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Review Article

Plastic Pollution and Global Sustainability: A Deep Dive into Environmental, Health, and Economic Challenges

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ABSTRACT

Plastic pollution is one of the most pressing environmental challenges of the 21st century, leaving lasting impacts on ecosystems, human health, and economies. Since the mid-20th century, the widespread use of artificial polymers has led to plastics and their by-products macroplastics, microplastics, and nanoplastics contaminating every corner of the planet. Due to their resistance to decomposition, plastics accumulate in terrestrial and aquatic environments, polluting freshwater sources, choking marine ecosystems, and disrupting wildlife. Many organisms ingest plastic fragments, affecting their feeding, reproduction, and survival. Additionally, persistent organic pollutants (POPs) like PCBs, BPA, and phthalates bioaccumulate in food chains, posing risks to biodiversity and human health. Studies confirm that plastics harm various species, from seabirds to whales, while their presence in soil affects microbial populations and contributes to degradation. Water pollution worsens due to plastic waste, reducing quality and threatening aquatic life. Humans are exposed through ingestion, inhalation, and dermal contact, with research linking plastics to endocrine disruption, reproductive issues, and diseases. Economically, plastic waste burdens fisheries, tourism, and waste management industries. Natural forces such as wind and water spread plastics worldwide, creating a global plastic cycle. Efforts to mitigate the crisis include regulatory measures, alternative materials, and public awareness campaigns. Coordinated international action is essential, requiring collaboration among experts to assess health impacts and develop sustainable solutions. Addressing plastic pollution necessitates scientific policies, technological advancements, and behavioral changes, framing it alongside climate change and biodiversity loss as a "triple crisis" demanding urgent intervention.

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INTRODUCTION

Environmental pollution has emerged as a major global concern, driven by rapid industrialization, modern agricultural practices, and urbanization. It involves the introduction of harmful contaminants into the environment, leading to ecological imbalance, discomfort, and damage to ecosystems (Liaqat *et al*., 2022; Ramzan *et al*., 2022; Zahra *et al*., 2023; Zahra *et al*., 2024). However, in the current decade, plastic pollution has become a greater concern. Traces of polymer synthesis and various related chemicals, both overtly added, such as additives and plasticizers, and covertly present as by-products and impurities, belongs to the category of synthetic polymers that form plastic pollution (Schmidt *et al*., 2024). Evolving since the 1950s, the plastic industry has seen exponential growth and further exacerbated societal issues by capitalizing on improper disposal methods and resulting devastation from pollution. Due to being durable, versatile, and low-cost, plastic has found its way into every aspect of modern society. These unprecedented benefits, however, obstruct plastic's dangerous environmental impact and long-term hazards, most notably, undying degradation throughout nature. The worst of all consequences looming on the horizon is the destructive weathering of larger plastic materials, leading to the formation of microplastics (MPs) and nanoplastics (NPs). These minuscule ogres, most of which are 5 mm or less, are now infiltrating our atmosphere, soil, freshwater, and even the bodies of humans (Bashir *et al*., 2024).

Expansion of microplastics: The ND threat

One issue of specific interest is the proliferation of tiny plastic pieces, with microplastics. In some marine environments, parts as small as 1.6 mm have been located,

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and it appears probable that there are smaller bits beneath current detection levels. A recent workshop convened in the USA by the National Oceanic and Atmospheric Administration (NOAA) concluded that microplastics should be defined as pieces 5 mm with a suggested lower size boundary of 333 micrometers to focus on microplastics that will be captured using conventional sampling approaches (Arthur *et al*., 2009). Nonetheless, it remains the responsibility of those defining microplastics not to overlook the vast abundance of smaller fragments. It seems likely that plastic fragments arise from some form of mechanical and chemical breakdown of larger plastic items. Alternative ways for microplastics to enter the environment include the outright dumping of litter, abrasively used plastic in industrial and household cleaning, such as in shot blasting and scrubbers in proprietary hand cleansers, and spillages of feedstock plastic pellets and powders from which most plastic products are manufactured. Shore data combined with data from open oceans and sea bird debris indicate that the volume of plastic fragments is increasing over time. Some coastal regions have reported worrying amounts of plastic debris, over 10% by mass of strandline material, as reported by Barnes *et al*. (2009).

Laboratory experiments demonstrate this phenomenon, showing that tiny fragments are indiscriminately swallowed by smaller marine organisms devoid of backbone creatures, including filter, deposit, and detritivore feeders (Thompson *et al*., 2004) while also showcasing the retention of plastic for over 48 days by mussels (Browne *et al*., 2008). The scale of microplastic consumption, particularly amongst natural populations, remains an enigma. Beyond the physical dangers that plastic waste incurs, there is much conjecture that ingestible plastic could relatively easily, if not seamlessly, contaminate the food chain by transferring poisons (Teuten *et al*., 2009).

The plastic debris from oceans in forms of pellets, fragments and microplastics have shown presence of organic contaminants such as polychlorinated biphenyls (PCBs), in addition to hexachlorinated hexane (HCH), polybrominated diphenylethers (PBDEs), alkylphenols, and BPA, at concentrations from ng g^{-1} to μg g^{-1}. There are subsets of these chemicals, some of which are deliberately added to the manufacture of plastics, while the others are attracted onto plastic debris as they encounter environmental conditions. Research in Japan has shown how plastics are capable of accumulating and retaining persistent organic pollutants produced in other parts of the environment. These contaminants may accumulate thousands of fold higher concentrations on plastic debris than are available in the contiguous seawater (Mato *et al*., 2001).

In the 2009 study, Teuten and colleagues focused on learning how these contaminants transfer from plastic waste into seabirds and other marine animals. The quality of the transport processes depends on the properties of the pollutants and the specific types of polymers that are affected. It may also be influenced by the physical state of the plastic debris after exposure to the environment. Recent modeling work has shown that even low levels of plastics can enhance the uptake of contaminants since, as they are ingested, they are transferred from the plastic to animals. Such a transfer could form a direct and critical way of getting chemicals into upper marine species like seabirds (Teuten *et al*., 2009), but it depends on things like the type of habitat, amount, and quality of plastic debris. In illustration, the contribution of plastic particles to total contaminant accumulation in organisms is largely determined by interactions with other particles that compete for the sorption and transport of contaminants. There are increasing amounts of plastic fragments found in regions all over the world. Microscopic fragments smaller than the 333 μm threshold of the NOAA have a great surface-to-volume ratio that improves the movement of contaminants, while their minuscule size allows for ingestion by various organisms. It has, therefore, given rise to an important problem of the capacity of plastics to deliver and expose wildlife to chemical contaminants. Extra discussion is required to come to terms with the magnitude of contribution by plastics to the movement of contaminants in natural settings and the extent these contaminants could be passed through food webs. However, chemicals associated with plastic threaten wildlife. The findings of laboratory-based research, which are the roots of this summary, are based on Oehlmann *et al*. (2009). These studies show that phthalates and bisphenol A (BPA) affect reproduction in all examined animal groups and stymie development in crustaceans and amphibians. These chemicals adversely affect molluscs and amphibians disproportionately. On the other hand, most observable impacts on fish are seen under increased concentrations. Most of these plasticizers seem to affect hormone function in a variety of ways, based on research conducted by Hu *et al*. (2009).

Plastic pollution is now one of the stubborn and widespread environmental problems. The consequences of plastic pollution extend both into the environment and our health, and even have economic implications, and therefore, an urgent need to identify the full extent of its impact on our planet is in order.

ENVIRONMENTAL IMPACTS OF PLASTIC POLLUTION

Terrestrial ecosystems: Silent contamination beneath our feet

Though it is rather invisible in soil than it is in oceans,

plastic's impact is just as important. Agricultural land has increased risks driven by plastic mulching and the use of wastewater irrigation, sewage sludge. Small plastic fragments confound the structure of the soil and create an issue regarding the movement of air and water storage. Such changes distort the useful microbial populations required for nutrient processing and plant vitality. Also, microplastics in the soil are prone to dissolve in solutions containing toxins such as phthalates and BPA, polluting edible plants and aquifers. Both public health and the future ability to farm are put at risk by these contaminants. Although less spotlighted, terrestrial effects are equally harmful. Plastic contamination within farming areas develops a variety of chemical and physical impacts that wear the earth down over time (Rillig *et al*., 2019).

Fig. 1. The adverse implications of plastic pollution in coastal and marine areas (photo credit J.D.M. Senevirathna).

Marine ecosystems: An ocean choked in plastic

Plastic pollution in beaches and marine environments reduces aesthetic value and ecosystem health, leading to a significant decline in tourism (Fig. 1). It is estimated that approximately 14 million tons of plastic get into the world's oceans every year (Jambeck *et al*., 2015). Plastic fragments are regularly confused for food by marine creatures, causing damage through ingestion-related injuries, reproductive problems, or even death. Rodrigues *et al*. (2019) show that plastics allow for the transportation of persistent organic pollutants, invasive species, and pathogens to marine environments. The interactions level off downstream effects into marine ecosystems, which ultimately reduce the well-being of both fish populations and coral reefs. Plastic breakdown makes bioavailable toxins more dangerous to marine life (Massos and Turner, 2017). Damage to scenic values and maritime services like fishery and tourism, among others, constitutes the effects of marine plastic waste (Moore, 2008; Gregory, 2009). Residual fishing nets called ghost fishing gear result in improperly caught fish and severe harm to commercial fisheries (Moore, 2008; Brown and Macfadyen, 2007). Plastic pollution has extended in the marine realm from the surface toward the deep waters of the ocean, threatening biotic diversity, which is Earth's most fragile ecological system.

Freshwater systems: Rivers and lakes

People's living areas, such as freshwaters, are at the cut-off point of plastic consumption. Plastic debris occupies significant rivers, lakes, and streams. The extensive contamination of freshwater habitats by microplastics (MPs) threatens filter feeders like mussels and fish, as well as decreases the performance of municipal water treatment plants. This presents major threats to people's safe access to clean water and the overall public good. Tropical ecosystems transcend ecological boundaries with disparate ecological histories that link terrestrial sources of plastic to freshwater ecosystems and then to the ocean; they are transitional corridors linking terrestrial sources of plastic to the ocean. Along this way, these water bodies collect pollutants and endanger both wildlife and societies dependent on them (Bhardwaj *et al*., 2024).

WILDLIFE HARM: THE INFLUENCE ON SPECIES AND THEIR TERRITORIES

Some of the cases where terrestrial debris is consumed by wildlife include situations where endangered California condors (*Gymnogyps californianus*) were discovered to take such objects (Mee *et al*., 2007). Marine creatures can rapidly attach to floating plastic debris, and since they stay in the sea for extended periods, they can transport non-native species across oceans (Barnes *et al*., 2009; Gregory, 2009). However, the wildlife problems that remain the most media and public favorites are ingestion and entanglement. Many species, including invertebrates, turtles, fish, seabirds, and mammals, have been reported to have ingested or been entangled in plastic debris, thereby impairing their movement and feeding, reducing reproductive success, and causing injuries and death (Laist *et al*., 1997). Monitoring data that already exist indicates an increasing number of animals entangled over time (Ryan *et al*., 2009).

Many species that use various methods of feeding, such as filter feeding, deposit feeding, or detritivory, have been accounted for in ingesting plastics. It is possible for those species that have intentionally selected plastic debris as a food source to be particularly susceptible to ingestion. As such, the number of persons ingesting plastic can at times be extremely high in specific groups. For instance, 95% of dead fulmars in the North Sea. Reliable

and substantial figures about debris consumed by seabirds can be drawn by looking at the carcasses of dead birds. Using this strategy, scientists have managed to measure temporal and spatial plastic debris concentration in European seas (Van Franeker *et al*., 2005). The negative impacts of plastic pollution on wildlife have increased, most obviously in terms of ingestion, entanglement, and loss of critical habitats. There are between 260 and several hundred species of invertebrates, fish, seabirds, turtles, and marine mammals whose populations have been impacted by plastic interactions (Derraik, 2002). For example, the examination of fulmars found dead on North Sea shores showed that 95% had plastic in their stomachs. Sperm whales are among the most hit, with some even being found with copious ingestion of plastics and stomach rupture (Jacobsen *et al*., 2010).

Entanglement is equally alarming. Fishing nets and different plastic waste remain to entangle marine wildlife, leading to injuries, and heightening the possibility of infection or death as the priority for seabirds and marine mammals (Macfadyen, 2007; Stelfox *et al*., 2016, Fig. 2). These interact ions threaten local ecosystems and result in considerable economic damage for coastal inhabitants who rely on fisheries and tourism as their main source of income. Consistent recording of entanglement patterns indicates a worsening of the problem, which threatens the milieu of marine ecosystems, as indicated by Ryan *et al*. (2009).

Fig. 2. Effects of plastics on coastal and marine biota: (a) Plastics ingestion by a blueshark: Priona ceglauca of Carlos Canales-Cerro (Thiel *et al*., 2018; photo authorship: Dr. Carlos Canales-Cerro), (b) Attachment on plastic debris by Goose Barnacle, *Lepas anserifera* (photo authorship: J.D.M. Senevirathna), (c) Partial cover of macroplastic pollutants on Rock Oyster: *Saccostrea forskalii* colony (photo authorship: J.D.M. Senevirathna), (d) Entanglement of nestling in a synthetic plastic string (photo authorship: Townsend and Barker, 2014).

Bioaccumulation and trophic transfer

When microplastics are consumed by organisms, they are transferred up the food chain. As reported by Setälä *et al*. (2016), trophic transfer has been recorded in both controlled and natural settings. In addition, the contaminants, such as BPA and heavy metals, bind to plastics, penetrating organisms, and increasing the risk of toxicity (Bakir *et al*., 2016).

HUMAN EXPOSURE AND HEALTH RISKS

As far as public exposure to plastics, it comes about by consuming seafood and salt, breathing plastic-based particles, and skin contact with plastics. It is believed that the average individual consumes around 70,000 to 120,000 micrometer-sized plastic pieces per annum (Cox *et al*., 2019). Nanoplastics have been found to react with human cells, causing inflammation, oxidative stress, and endocrine disruption (Lehner *et al*., 2019). Despite the absence of long-term epidemiological research, the current data indicate the possibility of severe health risks forcing professionals to call for plastic waste to be regarded as a dangerous pollutant (Rochman *et al*., 2013). Impacts of plastics do not only extend past the environment, but are also specifically emerging as threats to human health as well. Evidence is building on the ground that highlights the problems associated with the wide use of chemical compounds in plastics, such as that plastics can be toxic or hormone-like in their effects. Investigations by biomonitoring methods detected residues of phthalate additives, bisphenol A (BPA), and tetrabromobisphenol A (TBBPA) in humans. With these approaches, scientists can assess the overall exposure, including ingestion, direct skin contact, and air inhalation (Talsness *et al*., 2009).

Existing data imply that this substance exposure is widespread and associated with various unwanted health outcomes. Studies reveal that exposure to phthalates and BPA triggers developmental defects related to reproduction, especially among males. According to reports, an inverse relation exists between maternal urinary concentrations of Di(2-ethylhexyl) phthalate (DEHP) metabolites and indices of male genital development, viz, anogenital distance, penile width, and testicular descent (Swan, 2005). Research has also identified that exposure to phthalates is linked with poor semen quality in adults and reduced levels of testosterone in persons working in factories that produce high levels of phthalates, such as in PVC flooring factories. While average levels of these chemicals appear similar for global populations, large disparities occur among individuals and even in the same individual over time (Meeker and Sathyanarayana, 2008).

According to Koch and Calafat (2009), average exposure to di-ethyl phthalate and di-butyl phthalate is,

in general terms, below the reference dose of the U.S. Environmental Protection Agency as well as the tolerance daily dose of the European Union but in some cases there are individuals located at the variability observed in the finding points to the fact that the current notion of "safe" levels of exposure (based on outdated assessments) can dramatically misestimate the actual risks, especially for sensitive groups like children, pregnant women or infants.

People encounter PHIPs by eating contaminated food, breathing in dust, or absorbing chemicals through their skin (Adibi *et al*., 2003). The main source of DEHP and comparable compounds in households appears to be food, according to Wormuth *et al*. (2006), while exposure via house dust is especially notable. Although fewer studies have focused on BPA, it appears that it enters the body using routes similar to those of phthalates and may remain in the system for a long time. Taking into account recent research by Lang *et al*. (2008), BPA concentrations in urine appear connected to the risk of cardiovascular disease, type 2 diabetes, and liver enzyme abnormalities. The high degree of exposure found in premature infants under care in neonatal intensive care units is of special concern because of the potential for acute and damaging contact with BPA and phthalates from these medical devices (Calafat *et al*., 2009). Even though studies are needed to determine how plastics contribute to the spread of these toxic compounds, it is obvious that existing toxicological models fail to capture all relevant aspects of risk. Current understanding questions the earlier assumption that toxicity occurs in a straight, increasing fashion with increasing dosage. Conventional toxicological methods frequently fail to pick up hormonal activities that occur at very low doses associated with endocrine disruption (Myers *et al*., 2009). Cellular programming by these disruptions in critical growth times may result in persistent physiological disruptions. Talsness *et al*. (2009) point out that developing alternative methods and standards for risk evaluation is now essential, especially since they must reflect endocrinological aspects, handle chronic low-level exposures, and model complex mixtures.

Animal experiments continue to be an essential source of information. The male reproductive tract, for example, appears particularly affected by phthalates not because of the parent compounds, but owing to the formation of biologically active monoester metabolites in the liver. While rats have been the primary model organism in these studies, there are uncertainties about how well results can be applied to humans, however, the basic hormonal processes are very relevant. Taken together, the growing body of research implies that human health may face serious and probably underestimated risks from prolonged, low-dose exposures as well as occasional, high-dose contacts with chemicals from plastic. The need to develop more refined toxicological evaluation approaches and adapt current regulatory strategies is now of great importance (National Research Council, 2009).

SOCIO-ECONOMIC IMPLICATIONS OF PLASTIC POLLUTION

Economic sectors, as well as ecosystems and human health, are all affected by the severe costs associated with plastic pollution. Its consequences are most evident in coastal districts, fishery operations, the tourism sector, and waste disposal arrangements.

Impacts on fisheries and livelihoods

Plastic in the oceans creates severe difficulties for both industrial and local fishing enterprises. Declining fish numbers caused by ingestion and environmental modification lower the overall productivity and net income of fisheries. In addition, consumer trust in seafood is eroded by contamination, which reduces participation in global seafood transactions. Communities and small-scale fishers who mainly live off fishing are particularly sensitive to such economic fluctuations (Galgani *et al*., 2010).

Impact on tourism

Tourism along the coast suffers important consequences from the environmental damage and sight pollution caused by plastic waste. Large quantities of plastic on shorelines serve to exclude tourists, hence lowering the income and job creation in tourism. On several occasions, local administrations, together with private successful policies result from responsible for addressing the issue of cleanup. Each year, according to the UN Environment Programme, cleaning up plastic from Caribbean beaches can require as much as $10 million in funding. Therefore, the ongoing degradation of coral reefs because of plastic endangers ecotourism initiatives in places with diverse underwater species (Reddy *et al*., 2020).

Waste management and infrastructure costs

An increase in plastic waste levels in some nations puts heavy stress on waste management operations like collection, processing, and landfilling, especially where poverty levels are high. Weak infrastructure for managing waste in many countries allows plastics to repeatedly enter natural settings, repeatedly damaging ecosystems and exacerbating mismanagement. Constructing effective plastic management systems in such contexts is expensive. Still, achieving long-term environmental stability depends on this. Attaining international environmental standards is more challenging for countries whose major sources of wealth are aid or tourism, as plastic pollution grows (Koelmans *et al*., 2019).

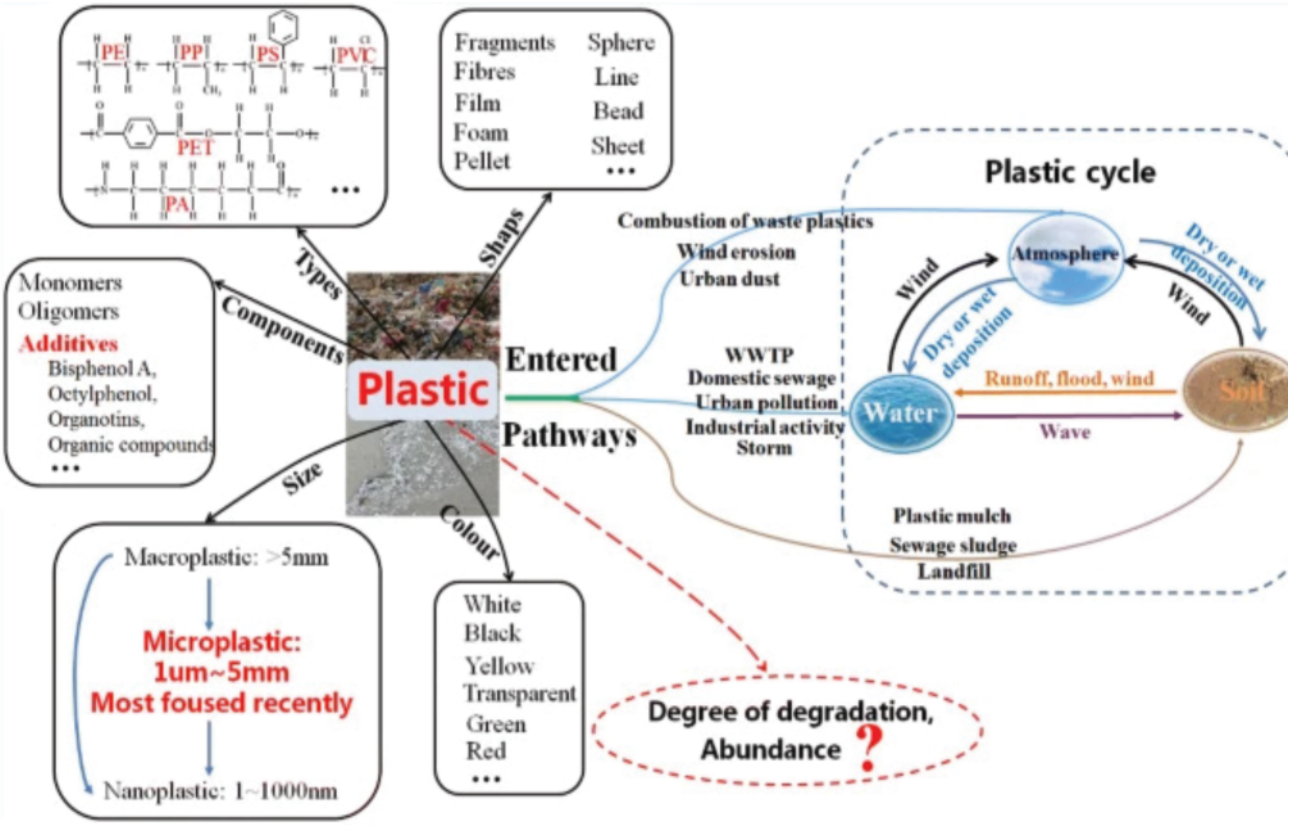

Fig. 3. Diversity of plastic pollution and cycles of plastic in the environment. (Based on Refers Teuten *et al*., 2009; Geyer *et al*., 2017; Koelmans *et al*., 2019).

GLOBAL DISSEMINATION AND PLASTIC CYCLE

Plastic pollution is now a global problem because plastics last a long time, can move easily, and are found almost everywhere. Most substances that pollute the environment either break down or settle, yet plastics can move over long distances because of wind, water, and animals (Fig. 3).

Ubiquity in environmental compartments

Samples from the seemingly untouched areas of both the Arctic Sea ice and the Mariana Trench have yielded traces of plastic. Atmospheric transfer, riverine runoff, and global ocean transport all help to drive the worldwide motion of plastics among land, water, and air systems (Allen *et al*., 2019). A global cycle of plastics, analogous to carbon and nitrogen cycles, is possible now due to human actions, according to Bjerrum and Canfield (2011). Reports found that polyethylene and polypropylene are present in rainwater, alpine soils, as well as in polar snow (Peeken *et al*., 2018).

Plastics in agricultural and urban areas

In land-based ecosystems, plastic litter predominantly is a result of Sewage sludge used as fertilizer, plastic mulch films in agriculture, urban runoff and industrial discharges, and improper landfill practices. These additions ultimately cause plastic to gather in soils and waterways, which has the potential to harm urban health and the safety of food (Zhu *et al*., 2019).

MAINTENANCE AND PRESERVATION OF THE MARINE ENVIRONMENT

Biodiversity hotspots and ecosystem services

Biodiversity hotspots in marine and coastal settings, for example, coral reefs, estuaries, and seagrass meadows, play a major role in the provision of environmental services. Safeguarding marine and shore environments is important in conservation. Biodiversity hotspots and ecosystem services coral reefs, estuaries, and seagrasses, along with other marine and coastal systems, are hotspots of biodiversity, supplying essential ecological services. Such services include the provision of food, the storage of

carbon, the regulation of local climate, and the support of tourism (Reddy *et al*., 2020).

Approximately three-quarters of the global surface is ocean, home to many species for which taxonomic details are still incomplete. Problems caused by plastic debris are evident in the damage it inflicts on ecosystem structures and in the interference with different species' interactions. Filter feeders' feeding and reproductive ability are shown to be reduced by the presence of microplastics, as found by Cole *et al*. (2015).

Emerging threats

Rising levels of development on coasts, together with less effective tourism management, have resulted in more plastics entering these fragile environments. Because plastics have natural stability and buoyancy, they spread effortlessly and are tough to regulate. Given that salinity has a major impact on plastic resilience, strategies for conserving the marine environment must combine environmental care with pollution reduction (Matavos-Aramyan, 2024).

Call for integrated management

Proper safeguarding of marine and coastal ecosystems is contingent upon teamwork between governments at different levels.

1. MARPOL and the Basel Convention are two international agreements that create standards that help determine effective policies (Julian, 2000).
2. Developing national regulations for both the manufacturing and handling of plastic materials is very important.
3. Initiatives such as beach cleanups and resident education are both essential measures in local areas.
4. According to Law's (2017) report, successful policies result from combining public awareness efforts and the direct participation of stakeholders.

MITIGATION STRATEGIES AND RECOMMENDATIONS

Policy measures and international cooperation

Various countries have embarked on the ban of single-use plastics, promoting the use of biodegradable plastics and introducing extended producer responsibility (EPR) schemes. Policy enforcement, however, is usually lax. Nevertheless, a major challenge remains due to the considerable variations and lack of sufficient adoption of these strategies. Having the UN plastics treaty in place, together with parallel international commitments, can make rules more even and inspire higher levels of responsibility. Very recently, the basel convention brought particular plastic waste forms under its international trade regulations (Leal-Filho *et al*., 2019).

Innovations in material science

Both bioplastics and real biodegradable materials are becoming more widely appreciated (Chek *et al*, 2020). Although this is the case, considerable testing remains necessary to ensure these materials do not cause new kinds of harm to the environment. Both circular systems and chemical recycling have received increased attention in research studies recently (Moshood *et al*., 2022).

Public awareness and behavior change

Promoting public awareness about plastic consumption is of great importance. Educational campaigns on reducing plastic use at the consumer end are necessary. Source-based reduction of plastic leakage can be achieved by promoting reusable packaging measures, deposit return schemes, and corporate responsibility initiatives (Kumar *et al*., 2021).

Scientific monitoring and research

Addressing plastic pollution requires the establishment of a unified global system for monitoring contamination across multiple locations, ensuring consistent tracking and analysis. Research focusing on the health impacts of micro- and nanoplastics on humans should be prioritized, as their long-term effects remain largely uncertain. Additionally, studies exploring the interconnectedness of ecological, health, and economic factors are essential to fully understand the broader consequences of plastic waste. Cox *et al*. (2019) and Li *et al*. (2024) emphasize that comprehensive data accessibility plays a pivotal role in facilitating effective problem-solving, reinforcing the need for open and extensive research collaborations.

CONCLUISON

Plastic pollution results in interconnected problems for both natural environments and the health and development of human society. Even after years of expert caution, plastic is still building up in every region of the world. All environments, from agricultural soils to deep marine regions, are experiencing plastic pollution, with most of the accompanying hazards incompletely understood. Because of the complexity involved, multiple types of action, ranging from international control to inventive technology, community programs, and changed personal habits, are all required. If plastic pollution continues unchecked, it would endanger both natural biodiversity and ecosystem functions, as well as the resilience of future economic and social systems. A radical change in how we think about

and handle this problem is required immediately, from a throwaway lifestyle to a model of regenerative design, from merely reacting to contamination to anticipating and avoiding it, and from disconnected regulations to science-supported overall action plans.

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Data availability

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The authors have declared no conflict of interest.

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Exploring the Anticancer Potential of Marine Brown Algae as Estrogen Receptor Alpha Inhibitor

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AJ conducted the methodology and drafted the manuscript. AB and AA contributed to figure preparation and manuscript revisions. AQ did the software. EZ proposed the study, supervised the research work, and provided critical review. MK contributed to manuscript review and guidance.

Key words
Breast cancer, Estrogen receptor alpha, Molecular docking, Drug discovery, Marine phytoactive compounds, Brown algae

ABSTRACT

Cancer is currently the second leading cause of death worldwide and continues to be a major global health concern. Breast cancer is one of the most prevalent cancers among women. Although the current breast cancer treatments are effective, they have disadvantages, including the possibility of cancer recurrence, negative side effects, and tamoxifen resistance. Novel targeted therapies are therefore always needed. This study aims to examine how phlorotannins from marine brown algae affect the estrogen receptor alpha (ERα) function in breast cancer cells. To predict the binding affinities between ERα (3ERT) and specific ligands, molecular docking studies were performed using AutoDock Vina. The binding pockets were identified with the help of CASTp analysis, which focuses on ligand-protein interactions. Using BIOVIA discovery studio, ligand-protein complexes were structurally visualized. The binding energies lie between -5.1 and -7.6 kcal/mol. Lowest binding energies of -7.6 and -7.3 kcal/mol, respectively, Eckol (PubChem ID: 145937) and 2, 4-diisobutyryl phloroglucinol (PubChem ID: 15659412) showed the strongest binding affinity to the ERα protein. Positive drug-likeness was predicted by ADMET by checking their properties. The docking results open the door for further *in vitro* and *in vivo* validation by highlighting the potential of plant-derived photoactive compounds as ERα inhibitors.

INTRODUCTION

Breast cancer, one of the most common cancers in women worldwide, is a cancerous tumor that is formed from breast cells, but also occurs in men (Cowin *et al*., 2005). Breast cancer, one of the most common types of cancer worldwide, is a condition of uncontrolled development of malignant cells in mammary epithelial tissue with gender variation and an abrupt rise with age (do Nascimento *et al*., 2020). Breast cancer is modulated by environmental, lifestyle and social-psychological variables with a contribution of genetic mutations and family background to 5%-10% of the cases (Obeagu and Obeagu, 2024). Cancer is a heavy worldwide health burden, with traditional treatments having limitations. Fungal, plant and sea creatures natural products have therapeutic activity like antioxidant, anti-inflammatory and anticancer action (Manzari-Tavakoli *et al*., 2024).

