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Larvicidal, Adulticidal and Lethal Time Activity of Indigenous Trichoderma spp. Against Housefly (Musca domestica L.) in Katsina State, Nigeria

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ABSTRACT
The housefly (Musca domestica L.) is an important mechanical vector of numerous pathogens affecting both humans and animals. The increasing development of resistance to synthetic insecticides and growing environmental concerns have stimulated interest in biological control agents such as entomopathogenic fungi. This study evaluated the larvicidal and adulticidal activities of an indigenous isolate of Trichoderma spp. against housefly larvae and adults under laboratory conditions. Different concentrations of fungal conidial suspensions ($1x10^6$, $1x10^7$, and $1x10^8$ conidia/mL) were prepared and applied to 2nd instar larvae and 10-day old adults of housefly. Mortality was recorded at 24-, 48-, and 72hour intervals and lethal time values (LT_{50}) were determined. Results showed that Trichoderma spp. caused significant mortality in both larval and adult stages of Musca domestica, with mortality increasing with concentration and exposure period. Higher concentrations (75%) produced significantly greater mortality and shorter lethal times than lower concentrations. The findings indicate that indigenous Trichoderma spp. possesses considerable entomopathogenic potential against houseflies and may serve as an environmentally friendly alternative for housefly management. Further studies are recommended to evaluate its field performance and formulation for large-scale application.

Keywords: Trichoderma spp., *Musca domestica*, Entomopathogenic fungi, biological control, Larvicidal activity, Adulticidal activity, Lethal time.

INTRODUCTION

The housefly (*Musca domestica* L.) is one of the most common insect pests associated with human settlements and livestock production systems worldwide (Hussein, 2014). Due to its close association with decomposing organic matter, animal wastes, refuse dumps, and food sources, the housefly plays a significant role in the transmission of numerous pathogenic microorganisms (Phoku, 2016) It has been implicated in the spread of bacterial, viral, protozoan, and helminth infections affecting both humans and animals (Yap *et. al.*, 2008)

Control of housefly populations relies heavily on chemical insecticides. However, extensive and indiscriminate use of these chemicals has resulted in the development of insecticide resistance, environmental pollution, contamination of food products, and adverse effects on non-target organisms (Yap *et. al.*, 2008). Consequently, there is increasing demand for environmentally sustainable alternatives that can effectively suppress housefly population while minimizing ecological risks (Malik *et. al.*, 2007)

Entomopathogenic fungi are important microbial control agents capable of infecting insects through direct penetration of the cuticle (Khan, 2012). Unlike bacterial pathogens that generally require ingestion to initiate infection, entomopathogenic fungi can infect insects through direct contact with the cuticle. Following attachment, fungal conidia germinate and penetrate the cuticle, allowing infection to occur without ingestion and making these pathogens potentially effective against different developmental stages of insect pests (Fan et al., 2007). Species belonging to Beauveria, Metarhizium, Lecanicillium, and Trichoderma have demonstrated varying degrees of pathogenicity against several insect pests (Sarwar 2015).

Although Trichoderma species are widely recognized for their antagonistic effects against plant pathogens and their role in agricultural biocontrol, emerging evidence suggests that certain isolates may possess insecticidal properties (Zarrin *et. al.*, 2007). Indigenous isolates adapted to local environmental conditions may provide effective and sustainable options for biological control programmes (Ownley, 2010).

Therefore, this study was conducted to evaluate the larvicidal and adulticidal efficacy of indigenous Trichoderma spp. isolated in Katsina State, Nigeria, and to determine their lethal time against larval and adult stages of Musca domestica.

MATERIALS AND METHODS

Study Design

Houseflies that were naturally infected were gathered from variety of habitats. The infected houseflies that show outward signs of mycosis were gathered and brought the laboratory at biological science Department, Umaru Musa Yar'adua University, Katsina. The experiment was conducted under laboratory conditions to evaluate the pathogenicity of Trichoderma spp. against larvae and adults of *Musca domestica.*

Isolation of Entomopathogenic Fungi

To isolate fungal strains, the sample of *Musca domestica* were surface sterilized with 70% ethanol for 5 minutes and washed 5 times with sterile distilled water in a laminar flow cabinet. The treated samples were transferred into a surface-sterilized petri dish, and then picked using a sterile forceps and placed directly onto the potato dextrose agar supplemented with chloramphenicol (250mg/L) (Humber, 2012; Bilgo *et. al.*, 2018). Two samples were placed in the opposite direction on each plate and replicated 5 times. The plates were incubated at 25±2℃ for 7 days and the presence or absence of Entomopathogenic fungi on the plates inoculated with housefly cadavers was observed daily until the fungal growth was observed (Zarrin *et al*., 2007; Sani *et al*., 2023).

