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Laboratory Evaluation of Native and Commercial Entomopathogenic Fungi Against *Macrodactylus nigripes* (Coleoptera: Melolonthidae) Associated with Maize Crops

Omar Kareem Martínez Bonilla [1], Omar Romero-Arenas [2,*], Conrado Parraguirre Lezama [2], Raúl Ávila Sosa [3], Rolando Rueda Luna [4], Ricardo Munguía Pérez [5] and Manuel Huerta Lara [5,*]

[1] Doctorado en Ciencias Ambientales, Instituto de Ciencias, Benemérita Universidad Autónoma de Puebla, Puebla 72570, Mexico; omar.martinezbonilla@alumno.buap.mx
[2] Centro de Agroecología, Instituto de Ciencias, Benemérita Universidad Autónoma de Puebla, Edificio VAL 1, Km 1.7 Carretera a San Baltazar Tetela, San Pedro Zacachimalpa, Puebla 72960, Mexico; conrado.parraguirre@correo.buap.mx
[3] Facultad de Ciencias Químicas, Benemérita Universidad Autónoma de Puebla, Puebla 72570, Mexico; raul.avila@correo.buap.mx
[4] Centro de Investigación en Ciencias Agrícolas, Instituto de Ciencias, Benemérita Universidad Autónoma de Puebla, Puebla 72570, Mexico; rolandorueda@hotmail.com
[5] Departamento de Desarrollo Sustentable, Instituto de Ciencias, Benemérita Universidad Autónoma de Puebla, Puebla 72570, Mexico; ricardo.munguia@correo.buap.mx
* Correspondence: biol.ora@hotmail.com (O.R.-A.); manuel.huerta@correo.buap.mx (M.H.L.)

Abstract

Maize in Mexico suffers severe economic losses due to root-feeding and defoliating pests, among which *Macrodactylus nigripes* is particularly damaging, attacking roots during its larval stage and reducing pollination in adults. The intensive use of chemical insecticides has generated environmental impacts and resistance risks, driving the search for sustainable alternatives. In this study, the efficacy of entomopathogenic fungi (*Beauveria bassiana* and *Metarhizium anisopliae*), diatomaceous earth, and a chemical control was evaluated against larvae and adults of *M. nigripes* under laboratory (controlled) conditions. A completely randomized factorial design was employed, and mortality and survival were assessed using Abbott's correction, Kaplan–Meier survival analysis, and Probit regression. Results revealed highly significant differences among treatments. The native strain of *B. bassiana* (MA-Bb1) achieved the highest Abbott-corrected cumulative mortality in L_1 larvae (99.4 ± 1.0%) and adults (75.3 ± 3.9%), with lethal times (LT_{50}) of approximately 1.1–1.3 days in larvae and 1.6 days in adults, outperforming commercial strains and diatomaceous earth. Chemical control exhibited immediate action ($LT_{50} < 0.4$ days). These findings highlight the potential of entomopathogenic fungi, particularly the native strain MA-Bb1 exhibited high pathogenicity against *M. nigripes* under laboratory conditions and represents a promising candidate for future biological control programs. Additional studies under semi-field and field conditions are required to confirm its effectiveness under agricultural production systems.

Keywords: root-feeding; defoliating pests; biological control; maize; agroecology

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1. Introduction

Maize (*Zea mays* L.) constitutes the foundation of food security and economic stability in Mexico, with more than 19 million ha cultivated and an annual production exceeding 24 million tons [1]. The leading states in maize production are Michoacán, Jalisco, Veracruz, Chihuahua, Guanajuato, and Puebla, the latter with 513,101.80 has sown to maize in 2021 (74.8% of the state's agricultural surface, tenth place nationally), a production of 1,151,896.04 tons and an average yield of 2.32 t ha^{-1} [2]. However, maize productivity faces severe threats from agricultural pests such as the fall armyworm (*Spodoptera frugiperda*), white grubs (*Phyllophaga* spp.), wireworms (*Elateridae*) and the beetle *M. nigripes*, commonly known in Mexico as the "falso torito" [3], which causes considerable economic losses for small and medium-sized rural producers.

M. nigripes causes critical damage to maize crops; in its larval stage, it destroys the root system, reducing water and nutrient uptake, while adults feed on maize silks, impairing pollination and directly decreasing yield. This scenario has driven a significant increase in the use of chemical insecticides in recent decades [3]. In Durango, the economic and environmental costs associated with pesticide use in maize are estimated at 24 million pesos annually [4]. Recent studies demonstrate that insecticides exert the greatest environmental impact compared to herbicides and fungicides, contributing to soil and water contamination and biodiversity loss [5]. This trend reflects a growing dependence on agrochemicals, with documented risks of pest resistance and adverse effects on human health [6].

In response, biological control using entomopathogenic fungi has emerged as a viable alternative within organic agriculture and agroecological management [7]. *B. bassiana* and *M. anisopliae* have demonstrated efficacy across diverse agricultural systems [8]. We can mention that in greenhouse tomato, biopesticides including *B. bassiana* proved as efficient as, or better than, a commercial insecticide for the control of whiteflies (*Trialeurodes vaporariorum*) [9]. In Guanajuato, native strains of *M. anisopliae* achieved 100% mortality in *Plutella xylostella* larvae, while *B. bassiana* reached 86% [10]. In aquaponic systems, the application of *B. bassiana* and *M. anisopliae* induced mortalities of 73% and 79% on *Bemisia tabaci* in a period of six days under controlled conditions [11]. In central Mexico, *M. anisopliae* strains isolated from agricultural soils have shown adequate conidial production and quality, supporting the use of locally adapted isolates for biocontrol programs [12]. These fungi offer potential advantages against arthropod pests, including biodegradability and compatibility with organic farming systems [7]; however, their efficacy under field conditions may be limited by environmental factors. Their infection mechanism, penetration through the insect cuticle, unlike bacteria and viruses that require ingestion; makes them particularly effective against root-feeding coleopterans [13]. Despite these advances, challenges remain for large-scale application under field conditions, particularly in rainfed systems, where environmental factors such as temperature and relative humidity limit efficacy [14]. In the specific case of *M. nigripes*, information on the potential of entomopathogenic fungi remains scarce, especially in the context of the town of Huejotzingo, Puebla, Mexico.

The present study aims to evaluate the laboratory pathogenicity of entomopathogenic fungi against *M. nigripes* under controlled conditions, as a first step toward the development of sustainable alternatives to chemical insecticides.

2. Materials and Methods

2.1. Biological Material

The study was conducted at the Plant Pathology Laboratory (Lab 204) of the Agroecology Center at the Benemérita Universidad Autónoma de Puebla (BUAP),

Mexico. *M. nigripes* specimens at various developmental stages (L_1, L_2, L_3, and adults) were collected via manual excavation at depths of 15–25 cm around plants exhibiting root damage symptoms; the samples were taken directly from rain-fed maize plots in Huejotzingo, Puebla, Mexico (19°09′ N, 98°24′ W; altitude 2270 m a.s.l.), a region characterized by traditional agricultural practices and continuous annual maize cultivation. Specimens were transported in individual containers with moist substrate to the laboratory for processing and bioassay establishment. Larvae were identified following the taxonomic keys for *Phyllophaga* larvae [15,16] using the palidia and epipharynx as primary diagnostic structures. Larval instars were distinguished by cephalic capsule width. Adults were identified using the morphological criteria for the genus [17] and sex was determined by the length of the antennal club, which is distinctly longer in males. Collection dates followed the biological cycle of *M. nigripes* (Figure 1): L_1 in November 2024, L_2 and L_3 in January–March, and adults in May from July 2025. Insects were randomly allocated to treatments using a completely randomized design, with three replicate trays per treatment and developmental stage.