Marine macro algae, which contain anti-inflammatory, anti-cancer, immunomodulatory and antioxidant activities, hold vast potential in the application of the biomedical sector as a result of their peculiar chemical composition and capacity for adapting to the environment (Nova *et al*., 2024). Botanicals, rationally and wisely applied, can be a therapeutic system that is effective, economical, and less side-effect-causing in contemporary medicine (Mohd Zaid *et al*., 2023).

Although the rates of incidence are diverse within and among populations and geographic regions, breast cancer remains a significant adverse burden to both health services and individuals (Wilkerson *et al*., 2024), while underlining the need for continued prevention, early detection, and treatment efforts. The steroid hormone family known as estrogens regulates human reproductive system physiology, development, and growth. Estrogens also affect the skeletal, adipogenic, neuroendocrine and cardiovascular systems. The activity of estrogen receptor alpha (ERα) or ERβ in target organs balances to ensure

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selective activation or inhibition of estrogen signaling pathways (Lee *et al*., 2012). Estrogen plays an essential role in normal mammary gland development and growth, stimulating 50% of primary breast tumors, so Tamoxifen is the first-line treatment for ERα cancers (Palmieri *et al*., 2002). Additionally, through membrane-associated receptors, ERα can stimulate non-genomic signaling cascades that affect cell signaling pathways, including the PI3K and MAPK pathways (Arnal *et al*., 2017; Yin *et al*., 2024).

Scientists highlight the need to enhance breast cancer risk factors, backing screening programs with molecular docking in drug design and structural biology (Morris *et al*., 2009). Molecular docking is a method used to predict the favored position of a ligand in relation to a protein to create a stable compound. It plays a crucial role in drug design and drug discovery since it predicts drug affinity and activity towards protein targets (Chaudhary and Mishra, 2016).

Brown algae, bioactive chemicals such as phlorotannins, possess anti-inflammatory, anti-cancer, and antioxidant activities and can serve as therapeutic agents for alternative cancer therapies via apoptosis and angiogenesis (Pradhan and Ki, 2023; Remya *et al*., 2022). Recent studies have indicated that phenol-dense extracts of *Ecklonia cava*, a brown alga, suppress breast cancer cell growth and induce apoptosis through NF-κB activation repression (Nho *et al*., 2020). Dieckol, a phlorotannin molecule, had high anticancer activity against breast cancer cells by suppressing various molecular pathways related to carcinogenesis (Chen *et al*., 2018; Hakim and Patel, 2020). The study investigates phlorotannins of marine brown algae, like diphlorethol pentacetate, fucophloroethol and phloroglucinol triacetate, to prevent cell growth in breast cancer with web and desktop docking software.

MATERIALS AND METHODS

Protein preparation

The protein selected is 3ERT (human estrogen receptor alpha ligand-binding domain in complex with 4-hydroxytamoxifen) for docking. The structure of the PDB was converted from PDB to PDBQT format by AutoDock Tool (1.5.6). The already attached ligands were removed and water molecule were and adding polar hydrogen, Kollman charges were added to give the required structure.

Ligand preparation

The PubChem web database was queried for bioactive compounds, and the search results were downloaded in SDF format. Conversion of the 3D and 2D structures to PDB format was done using PyMol and converting them to PDBQT format using AutoDock Tool 1.5.6.

Molecular visualization of ERα complex

The ERα complexes were visualized via Discovery Studio 2025 and PyMOL. PyMOL is a molecular visualization program that can be employed to depict small molecule structures and also finds use in structural biology research and publications (Verma *et al*., 2025). A suite of molecular modeling biotechnology and pharmaceuticals, BIOVIA Discovery Studio Visualizer enables collaboration, speeds up research, simplifies procedures and provides predictive analytics (Naithani *et al*., 2024).

Grid box configuration

Ligand and protein, which are present in PDBQT format, are uploaded in Autodock Tool (1.5.6) Grid box was created with x, y, and z center as 22.396, 5.644 and 21.988 and size 40, 40, and 40 Å, respectively.

Active site prediction

The CASTp server (Computerized Atlas of Surface Topography of Proteins) application was utilized to assess the reactive sites of ERα. Additionally, it provides a visual representation of the binding pocket's volume and dimensions based on crystal structures (Dariya *et al*., 2021).

A total of 33 binding sites were predicted with the effective interactive amino acids including GLU353, LEU346, MET343, GLY390, THR347, LEU346, Pro324, LEU525, LEU346, THR347, ALA350, PRO324, LEU391, MET343 etc. The majority of ligands bind to Pocket 1 and a few bind to Pocket 2 and 3. Three binding pockets for the 3ERT as shown in the Figure 1. The majority of ligands bind to Pocket 1 and a few bind to Pocket 2 and 3.

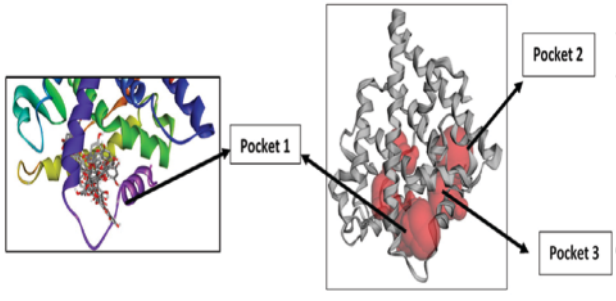

Fig. 1. Image obtained via DSV indicates that all the studied ligands are docked in binding pocket 1 on the left. CASTp predicted a total of 33 binding pockets of the protein (3ERT), of which three binding pockets 1, 2, and 3 are shown highlighted in red color on the right.

Physiochemical properties

The web tools pkCSM and Swiss ADME were utilized to calculate the Lipinski rule of five, ADMET analysis and the drug toxicity. Unique canonical smiles for each of the ligands were retrieved from PubChem, submitted to the ADMET tools and the ADMET evaluation and toxicity results were retrieved.

RESULTS

Molecular docking analysis

The molecular docking analysis of the selected ligands against the estrogen receptor alpha (PDB ID: 3ERT) revealed varying binding affinities and interactions with key amino acid residues in the active site (Table I).

Phloroglucinol (PubChem ID: 359) showed a binding energy of -5.1 kcal/mol with a predicted inhibition constant of 1.76 µM. It formed hydrogen bonds with GLU353 (2.84 Å, 2.22 Å) and displayed hydrophobic interactions with PRO324 (4.15 Å) and LYS499 (4.99 Å). Phloroglucinol triacetate (PubChem ID: 76347) exhibited a better binding affinity of -6.3 kcal/mol (Ki 2.3 µM), forming hydrogen bonds with HIS524 (2.23 Å) and hydrophobic interactions with LEU346 (3.84 Å) as shown in Figure 2.

Table I. Protein (3ERT) interaction with respective ligands and amino acid residues with their distances and binding energies.

Ligand name (PubChem ID)	Interaction amino acid residues (Distance: Å)				Binding energy (Kcal/mol)	Inhibition constant (µM)
	Hydrogen bond	Hydrophobic	Electrostatic interaction	Other		
Phloroglucinol (359)	GLU353 (2.84 Å) GLU353(2.22 Å)	PRO324(4.15 Å)	LYS 499(4.99 Å) GLU353(3.61 Å)		-5.1	1.76
Phloroglucinol triacetate (76347)	HIS524 (2.23)	LEU346 (3.84 Å)			-6.3	2.3
Eckol (145937)	LEU346(2.22 Å)	LEU525(3.81 Å)		MET343(5.59 Å)	-7.6	2.55
	MET A:343(2.85 Å)	LEU346 (4.57 Å)		MET343(4.72 Å)		
		LEU346(5.32 Å) LEU384(5.46 Å) LEU391(5.12 Å) LEU387(4.47 Å) ALA 350(5.10 Å) ALA350(4.37 Å)				
Phloroglucinol dihydrate (80196)	PHE404 (2.68 Å)	LEU346 (5.18 Å) ALA350 (5.31 Å) LEU387 (5.04 Å) LEU391(4.98 Å)	GLU353 (4.38 Å)		-5.1	1.76
2,4-Diisobutyryl phloroglucinol (15659412)	ASP351(2.13 Å)	TRP383(3.93 Å) TRP383(3.54 Å) TRP383(5.43 Å) LEU354(4.10 Å) LEU536(4.01 Å) LEU525(5.13 Å) MET522(4.65 Å) TRP383(5.40 Å) LEU525(4.98 Å)			-7.3	4.23
Diphlorethol (14020558)	ARG394(2.67 Å) ILE386(2.47 Å) PRO325(2.1 Å) PRO324(1.70 Å) GLY390(3.65 Å)	PRO324(4.18 Å) PRO324(5.49 Å) ILE326(4.84 Å)	GLU353(3.54 Å)		-7.4	3.79

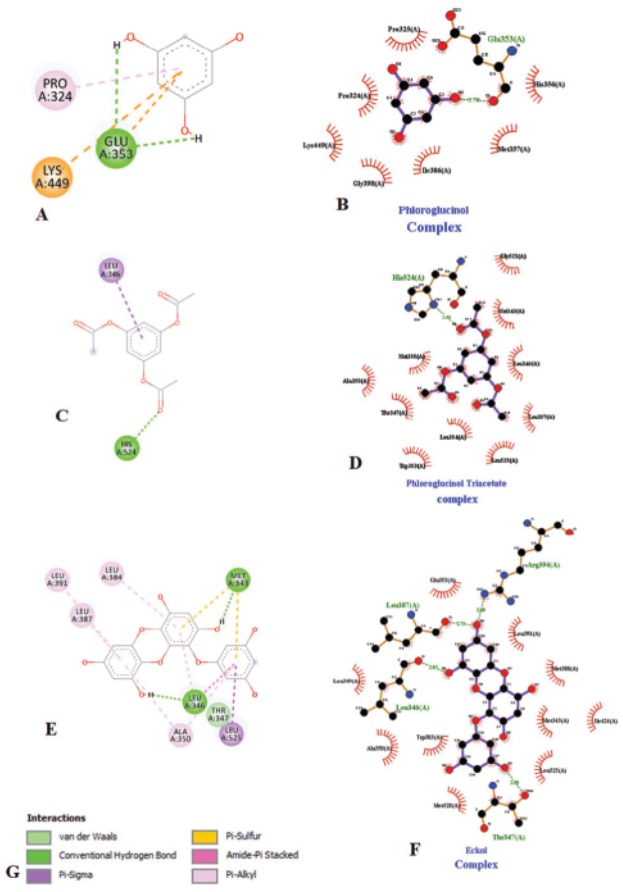

Fig. 2. Illustrating docked complexes. Phloroglucinol docked complex and its visualization (A and B). Phloroglucinol triacetate docked complex and its visualization (C and D). Eckol docked complex and its visualization (E and F). The different interaction types are shown in different colors (G).

Eckol (PubChem ID: 145937) demonstrated the strongest binding among the tested compounds, with a binding energy of -7.6 kcal/mol and an inhibition constant of 2.55 µM. It formed hydrogen bonds with LEU346 (2.22 Å) and hydrophobic contacts with residues including LEU525 (3.81 Å) and MET343 (5.59 Å) (Fig. 2). Phloroglucinol Dihydrate (PubChem ID: 80196) bound with an energy of -5.1 kcal/mol **(**Ki 1.76 µM**),** forming a hydrogen bond with PHE404 (2.68 Å) and hydrophobic interactions with LEU346 (5.18 Å) and ALA350 (5.31 Å) as shown in Figure 3.

2,4-Diisobutyryl Phloroglucinol (PubChem ID: 15659412) showed good affinity with -7.3 kcal/mol **(**Ki 4.23 µM**),** interacting via hydrogen bonding with ASP351 (2.13 Å) and hydrophobic contacts with TRP383 (3.93 Å) and LEU354 (4.10 Å). Diphlorethol (PubChem ID: 14020558) had a binding energy of -7.4 kcal/mol and a calculated inhibition constant of 3.79 µM**.** It interacted with ARG394 (2.67 Å) and ILE386 (2.47 Å) via hydrogen bonding, and displayed hydrophobic interactions with PRO324 (4.18 Å) and GLU353 (3.54 Å) (Fig. 3).

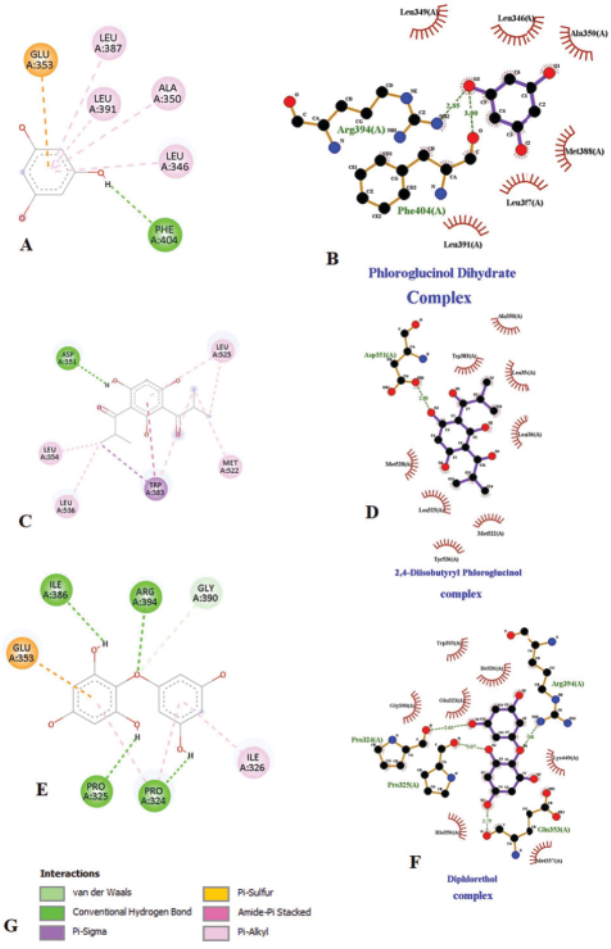

Fig. 3. Illustrating docked complexes. Phloroglucinol dehydrate docked complex and its visualization (A and B). 2,4-Diisobutyryl Phloroglucinol docked complex and its visualization (C and D). Diphlorethol docked complex and its visualization (E and F). The different interaction types are shown in different colors (G).

Lipinski's rule of five

The selected ligands were tested for drug-likeness according to Lipinski's Rule of Five, keeping in view molecular weight (<500 g/mol), log P (<5), H-bond donors (<5) and H-bond acceptors (<10). From the table, we can see that all five ligands fall under the drug-ability criterion of molecular weight, log P and H-bond acceptor. Eckol only violates one rule despite having six hydrogen bond donors, which is slightly more than is advised. There are

also considerable variations in polar surface area (PSA), which affect drug absorption. While phloroglucinol has the lowest PSA (60.69 Å²), suggesting greater permeability, Eckol has the largest PSA (149.07 Å²), which can hinder absorption. In general, most ligands exhibit suitable PSA values and satisfy Lipinski's criterion, suggesting that they have the potential to be drug-like substances (Table II).

Drug likeness and toxicity

To confirm if the potential ligands were potential drugs, their ADMET properties were investigated. ADMET properties were predicted using the pkCSM web tool. The five ligands were well absorbed with a moderate to high level of human intestinal absorption (HIA). Highest absorption rates were for 2, 4-diisobutyryl phloroglucinol and phloroglucinol triacetate. All the compounds possessed low central nervous system (CNS) and blood-brain barrier (BBB) permeability, hence reducing the likelihood of CNS adverse effects. Drug-drug interaction risks were reduced by metabolic predictions that presented negligible inhibition of the CYP450 enzyme. Good renal or total clearance rates were presented by excretion parameters. Except for Eckol, which presented hepatotoxicity and one infringement of the Lipinski rule, most of the compounds possessed viable toxicity profiles and were AMES-negative and non-hepatotoxic. Drug-likeness of most of the ligands was generally validated by ADMET analysis, i.e., that they possessed the potential for further development as safe and effective oral therapeutic agents (Table III).

Table II. Lipinski rule of five analysis of selected ligands.

S. No	Ligands	Molecular weight < 500 (g/mol)	H-Bond acceptor < 10	H-Bond donors < 5	logP < 5 (WLOGP)	Polar surface area (Å2)
1	Phloroglucinol	126.11	3	3	0.8	60.69
2	Phloroglucinol triacetate	252.22	6	0	1.46	78.9
3	Eckol	372.28	9	6	3.61	149.07
4	Phloroglucinol dihydrate	162.14	5	5	0.67	79.15
5	2,4-Diisobutyryl phloroglucinol	266.29	5	3	2.48	94.83
6	Diphlorethol (14020558)	250.20	6	5	2.01	110.38

Table III. Drug likeness prediction using pkCSM online database server for the selected ligands.

ADMET		Phloro-glucinol	Phloroglucinol triacetate	Eckol	Phloroglucinol dihydrate	2,4-diisobutyryl phloroglucinol	Diphlore-thol
Absorption	Water solubility (LogS)ml/L	-1.408	-2.37	-2.899	-1.615	-2.43	-3.04
	Intestinal absorption (human)(%Ab-sorption)	83.55	96.67	67.17	61.7	73.88	70.93
	P-Glycoprptein I Inhibitors	No	No	No	No	No	No
	P-Glycoprptein II inhibitors	No	No	Yes	No	No	No
Distribution	VDss (human) (logL/kg)	0.13	-0.679	0.595	-0.136	0.102	0.99
	BBB permeability	-0.466	-0.686	-1.399	-0.72	-0.826	-1.36
	CNS permeability	-3.252	-3.114	-3.259	-3.392	-2.478	-3.23
Metabolism	CYP2D6 substrate	No	No	No	No	No	No
	CYP3A4 substrate	No	No	No	No	No	No
	CYP2D6 inhibitor	No	No	No	No	No	No
	CYP 3A4 inhibitor	No	No	No	No	No	No
Excretion	Total clearance (Log ml/min / kg)	0.58	0.99	0.23	0.66	0.29	0.47
	Renal OCT 2 substrate	No	No	No	No	No	No
Toxicity	AMES toxicity categorical (Yes No/)	No	No	No	No	No	No
	Max. tolerable dose (log mg/kg/day)	0.107	Yes	0.476	0.172	0.591	0.50
	Hepatotoxicity	No	1.207	No	No	No	No

DISCUSSION

A comprehensive molecular docking and *in silico* evaluation were conducted on five ligands: phloroglucinol, phloroglucinol triacetate, eckol, phloroglucinol dihydrate, and 2,4-diisobutyryl phloroglucinol. These compounds were selected based on their reported therapeutic potential, particularly their anti-cancer and estrogen receptor-modulating activities (Kim *et al*., 2015). Their interactions with estrogen receptor alpha (PDB ID: 3ERT) were analyzed alongside drug-likeness predictions using Lipinski's Rule of Five and ADMET properties (pkCSM server).

Among the tested ligands, eckol exhibited the highest binding affinity (–7.6 kcal/mol), suggesting strong and stable interactions with the receptor's active site. Eckol formed key hydrogen bonds with LEU346 and MET343, and hydrophobic interactions with LEU384, ALA350, and LEU387. Similar interactions between eckol and estrogen receptor-related targets have been reported previously, supporting its potential as a natural anti-cancer agent (Zhang *et al*., 2019). These interactions likely contribute to eckol's enhanced binding within the ligand-binding pocket of 3ERT, potentially modulating its biological activity.

All compounds had molecular weights within the acceptable range for oral drugs (<500 g/mol), consistent with desirable pharmacokinetic properties. However, eckol exceeded the recommended limit for hydrogen bond donors (six donors), which may hinder membrane permeability and oral bioavailability. Prior reports also noted that polyphenols with high polar surface area and multiple hydrogen bond donors often exhibit limited absorption through biological membranes (Teng and Chen, 2019). The low logP (–0.466) and high polar surface area (149.07 Å²) of eckol indicate good water solubility but poor lipophilicity, factors that may further limit its absorption in the gastrointestinal tract (Karami *et al*., 2022).

In contrast, phloroglucinol and its derivatives complied with Lipinski's criteria, demonstrating favorable lipophilicity (logP 0.67–2.48) and hydrogen bond donor/acceptor profiles. These properties suggest better membrane permeability and oral absorption potential. 2,4-diisobutyryl phloroglucinol, in particular, exhibited a balanced profile with moderate lipophilicity and three hydrogen bond donors, indicating promising oral bioavailability. Similar derivatives of Phloroglucinol have shown good pharmacokinetics and bioactivity in earlier studies targeting cancer-related pathways (Kim *et al*., 2015; Sadeghi *et al*., 2024).

The ADMET analysis further supported the drug-likeness and safety profiles of the studied compounds. All ligands demonstrated acceptable water solubility, with Phloroglucinol showing the highest solubility (LogS –1.408) and diphlorethol the lowest (LogS –3.04). Intestinal absorption predictions indicated that phloroglucinol triacetate had the best absorption (96.67%) followed by phloroglucinol (83.55%), while eckol and phloroglucinol dihydrate exhibited relatively lower absorption (67.17% and 61.7%, respectively). None of the compounds were predicted to inhibit P-glycoprotein I, although eckol was identified as a P-glycoprotein II inhibitor, which may affect its efflux and distribution. Distribution data revealed that all compounds are unlikely to cross the blood-brain barrier (e.g., Eckol BBB permeability –1.399), minimizing potential central nervous system side effects. Importantly, none of the ligands were predicted to act as substrates or inhibitors of CYP2D6 or CYP3A4, suggesting a low risk of cytochrome P450-mediated drug-drug interactions. Excretion profiles indicated moderate clearance rates, with Phloroglucinol Triacetate showing the highest predicted clearance (log 0.99 ml/min/kg). Toxicity predictions were favorable across all compounds, as no AMES toxicity or hepatotoxicity was detected, and all ligands fell within a safe range of the maximum tolerable dose. Together, these ADMET characteristics support the potential of these compounds, particularly the Phloroglucinol derivatives, as promising candidates for further pharmacological evaluation.

CONCLUSION

In summary, the present study highlights the potential of marine-derived phlorotannins, particularly phloroglucinol derivatives and eckol, as promising candidates for targeting estrogen receptor alpha (ERα) in breast cancer therapy. Consistent with previous reports on the cytotoxic and anticancer activities of phlorotannins from brown algae, our molecular docking results suggest that these compounds can effectively interact with key residues within the ERα binding pocket, possibly modulating its activity. Among the tested ligands, phloroglucinol triacetate and 2,4-diisobutyryl phloroglucinol exhibited favorable drug-likeness profiles according to Lipinski's rule of five and ADMET predictions, supporting their potential as orally bioavailable therapeutic agents. However, the predictive nature of *in silico* analyses warrants validation through comprehensive *in vitro* and *in vivo* studies. Such experimental work will be essential to confirm the molecular mechanisms, efficacy, and safety of these compounds and to advance them towards clinical application in breast cancer treatment.

DECLARATIONS

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Generative AI or AI-assisted technology statement

No genarative AI or AI-assisted technology was used for this study.

Statement of conflict of interest

The authors have declared no conflict of interest.

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Mitochondrial ND3 Gene Mutations in Breast Cancer Patients: Experimental and Computational Analysis of Mt: A10398G

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ABSTRACT

Breast cancer is the most common malignancy in women and a leading cause of mortality, despite advancements in screening and treatment. The mitochondrial genome shows high variability in breast cancer patients, with mutations linked to altered energy production in tumor cells. This study investigates molecular alterations in the MT-ND3 gene, part of NADH dehydrogenase (Complex I of the respiratory chain), in women with breast cancer. Blood and tissue samples were collected from breast cancer patients, with blood samples from selected healthy individuals. The MT-ND3 gene was amplified using PCR and analyzed via Sanger sequencing. Bioinformatics tools, including PolyPhen-2, PhD-SNP, PANTHER, Align-GVGD, and SNPs and GO, evaluated the pathogenicity of the mutations. Sequencing revealed a mutation at position 10398 (A>G), resulting in a threonine to alanine substitution, along with two synonymous mutations (MT: 10400 and MT: 10253). This mutation was found in 75% of breast cancer patients, consistent with studies from Sri Lanka and Bangladesh, but absent in Polish women. *In silico* analysis indicated this mutation is likely benign but decreases protein stability (free energy change of -0.5 kcal/mol) and increases hydrophobicity. These findings suggest that the MT-ND3 mutation may contribute to breast cancer development and underscore the need for further research to clarify the relationship between MT-ND3 mutations and breast cancer, particularly regarding gene expression and cancer biology implications.

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Authors' Contribution
MM conceived the idea and supervised the study, BR performed experimentation and drafted the manuscript, ZH assisted with the manuscript and evaluated results, HM performed the computational analysis.

Key words
Breast cancer, MT-ND3, A > G mutation, Synonymous mutation

INTRODUCTION

Carcinogenesis is characterized by the uncontrolled proliferation of cells that have the capacity to invade surrounding tissues (Azamjah *et al*., 2019). Among all malignancies, breast cancer is the most lethal cancer affecting women globally (Brown *et al*., 2023). The 2020 GLOBOCAN report recorded 2,261,419 diagnosed cases of breast cancer in women worldwide, resulting in 684,996 fatalities. In Pakistan, breast cancer is often diagnosed approximately a decade earlier than in Western countries (Castañeda *et al*., 2022). The same report indicates that Pakistan had 25,928 new cases of breast cancer and 13,725 deaths among women, representing a higher incidence than all other cancers affecting women in the country. Currently, nearly 79% of breast cancer cases occur in women aged 50 and older, with more than 40% in those over 65. The risk of developing breast cancer increases with age: approximately 1.5% after 40 years, 3% after 50, and over 4% after 70 (Chomyn *et al*., 1986). The incidence peaks around menopause, subsequently either declining or stabilizing (Francis *et al*., 2013).

The mitochondrial genome is particularly susceptible to mutations due to the absence of introns and histone proteins. Single deletions in mitochondrial DNA (mtDNA) occur randomly, typically resulting from errors in replication or repair, while multiple deletions are less common (Grzybowska *et al*., 2014). Mitochondria contain a unique genome known as mitochondrial DNA (mtDNA), which encodes essential components such as adenosine triphosphate (ATP) synthase, transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), and core elements of the respiratory chain across most species. mtDNA is maternally inherited, passed down exclusively through gametogenesis and embryogenesis (Habbane *et al*., 2021). Mitochondria have long been implicated in carcinogenesis, as disrupted energy metabolism is a hallmark of cancer (Jayasekera *et al*., 2023). Beyond energy metabolism, mitochondria are crucial for various processes, including biosynthesis, signaling, cellular differentiation, apoptosis, cell proliferation, and cell cycle regulation, all of which

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are intricately linked to cancer (Jin *et al*., 2018).

An emerging field in cancer research examines mitochondrial gene variations and their links to breast cancer. The MT-ND3 gene, which encodes mitochondrial NADH dehydrogenase 3, is a key component of Complex I in the electron transport chain. Investigations into the ND3 A10389G polymorphism have shown inconsistent results regarding its association with breast cancer risk, although it has been noted to affect mitochondrial pH and intracellular calcium levels (Kim *et al*., 2015).

Variations in MT-ND3 have been reported across diverse populations, including those in Bangladesh, Sri Lanka, Poland, and South India, suggesting potential connections to breast cancer (Li *et al*., 2019). Additionally, mutations in the MT-ND3 gene have been documented in African, American, and Asian cohorts, with the highest mutation frequencies observed in the *CO1* and *ATP6* genes. However, these studies have not established significant associations between the mutations and specific breast cancer subtypes (May *et al*., 2021). In Bangladesh, the A8812C variation in the *ATPase 6* gene leads to a threonine-to-proline substitution, proposed as a potential biomarker for breast cancer (McGuire *et al*., 2015). Pérez *et al*. (2020) identified 709 variants in breast cancer patients across various positions in the mitochondrial genome, including 685 single nucleotide polymorphisms (SNPs), 12 small deletions, and 12 small insertions. Notably, 438 variants were found in coding regions, with 17 variations located in the MT-ND3 gene. Their meta-analysis found no significant association between the MT-ND3 10398 polymorphism and breast cancer risk (Otaegui *et al*., 2004). In Sri Lanka, however, mutations such as A10398G in MT-ND3 and A8701G and A8860G in MT-ATP6 were present in over 50% of both breast cancer patients and control subjects (Li *et al*., 2019).

In this study, we collected breast tissue and blood samples from breast cancer patients to analyze the mutational landscape of MT-ND3 and further elucidate its relationship with breast cancer.

MATERIALS AND METHODS

Sample collection

Tissue samples were collected immediately after surgery and stored in biopsy jars at -22°C without any chemicals. Blood samples were collected in EDTA tubes and similarly stored at -22°C. Tissue samples were specifically used to identify somatic mutations, while blood samples were analyzed for germline mutations. All patient cases involved ductal carcinoma. The patients were from various regions of Punjab, representing a diverse demographic across different age groups. Each patient was diagnosed with varying grades and symptoms of cancer, but all had undergone modified radical mastectomy (MRM). Control samples were also collected from a random population and the family members of the patients to establish a comparative baseline.

DNA extraction

DNA extraction from tissue samples was performed using the organic method, involving stages of cell lysis, protein precipitation, and alcohol purification. For blood samples, DNA was isolated using a commercial DNA isolation kit following the manufacturer's protocols. The extracted DNA from both control and breast cancer samples was quantified via 2% agarose gel electrophoresis and subsequently visualized using a UV gel documentation system. The reaction mixture for PCR included 2µl of template DNA from breast tissue or blood samples. 1.5 µl of each primer,15µl injection water and 10µl of prepared 2X PCR Master Mix added to reach a final volume of 30µl. A negative control was also used to check the credibility with same concentrations but rather than DNA template water template was added. The thermal cycling program consisted of an initial denaturation at 95^{o} for 5 min, followed by 35 cycles of denaturation at 94^{o} for 45 seconds, annealing at 59^{0} for 45 seconds, and extension at 72^{o} for one min. A final extension step was conducted at 72^{o} for 10 min.

The PCR products were then analyzed using gel electrophoresis to verify amplification success and check fragment size. The amplified products were mixed with loading dye and loaded onto a 2% agarose gel in 1X TAE buffer stained with ethidium bromide for visualization. Electrophoresis was performed at 120 volts for 25-35 min, and the gel was examined under UV light.

The size of the PCR products was observed to be almost 450 base pairs and compared to a DNA ladder to ensure they matched the expected size of the MT-ND3 fragment. Successful PCR products were subsequently purified using a purification kit (Invitrogen by thermo fisher scientific Pure Link PCR Purification Kit).The forward primer was used for the sequencing and commercial unidirectional sanger sequencing was done.

Bioinformatics analysis

The amplification products were subjected to comprehensive bioinformatics analysis to identify potential mutations within the MT-ND3 gene and their implications in breast cancer. The analysis commenced with sequence alignment using the Basic Local Alignment Search Tool (BLAST), which allowed for the comparison of the obtained sequences against reference sequences in the nucleotide database. This step was crucial for

identifying any discrepancies or mutations present in the patient samples.

