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# Impact of magnetic fields on the entomopathogenic activity of *Beauveria bassiana* and the biological and biochemical responses of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae)

*Mervat A. Kandil*, Rania M. El-Shennawy, Dalia E. Lotfy; Hanady S. E. Nasr*

***Address:***

*Plant Protection Research Institute, Agriculture Research Centre, Dokki, Giza, Egypt.*

*Corresponding author: ***Mervat A. Kandil*** email: dr.mervatkandil@yahoo,com

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## ABSTRACT

Using magnetic fields for pest management requires a radical knowledge of their effects on insect behavior and anatomy. This study evaluated the impact of magnetic fields on pathogenic efficacy of the white muscarinic fungus, *Beauveria bassiana,* against the cotton leafworm, *Spodoptera littoralis* (Boisd.) second-instar larvae. The fungus suspension was prepared using sterile water with 0.05% Tween 80, before and after magnetization. Magnetized suspensions of fungi displayed improved bioactivity with higher larval mortality (71.7%) compared to their non-magnetized ones (58%) and untreated controls (7%). Toxicity varied for $LC_{50}$ values, it were (9x105 conidia/mL for un- magnetized fungus vs. $2x10^5$ conidia/mL for magnetized fungus.), with longer developmental periods for *S. littoralis* immatures with magnetized fungi (39.0 days) than unmagnified fungi (34.6 days). Biochemical assays showed significant reductions in treated larvae for total proteins, lipids, and carbohydrates, the maximum depletion being that under magnetized fungal treatment. Significant reductions in total proteins (9.33 mg/g), lipids (16.63 mg/g), and carbohydrates (12.9 mg/g) for magnetized fungus treatments in comparison with both non-magnetized fungi (11.2, 14.93, and 26.63 mg/g, respectively) and untreated larvae (14.3, 18.77, and 29.0 mg/g, respectively). Simultaneously, heightened activities of transaminase enzymes (AST and ALT) were observed, reflecting physiological stress responses. The results demonstrate that magnetization enhances *B. bassiana's* pathogenic impacts, supporting its potential as a novel and efficient tool for sustainable pests' management.

**Keywords:** *Spodoptera. littoralis*, *Beauveria bassiana*, electromagnetic fields, biological and biochemical processes.

## INTRODUCTION

The caterpillar cotton leafworm, *Spodoptera littoralis*, is recognized as one of the most destructive agricultural pests in tropical and subtropical regions worldwide (Hill, 1987). It has been documented to infest over 112 species of plants across 44 families, many of which hold significant economic value (Sarto and Monteys, 1988). Among larval instars, the second through sixth instars are particularly important, as they can induce yield losses of up to 50% (Russell *et al.,* 1993). Lepidoptera is the largest group of plant-feeding insects, and its immune function has been extensively studied. Entomopathogenic is gradually more recognized for its environmentally friendly characteristics and prominence in combined pest management (Tang *et al.,* 2022).

Entomopathogenic fungi (EPF) are myco-biocontrol agents that are widely distributed and exhibit both restricted and broad host ranges, demonstrating varying biocontrol potentials against different species of lepidopteran insects. The entomopathogenic lifestyle of these fungi is believed to have evolved multiple times from a common saprophytic ancestor inhabiting soil and leaf litter (Spatafora and Blackwell, 1993).

Among the commercially existing bio-insecticides, formulations derived from *bassiana* are excellent for their balance compared to different biological manipulation mechanisms for many agricultural pests, including Lepidopteran pests (Hazaa, *et al.,* 2019). Previous research has established the potential of fungi as biological control agents due to their high reproductive capabilities, target-specific activity, short generation times, and saprobic phases (Faria and Wraight, 2001; Garrido-Jurado *et al*., 2020). Also, (El-Katatny 2010) Studies that the Virulence perspective of some fungal isolates and their control-promise in contradiction of the Egyptian cotton leafworm, *S. littoralis* Notably, *B. bassiana* (Balsamo) accounts for 33.9% of the 171 products available globally, underscoring its significant advantages for insect biological control (McCoy *et al.,* 1988; Fuxa; 1997). The spore suspensions and metabolites of this fungus demonstrate high pathogenicity against *S. littoralis* across various instar larvae, indicating its potential application in biocontrol strategies (Shah and Pell, 2003).