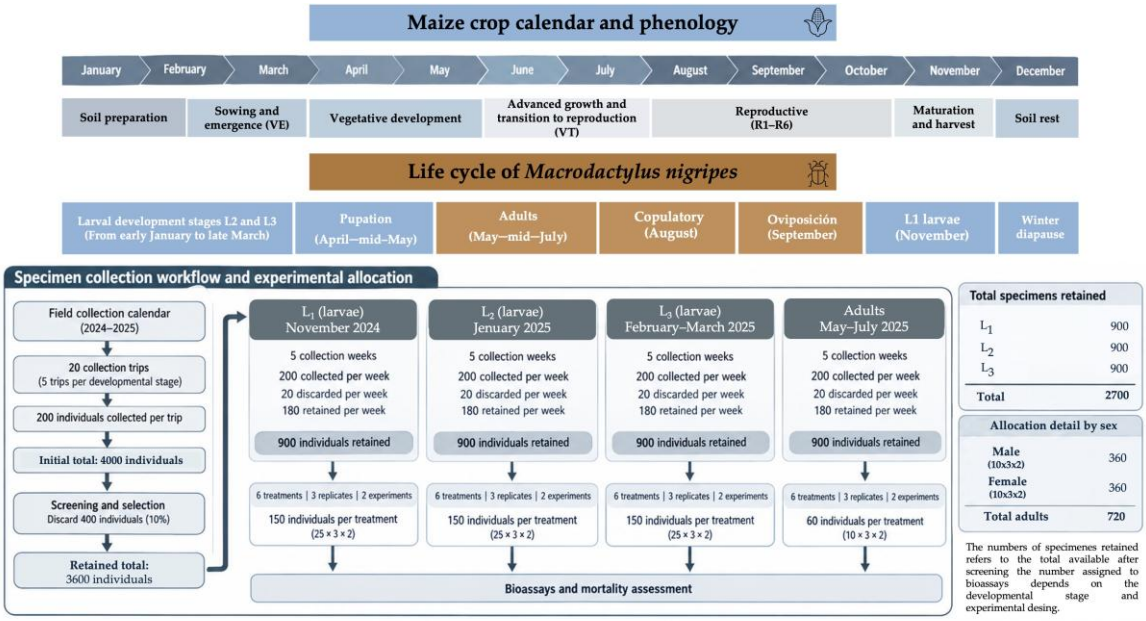


Figure 1. Seasonal synchronization of the *M. nigripes* life cycle, maize phenology, and specimen collection periods in Huejotzingo, Puebla, Mexico, for the 2024–2025 period.

A group of 25 individuals from each stage was quarantined for 72 h to detect possible fungal or microbial contamination that could interfere with experimental results. Larvae of different instars were maintained under controlled conditions of temperature (24 ± 2 °C), relative humidity (65–80%), and photoperiod (12 h light/12 h dark) during the acclimatization period prior to bioassays.

2.2. Diet and Feeding

Larvae were placed individually in rectangular plastic containers (35.9 × 19.4 × 12.4 cm; area = 696.46 cm^2), internally divided into 5 × 5 cm cells (25 cm^2) by removable panels, to house 25 larvae per tray and prevent cannibalism; a behavior documented in melolonthid larvae [18], and fed a solid maintenance diet (5% agar, 1% peptone, 0.1% yeast extract), following standardized laboratory protocols for bioassays with entomopathogenic fungi [19]. The mixture was heated in a microwave oven for 6 min, sterilized in an autoclave at 120 °C for 15 min, and stored in 90 mm Petri dishes at 4 °C until use. This substrate was designed as a short-term maintenance diet to standardize

moisture and nutritional conditions during the bioassay rather than to sustain long-term larval development; its adequacy is supported by the survival of negative control (85% over the 20-day observation period).

For adult bioassays, the same trays were used but with larger cells (7 × 7 cm ≈ 50 cm^2), housing 10 adults per tray to reduce stress. Each experimental unit consisted of 10 adults of the same sex, identified morphologically: females with larger body size and more developed elytra; males with longer antennae and differentiated abdominal segments. Adults were fed fresh maize silks, apples, pear, and *Hibiscus* spp. flowers, following protocols for melolonthid rearing [20]. Food was renewed daily. In both cases, each cell contained sterile substrate (sand:peat 1:1) moistened with distilled water (12% gravimetric moisture). Containers were covered with perforated lids to allow gas exchange and incubated in bioclimatic chambers at 24 ± 2 °C, 70 ± 5% relative humidity, and a 12 h light/12 h dark photoperiod.

2.3. Entomopathogenic Fungi

A native strain MA-Bb1 of *B. bassiana* (GenBank: MN209825.1) was used. The molecular identification of MA-Bb1 was based on the internal transcribed spacer (ITS) region, which showed 100% homology with *B. bassiana*. The strain is deposited in the culture collection of the Centro de Agroecología, ICUAP-BUAP (Puebla, Mexico). The strain was previously isolated from *Sphenarium purpurascens* in maize crops of Huejotzingo, Puebla, Mexico.

The strain was reactivated on Sabouraud dextrose agar (SDA) (BD Bioxon, Mexico City, Mexico) supplemented with 0.5% yeast extract, incubated at 26 ± 1 °C for 14 days under a 12 h light/12 h dark photoperiod [21]. Conidia were harvested by surface scraping with a sterile spatula and suspended in a sterile aqueous solution of 0.01% Tween 80 (Sigma, St. Louis, MO, USA).

Subsequently, the suspension was adjusted to 1 × 10^8 conidia mL^{-1} using a Neubauer chamber (Thermo Fisher Scientific, Waltham, MA USA). Conidial viability was assessed on 2% water agar using three replicates, considering a conidium viable when the germ tube length exceeded the conidial diameter after 24 h of incubation [22]. The native strain MA-Bb1 exhibited 94% germination. Two commercial liquid formulations were also evaluated: commercial *B. bassiana* (CHE-CNRCB 497) and commercial *M. anisopliae* (CHE-CNRCB 535), obtained from the National Reference Centre for Biological Control (CNRCB). Both formulations were applied at 1 × 10^8 conidia mL^{-1}, and conidial viability was determined following the same protocol, resulting in germination percentages of 73% and 68%, respectively [19].

Diatomaceous earth (Diatomin®), composed mainly of silicon dioxide (SiO_2) from fossilized diatoms, was included due to its ability to disrupt cuticular lipids, facilitating penetration through the insect exoskeleton, and potentially enhancing fungal efficacy [23]. The product was diluted at 10% (*w*/*v*) in distilled water (10 g Diatomin®: 100 mL water) and stirred for 5 min before application.

As positive control, the synthetic insecticide GallinaTox® 500 mg (active ingredient: chlorpyrifos) was applied at the manufacturer's recommended dose (2 g/L water), selected to ensure rapid and complete mortality under laboratory conditions and thus serve as a reference for the biological treatments. Sterile distilled water with 0.01% Tween 80 (*v*/*v*) served as negative control.

2.4. Experimental Design

Bioassays were conducted using a completely randomized factorial design comprising six treatments and four developmental stages (L_1, L_2, L_3, and adults) with three replicate trays per treatment-stage combination (Table 1). Each tray contained 25

individually isolated insects of the same developmental stage housed in separate compartments, resulting in 75 larvae per treatment in each temporal run. For adult bioassays, each treatment consisted of three trays per sex, with 10 adults per tray, resulting in 30 males and 30 females per treatment in each temporal run (60 adults per treatment per run). The experiment was repeated in two independent temporal runs conducted under identical environmental conditions.

The final sample size per treatment across the complete experiment was 150 larvae (75 insects × 2 runs). These individuals were distributed across 12 trays per treatment (6 trays × 2 runs), with two temporal runs. The tray was considered the experimental unit throughout the study, whereas individual insects represented subsamples within each tray.

