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ORIGINAL ARTICLE

Enhancement of Taxol Production by Endophytic fungi from *Hibiscus* and *Moringa* plant using Gamma irradiation

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Key words:
Moringa and Hibiscus plant, Fungal endophytes, Taxol, Gamma irradiation

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ABSTRACT

Background*: Taxol is a commercial anticancer drug, with broad spectrum towards different cancer cell as breast, lung, head and uterine cancers. Taxol producing by endophytes recovered from the medicinal plants.* ***Objective:*** *This evaluating of Taxol production by endophytes isolated from Moringa and Hibiscus plants. Enhancement of Taxol production by different types of media and Gamma irradiation were studied. The antimicrobial activity of Taxol was observed.* ***Methodology:*** *Fungal isolates were recovered from Moringa and Hibiscus plant. The samples were sterilized with ethanol (70%), wash with sterile water, sterilized tissue by sodium hypochlorite 2.5% then wash with sterile water. The isolates were grown on PDB media, after incubation, the cultures were filtered and characterized by UV-Vis., TLC, HPLC and FTIR.* ***Results:*** *Twenty seven isolates were separated from Moringa (16 isolates) and Hibiscus plant (11 isolates). These organisms were observed from barks (seven isolates), twigs (eight isolates), leaves (five isolates) and buds (seven isolates) of plants. Purified fungal isolates were Aspergillus, Penicillium, Cladosporium and Fusarium. Between these isolates, the prevalence of genus Aspergillus was detected (70.3%) while Fusarium remark to be (14.8%); also Penicillium was reported (11.1%) and finally Cladosporium (3.7%). From HPLC results the optimum Taxol production was Penicillium sp.$_5$ (54.42µg/L), Aspergillus niger$_{10}$ (43.95µg/L) and Fusarium sp.$_8$ (26.8µg/L) on potato dextrose agar medium.* ***Conclusion:*** *Improvement of Taxol harvest by Penicillium sp. from (54.42µg/l) to (184.3µg/l) on Dox medium, maximum yield of Taxol was appeared at 1.25 kGy (274.6µg/l). Finally a significant antimicrobial of Taxol towards E. coli 15.0mm and Ent. cloacae 22.0mm.*

INTRODUCTION

A medicinal plants one of the common important plants contains substances which is useful for medical purposes and synthesis of useful drugs. Medicinal plant species of *Taxus* harbor fungal endophytes in various parts such as leaves and stems[26]**.** Endophytes isolates are a division of molds that colonize living, internal tissues of plants without any apparent pathogenic infection to their host plants[13]. Endophytic fungi are exporters of a new bioactive mixture; the bioactive metabolites produced by fungal isolates generated from different biosynthetic pathways and belong to different structural groups such as terpenoids, quinones, steroids and phenols[15]. There is a great potential of finding new drugs from fungal isolates for treating new diseases in humans and animals[17]. There are large numbers of anticancer products by fungi populating various medicinal plants[16]. Endophytic fungi have been confirmed to be rich sources of different original composites with a wide spectrum of biological actions and a large level of structural variety. Taxol as an anticancer product can be useful against mammary and ovarian cancers and be used in hospitals and clinics. The most common origin of Taxol is the bark of trees belonging to the Taxus family including *Yew trees*[14]. The action of Taxol develops from its individual specificity for connecting with tubulin β-subunits heterodimer, increasing tubulin polymerization and disrupting mitotic part of the objective cells[5]. The drug has been established for the therapy of ovarian tumor by the Food and Drug Administration (FDA). Its action against lung cancer, head cancer, breast and neck cancer had been explained[18]**.** Gamma radiation has been proved to be a simple and efficient method for biosynthetic method of Taxol. The Taxol crop by fungi has been extra optimized with gamma-rays more than control.

In this work, screening of Taxol extract from fungal endophytes recovered from different medical plants was investigated. Taxol characterized by using UV-Visible

spectrophotometer, FTIR, TLC and HPLC analysis were recorded. The study of different nutritional factors and Gamma irradiation on Taxol crops by selected highly producer isolate were evaluated.

