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Original article

Dual biocontrol potential of the entomopathogenic fungus *Akanthomyces muscarius* against *Thaumetopoea pityocampa* and plant pathogenic fungi

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ABSTRACT

Akanthomyces spp. species are known for their capacity to biocontrol of certain insects and plant pathogens; however, their ability to biocontrol the pine processionary (*Thaumetopoea pityocampa*) and certain phytopathogenic fungi belonging to the genera *Fusarium* and *Curvularia* have not been studied before. In this study, a strain from *Akanthomyces muscarius* was isolated from wheat grains and then identified by morphological and molecular tests. The strain was further studied for its capacity to control *Thaumetopoea pityocampa* larvae through dose-mortality tests, and its ability to control some phytopathogenic fungi strains of the genera *Fusarium* and *Curvularia* was studied through direct confrontation tests. Dose-mortality tests at three concentrations of *Akanthomyces muscarius* against the first instar larvae revealed a mortality of 92.15% after 11 days for the concentration of 2.3×10^6 conidia.ml^{-1}, with a median lethal concentration of 7.6 x10^3 conidia.$ml^{1\cdot}$ Our isolate also showed antifungal activity against these phytopathogenic fungi with inhibition rates ranging from 39.61% to 52.94%. *Akanthomyces muscarius* proved to be a promising biocontrol agent for plant pests and diseases.

1. Introduction

The search for new efficient compounds to protect plants against pests and diseases became a primary need to ensure sustainable plant and food production (Kumar et al., 2008). However, the interest in employing entomopathogenic fungi as mycopesticides has increased in numerous regions of the world due to the resurgence of insecticide-resistant populations and the fact that insecticides are ineffective against certain pests (Avery et al., 2013). Furthermore, extensive use of conventional pesticides leads to adverse effects, including harmful consequences on human and animal health (Arcury et al., 2007; Michalakis et al., 2014), contamination of surface and groundwater, and soil biodiversity loss (-Ondráčková et al., 2019).

Entomopathogenic fungi are mostly isolated from insects, but they can be found in soils (Ondráčková et al., 2019). Among them can colonize plants as endophytic fungi, or phytopathogenic fungi as mycoparasites (Ownley et al., 2010). They can also colonize grains of several crops such as corn (Błaszczyk et al., 2021). The microbiome of plants is a crucial side of sustainable agriculture, and endophytic entomopathogens play a significant role in it (Nicoletti et al., 2020).

For insects' control, numerous entomopathogenic fungal isolates have been sold globally in a variety of formulations (de Faria and Wraight, 2007). While some entomopathogenic fungi are able directly to suppress plant infections, promote systemic resistance, and stimulate plant development (Jaber and Ownley, 2018).

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It's important to note that some plant endophytes with entomopathogenic potentials, such as species of *Lecanicillum, Beauveria,* and *Metarhizium*, have the capacity to control plant diseases (Ownley et al., 2010; Sasan and Bidochka, 2013; Jaber and Ownley, 2018).

Akanthomyces species are closely linked to the genus *Verticillium* and are known as *Lecanicillium* (Kepler et al., 2017). These species are entomopathogenic fungi that infect a range of insects (mostly whiteflies and aphids), plant pathogenic fungi that cause powdery mildew (for example *Sphaerotheca fuliginea*), and rusts (for example *Hemileia vastatrix*), and they can also infect plant-parasitic nematodes such as *Heterodera glycines* (Askary and Yarmand 2007). The species most investigated and employed in the pests' management for insects are *Akanthomyces muscarius* (Petch) and *A. lecanii* (Zimm) (Upadhyay et al., 2014).

Akanthomyces muscarius (Petch) (formerly *Lecanicillium muscarium*) is a promising biological control agent. It has been commercialized as Verticillium and Mycotal for the biocontrol of aphids and whiteflies (Güçlü et al., 2010; Cuthbertson and Walters 2005). *A. muscarius* can form a mucilaginous matrix containing extracellular enzymes that facilitates cuticle penetration on its hosts (Askary and Yarmand 2007). It has already been documented that this fungus has a harmful effect on various insect pests such as cotton whitefly *Bemisia tabaci* (Cuthbertson and Walters 2005; Broumandnia et al., 2021), Aphids *Myzus persicae* (Erdos et al., 2021), the fruit fly; *Anastrepha fraterculus* (Danilovich et al., 2020), and the rice insect pest *Chilo suppressalis* (Shahriari et al., 2021).

Accordingly, our objective was to assess, under lab conditions, the ability of a strain of A muscarius endophyte of wheat grain to control the larvae of one of the most destructive pine pests (T. pityocampa), and its ability to inhibit the growth of some strains of wheat phytopathogenic fungi. Whether this isolate has dual biocontrol activity. Moreover, this study presents morphological and molecular identification of this isolate.

