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Enzymatic, cellular breakdown and lysis in treatment of *Beauveria Brongniartii* on *Spodoptera litura* (Fabricius, 1775)

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The current study aimed to isolate *Beauveria brongniartii* conidia from forest soils, identify the fungus, and evaluate its effectiveness on the eggs, larvae, pupae, and adults of *Spodoptera litura*. Insect mortality rates were recorded every 3, 6, 9, and 12 days. The identification of entomopathogenic fungi was carried out using molecular techniques, including PCR, DNA sequencing, and molecular markers, to detect species-specific 18 S rDNA genetic sequences, all performed under aseptic conditions. The results indicated that higher conidia concentrations (2.7×10^9 conidia/mL) exhibited greater virulence, with eggs showing a mortality rate of 98.66%, followed by larvae 96%, adults 90.66%, and pupae 77.33% after 12 days. Probit analysis revealed minimal LC_{50} and LC_{90} values: eggs (5.5×10^2; 1.0×10^6 spores/mL), larvae (8.2×10^2; 1.2×10^7 spores/mL), pupae (9.6×10^4; 7.3×10^{10} spores/mL), and adults (1.0×10^3; 2.0×10^8 spores/mL). The total hemocyte counts and detailed observational results revealed that *B. brongniartii* induces cellular breakdown and cell lysis in *S. litura* larvae by producing enzymes that degrade the cuticle and cell membranes. Earthworm bioindicator studies showed minimal effects from *B. brongniartii* conidia compared to controls, while chemical treatments resulted in 96% mortality at 100 ppm. Histopathological examinations revealed no significant differences in gut tissue between earthworms treated with fungal conidia and those in the control group, unlike the substantial damage caused by chemical treatments. Biochemical analysis revealed significant alterations in enzyme activity, including reduced levels of phosphatase and catalase, as well as increased levels of lipid peroxides and superoxide dismutase. This study highlights the effectiveness of *B. brongniartii* in controlling *S. litura*, demonstrating its potential as a viable biocontrol agent and promoting eco-friendly alternatives to chemical pesticides, with no risk to non-target species or the environment.

Keywords *Spodoptera litura*, Insect immune responses, Insect immunity, *Beauveria Brongniartii*, *No Poverty*, Climate Action

Spodoptera litura, known as the tobacco cutworm or cotton leafworm, is a highly destructive pest affecting various crops, including maize, cotton, soybean, tomato, tobacco, and vegetables[1–3]. This polyphagous pest causes extensive damage through defoliation and feeding on leaves, stems, and reproductive parts, leading to significant economic losses[4,5]. In maize, *S. litura*reduces grain production by damaging foliage, while in cotton, it affects fibre quality and quantity. In vegetables, the pest leads to direct loss and aesthetic damage, impacting market value[6,7]. Chemical control methods are commonly used but can lead to resistance, environmental contamination, and harm to non-target organisms[8–10].

Entomopathogenic fungi (EPF), such as *Beauveria bassiana*, *Metarhizium majus*, *Metarhizium flavoviride*, *Metarhizium rileyi*, *Metarhizium anisopliae*, and *Isaria fumosorosea*, provide a sustainable alternative to chemical pesticides[11–15]. These fungi infect insects by adhering to their cuticle, penetrating it, and proliferating within, ultimately causing death through toxin production and physical disruption[16–23]. Recent studies have demonstrated the effectiveness of EPF against various pests, including aphids, whiteflies, termites, thrips, and mealybugs, with minimal impact on beneficial insects[24–26]. Integration of EPF into pest management strategies reduces reliance on chemical pesticides, thereby promoting sustainable agriculture and enhancing ecosystem health[27–30].

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Entomopathogenic fungal species, including *Beauveria bassiana*, *Metarhizium anisopliae*, *Metarhizium majus*, *Metarhizium rileyi*, *Isaria fumosorosea*, *Breviphaeosphaeria* sp., *Entomophthora muscae*, *Cordyceps militaris*, *Nomuraea rileyi*, *Verticillium lecanii*, *Aspergillus flavus*, *Paecilomyces variotii*, *Conidiobolus* spp., *Metarhizium flavoviride*, *Hirsutella thompsonii*, *Ophiocordyceps unilateralis*, and *Tolypocladium inflatum*, exhibit significant insecticidal activity against a wide range of pests[13–15,20,25,23]. These pests include *Helicoverpa armigera*, *Spodoptera litura*, *Camponotini* spp., *Frankliniella occidentalis*, *Bemisia tabaci*, *Coptotermes formosanus*, *Blattella germanica*, *Otiorhynchus sulcatus*, *Sitophilus oryzae*, *Tetranychus* spp., *Aphis* spp., *Spodoptera frugiperda*, *Anticarsia gemmatalis*, *Bombyx mori*, *Drosophila melanogaster*, *Musca domestica*, *Aphis gossypii*, *Planococcus citri*, *Locusta migratoria*, and *Periplaneta americana*[18–23,27,31–37].

Enzymes like acid phosphatase (ACP), alkaline phosphatase (ALP), catalase (CAT), and superoxide dismutase (SOD) are crucial in insects' response to EPF. ACP and ALP are involved in metabolic processes and stress responses in insect pest[1]. Infection with EPF often disrupts ACP and ALP activity, indicating metabolic disturbances[1]. Catalase protects cells from oxidative damage by decomposing hydrogen peroxide, and decreased CAT activity in infected insects leads to ROS accumulation[1,38]. SOD mitigates oxidative stress by converting superoxide radicals into less harmful molecules, often increasing in response to fungal infection[1,39,40]. The raised lipid peroxide levels further confirm oxidative damage and stress caused by fungal infections[1]. *Eudrilus eugeniae*, or the African nightcrawler, is used as a soil bioindicator due to its sensitivity to soil conditions and pollutants. It provides insights into soil health, nutrient content[18–23]. This study aimed to isolate, identify, and evaluate *Beauveria brongniartii* conidia against *Spodoptera frugiperda* in various life stages and assess the enzymatic responses. The impact on the non-target earthworm *Eudrilus eugeniae* was also examined.

Materials and methods

Spodoptera litura insect

The culture of *S. litura*was maintained under controlled conditions in the Insect Pathology Laboratory for multiple generations[1]. The rearing environment consisted of a 12:12 (light: dark) photoperiod at 27 ± 1 °C with a relative humidity of 75–80%. To prevent excess moisture, water content was carefully managed to avoid water droplets in the rearing containers and on *Brassica oleracea* leaves. The larvae were fed fresh *B. oleracea* leaves. Pupae and adults were placed in plastic cages (11 × 12 × 9 cm) with *B. oleracea* leaves for mating, using a ratio of 3 males to 15 females. Adults were provided with a 10% honey solution to facilitate oviposition, and the containers were covered with black sanitary cloth.

Soil samples

Fifteen soil samples were gathered from forested regions in Chiang Mai Province, Thailand, located at 18.7965° N latitude and 98.6601° E longitude. Each sample, weighing 1 kg, was collected from depths ranging from 1 to 16 cm, as determined by previous experiments[18]. The soil samples were stored in clean plastic bags at 4 °C for subsequent analysis.

Insect bait method

Soil samples had been subjected to insect bait techniques to isolate entomopathogenic fungi, following our previous protocols[18,19]. This approach employs *Tenebrio molitor* larvae to effectively isolate insect-pathogenic fungi from the soil. Fifteen third-instar larvae of *T. molitor* had been transferred to a plastic box containing 250 g of soil, with dimensions of 15 cm in length, 10 cm in width, and 10 cm in height. The box changed into sealed and stored in an incubator set to 26 ± 1 °C and 85% relative humidity. Observations had been made two times day by day for 12 days. Following the experiment, the cadavers of the larvae had been accrued and sterilized with 70% ethanol for two to a few minutes. They had been then positioned on Petri dishes (ninety mm × 15 mm) containing potato dextrose agar (PDA) medium (HiMedia, Thailand) as defined via way of means of Perumal et al.[28]. The Petri dishes had been stored at 85% relative humidity and 26 ± 2 °C for a duration of seven to ten days. Fungal cultures had been sooner or later remoted from the cadavers and saved in an incubator at 26 ± 2 °C for destiny use.

Microscopic observation of fungi

The morphology of the isolated entomopathogenic fungi was confirmed through microscopic examination of the fungal colonies. This involved evaluating characteristics such as pigment production, mycelial structure, and spore morphology, using the procedures described by Vivekanandhan et al.[41]. In order to accomplish this, a small amount of lactophenol cotton blue (LCB) stain (HiMedia, Thailand) was applied to the fungal conidia on a sterile slide. The slides were subsequently analyzed using an Olympus CH20i light microscope with a magnification of 40×.

Culturing of fungi in broth

The genomic DNA extraction from entomopathogenic fungi was conducted using a cultivation procedure that adhered to the methods outlined in previous studies[18–20]. A 100-mL conical flask manufactured by Borosil in Thailand was filled with 75 mL of Potato Dextrose Broth (PDB) produced by HiMedia in Thailand. The flask was then sterilized using an autoclave at a temperature of 120 °C for a duration of 15 min. Following the sterilization process, the broth was cooled in a laminar flow hood while maintaining sterile conditions. Subsequently, a concentration of 1×10^7 conidia/mL was introduced into the medium, accompanied by the addition of 1 mL of chloramphenicol to suppress bacterial proliferation. The culture was subsequently maintained at a temperature of 26 ± 1 °C for a period of 5 to 7 days in order to facilitate the growth and development of fungi.

Molecular study

DNA isolation

After a 5–7 day incubation period, the mycelia were separated by filtration using Whatman No. 1 filter paper (HiMedia, Thailand). Genomic DNA was extracted following the protocol by Vivekanandhan et al.[18], which utilizes cetyltrimethylammonium bromide (CTAB) for effective contamination-free results. The fungal biomass was ground to a fine powder with liquid nitrogen in a sterile mortar, yielding one gram of ground material.

Next, 2.5 mL of CTAB lysis buffer was added to the powdered mycelium, and the mixture was incubated at 60 °C for 1 h. Following incubation, the samples were centrifuged at 8500 RPM for 15 min, then cooled to 4 °C for 18 min. The supernatant was carefully transferred to fresh tubes and mixed with isoamyl alcohol and chloroform in a 24:1 ratio. This mixture was centrifuged at 13,000 RPM for 20 min. The resulting supernatant was then processed by adding a mixture of 90% ethanol and an equal volume of cold isopropanol, followed by incubation at 25 °C for 1 h to precipitate the DNA. The DNA pellet was obtained by centrifugation at 13,500 RPM for 20 min, rinsed with 70% ethanol, and assessed for purity using 0.8% agarose gel electrophoresis.

