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Antimicrobial Activities of Fungal Strains from Fungus-Growing *Macrotermes bellicosus* Mound Materials

Joseph B. Sawadogo[1, 2]*, Salimata Traoré[2], Sandrine E. T. Hien[2], Dagoro Palé[2], Ynoussa Maïga[2], Dayéri Dianou[3] and Aboubakar S. Ouattara[2]

[1]University Nazi BONI, 01 BP 1091 Bobo-Dioulasso, Burkina Faso
[2]University Joseph KI-ZERBO, 03 BP 7131 Ouagadougou, Burkina Faso
[3]Centre National de la Recherche Scientifique et Technologique, 03 BP 7192, Ouagadougou, Burkina Faso

**Corresponding author*

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A B S T R A C T

Increasing world antimicrobial resistance requires to search for new microbes-derived biomolecules with higher antimicrobial activity from natural environments. This study aimed to evaluate the antimicrobial activity of fungal isolates associated with termite mounds. Isolates were obtained from soil and fungi comb samples of active *Macrotermes bellicosus* termite mounds. The antimicrobial bioassays of fungal isolates against test pathogenic bacteria and the fungus *Aspergillus niger* by dual culture, agar well diffusion, and disc diffusion methods were performed. The isolates with potent antimicrobial activity were phenotypically identified. Of over 65 fungal isolates, 48 exhibited an antagonistic effect against at least one test germ. Fifteen isolates (9 and 6 from soil and comb, respectively) that interestingly 3 (IM2s, IM15s, and IM51s belonging to *Aspergillus* sp.) had exclusively broad-spectrum antibacterial activity (10-35 mm) against all bacteria. Only the isolate IM51s inhibited Gram-positive and Gram-negative bacteria by dual culture and agar well diffusion. Six other isolates IM1s, IM61s (*Rhizopus* sp.), and IM3s, IM31m, IM34m, and IM40m (as *Aspergillus* sp.) had solely high antifungal activity (53-60% inhibition) against *A. niger*. This study indicated that the *M. bellicosus* mound material-associated fungal isolates can be of potential interest in discovering novel fungal species and antimicrobials, and their various applications.

Introduction

Antimicrobial resistance constitutes a major challenge in public health. The emergence of resistant and multidrug-resistant microbial pathogens, face to existing antimicrobials intensively and/or wrongly used, deserves full attention in the search for new biomolecules. Thus, there is an urgency to find new natural products-based antimicrobial agents against putative

pathogens. This interest in natural products of microbial origin is due to their diversity (toxins, antimicrobial peptides/proteins, hormones, vitamins, amino acids). These products are also easily synthesized by skilled microbes, readily biodegradable, specific, and generally have low toxicity (Fatope *et al.,* 2000; Newman *et al.,* 2016). Moreover, over more than 22,500 antibiotics arising from microbes, 45, 38, and 17 % are attributed to actinomycetes, fungi, and non-actinomycetes bacteria, respectively (Kumari *et al.,* 2020).

Ecological niches constitute a huge source of natural products that are the origin of most antimicrobial compounds. Natural environments and insect symbionts consist of high sources of producers of antimicrobial agents (Beemelmanns *et al.,* 2016; Dimri *et al.,* 2020). Among these targeted environments, termite mounds have been subjected to several studies. Termites are eusocial insects widespread in tropical and subtropical areas. In West Africa, particularly Burkina Faso, the *Macrotermes* and *Odontotermes* genera are mostly found in fields. On a comb, these termites grow and maintain a predominant fungus *Termitomyces* monoculture which degrades and recycles the organic vegetable matter. Thus, the fungus provides nutrients to the termites and protects them against invading entomopathogens, phytopathogens, and other fungal pathogens, consequently maintaining the colony's well-being.

Furthermore, it was reported that the growing-fungus termites produce a range of interesting bio-substances with various properties such as antimicrobial, antitumoral, immune regulatory, and neurogenesis (Hsieh and Ju, 2018). The fungus-growing termites were subjected to recent studies that demonstrated the potent antimicrobial activity of crude and pure extracts, and that of microorganisms isolated from termite mound materials (Otani *et al.,* 2019; Nandika *et al.,* 2021; Witasari *et al.,* 2022). Indeed, Mahdi *et al.,* (2020) and Witasari *et al.,* (2022) evidenced the antimicrobial activities of extracts derived from *Macrotermes bellicosus* and *M. gilvus* mound materials (termite gut, comb, and soil). However, studies isolated numerous actinobacteria (*Streptomyces* sp.), bacteria (*Bacillus* sp.), and fungi (*Termitomyces, Pseudoxylaria, Xylaria*) having potent antibacterial and/or antifungal activity from *Macrotermes barneyi* (Nagam *et al.,* 2021), *M. natalensis* (Um *et al.,* 2013), *Odontotermes formosanus* (Xu *et al.,* 2020), *O. obesus* (Agarwal *et al.,* 2023).