METHODOLOGY

Isolation and culture of the fungal isolates:

Various pieces of medicinal plant (*Moringa* and *Hibiscus*) as leaves, barks, twigs and buds were obtained from Faculty of Agriculture, Cairo University then utilized as a source of fungal endophytes. The medicinal plants were collected and cleaned under tap water to remove attached particles of soil. The samples were surface-sterilized successively through ethanol (70%) for 1 minute and then rinsed with sterile water. Then the tissue was sterilized by sodium hypochlorite 2.5% for 3 minute and again rinsed with sterile water five times[1]. The surface-sterilized part of plant was cut into about 0.5×1 cm pieces and located on the surface of sterilized (PDA) plates with ampicillin (1µg/ml) added to the media prior pouring into the plates[22]. Cultivated dishes were incubated at 30°C for seven days. The appeared fungal mycelium were picked up and purified by cultivating on new (PDA) plates. The purified fungal endophytes isolates were subcultured on PDA slants for seven days and storage at 4°C.

Screening of fungal endophytes producing Taxol:

The obtained endophytic fungi populating *Moringa* and *Hibiscus* plants were chosen for Taxol production by utilizing potato dextrose broth (PDB)[27]. The fungal isolates were cultivated on PDA media for seven days at 30°C. Two agar plug (approximately 1cm diameter) containing mycelia of every fungal isolate were injected in 100 ml of the potato dextrose broth in 250ml Erlenmeyer flasks, then incubation of cultures at 30°C below shaking statuses (120 RPM) for 15 days. After incubation, the fungal isolates were purified with filter paper; the filtrates were mixed with sodium bicarbonate (0.2%) for precipitation of fatty acids. Taxol has been separated with double volume of dichloromethane (DCM), and the organic condition was collected and fumigated to dryness, and the precipitate was re-dissolved in three ml of methanol[12].

Characterization of endophytic Taxol by U.V, FTIR, TLC and HPLC:

The UV absorption of purified Taxol was detected at λ 227 nm (RIGOL, Ultra-3000 Series) comparing to standard Taxol. Taxol was separated and known by TLC study working Merck 1mm (20×20 cm) pre-coated silica gel plates (TLC Silica gel 60 F254, Merck KGaA, Darmstadt, Germany)[23]. From the TLC data, The precipitated silica particles were removed and the supernatant was taken for Taxol purification checking by HPLC (Agilent Technology, G1315D) of C18 reverse phase column (Eclipse Plus C18 4.6×150 mm, 3.5 µm, Cat.# 959963-902). The mobile phase was methanol/ acetonitrile/water (25:35:40, v/v/v) at movement rate 1.0 ml/min for 20 min[5]. FT-IR spectrum of the cleaned Taxol sample was examined by JASCO FT-IR 3600 Spectrophotometer, the absorption was estimated in the district 500 to 4000 cm^{-1} [18,26].

Morphological description of the endophytic fungal isolates:

The purified fungal isolates were observed daily based on the microscopic examination as colony diameter, extracellular exudates, mycelium color, conidial heads, pigmentation, fruiting bodies and sporulation. The cleaned fungal isolates were classified to the genus and species levels according with the morphological features[21,3]. Photo of fungal conidia were taken with light microscope at 1000× magnification.

Impact of various types of media on Taxol yield:

The chosen endophytes isolates were cultivated on four types of broth media, Malt Extract broth, Potato Dextrose broth (PDB), Czapek's Dox broth and MID broth media. Two agar plugs old pure cultures of fungal isolate were injected in 100 ml broth/250 ml Erlenmeyer flask of chosen broth media matching to the negative control. The flasks were incubated at 30°C for 15 days under shaking (120 RPM). After incubation, broth cultures were filtered. Taxol was separated and determined by method mentioned above.

Optimization of fungal isolate to maximize of Taxol yield by Gamma irradiation:

Gamma irradiation was applied at NCRRT on plates of endophytic fungal culture using ^{60}Cobalt source (Gamma cell 4000-A-India) at varying doses (0.25, 0.50, 0.75, 1.0, 1.25, 1.50, 2.0 and 3.0 kGy) matching to the control (un-irradiated) cultures, at dose rate 1.2 kGy/h. The irradiated fungal germination ware inoculated as mention above matching to the cultural (non-irradiated). The fungal cultures were incubated; Taxol was extracted, purified and determined by UV-Visible spectrophotometer as described above[6].