Polymerase chain reaction analysis

The amplification of fungal genomic DNA was performed using the universal primers NS1 (GTAGTCATATGC TTGTCTC) and NS2 (CTTCCGTCAATTCCTTTAAG), following the protocol by Vivekanandhan et al.[18]. The polymerase chain reaction (PCR) was carried out in a total reaction volume of 20 µL, which included 1 µL of genomic DNA, 0.2 µL of DNA polymerase, 0.1 mg/mL of bovine serum albumin (BSA), 3% dimethyl sulfoxide (DMSO), 0.5 M betaine, and 0.2 mM of each deoxyribonucleotide triphosphate (dNTP) (dATP, dGTP, dCTP, and dTTP). The PCR cycling conditions consisted of an initial denaturation step, followed by an annealing phase at 50 °C for 30 s, an extension phase at 72 °C for 7 min, and concluded with a final extension phase at 72 °C for 2 min.

Fungal sequence analysis

The DNA of the entomopathogenic fungus was sequenced, and the resulting sequences were compared to the GenBank database using BLAST analysis to identify the species. Sequence alignment was performed using CLUSTAL W (BioEdit) 42, and nucleotide and amino acid sequence similarities were assessed with MegAlign (DNA Star, Inc., Madison, WI, USA). The fungal sequence data were then uploaded to GenBank (NCBI). Phylogenetic analysis was conducted using MEGA5 software43, employing the neighbor-joining method44 to create a distance matrix.

Beauveria brongniartii *culture*

The entomopathogenic fungus *B. brongniartii* was grown on potato dextrose agar (PDA) plates at 26 ± 1 °C for 14 days to achieve a spore concentration of 1.5×10^6spores/mL. After incubation, conidia were collected by gently scraping the agar surface with a sterile loop and suspended in a 0.5% Tween 80 solution, following the procedure outlined by Perumal et al.[22]. The suspension was vigorously mixed for 3 min, and hyphal debris was removed using sterile Millipore cloth. The conidia were then centrifuged for 20 min to eliminate clumps. Using a Neubauer hemocytometer, the conidia were counted, and their concentration was verified with an Olympus DSX510i light microscope at 40× magnification. Based on our previous study[28], different concentrations were prepared by diluting the original solution with sterile distilled water to obtain five levels: 2.7×10^4, 2.7×10^5, 2.7×10^6, 2.7×10^7, and 2.7×10^8 conidia/mL. These concentrations were used for insecticidal bioassays.

Effects fungal spores on egg

Twenty-five eggs of *S. litura* (1 days old) were collected from adult rearing cages and transferred to bioassay containers using a camel hairbrush. The eggs were then sprayed with a fungal spore solution at concentrations of 2.7×10^4, 2.7×10^5, 2.7×10^6, 2.7×10^7, and 2.7×10^8 conidia/mL. Following treatment, the eggs were allowed to air-dry at room temperature (26 ± 2 °C) under a laminar flow hood for one hour. They were then placed in Petri dishes and incubated at 26 ± 2 °C with 70–75% relative humidity. A negative control was prepared using sterile distilled water with 0.5% Tween-80. Each concentration was tested in triplicate, with each replicate consisting of 25 eggs. The number of hatched and unhatched eggs was recorded every three days over a 12-day period. Newly hatched larvae were counted and provided with freshly surface-sterilized *B. oleracea*leaves. They were then incubated at 27 ± 2 °C for 12 days in ventilated boxes lined with moist filter paper (25 × 15 × 16 cm), with observations made every three days. After the larvae had died, a mycosis test was conducted according to the methods described by Vivekanandhan et al.[18–20].

Effects of fungal spores on larvae

The *B. brongniartii* spore solution was tested against second-instar larvae of *S. litura*. Fresh *B. oleracea* leaves were placed in ventilated plastic boxes (10 × 7 × 5 cm), and 25 s-instar *S. litura* larvae were individually transferred to each box. The larvae were then dipped in 1 mL of a fungal spore suspension at concentrations of 2.7×10^4, 2.7×10^5, 2.7×10^6, 2.7×10^7, and 2.7×10^8 conidia/mL and the control treatments were treated with distilled water and incubated at 26 ± 2 °C. They were provided with surface-sterilized fresh *B. oleracea* leaves as food. Larval mortality was monitored every three days over a period of 12 days. The experiment followed a completely randomized design (CRD), with each treatment conducted in triplicate, and each replicate containing 25 larvae.

Total haemocyte count (THC)

A blood cell pipette was used to collect 0.5 mL of hemolymph, which was then diluted with Tauber-Yeager fluid (4.65 g NaCl, 0.15 g KCl, 0.11 g CaCl2, 0.005 g Gentian violet, 0.125 mL acetic acid, and distilled water to a

total volume of 100 mL). The mixture was gently stirred for a few minutes. Hemocytes were counted using an Olympus light microscope at 40× magnification.

$$\text{Number of hemocytes/mm}^3 = \frac{\text{X} \times \text{dilution} \times 10 \times 100}{\text{Number of smallest squares counted}}$$

Homogenate preparation
In order to assess the detoxification and antioxidant enzyme reactions, such as superoxide dismutase (SOD), catalase (CAT), alkaline phosphatase (ALP), acid phosphatase (ACP), and lipid peroxidation (LPO), a mixture of the midgut of 15 third-instar larvae of S. litura that had been exposed to a solution of fungal spores was prepared.

Acid phosphatase (ACP) and alkaline phosphatase (ALP) assays
A modified version of Asakura's[45] method was used to quantify the activities of acid phosphatase (ACP) and alkaline phosphatase (ALP) in larval homogenate samples. For the ACP activity, 50 µL of larval homogenate were mixed with 450 µL of 50 mM sodium acetate buffer at either pH 4.6 or pH 4.0. In order to measure the activity of alkaline phosphatase (ALP), 20 µL of the larval homogenate were mixed with 500 µL of a Tris-HCl buffer solution (pH 8.0) and 500 µL of a buffer solution containing 12.5 mM p-nitrophenyl phosphate. The resulting mixture was subsequently subjected to absorbance measurement at a wavelength of 440 nm.

Lipid peroxidation (LPO) assay
Lipid peroxidation was measured using the method of Ohkawa et al.[46]. A 0.1 mL aliquot of larval midgut tissue homogenate was mixed with 1.9 mL of 0.1 M sodium phosphate buffer (pH 7.4) and incubated at 37 °C for 1 h. The mixture was precipitated with 10% trichloroacetic acid (TCA), centrifuged at 5000 rpm for 15 min, and the supernatant was collected. To this supernatant, 1 mL of 1% thiobarbituric acid (TBA) was added. The sample was then boiled in a water bath for 15 min, cooled, and the absorbance was measured at 532 nm using a UV-visible spectrophotometer.

Superoxide dismutase (SOD) assay SOD activity was measured using the method of Marklund and Marklund[47]. A 50 µL aliquot of larval midgut tissue homogenate was added to a test tube, and the final volume was adjusted to 3.0 mL with 50 mM Tris and 10 mM EDTA (pH 8.2). Then, 100 µL of pyrogallol solution was added, and the mixture was thoroughly mixed. Absorbance was recorded at 440 nm using a UV-visible spectrophotometer.

Catalase (CAT) assay
CAT activity was measured using the method of Wang et al.[48]. A 0.1 mL aliquot of larval midgut tissue homogenate was mixed with 2.9 mL of 50 mM potassium phosphate buffer (pH 7.0) and 0.03 mL of 0.036% H_2O_2. Absorbance was measured at 240 nm against a control containing only H_2O_2 and phosphate buffer.

Effects of fungal spores on pupae
For the pupicidal bioassay, 25 pupae of *S. litura* were placed in plastic cups lined with filter paper. Fungal suspensions were sprayed onto the surface of the *S. litura* pupae. Each cup received a 1 mL dose of *B. brongniartii* suspension at concentrations of 2.7×10^4, 2.7×10^5, 2.7×10^6, 2.7×10^7, and 2.7×10^8 conidia/mL. The control group was administered 1 mL of distilled water. Each concentration was tested in three separate trials, with each trial involving 25 pupae. The efficacy of the treatment was assessed by monitoring pupal mortality at three-day intervals over a 12-day period.

Effects of fungal spores on adult
In the adulticidal bioassays, 1000 mL glass screw-cap bottles with a small hole in the cap were exposed to fungal conidial suspensions at various concentrations, ranging from 2.7×10^4 to 2.7×10^8 conidia per mL. Following the administration of the suspensions, 25 mature insects were placed in each individual bottle. Mortality rates were recorded every three days over a 12-day period. The insects were provided with a 10% honey solution, while the negative control group received distilled water and 10% honey. Each concentration was tested three times, using 25 healthy adult S. litura in each replicate.

Earthworm culture
The *Eudrilus eugeniae*culture was used for the toxicological bioassay and maintained at the Insect Pathology Laboratory, Chiang Mai University, at 28 ± 2 °C, according to the procedures by Vivekanandhan et al.[1]. Crop residue, dried in a hot air oven at 60 °C and sieved, was used as bedding and food in three forms: dried crop residue, pure cow dung, and a mixture of both. The earthworms were housed in 25 × 25 × 10 cm containers with aeration lids and maintained in a humid, dark chamber at 28 ± 2 °C with 70–75% moisture. Every 60 days, 100 mg of old substrate was replaced with 200 mg of fresh substrate. Earthworms and cocoons were separated, cleaned with tap water, and transferred to new containers with fresh substrate.

Artificial soil test (AST)
Artificial soil tests were conducted according to the Spurgeon and Hopkin[49]standard methodology, with minor modifications[41]. Soil samples were prepared with Monocrotophos at 100 ppm/kg and fungal spore solutions at concentrations of 2.7×10^4, 2.7×10^5, 2.7×10^6, 2.7×10^7, and 2.7×10^8 conidia/mL. Twenty-five adults *E. eugeniae* earthworms were introduced into each test container. A negative control with distilled water containing 0.05%

DMSO (without chemical or fungal spore solution) was treated. Each experiment was replicated three times, with each replicate containing 25 earthworms. Mortality was calculated after 12 days of treatment.

Histological study

Control and treated *E. eugeniae*earthworms were dissected and fixed in Bouin's solution. The gut tissues were cut into small pieces, embedded in paraffin, and chilled for three hours at 27 ± 1 °C. The paraffin blocks were sectioned into 1.5 µm ribbons using a microtome (Leica, Germany). The sections were stained with hematoxylin and counterstained with eosin, then mounted and examined under a light microscope (Optika Vision LITE 2.0 ML). Histological examination followed the method described by Vivekanandhan et al.[1].

Statistical analysis

The Abbott formula[50] was employed to adjust for background mortality rates in the insects (eggs, larvae, pupae, and adults). Mortality data were recorded every three days to determine the lethal concentrations (LC_{50} and LC_{90}) for *S. litura*. Statistical comparisons of insect mortality were conducted using Analysis of Variance (ANOVA) followed by Tukey's test ($p \leq 0.05$). Additionally, correlation analysis and other relevant metrics were evaluated. All statistical analyses were conducted using SPSS Software Version 23.0.

Results

Morphological level confirmation

The isolated entomopathogenic fungus *B. brongniartii* displayed distinct morphological features. The mycelium is white, and the conidia are oval to ellipsoidal, measuring approximately 2.5–4.5 µm in length (Fig. 1). This morphology is essential for accurate identification and differentiation from other fungal species.

