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Original article

Isolation, molecular identification, and biocontrol efficacy of potential indigenous fungi against the *Pentalonia nigronervosa* in selected provinces of Eastern Visayas, Philippines

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PRE-PROOF PUBLICATION

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Abstract

Abaca bunchy top virus (ABTV) is a major constraint to abaca (*Musa textilis* Nee) production in the Philippines. As the disease is transmitted by the banana aphid, *Pentalonia nigronervosa*, effective vector management is critical for limiting virus transmission and disease spread. Although chemical insecticides are widely used, their prolonged application may lead to insecticide resistance, environmental contamination, and pest resurgence. This study evaluated indigenous fungi as potential biological control agents against the banana aphid. Field surveys were conducted in selected abaca-growing areas of Biliran, Leyte, and Southern Leyte, Philippines, where aphids were collected from symptomatic plants and examined for natural fungal infection. Fungal isolates recovered from infected aphid cadavers were identified using morphological and cultural characteristics and internal transcribed spacer rDNA sequence analysis. Molecular identification revealed a diverse assemblage of indigenous fungi, with *Fusarium* spp. being the predominant group recovered from aphid cadavers. Pathogenicity assays conducted under laboratory and screenhouse conditions demonstrated that all tested fungal isolates exhibited pathogenicity against the banana aphid, although their efficacy varied significantly between isolates. Among the indigenous isolates, *Aspergillus nomiae and Fusarium proliferatum,* showed the highest laboratory pathogenicity, each causing 86.67% mortality at 96 hours after treatment initiation, while *Purpureocillium lilacinum* caused 80.00% mortality. Under screenhouse conditions, *P. lilacinum* produced the highest corrected mortality among the indigenous isolates (36.61%) by Day 7, followed by *Fusarium* sp. (32.86%) and *F. proliferatum* (31.23%). These findings identified indigenous fungi, particularly *A. nomiae, F. proliferatum*, and *P. lilacinum,* as potential candidates for further evaluation as biological control agents against *P. nigronervosa* in selected provinces of Eastern Visayas, Philippines. However, further assessment of plant pathogenicity, biosafety, and effects on non-target organisms is necessary before these isolates can be considered for field application.

Keywords: abaca, aphids, Biliran, Bunchy Top Virus, entomopathogenic fungi

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Introduction

Abaca (*Musa textilis* Nee) is a fiber crop native to the Philippines and is widely recognized for producing one of the world's strongest natural fibers. Abaca is highly valued for its exceptionally strong fiber, approximately three times stronger than cotton and sisal, which supports its use in ropes, cordage, specialty papers, and other industrial applications (Armecin *et al*. 2014). It also provides an important source of pulp for specialty papers and other high-value products (Parac *et al*. 2020). The Philippines remains the world's leading producer and exporter of abaca fiber, supplying approximately 87% of the global market (PCAARRD 2025). Eastern Visayas is one of the country's major abaca-producing regions and consistently contributes substantially to national production (PSA 2026). Despite its economic importance, abaca production is continually threatened by natural disasters, insect pests, and plant diseases. Among the most destructive diseases is Abaca Bunchy Top Disease (ABTD), which is caused by the *Abaca bunchy top virus* (ABTV) (Furuya *et al*. 2006). The disease has caused substantial production losses in several abaca-growing regions of the Philippines, including Eastern Visayas (Amazona 2020). Initially confined to the Bicol Region, ABTV has gradually spread to other major abaca-producing areas throughout the country (Bajet and Magnaye 2002; Mati-om *et al*. 2024). The virus is transmitted by the banana aphid, *Pentalonia nigronervosa*, in a circulative, non-propagative manner during feeding (Nelson 2004). Aphids acquire the virus after feeding on infected plants and subsequently transmit it to healthy plants following a latent period (Watanabe *et al*. 2013). Infection may reduce fiber yield by 13–77%, making ABTD one of the most economically important diseases affecting abaca production in the Philippines (Patayon and Crisostomo 2021). In Eastern Visayas, aphid transmission has been reported to cause up to 100% disease severity in the susceptible abaca variety Inosa (Mati-om *et al*. 2022). Since no effective cure is currently available for ABTD, management primarily relies on the removal of infected plants and suppression of the aphid vector.

Chemical insecticides remain the most common approach for aphid management; however, their continuous use may result in insecticide resistance, environmental contamination, pest resurgence, and increased production costs, particularly for smallholder farmers cultivating abaca in upland areas. Biological control has therefore emerged as a sustainable alternative for aphid management. Entomopathogenic fungi (EPF) are among the most important microbial biological control agents because of their ability to infect and kill insect pests naturally. Their use has also been shown to be economically competitive with conventional insecticide-based control strategies

PRE-PROOF PUBLICATION

PRE-PROOF PUBLICATION JOURNAL OF PLANT PROTECTION RESEARCH

(Bale *et al*. 2007). Naturally occurring aphid pathogens belonging to the order Entomophthorales can cause epizootics under favorable environmental conditions (Volkl *et al*. 2007). However, these fungi are difficult to culture for large-scale production. Consequently, commercially available mycoinsecticides are predominantly based on species in the order Hypocreales, including *Beauveria*, *Metarhizium*, *Cordyceps* (*Isaria*), and *Lecanicillium*, owing to their ease of cultivation and formulation (Reddy 2020; Jasman *et al.* 2026).

Although numerous EPF have been reported against aphids and other insect pests, information on indigenous fungal species naturally associated with *P. nigronervosa* remains scarce. This knowledge gap is particularly evident in Eastern Visayas, where bunchy top disease continues to threaten one of the country's major abaca-producing regions. Identifying indigenous entomopathogenic fungi is important because locally adapted isolates are often better suited to environmental conditions and may provide more effective and sustainable biological control. Furthermore, molecular identification combined with pathogenicity evaluation provides a robust approach for selecting promising fungal candidates for biological control. Therefore, this study aimed to isolate, characterize, and molecularly identify indigenous fungi associated with *P. nigronervosa* collected from selected abaca-growing areas of Biliran, Leyte, and Southern Leyte, Philippines, and to evaluate their pathogenicity under laboratory and screenhouse conditions. Based on available information this is the first comprehensive study in Eastern Visayas, Philippines, to integrate field surveys, morpho-cultural characterization, ITS-based molecular identification, phylogenetic analysis, laboratory bioassays, and screenhouse evaluation of indigenous EPF associated with the banana aphid, *P. nigronervosa*.

Materials and Methods

Survey, collection, and isolation of entomopathogenic fungi from abaca aphids

Prior to field sampling, formal letters were sent to the concerned municipalities, cities, and Provincial Fiber Officers to obtain permission and coordinate sampling activities. Surveys were conducted in selected abaca-growing areas in Biliran, Leyte, and Southern Leyte, Eastern Visayas, Philippines. Sampling sites included the municipalities of Naval, Almeria, and Caibiran in Biliran; Baybay City, Ormoc City, and Mahaplag in Leyte, and Sogod in Southern Leyte. Abaca plants exhibiting symptoms associated with ABTD were purposively selected for sampling. Aphid cadavers showing signs of fungal infection were processed for fungal isolation. Each cadaver was

PRE-PROOF PUBLICATION

PRE-PROOF PUBLICATION JOURNAL OF PLANT PROTECTION RESEARCH

surface sterilized following standard procedures and aseptically transferred onto potato dextrose agar using a sterile inoculation needle. Culture plates were sealed, labeled according to collection site, and incubated under controlled laboratory conditions. The plates were monitored daily for fungal growth, and emerging colonies were subcultured repeatedly to obtain pure isolates.

Morpho-cultural characteristics of entomopathogenic fungi

A 5 mm agar disc from 11 potential EPF was placed at the center of PDA plates and incubated for seven days. Cultural characteristics of the fungal isolates, including colony size, pigmentation, texture, margin and sporulation, were recorded during incubation. The agar block technique was subsequently used to facilitate detailed morphological characterization based on mycelial appearance, and spore characteristics, including type, size, and shape.