Several studies have demonstrated the efficacy of *B. bassiana* against *S. littoralis* when applied as a soil treatment. The fungus can suppress the late larval stage that otherwise burrows into the surface soil to form a cocoon and pupate in 5–6 days (Pinhey, 1975). The biological control approach inhibits the emergence of adults and disrupts the pest's life cycle. Furthermore, the cryptic nature of *S. littoralis* characterised by leaf-feeding and subterranean pupation—combined with increased infestation following heavy rainfall, enhances the potential of *B. bassiana* as a high-quality entomopathogenic agent for managing the soil-dwelling stages of the pest (Garrido-Jurado *et al.,* 2020).

Magnetic flux, as a form of environmental pressure, is known to influence various biological systems (Starick *et al*., 2005). Our prior investigations have highlighted the role of magnetic fields in the response and adaptation of organisms to stressful conditions. Kandil *et al*., (2018); El-Shennawy *et al*., (2019) reported an adverse increase in the generation time of *Pectinophora gossypiella* and *Earias insulana* insects after magnetic field exposure. Despite extensive research into the behavioural effects of magnetic fields and/or materials, the mechanisms through which different insects detect geomagnetic fields remain largely unexplored. The biological effects of extremely low-frequency electromagnetic fields (EMF) on various organisms have been the subject of numerous studies; however, the impact of static magnetic fields (SMF) on different organisms has received less attention, yielding notable yet contradictory results (Ghodbanea *et al*., 2011).

For instance, (Pan and Liu, 2004) reported that larval development was slower under strong static magnetic fields than under geomagnetic fields. Conversely, (El-Shennawy *et al*., 2019; Kandil *et al*., 2019), indicated that high magnetic fields significantly affected the duration of larval and pupal development of *P. gossypiella.*

Therefore, the present research aims to clarify significant enquiries regarding the effectiveness of magnetic fields combined with the potential of *B. bassiana* strains as a pre-emptive tool in strategies for biocontrol against *S. littoralis*.

## MATERIALS AND METHODS

**Materials used:**

**1. Insect Strains Used:**

Second instar larvae of the cotton leafworm, *S. littoralis*, were obtained from a laboratory strain maintained at the Cotton Leafworm Research Department, Plant Protection Research Institute, Agricultural Research Centre, Dokki, Giza. The larvae were reared on fresh castor bean leaves (*Ricinus communis*) under laboratory conditions (25 ± 1 °C, and 60-65 % RH) according to (El-Dafrawy *et al.,* 1964).

**2. Fungal Culture:**

The *B. bassiana* isolate used in this study was obtained from the Mycological Centre at Assiut University, Faculty of Science. The fungal isolate was cultured on Sabouraud dextrose yeast agar (SDYA) medium, which comprises 40 g of glucose, 20 g of peptone, 20 g of agar, and 2 g of yeast extract in 1000 mL of distilled water, as first described by (Sabouraud, 1892). The medium was autoclaved at 21 °C for 15 to 20 minutes.

**3. Magnetic field preparation:**

The magnetic field was generated using nine similar magnet pieces placed in pure water at room temperature. Each magnet was measured with a tesla meter at the Faculty of Engineering, Menoufia University.

**4. Preparation of spore suspension:**

Fungal cultures grown on SDYA were incubated in the dark at 25 ± 1 °C, and 60-65 % RH for 14 days. Conidial suspensions were prepared by scraping the cultures with a sterile glass rod and transferring the material to 10 mL of sterile magnetized and/ or non-magnetized water (containing 0.05% Tween 80, which was used to help stabilize the product), within a laminar flow chamber. The conidia were harvested by scraping the culture surface with an inoculation needle. The ensuing mixture of spores and hyphae became agitated for 10 minutes, and then the hyphae were removed by filtration through a satisfactory mesh sieve. The conidial concentration of the final suspension was adjusted to $1 \times 10^8$ viable conidia, as determined by direct counting using a haemocytometer.

Serial dilutions were prepared and saved at 5 °C until wished for experimentation. For the fungi isolate, conidia suspensions contained $10^4$, $10^5$, $10^6$, $10^7$ and $10^8$ conidia/mL viable conidia were directly administered to *S. littoralis.*

**5. Bioassays of *S. littoralis* larvae:**

Fungal suspensions of different concentrations of conidia ($10^4$–$10^8$ conidia/mL) were prepared in both magnetized and non-magnetized sterile water. Castor bean leaves were treated by dipping in such suspensions

before being fed to *S. littoralis* larvae. Three replicates each of fifty second instar larvae were kept singly in cylinder plastic containers (3X6 cm**)** dedicated to insect rearing**,** and allowed to feed on treated leaves for 48 hr. while untreated leaves were provided to the controls. Larval mortality was assessed after 24 hours, 3, 5, and 7 days of treatment and mortality values were corrected with (Abbott's formula, 1925). Subsequent lethal concentration values ($LC_{50}$) were calculated using probit analysis (LDP line program). The study design enabled direct comparison of magnetized and non-magnetized fungal suspension effectiveness against larval mortality.