Table 1. Description of experimental treatments and concentrations used for the evaluation of control agents.

Treatment	Active Ingredient/Formulation	Concentration	Viability
Native *B. bassiana* (MA-Bb1)	Conidial suspension	1×10^8 conidia mL^{-1}	94%
Commercial *B. bassiana* (CHE-CNRCB 497)	Conidial suspension	1×10^8 conidia mL^{-1}	73%
Commercial *M. anisopliae* (CHE-CNRCB 535)	Conidial suspension	1×10^8 conidia mL^{-1}	68%
Diatomaceous earth (Diatomin®)	Diatomaceous earth suspension	100 mg mL^{-1}	NA
Chemical control (GallinaTox®)	Chlorpyrifos ethyl formulation 3%	2 mg mL^{-1}	NA
Negative control	Sterile distilled water + 0.01% Tween 80	—	NA

Note: NA = not applicable; — = no active ingredient concentration applicable to the negative control.

Bioassays on adults were conducted as a separate experiment under the same controlled conditions, using the same six treatments. Each treatment consisted of three trays per sex, with 10 adults per tray (30 males and 30 females per treatment; n = 60 adults per run). The experiment was likewise repeated in two independent temporal runs.

2.5. Treatment Application

Applications were performed using a Potter spray tower (Burkard Manufacturing Co., Ltd., Rickmansworth, UK) at a constant pressure of 15 psi (approximately 103.4 kPa or 1.05 kg cm^{-2}), following standardized bioassay protocols [24]. Each experimental unit (one tray with 25 larvae or 10 adults) received 1000 µL of the corresponding treatment. For fungal treatments, conidial suspensions were adjusted to 1×10^8 conidia mL^{-1}, giving a nominal dose of 1×10^8 conidia per tray (arithmetic equivalent: 4×10^6 conidia $larva^{-1}$ or 1×10^7 conidia $adult^{-1}$). These values refer to the applied dose per tray and not to measured deposition on individual insects. Diatomin® was applied as a 10% (*w*/*v*) aqueous suspension (100 mg product mL^{-1}), delivering 100 mg per tray (4 mg $larva^{-1}$ or 10 mg $adult^{-1}$). GallinaTox® was prepared by dispersing 2 g in 1 L water (2 mg product mL^{-1}; 0.06 mg chlorpyrifos ethyl mL^{-1}), resulting in a nominal dose of 2 mg product and 60 µg chlorpyrifos ethyl per tray (0.08 mg product and 2.4 µg chlorpyrifos ethyl $larva^{-1}$; 0.2 mg product and 6 µg chlorpyrifos ethyl $adult^{-1}$). The negative control was sterile distilled water with 0.01% (*v*/*v*) Tween 80. Before each treatment, the spray tower reservoir was cleaned with 1.2 mL of 70% ethanol, rinsed twice with 1.2 mL of 0.01% TRITON™ X-100 (Merck, Darmstadt, Germany), and air-dried. Trays were kept at 24 ± 2 °C, 70 ± 5% RH, and a 12 h light/12 h dark photoperiod, with mortality recorded over 20 days.

2.6. Evaluation of Insecticidal Activity

Mortality was recorded at 0.4 days post-application (10 h after spraying) and subsequently every 12 h (08:00 and 20:00 h) for the 20-day observation period. After application, all experimental units were maintained for the 20-day observation period

under the same controlled conditions used during rearing (24 ± 2 °C, 70 ± 5% RH, 12 h light/12 h dark photoperiod). Individuals were considered dead if unresponsive to stimulation with an entomological needle. Dead insects were transferred to humid chambers at 20 °C for 48 h to confirm fungal sporulation and determine cause of death [25]. Presence of mycelium and conidia on the body surface was considered evidence of fungal infection.

Corrected mortality was calculated using Abbott's formula [26] to account for natural mortality in the negative control:

$$MC\% = [(M_t - M_c)/(100 - M_c)] \times 100 \quad (1)$$

where
MC = corrected mortality (%)
M_t = mortality in treatment (%)
M_c = mortality in negative control (%)

Abbott's correction was applied time-by-time for each treatment and replicate, using the mortality of the negative control at the same observation time. When the negative control mortality was zero, the observed mortality was used without correction. When the negative control mortality was equal to or greater than the treatment mortality at a given time, corrected mortality was set to zero.

Lethal times (LT_{50} and LT_{80}) were estimated by Probit analysis [27] on Abbott-corrected mortality data. The Probit model was fitted using a log-logistic link function:

$$Y = a + b\ ln(t) \quad (2)$$

where
Y = Probit of corrected mortality
a = Intercept
b = Slope of regression line
t = Time (days)

In the Probit analysis, insects that remained alive at the end of the 20-day observation period were treated as censored observations.

The average specific mortality rate *k* (day^{-1}) was calculated over the 20-day observation period based on the final Abbott-corrected cumulative mortality (*MC%*) using the formula [28]:

$$k = -ln(1 - MC)/20 \quad (3)$$

where
k = Average specific mortality rate (day^{-1})
MC% = Final Abbott-corrected cumulative mortality
20 = Observation period (days)

Additionally, survival curves were fitted to a Weibull model to obtain the shape (ρ) and scale (λ) parameters, which describe the progression of mortality over time.

Dead insects from fungal treatments were transferred to humid chambers for qualitative confirmation of fungal infection. Cadavers were examined for external mycelial growth and sporulation characteristic of the inoculated fungi. All cadavers obtained from treatments with the native *B. bassiana* strain MA-Bb1, commercial *B. bassiana*, and commercial *M. anisopliae*, exhibited characteristic fungal outgrowth during incubation in humid chambers, confirming the presence of the inoculated entomopathogens. Consequently, all mortality observed in fungal treatments was considered pathogen associated, and no cases were recorded as unconfirmed fungal infection.

2.7. Statistical Analysis

Mortality and survival data were analyzed using the tray as the experimental unit, with individual insects treated as subsamples within each tray (see Section 2.4 for sample sizes). Three trays were established per treatment and developmental stage in each temporal run, and the experiment was repeated in two independent temporal runs conducted under identical environmental conditions. The two temporal runs were combined using a mixed-effects model with temporal run as a random factor and treatment, developmental stage, and their interaction as fixed factors. This approach accounts for the nested structure of the data (insects within trays, trays within runs) and avoids pseudoreplication. When significant treatment effects were detected, means were compared using Tukey's HSD test at $p < 0.05$.

Survival probabilities were estimated using the Kaplan–Meier method. To account for the nested clustering structure of the data, differences among treatments and developmental stages were evaluated using a Cox proportional hazards model with shared frailty, with tray and run as random effects. The frailty variance was highly significant for all developmental stages (L_1: $\chi^2 = 663.7$, $p = 3.6 \times 10^{-140}$; L_2: $\chi^2 = 547.0$, $p = 5.3 \times 10^{-115}$; L_3: $\chi^2 = 515.7$, $p = 2.9 \times 10^{-108}$; adults: $\chi^2 = 317.8$, $p = 1.0 \times 10^{-65}$), confirming the need to account for clustering. The significance of treatment effects was maintained after accounting for clustering, indicating that the conclusions are robust. For adult bioassays, mortality responses between males and females were compared using Student's *t*-test for independent samples after confirming variance homogeneity with Levene's test.

Lethal times causing 50% (LT_{50}) and 80% (LT_{80}) mortality were estimated by Probit analysis, and their corresponding 95% confidence intervals (CI) were calculated using the delta method. Survival data were also fitted to a Weibull model to obtain the shape (ρ) and scale (λ) parameters, which describe the progression of mortality over time. The mean specific mortality rate (k) was calculated based on the final cumulative mortality "corrected using Abbott's formula" at 20 days; additionally, for each treatment and developmental stage combination, the associated standard deviation (SD) and 95% confidence intervals were determined.

