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ENTOMOPATHOGENIC FUNGI FROM PAKISTAN AS POTENTIAL BIOCONTROL AGAINST APHID

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Abstract

Rose is a versatile plant with varying colors and fragrant flowers. This study was conducted to assess the biocontrol efficacy of various indigenous entomopathogenic fungi from Pakistan in controlling aphid collected from different plants and reared in the lab. Different concentrations (1×105, 1×106, 1×107, 1×108 and 1×109 conidia mL^{-1}) of four fungal species namely *Beauveria bassiana, Metarhizium anixpoli, M. attenuatum* and *Verticillium lecani* were applied on aphids by means of filter paper technique. The highest aphid mortality was due to *B. bassiana* and *M. anixpoli*. The later fungal species caused up to 84% aphid mortality after 72 hours incubation period. The minimum lethal time (LT), LT50 and LT90 values for *B. bassiana* were 71.69 and 149.7 days, respectively, while the lowest lethal concentration (LC), LC50 and LC90 values were 8.6 × 108 and 8.7 × 1010 conidia mL^{-1} at 72 hours. In case of *M. anixpoli*, the minimum LT50 and LT90 values were 76.02 and 150.02 days, , and the minimum LC50 and LC90 values were 1.0×106 and 2.23×1013 conidia mL^{-1}, respectively. This study demonstrates that *M. anixpoli* and *B. bassiana* can be used as effective biocontrol agents against aphids in Pakistan.

Keywords: Aphids, entomopathogens, fungi, management, mortality

INTRODUCTION

Rose is an important flowering plant. There are about three hundred species of roses. There are many uses of rose plant viz. rose oil, as medicinal herbs, as good sources of nutrients, beneficial substances and phenolic chemicals, ethanol production, and jams and jelly preservation. Pakistan economy status improved due to rose culture (Erper *et al.,* 2016; Vasilev and Atanasova, 2024). Different insects such as mites, aphid and thrips attack on rose plants. Aphid (*Macrosiphum rosae* L.) can cause significant damage to crop and landscapes (Saurabh *et al.,* 2021; Vasilev and Atanasova, 2024). These pests can feed on the sap of plants, causing deformation, stunted growth, and reduced yield. In addition, they can transmit viruses and other pathogens to plants, further exacerbating the damage. When aphids attack the rose plant, it sucks sap from plant and secretes sugary material. As a result, black mold is formed. Approximately 60 plant species are the host for green aphids (Bhat and Kumar, 2024; Vasilev and Atanasova, 2024).

Application of insecticides has been the primary method of controlling insect populations (Ali *et al.,* 2023; Rehman *et al.,* 2024). However, this tactic has numerous drawbacks such as potential for harm to non-target organisms, development of resistance, and negative effects on the environment (Rousselin *et al.,* 2017; Luo *et al.,* 2022). Using pesticides carelessly also results in the development of insecticide

resistance in pest insects, negative impacts on beneficial organisms, environmental contamination, the buildup of toxic substances in food, and eventually, diseases like cancer, liver and kidney failure, and genetic disorders in humans brought on by pesticide residues (Ambethgar, 2009; Luo *et al.,* 2022; Sonhafouo-Chiana *et al.,* 2022). Therefore, there is a need to search for nature friendly alternative strategies for the control of insect pests (Ahmad *et al.,* 2023; Hamza *et al.,* 2023; Masood *et al.,* 2024).

One of the potential alternative control strategies is the biological control of insects using entomopathogenic fungi (Ahmad *et al.,* 2022). Entomopathogens control insect pests naturally and do not harm our environment. Additionally, the likelihood of pesticide resistance in insects and pesticide residues in food is decreased, while biodiversity and other natural enemies are preserved in managed environments (Diez *et al.,* 2021; Irsad *et al.,* 2023; Quesada-Moraga *et al.,* 2024). Entomopathogenic fungi cause serious illness in insect pest and reduce its population and helpful for environment (Islam *et al.,* 2021; Zhang *et al.,* 2021). Large research articles were published on fungal infection against insect pests especially aphid Zhang *et al.,* 2021; Hong *et al.,* 2024; Ma *et al.,* 2024). EPF like *B. bassiana* used for large number of pest control in all over the world (Dannon *et al.,* 2020; Swathy *et al.,* 2024). On the other hand, fungus like *M. anisopliae* and *V. lacanni* was also used as biocontrol agent against aphid and other soft body insects (Wang *et al.,* 2021; Ye *et al.,* 2022).

Effective control of aphids by different EPF stains depends on number of factors include stage of aphid, number of aphids, fungal strains to be used and more dominant factor was environmental conditions (Boni *et al.,* 2021; Francis *et al.,* 2022; Swathy *et al.,* 2024). Present study was carried out to evaluate the virulence efficacy of different EPF strains against aphids under laboratory conditions.

Materials and Methods

Lab rearing

Adult and nymph of rose plant was captured from field and brought into laboratory and reared according to methods describe by (Usman *et al.,* 2021). Aphids were removed from the shoots with help of camel hairbrush. They were fed on portions of young, fragile apical rose plants every day, which were first housed in big beakers and then transferred to glass rearing jars covered with muslin cloth for proper aeration.

Rearing of aphid

For the rearing of aphids, 25-mesh and 30-mesh sieve were used. Eggs were collected using a no. 50 mesh size. Each adult was gently handled and put into a glass jar using a camel hairbrush.