**5.1. Assessment of biological aspects of *S. littoralis*:**

Following the $LC_{50}$ determination, castor bean leaves were treated, and three replicates of fifty second instar larvae were allowed to feed on these leaves for three days before being transferred to fresh untreated leaves until pupation. Control larvae were maintained on untreated leaves. Mortality and malformation percentages were recorded. Additionally, biological aspects such as larval, pupal, and total immature (larvae, prepupa and pupae) durations were calculated.

**5.2. Resolution of fungus from infected larvae and pupae:**

Under laboratory, conditions of 25 ± 1 °C, and 60-65 % RH, dead and/or malformed larvae and pupae were isolated in Petri dishes for examination. After 7 to 12 days, examined individuals displayed surface mycosis, which was indicated by profuse growth of mycelia from the respective fungi (Fig. 1).

The pure cultures of the fungal pathogens utilized in the bioassays were successfully recovered from the infected larvae and pupae on Sabouraud Dextrose Agar Yeast (SDAY) plates according to (Sabouraud, 1892).

**6. Biochemical assays:**

**6.1. Preparation of insects for biochemical assays:**

Biochemical assays were conducted on two groups: one treated with the $LC_{50}$ of magnetized *B. bassiana* and the other with the $LC_{50}$ of non-magnetized *B. bassiana*. Each group consisted of 150-second instar larvae of *S. littoralis*. Total soluble lipids, proteins, and carbohydrates in the homogenates of various magnetized compared to non-magnetized and untreated control larvae were analysed at the Physiological Department of the Plant Protection Research Institute (PPRI). Samples of *S. littoralis* larvae were collected 10 days post-treatment and stored under controlled refrigeration until the biochemical analyses were performed. Larval specimens had been homogenized in distilled water, followed by centrifugation of the homogenates at 5000 rpm and 5 °C in a refrigerated centrifuge. The supernatants have been ultimately stored at -20 °C until further evaluation.

The total soluble lipids and carbohydrates were estimated using the method described by (Knight *et al*., 1972), while total soluble protein and free amino acid were quantified using the (Bradford, 1976). Each of Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzyme activities was measured according to the method of (Reitman and Frankel, 1957).

**7. Statistical Analysis:**

Biological and biochemical data were analysed statistically using one-way analysis of variances (ANOVA) via the Costat statistical program (1990). Significant differences were assessed using the least significant difference (LSD) test, with a probability level of $P < 0.05$ as articulated through (Duncan, 1955).

## RESULTS

**Virulence bioassay of magnetized and non-magnetized fungal suspensions**:

The evaluation of *B. bassiana* pathogenicity on 2$^{nd}$-instar larvae of *S. littoralis* at various time intervals post-treatment is illustrated in (Table 1). The findings indicate that the magnetized fungal suspension exhibited tremendous toxicity on *S. Littoralis*. Mortality rates showed a progressive increase from the 24-hour mark, extending to 3 days and continued to rise over the subsequent 7 days. Additionally, the results revealed that the mortality percentage of the larvae was positively correlated with the concentration of the tested *B. bassiana* pathogen. Fungus applied with the magnetized water suspension shows the highest efficacy against *S. littoralis* larvae. It is especially noteworthy that *B. Bassiana* prepared with magnetized water at an elevated concentration of 1 $\times 10^8$ conidia caused a mortality percentage of 99.17%, as compared to a mortality percentage of 94.54% recorded for the non-magnetized suspension at the same concentration after 7 days of treatment. The $LC_{50}$ values were $3x10^6$, $9x10^5$ and $22x10^5$ versus $1x10^5$, $2.1x10^5$ and $3x10^4$ at 1,3 and 7 days before and after magnetization, respectively.