Molecular identification of fungal isolates

Pure cultures of the potential EPF isolates obtained from aphid cadavers were subjected to molecular identification through internal transcribed spacer (ITS) rDNA sequencing. Actively growing fungal mycelia were transferred into sterile 1 mL microtubes and submitted to Kinovett Scientific Solutions Co. for molecular analysis. Total genomic DNA was extracted and used as template for polymerase chain reaction (PCR) amplification targeting the ITS region. Amplification and sequencing were performed using universal fungal primers ITS5 (5′–GGA AGT AAA AGT CGT AAC AAG G–3′) and ITS4 (5′–TCC TCC GCT TAT TGA TAT GC–3′). Amplified products were verified and subjected to DNA sequencing. Generated sequences were compared with reference sequences available in the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST). Fungal identification was assigned based on sequence similarity, query coverage, and alignment scores. The closest matching reference sequences and their corresponding GenBank accession numbers were recorded for taxonomic identification. Taxonomic classification was determined at the genus or species level and used as the basis for phylogenetic analysis.

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PRE-PROOF PUBLICATION JOURNAL OF PLANT PROTECTION RESEARCH

Mass rearing of banana aphids

Banana aphids were mass reared using abaca stalks and potted abaca seedlings as host materials. Three-month-old potted abaca seedlings were artificially infested with aphids and enclosed with ventilated transparent plastic covers. Water was placed beneath the seedlings to maintain humidity and support aphid multiplication. Both systems were maintained to produce sufficient aphid populations for the experiments.

Pathogenicity of entomopathogenic fungi

Laboratory conditions

All materials used in the experiment were sterilized prior to use. Glassware was sterilized in a dry oven at 160°C for 3 h, while plastic Petri plates, brushes, and atomizers were exposed to ultraviolet light inside a laminar flow hood for 20 min. The working area was disinfected using 70% alcohol before and after all procedures. Healthy abaca leaf discs (3 cm diameter) were prepared and surface sterilized before use. All materials were handled aseptically to minimize contamination and maintain fungal culture integrity. Seven-day-old fungal cultures grown on agar media were used for preparation of conidial suspensions. Approximately 10 mL of sterile distilled water containing 0.01% Tween 80 was added to each plate, and conidia were gently scraped using a sterile wire loop. Suspensions were filtered through sterile nylon tulle to remove mycelial fragments. The conidial suspension was adjusted to 1×10^8 conidia mL^{-1} using a hemocytometer. This concentration was selected as the single screening dosage because concentrations within this range are commonly employed in laboratory bioassays to evaluate the pathogenicity and virulence of entomopathogenic fungi against insect pests, providing sufficient conidial deposition and facilitating comparison with previous studies (de Faria and Wraight 2007; Şahin and Yanar 2021). It was selected for comparative screening rather than as a standardized or optimal dose for all isolates since no preliminary dose-response assay was conducted. Fresh suspensions were prepared immediately prior to application. Pathogenicity was evaluated using the leaf disc assay. Sterile abaca leaf discs were placed on the Petri plates lined with moist cotton to maintain humidity. Ten adult aphids were transferred onto each leaf disc using a fine brush. The experiment was arranged in a Completely Randomized Design (CRD) with three replications per treatment and 10 aphids per replicate. Treatments consisted of 11 indigenous fungi, commercial EPF controls, negative control, and positive control. Applications were performed using a sterile hand atomizer. Sterile distilled water

PRE-PROOF PUBLICATION

PRE-PROOF PUBLICATION JOURNAL OF PLANT PROTECTION RESEARCH

containing 0.1% Tween 80 served as the negative control, while Brodan EC served as the positive control. Commercial isolates of *Beauveria bassiana* and *Metarhizium anisopliae* obtained from the Regional Crop Protection Center (RCPC) Eastern Visayas and *Lecanicillium tenuipes* from Project 2 were included as reference fungal treatments.

Screenhouse conditions

The laboratory pathogenicity assay was initially conducted using 11 potential EPF isolates prior to molecular identification. Subsequent ITS-based molecular identification revealed that some of these isolates belonged to the same fungal taxon (Table 3). To avoid redundant testing of isolates representing the same identified taxon, seven representative isolates were selected for the subsequent screenhouse experiment. Thus, the laboratory assay provided an initial assessment of the recovered isolates, whereas the screenhouse assay evaluated representative fungal taxa under semi-controlled conditions.

Three-month-old potted abaca plants of uniform age were grown in identical polyethylene bags (6 × 6 × 11 in.) and maintained under standardized watering and fertilization regimes to ensure homogeneous experimental conditions. Each plant was artificially infested with 20 adult abaca aphids and allowed to acclimatize for 24 h under screenhouse experiments. The experiment was arranged in a Completely Randomized Design (CRD) consisting of 12 treatments and three replications, as the experimental units were considered sufficiently homogeneous and no systematic environmental gradients were evident within the screenhouse. Treatments were comprised of seven potential fungal isolates, three reference entomopathogenic fungi, sterile distilled water as the negative control, and Brodan EC insecticide as the positive control. Fungal suspensions (1×10^8 conidia mL^{-1} containing 0.01% Tween 80) were applied using a hand atomizer. Aphid mortality was recorded daily for seven days, and dead aphids were incubated to assess mycosis. Fungal identity was confirmed by microscopic examination and re-isolation on potato dextrose agar to fulfill Koch's postulates. Temperature and relative humidity were monitored throughout the experiment.

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Data collection and statistical analysis

The percentage of infection was assessed under both laboratory and screenhouse conditions, whereas mortality (%) was recorded under laboratory conditions and corrected mortality under screenhouse conditions using Abbott's formula (Abbott 1925). Corrected mortality was calculated based on observed aphid mortality and not on infection percentage, with mortality in the untreated control used to account for background mortality. Infection percentage was analyzed separately as an indicator of fungal infection. Mortality was defined as the absence of movement or response to gentle stimulation, whereas infection was recorded only when fungal growth or sporulation characteristic of the inoculated isolate was observed on the cadaver following incubation. Thus, mortality and infection were treated as separate response variables, and aphids that died without visible external fungal growth were counted as mortality but not as confirmed infection. Laboratory observations were made 24, 48, 72, and 96 h after treatment initiation (HATI), while screenhouse observations were recorded daily for seven days after treatment application. Mortality was recorded as cumulative mortality at each observation time, with the total number of dead individuals determined based on all mortality occurring up to that time point. Individuals previously recorded as dead were retained in the cumulative mortality count and were not counted as new mortality during subsequent observations. The same experimental units were monitored throughout the observation period, with mortality assessed repeatedly at each designated observation time. Thus, successive mortality observations represented repeated measurements from the same experimental units over time.

Data were analyzed using Jeffrey's Amazing Statistics Program (JASP) version 0.19.3.0. Laboratory and screenhouse data including mortality (%) and infection (%) were analyzed separately using repeated-measures ANOVA, with treatment as the between-subject factor and observation time as the within-subject factor. The mortality and infection data were analyzed using their original, untransformed percentage values. Prior to interpretation of the ANOVA results, the assumptions of normality and homogeneity of variance were assessed using appropriate diagnostic procedures. The assumption of sphericity was assessed for the within-subject factor, and when sphericity was violated, Greenhouse–Geisser corrections were applied. Treatment means were compared using Tukey's honestly significant difference (HSD) test at $P < 0.05$. Pearson's correlation and multiple linear regression analyses were performed to examine the relationships

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PRE-PROOF PUBLICATION JOURNAL OF PLANT PROTECTION RESEARCH

and effects of temperature and relative humidity on corrected mortality and infection (%) in the screenhouse experiment.