Given that *M. bellicosus* is used in traditional medicine against infectious inflammatory diseases in Benin (West Africa), Mahdi *et al.,* (2020) demonstrated the high antimicrobial activity of termite gut-, mound soil-, and fungi comb-derived extracts. However, the bioactive compounds-producing microorganisms from *M. bellicosus* mound materials were poorly documented. Therefore, we have recently undertaken an investigation of bioactive strains up to the present based on the bacterial isolates from *M. bellicosus* termite gut and mound soil (Sawadogo *et al.,* 2022, 2023). To the best of our knowledge, no fungal strain with antimicrobial activity was isolated from the *M. bellicosus* mound. Here this is the first study that aimed to screen and phenotypically identify the fungi strains isolated from *M. bellicosus* for their high antibacterial and antifungal activities against test pathogenic bacteria and fungi.

Materials and Methods

Collection of termite mound material

Mound materials consisted of soil and fungi combs. They were sampled from four active *Macrotermes bellicosus* termite mounds in the botanic reserve of Somgandé (12°24'30N, 1°29'30W and altitude 294) in Ouagadougou, Burkina Faso during the rainy season in June and July 2022. Sampling was carried out by excavating the mound using a 70% alcohol solution-sterilized hoe. Two samples of soil (200 g per sample) and fungi comb (50 g per sample) were aseptically withdrawn per termite mound using a sterile spatula as described by Sawadogo *et al.,* (2023). Samples were placed into sterile plastic

bags, kept in an icebox, and immediately transported to the Laboratory of Microbiology and Microbial Biotechnologies (LAMBM), University Joseph KI-ZERBO. Samples were stored at 4°C until use.

Isolation of fungi

Composite samples of fresh soils and fungi combs (1:1, w/w) were prepared separately in aseptic conditions. Ten grams (10 g) of each composite sample was suspended in 90 mL of sterile physiological saline solution (0.9% NaCl) in 120 mL flasks containing sterile glass beads. The mixtures were thoroughly vortexed for 2-15 min with regards to the type of sample. The suspensions were serially diluted 10-fold until 10^{-8}. Hundred µL aliquots of 10^{-5}-10^{-8} dilutions were spread onto duplicate Sabouraud Chloramphenicol Agar (SCA) plates in sterile conditions. Plates were incubated at 30°C for 2-5 days in aerobic conditions (Hamid *et al.*, 2015). The mycelia of single colonies were picked up and purified on Potato Dextrose Agar (PDA) plates at 30°C for 7 days, repeatedly until pure cultures were obtained. The pure fungal isolates were macroscopically and microscopically checked and then kept on PDA plates for short-term storage at 4°C as working stock.

Culture of test microorganisms

The test pathogenic microorganisms include *Bacillus subtilis* ATCC 6051, *Micrococcus luteus* SKN 624, *Staphylococcus aureus* ATCC 2523 (as Gram-positive bacteria), *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027 (as Gram-negative bacteria), and *Aspergillus niger* ATCC 16404(a fungus). They were obtained from a microbial collection of the Département de Technologie Alimentaire (DTA) at the Institut de Recherche en Sciences Appliquées et Technologies (IRSAT) in Ouagadougou, and maintained in pure cultures at the laboratory (LAMBM). Bacterial cultures were grown and maintained on Nutrient Agar (NA) and Nutrient Broth (NB) media at 37°C for 24 h, while the fungus *A. niger* was cultured on PDA and Potato Dextrose Broth (PDB) media and incubated at 30°C for 5-7 days (Sujada *et al.*, 2014). Test microorganisms were stored in NA and PDA media at 4°C as working stock, and in NB and PDB with 20% glycerol (v/v) for long-term storage at -20°C.

Test bacteria were standardized to 0.5 McFarland ($1.5x10^8$ CFU/mL) by using the sterile physiological saline solution before the inhibition test (Quintana *et al.*, 2015). Meanwhile, the mycelia of *A. niger* from PDA cultures were used for the inhibition test on agar plates (Salehghamari and Najafi, 2016).