Fresh rose leaves were replaced every day to feed the aphids. The nymph and adult prey on the leaves, stems, buds, and flowers of rose plants to consume their fluids. A 25°C temperature, 75% humidity and a 16:8 hours (L:D) photoperiod was maintained in an incubator. Aphid individuals were checked on a daily basis.

Development of fungal cultures

For this investigation, four entomopathogenic fungi *M. anixpoli*, *V. lecanii*, *B. bassiana*, and *M. attenuatum* were obtained from the University of Agriculture Faisalabad (Sayed *et al.,* 2018). Every entomopathogen was grown on potato dextrose agar (PDA) in 90 mm Petri dishes, covered with parafilm, and kept in an incubator set at 25 °C with a 14:10 h (Light:Dark) photoperiod for 10days.

A sterile scalpel was used to extract the dry conidia after the incubation time, and they were then

placed in 50 mL sterile falcon tubes. To guarantee homogeneity, eight glass beads were vortexed for five minutes after thirty milliliters of 0.05% tween solution was added. In order to determine concentrations of EPF, 10 µL of each suspension were placed onto side of the hemocytometer and conidia were counted under a 400x microscope. To determine conidia viability, 100 µL of each suspension was spread on Sabouraud dextrose agar with 1% yeast (SDAY) media in Petri plates and inoculated at 25 °C for 16hours. The conidia were considered germinated if the length of germ tube was about twice as the length of a conidium. The conidial viability of each isolate was above 90%.

PREPARATION OF CONCENTRATIONS

The cultures of four selected fungal species were prepared in varying concentrations (1×10^5, 1×10^6, 1×10^7, 1×10^8, and 1×10^9 spores mL^{-1}). To maintain homogeneity of the suspensions, these were vortexed five times. A 0.02% tween 80 were added to the suspensions (Selvaraj *et al.,* 2012).

The final volume of stock solution required to prepare each concentration was determined by the following formula:

$$V\,final = \frac{V\,stock \times C\,stock}{C\,final}$$

V_{Final} = Final volume of stock solution needed to prepare required concentration; V_{Stock} = Volume of stock solution; C_{Stock} = Concentration of stock solution; C_{Final} = Final concentration to be prepared.

EXPERIMENT METHOD

A total of 100 aphids (5 replicates, each containing 20 insects) were employed for each concentration. Using a micro pipette, a 1 µL drop of each concentration was directly applied to the insect body as part of the bioassay (Eidy *et al.,* 2015). Aphids that are 21 days old were placed in each Petri dish. Distilled water was used to treat the adult aphids in control contains 80 percent in between (0.02%). To feed the aphids, we swapped over the rose leaves every day. All the treatments were maintained at 26°C, 70% relative humidity, and a photo period of 16:8 hours (L:D) in an incubator. Each individual aphid was checked for death daily.

EXPERIMENT 1: EPF SCREENING BIOASSAY AGAINST NYMPH

The aim of this laboratory investigation was to examine the virulence of 21 entomopathogenic fungal isolates against aphid nymphs. The bioassay area consists of petri plates with filter paper that we dipped in distilled water for each concentration and rose leaves. Using a camel hairbrush on rose leaf, twenty nymph aphids were transferred into each Petri dish. Three replications of each treatment were maintained.

EXPERIMENT 2. EPF SCREENING BIOASSAY AGAINST ADULTS

Adult aphids were transferred to a Petri dish using a camel hairbrush before being covered with a lid. Three Petri plates were utilized in each experiment, and 60 aphids in total were employed for each treatment. For the first, second, and third day, aphids were exposed to fungal conidia inside a Petri dish. Mortality of the aphids was noted daily.

Data analysis: The mortality data were transformed into percent corrected mortality by Abbot' formula (Abott, 1925).

$$\text{Corrected \% mortality} = 1 - \frac{n \text{ in C before treatment} \times n \text{ in T after treatment}}{n \text{ in C after treatment} \times n \text{ in T before treatment}} \times 100$$

Where: n = Insect population, T = treated, C = control

Statistical analysis

To calculate different ANOVA parameters and averages for different independent variables (treatments), this transferred corrected mortality data was subjected to an ANOVA at a 5% probability value using STATISTICS -10 software. Tukey's honestly

significant difference test was used to compare the means of significant treatments (Danho *et al.,* 2002). For each microbial pesticide, LC50 and LT50 values as well as the relevant descriptive parameters (values of degree of freedom, P value fiducial limit, chi-square, and slope) were determined by applying probit analysis to mortality data using the Minitab Statistical Program (Finney, 1971). The effectiveness of the products was assessed using their LC_{50} and LT_{50} values. To establish regression between *M. rosae* mortality and concentrations, linear regression and Pearson correlation analyses were also conducted at a 5% α-value. The determination coefficient (R_2). To determine the kind and degree of relationship between concentrations of each microbial pesticide and *M. rosae* nymph and adult mortality, the linear regression equation and coefficient of correlation were calculated. To ascertain the trend of the fitted-simple regression line of Y (mortality) on X (concentration) of each microbial pesticide, scatter diagrams were also generated for each one.

