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Environmental and Applied Perspectives

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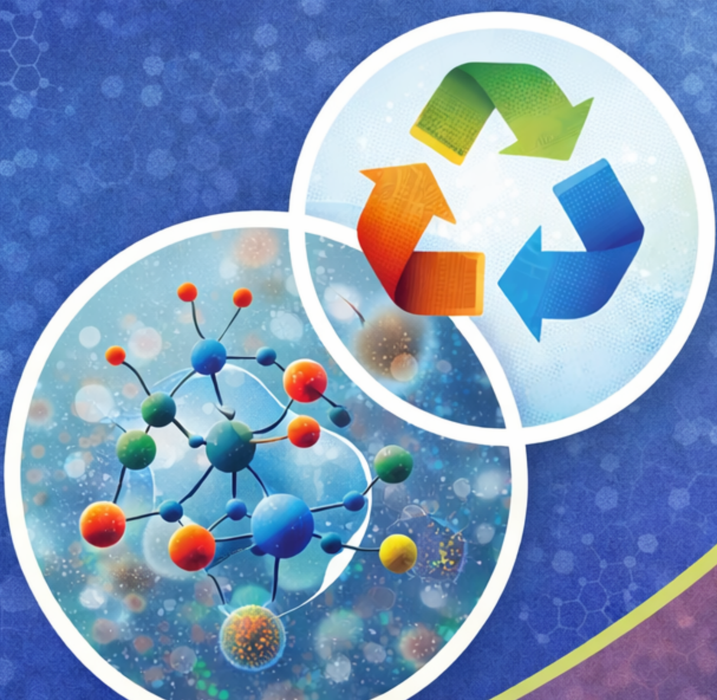

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PREFACE

This volume brings together interdisciplinary research that addresses critical issues at the intersection of agriculture, ecology, environmental quality, and public health. The chapters collectively highlight practical and scientific approaches aimed at promoting sustainability, biological safety, and effective pest and vector management.

The first chapter explores strategies for reducing post-harvest losses through the botanical control of the Angoumois grain moth in rice. By emphasizing plant-derived pest control methods, it contributes to sustainable agricultural practices that enhance food security while minimizing environmental impact.

The second and third chapters extend the discussion to aquatic ecology and environmental health. The study of Nereididae (Polychaeta) from Indonesian coastal waters examines their biological traits, ecological roles, and culture potential, while the microbiological air quality assessment of laboratories at the Federal University, Wukari, underscores the importance of environmental monitoring in maintaining safe research and learning environments.

The final chapter focuses on vector control, evaluating the time- and dose-dependent larvicidal efficacy of a novel imine-based compound against *Culex quinquefasciatus* using probit-based toxicological analysis. Together, these contributions reinforce the role of applied biological research in addressing real-world challenges in health, agriculture, and environmental management.

Editorial Team
February 17, 2026
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CHAPTER 1
REDUCING POST-HARVEST LOSSES: BOTANICAL CONTROL OF ANGOUMOIS GRAIN MOTH IN RICE

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INTRODUCTION

Rice is a staple grain for many people, and it contributes to food security, economic stability, and cultural heritage, especially in Asia and Africa (Biswas *et al*., 2021; FAO, 2021). In Bangladesh, millions of farmers depend on growing and storing rice, thus people there rely on it for both food and revenue (Islam *et al*., 2020; Mahmud *et al*., 2020). But when crops are harvested, there are a number of losses that reduce the amount of food that is kept in the nation and endanger the nutrition of households (Bhattacharjee *et al*., 2020; Dutta & Rahman, 2023). The most severe threat to rice kept in warehouses is insect pests, which can cause both visible and invisible harm that can rapidly worsen even in the presence of favorable storage conditions (Chowdhury *et al*., 2022; Rahman *et al*., 2021).

The Angoumois grain moth (*Sitotroga cerealella*) is the primary cause of the problem. This pest is made more problematic in the warm, humid climates found in South Asia where rice is grown, and its global spread is aided by grain exports (Ali *et al*., 2019; Hossien *et al*., 2018). Substantial paddy stocks can be consumed by the infestation a few generations after a gravid female lays her eggs (Ghosh & Roy, 2021; Mathew *et al*., 2019). The developing larvae become stuck in the grains, causing them to become light inside, webbed, and odorous. This destroys the grains and reduces their marketability and value (Ahmed *et al*., 2018; Sarker & Ahmed, 2023). In Bangladesh, *S. cerealella* is responsible for substantial financial losses, necessitating frequent fumigation and pest control interventions that drive up storage costs and threaten food safety (Islam *et al*., 2020; Rahman *et al*., 2021).

The primary method of controlling *S. cerealella* has been chemical pesticides and fumigants, which may be helpful in the short term but are linked to issues like pests developing resistance, damaging the environment, and endangering farmers and food consumers (Debnath & Sultana, 2020; Saha & Bhattacharjee, 2021). Awareness in botanical insecticides, which are plant-based substitutes for conventional pesticides, has grown as more people examine for safer and more sustainable methods of growing food (Das & Saha, 2019; Sharma & Singh, 2020).

Recent investigation has demonstrated that garlic, marigold, and lemongrass are plants that have anti-storage pest properties; however, these findings lack numerous in-depth analyses that compare different plants under comparable storage conditions (Hasan & Islam, 2020; Haque *et al*., 2021; Uddin & Rahman, 2024). To determine what increases resistance, determine the ideal dosages, and advise smallholder farmers on what works best, systematic research concentrating on a variety of botanicals is essential (Khatun & Rahman, 2021; Zhang *et al*., 2022).

The primary goal of this study is to evaluate the effectiveness of six botanicals - *Codiaeum variegatum*, *Calendula officinalis*, *Allium sativum*, *Mentha piperita*, *Cymbopogon citratus*, and *Nephrolepis exaltata*, in preventing *S. cerealella* from growing in rice that has been stored in a laboratory environment. It also compares the effects of these botanicals on significant pest lifespans and chooses the best ones for use in sustainable rice pest management. In Bangladesh and elsewhere, this study promises to generate reliable information that will be used to minimize the use of threatening pesticides, ensure food safety, and promote rice-based agriculture for several decades in the future.

1. MATERIALS AND METHODS

The experiment was conducted in the Integrated Pest Management Laboratory, Department of Entomology, Bangladesh Agriculture University, Mymensingh. The laboratory conditions were maintained at 28° ± 3ºC and 66 ± 2% relative humidity (RH). The experiment was laid out with six treatments including control and each treatment had six replications. Six effective botanicals, viz., *Codiaeum variegatum*, *Calendula officinalis*, *Allium sativum*, *Mentha piperita*, *Cymbopogon citratus*, and *Nephrolepis exaltata*, were applied against Angoumois grain moth (*S. cerealella)* infesting stored rice under laboratory conditions.

Collection of Paddy Seed

To assess the efficacy of botanicals against the Angoumois grain moth, fresh and healthy harvested paddy seeds were collected from Bangladesh Agricultural Development Corporation (BADC), Mymensingh.

Before the experiment, the collected seed was treated with aluminium phosphide in an airtight container to eradicate any parasites.

Rearing of Angoumois Grain Moth (Sitotroga Cerealella)

The adult moths of *Sitotroga cerealella* were collected from local storage. The eight pairs of *S. cerealella* were released into each clay pot. The mouth of the clay pots was covered with muslin cloth and tied by a rubber band for proper ventilation. The pots were kept at a temperature of 25°-28°C with 65-75% relative humidity (RH) throughout the experiment. All recently emerged adults were removed as the parental population for additional experimental investigation after they had finished rising.

Treatments

The selected botanicals were carefully chosen for their availability in the surrounding environment. The botanical parts were collected from their natural habitats and dried at 60°C for 12 hours. After the drying process, they were ground into a fine powder. The control treatment serves as a baseline for evaluating the effectiveness of the botanical treatments; it consists of using untreated rice grains to observe the natural infestation and life cycle progression of *S. cerealella* without any influence from the botanical treatments. The treatments were placed in the grain during all the period of storage lifespan. These powders were used to make the actual treatments. The study's six chosen treatments, along with the corresponding doses are listed below:

Table 1. Name of the Botanical Treatments and Dosages

Sl No.	Botanicals	Scientific name of the botanicals	Doses (mg/100 g paddy grains)
1.	Croton	*Codiaeum variegatum*	5
2.	Fern	*Nephrolepisexaltata*	5
3.	Lemongrass	*Cymbopogon citratus*	5
4.	Garlic bulb	*Allium sativum*	5
5.	Marigold	*Calendula officinalis*	5
6.	Peppermint	*Mentha piperita*	5

Biological Parameters

Oviposition: Female moths deposit eggs on the grain surface or in crevices. Each female is capable of depositing from 35 to 155 eggs. The eggs are whitish, tiny, and oval in shape. Oviposition takes place nearly exclusively at 25° to 30°C and in the presence of high humidity.

Egg Hatching: Eggs generally hatch from 5 to 10 days later, depending on the temperature and humidity. High hatch rates are obtained under optimum conditions, but they decrease markedly under extreme conditions.

Larval Mortality: Larvae pass through several instars before entering the pupal stage. Larval mortality is influenced by many factors including temperature, humidity, grain moisture, and natural predators or parasitoids. Larval mortality is high due to poor conditions, predation, and parasitism. Botanical treatments also heavily impact larval survival rates.

Adult Emergence: After 7 to 15 days the adults will emerge from the pupae. Successful emergence depends on optimal temperature and humidity levels. High emergence success is realized under favorable conditions and emergence success is reduced under suboptimal conditions.

Reduction over Control: Infestation of paddy by *S. cerealella* causes both quantitative and qualitative losses. Damage is indicated by the presence of frass (insect excrement), webbing, or hollow grains. The damage percentage can go up to 20-50% in case of severe infestation when not controlled. The formulae have been given below:

$$\text{Reduction over control (\%)} = \frac{\textit{Number of damaged grains in treatment} - \textit{Number of damaged grains in control}}{\textit{Number of damaged grains in control}} \times 100$$

Data Collection

Data was collected depending on the different developmental stages of the Angoumois grain moth. So the data collection interval and days vary with the egg, larva, pupa, and adult stages.

Statistical Analysis

For statistical analysis, all recorded data were compiled and tabulated. In this study, Analysis of Variance (ANOVA) was carried out using R version 3.5.3. Least Significant Differences (LSD) and Duncan's Multiple Range Tests were carried out when necessary to determine treatment means differences.

2. RESULTS

Oviposition and Hatching

The exact and calculated process by which female insects or other oviparous species lay their eggs is known as oviposition. It involves careful planning and environmental preparation to ensure the best conditions possible for the growth and survival of the progeny in the future. The act of an organism emerging from an egg and becoming autonomous is known as hatching, which marks the passage from a stage of development to a state of maturation. In the study, the lowest oviposition rate has been observed in *Cymbopogon citratus*, which is 68.4±3.75 (Table 2). This is followed by *Allium sativum* treatment, which has an oviposition rate of 76.8±3.76 (Table 2). The highest oviposition rate was observed in the control group.

Table 2. Effect Of Botanicals Against Oviposition and Egg Hatching of Angoumois Grain Moth (*Sitotroga Cerealella*)

Treatment	**Oviposition**	**Hatched**
Codiaeum variegatum	78±1.82	72.2±2.56
Nephrolepisexaltata	105±3.78	85.4±6.9
Cymbopogon citratus	76.8±3.76	65±3.82
Allium sativum	68.4±3.75	55±2.92
Calendula officinalis	107.8±3.71	66.4±6.38
Mentha piperita	90.2±3.80	74±5.21
7. Control	119.4±5.33	117.6±2.5
CV (%)	8.98	12.64
Level of Significance	<0.01	<0.01

Larval Mortality

There is a wide variation in the responses regardingtreating larval mortality rates.

Allium sativum (42.4 ±1.43%) caused a high level of larval mortality and was the most efficient treatment among the other competing treatments (Table 3). Based on this, it is probably safe to conclude that *Allium sativum* has strong larvicidal active compounds contained in it and deserves more exhaustive study as a natural pesticide. On the other, *Nephrolepisexaltata* (14.4 ± 0.92%) and *Mentha piperita* (15.0 ± 1.04%) have shown low mortality rates in the larvae owing, to lower potential in larval stage mortality (Table 3).

Adult Emergence

An understanding of the treatments' overall efficacy can be gained from the adult emergence rates. With a final developmental stage that showed a significant level of inhibition, *Allium sativum* (12.6 ± 2.804%) produced the lowest adult emergence (Table 3). Given its high incidence of larval mortality, this finding supports the substance's potential to be an effective deterrent to both larval and adult stages. Conversely, more adult emergence is permitted by *Nephrolepis exaltata* (63.0 ± 6.573%) and *Cymbopogon citrates* (51.4 ± 1.72%) (Table 3), indicating that these treatments might be less successful in stopping pests from reaching their full maturity.

Table 3. Effect of Botanicals Against Larval Mortality, Pupal Mortality, and Adult Emergence of Angoumois Grain Moth (*Sitotroga Cerealella*)

Treatment	Larval Mortality	Adult Emergence
Codiaeum variegatum	14.8±2.332	57.2±2.289
Nephrolepis exaltata	14.4±0.927	63±6.573
Cymbopogon citratus	15.2±2.059	51.4±1.72
Allium sativum	42.4±1.435	12.6±2.804
Calendula officinalis	35±4.848	37.8±1.855
Mentha piperita	15±1.049	54.6±1.249
Control	9.4±1.364	99.4±3.932
CV (%)	19.33	12.14
Level of Significance	<0.01	<0.01

The most promising option is *Allium sativum* which shows great overall efficacy with low adult emergence rates and significant larval mortality, especially when it comes to working with pupae. While *Calendula officinalis* is efficient in controlling pests, their performance may need to be optimized or combined with additional treatments.

The Reduction Rates Over Control

Seven treatments along with a control group were examined in a study evaluating the effectiveness of several botanical treatments for insect reduction. For every treatment, the percentage decrease in pest presence compared to the control was recorded. With an 84% decrease, the *Allium sativum* showed the most efficacy of all the treatments (Fig. 1). *Cymbopogon citratus* reduced insect prevalence by 49%, while *Calendula officinalis* followed with a drop of 61.5%. With decreases of 41% and 34% (Fig. 1), respectively, *Codiaeum variegatum* and *Nephrolepis exaltata* exhibited less efficacy than *Mentha piperita*, which displayed a 46.5% reduction (Fig. 1). Hence, control has been taken as the standard treatment acting as a check. These findings show that *Allium sativum* has the potential to be a great botanical treatment for managing pests, whereas other treatments have moderate to low levels of effectiveness.

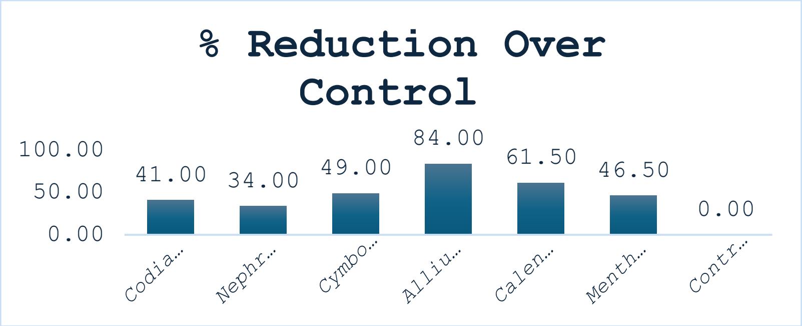

Figure 1. The Reduction Rates Over Control (Botanical Treatments) Against Angoumois Grain Moth (*S. Cerealella)*

3. DISCUSSION

The insecticidal properties of *Allium sativum* essential oil and its major components were thoroughly observed by Yang et al., (2012). The results demonstrated that both garlic essential oil and its two major components diallyl disulfide and diallyl trisulfide exhibit significant activities related to behavioral deterrence and oviposition inhibition against *S. cerealella.* These findings suggest that the essential oil of *Allium sativum*, along with its active compounds, could serve as a potent natural alternative for pest management in agricultural practices.

Additionally, Shah et al., (2023) stated 0.01 µL/L dosage of di-allyl trisulfide (DAT), efficiently inhibits oviposition. This implies that it might be useful as a non-disruptive pesticide. According to Bushra & Aslam, (2014), diallyl disulfide diallyl trisulphide, and garlic essential oil act against *S. cerealella* by inhibiting oviposition and acting as a behavioural deterrent.

Rhetso et al., 2020 demonstrated that the prominent oorgano-sulfurcompounds and saponins found in *Allium chinense* extracts are responsible for some of its insecticidal qualities. Because of its bioactive ingredients, *A. Chinese*juice has moth-repelling properties. Especially saponins have a variety of insecticidal actions, such as inhibiting the hatching of eggs, increasing mortality, and upsetting moth feeding behaviour and growth regulation.

Wu et al., (2020) studied garlic essential oil as an effective botanical insecticide against pests of stored grains through a variety of ways. It is a useful tool in pest management methods because of its effects, which include contact toxicity, repellency, growth inhibition, and mortality. Also, (Wu et al., 2020) that diallyl trisulfide (DAT, 46.66%), diallyl disulfide (20.31%), and methyl allyl trisulfide (8.95%) are the main active ingredients of garlic essential oil. Its biological activity may be due to these sulfur-containing compounds, especially the high percentage of DAT, which may be important for its effectiveness against various species of insects.

The efficient reduction of *Sitotroga cerealella* by extracts from *Allium chinense* has been observed by Rhetso et al., (2020). The use of extracts from the bulbs containing 2% ethanol and 2% petroleum ether, in particular, entirely inhibited adult emergence. This implies that for controlling pest populations in stored grains, extracts from *A. Chinese* bulbs present an effective alternative to natural insecticides.

CONCLUSIONS

The study concludes that treating *Allium sativum* can effectively control the Angoumois grain moth *(S. cerealella*) without injuring the environment, in contrast to the application of chemical pesticides. The findings show that applying garlic bulbs, in contrast to other botanical treatments, significantly decreased oviposition, larval mortality, and adult emergence.

Because *Allium sativum* contains a high concentration of diallyl disulfide and diallyl trisulfide, it possesses insecticidal qualities that help to decrease the control of pests. In comparison to other botanicals, Garlic bulbs appear as a viable alternative that aligns with sustainable development and food safety concerns while simultaneously controlling insect populations, garlic bulbs appear as a viable alternative that aligns with sustainable development and food safety concerns while simultaneously controlling insect populations. As a result, the implementation of garlic bulb treatment in sustainable pest management strategies possesses significant potential. However, the effect of *Allium sativum* on pupal stages needs to be further studied to gain more insightful results.

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Declaration

The authors declare that the research findings reported in this article do not have any conflicting interest.

Authors' Contributions

Saleh Ahmed-Conceptualization, Data Analysis, Manuscript Writing. Md. Sohel Rana-Manuscript writing and editing. Maimuna Koli- Data collection and review, Sumaiya Akter- Manuscript writing and editing.