**Table 1.** Accumulative mortality percentage and $LC_{50}$ values of *B. bassiana* on second instar larvae of *S. littoralis* under laboratory conditions.

| Treatment conc. (conidia/ml)* | | | Accumulative mortality % after | | |
|---|---|---|---|---|---|
| | | | 1 days | 3 days | 7 days |
| *S. littoralis* | *B. bassiana* non-magnetized suspension | $10^8$ | 78.12 | 85.62 | 94.54 |
| | | $10^7$ | 67.13 | 72.17 | 84.41 |
| | | $10^6$ | 27.36 | 34.42 | 67.61 |
| | | $10^5$ | 13.82 | 35.74 | 46.36 |
| | | $10^4$ | 12.9 | 33.8 | 40.2 |
| | Slope | | 0.63±0.14 | 0.0.5±0.12 | 0.38±0.14 |
| | *$LC_{50}$ | | $3x10^6$ | $9x10^5$ | $22x\ 10^5$ |
| | The times exposed | | 1 days | 3 days | 7 days |
| | *B. bassiana* magnetized suspension | $10^8$ | 80.0 | 99.12 | 99.17 |
| | | $10^7$ | 83.07 | 94.15 | 96.53 |
| | | $10^6$ | 58.90 | 70.69 | 76.85 |
| | | $10^5$ | 31.98 | 34.45 | 37.88 |
| | | $10^4$ | 29.99 | 33.11 | 35.00 |
| | Slope | | 0.47±0.06 | 1.06±0.23 | 1.11±0.21 |
| | *$LC_{50}$ | | $1x10^5$ | $2.1x10^5$ | $3x10^4$ |

* LC: Lethal Concentrations of conidia spores.

**Biological Effects:**

**Cumulative Mortality:**

Data presented in (Table 2) illustrate the effects of the $LC_{50}$ toxicity of *B. bassiana*, prepared with and without water magnetization, on the second instar larvae of *S. littoralis.* The findings reveal a clear correlation between larval mortality and the two treatment modalities across various time intervals, specifically at 1, 2 and 7 days post-treatment, and extending to the conclusion of the larval stage.

The mean cumulative mortality percentage significantly increased with the magnetized formulation, reaching 71.7% larval mortality over 20 days. In contrast, the non-magnetized *B. bassiana* formulation resulted in a lower cumulative mortality of 58%, compared to the markedly lower cumulative mortalities of 7% for the control group. These findings indicate that the magnetization of *B. bassiana* enhances its efficacy against *S. littoralis* larvae.

**Table 2.** Mortality and malformation of *S. littoralis* larvae treated with the $LC_{50}$ of magnetized and non-magnetized *B. bassiana* suspension under laboratory conditions.

| Compound used | Larval mortality % after | | | | *% Larval malformation |
|---|---|---|---|---|---|
| | 1 (days) | 3 (days) | 7 (days) | Total larval mortality | |
| ***B. bassiana* magnetized suspension( $Lc_{50}$= $2x10^5$ conidia/mL)** | $49^a$ | $11^b$ | $6^a$ | 71.7a | $23.4^a$ |
| ***B. bassiana* non-magnetized suspension ($Lc_{50}$ =$9x10^5$** conidia/mL) | $43^b$ | $11^a$ | $4^b$ | $58^b$ | $19.3^b$ |
| **Control** | 00 | 0.0 | $4^b$ | $7^c$ | $5.0^c$ |
| **LSD** | 0.70 | 1.10 | 4.67 | 9.22 | 2.38 |
| **P** | ** | ** | * | *** | *** |

*Larval mortality percentage includes calculated larval deformity percentage.

**Immature durations (larva- pre-pupae and pupal stage):**

Data presented in (Table 3) indicate that the tested fungal suspension of *B. bassiana*, before and after magnetization, significantly influenced the development of the resulting stages of *S. littoralis*. Notably, there was an extensive increase in the duration of the larval, pre-pupal and pupal stages, and subsequently, the total duration of immature stages (larvae, prepupae and pupae). Specifically, the larval period within the untreated control larvae was 17.6 days, expanded to 21.7 days for larvae treated with the fungus before magnetization, and 24.3 days when subjected to the fungus after magnetization.

**Table 3.** Effect of tested magnetized and non-magnetized suspension of *B. bassiana* on *S. littoralis* different immature durations under laboratory conditions.

| Compound used | $LC_{50}$ conidia/mL | (Duration times in days) (mean± SE) | | | |
|---|---|---|---|---|---|
| | | Larvae | Pre-pupae | pupae | Total immature stages |
| ***B. bassiana* magnetized suspension** | $2x10^5$ | 24.3±0.57[a] | 4.1±0.2[a] | 9.6±0.5[a] | 39.0±0.33[a] |
| ***B. bassiana* non-magnetized suspension** | $9x10^5$ | 21.7±1.33[b] | 3.8±0.33[a] | 9.1±0.3[b] | 34.6±0.33[b] |
| **Control** | **0.0** | 17.6±1.5[c] | 2.6±.1[b] | 8.00±0.33c | 28.2±0.33[C] |
| **LSD** | | 1.338 | 0.4110 | 0.1088 | 2.041 |
| **P*** | | *** | ** | * | *** |

*Values are mean ± SE of three replicates (**50 larvae /**replicate) **for each treatment.**

*Values within the same column having the same letters are not significantly different.