Molecular level confirmation

Entomopathogenic fungal genomic DNA was amplified using a universal primer targeting the 18 S rDNA gene, and the DNA fragments were analyzed with a gel documentation unit. The obtained DNA fragment was 534 bp in length (https://www.ncbi.nlm.nih.gov/nuccore/pp344626.1).

The sequence quality of the amplified DNA was assessed, and the sequence was submitted to the GenBank database with the accession number PP344626.1. A BLAST search of the 18 S rRNA sequence showed a 100% match with previously reported *B. brongniartii* cultures (Nishi et al. 2015). The neighbor-joining tree method was employed to determine the evolutionary relationships of the isolated entomopathogenic fungi (Fig. 2).

Ovicidal activity

Following treatment with a concentration of 2.7×10^8 conidia/mL, mortality rates were observed as follows: 42.66% at 3 days post-treatment ($F_{(5,12)} = 18.640$; $p < 0.01$), and the LC_{50} and LC_{90} values were observed as follows: at 3 days (1.2×10^9 ; 2.3×10^{17} spores/mL), 61.33% at 6 days post-treatment ($F_{(5,12)} = 38.659$; $p < 0.01$) and the LC_{50} and LC_{90} values were (2.7×10^6; 9.6×10^{15} spores/mL), 89.33% at 9 days post-treatment ($F_{(5,12)} = 289.950$;

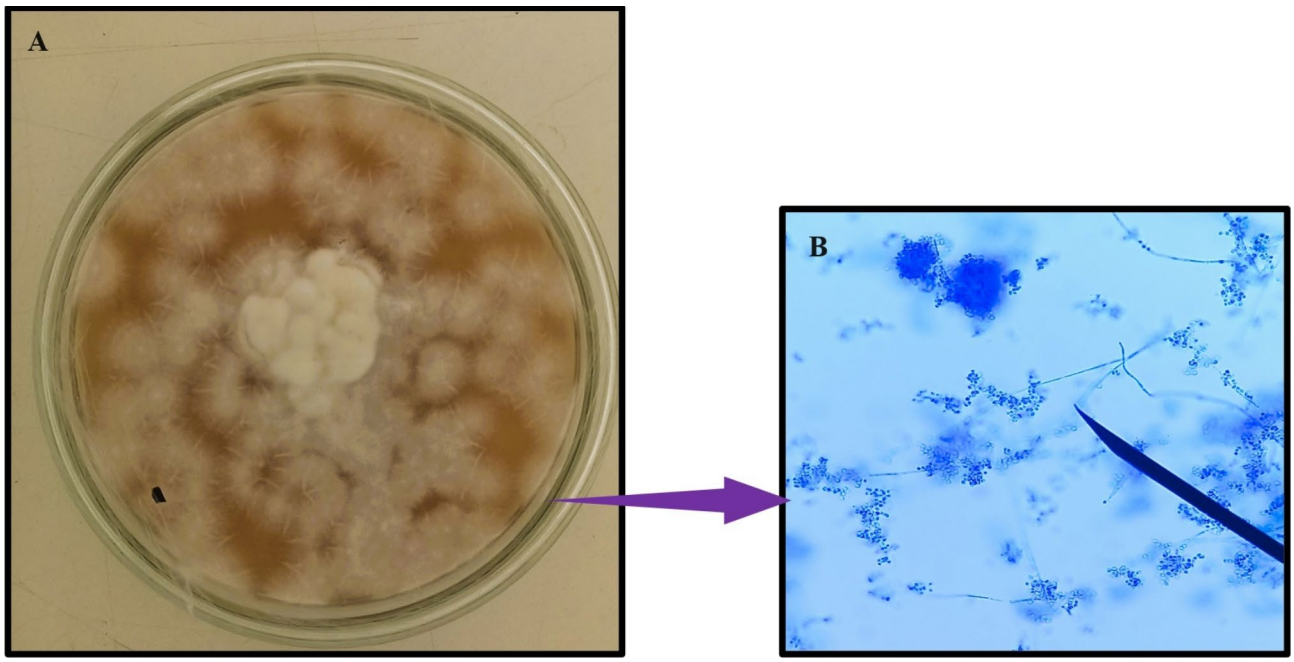

Fig. 1. The 10 days old culture of entomopathogenic fungi culture of *B. brongniartii* on Potato Dextrose Agar media (GenBank: PP344626.1). (**A**) Fungal culture. (**B**) Fungal morphological charectristics.

Fig. 2. The neighbor-joining method was used to create a phylogenetic tree of the entomopathogenic fungi that were isolated. The fungal strains we isolated were genetically identical to *B. brongniartii* (GenBank: PP344626.1).

$p < 0.01$), and the LC_{50} and LC_{90} values were (1.9×10^3; 3.1×10^8 spores/mL), and 98.66% at 12 days post-treatment ($F_{(5,12)} = 105.967$; $p < 0.01$), and the LC_{50} and LC_{90} values were (5.5×10^2; 1.0×10^6 spores/mL) (Fig. 3).

Larvicidal efficacy

After treatment with a concentration of 2.7×10^8 conidia/mL, the observed mortality rates were as follows: At 3 days post-treatment, the efficacy was 38.66% ($F_{(5,12)} = 55.483$; $p < 0.01$), with LC_{50} and LC_{90} values of 1.8×10^9 and 2.0×10^{16} spores/mL, respectively. At 6 days post-treatment, the efficacy increased to 58.66% ($F_{(5,12)} = 40.028$; $p < 0.01$), with LC_{50} and LC_{90} values of 1.0×10^7 and 4.4×10^{15} spores/mL. By 9 days post-treatment, the efficacy reached 85.33% ($F_{(5,12)} = 71.143$; $p < 0.01$), with LC_{50} and LC_{90} values of 1.4×10^4 and 3.4×10^9 spores/mL. Finally, at 12 days post-treatment, the efficacy was 96% ($F_{(5,12)} = 72.105$; $p < 0.01$), with LC_{50} and LC_{90} values of 8.2×10^2 and 1.2×10^7 spores/mL (Fig. 4).

Neurobehavioral toxicity

After 6 days of treatment with *B. brongniartii* conidia, the movement of *S.litura* larvae was significantly impaired compared to the control group. The larvae exhibited pronounced lethargy, characterized by a marked reduction in activity levels and a complete cessation of feeding behavior. This inactivity and the absence of feeding indicate a severe detriment to their overall health and vitality. Following an additional 1 to 2 days, the majority of the treated larvae succumbed to the infection, resulting in a substantial mortality rate. These findings indicate that *B. brongniartii* conidia effectively disrupt the normal physiological functions of *S. litura* larvae, leading to significant mortality.

Total haemocyte count

S. litura larvae were exposed to various concentrations of *B. brongniartii* (2.7×10^4, 2.7×10^5, 2.7×10^6, 2.7×10^7, and 2.7×10^8 conidia/mL). The levels of haemocytes increased at 3 days post-treatment, indicating that the host produced more blood cells to fight against the entomopathogenic fungus *B. brongniartii*. Three days into the treatment, the highest concentration of 2.7×10^8 conidia/mL led to a significantly increased 58.74% increase in haemocyte count compared to the control. This result was statistically non-significant ($F_{(5,12)} = 292.467$; $p \leq 0.01$) (see Figs. 5 and 6).

Acid phosphatase (ACP) and alkaline phosphatase (ALP)

Compared to control treatments, *B. brongniartii* treatment reduced Acid Phosphatase (ACP) activity by up to -55%. Fungal conidia significantly inhibited larvae ACP enzyme activity. The highest ACP suppression occurred at 3 days post-treatment with 2.7×10^8 conidia/mL ($F_{(5,12)} = 443.584$; $p \leq 0.01$) (Fig. 7A). In control larvae, enzyme levels were stable, but higher fungal conidia concentrations reduced ACP activity non-significantly (Fig. 7A).

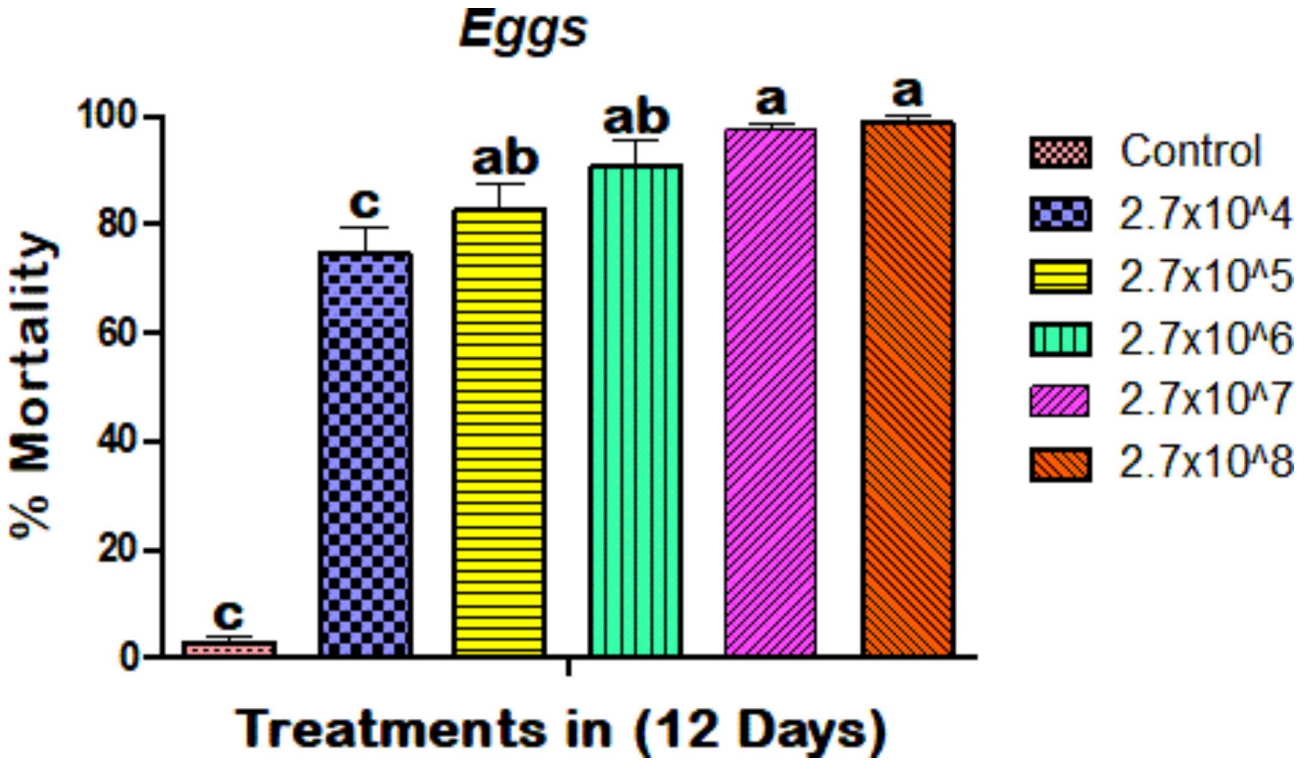

Fig. 3. *Beauveria brongniartii* spores exhibited 98.66% ovicidal activity against *Spodoptera litura* eggs at 12 days post-treatment. In a Tukey test, means (± SE, standard error) that are labeled with the same letters above bars indicate that there is no statistically significant difference ($p \leq 0.05$).