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CHAPTER 2
BIOLOGICAL CHARACTERISTICS, ECOLOGICAL ROLES, AND CULTURE POTENTIAL OF NEREIDIDAE (POLYCHAETA) FROM INDONESIAN COASTAL WATERS

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INTRODUCTION

The diversity of marine worms, particularly Polychaeta, in Indonesian waters is substantial. Within this tropical archipelago, members of the family *Nereididae* comprise 22 genera and 85 species (Pamungkas & Glasby, 2015). Species belonging to Nereididae are characterized by an eversible pharynx bearing paragnaths, a pair of cirriform antennae, four pairs of elongated tentacular cirri, and four distinct eyes located on the head. Each body segment bears parapodia, which support locomotion and respiration (Wilson et al, 2023). These errant polychaetes typically inhabit mucous-lined burrows and are widely distributed across Indonesian coastal and marine ecosystems, particularly in muddy and sandy substrates (Siregar & Yuwono, 2005; Asnawi et al., 2018; Wibowo et al., 2022). They are frequently reported as dominant taxa in benthic assemblages collected from estuaries and marine environments throughout Java, Sumatera, Sulawesi, and several other regions of the country (Sawestri, 2013; Glasby et al., 2007; Pamungkas, 2020; Ramses et al., 2020).

Members of the Nereididae are also regarded as economically important organisms due to their potential use as bait for recreational fisheries and as a valuable nutritional resource for aquaculture species. Their tissues contain essential proteins, lipids, amino acids, and vitamins, and they have been proposed as an alternative to fishmeal and fish oil in aquaculture feeds (Yuwono et al., 2001; Hadiyanto, 2013; Herawati et al., 2020).

In shrimp aquaculture, nereidid worms are commonly administered to broodstock diets, either singly or in combination with other natural feeds (Yuwono, 2005; Wibowo et al., 2020). They are widely recognized for their effectiveness in stimulating ovarian maturation and spawning (Subaidah et al., 2017), and diets containing Nereididae have been shown to enhance reproductive performance in *Litopenaeus vannamei* broodstock (Yang et al., 2022). This response is associated with the relatively high lipid content of nereidid tissues, which supports final gamete maturation in shrimp that exhibit limited capacity for *de novo* cholesterol synthesis (Nguyen et al., 2012; Palmer et al., 2014; Wang et al., 2019; Wibowo et al., 2020).

Several nereidid species, including *Perinereis* sp. (Meunpol et al., 2005), *Nereis diversicolor* (Wang et al., 2020), and *Namalycastis rhodochorde* (Junardi et al., 2022), have also been reported to contain progesterone, which is thought to contribute to broodstock sexual maturation (Nguyen et al., 2012). In addition, broodstock fed live Nereididae have been shown to produce juveniles with improved survival rates (Muli et al., 2016).

Nereididae, particularly the giant nypa worm, are predominantly harvested from natural habitats through manual digging, as documented among marine worm collectors in Situbondo, Banyuwangi, and Tuban (Rasidi, 2013). Similar harvesting activities occur in the Kapuas River estuary, Kalimantan, where the worms are collected for use as bait in both local and international markets (Junardi, 2021). However, intensive harvesting poses potential ecological risks. Excessive excavation may threaten population sustainability and disrupt ecosystem functioning, as nereidid burrowing activity plays a critical role in sediment reworking, bioturbation, and nutrient cycling in coastal environments (Rodrigues-Filho et al., 2023). For this reason, detailed studies of the ecology and broader biological attributes of Nereididae are urgently required.

A considerable number of research outputs concerning the biology of Indonesian Nereididae have been disseminated in national and international scientific publications. A synthesis of these studies is needed to evaluate the current state of knowledge and to identify research gaps that remain to be addressed. In particular, recent molecular phylogenetic work has revealed fundamental implications for nereidid classification. Pamungkas et al. (2025) demonstrated that traditional subfamily groupings within Nereididae, such as *Dendronereidinae*, are not uniformly supported by genetic data, prompting the establishment of a new subfamily, *Tylorrhynchinae* subfam. nov., and the description of previously unrecognized species from Indonesian waters. These findings underscore how molecular systematics can refine our understanding of nereidid diversity and evolutionary relationships, with direct relevance to biodiversity assessment and conservation planning. This chapter, therefore, provides an overview of the progress of research on the biological characteristics of Nereididae inhabiting Indonesian waters, integrating both classical morphological studies and contemporary molecular insights.

1. HISTORICAL AND CONTEMPORARY TAXONOMIC STUDIES OF INDONESIAN NEREIDIDAE

Taxonomy plays a crucial role in documenting biodiversity and supports both conservation initiatives and the sustainable use of biological resources (Sekar et al., 2021). Taxonomic investigations traditionally rely on morphological characters to distinguish among taxa (Jost, 2017). In Polychaeta, species are typically characterized by the presence of parapodia - paired appendages with dorsal and ventral lobes bearing bundles of chaetae - which function primarily in locomotion and respiration (Read & Fauchald, 2023). More than 80 families are currently recognized within the class, each defined by distinctive body forms and chaetal structures. Members of the family Nereididae are characterized by a prostomium fused with the peristomium around the mouth region, a pair of lateral antennae positioned between two palps, two pairs of tentacular cirri, and two pairs of well-developed eyes (Wilson et al., 2023). The eversible pharynx consists of a proximal oral ring and a distal maxillary ring bearing two jaws and, in some taxa, paragnath groups. The peristomium lacks parapodia but carries four pairs of tentacular cirri; parapodia are uniramous in the first two setigers and biramous thereafter, each equipped with a dorsal and a ventral cirrus. Setae are generally compound in both notopodia and neuropodia, although simple falcigers occur in some genera, and branched gills may arise on the mid-anterior segments in certain species. The pygidium terminates in a pair of cirri, and body length ranges from a few centimeters to over one meter in the largest species (Glasby et al., 2007).

Historical and contemporary research indicates that studies of Indonesian marine polychaetes have been conducted for more than two and a half centuries. The earliest documented description was Amphinome rostrata (Amphinomidae), described by Pallas in 1766 from material collected in Ambon (Pamungkas & Glasby, 2019). More recent assessments by the same authors recorded 713 species representing 55 families within Indonesian waters. Subsequent work by Villalobos-Guerrero and Idris (2021) included the redescription of several nereidid species listed in the World Polychaeta Database (Read & Fauchald, 2018), including *Neanthes gisserana* from eastern Seram Island, *Neanthes vandersandei* from East Flores, and *Neanthes thysanota* from Pulo Damar Besar, Jakarta Bay.

Comparative examinations of historical museum collections alongside recently acquired specimens have further demonstrated the exceptionally high diversity of Polychaeta in Indonesia's marine and coastal ecosystems. Nevertheless, the true richness of these taxa is likely underestimated due to gaps in systematic sampling and dedicated taxonomic research. Although Indonesia is believed to harbor polychaete diversity exceeding that of many neighboring regions, only a fraction has been formally documented - approximately 11,500 examined specimens representing 85 families (Pamungkas et al., 2019). Mass-spawning nereidid worms observed in Ambon were shown to consist of four species - *Ceratonereis singularis australis, Composetia marmorata, Nereis gisserana,* and *Perinereis nigropunctata* - identified for the first time from preserved epitoke specimens collected from the Banda Sea (Pamungkas & Glasby, 2015). These findings highlight the importance of continued taxonomic investigation of nereidid populations across diverse Indonesian habitats.

Recent molecular phylogenetic work has further advanced nereidid systematics. Using mitochondrial and nuclear genetic markers, Pamungkas et al. (2025) reassessed relationships within the subfamily Dendronereidinae and showed that several traditional subfamily boundaries are not consistently supported by molecular phylogenetic evidence. Their analyses indicated that only the type genus, *Dendronereis*, forms a coherent clade, whereas other genera previously assigned to Dendronereidinae are more appropriately classified elsewhere. To accommodate these distinct evolutionary lineages, the authors erected a new subfamily, Tylorrhynchinae subfam. nov., to include clades comprising *Dendronereides* and *Tylorrhynchus*. The study also described two new Indonesian species, underscoring the continuing discovery of nereidid diversity even within relatively well-studied regions.

Additional taxonomic contributions have emerged in recent years. Pamungkas (2020) noted that comprehensive studies on Nereididae in Indonesia have only recently gained significant momentum. A new species, Namalycastis rhodochorde, was described from mangrove habitats in the Kakap Estuary near Pontianak, West Kalimantan (Glasby et al., 2007), while Perinereis aff. arabica was reported from Gunungkidul, Yogyakarta (Hadiyanto, 2013).

Pamungkas and Glasby (2015) also conducted a taxonomic revision of Nereididae from Ambon, based on specimens collected in 1866 alongside samples obtained in 2009 and 2014. Their work documented the presence of multiple species, including *Ceratonereis sp. cf. C. perkinsi, Ceratonereis singularis australis, Composetia marmorata, Neanthes pachychaeta, Neanthes unifasciata, Neanthes sp. cf. N. gisserana, Neanthes sp. cf. N. masalacensis, Nereis sp., Perinereis helleri, Perinereis nigropunctata*, *Solomononereis marauensis*, and *Websterinereis foli*. These findings challenge earlier assumptions that the mass-spawning “wawo” or “laor” in Ambon represented a single species and further emphasize the significance of taxonomic research in elucidating nereidid diversity in Indonesia. Despite these advances, specialized taxonomic studies on Polychaeta remain limited, as many investigations have been embedded within broader ecological research frameworks — including studies in Jakarta Bay (Abdurrahman et al., 1990), the Mahakam Delta, East Kalimantan (Finishia et al., 2014), mangrove forests of North Sumatra (Rabiah et al., 2017), the Prawean Coast of Jepara, Central Java (Apriyanti & Tumiran, 2018), Tahura Ngurah Rai, Bali (Priyandayani et al., 2018), and Gresik, East Java (Madyowati & Kusyairi, 2020). Dedicated, integrative taxonomic research on Polychaeta — particularly Nereididae — is therefore essential as a scientific foundation for biodiversity assessment, sustainable utilization, and conservation planning. At the same time, expansion of comprehensive ecological investigations is urgently needed to deepen understanding of nereidid biodiversity, functional roles, and potential applications

2. ECOLOGY

2.1 Distribution, Community Structure, and Functional Roles

Ecological studies on the family Nereididae (Polychaeta, Annelida) in Indonesia have been conducted across a variety of coastal settings, gradually building a broader understanding of their habitat preferences and ecological significance. Early work along the northern coast of Java (Siregar & Yuwono, 2005) and in the mangrove forests of West Kalimantan (Junardi & Wardoyo, 2008) first highlighted the prominence of nereidid worms in intertidal benthic communities.

Subsequent research expanded this geographical scope to the southern coast of Central Java (Wibowo et al., 2017) and Manado Bay in Sulawesi (Lumingas et al., 2022), thereby encompassing regions with contrasting hydrodynamic conditions, sediment characteristics, and levels of human influence.

Collectively, these investigations demonstrate that nereidid polychaetes inhabit a broad range of coastal environments, including mudflats, sandy shores, estuarine channels, and mangrove sediments. In many of these habitats they occur at relatively high densities and often represent a dominant component of the benthic macrofauna, contributing to processes such as sediment turnover and organic matter transformation. Their distribution and abundance are strongly shaped by environmental gradients—particularly sediment composition, salinity, organic enrichment, and tidal exposure—indicating a close ecological association with local habitat conditions.

This pronounced sensitivity to environmental variation has important implications for coastal research and management. Patterns emerging from existing studies suggest that members of the Nereididae possess considerable potential as biological indicators for assessing habitat quality and detecting anthropogenic disturbance within Indonesian coastal ecosystems.

More broadly, ecological research on Indonesian nereidids has focused primarily on species diversity, community composition, and abundance in relation to environmental and habitat characteristics (Yusron, 1985; Finishia et al., 2014; Rabiah et al., 2017; Priyandayani et al., 2018; Madyowati & Kusyairi, 2020). These studies have sought to characterise spatial and temporal variability in nereidid assemblages, revealing fluctuations in population density and community structure in response to seasonal cycles, hydrological dynamics, and substrate stability. This body of work has provided valuable insight into the factors shaping the distribution and persistence of nereidid populations across Indonesian coastal habitats.

Beyond patterns of diversity and community structure, several studies have also highlighted the functional roles of nereidids in benthic ecosystems. Through activities such as sediment bioturbation, nutrient recycling, and participation in trophic interactions, these worms influence the organisation and productivity of benthic food webs.

However, these functional and mechanistic dimensions have received comparatively less attention than taxonomic and community-level surveys, leaving important questions regarding the behavioural, physiological, and ecosystem-level drivers of their ecological roles largely unresolved.

Taken together, the available literature emphasises both the ecological importance of nereidid polychaetes and the need for more integrative approaches. Future research would benefit from combining detailed taxonomic surveys—using both morphological and molecular techniques—with studies of life-history traits, reproductive strategies, and behavioural ecology to better understand population dynamics and responses to environmental variability. Equally important are investigations that explicitly link nereidid activity to ecosystem-level processes such as bioturbation, nutrient fluxes, and trophic pathways, supported by long-term monitoring across seasons and habitat types. Such multidisciplinary efforts are essential for fully elucidating the ecological significance of nereidids and for informing management and conservation strategies aimed at sustaining the resilience of Indonesian coastal ecosystems.

2.2 Substrate Productivity and Its Role in Supporting Nereidid Diversity

The diversity of Polychaeta in Indonesian coastal and mangrove ecosystems is closely linked to environmental characteristics, particularly sediment composition and the availability of organic matter. Sediment conditions influence not only the physical stability of the benthic habitat but also the quantity and quality of trophic resources that sustain detritivorous and deposit-feeding taxa, such as nereidid worms. Evidence from the Peniti mangrove forest in West Kalimantan exemplifies this relationship, where high polychaete diversity has been recorded in clay-mud substrates enriched with organic carbon (Junardi & Wardoyo, 2008). Within this habitat, species richness was higher in stands dominated by *Sonneratia alba* compared with those dominated by *Rhizophora mucronata*, a pattern attributed to the greater organic-carbon content of *Sonneratia* sediments, which supports enhanced microbial activity and detrital food production for benthic invertebrates.

Comparable ecological trends have been documented in other regions; for example, Sweetman et al. (2010) reported that elevated substrate organic-carbon levels increase resource availability and promote the development of structurally complex benthic communities. Differences in litter quality further reinforce habitat suitability patterns. Leaves of *Sonneratia alba* possess higher nutritional value and a lower C/N ratio compared with *Rizhophora mucronata,* resulting in faster decomposition and more efficient transfer of organic matter into the benthic food web (Pendleton et al., 2012). In combination with favorable physicochemical conditions and the stabilizing influence of mangrove root structures, these factors create productive and ecologically supportive environments for the settlement, growth, and persistence of nereidid worms. Consequently, the interaction between sediment geochemistry, detrital resource pathways, and habitat structure plays a pivotal role in shaping the diversity, distribution, and ecological success of Polychaeta in Indonesian mangrove ecosystems.

Despite these insights, several knowledge gaps remain. Current studies are largely descriptive and site-specific, leaving a limited understanding of the broader spatial and temporal dynamics of polychaete communities across Indonesia's diverse mangrove and coastal systems. In particular, the functional roles of nereidid worms in nutrient cycling, sediment reworking, and energy transfer within benthic food webs remain poorly quantified. Moreover, the influence of environmental stressors—including pollution, habitat alteration, and climate-related changes—on polychaete diversity and ecosystem functioning is insufficiently understood.

To address these gaps, future research should adopt integrative and multidisciplinary approaches. Priority areas include: (i) broad-scale surveys combining morphological and molecular taxonomy to capture species diversity and biogeographic patterns; (ii) experimental and observational studies quantifying the functional contributions of nereidids to sediment biogeochemistry, organic matter processing, and trophic dynamics; (iii) assessments of the effects of environmental gradients and anthropogenic disturbances on polychaete community structure; and (iv) long-term monitoring to understand temporal variability and resilience of benthic assemblages.

Such research would not only advance ecological theory regarding benthic invertebrates in mangrove and coastal ecosystems but also inform sustainable management and conservation strategies, highlighting the critical roles of Polychaeta in maintaining ecosystem health and productivity.

2.3 Influence of Sediment Composition and Organic Matter on Polychaete Diversity

Members of the family Nereididae exhibit a remarkably broad ecological distribution, inhabiting a wide spectrum of marine environments that range from soft muddy and sandy bottoms to rocky coastal substrates (Siregar & Yuwono, 2005; Finishia et al., 2014; Jumars et al., 2015; Asnawi et al., 2018; Balakrishnan & Tudu, 2021). In Indonesia, tropical coastal systems across Java, Sumatra, Sulawesi, and Kalimantan represent major centres of nereidid occurrence, where these polychaetes form integral components of benthic communities spanning estuarine channels, intertidal flats, and mangrove forests (Yuwono et al., 1994; Siregar & Yuwono, 2005; Junardi, 2008; Sawestri, 2013; Apriyanti & Tumiran, 2018; Junardi, 2018; Lumingas et al., 2022; Sari et al., 2022; Wibowo et al., 2022). Their widespread presence across diverse habitat types highlights their adaptability to environmental variability, including differences in sediment composition, salinity gradients, tidal regimes, and hydrodynamic exposure.

Within these habitats, nereidid polychaetes frequently dominate soft-muddy and sandy-mud substrates, environments that are particularly suitable for their burrowing lifestyle and deposit-feeding behaviour (Asnawi et al., 2018; Hajializadeh et al., 2020). Fine-grained sediments provide structural stability essential for tube-building, burrow excavation, and maintenance, while the upper layers of sediment are typically enriched in labile organic matter that can be readily ingested and assimilated to support growth and metabolism (Jumars et al., 2015). The combination of favourable substrate properties, abundant food resources, and stable hydrological conditions in coastal and mangrove ecosystems creates optimal conditions for the settlement, development, and long-term persistence of nereidid populations.

This broad ecological tolerance and habitat flexibility not only underpins the high abundance and widespread distribution of nereidids in tropical coastal systems but also allows them to perform important ecological roles, including sediment reworking, nutrient cycling, and energy transfer within benthic food webs. By thriving in habitats that vary in sediment type, organic content, and tidal influence, nereidid polychaetes exemplify the complex interactions between benthic fauna and their physical and chemical environment, making them valuable model organisms for studies of tropical coastal ecology.

The ecological significance of the family Nereididae extends far beyond their numerical abundance and spatial distribution. Through their continuous burrowing and foraging activities, nereidid polychaetes exert profound influences on sediment structure, physical stability, and benthic biogeochemical processes. Species such as *Nereis virens*, *Nereis diversicolor*, *Nereis falsa*, and *Nereis pelagica* act as principal agents of bioturbation, actively reworking sediments through processes that include ventilation, selective feeding, and burrow construction and maintenance (Mustajärvi et al., 2019; He et al., 2022). By ingesting organic-rich deposits and recycling particulate matter within the substrate (Asnawi et al., 2018; Kanaya, 2023), these worms play a crucial role in mitigating excessive organic accumulation, enhancing sediment aeration, and promoting the overall quality and stability of benthic habitats (Bergström et al., 2019).