* (ANOVA, Duncan's multiple range tests, P < 0.05).

Table (3) further clarifies that the duration necessary for the pupal stage was 8.0 days in the control group, compared to 9.1 days for the fungal treatment before magnetization and 9.6 days after magnetization. Additionally, the total immature duration was appreciably elevated to 34.6 days and 39.0 days for the fungal treatment before and after magnetization, respectively, in contrast to 28.2 days for the water treatments.

**Re-isolation of *B. bassiana* from *S. littoralis*-infected larvae and pupae:**

Under laboratory conditions, the bioassay tests demonstrated that the treated larvae and their resulting pupae exhibited notable mortality and malformation rates. Treated larvae displayed surface mycosis, which indicated profuse growth of mycelia from the respective fungi (Fig. 1).

The pure cultures of the fungal pathogens utilized in the bioassays were successfully recovered from the infected larvae and pupae. The isolated cultures exhibited typical mycelial and conidial characteristics of the entomopathogenic fungi within 2-5 days and 7-12 days of incubation for magnetized and non-magnetized suspensions, respectively. Although a few fungal contaminants were noted during the culturing process, their occurrence was minimal and did not significantly affect the results.

Furthermore, a distinct white conidial mass feature of *B. Bassiana* becomes prominently observable within the infected larvae and pupae of magnetized suspensions, as opposed to the less clearly defined layer in the non-magnetized and normal control ones (Fig. 3a, 3b, 3c, and 3d).

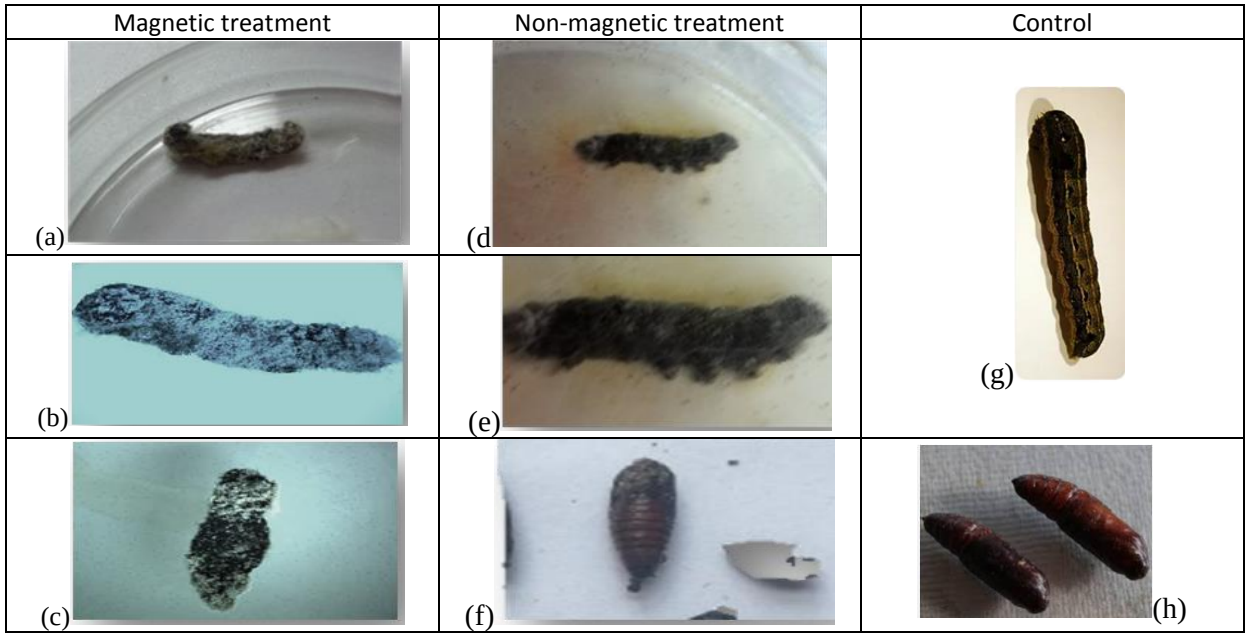


**Fig. 1.** Growth of *B. Bassiana* mycelia on *S. littoralis*-infected larvae and pupae

(a): Larvae of magnetic treatment, (b): Larvae result from fungi + magnetic treatment,

(c): pupa resulted from magnetic treatment, (d): Amplified photo of larva treated with fungi only, (e) larva after fungi treatment, (f): Pupa resulted from treated fungi only, (g): Normal larva and (h): Normal pupa.