Results

Collection of aphids and recovery of potential entomopathogenic fungi

Table 1 summarizes the distribution of the EPF isolates re-isolated from naturally infected *P. nigronervosa* collected in selected abaca-growing areas of Biliran, Leyte, and Southern Leyte. A total of 61 fungal isolates were recovered, from which 11 representative isolates were selected based on distinct morpho-cultural characteristics and frequency of occurrence to capture the diversity of fungi associated with infected aphid cadavers. The selected isolates represented distinct colony characteristics and sporulation patterns and were subsequently subjected to molecular identification and pathogenicity evaluation. Southern Leyte, particularly Brgy, Pancho Villa, and Sogod, yielded the highest numbers of representative isolates, followed by Biliran and Leyte. The recovery of these diverse fungal isolates demonstrated the occurrence of naturally associated EPF in abaca-growing areas of Eastern Visayas and provided the basis for subsequent morphological, molecular, and bioefficacy assessments.

Morphological characterization and preliminary identification of fungal isolates

The fungal isolates exhibited distinct colony morphology and growth characteristics on Potato Dextrose Agar (PDA), facilitating their preliminary identification (Figure 1; Table 2). Most isolates showed rapid mycelial growth and abundant sporulation within 3–7 days of incubation. Colony characteristics varied in color, texture, margin, and pigmentation among the isolates. EPF-2 exhibited the fastest growth and highest sporulation, producing white mycelia that later turned dark green due to abundant conidia formation. EPF-1 and EPF-3 to EPF-7 developed cottony to woolly colonies with white aerial mycelia that became pink to purple with age, whereas EPF-8, EPF-9, and EPF-11 formed powdery to granular colonies with yellow brown to olive-green pigmentation. EPF-10 was distinguished by its lilac to violet colonies and velvety to powdery texture. These morpho-cultural characteristics supported the differentiation of the fungal isolates prior to molecular identification.

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Molecular identification of fungal isolates from aphids in abaca-growing areas

Molecular identification based on internal transcribed spacer (ITS) sequencing was used for the preliminary identification of the fungal isolates recovered from banana aphids collected in Biliran, Leyte, and Southern Leyte (Table 3). Sequence analysis revealed high similarity (99.33–100%) and query coverage (99–100%) with reference sequences in the NCBI GenBank database. The isolates belonged to four genera, namely *Fusarium*, *Aspergillus*, *Trichoderma*, and *Purpureocillium* representing four families within the phylum Ascomycota. *Fusarium* was the predominant genus, with several isolates identified as *Fusarium proliferatum*, suggesting its widespread occurrence among aphid-associated fungi in the study area. The presented findings showed the diversity of fungi associated with abaca aphids and provided a basis for selecting potential EPF isolates for biological control evaluation.

Phylogenetic characterization of potential entomopathogenic fungal isolates

Molecular identification based on ITS rDNA sequencing confirmed the taxonomic identity of the fungal isolates recovered from abaca aphids (Figure 2; Table 3). Sequence similarity with reference sequences in the NCBI GenBank database ranged from 99.33% to 100%, supporting reliable species-level identification. The isolates were identified as *Fusarium proliferatum*, *Fusarium solani*, *Fusarium* sp., *Trichoderma hamatum*, *Aspergillus tamarii*, *Aspergillus nomiae*, and *Purpureocillium lilacinum*. Phylogenetic analyses further supported these identifications, with the isolates clustering with their corresponding reference sequences and receiving high bootstrap support, confirming their taxonomic placement. The ITS nucleotide sequences generated in this study have been deposited in GeneBank, and the corresponding accession numbers are provided in Figure 2.

Laboratory conditions of potential EPF against abaca aphids

Aphid infection and mortality increased progressively with exposure time, with significant differences between fungal isolates. At 96 h after treatment initiation (HATI), *M. anisopliae* produced the highest infection (86.67%) and complete mortality (100%). Among the indigenous isolates, *A. nomiae* and *F. proliferatum* were the most effective, causing 76.67% and 70.00% infection, respectively, and 86.67% mortality each. *P. lilacinum* and *T. hamatum* also showed high efficacy, with 70.00% infection and 80.00% mortality. In contrast, *F. solani* exhibited the lowest

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pathogenicity, with only 23.33% infection and 43.33% mortality, respectively. No fungal infection was observed in the water and insecticide controls, confirming that mycosis resulted from fungal inoculation (Fig. 4-5).

Although some fungal isolates exhibited relatively low infection during the early observation periods, mortality continued to increase throughout the evaluation period, suggesting slower infection processes and delayed pathogenic activity. Among the isolates, *A. nomiae*, *F. proliferatum*, *P. lilacinum*, and *T. hamatum* consistently produced higher aphid mortality and infection than the other tested isolates and performed comparably to the reference EPF, indicating their potential as biological control agents. Repeated measures ANOVA further showed that aphid infection and mortality were significantly affected by observation time, treatment, and their interaction (all $p < 0.001$), indicating that fungal pathogenicity increased with exposure period and varied among isolates (data not presented).

Relationship between fungal infection and aphid mortality under laboratory conditions

Pearson correlation analysis revealed a moderate positive correlation between fungal infection and aphid mortality under laboratory conditions ($r = 0.5296$, $p < 0.001$), indicating that mortality generally increased with increasing fungal infection (Fig. 5). Although fungal infection contributed significantly to aphid mortality, the moderate correlation suggests that mortality was also influenced by species-specific pathogenic mechanisms beyond visible mycosis.

Corrected mortality and percent infection of abaca aphids treated with different fungal isolates under screenhouse conditions

Corrected mortality of banana aphids increased progressively throughout the seven-day observation period across all fungal treatments (Fig. 6). Among the treatments, *M. anisopliae* was the most effective, achieving the highest corrected mortality (53.51%) on Day 7, followed by *P. lilacinum* (36.61%), *Fusarium* sp. (32.86%), and *F. proliferatum (*31.23%). The remaining isolates produced comparatively lower corrected mortality. Percent infection varied between fungal isolates throughout the evaluation period (Fig. 7). *L. tenuipes* exhibited the highest initial infection (100% on Day 1), while *A. nomiae*, *Fusarium* sp., *T. hamatum*, *P. lilacinum*, and *F. proliferatum* also showed relatively high infection during the early stages of the experiment. In contrast, *F. solani* exhibited delayed infection, reaching its highest level on Day 7. Fungal colonization of aphid

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cadavers and sporulation on treated abaca plants confirmed successful establishment of the fungal isolates under the screenhouse experiment. Repeated measures ANOVA showed that corrected mortality and percent infection were significantly affected by observation period, fungal isolate, and their interaction (all $p < 0.01$), indicating that pathogenicity differed between isolates and became more pronounced with increasing exposure time.

Relationship between fungal infection and corrected mortality under screenhouse conditions

Pearson correlation analysis showed a weak, non-significant negative relationship between fungal infection and corrected mortality of banana aphids under screenhouse conditions ($r = -0.212$, $p = 0.054$), indicating that visible fungal infection was not directly associated with increased mortality (Fig. 8). Likewise, simple linear regression showed that percent infection was a poor predictor of corrected mortality, explaining only 4.5% of the observed variation ($R^2 = 0.045$), and the regression model was not statistically significant ($p = 0.054$).

The influence of environmental factors on percent infection and corrected mortality of abaca aphids under screenhouse experiments

Multiple linear regression analysis showed that mean relative humidity and temperature had no significant effect on corrected mortality or percent infection of abaca aphids under screenhouse experiments. The regression models were not statistically significant for corrected mortality ($F = 1.434$, $p = 0.244$, $R^2 = 0.034$) or percent infection ($F = 1.050$, $p = 0.355$, $R^2 = 0.026$), indicating that these environmental variables explained only 3.4% and 2.6% of the observed variation, respectively. Residual plots showed no systematic patterns, supporting the adequacy of the regression models (Fig. 9). Our findings indicated that the range of temperature and relative humidity recorded during the study had minimal influence on fungal infection and corrected mortality of abaca aphids under screenhouse conditions.