In addition to their roles as sediment engineers, many nereidid species function as omnivorous or opportunistic predators, feeding on a variety of small crustaceans, diatoms, detritus, and other benthic invertebrates (Belfetmi et al., 2021). Through these trophic interactions, nereidid polychaetes occupy a central position within coastal food webs, linking primary producers and detrital resources to higher trophic levels such as fishes and crustaceans. Their dual function as both ecosystem engineers and trophic mediators underscores the integral role, they play in maintaining ecological balance and supporting the productivity of tropical coastal ecosystems.

The combination of wide habitat tolerance, functional versatility, and strong interactions with both biotic and abiotic components of their environment has established nereidid polychaetes as valuable model organisms for ecological research.

Their high abundance, distinct habitat preferences, and sensitivity to environmental change further highlight their utility as bioindicators, capable of reflecting the condition of coastal ecosystems and signalling the impacts of anthropogenic disturbances such as pollution, habitat modification, and hydrological alteration.

Despite extensive ecological documentation, several knowledge gaps remain. Most studies in Indonesia have been descriptive and limited to specific sites, leaving the spatial and temporal dynamics of nereidid populations across diverse habitats poorly understood. Functional roles in sediment biogeochemistry, nutrient fluxes, and ecosystem productivity remain only partially quantified, particularly under variable environmental and anthropogenic stressors. Additionally, the interactions between nereidid worms and other benthic fauna, as well as their responses to climate-related changes such as sea-level rise, temperature fluctuations, and salinity shifts, are still poorly explored

2.4 Trophic Interactions, Host–Pathogen Relationships, and Bioindicator Roles

Polychaetes of the family Nereididae interact with a broad spectrum of organisms within their habitats and frequently occupy dual ecological roles as both predators and prey. Several studies have identified nereidid worms as an important natural food source for shrimp (Rajuansah et al., 2021; Herawati et al., 2022). However, their consumption may also facilitate the transmission of marine pathogens. For example, outbreaks of White Spot Syndrome Virus (WSSV) in *Litopenaeus vannamei* have been linked to the ingestion of infected nereidid worms collected from natural habitats such as the Mahakam Delta (Kalimantan) and the coastal waters of Semarang, Central Java. Individuals of *Dendronereis* spp. were shown to harbor active WSSV infections, with viral replication occurring in the intestinal tissues and transmission to shrimp occurring through oral exposure (Desrina et al., 2014). Beyond pathogen dynamics, nereidid polychaetes form a key component of estuarine and marine food webs (Balakrishnan et al., 2019).

They represent a major dietary resource for numerous demersal fish and shorebird species (Wibowo et al., 2022), while themselves functioning primarily as deposit feeders that process organic material within sediments (Bergström et al., 2019). The life histories of some species, such as *Namalycastis rhodochorde*, include a planktonic larval stage followed by a transition to a sedentary benthic phase, rendering them particularly sensitive to long-term environmental fluctuations (Junardi et al., 2020). At the same time, many nereidids exhibit notable physiological tolerance and adaptive capacity (Alter et al., 2021). For instance, *Namalycastis abiuma* from coastal regions of Java and Sumatra is capable of surviving under low dissolved-oxygen conditions, yet displays morphological abnormalities when exposed to mercury contamination (Sawestri, 2013).

These biological responses, together with their strong association with substrate conditions and organic enrichment, have contributed to the widespread use of nereidid worms as bioindicators of environmental change in coastal and marine ecosystems (Sabha et al., 2022). Accordingly, many coastal monitoring programs employ quantitative assessments of macroinvertebrate assemblages -including Nereididae - in relation to physicochemical and habitat variables (Abdullah et al., 2022; Sahidin et al., 2018), thereby reinforcing their value as indicator taxa for evaluating anthropogenic disturbance and overall environmental quality.

According to Schmeller et al. (2018), a bioindicator is defined as a species or taxonomic group that exhibits measurable biological responses to anthropogenic stressors, including industrial and chemical pollution. Macrozoobenthic organisms such as nereidid polychaetes are particularly responsive to shifts in environmental conditions because they inhabit the sediment–water interface, where contaminant accumulation and physicochemical fluctuations are most pronounced (De Marchi et al., 2018; Ramses et al., 2020). Their relatively short life cycles and rapid generation turnover enable timely detection of environmental disturbance (Sanchis et al., 2021), making them suitable indicators for assessing ecosystem integrity in marine and coastal habitats (Dean, 2008; Sawestri, 2013; Wardiatno et al., 2017; Sahidin et al., 2018; Ramses et al., 2020; Abdullah et al., 2022; Sabha et al., 2022).

Correspondingly, nereidid assemblages have been widely incorporated into coastal monitoring programs and pollution assessments (Wibowo et al., 2017). Industrial and urban pollution can modify both the physicochemical properties of sediments and the community structure of nereidid populations, as documented in coastal environments such as Sayung District, Demak, Indonesia (Iqbal et al., 2020). Because nereidid worms are largely sedentary and inhabit burrows or attach to hard substrates, they experience prolonged exposure to contaminants and can reflect the historical accumulation of pollutants in benthic habitats (Dsikowitzky et al., 2011). Consequently, the bioaccumulation of heavy metals—including cadmium (Cd), lead (Pb), and mercury (Hg) - in nereidid tissues has been widely applied as an indicator of environmental contamination (Liline & Rumahlatu, 2019). Similar applications have been reported internationally, where members of Nereididae have been used to evaluate exposure to Cd, Zn, and Cu in coastal ecosystems (Dean, 2008).

Beyond metal accumulation, several studies have examined physiological and biochemical biomarkers in nereidid species. For instance, oxidative stress responses - including glutathione, glutamate cysteine ligase, and glutathione-S-transferase activity - have been evaluated in *Perinereis gualpensis* as indicators of contaminant-induced cellular stress (Díaz-Jaramillo et al., 2011). Such biomarker approaches complement abundance- and distribution-based assessments, providing mechanistic insight into how pollutants influence organismal health and population dynamics.

Taken together, the abundance, spatial distribution, contaminant burden, and physiological status of nereidid polychaetes collectively provide robust indicators of environmental quality in coastal and estuarine ecosystems (Sahidin et al., 2014; Wardiatno et al., 2017; Sahidin et al., 2018). Their widespread use—both in Indonesia and globally—reflects their ecological importance, broad habitat representation, and rapid yet measurable responses to environmental disturbance.

3. REPRODUCTION

3.1 Reproductive Biology, Developmental Stages, and Epitoky Variation

Species of Nereididae inhabiting Indonesian coastal and estuarine environments generally exhibit gonochoristic reproduction, with males and females occurring as separate individuals that release sperm and oocytes into the surrounding water, where fertilization takes place externally during synchronized spawning events (Yuwono et al., 1994; Junardi et al., 2010; Pamungkas & Glasby, 2015). Such reproductive behavior is typical of broadcast-spawning polychaetes and is closely linked to tidal cycles, lunar periodicity, and local hydrodynamic conditions that enhance fertilization success and larval dispersal. Observations of sea worms previously identified as *Nereis* sp. (Yuwono et al., 1994) and *Dendronereis javaensis* (Yuwono, 2008), and more recently described as *Dendronereis javaensis* by Pamungkas et al. (2025) - the name adopted in this manuscript - show that spawning occurs year-round, peaking in February–March, after which adults die, indicating a semelparous life-history strategy. Comparable spawning periodicity and post-reproductive mortality have also been documented in nereidid populations from the southern coast of Lombok (Bachtiar & Bachtiar, 2019), suggesting that semelparity may be a prevalent reproductive trait among several Indonesian taxa. Following external fertilization, embryos develop into planktonic trochophore larvae, which form the earliest free-swimming developmental stage (Fig. 1). In *Dendronereis javaensis*, the trochophore stage persists for approximately one to two days before transitioning into a three-setiger benthic larva (Yuwono et al., 1994). During subsequent ontogenetic progression, the head becomes fully differentiated, parapodia develop on successive segments, and anal cirri form at the posterior end, marking the establishment of key morphological features associated with benthic life. Within roughly ten weeks, larvae metamorphose into juveniles capable of burrowing and sediment feeding, and these individuals typically attain reproductive maturity within one to two years, depending on environmental conditions and resource availability.

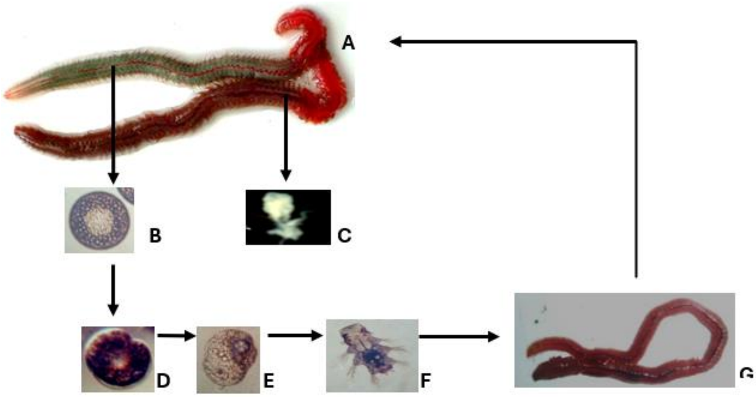

Figure 1. Life Cycle of *Dendronereis Javaensis* (Polychaeta, Nereididae). Adult Worms (A) Produce Oocytes (B) That Are Fertilized by Spermatozoa (C). The Fertilized Oocyte (D) Undergoes Division and Spirally Cleaving Embryos Developing into Planktonic Trochophore (E), Which Then Develops into Benthic Larvae With 3 Setigers (F) and Further Develops into an Immature Worm (G).

In many nereidid species, reproductive maturation is accompanied by epitoky, a metamorphic transition in which benthic, non-reproductive atoke forms transform into pelagic epitokes specialized for spawning (Fischer, 1999). This process often involves enlargement of the eyes, reconfiguration of parapodia for swimming, and alterations in body pigmentation and musculature. For example, *Perinereis cultrifera* from Maluku waters exhibits pronounced epitokous modification of parapodia and enhanced swimming capacity during the reproductive phase (Rettob, 2012). However, epitoky expression is not universal among Indonesian nereidids. Several species - such as *Dendronereis javaensis* from northern Java (Yuwono et al., 1994), *Nereis* sp. from Ambon (Pamungkas, 2009), and *Namalycastis abiuma* from Kalimantan (Junardi et al., 2014) - display minimal or no conspicuous epitokous transformation, indicating variability in reproductive strategies within the family.

In *Namalycastis rhodochorde*, maturation is instead characterized by distinctive physiological and chromatic changes, including a shift in body coloration (pink-to-reddish in females and greenish in males) and softening of the body wall, which subsequently ruptures during gamete release (Junardi et al., 2014).

These contrasting reproductive modes underscore the diversity of life-history strategies among Indonesian Nereididae and highlight the influence of evolutionary lineage, habitat characteristics, and reproductive ecology on the expression of epitoky and spawning behavior.

3.2 Reproductive Strategy and Life-History Patterns

The reproductive biology of nereidid polychaetes represents a distinctive life-history strategy shaped by physiological specialization and environmental adaptation. In *Dendronereis javaensis*, as in many members of Nereididae, reproduction encompasses several sequential processes, beginning with gametogenesis (oogenesis in females and spermatogenesis in males), followed by gamete maturation, spawning, and the subsequent development of larvae and juveniles into fully formed adults (Yuwono, 2008). Field observations across multiple coastal and estuarine habitats in Indonesia indicate that reproductive activity can occur throughout the year, although pronounced spawning peaks are typically recorded during specific months, notably February, March, and June (Yuwono et al., 1994; Junardi et al., 2010, 2014; Bachtiar & Bachtiar, 2019). These temporal patterns suggest that local environmental conditions—such as temperature, salinity, tidal cycles, and organic-matter availability—may act as reproductive cues that synchronize spawning within populations.

Consistent with earlier accounts, nereidid polychaetes are dioecious and exhibit semelparous reproduction, meaning that individuals reproduce only once before death. Species such as *Dendronereis javaensis, Nereis sp., Namalycastis abiuma,* and *Namalycastis rhodochorde* undergo a terminal reproductive event in which adults allocate substantial physiological resources to gamete production during gametogenesis. Upon release of mature gametes during spawning, somatic degeneration ensues and the adults die shortly thereafter (Yuwono et al., 1994; Pamungkas, 2009; Junardi et al., 2014, 2021). This strategy reflects a trade-off between survival and reproductive investment, maximizing reproductive output at the expense of post-spawning survival. The completion of the life cycle is thus transferred to the next generation, with larval development and recruitment playing a pivotal role in sustaining population continuity within nereidid communities

3.3 Gametogenesis, Spawning Regulation, and Reproductive Cycles

The reproductive system of nereidid polychaetes displays remarkable diversity in gamete origin and developmental pathways, reflecting both phylogenetic differentiation and adaptation to environmental conditions. Gametes may arise within discrete gonadal structures, originate from proliferative cells that differentiate freely within the coelomic cavity, or develop through a combination of these mechanisms (Eckelbarger, 2005). Several tropical Indo-Pacific taxa, including *Dendronereis javaensis* (Yuwono et al., 1994) and *Namalycastis rhodochorde* (Junardi, 2021), lack true gonads; instead, their gametes develop extra-gonadally within the coelom, where differentiation proceeds in association with coelomic tissues and nutrient-transporting cells.

Male gametogenesis (spermatogenesis) in nereidids follows a conserved morphological sequence. Spermatogonial clusters proliferate during the premeiotic phase and subsequently segregate during meiotic prophase to form characteristic spermatid tetrads. These tetrads differentiate into fully developed spermatozoa as maturation progresses (Yuwono et al., 1994). In *Namalycastis rhodochorde*, immature males contain undifferentiated spermatocytes dispersed within the coelomic fluid (Fig. 2A, 2B). These develop into spermatid tetrads during the submature phase (Fig. 2C) and eventually transform into motile spermatozoa at sexual maturity (Fig. 2D) (Junardi et al., 2022). Morphologically, the spermatozoa are characterized by a cone-shaped acrosome enclosing the nucleus and an elongated posterior flagellum, structural features that enhance motility and facilitate fertilization of free-spawning oocytes (Yuwono, 1992). Consistent with semelparous life histories, males typically die following gamete release during spawning (Yuwono et al., 1994).

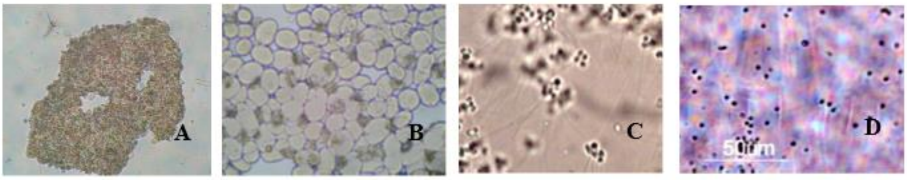

Figure 2. Spermatogenesis in *Namalycastis rhodochorde* (Polychaeta, Nereididae). (A) Undifferentiated spermatocytes in immature male coelom; (B) Spermatocyte clusters in immature stage; (C) Spermatid tetrads at submature stage; (D) Mature spermatozoa in coelomic fluid, exhibiting cone-shaped acrosome and posterior flagellum. Adapted from Junardi et al. (2022).

Female gametogenesis (oogenesis) in nereidids occurs via either intraovarian or extraovarian pathways (Eckelbarger, 2005). In *Dendronereis javaensis*, oogenesis is extraovarian: early oocytes are released into the coelom, where they proliferate, increase in size, and undergo sequential differentiation (Yuwono et al., 1994). Oocyte development progresses through proliferative, previtellogenic, vitellogenic, and corticogenic stages. Vitellogenesis—marked by the accumulation of yolk proteins—initiates when oocytes reach diameters of approximately 50–60 µm, followed by cortical alveoli formation during corticogenesis. Fully mature oocytes attain uniform sizes ranging from 140–160 µm. Notably, tropical nereidids tend to produce smaller mature oocytes compared with temperate representatives such as *Perinereis cultrifera,* whose oocytes may reach 280–320 µm (Bharathidasan et al., 2017), suggesting ecological or energetic trade-offs in reproductive allocation. Immature female individuals typically contain oogonia and developing oocytes of varying sizes within the coelom. The previtellogenic stage concludes when oocytes detach from cluster arrangements and disperse individually. During vitellogenesis, yolk deposition is facilitated by eleocytes present in the coelomic fluid (Eckelbarger, 2005), after which corticogenesis produces submature oocytes of relatively uniform maximum size (Lawrence & Soame, 2009; Yuwono et al., 1994). In mature females, lipid droplets frequently appear prior to spawning, a pattern documented in N. rhodochorde (Junardi et al., 2014). Overall, female gametogenesis follows a well-ordered trajectory from undifferentiated to immature, submature, and finally mature oocyte stages (Fig. 3).

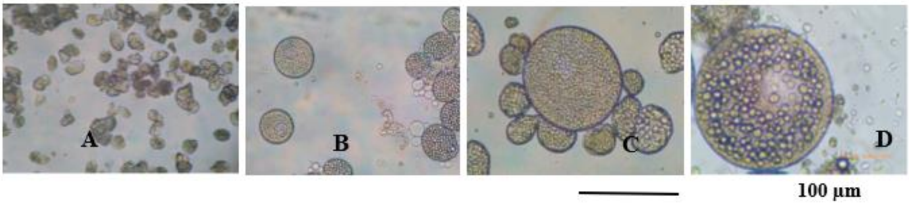

Figure 3. Oogenesis in Nereididae (Polychaeta). Stages of female gametogenesis (A). Undifferentiated oocyte; (B). Immature oocyte during previtellogenesis; (C). Submature oocyte during corticogenesis; (D). Mature oocytewith yolk accumulation and lipid droplets prior to spawning. Adapted from Yuwono et al. (1994) and Junardi et al. (2014)

Spawning in nereidids occurs through the synchronized release of mature gametes into the surrounding water column, a process governed by the interaction of environmental cues and internal regulatory mechanisms. Exogenous drivers include tidal, lunar, and seasonal temperature cycles, which collectively modulate reproductive timing and larval survival prospects (Bachtiar & Odani, 2021). In many tropical systems, seasonal temperature peaks—particularly during March—are associated with enhanced plankton availability, thereby increasing post-spawning recruitment success (Mulyadi & Saputra, 2019). Fecundity appears to be influenced more strongly by environmental conditions and food resources than by body size in some species, including *P. erinereis cultrifera* (Rettob, 2012).