BIOASSAY STUDY

Using a micro pipette, a 1 µL drop of each concentration was directly applied to the insect body as part of the bioassay (Eidy *et al.,* 2015). Aphids that are 21 days old are placed in each Petri dish using a camel hairbrush on rose leaf. The distilled water used to treat the adult aphids under control contains 80% tween (0.02%). To feed the aphids, we swapped over the rose leaves every day. To feed the aphids, we swapped over the rose leaves every day. All treatments were maintained at 28±2 °C, 70% relative humidity, and a photo period of 16:8 hours (L:D) in an incubator. Up until the third day, each individual aphid was checked for death every day.

maximum mortality rate, 83.67%, was achieved by *M. anixpoli*.

RESULTS

In the first experiment using *B. bassiana*, the results showed that the mortality rates for both nymph and adult stages increased with increasing concentration and post-treatment times. The observed highest mortality rates were 78.12% for adults and 63.21% for nymphs, measured by 72 hours post-treatment with the maximum concentration of *B. bassiana* (1 × 10^9 conidia mL^-1) (Figure. 1a-b). In the subsequent trial involving *M. anisopliae*, the mortality rates for both nymph and adult stages increased with escalating concentrations and extended post-treatment durations. The maximal recorded mortality rates were 83.67% for adults and 76.76% for nymphs, following a 72-hour treatment with the highest concentration of *M. anisopliae* (Figure 1 c-d).

In the third study concerning *V. lecanii*, mortality rates also exhibited an upward trend with increasing concentrations and longer post-treatment durations for both nymphs and adults. At 72 hours after treatment with the maximum concentration of *V. lecanii*, the mortality rate for adults was 61.67%, while for nymphs, it was 75.76% (Figure 2 a-b).

In the fourth study utilizing *M. attenuatum*, mortality rates similarly rose with increased concentrations and extended post-treatment durations for both nymph and adult stages. After 72 hours, the adult mortality rate reached 83.45%, and the nymph mortality rate was 76.56% following treatment with the maximum concentration of *M. attenuatum* (Figure 2 c-d). The nymph mortality rate was 76.56% following treatment with the maximum concentration of *M. attenuatum*.

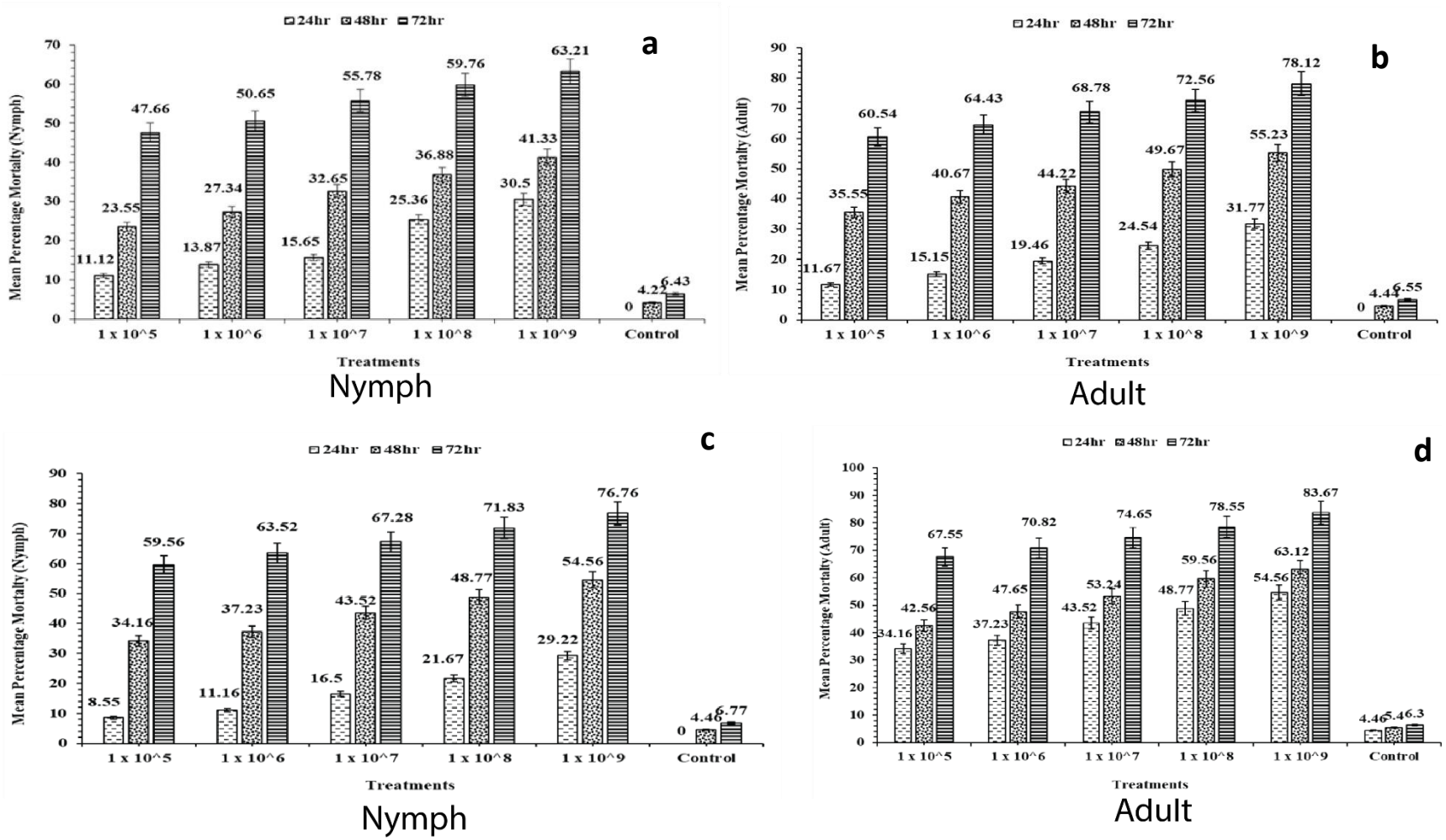

Figure 1: Mean percentage mortality of aphid (*Macrosiphum rosae*) after 24, 48 and 72 hours of post treatment application for different concentrations of (a-b) *B. bassiana* (c-d) of *M. anisopliae*