Screening of fungal isolates with antimicrobial activities by dual culture method

The antibacterial test was performed by disc technique. A volume of 0.1 mL bacterial suspension was spread on Mueller Hinton Agar (MHA) plates. After drying plates, sterile 6 mm-diameter paper discs were imbued with the mycelia of 5- to 7-day fungal isolates and then deposited on the surface of MHA plates. All the plates were incubated in duplicate at 37°C for 48 h. Control plates without fungal isolates were prepared to check the normal growth of the test bacteria on MHA plates. Antibacterial activity was positive when a clear zone (inhibition zone) between test bacteria and isolate appeared, and the diameter of inhibition zone was measured.

The antifungal activity of fungal isolates was tested against *A. niger* in dual culture according to the adapted methods of An *et al.*, (2020) and Axanbayev and Ismailov (2022). The fungal isolates and *A. niger* were cultured individually on PDA plates at 30°C for 7 days. Afterward, a mycelial plug withdrawn from the edge of each fungal isolate was placed at 3 cm from the plate center. Four fungal isolates (mycelial plugs) were deposited at equal distances between themselves and around the center containing a mycelial plug of *A. niger*. The PDA plates inoculated only with *A. niger* were considered as negative controls. All cultures were carried out in duplicate and incubated at 30°C for at least 3 days until a margin of inhibition was observed. The

percent inhibition (PI %) of radius growth of *A. niger* was calculated according to Devi *et al.,* (2018) as follows:

$$PI\ \% = 100 \times (R-r)/R$$

Where R: Diameter of mycelial growth of *A. niger* in control plate (mm); r: Diameter of mycelial growth of *A. niger* in test plate (mm).

Production of antimicrobials from culture of fungal isolates

Based on their capacity to inhibit the growth of at least one test microorganism from the previous antimicrobial assays, the fungal isolates showing an inhibitory activity were subjected to fermentation culture. These isolates were used to evaluate their potential to produce antimicrobials in a broth medium according to the adapted methods of An *et al.*, (2020) and Xu *et al.*, (2020). Pure colonies of each fungal isolate were inoculated in 10 mL PDB and incubated at 30°C under shaking at 150 rpm for 48 h. Then the precultures were transferred into 250 mL flasks containing 90 mL fresh PDB and incubated again at 30°C at 150 rpm for 10 days.

Afterward, the fermentation broths were centrifuged at 6000 rpm for 15 min. The supernatants were taken and filtered using a sterile Millipore syringe-filter (0.20 µm diameter). The fermentation filtrates were collected into sterile tubes and stored at 4°C to be used for the second test of antimicrobial activity.

Antimicrobial assays from fungal culture filtrates

The agar well diffusion and disc diffusion techniques were used for the inhibition test against pathogenic strains from fermentation filtrates. In both techniques, a volume of 0.1 mL test standardized bacterial inoculum or 3-day *A. niger* broth (PDB) at 30°C was spread over the whole surface of MHA and PDA plates, respectively.

As for the agar well diffusion, holes with a 6 mm diameter were then punched aseptically in the plates with a sterile glass tip, and 10 µL fermentation filtrates were poured into the wells of agar plates. Whereas in the Kirby-Bauer disc diffusion technique (Bauer *et al.,* 1966), the sterile 6 mm filter paper discs were imbued with different filtrates (10 µL) and placed on the agar media surface.

All plates were incubated at 37°C for 24-48 h for the pathogenic bacteria or 30°C for 48-72 h for *A. niger*. Experiments were carried out in duplicate. Gentamicin (CN 10 µg/mL) and Nystatin (NY 100 IU) were used as a positive control against test bacteria and fungus, respectively. The antimicrobial activity of each filtrate was evaluated by measuring the diameter of inhibition zones around wells and discs using a transparent ruler.

Phenotypic characterization of fungal isolates

The pure fungal isolates with higher antimicrobial activity were grown onto PDA plates at 30°C for 3-5 days to determine their phenotypic features. Whenever fungal growth was observed, the morphology of colonies was recorded. Then, the hyphae of each isolate were microscopically observed as to the shape, container and structure of conidia/spores. The isolates were identified until the genus level using the guides of classification for fungi based on macro- and micro-observations (Williams-Woodward, 2001).