Endogenous regulation involves neuroendocrine and hormonal controls that mediate gamete maturation and reproductive readiness (Ram et al., 1999; Andries, 2001). Additional environmental signals include photoperiod (Fischer, 1999), short-term light–dark cycles (Hardege et al., 1998; Zantke et al., 2013), water temperature (Hardege et al., 1994), salinity (Fong, 1993), climate variability (Lawrence & Soame, 2004), and tidal rhythms (Wilcockson & Zhang, 2008). Temperature acts as a particularly strong trigger: in cultivated Nereis diversicolor, spawning is initiated either by rising temperatures from 6°C to 12°C or by declining temperatures from 12°C to 6°C (Bartels-Hardege & Zeeck, 1990).

In natural habitats, spawning thresholds vary geographically; for instance, Nereis falsa in tropical East Algeria spawns at 20–23°C (Daas et al., 2011). Lunar periodicity functions as a key zeitgeber in many nereidids, influencing tidal regimes and synchronizing reproductive events within populations (Bentley et al., 1999). In West Kalimantan estuaries, N. rhodochorde displays a distinct spawning window from May to July, with maximum oocyte diameters observed in June (Junardi et al., 2014), paralleling reproductive peaks in Perinereis nuntia brevicirris in Malaysian waters (Ong, 1996). By contrast, N. abiuma exhibits year-round reproduction with pronounced peaks in March and July (Junardi et al., 2014), a pattern reminiscent of the 28-day spawning cycle documented in Platynereis dumerilii (Olive et al., 2005).

Spawning duration varies considerably among species and appears to reflect differences in maturation synchrony within populations. In N. rhodochorde, mature individuals persist for approximately nine weeks, whereas *Ceratonereis limnetica* and *Hediste atoka* exhibit far shorter reproductive windows of only 3–5 days (Glasby, 1986; Kikuchi & Yasuda, 2006). Extended spawning periods, such as the 13-week duration reported in P. nuntia brevicirris, are typically associated with asynchronous maturation across individuals (Hardege et al., 1994). Taken together, these observations indicate that annual reproductive cycles in nereidids are shaped by a combination of genetic regulatory mechanisms, fecundity, body size, and the interplay of environmental drivers (Tessmar-Raible et al., 2011).

3.4 Early Embryogenesis and Larval Development

Artificial fertilization has provided an important experimental approach for elucidating the early developmental processes of nereidid polychaetes, particularly in tropical species (Table 1). In *Dendronereis javaensis*, mature oocytes and spermatozoa have been successfully fertilized in vitro using seawater media of 15–17 ppt salinity at room temperature (Yuwono et al., 2002). Fertilized eggs of both *Dendronereis javaensis* and *Namalycastis rhodochorde* have also been obtained from mature individuals collected directly from their natural habitats, allowing comparative observation of laboratory-induced and naturally spawned embryos.

Successful fertilization is indicated by the rapid formation of a fertilization membrane (Sawada, 1984), which in *Dendronereis javaensis* appears within 2–3 minutes following exposure to spermatozoa (Yuwono et al., 2002). This developmental response is notably faster than that documented for the temperate species *Perinereis cultrifera*, in which membrane formation requires 10–20 minutes (Bharathidasan et al., 2017), suggesting that tropical nereidids may possess accelerated early cellular activation mechanisms. Following fertilization, the zygote undergoes rapid cleavage divisions.

In *Dendronereis javaensis*, the 2-cell stage is reached approximately 40 minutes post-fertilization, and rotating larvae develop within 2.5–3 hours (Yuwono et al., 2002). A similar pattern is observed in *N. rhodochorde*, where rotating larvae also appear within 2.5–3 hours (Junardi et al., 2020). These developmental rates are substantially faster than those of *P. cultrifera*, in which the 2-cell stage occurs 1.5–2 hours after fertilization and trochophore larvae do not appear until 60–63 hours post-fertilization (Bharathidasan et al., 2017). The comparison highlights a consistent trend: tropical nereidid species exhibit markedly accelerated embryogenesis and larval formation relative to temperate taxa.

Table 1. Comparative Developmental Stages of Fertilized Mature Oocytes in *Dendronereis Javaensis* (Previously Referred to As *Nereis* Sp.; Yuwono Et Al., 2002) And *Namalycastis Rhodochorde* (Junardi et al., 2020)

Development of the fertilized egg	Age	
	Dendronereis javaensis	*Namalycastis rhodochorde*
Formation of fertilization membrane	2 minutes	-
Beginning of cell division	20 minutes	-
2-cell stage	40 minutes	36 minutes
4-cell stage	50 minutes	42 minutes
8-cell stage	1 hour	1 hour
16-cell stage	1.5 – 2 hours	1.5 – 2 hours
32-cell stage	2 – 2.5 hours	2 – 2.5 hours

Broader interspecific comparisons further support this latitudinal pattern. In the temperate polychaete *Nereis diversicolor*, fertilization membrane formation takes approximately 30 minutes, the 8-cell stage is achieved only after 16 hours, and trochophore larvae emerge around five days post-fertilization (Wang et al., 2020). By contrast, the developmental sequence of *N. rhodochorde* more closely parallels that of *Dendronereis javaensis* and, to a lesser extent, *P. cultrifera*: fertilized eggs progress rapidly through the 2-, 4-, 8-, 16-, and 32-cell stages, followed by rotating embryos, trochophore larvae with paired eyes, metatrochophore stages, and three-setiger early nectochaeta Following fertilization, the zygote undergoes rapid cleavage divisions. In *Dendronereis javaensis*, the 2-cell stage is reached approximately 40 minutes post-fertilization, and rotating larvae develop within 2.5–3 hours (Yuwono et al., 2002). A similar pattern is observed in *N. rhodochorde*, where rotating larvae also appear within 2.5–3 hours (Junardi et al., 2020). These developmental rates are substantially faster than those of *P. cultrifera*, in which the 2-cell stage occurs 1.5–2 hours after fertilization and trochophore larvae do not appear until 60–63 hours post-fertilization (Bharathidasan et al., 2017). The comparison highlights a consistent trend: tropical nereidid species exhibit markedly accelerated embryogenesis and larval formation relative to temperate taxa.

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The use of artificial fertilization techniques has thus been instrumental in revealing fine-scale developmental differences across species and climatic regions, while providing baseline data for future studies on larval ecology, reproductive biology, and potential aquaculture applications.

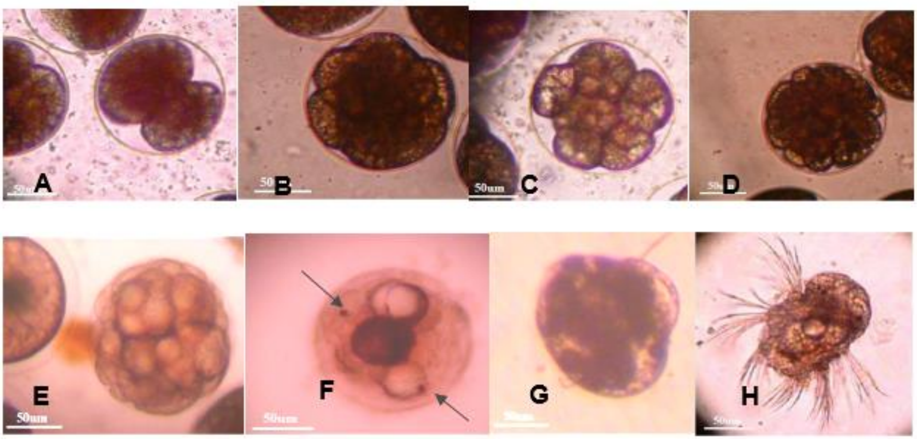

Figure 4. Embryonic development of *Namalycastis rhodochorde* (Junardi et al, 2020): A. 2-cell division (36 minutes); B. 4-cell division (42 minutes); C. 8-cell division (53 minutes); D. 16-cell division (120 minutes); E. 32-cell division (150 minutes, rotating larvae); F. trochophore larvae with two eyes (240 minutes, free-swimming larvae, black arrows indicated eyes); G. metatrochophore (22 hours); H. 3-setiger early nectochaeta (3 days).

3.5 Endocrine Regulation of Reproduction

Endocrinological research on nereidid polychaetes has played a pivotal role in advancing understanding of reproductive regulation and has provided a physiological basis for managing maturation and spawning in aquaculture contexts (Olive, 1999). Early investigations into nereidid neuroendocrinology began with Scharer's pioneering work in 1936, which identified neurosecretory cells in the cerebral ganglia of *Nereis virens*, a temperate-zone species (Baskin, 1976). Subsequent studies demonstrated that hormones produced in the brain exert regulatory control over both gametogenesis and the transition to reproductive metamorphosis (Durchon & Hauenschild, as cited in Pfannenstiel, 1978), thereby establishing the conceptual foundation for a neuroendocrine model of reproduction in the group.

Experimental studies on tropical nereidids have since expanded this framework. In *Dendronereis javaensis* from the Brebes coast of Central Java, surgical removal of the cerebral ganglia accelerates sexual maturation while simultaneously suppressing posterior segment regeneration, indicating that brain hormonal activity diminishes as worms transition from submaturity to full maturity (Yuwono et al., 1994). Comparable findings have been reported in *Nereis diversicolor*, where brain hormone concentrations are elevated in immature individuals but progressively decline during maturation, eventually ceasing upon attainment of full reproductive condition (Golding, 1983; Yuwono, 1992; Golding & Yuwono, 1994).

Implantation experiments—where cerebral ganglia from immature worms are transplanted into sexually mature individuals—reverse the maturation process, lending strong support to a single-hormone model in which the brain-derived factor nereidin inhibits sexual maturation while maintaining gametogenic processes (Andries, 2001; Schenk et al., 2016). The regulatory influence of nereidin on oocyte development has been confirmed through histological and experimental analyses. In decerebrated *Dendronereis javaensis*, oocyte growth proceeds rapidly, but this acceleration is halted when cerebral ganglia are re-implanted, demonstrating the inhibitory role of the hormone in controlling vitellogenesis and maturation timing (Yuwono et al., 1994, 1998). In addition to endocrine control, reproductive synchronization in nereidids is mediated by chemical communication.

Female-derived sex pheromones—such as 5-methyl-3-heptanone in *Platynereis dumerilii* and *Nereis succinea*—induce male spawning responses, while bioactive peptides such as "nereithione" facilitate mate recognition and coordinated gamete release (Zeeck et al., 1990; Ram et al., 1999; Hardege et al., 2004). These findings underscore the integration of endocrine and pheromonal signaling in reproductive behavior. More recent research, however, has challenged the sufficiency of the classical single-hormone nereidin model and points toward a more complex, multi-hormonal regulatory system.

In *N. virens*, the steroid hormone 17β-estradiol has been shown to promote vitellogenesis (Garcia-Alonso et al., 2006), while additional hormones including oxytocin, serotonin, progesterone, and testosterone have been detected in both temperate and tropical nereidids such as *Perinereis* sp., *N. diversicolor*, and *N. rhodochorde* (Durou & Mouneyrac, 2007; Meunpol et al., 2007; Junardi et al., 2014, 2022). These discoveries suggest that nereidid reproduction is governed not by a single inhibitory neurohormone alone, but through a coordinated network of interacting endocrine pathways that respond to both internal physiological status and external environmental cues.

Accordingly, contemporary perspectives increasingly favor a multi-hormone framework that integrates neurosecretory control, steroidal modulation, and environmentally driven endocrine signaling (Lawrence & Soame, 2009). Despite these advances, detailed mechanistic studies—particularly on tropical nereidid taxa—remain limited. Further research combining endocrinology, molecular biology, and experimental ecology will therefore be essential to validate and refine this emerging model of reproductive regulation in Nereididae.

CONCLUSION

Nereidid polychaetes are widely distributed and ecologically important in Indonesian waters, and research over recent decades has generated valuable knowledge on their ecology, reproduction, endocrinology, and taxonomy. These insights underpin their growing relevance in aquaculture, particularly as fishing bait and as a nutritious feed source for shrimp and fish, while emerging evidence also indicates potential roles in bioremediation, cosmetic applications, and human nutrition.

Despite this progress, taxonomic understanding of Indonesian Nereididae remains limited and warrants greater research attention, alongside integrative studies of reproductive mechanisms, endocrine regulation, and ecological functioning. By synthesising current knowledge of the biology and ecology of nereidid worms, this chapter highlights both their ecological significance and applied potential, while identifying key research priorities to support future scientific development, conservation, and sustainable utilisation.

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CHAPTER 3
MICROBIOLOGICAL AIR QUALITY ASSESSMENT OF LABORATORIES IN FEDERAL UNIVERSITY, WUKARI, TARABA STATE

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INTRODUCTION

Microorganisms are found everywhere in our environment and they impact the whole ecosphere in one way or the other. They also influence human in different ways, including nutrient cycling, biotransformation, and disease causation. Hence, microorganisms can be sensitive indicators of environmental quality. Air serves as a very good dispersal medium for microbiota. The types of species and number of organisms present in the air depend on several physico-chemical factors such as temperature, viscosity, suspension of organic and inorganic materials and food availability. Human activities are also an important determining factor for the diversity of microbes in an area (Umana *et al*., 2018). The quality of air inhaled by an individual within his environment determines to a greater degree of the well-being of that individual as infectious agents are suspended in the air (Agwaranze *et al*., 2020).

The transmission of pathogens by air can be direct or indirect. In indirect transmission, droplets containing microbes from infected persons are propelled through the air and deposited on the host body. These Droplets are generated from the source mainly when a person coughs, sneezes, and during the performance of certain procedures such as bronchoscopy (Agwaranze *et al*., 2020). The air inhaled by people is abundantly loaded with microorganisms in the form of bio-aerosols (Kotgire *et al*., 2020). Human activities such as sweeping, walking, waving of clothes etc., can raise dust which is a good vehicle for airborne contamination. Droplets containing airborne microorganisms can be generated through sneezing (Awosika *et al*., 2012).

Fungi are commonly associated with indoor as well as outdoor environments, causing allergy in nearly 10% of people worldwide (Agwaranze *et al*., 2020). People exposed to aerosol with airborne microorganisms and their by-products can develop respiratory disorders, hypersensitivity pneumonitis and toxic syndromes (Agwaranze *et al*., 2020). Fungi and bacteria that cause damage in indoor and outdoor environments may play important role in the biogeochemical transformation of the area. However, the relative humidity of an environment determines to what extent different types of microorganisms are able to grow and multiply on indoor or outdoor materials or environment (Agwaranze *et al*., 2020).

According to World Health Organization (W.H.O) guidelines, today's societies should live in a safer environment, with exposure to contaminants hazardous to health at levels not exceeding internationally agreed standards. Humans also have the right to breathe healthy air, to receive adequate information about potentially harmful exposures, and to be provided with effective containment measures. Nevertheless, exposure to microbial aerosols is still common in many different environments and is often the cause of many adverse health effects. The protection of human health against the risks associated with this type of exposure is not only a legal requirement but also a logical consequence of today's state of knowledge, both the control of exposure to microorganisms and the resultant assurance of safe living and working conditions are not treated with due seriousness. This situation should change quickly, and the knowledge already available in this area and the increasingly widespread use of tools for the precise quantification of exposure to airborne microorganisms should result, if not in elimination, at least in a significant reduction of microbiological hazards. (Rafal *et al.*, 2022).

Statement of Problem

The air inhaled by people is abundantly loaded with microorganisms in form of bio-aerosols (Kotgire *et al.*, 2020). Human activities such as sweeping, walking, waving of clothes etc., can raise dust which is a good vehicle of airborne contamination. Droplets containing airborne microorganisms can also be generated through sneezing (Awosika *et al.*, 2012). Fungi are commonly associated with indoor as well as outdoor environments, causing allergies in nearly 10% of people worldwide (Agwaranze *et al.*, 2020). People exposed to aerosol with airborne microorganisms and their by-products can develop respiratory disorders, hypersensitivity pneumonitis and toxic syndromes (Agwaranze *et al.*, 2020). Fungi and bacteria that cause damage in indoor and outdoor environments may play important role in the biogeochemical transformation of the area. However, the relative humidity of an environment determines to what extent different types of microorganisms are able to grow and multiply on indoor or outdoor materials or environment (Agwaranze *et al.*, 2020).

Justification of Study

Microbiological air quality is an important criterion that must be taken into account when laboratories are designed to provide a safe environment. Hence, this project will provide data and information on microorganisms contaminating the air environment of laboratories in Federal University Wukari, Taraba state Nigeria.

Aim of Study

To determine the Microbiological quality of the air environment of laboratories in Federal University Wukari, Taraba state.

Objectives of The Study

- To isolate and identify bacteria contaminating the air environment of laboratories.
- To isolate and identify fungi contaminating the air environment of laboratories.

1. LITERATURE REVIEW

1.1 Microbial Air Quality

Microorganisms are essential to human survival, health and disease, and hence their environmental abundance and diversity are of great practical importance (Gorny *et al*., 2020). A look at the world of organisms through their genomes has reformulated our perception of the natural system. Life's diversity seen as comprising 3 domains, i.e. bacteria, archaea and eucarya, has been dramatically broadened taking into account both community and ecosystem interrelations (Hug *et al*., 2016).

Bioaerosols are crucial indicators of air pollution and play an instrumental role as risk factors when it comes to the adverse health outcome. These indicators, also known as primary biological airborne particles (PBAPs), have been linked to various health effects, from allergic, through infections, to toxic reactions. PBAPs include all particles having a biological source that is in suspension in the air (bacteria, fungi, viruses, pollen) as well as biomolecules (toxins, debris from membranes such as lipids and proteins).

Rapid urbanization and high population growth rates have made air pollution a global issue. Depending upon exposure time and concentration of pollutants, both indoor and outdoor air pollutants are reported to be detrimental to human health (Salthammer *et al*., 2016). Airborne microorganisms contribute approximately 5-34% of total indoor air pollution (Kim *et al.,* 2017; Awad *et al*., 2018). Moreover, humans spend more time in indoor than outdoor (Yang *et al*., 2015), which makes the indoor air quality (IAQ) a bigger concern to ensure public health protection (Branco *et al*., 2015; Madureira *et al*., 2015a Salthammer *et al*., 2016;). In recent years it has been observed that air pollution is a Global problem. However, air quality policy is not the same in every country/region. Developed countries are introducing more strengthen standards and advanced strategies to reduce air pollution. whereas, developing countries such as China and India, which now suffer from serious air pollution, are beginning to build their environmental management systems. Probably these countries will benefit from existing standards and the experience of the leaders the U.S. and EU. Therefore, the aim of this study was to compare the air quality of the U.S. and the EU. Both regions have long– standing experience in the management of air quality and have a similar economic and technological status. Despite these similarities, these two regions have different approaches to the protection of the environment.

1.2 Behavior and Dynamics in The Air

In the physical sense, microbiological agents can be treated as solid particles. Several physical (size, shape, density, electrical charge), chemical (composition, hygroscopicity), and biological (breathing pattern, route of breathing, anatomy of the airways) factors condition their behavior both in the air and, if inhaled, in the lungs. The basic and one of the most important characteristics of microbial particles is their diameter. The sizes of microbial aerosol particles range from ~0.02 μm up to tens of micrometers. Bioaerosol particles are rarely spherical and, when studied, their usually irregular shapes are characterized using certain equivalents of particle diameter (in aerobiology, the most frequently used are aerodynamic and optical approximations). It is worth mentioning that data from remote measurements suggest that microbial particles usually tend to form aggregates larger than 2.5 μm in diameter.