Identification and Rearing of Housefly

The house flies were identified using their distinct characteristics Larvae and adults of Musca domestica were maintained under laboratory conditions and provided with suitable diet throughout the experimental period. (Hogsette *et al*. 2021; Khan *et al*. 2023).

Adult houseflies (*Musca domestica*) were collected from refuse dumps within the study area using sweep nets and baited traps. The flies were transferred into screened rearing cages (approximately 30 × 30 × 30 cm) and maintained under laboratory conditions at a temperature of 27 ± 2°C, relative humidity of 60–70%, and a 12:12-hour light-dark photoperiod.

The adult flies were provided with a diet consisting of granulated sugar and powdered milk (1:1) with clean water supplied on cotton wool. An oviposition medium comprising fresh wheat bran moistened with milk or water and supplemented with a small quantity of fish meal was placed inside the cage to encourage egg laying (Khan *et al*., 2023). Eggs deposited by the females were collected and transferred to plastic containers containing fresh larval diet. The larvae were reared until the desired developmental stage for bioassay. The second instar larvae were identified approximately 48–72 hours after egg hatching under the laboratory conditions used. Identification was based on morphological characteristics.

Adult houseflies emerging from pupae were collected daily and maintained in separate rearing cages. Their age was recorded from the day of adult emergence. Adults that reached 10 days of age were selected for the adulticidal experiments. Only healthy, active, and undamaged insects of standardized age were used throughout the bioassays to ensure consistency and reliability of the experimental results (Miller et al., 2010).

Preparation of Conidial Suspension

Conidia were harvested from actively growing cultures of Trichoderma and the conidia was scraped using a sterile spatula suspended in sterile distilled water containing 0.01% Tween 80. The suspension was filtered and adjusted to the required concentrations using a haemocytometer under x400 magnification (Butt *et al*., 2001)

Larvicidal and Adulticidal Bioassay

There were four treatment groups created. Three distinct conidial concentrations ($1x10^6$, $1x10^7$, $1x10^8$) were tested for each treatment group. A matching control group received a sterile 0.01% Tween-80 solution for each treatment (Bilgo *et al.,* 2018). Second-instar larvae and 10-day-old adults of *Musca domestica* were used to test the pathogenicity of the isolate of *Trichoderma spp.* Twenty larvae or twenty adults were put in separate plastic containers for each treatment and control group (Butler, 2013). To ensure even coverage, conidia suspensions were sprayed onto each replicate containing 20 houseflies using about 1ml of suspension. Following treatment, the containers were kept in a lab setting and covered with fine mesh to allow for ventilation. At 24, 48, and 72hours after treatment, mortality was noted. The dead were brought to the surface and cleaned. And put in a petri dish with wet filter paper to verify fungal infection by looking for visible mycosis.

Determination of Lethal Time

Mortality data obtained from the bioassays were subjected to probit analysis for estimation of LT_{50} values for each concentration tested. The median lethal time (LT_{50}) of each entomopathogenic fungal isolate at each concentration was estimated from the cumulative mortality data recorded at 24, 48, and 72 hours which determines the time required to achieve 50% mortality between observation periods.

RESULTS AND DISCUSSION

Larval Mortality of Housefly Exposed to *Trichoderma spp.*

Table 1 shows mortality of housefly larvae exposed to different concentrations of Trichoderma spp. having 20% as its lowest concentration ($1x10^6$) and 75% at its highest concentration (1×10^8). Mortality increased with both concentration and exposure time, indicating dose-dependent and time-dependent effect.

Table 1: Percentage Effect of *Trichoderma spp*. on Larval Mortality of *Musca Domestica*

Fungus	Conidia/ml	% Larval Mortality Exposure Period (Hour)		
		24	48	72
Trichoderma spp	1×10^6	20%	40%	65%
	1×10^7	25%	40%	70%
	1×10^8	25%	45%	75%

Adult Mortality of Housefly Exposed to *Trichoderma spp*

It can be seen in Table 2 that higher mortality was observed in adults than in larvae, Results show that the mortality increased with longer exposure time. At 24hrs, mortality was (25-45%) and at 72hrs it reached (75-85%)

Table 2: Percentage Effect of *Trichoderma spp*. on Adult Mortality of *Musca Domestica*

Fungus	Conidia/ml	% Larval Mortality Exposure Period (Hour)		
		24	48	72
Trichoderma spp	1×10^6	25%	45%	75%
	1×10^7	30%	55%	80%
	1×10^8	45%	60%	85%

Influence of Concentration on Mortality Response

The concentration of the entomopathogenic fungi had a marked influence on the mortality of *Musca domestica* larvae and adults. Mortality increased progressively with increasing fungal concentration across all exposure periods. The highest concentration (1×10^8 conidia/mL) consistently produced the greatest mortality, followed by 1×10^7 conidia/mL, while the lowest concentration (1×10^6 conidia/mL) resulted in the least mortality. No mortality was observed in the untreated control throughout the experiment.