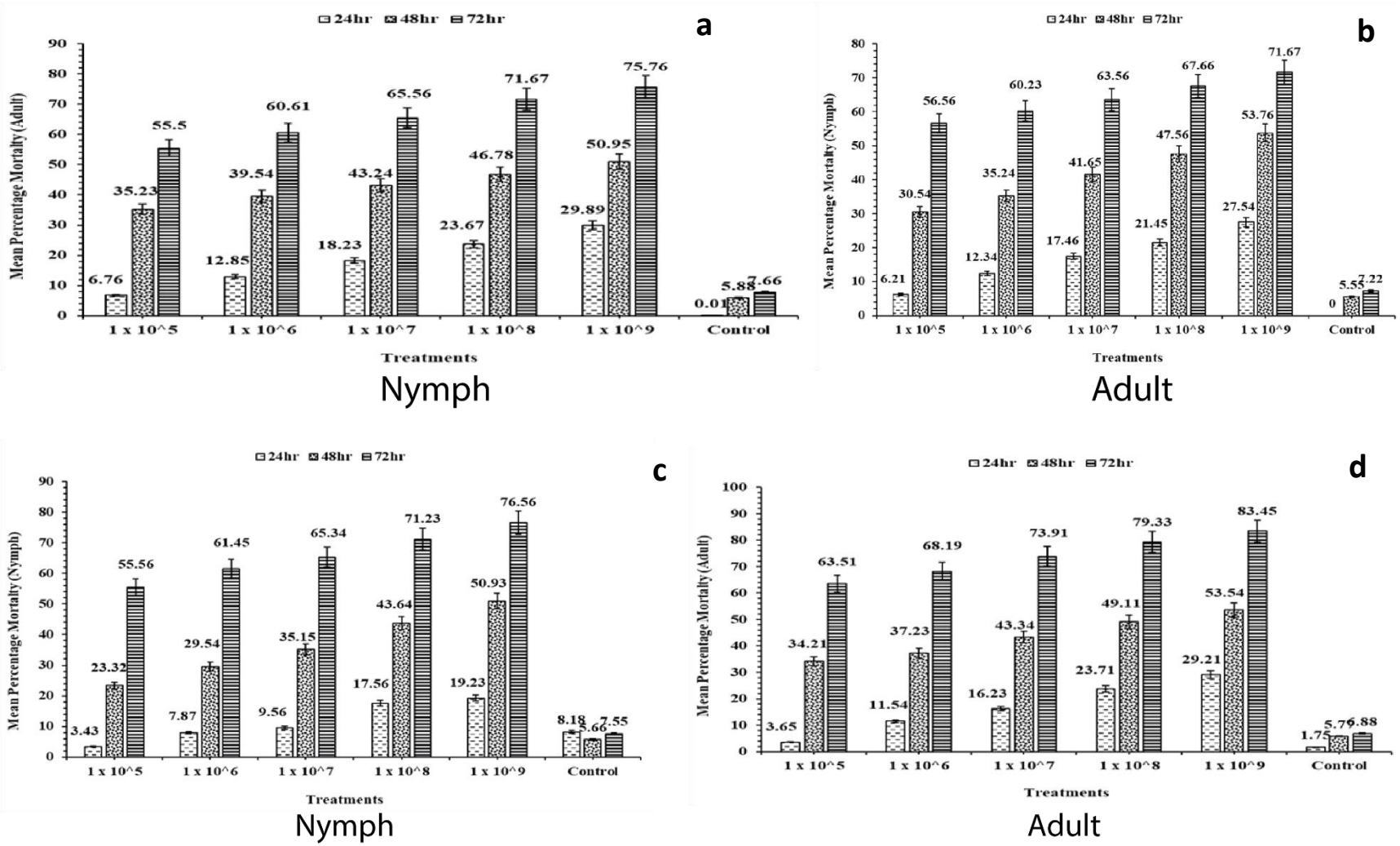

Figure 2: Mean percentage mortality of aphid (*Macrosiphum rosae*) after 24, 48 and 72 hours of post treatment application for different concentrations (a-b) of *V. lecanii* (c-d) *M. attenuatum*

Although to varying degrees, the results show that all four fungi can successfully control aphids, depending on the specific fungal species and concentration used. Higher fungal concentrations and longer post-treatment periods were often linked to higher mortality rates. *B. bassiana* and *M. anixpoli* were the two species that killed the most aphids, according to our research. After 72 hours, the maximum mortality rate, 83.67%, was achieved by *M. anixpoli*.