Both equivalent diameters may be significantly distinct from their physical dimensions. For example, the dimensions of *Bacillus subtilis* endospore-forming gram-positive rods and *Cladosporium* cladosporioides fungal conidia, whose physical sizes of 0.7–0.8 × 1.5–1.8 μm and 3–7 × 2–4 μm correspond with aerodynamic diameters of 0.9 μm and 1.8 μm, respectively. To be inhaled, microbial particles must be airborne for a sufficient period of time. In the vast majority of environmental situations, when the air is almost permanently turbulent, such a time frame is characterized by the 'half-life' term. In brief: as rarely spherical, the sedimentation velocity of airborne microbial particles depends inter alia on the square of the particle diameter. (Kulkarni *et al*., 2011).

1.3 Qualitative Techniques and Analyses for Microbial Propagules

A recently observed tremendous development of analytical techniques enables a comprehensive characterization of the biological, chemical and physical features of microbial aerosol particles. This is particularly evident in the case of deoxyribonucleic acid (DNA)-based and ribonucleic acid (RNA)-based methods including taxonspecific quantitative polymerase chain reaction (qPCR) or droplet digital PCR (ddPCR). The use of these methods in both Sanger sequencing-based and Next Generation Sequencing (NGS) analyses enables not only the identification of individual genera or species but also the characterization of diversity and metabolic potential of airborne microbiota. (Madsen *et al*. 2015).

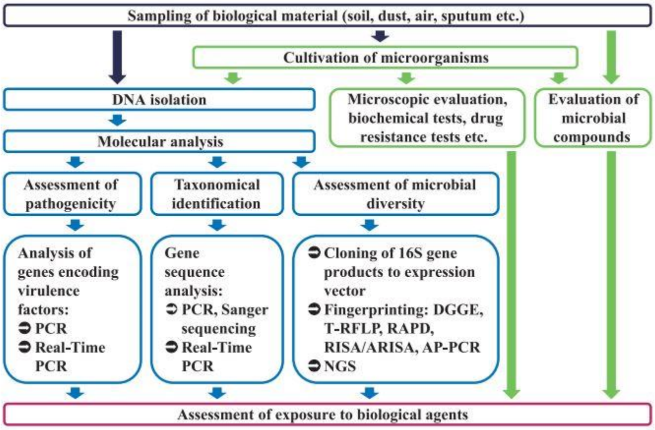

Figure 1. Procedure in The Assessment of Exposure to Harmful Biological Agents

1.4 Global Air Report

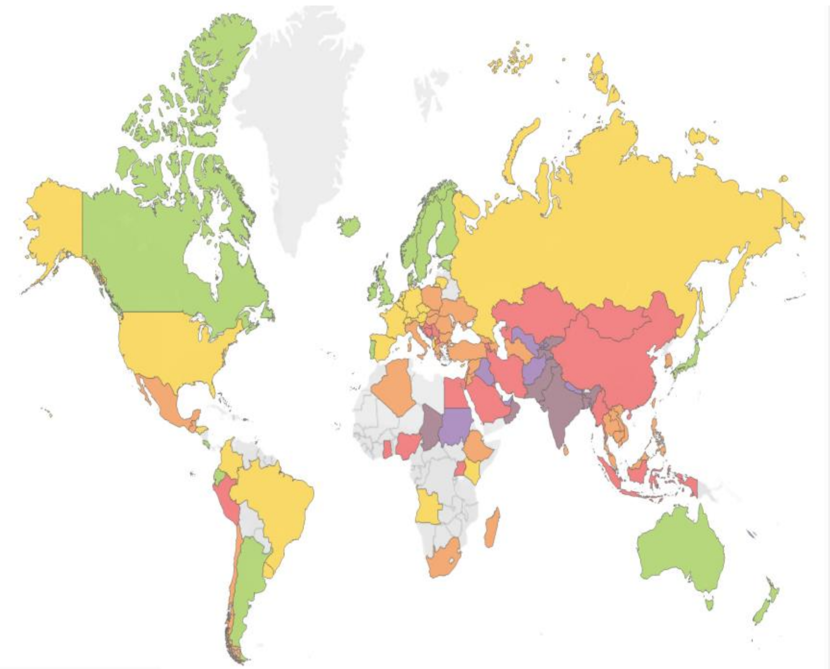

Figure 2. Global Air Quality Report

Countries and regions in East Asia, Southeast Asia, and South Asia suffered from the highest annual average PM2.5 concentration weighted by population. Notably, the African continent had only 13 out of 54 countries with sufficient public air quality monitoring data, resulting in a majority of countries showing in gray. Additionally, the Latin America and Caribbean region also lacked sufficient monitoring. Publicly available air quality monitoring station data continued to increase in 2021. India, New Zealand, and Canada, in particular, saw significant increases. Government air quality monitoring networks continue to be most commonly found in China, the United States, Japan, and in Europe.

It should be noted that developed countries have a higher density of air quality monitoring stations than developing countries and regions. Latin America, Africa, and Central Asia remain sparsely monitored, despite the high population density in many of these regional areas. Given the low-cost point, ease of deployment, and significant reduction in operational costs, low-cost air quality monitors are increasingly becoming a viable public alternative to those countries, regions, and territories that lack government-operated air quality monitoring stations. In 2021, non-governmental low-cost air quality monitoring provided the only real-time air quality data for countries like Albania, Angola, Azerbaijan, the Bahamas, Belize, Benin, Bermuda, Cambodia, Cameroon, Cape Verde, Democratic Republic of the Congo, Dominican Republic, Egypt, Ethiopia, French Polynesia, Gambia, Grenada, Guam, Guyana, Honduras, Jamaica, Jersey, Lebanon, the Maldives, Moldova, Morocco, Nicaragua, Niger, Panama, Qatar, the Republic of the Congo, Rwanda, Saba, Senegal, Sierra Leone, South Sudan, Suriname, Timor-Leste, Togo, Uruguay, U.S. Virgin Islands, Venezuela, Zambia, and Zimbabwe.

1.5 Air Quality Index (AQI)

An air quality index (AQI) is used by government agencies to communicate to the public how polluted the air currently is or how polluted it is forecast to become. AQI information is obtained by averaging readings from an air quality sensor, which can increase due to vehicle traffic, forest fires, or anything that can increase air pollution. Pollutants tested include ozone, nitrogen dioxide, and sulphurdioxide, among others.

Public health risks increase as the AQI rises, especially affecting children, the elderly, and individuals with respiratory or cardiovascular issues. During these times, governmental bodies generally encourage people to reduce physical activity outdoors, or even avoid going out altogether. The use of face masks such as cloth masks may also be recommended. Different countries have their own air quality indices, corresponding to different national air quality standards. Some of these are the Air Quality Health Index (Canada), the Air Pollution Index (Malaysia), and the Pollutant Standards Index (Singapore). (WHO, 2016a).

Atmospheric air pollution is the 4th largest health threat worldwide and the top most environmental risk to human health. Outdoor air pollution contributes about 4.2 million premature deaths annually, with particulate matter noted as the major contributor to air pollution and having the greatest health risk among the air pollutants (WHO, 2020). Globally, nine out of ten people breathe unsafe polluted air; resulting to approximately 7 million deaths annually, as more than 90% of people live in settlements with unhealthy`y air quality (WHO, 2016a; WHO, 2018a). An increase in ambient particulate matter (PM10) load by 10 μg/m3 have been reported to reduce life expectancy by 0.64 years (Ebenstein, Greenstone, & Zhou, 2017; Edokpa & Ede, 2019). It is specified that reducing PM10 pollution from 70 to 20 (μg/m3) and annual PM2.5 from levels 35 (μg/m3) commonly noted in developing countries to 10 (μg/m3) can reduce air pollution related death by 15% (WHO, 2018b). Urban air quality is noted to be improving in cities of developed countries as against those of low- and middle-income countries such as Abuja in Nigeria (WHO, 2016b).

Nigeria is said to have the highest burden of mortalities from poor air quality in Africa and 4th globally (Health Effects Institute, 2018). The country was ranked 150th out of 180 countries for poor environmental performance index on air quality (Yale Center for Environmental law and Policy, 2018). Some cities across Nigeria have been noted to have poor air quality (WHO, 2016c; Yakubu, 2017; Ede & Edokpa, 2017; Akinfolarin *et al*., 2017; Edokpa & Ede, 2019) and with continuous increase in population, urbanization, Anthropogenic activities and climate change, concern on the state of air quality in Abuja and other cities across the world such remains important discuss (Petkova *et al*., 2013).

The study assessed suspended particulate matter of size PM10 and PM2.5 during dry and wet seasons, through which prevalent ambient air quality of the selected locations was evaluated and air quality index (AQI) computed. (WHO, 2016a).

1.6 Computing Air Quality Index

The air quality index is a piece wise linear function of the pollutant concentration. At the boundary between AQI categories, there is a discontinuous jump of one AQI unit. To convert from concentration to AQI this equation is used:

(If multiple pollutants are measured, the calculated AQI is the highest value calculated from the equation applied for each pollutant).

$$I = \frac{I_{high} - I_{low}}{C_{high} - C_{low}}(C - C_{low}) + I_{low}$$

"International Air Quality" 2020

where:

I= the (Air Quality) index,

C = the pollutant concentration,

C_{low}= the concentration breakpoint that is ≤ ,

C_{high}= the concentration breakpoint that is ≥ ,

I_{low}= the index breakpoint corresponding to C_{low}

I_{high}= the index breakpoint corresponding to C_{high}

1.7 Air Quality Health Index

A new Air Quality health index (AQHI) was developed in Canada to understand the state of local air quality with respect of public health. AQHI is available for about ten communities in Canada, including Vancouver and

Victoria. The AQHI is constructed as the sum of excess mortality risk associated with NO2, ground-level O3, and PM2.5 at certain concentrations.

It is calculated hourly based on 3-hour rolling average pollutant concentrations, and is then adjusted to a scale of 1 to 10. The value of 10 corresponds to the highest observed weighted average in an initial data set covering a reference period from 1998 to 2000 (Stieb *et al.*, 2008; Taylor, 2008). The scientific basis for the formulation of AQHI is based on the epidemiological research undertaken at Health Canada. Relative risk (RR) values are estimated, based on the local time series analyses of air pollution and mortality (Stieb *et al.*, 2008; Taylor, 2008).

Air Quality Health Index is determined by the eqn. as shown below:

A Q H I = - 1--0-- Σ *c i*=1….*p* 100(*eβiXi*-1) (18)

Where, βi is the regression coefficient from same Poisson model linking the ith air pollutant with mortality, xi is the concentration of the ith pollutant, and c is the Scaling factor.

1.8 Indoor Air Quality

Indoor air quality (IAQ) usually refers to the air quality within and around buildings and structures that are other than industrial environments. These building premises include; colleges, hospitals, offices, restaurants, homes, and similar partially closed settings. (Hulin *et al.*, 2012), where people stay inside more than 90% of their time. (Larrey *et al.*, 2020).

IAQ is a major public health concern and known to affect the health, comfort, and well-being of the residents, especially those individuals with comorbidity from respiratory and allergic problems and who have suppressed immunity level. Pollution of the interior environment has been linked to many human health problems. Sick building syndrome and building-related diseases are the two major categories that can lead to many other socio-economic problems like reduced productivity and impaired learning in schools. (Larrey *et al.*, 2020). We can divide the health effects of indoor air pollution into acute and chronic cases based on when the issue first appeared. Some illnesses develop quickly following a single or severe exposure to the pollutants. The eye, nose and throat irritation, headaches, dizziness and fatigue are categorized under this group and are treatable. Sometimes the treatment is simply avoiding the person's exposure to the source of the pollution and concentrating on the contributing factors if they are identified.

Soon after such exposure to these pollutants, symptoms of some diseases such as asthma can also be occurred, aggravate or worsen (Fujiyoshi *et al.*, 2020) Another health problem of indoor air pollution is chronic health cases that could happen after a long period or repeated exposure. These health defects include some respiratory diseases, heart disease, and cancer. They can be severely impairment or leads to life-threatening consequences unless preventive measures are taken. This can be achieved by improving the IAQ, even though the signs and symptoms of the cases are not noticeable. (Larrey *et al.*, 2020).

Factors Affecting Indoor Air Quality

Many physical and environmental factors influence IAQ. These factors affect the fresh air coming into the building and include human activities, poor ventilation (lack of outside air or poor indoor air circulation), fluctuation in temperature and humidity and other activities in or around the interior environment of the building premises. The entry of contaminants from the outdoor environment like dust from different activities and chemical emissions (construction or renovation, cleaning supplies, pesticides, etc.) may also contribute to poor IAQ. (Kumar *et al.*, 2020).

Bacteria, fungi, and viruses, collectively microorganisms are the key elements of indoor air pollutants and contribute about 5% to 34% to indoor air pollution health problems. Particular activities like talking, sneezing, coughing, walking, and washing can majorly release these airborne biological contaminants and form air suspensions that increase exposure for the inmates. Indoor air quality problems for prisoners can be even more serious, due to their confinement and exposure level, duration, and frequency. (Landry KG *et al.*,2020).

Outdoor Air Quality

Outdoor air is the air outside buildings, from ground level to several miles above theEarth's surface is a valuable resource for current and future generations because it provides essential gases to sustain life and it shields the

Earth from harmful radiation. Air pollution can compromise human health and the environment in many ways. For example, outdoor air pollution:

Is associated with a number of human health effect including heart attacks, asthma attacks, bronchitis, hospital and emergency room visits, work and school days lost, restricted activity days, respiratory symptoms, and premature mortality. Outdoor air quality may be affected by car exhaust, emissions from factory smoke stacks, and road dust. Pollen in the atmosphere also contributes to a reduction in outdoor air quality. A range of instruments are available for measuring outdoor air quality using sensor-based instruments. The transmission of pathogens by air can be direct or indirect. Indirect transmission, droplets containing microbes from infected persons are propelled through the air and deposited on the host's body. Droplets are generated from the source mainly when the person coughs, sneezes and during the performance of certain procedures such as bronchoscopy. Dissemination can be withered by air-borne droplets containing microorganisms that remain suspended in the air for a long period of time or dust particles containing the infectious agents (Jain, *et al*., 2005).

Sources of Indoor and Outdoor Air Pollutants

Generally, pollutants, especially animals' excreta and secretions, are the main source of microbial air contamination (Chmielowiec-Korzeniowska *et al*., 2018). Molecular biology methods used by Nehme *et al*. (2008) confirm that animal faeces are the main source of microbial air contamination in houses. Enterohaemorrhagic E. coli O157:H7 serotypes, which cause haemorrhagic colitis in humans, may also be found in the solid or liquid manure of cattle, and less often of pigs and poultry. Pathogenic microorganisms such as *Salmonella spp*., *Campylobacter spp*., *Listeria monocytogenes*, or *Yersinia enterocolitica* from diseased animals or carriers can also be found here (Manyi-Loh *et al*., 2016).

Microorganisms also enter the air from feed and microbiologically contaminated water. Research confirms that feed and drinking water contaminated with faeces of sick animals or carriers can be an important source of pathogenic strains of Salmonella spp. and E. coli (Truszczyński and Pejsak, 2015). High temperature and high humidity are conducive to their multiplication (Budzińska *et al.*, 2014).

Factors affecting the Spread of Microbes in Air

As the locations of microbial aerosol sources are (in an obvious way) not spatially uniformly distributed and their productivity differs, both temporal (including diurnal, seasonal, and annual) and environmental changes in bio aerosol structure can be observed. Moreover, microbial sources do not release bio aerosol particles continuously as numerous physical (temperature, relative humidity, availability of water and nutrients, radiation, environmental oxygen presence, etc.) and biological parameters. (Bhangar *et al.*, 2016). From the above-mentioned physical factors, one of the major factors is temperature. It directly affects the rate of microbial metabolism, reproduction, and cultivability. It also correlates with a number of important meteorological and climatic variables (e.g. air turbulence, time of day and season) that may affect concentrations of microorganisms in the air. For both of these parameters, however, their proper environmental levels are strictly connected with specific microbial strains determining the issues of survival in the air, (if deposited) conditioning the possibilities to colonize the surfaces and as such should be individually considered. Regarding radiation, each type acts destructively on microbial particles. (Meadow *et al.*, 2015).

Sunlight has a known bactericidal effect due to the part of the spectrum that includes short, violet and ultraviolet waves. For example, the vegetative bacterial forms (including Mycobacterium tuberculosis) die after several hours of irradiation, especially in the summer (Carroll *et al.*, 2016). Also, artificial (xenon) radiation with the same wavelength spectrum as solar light reduces the quantity of viable microorganisms (which was shown in laboratory experiments with *Escherichia coli* or *Serratia marcescens* aerosols) (Carroll *et al.*, 2016). Ultraviolet rays (with wavelengths from 250 nm to 280 nm), just as other high-energy radiation types (such as gamma and X rays), penetrate into microbial cells and induce a number of reactions, including those with free radicals. Such processes damage the structure of nucleic acids, proteins, carbohydrates, lipids, and cell membranes, leading to changes in cell functioning and/or death. (Carroll *et al.*, 2016). Microorganisms have different requirements for the atmospheric presence (or absence) of oxygen. Depending on the degree of oxygen tolerance, they are divided into four groups: absolute or relative anaerobes, microaerophiles (tolerant anaerobes), and absolute aerobes.

The influence of oxygen on microbial aerosols may reveal both toxic and protective effects. The positive effect of oxygen is hypothesized based on laboratory experiments with the suppression of activity of toxic substances, whereas the toxicity is due to its enzymatic reduction inside the cell to hydrogen peroxide and toxic free radicals (O2 –). Aerobes and relative anaerobes are protected against these products by the presence of superoxide dismutase or catalase enzymes. The oxygen toxicity to the microbial cell depends on the degree of cell hydration and is observed at a relative humidity below 65–70 % when the oxygen content is about 30 % (further increase in oxygen content no longer produces an additional toxic effect). (Carroll *et al.*, 2016).

Effects of Air Quality

The effects of air pollutants on the respiratory system are well known and include the development and exacerbation of chronic obstructive pulmonary disease, asthma, and lower respiratory tract infections including pneumonia (Gabriela et'al.,2021). Some initial research has suggested that air pollution may even have been a contributor to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and mortality related to coronavirus disease 2019 (COVID-19). Another matter for concern is the fact that the effects of air pollution appear to be influenced by global warming and climate change; rising greenhouse gas levels are pushing up temperatures and thus increasing the impact of air pollution on health, and are changing the seasonality and distribution of many infectious diseases worldwide (De Bruyne et'al.,2021). Among microbial aerosol particles, one can find: infectious agents, allergens, toxins and other biological compounds that are able to provoke similar toxic effects (e.g. endotoxins, β-glucans), carcinogens (e.g. mycotoxins), and biologically active (submicrometric and nanometric) fragments of microorganisms. All these particles may perform a causative role of many adverse health conditions. (Rafal *et al.*, 2022).