2. Materials and methods

2.1. Isolation and identification of A. muscarius

The strain *TA01* was obtained from soft wheat grains *Triticum aestivum* in Bouira province (Algeria) during the 2018/2019 agricultural season. Freshly harvested grains samples were collected from 10 fields (1 kg for each sample) situated in three localities El Hachimia (36°14′00″N, 3°50′00″E;3 fields), El Asnam (36°19′16″N, 4°00′50″ E; 4 fields) and Sour EL Ghozlane (36°08′50″N, 3°41′26″E; 3fields). Isolation of the strain was done according to Tabuc et al (2011): Twenty grams were added to 180 ml of a 0.05% Tween 80, and the solution obtained was stirred for 30 min. Subsequently, 1 ml of all 1/10 dilutions was placed on malt agar (2% agar + 50 ppm chloramphenicol + 2% malt). Subcultures from the margin colonies resembling *A. muscarius* were transferred on malt agar medium. Purification was performed following the method of monosporic culture. The morphological identification was performed according to some general and specific identification guides (Zare and Gams 2001, 2003; Kepler et al., 2017). The strain *TA01* isolated from a field located in El Hachimia is the only one that presented the morphological characteristics of the genus *Akanthomyces.*

2.2. Molecular identification

2.2.1. DNA extraction, amplification, and sequencing

DNA extraction of the strain *TA01* was performed from 10-day-old PDA medium culture utilizing a commercial NucleoSpin Plant II fungal genomic DNA extraction kit (Macherey-Nagel, Germany), according to the manufacturer's instructions. The ITS (Internal Transcribed Spacer) and EF1-α (Elongation factor 1-alpha 1) region were amplified with primer pairs ITS1 (5′CTT GGT CAT TTA GAG GAA GTA A3′)/ ITS4 (5′TCCTCCGCTTATTGATATGC3′) (Gardes and Bruns, 1993) and EF1-728F (5′ CAT CGA GAA GTT CGA GAA GG)/ EF2 (5′ TAC TTG AAG GAA CCC TTA) (Carbone and Kohn, 1999). 25 µL were used for the amplification, which included 2 µL of genomic DNA, 1 µL of 10 µM primers (sense and antisense), 5 µL of 5X Taq buffer (Promega Corporation, USA), 1.5 µL of 25 mM MgCl2, 0.2 µL of d'NTP mixture (25 mM), 0.2 µL of 5U/ µL of Taq DNA polymerase (Promega Corporation, USA), and 14.10 µL of double-distilled sterile water. The following PCR conditions were used for the ITS region: Initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at this temperature for 30 s each, hybridization at 55 °C for 30 s, extension at 72 °C for 45 s, and finally, a final extension at 72 °C for 7 min. The EF1- α region was subjected to the same conditions, but with a 52 °C hybridization temperature. The PCR result had been separated into a 1.5% agarose gel (Sigma-Aldrich, USA) following the PCR reaction, and it was then stained with 0.5 g ml-1 ethidium bromide. A molecular weight marker of 100 bp (PCR 100 bp low Ladder, Sigma-Aldrich) was utilizing DNA fingerprints that were visualized following exposure to UV light using the Gel Documentation System (Bio-Rad, USA). The PCR results were then purified using the NucleoSpin® Gel and PCR Clean-up kit from Macherey-Nagel (Germany), and sequenced using the Sanger approach (Sanger et al., 1977) using the BigDye v3.1 kit from Applied Biosystems and the identical primers used in PCR. The obtained sequences were analyzed and corrected using the CHROMAS PRO software.

2.2.2. Phylogenetic analysis

Once the final sequences are obtained, they were deposited at the NCBI GeneBank, and compared to those found on NCBI using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi Blast), and all sequences that displayed a high degree of affinity with our sequences were retrieved. (Sedrati et al., 2023). Multiple sequence alignment was started for the phylogenetic analysis using MEGA7 software's muscle option, followed by manual adjustments. The aligned regions were combined using Mesquite 3.6 software to create a single sequence. Maximum likelihood analysis was then applied to both individual alignments and the concatenated sequence using the "Tamurai-Nei" model in MEGA7. To assess the strength of the internal branches of the built tree, the bootstrap method was used with one thousand replications. (Visagie et al., 2013; Houbraken et al., 2014; Siqueira et al., 2017).

2.3. Evaluation of the growth and sporulation of A. muscarius

Mycelial discs of 5 mm diameter of *A. muscarius* were inoculated into 90 mm Petri dishes containing six different culture media: PDA (potato dextrose agar), SDA (Sabouraud Dextrose Agar), NA (Nutrient Agar), SNA (Synthetisher Nährstoffärmer Agar), MA (malt agar), and DRBC (Dichloran Rose Bengal Chloramphenicol). After ten days of incubation at 27 °C, the colonies' average diameter (mm) and spore concentration (conidia ml-1) of the isolates was measured. For this purpose, 10 ml of sterilized distilled water with 0.01% Tween 80 was poured into the dishes containing the culture. After shaking, filtration of the suspension was performed through six layers made with sterile cheesecloth to eliminate mycelium fragments, and the outcome was then diluted. The concentration of conidia per ml was finally evaluated with the aid of a hemocytometer. Three copies of each treatment were performed.

2.4. Bioassay tests against Thaumetopoea pityocampa

The first instar larvae of *T. pityocampa* were obtained on October 2021 from two Mariwet and Sidi Mebarek forests located in the west of Bordj Bou Arreridj province (Algeria) characterized by a semi-arid climate. Each, twenty larvae were deposited in Petri dishes whose bottom was lined by a wet filter paper, and pine needles were deposited to feed the larvae (Abou-Jawdah et al., 2008).

