



## Research Article

# Toxicity of entomopathogenic fungi against *Spodoptera frugiperda* larvae under laboratory conditions

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**Received:** 25 October, 2021

**Accepted:** 16 November, 2021

**Published:** 17 November, 2021

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**Keywords:** *Spodoptera frugiperda*; Invasive alien species; Maize; Biopesticides; Pakistan

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## Abstract

Maize Fall Armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is considered an economically important pest and becoming the main threat to food security. This polyphagous pest is widely distributed in various countries of the world especially tropical and subtropical regions. The toxicity of two entomopathogenic fungi such as *Metarhizium anisopliae* and *Beauveria bassiana* were evaluated against 2<sup>nd</sup> instar larvae of FAW under controlled conditions. The results showed that among tested entomopathogenic fungi, *B. bassiana* was found more toxic than *M. anisopliae*. *B. bassiana* caused 79% larval mortality while *M. anisopliae* 59%. *M. anisopliae* and *B. bassiana* were showed LT<sub>50</sub> of 84.01 h and 80.99 h, respectively. *M. anisopliae* and *B. bassiana* were showed LC<sub>50</sub> of 1.3×10<sup>7</sup> and 1.8×10<sup>7</sup> spores ml<sup>-1</sup>, respectively. The current study concluded that Entomopathogenic fungi can give effective control against early instar and further studies are needed to check the efficacy against older instars under field and laboratory conditions.

## Introduction

Fall armyworm, *Spodoptera frugiperda* belongs to order Lepidoptera and Family Noctuidae has reported from several countries of the globe. It has been reported from Africa in 2016 [1], Pakistan in 2017, India in 2018 (29), China in 2019 [2,3] while native to tropical and subtropical regions of the world [4-6]. *Spodoptera frugiperda* larvae causes damage to other host plant species belonging to following families; Asteraceae, Fabaceae and Poaceae.

The most important crops that infested with larvae are rice, sorghum, cabbage, cotton, wheat, tomato, millet, beet, groundnut, onion, potato and soyabean [7-10]. Maize, *Zea mays* commonly known as queen of cereal is an important cereal crop with high reproductivity all over the world [11], source of livestock feed, highly infested with invasive alien insect

species, Fall armyworm (FAW) *Spodoptera frugiperda* [12]. The larvae attack on the tender parts of plants, mostly feed on the young plant parts and even cob [13]. The severe attack of larvae has caused 8–20 million tons per annum maize yield losses in Africa [14]. It is becoming major threat for maize production in Pakistan and as polyphagous in nature, pest is spreading to other host plants.

Therefore, quick actions are needed to control the current pest at early stage to minimize the yield losses. Several management approaches have been adopted to control insect pests such as chemical, biological and botanicals throughout the globe [15]. Chemicals (insecticides) are best methods that give quick and positive response against insect pests but negative impacts on environment, natural enemies and cause resistance to insect pests.

An ecofriendly and safe management practice should be developed against insect pests to minimize insecticides resistance and environmental pollution in Pakistan [15]. The entomopathogenic microorganisms (fungi, nematodes, bacteria and viruses) are ecofriendly and safe for biological fauna but larvae of *Spodoptera frugiperda* susceptible to these microbes [16]. However, no entomopathogenic fungi related studies have been conducted against this notorious pest of agricultural crops in Pakistan. Therefore, the current study was conducted to check the toxicity of entomopathogenic fungi against larvae under laboratory conditions.

## Material and methods

### Insect collection

*Spodoptera frugiperda* larvae were collected from different unsprayed fields of maize crops cultivated in district Multan. The collected larvae with maize leaves were shifted into Rearing Laboratory at Institute of Plant Protection.

### Maintenance of mass culture

The culture was maintained in Rearing Laboratory at 26.2°C, 75% relative humidity (RH), and 14:10 h day–light photoperiod.

### Toxicity

Two entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana* were tested against 2<sup>nd</sup> instar larvae of *S. frugiperda*. The different spore concentrations ( $1 \times 10^4$ ,  $1 \times 10^5$ ,  $1 \times 10^6$ ,  $1 \times 10^7$  and  $1 \times 10^8$  spores/ml) were prepared using Neubauer's improved hemocytometer. The equal age and equal size 2<sup>nd</sup> instar larvae of second generations were collected from culture and used to perform the current study. There were three replications with 10 larvae per replication which dipped in suspension of  $1 \times 10^8$  spores/ml for 20s. The treated larvae were shifted into petri dishes containing clean and equal size fresh maize leaves as a food to the larvae. On daily basis, new and fresh maize leaves were provided to larvae for feed. Nothing was used in controls except distilled water. The dose and time mortality responses caused due to fungal infection were observed and recorded or noted.

### Statistical analysis

Percentage mortality of larvae was calculated by using Abbott's formula (Abbott, 1925). Data were statistically analyzed to one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) software windows version 20.0.