**Effect of *B. bassiana* treatments on subsequent stages of *S. littoralis*:**

**Pupal Stage:**

Table (4) show that using the magnetized suspension of *B. bassiana* resulted in a pupation rate of 28.3%, significantly lower than the 42% observed with the non-magnetized suspension and markedly below the control group's rate of 93%. Furthermore, the magnetized suspension treatment led to a 25.33% malformation rate in pupae, compared to 14.67% for the non-magnetized suspension, while the control group exhibited no malformations (0.0%) (Table 4).

**Table 4. Effect of *B. bassiana* ($LC_{50}$ conidia/ml) *on S. littoralis* resulting stages.**

| Compound used | $LC_{50}$ conidia/ml | Pupal stage* | | Adult emergence* | |
|---|---|---|---|---|---|
| | | % Pupation | % Malformation | % Emergence | % Malformation |
| ***B. bassiana* magnetized suspension** | **$2x10^5$** | $28.3^c$ | $25.33^b$ | $74.67^c$ | $19.0^a$ |
| ***B. bassiana* non-agnetized suspension** | **$9x10^5$** | $42^b$ | $14.67^a$ | $83.67^b$ | $13.00^b$ |
| **Control** | **0.0** | $93^a$ | 0.0 | $95.00^a$ | $3.00^c$ |
| **LSD** | | 9.22 | 6.21 | 7.98 | 3.22 |
| **P** | | ** | ** | *** | *** |

*Values within the same column having the same letters are not significantly different

**(ANOVA, Duncan's multiple range tests, P < 0.05)

**Adult stage**:

In the adult stage, the emergence rate was 74.67% for the magnetized suspension, which is lower than 83.67% for the non-magnetized suspension and significantly below the control group's emergence rate of 95% (Table 4). Additionally, the magnetized suspension treatment resulted in a higher malformation percentage (19.0%) compared to the non-magnetized group (13.0%), which was significantly higher than the control group (3.00%) (Table 4).

**Physiological assays measurement:**

**Total protein:**

Data presented in (Table 5) indicate that larval stages exposed to fungi prepared with water magnetization exhibited a significant reduction in the level of total soluble protein, measuring 9.33 mg/g body weight. The larvae treated with fungus alone reduced the total soluble protein level to 11.2 mg/g body weight compared to 14.33 mg/g b.w. in the untreated group.

**Table 5.** Physiological effect of the tested *B. bassiana* ($LC_{50}$ conidia/ml) *on S. littoralis* treated larvae under laboratory conditions

| Substrates / Treatments $LC_{50}$ | | Protein (mg/g b.w.) | Carbohydrate (mg/g b.w.) | Lipid (mg/g b.w.) | Free amino-acid (µg ) | ALT (mg/g b.w.) | AST (mg/g b.w.) |
|---|---|---|---|---|---|---|---|
| ***B. bassiana* magnetized suspension** | $2x10^5$ conidia/mL | $9.33^c$ | $12.23^b$ | $16.63^b$ | $191.67^b$ | $2262.00^a$ | $1315.33^a$ |
| ***B. bassiana* non-magnetized suspension** | $9x10^5$ conidia/mL | $11.20^b$ | $26.63^a$ | $14.93^c$ | $202.00^a$ | $1788.88^b$ | $1130.33^c$ |
| **Control** | **0.0** | $14.33^a$ | $29.00^a$ | $18.77^a$ | $182.33^c$ | $1617.67^c$ | $1064.33^c$ |
| **LSD** | | 1.453 | 3.01 | 0.37 | 7.68 | 26.001 | 10.00 |
| **P** | | ** | * | ** | *** | *** | *** |

*Values within the same column having the same letters are not significantly different (ANOVA, Duncan's multiple range tests, P < 0.05)

**Total Carbohydrates:**

Data in (Table 5) reveal a substantial decrease in total carbohydrate levels, with larvae treated with fungi and magnetic fields showing an approximate halving of carbohydrate content, estimated by 12.23 mg/g body weight. In contrast, larvae treated with fungi alone exhibited a moderate decrease to 26.63 mg/g body weight, compared to 29.00 mg/g b.w. in the control group.