The *A. muscarius* spore suspension used for the treatment of *T. pityocampa* larvae was made by mixing 10 ml of sterilized distilled water and Tween 80 (0.01%) to sporulating colonies from 15 days of culture. After shaking, the obtained suspension was filtered through six layers of sterile cheesecloth to eliminate any mycelial fragments. (Abou-Jawdah et al., 2008). The obtained spore suspension (stock solution) was used to make diluted solutions with the following concentrations: 2.3x10^4 conidia.ml^{-1}, 2.3x 10^{5}conidia. ml^{-1}, and 2.3x106 conidia.ml^{-1}. For the treatment, 1 ml of each solution was sprayed per Petri dish (at a rate of 50 µL of the spore suspension/larva) at 27 °C and 100% relative humidity. Each treatment was replicated three times. For the negative control,1 ml of sterile water with Tween 80 (0.01%) was sprayed instead of spore suspension. A daily follow-up of the number of dead individuals is carried out until the death of all the individuals. The mortality rate was calculated using the following formula:

Observed mortality = (Number of dead individuals/total number of individuals) × 100.

The noticed mortality was then modified as proposed by Abbot (1925):

$$MC = \frac{(M2 - M1)}{(100 - M1)} * 100$$

In the formula above, M1 is the mortality proportion in controls; M2 is the relative mortality in the treated group and MC is the corrected mortality percentage.

2.5. Antagonistic potential of A. muscarius at odds with phytopathogenic fungi

The antagonistic effect of *A. muscarius* on the growth of five fungal strains was carried out by the direct confrontation technique described by Sivan and Chet (1989). *A. muscarius* and the cultures of the target fungal strain were grown for 7 days, and 6 mm-diameter discs from these cultures were placed 4 cm apart on PDA Petri dishes with a 9 cm diameter. The target fungi tested in this study were *Fusarium verticillioides, F. oxysporum, F. solani, Fusarium* sp., and *Curvularia lunata*. The controls consist of the antagonist or the target fungal strain plated into the center of a PDA Petri dish with a 9 cm diameter. After then, the plates were incubated at 26 ± 2 °C. Radial growth measurements of the target and antagonist fungi are performed daily. The percentage inhibition of the pathogen's radial growth is determined using the formula established by Datta et al. (2004).

$$\text{Inhibition rate} = \frac{(\text{D control} - \text{D test})}{(\text{D control})} * 100$$

Here D control represents the diameter of the radial growth for the target fungus in the control box, and D test is the diameter of the radial growth for the target fungus in the test box of *A. muscarius.*

2.6. Statistical analyses

The R software version 2022 and XLSTAT version 2000 were employed to conduct all of the analyses. Mortality data were converted to percentages, and probit analysis was started to determine the median lethal concentration and time (LC50 and LT50, respectively) (Finney et al., 1971). Analysis of variance (ANOVA) followed by Tukey's HSD test (95% confidence interval) was applied to all numerical series collected from this study.

3. Results

3.1. Morphological and molecular identification

On PDA medium (10 days, 26 °C), colonies of strain *TA01* were round with a 21 to 25 mm diameter, compact, raised in the center, fluffy with a white color on the front and a pale-yellow color on the reverse Fig. 1 A-B. Under the microscope, this fungus has smooth, hyaline, septate hyphae with erect conidiophores bearing solitary or mostly whorled phialides. The phialides are erect, usually, punch-shaped measuring 25 to 45 µm and the conidia are smooth-walled, hyaline, and unicellular, subcylindrical to cylindrical and ellipsoidal in shape, varying in size from 2.5 to 6 µm. The conidia are produced in false heads: Fig. 1C.

According to the megablast results on the NCBI GenBank nucleotide sequence database, the closest species to the *TA01* isolate was *A. muscarius* strain CBS 143.62, with a very high similarity percentage (99.63%) for ITS. However, for TEF1 the closest species was *Beauveria malawiensis* strain IMI 228343 with a percentage similarity of 85.90% (Fig. 2). The aligned sequence data of ITS and TEF1 from 39 taxa, including *TA01* isolate, reference strains of related species; 10 *Beauveria,* 2 *Lecanicillium*, 3 *Samsoniella*, 22 *Akanthomyces* strains, 2 strains of *Hevansia novoguineensis* CBS 610.80, and *Hevansia novoguineensis* BCC36270 as an out-group were utilized for phylogenetic analysis. Maximum likelihood analysis showed that *TA01* isolate was closely related to *Akanthomyces muscarius* strain CBS 143.62 supported by a very high bootstrap value (ML = 97%) (Fig. 2). Based on morphological identification and the results of the phylogenetic analyses of the concatenated data set of ITS and TEF1 sequences, TA01 isolate was therefore identified as *A. muscarius* belonging to the Cordycypitaceae family.