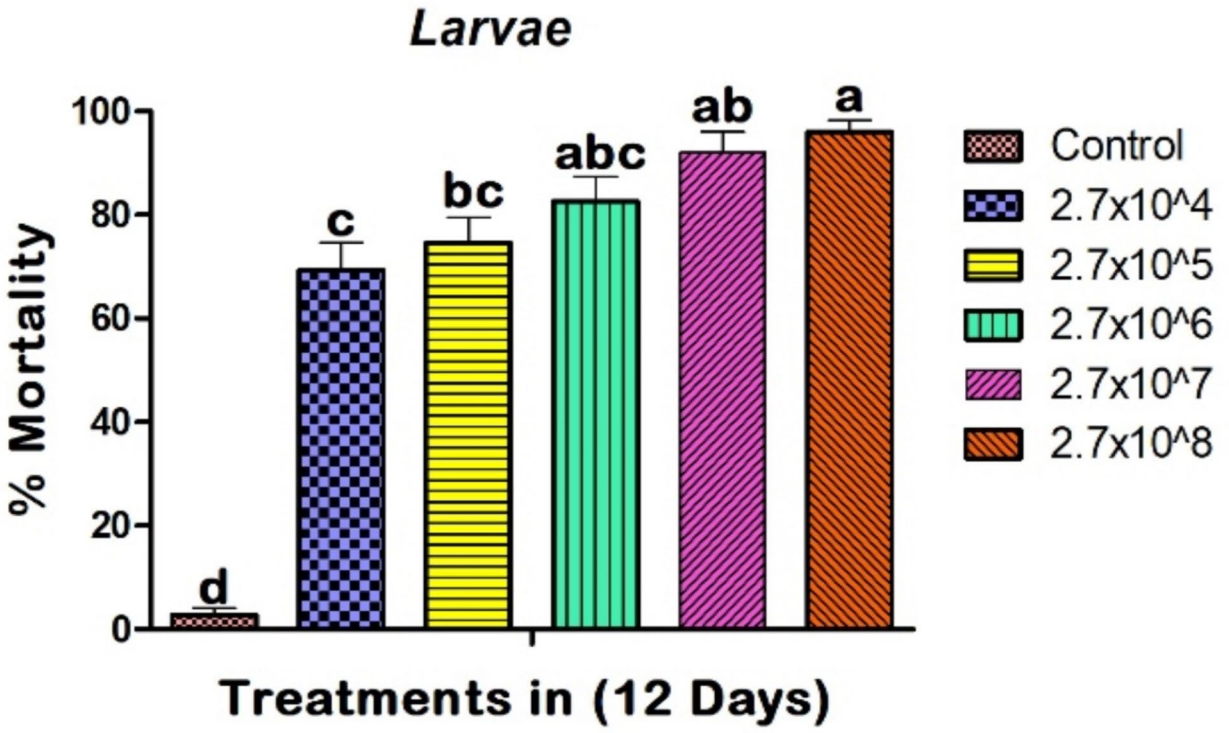

Fig. 4. *Beauveria brongniartii* spores exhibited 96% larvicidal activity against *S. litura* larvae at 12 days post-treatment. In a Tukey test, means (± SE, standard error) that are labeled with the same letters above bars indicate that there is no statistically significant difference ($p \leq 0.05$).

Following *B. brongniartii* treatment, ALP activity dropped by up to -61.72% compared to the control. At a concentration of 2.7×10^8 conidia/mL, fungal conidia significantly inhibited ALP enzyme activity at 3 days post-treatment ($F_{(5,12)} = 515.118$; $p \leq 0.01$) (see Fig. 7B). These findings showed dose-dependent ALP enzyme activity. Control larvae had normal enzyme levels, but those exposed to higher fungal conidia concentrations had significantly lower ALP activity (Fig. 7B). Lethargy and death in *S. litura* larvae treated with *B. brongniartii* may be caused by fungal metabolites inhibiting enzyme activity, physiological stress from infection, and impaired digestive processes.

Lipid peroxidation (LPO)

Lipid peroxidation (LPO) activity in *S. litura* larvae increased significantly after treatment in the third instar (Fig. 8), peaking three days later. The enzyme activity was dose-dependent to *B. brongniartii* conidia. Compared to the control group, larvae treated with fungal conidia showed a significant increase in LPO activity, increasing from 0.4 to 1.56 mg protein/mL of homogenate ($F_{(5,12)} = 18.400$; $p \leq 0.01$) (see Fig. 8). Due to oxidative stress from *B. brongniartii*, *S. litura* larvae treated with the fungal infection have significantly higher LPO activity. Fungal conidia generate reactive oxygen species, which raise LPO levels, indicating cellular membrane damage and larval illness.

Superoxide dismutase (SOD)

Exposure to *B. brongniartii* conidia dose-dependently affected larvae SOD levels. Exposure to *B. brongniartii* fungal conidia significantly increased superoxide dismutase (SOD) enzyme activity in larvae (from 4.06 to 8.0 mg protein/mL of homogenate) ($F_{(5,12)} = 113.565$; $p \leq 0.01$) (see Fig. 9). The increase in SOD enzyme activity in *S. litura* larvae treated with *B. brongniartii* is a defense against oxidative stress. Larvae increase SOD levels to neutralize reactive oxygen species from the fungal infection and protect cellular structures.

Catalase (CAT)

In *S. litura* larvae treated with the entomopathogenic fungus *B. brongniartii*, catalase (CAT) enzyme activity dropped significantly during the third instar phase (Fig. 10), reaching its lowest point within three days. Higher concentrations of B. brongniartii conidia reduced CAT enzyme activity more. The larvae treated with fungal conidia showed a significant decrease in catalase activity, from 3.4 to 1.6 mg protein/mL of homogenate ($F_{(5,12)} = 12.672$; $p \leq 0.01$) compared to the control group (Fig. 10). Spodoptera litura larvae treated with Beauveria brongniartii had significantly lower catalase (CAT) enzyme activity, suggesting an impaired antioxidant

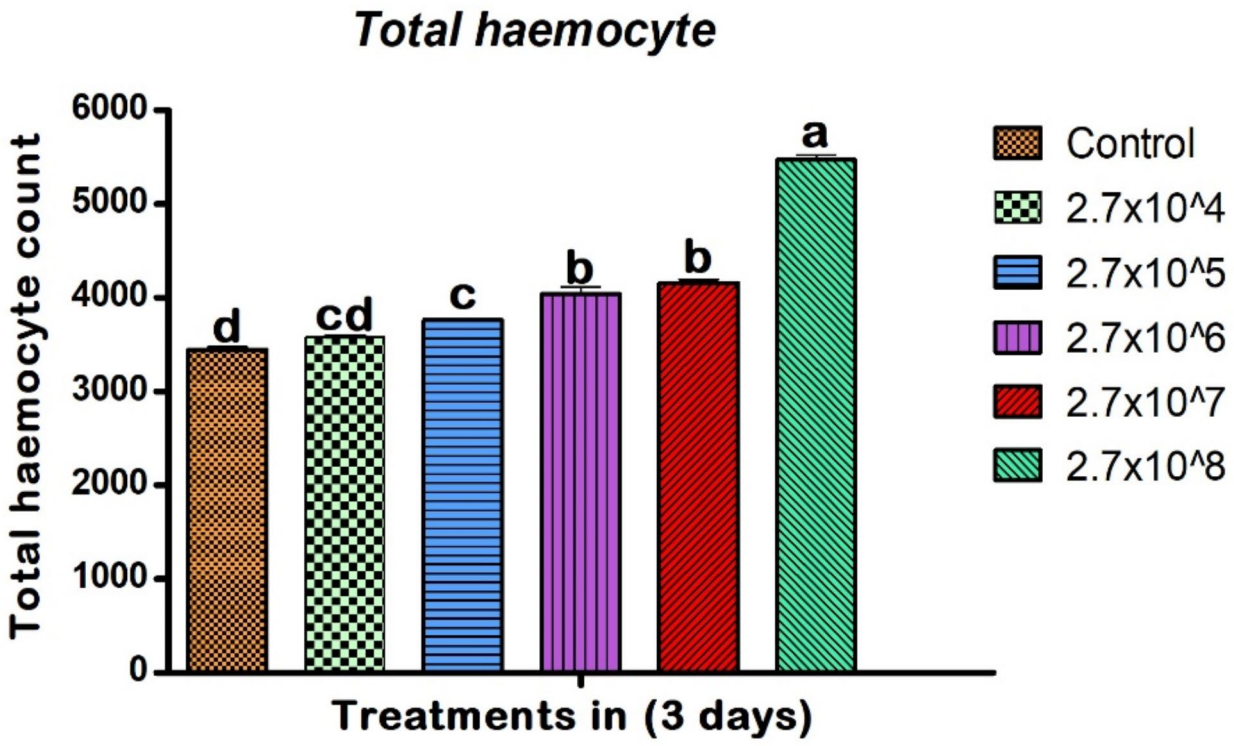

Fig. 5. Total haemocyte count (THC) increased in *S. litura* second instar larvae after treatment with *B. brongniartii* spores, compared to untreated (control) larvae. In a Tukey test, means (± SE, standard error) that are labeled with the same letters above bars indicate that there is no statistically significant difference ($p \leq 0.05$).

response to oxidative stress. Reduced CAT levels may disrupt the larvae's ability to neutralize hydrogen peroxide, increasing oxidative damage at higher fungal concentrations.

Pupicidal efficacy

B. brongniartii demonstrated a negligible effect on *S. litura* pupae relative to other developmental stages (egg, larvae, and adult), resulting in low mortality rates by 12 days post-treatment. After treatment with a concentration of 2.7×10^8 conidia/mL, the observed mortality rates were as follows: At 3 days post-treatment, the observed effect was 18.66% ($F_{(5,12)} = 10.969$; $p < 0.01$), with LC_{50} and LC_{90} values of 4.3×10^{14} and 1.1×10^{21} spores/mL, respectively. At 6 days post-treatment, the effect increased to 37.33% ($F_{(5,12)} = 57.040$; $p < 0.01$), with LC_{50} and LC_{90} values of 4.4×10^9 and 1.1×10^{17} spores/mL. By 9 days post-treatment, the effect reached 62.66% ($F_{(5,12)} = 57.333$; $p < 0.01$), with LC_{50} and LC_{90} values of 4.9×10^6 and 1.7×10^{14} spores/mL. Finally, at 12 days post-treatment, the effect was 73.33% ($F_{(5,12)} = 234.880$; $p < 0.01$), with LC_{50} and LC_{90} values of 9.6×10^4 and 7.3×10^{10} spores/mL (Fig. 11).