Following the initial alignment, various specialized bioinformatics tools were employed to further evaluate the identified mutations. Panther was utilized to classify the mutations based on their functional characteristics and to predict their potential impact on protein function. This tool provided insights into the biological pathways affected by the mutations, highlighting their relevance in cancer biology. PhD-SNP and SNAP2 were then used to assess the potential pathogenicity of the mutations. PhD-SNP predicts the effect of amino acid substitutions on protein stability, while SNAP2 evaluates the impact of non-synonymous SNPs on protein function and structure. Both tools contribute valuable information regarding which mutations may influence disease progression or treatment response.

To further predict the functional consequences of the mutations, PolyPhen-2 and SIFT were employed. PolyPhen-2 analyzes the possible impact of an amino acid substitution on the structure and function of a protein, categorizing mutations as benign, possibly damaging, or probably damaging. SIFT, on the other hand, predicts whether an amino acid substitution affects protein function based on sequence homology and the physical properties of amino acids. Together, these analyses help prioritize mutations for further investigation. Finally, Align-GVGD and SNPs and GO were used to assess the potential clinical significance of the mutations. Align-GVGD classifies variants based on their alignments with known disease-associated mutations, while SNPs and GO provides insights into the functional annotations of SNPs and their potential associations with diseases. The combination of these tools resulted in a comprehensive understanding of the mutations identified in the MT-ND3 gene, offering valuable insights into their roles in breast cancer growth, proliferation, development, aggression, and progression.

RESULTS

Sequencing analysis

Sequencing analysis revealed a missense mutation, A to G at MT: A10398G, present in tissue samples from three patients with varying grades of breast cancer. This A>G variation resulted in an amino acid substitution, changing threonine to alanine. Initially presumed to be a somatic mutation, its presence in the blood samples confirmed it as a germline mutation. Notably, the same mutation was detected in a family member (control sample) of one patient, suggesting a hereditary component. Additionally, a single nucleotide polymorphism (SNP), C to T at MT: T10400C, was identified in both tissue and blood samples from these patients; however, this SNP did not lead to any protein change. Another variation was observed in a different patient's tissue sample at MT: 10253, characterized as a synonymous substitution where both TTT and TTC code for phenylalanine. Importantly, no mutations were found in the blood samples of the random control population.

In silico *analysis of mutation MT:10398*

To assess the pathogenicity of the mutation MT: 10398, five bioinformatics tools were employed: PolyPhen-2, PhD-SNP, PANTHER, Align-GVGD, and SNPs and GO (Table I). These tools collectively predicted that the missense variation would have a neutral impact on the protein. Align-GVGD classified the mutation as Class C55, indicating no functional impact. Moreover, both PhD-SNP and SNPs and GO corroborated these findings, predicting the effect of the mutation to be neutral as well.

Table I. Predicted effect of MT: 10398 (MTND3:T114A) variation on protein stability using different *in silico* approaches.

Bioinformatic tools	Predicted impact
PANTHER	Probably benign
PhD-SNP	Neutral
ALIGN GVGD	Probably benign
PolyPhen-2	Benign
SNPS AND GO	Neutral

Assessment of protein stability change

Further analysis using bioinformatics tools like I-Mutant, mCSM, and MUpro suggested that the mutation MT: 10398 decreases the protein's stability (Table II). These tools estimated the free energy change associated with the mutation, indicating a potential destabilizing effect on the protein structure.

Table II. Predicted effect of observed variations on protein stability by in silico approaches.

BI Approaches	Stability	$\triangle\triangle$G value (kcal/mol)
mCSM	Destabilizing	-0.564
MUpro	Decrease	-0.928
I-Mutant	Decrease	0.02

Conservatory role of deleterious variations in the MT-ND3 gene

The conserved regions within the MT-ND3 protein sequence were identified using the ConSurf technique, which assesses evolutionary conservation at each position

in the sequence (Fig. 1). The detected mutation MT: 10398 (MTND3: T114A) was located in a region of average conservation, as indicated by the scale provided. This position, T114A, was represented in white in the conservation map, suggesting that while it is not highly conserved, it may still play a role in protein function.

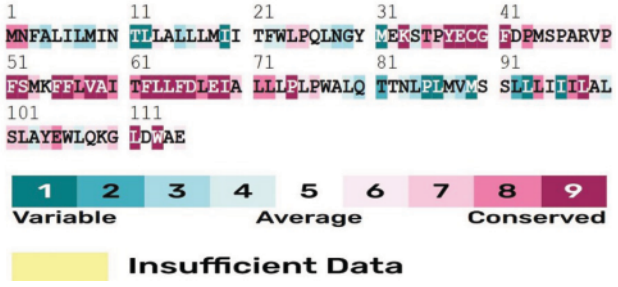

Fig. 1. Consurf mutant residue of MRND3.

Evaluation of protein by 3D structural models

Using Swiss Model, automated 3D models of both the mutant and wild-type MTND3 proteins were generated. The structural comparison revealed slight variations between the two models, each with different confidence levels concerning their reliability. The 3D protein structures for both the wild-type and mutant proteins were visualized using PyMOL. This structural visualization provided insights into the conformational differences between the two variants (Fig. 2).

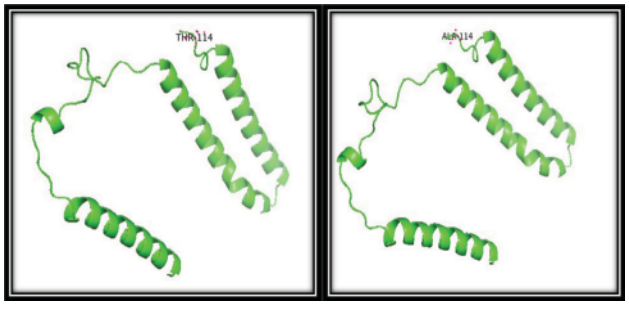

Fig. 2. Structure of wild (A) and mutant (B) MTND3 obtained from pyMOL.

Fig. 3. HOPE structural assessment (protein change from Threonine to Alanine).

HOPE analysis indicated that the mutation creates an empty space within the protein core. The mutant residue is smaller than the wild-type residue, resulting in the loss of hydrogen bonds that are vital for maintaining proper folding. Consequently, the mutant protein exhibited increased hydrophobicity, which could further disrupt its stability and function (Fig. 3).

Analyzing the interaction of ND3 by STRING

STRING database analysis revealed that MTND3 interacts with several proteins, including NDUFB10, NDUFA2, NDUFS4, NDUFS6, NDUFB7, NDUFS3, NDUFA10, NDUFA9, NDUFS7, and NDUFB9 (Fig. 4). Most of these proteins are accessory subunits of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I), which plays a crucial role in catalysis. Any alterations in these interacting proteins may impact the structural integrity and functional efficacy of the MTND3 protein, potentially influencing mitochondrial respiration and energy production.

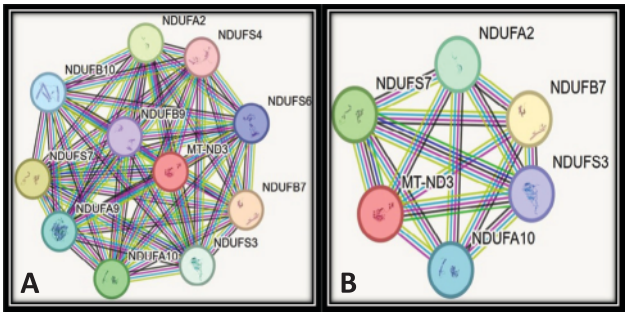

Fig. 4. Protein-Protein Interaction shown by STRING Database (A) interaction of MTND3 with naturally added proteins (B) Protein-protein interaction of MTND3 with different manually added proteins.

DISCUSSION

Mitochondria play a crucial role in energy production through respiration and oxidative phosphorylation, as well as in the production of reactive oxygen species (ROS) and the regulation of apoptosis. Cytotoxic by-products, such as ROS, can damage both cellular DNA and the mitochondrial genome, potentially contributing to tumorigenesis, as well as the growth, invasion, and metastasis of cancer cells (Otaegui *et al*., 2004). Furthermore, alterations in the oxidative phosphorylation pathway, modifications to mitochondrial proteins, and increased ROS production, along with inherited variations in mitochondrial genes, may significantly influence the development of cancer (Richard *et al*., 2000).

The circular mitochondrial genome, although small

at 16.6 kilobases, encodes 13 proteins that interact with nuclear-encoded proteins to form respiratory chain complexes. Among these, the mitochondrial NADH dehydrogenase subunit 3 (ND3) is one of seven mitochondrial-encoded proteins (ND1, ND2, ND3, ND4, ND5, ND6, and ND7) involved in the respiratory chain (Smullen *et al*., 2023).

In the present study, we identified a missense mutation at MT: A10398G, which results in a protein change from threonine to alanine, along with two single nucleotide polymorphisms (SNPs) at MT: 10400 and MT: 10253 in breast cancer patients. These findings contrast with those of Grzybowska *et al*. (2014), who reported no correlation between changes in the MT-ND3 gene and breast cancer. Their research, which involved fifty Polish patients, identified 28 changes in the mitochondrial genome but found none in the MTND3 gene (Soomro *et al*., 2018). Smullen *et al*. (2023) noted that the mutation MT: A10398G is significantly linked to heteroplasmy at five different loci. Their study utilized MITOMAP to examine each variant for associated disease phenotypes, revealing that among all reported MT variants, 10398A>G was linked to the greatest number of unique symptoms, including Type 2 diabetes, breast cancer, Parkinson's disease, and Alzheimer's disease. The presence of this same mutation in the breast cancer patients in our study aligns with the findings documented in MITOMAP (Sultana *et al*., 2011).

Interestingly, both breast cancer and Parkinson's disease patients share the mitochondrial 10398 variation. In the context of Parkinson's disease, this polymorphism has been identified as a protective factor (Touhidul Islam *et al*., 2021). A meta-analysis indicated that this variation was present in both diseased patients and control samples across most populations. However, in the Basque population, it was identified as a risk factor, appearing more frequently in diseased individuals than in controls (Yuan *et al*., 2020).

Additionally, our study found that mitochondrial variants 10398 and SNP 10400 are common among gastric cancer patients within the Korean population, suggesting an increased susceptibility to gastric cancer associated with these variants (Zong *et al*., 2016). According to NCBI, numerous variations have been reported in the MT-ND3 gene among patients with Leigh's syndrome, a neurological disorder. While variations in this gene have also been observed in breast cancer patients, the correlation between these mutations and breast cancer remains unclear due to a lack of expression studies. A related study that analyzed both pre- and postmenopausal breast cancer patients and control samples from women in South Asia found no correlation between breast cancer and the MT-ND3 10398 mutation, echoing our findings. Their meta-analysis similarly concluded that there was no association between this polymorphism and an increased risk of breast cancer (Pérez *et al*., 2020).

The results of Sultana *et al*. (2011) who conducted a meta-analysis investigating differences in the mitochondrial ND3 gene in breast cancer patients are consistent with our study. They sequenced 24 blood samples from individuals with breast cancer using MTND3 gene primers and identified two mutations, MT: A10398G and MT: 10400 (C>T), in 18 of the samples. Notably, three affected patients in our current investigation also exhibited the same mutations, 10398 and 10400. The presence of these mutations in both tissue and blood samples suggests that these variants represent germline mutations. Sultana *et al*. also found that this variation is common in Asian and African populations (Li *et al*., 2019).

Our findings align with those of Jayasekera *et al*. (2023), who studied 60 patients with sporadic breast cancer alongside control groups in Sri Lanka. In their study, 75% of the cases were invasive ductal carcinoma, consistent with our findings where all patients had invasive ductal carcinoma (Mao *et al*., 2013). Over half of the patients and controls in their study carried the MTND3 variants 10398 and 10400, which were also present in our study. Additionally, a blood sample from a patient's sister, who was older and healthy, revealed that she also carried the MT: 10398 mutation, although their study did not sequence family members' samples as a control group.

To date, no computational analysis of the MT: 10398 variation has been reported. In this study, we employed several in-silico tools to analyze the variation's potential impact. The deleterious effects of the protein variation were assessed using five different tools: SNPs and GO, PolyPhen-2, PhD-SNP, PANTHER, and Align GVGD. All tools predicted that the missense variation MT: 10398 has a neutral impact on the protein, with PANTHER, PolyPhen-2, and Align GVGD classifying the mutation as probably benign.

To evaluate the impact of the identified variation on protein stability, we utilized three web servers: I-mutant2.0, mCSM, and MUpro. These servers predicted that the mutation would decrease the protein's stability by estimating changes in free energy. HOPE analysis further revealed that this mutation reduced the protein's size, created empty spaces within the protein core, and increased its hydrophobicity. Finally, we modeled the 3D structures of both the wild-type and mutant proteins using Swiss Model and PyMOL, while the STRING database provided insights into the proteins interacting with MT-ND3.

CONCLUSION

This study concludes that mutations in the MTND3 gene are common among patients with invasive ductal carcinoma. Sequencing results revealed the MT: A10398G variation in both tissue and blood samples, alongside two single nucleotide polymorphisms (SNPs), MT: 10400 and MT: 10253. These variants appear to be more prevalent in Asian and African populations, as evidenced by their discovery in studies conducted in Bangladesh and Sri Lanka, while being absent in Polish women with breast cancer. Additionally, these mutations have been noted in patients with Parkinson's disease and may also increase susceptibility to gastric cancer. The presence of the detected variants in both blood and tissue samples suggests that they are germline mutations rather than localized somatic mutations. Although results from various in-silico tools indicate that the MT: A10398G mutation is likely benign, it also suggests a decrease in protein stability and an increase in hydrophobicity. The relationship between MTND3 mutations and breast cancer remains unclear, highlighting the need for further studies focused on the gene's expression and its potential role in cancer development.

DECLARATIONS

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IRB approval

The Advance Study and Research Board at University of Education, Lahore approved the protocol of the present study.

Ethical statement

To identify mutations, blood and tissue samples were collected from breast cancer patients after obtaining informed consent. The study was approved by the Ethical Review Board of the University of Education, Lahore. The informed consent process included detailed explanations of the study's objectives, procedures, and potential risks to ensure that participants were fully aware of their involvement.

Permission from Ethical Committee, University of Education, Lahore, was taken for the research work.

Generative AI or AI-assisted technology statement

No genarative AI or AI-assisted technology was used for this study.

Statement of conflict of interest

The authors have declared no conflict of interest.

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In Silico Investigation of Novel Phytoactive Compounds as ER-α Inhibitors

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ABSTRACT

Breast cancer is one of the most common types of cancer worldwide, and it's a leading cause of cancer-related deaths, especially in women. It's classified into several subtypes, with hormone-dependent breast cancer being particularly tough to treat. ERα is a key player in the growth and survival of breast cancer cells, making it a prime target for treatment. Traditional therapies include selective estrogen receptor modulators (SERMs) and aromatase inhibitors, but these often lead to resistance and unwanted side effects. As a result, researchers are looking for natural, plant-based inhibitors as a promising alternative. This study explores the potential of plant-derived photoactive compounds, including Butein, Leonurine, Nobiletin, Genistein, and Luteolin, as ERα inhibitors through computational approaches. Molecular docking studies were performed using AutoDock Vina to predict binding affinities between ERα (3ERT) and selected bioactive compounds. Active sites of the ERα were determined by the CASTp analysis to obtain deep insights into ligand-protein interactions. To visualize the interaction of ligand-protein complexes Discovery Studio Visualizer was used. This docking studies showed strong binding interactions between ERα and the selected ligands. Butein and Genistein had the highest binding affinity, with -8.7 kcal/mol and -7.8 kcal/mol, respectively, with ERα. CASTp analysis confirmed that these ligands occupy key binding pockets of ERα, suggesting they may have an inhibitory role. Additionally, ADMET predictions indicated favorable pharmacokinetic properties, supporting their potential as drugs. The computational findings point to the potential of plant-derived photoactive compounds as ERα inhibitors, setting the stage for future in vitro and in vivo validation. These compounds show promising therapeutic potential against hormone-dependent breast cancer, making them worth exploring further for clinical applications.

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AB conducted the methodology and drafted the manuscript. AJ and FM contributed to figure preparation and manuscript revisions. EZ proposed the study, supervised the research work, and provided critical review. MK contributed to manuscript review and guidance.

Key words
Estrogen receptor α, Nuclear receptor, Breast cancer, Molecular docking, Genistein, Butein, Luteolin

INTRODUCTION

Breast cancer remains one of the most prevalent and significant health challenges worldwide, with an alarming rise in both incidence and mortality rates. As of 2020, there were an estimated 2.26 million new cases of breast cancer globally, with the disease becoming the leading cause of cancer-related deaths in women (Sung *et al*., 2020). This surge is strongly linked to human development, with regions undergoing economic transition experiencing a notable rise in cases. Breast cancer remains a leading cause of cancer-related mortality among women worldwide, with significantly poorer survival outcomes observed in low- and middle-income countries. These disparities are largely attributed to delayed diagnoses and limited access to effective treatments. Addressing this global challenge, the World Health Organization introduced the Global Breast Cancer Initiative (2022), aiming to improve survival through public health education, early detection, and integrated care strategies (Wilkinson *et al*., 2022).

Importantly, breast cancer is not a uniform disease; rather, it encompasses a spectrum of biologically distinct subtypes that differ in clinical presentation, molecular features, and genetic profiles (Sung *et al*., 2020). These subtypes heavily influence the selection of therapeutic interventions, which may include surgical procedures, radiation, chemotherapy, hormone therapy, or targeted biologics. For example, HER2-positive breast cancers are typically treated with chemotherapy and anti-HER2 agents, while hormone receptor-positive (ER+) cancers benefit from endocrine therapies. Despite these advances, the rising incidence particularly in younger women underscores the need for more precise diagnostic tools and innovative therapeutic strategies (Siegel *et al*., 2022). Estrogen is known to play a key role in the pathophysiology

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of hormone-responsive breast tumors, with estrogen receptor alpha (ERα) serving as a crucial driver of tumor proliferation by modulating gene expression linked to cell growth (Klinge, 2001). This makes ERα a prime target for therapeutic intervention in breast cancer treatment. This makes ERα a central target in current breast cancer treatments. Nonetheless, resistance to existing therapies such as selective estrogen receptor modulators (SERMs) and aromatase inhibitors remained a significant hurdle, often leading to treatment failure or recurrence (Russo, 2007).

In recent years, plant-derived bioactive compounds have garnered significant interest as potential inhibitors of estrogen receptor alpha (ERα) in breast cancer therapy. Several phytochemicals, including those derived from marine sources such as brown algae, exhibit promising anticancer effects, particularly by modulating estrogen receptor signaling pathways (Holdt and Kraan, 2011). Among these, photoactive compounds which become biologically active upon exposure to light are being explored for their ability to selectively disrupt estrogen signaling with enhanced specificity toward cancer cells (Nooreen *et al*., 2024). The investigation of such phytoactive compounds represents a novel and promising strategy in the treatment of ER-positive (ER+) breast cancer. By employing approaches such as molecular docking**,** virtual screening**,** and advanced drug delivery systems, researchers aim to identify new therapeutic candidates that can overcome limitations of current endocrine therapies. This study focuses on evaluating the inhibitory potential of selected natural compounds on Butein, Lenurine, Genistein, Nobiletin, and Luteolin against ERα through *in silico* docking techniques**,** thereby contributing to the development of more targeted and personalized breast cancer therapies.

MATERIALS AND METHODS

Protein preparation

The estrogen alpha receptor was dowloaded with the PDB ID 3ERT. AutoDock MGL tools were used to prepare the protein for docking studies. The protein preparation was performed using the standard protocol as discussed in our previous study (Hassan *et al*., 2024).

Molecular visualization of ERα complex

The Docked ERα complexes are visualized using Pymol, Biovia Discovery Studio Visualizer (DSV), and MGL tools. The 2D and 3D visualization of the structures were generated. The steps for visualization are as per the standard protocol given in our previous studies (Muhammad *et al*., 2021; Hassan *et al*., 2024).

Docking analysis

AutoDock vina was used for molecular docking analysis by keeping the receptor as a rigid molecule and ligands as flexible molecule for rotatable bonds. Grid box with the size of 40 (x, y, z) was used, the center of the grid box was set at coordinates x = 22.396, y = 5.644, and z = 21.988. After docking the nine poses for each ligand was generated. The ligand with the lowest binding energies was selected and were further subjected to visualization.

Active site prediction

The CASTp server was used to predict the effective sites of the studied protein. This software calculates the volume and surface area of the binding domains and its potential catalytic sites (Tian *et al*., 2018).

Physiochemical properties

The Lipinski rule of five and the ADMET analysis and drug toxicity was performed using the online available tools pkCSM and SwissADME. The specific canonical smiles for each ligand was retrieved from Pubchem and then was submitted to the ADMET tools used and the ADMET evaluation and toxicity results were obtained.

RESULTS

Molecular docking

In this study, molecular docking was performed to evaluate the interaction of plant-derived photoactive compounds with the ERα. The primary goal was to identify potential inhibitors that can modulate ERα activity, which is relevant to various hormone-dependent diseases, including breast cancer. We utilized offline tools that are PyMOL, Auto Dock Vina, and DSV to conduct and visualize the docking analysis. The docking results gave the binding affinities and interactions of docked compounds within the receptor's active site. The protein structure 3ERT was downloaded from the Protein Data Bank (PDB) in pdb format. Using Auto Dock Tools, the protein was prepared by removing water molecules, adding Kolman charges and only polar hydrogens, and converting it into .pdbqt format (Fig. 1). The ligand was downloaded from PubChem in SDF format. It was then converted to .pdb format using PyMOL and further processed in Auto Dock Tools and converted into .pdbqt format for docking studies. The resulting PDBQT files were then used as input for docking analysis in Auto Dock Vina 1.5.6.

Protein active sites prediction

Using the online available tool CASTp was used to predict the binding pockets of the respective protein 3ERT. A total of 33 binding sites were predicted with the effective

interactive amino acids including GLY521, GLY420, His 420, GLU353, LEU349, ILE326, ARG394, PRO324, MET522, LEU536, GLY 390, LYS 449 etc. Most of studied ligands are docked to the binding pocket 1 while some at binding pocket 3 as shown in Figure 2.

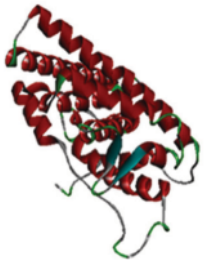

Fig. 1. 3ERT protein in PDBQT format.

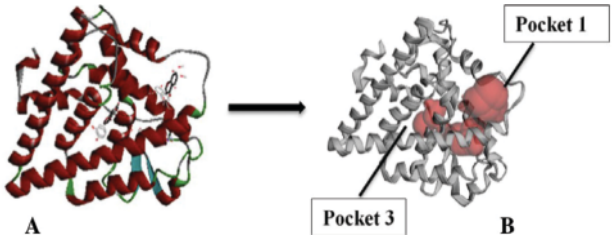

Fig. 2. (A) Image obtained via DSV indicates that all the studies are docked in binding pocket 1 and 3. (B) CASTp predicted a total of 33 binding pockets of the protein (3ERT) of which the two binding pockets (1 and 3) are shown highlighted in red color.

Molecular docking analysis

Out of the screened plant extracted compounds the selected compounds were subject to molecular docking analysis and visualization based on lowest binding energies that are Leonurine (Ligand 1), Butein (Ligand 2), Genistein (Ligand 3), Nobiletin (Ligand 4), Luteolin (Ligand 5) Table I. Ligand 1 and 2 exhibit the lowest binding energies and inhibition constant -6.2 kcal/mol, -7.8kcal/mol, and 2.731uM and 1.813uM, respectively. Ligand 1 showed hydrogen bonds with GLY521 and GLY420 amino acid residues while LEU525 and ALA350 showed hydrophobic interactions. However, Ligand 2 showed hydrophobic interactions with LEU387, LEU346, LEU525 and LEU349 (Fig. 3).

Ligand 4 and 5 showed the lowest binding energies and inhibition constant -6.6kcal/mol, -7.9kcal/mol, and 1.386uM and 1.531uM, respectively. Ligand 4 showed pi-sulfur bond interaction with MET522 amino acid residues and LEU536 with pi-alkyl interactions. However, ligand 5 showed hydrogen bond with GLY390 while electrostatic interactions was observed with ARG394, LYS449 AND GLU353 amino acid residues. Ligand 3 showed the highest binding affinity with lowest binding energy -8.7kcal/mol and inhibition constant 3.94uM. The docked complex exhibit hydrogen bond with GLY390 amino acid residue while ARG394, LYS449 and GLU353 showed electrostatic interactions (pi-cation and pi-ananion). However, PRO324 showed hydrophobic interactions (Fig. 4).

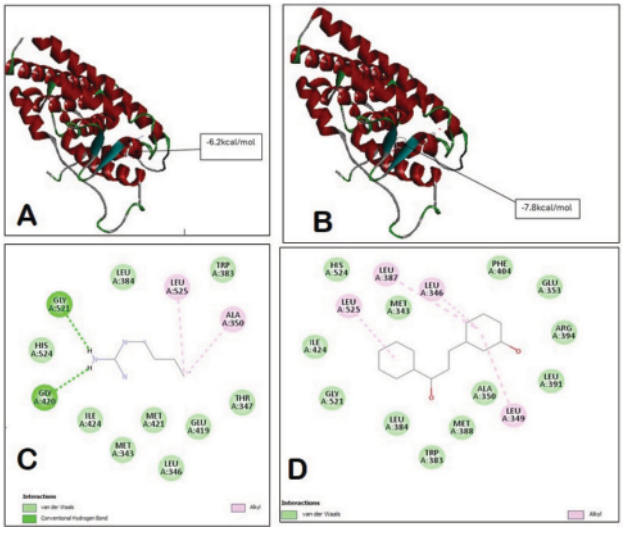

Fig. 3. Figure illustrating docked complexes. Ligand 1 docked complex and its visualization (A and C). Ligand 2 docked complex and its visualization (B and D).

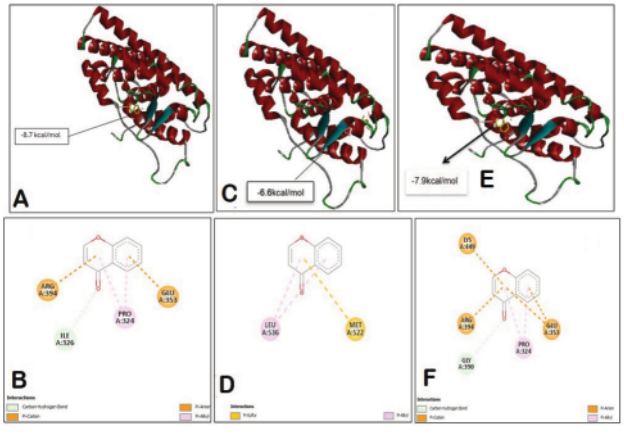

Fig. 4. Figure illustrating docked complexes. Ligand 3 docked complex and its visualization (A and B). Ligand 4 docked complex and its visualization (B and D). Ligand 5 docked complex and its visualization (E and F).

Physiochemical properties

Lipinski rule of five is the estimation of the compound's physiochemichal properties. Each ligand was subjected to SwissADME for Lipinski's rule of five.

Table I. Phytoactive compounds along with binding energies, amino acid residues and interaction types.

Ligand	CID	Protein	Binding energies (kcal/mol)	Amino acid residue	Interaction	Inhibition constant (uM)
Ligand 1	161464	3ERT	-6.2	GLY521 GLY420 LEU525 ALA350	Hydrogen Hydrogen Hydrophobic Hydrophobic	2.731
Ligand 2	5281222		-7.8	LEU387 LEU346 LEU525 LEU349	 Hydrophobic	1.813
Ligand 3	5280961		-8.7	ILE326 ARG394 GLU 353 PRO324	Hydrogen Electrostatic Electrostatic Hydrophobic	3.94
Ligand 4	72344		-6.6	MET522 LEU536	Pi-sulfur Hydrophobic	1.386
Ligand 5	5280445		-7.9	GLY390 ARG394 LYS449 GLU353 PRO324	Hydrogen Electrostatic Electrostatic Electrostatic Hydrophobic	1.531

All compounds meet the criteria, including molecular weight under 500 g/mol, fewer than 10 hydrogen bond acceptors, fewer than 5 hydrogen bond donors, and a LogP below 5, indicating good oral bioavailability. Leonurine shows favorable water solubility with a LogP of 0.62 and a polar surface area (PSA) of 129.39 Å². Butein and Genistein also fall within optimal ranges with moderate lipophilicity and PSA values supportive of membrane permeability. Nobiletin, despite having zero hydrogen bond donors, meets all the rules with a LogP of 3.51. Luteolin exhibits strong compliance, suggesting that all five compounds are suitable for further drug development studies targeting estrogen receptors (Table II).

Table II. Lipinski rule of five analysis of selected ligands.

Ligands	Moleular weight <500(g/mol)	H-Bond acceptor <10	H-bond donor <5	LogP <5	Polar surface area (Å²)
Ligand 1	311.33	6	3	0.62	129.39
Ligand 2	272.25	5	4	2.39	97.99
Ligand 3	270.24	5	3	2.58	90.90
Ligand 4	402.39	8	0	3.51	85.59
Ligand 5	286.24	6	4	2.28	111.13

Drug likeness and toxicity

The ADMET analysis of selected ligands; Leonurine, Butein, Genistein, Nobiletin, and Luteolin was conducted using the pkCSM online database, evaluating their absorption, distribution, metabolism, excretion, and toxicity profiles. All five compounds showed good intestinal absorption, with Genistein having the highest (87.13%) and Leonurine the lowest (70.33%). None of the ligands were identified as substrates or inhibitors of major cytochrome P450 enzymes (CYP2D6 and CYP3A4), indicating a low potential for drug–drug interactions. Water solubility values ranged from –2.317 to –3.251 LogS, suggesting moderate solubility, with Leonurine being the most soluble. Brain penetration, indicated by BBB permeability, was low in all compounds except Nobiletin, which showed a positive value (0.695), suggesting potential CNS activity. All ligands exhibited non-hepatotoxic and non-AMES toxic properties, except Nobiletin, which tested positive for AMES toxicity. Their total clearance values and non-inhibitory profiles for P-glycoprotein suggest good excretion characteristics and limited risk of efflux. Collectively, these findings support the drug-likeness and pharmacokinetic suitability of the selected ligands for further investigation in therapeutic development (Table III).

Table III. Drug likeness using pkCSM and SwissADME and ADMETlab 2.0 online databases server for the selected ligands.