3.2. Growth and sporulation of A. muscarius

The results of adopting different media of culture on mycelial growth and spore production of *A. muscarius* are summarized in Table 1. After 10 days of incubation, a very highly discernible impact of the medium on conidial concentrations. The highest conidial concentration of *A. muscarius* was obtained for the NA medium. For radial growth, five of the six media tested had the same effect with mean diameters between 21.6 and 25.3 mm. The lowest value of colony diameter of *A. muscarius* was observed on the SNA medium.

3.3. Biocontrol assay against Thaumetopoea pityocampa larvae

The results of the corrected mortality percentages of *T. pityocampa* larvae treated with three concentrations of *A. muscarius* strain (C_1 = 2.3 × 10^4, C_2 = 2.3x 10^5 and C_3 = 2.3x 10^{6}conidia. ml^{-1}) calculated during the period from day 6 to day 11 are summarized in Table 2. It was noted that the corrected mortality of larvae for the three concentrations studied was time-dependent and increased with time. Indeed, corrected mortality increased from 16% on day 6 to 63% on day 11 for the lowest *A. muscarius* concentration (C_1), and from 46% to 92% for the same period at the highest concentration (C_3). After death, larvae treated with the fungus are covered with a whitish mycelium (Fig. 3). Analysis of variance showed a very highly significant effect of time and dose ($P < 0.001$). The 100% mortality of the larvae was observed after

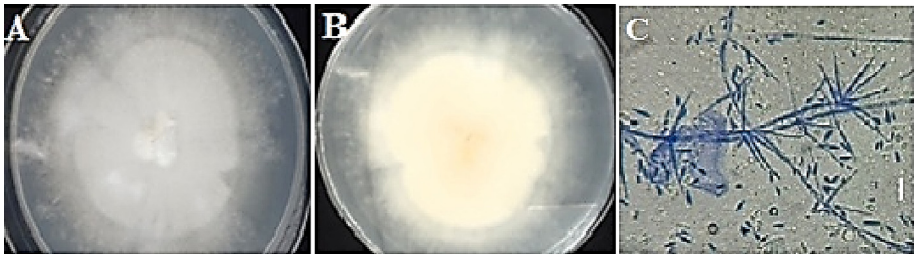

Fig. 1. (A-B) Macroscopic aspect of the strain *TA01* on PDA medium, (C) microscopic aspect of the strain *TA01* (scale = 15 µm).

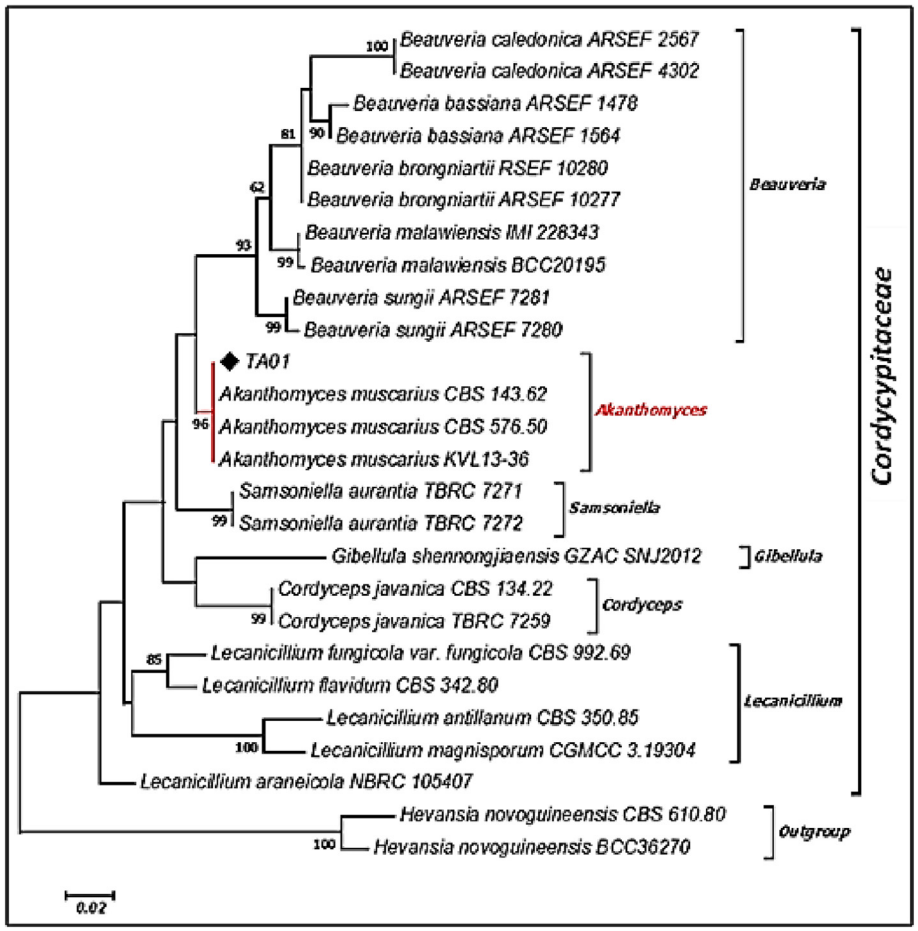

Fig. 2. Maximum likelihood (ML) phylogenetic tree of isolated strain *TA01* and related *Cordycypitaceae* species based on a combined dataset of two loci sequences (internal transcribed spacer (ITS) + translation elongation factor-1a (TEF). Only bootstraps over 60% are shown at nodes.