Adulticidal efficacy

B. brongniartii showed an extensive impact on *S. litura* adults, resulting in significant mortality rates by 12 days following treatment. After treatment with a concentration of 2.7×10^8 conidia/mL, the observed mortality rates were as follows: At 3 days post-treatment, the efficacy was 45.33% ($F_{(5,12)} = 33.376$; $p < 0.01$), with LC_{50} and LC_{90} values of 6.2×10^8 and 6.9×10^{16} spores/mL, respectively. At 6 days post-treatment, efficacy increased to 62.66% ($F_{(5,12)} = 88.189$; $p < 0.01$), with LC_{50} and LC_{90} values of 4.7×10^6 and 1.4×10^{14} spores/mL. At 9 days post-treatment, efficacy was 77.33% ($F_{(5,12)} = 24.050$; $p < 0.01$), with LC_{50} and LC_{90} values of 5.2×10^4 and 7.0×10^{10} spores/mL. Finally, at 12 days post-treatment, efficacy reached 90.66% ($F_{(5,12)} = 105.139$; $p < 0.01$), with LC_{50} and LC_{90} values of 1.0×10^3 and 2.0×10^8 spores/mL (Fig. 12).

Fungi colonization assessment

After being treated with the entomopathogenic fungus *B. brongniartii*, all stages of development of *S. litura*, including eggs, larvae, pupae, and adults, were heavily infested by the fungus, which was clearly visible as white mycelium. On the other hand, the control insects did not experience any impact and showed no indications of fungal infection. The mycelium of B. brongniartii was observed to proliferate and spread extensively within the host tissues, resulting in mortality that varied depending on the dosage, affecting all developmental stages. The highest level of pathogenicity was observed at a concentration of 2.7×10^8 conidia/mL.

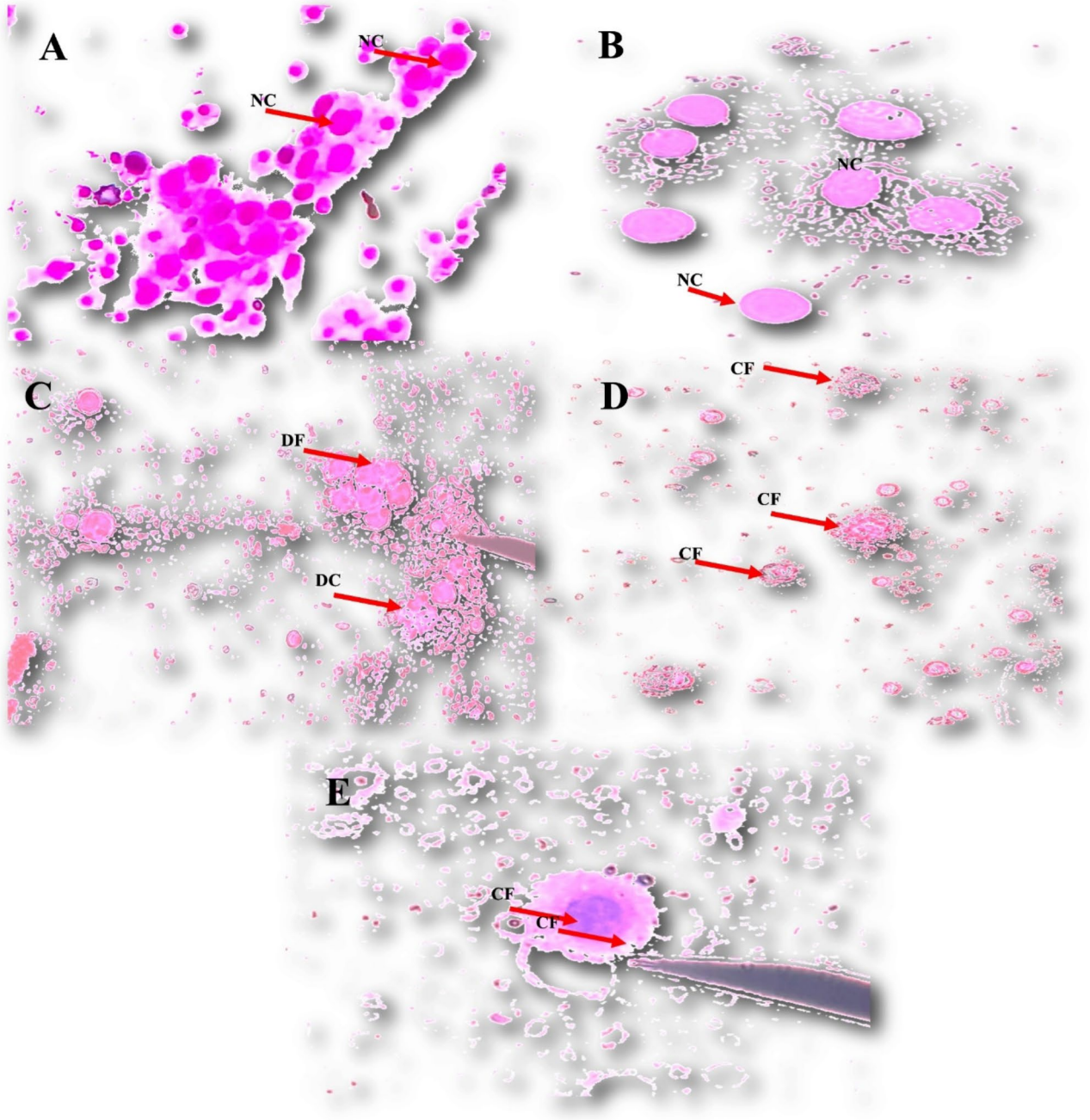

Fig. 6. After treatment with *B. brongniartii* spores, *S. litura* larvae exhibited significant haemocyte discoloration, deformation, and fragmentation three days post-treatment, compared to untreated (control) larvae. In the treated groups, altered cell morphology, cellular breakdown, and cell lysis were observed.

Toxicity on non-target earthworms

Monocrotophos is widely used chemical pesticide in agriculture and proved to be more toxic to earthworms compared to *B. brongniartii* conidia at a concentration of 2.7×10^8. After a 12-day treatment period, the *B. brongniartii* conidia showed no harmful effects on earthworms. There was significant difference in mortality rates between the groups treated with the fungal conidia and the control group (distilled water with 0.05% DMSO), as confirmed by statistical analysis ($F_{(5,12)} = 12.900$; $p \leq 0.01$). In the control group (distilled water with 0.05% DMSO) Monocrotophos was determined to be the most toxic substance to earthworms in comparison to both the fungal conidia and the negative control. Earthworms that were exposed to fungal spores did not experience any increase in death rate during the 12-day period. However, when a dose of Monocrotophos at a concentration of 100ppm was administered, a high number of earthworms died, with a mortality rate of 96% recorded just 12 h after treatment (see Fig. 13).

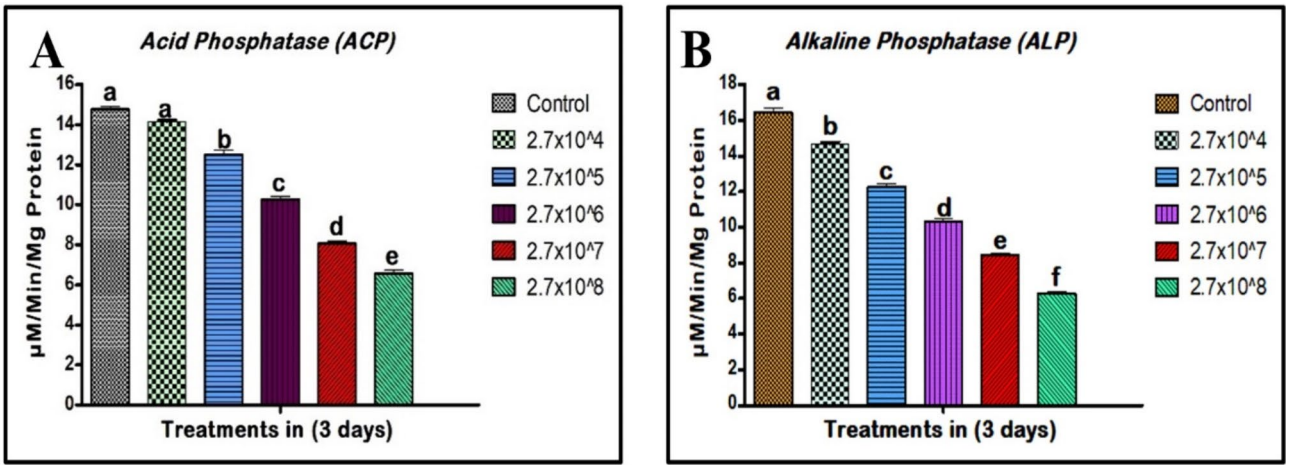

Fig. 7. (**A**) Biochemical analysis revealed that acid phosphatase (ACP) and (**B**) Alkaline phosphatase (ALP) enzyme levels were decreased in *S. litura* second instar larvae after treatment with *B. brongniartii* spores compared to control groups. In a Tukey test, means (± SE, standard error) that are labeled with the same letters above bars indicate that there is no statistically significant difference ($p \leq 0.05$).

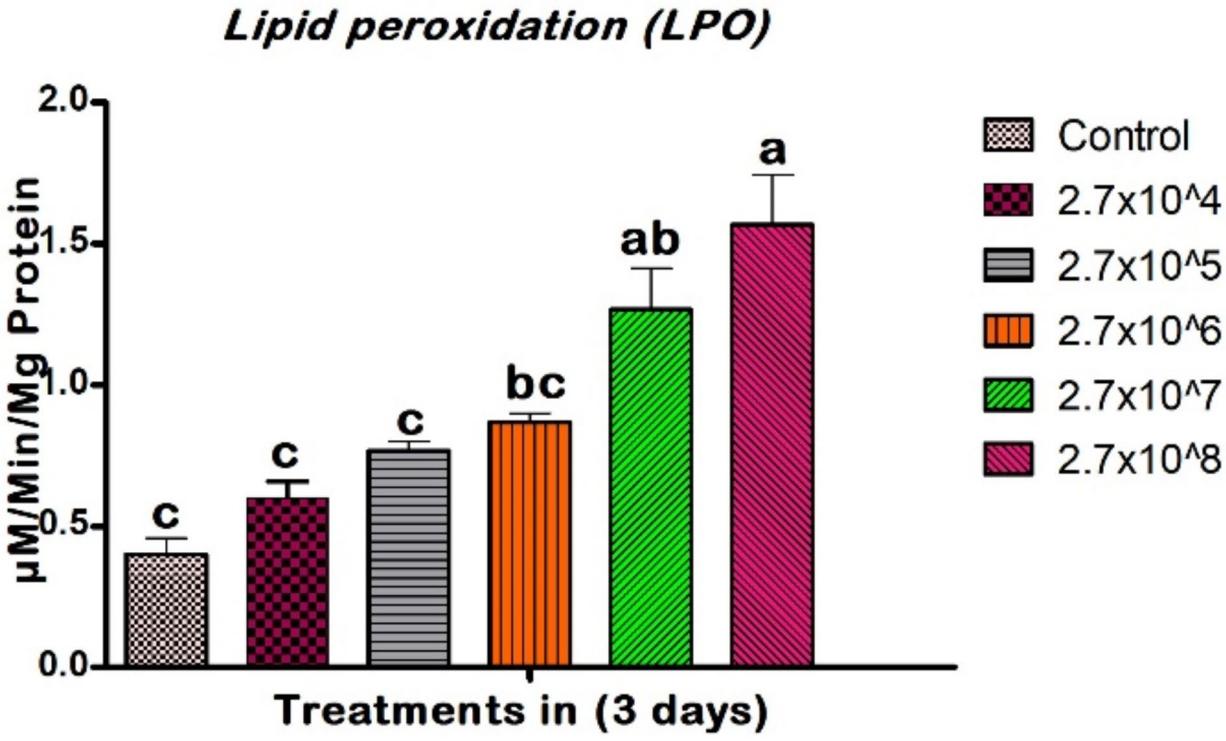

Fig. 8. Biochemical analysis revealed that lipid peroxidase enzyme levels were increased in *S. litura* second instar larvae after treatment with *B. brongniartii* spores compared to control groups. In a Tukey test, means (± SE, standard error) that are labeled with the same letters above bars indicate that there is no statistically significant difference ($p \leq 0.05$).