ADMET		Ligand 1	Ligand 2	Ligand 3	Ligand 4	Ligand 5
Absorption	Water solubility (LogS) ml/L	-2.317	-3.177	-2.892	-2.892	-3.251
	Intestinal absorptipn (human) (% Absorption)	70.339	72.567	87.135	81.13	81.13
	P-Glycoprptein substrate	Yes	Yes	No	No	Yes
	P-Glycoprptein I inhibitors	No	No	No	No	No
	P-Glycoprptein II inhibitors	No	No	No	No	No
Distribution	VDss (human) (logL/kg)	0.442	0.741	0.011	0.011	1.153
	BBB permeability	-1.217	-0.895	-1.086	0.695	-0.907
	CNS permeability	-3.398	-2.395	-2.994	-1.317	-2.251
Metabolism	CYP2D6 substrate	No	No	No	No	No
	CYP3A4 substrate	No	No	No	No	No
	CYP2D6 inhibitor	No	No	No	No	No
	CYP 3A4 inhibitor	No	No	No	No	No
Excretion	Total clearance (Log ml/min / kg)	0.716	0.015	25.296	0.354	0.495
	Renal OCT 2 substrate	No	No	No	No	No
Toxicity	AMES toxicity categorical (Yes No/)	No	No	Yes	Yes	No
	Max. tolerable dose (log mg/kg/day)	0.486	0.117	0.438	0.438	0.499
	Hepatoxicity	No	No	No	No	No

DISCUSSION

Estrogen receptor alpha (ERα) plays a central role in the pathogenesis of hormone-dependent breast cancer and remains a prime target in endocrine therapy. However, resistance to existing agents like tamoxifen and aromatase inhibitors continues to limit their long-term efficacy (Manna and Holz, 2016). In this study, we employed molecular docking and ADMET prediction to evaluate five plant-derived photoactive compounds Leonurine, Butein, Genistein, Nobiletin, and Luteolin for their inhibitory interaction with ERα.

Among the tested ligands, Genistein exhibited the strongest binding affinity (-8.7 kcal/mol), followed by Luteolin and Butein, which also showed favorable docking scores and interaction profiles with key residues in the ERα binding pocket particularly GLU353, ARG394, and PRO324. These residues are critical for ligand binding and receptor activation. The CASTp analysis confirmed that these compounds occupied well-defined binding pockets, indicating potential allosteric or competitive inhibition (Tian *et al*., 2018).

Genistein, a soy isoflavone, has long been studied for its dual role as a phytoestrogen and anticancer agent. Recent work confirms that it not only binds ERα but also sensitizes cancer cells to hormonal therapies by modulating apoptotic and proliferative pathways (Mai *et al*., 2007). Similarly, Butein has been shown to interfere with Akt/mTOR signaling, reduce cell migration, and downregulate inflammatory cytokines, thereby enhancing its appeal as a multi-targeted anticancer compound (Golmei *et al*., 2024). Luteolin, widely distributed in many medicinal plants, inhibits breast cancer cell proliferation by modulating NF-κB and MAPK signaling cascades (Cook, 2018).

Our ADMET analysis supports the pharmacokinetic suitability of all five compounds. They exhibited high intestinal absorption, low hepatotoxicity, and no significant interaction with CYP450 enzymes essential features for oral bioavailability and low-risk drug interactions. Notably, Leonurine displayed the most favorable solubility and clearance profile, whereas Nobiletin, despite moderate docking performance, showed AMES toxicity, suggesting a need for further optimization or modification before therapeutic development. These findings are consistent with broader computational research efforts that explore dietary polyphenols and flavonoids as selective estrogen receptor modulators (SERMs). In a recent systematic review, flavonoids were found to engage ERα and HER2 targets with comparable or superior binding to synthetic ligands (Zand *et al*., 2002).

The potential photoactivity of some of these compounds opens up new possibilities for photoactivated

therapies that offer temporal and spatial control over drug action, especially relevant for localized tumor sites. This aligns with emerging interest in photodynamic estrogen receptor modulation, which could complement or replace conventional endocrine therapies with fewer side effects (Muniyandi *et al*., 2020).

Our study provides compelling computational evidence supporting the role of plant-derived compounds as ERα inhibitors. With favorable docking scores, strong interactions at biologically relevant binding sites, and drug-like ADMET profiles, the selected compounds especially Genistein, Luteolin, and Butein represent promising candidates for further *in-vitro*, *in-vivo*, and clinical exploration. These findings strengthen the case for integrating ethnopharmacology and molecular modeling in next-generation endocrine therapy development, particularly for resistant or relapsing breast cancer cases.

CONCLUSION

This study underscores the structural diversity of plant-derived compounds and their ability to bind effectively to estrogen receptor alpha (ERα), thereby contributing to their varied therapeutic potentials. Among the screened compounds, Genistein demonstrated the highest binding affinity, positioning it as the most promising candidate for ERα inhibition, followed by Luteolin and Butein. These findings support the potential of natural phytoactive compounds as safer and less toxic alternatives or adjuncts to conventional breast cancer therapies. The identification of these ERα-targeting compounds reinforces the growing interest in plant-based drug discovery, especially for the development of targeted therapies against hormone-dependent breast cancers.

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Generative AI or AI-assisted technology statement

No generative AI or AI-assisted technologies were used in the preparation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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Scanning Electron Microscopic Analysis and Biocontrol Potential of Entomopathogenic Fungi Against Grasshoppers

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SK conceived and designed the study, conducted fieldwork and laboratory experiments. RS supervised the research project, provided critical revisions to the manuscript, and approved the final version for submission.

Key words
***Entomopathogenic fungi, Aspergillus,* Grasshopper, Mortality, Biocontrol, SEM, IPM**

ABSTRACT

The present study evaluated the pathogenicity of five native strains of entomopathogenic fungi, including *Aspergillus niger, A. flavus, A. fumigatus*, and two unidentified fungal strains, against various pest species of grasshoppers. To confirm fungal identification and assess structural characteristics, scanning electron microscopy (SEM) analysis was conducted. Significant differences were observed among the strains in terms of phialide coloration, spore morphology, and growth patterns. Elemental composition determined through SEM spectrum acquisition revealed that in *A. niger*, the normal weight percentage of oxygen (O_2) was the highest at 56.19±17.5%, followed by carbon (C) at 42.60±13.1%, while sodium (Na) was present in the least amount (1.21 ±0.1%). For *A. flavus*, carbon content was highest at 52.33±16.1, followed by oxygen at 46.84±14.5%, with sodium again recorded in minimal quantity. In *A. fumigatus*, oxygen was dominant at 54.61±17.1%, followed by carbon at 43.92±13.6%, with minor concentrations of sodium (0.92%), sulfur (0.35%), and phosphorus (0.20%) detected. Biologically, insect mortality was most pronounced in treatments involving *Aspergillus spp*., which caused rapid declines in grasshopper populations, particularly among nymphal stages (N1 to N3), with only a few individuals surviving. The highest mortality rate was recorded on the first day of treatment [F_0.48 = 84.65, $P < 0.05$], followed by day four [F_0.35 = 61.96, $P < 0.05$] and day two [F_0.27 = 48.00, $P < 0.05$]. This study is the first to report the efficacy of *Aspergillus* spp. against both nymphal and adult stages of grasshoppers, highlighting their potential as effective biological control agents within integrated pest management strategies.

INTRODUCTION

Diseases caused by entomopathogenic fungi in insect fauna are widespread and have been extensively studied worldwide (Ferron, 1985; Goettel *et al*., 1990; Assaf *et al*., 2011). These fungi can rapidly decimate insect populations through spectacular epizootics. In natural field conditions, most insect populations are highly susceptible to such pathogenic fungi. Since insect orders like Diptera and Orthoptera are among the most damaging to agriculture, entomopathogenic fungi should be strategically employed against them. When fungal pathogens are abundant in the field, they significantly reduce insect populations and often contribute to their natural regulation (Samson *et al*., 1988).

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Entomopathogenic fungi can lethally affect all developmental stages of grasshoppers, from the embryonic stage to adulthood. For instance, Hemiptera overwinter in plant root zones and are often affected by these fungi (Kubatova and Dvorak, 2005). Kilic (1976) reported that *Beauveria bassiana* can kill up to 80% of Sunn pests. While several entomopathogenic fungal species such as *Beauveria*, *Aspergillus*, and *Metarhizium* are commercially available for the control of flies, aphids, and thrips (Upadhyay, 2003), their application as biopesticides against grasshoppers has not yet been widely explored. Therefore, the present study aims to expand our understanding of the occurrence and pathogenic potential of *Aspergillus* species for controlling grasshopper populations in the field. The microbial agent studied proved to be highly effective in reducing major pest species (Table I). This study suggests that introducing a novel biocontrol agent one not previously encountered by the target pest may offer a more effective and sustainable approach to pest management. Recent studies have renewed interest in the use of entomopathogenic fungi as environmentally sustainable alternatives to chemical pesticides, particularly in the context of increasing resistance and ecological concerns.

Table I. Important pest species of Acrididae occurring in Sindh.

Species
Subfamily: Acridinae
Acrida exaltata (Walker, 1859)
A. gigantea (Herbst, 1786)
Duroniella laticornis (Krauss, 1909)
Gelastorhinus semipictus (Walker, 1870)
Phlaeoba infumata Brunner von Wattenwyl, 1893
P. tenebrosa Walker, 1871
Truxalis exmia exmia Eichwald, 1830
T. fitzgeraldi Drish, 1950
Subfamily: Calliptaminae
Acorypha glaucopsis (Walker, 1870)
Sphodromerus undulatus undulatus (Kirby, 1914)
Subfamily: Gomphocerinae
Chorthippus indus Uvarov, 1942
Ch. dorsatus (Zetterstedt, 1821)
Gonista rotundata (Uvarov, 1933)
Ochrilidia geniculata (Bolivar, 1913)
Oxypterna afghana Ramme, 1952
Subfamily: Hemiacridinae
Hieroglyphus banian (Fabricius, 1798)
H. nigrorepletus Bolivar, 1912
H. oryzivorus Carl, 1916
H. perpolita (Uvarov, 1933)
Spathosternum prasiniferum (Walker, 1871)
Subfamily: Oedipodinae
Acrotylus humbertianus Saussure, 1884
A. longipes longipes (Charpentier 1845)
Aiolopus thalassinus thalassinus (Fabricius, 1781)
Hilethera aeolopoides (Uvarov, 1922)
Locusta migratoria (Linnaeus, 1758)
Oedaleus rosescens Uvarov, 1942
O. senegalensis (Krauss, 1877)
Trilophidia annulata (Thunberg, 1815)
Subfamily: Oxyinae
Oxya bidentata (Willemse, 1925)
O. fuscovittata (Marschall, 1836)
O. hyla hyla Serville, 1831
O. velox (Fabricius, 1787)

Fungal strains, formulations and application methods have progressively improved field performance of entomopathogenic fungi against a range of insect pests (Mascarin and Jaronski, 2016). In addition, Shahid *et al*. (2023). With over 92% mortality in a semi-arid field, Bankola *et al*. (2023) showed the efficacy of Aspergillus and Metarhizium species against Orthopteran pests (grasshoppers) with the advantages of low non-target effects. This support is pivotal in a modern integrated pest management (IPM) approach, especially in areas where ecological imbalance has been exacerbated due to synthetic insecticide misuse. Thus presently, the study provides timely evidence that Aspergillus species could be useful organisms as potential biological control agents of grasshoppers in agroecosystems undergoing pest outbreak situations.

According to Mascarin and Jaronski (2016), advances in fungal strain selection, formulation technologies, and application methods have significantly improved the field efficacy of entomopathogenic fungi against various insect pests. Furthermore, a study by Shahid *et al*. (2012) demonstrated the successful application of *Aspergillus* and *Metarhizium* species under semi-arid conditions for the suppression of Orthopteran pests, including grasshoppers, with notable mortality rates and minimal non-target effects. These findings support the integration of entomopathogenic fungi into modern integrated pest management (IPM) programs, especially in regions facing ecological imbalance due to overuse of synthetic insecticides. Therefore, the current study contributes timely evidence that *Aspergillus* species can serve as viable biological control agents against grasshoppers in agroecosystems facing pest outbreaks.

MATERIALS AND METHODS

Insect sampling

Grasshoppers (both nymphs and adults) were collected from various districts of Sindh (Table I). Specimens were captured using a sweep net (diameter: 8.89 cm; length: 50.8 cm). Larger individuals were collected using forceps, while first instar nymphs were picked by hand. The collected insects were transported to the laboratory, where they were placed in two rearing cages of different dimensions (42 cm × 30 cm and 35 cm × 32.5 cm). Groups of 50 individuals were housed per cage and provided with fresh Zea mays leaves as food. This methodology, with slight modifications, was adapted from Prior *et al*. (1995) and Sultana *et al*. (2013) and Jamil and Sultana (2025). For species identification, the classification scheme developed by Sultana and Wagan (2008, 2015) was followed.

Collection of infected specimens

Grasshoppers showing clear symptoms of fungal infection (mycoses) were selectively collected. These

individuals exhibited sluggish behavior and minimal resistance upon capture. Infected specimens were reared separately in cages and jars to closely observe the progression of fungal growth on the host body. Observations were made for a duration of 24–72 h.

Insect rearing

Various species of Acrididae were grouped into sets of approximately 50 individuals, regardless of age, sex, or developmental stage. The collections were maintained under laboratory conditions in wooden cages, with temperatures ranging from 28 ± 2°C to 41 ± 2°C and relative humidity (RH) from 26.5% to 60.5%. All developmental stages of field-collected grasshoppers were maintained at the Entomology and Bio-Control Research (EBCRL), Department of Zoology, University of Sindh, Jamshoro (25°-23/N, 68°-24/E).

Fungal isolation and sporulation test

Sporulating entomopathogenic fungi were isolated in pure culture using Sabouraud Dextrose Agar (SDA), a medium favorable for fungal growth. The isolated cultures were formulated into a coconut oil-based suspension. To ensure uniform dispersion and breakage of conidial chains, the formulation was sonicated for 60 sec. Conidial concentration was then quantified using a hemocytometer, following standardized procedures adopted and modified from Poinar and Thomas (1984), Kumar *et al*. (2013), and more recently from Qayyum *et al*. (2020) and Ullah *et al*. (2021). Identification of Aspergillus species was conducted based on morphological characteristics and culture characteristics according to the recent taxonomic descriptions of Samson *et al*. (2017), the databases of the Westerdijk Fungal Biodiversity Institute.

Observations under scanning electron microscopy (SEM-EDS)

Fungal spores were subjected to SEM coupled with EDS for elemental composition at the Centre for Pure and Applied Geology, University of Sindh, Jamshoro. Three known species as *Aspergillus niger, A. flavus*, and *A. fumigatus*, as well as two unidentified fungal isolates (Uk FI and Uk FII), were included to analyze five fungal samples. Infected specimens in both isolation and co-isolation were killed, and fungal spores were subsequently recovered from cadavers using the protocol of Kumar *et al*. (2014) subsequently improved by Sharma *et al*. (2019) and Ahmad *et al*. (2022). Each sample was kept under natural sunlight 12–14 h to obtain full dehydration. Dried spore samples were then mounted for SEM-EDS analysis, to assess qualitative and quantitative elemental composition. The data on the mineral uptake and surface morphology of entomopathogenic fungi has been discussed.

The experimental procedure for SEM-EDS analysis was started by manually cutting fungal core chips with distinct structures, followed by stepwise mounting on SEM sample stubs with conductive double-sided carbon adhesive tape. SEM images of all samples were taken within the chamber of a JEOL JSM-6490LV SEM fitted with a Bruker Energy Dispersive X-ray Spectroscopy (EDS) unit for elemental composition analysis. The sample was left inside the SEM, and it took 15–20 min to get required vacuum level achieved inside the SEM. After a stable vacuum was shown on the interface of the system, specific portions of each sample were chosen for imaging and elemental analysis using EDS. Each Aspergillus sample was mounted in a consistent manner on the conductive carbon tape, labeled with a unique identifier, and analyzed in sequence. High-resolution magnified images were captured, and EDS analysis was conducted to determine the elemental composition of each fungal sample. Optimal SEM operational parameters were configured to ensure fine focusing and desired magnification levels. Once an ideal magnification typically around ×80 was achieved, the samples were subjected to both qualitative and quantitative elemental analysis. Elemental composition was determined by examining the characteristic X-ray peaks produced during EDS, where peak height indicated the presence of specific elements. Finally, the results were compiled in both tabular and graphical formats to clearly present the qualitative identities and quantitative proportions of elements found in the fungal spores.

RESULTS

During the present study, five subfamilies of Acrididae namely *Acridinae*, *Calliptaminae*, *Gomphocerinae*, *Hemiacridinae*, *Oedipodinae*, and *Oxyinae* were represented by 32 pest species of grasshoppers (Table I). These were treated with various entomopathogenic fungi. Fungal identification and elemental analysis were carried out using SEM coupled with EDS (Table II). Spectrum acquisition for *Aspergillus niger* revealed that oxygen (O_2) had the highest normal weight percentage at 56.19±17.5%, followed by carbon (C) at 42.60±13.1%, while sodium (Na) was present in the lowest amount at 1.21±0.1%. In the case of *Aspergillus flavus*, the highest elemental composition was observed for carbon (C) at 52.33±16.1%, followed by oxygen (O_2) at 46.84±14.5%. Sodium was again detected in the lowest proportion. Similarly, *Aspergillus fumigatus* showed a greater percentage of oxygen (O_2) at 54.61±17.1%, followed by carbon (C) at 43.92±13.6%. Other elements detected included sodium

(Na) at 0.92%, sulfur (S) at 0.35% and phosphorus (P) at 0.20%, all with minimal error values (Table I; Fig. 1, 2). Two unidentified fungal isolates (Uk FI and Uk FII) were also analyzed. In Uk FI, carbon (C) content was the highest at 62.82±19.2%, followed by oxygen (O_2) at 36.82 ±11.4%, while sodium was detected in trace amounts. Uk FII presented five elements, with carbon (C) again being dominant at 54.00±16.6%, followed by oxygen (O_2) at 43.53±13.6%. The lowest values were recorded for sulfur (S) and sodium (Na) at 0.79% and 0.1%, respectively. Overall, the elemental composition of the three known *Aspergillus* species and the two unidentified fungi showed significant variation, indicating possible species-specific physiological traits (Table II; Figs. 1, 2). Biological assays showed that insects treated with *Aspergillus* species exhibited rapid mortality, especially in early developmental stages (N1 to N3). Most immature stages succumbed, with only a few individuals surviving the fungal exposure. Mortality was highest on Day 1, showing statistically significant results [F0.48=84.65, P<0.05], followed by Day 4 [F0.35=61.96, P<0.05], and Day 2 [F0.27=48.00,P<0.05]. The lowest mortality was recorded on Day 3, but it was still statistically significant [F0.17=30.54, P<0.05] (Table II).

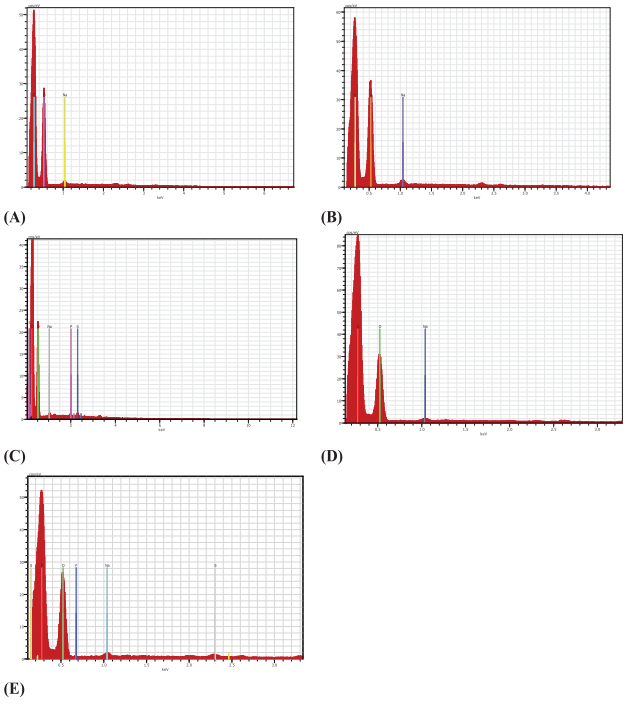

Fig. 1. Element concentrations under scanning electron microscope (SEM): A, *Aspergillus niger*; B, *A. flavus*; C, *A. fumigatus*; D, unknown Fungi I; E, unknown Fungi II.

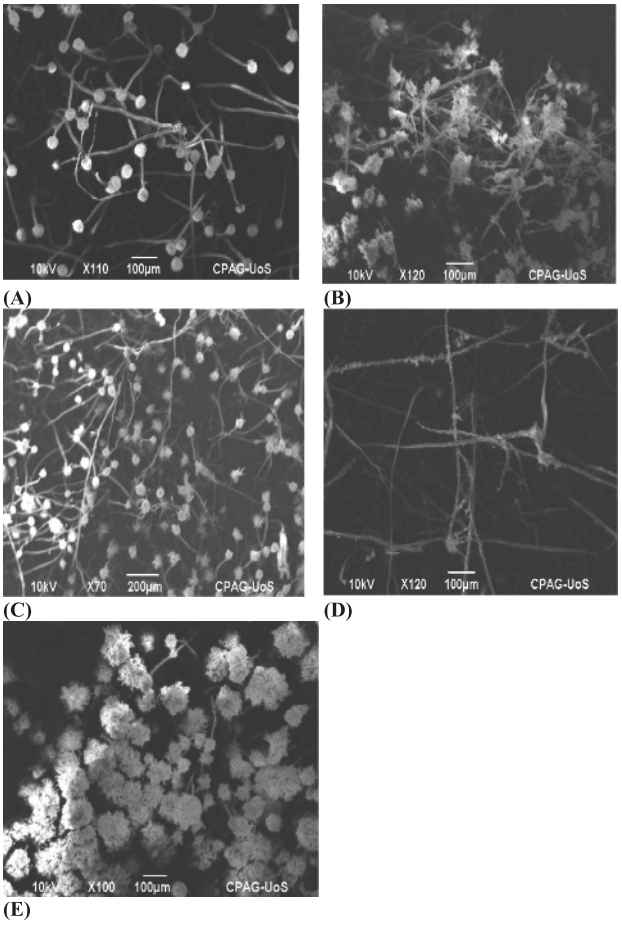

Fig. 2. Electron microscopy: A, *Aspergillus niger*; B, *A. flavus*; C, *A. fumigatus*; D, unknown Fungi I; E, unknown Fungi II.

Mortality trends and pathogenic effects

In the control replicates, the mortality ratio for nymphal stages (N4–N6) was highest on Day 2 [F10.7=18.33, P<0.05], followed by Day 4 [F4.20=7.85, P<0.05] and Day 3 [F3.77=6.11, P<0.05]. Conversely, the lowest mortality was recorded on Day 1 [F0.48=84.65, P<0.05] (Table III). Throughout the experiment, it was consistently observed that fungal-infected individuals showed reduced feeding activity and suffered from multiple pathological symptoms. Regarding nymphal populations kept in large cages and treated with conidial suspensions prepared in water, the highest mortality was observed on Day 6 [F0.82=43.99, P<0.05] (Table IV). However, the mortality was statistically non-significant on Day 2 [F8.5=14.84, P<0.05] and Day 3 [F7.25=13.09,P<0.05], while significantly lower mortality was noted on Day 5 [F3.32=6.11, P<0.05] (Table IV). In adult *Acrididae*, the maximum mortality occurred on Day 7 [F13.7=23.56, P<0.05], followed by Day 6 [F12.5=21.82,

Table II. Spectrum acquisition under SEM.

Element	Series	unn. C (wt. %)	Norm. C (wt. %)	Atom. C	Error (%)
Aspergillus niger					
Carbon (C)	K-series	42.60[b]	42.60[b]	49.88[a]	13.1[b]
Oxygen (O_2)	K-series	56.19[a]	56.19[a]	49.88[a]	17.5[a]
Sodium (Na)	K-series	1.21[c]	1.21[c]	0.74[c]	0.1[c]
	Total	100.00	100.00	100.00	
Aspergillus flavus					
Carbon (C)	K-series	52.33[a]	52.33[a]	59.88[a]	16.1[a]
Oxygen (O_2)	K-series	46.84[b]	46.84[b]	39.88[b]	14.5[b]
Sodium (Na)	K-series	0.83[c]	0.83[c]	0.49[c]	0.1[c]
	Total	100.00	100.00	100.00	
Aspergillus fumigatus					
Carbon (C)	K-series	43.92[b]	43.92[b]	51.31[a]	13.6[b]
Oxygen (O_2)	K-series	54.61[a]	54.61[a]	47.89[b]	17.1[a]
Sulfur (S)	K-series	0.35[d]	0.35[d]	0.15[d]	0.0[d]
Sodium (Na)	K-series	0.92[c]	0.92[c]	0.56[c]	0.1[c]
Phosphorus (P)	K-series	0.20[d]	0.20[d]	0.09[d]	0.0[d]
	Total	100.00	100.00	100.00	
Unknown Fungi I.					
Carbon (C)	K-series	62.82[a]	62.82[a]	69.30[a]	19.2[a]
Oxygen (O_2)	K-series	36.82[b]	36.82[b]	30.49[b]	11.4[b]
Sodium (Na)	K-series	0.36[c]	0.36[c]	0.21[c]	0.1[c]
	Total	100.00	100.00	100.00	
Unknown Fungi II					
Carbon (C)	K-series	54.00[a]	54.00[a]	69.38[a]	16.6[a]
Oxygen (O_2)	K-series	43.53[b]	43.53[b]	30.14[b]	13.6[b]
Fluorine (F)	K-series	1.17[c]	1.17[c]	0.84[c]	0.5[d]
Sodium (Na)	K-series	0.52[e]	0.52[e]	69.31[a]	0.1[c]
Sulfur (S)	K-series	0.79[d]	0.79[d]	30.34[b]	0.1[c]
	Total	100.00	100.00	100.00	

P<0.05] (Table V). In contrast, mortality was at its lowest on Day 1 [F0.44=77.67, P<0.05], followed by Day 3 [F0.77=35.26, P<0.05] . Mortality on Days 4 and 5 was statistically non-significant (Table V). Additionally, a significant difference was observed in the cumulative fecal output of infected insects compared to control groups. Among various treatments, the H_2O-based formulation of conidia showed the most pronounced impact on adult mortality, with a peak observed on Day 8 [F1.00=2.62, P<0.05]. Mortality was also significantly higher on Day 2 [F0.02=4.36, P<0.05], though lower than the peak, and least on Day 1 [F0.06=11.34, P<0.05]. However, from Day 3 to Day 7, the differences in mortality were statistically non-significant (Table VI).

DISCUSSION

Entomopathogenic fungi (EPFs) are increasingly recognized for their wide-ranging potential in Integrated Pest Management (IPM) due to their eco-friendly, host-specific, and biodegradable nature. These microbial agents can effectively target and control various insect pests, including grasshoppers, with minimal impact on non-target organisms and the environment (Sánchez-Ramos *et al*., 2022). In the present study, high mortality rates were observed in acridid populations, and susceptibility to fungal infection was closely linked to the host's developmental stage. Early instars (N1–N3) experienced significantly higher mortality than the older stages, which supports the results of stage-specific efficacy of EPFs Wakil *et al*. (2019). All fungal isolates tested showed efficacy, especially *Aspergillus* species against grasshoppers but varied with life stage. The differences we observe are likely due to structural and physiological defenses at later development stages, with thicker cuticles and more activated immunity (Fernandes *et al*., 2021). Under SEM imaging, the conidia showed considerable morphological diversity, shape, ontogeny and in pigmentation. Conidia varied from monocellular to multicellular and appeared in various shapes including globose, ovoid, cylindrical, and spirally coiled with colours from hyaline to melanised dark spores. These features are important for fungal classification and pathogenicity as melanin has been correlated with higher virulence and resistance to unfavorable environmental conditions (Rangel *et al*., 2020). Saccardo's conventional classification scheme based on color and morphology of the conidia is of historical interest but is now seen as restricted. Molecular approaches have been given priority over traditional mycological methods as they are vital for refining species boundaries, which results in improvement of taxonomic resolution (Ramanpreet *et al*., 2012; Kumar *et al*., 2022; Sultana *et al*., 2021). Here, we show that the differentiation based on SEM corroborates the integrated taxonomic approach we have performed. Magalhães *et al*. (2001) stated in support of our results, the use of *Metarhizium anisopliae* var was successfully demonstrated by Wraight *et al*. (2001). A review of the use of *Metarhizium acridum* as a biopesticide for grasshopper control in Brazil, although noted limitations including short storage life and variable field performance. Studies done recently, though, are trying to overcome this barrier with better formulations and cold storage (Qayyum *et al*., 2020; Ullah *et al*., 2023). We also noted reduced feeding and movement, reproductive abnormalities such as behavioral

Table III. Mortality of Acridid (Nymphs) population cultured in small jars under laboratory conditions (after treatment of *Aspergillus* oil formation).

Treatments	Days of observation (Mean ± SE)						
	1st	2nd	3rd	4th	5th	6th	7th
Nymphs stages 1st to 3rd							
A. flavus	0.45±0.23^{c}	0.35±0.21^{a}	0.20±0.21^{a}	0.8±0.23^{a}	0.00±0.00	0.00±0.00	0.00±0.00
A. fumigatus	0.62±0.01^{a}	0.22±0.01^{c}	0.16±0.01^{b}	0.2±0.01^{c}	0.00±0.00	0.00±0.00	0.00±0.00
A. niger	0.55±0.2^{b}	0.32±0.23^{b}	0.13±0.1^{c}	0.00±0.00^{d}	0.00±0.00	0.00±0.00	0.00±0.00
Control	0.3±0.1^{d}	0.2±0.1^{d}	0.2±0.23^{a}	0.4±0.2^{b}	0.00±0.00	0.00±0.00	0.00±0.00
$F_{.(0.05)}$	$_{(0.48)}$84.65	$_{(0.27)}$48.00	$_{(0.17)}$30.54	$_{(0.35)}$61.96	-------	-------	-------
Nymphs Stages 4th to 6th							
A. flavus	0.29±0.1^{c}	42±0.2^{a}	13±0.2^{a}	16±0.1^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
A.fumigatus	0.43±0.1^{b}	0.38±0.1^{c}	0.7±0.32^{d}	0.12±0.2^{d}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
A.niger	0.62±0.2^{a}	0.28±0.1^{d}	0.8±0.2^{c}	0.2±0.1^{c}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
Control	0.6±0.1^{a}	0.4±0.3^{b}	0.6±0.2^{b}	0.5±0.3^{b}	0.7±0.1^{a}	0.6±0.1^{a}	0.5±0.1^{a}
$F_{.(0.05)}$	$_{(0.48)}$84.65	$_{(10.7)}$18.33	$_{(3.77)}$06.11	$_{(4.20)}$07.85	-------	-------	-------

Note: Mean in the same column followed by the same letters is not significantly different from one another at 5% level of probability.

Table IV. Mortality of Acridid (Nymphs) population treated with conidial concentration in H_2O cultured maintained in the large cage.