Table 1
Conidial concentrations and colony diameter of *Akanthomyces muscarius* grown in six media after 10 days incubation.

	Media	Concentrations (conidia.ml^{-1}) Mean ± SE (n = 3)	Colony diameter (mm) Mean ± SE (n = 3)
1	NA	$2.1 \times 10^6 \pm 10^{6a}$	24.6 ± 1.3^a
2	PDA	$1.3 \times 10^6 \pm 3.3 \times 10^{4b}$	25.3 ± 2.6^a
3	MA	$1.3 \times 10^6 \pm 3.3 \times 10^{4b}$	23.6 ± 1.4^a
4	SDA	$1.0 \times 10^6 \pm 5.7 \times 10^{4c}$	23.3 ± 0.3^a
5	DRBC	$6.3 \times 10^4 \pm 6.3 \times 10^{3d}$	21.6 ± 0.3^a
6	SNA	$6.0 \times 10^4 \pm 1.7 \times 10^{4\ d}$	11 ± 1.1^b

The same letters in each column indicate a non-significant difference.

14 days in the case of the highest spore concentration (C_3), and after 18 and 19 days for concentrations C_2 and C_1, respectively. Comparison of means by Turkey test revealed no significant difference in corrected mortality between concentrations C_2; 2.3x 10^5 and C_1; 2.3x10^4conidia.ml^{-1}.

The LC50 and LC90 values of *A. muscarius* against *T. pityocampa* were calculated from the 6th day after treatment to the 11th day and are summarized in Table 3. These values decreased with time, this LC50 decreased from 2.5x10^6 conidia.ml^{-1} on the 6th day to 3.5x10^3 on the 11th day after treatment. Similarly, LC90 decreased from 1.06x 10^{10} to 1.4x10^6 conidia.ml^{-1}during the same period.

The values of LT50, and LT90 were also calculated as shown in Table 4. The lowest lethal times 50 and 90 (6.1 days and 10.4 days respectively) were observed for the highest *A. muscarius* spore concentration (C_3). For concentrations C_1 and C_2, the values of these two parameters were higher than those recorded in the case of C_3. However, it should be noted that there were no significant differences between the LT50 and LT90 values for these two concentrations.

Table 2
Corrected mortality of *Thaumetopoea pityocampa* with different conidial concentrations of the *Akanthomyces muscarius* isolate.

Time (days)	Traitement (conidia.ml^{-1})	Corrected mortality (%)	F value	*P*-value
6	$C_1 = 2.3 \times 10^4$	16.29 ± 13.59^b	8.948	< 0.01
	$C_2 = 2.3 \times 10^5$	21.95 ± 8.85^b		
	$C_3 = 2.3 \times 10^6$	46.19 ± 6.91^a		
7	$C_1 = 2.3 \times 10^4$	16.88 ± 9.43^b	31.41	< 0.001
	$C_2 = 2.3 \times 10^5$	26.25 ± 7.95^b		
	$C_3 = 2.3 \times 10^6$	58.49 ± 2.79^a		
8	$C_1 = 2.3 \times 10^4$	20.69 ± 11.42^b	33.75	< 0.001
	$C_2 = 2.3 \times 10^5$	30.06 ± 7.93^b		
	$C_3 = 2.3 \times 10^6$	67.86 ± 3.89^a		
9	$C_1 = 2.3 \times 10^4$	37.90 ± 13.64^b	41.92	< 0.001
	$C_2 = 2.3 \times 10^5$	41.39 ± 5.28^b		
	$C_3 = 2.3 \times 10^6$	83.11 ± 5.23^a		
10	$C_1 = 2.3 \times 10^4$	54.90 ± 12.24^b	33.9	< 0.001
	$C_2 = 2.3 \times 10^5$	66.66 ± 6.79^{ab}		
	$C_3 = 2.3 \times 10^6$	88.23 ± 11.76^a		
11	$C_1 = 2.3 \times 10^4$	62.74 ± 12.24^b	47.28	< 0.001
	$C_2 = 2.3 \times 10^5$	74.50 ± 8.98^{ab}		
	$C_3 = 2.3 \times 10^6$	92.15 ± 6.79^a		

Results are expressed as means of triplicates ± standard deviation; values in the same column with different superscript letters are significantly different at P < 0.05.

Fig. 3. Morphological aspect of *Thaumetopoea pityocampa* larvae treated with *Akanthomyces muscarius* (10 days after treatment at the concentration C_3).