PRE-PROOF PUBLICATION

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Discussion

The recovery of fungal isolates from aphids collected from symptomatic abaca plants supported the natural occurrence of aphid-associated fungi in abaca-growing ecosystems of Eastern Visayas. Their recovery from aphids across multiple sampling sites indicated that these fungi were prevalent among indigenous aphid populations and may represent naturally occurring EPF associated with *P. nigronervosa*. Their occurrence suggests a potential role in the natural suppression of aphid populations, consistent with previous reports on naturally occurring EPF in insect populations (Lacey *et al.* 2015; Uzman *et al*. 2019). However, fungal isolation from insect cadavers alone did not confirm entomopathogenicity because cadavers may also be colonized by saprophytic or opportunistic fungi. Therefore, molecular identification and pathogenicity bioassays were necessary to distinguish true entomopathogens from non-pathogenic fungal associates. ITS sequencing and phylogenetic analysis identified a diverse assemblage of fungi associated with *P. nigronervosa*, although two isolates could only be assigned to *Fusarium* sp. because of the limited taxonomic resolution of the ITS region. Although the ITS region is the universal fungal DNA barcode, it provides limited resolution for species delimitation within the *Fusarium fujikuroi* species complex (FFSC) (O'Donnell *et al*. 1998). Consequently, multilocus sequence analysis using translation elongation factor 1-alpha (TEF1-α), the second largest subunit of RNA polymerase II (RPB2), and β-tubulin (TUB2) can be recommended to improve species identification and phylogenetic resolution (Geiser *et al*. 2004; Aoki *et al*. 2014). Future studies should incorporate these loci to confirm the identity of the indigenous *Fusarium* isolates recovered in this study.

The predominance of *Fusarium* among the recovered isolates suggests that members of this genus were common fungal associates of banana aphids. Although *Fusarium* species are primarily recognized as plant pathogens, increasing evidence indicates that certain isolates also possess entomopathogenic activity (Ganassi *et al*. 2001; Tosi *et al*. 2015; Ahmad *et al*. 2019; Leslie *et al.* 2006; Summerell 2019; Nkuwi *et al*. 2023). Although *F. proliferatum* exhibited insecticidal activity against *P. nigronervosa* under the conditions tested, its potential as a biological control agent warrants caution because the species is associated with plant diseases and has also been reported in human infections (Sun *et al.* 2018). Thus, the observed pathogenicity should not be interpreted as evidence of biosafety or suitability for field application. Further assessment of pathogenicity toward abaca, host specificity, non-target effects, and biosafety is necessary before this isolate can be considered for biocontrol development. However, because pathogenicity within the genus is

PRE-PROOF PUBLICATION

PRE-PROOF PUBLICATION JOURNAL OF PLANT PROTECTION RESEARCH

species-dependent and their effects on abaca plants were not evaluated, further studies on plant pathogenicity, host range, multilocus characterization, production feasibility and biosafety are required before these indigenous isolates can be considered for field application as biological control agents. The absence of commonly reported entomopathogenic fungi such as *Beauveria* and *Metarhizium* species was unexpected. It may be attributed to the sampling techniques used in the present study, which primarily focused on naturally infected aphid cadavers. Although genera such as *Beauveria* and *Metarhizium* are widely reported as entomopathogenic fungi, their absence from the recovered isolates did not necessarily indicate their absence from abaca-growing areas. Fungal recovery may be influenced by host infection prevalence, environmental conditions, sampling time, host stage, and the isolation and culturing methods used. In the present study, only fungi recovered from symptomatic aphid cadavers under the sampling and isolation conditions employed were characterized; therefore, the survey may not represent the complete entomopathogenic fungal community associated with *P. nigronervosa*. The recovery of *P. lilacinum*, *T. hamatum*, and *Aspergillus* spp. further demonstrated the diversity of fungi naturally associated with abaca aphids. While *P. lilacinum* is a well-established entomopathogen, several *Trichoderma* and *Aspergillus* species have also been reported to exhibit entomopathogenic or insect-suppressive activities (Coppola *et al*. 2017; Liu *et al*. 2022; Wang *et al*. 2023). These findings further emphasize that molecular identification alone is insufficient to establish insect pathogenicity and should be complemented by pathogenicity bioassays. Although *P. lilacinum* exhibited relatively high pathogenicity against *P. nigronervosa*, its use as a biological control agent warrants caution because it has also been reported as an opportunistic pathogen of humans and vertebrates, particularly susceptible individuals (Usuda *et al.* 2025). Thus, the observed insecticidal activity did not establish its biosafety or suitability of the isolates for field application, and further species-specific assessment of host range, non-target effects, and biosafety is necessary.

Laboratory bioassays demonstrated progressive increases in aphid infection and mortality throughout the evaluation period, highlighting the potential of the indigenous fungal isolates as biological control agents against *P. nigronervosa*. EPF infect insects through a conserved process involving conidial adhesion, germination, cuticle penetration, proliferation within the hemocoel, and production of secondary metabolites that ultimately kill the host (Leger *et al*. 1997; Vestergaard *et al*. 1999; Chang *et al*. 2021). Under favorable humidity, the fungi emerge from insect cadavers and sporulate, enabling dissemination of infective conidia (Goettel *et al*. 2010; Butt *et al*. 2016).

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Differences in the efficiency of these infection processes likely explain the variation in pathogenicity observed between the fungal isolates. Among the indigenous isolates, *P. lilacinum* consistently exhibited one of the highest levels of pathogenicity, likely due to its efficient production of cuticle-degrading enzymes and bioactive secondary metabolites that facilitate host penetration and colonization (Liu *et al*. 2022; Goettel *et al*. 2010; Butt *et al*. 2016). Likewise, *T. hamatum* demonstrated promising pathogenicity, supporting previous reports that selected *Trichoderma* species suppress insects through hydrolytic enzymes, insecticidal metabolites, and endophytic colonization that enhances plant resistance (Coppola *et al*. 2017; Poveda 2021). The superior performance of *M. anisopliae* under both laboratory and screenhouse conditions was consistent with its well-established entomopathogenic characteristics. Its rapid conidial germination, efficient cuticle penetration, and production of destruxins and other toxic metabolites contribute to its broad host range and high virulence (Zimmermann 2007; de Faria and Wraight 2007; Butt *et al*. 2016). Consequently, *M. anisopliae* consistently outperformed the indigenous isolates and served as an effective reference entomopathogen. Although *F. proliferatum* is primarily recognized as a plant-associated fungus, increasing evidence indicates that some isolates also possess entomopathogenic properties. The relatively high pathogenicity observed in this study supports its potential as an insect-associated fungus; however, its pathogenicity toward abaca plants and biosafety require further evaluation because virulence and host specificity vary considerably between *Fusarium* isolates (Ganassi *et al*. 2001; Tosi *et al*. 2015; Nkuwi *et al*. 2023; Diniz *et al*. 2024). Overall, the reference isolate *M. anisopliae* exhibited the greatest pathogenicity, consistent with previous reports describing it as one of the most effective entomopathogenic fungi against aphids (Qubbaj and Samara 2022). Among the indigenous isolates *A. nomiae*, *F. proliferatum*, *P. lilacinum*, and *T. hamatum* also produced relatively high levels of infection and mortality under laboratory conditions. Their efficacy was further demonstrated under screenhouse conditions, where *P. lilacinum*, *F. proliferatum*, and *Fusarium* sp. achieved moderate corrected mortality, supporting their potential as biological control agents against *P. nigronervosa*. These findings agree with previous reports attributing the insecticidal activity of these fungi to efficient host penetration, extracellular enzyme production, and bioactive secondary metabolites (Ganassi *et al*. 2001; Goettel *et al*. 2010; Jaber and Alananbeh 2018; Liu *et al*. 2022; Wang *et al*. 2023; Mukherjee *et al.* 2020; Diniz *et al*. 2024).