All statistical analyses were performed using IBM SPSS Statistics v25 (IBM Corp., Armonk, NY, USA) and R v4.3.1. Survival analyses were conducted using the survival and survminer packages, whereas Probit and dose–response analyses were performed using the drc package. Weibull survival models were fitted using the flexsurv package [29].

3. Results

Throughout this section, three metrics are distinguished: (i) observed mortality, defined as the raw mortality recorded in each treatment, including natural mortality; (ii) Abbott-corrected mortality, defined as mortality after removing the natural mortality of the negative control; and (iii) uncorrected survival, defined as survival probabilities estimated by Kaplan–Meier without Abbott correction.

3.1. Cumulative Mortality (%) of M. nigripes

The evaluation of mortality induced by entomopathogenic fungi, physical treatments, and a chemical control against *M. nigripes* revealed statistically significant differences among treatments across all developmental stages (one-way ANOVA; L_1: $F_{5,12} = 48.73$, $p < 0.001$; L_2: $F_{5,12} = 42.15$, $p < 0.001$; L_3: $F_{5,12} = 36.82$, $p < 0.001$; Adults: $F_{5,12} = 31.44$, $p < 0.001$). A consistent hierarchy of efficacy was observed, favoring biological agents over physical treatments, although still inferior to the chemical control (Figure 2).

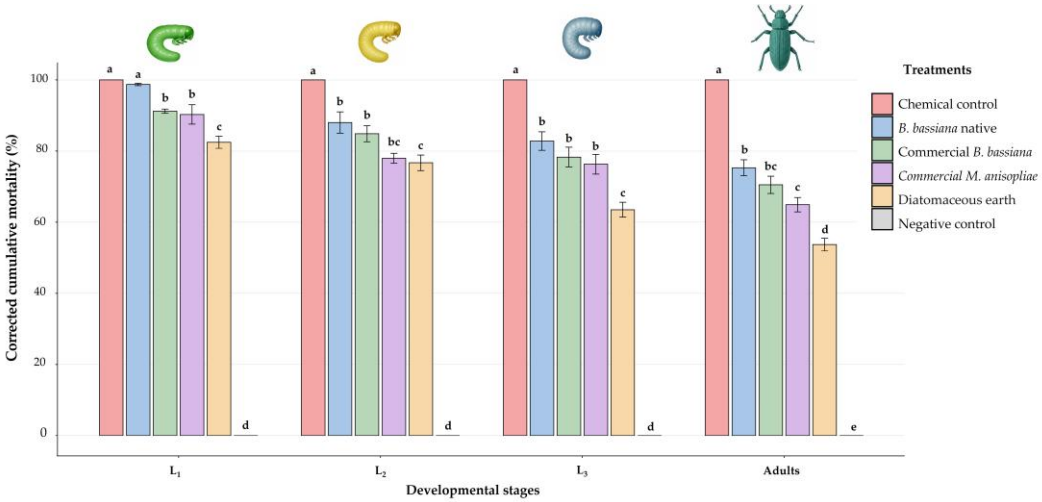


Figure 2. Abbott-corrected cumulative mortality (%) of *M. nigripes* exposed to different treatments in L_1, L_2, L_3 larvae and adults 20 days after application. Error bars represent standard deviation (SD). Larval assays were based on n = 75 insects per treatment per temporal run; two independent runs were pooled for analysis. Different letters indicate significant differences among treatments within each developmental stage (Tukey, $p < 0.05$).

In first-instar larvae (L_1), native *B. bassiana* (MA-Bb1) exhibited the highest relative cumulative mortality among biological treatments (99.4 ± 1.0%), followed by commercial *B. bassiana* (91.2 ± 1.0%) and commercial *M. anisopliae* (90.3 ± 4.7%). Diatomaceous earth produced an intermediate effect (85.8 ± 4.4%), while the negative control (sterile distilled water) showed zero corrected mortality after Abbott correction, although 15% observed mortality was recorded over the 20-day observation period, confirming the validity of the bioassay. Chemical treatment (GallinaTox® 500 mg) achieved maximum lethality (100%), consolidating its role as the positive control.

In second-instar larvae (L_2), the trend observed in L_1 persisted, although susceptibility decreased, likely due to greater physiological development and increased tolerance. Nevertheless, the native *B. bassiana* (MA-Bb1) maintained exceptionally high lethality (88.0 ± 5.2%), surpassing commercial *B. bassiana* (84.1 ± 4.9%) and commercial *M. anisopliae* (80.3 ± 4.9%), while diatomaceous earth recorded 76.7 ± 3.8%. The chemical control consistently exhibited the highest mortality (100%).

Third-instar larvae (L_3), prior to pupation, an additional increase in larval tolerance was evident, associated with more robust defense mechanisms, resulting in a general reduction in susceptibility compared to earlier instars. Nevertheless, entomopathogenic fungi maintained consistent lethal effects, outperforming physical treatments: the native strain (82.8 ± 4.5%), commercial *B. bassiana* (78.3 ± 4.8%), and commercial *M. anisopliae* (76.3 ± 4.8%) compared to 63.5 ± 3.6% for diatomaceous earth, while the chemical control again reached 100% mortality.

In adults, the general patterns observed in larval stages persisted, although with variations in differential responses to control agents. Native *B. bassiana* (MA-Bb1) exhibited the highest entomopathogenic effect in adults (75.3 ± 3.9%), followed by commercial *B. bassiana* (70.5 ± 4.2%) and commercial *M. anisopliae* (64.9 ± 3.5%), surpassing diatomaceous earth (53.7 ± 3.1%).

These results suggest an underexplored biological potential of the native strain against soil-dwelling coleopterans. The chemical treatment once again maintained maximum lethality (100%). Taken together, the integrated data across larval instars and adults demonstrate that, although the efficacy of control agents decreases with the ontogenetic development of the pest, biological control—particularly through

entomopathogenic fungi—retains practical relevance within integrated and sustainable management schemes for maize agroecosystems in Huejotzingo, Puebla, Mexico.

3.2. Kaplan–Meier Survival Analysis

Survival analysis of L_1 larvae showed highly significant differences between treatments (log-rank $\chi^2 = 441.5$, $p < 0.0001$; Figure 3). Chemical control (GallinaTox® 500 mg) caused immediate mortality, with survival reduced to zero in the first observation interval (0.4 days). The native strain MA-Bb1 also showed rapid action, decreasing survival to 0.053 by day 2.4 and reaching virtually zero by day 14. Commercial strains showed intermediate patterns, with final values close to 0.08 on day 20. Diatomaceous earth showed the most gradual effect, reducing survival from 0.507 to 0.12 on day 20. Negative control maintained high survival (0.98 on day 2.4; 0.85 on day 20), confirming low background mortality under the experimental conditions.

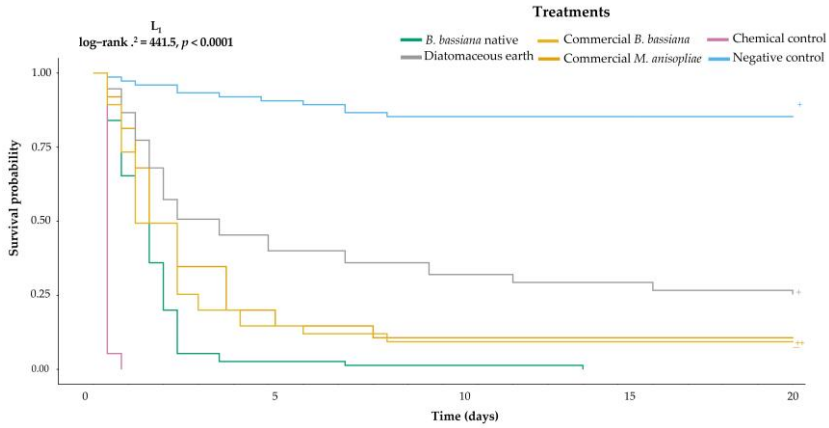


Figure 3. Kaplan–Meier survival curves (uncorrected data) for L_1 larvae of *M. nigripes* exposed to different treatments.