Table 3
LC50 and LC90 values of *Akanthomyces muscarius* against 1st instar larvae of *Thaumetopoea pityocampa.*

T (Days)	LC (conidia.ml^{-1})		Confidence intervals	χ^2Df = 14	Intercept	Slope
6	LC50	2.5×10^6	-1.9×10^6 -6.9×10^6	12	5.2 ± 0.5	2.2 ± 0.4
	LC90	1.06×10^{10}	-6.06×10^{10}–8.2×10^{10}			
7	LC50	6.7×10^5	-2.9×10^5–1.3×10^6	15	5.7 ± 0.4	3.02 ± 0.3
	LC90	3.9×10^8	-1.05×10^8-1.8×10^9			
8	LC50	3.4×10^5	6.2×10^4–-6.1×10^5	18	6.3 ± 0.5	3.4 ± 0.4
	LC90	9.9×10^7	-1.7×10^8 -3.7×10^8			
9	LC50	7.4×10^4	6.6×10^3-1.4×10^5	14	8.8 ± 0.6	3.5 ± 0.5
	LC90	2.3×10^7	-3.07×10^7- 7.6×10^7			
10	LC50	7.6×10^3	-6.8×10^3–-2.2×10^4	15	13.02 ± 0.7	2.1 ± 0.5
	LC90	4.5×10^6	-5.7×10^6–-1.4×10^7			
11	LC50	3.5×10^3	-4.4×10^3–-1.1×10^4	15	14.3 ± 0.7	1.8 ± 0.5
	LC90	1.4×10^6	-1.2×10^6- 4 × 08 10^6			

Table 4
LT50 and LT90 values of *Akanthomyces muscarius* against 1st instar larvae of *Thaumetopoea pityocampa.*

Dose	LT50 (Days)	LT90 (days)	χ^2	df	Intercept	Slope
$C_1 = 2.3 \times 10^4$	9,7	16.5	24	21	1.7 ± 0.03	−0.14 ± 0.004
$C_2 = 2.3 \times 10^5$	9.5	13.7	20	18	1.3 ± 0.05	−0.12 ± 0.006
$C_3 = 2.3 \times 10^6$	6.1	10.4	20	18	0.8 ± 0.17	−0.08 ± 0.020

3.4. Antagonist test against phytopathogenic fungi

In order to evaluate the antagonistic capacity of *A. muscarius* (strain *TA01*) towards phytopathogenic fungi, direct confrontation tests against 5 strains of phytopathogenic fungi (*F. verticillioides, F. oxysporum, F. solani, Fusarium* sp., and *Curvularia lunata*) were conducted (Fig. 4).

Daily measurements of the radial growth of each of the five phytopathogenic fungal strains in the absence and presence of the antagonistic strain *A. muscarius*, as well as daily measurements of the radial growth of the antagonistic strain in the absence and presence of each of the plant pathogenic fungal strains, are shown in Fig. 5.

A. muscarius caused a discernible decrease in the radial growth of all fungal strains tested. The maximum inhibition rate observed for each strain was 52.94% on the 5th day for *C. lunata,* 52.90% on the 7th day for *Fusarium* sp., 45.83% on the 9th day for *F. oxysporum*, 43.92% on the 6th day for *F. solani* and 39.61% on 7th day for *F. verticillioides.* At the same time, the radial growth of *A. muscarius* was not affected by the presence of phytopathogenic fungal strains.

4. Discussion

The strain of *A. muscarius* studied in this work was isolated from Algerian soft wheat grains, it is worth noting that the species has only been previously obtained from insects or other plant parts such as *Nypa fruticans* leaves (Vinit et al., 2018), and stored apples in Romania (Roxana et al., 2019). The macro and microscopic characteristics of our strain were assembled to those found by Vinit et al (2018) and Roxana et al (2019). Morphologically, *Akanthomyces* is similar to *Lecanicillium*. However, the conidia of *Lecanicillium* have an ellipsoidal to oblong-oval and oval shape, while the majority of conidia in *Akanthomyces* are ellipsoidal to cylindrical in shape (Chiriví-Salomón et al., 2015).

As reported by Hillis and Dixon (1991); Salazar et al (1999); Arenal et al (2000), the utilization of the ITS region as a molecular marker for the purpose of fungi classification is commonly practiced in phylogenetic analysis. Thus, our strain was subjected to a complete phylogenetic analysis and was grouped in a different clade from other *Akanthomyces* in the phylogenetic tree. The percentage of similarity with the species *A. muscarius* was very high (99.63%).

According to our results, PDA, MA, SDA, NA, and DRBC media promoted the vegetative growth of *A. muscarius* in the same way. Regarding spore production, the NA medium was the optimal medium. However, Broumandnia et al. (2021) found that the PDA medium was the medium that best promotes radial growth, while the SDA medium was the optimal medium for conidial production of *A. muscarius*.

The results of the biological control assay against *T. pityocampa* larvae indicated that the *Akanthomyces* isolate *TA01* is an effective controlling agent for these larvae. As far as we are aware, this is the first investigation into the efficacy of *A. muscarius versus T. pityocampa.* This strain is extremely pathogenic to the first instar larvae of *T. pityocampa.* The susceptibility of pine processionary moth larvae at different instars varied, as noted by Sonmez et al. (2017). Due to their thicker cuticles, the older instar larvae were undoubtedly more able to fend off infections.