## Results and discussion

Fall armyworm, *S. frugiperda* is the most destructive pest of many agricultural crops especially maize. It is an invasive species and becoming threat for food security in Pakistan. Chemical control, insecticides is extensively use practice that adopted by majority of farmers against this notorious and emerging pest in the study area that become the cause of environmental pollution and harmful for biological fauna (predators and parasitoids). The negative impacts of insecticides lead to

develop an ecofriendly and alternative approach to manage the insect pests especially FAW, *S. frugiperda* [17]. Microbial control (fungi, bacteria, viruses, protozoa) is an alternative method, larvae of *S. frugiperda* are susceptible to these and consider an important part of integrated pest management [18,19]. Isolation of fungi from different stages of *S. frugiperda* (eggs, larvae, pupae, adults) and their infection or toxicity had been reported by many researchers from different countries except Pakistan.

Among tested entomopathogenic fungi, *B. bassiana* was found more toxic than *M. anisopliae*. *B. bassiana* caused 79% larval mortality while *M. anisopliae* 59% (Table 1).

According to [20], *M. anisopliae* and *B. bassiana* strains had showed 78.6 and 96.6% larval mortality at  $1 \times 10^9$  conidia/ml. Our findings are different to many previous researchers like GARCÍA and BAUTISTA, 2011 [20] who had reported that *M. anisopliae* isolates cause 97% mortality while 30% with *B. bassiana* while *B. bassiana* showed maximum pathogenicity as compared to *M. anisopliae* in the current study. Our current study findings are different from others researchers may be due to variation in larval genetic makeup. The current and previous results variations are due to isolation of fungus strains from different sources. The pathogenicity of microbial agents can vary according to agricultural practices and geographical location. Lezama-Gutierrez R., et al 2001 [21] had reported the similar findings about virulence of fungi. Some researchers had reported 97–100% larval mortality infected with entomopathogenic fungi [22]. It has already discussed that entomopathogenic fungi show high mortality at early instars and high dose [23]. During the study, only 4% larval mortality was recorded in control. entomopathogenic fungi

*M. anisopliae* and *B. bassiana* were showed  $LT_{50}$  of 84.01h and 80.99 h, respectively. *M. anisopliae* and *B. bassiana* were showed  $LC_{50}$  of  $1.3 \times 10^7$  and  $1.8 \times 10^7$  spores  $ml^{-1}$ , respectively as shown (Tables 2,3). [22] had reported that *B. bassiana* show 19% mortality and  $LC_{50}$  in the range of  $7.4 \times 10^4$  conidia $^{-1}$  on new hatch larvae of *S. frugiperda* while *M. anisopliae* isolate CP-MA1 showed 72.5% mortality and  $LC_{50}$  at  $5.3 \times 10^5$  conidia/ml [24–30].

## Conclusion

Toxicity of entomopathogenic fungi against different instar of larvae was conducted in the present study. Each and every concentration of fungus had significant effect against larvae of *S. litura*. The mortality rate of larvae was increased with

**Table 1:** Toxicity of *Metarhizium anisopliae* and *Beauveria bassiana* against 2<sup>nd</sup> instar larvae of *Spodoptera frugiperda*.

1	Isolates	Percentage (%age) mortality of larvae	Source of isolates
2	<i>B. bassiana</i>	79.10±0.11 <sup>a</sup>	Armyworm, <i>Spodoptera exigua</i>
3	<i>M. anisopliae</i>	59.20±0.00 <sup>bc</sup>	Armyworm, <i>Spodoptera litura</i>
4	Control	4.99±0.79 <sup>c</sup>	—
5	CD@1%	0.337	—

**Notes:** Values with different letters in columns are significantly ( $P < 0.01$ ) different with each other



**Table 2:** Dose mortality response of entomopathogenic fungi against *Spodoptera frugiperda* larvae.

No.	Isolates	LC <sub>50</sub> spores/ml	95% fiducial limit	Slope±SE	χ <sup>2</sup>	P value	df
1	<i>M. anisopliae</i>	1.3×10 <sup>7</sup>	4.0×10 <sup>9</sup> –2.9×10 <sup>7</sup>	0.599±0.104	1.124	0.669	2
2	<i>B. bassiana</i>	1.8×10 <sup>7</sup>	6.1×10 <sup>4</sup> –9.3×10 <sup>7</sup>	0.454±0.101	1.981	0.589	2

LC: Lethal concentration, SE: Standard error, χ<sup>2</sup>: Chi square, df: Degree of freedom

**Table 3:** Time mortality response of entomopathogenic fungi against *Spodoptera frugiperda* larvae.

No.	Isolates	LT <sub>50</sub> hours	95% fiducial limit	Slope±SE	χ <sup>2</sup>	P value	df
1	<i>M. anisopliae</i>	84.01 h	70.49–110.10	6.612±1.299	1.500	0.539	2
2	<i>B. bassiana</i>	80.99 h	75.91–199.12	7.922±1.143	1.999	0.601	2

LT: Lethal Time, SE: Standard Error, χ<sup>2</sup>: Chi square, df: degree of freedom

increase in concentration of fungus. The current study findings are very important for future studies and helpful in controlling the pest population at laboratory and field conditions.

## Acknowledgements

All authors are highly thankful to concern institutes especially department of Entomology.

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