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Pathogenic potential of native entomopathogenic fungi against Corn earworm, *Helicoverpa zea* (Lepidoptera: Noctuidae)

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ABSTRACT

The current experiment was conducted to evaluate the pathogenic potential of native isolates of entomopathogenic fungi viz., *Nomuraea rileyi*, *Aspergillus parasiticus*, *A. niger* and *A. flavus* against corn earworm, *Helicoverpa zea* under laboratory conditions. The spore concentration of each tested fungi were adjusted to 1×10^8 spores/ mL and 10 µL volume of each was topically inoculated on dorsal side of the 3rd instar larvae. The experimental results revealed that the *A. parasiticus* and *N. rileyi* was found highly virulent to cause earliest first mortality, maximum total mortality and minimum adult emergence of *H. zea*. Larval and pupal duration of *H. zea* was not affected by the tested entomopathogenic fungi. With respect to lethal time to kill 50% population of *H. zea* larvae, the lowest LT_{50} value was recorded for *A. parasiticus* (7.14 days) as compared to other tested fungi. It is concluded that the *A. parasiticus* and *N. rileyi* have the potential to combat *H. zea* populations and could be used in successful Integrated Pest Management programs after ensuring biosafety evaluations.

Keywords: *Aspergillus parasiticus*, corn earworm, *Helicoverpa zea*, *Nomuraea rileyi*, lethal time

INTRODUCTION

Maize (*Zea mays* L.) is the most important cereal crop worldwide and ranked 3rd after rice and wheat. It serves as a staple food for humans, a vital source of livestock feed and a raw material for industrial products including starch, sweeteners and biofuels [1]. Global demand for maize is steadily increasing due to rise in population growth and increasing use in ethanol production. Nevertheless, maize productivity is constantly threatened by insect pests which significantly reduce yields range from 16-41% and increase management costs [2,3]. Many insect species have been reported to infest maize worldwide, among which the *Helicoverpa zea* (Boddie) also known as corn earworm or New World bollworm is recognized as one of the most important destructive pests. It attacks over 180 plant species including sorghum, maize, chickpea, cotton, sunflower, tobacco, pigeonpea, tomato and several fruits and vegetables. The larvae feed primarily on reproductive parts of the plant which lead to substantial yield losses especially during the flowering or pod formation [4,5,6]. The wide host range, high dispersal ability and capacity to thrive year-round in tropical and subtropical regions make *H. zea* a persistent and challenging pest for management. In temperate regions, populations survive through overwintering or seasonal migration, with outbreaks strongly influenced by climatic conditions such as temperature and rainfall [7].

Climate change is expected to further enhance the threat posed by *H. zea* by promoting its population growth and enabling expansion into new regions. Modeling studies predict that the rising temperatures, altered precipitation patterns, and elevated atmospheric CO□ levels may increase the geographic distribution, survival and reproduction of pest [8]. Consequently, *H. zea* poses an increasing threat to maize production under future climate scenarios, demanding the development of sustainable and eco-friendly control strategies.

Conventional chemical insecticides while providing partial suppression of *H. zea* and other agricultural pests, have been shown to negatively impact the soil and microbial communities which reduce the soil fertility and hinder plant-microbe symbiosis essential for healthy plant growth [9,10]. Alternatively, biological control using microbial bio control agents have gained attention as a sustainable and eco-friendly technique for managing agricultural pests [11]. Globally, more than 750 species of fungi belongs to 90 genera are known to be pathogenic to thousands of insect species with notable genera of *Beauveria*, *Cordyceps*, *Isaria* and

Metarhizium. These entomopathogenic fungi play significant role in suppressing or regulating the pest population naturally [12,13,14]. Entomopathogenic fungi infect insect when conidia adhere to the cuticle, followed by penetration aided by cuticle-degrading enzymes such as proteases, chitinases, and lipases. Once the fungal hyphae breach the cuticle, it proliferate within the insect body's cavity and release toxins into the hemolymph, ultimately killing the host [15,16]. In Pakistan, the comparative assessments of native isolates of entomopathogenic fungi against *Helicoverpa zea* are scarce and necessitates studies to determine their effectiveness which could provide scientific evidence for developing sustainable biocontrol strategies. Therefore, the current study was conducted to evaluate the effect of four native entomopathogenic fungi on the biological parameters (larval mortality, larval duration, pupal recovery, pupal duration and adult emergence) of *H. zea* under controlled laboratory conditions.