Significant differences among treatments were also recorded in L_2 larvae (log-rank test: $\chi^2 = 414.4$, $p < 0.0001$; n = 75 per treatment; Figure 4). As in L_1, the chemical control eliminated all larvae by 0.4 days post-application. The native strain showed greatest survival reduction, decreasing from 0.10 at day 20, followed by commercial *B. bassiana* from 0.32 to 0.14 and commercial *M. anisopliae* from 0.21 to 0.17. Diatomaceous earth reduced survival from 0.20 at day 20. The negative control again showed minimal mortality.

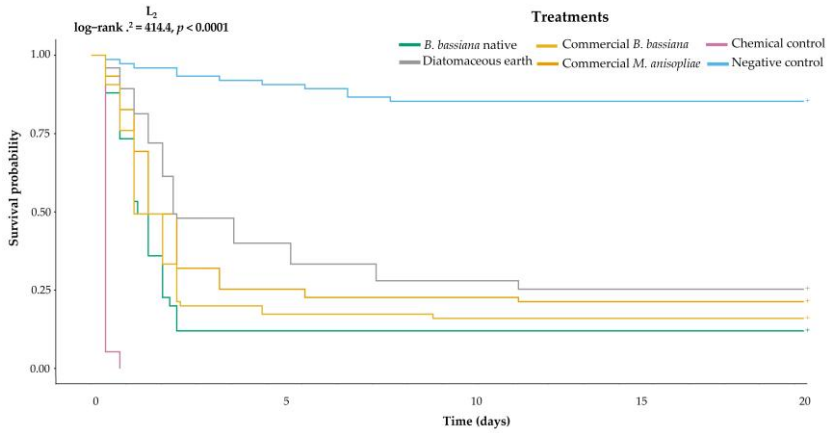


Figure 4. Kaplan–Meier survival curves (uncorrected data) for L_2 larvae of *M. nigripes* exposed to different treatments.

The survival curves of L3 larvae differed significantly among treatments (log-rank test: $\chi^2 = 405.2$, $p < 0.0001$; n = 75 per treatment; Figure 5). The chemical control caused complete mortality within 0.4 days. Native *B. bassiana* (MA-Bb1) produced the lowest final survival (from 0.24 at day 2.4 to 0.15 at day 20), followed by commercial *B. bassiana* (from 0.43 to 0.18) and commercial *M. anisopliae* (from 0.35 to 0.20). Diatomaceous earth was the least effective treatment, with survival decreasing from 0.55 at day 2.4 to 0.31 at day 20. As in the other larval stages, negative control-maintained survival close to 0.85 at day 20 (0.98 at day 2.4).

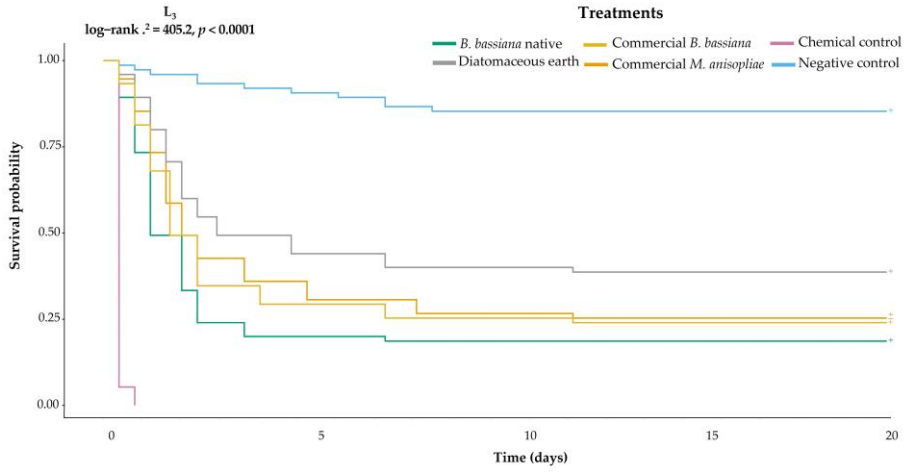


Figure 5. Kaplan–Meier survival curves (uncorrected data) for L3 larvae of *M. nigripes* exposed to different treatments.

In adults, survival curves differed significantly between treatments (log-rank test: $\chi^2 = 308.7$, $p < 0.0001$; Figure 6). Chemical control again caused 100% mortality within the first observation interval (0.4 days). The native strain (MA-Bb1) showed the greatest reduction in survival (0.21 at day 20), while commercial *B. bassiana* reached a final survival of 0.32 (from 0.40) and commercial *M. anisopliae* of 0.38 (from 0.43). Diatomaceous earth was the least effective, with survival declining to 0.50 by day 20. The negative control maintained high survival (0.83 at day 20).

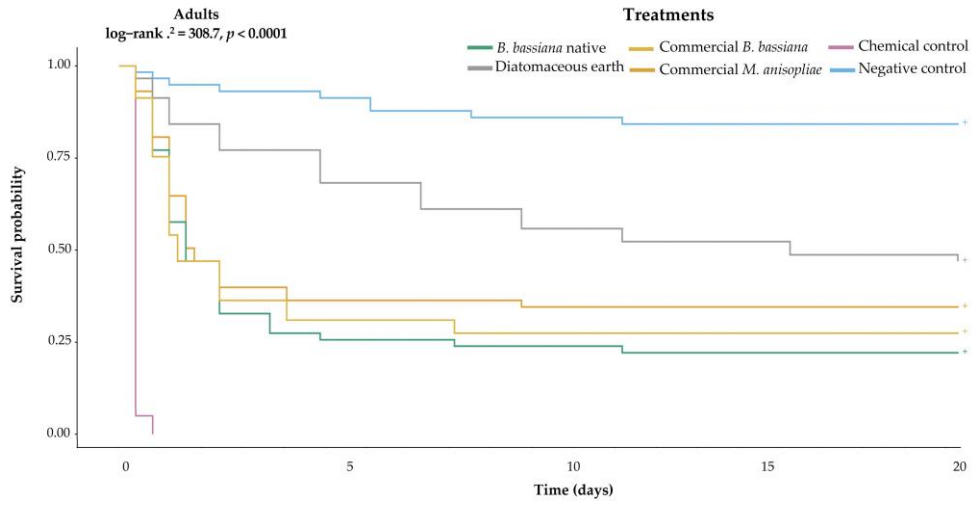


Figure 6. Kaplan–Meier survival curves (uncorrected data) for adults of *M. nigripes* exposed to different treatments.

The uncorrected survival values in Figure 6 (0.21 at day 20 for native *B. bassiana*) correspond to 79.0% observed mortality. After Abbott correction using the negative control (15% observed mortality), the corrected mortality was 75.3%, consistent with the value reported in Figure 2. The difference between observed and corrected mortality reflects the natural mortality recorded in the negative control.

3.3. Lethal Times (LT_{50} and LT_{80})

Probit analysis revealed significant differences in LT_{50} and LT_{80} values among treatments and developmental stages (Table 2). Native *B. bassiana* (MA-Bb1) exhibited the shortest lethal times in L_1 and L_3 and the highest specific mortality rates in all stages, confirming its higher virulence compared with commercial strains and diatomaceous earth.