Histopathology evaluation

The histopathological analysis revealed that earthworms exhibit resistance to the entomopathogenic fungus *B. brongniartii*, particularly when compared to the chemical pesticide Monocrotophos. Treatment with fungal conidia caused no visible damage to the gut tissue, epidermis, circular muscle, setae, mitochondria, or intestinal lumen. This finding aligns with previous research indicating that *E. eugeniae*secretes mucus during

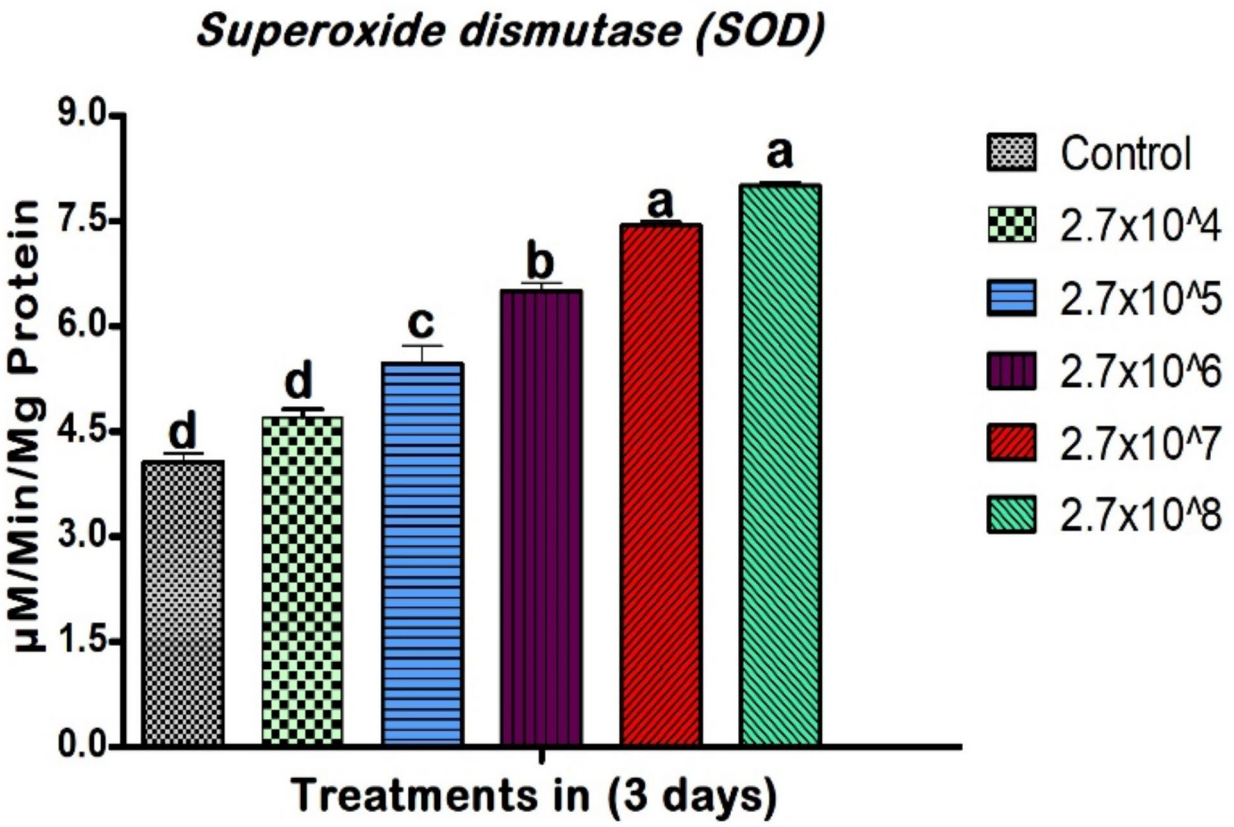

Fig. 9. Biochemical analysis revealed that superoxide dismutase (SOD) enzyme levels were increased in *S. litura* second instar larvae after treatment with *B. brongniartii* spores compared to control groups. In a Tukey test, means (± SE, standard error) that are labeled with the same letters above bars indicate that there is no statistically significant difference ($p \leq 0.05$).

entomopathogenic fungal infections, which acts as a protective adhesive barrier and possesses antifungal properties[22]. In contrast, exposure to Monocrotophos resulted in significant histopathological damage to gut tissue, epidermis, circular muscle, setae, mitochondria, or intestinal lumen of E. eugeniae, with effects observable within 12 h of treatment, as illustrated in Fig. 14. This underscores the critical role of mucus in safeguarding against fungal infections, as well as the detrimental impact of chemical pesticides on earthworm health.

Discussion

In recent years, chemical pesticides have resulted in numerous adverse effects on green ecosystems and non-target species[9]. The repeated application of various types, or even the same type, of chemicals has led to increased impacts on humans and other vertebrates[9]. Additionally, insects have developed resistance to multiple insecticides[9]. Consequently, the use of entomopathogenic fungi (EPF), such as *Beauveria bassiana* and *Metarhizium anisopliae*, presents a viable solution[1,16–21]. These fungi penetrate the insect cuticle, proliferate within the host, and release toxins that ultimately lead to death[18]. This method specifically targets *Spodoptera litura*, minimizing harm to non-target organisms and promoting sustainable pest management practices[1]. In the current days the chemical control of *S. litura*involves using pesticides to manage pest infestations worldwide. However, drawbacks include pesticide resistance, environmental damage, and harm to non-target organisms[1,18–21]. Entomopathogenic fungi, such as *B. brongniartii*, offer a viable alternative by providing effective pest control with minimal ecological impact and reduced risk of resistance development. The results of this study provide compelling evidence for the effectiveness of *B. brongniartii* as a biological control agent against *S. litura*, a major pest affecting broad range of crops worldwide. The presence of entomopathogenic fungi in soils, as demonstrated in Figs. 1 and 2, highlights their prevalence and potential effectiveness compared to fungi from other sources[12–21]. Similar to the present study, soil-derived *B. bassiana*has shown high insecticidal efficacy against several agricultural insect pests[17]. The insecticidal bioassay results clearly demonstrated the high efficacy of *B. brongniartii* in this study. At the highest concentration of 2.7×10^9 conidia/mL, the mortality rates were 98.66% for eggs, 96% for larvae, 77.33% for pupae, and 90.66% for adults. These results highlight *B. brongniartii*as a potent biocontrol agent. The high virulence observed in this study may be attributed to the fact that the entomopathogenic fungi were isolated from unpolluted forest environments, which appear to yield more virulent strains compared to those

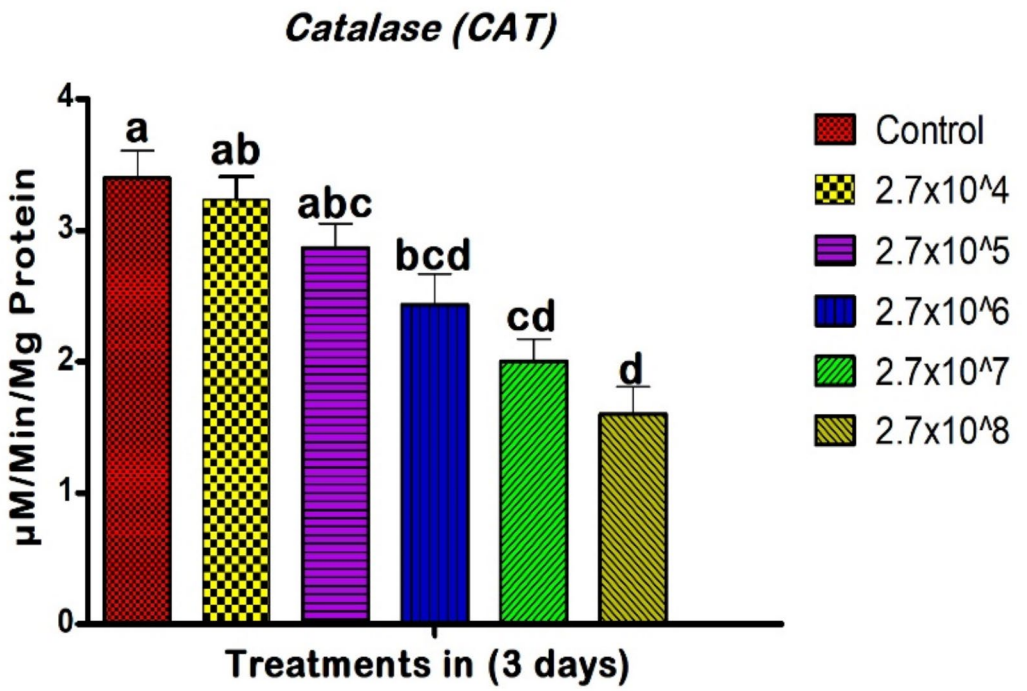

Fig. 10. Biochemical analysis revealed that catalase enzyme levels were reduced in *S. litura* second instar larvae after treatment with *B. brongniartii* spores compared to control groups. In a Tukey test, means (± SE, standard error) that are labeled with the same letters above bars indicate that there is no statistically significant difference ($p \leq 0.05$).

sourced from other environments. These findings align with previous studies indicating the effectiveness of entomopathogenic fungi in managing lepidopteran pests[1,20,21].