BIOASSAY STUDY

The efficiency of a fungus species called *B. bassiana* against aphids at varying exposure intervals is shown in Table 1. *B. bassiana* LC_{50} and LC_{90} values were measured for adult and nymph aphids at 24, 48, and 72 hours of exposure. At 24 hours of exposure, the adult aphids' maximal LC_{50} value was 1.45 × 10^11 conidia mL^{-1}; at 48 and 72 hours, the values decreased. At 24 hours of exposure, the nymphs' maximal LC_{50} value was 2.6×10^{10} conidia mL^{-1}; at 48 and 72 hours, the values decreased. Furthermore, LC_{90} values for both adults and nymphs were typically higher than LC_{50} values, indicating that higher fungal concentrations were needed to kill 90% of the test population rather than 50%. The study also revealed the slope and significance level of the regression analysis, proving *B. bassiana* effectiveness against aphids. Slope values ranging from 0.06 to 0.13 indicated a relatively positive relationship between aphid mortality and fungal concentration. The significance levels were high ($P > 0.67$) for all exposure intervals, indicating a statistically significant relationship between aphid mortality and fungal concentration.

LC_{50} values for adult and nymph aphid was 1.3×10^{11} conidia mL^{-1} and 2.2×10^{9} conidia mL^{-1} after 24 hours of exposure and LC_{50} values decreased after exposure to 48 hours and 72 hours. LC_{90} values was higher compared to LC_{50} values at same exposure intervals. Regression analyzed show3d significant effect for virulence efficacy for *M. anisopliae.*

LC_{50} and LC_{90} values for *V. lecanii* ranged from 6.8×10^{8} to 1.45×10^{11} conidia mL^{-1} and 7.2×10^{15} to 2.16×10^{15} conidia mL^{-1} for different exposure intervals. The LC_{50} values for nymph aphids range from 9.3×10^{2} to 6.0×10^{10} conidia mL^{-1}, while the LC_{90} values range from 1.5×10^{16} to 2.2×10^{15} conidia mL^{-1}, depending on the exposure duration. The concentration-mortality response curve has a slope between 0.05 and 0.11 ± 0.02. The FD limit and χ^2 values are also provided for each concentration and exposure time. The FD limit represents the 95% fiducial limit for the estimated LC_{50} or LC_{90} value, while χ^2 is the goodness-of-fit statistic for the concentration-mortality response curve. When the *M. attenuatum* was used against aphid after different exposure intervals. LC_{50} is the concentration of a substance required to kill 50% of a population, while LC_{90} is the concentration required to kill 90% of the population.

The table has two sections: one for nymphs and one for adults. The durations of exposure are 24, 48, and 72 hours. The LC_{50} values for adult aphids vary based on the exposure interval, ranging from 7.8×10^{10} to 1.0×10^{6} conidia mL^{-1}. The range of the LC_{90} values is 9.7×10^{15} to 4.5×10^{26} conidia mL^{-1}. This concentration-response curve has a rather steep slope (Slope ± S.E.) that runs from 0.06 to 0.10. The chi-square (χ^2) values, which range from 0.00 to 0.13, and the p-values, which range from 0.98 to 1.00, show that the data and the model match satisfactorily. The LC_{50} and LC_{90} values of nymphs range from 2.5×10^{12} to

1.0×10^{9} conidia mL^{-1} and 1.04×10^{20} to 1.08×10^{78} conidia mL^{-1}, respectively, depending on the duration of exposure. From 0.03 to 0.06 is the comparatively shallow slope of the concentration-response curve. Chi-square (χ^2) values range from 0.05 to 0.36, and P-values range from 0.94 to 1.00, indicating a reasonable match between the data and the model.

M. attenuatum has a strong insecticidal effect on aphids, according to the results, despite a very steep concentration-response curve. Aphids' efficiency is influenced by their age; nymphs are more susceptible than adults. Also, the results demonstrate that lower LC_{50} and LC_{90} values during extended exposure times suggest a more powerful fungus.

LETHAL TIME (LT)

LT_{50} and LT_{90} values for adult and nymph aphids at different fungal concentrations for Beauveria bassiana are listed in Table 2. The LT_{50} values represent the amount of time needed to eradicate 50% of the population, while the LT90 values reveal the amount of time needed to eradicate 90% of the population. Along with the χ^2 and P values, the table also shows the slope ± S.E. for the LT_{50} and LT_{90} values.

At the maximum concentration of 1×10^9 conidia/ml, the LT_{50} values for adult aphids were 42.18 days, while at the lowest concentration of 1 x 10^5 conidia mL^{-1}, they were 71.69 days. At the greatest concentration, the LT_{90} values were 99.51 days, while at the lowest concentration, they were 145.097 days. The slope of the LT_{90} values ranged from 1.3±0.21 to 1.4±0.2, whereas the LT_{50} values ranged from 1.2±0.2 to 1.7±0.3. The LT_{50} values for nymph aphids were 41.51 days at the highest concentration of 1×10^9 conidia mL^{-1} and 64.75 days at the lowest concentration of 1 × 10^5 conidia mL^{-1}. The LT_{90} values ranged from 106.781 days at the highest concentration to 165.96 days at the lowest concentration. The slope of the LT_{90} values ranged from 1.27±0.2 to 1.7±0.24, whereas the LT_{50} values ranged from 1.3±0.20 to 1.7±0.26.

