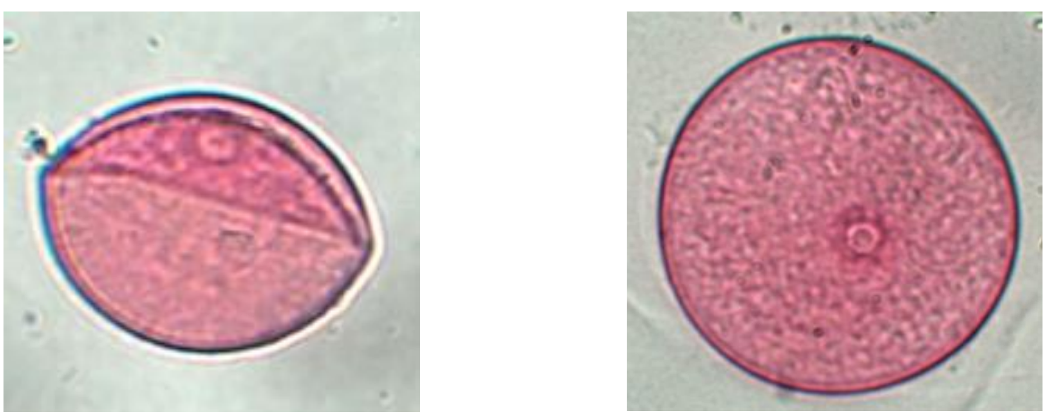


Fig. 1: Percentage of pollen taxa/family in fresh and branded honeys



Pollens of Poacea

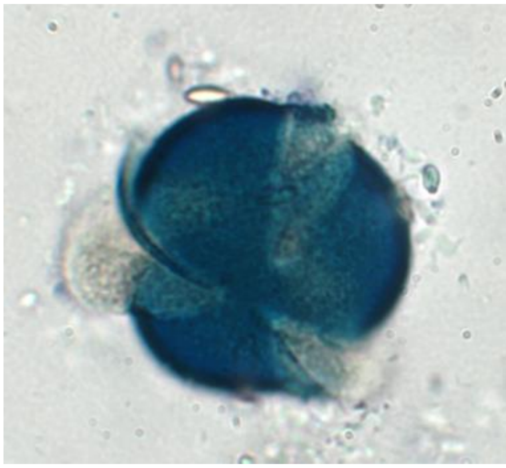
Azadirachta spp.

Citrus spp.

Pisum spp.

Ziziphus spp.

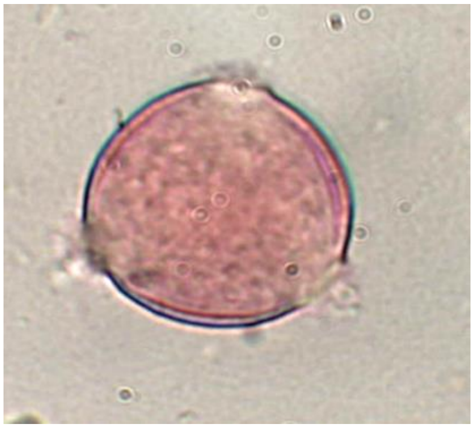
Prosopis spp.



Brassica spp.

Malvacea

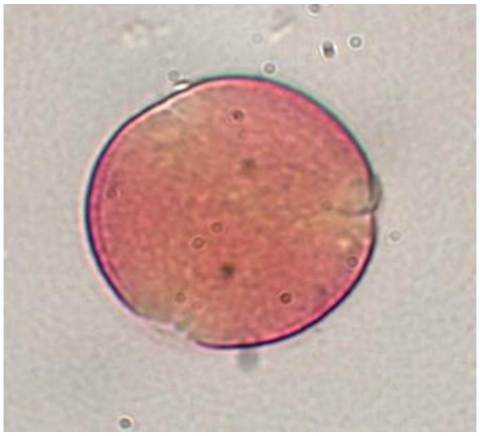
Syzygium spp.