Lethal Time LT_{50} Mortality of Musca Domestica caused by *Trichoderma spp*

The LT_{50} values (lethal time to 50% mortality) varied by fungal species, life stage of the insect, and conidial concentration. In general, increasing fungal concentration resulted in a shorter LT_{50}, indicating faster mortality. Trichoderma spp. exhibited longer LT_{50} values. For larvae, the LT_{50} ranged from 57.6 hours at 1×10^6 conidia/mL to 52.0 hours at 1×10^8 conidia/ml. In adults, the LT_{50} was 52.0, 43.2, and 32.0 hours at the increasing concentrations, respectively.

Table 3: Lethal Time for 50% Mortality of *Musca Domestica* caused by *Trichodrma spp.*

Life stage	Concentration (Conidia/ml)	LT_{50} (hours)
Larvae	1×10^6	57.6
	1×10^7	56.0
	1×10^8	52.0
Adults	1×10^6	52.0
	1×10^7	43.2
	1×10^8	32.0

Discussion

This research aims at assessing the pathogenicity of Trichoderma spp against Larvae and adult housefly and also determining its lethal time after being exposed over 72 hours at different conidial concentration. The results shows that the higher the concentration and longer exposure time the higher the mortality rate (65% - 85%). Adult flies showed slightly faster mortality compared to larvae at all concentrations which might be as a result of cuticle composition, immune defence or fungal penetration efficiency between the life stages.

The relatively lower efficacy of Trichoderma spp. is notable when compared to Beauveria *bassiana* (Sani *et al.,* 2023), as this fungus is typically recognized more for its antagonistic activity against plant pathogens and soil-borne pests rather than direct entomopathogenicity. However, some strains of Trichoderma have shown insecticidal activity, potentially through secondary metabolites or mechanical disruption. The moderate effectiveness observed in this study suggests Trichoderma spp. may have some potential for control of *M. Domestica* (Al-Olayan, 2013).

In comparable with *Beauveria bassiana, Trichoderma spp* showed slow action and lower mortality as Beauveria bassiana acts higher and more rapid than Trichoderma. Sani et al., (2023) conducted similar studies on *Beauveria bassiana*

The infection process of EPF involves the attachment of spores to adhere to the insect's cuticle using hydrophobic and electrostatic interactions. The spores germinate under favourable environmental conditions, producing germ tubes (Bilgo *et al*., 2018). The fungus releases enzymes, such as chitinases, proteases, and lipases, that degrade the insect's cuticle. Once inside the insect, the fungus proliferates in the hemocoel (body cavity), disrupting internal organs and physiological processes. The insect typically dies within 3–10 days, depending on the fungal species, environmental conditions, and host susceptibility. After death, the fungus sporulates, releasing new spores into the environment to infect other hosts. (Khan *et al*., 2014; Lacey *et al*., 2015).

The reduction in LT_{50} observed at higher fungal concentrations is attributed to the increased number of infective conidia available to initiate infection. Higher conidial concentrations increase the likelihood of spore attachment, germination, and penetration through the insect cuticle, resulting in faster colonization of host tissues and earlier death. Consequently, treatments that produced higher mortality also exhibited lower LT_{50} values, indicating greater pathogenicity and a faster speed of kill. This inverse relationship between mortality and LT_{50} demonstrates that increasing fungal concentration enhances the efficacy of the entomopathogenic fungi against *Musca domestica*. The findings support its use in integrated pest management (IPM) programs as an alternative to chemical insecticides, especially in environments like livestock facilities and waste disposal areas where houseflies are a major concern.

CONCLUSION

The indigenous Trichoderma spp. isolate demonstrated significant larvicidal and adulticidal activity against *Musca domestica* under laboratory conditions. Mortality increased with increasing fungal concentration and exposure period, while lethal time decreased as concentration increased. These findings suggest that Trichoderma spp. possesses promising potential as a biological control agent for the sustainable management of housefly populations in Katsina. Further studies under semi-field and field conditions are recommended to validate its practical application.

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