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Although several indigenous isolates exhibited promising pathogenicity under screenhouse conditions, corrected mortality was lower than that observed under laboratory conditions. This reduction was expected because semi-controlled environments expose fungal propagules to fluctuating abiotic factors that reduce infection efficiency (Butt *et al*. 2016). Variations in temperature, relative humidity, and solar ultraviolet (UV) radiation can reduce conidial germination, viability, and persistence, while uneven deposition or removal of conidia from foliage may limit contact with aphid colonies (Jaronski 2010; Inglis *et al*. 2001). In addition, aphid aggregation within protected feeding sites inside leaf sheaths may further reduce exposure to fungal propagules (de Faria and Wraight 2007; Lacey *et al.* 2015). Consequently, the moderate corrected mortality observed under screenhouse conditions likely reflected environmental and behavioral constraints rather than low virulence of the indigenous fungal isolates. Visible fungal infection was not always proportional to aphid mortality, indicating that external mycosis alone was not a reliable indicator of fungal virulence. For example, *L. tenuipes* produced extensive external sporulation but only moderate mortality, whereas *M. anisopliae* caused the highest mortality despite comparatively limited visible fungal growth. Similar observations have been reported previously, suggesting that successful host penetration, internal colonization, secondary metabolite production, and isolate-specific virulence are more important determinants of pathogenicity than external mycosis (Butt *et al*. 2016; Francis *et al*. 2022; Zhang *et al*. 2024). Regression and correlation analyses further showed that temperature (31.5–36.5°C) and relative humidity (54.5–79.5%) did not significantly influence aphid infection or corrected mortality within the environmental ranges recorded during the screenhouse conditions, indicating that treatment differences were primarily associated with the biological characteristics of the fungal isolates. Consistent re-isolation of the inoculated fungi from aphid cadavers provided further evidence supporting their pathogenicity toward *P. nigronervosa*. However, because some cadavers did not exhibit external sporulation, internal examination or histopathological assessment of non-sporulating cadavers would provide additional confirmation of fungal infection and should be considered in future studies.

The findings presented here demonstrated that indigenous fungi associated with banana aphids are promising candidates for the biological control of *P. nigronervosa*. Among the recovered isolates, *A. nomiae, F. proliferatum,* and *P. lilacinum* showed the highest pathogenicity under both laboratory conditions, whereas *P. lilacinum* demonstrated the highest efficacy under screenhouse conditions. The *M. anisopliae* served as an effective reference standard. These results provided a

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strong foundation for the development and field evaluation of indigenous fungal biocontrol agents for sustainable management of *P. nigronervosa*, the vector of abaca bunchy top disease in Eastern Visayas, Philippines. Despite these promising findings, several limitations should be acknowledged. Pathogenicity was evaluated only under laboratory and screenhouse conditions, and field validation is required to confirm efficacy under natural environments. Bioassays were conducted using a single conidial concentration and only adult *P. nigronervosa*, preventing assessment of dose-response relationships and susceptibility across developmental stages.

Conclusions

This study confirmed the occurrence and diversity of indigenous fungal isolates associated with *P. nigronervosa* in abaca-growing areas of Eastern Visayas. Molecular identification using ITS sequencing established the taxonomic identity of the recovered isolates, while laboratory and screenhouse bioassays demonstrated significant differences in their pathogenicity against the banana aphid. Among the indigenous isolates, *A. nomiae, F. proliferatum,* and *P. lilacinum* exhibited the highest pathogenic potential under both laboratory conditions, whereas *P. lilacinum* demonstrated the highest efficacy among tested isolates. The *M. anisopliae* served as an effective reference standard. Although several indigenous isolates exhibited insecticidal activity against *P. nigronervosa*, these findings represented preliminary evidence of pathogenicity under controlled conditions and did not establish their safety or suitability as biological control agents. In particular, the opportunistic pathogenicity concerns associated with *P. lilacinum* and the plant pathogenic potential of *F. proliferatum* warrant caution in considering these isolates for pest management. Therefore, their observed insecticidal activity should not be interpreted as evidence of suitability for field application. Future research should also focus on multilocus molecular characterization, formulation development, mass production, application optimization, and field validation to facilitate the practical implementation of these indigenous fungal isolates as biological control agents.

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Table 1. Distribution of re-isolated potential EPF from *P. nigronervosa* across selected collection sites

Province	Municipality/City	Barangay	No. of Re-isolated EPF	EPF Code
Biliran	Naval	Libtong	2	EPF 5 and EPF 7
		Villaconsuelo	2	EPF-8 and EPF-9
Leyte	Caibiran	Cabibihan	1	EPF 11 (CC1)
	Baybay City (VSU)	NARC Nursery 3	2	EPF 4 and EPF-10 (NN3)
Southern Leyte	Sogod	Pancho Villa	4	EPF-1, EPF-2, EPF-3 and EPF-6
		Total	**11**	

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Table 2. Cultural and morphological characteristics of fungal isolates based on colony appearance, sporulation, and growth rate

EPF	Colony Color	Texture	Margin	Sporulation	Growth Rate	Colony Diameter (cm)	Conidial Size
1	White to pink	Cottony	Irregular to slightly entire margin	Abundant	Fast	6	*6.16 : 1.99 µm
2	White to green	Cottony	Entire and regular	Abundant	Fast	9.1	*5.11 : 2.94 µm
3	White to pink	Cottony	Irregular to slightly entire margin	Abundant	Fast	6	*6.16 : 1.99 µm
4	White to cream	Cottony	Entire	Moderate	Fast	5	*17.39 : 3.97 µm
5	White to pink	Cottony	Irregular to slightly entire margin	Abundant	Fast	5.9	*6.16 : 1.99 µm
6	White to pink	Cottony	Relatively regular	Moderate	Moderate	5.9	*15.96 : 3.41 µm
7	White to pink	Cottony	Relatively regular	Moderate	Moderate	5.9	*15.96 : 3.41 µm
8	Yellowish-brown	Powdery	Circular with irregular	Abundant	Fast	8.8	6.71 µm
9	Yellowish-brown	Powdery	Circular with irregular	Abundant	Fast	8.8	6.71 µm
10	White to violet	Powdery	Regular	Abundant	Fast	2.8	*15.96 : 3.41 µm
11	Yellow to green	Powdery	Irregular and confluent	Abundant	Moderate	9.8	5.06 µm

**Represents the length and width of the micronidia*

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Table 3. Molecular Characterization of Fungal Isolates from *P. nigronervosa* with Corresponding GenBank Accession Numbers

EPF No.	Place of Collection	Closest Species Match	Sequence Identity (%)	Fungal Family
1	Sogod, Southern Leyte	*Fusarium proliferatum*	100	Nectriaceae
2	Sogod, Southern Leyte	*Trichoderma hamatum*	99.66	Hypocreaceae
3	Sogod, Southern Leyte	*Fusarium proliferatum*	100	Nectriaceae
4	Baybay City, Leyte	*Fusarium solani*	100	Nectriaceae
5	Naval, Biliran	*Fusarium proliferatum*	100	Nectriaceae
6	Sogod, Southern Leyte	*Fusarium* sp.	99.47	Nectriaceae
7	Naval, Biliran	*Fusarium* sp.	99.64	Nectriaceae
8	Naval, Biliran	*Aspergillus tamarii*	100	Aspergillaceae
9	Naval, Biliran	*Aspergillus tamarii*	100	Aspergillaceae
10	Baybay City, Leyte	*Purpureocillium lilacinum*	100	Ophiocordycipitaceae
11	Caibiran, Biliran	*Aspergillus nomiae*	99.33	Aspergillaceae