**Total Lipids:**

Conversely, the total lipid content significantly varied among the treatment groups. The larvae exposed to fungi prepared with magnetized water had a total lipid content of 16.63 mg/g body weight, which decreased markedly to 14.93 mg/g body weight in the solely treated fungi, compared to 18.77 mg/g body weight in the untreated control (Table 5).

**Free Amino Acids:**

Data presented in (Table 5) indicate a significant increase in free amino acids in larvae treated with non-magnetized fungi suspension, recording 202.0 µg D, L-alanine per gram of body weight. This was followed by larvae treated with fungi prepared using magnetized water, which recorded 191.67 µg D, L-alanine per gram of body weight, compared to 182.33 µg D, L-alanine per gram of body weight in the untreated control group.

**Transaminase Enzymes:**

**AST (aspartate transaminase) and ALT (alanine transaminase):**

Data in (Table 5) indicate a significant increase in transaminase enzymes, specifically AST/GOT and ALT/GPT, in larvae treated with magnetized fungi suspension, recording 2262.0 and 1315.33 mg/g body weight, respectively. In contrast, larvae treated with non-magnetized fungi suspension showed moderate increases to 1788.88 and 1130.33 mg/g body weight, compared to 1617.67 and 1064.33 mg/g b.w. in the control group.

## DISCUSSION

The current study found that magnetising *B. bassiana* significantly increased its virulence, biological efficacy, and physiological effects against the cotton leaf worm *S. littoralis*. Results showed a strong positive correlation between fungal concentration, exposure time, and larval mortality, indicating that magnetized suspensions markedly boosted the pathogens potential under laboratory conditions.

Magnetization restructures water molecules, reducing surface tension and enhancing ion mobility (Quiun *et al.,* 1997; Zhou *et al.,* 2000). This improves *B. bassiana* spore hydration, dispersion, and adhesion to the insect cuticle. Enhanced enzymatic activity (proteases, lipases, chitinases) promotes faster penetration and colonization of *S. littoralis*. The result is increased larval mortality and metabolic depletion. Thus, magnetization enhances fungal virulence by optimizing the physicochemical environment and accelerating pathogen–host interaction.

**Enhanced Virulence through Magnetic Exposure:**

Virulence augmentation through magnetization is well-documented in microbial physiology, where exposure to a magnetic field can alter cell membrane permeability and stimulate enzymatic activity (Pan and Liu, 2004). The mortality rate of the magnetized *B. bassiana* suspension was considerably greater than that of the non-magnetized suspension. Maximum mortality of 99.17% was recorded at $1 \times 10^8$ conidia/ml after 5–7 days, and the corresponding non-magnetized suspension resulted in 94.54% mortality. Additionally, $LC_{50}$ values were considerably lower following magnetization ($1\times10^5$, $2.1\times10^5$, and $3\times10^4$ conidia/ml at 1, 3, and 7 days, respectively), confirming a remarkable increase in pathogenic efficacy. The observed reduction in the values of $LC_{50}$ for magnetized compared to non-magnetized fungi indicates significant improvement in using lower fungal inoculum concentrations for increased fungal virulence. The *B. bassiana* with electromagnetic treatment improves fungal bioactivity, a phenomenon that is corroborated by (Pietruszewski and Martínez, 2015), who observed that magnetic field exposure has been reported to increase metabolic and enzymatic activity in microbial systems, including fungi, by increasing the intracellular ion transport, enzymatic efficiency. Similar improvement in fungal metabolism has been observed by (Bordalo *et al.*, 2019), who observed improved spore germination and metabolic efficiency in magnetically treated conidia.

**Biological and Developmental Disruptions:**

The profuse mycelial growth over the infected larvae and pupae clearly indicates that *B. bassiana* successfully colonized its hosts, leading to mortality (Hajek and St. Leger, 1994). Accordingly, (Vega and Kaya, 2012) emphasized that mycelia growth on the host's surface is an indicator of successful colonization by *B. bassiana*. (Sehroon and Shamsi, 2016) highlighted the potential applications of magnetization in enhancing microbial performance in agricultural and environmental contexts. Zhang and Zhang, (2020) revealed improvements in the growth and metabolic characteristics of *Bacillus subtilis* due to magnetic field treatment, which may contribute to enhanced efficacy in various applications. The magnetized suspension caused higher cumulative mortality (71.7%) compared to the non-magnetized one (58%) after 20 to 26 days. This increase indicates an elevated infection establishment rate and a faster fungal colonization of host tissues. Such morphological indications support the hypothesis that magnetic impact can enhance the pathogenic potential of the fungus by improving the early stages of host invasion, i.e., adhesion, germination, and spore penetration. This enhanced pathogenicity has been correlated with physicochemical changes in the magnetized medium,