Treatments	Days of observation (Mean ± SE)									
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
A. flavus	13±0.1^{c}	11±0.2^{b}	7±0.1^{c}	6±0.2^{b}	5±0.2^{a}	2±0.1^{a}	6±0.2^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
A. fumigatus	15±0.1^{b}	12±0.1^{a}	9±0.2^{b}	7±0.2^{a}	5±0.1^{a}	1.0±0.1^{b}	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
A. niger	21±0.1^{a}	7±0.2^{c}	10±0.1^{a}	7±0.2^{a}	3±0.1^{b}	0.2±0.1^{c}	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
Control	0.2±0.1^{d}	4±0.2^{d}	3±0.1^{d}	6±0.1^{b}	0.3±0.00^{c}	0.1±0.00^{c}	0.1±0.3^{b}	0.43±0.1^{a}	0.21±0.2^{a}	0.22±0.1^{a}
$F_{.(0.05)}$	$_{(12.3)}$21.82	$_{(8.5)}$14.84	$_{(7.25.)}$13.09	$_{(6.5)}$11.34	$_{(3.32)}$06.11	$_{(0.82)}$43.99	--	--	--	--

Note: Mean in the same column followed by the same letters is not significantly different from one another at 5% level of probability.

Table V. Mortality of Acridid (Adults) population cultured in small jars under laboratory conditions (after treatment of *Aspergillus* oil formation).

Treatments	Days of observation (Mean ± SE)						
	1st	2nd	3rd	4th	5th	6th	7th
A. flavus	0.35±0.32^{b}	0.00±0.00^{d}	1.5±0.47^{a}	6.9±1.41^{a}	11.0±2.10^{a}	27.8±1.30^{a}	3.4±2.00^{a}
A. fumigatus	0.00±0.00^{c}	2.5±1.00^{a}	0.61±0.32^{c}	3.8±1.32^{b}	5.8±0.43^{b}	9.8±1.20^{c}	27.0±3.9^{b}
A. niger	1.42±0.31^{a}	1.00±0.58^{b}	1.00±0.43^{b}	4.5±0.53^{b}	4.9±1.02^{c}	11.42±1.30^{b}	22.8±1.90^{c}
Control	0.00±0.00^{c}	0.75±0.31^{c}	0.00±0.00^{d}	1.9±0.46^{c}	0.00±0.00^{d}	1.00±0.57^{d}	1.8±0.00^{d}
$F_{.(0.05)}$	$_{(0.44)}$77.67	$_{(1.06)}$02.62	$_{(0.77)}$35.26	$_{(4.27)}$07.85	$_{(5.42)}$09.60	$_{(12.5)}$21.82	$_{(13.7)}$23.56

Note: Mean in the same column followed by the same letters is not significantly different from one another at 5% level of probability.

Table VI. Mortality of Acridid (Adult) population treated with conidial concentration in H_2O cultured maintained in the large cage.

Treatments	Days of observation (Mean ± SE)									
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
A. flavus	0.02±0.32^{b}	0.01±0.02^{b}	1.2±0.01^{a}	0.23±0.15^{b}	1.5±0.03^{a}	1.5±0.01^{a}	1.2±0.32^{b}	1.2±0.13^{c}	1.3±0.14^{b}	1.5±0.01^{a}
A. fumigatus	0.01±0.21^{c}	0.00±0.00^{c}	0.3±0.04^{c}	1.6±0.01^{a}	1.2±0.03^{b}	0.5±0.04^{c}	1.3±0.01^{a}	1.5±0.02^{a}	0.00±0.00^{c}	0.00±0.00^{b}
A. niger	0.2±0.23^{a}	0.1±0.12^{a}	0.1±0.15^{d}	0.23±0.1^{b}	0.5±0.7^{d}	0.2±0.15^{d}	0.01±0.2^{c}	0.00±0.00^{d}	0.00±0.00^{c}	0.00±0.00^{b}
Control	0.01±0.02^{d}	0.00±0.00^{c}	0.5±0.23^{b}	0.02±0.1^{c}	0.6±0.22^{c}	0.7±0.23^{b}	0.00±0.00^{d}	1.3±0.01^{b}	1.4±0.01^{a}	1.5±0.04^{a}
$F_{.(0.05)}$	$_{(0.06.)}$11.34	$_{(0.02)}$04.36	$_{(0.52)}$91.63	$_{(0.52)}$91.63	$_{(0.95)}$16.58	$_{(0.72)}$26.54	$_{(0.62)}$09.08	$_{(1.00)}$02.62	-------	-------

Note: Mean in the same column followed by the same letters is not significantly different from one another at 5% level of probability.

mating (aberrant mating practice), and oviposition behavior, typical behavioral responses to entomopathogenic infections (Mascarin *et al*., 2019), in infected insects. In addition, the spore dissemination through air, soil and water which we observed in this study confirms the earlier reports by Nawaz *et al*. (2024) investigations done by Ali *et al*. (2014) which provides some evidence of such a natural epizootic due to their apparent environmental adaptability. The choice of *Aspergillus* spp. considerations of their cosmopolitan distribution, speed of sporulation, and ease in culture, form the basis of this study. The choice of *Aspergillus* spp. in this study was based on their cosmopolitan distribution, rapid sporulation, and ease of culture. Although less commonly used in commercial biocontrol products compared to *Metarhizium*, *Beauveria* and *Aspergillus* strains have shown promising virulence in tropical conditions (Khan *et al*., 2022). In Pakistan, especially Sindh, their integration into IPM remains understudied and holds potential for sustainable pest suppression. This study advocates the use of fungal biopesticides as alternatives to chemical insecticides, particularly in sensitive agricultural ecosystems. However, the development of safe, targeted formulations is critical to avoid unintended effects on pollinators and other beneficial arthropods (Batista *et al*., 2021). Additionally, efforts must be made to promote mass production of conidia, molecular profiling of fungal strains, and field validation in diverse agroecological zones to ensure their effectiveness and regulatory acceptance.

CONCLUSION

Present study recommends that where indigenous *Aspergillus* spp. strains can be used as potential bio-control agents against various economical important grasshopper species in Pakistan. The significant morphologies and elemental composition differences of fungal isolates were displayed in scanning electron microscope (SEM) at a good extent and these upright features were useful in species identification and support their structural integrity. It was also observed that all the assayed Aspergillus strains were pathogenic, especially when used at initial stages of nymph stages, which were causing instant death. *Aspergillus niger*, *A. flavus* and *A. fumigatus* were characterized by high virulence and the highest mortality occurred in the first part of days before and after treatment in the laboratory and semi-field conditions. Also, the infected insects had decreased feeding behavior and anomalies in reproduction, which suppressed the physiological effects of the fungi. The results highlight the possibility of using *Aspergillus* spp. as potential biopesticides in the biosecurity pest management (IPM) strategies particularly in areas with mid-scale ecological imbalances due to the overuse of chemical pesticides. Still, to apply their wider applicability, additional studies of formulation optimization, field validation, and the safety of non-target organisms should be carried out. This study is important in the creation of sustainable and environmentally friendly pest interventions in the agro eco-systems.

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Data availability

Data presented in this study will be available on a fair request to the corresponding author.

Generative AI or AI-assisted technology statement

The authors declare that no generative AI or AI-assisted technologies were used in this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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Could Orthoptera unlock Nutritional Potential for Humans and Livestock?

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Protein, insects, Awareness, Nutritional, Livestock, Cost-effectiveness

ABSTRACT

The world is currently grappling with a substantial challenge in meeting the global demand for meat protein, particularly in arid regions where persistent food and water shortages render the population highly vulnerable. Scarce food resources contribute to severe malnutrition, including protein deficiency, resulting in elevated maternal mortality rates. To address these pressing issues, the consumption of insects, known for their protein richness, emerges as a valuable strategy to combat malnutrition in these communities. Unfortunately, the concept of entomophagy is poorly understood in Pakistan. To raise awareness and foster acceptance, an awareness campaign and a diversity-exploring survey have been planned for 2023-2024 in selected desert localities. To achieve our goals, the sensitization program aimed to: (1) To increase awareness among villagers about the benefits of using insects as a protein source in their diet. (2) To alleviate reluctance by highlighting that consuming locust adheres to halal principles in Islam. (3) To improve the nutritional content of livestock feed, emphasizing its cost-effectiveness compared to alternatives

INTRODUCTION

Orthoptera are the most diverse group of insects: With 30388 valid species and 2821 sub-species and many more still waiting to be discovered (Cigliano *et al*., 2025). Orthoptera stands out among alternative food sources due to various qualities. Ecologically, they function as primary consumers. Additionally, despite being univoltines, they represent a substantial biomass that people globally prepare and consume (Ramos-Elorduy, 2009; Blásquez *et al*., 2012). Moreover, they are often forward sold or stored, especially in their dried form. The consumption of swarming locusts is a prevalent practice in regions affected by locust plagues. Locusts and grasshoppers have been a part of human diets for centuries and continue to be consumed in certain areas today. This practice, known as entomophagy, holds significant nutritional benefits and plays a crucial role in regions within the habitat range of the desert locust, particularly in specific areas of Pakistan. In the Thar, Nara, Cholistan desert regions, the population has long grappled with persistent food and water shortages, rendering them highly susceptible to vulnerabilities. Scarce food resources contribute to severe malnutrition, notably protein deficiency, and contribute to elevated maternal mortality rates. The consumption of desert locusts, rich in protein, emerges as a valuable strategy to combat malnutrition in these communities. Therefore, viewing locust collection as an intriguing food source becomes imperative, especially for impoverished and undernourished rural populations. Insect protein is indeed much more sustainable than protein from other conventional sources, primarily due to their minimal environmental impact and limited use of natural resources (Oonincx and de Boer, 2012). Notably, the inclusion of insects in the market is driven by these environmentally friendly characteristics. Insects are also proposed as a remedy for world hunger, with their potential contribution to ensuring food security in developing countries being emphasized (Kari and Gates 2013; Kelemu *et al*., 2015). Locusts are mentioned in religious holy books, the Bible and The Holy Quran (7:133). The Quran refers to them two times, while The Holy Bible (Leviticus 11:20–25) contains as many as 36 references.

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The world is currently facing a significant challenge in meeting the global demand for meat protein. According to the United Nations (2022) the global population could grow to around 8.5 billion in 2030, and add 1.18 billion in the following two decades, reaching 9.7 billion in 2050 (World Population Prospects, 2022), the need for protein-rich food sources, particularly meat, is escalating. Traditional methods of meat production, such as livestock farming, are struggling to keep pace with this growing demand. The scarcity of meat protein is aggravated by resource scarcity, environmental issues, and inefficiency of feed-to-protein conversion. The shortfall not only threatens nutritional needs for a growing population but also creates potential problems with environmental sustainability. Therefore, the gap between demand and supply in the global meat protein market is gradually widening, and exploring alternative protein sources to meet the increasing demand is urgent. The response, entomophagy (the consumption of insects), presents as a sustainable option. Given that entomophagy is practiced in many cultures globally, over 2,100 species 66 consumed as food in more than 110 countries (Jongema, 2015). Despite certain limitations, entomophagy presents a promising opportunity to help bridge the protein gap in human diets. Within the domain of food security, it is particularly pressing that a broader evaluation and re-evaluations of entomophagy be conducted through the lens of contemporary living. Hinted as a potential solution to a range of critical environmental and human health issues from climate change to malnutrition, food insecurity, and the environmental degradation associated with agro-industrial production (Davis *et al*., 2015; van Huis and Oonincx, 2017; Godfray *et al*., 2018; Dickie *et al*., 2019; van Huis, 2020) edible insects Likewise, Chuanhui *et al*. (2010), Lesnik (2019) explored the editable insect from China and Florida and Chakravorty *et al*. (2011) from India and later reported that approximately 255 insect's species are consumed in India. In Pakistan, the practice of entomophagy, or consuming insects, is presently not widely acknowledged or embraced. Nonetheless, there is an innovative initiative underway to introduce and normalize this practice, particularly by tapping into the nutritional potential of orthoptera for both human and livestock consumption. With an estimated 1.5 billion chickens and numerous fish farms in the country, there exists a substantial market that could potentially benefit from the incorporation of high-protein locust meat (www.veterinariadigital.com). As part of our awareness campaign, we underscored the permissibility of consuming locusts in Islam, fostering a positive response from poultry and fish farm owners. This religious endorsement encouraged active engagement and advocacy for the inclusion of locusts, grasshoppers, and crickets in livestock feed. Through persistent efforts, we successfully heightened awareness among the Thari people regarding the nutritional advantages of incorporating locusts into their diets, addressing protein deficiencies, and promoting improved nutrition within the community.

MATERIALS AND METHODS

Village and farming facility selection

Fifteen villages across three districts Mithi, Tharparkar, (24.7436° N, 69.8061° E), and Choondiko, Khairpur, (27.1606° N, 68.9569° E) Sindh, as well as CUVAS Bahawalpur, Punjab (29.3544° N, 71.6911° E) were selected for a human awareness campaign (Figure 1). Additionally, two farming facilities: The Government Poultry Farm and the Fish Hatcheries and Nurseries in Bahawalpur,

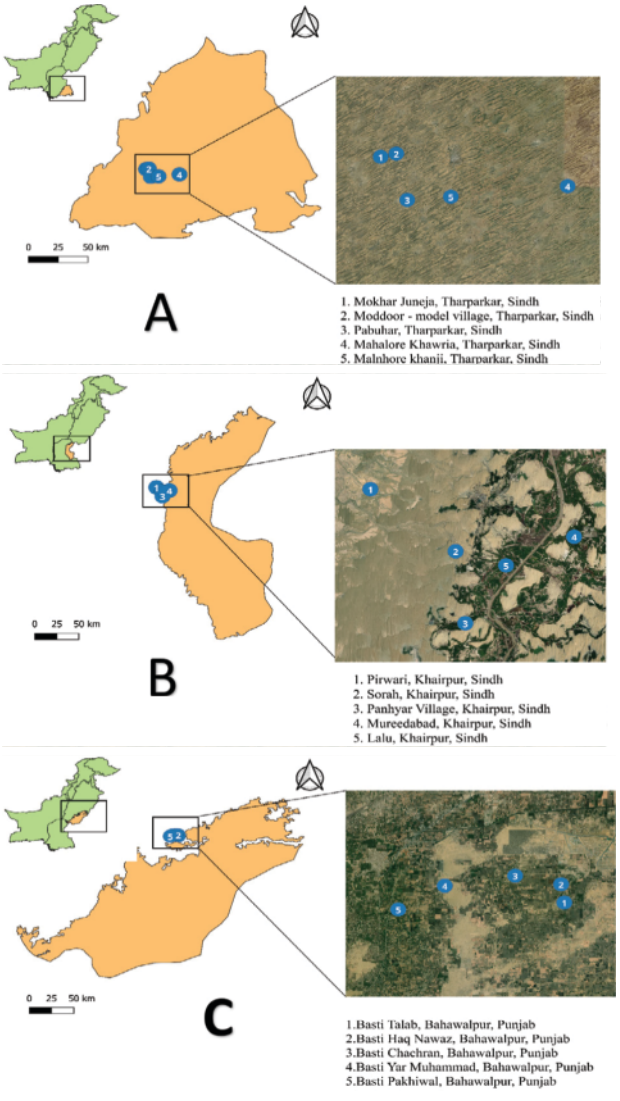

Fig. 1. Surveying areas of selected villages during 2023 in Sindh-Punjab.

Fig. 2. The Government Poultry Farm, Bahawalpur.

were included in the program for livestock feed supplementation (Figure 2). The sensitization program took place between June and August 2023, followed by another phase from May to July 2024.

Sampling and identification

All specimens were collected from different agricultural crops in various selected localities (Figure 1). Material was brought to Entomology and Bio-control Research Lab (EBCRL), Department of Zoology, University of Sindh, Jamshoro. The mounting, labeling, and taxonomic identification of collected Orthoptera were conducted following appropriate keys given by Otte (1995) and Sultana and Wagan (2015), Sultana *et al*. (2021), Sultana and Song (2024) and taxonomic status and validity of Orthoptera were also verified by consulting with OSF Cigliano *et al*. (2025).

Empowering desert communities

During the awareness campaign, individuals were adeptly trained in the collection and cleaning of insects. This hands-on approach allowed participants to engage in practical experiences, experimenting with diverse recipes. The culmination of these efforts resulted in the preparation and serving of delightful insect-based dishes (Figure 3). The shared recipes were disseminated to encourage ongoing exploration and adoption of insect-based cuisine. Simultaneously, proprietors of farming facilities were actively involved in discussions that illuminated the nutritional value inherent in insects, Emphasizing the cost-effectiveness and nutritional impact of these insect-based options, we underscored their potential as alternatives to the conventional livestock feed currently provided. In regions characterized by arid conditions and widespread financial constraints, where conventional meat sources are not easily accessible, our campaign endeavors to introduce sustainable alternatives. By promoting the utilization of locally available insects such as locusts, grasshoppers, and crickets, we aim to mitigate the economic challenges faced by these communities. This dual approach seeks to alleviate financial burdens while enriching diets with valuable protein sources, thereby contributing to the overall well-being of these communities.

Fig. 3. Different locust dishes were cooked during locust swarm 2019-2020 (after Samejo *et al.,* 2021).

RESULTS AND DISCUSSION

Table I illustrates a comprehensive survey of Orthoptera (mostly we focused on locust, grasshoppers, and crickets) diversity, including both nymphs and adults, in selected villages across three regions. The survey occurred in Mithi, Tharparkar (Sindh) during the last week of June 2023, Choondiko, Khairpur (Sindh) in mid-July 2023, and CUVAS, Bahawalpur (Punjab) in mid-August 2023. The counts of three Orthoptera groups grasshoppers, locusts, and crickets over three days reveal variability across villages, highlighting notable diversity in the selected areas. In the villages of Mithi, Tharparkar, total Orthoptera counts range from 1377 to 1959 across the three survey days. Examining the counts for each group exposes specific patterns within each village; for example, Malnhore Khanji displays a relatively balanced distribution, while Moddoor model village exhibits a

Table I. Surveying orthoptera diversity in respective areas of selected villages during 2022.

Villages	First day			2nd Day			3rd Day			Total	%
	G.	L.	C.	G.	L.	C.	G.	L.	C.		
A. Mithi, Tharparkar (Sindh) (Last week June-2023)											
1. Mokhar Juneja,	274	39	323	189	21	301	193	13	289	1642	7.64%
2. Moddoor - model village	203	49	198	274	72	287	293	49	303	1728	8.04%
3. Pabuhar	187	17	349	399	53	403	177	87	287	1959	9.11%
4. Mahalore Khawria	162	19	271	173	69	194	208	92	189	1377	6.40%
5. Malnhore khanji	233	51	133	151	182	309	79	56	167	1361	6.33%
B. Choondiko, Khairpur (Sindh) (Mid-July-2023)											
1. Pirwari	122	23	204	203	23	186	55	83	173	1072	4.98%
2. Sorah	176	14	387	197	47	169	124	18	203	1335	6.21%
3. Panhyar Village	78	35	179	238	77	201	206	13	289	1316	6.12%
4. Mureedabad	103	5	134	134	37	302	157	---	303	1175	5.46%
5. Lalu	157	19	129	73	11	382	149	29	204	1153	5.36%
C. CUVAS, Bahawalpur (Punjab) (Mid-August-2023)											
1. Basti Talab	307	41	204	298	11	189	207	49	199	1505	7.00%
2. Basti Haq Nawaz	208	32	186	203	--	203	301	19	205	1357	6.31%
3. Basti Chachran	197	--	132	305	7	246	153	31	308	1379	6.41%
4. Basti Yar Muhammad	243	9	309	109	37	287	178	44	401	1617	7.52%
5. Basti Pakhiwal	181	41	402	137	23	389	128	7	203	1511	7.03%

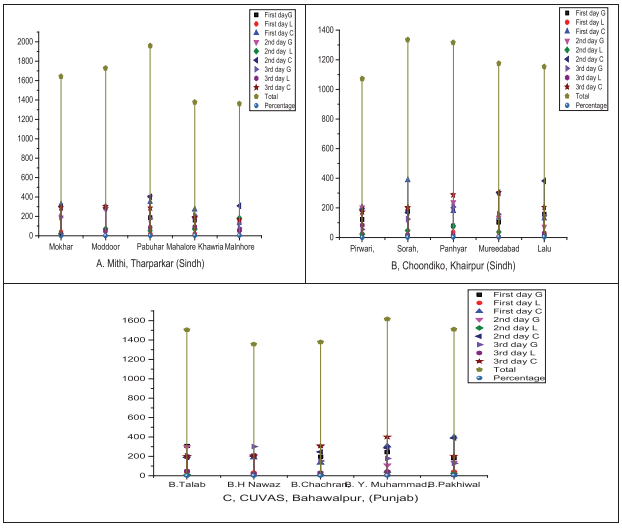

Fig. 4. Surveying orthoptera diversity in respective areas of selected villages during 2023.

higher count of locusts (this area was not surveyed and sprayed by the plant protection during the 2019-2020 locust-swarm-control operation (Sultana *et al*., 2021). In Choondiko, Khairpur, Orthoptera counts vary significantly across villages, with Panhyar totaling 1316 and Pirwari recording 1072. Besides this, in Bahawalpur villages, orthoptera counts range from 1357 to 1617. However, it's noteworthy that no locusts were collected from Mureedabad on the third day and Basti Haq Nawaz on the second day. Overall, the observed low percentages of locusts in all localities may be attributed to extensive insecticide spraying during the 2019-2020 locust swarm, coupled with continuous monitoring of locust emergence/ hatching in different areas of Pakistan, resulting in fewer reported locust numbers during our survey days (Table I, Fig. 4). Table II provides information about common Orthoptera species predominantly consumed by invertebrates and vertebrates during field surveys. A total of 26 species were observed, belonging to 18 genera, 11 subfamilies, and 4 families. Regrettably, *Schizodactylus* was captured by younger individuals for their domestic pets, but unfortunately, its population is declining at the regional level. This study underscores the urgent need for counseling to discourage hunting of *Schizodactylus* in Pakistan. Within the Gryllidae family, 22 species from 12 genera and 2 subfamilies were reported as frequently consumable by domestic pets (Table III). In the case of fish, common species were highlighted, providing information on their habitat, common coloration, and season of hatching. A total of 6 families, including 11 subfamilies and 13 species, were listed (Table IV). Table V

presents common predators of grasshoppers and crickets, revealing that globally, 11 bird species, 5 mammal species, and 7 other insect species are known to feed on adults, nymphs, larvae, and eggs of these insects.

Table II. Taxonomy of some common edible Orthoptera of Pakistan.

Genus	Species	Consumption stage
Family: Acrididae		
Subfamily: Acridinae		
Acrida	*Acrida exaltata*	Nymphs/ Adults
Acrida	*Acrida gigantea*	Nymphs/ Adults
Truxalis	*Truxalis eximia*	Nymphs/ Adults
Subfamily: Calliptaminae		
Subfamily: Cyrtacanthacridinae		
Acorypha	*Acorypha glaucopsis*	Nymphs/ Adults
Anacridium	*Anacridium aegyptium*	Nymphs/ Adults
Anacridium	*Anacridium rubrispinum*	Nymphs/ Adults
Schistocerca	*Schistocerca gregaria gregaria*	Nymphs/ Adults
Subfamily: Eyprepocnemidinae		
Eyprepocnemis	*Eyprepocnemis alacris alacris*	Nymphs/ Adults
Tylotropidius	*Tylotropidius varicornis*	Nymphs/ Adults
Subfamily: Gomphocerinae		
Leva	*Leva indica*	Nymphs/ Adults
Ochrilidia	*Ochrilidia geniculata*	Nymphs/ Adults
Hieroglyphus	*Hieroglyphus banian*	Nymphs/ Adults
Hieroglyphus	*Hieroglyphus nigrorepletus*	Nymphs/ Adults
Hieroglyphus	*Hieroglyphus oryzivorus*	Nymphs/ Adults
Subfamily: Oedipodinae		
Acrotylus	*Acrotylus humbertianus*	Nymphs/ Adults
Aiolopus	*Aiolopus thalassinus tamulus*	Nymphs/ Adults
Phlaeoba	*Phlaeoba tenebrosa*	Nymphs/ Adults
Subfamily: Oxyinae		
Oxya	*Oxya hyla hyla*	Nymphs/ Adults
Oxya	*Oxya fuscovittata*	Nymphs/ Adults
Subfamily: Pyrgomorphinae		
Chrotogonus	*Chrotogonus trachypterus trachypterus*	Nymphs/ Adults
Chrotogonus	*Chrotogonus homalodemus homalodemus*	Nymphs/ Adults
Family: Tettigoniidae		
Subfamily: Phaneropterinae		
Trigonocorypha	*Trigonocorypha unicolor*	Nymphs/ Adults
Phaneroptera	*Phaneroptera (Phaneroptera) spinosa*	Nymphs/ Adults
Phaneroptera	*Phaneroptera (Phaneroptera) gracilis*	Nymphs/ Adults
Conocephalus	*Conocephalus (Anisoptera) maculatus*	Nymphs/ Adults
Schizodactylus	*Schizodactylus minor*	Nymphs/ Adults

Note: In Pakistan, people use these species as their domestic pet's diet. *Indicate that species within this family are becoming endangered worldwide, emphasizing the urgent need for counseling to discourage people from hunting these species.

Table III. Taxonomy of some common edible Gryllidae of Pakistan.

Genus	Species	Consumption stage
Family: Gryllidae		
Subfamily: Gryllinae		
Acheta	*Acheta chudeaui* (Chopard, 1927)	Adults
	A. domesticus (Linnaeus, 1758)	Adults
	A. meridionalis (Uvarov, 1921)	Adults
	A. hispanicus Rambur, 1838	Adults and Larvae
Gryllus	*Gryllus* (*Gryllus*) *multipulsator* Weissman, 2009	Adults
Gryllodes	*Gryllodes sigillatus* (Walker, 1869)	Adults
	G. supplicans (Walker, 1859)	Adults
Callogryllus	*Callogryllus ovilongus* Saeed, Saeed and Yousuf, 2000	Adults
	Callogryllus saeedi Malik, *et al.,* 2013	Adults and Larvae
	*C. bilineatus (*Bolívar, 1900)	Adults
Modicogryllus	*Modicogryllus sindhensis* Sultana *et al*, 2021,	Adults
Phonarellus	*Phonarellus* (*Phonarellus*) *minor* (Chopard, 1959)	Adults
	P. (Phonarellus) humeralis (Walker, 1871)	Adults and Larvae
Plebeiogryllus	*Plebeiogryllus retiregularis* Saeed, Saeed and Yousuf, 2000	Adults
Tartarogryllus	*Tartarogryllus tartarus* (Saussure, 1874)	Adults
Gryllopsis	*Gryllopsis pubescens* Chopard, 1928	Adults
Eumodicogryllus	*Eumodicogryllus bordigalensis* (Latreille, 1804)	Adults and Larvae
Teleogryllus	*Teleogryllus (Brachyteleogryllus) occipitalis (*Serville, 1838)	Field crickets
Subfamily: Nemobiinae		
Pteronemobius	*Pteronemobius concolor* Walker 1871	Adults
	P. (Pteronemobius) indicus (Walker, 1869)	Adults and Larvae
Loxoblemmus	*Loxoblemmus (Loxoblemmus) formosanus* Shiraki, 1930	Adults

Table IV. Taxonomy and habitat of common occurring fish of Pakistan.

Family	Genus	Species	Habitat	Color	Common name	Spawning period
Anabantidae	*Anabas*	*Anabas testudineus*	Freshwater	Greenish to Brownish,	Anabas	April-August
Ariidae	*Arius*	*Arius arius.*	Freshwater	*Gray to Grayish Brown*	Khaga	April -October
Bagridae	*Sperata*	*Sperata seenghala*	Rivers, canals ditches	Brownish Gray	Singhara	April-August
Belonidae	*Xenentodon*	*Xenentodon cancila*	Freshwater	Green, Silver, Whitish	Garfish	June-July
Cichlidae	*Oreochro-mis*	*Oreochromis niloticus*	Fresh and Brackish water	Gray and Light Pink	Tilapia	April -December
Clariidae	*Clarias*	*Clarias batrachus*	Freshwater	Gray or Grayish Brown	Mangur, walk-ing catfish	June-August
Channidae	*Channa*	*Channa striataus*	Ponds, streams and rivers	Brown and Black	Sowra	April-August
Channidae	*Channa*	*Channa punctatus*	Ponds and Brackish	Tan to Black	Gurrie	April-September
Channidae	*Channa*	*Channa gachua*	Freshwater	Grey, Brown	Sauri	December-February
Channidae	*Channa*	*Channa marulius*	Freshwater	Deep Black	Saul	June-July
Cyprinidae	*Cyprinus*	*Cyprinus carpio*	Freshwater	Brownish green	Gulfam com-mon carp	Jan-August

Table contines on next page.....

Family	Genus	Species	Habitat	Color	Common name	Spawning period
Cyprinidae	*Catla*	*Catla catla*	Freshwater brackish water	Grayish	Theila	June – August.
Cyprinidae	*Labeo*	*Labeo rohita*	Fresh water brackish water.	Bluish or Brown	Rohu	June – August.
Cyprinidae	*Labeo*	*Labeo callasu*	Freshwater	Bluish	Kalbans	June – August.
Cyprinidae	*Cirrhina*	*Cirrhina mrigala*	Freshwater	Silvery Dark Grey	Mirgal Mori	June – August.
Cyprinidae	*Barbus*	*Barbus eputitora*	Cold waters of hilly areas	Greenish and Whitish	Mahaseer	April – September
Cyprinidae	*Cteno-pharyngoden*	*Ctenopharyngoden idellus*	Freshwater river and lake fish	Bluish to Grey	Mullee	Monsoon
Cyprinidae	*Barbut*	*Barbut tor*	Hilly streams and river	Silvery Grey with Red Fins	Bhor	April -September
Salmonidae	*Salmo*	*Salmo* sp.	Clear, cold and fast flowing water.	Ventral Dark Grey,	Trout	October - March
Siluridae	*Wallago*	*Wallago attu*	Freshwater river and lake fish	Bluish to Grey	Mullee, Parhin	Monsoon
Mugilidae	*Mugil*	*Mugil corsula*	Rivers	Silver or Gray,	Corsula Mullet	June -February
Mastacem-belidae	*Mastacem-belus*	*mastacembelus armatus*	Stream and rivers	Dark-Sliver	Baam	September-Oct
Notopteri-dae	*Notopterus*	*Notopterus chitala*	Standing and sluggish waters	Silvery Dark or Green-ish	Chital	May-August

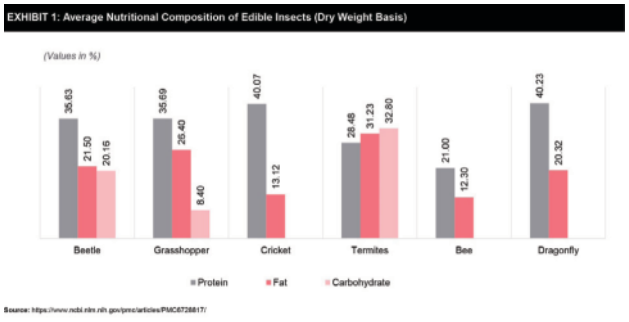

Fig. 5. Average nutritional composition of editable insects (Dry weight basis). (Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6728817/).