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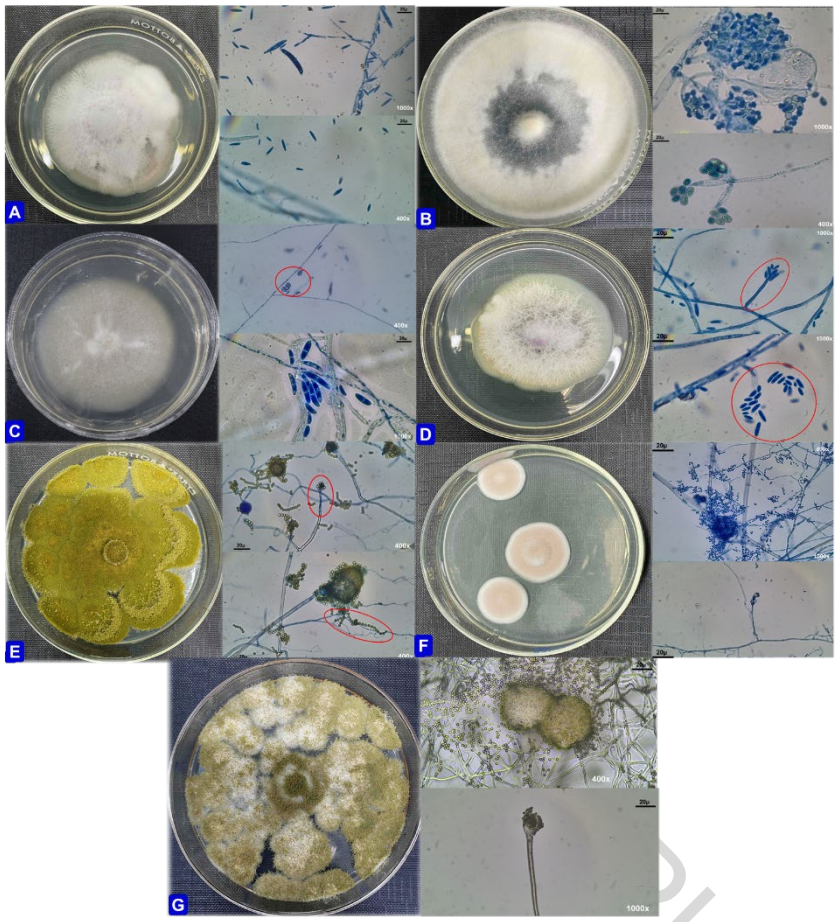


Fig. 1. Colony morphology of potential EPF isolates cultured on Potato Dextrose Agar (PDA): (A) EPF-1, EPF-3, and EPF-5; (B) EPF-2; (C) EPF-4; (D) EPF-6 and EPF-7; (E) EPF-8 and EPF-9; (F) EPF-10; and (G) EPF-11

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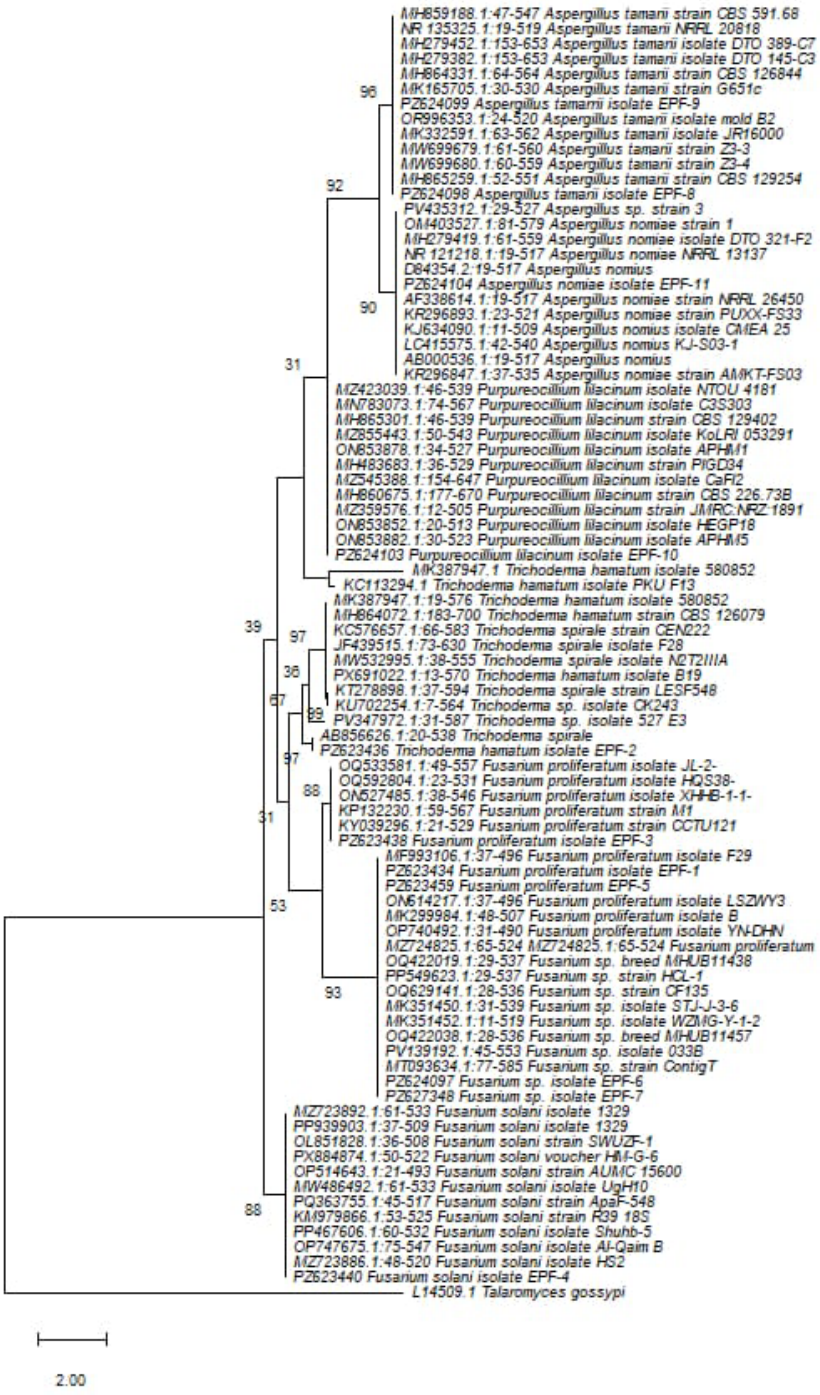


Fig. 2. Phylogenetic relationships among the fungal isolates were inferred using the Maximum Likelihood method implemented in MEGA X (Kumar *et al*. 2018). Evolutionary distances were estimated using the Tamura–Nei substitution model, and branch support was assessed using 1,000 bootstrap replicates. *Talaromyces gossypii* (L14509.1) was used as out-group