MATERIAL AND METHOD

Insect culture

A visit of maize field was conducted at University of Agriculture, Peshawar for the collection of *Helicoverpa zea* larvae. To prevent cannibalism, larvae were individually reared in disposable plastic cups and provided with fresh maize leaves daily until pupation. Pupae were then transferred to other disposable plastic cups for the adult emergence. Newly emerged adults were transferred to 2.5 L plastic jars, with the top covered by a muslin cloth and the inner walls lined with strips of muslin cloth to facilitate oviposition. A 10% sugar solution was provided as a food source for adults in the plastic jars. Strips of muslin cloth with eggs were replaced daily and stored in zip lock bag until hatching. Upon hatching, neonate larvae were supplied with tender and fresh maize leaves. Culture of *H. zea* was reared under controlled conditions i.e., 25±2 °C, 50±10% R.H. and 08:16 h light and dark period. Third instar larvae of *H. zea* was used in the experiment.

Fungal material

Four fungal isolates viz., *Noumeria rileyi*, *Aspergillus niger*, *A. parasiticus* and *A. flavus* were acquired from the already established culture in Plant Protection Division, NIFA Peshawar and sub-cultured on Sabouraud Dextrose Agar Yeast media in sterile petri plates. For smooth growth

of the fungi, the petri plates were incubated at 25±2°C under dark condition. After two to three weeks, the fungal conidia were isolated from sporulated cultures by suspended 10 mL distilled water with 0.05% Tween-80 in the cultural plates. Spatula was used to detach the spores from the media and suspension was collected in test tubes. Utilizing a hemocytometer, conidial suspensions were serially diluted to adjust the concentration at 1×10^8 conidia per mL before to the bioassay.

Bioassay: Effect of entomopathogenic fungi on biological parameters of *H. zea*

All the four fungal isolates *N. rielyi, A. flavus, A. parasiticus* and *A. niger* were assessed against 3rd instars of *H. zea* under laboratory conditions. Larvae were obtained from already established colony and were shifted into the clean disposable plastic cups containing filter paper at the bottom to absorb the excess fungal suspension. Larvae were topically inoculated with 10 µL of entomopathogenic fungi @ 1×10^8 spores per mL using micropipette (range: 10-100 µL) and provided with fresh maize leaves as a diet. Larvae treated with distilled water only was considered as control treatment. The experiment included ten larvae per replication and 30 larvae per treatment by following complete randomized design (CRD) with 3 replication of each treatment. All the larvae were effectively exposed to the fungal spore suspension to prevent larval escape from the inoculums. The cups containing treated larvae were placed in insect growth chamber under controlled conditions at 25±2°C temp. 50±10% R.H. and 08:16 H light and dark period. Diet was changed daily with fresh maize leaves to ensure the provision of healthy diet. Larval mortality data was recorded daily until the pupae formation.

First mortality

Post application of tested entomopathogenic fungi, the mortality data was recorded daily. The first mortality of *H. zea* larvae was recorded in replications of each treatment. Larvae was considered dead when no sign of movement was observed upon disturbance with camel hairbrush.

Total mortality

After topical bioassay, daily mortality data was noted until the death of the last larva. Total mortality is converted into percent mortality by following formula:

$$\text{Total mortality (\%)} = \frac{\text{Total dead larvae}}{\text{Total number of larvae}} \times 100$$

Larval duration

In order to determine the duration of larval development from 3rd instar to pupal formation, the treated larvae was individually placed in disposable plastic cups having perforated lids with the help of camel hair brush and provided with fresh maize leaves as diet. Larval period was monitored regularly till pupation and data recorded.