Results and Discussion

Isolation and antibacterial activity of fungal isolates

A total of 65 fungal strains were isolated from *M. bellicosus* mound materials among which 48 and 17 strains were obtained from mound soils and fungi combs, respectively. Based on the direct dual cultures of fungal isolates with test pathogenic bacteria, 48 (73.84%) isolates exhibited an antagonistic effect against at least one tested germ (Fig. 1, Table 1). 19 and 20 fungal isolates were bioactive against test Gram-positive (*B. subtilis*, *M. luteus,* and *S. aureus*) bacteria with the diameters of

inhibition ranging from 10-30 mm, and Gram-negative (*E. coli* and *P. aeruginosa*) bacteria with 7-25 mm of inhibition diameter, respectively. Interestingly among bioactive strains, 15 fungal isolates (31.25%) whose 9 (IM2s, IM5s, IM13s, IM15s, IM19s, IM20s, IM26s, IM41s and IM51s) and 6 (IM33m, IM36m, IM54m, IM55m, IM56m and IM60m) from soil and comb samples, respectively, exhibited broad-spectrum inhibitory activity against all test bacteria.

From all bioactive fungal isolates, 35 (72.91%), 30 (62.5%), 30 (62.5%), 26 (54.17%) and 26 (54.17%) strains highlighted a potent antibacterial effect against *M. luteus* (10-30 mm), *B. subtilis* (10-17 mm), *P. aeruginosa* (10-20 mm), *S. aureus* (10-25 mm) and *E. coli* (8-20 mm), respectively.

The isolate IM51s was the best candidate that has a large-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria with high inhibition diameters ranging from 10-30 mm.

Based on the results of the dual culture bioassay, the second screening of the antibacterial test was carried out with the filtrates from the cultivation of 48 bioactive fungal isolates.

Out of them, 14 (29.17%) isolates showed an inhibitory activity against at least one of the test bacteria (Table 2). Most filtrates of fungal isolates (12) had a potent inhibitory effect (10-35 mm) on *M. luteus*, whereas no or low inhibition (7-10 mm) was noted against other test bacteria.

The highest antibacterial activity was shown by IM2s (35 mm), IM15s (30 mm), and IM21s (30 mm) as higher or similar to the reference antibiotic Gentamicin (30 mm) face to *M. luteus* (Table 2, Fig. 2).

However, IM51s was the main isolate having displayed a more and less high inhibition against both *M. luteus* (25 mm), *B. subtilis* (10 mm), *P. aeruginosa* (10 mm), and *S. aureus* (7 mm) in this experiment. On the other hand, no isolate filtrate was effective against *E. coli*. In contrast to the paper disc diffusion method, the antibacterial bioassay using agar well diffusion technique with isolate culture filtrates revealed no inhibition regardless of bacteria tested overall, except for some rare cases of low inhibition (7-10 mm) such as IM13s against *M. luteus* and *S. aureus* (data unpublished).

This finding is supported by Arenas *et al.*, (2022) who found no or weak antibacterial activity of their endophytic and rhizospheric fungal isolates against *B. subtilis*, *S. aureus*, and four types of *E. coli* strains by agar well diffusion method.

Antifungal activities of fungal isolates

From dual cultures, 20 (30.77%) fungal isolates exhibited 13-60% inhibition against the strain *A. niger* (Table 3). Out of them, 6 isolates namely, IM1s, IM3s, IM31m, IM34m, IM40m, and IM61s inhibited 50-63% of the *A. niger* growth in this experiment.

Although elsewhere researchers found bioactive bacteria instead of fungi strains from termite mounds, their bacterial isolates have high antifungal activity against phytopathogenic fungi (Devi *et al.*, 2018) and *A. niger* (Sawadogo *et al.*, 2023) with 50-67.44% and 25-100% inhibition, respectively, by dual cultures. Our fungal isolates have inhibition values in this range. However, the antifungal activity of our fungal isolates is relatively lower than that of bacterial isolates of those authors.

No inhibition was noted whatever the filtrates of isolate culture were used through the disc diffusion method (Table 3).

Table.1 Inhibition diameter of fungal isolates against test bacteria in dual cultures.