The studies conducted so far for the biocontrol of *T. pityocampa* tested by some bacteria, viruses, and entomopathogenic fungi. The study of Latifa et al (2012) showed that *Bacillus* thuringiensis can cause 100% mortality of *T. pityocampa* larvae. Bonnet et al., 2013 found that the species *Beauveria bassiana* was efficient in the control of this pest insect, with mortality rates of 82 to 86%. However, it has been reported that chrysalids and larvae of *T. pityocampa* were naturally found infected by *B. bassiana* in Italy (Battisti et al., 2000) and in the Andulacia region in Spain (Vargas-Osuna et al., 1994). Several other studies have tested other entomopathogenic fungi including *Metarhizium anisopliae*, *Isaria farinose*, and *Lecanicillium lecanii,* and reported optimistic results against *T. pityocampa* larvae (Er et al., 2007; Sönmez et al., 2017; Ozdemir et al., 2019). Furthermore, using three separate approaches, Akinci et al., 2017 put in *B. bassiana* (strain TP-153) at 10^8 conidia.ml^{-1} against the pest insect's fourth instar larvae. The most successful technique was immersion, with a mortality rate of 13% to 31%. In our investigation, ten days after the treatment of 2.3 10^6 conidia.ml^{-1} of *A. muscarius*, a mortality rate of 88.23% was observed using the spraying approach.

The LC50 values found in the present study (LC50 = $3.5x10^3$ – $2.5x10^6$ conidia.ml^{-1}) were relatively lower than the values reported in the study by Ren et al (2010). These authors tested isolates of *A. muscarius* (V20, V26, V07, and V17) against *B. tabaci*

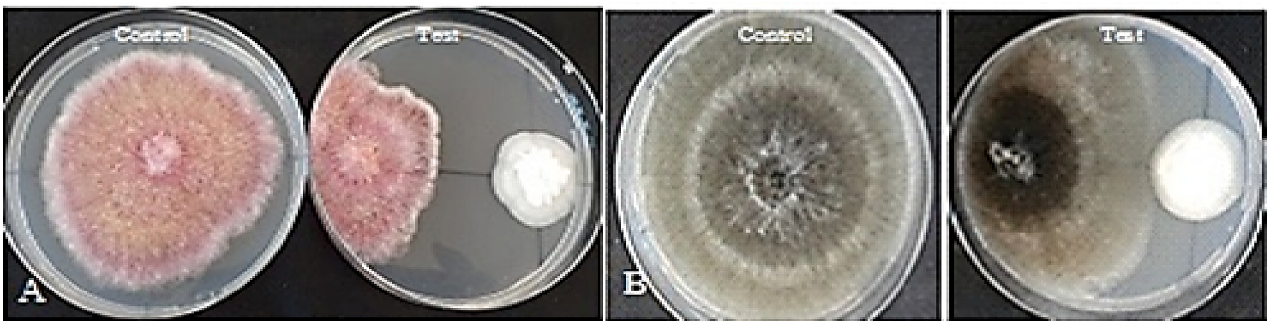

Fig. 4. Growth inhibition of plant pathogens caused by *Akanthomyces muscarius* when grown in dual cultures on potato dextrose agar. (A) *Fusarium oxysporum;* (B) *Curvularia lunata*.