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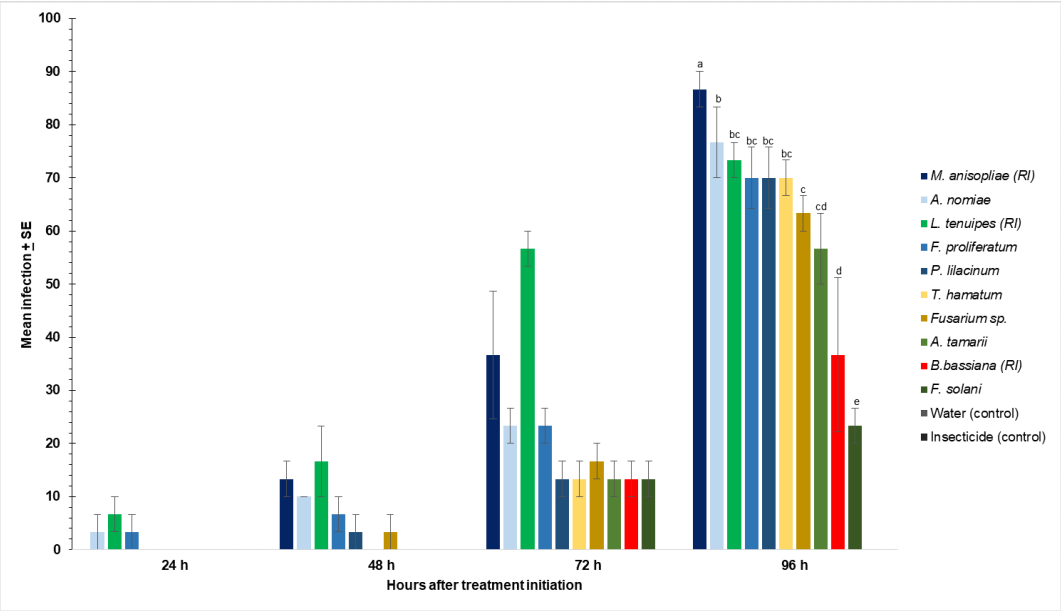


Fig. 3. Mean (± SE) percent infection of *P. nigronervosa* treated with potential EPF isolates under laboratory conditions at 24, 48, 72, and 96 hours after treatment initiation (HATI). Bars within the same observation period followed by the same letter are not significantly different according to Tukey's HSD test ($p \leq 0.05$) (RI=Reference isolate)

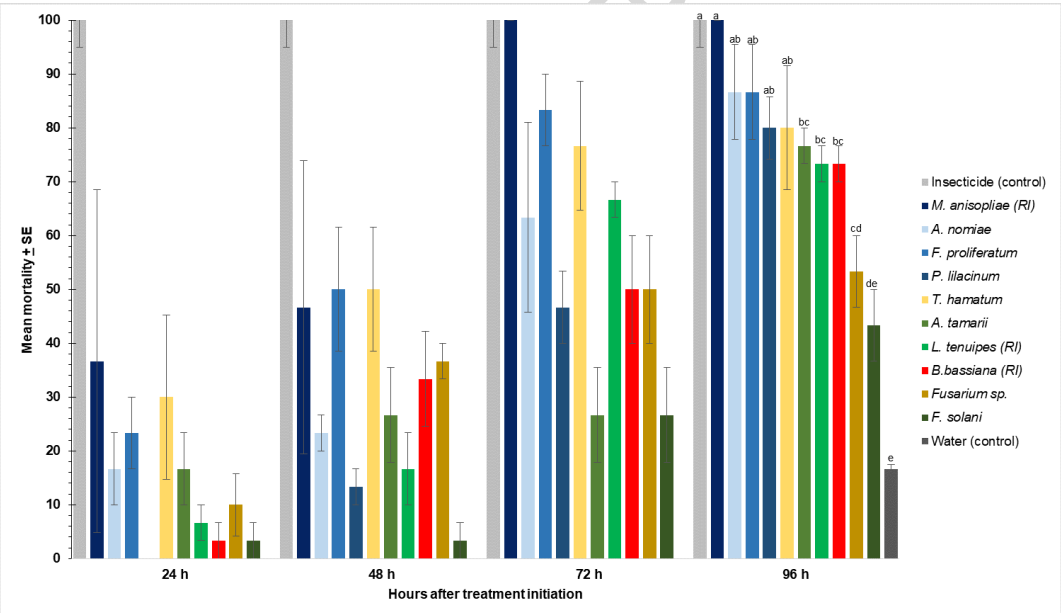


Fig. 4. Mean (± SE) percent mortality of *P. nigronervosa* treated with potential EPF isolates under laboratory conditions at 24, 48, 72, and 96 hours after treatment initiation (HATI). Bars within the same observation period followed by the same letter are not significantly different according to Tukey's HSD test ($p \leq 0.05$) (RI=Reference isolate)

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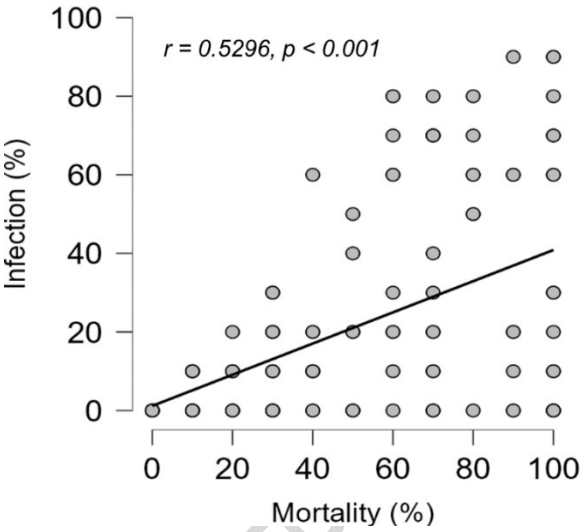


Fig. 5. Scatter Plot showing the relationship between infection (%) and mortality (%) of *P. nigronervosa* under laboratory conditions

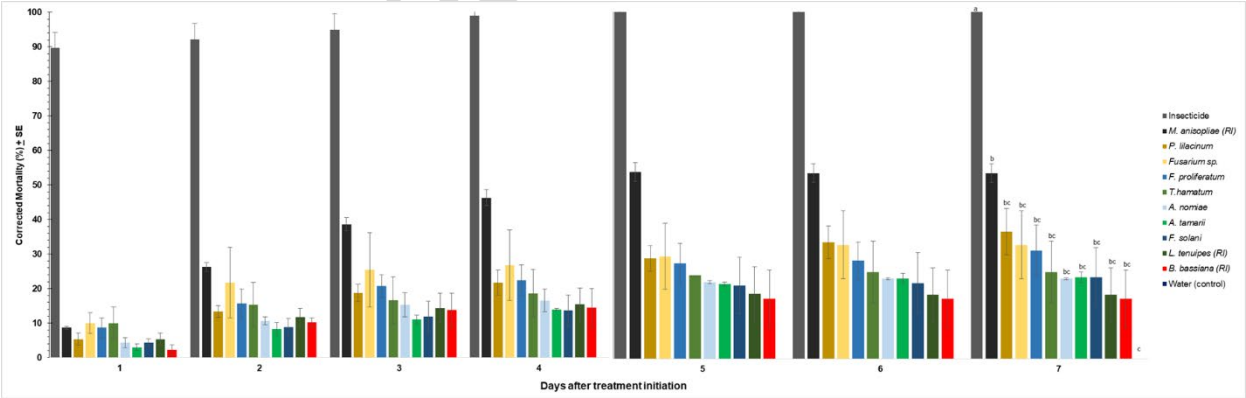


Fig. 6. Mean corrected mortality (± SE) of *P. nigronervosa* treated with potential EPF isolates under screenhouse conditions from 1 to 7 days after treatment initiation. Bars at 7 days followed by the same letter are not significantly different according to Tukey's HSD test ($p \leq 0.05$). Corrected mortality was calculated using Abbott's formula. (RI – reference isolate)