Isolate code	Isolation source	Diameter of inhibition zone (mm)				
		Gram-negative bacteria		Gram-positive bacteria		
		E. coli	*P. aeruginosa*	*B. subtilis*	*S. aureus*	*M. luteus*
IM1s	soil	-	10	-	-	-
IM2s	soil	20	13	16	15	15
IM3s	soil	17	-	-	-	15
IM4s	soil	-	-	13	15	15
IM5s	soil	12	25	15	16	12
IM6s	soil	11	-	15	-	-
IM8s	soil	15	10	12	12	-
IM11s	soil	-	10	12	12	-
IM12s	soil	-	15	13	25	-
IM13s	soil	12	13	13	17	26
IM14s	soil	-	-	-	-	10
IM15s	soil	13	12	15	20	16
IM16s	soil	15	-	12	10	10
IM17s	soil	-	-	-	-	12
IM18s	soil	-	-	-	-	12
IM19s	soil	13	15	15	13	10
IM20s	soil	12	13	10	14	15
IM21s	soil	15	12	16	-	15
IM26s	soil	10	15	15	13	13
IM27s	soil	-	-	-	-	10
IM28s	soil	-	-	-	-	22
IM29s	soil	-	-	-	-	10
IM30m	comb	15	-	13	10	-
IM31m	comb	15	-	-	-	10
IM33m	comb	17	13	12	15	17
IM34m	comb	-	12	-	-	-
IM35m	comb	-	-	-	-	15
IM36m	comb	8	10	12	15	10
IM38m	comb	10	-	-	-	12
IM39m	comb	-	-	-	-	12
IM41s	soil	12	18	17	20	22
IM43s	soil	-	7	-	-	-
IM44s	soil	-	12	16	-	-
IM45s	soil	-	-	-	13	15
IM46s	soil	-	10	-	-	12
IM48s	soil	-	12	26	14	15
IM49s	soil	-	-	-	-	11
IM51s	soil	13	12	15	10	30

IM52s	soil	-	10	12	15	-
IM53s	soil	18	15	10	20	-
IM54s	soil	15	15	15	20	25
IM55m	comb	15	20	17	12	20
IM56m	comb	12	12	12	12	15
IM57m	comb	-	-	10	-	-
IM58m	comb	15	10	-	-	20
IM59m	comb	20	10	10	-	-
IM60m	comb	20	20	15	13	21
IM63s	soil	-	13	10	13	24
Total of bioactive isolates		26	30	30	26	35

-: no inhibition; IM..s and IM..m: codes of fungal isolate originating from mound soil and fungi comb samples, respectively.

Table.2 Inhibition diameter of filtrates from fungal isolates culture against test bacteria.

Filtrate code	**Diameter of inhibition zone (mm)**				
	Gram-negative bacteria		**Gram-positive bacteria**		
	E. coli	***P. aeruginosa***	***B. subtilis***	***S. aureus***	***M. luteus***
IM2s	-	-	-	-	35
IM4s	-	-	-	-	10
IM13s	-	-	-	7	-
IM15s	-	-	-	7	30
IM18s	-	-	-	-	12
IM20s	-	-	-	-	25
IM21s	-	-	-	-	30
IM31m	-	-	-	-	13
IM33m	-	-	-	7	-
IM51s	-	10	10	7	25
IM55m	-	-	-	-	12
IM60m	-	-	-	-	20
IM63s	-	-	-	-	20
IM65s	-	-	-	-	15
Gentamicin (control)	ND	ND	20	25	30
Total of bioactive isolates	0	1	1	4	12

-: no inhibition; ND: not determined.

Table.3 Inhibitory activity of fungal isolates against *A. niger* by dual culture and disc diffusion methods.

Isolate code	Dual cultures			Paper disc diffusion
	Radius mycelia control (mm)	Radius mycelia test (mm)	Percent inhibition (%)	Inhibition diameter (mm)
IM1s	15	6	60	-
IM3s	15	6	60	-
IM4s	15	8	48	-
IM5s	15	12	20	-
IM9s	15	12	20	-
IM13s	15	10	33	-
IM20s	15	13	13	-
IM26s	15	10	33	-
IM31m	15	6	60	-
IM32m	15	10	33	-
IM34m	15	7	53	-
IM37m	15	10	33	-
IM38m	15	10	33	-
IM40m	15	7	53	-
IM52s	15	12	20	-
IM58m	15	12	20	-
IM61s	15	6	60	-
IM63s	15	10	33	-
IM64s	15	10	33	-
IM65s	15	12	20	-
Nystatin(control)	NA	NA	NA	24

-: no inhibition; NA: not applied

Agar well diffusion and disc diffusion methods are used for testing the antimicrobial activity of compounds-containing filtrates (originating from mycelial cultures and/or extracts). However, it is well-known that these techniques allow us to determine whether the inhibitory activity of microbial isolates has an extracellular origin, *i.e.*, an antimicrobial compound is released in a medium (Sabdaningsil *et al.*, 2017). That suggests that our fungal isolates released less or no bioactive chemical compounds in the growth medium.

Furthermore, the low volume of filtrates (10 µL) in agar well and disc diffusion techniques, and the appropriate growth conditions of fungal isolates (pH, temperature, nutrients) not determined in our experiment could influence the optimal production and efficiency of antimicrobial substances. Thus, further studies are needed to elucidate the best conditions for both the growth of isolates and the production of antimicrobials followed by compounds extraction for further knowledge related to the minimum inhibitory concentration (MIC), minimum bactericidal or fungicidal concentration (MBC, MFC), and chemical composition of bioactive extracts.