Consultation with communities

Our three teams conducted visits to selected areas, organizing separate meetings and seminars for men and women to inform the community about the nutritional value of orthoptera as a protein source in their diet. During these sessions, we distributed informative pamphlets and visual materials illustrating the benefits of incorporating orthoptera into livestock diets. To amplify our message, we strategically engaged local influencers, community leaders, and religious figures, seeking their endorsement of the campaign and their assistance in emphasizing the positive impact on agriculture and nutrition. Recognizing the importance of aligning with cultural and religious values, we collaborated closely with Islamic scholars and local religious authorities, resulting in the development of educational content focused on showcasing the compatibility of locust consumption with halal principles in Islam (Fig. 5). To deepen understanding and acceptance, we conducted nutritional awareness campaigns emphasizing the benefits of orthoptera-based protein, encompassing high protein content, essential amino acids, and vitamins. Moreover, we conducted a comparative analysis to demonstrate the cost-effectiveness of locusts, grasshoppers, and crickets compared to traditional livestock feed ingredients, highlighting the potential economic advantages for farmers. Besides this, for a tangible and experiential dimension to our efforts, we presented a documentary from abroad where villagers could directly observe the positive effects of integrating locusts into animal feed, benefiting both livestock health and economic returns. This comprehensive and forward-thinking approach not only addresses immediate needs but also lays the foundation for enduring positive change, fostering community acceptance, and promoting sustainable practices deeply rooted in the values of the communities we aim to empower. Many delicious dishes, such as burgers, candies, chocolate powders, cookies, drinks, flours, granola and musli, pasta, pasta sauce, protein bars, protein powders, snacks, spreads, tofu, whole fire and

Table V. Common predators of grasshoppers and crickets.

Common names	Scientific names	Regions
Birds		
Crow	*Carvus corax*	North Hemisphere
Collared King fishers	*Todiramphus chloris*	Tropical region of Africa and Asia
House Sparrow	*Passer domesticus*	Europe, Mediterranean, Basin, Asia
Duck	*Anas platyrhyncos*	Asia, Europe, and other countries
Parrots	*Psittaciformes*	Australia, Oceania, South Asia, America and Africa
Squacco heron	*Ardeola ralloides*	Europe, Africa, Iran
Myna	*Acridotheres tristis*	Asia, Iran, Pakistan, India and other countries
Hen	*Gallus domesticus*	Mostly Asia, Europe, and Africa
Brown partridge (Teether)	*Pondicerianus splenden*	Asia, Europe, and Africa
House crow	*Carvus splenden*	Native to the Indian subcontinent, including all of India, Pakistan, the Maldives, and Sri Lanka.
Rusty black bird	*Euphagus, carolinus*	Canadian provinces and territories, the state of Alaska, several Great Lakes states and most New England states
Mammals		
Giant anteaters	*Myrmecophaga tridactyla*	Central America and Northern South America
Giant Armadillos	*Priodontes maximus*	Central America and Northern South America
Common Shrews	*Sorex araneus.*	Northern Europe
Numbat	*Myrmecobius fasciatus)*	Australia
Wongai Ningaui	*Ningaui ridei*	Central Asia
Echidina	*Tachyglossus aculeatu*	Australia
Insects eat grasshoppers and crickets' eggs		
Dragonflies,	*Odonata*	Tropical region
Hornets,	*Hymenoptera.*	Asia and Europe
Ladybugs,	*Coleoptera*	Africa and other countries
Robber flies,	*Diptera*	All countries except Antartica
Praying mantides	*Mantodea*	All countries except Antartica
Ants	*Hymenoptera,*	Asia, Africa and other countries
Mosquito	*Diptera*	All countries, except Antartica

boil insects, locust fried, locust biryani, cricket cake, locust karahi, fried bar-bi-qab locust, and locust Chinese rice, made from insects are very famous in many countries like Mexico, Thailand, China, Zimbabwe, Brazil, Kenya, Australia, Cambodia, India, China, and Thailand, including Tokyo, Japan (Hanboonsong *et al*., 2000; Bugs Feed, 2016). In Pakistan, locusts were consumed during the historical swarm of 2019-2020 and people first tasted locusts specifically in Karachi and Thar, Sindh (Samajo *et al*., 2021).

Engagement with live-stock holders

A comprehensive meeting was organized with fish and poultry Farm's owners/in-charge, focusing on the discussion of insect protein ratios in feed. It was emphasized that Pakistan currently imports 0.3 million tonnes of soybeans, utilizing the crushed residue for animal feed after oil extraction. Notably, soybeans contain 45% protein, while locusts boast an impressive 70% protein content. Introducing a rich protein diet, such as locusts, grasshoppers, crickets could potentially enhance the growth and flavor of farming products. Various valuable fish varieties in Pakistan, including Gulfam, Seengari, Jarka, Khaga (Rohu fish), Tilapia (dayo), Theeli, and others, hold significant commercial value. Despite being rich in protein, these fish face high production costs. Pakistan's global reputation for delicious fish dishes is a testament to its culinary heritage, blending traditional

Fig. 6. Fish Bar B Q at University of Sindh Jamshoro Pakistan 2023.

recipes with modern innovations, making it a culinary destination for fish enthusiasts (Fig. 6). Similarly, poultry feeding is a critical aspect of industry, influencing chicken growth, health, and productivity. An impassable issue in this process is the dependence on traditional, often low diversity and unbalanced feed components. Changes in the prices of these poultry feed ingredients can therefore create economic difficulties of poultry farms, reducing profitability. Moreover, probing contamination and feeding of adulterated feeds is a risk to poultry flock health and product quality. Inadequate awareness among some farmers regarding proper feed management practices such as appropriate storage and feed formulation further exacerbates poultry feeding challenges. A farmer who switches to an insect-based diet for its livestock can achieve better feeding efficiency and maximum acceptability level by the livestock. This saves time and money but indeed enhances the protein content of fish and chicken also making it a powerful method for effective and healthy farming at lower costs. Therefore, solving these problems is fundamental for the development of the poultry industry and the production of healthy and high-quality poultry products. There is a clear need to simply promote the use of insects in poultry feeding to solve these problems and augment nutritional profile of poultry and fish as a feed. This move makes the diet plan more balanced with a proper ratio of protein with more awareness and promotion of sustainable practices, the poultry and fish industry in Pakistan could (i) overcome the existing challenges faced and (ii) contribute substantially to the nutritional requirements of its population. While researchers encounter innumerable obstacles in combating these problems, the adoption of entomophagy projects is practical and necessary to create a paradigm change in our diet. Such a transition is imperative to fulfil the increasing food demand and curtail the looming protein crisis soon.

Current hurdles

Entomophagy is receiving increasing acceptance in many developed nations due to the nutritional ability of insects, but its acceptance remains a great hurdle in areas where there is little or no knowledge of the concept. Cultural and religious beliefs frequently preclude insects from national or traditional diets, and hesitation is drawn into their consumption or their use as feed for livestock. Humans like familiar foods, and the ignorance about the nutritional and ecological advantages of insects reinforce this resistance. Widespread myths about insects being pests or vectors for disease also live on, so community education about how to farm or eat bugs safely is vital. A lack of clear regulations and standards in terms of safety, in conjunction with communication gaps regarding the benefits of entomophagy, also explains public scepticism. Insect-based feed, as a relatively new concept, is not well known among livestock farmers who may also worry about its implementation in their systems. In addition, uncertainty over consumer acceptance of livestock products from animals fed insects, together with concerns over potential effects on animal health and productivity, also limit investment and may require awareness, education and policy support. It requires a combination of approaches to normalizing eating insects and feeding livestock with insects. Even better is to work with cultural and religious leaders so that such practices can be consistent with what is generally accepted in the community at local and national levels. Food targeting communication strategies need to be developed to inform the public about the nutritional, economic, and environmental benefits associated with eating insects. Addressing misconceptions with varied media channels will broaden reach and dispel myths, whilst awareness campaigns can educate on the safety and advantages of insect farming which is done under controlled practices. Concurrently, partnerships should be built with relevant stakeholders of the food industry on the development of insect-based products that are attractive for consumers. Moreover, help programs for insurance plans or incentives from the state might mitigate the perceived threats for the livestock holders. Stakeholders can consequently work together to strategically solve major roadblocks along the path toward a sustainable and culturally appropriate implementation of insect-based food and feed systems.

CONCLUSIONS

This indicates the diversity of Orthoptera in Pakistan

and their prospects as sustainable protein to humans and livestock. The community engagement showed increasing interest, alongside cultural, regulatory and awareness challenges. Educating the population, developing relevant policies, and working together with community leaders can help promote insect-based diets as an affordable, healthy and environmentally sustainable option to meet protein demands and promote agricultural sustainability in Pakistan.

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Availability of data and material (data transparency)

The data that support the findings of this study are made available by contacting the author (riffat.sultana@usindh.edu.pk)

Generative AI or AI-assisted technology statement

The authors declare that no generative AI or AI-assisted technologies were used in this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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Alterations in Hepatorenal Profile of Occupationally Exposed Workers to Welding Fumes

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ABSTRACT

Welding fumes contain enormous harmful toxicants including Cadmium (Cd^{2+}), iron (Fe^{2+}), aluminium (Al^{3+}), manganese (Mn^{2+}), zinc (Zn^{2+}) and their derivatives that can give rise to multiple chronic ailments in individuals exposed to them. Present investigation was designed to estimate the harmful effects of welding environments on hepatic and renal profile of welders. Blood samples of welders (n=30) and controls (n=30) were collected from Shakargarh-Punjab. Hepatorenal parameters were assessed by the medical chemistry analyser using commercially available diagnostic kits for each parameter. At confidence interval (CI) of 95% ($P \leq 0.05$), independent sample t-test was applied for statistical analysis. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and showed non-significant decrease in welders as compared to controls. While, Alkaline phosphatase (ALP) and bilirubin exhibited highly significant increase ($P \leq 0.0001$ and $P \leq 0.01$, respectively) in welders as compared to controls, respectively. A prominent decrease was observed in creatinine ($P \leq 0.02$) and urea ($P \leq 0.0004$) in labours of the welding industry as compared to controls. Whereas, in serum electrolytes; K^+ ($P \leq 0.005$) and Cl^- ($P \leq 0.002$) demonstrated a considerable elevation, while Na^+ showed a non-significant difference in welders when compared to controls. Thus, our investigation showed that welding fumes and its related constituent's exposure bring disturbance in hepatic and renal physiology due to variations in respective parameters in welders. Therefore, welders are recommended to use safe techniques such as masks on face to prevent inspiration of harmful gases and related toxic compounds, glasses on eyes, gloves on hands for good health.

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ZS: Conceptualization of study
MAI: Writing original draft and statistical analysis
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NR: Supervision of the investigation
FL: Biochemical analysis
HA: Acquisition of data from participants

Key words
ALT, AST, ALP, Creatinine, Liver function enzymes, Heavy Metals, Renal function test

INTRODUCTION

Welding is an industrial practice in which joining of metallic objects is performed by using metal as a filler. The filler, in turn, is created from a wire of electrode utilized during the welding fusion method (Antonini, 2003). Workers in the welding industry are exposed to fumes, primarily through skin contact and inhalation. These fumes are chief contributors to deviations in various bodily mechanisms that could ultimately lead to the manifestation of different ailments (Liden and Surakka, 2009) Welders can also get a considerable quantity of these toxicants, while, eating or drinking with dirty hands through contaminating their food or liquid.

The materials typically found in welding fumes include chromium (Cr), aluminium (Al), nickel (Ni), copper (Cu), beryllium (Be), lead (Pb), manganese (Mn), molybdenum (Mo), vanadium (V), fluoride (F^-), oxides of cadmium (CdO), iron oxides (FeO) and zinc oxides (ZnO). Gases are also produced during the welding process. Main gases are hydrogen fluoride (HF), nitrogen oxides (NO_x), carbon monoxide (CO), fluorine (F) and ozone (O_3) (Liu *et al*., 2007).

A variety of mechanisms could be involved in generating welding fumes. More than 90% of the particles of fumes in welding are produced by the electrode filling metal vaporization, core and coatings as in arc welding and to a lesser degree, in laser beam welding (Schnick *et al*., 2010). Welding fumes can be of various forms. Particulates in the welding fumes can be classified into three groups based on their sizes: coarse ($d > 2.5\mu m$), fine ($0.1 < d < 2.5\mu m$) and ultrafine ($0.01 < d < 0.1\mu m$) (Jenkins, 2003). Overall, exposure of these environmental toxicants can induce epigenetic changes in these occupationally exposed subjects (Feinberg, 2018). Pathological symptoms that appear due to an exposure to welding fumes have been associated with systemic and respiratory health effects,

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such as a decline in the lung function, enlarged air passage sensitivity, bronchitis, lung cirrhosis, fibrosis, and a large number of pulmonary disorders including emphysema, chronic bronchitis and asthma (Antonini *et al*., 2013; Kim *et al*., 2005). These systemic conditions are produced by inspiration of oxides of zinc (Draznin and Epstein, 2011) in fumes and are categorized by onset of acute flu-like sickness with dry cough, muscle aches, headaches, dyspnea and fever (McHugh, 2010).

Additionally, several respiratory dysfunctions such as asthma, bronchitis, changes in the lung function as well as cardiovascular complications have been attributed to the chronic exposure to welding fumes (Leso *et al*., 2019). Welding fumes including Be and Cr may get absorbed by the skin causing allergy and irritation. Absorption by skin is further boosted by ultrafine particles and via cuts or other injuries to the skin (Nygren, 2006). Welding fumes are also linked to the metal fume fever (MFF), inflamed oral and nasal cavity (El-Zain *et al*., 2003; Greenberg and Vearrier, 2015) and cardiovascular abnormalities. Many investigations have also demonstrated an increased mortality due to ischemic heart disease in the welders, who have had a prolonged exposure to welding fumes (Ibfelt *et al*., 2010; Fan *et al*., 2014; Li *et al*., 2015).

Biomonitoring of blood is essential to assess the level of toxicity in exposed workers. This study was designed to evaluate sickness pattern, attentiveness of work-related threats such as the effects of long-term welding fume exposure on hepatorenal profile of the welders working in such environment.

MATERIALS AND METHODS

A case-control study was designed for the welding fume workers. For this purpose, blood samples of age matched (20-45) welders (N=30) and controls (N=30) were collected from Shakargarh, Punjab, Pakistan. It was assured that samples were collected after 8-12 h of fasting with their consent. Only male workers were recruited for the present investigation as in Pakistan welding process is specifically associated with males.

A systemised proforma was made to document demographic variables of both groups. Participants noticeables such as sex, height, weight, waist, blood pressure, hip circumference, drug usage, family history of illness, smoking history, working experience were documented. Parameters regarding the health perspective of all individuals were attained before phlebotomy. Reliable and sanitized syringes of Becton Dickinson (BD) Pakistan were utilised for withdrawing the blood from all participants. For this purpose a registered medical laboratory technician was hired.

Blood samples were centrifuged (Model; Hettich, Zentrifugein D-7200 Tuttligen, Germany) at 2500-3000 rpm for about 10 min, for separation of blood serum which was stored at -80 ^{o}C for future biochemical investigations.

Commercially available kits of Beckman Coulter USA, were used for determination of hepatic and renal metabolites through Chemistry analyser (Beckman Coulter USA, Model AU480).

Two tailed independent sample t-test at Confidence Interval (CI): 95%; $P \leq 0.05$, was applied for statistical analysis. All Statistical exploration was performed on GraphPad Prism 6.00 software.

RESULTS

Body mass index expressed highly significant ($P \leq 0.0005$) decrease of 17 % in welders as compared to control group. In hepatic profile, alanine aminotransferase (ALT) presented a non-significant decrease of 15% in welders as compared to controls. Moreover, the same trend was witnessed in the aspartate aminotransferase (AST) levels in comparison of welders and control group. The level of alkaline phosphatase (ALP) was significantly ($P \leq 0.0001$) increased in welders as compared to control group. Similarly, concentration of bilirubin expressed a significant ($P \leq 0.01$) increase of 37% in welders as compared to control group (Table I). In renal metabolites, creatinine levels demonstrated significant ($P \leq 0.02$) decrease of 20% in welders as compared to control group. Moreover, serum concentration of urea depicted a prominent ($P \leq 0.0004$) decrease of 32% in welders as compared to control group. In serum electrloytes, workers of welding industry presented slight increase of 1% of Na^{+} concentration as compared to control group. In addition to that, K^{+} expressed significant ($P \leq 0.005$) increase of 8% in welders' group as compared to healthy controls. Comparing serum chloride Cl^{-} levels indicated a considerable ($P \leq 0.002$) elevation of 8% in welders when compared to controls (Table I).

DISCUSSION

Liver diseases are the most common health conditions in all over the world. A massive diversity of chemicals, microbes and especially viruses are renowned liver damaging agents (Stankova *et al*., 2010).

Under occupationally exposed conditions, inhalation of certain hazardous substances cause damage to the hepatic lobules that contain parenchymal cells which ultimately are unable to perform vital roles, resulting in distressed and unprovoked intermediate metabolism. It can lead to cellular destruction and tissue necrosis. These substances enhance the permeability of hepatic bio-membranes,

Table I. Effect of metal welding funes on hepatorenal profile concentrations in blood samples of control and welders.

Parameters	Control (N=30)	Welders (N=30)	t value	p value	Difference %
ALT (U/L)	50.62±9.96	43.23±3.93	0.80	0.4	15 ↓
AST (U/L)	37.31±4.65	35.45±2.47	0.38	0.7	5 ↓
ALP (U/L)	103.20±6.41	215.10±7.20	10.54	< 0.0001***	108 ↑
Bilirubin (mg/dL)	0.63±0.04	0.86±0.06	2.53	0.01**	37 ↑
BMI (kg/m^2)	25.13±1.00	20.92±0.59	3.85	0.0005***	17 ↓
Creatinine (mg/dL)	0.79±0.08	0.63±0.01	2.37	0.02*	20 ↓
Urea (mg/dL)	30.54±2.74	20.91±0.99	3.91	0.0004***	32 ↓
Na^+ (mmol/L)	139.5±0.64	141.1±0.61	1.70	0.09	1 ↑
K^+ (mmol/L)	3.73±0.09	4.02±0.04	3.01	0.005**	8 ↑
Cl^- (mmol/L)	98.08±0.78	100.8±0.43	3.25	0.002**	8 ↑

↑ and ↓ represents increase and decrease, respectively, mmol/L = millimole per litre, mg/dL = milligram per decilitre. ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; BMI, body mass index.

ultimately leading to elevated levels of hepatocellular enzymes in plasma (Center, 2007). Elevation of these enzymes in serum above a normal range presents degree of destruction in liver morphology as well as hepatocytes malfunction (Louvet and Mathurim, 2015).

Both ALT and AST are related to the transaminase group of enzymes. The main sites for the production of ALT is generally are hepatocytes. It is also produced in minute quantity in extrahepatic tissue comprising kidney, heart and muscle mass. While, AST is generally produced in several body organs such as kidney, lungs, heart, muscle and brain (Louvet and Mathurim, 2015; Afalobi *et al*., 2018, 2020). Estimation of the activity of ALT, AST, ALP as well as total bilirubin are renowned markers for assessment of liver function (Adaramoye *et al*., 2008). Current study was intended to analyse the effect of welding fumes exposure on hepatic and renal profile of experienced welders in comparison with controls. In our study, ALT and AST of welders showed non-significant decrease as compared with control. However, ALP level of welders demonstrated highly significant increase as compared with control, which may indicate the presence of hepatocyte injury.

In an investigation, workers facing metal fumes showed significant increase in biomarkers of liver physiology (Orisakwe and Nduka, 2009) similar to the contaminated metallic fumes of welding exposure. Heme-oxygenase metabolic activity in our body yields a natural product known as bilirubin, which has been documented as an effective antioxidant, whether it is unconjugated or conjugated, free or albumin bound (Neuzil and Stocken, 1994; Wu *et al*. 1996).

In our study, bilirubin of welders depicted prominent increase as compared with controls. This increase of total bilirubin level may indicate the chances of haemolytic jaundice, cardiovascular diseases and malaria. Gul *et al*. (2013) presented that high bilirubin concentration in serum can independently results in acute myocardial infarction in patients having ST-segment elevation in hospital, who undergo primary percutaneous coronary interference.

Welding fumes containing diverse chemical constituent have been revealed to bring DNA destruction through ROS generation in animals as well as in humans (Antonini *et al*., 2005; Singh and Chadha, 2016). Another research revealed that inspiration of Mn present in welding fumes result in higher brain Mn concentration which causes oxidative stress and neurodegeneration in rats (Erikson *et al*., 2004; Sriram *et al*., 2010). Overall welding fumes can be regarded as oxidative contaminants and can bring detrimental effects on liver profile of welders through increased oxidative stress (Bagchi *et al*., 2002).

Welding fumes are composed of numerous fine and ultrafine metallic particles and a variety of gases. Inspiration of these particles and gases bring alternations in the respiratory, hepatic and renal physiology (Dumkova *et al*., 2016). As far as renal profile is concerned both urea and creatinine were assessed in present investigation. These metabolites play an important role in several mechanisms, such as, urea metabolism, oxidation and/or reduction pathways, carbohydrate and amino acid metabolism (Kuo *et al*., 2012). Renal function analysis demonstrated a significant decrease in creatinine and urea levels in welders as compared to healthy individuals, which could be an indication of a possibly disturbed renal functioning.

Several studies revealed significant urea concentration in serum of occupationally exposed subjects of different industrial units as compared with healthy controls (Wang *et al*., 2002). Patil *et al*. (2007) have reported marked elevation of blood urea concentration in workers of

battery manufacturing sector having an exposure of fumes containing toxicants. Main cause of reduced creatinine and urea level in our study could be attributed to lean body mass as welders depicted reduced body mass index (BMI) possibly due to occupational stress (Fohr *et al*., 2016). So, it is conceivable that reduced creatinine and urea may be associated with lower BMI. Previous investigations have documented renal tubular epithelial damage and proximal tubular defects that occurs via the exposure to ferrochromium particles, bringing variations in the renal functions of workers in the welding industry (Lee *et al*., 2016). Moreover, Casalegono *et al*. (2015) have documented association of nickel and chromium fumes exposure with renal defects and immunosuppression activities in experimentally exposed animals. Hence, this phenomenon advocates the possibility of renal defects in workers of welding industry.

A significant decline in creatinine level of workers was observed in an investigation where workers had an exposure to air borne toxicants during their job activities parallel to those who didn't face toxicants (Ehrlich, 1998). Welding fumes have also been demonstrated to increase lipid peroxidation in plasma, lungs and liver tissue in rats (Chuang *et al*., 2010). Because welding fumes produce oxidative stress, we suspect that ROS decreased creatine kinase and therefore caused a decrease in creatinine concentration (Chuang *et al*., 2010).

Serum electrolyte analysis revealed that Na^+ level of welders showed non-significant increase, while, K^+ and Cl^- levels of welders depicted a significant increase as compared with controls. The electrolyte disturbances should be monitored and treated appropriately to avoid ill effects e.g., prolonged diarrhoea or vomiting, increased secretion of mineralocorticosteroids and urinary obstruction etc (Wu *et al*., 2012).

An earlier investigation revealed non-significant differences in Na^+ and K^+ concentration of blood among designated industrial employees of Ewekoro, Abeokuta and Ogun states in Nigeria. The study further suggested it to be a consequence of an exposure to similar kind of metals as found in welding fumes (Babalola and Babejide, 2009).

Due to high Cd exposure, primarily through respiratory pathways, decline in the efficacy of the kidneys to eliminate acids from the blood due to dysfunction in proximal renal tubule have been reported (Vallero, 2011).

Previous investigations have described high blood Cd levels in battery recyclers, welders and spray painters due to Cd being a joint constituent of Cd-batteries, welding fumes and spray paint pigments.

Another report exhibit that exposure to welding fumes can lead to enhanced Cd concentration in urine and indicates renal tubular dysfunction (Ding *et al*., 2011). It is worth mentioning that higher levels of electrolytes in blood are symbol of gradual progression to kidney disorders (Pachal *et al*., 2000). Overall, welding fumes can be regarded as oxidative contaminants and can bring detrimental effects on kidney profile of welders through increased oxidative stress (Bagchi *et al*., 2002).

Based on above findings, we suggest that both hepatic and renal injury can be a manifestation of welding fume exposure.

In conclusion, the statistics which were obtained during our study entirely suggest hepatocyte injury and oxidative stress production in hepatocytes in response to welding fume exposure.

Specially, indication of highly increased ALP level reflected a hepatic injury, rickets or renal tubular dystrophies. Increased bilirubin concentration may be the sign of hemolytic jaundice, internal haemorrhage, acute hemolytic anemia, malaria and septicaemia.

Precautionary measure such as use of masks on face to prevent inspiration of poisonous fumes, glasses on eyes and gloves on hands are suggested to welders for maintenance of appropriate health. A well-managed ventilation system and methods to dispose off wastes must be necessary during welding operations to prevent health related complications.

More investigations are necessary to evaluate other health vulnerabilities occurring because of exposure to welding fumes and related toxic species on multiple organs, because this kind of exposure may bring huge hazards to the human organs particularly those which are related with manufacturing and removal of waste such as the liver and kidney.

DECLARATIONS

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IRB approval

Institutional ethical review committee of the Institute of Zoology, University of the Punjab, Lahore, endorsed the study plan.

Generative AI and AI-assisted technology statement

The authors have declared that no generative AI or AI-

assisted technologies were used to create this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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Department of Zoology, University of Okara hosted the 43rd Pakistan Congress of Zoology (International).

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PROGRAMME

43rd PAKISTAN CONGRESS OF ZOOLOGY

(INTERNATIONAL)

UNIVERSITY OF OKARA, OKARA
June 23-25, 2025

DAY ONE **MONDAY, JUNE 23, 2025**

08:15 AM	**REGISTRATION**
09:00 AM	**Inauguration: Recitation from the Holy Quran**
09:05 AM	**Welcome address by Chairperson, Department of Zoology, University of Okara, Okara**
09:10 AM	**Address by the Secretory General, Zoological Society of Pakistan**
09:15 AM	**Address by the President, Zoological Society of Pakistan**
09:25 AM	**Address by the Vice Chancellor, University of Okara, Okara.**
09:35 AM	**Distribution of Medals and Awards**
10:10 AM	**Address by the Chief Guest**
10:25 AM	**Vote of Thanks**
10:30 AM	**Refreshment**

11:30 AM, JOINT SESSION I: PLENARY LECTURES
CHAIRPERSON: Prof. Dr. Muhammad Ali Shah
CO-CHAIRPERSON: Prof. Dr. A.R. Shakoori

1. **Prof. Dr. Anwar Hussain Gilani**, *Distinguished National Professor and Consultant/Incharge (Quality Assurance/PR& I), Higher Education Commission, Sector H-9, Islamabad.*
   Health Challenges of 21st Century and Treating Life-Style Medicine

2. **Prof. Dr. Sadaf Naz,** *School of Biological Sciences, University of the Punjab, Quaid-i-Azam Campus, Lahore 54590, Lahore*
   Inherited schizophrenia in consanguineous Pakistani families.