Cassia spp.

Accacia spp.

Eucalyptus spp.



Moringa spp.

Psidium spp.

Bombax spp.

Mangifera spp.

Alfa spp.

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Short communication

BRIEF HISTORY AND JUDGE OF KOKAH PIGEONS

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ABSTRACT

As a distinctively vocal avian species, 'kokah' pigeons stand out as an exceptional breed in Bangladesh, particularly in the regions of Rajshahi, Natore, and Rangpur. Despite their prevalence, there are currently no established standards for judging this unique pigeon breed. Following discussions with numerous breeders in Bangladesh, there is a recognized need to establish standardized criteria for future judging.

1. INTRODUCTION

The history of the laugher pigeon is known but kokah pigeon is completely unknown in any books (Kabir, 2022). Kokah is a pigeon breed of the Indian subcontinent. Levi (Levi, 1992) mentioned Abul Fazl's quote on some Indian pigeon breeds. He described some koka/kokah pigeons with their melodious voice as laughers or yahu pigeons. There are several breeds of Arabian trumpeters which are known for their vocal cooing which sounds similar to laugher or trumpeting. There was a possibility to come to this name was after Zayn Khan Kokah (c. 1542-1601), Governor of Kabul (Asian & African Studies Blog). After this Rahman (Rahman, 1999) described kokah pigeon in his book named ***'Kingdom of Pigeon'*** (in Bangla) with some major characteristics. Finally, Kabir (Kabir, 2022) focused on some profitable pigeon breeds with kokah breeds as well. The actual markings especially the voice quality of kokah pigeons is needed to get good offspring from them. Among all fancy pigeons, kokah pigeons are considered voice or trumpeter pigeons in the country.

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Emperor Akbar kept these kokah pigeons in his court of Delhi (Kabir, 2014). Many breeders of Bangladesh cross this pigeon with other pigeons and ultimately get impure pigeons. In this way, breeders do not get benefits by rearing kokah pigeons. In this sense, there is no shortcut to collecting pure pigeon breeds through proper judging. From the champion pigeons, we could get a first-graded kokah pigeon and a pigeon show could be a good option for purchasing good-graded pigeons (Table 1; Plates 1-2).

2. OBSERVATION FOR ISOLATING QUALITY KOKAH PIGEONS

The price of this pigeon is moderately high, depending on its voice quality (Kabir, 2022). In most cases, after hatching, they fight with each other, so alternative use of parents could be a solution (Rahman, 1999).

Following disqualifications could be implemented during judging:

1. Irregular voice
2. Excessive parasites
3. Large size
4. Both eyes are different in colour
5. Tail feathers—crooked or slanted, any lacking
6. Leg-feathered

3. SUMMARY

These kokah pigeons are the most acceptable breed for everybody, especially on their voice quality. Some breeders are rearing this nice breed with Arabian trumpeters. The market value of these pigeons always accepts the squab of adult pigeons. Some breeders do not know its standard, so they collect this bird wrongly. To get profit and maintain their genetic material without avoiding non-judicious breeding, need to settle a standard immediately.

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Table 1. Features and marking points of kokah pigeon.

Features	Characteristics	Points
Voice quality	Very narrow (continuous coo-coo-coo)	20
Condition	Highly energetic bird	5
Station	Horizontal posture (45° angle)	10
Body size	Small; elongated body; light weighted	10
Primaries:Tail	Will be the same length	5
Beak	Narrow and nearly straight; blackish in colour	10
Plumage colour	Mostly brick-red; primaries and tail feathers will be yellowish; loose feathers	15
Head, eyes, neck	Frontal high; eyes are orange coloured; comparatively short neck	15
Crest	Mostly peak crested	5
Legs and toenails	Clean-legged; blackish toenails	5
	Total	100

Plate 1. Pairs of kokah pigeon

Plate 2. Kokah pigeon farm at Durgapur, Rajshahi

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