Nevertheless, the dual culture of our fungal isolates showed better antagonistic activity against both bacteria and *A. niger* tested overall. This method has a similar effect to the agar plug diffusion method that permits to determine if the antimicrobial activity of the isolate has an intracellular origin (Manikkam *et al.*, 2014). That means our fungal isolates had more ability to produce antimicrobials by their mycelia as shown by IM2s, IM15s, IM51s, and IM60m for their potent antibacterial activity, and by IM1s, IM3s, IM31m, IM34m, IM40m, and IM61s for their high antifungal activity.

Table.4 Morphological characteristics and identification of fungal isolates.

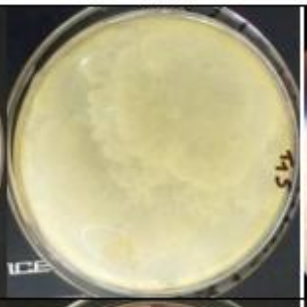
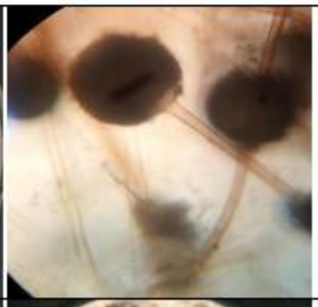
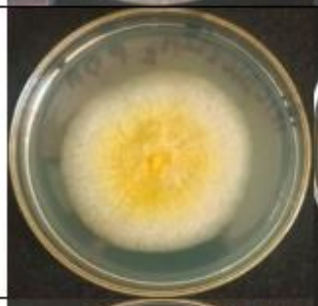
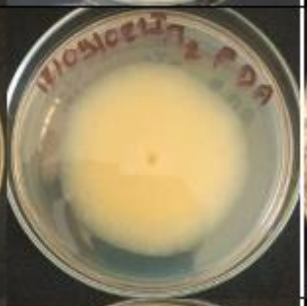
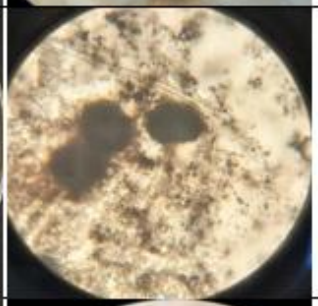
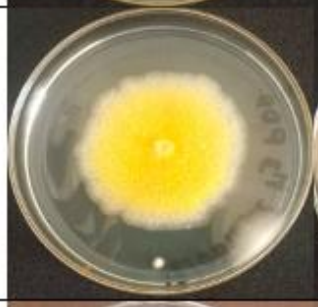
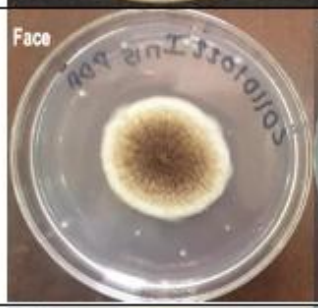
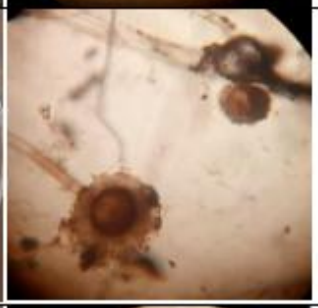
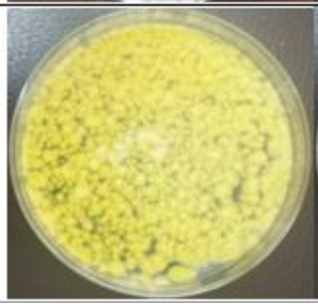
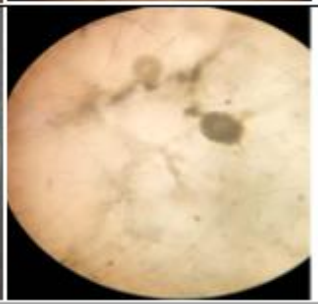
Isolate code	PDA plate culture		Hyphae structure	Phenotypic identification
	Front	Back		
IM1s				*Rhizopus* sp.
IM2s				*Aspergillus* sp.
IM3s				*Aspergillus* sp.
IM15s				*Aspergillus* sp.
IM31m				*Aspergillus* sp.