Antimicrobial activity of Taxol toward several microorganisms:

Taxol activity against wound infectious bacterial isolates was observed collected from Drug Microbiology Lab, Drug Radiation Research Dep, NCRRT, Cairo, Egypt. *Staphylococcus epidermidis* and *Staphylococcus aureus* as a model of Gram-positive and *Pseudomonas aeruginosna* and *Escherichia coli* as a model of Gram-negative. These microorganisms were freshly streaked, incubated at 37°C for 24 h. Bacterial were suspended in pure peptone water to obtained suitable inoculum. The growth inhibition (mm) of bacteria was evaluated by the disc diffusion technique. Sterile paper discs (6.0 mm) were immersed in 20 µl of methanol as negative control whereas positive control was amoxicillin-clavulanic acid (AMC). 20 µl from Taxol concentration was loaded on discs. The plates were observed at 37°C for 24 hours, and the inhibition

zones were measured. The inhibition zone of growth was measurement by a Vernier caliper (mm)[4].

RESULTS

Isolation of endophytes isolates from *Moringa* and *Hibiscus* plant:

Twenty seven endophytic isolates were separated from the twigs, barks, leaves and buds of *Hibiscus* plant (11 isolates) (Figure 1a) and *Moringa (*16 isolates) (Figure 1b;1c) on suitable medium as recorded in table (1), these isolates were found in barks (seven isolates), twigs (eight isolates), leaves (five isolates) and buds (seven isolates) of plant as noted in table (2) and (Figure 1d). The purification of fungal isolates was remarked in (Figure 2a;2b) and classified into the species level according to their morphological features by universal keys and were reported to belongs to 4 genera namely; *Aspergillus*, *Penicillium*, *Cladosporium* and *Fusarium.* Out of them, prevalence of genus *Aspergillus* was related to be (70.3%) while *Fusarium* remark to be (14.8%); also *Penicillium* reported (11.1%) and finally *Cladosporium* (3.7%). *Aspergillus* was recorded by four species namely; *Aspergillus flavus* (tow isolates), *Aspergillus oryzae* (six isolates), *Aspergillus niger* (five isolates), *Aspergillus fumigatus* (three isolates), and *Aspergillus terreus* (three isolates). *Penicillium* was reported three species, one species of *Cladosporium* and finally *Fusarium* noted four species. Taxol yield by the fungal endophytes was evaluated by cultivating on PDB, incubation at the suitable conditions, extraction and characterized by UV-Vis. spectrum (Figure 2c) TLC and HPLC as shown (Figure 3).