such as reductions in surface tension and viscosity, increases in ionic conductivity, and an increase in wettability, all of which collectively facilitate more efficient spore dispersion, adhesion, and germination on the cuticle of the insect (Alattar *et al.,* 2022). Results are in accordance with those of (Kaur *et al.*, 2011), in which *B. bassiana* induced concentration-dependent significant mortality of *S. litura* larvae. Concentration and mortality were positively correlated.

Besides, results show that entomopathogenic fungi, particularly when enhanced by magnetization, significantly influence developmental times of various instar larval and pupal stages of *S. littoralis*. The larval period was prolonged from 17.6 days in the control to 24.3 days under magnetized fungal treatment, representing a considerable delay in development. Such interruptions may be attributed to the physiological stress imposed by fungal toxins such as beauvericin and oosporein, which interfere with molting hormones, nutrient assimilation, and chitin biosynthesis (Perumal *et al*., 2018; Berestetskiy and Hu, 2021). This developmental delay can ultimately reduce pest fitness, survival, and reproductive success, suggesting the potential of magnetized *B. bassiana* as an eco-friendly pest control agent.

**Physiological and Biochemical Changes:**

The significant decrease in total proteins, lipids, and carbohydrates in magnetized *B. bassiana*-treated larvae (9.33, 16.63, and 12.9 mg/g body weight, respectively) compared to non-magnetized (11.2, 14.93, and 26.63 mg/g b.w.) and control treatments (14.3, 18.77, and 29.0 mg/g b.w.) validates that magnetically treated fungi cause greater metabolic decline. These reductions may result from increased fungal enzymatic secretion, particularly proteases and lipases, which increase nutrient acquisition from host tissues (El-Dafrawy *et al.,* 1964). Magnetized suspensions may possess greater spore hydration, germination, and enzymatic diffusion due to increased solvent polarity and ion mobility, as demonstrated by (Esmaeilnezhad *et al*., 2018). The high elevation of transaminase enzyme activities (AST and ALT) in magnetized fungus-treated larvae depicts the cytotoxic effects and physiological stress brought about by fungal infection. Such enzymes are excellent indicators of cellular damage and metabolic disorder in insects and are likely to increase as a response to toxin-induced tissue damage and oxidative stress (El-Katatny, 2010). The increased transaminase activity in this research, therefore, supports the enhanced physiological burden by magnetized fungal inocula. Previous findings also revealed that magnetic fields could influence microbial biosynthetic pathways and stress-related proteins, potentially contributing to enhanced virulence and host susceptibility. These findings are in compatible with (Khan and Zafar, 2019), who observed that infection by fungi disrupts amino acid metabolism and lipid mobilization in lepidopteran larvae. Practically, these results suggest the promising potential of magnetic field treatment as a new approach to boost the efficacy of entomopathogenic fungi as an IPM component. Magnetic stimulation can act as a biophysical stimulant that improves spore virulence, bioavailability, and host-pathogen interaction efficiency without invoking chemicals.

Overall, these results suggest that magnetization can enhance in vivo pathogenicity of *B. bassiana* by several synergistic mechanisms: Improved physicochemical characteristics of the carrier medium leading to enhanced spore adherence and germination. Enhanced fungal enzymatic activity and metabolite diffusion, maybe induced by altered ionic mobility in magnetized suspensions. Facilitated host penetration and nutrition depletion by augmented metabolic stress upon the insect host. The findings are consistent with broader literature indicating that magnetic fields can enhance the physiological and biochemical functioning of biological systems through the modulation of hydrogen-bond networks, ionic polarization, and enzyme kinetics (Kou, 2024).

## CONCLUSION

The cumulative evidence from virulence, biological, and biochemical assays of the current study demonstrates the entomopathogenic potential of *B. bassiana* against *S. littoralis*. The synergistic effect, as measured by elevated mortality, postponed development, and higher metabolic depletion, highlights the potential for the inclusion of magnetic treatment in biocontrol applications against pest control. A combination of physical treatments (magnetization) with biological agents (*B. bassiana*) represents a promising, eco-friendly alternative to chemical insecticides, supporting sustainable agriculture and integrated pest management programs. Future studies should aim to elucidate the molecular mechanisms underlying this enhancement.

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