Pupal recovery

Surviving larvae at 6th instar stage from each treatment was individually transferred in separate disposable plastic cups having perforated lids to allow pupation. The percentage of pupae recovery was calculated by using the following formula:

$$\text{Pupae recovery (\%)} = \frac{\text{Number of pupae formed}}{\text{Total number of larvae}} \times 100$$

Pupal duration

In order to determine the pupal duration (time period between pupal formations to adult emergence) of *H. zea*, the newly formed pupae was placed individually in disposable plastic cups having perforated lids with the help of forceps. Pupal period was monitored regularly till the emergence of adult moths (eclosion) and data recorded.

Adult emergence

After emergence of adults from pupae, percentage of adult emergence was calculated by following formula:

$$\text{Adult emergence (\%)} = \frac{\text{Number of emerged adults}}{\text{Total number of pupae}} \times 100$$

Statistical analysis

Recorded data was analyzed by using statistical software “Statistix 8.1” through ANOVA and mean were compared by using Tukey Honestly Significance Difference test at 5% level of

significance. Probit analysis was performed to calculate lethal time (LT_{50}) of tested entomopathogenic fungi by using statistical software "SPSS v16".

RESULTS

First mortality of *Helicoverpa zea*

In fungal topical bioassay, the fungal treatments showed highly significant results in first mortality of *H. zea* (F = 29.5; *P* = 0.0000). The first mortality was ranged from 3.0 to 5.0 days with earliest mean mortality was recorded in *N. rileyi* and *A. parasiticus* i.e., 3.0 days as compared to other fungal treatments and control (Table 1).

Total mortality of *Helicoverpa zea*

The total mortality of *H. zea* was significantly affected by the tested entomopathogenic fungi (F = 41.1; *P* = 0.0000) result in last mortality after exposure to fungal treatments (4.2.1). The total mortality in fungal treatments ranged from 56.7 to 80.0% with maximum mortality was recorded in *A. parasiticus* i.e., 80% as compared to other treatments and control (Table 1).

Table 1: Effect of native EPFs on first mortality and total mortality of *Helicoverpa zea*

Fungal treatment	First mortality (days)	Total mortality (%)
Aspergillus niger	5.0 ± 0.58 B	60.0 ± 0.33 B
Aspergillus parasiticus	3.0 ± 0.00 C	80.0 ± 0.3 A
Noumarea rielyi	3.0 ± 0.00 C	63.3 ± 0.33 AB
Aspergillus flavus	3.3 ± 0.33 BC	56.7 ± 0.33 B
Control	8.0 ± 0.58 A	16.7 ± 3.33 C
Tukey-HSD value	1.8	16.9

The means in the column with different uppercase letters revealed statistically significant differences at 0.05% of probability (Statistix v8.1).

Larval duration of *Helicoverpa zea*

The fungal treatment showed non-significant result in larval duration of *H. zea* after exposure to fungal treatment (F = 1.65; *P* = 0.2362). However, the mean of larval duration (from 3rd instar to pupa formation) was ranged from 8.0 to 9.7 days (Table 2). It means that there is no effect of

entomopathogenic fungi on the development of surviving larvae of *H. zea* after treatment exposure.

Pupal duration of *Helicoverpa zea*

The pupal duration of *H. zea* was not affected by the fungal treatments significantly (F = 2.60; *P* = 0.1001). However, the mean pupal duration was ranged from 9.0 to 13.3 days (Table 2). It means that there is no effect of entomopathogenic fungi on the development of surviving pupae of *H. zea* after treatment exposure.