During the inhalation of microbial aerosols, both the upper and lower airways are exposed. The diseases (if they occur) most often appear within the part of respiratory tract into which the particular microbial agent penetrates and deposits.

For example, the influenza virus (representing the Orthomyxoviridae family) together with other rhino-, adeno-, and coronaviruses that are responsible for the common cold usually infect the upper respiratory tract.

Also, sensitization diseases (such as allergic rhinitis or sinusitis) are located in the upper airways (Rafal et'al.,2022). In turn, lower respiratory tract diseases (such as bronchitis or pneumonia) are mainly provoked by aerodynamically bigger structures such as bacteria (e.g. *Legionella spp*., *Streptococcus spp*., *Haemophilus influenzae*) (Gabriela et'al.,2021).

Control of Air Quality

Mechanical ventilation: Mechanical ventilation is essential for diluting indoor air pollutants by exhausting the contaminated indoor air and introducing clean outdoor air into an air-conditioned building. Moreover, local exhaust ventilation by means of chemical fume hoods and biological safety cabinets should be used to control critical emission sources of contaminants in places like rooms, laboratories, tissue processing rooms e.t.c (Alan H.S. Chan,2020).

Filtration: Filters can effectively trap particulate contaminants, including microbiological pathogens, and remove them from the circulating air. Various grades of filters can be used to achieve different degrees of cleanliness. (Alan H.S. Chan,2020).

Directional airflow control: Besides air leakage between two adjacent areas, the cleanto-less-clean principle is applied to the airfl ow within an enclosed environment. The air movement should be from clean zones to zones of progressively greater contamination. (Alan H.S. Chan,2020).

Ultraviolet germicidal irradiation (UVGI) disinfection: UV radiation of wavelength 220–300 nm can penetrate cell walls and inactivate tiny airborne droplet nuclei by disrupting their reproductive mechanisms. Airborne pathogens, such as multidrug-resistant *Mycobacterium tuberculosis* bacteria, *Legionella* bacteria, and measles viruses, can be killed by UVGI. (Alan H.S. Chan,2020).

2. MATERIALS AND METHODS

Study area

The study was carried out in Federal University Wukari, Taraba State, Nigeria. Wukari is located at southern guinea savanna with coordinates latitude 7E, 51'N-7.850EN and longitude 9E, 47'E-9.783EE, annual precipitation of 1205 mm and it has an average temperature of 26.8E. Federal University Wukari is located along Katsina-Ala road in Wukari local Government Area. The samples were collected from various Laboratories in Federal University Wukari: Biology, Physics, Chemistry, and Biochemistry Laboratories respectively. An informed consent was sort and granted by the Laboratory attendants before sampling was done.

Materials Used

The materials used in this study comprises of the following; universal container, petri dishes, beaker, conical flask, measuring cylinder, glass slide, wire loop, Bunsen burner, hydrogen peroxide, biochemical test reagents, disinfectant, Gram staining reagents, Masking tape, hand glove, nose mask, incubator, microscope, autoclave, Nutrient Agar, Sabroaud Dextrose Agar, MacConkey Agar, Chloramphenicol, e.t.c

Sample collection

50 Samples were collected at strategic points in the Laboratories by exposing freshly prepared nutrient agar and MacConkey agar plates to the air for 60 minutes. Plates were then incubated immediately for 18-24 hours at 37ºC.

Media preparation

The media used include; Nutrient agar, Sabouraud Dextrose agar, triple sugar iron agar and MacConkey Agar. The media were prepared according to the manufacturer's instructions from ready-to-use dehydrated agar powder. All media were sterilized for 15 minutes at 121^0C and 15psi in an autoclave. Prepared molten media were allowed to cool to ambient temperature before aseptically dispensing into Petri dishes and test tubes based on the required consistency.

Identification of Isolates

Distinct colonies were sub-cultured on freshly prepared nutrient agar and successively streaked to obtain pure cultures while pure fungal cultures were obtained using spot inoculation. Pure cultures of bacteria were characterized and identified using, colonial characteristics, Gram staining, and Catalase, oxidase and Triple sugar ion tests. Fungi were identified based on their cultural characteristics and lactophenol cotton blue stain.

Gram Staining

Each representative colony was used to form a smear on a slide. The heat-fixed smear was laid across a staining rack and placed over a sink. Dry smear was flooded with crystal violet and allowed to stand for 1 min, afterwards the smear was washed off with water. Lugols iodine was now used to flood the smear to remove excess stain for 45 seconds. Thereafter, the smear was now washed with water and flooded with safaranin for 60 seconds. The smear was now washed off after 30 seconds and allowed to air dry. After drying the slide will be viewed using oil immersion lens:

Principle of gram staining: The principle of gram staining is based on the ability of Bacteria cell wall to retain the crystal violet dye during solvent treatment.

Materials and equipment's for gram staining:

- The materials/equipment's used for gram staining used include:
- Alcohol-cleaned microscope slide
- Slide rack
- Microscope

Reagents used include:

- Crystal violet (primary stain)
- Gram's iodine solution (the mordant)
- Ethanol (50:50 v:v) (decolorizer)
- 0.1% basic fuchsin solution (the counter stain)
- Water.

Procedure for gram staining: Each representative colony was used to make a thin smear on a slide with the aid of a wire loop and left to air dry and after which it was heat fixed and allowed to cool. The air-dried smears were covered with crystal violet stain for 30-60seconds and rapidly washed off with clean water. Then it was covered with gram's iodine for 30-60seconds and rapidly washed off with clean water. The smears were decolorized rapidly with alcohol and washed out immediately with clean water. After which the smears were covered with 0.1% basic fuchsin solution for 30-60seconds and washed immediately with clean water. The stained smears were then allowed to air-dry. After drying, a few drops of oil immersion were dropped on the stained smears and viewed with the aid of a microscope (×100 oil objective lens) to check for the microscopic properties of the organisms like the Gram reaction and morphology (Taofeeq and Joy, 2021).

Expected result: Gram-positive bacterium appears purple while Gram-negative bacterium appeared Pink

Catalase Test

The test is used to distinguish those bacteria that produce the enzyme catalase, such as *staphylococcus* species from non-producing ones such as *streptococcus* species (Cheesebrough, 2015).

Principle of catalase activity: The principle of catalase activity is based on the ability of the enzyme catalase to acts as a catalyst in the breakdown of hydrogen peroxides to oxygen and water.

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2 \text{ (gas bubbles)}$$

Materials for catalase activity test:

- Microscope glass slide
- Applicator stick

Reagent used include;

- Hydrogen peroxide (H_2O_2)

Procedure for catalase activity test:

- A drop of hydrogen peroxide was placed on a microscopic clean grease free slide, using an applicator stick.
- A 24hr colony of the bacterial isolate was emulsified onto the hydrogen peroxide and reaction was observed.

Test result: A positive result was indicated by the immediate formation of gas bubble.

Oxidase Test

The oxidase test detects the presence of a cytochrome oxidase system that will catalyze the transport of electrons between electron donors in the bacteria and a redox dye- tetramethyl-*p*-phenylene-diamine.

Principle of oxidase test: The principle of oxidase test is based on the oxidization of the substrate "tetramethyl-p-phenylene-diamine dihydrochloride" to indophenol using bacteria cytochrome oxidase enzyme. Electrons are transferred from the reagent to cytochrome *c* and thence, via cytochrome *c* oxidase, to molecular oxygen (Varghese *et al.,* 2014).

Materials used for oxidase test:

- Filter paper
- Platinum wire loop

Reagent used include:

- Oxidase reagent

Procedure for oxidase test:

- The filter paper method was adopted for this work.
- A clean filter paper was moistened with few drops of oxidase reagent, and bacterial growth was smeared onto the moist filter paper with a platinum loop (Nichrome or iron-containing loops may give a false-positive reaction).

Result: In a positive test, a purple coloration (due to oxidation of the reagent) develops immediately or within 10 seconds.

Tripple Sugar Iron (TSI)

The Triple Sugar Iron (TSI) test is a microbiological test roughly named for its ability to test a microorganism's ability to ferment sugars to produce hydrogen sulphide.

Principle of triple sugar ion test: The principle of triple sugar ion test is based on the differences in carbohydrate fermentation patterns and hydrogen sulfide production among different bacteria employing the triple sugar ion agar.

Materials used in triple sugar ion test:

- Triple sugar ion agar
- Wire loop

Procedure for triple sugar ion (TSI) test:

- A sterilized straight wire loop was used to touch the top of a well-isolated colony
- It was then inoculated into a TSI Agar by first stabbing through the center of the medium to the bottom of the tube and then streaking on the surface of the agar slant
- After which it was incubated for 24 hours at 37^0C.

Result: The development of yellow color with butt, slant, gas and hydrogen sulphide indicates a positive result and black coloration indicate a negative reaction (Regina *et al.*,2020).

3. RESULT

Fifty (50) samples from air were collected from Laboratories at Federal University using the settled plate methods. The result revealed the isolation of 5 fungal and 3 bacteria isolates. These include *Aspergillus niger Aspergillus flavus*, *Fusarium oxysporium*, *Geotrichum candidum*, *Aspergillus fumigatus*, *Pseudomona aeruginosa*, *Staphylococcus* spp, *Klebsiella pneumonia* (Table 1 and 4). Table 2 shows the percentage occurrence of bacteria isolates on the samples. The result showed that *Staphylococcus* species (52.94%) was the most prevalent followed by *Klebsiella pneumoniae* (35.29%) while *Pseudomona aeruginosa* (11.76%) was the least. Table 4 shows the percentage occurrence of fungi isolates on the samples. The shows that *Aspergillus niger* and *Fusarium oxysporium* (28.57%) were shown to be the most frequently isolated airborne fungal organisms while *Geotrichum candidum* (14.28%) was the least. Figure 1 shows the frequency distribution of bacteria in Federal University, Wuakri laboratories. Figure 2 shows the frequency distribution of bacteria in Federal University, Wuakri laboratories.

Table 1. Bacteria Isolate from Air and Their Identification

		Microscopy		**Biochemical Test**				**Sugar Fermentation**
S/N	Colony Characteristics	Morphology	Gram Stain	Catalase	Oxidase	T.S.I		Bacteria Isolate
1.	Shining, yellow, spherical colonies	Cocci in cluster	+	+	-			*Staphylococcus* spp
2.	Green colonies with a typical matte surface and rough periphery	Rods	+	-	-			*Pseudomonas aeruginosa*
3.	Extremely mucoid, whitish colonies	Rods	-	+	-			*Klebsiella pneumoniae*

\+ = Positive, spp = species, Coagula =Coagulase

\- = Negative, spp = species.

Table 2. Percentage Occurrence of Bacteria Isolates on The Sample*s*

Organisms	Number of positive	Percentage
Pseudomona aeruginosa	2	11.76%
Staphylococcus spp	9	52.94%
Klebsiella pneumoniae	6	35.29%
Total	17	99.99%

Table 3. Morphological Characteristics of Fungi Isolate from Air

S/NO	Colony Characteristics at the surface	Reverse	Microscopic Appearance	Probable Organisms
1	Wooly at first white to yellow, then turning to dark brown black	White to yellow	Double cover, entire vesicle form "radiate" head	*Aspergillus niger*
2	Velvety, yellow to green brown	Golden to red Single and double cover, entire	Single and double cover, entire vesicle point out in all directions	*Aspergillus flavus*
3	Blue-green pigmentation with suede- like surface consisting of a dense felt of conidiophores	Pale yellow	A single series of phialides, rounded and rarely conidia	*Aspergillus fumigatus*
4	pale, or bright-colored	Yellow to brown	Macroconidia are hyaline, two to several-celled, fusiform to sickle-shaped	*Fusarium oxysporium*
5	Flat, white to cream colony	Colourless	Hyphae are hyaline, septate, branched	*Geotrichum candidum*

Table 4. Percentage Occurrence of Fungi Isolates on The Sample*s*

Onisms	Number of positive	Percentage
Aspergillus niger	2	28.57%
Aspergillus fumigatus	1	14.28%
Aspergillus flavus	1	14.28%
Fusarium oxysporium	2	28.57%
Geotrichum candidum	1	14.28%
Total	7	99.98%

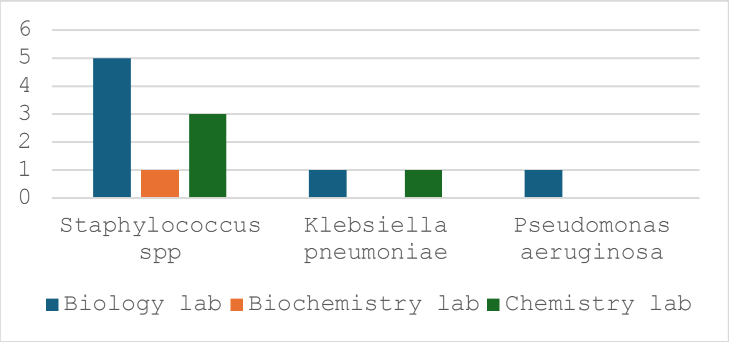

Figure 1. Frequency Distribution of Bacteria in Federal University Laboratories

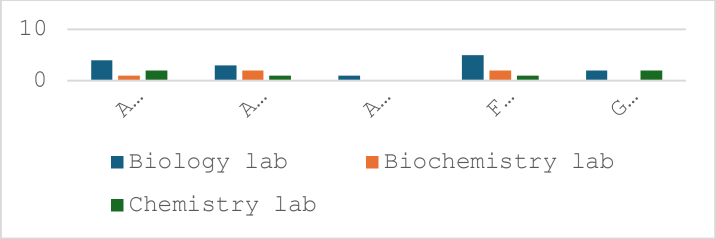

Figure 2. Frequency Distribution of Fungi in Federal University Laboratories

4. DISCUSSION, CONCLUSION AND RECOMMENDATION

Discussion

One of the most important studies to identify the level of microbial air pollution is the microbiological assessment of air study. To evaluate the health risks and establish standards for indoor air quality control, information on the indoor microbiological concentrations of airborne bacteria and fungi is required.

In this study, the bacteria isolated include *Staphylococcus* species, *Klebsiella pneumonia, Pseudomonas aeruginosa*. Similar findings and isolates have been reported by Ekhaise and Ogboghodo (2011) in their investigation of airborne microflora in the atmosphere of a hospital environment at the University of Benin Teaching Hospital (UBTH), Benin City, Nigeria. Bacterial isolates include *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli, Bacillus cereus, Serratia marcescens*, and *Micrococcus* species.

Fungal Isolates such as *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporium*, *Geotrichum candidum*, *niger* and *Fusarium oxysporium* were the fungi species isolated.

Additionally, comparable results and isolates have been reported by Agwaranze *et al*. (2020), in their investigation of Microbiological Assessment of Indoor and Outdoor Air Quality in a General Hospital in North-East Nigeria. These isolates included *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus* spp., *Staphylococcus* spp., *Escherichia coli*, *Micrococcus* spp., *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus, Aspergillus verssicolor, Trycophyton spp. and Ulacladium spp. Aspergillus niger and Aspergillus flavus*

The quantity and variety of airborne microbes can also be used to gauge how clean an area or environment is, according to Agwaranze *et al*. (2020). Therefore, there is a need for interventions to reduce the conditions that encourage the development and spread of airborne microorganisms in the research region. These microorganisms are known to be associated with nosocomial infections. *Staphylococcus* spp is known into cause infections of the skin, deeper tissue and organs as well as pneumonia (Agwaranze *et al*., 2020). These bacteria in the wards could originate from the staff, visitors, ventilation and air conditioning systems or patients. *Aspergillus* species is a common mould that causes the infection aspergillosis. Most people breath in *Aspergillus* spore every day without getting sick but it does affect people with wreaked immune system or lungs disease that have high risk of developing problems due to *Aspergillus* (Abu *et al*., 2016)

CONCLUSION

According to the results of the current investigation, there were potential harmful microorganisms in the labs at Federal University in Wukari, and there was a correlation between the species and number of the organisms.

The bacteria and fungi from air that were collected for this investigation could provide risks for the spread of infection in the laboratory.

Recommendation

Regular air quality monitoring is required to spot and manage environmental elements that encourage the development and proliferation of microorganisms.

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CHAPTER 4
TIME AND DOSE DEPENDENT LARVICIDAL EFFICACY OF A NOVEL IMINE-BASED COMPOUND AGAINST *CULEX QUINQUEFASCIATUS*: PROBIT-BASED TOXICOLOGICAL ASSESSMENT

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INTRODUCTION

Mosquitoes are among the most important vectors of human diseases, transmitting pathogens responsible for malaria, filariasis, dengue, chikungunya, and other life-threatening illnesses. *Culex quinquefasciatus* is a well-known vector of lymphatic filariasis and is widely distributed in urban and semi-urban (Munusamy et al., 2016) areas of Bangladesh. Therefore, mosquitoes and mosquito-borne diseases have become challenging problems that have social and economic impacts (Raveen et al., 2014).

Adult and larval mosquito control have been undertaken by many vector control programs to suppress mosquito-borne diseases in many countries (Miah, 2021). Effective control of mosquito populations (Culver, 1925), particularly at the larval stage, is considered one of the most practical and environmentally manageable approaches for reducing disease transmission (Mozhiyarasi & Anuradha, 2018).

In recent years, *Bacillus thuringiensis israelensis* (Bti) and insect growth regulators (IGRs) have been used widely to control mosquito larvae which are comparatively expensive, and they have some limitations too. Copper sulphate pentahy-drate (CuSO4·5H2O) solution has been used as a mosquito larvicide against 3rd in-star larvae of *Ae. aegypti, An. quadrimaculatus*, and *Cx. Quinquefasciatus* (Miah, 2021).

The compound 2-((2-Hydroxybenzylidene) amino)-2-(hydroxymethyl) propane-1,3-diol, is a derivative of a polyol that may exhibit interesting chemical properties due to the presence of both hydroxymethyl and imine functional groups. In the case of benzylidene derivatives, studies have shown that these compounds can be synthesized through the reaction of phenolic compounds with aldehydes, leading to products with potential biological activity (Kumar et al., 2015). Polyols like propane-1,3-diol derivatives are often used in the synthesis of polymers and other materials and their ability to act as cross-linking agents or as building blocks for more complex structures has been explored in various studies (Zhang et al., 2018). The chemistry of imines is well-documented, with applications ranging from organic synthesis to catalysis. Imines are known to participate in various reactions, including nucleophilic additions and cycloadditions (Meyer et al., 2016).