The time it takes to kill 50% of the population is known as the LT_{50} of *M. anisopliae*, while the time it takes to kill 90% of the population is known as the LT_{90}. With the highest concentration of 1×10^9 conidia mL^{-1}, the LT_{50} values for adult aphids were 49.39 days, whereas the lowest concentration of 1 × 10^5 conidia/ml was 76.02 days. At the greatest concentration, the LT_{90} values were 122.119 days, while at the lowest concentration, they were 133.82 days. The LT_{50} values had a slope between 1.2±0.2 and 2.1±0.3, and the LT_{90} values had a slope between 1.3±0.22 and 1.7±0.3. For nymph Aphids, the LT_{50} values ranged from 51.34 days at the highest concentration to 76.40 days at the lowest concentration. The LT_{90} values ranged from 138.64 days at the highest concentration to 165.96 days at the lowest concentration. The slope for the LT50 values ranged from 1.1±0.2 to 1.8±0.3, while the slope for the LT_{90} values ranged from 1.1±0.2 to 1.4±0.2.

In case of *Verticillium lecanii,* LT_{50} and LT_{90} values (conidia mL^{-1}) against aphids following varying exposure times for both adults and nymphs. Slope, χ^2, D.F., and P-values for every tested concentration are also included in the table. For adults, the LT_{50} values range from 47.65 days at a concentration of 1×10^9 conidia mL^{-1} to 83.13 days at a concentration of 1×10^5 conidia mL^{-1}, while the LT_{90} values range from 194.85 days to 218.91 days, respectively. For nymphs, the LT_{50} values range from 44.18 days at a concentration of 1×10^9 conidia/ml to 66.6 days at a concentration of 1 x 10^5 conidia mL^{-1}, while the LT_{90}

values range from 120.09 days to 135.16 days, respectively. The slope values for adults and nymphs range from 0.85 to 1.8 and from 1.20 to 1.8, respectively. The χ^2 values range from 0.01 to 3.2 for adults and from 0.04 to 0.24 for nymphs, while the P-values range from 0.07 to 0.96 for adults and from 0.61 to 0.82 for nymphs. The FD limit values are also provided for each concentration tested.

The LT_{50} and LT_{90} values (conidia mL^{-1}) of *Metarhizium attenuatum* against aphids after different exposure intervals. The table is divided into two sections, one for adults and one for nymphs, and each section includes data on different concentrations of conidia mL^{-1}. For adults, the table shows that the LT_{50} values range from 44.7779 days for the concentration of 1 x 10^9 conidia mL^{-1} to 82.2021 days for the concentration of 1 x 10^5 conidia mL^{-1}. The LT_{90} values range from 196.743 days for the concentration of 1 x 10^9 conidia mL^{-1} to 225.03 days for the concentration of 1 × 10^5 conidia mL^{-1}. The slope ± S.E. values range from 0.81±0.19 to 1.19±0.27, and the χ^2 values range from 0.024 to 0.23. For the nymphs, the table shows that the LT50 values range from 41.40 days for the concentration of 1 × 10^9 conidia mL^{-1} to 73.92 days for the concentration of 1 × 10^5 conidia mL^{-1}. The LT_{90} values range from 192.65 days for the concentration of 1 × 10^9 conidia mL^{-1} to 298.89 days for the concentration of 1 × 10^7 conidia mL^{-1}. The slope ± S.E. values range from 0.72±0.1 to 1.3±0.27, and the χ^2 values range from 0.001 to 2.5. The FD Limit column provides the confidence interval for the LT_{50} and LT_{90} values, and the D.F. column shows the degrees of freedom. The P column shows the probability level for the goodness-of-fit test, where a value less than 0.05 indicates a good fit.

Entomopathogenic fungi are an efficient way to reduce the amount of insecticide used on rose plants to control aphids. In the instance of *Beauveria bassiana*, the lowest LC_{50} and LC_{90} values were 8.6 ×10^8 conidia mL^{-1} and 8.7 × 10^{10} conidia mL^{-1} after 72 hours, whereas the lowest LT_{50} and LT_{90} values were 71.69 days and 149.7 days, respectively. The minimum LT_{50} and LT_{90} values for *M. anixpoli* were 76.02 days and 150.02 days, respectively, whereas the minimum LC_{50} and LC_{90} values were 1.0 ×10^6 conidia mL^{-1} and 2.23 ×10^{13} conidia mL^{-1}.

DISCUSSION

Insect pests are the primary challenge in the post-green revolution age, which can lead to 10.80% global crop losses (Litwin *et al.,* 2020). According to Russo *et al.* (2021), there has been a recent loss of $470 billion, or 18 to 26% of the world's yearly crop production. Since insecticides lower insect populations, they can help offset these losses. However, excessive pesticide use has led to up to 500 distinct pest species becoming resistant to one or more insecticide types.

We should use entomopathogens, which comprise various viruses, bacterial, fungal, nematode, and protozoan species, to prevent insecticidal resistance. In addition to being efficient against insect pests, this method is safe for the environment, both for humans (owing to lower pesticide residues) and non-target organisms (Singh and Tanjot, 2020). This investigation employed four strains of entomopathogenic fungi: *B. bassiana*, *M. anixpoli*, *V. lecani*, and *M. attenuatum*. The percentage adjusted mortality data suggests that the maximum percentage mortality of aphid nymphs against *B. bassiana* 109 concentration was 30.5% after 24 hours of treatment application. The highest mortality rate in adult aphids against 10^9 concentration was 31.77%.