Table 2: Effect of native EPFs on larval and pupal duration of *Helicoverpa zea*

Fungal treatment	Larval duration (days)	Pupal duration (days)
Aspergillus niger	9.3 ± 0.58 A	13.3 ± 0.88 A
Aspergillus parasiticus	9.7 ± 0.00 A	12.7 ± 0.33 A
Noumarea rielyi	9.7 ± 0.33 A	12.0 ± 2.00 A
Aspergillus flavus	9.3 ± 0.33 A	11.3 ± 0.66 A
Control	8.0 ± 0.33 A	9.0 ± 0.00 A
Tukey-HSD value	2.5	4.8

The means in the column with different uppercase letters revealed statistically significant differences at 0.05% of probability (Statistix v8.1).

Pupal recovery of *Helicoverpa zea*

The pupal recovery of *H. zea* was significantly affected by the fugal treatment applications (F = 8.08; *P* = 0.0035). Minimum pupal recovery was recorded in *A. parasiticus* i.e., 20.0% followed by *N. rielyi, A. niger* and *A. flavus* with 36.7, 40.0 and 43.3%, respectively while maximum was recorded in control (46.7%) (Table 3).

Adult Emergence of *Helicoverpa zea*

The fungal treatments showed highly significant results in adult emergence of *H. zea* applications (F = 85.2; *P* = 0.0000). The adult emergence ranged from 30.0 to 83.3% with least adult emergence was recorded in *A. flavus* (30.0%) as compared to other treatments (Table 3).

Table 3: Effect of native EPFs on pupal recovery and adult emergence of *Helicoverpa zea*

Fungal treatment	Pupal recovery (%)	Adult emergence (%)
Aspergillus niger	40.0 ± 0.58 A	13.3 ± 0.33 B
Aspergillus parasiticus	20.0 ± 0.00 B	16.7 ± 0.33 B
Noumarea rielyi	36.7 ± 0.33 AB	16.7 ±0.33 B
Aspergillus flavus	43.3 ± 0.33 A	13.3 ± 0.33 B
Control	46.7 ± 0.33 A	78.3 ± 0.33 AB
Tukey-HSD value	16.9	14.3

The means in the column with different uppercase letters revealed statistically significant differences at 0.05% of probability (Statistix v8.1).

LT_{50} of entomopathogenic fungi against *Helicoverpa zea*

With respect to the time taken to cause 50% mortality (LT_{50}) of the exposed larvae of *Helicoverpa zea*, *A. parasiticus* had an LT_{50} of 7.14 days followed by *A. niger* (7.71 days) and *A. flavus* (8.45 days). Whereas *N. rielyi* took the longest time (9.32 days) to cause 50% mortality of the exposed larvae (Figure 1).

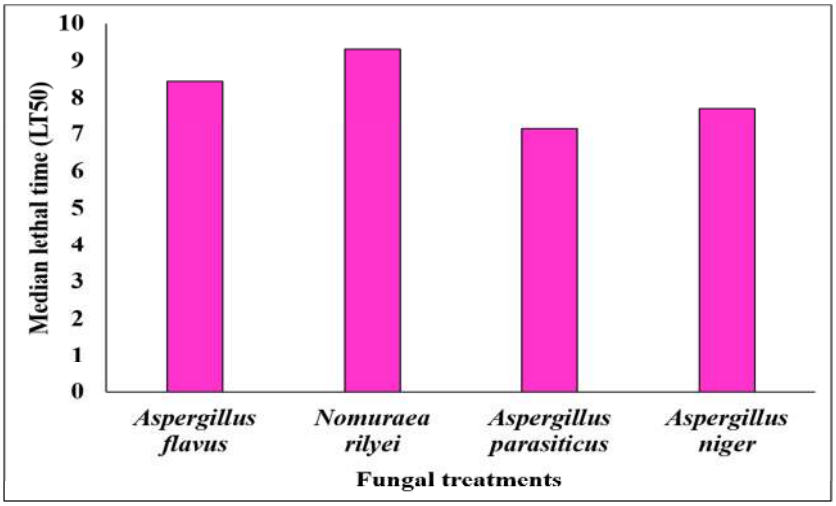

Figure 1: Lethal time (days) of different entomopathogenic fungi to kill 50% population of the exposed *Helicoverpa zea* larvae.