Determination of LC_{50} values at different exposure times provides a reliable measure of larvicidal efficacy (Ganesan et al., 2023). Therefore, the present study was designed to assess the larvicidal activity (Senthilkumar et al., 2008) of a newly synthesized compound, 2-((2-Hydroxybenzylidene)amino)-2-(hydroxymethyl)propane-1,3-diol, against *Culex quinquefasciatus* larvae under laboratory conditions and to determine the dose- and time-dependent mortality of mosquito larvae exposed to different concentrations of this compound. The extensive use of conventional chemical insecticides has resulted in the development of insecticide resistance (Pushpanathan et al., 2008), environmental pollution, and adverse effects on non-target organisms (Noosidum et al., 2008). These limitations have increased the demand for alternative mosquito control agents that are effective, selective, and environmentally safer. In this context, newly synthesized chemical compounds with larvicidal properties are receiving increasing attention.

1. LIFE CYCLE OF *CULEX QUINQUEFASCIATUS*

The *Culex quinquefasciatus* life cycle involves four stages: **Egg, Larva, Pupa, and Adult**, requiring water for development. Females lay "rafts" of 100-300 eggs on stagnant, often polluted, water, which hatch into larvae that filter feed in the water. Larvae develop into non-feeding pupae, from which winged adults emerge, with females needing blood meals for egg production, completing the cycle.

Egg Stage:

- **Description:** Females lay eggs in clusters (rafts) on the surface of fresh or stagnant water.
- **Habitat:** Breeds in dirty water like drains, cesspools, septic tanks, and water barrels.
- **Duration:** 1-10 days to hatch, depending on temperature.

Larval Stage (Wiggler):

- **Description:** Hatches from the egg, lives in water, breathes through a siphon, and feeds on organic matter.
- **Development:** Molts (sheds skin) several times as they grow through four instars.
- **Duration:**5-8 days under ideal conditions.

Pupal Stage (Tumbler):

- **Description:** A non-feeding, active stage where the larva transforms into an adult.
- **Activity:** Tumbles when disturbed but don't eat.
- **Duration:** About 36 hours at 27°C (81°F).

Adult Stage:

- **Emergence:** Adults emerge from pupae, dry their wings, and fly away.
- **Feeding:** Both sexes feed on plant sugars; females need a blood meal (birds, humans, animals) for egg development.
- **Reproduction:** After a blood meal, females seek water to lay eggs, repeating the cycle.
- **Lifespan:** Adults live for weeks to months; late-summer females may hibernate.

Key Feature: The entire cycle can be completed in as little as 7 days in optimally warm, humid conditions, allowing for rapid population growth in polluted water.

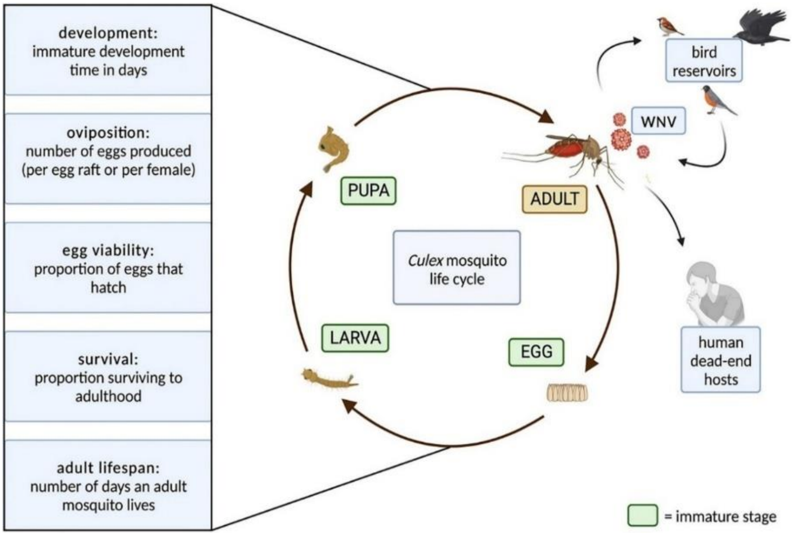
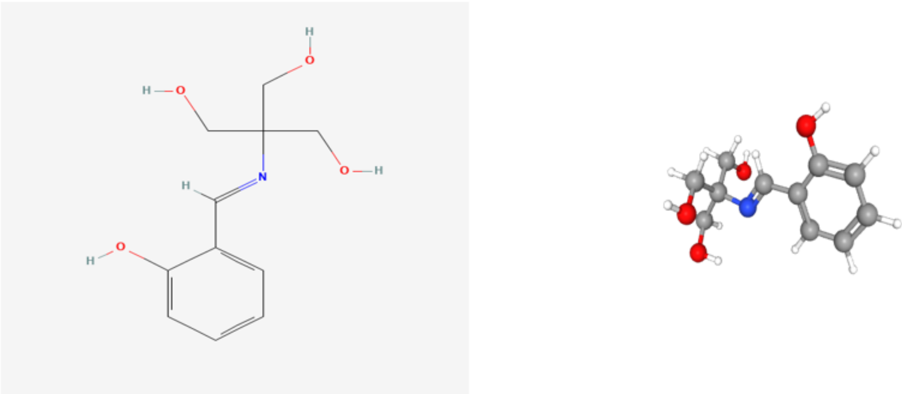


Figure 1. Life Cycle of *C. Quinquefasciatus*



(A) **(B)**

Figure 2. 2D Structure(A) & 3D Structure(B) Of Compound

Tablo 1. SwissADME Analysis of $C_{11}H_{15}NO_4$

Physicochemical Properties	
Formula	$C_{11}H_{15}NO_4$
Molecular weight	225.24 g/mol
Num. heavy atoms	16
Num. arom. heavy atoms	6
Num. H-bond acceptors	5
Num. H-bond donors	4
TPSA	93.28 Å²
Molar Refractivity	59.87
Fraction Csp3	0.36
Num. rotatable bonds	5

Water Solubility	
Solubility (Water)	Very Soluble
Pharmacokinetics	
GI absorption	High
P-gp substrate	No
CYP2C19 inhibitor	No
Druglikeness	
Lipinski	Yes; 0 violation
Bioavailability Score	0.55
Leadlikeness	No; 1 violation:

Lipophilicity	
Log $P_{o/w}$ (iLOGP)	0.85
Log $P_{o/w}$ (XLOGP3)	-0.16
Log $P_{o/w}$ (WLOGP)	-0.47
Log $P_{o/w}$ (MLOGP)	-0.53
Log $P_{o/w}$ (SILICOS-IT)	1.09
Consensus Log $P_{o/w}$	0.15

2. MATERIALS AND METHODS

Study Area and Period

This experimental study was conducted at the Crop Protection and Toxicology Laboratory of the Department of Zoology at Rajshahi University, Bangladesh. All bioassays and laboratory observations were performed under controlled conditions, in accordance with standard toxicological protocols.

The experimental work took place over a three-month period, from October to December 2025. During this period, all procedures, including the preparation of the test compound, the larvicidal bioassay, mortality observation, and data recording, were carried out systematically to ensure the accuracy and reproducibility of the results.

Test Organism (Mosquito Larvae)

The mosquito species selected for this larvicide bioassay was *Culex quinquefasciatus*. This choice was based on its importance of public health and its suitability for toxicological evaluation in the laboratory. *C. quinquefasciatus* larvae were collected on the Rajshahi University campus, where natural larval habitats are frequently found. After collecting, the larvae were carefully transported to the laboratory and used for experimental purposes after appropriate acclimatization. Larvae in the larval stage were selected for the bioassay, as these developmental stages are considered ideal for larvicidal studies due to their intense feeding activity and greater sensitivity to toxic compounds. All larvae were maintained under laboratory conditions suitable for the experimental bioassay prior to exposure. Only healthy and active larvae were used in the experiments to ensure the reliability and consistency of the observed mortality data.

Test Compound

The substance tested in this study was a newly synthesized chemical compound, developed specifically to evaluate its larvicidal potential against mosquito larvae. This compound was prepared in the BCSIR laboratories in Rajshahi prior to the biological tests and was used exclusively for toxicological evaluation in this study. The synthesized compound was in solid form and proved to be physically stable under laboratory conditions. For experimental application, it was dissolved in distilled water, which served as the solvent to ensure homogeneous dispersion of the test substance and to avoid any toxic effects of the solvent on the test organism. A 1000 ppm stock solution was prepared by dissolving an appropriate amount of the synthesized compound in distilled water.

From this stock solution, different concentrations were prepared by successive dilutions for the larvicidal test. All solutions were prepared fresh to ensure the accuracy and efficacy of the tested compound.

Preparation for Test Concentrations

A series of experimental solutions at different concentrations were prepared from the stock solution of the compound synthesized for the larvicidal bioassay. The concentrations selected for the experiment were 300 ppm, 400 ppm, 600 ppm, 800 ppm, and 1000 ppm, covering a wide range to assess mosquito larval mortality as a function of dose. All experimental solutions were prepared by diluting the stock solution with distilled water just before the experiment to ensure the stability and consistency of the compound (Corbel et al., 2023). A control group was maintained concurrently with distilled water only, without the addition of the compound. This control group allowed for the assessment of natural mortality and ensured that any larval mortality observed in the treated groups was solely due to the toxic effect of the synthesized compound.

Larvicidal Bioassay Procedure

The larvicidal test was performed according to standard laboratory procedures to evaluate the toxicity of the synthesized compound on mosquito larvae. For each concentration tested, the experiment was conducted in three replicates, each replicate comprising 10 larvae, for a total of 30 larvae per concentration. Each replicate was placed in a separate experimental container containing 20 mL of the solution being tested at the corresponding concentration. The larvae were gently introduced into the solution to avoid any mechanical stress, and no food was provided during the exposure period to avoid interfering with the toxic action of the tested compound. Larval mortality was recorded at various exposure intervals, specifically after 6, 12, 18, 24, 30, 36, 42, and 48 hours of treatment. This temporal monitoring allowed for a precise assessment of the early and late toxic effects of the synthesized compound. Larvae were considered dead if they showed no movement, even after gentle stimulation with a fine needle or glass rod. All mortality observations were carefully recorded (Not Provided, 2020).

Data Analysis

Larval bioassay and probit analysis are widely accepted methods (Rahman & Razzak, 2022) for evaluating the toxicity and dose–response relationship of candidate larvicides (Pramodini et al., 2019). The larval mortality data observed during the bioassays were first corrected using Abbott's formula to account for the natural mortality recorded in the control group (Kim et al., 2015). This correction ensured that the calculated mortality percentages accurately reflected the toxic effect of the synthesized compound. Median lethal concentration (LC_{50}) values (Aremu et al., 2022) were determined by probit analysis (Ganesan et al., 2023), a widely recognized statistical method for analyzing dose-response relationships in toxicological studies. The probit model was applied to evaluate the relationship between the tested concentrations and the mosquito larval mortality percentage. All statistical analyses were performed using the Python programming language, which was used for data processing, probit regression analysis, and graphical representation of the dose-mortality relationship. The results are expressed as LC_{50} values with appropriate confidence intervals to ensure their statistical reliability.

3. RESULT

Larvicidal Activity of the Synthesized Compound

The larvicidal activity of the newly synthesized chemical compound against *C. quinquefasciatus* larvae was evaluated under controlled laboratory conditions using dose-dependent and time-dependent bioassays (Gope et al., 2023). Larval mortality was recorded at various exposure intervals to assess the intensity and progression of toxic effects.The results clearly demonstrated that larval mortality increased with compound concentration and exposure time (Mandal et al., 2024). The control group exhibited negligible mortality throughout the experimental period, confirming that the larval mortality observed in the treated groups was solely due to the toxic action of the synthesized compound and not to environmental stress or larval handling. These results indicate that the compound possesses significant larvicidal potential against *C. quinquefasciatus* larvae.

Time- and Dose-Dependent Larval Mortality

Table 2 represents the cumulative larval mortality recorded at different exposure intervals for each tested concentration. A progressive increase in mortality was observed for all concentrations with increasing exposure time.

At the lowest concentrations (300 and 400 ppm), larval mortality remained relatively low during the initial exposure periods, then increased steadily after 30 hours, indicating a delayed toxic response (Bello et al., 2022). In contrast, the highest concentrations (800 and 1000 ppm) induced rapid and marked larval mortality, particularly after 36 to 48 hours of exposure. Near-total mortality was observed at 1000 ppm at the end of the experimental period, demonstrating the high larvicidal efficacy of the synthesized compound. The minimal mortality observed in the control group confirms the validity of the experimental protocol and the dose-dependent toxic effect of the compound.

Hourly Probit Analysis of Larval Mortality

To summarize the temporal variation of toxicity, an hourly probit analysis was performed, and the main statistical parameters were compared for different exposure durations. The LC_{50} values showed a progressive decrease with increasing exposure time, indicating increased larvicidal efficacy of the synthesized compound during prolonged exposure. Regression equations demonstrated a consistent dose-response relationship at each hour of exposure, while the χ^2 values indicated good agreement between observed and expected mortality data. 95% confidence intervals confirmed the reliability and accuracy of the LC_{50} estimates for different exposure durations (Sharma et al., 2022).

Table 2. Mosquito Larvae Mortality Over Time, Exposed to Different Concentrations of The Synthesized Compound ($C_{11}H_{15}NO_4$))

Concentration (ppm)	Number of larvae (per replicant)		Mortality in different exposure time (hour)							
			6	12	18	24	30	36	42	48
Control	R1	10	0	0	0	0	0	0	0	0
	R2	10	0	0	0	0	0	0	0	0
	R3	10	0	0	0	0	0	2	2	2
300	R1	10	0	0	0	0	1	1	3	3
	R2	10	0	0	1	1	1	1	3	4
	R3	10	0	0	0	1	2	3	3	3
400	R1	10	0	0	0	0	2	2	5	5
	R2	10	0	0	1	1	1	1	2	3
	R3	10	0	0	0	0	2	3	4	4
600	R1	10	0	0	0	2	2	3	3	6
	R2	10	0	1	1	1	1	3	3	4
	R3	10	0	0	0	1	1	3	6	8
800	R1	10	0	0	0	2	3	5	6	10
	R2	10	0	0	0	2	2	2	5	7
	R3	10	0	0	0	1	5	10	10	10
1000	R1	10	0	1	1	1	5	10	10	10
	R2	10	0	0	0	2	2	4	8	8
	R3	10	0	0	0	1	2	7	10	10

Table 3. Larvicidal Effect of New Synthesized Chemical Compound

Exposure time (Hour)	LC_{50} value (ppm)	95% confidence limits		Regression equation	Chi-square value	df
		Upper	Lower			
12	13416.45	5871115.62	30.66	Y = -6.911708 + 1.6744948 X	2.019	3
18	0.0156	2930847367665647353856.00	0.00	Y = -0.767032 + -0.4245307 X	0.9916389	3
24	6368.6082	115000.77	352.69	Y = -4.620718 + 1.2146856 X	1.2987390	3
30	2441.9155	9818.33	607.33	Y = -4.365177 + 1.2885254 X	1.5321910	3
36	785.6048	930.22	663.47	Y = -10.089175 + 3.4847889 X	0.9411514	3
42	555.1354	641.98	480.04	Y = -9.426025 + 3.4346411 X	3.7209369	3
48	461.7700	528.62	403.37	Y = -10.813783 + 4.0585792 X	1.5858249	3

Dose-Response and Probit Analyses Over Time of C. Quinquefasciatus

Dose-response relationships were analyzed for different exposure durations (12, 18, 24, 30, 36, 42, and 48 h) to assess the toxicity of the tested compound over time. Probit regression models were fitted to mortality data at each time point to estimate lethal concentrations (LCs) and their confidence intervals. Linear and logarithmic dose-response curves, probit plots, residue diagnostics, and LC variance estimates were used to assess model accuracy and temporal trends in toxicity(M. M. Rahman et al., 2024).