Similarly, *B. brongniartii*-derived Fe0 nanoparticles (Fe0NPs) showed remarkable toxic effects against *S. litura*at 500 ppm concentrations[51], and *B. brongniartii* (Sacc.) Petch showed high insecticidal activity against *Holotrichia serrata*F. (Coleoptera: Scarabaeidae) infesting sugarcane fields[52]. Likewise, *B. brongniartii* showed high insecticidal activity in field efficacy tests against *E. rufipes* and *E. orientalis*larvae[53]. Konstantopoulou and Mazomenos[54] reported that entomopathogenic fungi *B. bassiana* and *B. brongniartii* exhibited promising insecticidal activity against adults of *Bactrocera oleae* and *Ceratitis capitata*. In addition, *B. brongniartii* (Saccardo) Petch was found to be highly virulent against the melolonthid *Hypopholis sommeri*(Coleoptera: Scarabaeidae)[55], and *B. brongniartii* spores demonstrated more larvicidal activity against 2nd instar larvae of *S. litura*[56].

In the present study, the variability in mortality rates across developmental stages (egg, larvae, pupae, and adult) highlights the differential susceptibility of *S. litura* to *B. brongniartii* (Figs. 3, 4, 11 and 12). The higher mortality observed in eggs and larvae compared to pupae and adults suggests that the earlier developmental stages are more vulnerable to fungal infection (Figs. 3, 4, 11 and 12). This finding aligns with the understanding that fungal pathogens often exhibit greater efficacy against younger insects due to their less developed immune systems. Consequently, earlier developmental stages are more susceptible than later stages. *B. brongniartii* exhibited minimal impact on *S. litura*pupae because pupae have a hardened cuticle that offers better protection against fungal invasion. Additionally, the pupal stage has reduced metabolic activity, limiting fungal effectiveness and may be causing lower mortality rates. These results are consistent with previous studies[57]. Several previous studies have shown that entomopathogenic fungi, such as Beauveria and Metarhizium species, are more pathogenic to earlier stages of insect pests, supporting the present study[18–21,58–63]. This study demonstrated that the minimal LC_{50} and LC_{90} values further support the high virulence of *B. brongniartii*, indicating its potential for effective pest management (Figs. 3, 4, 11 and 12). Similarly, Swathy et al.[16] and Vivekanandhan et al.[18] reported that entomopathogenic fungi *B. bassiana* and *M. anisopliae* showed lower LC_{50} and LC_{90} values within nine days post-treatment against eggs, larvae, pupae, and adults of *Spodoptera frugiperda*, *Halyomorpha halys*, *Tenebrio molitor*, and *Popillia japonica*.

In this study, *S. litura* hemocyte counts significantly increased after treatment with *B. brongniartii*(Fig. 5). After entomopathogenic fungi treatment, the insect's immune system responds by increasing hemocyte production, as these cells play a key role in defending against fungal infection through phagocytosis and encapsulation. Similarly, Vivekanandhan et al.[1] reported that treatment with *M. anisopliae* led to elevated hemocyte levels in *S. litura* larvae within 3 days. These findings indicate that increased hemocyte levels following treatment with entomopathogenic fungi reflect an immune response, as the insect's immune system mobilizes hemocytes to combat fungal infection and address cellular damage. The biochemical analyses in this study indicated increased

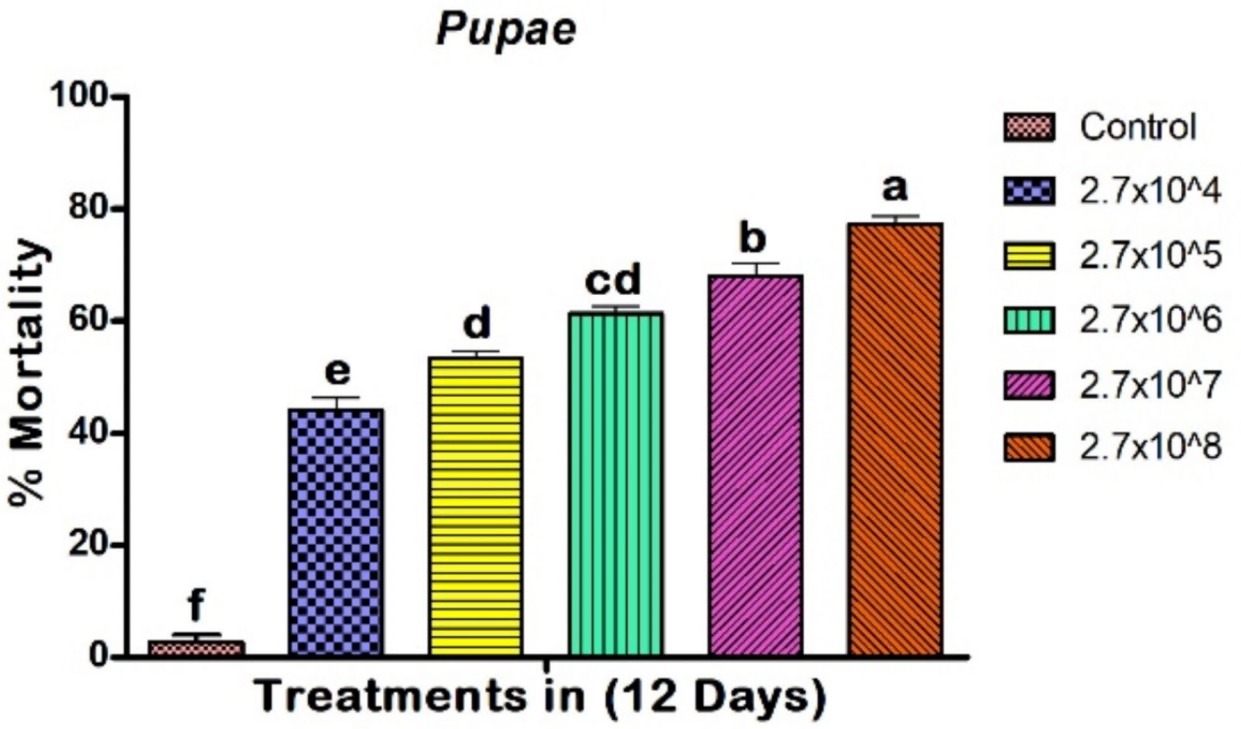

Fig. 11. *Beauveria brongniartii* fungal spores exhibited pupicidal effects against *S. litura*, with 77.3% mortality observed 12 days post-treatment. In a Tukey test, means (± SE, standard error) that are labeled with the same letters above bars indicate that there is no statistically significant difference ($p \leq 0.05$).

oxidative stress and altered enzyme levels in *S. litura* larvae exposed to *B. brongniartii*, suggesting that the fungus disrupts the pest's physiological processes.

Treatment with *B. brongniartii* reduced Acid Phosphatase (ACP) activity by up to − 55% compared to controls, with the most significant suppression observed 3 days post-treatment at 2.7×10^8 conidia/mL ($F_{(5,12)} = 443.584$; $p \leq 0.01$) (see Fig. 7A). Similarly, Alkaline Phosphatase (ALP) activity decreased by up to − 61.72% ($F_{(5,12)} = 515.118$; $p \leq 0.01$) (see Fig. 7B). Higher fungal conidia concentrations led to a marked reduction in both ACP and ALP activities. Larvae treated with fungal conidia exhibited a marked rise in LPO activity compared to the control group, with levels escalating from 0.4 to 1.56 mg protein/mL of homogenate (see Fig. 8). Larvae were exposed to *B. brongniartii* fungal conidia, their superoxide dismutase (SOD) enzyme activity increased significantly compared to the control group (from 4.06 to 8.0 mg protein/mL of homogenate) ($F_{(5,12)} = 113.565$; $p \leq 0.01$) (see Fig. 9), the increase in SOD activity indicates an oxidative stress response to fungal infection. This enzyme neutralizes reactive oxygen species, but excessive SOD activity can damage cellular components, impairing the insect's physiology. Catalase (CAT) activity in *S. litura* larvae decreased significantly, reaching its lowest level within 3 days of *B. brongniartii* treatment, with higher conidia concentrations causing greater decreases ($F_{(5,12)} = 12.672$; $p \leq 0.01$), dropping from 3.4 to 1.6 mg protein/mL of homogenate (see Fig. 10). This study decrease in catalase activity likely results from fungal toxins impairing the enzyme's function. Reduced catalase activity can lead to accumulated hydrogen peroxide, causing oxidative damage and disrupting cellular processes. Similar to the present study, the spores of entomopathogenic fungi *M. anisopliae* and *B. bassiana*also affect antioxidant and detoxification enzymes during the infection of insect pests[1,16–23]. Similar to the present study, the spores of the entomopathogenic fungus *M. anisopliae* can affect the acetylcholinesterase, α-carboxylesterase, and β-carboxylesterase enzymes in *S. frugiperda*larvae[22]. Similar to the present study, *(A) niger*α-1,3-glucan inhibits insect phenoloxidase (PO) activity in the hemolymph shortly after injection. This effect differs from that of β-1,3/1,6-glucan and acts as a virulence factor[63]. In this present study showed that *(B) brongniartii* induces oxidative stress in larvae by increasing reactive oxygen species, leading to elevated lipid peroxidation and SOD, and decreased phosphatase and catalase. Molecular interactions involve ROS damaging cellular components.

The lack of an effect of the entomopathogenic fungus *B. brongniartii* on the non-target organism *E. eugeniae*, as demonstrated by earthworm bioindicator studies, is a significant finding. In contrast to the considerable mortality caused by chemical treatments with monocrotophos (Figs. 13 and 14), *B. brongniartii* exhibited no impact on earthworms. The lower susceptibility of earthworms to *B. brongniartii* compared to Monocrotophos is due to the fungus's selective action on insects, causing minimal harm to non-target tissues. Additionally, earthworms secrete antimicrobial mucus that destroys fungal spores. In contrast, Monocrotophos, a chemical