For first-instar larvae (L_1), the native strain reached LT_{50} in 1.1 days and LT_{80} in 2.0 days, whereas commercial *M. anisopliae* required 1.2 and 3.0 days and commercial *B. bassiana* 1.6 and 3.8 days, respectively. In L_2, the native strain recorded LT_{50} and LT_{80} of 1.3 and 2.2 days, respectively. Commercial *M. anisopliae* showed LT_{50} and LT_{80} of 1.2 and 2.5 days, while commercial *B. bassiana* showed LT_{50} and LT_{80} of 1.6 and 2.45 days.

Table 2. Lethal times (LT_{50} and LT_{80}) in days for developmental stages of *M. nigripes* exposed to different treatments, estimated by Probit analysis on Abbott-corrected mortality data.

Treatment	Developmental Stages	LT_{50} (Days)	SE	95% CI	LT_{80} (Days)	SE	95% CI
Native *B. bassiana* (MA-Bb1)	L_1	1.1	0.08	0.9–1.4	2.0	0.15	1.5–2.7
	L_2	1.3	0.09	1.0–1.7	2.2	0.18	1.6–3.0
	L_3	1.2	0.09	0.9–1.6	2.34	0.19	1.76–3.12
	Adults	1.6	0.11	1.2–2.1	>20	—	—
Commercial *B. bassiana*	L_1	1.6	0.11	1.2–2.1	3.8	0.33	2.7–5.4
	L_2	1.6	0.11	1.2–2.1	2.45	0.19	1.84–3.27
	L_3	2.0	0.14	1.5–2.7	>20	—	—
	Adults	1.8	0.13	1.4–2.3	>20	—	—
Commercial *M. anisopliae*	L_1	1.2	0.09	0.9–1.6	3.0	0.24	2.2–4.1
	L_2	1.2	0.09	0.9–1.6	2.5	0.19	1.9–3.3
	L_3	1.7	0.12	1.3–2.2	>20	—	—
	Adults	1.4	0.10	1.1–1.8	>20	—	—
Diatomaceous earth	L_1	2.4	0.19	1.8–3.2	13.8	1.43	9.5–20.0
	L_2	2.3	0.18	1.7–3.1	19.6	2.14	13.0–29.6
	L_3	2.9	0.24	2.1–4.0	>20	—	—
	Adults	>20	—	—	>20	—	—
Chemical control	All stages	<0.4	—	—	<0.4	—	—
Negative control	All stages	>20	—	—	>20	—	—

Note: LT_{50} and LT_{80} were estimated by Probit analysis on Abbott-corrected mortality data, with insects alive at the end of the 20-day period treated as censored observations. Standard errors (SE) and 95% confidence intervals (CI), calculated using the delta method, are provided for each estimate. Goodness-of-fit was assessed using Pearson's χ^2 statistic, and all models showed adequate fit ($p > 0.05$). Values > 20 indicate that mortality did not reach the specified threshold during the 20-day observation period. Values < 0.4 indicate complete mortality before the first observation interval and therefore do not represent fitted estimates. Chemical control produced complete mortality before the first assessment interval (0.4 days), preventing estimation of finite LT values.

In L_3, LT_{50} increased to 1.2, 1.7, and 2.0 days for the native strain, commercial *M. anisopliae*, and commercial *B. bassiana*, respectively, with LT_{80} values of 2.34 days for the

native strain and >20 days for the commercial strains. In adults, the native strain showed an LT_{50} of 1.6 days, while the commercial strains reached it at 1.4 (*M. anisopliae*) and 1.8 days (*B. bassiana*); LT_{80} was >20 days for all biological treatments in adults.

It is important to note that the relative ranking of commercial *B. bassiana* and commercial *M. anisopliae* differed between cumulative mortality and lethal times: *B. bassiana* reached higher final cumulative mortality (Figure 2), whereas *M. anisopliae* acted faster (lower LT_{50}; Table 2). This pattern indicates that these two parameters capture different aspects of virulence—speed of action versus final efficacy—and should not be interpreted as contradictory. For the chemical control, the value '<0.4 days' should not be interpreted as a fitted LT estimate. It indicates that complete mortality occurred before the first observation interval (0.4 days), and therefore no finite LT_{50} or LT_{80} could be estimated by Probit analysis.

Diatomaceous earth exhibited the longest lethal times among biological treatments, with LT_{50} of 2.4 (L_1), 2.3 (L_2), 2.9 (L_3), and >20 days (adults); its LT_{80} was 13.8 days in L_1 and 19.6 days in L_2, but was not reached within the 20-day observation window in L_3 and adults.

3.4. Specific Mortality Rate

The average specific mortality rate (k) was calculated over the 20-day observation period from the Abbott-corrected cumulative mortality (*MC%*). In first-instar larvae (L_1), the native strain exhibited the highest rate (0.256 ± 0.012 day^{-1}), followed by commercial *B. bassiana* (0.121 ± 0.006 day^{-1}) and commercial *M. anisopliae* (0.116 ± 0.006 day^{-1}). Diatomaceous earth recorded a lower rate (0.097 ± 0.005 day^{-1}).

In L_2, the native strain maintained the highest rate (0.106 ± 0.006 day^{-1}), followed by commercial *B. bassiana* (0.092 ± 0.005 day^{-1}), commercial *M. anisopliae* (0.082 ± 0.005 day^{-1}), and diatomaceous earth (0.073 ± 0.004 day^{-1}). For L_3, a general decrease in k was observed across treatments, reflecting reduced susceptibility associated with ontogenetic development; nevertheless, the native strain (0.088 ± 0.005 day^{-1}) maintained higher values than the commercial strains (0.076 ± 0.004 day^{-1} for commercial *B. bassiana* and 0.072 ± 0.004 day^{-1} for commercial *M. anisopliae*).

In adults, the native strain (0.070 ± 0.004 day^{-1}) continued to show the highest rate among biological treatments, surpassing commercial *B. bassiana* (0.061 ± 0.003 day^{-1}) and commercial *M. anisopliae* (0.052 ± 0.003 day^{-1}). Diatomaceous earth exhibited the lowest rates across all stages (0.039–0.097 day^{-1}), consistent with its gradual physical mechanism of action.

The k of the chemical control could not be estimated because survival reached zero within the first observation interval; its immediate action is expressed as $LT_{50} < 0.4$ days. The negative control exhibited a specific mortality rate of $k = 0.000$ day^{-1} after Abbott correction, although 15% observed mortality was recorded over the 20-day period. This background mortality was removed from the treatment mortality estimates using Abbott's correction. Therefore, the corrected cumulative mortality values (Figure 2) and the lethal times (Table 2) are expressed net of natural mortality. In contrast, the survival curves (Figures 3–6) represent the uncorrected survival of each treatment. The lethal times (LT_{50} and LT_{80}; Table 2) were estimated by Probit analysis on Abbott-corrected mortality data, whereas the specific mortality rate (k; Table 3) was calculated empirically from the final Abbott-corrected cumulative mortality over 20 days. The Weibull parameters (ρ and λ) are also reported in Table 3 for descriptive modeling of the survival curves.

Table 3. Specific mortality rate (k, days^{-1}) and Weibull parameters (ρ, λ) for developmental stages of *M. nigripes* exposed to different treatments.