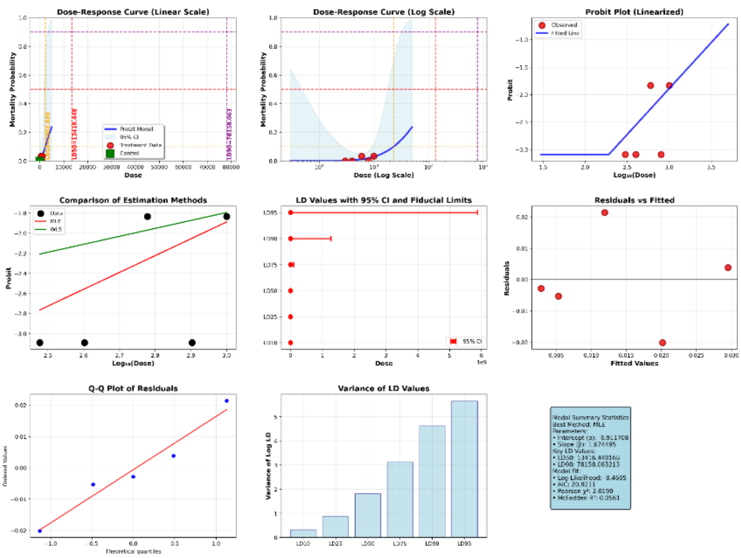


Figure 3. Dose–Response and Probit Analysis at 12 h Exposure

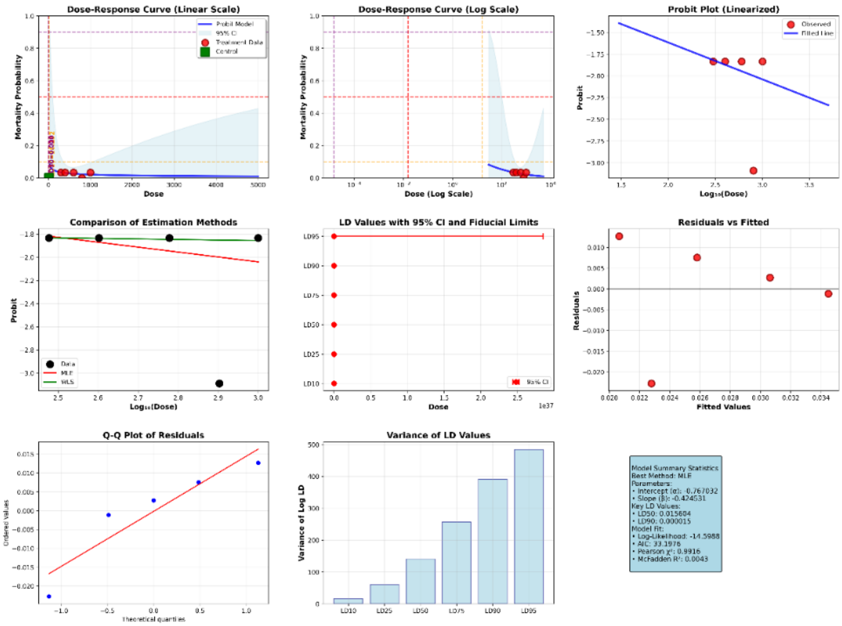


Figure 4. Dose–Response and Probit Analysis at 18 h Exposure

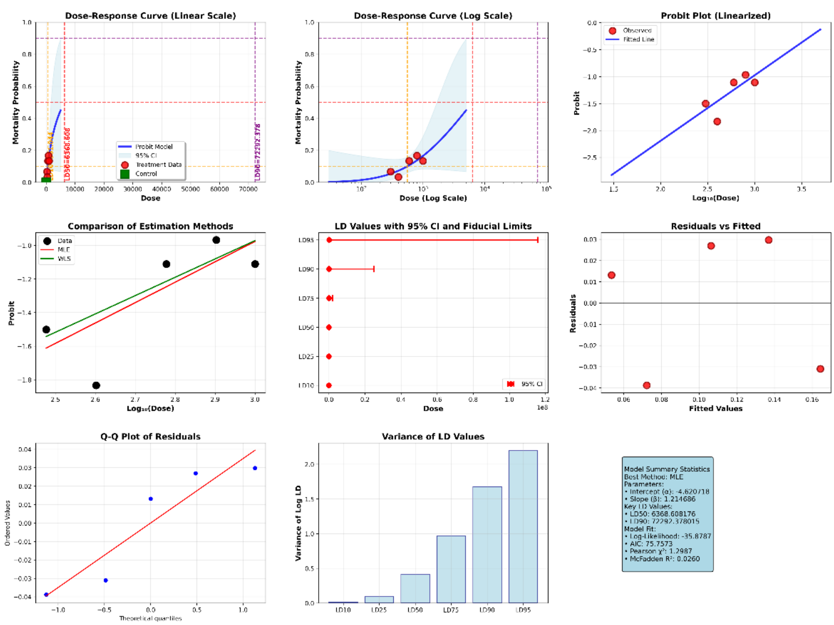


Figure 5. Dose-Response and Probit Analysis at 24 h Exposure

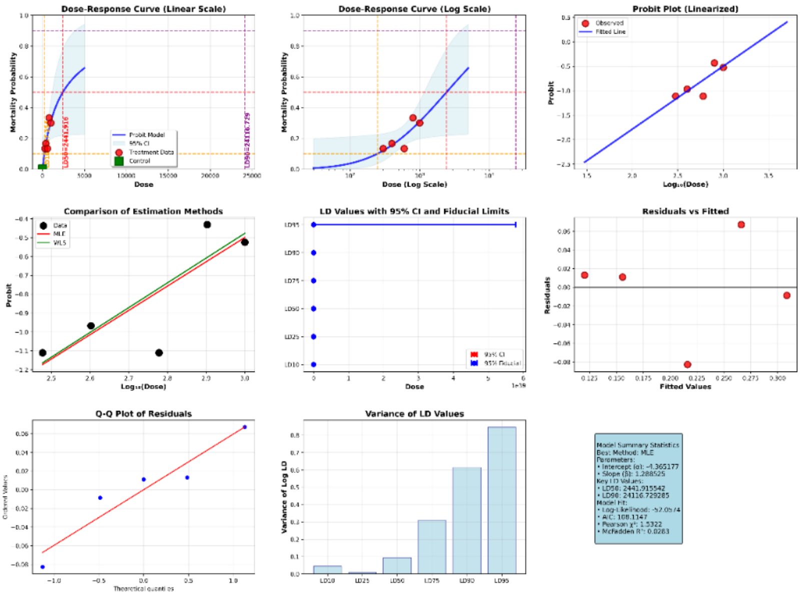


Figure 6. Dose–Response and Probit Analysis at 30 h Exposure

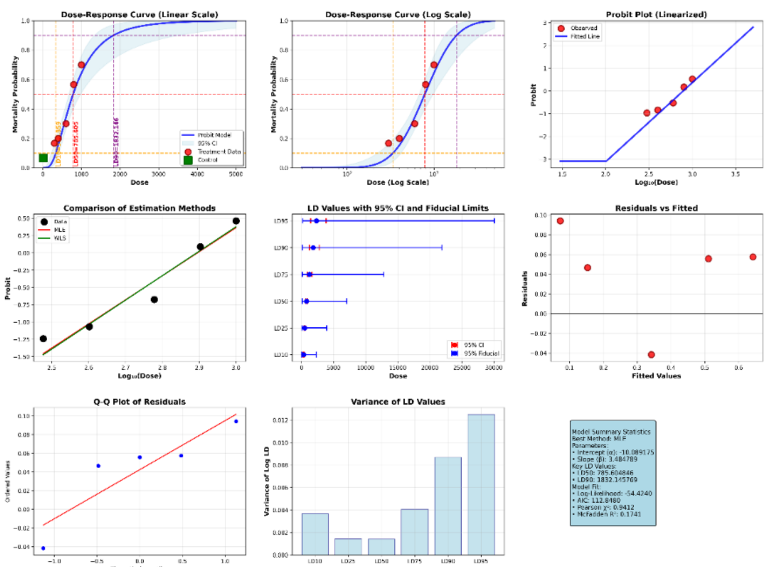


Figure 7. Dose–Response and Probit Analysis at 36 h Exposure

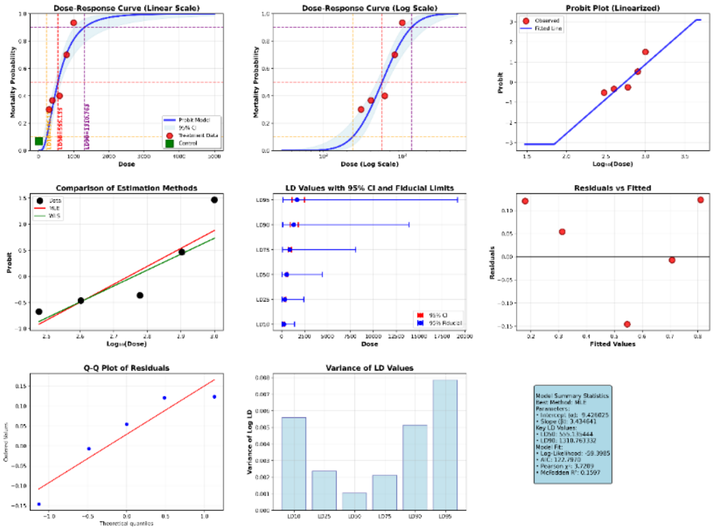


Figure 8. Dose–Response and Probit Analysis at 42 h Exposure

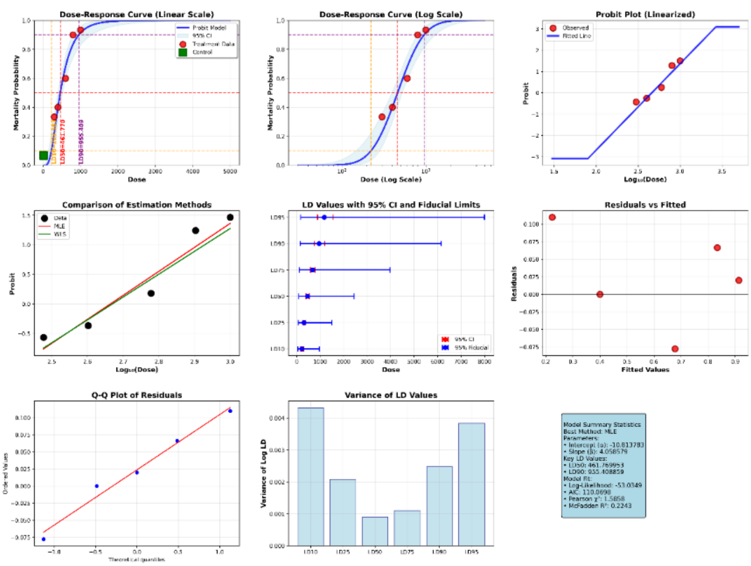


Figure 9. Dose–Response and Probit Analysis at 48 h Exposure

Probit Analysis of The Larvicidal Activity of The Newly Synthesized Compound

Table 4. Probit Analysis Showing the Larvicidal Effect of The Synthesized Compound Against *C. Quinquefasciatus* After 12 H Of Exposure.

Dose (ppm)	**Log Dose**	**No. of insects used**	**No. killed**	**% Kill**	**Corr. %**	**Emp. probit**	**Expt. probit**	**Work probit**	**Weight**	**Final Probit**
300	2.477	30	0	0.000	0.000	-3.090	-2.763	-2.975	0.027	-2.763
400	2.602	30	0	0.000	0.000	-3.090	-2.554	-2.837	0.044	-2.554
600	2.778	30	1	3.333	3	-1.833	-2.259	-1.570	0.082	-2.259
800	2.903	30	0	0.000	0	-3.090	-2.050	-2.443	0.120	-2.050
1000	3.000	30	1	3.333	3	-1.834	-1.888	-1.831	0.157	-1.888

- Y = -6.911708 + 1.6744948 X
- Log LC_{50} is 4.127638
- LC_{50} is 13416.4482
- χ^2 is 2.0189619 with 3 degrees of freedom
- No sig heterogeneity
- 95% conf. limits are 30.6587526 to 5871115.6202582

Table 5. Probit Analysis Showing the Larvicidal Effect of The Synthesized Compound Against *C. Quinquefasciatus* After 18 Hours of Exposure

Dose (ppm)	**Log Dose**	**No. of insects used**	**No. killed**	**% Kill**	**Corr. %**	**Emp. probit**	**Expt. probit**	**Work probit**	**Weight**	**Final Probit**
300	2.477	30	1	3.333	3	-1.834	-1.818	-1.834	0.175	-1.818
400	2.602	30	1	3.333	3	-1.834	-1.871	-1.832	0.161	-1.871
600	2.778	30	1	3.333	3	-1.833	-1.946	-1.820	0.143	-1.946
800	2.903	30	0	0.000	0	-3.090	-1.999	-2.402	0.131	-1.999
1000	3.000	30	1	3.333	3	-1.833	-2.040	-1.785	0.122	-2.040

- Y = -0.767032 + -0.4245307 X
- Log LC_{50} is -1.806776
- LC_{50} is 0.0156
- χ^2 is 0.9916389 with 3 degrees of freedom
- No sig heterogeneity
- 95% conf. limits are 0.0000000 to 2930847367665647353856.00

Table 6. Probit Analysis Showing the Larvicidal Effect of The Synthesized Compound Against *C. Quinquefasciatus* After 24 Hours of Exposure

Dose (ppm)	**Log Dose**	**No. of insects used**	**No. killed**	**% Kill**	**Corr. %**	**Emp. probit**	**Expt. probit**	**Work probit**	**Weight**	**Final Probit**
300	2.477	30	2	6.667	7	-1.501	-1.611	-1.490	0.234	-1.611

400	2.602	30	1	3.333	3	-1.833	-1.460	-1.742	0.282	-1.460
600	2.778	30	4	13.333	13	-1.110	-1.246	-1.099	0.354	-1.246
800	2.903	30	5	16.667	17	-0.967	-1.094	-0.958	0.407	-1.094
1000	3.000	30	4	13.33333	13	-1.110	-0.976	-1.101	0.446	-0.976

- Y = -4.620718 + 1.2146856 X
- Log LC_{50} is 3.804045
- LC_{50} is 6368.6082
- χ^2 is 1.2987390 with 3 degrees of freedom
- No sig heterogeneity
- 95% conf. limits are 352.6860564 to 115000.7757775

Table 7. Probit Analysis Showing the Larvicidal Effect of The Synthesized Compound Against *C. Quinquefasciatus* After 30 Hours of Exposure

Dose (ppm)	**Log Dose**	**No. of insects used**	**No. killed**	**% Kill**	**Corr. %**	**Emp. probit**	**Expt. probit**	**Work probit**	**Weight**	**Final Probit**
300	2.477	30	4	13.333	13	-1.111	-1.173	-1.108	0.380	-1.173
400	2.602	30	5	16.666	17	-0.967	-1.012	-0.966	0.434	-1.012
600	2.778	30	4	13.333	13	-1.110	-0.785	-1.067	0.507	-0.785
800	2.903	30	10	33.333	33	-0.430	-0.624	-0.419	0.552	-0.624
1000	3.00	30	9	30.00	30	-0.524	-0.499	-0.524	0.581	-0.499

- Y = -4.365177 + 1.2885254 X
- Log LC_{50} is 3.387731
- LC_{50} is 2441.9155
- χ^2 is 1.5321910 with 3 degrees of freedom
- No sig heterogeneity
- 95% conf. limits are 607.3283595 to 9818.3320751

Table 8. Probit Analysis Showing the Larvicidal Effect of The Synthesized Compound Against *C. Quinquefasciatus* After 36 H Of Exposure

Dose (ppm)	**Log Dose**	**No. of insects used**	**No. killed**	**% Kill**	**Corr. %**	**Emp. probit**	**Expt. probit**	**Work probit**	**Weight**	**Final Probit**
300	2.477	30	5	16.667	11	-1.241	-1.456	-1.206	0.283	-1.456
400	2.602	30	6	20.000	14	-1.067	-1.021	-1.066	0.431	-1.021
600	2.778	30	9	30.000	25	-0.674	-0.407	-0.657	0.599	-0.407
800	2.903	30	17	56.667	54	0.089	0.027	0.089	0.636	0.027
1000	3.000	30	21	70.000	68	0.463	0.365	0.461	0.606	0.365

- Y = -10.089175 + 3.4847889 X
- Log LC_{50} is 2.895204
- LC_{50} is 785.6048
- χ^2 is 0.9411514 with 3 degrees of freedom
- No sig heterogeneity
- 95% conf. limits 663.4723656 to 930.2195624

Table 9. Probit Analysis Showing the Larvicidal Effect of The Synthesized Compound Against *C. Quinquefasciatus* After 42 H Of Exposure

Dose (ppm)	Log Dose	No. of insects used	No. killed	% Kill	Corr. %	Emp. probit	Expt. probit	Work probit	Weight	Final Probit
300	2.477	30	9	30.000	25	-0.674	-0.918	-0.647	0.466	-0.918
400	2.602	30	11	36.667	32	-0.463	-0.488	-0.463	0.583	-0.488
600	2.778	30	12	40.000	36	-0.366	0.115	-0.361	0.634	0.115
800	2.903	30	21	70.000	68	0.463	0.545	0.461	0.571	0.545
1000	3.000	30	28	93.333	93	1.465	0.877	1.314	0.478	0.877

- Y = -9.426025 + 3.4346411 X
- Log LC_{50} is 2.744399
- LC_{50} is 555.1354
- χ^2 is 3.7209369 with 3 degrees of freedom
- No sig heterogeneity
- 95% conf. limits 480.0375953 to 641.9817205

Table 10. Probit Analysis Showing the Larvicidal Effect of The Synthesized Compound Against *C. Quinquefasciatus* After 48 H Of Exposure

Dose (ppm)	Log Dose	No. of insects used	No. killed	% Kill	Corr. %	Emp. probit	Expt. probit	Work probit	Weight	Final Probit
300	2.477	30	10	33.333	29	-0.565	-0.760	-0.552	0.514	-0.760
400	2.602	30	12	40.000	36	-0.366	-0.253	-0.364	0.622	-0.253
600	2.778	30	18	60.000	57	0.180	0.461	0.164	0.589	0.461
800	2.903	30	27	90.000	89	1.241	0.968	1.205	0.449	0.968

1000	3.000	30	28	93.333	93	1.465	1.361	1.458	0.315	1.361

- Y = -10.813783 + 4.0585792 X
- Log LC_{50} is 2.664426
- LC_{50} is 461.7700
- χ^2 is 1.5858249 with 3 degrees of freedom
- No sig heterogeneity
- 95% conf. limits 403.3703412 to 528.6246102

4. DISCUSSION

The present study demonstrated that the newly synthesized chemical compound exhibits significant larvicidal activity against *Culex quinquefasciatus* larvae(Ghaffari et al., 2014). A clear dose-dependent increase in larval mortality was observed, which is consistent with standard toxicological principles. Additionally, mortality increased progressively with exposure time, indicating cumulative toxic effects of the compound(Paixão et al., 2013). At lower concentrations (300–400 ppm), larval mortality was relatively low during early exposure periods but increased gradually after prolonged exposure, suggesting delayed toxicity. In contrast, higher concentrations (800–1000 ppm) produced rapid and pronounced larval mortality, particularly after 36–48 hours. The near-complete mortality observed at 1000 ppm highlights the strong larvicidal potential of the synthesized compound.

The probit analysis further confirmed the effectiveness of the compound, as evidenced by the progressive reduction of LC_{50} values with increasing exposure time. This decline in LC_{50} values indicates enhanced larvicidal efficiency upon prolonged exposure(Conti et al., 2012). The chi-square values obtained at different exposure periods showed no significant heterogeneity, suggesting a good fit between observed and expected mortality data.

The negligible mortality recorded in the control group confirms that the observed larval deaths were solely due to the toxic action of the synthesized compound and not due to environmental stress or handling effects. Similar dose- and time-dependent larvicidal responses have been reported for other synthetic and plant-derived larvicides, supporting the validity of the present findings. The LC_{50} value at 48 h (461.77 ppm) is comparable or lower than several reported synthetic larvicides, indicating moderate to strong efficacy(Rahuman et al., 2009). Overall, the results suggest that the synthesized compound has promising larvicidal properties and could be considered for further evaluation as a mosquito control agent.

CONCLUSION

The present study was undertaken to evaluate the larvicidal efficacy of a newly synthesized chemical compound against *Culex quinquefasciatus* larvae under controlled laboratory conditions. The results clearly demonstrated that the synthesized compound exhibited pronounced larvicidal activity, with larval mortality increasing significantly with both concentration and exposure duration. A clear dose–response relationship was observed throughout the experimental period, indicating that the toxic effect of the compound was concentration dependent.

Probit analysis further confirmed the effectiveness of the synthesized compound, as evidenced by the progressive decline in LC_{50} values with increasing exposure time. This reduction in LC_{50} values suggests that prolonged exposure enhances the toxic action of the compound against mosquito larvae. The goodness-of-fit of the probit models and the absence of significant heterogeneity indicate that the mortality data were statistically reliable and suitable for toxicological interpretation. The negligible mortality recorded in the control group confirms that the observed larval deaths were solely attributable to the toxic effect of the synthesized compound rather than environmental or handling-related factors. Overall, the findings of this study suggest that the newly synthesized compound possesses strong larvicidal potential against *Culex quinquefasciatus*. Although the present investigation was limited to laboratory-based bioassays, the compound may be considered a promising candidate for mosquito larval control.

Further studies focusing on environmental safety, non-target organism toxicity, and field-level evaluation are necessary before practical application. Thus, the synthesized compound shows promise as a larvicidal agent and warrants further field and ecotoxicological evaluation.

Author Contribution

Meherun Nesa & Md. Badrul Islam: Conceptualization, supervision, methodology, critical review and manuscript editing. Md. Shafiqul Islam & Md Abdullah: Study design, observation, methodology, data analysis, software, visualization, writing and original draft preparation. Tasnia Munir Elma: Data observation & collection, technical assistance, and supportive contributions.

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Data Availability

Data is provided within the manuscript.

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