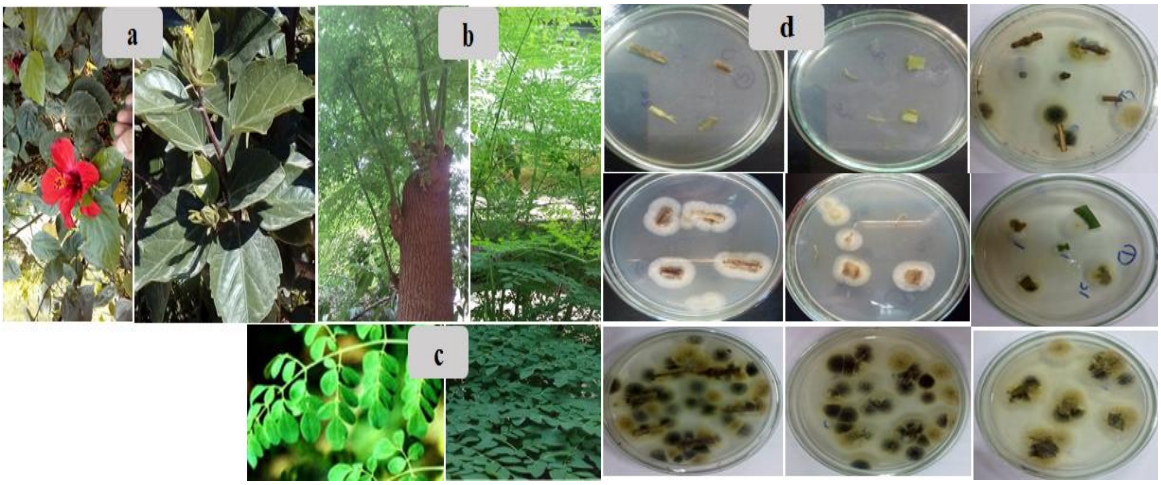

Fig. 1: a) Morphological views of Hibiscus; (b and c) Moringa plant; (d) appearance of fungal growth from inner leaves, barks, twigs and buds.

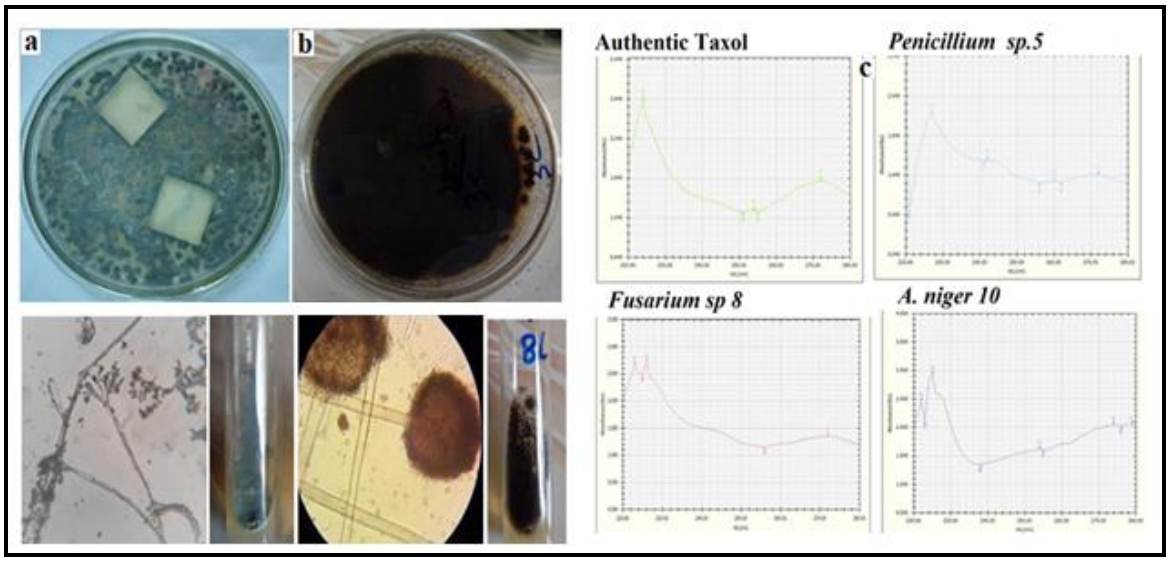

Fig. 2: Purification and microscopic features of *Penicillium sp*$_5$ (a), *A. niger*$_{10}$ (b) and c) UV-Vis. spectrum of extracted Taxol by endophytic fungi.

Table 1: Isolation of endophytic fungi from of Jojoba and Hibiscus:

Plant name	No of Endophytic Fungi	Endophytic Fungi
Moringa tree	16 isolates	*Aspergillus spp.* *Penicillium spp* *Fusarium spp.* *Cladosporium sp*
Hibiscus plant	11 isolates	*Aspergillus spp.* *Fusarium spp.*

Table 2: Screening for Taxol producing endophytic fungi of *Moringa* and *Hibiscus*:

Isolate No.	Fungal Isolate	Isolate Source	Taxol evaluation		
			UV Abs (227 nm) (µg/ ml)	TLC	HPLC (µg/L)
1	*Aspergillus niger*$_3$	Barks	9.08	+	-
2	*Aspergillus flavus*$_3$	Barks	9.08	+	-
3	*Aspergillus terreus*$_3$	Barks	-	-	-
4	*Penicillium* sp.$_2$	Barks	-	-	-
5	*Fusarium* sp.$_4$	Twigs	-	-	-
6	*Aspergillus oryzae*$_4$	Twigs	2.13	+	-
7	*Cladosporium* sp.$_2$	Twigs	-	-	-
8	*Aspergillus fumigatus* $_2$	Twigs	-	-	-
9	*Aspergillus niger*$_4$	Twigs	3.98	+	-
10	*Fusarium* sp.$_5$	Leaf	-	-	-
11	*Penicillium* sp.$_3$	Leaf	-	-	-
12	*Aspergillus terreus* $_4$	Leaf	2.35	++	10.34
13	*Aspergillus niger*$_5$	Buds	-	-	-
14	*Aspergillus oryzae* $_5$	Buds	-	-	-
15	*Fusarium* sp.$_6$	Buds	3.59	+	14.25
16	*Aspergillus oryzae* $_{13}$	Buds	-	-	-
17	*Aspergillus niger9*	Barks	3.46	+	13.51
18	*Aspergillus fumigatus5*	Barks	-	-	-
19	*Penicillium sp. 5*	Barks	22.93	++	54.42
20	*Aspergillus terreus7*	Twigs	-	-	-
21	*Fusarium sp.8*	Twigs	6.87	+	26.8
22	*Aspergillus oryzae8*	Twigs	-	-	-
23	*Aspergillus niger 10*	Leaf	11.25	++	43.95
24	*Aspergillus oryzae9*	Leaf	-	-	-
25	*Aspergillus fumigatus 6*	Buds	4.51	+	17.62
26	*Aspergillus oryzae 10*	Buds	-	-	-
27	*Aspergillus flavus 6*	Buds	3.62	+	14.15

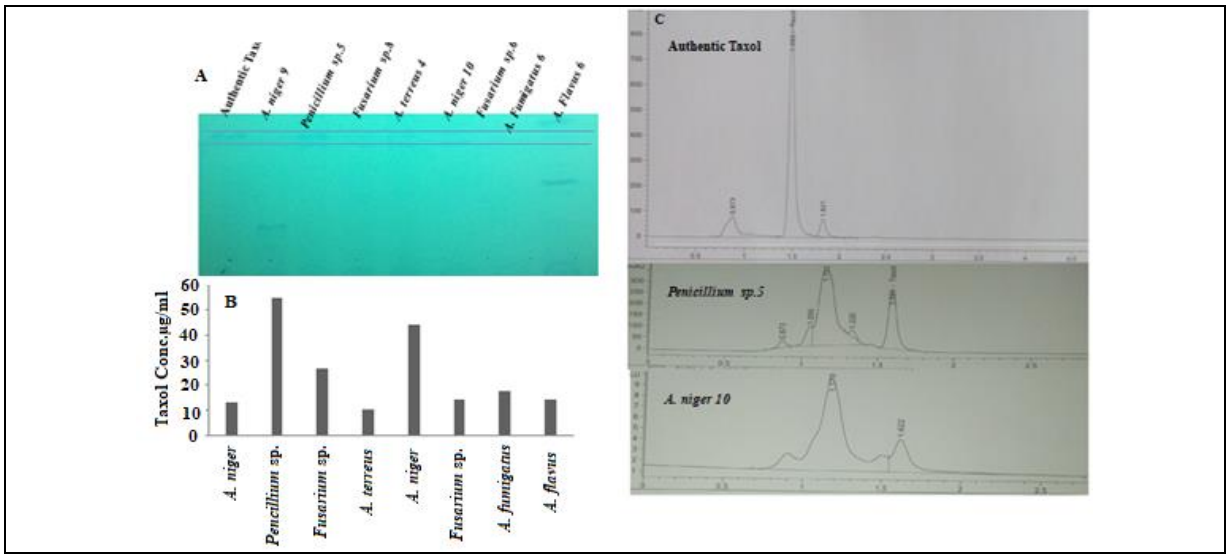

Fig. 3: Chromatographic analysis of the extracted Taxol from the potent fungal endophytes of *Moringa* and *Hibiscus* plant. a) TLC analysis comparing to authentic Taxol. b) The most potent fungal isolates for Taxol production. c) HPLC analysis for the highest endophytic fungi for Taxol production.