01:30 PM LUNCH AND PRAYER BREAK (ZUHAR)

A4

02:00 PM - HALL-1	02:00 PM - HALL-2	02:00 PM - HALL-3
SECTION I **CBGB** **BIOTECHNOLOGY** **SESSION 1** **Chairperson: Prof. Dr. Shahid Nadeem** **Co-Chairperson: Dr. Soumble Zulfiqar**	**SECTION III** **ENTOMOLOGY** **SESSION 1** **Chairperson: Prof. Dr. Riffat Sultana** **Co-Chairperson: Dr. Sajjad Ali Larik**	**SECTION V** **FEWFM** **FISHERIES, FRESHWATER BIOLOGY** **SESSION 1** **Chairperson: Prof. Dr. Naeem Tariq Narejo** **Co-Chairperson: Dr. Karim Gabol**
1. H.M.A. Sarwar (IZ, PU) CBGP-1 2. M. Anas (UVAS, Lahore) CBGP-2 3. A. Tabassum (UE, Lahore) CBGP-3 4. A. Bibi (UE, Lahore) CBGP-4 5. T. Tayyab (UVAS, Lahore) CBGP-5 6. S. Andleeb (UAJK) CBGP-6 7. A. Aziz (UAJK) CBGP-7 8. N. Shafi (UAJK) CBGP-8 9. S. Mulazim (UoL, Sargodha) CBGP-9 10. F. Fareed (UoL, Sargodha) CBGP-10 11. M. Attaullah (CUVAS) CBGP-11 12. Z.Bibi (U Malakand) CBGP-12 13. A. Umera (Baharia Uni Karachi) CBGP-13 14. A. Rehman (MMG, PU) CBGP-14 15. R.H. Haider (GCU, Lahore) CBGP-15	1. Q. Zaman (U Sawabi, Swabi) ENT-1 2. A. Ayaz (GCU, Hyderabad) ENT-2 3. K. Muhammad (Uni Swabi) ENT-3 4. M.A. Jakhrani (US, Jamshoro) ENT-5 5. M.F. Munir (MNSUA, Multan) ENT-6 6. R. Memon (US, Jamshoro) ENT-7 7. R.K. Israni (SALU, Khairpur) ENT-8 8. S.R. Soomro (SALU, Khairpur) ENT-9 9. R. Shah (US, Jamshoro) ENT-10 10. S. Hyder (US, Jamshoro) ENT-11 11. S. Soomro (US, Jamshoro) ENT-12 12. S. P. Memon (US, Jamshoro) ENT-13 13. S.A. Talpur (US, Jamshoro) ENT-14 14. N. Bhanger (US, Jamshoro) ENT-15 15. M. Ali (MNSUA, Multan) ENT-16	1. N. NaheeD (MNSUA, Multan) FEWFM-1 2. H.F. Rubab (UoL, Sargodha) FEWFM-2 3. A. Tariq (GCU, Lahore) FEWFM-3 4. B. Tooba (JUW, Karachi) FEWFM-4 5. Z.A. Muhamed (UVAS, Lahore) FEWFM-5 6. S. Jalil (UVAS, Lahore) FEWFM-6 7. T. Akhtar (UAJK) FEWFM-7 8. Z. Ameer (UoO) FEWFM-8 9. A.Z. Zafar (VU, Lahore) FEWFM-9 10. M.P. Narejo (CEMB, Karachi) FEWFM-10 11. S. Abbas (UVAS, Lahore) FEWFM-11 12. M. Usama (UAF, Faisalabad) FEWFM-12 13. T. Zulfiqar (UoO) FEWFM-13 14. Y. Samad (WU, Multan) FEWFM-14 15. N.N. Kanwal (UoL, Lahore) FEWFM-15 16. F. Noor (PU, Lahore) FEWFM-15a
04:30 PM TEA BREAK AND PRAYER BREAK (ASAR)		
05:00 PM - HALL-1	**05:00 PM - HALL-2**	**05:00 PM - HALL-3**
SECTION I **CBGP** **CELL BIOLOGY, MOLECULAR BIOLOGY** **SESSION 2** **Chairperson: Prof. Dr. Farah Rauf Shakoori** **Co-Chairperson: Dr. Asia Bibi**	**SECTION III** **ENTOMOLOGY** **SESSION 2** **Chairperson: Prof. Dr. Imran Khatri** **Co-Chairperson: Dr. Munawar Saleem Ahmad**	**SECTION V** **FEWFM** **MARINE BIOLOGY** **SESSION 2** **Chairperson: Prof. Dr. Noor us Saher** **Co-Chairperson: Dr. Roheela Yasmeen**
!1. F. Ali (IZ, PU) CBGP-16 2. J.A. Ujan (SALU, Khairpur) CBGP-17 3. M. Ruk (SALU, Khairpur) CBGP-18 4. S.N. Shabani (SALU, Khairpur) CBGP-19 5. N. Jamsheed (SALU, Khairpur) CBGP-20 6. M. Waqas (UE, Lahore) CBGP-21 7. A. Gull (UoL, Sargodha) CBGP-22 8. M. Hussain (UoL, Sargodha) CBGP-23 9. R. Aslam (UoL, Sargodha) CBGP-24 10. H. Masud (UO, Okara) CBGP-25 11. E. Sajjad (LCWU, Lahore) CBGP-26 12. M. Younas (CUVAS) CBGP-27 13. A. Rehman (CUVAS) CBGP-28	1. M. Jamil (US, Jamshoro) ENT-17 2. M.I. Bozdar (US, Jamshoro) ENT-18 3. S. Laraib (US, Jamshoro) ENT-19 4. F. Lashari (US, Jamshoro) ENT-20 5. F.A. Soomro (SALU, Khairpur) ENT-21 6. I.F. Buriro (US, Jamshoro) ENT-22 7. M.S. Dayo (US, Jamshoro) ENT-23 8. M. Ishaq (MNSUA, Multan) ENT-24 9. Y.A. Salam (US, Jamshoro) ENT-25 10. Z. Akbar (US, Jamshoro) ENT-26 11. V. Kumari (US, Jamshoro) ENT-27 12. L. Kainat (CUVAS, Bahawalpur) ENT-28 13. B. Mahar (US, Jamshoro) ENT-29 14. A.N. Soomro (US, Jamshoro) ENT-30 15. A. Chandio (US, Jamshoro) ENT-31	1. K. Gabol (UK, Karachi) FEWFM-16 2. M. Yousaf (CEMB, Karachi) FEWFM-17 3. R. Ali (CEMB, Karachi) FEWFM-18 4. M. Rasheed (CEMB, Karachi) FEWFM-19 5. S.B.A. Raza (CEMB, Karachi) FEWFM-20 6. Habibullah (LUAWMS, Uthal) FEWFM-21 7. S. Sakhawat (CEMB, Karachi) FEWFM-22 8. N. Ahmed (CEMB, Karachi) FEWFM-23 9. N. Shoaib (CEMB, Karachi) FEWFM-24 10. N. Saher (CEMB, Karachi) FEWFM-25 11. F. Naz (UK, Karachi) FEWFM-26 12. S.H. Noor (CEMB, Karachi) FEWFM-27 13. A. Mukhtiar (CEMB, Karachi FEWFM-28 14. W. Matanat (CEMB, Karachi) FEWFM-29 15. H. Atta (CEMB, Karachi) FEWFM-30 16. A. Hameed (CEMB, Karachi) FEWFM-31 17. N. Ashfaq (CEMB, Karachi) FEWFM-32 18. L. Tariq (CEMB), Karachi) FEWFM-33
6:25 PM PRAYER BREAK (MAGHRB)		

06:40 PM - HALL-1	06:40 PM - HALL-2	06:40 PM - HALL-3
SECTION I **CBGP** **CELL BIOLOGY, MOLECULAR BIOLOGY** **SESSION 3** **Chairperson: Prof. Dr. Abdul Rehman** **Co-Chairperson: Dr. Saba Irshad**	**SECTION III** **ENTOMOLOGY** **SESSION 3** **Chairperson: Dr. Asifa Hameed** **Co-Chairperson: Dr. M. Rafique Pitafi**	**SECTION V** **FEWFM** **PALAEONTOLOGY, WILDLIFE** **SESSION 3** **Chairperson: Dr. Abdul Aleem Chaudhary** **Co-Chairperson: Dr. Sangam Khalil**
1. A. Tariq (CUVAS) CBGP-29 2. U. Nadeem (CUVAS) CBGP-30 3. M. Munir (UVAS, Lahore) CBGP-31 4. W. Ahmad (AAU, R'pindi) CBGP-32 5. R. Kausar(UB, Quetta) CBGP-33 6. A.A. Abbasi (MUST, Mirpur) CBGP-34 7. Q. Amjad (SBS,PU) CBGP-35 8. H.A. Ahmad (SBKWU, Quetta) CBGP-36 9. Z. Jafar (UJ, Jhang) CBGP-37 10. T. Naheed (UJ, Jhang) CBGP-38 11. M.S. Ahmad (US, Swabi) CBGP-39 12. I. Yaseen (UAF, Faisalabad) CBGP-40 13. Q. Sattar (UAF, Faisalabad) CBGP-41	1. Afsa (US, Jamshoro) ENT-32 2. S. Sanam (US, Jamshoro) ENT-33 3. S. Sanam (US, Jamshoro) ENT-34 4. S. Mushtaque (US, Jamshoro) ENT-35 5. S. Naz (US, Jamshoro) ENT-36 6. A. Khan (US, Jamshoro) ENT-37 7. A. Jahangir (U Haripur, Haripur) ENT-38 8. E. Razia (CUVAS, Bahawalpur) ENT-39 9. R. Arshad (CUVAS, Bahawalpur) ENT-41 10. M.Z. Abedin (CUVAS, Bahawalpur) ENT-42 11. S. Hameed (CUVAS, Bahawalpur) ENT-43 12. S. Zubair (CUVAS, Bahawalpur) ENT-44 13. B. Khan (CUVAS, Bahawalpur) ENT-45 14. N. Sultan (CUVAS, Bahawalpur) ENT-46 15. M. Irfan (CUVAS, Bahawalpur) ENT-47	1. M. Akhtar (IZ, PU) FEWFM-34 2. K. Aftab (U Gujrat, Gujrat) FEWFM-35 3. S. Kanwal (PMASUAA, R'pindi) FEWFM-36 4. S. Siyal (SBBUVAS, Sakrand) FEWFM-37 5. K. Memon (SALU, Khairpur) FEWFM-38 6. N. Zahid (SALU, Khairpur) FEWFM-39 7. H. Imran (PMASUAA, R'pindi) FEWFM-40 8. R. Tahir (Zoology, UK) FEWFM-41 9. Zarina (US, Jamshoro) FEWFM-42 10. R. Zafar (UoL, Sargodha) FEWFM-43 11. M. Masood (UVAS, Lahore) FEWFM-44 12. S.A. Naqvi (UAJK) FEWFM-45 13. M. Waqas (U, Haripur) FEWFM-46
7:00 PM EXECUTIVE COUNCIL MEETING		
8:30 PM DINNER		

DAY TWO TUESDAY, JUNE 24, 2025

09:00 AM, JOINT SESSION II: PLENARY LECTURES
CHAIRPERSON: Prof. Dr. Muhammad Afzal
CO-CHAIRPERSON: Prof. Dr. Muhammad Wajid

1. **Dr. Rashad Hussain,** *Department of Neurology, Centre for Translational Neuromedicine, University of Rochester, 601 Elmwood Ave, Box 645, Rochester NY 14642, USA*
   Glymphatic dysfunction in traumatic brain injury: Implications for therapeutic strategies.

2. **Prof. Dr. Riffat Sultana,** *Department of Zoology, University of Sindh, Jamshoro, Sindh, Pakistan.*
   Global Orthoptera trends: Overcoming challenges and shaping the future.

3. **Prof. Dr. Muhammad Munir,** *Division of Biomedical and Life Sciences, Faculty of Health & Medicine, Lancaster University, Lancaster, U.K.*
   Zoonotic influenza viruses.

SHORT TALKS

1. **Prof. Dr. Imran Khatri,** *Department of Entomology, Sindh Agriculture University, Tandojam.*
   Insect identification: From classical taxonomy to AI-driven innovations

2. **Mr. M. Hassan Ziar,** *Tehsil Karezat, Khanozai, Balochistan.*
   The origin of species by means of formulas.

12:00 NOON - HALL-1

SECTION 1
CELL BIOLOGY, MOLECULAR BIOLOGY
SESSION 5
Chairperson: Dr. Muhammad Khan
Co-Chairperson: Dr. Durre-Samin Tahir

1.	M.A. Khan (PU, Lahore)	CBGP-55
2.	T. Noreen (UE, Lahore)	CBGP-56
3.	B. Riaz (UE, Lahore)	CBGP-57
4.	A. Jamshed (VU, Lahore)	CBGP-58
5.	I. Shehzadi (VU, Lahore)	CBGP-59
6.	Q. Fatima (VU, Lahore)	CBGP-60
7.	H. Saeed (SBS, PU)	CBGP-61
8.	N. Farooq (SBS, PU)	CBGP-62
9.	I. Zahra (PU, Lahore)	CBGP-63
10.	F.A. Hussain (US, Jamshoro)	CBGP-64
11.	S. Khalid (UAF, Faisalabad)	CBGP-65

12:00 NOON - HALL-2

SECTION III
ENTOMOLOGY
SESSION 5
Chairperson: Dr. Shafqat Saeed
Co-Chairperson: Dr. Zubair Ahmad

1.	S.A. Larik (SALU, Khairpur)	ENT-62
2.	S. Suhriani (SALU, Khairpur)	ENT-63
3.	Kaleemullah (US, Jamshoro)	ENT-64
4.	K.Q. Shah (US, Jamshoro)	ENT-65
5.	M.A. Akhtar (Zoology, UK)	ENT-66
6.	Z. Ahmed (FUUAST, Karachi)	ENT-67
7.	B. Mal (US, Jamshoro)	ENT-68
8.	A.N. Memon (GUBDC, Dadu)	ENT-69
9.	M.R. Pitafi (GDC, Hyderabad)	ENT-70
10.	M.M. Khan (US, Jamshoro)	ENT-71
11.	S. Muntha (US, Jamshoro)	ENT-72
12.	S. Khaskheli (US, Jamshoro)	ENT-73
13.	A.A. Babar (CUVAS, Bahawalpur)	ENT-74
14.	R.M. Mazhar Ali (UAF, Faisalabad)	ENT-75

12:00 NOON - HALL-3

SECTION I
CBGP
MICROBIOLOGY
SESSION 6
Chairperson: Prof. Dr. Javed Iqbal Qazi
Co-Chairperson: Dr. Iram Liaqat

1.	A. Iqbal (JUW, Karachi)	CBGP-67
2.	K. Malik (GGCW, Lahore)	CBGP-68
3.	N. Fayyaz (PU, Lahore)	CBGP-69
4.	S. Mahmood (PU, Lahore)	CBGP-70
5.	M. Jamil (UVAS, Lahore)	CBGP-71
6.	M. Jameel (UVAS, Lahore)	CBGP-72
7.	S. Solangi (SALU, Khairpur)	CBGP-73
8.	F. Ashraf (U Poonch, Rawalakot)	CBGP-74
9.	S. Younas (GCU, Lahore)	CBGP-75
10.	Husna (UVAS, Pattoki)	CBGP-76
11.	N. Ali (UVAS, Pattoki)	CBGP-77
12.	S. Ali (UOL, Sargodha Campus)	CBGP-78
13.	R. Shahid (UOL, Sargodha Campus)	CBGP-79
14.	A.M. Qureshi (CUVAS, Bahawalpur)	CBGP-80

01:30 PM LUNCH BREAK (ZUHAR)

10:30 AM - HALL-1

SECTION I
CBGP
CELL BIOLOGY, MOLECULAR BIOLOGY
SESSION 4
Chairperson: Prof. Dr. Rubina Mushtaq
Co-Chairperson: Dr. Javed Ahmad Ujjan

1.	U. Younis (UAF, Faisalabad)	CBGP-42
2.	I. Bibi (UAF, Faisalabad)	CBGP-43
3.	Q. Haneef (UAF, Faisalabad)	CBGP-44
4.	R. Yasmeen (LGU, Lahore)	CBGP-45
5.	S. Anjum (UM, Chakdara)	CBGP-46
6.	S.Z. Shah (AJKU)	CBGP-47
7.	A. Saeed (QAU, Islamabad)	CBGP-48
8.	A. Nisar (QAU, Islamabad)	CBGP-49
9.	A. Manzoor (QAU, Islamabad)	CBGP-50
10.	A. Fatima (WU, Multan)	CBGP-51
11.	S. Fatima (WU, Multan)	CBGP-52
12.	N. Asghar (WU, Multan)	CBGP-53
13.	H. Balqees (WU, Multan)	CBGP-54
12.	N. Asghar (WU, Multan)	CBGP-53
13.	H. Balqees (WU, Multan)	CBGP-54

10:30 AM - HALL-2

SECTION III
ENTOMOLOGY
SESSION 4
Chairperson: Dr. Zain ul Abdin
Co-Chairperson: Dr. Rashid Azad

1.	S. Iqbal (CUVAS, Bahawalpur)	ENT-48
2.	S. Kanwal (CUVAS, Bahawalpur)	ENT-49
3.	I. Ajmal (CUVAS, Bahawalpur)	ENT-50
4.	R. Khalil (CUVAS, Bahawalpur)	ENT-51
5.	M. Zeeshan (CUVAS, Bahawalpur)	ENT-52
6.	A.B. Mirbahar (CUVAS, Bahawalpur)	ENT-53
7.	A.A. Sarki (US, Jamshoro)	ENT-54
8.	A.A. Khalique (US, Jamshoro)	ENT-55
9.	Asama (US, Jamshoro)	ENT-56
10.	A. Udhejo (US, Jamshoro)	ENT-57
11.	N. Baloch (US, Jamshoro)	ENT-58
12.	H. Saund (US, Jamshoro)	ENT-59
13.	W.A. Panhwar (US, Jamshoro)	ENT-60
14.	M.A. Mahar (SALU, Khairpur)	ENT-61

10:30 AM - HALL-3

SECTION V
FEWFM
WILDLIFE
SESSION 4
Chairperson: Prof. Dr. M. Siddique Awan
Co-Chairperson: Dr. Muhammad Rais

1.	K. Sajjad (IUB, Bahawalpur)	FEWFM-47
2.	F. Hidayat (IUB, Bahawalpur)	FEWFM-48
3.	M. Saeed (Ministry of Climate Change)	FEWFM-49
4.	S. Ali (IWMB, Islamabad)	FEWFM-50
5.	Y. Ashfaq (IZ, PU)	FEWFM-51
6.	A. Zaman (MUST, Mirpur)	FEWFM-52
7.	W. Naz (Hazara U, Mansehra)	FEWFM-53
8.	E. Zahra (MUST, Mirpur)	FEWFM-54
9.	K. Mazhar (MUST, Mirpur)	FEWFM-55
10.	M. Rais (PMASUAA, R'pindi)	FEWFM-56
11.	Z. Memon (SALU, Kharpur)	FEWFM-57
12.	G. Ali (UAJK)	FEWFM-58

11:30 AM - TEA BREAK

02:00 PM - HALL-1

SECTION 1
CBGP
MICROBIOLOGY
SESSION 7
Chairperson: Dr. Shagufta Naz
Co-Chairperson: Dr. Faisal Siddiqui

1. A. Sagheer (CUVAS, Bahawalpur) CBGP-81
2. A. Fatima (CUVAS, Bahawalpur) CBGP-82
3. D. Hayat (CUVAS, Bahawalpur) CBGP-83
4. L. Tariq (CUVAS, Bahawalpur) CBGP-84
5. I. Naeem (CUVAS, Bahawalpur) CBGP-85
6. M. Nazir (CUVAS, Bahawalpur) CBGP-86
7. M. Malik (CUVAS, Bahawalpur) CBGP-87
8. M. Adnan (CUVAS, Bahawalpur) CBGP-88
9. M. Bhatti (CUVAS, Bahawalpur) CBGP-89
10. M. Rasheed (CUVAS, Bahawalpur) CBGP-90
11. S. Habib (CUVAS, Bahawalpur) CBGP-91
12. U.R. Zahra (CUVAS, Bahawalpur) CBGP-92
13. U. Gill, (CUVAS, Bahawalpur) CBGP-93

02:00 PM - HALL-2

SECTION II
PESTS AND PEST CONTROL
SESSION 1
Chairperson: Prof. Dr. Abida Butt
Co-Chairperson: Dr. Santosh Kumar

1. K. Saeed (U Swabi, Swabi) PC-1
2. I. Khoso (GCU, Hyderabad) PC-2
3. R. Khoso (GCU, Hyderabad) PC-3
4. J. Das (US, Jamshoro) PC-4
5. F. Abbasi (US, Jamshoro) PC-5
6. M. Muqeem (US, Jamshoro) PC-6
7. D. Khentio (US, Jamshoro) PC-7
8. B.A. Bhgio (US, Jamshoro) PC-8
9. H. Anjum (PU, Lahore) PC-9
10. B.K. Solangi (SAU, Tandojam) PC-10
11. A.B. Siddiq (CUVAS, Bahawalpur) PC-11
12. S. Kumar (CUVAS, Bahawalpur) PC-12

02:00 PM - HALL-3

SECTION I
CBGP
MICROBIOLOGY
SESSION 10
Chairperson: Prof. Dr. Nuzhat Shafi
Co-Chairperson: Dr. Bushra Allah Rakha

1. S.Y. Khan (GCU, Lahore) CBGP-120
2. K. Javed (UAA, Rawalpindi) CBGP-121
3. A. Riaz (UAA, Rawalpindi) CBGP-122
4. S. Nawaz (UAA, Rawalpindi) CBGP-123
5. A. Khan (GCU, Lahore) CBGP-124
6. M. Hamza (CUVAS, Bahawalpur) CBGP-125
7. T. Manzoor (GCU, Lahore) CBGP-126
8. T. Khalid (UO, Okara) CBGP-127
9. Noshaba (US, Jamshoro) CBGP-128
10. A. Sheraz (UAA, Rawalpindi) CBGP-129
11. S.A. Ujjan (SALU, Khairpur) CBGP-130
12. N. Asif (UAF, Faisalabad) CBGP-131
13. S. Rasheed (QAU, Islamabad) CBGP-132
14. Sumera (QAU, Islamabad) CBGP-133

04:30 PM TEA BREAK AND PRAYER BREAK (ASAR)

05:00 PM - HALL-1

SECTION I
CBGP
CELL BIOLOGY, MOLECULAR BIOLOGY
SESSION 8
Chairperson: Dr. Bushra Muneer
Co-Chairperson: Dr. Saiqa Andleeb

1. Z. Habib (CUVAS, Bahawalpur) CBGP-94
2. Z. Malik (CUVAS, Bahawalpur) CBGP-95
3. H. Jabeen (CUVAS, Bahawalpur) CBGP-96
4. A. Sarwar (CEMB, Karachi) CBGP-97
5. A. Tariq (Multan) CBGP-98
6. C.N.F. Zaheen (UAF, Faisalabad) CBGP-99
7. S.A. Shaikh (PCSIR, Karachi) CBGP-100
8. M. Bibi (UAJK) CBGP-101
9. S.T. Lashari (SALU, Khairpur) CBGP-102
10. M.W. Shafiat, (U Poonch, Rawalakot) CBGP-103
11. Z. Rafique (U Poonch, Rawalakot) CBGP-104
12. S. Akhtar (U Poonch, Rawalakot) CBGP-105
13. A. Ullah, (PU, Lahore) CBGP-106

05:00 PM - HALL-2

SECTION II
PESTS AND PEST CONTROL
SESSION 2
Chairperson: Dr. Bhai Khan Solongi
Co-Chairperson: Dr. Barkat Ali Bughio

1. M. Waseem (CUVAS, Bahawalpur) PC-13
2. K. Junaid (UA, Peshawar) PC-14
3. A. Ameer (GCU, Hyderabad) PC-15
4. S. Latif (U Haripur, Haripur) PC-16
5. M. Hanif (MNSUA, Multan) PC-17
6. Z. Abro (US, Jamshoro) PC-18
7. H.A.A. Khan (PU, Lahore) PC-19
8. T. Khan (PU, Lahore) PC-20
9. A. Abid (U Poonch, Rawlakot) PC-21
10. A. Munir (U Poonch, Rawlakot) PC-22
11. U. Sehar (NARC, Islamabad) PC-23
12. M.S. Abbas (MNSUA, Multan) PC-24

05:00 PM - HALL-3

SECTION I
CBGP
TOXICOLOGY
SESSION 11
Chairperson: Prof. Dr. Abdul Qadir
Co-Chairperson: Dr. Bushra Nisar Khan

1. A. Bibi (VU, Lahore) CBGP-134
2. M.A. Arain (US, Jamshoro) CBGP-135
3. M. Waris (UVAS, Lahore) CBGP-136
4. C. Raza (GCU, Lahore) CBGP-137
5. U.A. Khan (Lahore) CBGP-138
6. M. Batool (GCWU, Sialkot) CBGP-139
7. Q.ul Ain (GCWU, Sialkot) CBGP-140
8. S. Mumtaz (U Poonch, rawalakot) CBGP-141
9. A. Tanveer (UVAS, Lahore) CBGP-142
10. S. Ghayyur (Hazara U, Mansehra) CBGP-143
11. G. Muhayyaodin (UVAS, Pattoki) CBGP-144
12. M. Ahmad (UVAS, Lahore) CBGP-145
13. S. Abbas (U Jhang, jhang) CBGP-146
14. A. Hussain (UAF, Faisalabad) CBGP-147
15. M. Khalil (UAF, Faisalabad) CBGP-148
16. A. Tahir (UAF, Faisalabad) CBGP-149
17. M. Jameel (UAF, Faisalabad) CBGP-150
18. T. Fatima, (UAF, Faisalabad) CBGP-151

08:00 PM GENERAL BODY MEETING

08:30 PM DINNER/ CULTURE NIGHT

DAY THREE **WEDNESDAY, JUNE 25, 2025**

09:00 AM, JOINT SESSION III: PLENARY LECTURES
CHAIRPERSON: Prof. Dr. Amaël Borzée
CO-CHAIRPERSON: Mr. Abdul Aziz Khan

1. **Prof. Dr. AmaëlBorzée,** *Laboratory of Animal Behaviour and Conservation, College of Life Sciences, Nanjing Forestry University, Nanjing, People's Republic of China.*
   Variations in amphibian population sizes in Northeast Asia over the last millennia and in the proximate future
2. **Prof. Dr. Hyung-Goo Kim,** *Department of Neurosurgery, Robert Wood Johnson Medical School, Rutgers, the State University of New Jersey, USA.*
   Identification of disease genes in neurodevelopmental disorders.
3. **Prof. Dr. ShifaShaffique,** *College of Agriculture & Life Science, School of Applied Biosciences, Kyungpook National University, 80 Daehak-Ro, Buk-Gu, Daegu, 41566, South Korea*
   Cross Kingdom Communication

SHORT TALKS

1. **Dr. Abdul Rauf Janjua**, *Department of Zoology, University of Azad Jammu and Kashmir, Muzaffarabad*
   Alignment of Research With SDGS can help the academia overcome its upcoming challenges in Pakistan

2. **Dr. Ayesha Imtiaz,** *School of Biological Sciences, University of the Punjab, Lahore*
   Intrinsically disordered region of cdc14a is essential for hearing in human and mouse.

11:00 AM - HALL-1		11:00 AM - HALL-2		11:00 AM - HALL-3	
SECTION I CBGP CELL BIOLOGY, MOLECULAR BIOLOGY, SESSION 9 Chairperson: Dr. Irfan Zia Qureshi Co-Chairperson: Dr. Dil Ara Abbas Bukhari		SECTION IV PARASITOLOGY SESSION 1 Chairperson: Dr. Wali Khan Co-Chairperson: Dr. Sidra Abbas		SECTION I CBGP TOXICOLOGY SESSION 12 Chairperson: Prof. Dr. Mian Saeed Khan Co-Chairperson: Prof. Dr. M. Mansha	
1. I. Imtiaz (U Poonch, Rawalakot)	CBGP-107	1. M. S. Khan (U. Sawabi)	PAR-1	1. R. Azmat (UAF, Faisalabad)	CBGP-152
2. H. Islam (UVAS, Pattoki)	CBGP-108	2. W. Khan (U Malakand, Chakdara)	PAR-2	2. A. Zafar (UAF, Faisalabad)	CBGP-153
3. M. Nasrullah (IZ, PU)	CBGP-109	3. I. Khalid (U Jhang, Jhang)	PAR-3	3. G. Mustaja (UAF, Faisalabad)	CBGP-154
4. M. Junaid (IZ, PU)	CBGP-110	4. H. Lashari (U Sind, Jamshoro)	PAR-7	4. N. Ghafoor (UAF, Faisalabad)	CBGP-155
5. A. Mukhtar (IZ, PU)	CBGP-111	5. H. Moin (JUW, Karachi)	PAR-9	5. M.U. Ijaz (UAF, Faisalabad)	CBGP-156
6. S. Shabir (MUST, Mirpur)	CBGP-112	6. S. K. Pahooja (U Sind, Jamshoro)	PAR-10	6. M.Z. Salar (UAF, Faisalabad)	CBGP-157
7. A.W. Qureshi (GCWU, Sialkot)	CBGP-113	7. F. Shaikh (US, Jamshoro)	PAR-11	7. F. Shoukat (UAF, Faisalabad)	CBGP-158
8. M. Fatima (UVAS, Pattoki)	CBGP-114	8. S.S.B. Bukhari (UAJK)	PAR-13	8. A. Nawaz (U Jhang, Jhang)	CBGP-159
9. S. Arif (Rawalakot)	CBGP-115	9. H. Zulqarnain (U Gujrat)	PAR-14	9. M. Bushra (QAU, Islamabad)	CBGP-160
10. M.M. Raza (QAU, Islamabad)	CBGP-116			10. S. Eman (QAU, Islamabad)	CBGP-161
11. M.T. Chisti (U Kotli, AJK)	CBGP-117			11. R. Snaullah (QAU, Islamabad)	CBGP-162
12. H.A. Azam (GCU, Lahore)	CBGP-118			12. A. Khan (U Swabi, swabi)	CBGP-163
13. N. Bano, (MNSUA, Multan)	CBGP-119			13. M.K.A. Khan (UoO)	CBGP-164
				14. A. Raza (GCU, Lahore)	CBGP-165

11:45 AM - TEA BREAK

02:00 PM LUNCH BREAK (ZUHAR)

03:00 PM CONCLUDING CEREMONY

03:00 PM	Recitation
03:05 PM	Congress Report by President, ZSP
03:15 PM	Award Ceremony
03:45 PM	Concluding Remarks by the Chief Guest
03:55 PM	Vote of Thanks

04:00 PM REFRESHMENT

MEMBERS OF THE CONGRESS

BAHAWALPUR
Ahmad, J.
Ajmal, I.
Baboo, I. (Dr.)
Hidayat, F.
Kanwal, S.
Khalil, R.
Khalil, S. (Dr.)
Khan, S.
Kumar, S. (Dr.)
Ramzan, R.
Sajjad . K.
Sidique, A.B
Waseem, M.
Younus, M.
Zeshan, M.

CHINIOT
Ahmad, I.
Ahmad, W.
Ahmad. M. (Dr.)
Ashraf, A.
Beenish, A.W.
Gafoor, S.
Khokhar, S.A.
Manan, R.
Mashal
Mubashir, M.
Mujeeb, N.
Musawir, A.
Nadeem, A.
Nayab, T.
Nudrat, A.
Sadaf, N.
Shakoor, A.
Tahir, D.S. (Dr.)
Tahir, S.S.

DERA GHAZI KHAN
Ghani, G.M.A.

FAISALABAD
Abdin, Z. (Prof. Dr.)
Bashir, M.A.
Fatima, Q.
Hussain, A.
Ijaz, M.U. (Dr.)
Khan, S.M.D.
Nadeem, S. (Prof. Dr.)
Salar, M.Z.
Zaheer, C.N.F.

GUJRAT
Aftab, K. (Dr.)

HARIPUR, KPK
Azad, R. (Dr.)

ISLAMABAD
Abid, R.
Ali, S.
Habiba, U. (Dr.)
Khan, A.A. (Dr.)
Saeed, M. (Dr.)

JAMSHORO
Abbasi, F.
Afsa
Akber, Z.
Al-Hussain, F.
Bhanger, N.
Bozdar, M.I.
Bughio, B.A. (Dr.)
Chandio, A.
Das, J.R.
Dayo, M.S.
Fatima, S.M.
Hyder, S.
Jaffery, S.L.
Jakhrani, M.A.
Jakhrani, M.M.
Jamil, M.
Khani, A.K.K.
Khani, Y.K.
Khemtio, D.
Kumari, V.
Lashari, F.
Mahar, B.
Memon, A.A.
Memon, S.P.
Panhwar, W.A. (Dr.)
Shah, K.Q.
Shah, R.
Soomro, A.N.
Soomro, S.
Sultana, R. (Prof. Dr.)
Talpur, S.A.
Zarina

JHANG
Abbas, S. (Dr.)
Kanwal, N.N.
Khalil, I.
Naheed, T.

KABAL, SWAT
Wahab, A. (Dr.)

KARACHI
Qureshi, A.
Umer, A.
Nisa, N. (Dr.)
Shafique, S. (Dr.)
Ali, R.
Sarwar, A.
Sakhawat, S.
Bano, S.
Jabeen, H.
Yousuf, M.
Iqbal, A.
Gabol, K. (Dr.)
Khan, A. (Dr.)
Ahmed, Z. (Dr.)
Rasheed, M. (Dr.)

KHAIRPUR, SINDH
Akhtar, M. (Dr.)
Ali, F.
Ali, M.
Amjad, Q. (Dr.)
Anjum, H.
Asghar, T.
Ashfaq, Y.
Bibi, Z.
Butt, A. (Dr.)

Haider, R.H.
Iqbal, K.
Junaid, M.
Khan, B.N. (Dr.)
Khan, M.A.

LAHORE
Afzal, M. (Prof. Dr.)
Bukhari, D.A.A. (Dr.)
Butt, A. (Prof. Dr.)
Chaudhary, A.A. (Dr.)
Khan, B.N. (Dr.)
Khan, M. (Dr.)
Larik, S.A. (Dr.)
Mahar, U.A.
Malik, K.
Manzoor, T.
Maryam, A, (Dr.)
Mukhtar, A.
Muneer, B. (Dr.)
Naseer, A.
Nasrullah, M.
Nawaz, A.
Noreen, T.
Qadir, A. (Dr.)
Qazi, J.I. (Prof. Dr.)
Rehman, A. (Dr.)
Rehman, A. (Prof. Dr.)
Riaz, B.
Ruk, M. (Dr.)
Sajjad, E.
Samejo, B.A.
Saqib, Z.A. (Dr.)
Shakoori, A.R.(Prof. Dr.)
Shakoori, F.R. (Prof. Dr.)
Tasadduq, R. (Dr.)
Ujan, J.A. (Dr.)
Ullah, A.
Zafar, A.
Zahra, I. (Dr.)
Zulfiqar, S. (Dr.)