Table 1: LC_{50} and LC_{90} values (conidia mL^{-1}) of entomopathogenic fungi against rose aphid after different exposure intervals

Sex	Time (hours)	LC_{50} (conidia mL^{-1})	FD Limit	LC_{90} (conidia mL^{-1})	FD Limit	Slope ± S.E.	χ^2	D.F.	P
				Beauveria bassiana					
Adult	24	1.45×10^{11}	6.44×10^{9} - 1.02×10^{15}	2.16×10^{15}	4.4×10^{12} - 2.3×10^{23}	0.12 ± 0.03	0.29	3	0.96
	48	8.6×10^{8}	9.3×10^{7}- 8.17×10^{11}	1.7×10^{16}	5.7×10^{12} 2.9×10^{30}	0.07±0.02	0.02	3	0.99
	72	2.0×10^{5}	7.9×10^{3}- 1.0×10^{6}	8.7×10^{10}	5.9×10^{9} - 3.3×10^{13}	0.09±0.01	0.81	3	0.84
Nymph	24	2.6×10^{10}	2.4×10^{9}-9.1×10^{12}	2.6×10^{14}	1.63×10^{12} -1.4×10^{20}	0.13±0.02	0..22	3	0.97
	48	1.6×10^{8}	1.9×10^{7}-7.3 x10	4.7×10^{16}	6.2×10^{12} -1.1×10^{26}	0.06±0.02	0.13	3	0.98
	72	4.0×10^{4}	6.5×10^{2}-3.5×10^{5}	4.8×10^{10}	3.4×10^{9} -2.0×10^{13}	0.08±0.01	1.5	3	0.67
				Metarhizium anisopliae					
Adult	24	1.3×10^{11}	8.6×10^{9}-1.38×10^{14}	2.5×10^{14}	1.4×12 -7.8×10^{19}	0.16±0.03	0.81	3	0.84
	48	5.4×10^{9}	3.9×10^{8} -3.7×10^{13}	2.4×10^{16}	8.5×10^{11}-6.8×10^{29}	0.07±0.02	0.04	3	0.09
	72	1.0×10^{6}	3.0×10^{4}-5.9×10^{6}	2.23×10^{13}	1.0×10^{11}-2.3×10^{19}	0.07±0.01	0.37	3	0.94
Nymph	24	2.2×10^{9}	1.4×10^{9}-5.8×10^{9}	4.9×10^{9}	3.0×10^{9}-1.3×10^{10}	0.14±0.03	9.2	3	0.02
	48	1.0×10^{9}	6.3×10^{8} -5.0×10^{9}	4.7×10^{9}	2.72×10^{9}-2.5×10^{10}	0.07±0.02	4.0	3	0.25
	72	1.9×10^{7}	1.8×10^{6} -3.3×10^{8}	1.17×10^{14}	2.07×10^{7}-2.86×10^{25}	0.05±0.08	0.18	3	0.97
				Verticillium lecanii					
Adult	24	1.45×10^{11}	6.4×10^{9}-1.0×10^{1}	2.16×10^{15}	4.4×10^{1}-2.3×10^{2}	0.12±0.03	0.29	3	0,96
	48	6.8×10^{8}	1.4×10^{7}-8.2×10^{9}	7.2×10^{15}	3.0×10^{1}-9.4×10^{3}	0.06±0.02	0.01	3	1.00
	72	1.1×10^{7}	6.9×10^{5}-1.2×10^{8}	2.0×10^{15}	1.2×10^{1}-5.0×10^{2}	0.06±0.02	0.00	3	1.00
Nymph	24	6.0×10^{10}	3.3×10^{9}-2.0×10^{14}	2.2×10^{15}	4.3×10^{1}-2.6×10^{2}	0.11±0.02	0.28	3	0.96
	48	4.3×10^{7}	4.3×10^{6}-2.1×10^{9}	1.5×10^{16}	3.4×10^{1}-2.6×10^{2}	0.06±0.02	0.09	3	0.99
	72	928.636	3.0×10^{7}-6.6×10^{4}	2.3×10^{13}	7.4×10^{10}- 7.0×10^{2}	0.05±0.01	0.01	3	1.00
				Metarhizium attenatum					
Adult	24	7.8×10^{10}	3.32×10^{9}-1.56×10^{15}	9.7×10^{15}	8.05×10^{12} -1.75×10^{26}	0.10±0.02	0.13	3	0.98
	48	8.6×10^{8}	9.3×10^{7}-8.17×10^{11}	1.7×10^{16}	5.73×10^{12} -2.29×10^{30}	0.07±0.02	0.02	3	0.99
	72	1.0×10^{6}	5.5×10^{3} -7.9×10^{6}	3.7×10^{14}	4.56×10^{11} -4.50×10^{26}	0.06±0.01	0.00	3	1.00
Nymph	24	2.5×10^{12}	9.9×10^{9} -1.01×10^{31}	1.04×10^{20}	2.29×10^{14} -8.01×10^{65}	0.06±0.02	0.36	3	0.94
	48	1.0×10^{9}	5.52×10^{7}-2.31×10^{18}	1.8×10^{19}	5.10×10^{13}-1.08×10^{78}	0.05±0.02	0.01	3	1.00
	72	1.0×10^{3}	1.03×10^{1}-3.06×10^{4}	1.71×0^{17}	1.88×10^{12}-5.0×10^{137}	0.03±0.01	0.05	3	0.99

Table 2: LT_{50} and LT_{90} values of entomopathogenic fungi against rose aphid after different exposure intervals