Treatment	Developmental Stage	*MC* (%)	*k* (day^{-1})	SD	95% CI	ρ	λ
Native *B. bassiana* (MA-Bb1)	L_1	99.4	0.256 a	0.012	0.232–0.280	1.82	1.28
	L_2	88.0	0.106 a	0.006	0.094–0.118	1.65	1.55
	L_3	82.8	0.088 a	0.005	0.078–0.098	1.58	1.45
	Adults	75.3	0.070 a	0.004	0.062–0.078	1.42	2.05
Commercial *B. bassiana*	L_1	91.2	0.121 b	0.006	0.110–0.134	1.58	2.05
	L_2	84.1	0.092 b	0.005	0.082–0.102	1.52	2.10
	L_3	78.3	0.076 b	0.004	0.068–0.084	1.45	2.65
	Adults	70.5	0.061 b	0.003	0.055–0.067	1.38	2.45
Commercial *M. anisopliae*	L_1	90.3	0.116 b	0.006	0.105–0.129	1.72	1.52
	L_2	80.3	0.082 b	0.005	0.072–0.092	1.62	1.48
	L_3	76.3	0.072 b	0.004	0.064–0.080	1.48	2.20
	Adults	64.9	0.052 b	0.003	0.046–0.058	1.42	1.85
Diatomaceous earth	L_1	85.8	0.097 c	0.005	0.087–0.107	1.38	3.28
	L_2	76.7	0.073 c	0.004	0.065–0.081	1.35	3.15
	L_3	63.5	0.051 c	0.003	0.045–0.057	1.32	4.05
	Adults	53.7	0.039 c	0.002	0.035–0.043	1.25	8.50
Chemical control	All stages	100.0	—	—	—	—	—
Negative control	All stages	0.0	0.000 d	0.000	0.000–0.000	—	—

Note: The specific mortality rate (k) was calculated as the average daily mortality rate over the 20-day observation period: $k = -\ln(1 - MC)/20$, where *MC%* is the final Abbott-corrected cumulative mortality. The parameters ρ (shape) and λ (scale, in days) were obtained from fitting the Weibull survival model S(t) = exp [−(*t*/λ)^ρ] to the complete survival curves. Standard deviation (SD) and 95% confidence intervals (CI) are provided for each k estimate. Different letters indicate significant differences among treatments within each developmental stage (ANOVA followed by Tukey's test, $p < 0.05$). The chemical control caused complete mortality before the first assessment interval (0.4 days), preventing the estimation of a finite k value. The negative control showed zero corrected mortality ($k = 0$).

Overall, the results of the specific mortality rate corroborate the efficacy hierarchy observed in lethal times (LT_{50} and LT_{80}), highlighting native *B. bassiana* MA-Bb1 as the biological agent with the highest specific mortality rate and the most consistent performance across all developmental stages of *M. nigripes*.

3.5. Differential Susceptibility Between Sexes

The evaluation of corrected cumulative mortality in adult *M. nigripes* revealed sex-dependent differences in susceptibility among treatments (Figure 7). Native *B. bassiana* (MA-Bb1) produced significantly higher mortality in males (79.25 ± 3.9%) than in females (71.35 ± 3.9%) (t = 3.51, df = 10, p = 0.005). Likewise, diatomaceous earth caused significantly greater mortality in males (57.60 ± 3.1%) than in females (49.80 ± 3.1%) (t = 4.36, df = 10, p = 0.001). In contrast, commercial *B. bassiana* (72.50 ± 4.2% vs. 68.60 ± 4.2%; t = 1.61, df = 10, p = 0.139) and commercial *M. anisopliae* (66.90 ± 3.5% vs. 63.00 ± 3.5%; t = 1.93, df = 10, p = 0.083) did not show significant sex-related differences.

The chemical control (GallinaTox® 500 mg) caused complete mortality (100%) in both sexes, resulting in no detectable sex-related differences (t = 0.00, df = 10, p = 1.000). Similarly, no mortality was recorded in the negative control (0% in both sexes; t = 0.00, df = 10, p = 1.000). Across the biological and physical treatments, males consistently exhibited higher mortality than females, with differences ranging from 3.9 to 7.9 percentage points. Significant sex related differences were observed for the native *B. bassiana* (MA-Bb1)

isolate and diatomaceous earth, indicating greater male susceptibility, whereas commercial *B. bassiana* and commercial *M. anisopliae* produced comparable mortality responses between sexes. These differences may reflect sex specific physiological and morphological traits, including body size, cuticular permeability, and variation in energy allocation to reproductive and immune functions.

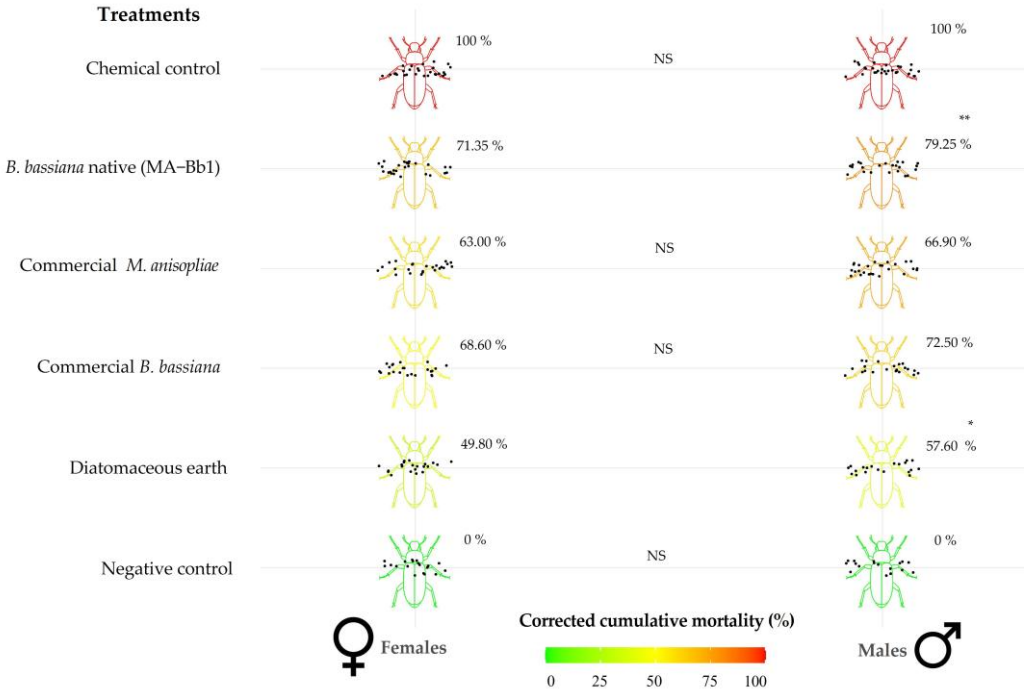


Figure 7. Abbott-corrected cumulative mortality (%) of adult male and female *M. nigripes* 20 days after exposure to different treatments. n = 30 males; n = 30 females per treatment. Error bars represent standard deviation (SD). * = indicate significant differences between sexes (Student's *t*-test; * $p < 0.05$; ** $p < 0.01$; NS = not significant).

The native strain *B. bassiana* MA-Bb1 again induced the highest mortality in both sexes, reaching 79.3% in males and 71.4% in females. Mortality in males was approximately 11.1% greater than in females, indicating a marked sex-dependent response and highlighting the high virulence of the native isolate against *M. nigripes*.

4. Discussion

Although García et al. [3] previously examined agroecological management strategies for *M. nigripes* in maize, their study focused on cultural and agroecological practices rather than on the direct evaluation of entomopathogenic fungi. To our knowledge, this is the first study to evaluate the laboratory pathogenicity of a native *B. bassiana* isolate (MA-Bb1) against all developmental stages and both sexes of *M. nigripes*, providing baseline data for future biological control programs. Therefore, the present study evaluated the laboratory efficacy of the native *B. bassiana* (MA-Bb1) across different developmental stages and sexes of *M. nigripes*.

The results obtained in this study indicate that the entomopathogenic fungi *B. bassiana* and *M. anisopliae* may have potential as biological control agents against *M. nigripes*. However, these findings are based on laboratory conditions, and their efficacy under field conditions remains to be evaluated.