MALAKAND, KPK
Khan, W. (Dr.)
Rahim, A. (Dr.)

MUZAFFARABAD
Awan, M.S. (Prof. Dr.)

OKARA
Abbas, A.
Abbas, M.
Abid, M.
Ahmad, K.
Ahmad, M.
Ahsan, M.M.
Akber, I.
Akram, S.
Ali, A.
Ali, M.
Ali, U.
Ameer, Z.
Amir, H.
Ashraf, M.Z.
Asif, A.
Ayman, Z.
Aza, M.
Batool, A.
Batool, A.
Batool, H.A.
Batool, R.
Bibi, F.
Bibi, T.
Farah, K.
Fatima, S.
Haer, A.
Haer, V.
Hameed, A.
Hameed, M.N.
Hussain, M.
Ijaz, M.
Imran, A.A.
Imran, M.
Iqbal, S.
Irshad, M.
Jabeen, W.
Javed, A.
Khadim, M.
Khan, I.
Khan, M.
Khan, S.
Khan, T.
Laiba, F.
Mariam, R.
Masood, A.
Masood, B.
Mehar, A.
Mehmood, M.
Mirza, M.F.
Munir, H.
Munir, M.U.
Mustafa, S.
Nasir, F.
Nawaz, F.S.
Nawaz, I.
Naz, N.
Nazir, H.Y.
Nazir, N.
Nida
Noreen, A.
Qamar, A.
Rana, L.
Rashid, I.
Rauf, A.
Rehman, T.
Riaz, M.
Sabir, N.
Sadaf, Z.
Saif, A.
Sajid, W.
Samavia
Sana
Sarwar, M.S. (Dr.)
Sattar, I.
Shaban, I.
Shafique, A.
Shahid, R.
Shahid, T.
Shahzadi, I.
Shakeel, M.
Hassan, S.A.
Shoukat, S.
Tahir, A.
Tahreem, R.
Tariq, L.
Wusqa, U.
Waheed, A.
Wajid, M. (Prof. Dr.)
Wasey, A.S.
Yamin, M.
Yaseen, L.
Yaseen, R.
Yousaf, T.
Zafar, U.
Zainab

Zain-ul-Abadin
Zain-ul-Abideen, M.Q.
Zubair, S.K.
Zubair, W.
Zulfiqar, T.

PATTOKI
Abbas, S. (Dr.)
Akhtar, N.
Ali, N.
Ali, S. (Dr.)
Aslam, H.
Baigh, B.
Fatima, M.
Hussain, M.
Masood, M.
Muhayyaodin, G.
Munir, M.
Riaz, R.

PESHAWAR
Khan, A.A.

QUETTA
Makai, G. (Dr.)

RAWALAKOT
Muhammad, A. (Dr.)

RAWALPINDI
Abbas, N.
Abbasi, M.
Ahmed, W.
Aziz, I.
Ejaz, M.
Fayyaz, K.
Hussain, S.A.
Javed, K.
Javed, M.
Junaid, A.
Kashaf, M.
Naseem, N.
Nawaz, S.
Qamar, H.
Riaz, M.M.

SAHIWAL
Zubiar, S.K.

SKARDU
Narejo, N.T. (Prof. Dr.)

SHAHEED BENAZIRABAD, SINDH
Rind, K.H. (Dr.)

SHAHKOT, NANKANA SAHIB
Ijaz, M.

SUKKUR, SINDH
Issrani, R.K.
Mahar, M.A. (Dr.)
Suhriani, S.N. (Dr.)

SWABI, KPK
Abdullah
Ali, Z.
Bibi, H.
Bibi, K.
Bibi, S.
Ghani, A.
Khan, M.S. (Dr.)
Khan, M.S. (Dr.)
Rahim, K.
Raziq, F.
Rehman, A.
Saeed, A.
Usman, A.
Usman, M.

SWAT
Riaz, M. (Dr.)

TANDOJAM
Khatri, I. (Dr.)
Solangi, B.K. (Dr.)

UTHAL, BALOCHISTAN
Bano, A. (Dr.)

Citations of Awardees

CITATIONS

Prof. Muzaffar Ahmad
Life Time Achievement Award
2025

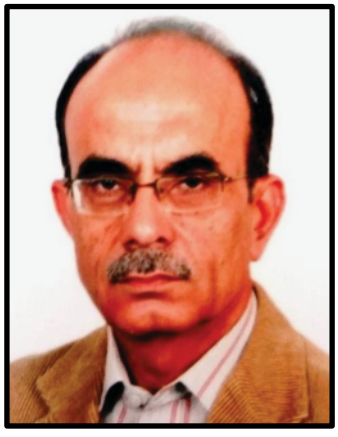
Mr. Ashiq Ahmad Khan

Ex-Conservation Director, Forest Institute, Peshawar
Ex-Director, WWF-Pakistan

Mr. Ashiq Ahmad Khan was born in Turangzai, a village in Charsadda (KP) in 1947. He has gotten three M.Sc. degrees in Zoology, Forestry and Natural Resource Management. He is therefore an Entomologist, Wildlife Ecologist and as a Resource Manager. During his earlier professional career Mr. Khan worked in the Entomology Branch of PakistanForest Institute dealing with insect and vertebrate pests related to the forests of Pakistan. During this period he visited almost all wetlands of Pakistan, collecting data about the waterfowl species, both resident and migratory, cranes and crocodiles, termite species and Musk deer.In 1992, he was inducted in WWF-Pakistan as Conservation Director with greater opportunities of translating his research and field reports to practical conservation projects in different ecosystems of Pakistan.He was instrumental in evolving Co-Management System to resolve decades old conflict between the GB Government and the park communities of Khunjerab National Park.This system also helped at protecting fishes, the birds of Chitral and also protecting medicinal plants from excessive exploitationand initiating trophy hunting programme that proved to be a big success in protecting species in specific habitats while, simultaneously, added a lot to the local economy. This also helped in protecting certain components of the Chilghoza Forest Ecosystem that is home to a variety of animal species, especially the endangered Suleiman Markhor and Striped Hyena.

Prof. Muzaffar Ahmad
Life Time Achievement Award
2025

Dr. Muhammad Ather Rafi
Ex-Principal Scientific Officer
Pakistan Agricultural Research Council
Islamabad



Dr. Muhammad Ather Rafi was Principal Scientific Officer, Pakistan Agricultural Research Council wherein he put in more 32 years of service as an Entomologist. One of his significant achievements was to establish a State-of-the-Art National Insect Museum at the NARC Campus of PARC, housing over 1 million insect specimens. It is a unique source bank for studies on biodiversity in Pakistan. He has reported four new insect species from Pakistan and recently an invasive cactus moth from Chakwal, Pakistan. Dr. Rafi also served as an Associate Professor and Coordinator in the Department of Plant & Environmental Protection at the PARC Institute of Advanced Studies in Agriculture, NARC. After his superamuation he served the Women's University Swabi, Khyber Pakhtunkhwa, as Professor/Chairperson of the Zoology Department. He has been co-chair of the South Asian Invertebrate Specialist Group of the IUCN Species Survival Commission from 2009 to 2025, and member Zoological Society of Pakistan, IUCN, the Invertebrate Conservation & Information Network of South Asia (ICINSA), and a life member of the Bombay Natural History Society (BNHS). Dr. Rafi supervised the research work of more than 42 students for their MPhil Degrees from various universities in Pakistan. He has published more than 117 research papers and written 7 books.

Prof. Muzaffar Ahmad
Life Time Achievement Award
2025

Prof. Dr. Naeem Tariq Narejo
Vice Chancellor
University of Baltistan,
Skardu.

Prof. N.T. Narejo obtained M.Sc and M.Phil degrees from the Department of Freshwater Biology and Fisheries, University of Sindh, Jamshoro, and Ph.D degree in Aquaculture (Fisheries) from Bangladesh Agriculture University, Mymensingh. He has made significant contributions in enhancing fish production through scientific fish culture in order to meet animal protein deficiency in the country. He has introduced freshwater eel culture practices of two big sized eel species and established new bookmark values of different parameters for commercial fish forming. Prof. Narejo has served University of Sindh, Jamshorofor more than 30 years. His sojournein Thailand under post-doc fellowship programmefunded by Network of Aquaculture Centers in Asia (NACA), Thailand in 2005 enriched has expertise as Fisheries scientist. Prof. Narejo has published 190 research articles, produced 30PhD and 60 MPhil scholars. He has executed several research projects as Principal Investigator funded by HEC, PARC, PSF, WWF Pakistan, Info-Fish Malaysia and World Bank. He has also published two text books for graduate and undergraduate students. In recognition of his outstanding research contribution in the field of Fisheries he was awarded two gold medals by Zoological Society of Pakistan and University of Sindh, Jamshoro in the year 2006 and 2013, respectively. HEC declared him as Best University Teacher for the year 2014. He received Zoologist of the Year Award in 2018by the Zoological Society of Pakistan.

Certificate of Appreciation in Recognition of this Contributions Towards Promotion of The Subject of Zoology
2025

Prof. Dr. Azizullah
Distinguished Professor, GCU, Lahore
Ex-Chairman, Department of Zoology
Government College University, Lahore.

Prof. Dr. Aziz Ullah has been engaged in teaching Zoology for the past 60 years. He was awarded academic Roll of honour by Govt. College, Lahore in 1963 on the basis of his first position in the B.Sc. honours exam of the Punjab University and Sir William Roberts Gold Medal for his first position in the M.Sc. exam of Punjab University in 1964. He was awarded Aizaz-e-Fazeelat of Govt. of Pakistan in 1996. During his career, he has produced hundreds of his students who are now engaged in teaching Zoology at various Universities of Pakistan and in a large number of Graduate and Post Graduate Colleges in Punjab. He has been engaged in carrying out research in Zoology during all these years and produced many research papers which have been published in local and foreign research journals. He has also contributed as one of the authors in the text books of Biology of middle, matric and intermediate classes.

Zoologist of the Year Award
2025

Prof. Dr. Abdul Rehman
Institute of Microbiology & Molecular Genetics
University of the Punjab,
Lahore.

Dr. Abdul Rehman is currently working as a Professor in the Institute of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan. He obtained his PhD degree in 2007 from the same university and did his postdoc from Washington State University USA in 2012-2013. His research interests are Cell Biology, Biochemistry, Microbiology, and Environmental Microbiology. Dr. Rehman has published more than 150 research articles in reputed scientific journals. He is working as an Assistant Editor, Pakistan Journal of Zoology since 2008. Based on scientific contributions, Dr. Rehman was awarded Research Productivity Allowance for the years 2005 to 2011, 2015, and 2016. He was awarded the Prof. Dr. Mirza Azhar Beg Gold Medal 2009 by the Zoological Society of Pakistan. Dr. Rehman was also awarded the PAS Gold Medal in 2010 and 2020 for outstanding Environmental Biology/Biological Sciences contributions by the Pakistan Academy of Sciences. Dr. Rehman was ranked among top 2% scientists of the world twice by Stanford University, USA.

*Other applicants of this award were Prof. Dr. Naheed Kaka, Prof. Dr. Noor Khan, Prof. Dr. Abdul Manan Shaikh, Dr. Waseem Ahmed Khan and Dr. Wali Khan.

Zoologist of the Year Award 2025

Prof. Dr. Farhat Jabeen
Dean, Faculty of Life Sciences
Government College University,
Faisalabad

Prof. Dr. Farhat Jabeen, earned her MPhil and a PhD degrees from Quid-i-Azam University, Islamabad followed by two Post Doctorates from Newcastle University, UK funded by the Islamic Development Bank Jedha, Saudi Arabia and HEC Pakistan. Her areas of research interest are Fisheries and Aquaculture, Toxicology, Nanotoxicology, Environmental Pollution, and Aquatic Ecology etc. She has six research projects as PI (03 International and 03 National) to her credit. She is the awardee of INSPIRE (International Strategic Partnerships in Research & Education) project funded by the British Council (UK) and HEC (Pak), SPEKE (Strategic Partnership Extension Knowledge Exchange) grants funded by the British Council (UK), Prof. Dr. Muhammad Ali Award 2017, 2016 & 2015 in recognition of services in research and organizing workshops and seminars at GCUF. She got Excellent Leadership Awards from Islamic Development Bank Jeddah Saudi Arabia for the years 2011 and 2015, HEC University Best Teacher Award-2016 confered in 2018. Dr. Jabeen is also awardee of Chancellor's Gold Medal for standing First in M.Phil, Quaid-i-Azam University Islamabad.

Dr. Jabeen has published over 176 research articles earned Web of Science Impct Factor of >230 and H-index: 24. Her Google Scholar Citation Citation Indices include 5341 total citations, i10 index: 113 and h-index: 34. She has also authored 01 book and 12 book chapters at International level. She Supervised 18 PhD& 69 MPhil scholars. Dr. Jabeen has attended more than 54 International scientific conferences in USA, UK, Egypt, Dubai, Sri Lanka, Denmark, Malsysia, Dublin, Italy, Turkey and Saudi Arabia and presented her research work. Beside this, she developed research collaborations with many prestigious universities like Newcastle University UK, Michigan University USA, Ankara University Turkiye, Islamic University Malaysia, and Oxford University UK.

Prof. A.R. Shakoori Gold Medal 2025

Dr. Muhammad Khan
Associate Professor
Institute of Zoology, University of the Punjab,
Quaid-e-Azam Campus, Lahore

Dr. Muhammad Khan received his master degree in Zoology from University of the Punjab in 2005 and Ph.D. in Cell Biology (Cancer Chemical Biology) from School of Genetics and Cytology, NENU, Changchun, Jilin in 2012.During his doctoral degree program, he identified and isolated several anticancer natural compounds from Traditional Chinese Medicinal Herbs and investigated their molecular mechanisms. Dr. Khan published 8 research articles as 1st author during his doctoral degree program and got incentive research award of 30,000 RMB from Jilin University. After obtaining doctoral degree, Dr. Khan joined Department of Zoology, University of the Punjab, Lahore where he served as Assistant Professor from September 4, 2012 to February 28, 2014. On July 15, 2014, Dr. Khan joined College of Basic Medical Sciences, Dalian Medical University, Dalian, China, as Associate Professor where he served till January 16, 2018. Currently, Dr. Khan is working as Associate Professor in Institute of Zoology, Punjab University, Lahore, Pakistan. Dr. Khan has authored and co-authored 112 published scientific research articles with cumulative Impact Factor >300, one book and 13 book chapter. His work has been cited over 4000 times in various world renowned journals with i10 index 65 and h-index 35. He is the recipient of 9 funded research grants with total granted amount of PKR >40 millions. Dr. Khan has supervised and trained 5 Ph.D., 42 MS/M.Phil., and 12 BS/M.Sc. students and has also evaluated 47 theses of MS/M.Phil. and Ph.D. students of various universities.

*Other applicants of this award were Dr. Muddasir Hassan Abbasi, Dr. Ali Hussain, Dr. Muhammad Shahid, Dr. Samia Afzal, Dr. Fouzia Tabssum, Dr. Shaukat Ali, Dr. Sajid Mahmood, Dr. Syeda Nadia Ahmad, Dr. Amjad Khan, Dr. Muhammad Ali, Dr. Rifat Ullah Khan, Dr. Tasleem Akhtar, Dr. Hafsa Memona and Dr. Shagufta Naz.

Dr. Khan is serving as Editor/EBM of various scientific journals. He is also serving as a Potential Reviewer of around 100 world renowned impact factor journals published by Elsevier, Wiley, Bentham Science, MDPI, IVYSPRING, Dove Press, Spandidos Publications, Hindawi Publications, Springer, PLOS, Taylor and Francis and Researcher link. To date Dr. Khan reviewed > 650 research articles from various impact factor journals. The cumulative Impact Factor of reviewed papers exceeds 3000 which clearly reflects Dr. Khan's outstanding review potential. Dr. Khan also reviewed HEC (Pakistan), RCAMS (KSA) and OPUS-2022 (Poland). Dr. Khan is recipient of various awards including Certificate of Excellence for research productivity in 2016 by DMU, Top 1% Publons Peer Review Awards in Clinical Medicines in 2018, Top 1% Publons Peer Review Awards in Croos-Field in 2019 and Top 1% Publons Peer Review Awards in Plants & Animal Sciences in 2019. Dr. Khan name has been Included in “World's Top 2% Scientist List” in 2024 by Stanford University.

Prof. A.R. Shakoori Gold Medal 2025

Prof. Dr. Muhammad Naveed
Head, Department of Biotechnology
The University of Central Punjab,
Johar Town, Lahore.

Prof. Dr. Muhammad Naveed earned his Ph.D. in Biotechnology with a specialization in Bioinformatics in 2015 from Quaid-i-Azam University, Islamabad and a Post-Doc in Bioinformatics in 2024 from Jiangsu University Zhenjiang, China. Dr. Naveed's research on vaccine design has introduced novel epitope prediction strategies, while his drug discovery research utilizes molecular docking, dynamics simulations, and Artificial Neural Networks (ANNs) to accelerate the identification and optimization of therapeutic candidates. Dr. Naveed has supervised over 150 BS/MSc., 80 MPhil, and 1 Ph.D. scholar. He has authored 230 research publications with a cumulative impact factor of 3970, H-Index of 38 and 13,660 citations. Besides these he has authored a book, 10 book chapters, and filed five patents, exemplifying his commitment to high-impact research and innovation. Beyond academia, Dr. Naveed 300+ recorded video lectures on YouTube have garnered over 4 million views worldwide, advancing open-access education. He has delivered over 100 invited talks, workshops, and training sessions at national and international platforms, actively sharing knowledge with academic, industrial, and research communities. Recognized among the world's top 2% scientists by Stanford University/ Elsevier.

*Other applicants of this award were Dr. Muddasir Hassan Abbasi, Dr. Ali Hussain, Dr. Muhammad Shahid, Dr. Samia Afzal, Dr. FouziaTabssum, Dr. Shaukat Ali, Dr. Sajid Mahmood, Dr. Syeda Nadia Ahmad, Dr. Amjad Khan, Dr. Muhammad Ali, Dr. RifatUllah Khan, Dr. Tasleem Akhtar, Dr. HafsaMemona and Dr. ShaguftaNaz.

Prof. Dr. Mirza Azhar Beg Gold Medal 2025

Dr. Irfan Baboo
Assistant Professor
Department of Zoology
Cholistan University of Veterinary & Animal Sciences, Bahawalpur.

Dr. Irfan Baboo obtained his Ph.D. degree in 2016 in the field of Wildlife and Ecology, with a specialization in Ornithology. Dr. Irfan Baboo has worked on the biodiversity of Class Aves and Mammalia, including conservation, behavioral studies, circadian variations, captive breeding, growth parameters, species identification at the molecular level, hematology and serum chemistry, parasitic prevalence, semen morphology, artificial insemination, hatchability, and egg quality parameters in birds. He has also worked on bird pollinator species, ecology, migratory routes, pet species, and mammalian diversity of the Cholistan Desert in Pakistan. Dr. Irfan has conducted various census surveys in collaboration with the Punjab Wildlife Department and the International Foundation for Houbara Conservation.Dr. Irfan has supervised and co-supervised more than 50 BS, M.Phil and Ph.D. students and has led nationally funded projects as a Principal Investigator. His work has focused on the conservation and welfare of animals, especially bird and mammal species. Dr. Irfan is a member of WWF (International), a fellow of the Zoological Society of Pakistan, and a life fellow of the UVAS Wildlife Society

*Other applicants of this award were Dr. Khalid Hussain Rind and Dr. SaimaNaz.

Dr. Abdul Aleem Chaudhary Gold Medal 2025

Dr. Shahzad Ali
Associate Professor
Department of Wildlife & Ecology
University of Veterinary & Animal Sciences
Ravi Campus, Pattoki.

Dr. Shahzad Ali earned his Ph.D. in Zoology in 2014, specializing in Wildlife Epidemiology. His research focuses on wildlife conservation through disease prevention, particularly infectious disease surveillance in wildlife. He received training from Duke University (USA), DUKE-NUS Medical School (Singapore), and the Friedrich Loeffler Institute (Germany). Dr. Ali has secured six national and international research grants, including from HEC, ALP-PARC, the German Federal Foreign Office, and the U.S. Department of Defense. He led Pakistan's research team in the Western Asia Bat Research Network (WAB-Net), identifying diverse genotypes of corona viruses in bats. He also explored the wildlife microbiome as part of the Earth Hologenome Initiative (EHI). He has supervised nine Ph.D. and 20 M.Phil. students on urban and rural wildlife topics.

*Other applicant of this award were Dr. Sana Ashraf.

Prof. Dr. Nasima M. Tirmizi Memorial Gold Medal 2025

Dr. Seema Shafique
Assistant Professor
Centre of Excellence in Marine Biology
University of Karachi, Karachi

Dr. Seema Shafique obtained her M.Sc. degree in Zoology and Ph.D. degree in Marine Biology from University of Karachi. She is working as Assistant Professor in CEMB for the past 15 years and has acquired extensive experience in the field of Marine Biology. She has published 33research articles on marine ecosystems, biodiversity, and environmental conservation in Marine Pollution Bulletin, Pakistan Journal of Pharmaceutical Sciences, Journal of Geo-Marine Sciences and Ocean and Coastal Management, making significant contributions to the field of marine biology and environmental management. Dr. Shafique has won national research projects of HEC, Islamabad under IPFP and NRPU programmes. She has actively participated and gained multidimensional experience in various professional development and training courses organized by HEC-BC, the Environmental Management Systems-ISO14001-2015 a & NIAB, Faisalabad, Quality Enhancement Cell, Ecologically Important Coastal Sites Workshop CEMB, University of Stirling, UK, under the DFID-funded DelPHE project. Four Ph.D. and twelve M.Phil students have successfully completed their degree under her guidance. She was awarded Productive Scientist of Pakistan during 2016-2018 by Ministry of Science and Technology, Pakistan.

*Other applicant of this award were Dr. Naveed Ahmad and Dr. Munawwer Rasheed

Z.B. Mirza Biodiversity (Faunal) Gold Medal 2025

Dr. Muhammad Kabir
Department of Forestry & Wildlife Management
The University of Haripur
Hattar Road, Haripur, KP

Dr. Muhammad Kabir obtained his Ph.D. in Wildlife Ecology in 2020. Dr. Kabir while working with Snow Leopard Foundation, Pakistan, in collaboration with the Norwegian University of Life Sciences, conducted first-ever extensive camera trapping surveys in Karakoram, Hindu Kush, and Himalayan mountain ranges in Pakistan. He also served in Zoological Sciences Division (PMNH), leading multiple avian studies in remote and previously unexplored regions of Pakistan. His contributions significantly enriched PMNH's reference collection of avian and small mammal specimens. His conservation projects on the Common leopard, Grey wolf, Pallas's cat, Asiatic black bear, and Indian pangolin in Pakistan addressed critical information gaps & conservation challenges in the region. Dr. Kabir is a representative member of the IUCN Species Survival Commission and actively contributes to the Pheasants Specialist Group, Pallas's Cat Conservation Alliance (PICA), and the Persian Leopard Working Group (PeLe) from Pakistan. He has supervised and co-supervised over 50 MS research students. His work aims to address complex ecological questions and contribute to evidence-based conservation strategies. Dr. Kabir has written 48 peer-reviewed articles and several book chapters. His work has garnered 794 citations, with an h-index of 12 and an i10-index of 16.

Prof. Imtiaz Ahmad Gold Medal 2025

Dr. Haroon Ahmed
Department of Biosciences
COMSATS University, Park Road,
Chak Shahzad, Islamabad

Dr. Haroon Ahmed currently working as Associate Professor of Biosciences in Department of Biosciences, COMSATS University, Islamabad. He did his PhD in Zoology (with specialization of Entomology) from department of Zoology, PMAS-Arid Agriculture University Rawalpindi, Pakistan. His major focus of the research is to investigate genetics of evolving insects and insect borne pathogens of public health importance. He has published 210 research publications in highly reputed national and international journals like The Lancet, Lancet Infectious Disease, Lancet Public Health, Clinical Microbiology Reviews, Travel Medicine and Infectious Disease, Parasitology, Journal of Infection and ActaTropica etc. He also published 08 book chapters, and his research work has more than 12000 citations and cumulative impact factor of 5260.He also won 04 research projects as "Principal Investigator" awarded by different national and international organizations. He has supervised more than 100 Research thesis at undergraduate level, 50 at post-graduate level and 05 at doctorate level.

Dr. Ahmed has worked as a visiting researcher in University of Santiago de Compostela (USC), Spain, Firat University, Turkey, WHO collaborating centre of cystic echinococcosis, University of Pavia, Italy and Henan Medical University, China etc. He has also worked as a visiting Professor inCCMAR, Faro, Portugal, University of Coimbra, Lisbon, Portugal, Istituto Superiore Di Sanità, Rome Italy, Cardiff University, UK, Natural History Museum, London, UK and University of Salford, UK.

*Other applicant of this award were Dr. Muhammad Fisal Shahzad and Dr. Zubair Ahmed.

He received meritorious awards at international (TUBİTAK (Turkey), CICIOP (Italy) and national level (such as PMAS-AAU) Rawalpindi, COMSATS University Islamabad and P AS). In recognition of his services in academia are recognized by Govt of Pakistan in the form of award of a Gold Medal in the field of "Health Sciences" from PAS, Gold Medal of Prof. Dr. Nazir Ahmad in the field of "Zoology" from GC University and Silver Medal from "PMAS-Arid Agriculture University, Rawalpindi Pakistan".Currently, he is working on different diseases caused by insect species of public health importance (Hypoderma, Citrus Leaf Miner and Leishmaniasis etc.) under the umbrella of one health. His studies on infectious diseases of humans and animals especially COVID-19 has very important impact on the society and welfare of human beings.

Prof. Dr. Riffat Sultana Gold Medal For Systematics & Applied Entomology 2025

Dr. Abid Ali
Associate Professor
Chairman, Department of Zoology
Abdul Wali Khan University, Mardan

Dr. Abid Ali obtained his PhD degree in 2014 in the field of Applied Entomology and One-Health with specialization of tick-borne diseases and control. During PhD training, he won awards including International Young Scientist Award for the world countries of sciences -2010, Best Research Award winning US$ 2000, Milan Italy, provided by United States Department of Agriculture, Agricultural Research Service National Animal Disease Centre 2013, and best research work (selected among 1250 works), XXIII Congresso Brasileiro de Parasitologia-2013-Brazil. Dr. Abid Ali has worked on the systematics, biodiversity of ticks which are important vectors of tick-borne diseases for humans and animals. He for the first time summarized an accurate key along with morphological descriptions for the ticks of Pakistan. Dr Ali has morphologically described several new tick species based on mitochondrial genomes. Furthermore, Dr Ali is also working on the control of ticks by using recombinant subunit vaccine. So far, Dr Ali has internationally or locally patented three anti-tick vaccines. Dr Abid Ali has published more than 160 publications in different international journals having above 600 IF, and above 5500 citations. Dr Ali has supervised 10 PhDs, 60 M.Phil and more than 150 undergraduate students and has completed 7 extramural projects from different organizations. He has the honour of receiving invitation from Kagoshima University Japan and UFRGS Brazil as invited professor.

*Other applicant of this award were Dr. Santosh Kumar and Dr. Waheed Ali Panhwar.

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Universith of the Punjab, Lahore

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Mr. Afaq Pervez Khan
University of Karachi, Karachi

M.A.H. Qadri Memorial Gold Medal 2025

Dr. Mehtab Ibrahim
University of Karachi, Karachi

Prof. Dr. Muhammad Ali Gold Medal 2025

Miss. Arbab
GC University, Faisalabad

Prof. Dr. S.S. Akbar Memorial Gold Medal 2025

Mr. Azad Ali
University of Sindh, Jamshoro

Ahmad Mohiuddin Memorial Gold Medal 2025

Mr. Azad Ali
University of Sindh, Jamshoro

Prof. Dr. Muhammad Saeed Wagan Gold Medal 2025

Ms. Syeda Auj Zehra
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University of Sindh, Jamshoro

Dr. Iftikhar Hussain Jafri Gold Medal 2024

Ms. Farheen
University of Sindh, Jamshoro

Prof. Dr. Naeem Tariq Narejo Gold Medal 2025

Ms. Fiza Muneer Bhutto
University of Sindh, Jamshoro

SOME GLIMPSES OF ACADEMIC SESSIONS AND THE CONGRESS PARTICIPANTS


43rd PAKISTAN CONGRESS
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Welcome


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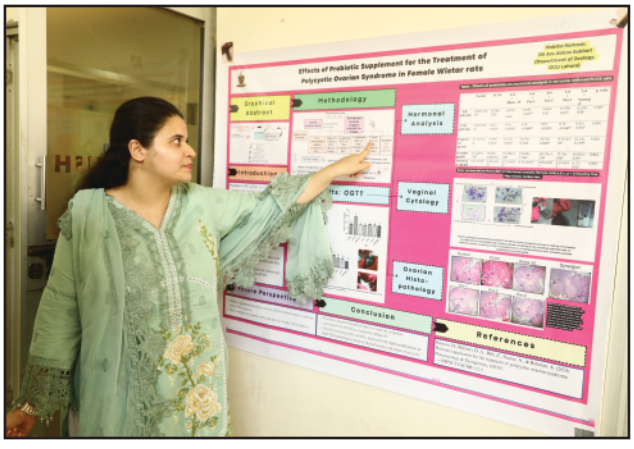
Effects of Probiotic Supplement for the Treatment of
Polycystic Ovarian Syndrome in Female Wistar rats
Methodology
Hormonal Analysis
Vaginal Cytology
Ovarian Histo-pathology
Conclusion
References


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INSTRUCTIONS TO CONTRIBUTORS

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Mohiuddin, A., 1957. Notes on a new strain of *Plasmodium inui. Riv. Malariol*., **36**: 203-208.

Schmid, C.W. and Paulson, K.E., 1984. Interspersed repeats in mammalian DNAs. A status report. In: *Genetics: new frontiers* (eds. V.L. Chopra, B.C. Joshi, R.P. Sharma, and H.C. Bansal), vol. 1, Bowker Publishing Co. Essex, UK, pp. 255-267.

Reference to a book

Smith, J.D., 1966. *The physiology of trematodes*. Butterworth, Edinburgh and London.

Reference to an article with no author given

(USDA) U.S. Department of Agriculture, 2001. Title. USDA, Beltsville, MD, USA

Reference to an article/Chapter in a Book or Proceedings of a Conference

Martin, P.D., Kuhlman, J. and Moore, S., 2001. Yield effects of European corn borer (Lepidoptera: Pyralidae) feeding, pp. 345-356,. In: Proceedings, 19th Illinois Cooperative Extension Service Spray School, 24-27 June 1985, Chicago, IL. Publisher, City, State

Reference to Thesis/Dissertation

James, H., 2001. Title of thesis. M.S. thesis or Ph.D. dissertation, University of Massachusetts Medical School, Worcester, MA, USA

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