Fungi	Stage	Concentrations	LT_{50} (days)	LT_{90} (days)	Slope ± S.E.	χ^2	D.F.	P
Beauveria bassiana	Adult	1×10^5	71.69	145.09	1.7±0.3	0.01	1	0.89
		1×10^6	65.68	149.77	1.4±0.2	0.01	1	0.89
		1×10^7	58.88	148.62	1.2±0.2	0.10	1	0.7
		1×10^8	49.39	122.11	1.3±0.22	0.86	1	0.3
		1×10^9	42.18	99.511	1.3±0.21	2.67	1	0.1
	Nymph	1×10^5	64.75	109.54	1.3±0.20	0.01	1	0.91
		1×10^6	59.69	120.95	1.7±0.26	0.63	1	0.421
		1×10^7	56.20	125.32	1.4±0.24	0.65	1	0.420
		1×10^8	49.42	121.72	1.3±0.22	0.17	1	0.67
		1×10^9	41.51	106.78	1.27±0.2	0.01	1	0.90
Metarhizium anisopliae	Adult	1×10^5	76.02	133.82	2.1±0.3	0.39	1	0.5
		1×10^6	71.69	145.09	1.7±0.3	0.01	1	0.8
		1×10^7	65.68	149.77	1.4±0.2	0.01	1	0.89
		1×10^8	58.88	148.62	1.2±0.2	0.10	1	0.7
		1×10^9	49.39	122.11	1.3±0.22	0.86	1	0.3
	Nymph	1×10^5	76.40	147.17	1.8±0.3	0.49	1	0.48
		1×10^6	71.24	158.50	1.5±0.2	0.05	1	0.82
		1×10^7	68.14	160.11	1.4±0.2	0.01	1	0.88
		1×10^8	60.91	165.96	1.1±0.2	0.16	1	0.68
		1×10^9	51.34	138.64	1.2±0.2	1.1	1	0.29
Verticillium lecanii	Adult	1×10^5	83.13	206.83	1.3±0.28	3.2	1	0.07
		1×10^6	75.21	218.15	1.1±0.25	2.36	1	0.12
		1×10^7	66.00	218.91	1.0±0.22	1.89	1	0.16
		1×10^8	56.58	210.47	0.91±0.21	1.60	1	0.20
		1×10^9	47.65	194.85	0.85±0.19	1.41	1	0.235
	Nymph	1×10^5	66.61	127.13	1.8±0.3	0.24	1	0.61
		1×10^6	62.01	129.01	1.6±0.26	0.14	1	0.70
		1×10^7	57.208	135.16	1.3±0.24	0.04	1	0.82
		1×10^8	51.11	126.62	1.3±0.2	0.08	1	0.77
		1×10^9	44.18	120.09	1.20±0.20	0.14	1	0.70
Metarhizium attenatum	Adult	1×10^5	82.20	225.03	1.19±0.27	0.024	1	0.87
		1×10^6	73.21	232.67	1.0±0.24	0.07	1	0.7
		1×10^7	63.37	229.16	0.93±0.22	0.12	1	0.72
		1×10^8	53.68	216.35	0.86±0.20	0.18	1	0.67
		1×10^9	44.77	196.74	0.81±0.19	0.23	1	0.62
	Nymph	1×10^5	73.92	175.68	1.3±0.27	0.12	1	0.71
		1×10^6	72.17	244.92	0.98±0.23	0.001	1	0.971
		1×10^7	57.48	298.89	0.77±0.2	1.8	1	0.16
		1×10^8	51.03	247.43	0.76±0.20	2.5	1	0.11
		1×10^9	41.40	192.65	0.72±0.1	2.2	1	0.13

Following a 48-hour treatment period, the mortality rate among aphid nymphs exposed to a concentration of *B. bassiana* at 10^9 was recorded at 41.3%. Maximum aphid mortality (55.23%) was observed at highest concentration. Similarly, 78.1% and 63.21% mortality rate was recorded for adult and nymph of aphid at concentration 1×10^9 spores/ml. Similar find also reported by Selvaraj et al. (2012) and Eidy et al. (2015) in his finding.

Maximum nymph and adult mortality (29.2%) and (38.01%) respectively were recorded at 1×10^9 spores/ml against *M. anixpoli*. Similarly (54.5%) and (63.1%) nymph and adult aphid mortality was observed at concentration 1×10^9 spores/ml against *Beauveria bassiana*. These results were also supported by Acosta et al. (2022). At concentration 1×10^9 spores/ml for EPF strains *V. lecanii* maximum mortality (27.54% and 29.89%), (53.76% and 590.01%) and (75.76% and 71.67%) was recorded for nymph and adult of rese aphid after 24h, 48h and 72hrs of post treatment application respectively. Alike results were also reported by Iqbal et al. (2018).

Mortality rate (19.23% and 29.21%), (50.93% and 53.54%) and (83.45% and 76.56%) for nymph and adult of aphid was observed for *M. attenuatum* at concentration 1×10^9 spores/ml was demonstrated by Sayed et al. (2019) in his finding,

CONCLUSION

Maximum mortality of aphids was observed at concentration 1×10^9 spores/ml for *B. bassiana*. Hence for effective and environment friendly aphid management tactics as well as for sustainable management of aphid *B. bassiana* as EPF was recommended. EPF strains was more reliable and economical biocontrol tool for IPM program.

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AUTHOR'S CONTRIBUTION:

All authors confirm that they have contributed equally and directly to all parts of this study, including planning, conducting experiments, analyzing data, and writing the manuscript. Each author has reviewed and approved the final version of the paper and agrees to its submission and publication in this journal. All authors also take responsibility for the accuracy and integrity of the work.

CONFLICT OF INTREST

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