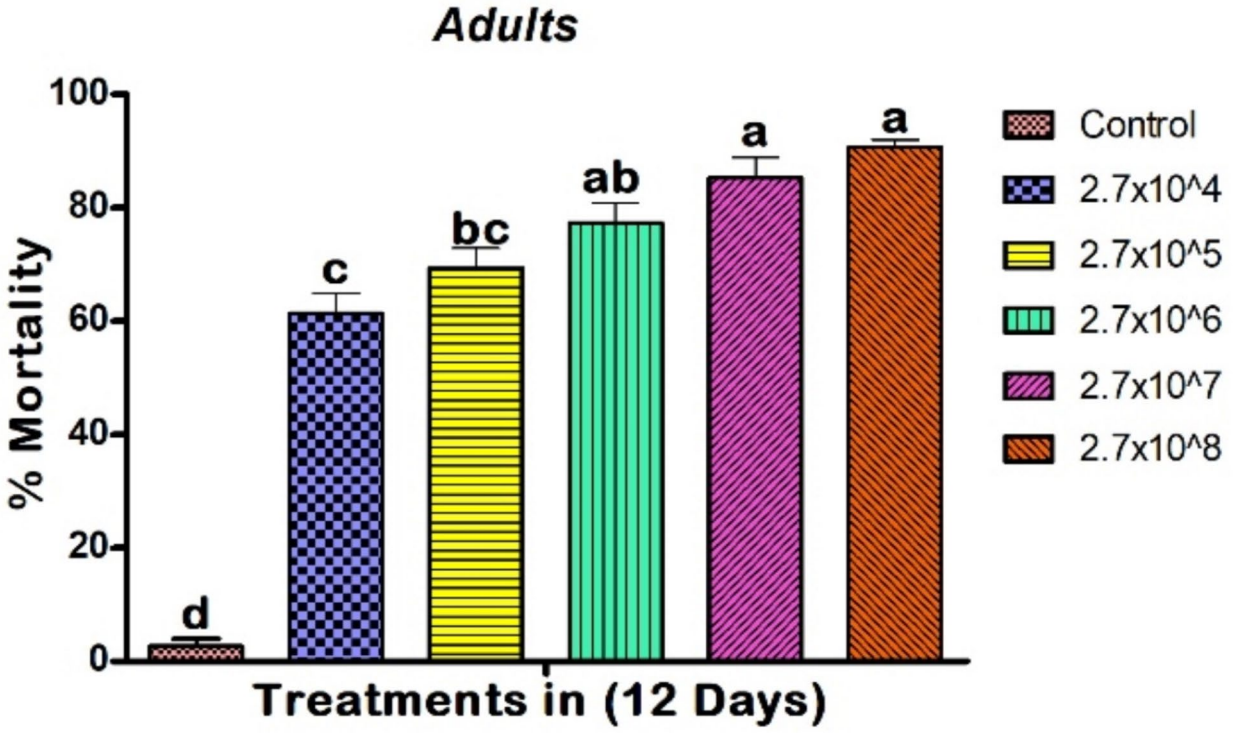

Fig. 12. *Beauveria brongniartii* fungal spores exhibited adulticidal effects against *S. litura*, with 90.66% mortality observed 12 days post-treatment. In a Tukey test, means (± SE, standard error) that are labeled with the same letters above bars indicate that there is no statistically significant difference ($p \leq 0.05$).

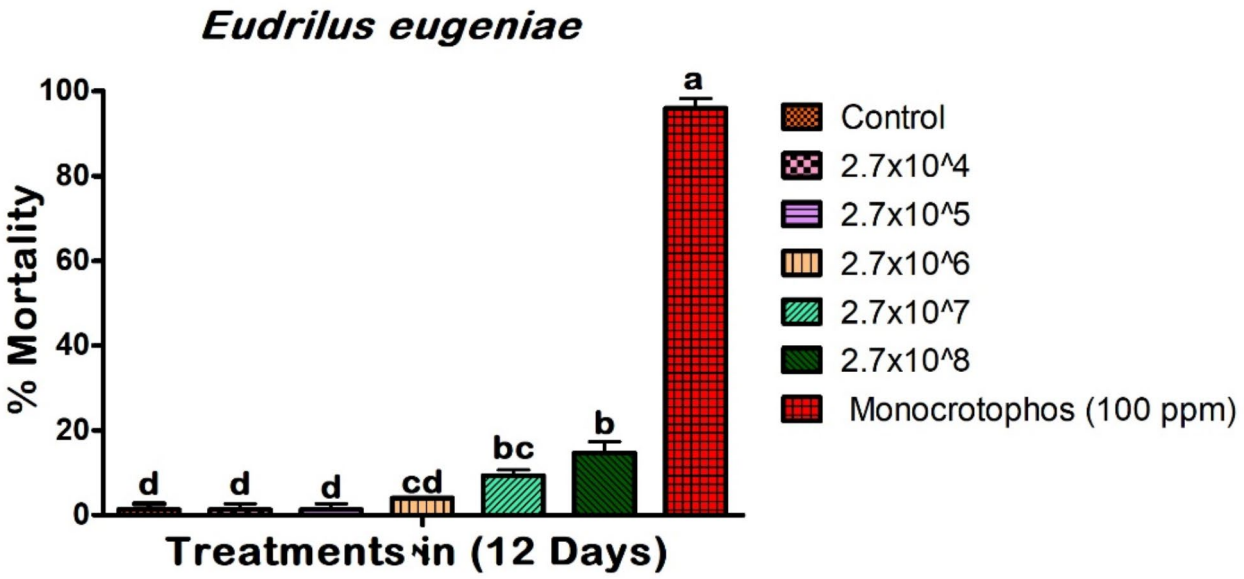

Fig. 13. *Beauveria brongniartii* spores exhibited similar toxicity effects on *E. eugeniae* as observed in the control groups, while the chemical pesticide monocrotophos showed a 96% mortality rate 12 days post-treatment. In a Tukey test, means (± SE, standard error) that are labeled with the same letters above bars indicate that there is no statistically significant difference ($p \leq 0.05$).

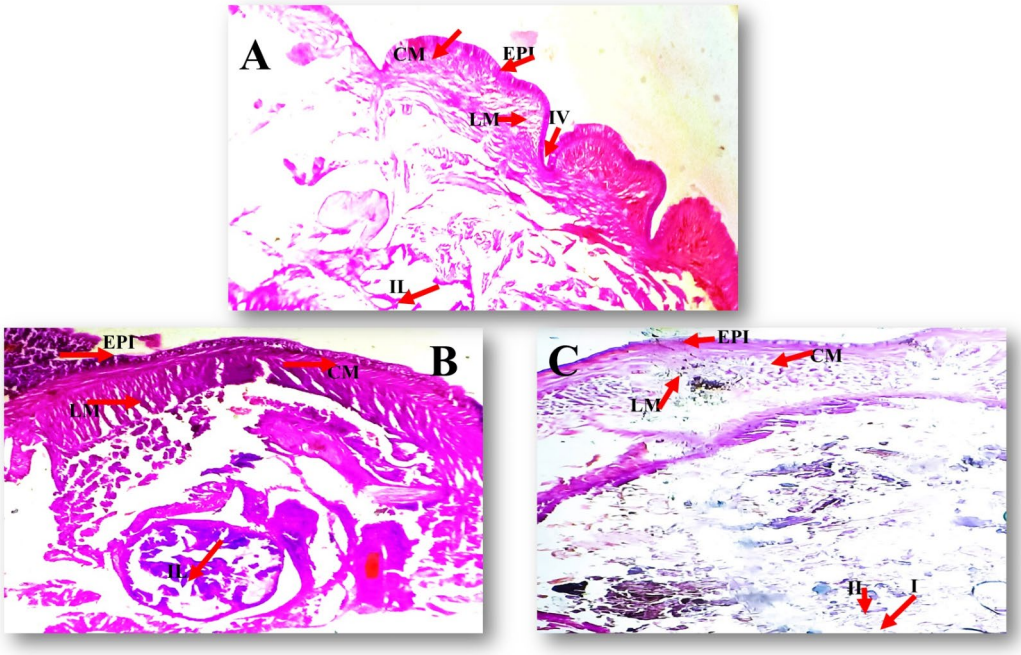

Fig. 14. After a 12-day treatment with *B. brongniartii*, histochemistry studies of *E. eugeniae* showed negligible toxicity effects, which were comparable to those observed in the control group. No adverse effects of the entomopathogenic fungi were observed in the gut tissues. The histological examinations revealed that the gut cells of earthworms treated with *B. brongniartii* were comparable to those of the negative control group. Both control and fungi-treated gut tissues exhibited an absence of cellular debris, and the nuclei maintained a spherical shape. Comparable outcomes were noted in the control group. On the other hand, the chemical insecticide monocrotophos (which was used as a positive control) resulted in substantial harm to the gut tissues. Here are some abbreviations commonly used in histological analysis: EPI stands for epidermis, SE stands for setae, IL stands for intestinal lumen, LM stands for longitudinal muscle, CO stands for coelom, CM stands for circular muscle, and MI stands for mitochondrion.

pesticide, causes extensive histopathological damage to earthworm tissues. This observation may elucidate why entomopathogenic fungi specifically target insect physiology and exploit vulnerabilities that are not present in earthworms. The protective mucus, robust cuticle, and distinct immune responses of earthworms limit the impact of fungi compared to insects. Since earthworms play a vital role in soil health and sustainable agriculture, their protection is essential. Previous research supports these findings, demonstrating that various entomopathogenic fungi do not harm non-target species such as *E. eugeniae*[16–23]. Additionally, histopathological examinations revealed no significant adverse effects on earthworm gut tissues, further affirming the ecological safety of *B. brongniartii* application (Fig. 14). Similar to the present study, different species of entomopathogenic fungi, such as *B. bassiana*, *M. anisopliae*, *M. rileyi*, *M. majus*, and *M. flavoviride*, do not affect non-target species like *E. eugeniae*earthworms, as confirmed through previous histochemistry studies[16–23].

Overall, this study demonstrates that *B. brongniartii* is a viable alternative to chemical pesticides for controlling *S. litura*. Its effectiveness across multiple developmental stages of the pest, combined with its no impact on non-target organisms and its ability to induce significant physiological stress in the pest, makes it an essential tool in integrated pest management strategies. By incorporating *B. brongniartii* into pest management programs, it is possible to reduce reliance on chemical pesticides, thereby lowering their environmental impact and promoting sustainable agricultural practices. Future research should include field trials to validate these laboratory findings and evaluate the long-term efficacy and safety of *B. brongniartii* in natural settings. Additionally, understanding the interactions between *B. brongniartii* and other components of the ecosystem is crucial for optimizing its application in integrated pest management.

Conclusion

This study demonstrates the high efficacy of *B. brongniartii* in managing *S. litura*, a significant pest of vegetables and grains. The results reveal that the entomopathogenic fungus exhibits considerable virulence across all developmental stages of *S. litura*, with the highest conidia concentration (2.7×10^9 conidia/mL) achieving up to 98.66% mortality in eggs, 96% in larvae, 77.33% in pupae, and 90.66% in adults over 12 days. The analysis of mortality rates, along with the minimal LC_{50} and LC_{90} values obtained for each developmental stage, highlights the effectiveness of *B. brongniartii* as a biological control agent. Earthworm bioindicator studies confirmed the safety of *B. brongniartii* for non-target organisms, showing minimal impact compared to chemical treatments, which resulted in a high mortality rate of 96% at 100 ppm.

Histopathological examinations of earthworm gut tissues revealed no significant adverse effects from the fungal conidia, in contrast to the considerable damage observed from chemical pesticide treatments. Additionally, biochemical analyses of *S. litura* larvae demonstrated altered enzyme levels and increased oxidative stress following exposure to *B. brongniartii*, indicating a physiological impact on the pest. Overall, this research highlights the potential of *B. brongniartii* as a promising biological control agent for *S. litura*, providing an effective and environmentally friendly alternative to conventional chemical pesticides in integrated pest management strategies.

Data availability

All the research data's used and/or analyzed in the present study are available from the corresponding author upon reasonable request.

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Declarations

Competing interests
The authors declare no competing interests.

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