Although their efficacy was lower than that of chemical control, it was significantly higher than the physical treatment with diatomaceous earth and the negative control. Native strain (MA-Bb1) consistently stood out as the biological agent with the fastest

action and highest lethality across all developmental stages, surpassing commercial strains of commercial *B. bassiana* and commercial *M. anisopliae*. The rapid action of native *B. bassiana* (MA-Bb1) was notable when compared with previous work on the same genus: in *Macrodactylus infuscatus*, enzymatic extracts of *B. bassiana* (rather than whole conidia) caused 75% mortality with a mean time to death of 7 days [30], whereas the native strain reached LT_{50} of 1.1–1.3 days in *M. nigripes* larvae and 1.6 days in adults, indicating that the application of whole infective conidia of a locally adapted strain considerably accelerates mortality. This behavior may suggest local adaptation of the native strain to environmental conditions and host physiology, although this hypothesis was not directly tested in the present study. Similar patterns have been documented in other systems, where native isolates of entomopathogenic fungi exhibit greater virulence than foreign strains against pests of the same ecological origin [31].

The higher virulence of the native strain MA-Bb1 against *M. nigripes* was associated with shorter lethal times (LT_{50} of 1.1 days in L_1), higher specific mortality rates, and a faster reduction in survival in Kaplan–Meier analyses. These findings are consistent with Nájera-Rincón et al. [32], who reported that native strains of *B. bassiana* and *M. anisopliae* isolated in Mexico showed virulence against *Phyllophaga crinita* larvae (Coleoptera: Melolonthidae). The hierarchy of efficacy observed in cumulative mortality (native *B. bassiana* > commercial *B. bassiana* > commercial *M. anisopliae* > diatomaceous earth) was maintained in all larval and adult stages, including lethal times and mortality rate (Tables 2 and 3). The effectiveness of *B. bassiana* against corn beetles is further supported by field cage studies in which *B. bassiana* suppressed populations of the adult western corn rootworm, *Diabrotica virgifera* [33].

The progressive decrease in the susceptibility of *M. nigripes* to entomopathogenic fungi with advancing developmental stages (L_1 > L_2 > L_3 > Adults) is one of the most relevant patterns in this study. First-instar larvae (L_1) exhibited the highest mortality (99.4% with the native strain), while adults showed the lowest susceptibility (75.3%). This pattern has been documented in multiple insect–fungus systems, where early instars are more vulnerable due to a less sclerotized cuticle and an underdeveloped immune system [34]. The hardening of the cuticle during larval ontogeny and the accumulation of defense mechanisms such as melanization and encapsulation represent physical and physiological barriers that hinder fungal penetration and establishment [35]. The greater tolerance observed in third-instar larvae (L_3) and adults suggests that biological control with entomopathogenic fungi may be more effective when applied during the early stages of the pest's life cycle, a finding with important practical implications for management strategies. Although larvae of other scarab beetle species can be remarkably resistant to *Beauveria* species, with susceptibility largely dependent on the developmental stage [36], the high cumulative mortality recorded in this study for the early instars of *M. nigripes* (99.4%) is consistent with the scientific literature, highlighting that the early larval stages are the most susceptible to fungal infection [37]. This result also reflects the use of a high dose of conidia (1×10^8 conidia/mL) applied via a spray tower under controlled humidity and temperature conditions that favor germination. In comparison, LT_{50} values of 2.9 to 3.0 days have been reported for *M. anisopliae* against third-instar larvae *of Phyllophaga crinita* and *Anomala flavipennis* [38], higher than the 1.2 to 1.7 days recorded in the present study for *M. nigripes* in its L_3 stage. The greater susceptibility of adult males compared to females is a relevant finding. This difference may reflect a combination of morphological and physiological factors, including smaller body size in males, which facilitates cuticular penetration, and a differential energy investment in reproduction, which may compromise immune responses [39]. However, sexual dimorphism in survival after *B. bassiana* infection in *Drosophila melanogaster* has been shown to depend intrinsically on core immune signaling pathways rather than solely on body size [40], indicating that sex

specific innate immune competence is a plausible driver of the differential susceptibility observed in *M. nigripes*. Susceptibility to *Beauveria* spp. in beetles is also strongly stage-dependent [36]. Recent research in Mexico has evaluated the use of entomopathogenic fungi for the management of *M. nigripes* [3], supporting the relevance of using these fungi for pest control. Chemical control, in contrast, showed no sex-based differences, confirming its non-specific action. From an integrated pest management perspective, the greater susceptibility of males could be exploited to reduce the reproductive population of this pest in maize crops

The intermediate efficacy of diatomaceous earth (85.8% in L_1, 76.7% in L_2, 63.5% in L_3, and 53.7% in adults) confirms its potential as a complementary tool within integrated pest management programs, although its effect is more gradual and less consistent than that of entomopathogenic fungi. Its mechanism of action, based on cuticle abrasion and insect desiccation [41], explains its slower action (LT_{50} of 2.3–2.9 days in larvae and >20 days in adults) compared to fungi, which combine physical penetration with toxic effects mediated by secondary metabolites such as beauvericin and destruxins [42]. In this context, combining diatomaceous earth with entomopathogenic fungi could improve control efficacy, since the cuticular abrasion induced by diatomaceous earth can facilitate conidia penetration and shorten the infection time. The efficacy of diatomaceous earth against other insect pests has been reported [43] and in combination with *B. bassiana* [44].

Chemical control (GallinaTox® 500 mg) showed the highest efficacy (100% mortality at all stages) and the fastest action (LT_{50} < 0.4 days), confirming its effectiveness as a positive control. However, the extensive use of chemical insecticides in maize cultivation raises environmental and public health concerns, as well as the risk of selecting for resistant populations [4]. Approximately 3000 tons of active ingredients are applied annually in Mexico to control the fall armyworm (*Spodoptera frugiperda*) [45], representing intensive pesticide use that has contributed to the elimination of natural enemies, widespread environmental contamination, and the emergence of insecticide resistance. This scenario underscores the urgent need for sustainable alternatives to chemical control. Furthermore, *Macrodactylus* spp. is among the most abundant insect pests documented in Mexican maize agroecosystems, further highlighting the importance of developing ecologically sound management strategies [46].

The observed laboratory pathogenicity suggests that native strain MA-Bb1 may have potential as a component of integrated pest management programs, although its effectiveness under field conditions remains to be evaluated.

5. Conclusions

Under laboratory conditions, the native *B. bassiana* MA-Bb1 showed the highest pathogenicity against all developmental stages of *M. nigripes*, outperforming the commercial formulations of *B. bassiana* and *M. anisopliae*, as well as diatomaceous earth. These results should be interpreted as evidence of laboratory pathogenicity and as a basis for future field evaluation

Abbott-corrected cumulative mortality reached 99.4%, 88.0%, 82.8%, and 75.3% for L_1, L_2, L_3 larvae and adults, respectively, 20 days after application. The native strain also produced the lowest survival probabilities and the shortest lethal times, indicating rapid and sustained pathogenic activity across the evaluated life stages.

The performance observed for strain MA-Bb1 suggests that native entomopathogenic fungal isolates may represent promising candidates for the biological control of *M. nigripes*, although their efficacy under field conditions remains to be evaluated.

Although the mechanisms underlying its enhanced efficacy were not directly evaluated, factors such as adaptation to local environmental conditions may have contributed to its performance relative to the commercial strains.

Because this study was conducted under controlled laboratory conditions using a single conidial concentration, the results should be interpreted as evidence of laboratory pathogenicity and not as direct validation of field efficacy. Nevertheless, the findings identify to strain MA-Bb1 as a promising candidate for the biological control of *M. nigripes* and provide a foundation for future studies aimed at evaluating dose–response relationships, persistence, formulation development, compatibility with integrated pest management strategies, and efficacy under field conditions.

This study also contributes novel information on the susceptibility of different developmental stages and sexes of *M. nigripes* to entomopathogenic fungi, providing baseline knowledge to support the future development of biologically based management strategies for this economically important maize pest.

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