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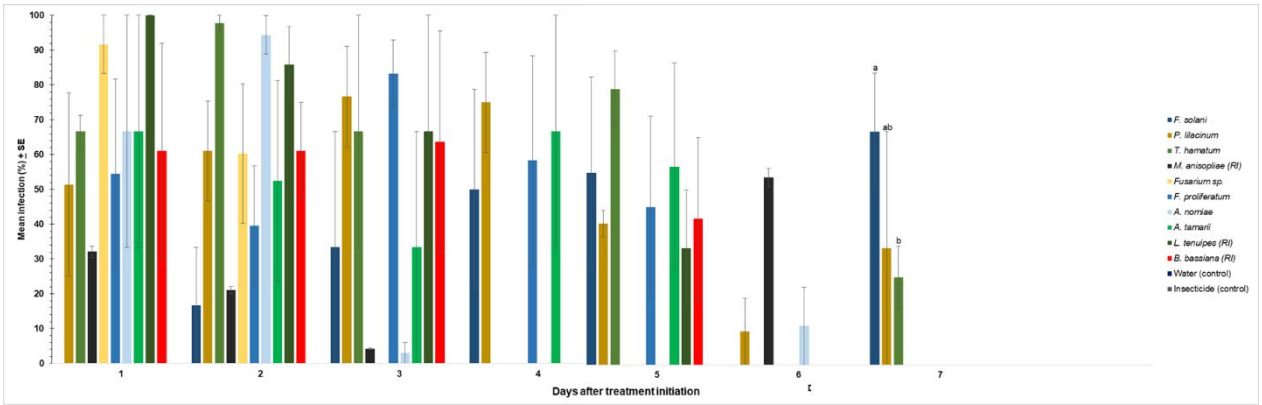


Fig. 7. Mean infection (± SE) of *P. nigronervosa* treated with potential EPF isolates under screenhouse conditions from 1 to 7 days after treatment initiation. Bars at 7 days followed by the same letter are not significantly different according to Tukey's HSD test ($p \leq 0.05$). Corrected mortality was calculated using Abbott's formula. (RI – reference isolate)

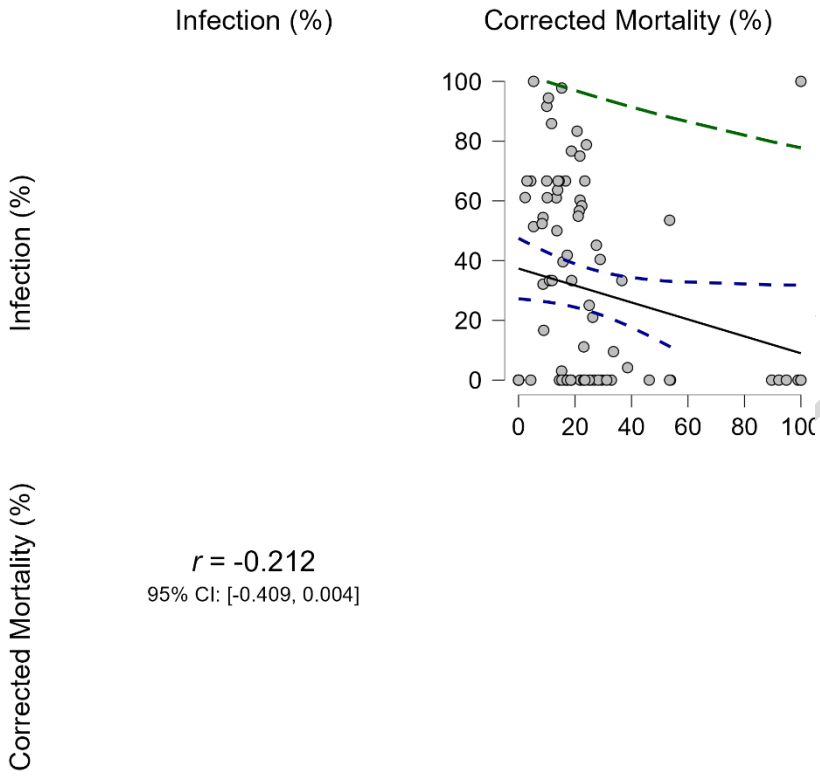


Fig. 8. Correlation between fungal infection (%) and corrected mortality (%) of *P. nigronervosa* under screenhouse experiments

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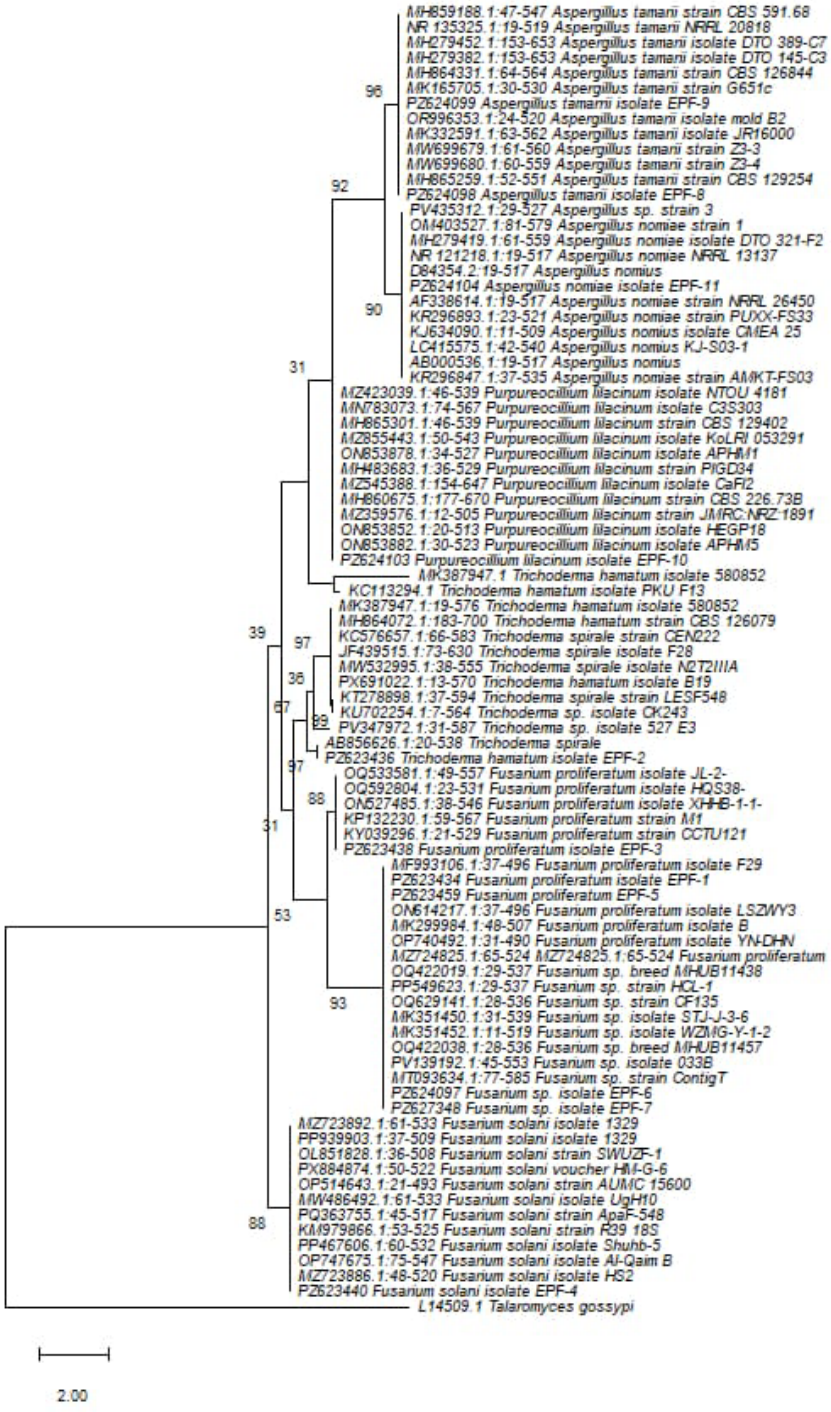


Fig. 9. Residual plots of corrected mortality (%) and infection (%) against mean relative humidity and mean temperature under screenhouse experiments. The absence of distinct residual patterns and the shallow fitted regression lines indicate weak associations between environmental variables and the mortality and infection responses *P. nigronervosa*

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