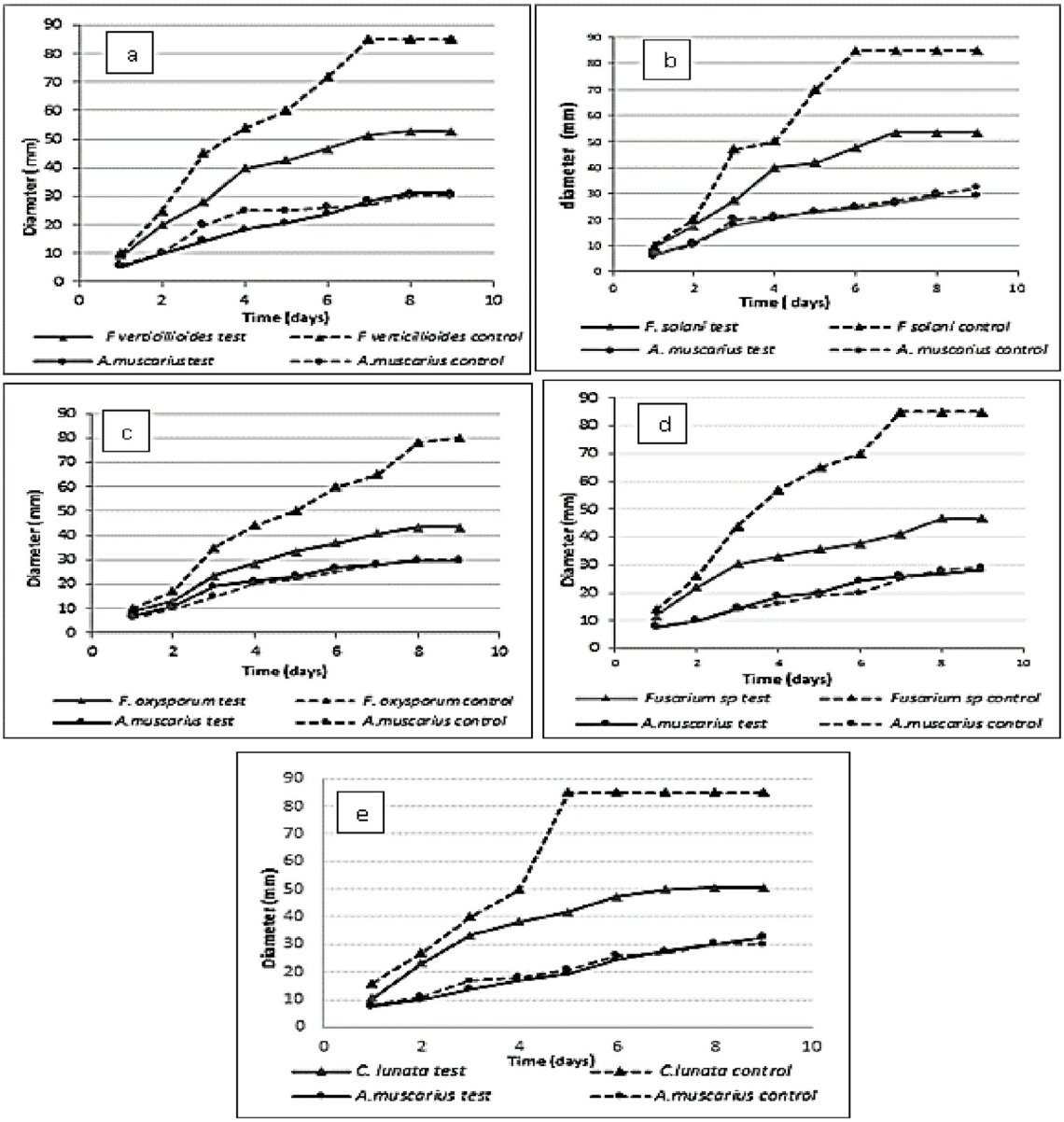

Fig. 5. Effect of the antagonist *Akanthomyces muscarius* on the radial growth of phytopatogenic fungi: (a) *Fusarium verticillioides*, (b) *F. solani*, (c) *F. oxysporum*, (d) *Fusarium* sp. (e) *Curvularia lunata.*

nymphs, and found LC50 values between 1.07×10^6 and 5.08×10^8-conidia.ml^{-1}.

The LT50 values in our study were 9.6, 9.5, and 6.1 days at concentrations of 2.3x10^4, 2.3x10^5, and 2.3x10^{6}conidia.ml^{-1}, respectively. These values are relatively higher than those reported for an Iranian strain *of A. muscarius obtained from Zeuzera pyrina* L. (Tabadkani et al., 2010). This strain was tested against *Trialeurodes vaporariorum* at these concentrations 10^5, 10^6, 10^7, and 10^8conidia. ml^{-1} and showed TL50 values of 8, 6, 6, and 4 days, respectively.

The strain *TA01* caused a significant reduction in the radial growth of all the fungal strains tested (four strains of *Fusarium* and one of *Curvularia*) with inhibition rates varying between 39.61% and 52.94%. According to the bibliography, the entomopathogenic fungus *A. muscarius* (*L. muscarium*), is one of the most hopeful natural parasites and is considered to have enormous potential as a biological control agent for an extensive variety of phytopathogenic fungi, such as *Sphaerotheca macularis*, *Puccinia coronata*, *Penicillium digitatum, Pythium ultimum* and *Fusarium moniliforme* (Benhamou and Brodeur, 2001; Benhamou, 2004; Miller et al., 2004; Yu et al., 2012). As *Akanthomyces* species are closely related to the genus *Verticillium*, they may also share a similar mode of action. In this context, the mode of action of *Verticillium lecanii,* may be related to a chitinase. Yu et al. (2015) succeeded in extracting and purifying a chitinase from this fungus, this enzyme has in vitro spore germination inhibitory action against various plant diseases, in particular *Fusarium moniliforme.* Other authors proved the phenomenon of parasitism of *Verticillium lecanii* towards biotrophic phytopathogenic fungi such as *Sphaerotheca fuliginea* (Askary et al., 1997).

In this research, it was found that the strain *TA01* of *A. muscarius* had a dual biocontrol potential against both *T. pityocampa* and plant pathogenic fungi. The study of Kim et al. (2007) demonstrated also that 3 taxa of *Lecanicillium* spp. (*Akanthomyces* spp.): *Lecanicillium* sp. (strain DAOM198499), *L. attenuatum* (strain CS625), and *L. longisporum* (Vertalec), possess dual effectiveness *versus* powdery mildew and aphids. Also, entomopathogenic

fungus, *Isaria javanica* had a dual biocontrol potential *versus* aphids and flora diseases (Kang et al., 2018).

5. Conclusions

The entophytic isolate *TA01* (*A. muscarius*) has a dual biocontrol activity *versus* insect pests and plant pathogenic fungi; our study showed a high insecticidal effect against the first instar *T. pityocampa* and a significant fungicidal effect against some phytopathogenic fungi of wheat under laboratory conditions. In conclusion, fungal endophytes are rapidly emerging as a distinctive class of microbial biocontrol agents. Besides their effectiveness against insect pests; fungal entomopathogens may provide protection against plant diseases such as endophytes. In future research, It is interesting to use the extracellular metabolites of this strain in order to evaluate their antifungal and insecticidal activity, more work is needed to confirm the efficacy of this strain for *in sit* circumstances and to develop optimal formulations for field application.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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