Table.4 Morphological characteristics and identification of fungal isolates. (continued)

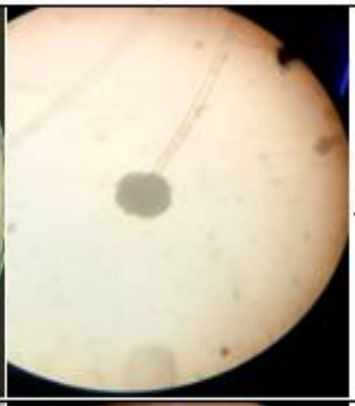
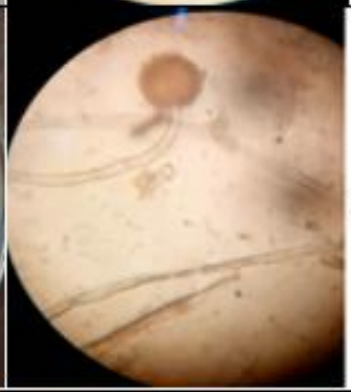
IM34m				*Aspergillus* sp.
IM40m				*Aspergillus* sp.
IM51s				*Aspergillus* sp.
IM61s				*Rhizopus* sp.

Fig.1 Antibacterial activity of soil- (IM..s) and comb (IM..m)-derived fungal isolates against *M. luteus* (A), *S. aureus* (B), *P. aeruginosa* (C), *B. subtilis* (D) and *E. coli* (E)

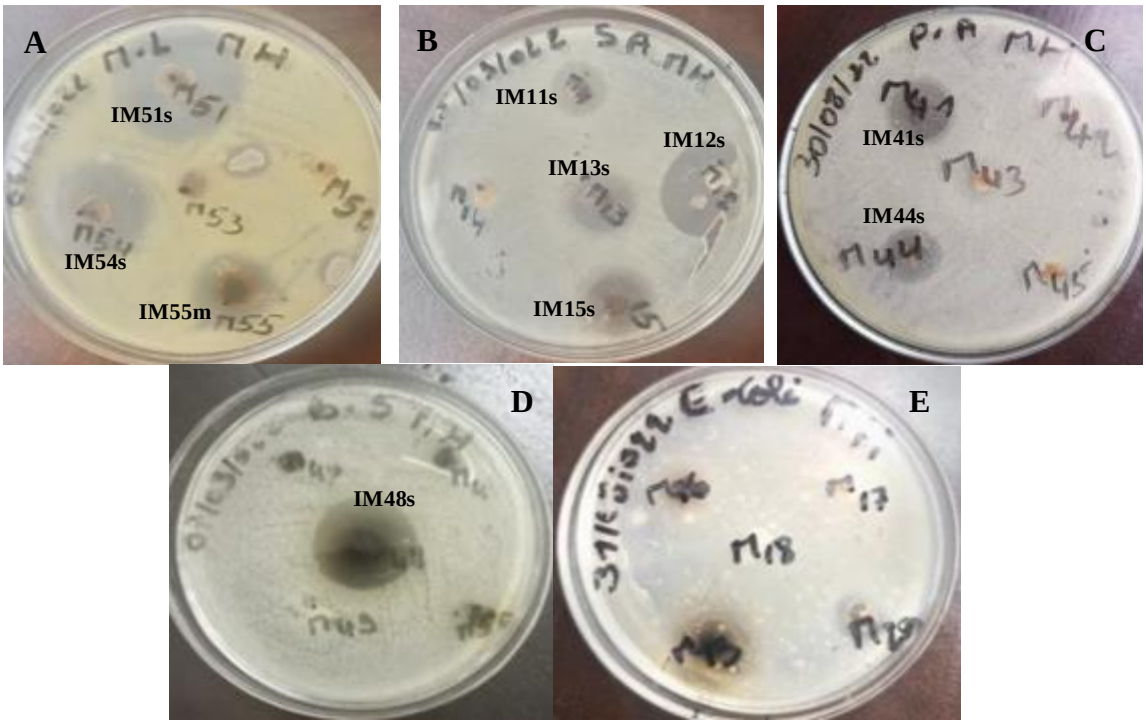
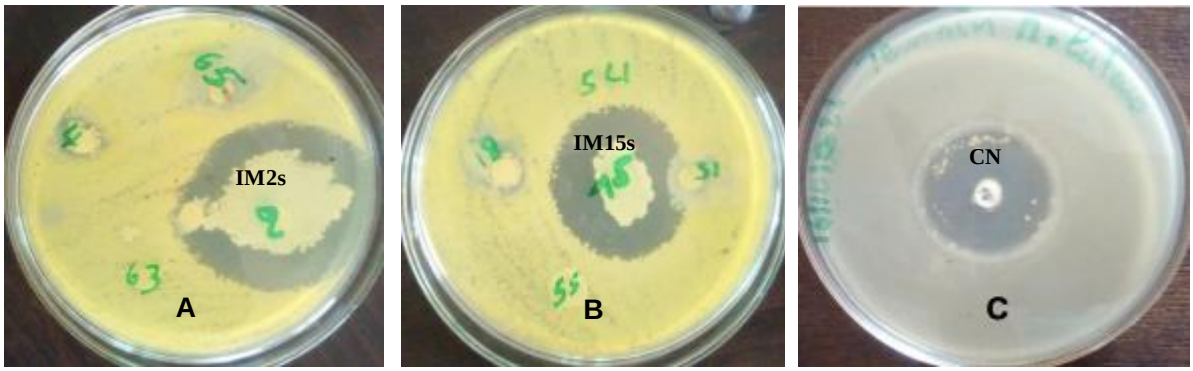