DISCUSSION

Helicoverpa zea is a highly significant pest that attacks various vegetable and cereal crops, causing serious financial harm and yield loss. Management of this pest heavily relies on

insecticides and the high cost of using chemical pesticides has resulted in insecticide resistance. The safety of entomopathogenic fungi toward humans, environment and non-target organisms is an important criterion for consideration and each insect-fungus system must be evaluated on a case-by-case basis. However, existing research suggests that the effects of entomopathogenic fungi on non-targets are minimal, offering a safer alternative for use in integrated pest management (IPM) compared to chemical insecticides [17]. To examine the native isolates of entomopathogenic fungi against *H. zea*, the present study was conducted.

Entomopathogenic fungi affects the developmental and reproductive parameters of insect pests (14,18]. Results of the current study revealed that the *A. parasiticus* caused 80% mortality of *H. zea* at concentrations of 1×10^8 spores/ mL after ten days of exposure. Our findings are agreed with the investigations of [19] who reported that the *Aspergillus sp.* effectively control the population of *Helicoverpa spp.* Our findings are also in line with those of [20,21] who reported that *A. parasiticus* and its aflatoxins are lethal to *H. zea* larvae, with mortality rates often exceeds 80% under continuous or high dose exposure. [22] reported that *N. rileyi* caused 78.9% mortality in 4^{th} instar larvae of *H. armigera* at concentrations of 1×10^8 spores/ ml. [17] investigated that the entomopathogenic fungi, *Cordyceps militaris* have potential to kill 95% population of early instars larvae of *H. zea* after ten days post exposure period.

[23] investigated that entomopathogenic fungus, particularly *N. rileyi*, showed high efficacy against *H. armigera* at second and third instar stages. The findings of the current study are contrary to those of [24], who investigated the efficacy of *N. rileyi* against the mortality of third instar larvae of *S. litura* and recorded a mortality rate of up to 96.7% at a spore concentration of 1×10^8 spores/mL. This discrepancy is due to the susceptibility of insect species to *N. rileyi*. In our study, *H. zea* was the targeted pest compared to *S. litura*.

In our observation, *A. parasiticus* have shortest LT_{50} value i.e., 7.14 days and highest in *N. rileyi* i.e., 9.32 days. Our findings are agreed with the investigations of [25], who reported the LT_{50} value (7.9-9.4 days) in *H. armigera* larvae. However, current results do not align with the findings of [24,26] who noted that the *N. rileyi* strain have lowest LT_{50} values ranging from 5.5 to 6.6 days in *Spodoptera litura*, respectively. The observed discrepancy may be attributed to the susceptibility of insect species to *N. rileyi*.

CONCLUSION

The present study demonstrated the effectiveness of different entomopathogenic fungi against *Helicoverpa zea* under laboratory conditions. Among the tested fungal species, *Aspergillus parasiticus* and *Nomuraea rileyi* emerged as the most potent biocontrol agents. They caused the earliest first mortality, highest total larval mortality, lowest pupal recovery and significantly reduced the adult emergence. Additionally, *A. parasiticus* exhibited the shortest lethal time, confirming its rapid pathogenicity. *Nomuraea rileyi* indicated a slower action compared to *A. parasiticus* with LT_{50} value of 9.32 days. Although *A. flavus* and *A. niger* displayed moderate efficacy in terms of larval mortality, pupal recovery and adult emergence. Larval and pupal durations were not significantly affected by fungal treatments, though slight prolongation was observed, indicating some sub-lethal effects on development. It is concluded that the *A. parasiticus* and *N. rileyi* demonstrated strong potential as eco-friendly biocontrol agents against *H. zea*, supporting their use in integrated pest management (IPM) programs aimed at reducing chemical pesticide reliance.

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