Fig.2 Antibacterial activity of the filtrate from fungal isolate cultures IM2s (**A**) and IM15s (**B**) and the antibiotic Gentamicin CN (**C**) against *M. luteus.*



Phenotypic identification of bioactive fungal isolates

Based on the greatest antibacterial or antifungal activity and the dichotomic key of fungi identification, a total of 9 bioactive fungal isolates were phenotypically identified (Table 4). Two isolates (IM1s and IM61s) were phenotypically identified as *Rhizopus* sp. and the 7 others (IM2s, IM3s, IM15s, IM31m, IM34m, IM40m, and IM51s) belonging to *Aspergillus* sp.

Generally, *Aspergillus* species are broadly well-known as endophytic and rhizospheric fungal strains associated with several ecological niches such as plants, sediments, and soil (Arenas *et al.*, 2022; Handayani *et al.*, 2018). The *Aspergillus* sp. are well-documented to produce numerous and potent antimicrobial agents (Suarez-Estrella *et al.*, 2007).

Afolami *et al.*, (2020) found more *Aspergillus niger* than other fungal strains (*Fusarium oxysporium, Fusarium fujikuroi,* and *Geotrichim condium*) isolated from termite nests on kola nut trees. Those fungal isolates displayed mild antimicrobial activities against both pediatric pathogenic bacteria and yeast with inhibition zones varying from 11.28 to 19.91 mm. Moreover, Arenas *et al.*, (2022) discovered numerous *Aspergillus* sp.-like fungal isolates inhibiting more test fungus *Cladosporium cladosporioides* than test bacteria. These latter findings corroborate more and less with our results where several of our *Aspergillus* sp.-aligned isolates highlighted inhibitory patterns against test pathogenic bacteria (10-35 mm) and fungus (53-60%). However, studies showed other fungal isolates, namely *Termitomyces*, *Pleosporales*, P*seudoxylaria*, and *Xylaria*, inhabiting *Macrotermes* and *Odontotermes* nests (Agarwal *et al.*, 2023; Nagam *et al.*, 2021; Otani *et al.*, 2019; Xu *et al.*, 2020). These isolates are recognized to play a crucial role in maintaining healthy colonies and survival by releasing antimicrobial compounds for suppressing putative competitive or antagonistic fungi of the cultivar fungus *Termitomyces* sp. and the fungal entomopathogens of termites. The molecular identification of our fungal isolates needs to be carried out to accurately define the species of isolates and strengthen the knowledge of different fungi with high antimicrobial potentiality inhabiting *M. bellicosus* mound material.

The fungal strains isolated from *M. bellicosus* mound soil and fungi comb are a potential source of antimicrobials. Nine fungal isolates were phenotypically identified among which seven as *Aspergillus* sp. and two *Rhizopus* sp. like. Most isolates exhibited either a potent antibacterial activity with 10-35 mm inhibition zones in the isolates IM2s, IM15s, IM51s or a strong antifungal activity (53-60% inhibition) in the isolates IM1s, IM3s, IM31m, IM34m, IM40m, and IM61s. The isolate IM51s identified as *Aspergillus* sp. showed a broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria, but no inhibitory effect on tested *A. niger*. The antimicrobial activity of these fungal isolates deserves further investigation by: (i) optimizing the conditions for the growth of isolates and the production of antimicrobials, (ii) determining the minimum inhibitory concentration and minimum bactericidal or fungicidal concentration of extracts, and (iii) determining the molecular diversity of fungal strains and the chemical composition of bioactive extracts. In conclusion, this study showed the fungal isolates associated with *M. bellicosus* materials have an antimicrobial potential that can serve as the resource of novel species and antimicrobials in various applications.

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