Screening of Taxol yield by fungal isolates:

From table (2); twenty seven isolates were captured from the twigs, barks, leaves and buds of *Moringa* tree and *Hibiscus* plant on PDA as illustrated table (2). Out of the total isolates, there were twelve isolates (44.4%) positive for Taxol crops and fifteen isolates (55.6%) were negative for Taxol yielding. In here research, Out of positive results eight isolates belonged to the genera *Aspergillus;* three of them were reported from Barks and two isolates from leaves, twigs and buds. However two isolates belonged to the genera *Fusarium sp.* and finally one isolate belonged to genera *Penicillium sp.* and *Cladosporium sp.*

Characterization of Taxol yield by UV-Vis, TLC, FTIR and HPLC analysis:

The UV-Vis. absorption spectrum of separated Taxol analysis showed a peak with absorption maxima at 230 nm, matching to authentic Taxol. Blank media was utilized as a negative control. The observed production of Taxol has been described by *A. niger*$_{3,}$ *A. flavus*$_{3,}$ *A. niger*$_{9}$, *Penicillium sp.*$_{5}$, *A. oryzae*$_{4,}$ *A. niger*$_{4,}$ *Fusarium sp*$_{8}$, *A. terreus*$_{4}$, *A. niger*$_{10}$, *Fusarium sp.*$_{6}$, *A. fumigatus*$_{6}$ and *A. flavus*$_{6}$ as recorded in table (2). Taxol was definite by TLC, which displaying line under UV lighting at 254 nm with R.f value 0.65 which was identical to standard Taxol (Figure 3a). Also, the Taxol spots showed a bluish spot when reacted including vanillin/sulfuric acid reagent alike to that of authentic Taxol. Out of the results from table (2) three fungal isolates presented the greatest potency for yield of Taxol, related to genus *A. niger*$_{10}$, *Fusarium sp.*$_{8}$ and *Penicillium sp.*$_{5.}$ The accepted Taxol spots were scrapped-off from the silica particles and moreover analyzed.

From the HPLC and TLC studies (Figure 3a; 3c) the maximum Taxol yield has been detailed by *Penicillium sp*$_{5}$. (54.42µg/l), followed by *Aspergillus niger*$_{10}$ (43.95µg/l), *Fusarium sp*$_{8}$ (26.8µg/l), *Aspergillus fumigatus*$_{6}$ (17.62µg/l). Different concentration of Taxol (10.34 - 14.25 µg/l) was recorded by *Aspergillus niger*$_{9,}$ *Aspergillus terreus*$_{4,}$ *Fusarium spp*$_{8}$ and *Aspergillus flavus*$_{6}$ as showed table (2). Whereas the other fungal isolates did not show any Taxol yield according to TLC and HPLC examination. Among the recuperated fungi, three isolates presented the greatest influence for Taxol yield, *Penicillium sp*$_{5,}$ *A. niger*$_{10}$ and *Fusarium sp*$_{8.}$ have been separated from barks while *Aspergillus niger*$_{10}$ have been recovered from leaves and the *Fusarium spp*$_{8}$ has been observed from twigs.

Taxol of *Penicillium spp*$_{5}$ and *A. niger*$_{10}$ agree with FTIR peaks of authentic Taxol. The peak at 3315.42 cm^{-1} was assigned for the hydroxyl (OH). While, the peaks at 2941.28 were assigned to the aliphatic CH stretch, the peaks at 1854.09 cm^{-1} was corresponding to C=O stretching frequency. The registration peak perceived at 1449.6 cm^{-1} and 1415.72 were due to the NH stretching frequency. The carbonyl group-oxygen stretching frequency was watched at 1114.42 cm^{-1}. Finally the peak 1021.55 cm^{-1} was due to the occurrence of aromatic C and H bends. FTIR of the produced Taxol was also similar in comparison with standard Taxol and literatures. So, it was apparent that this isolates displayed positive results for taxol yielding in PDB media (Figure 4a).

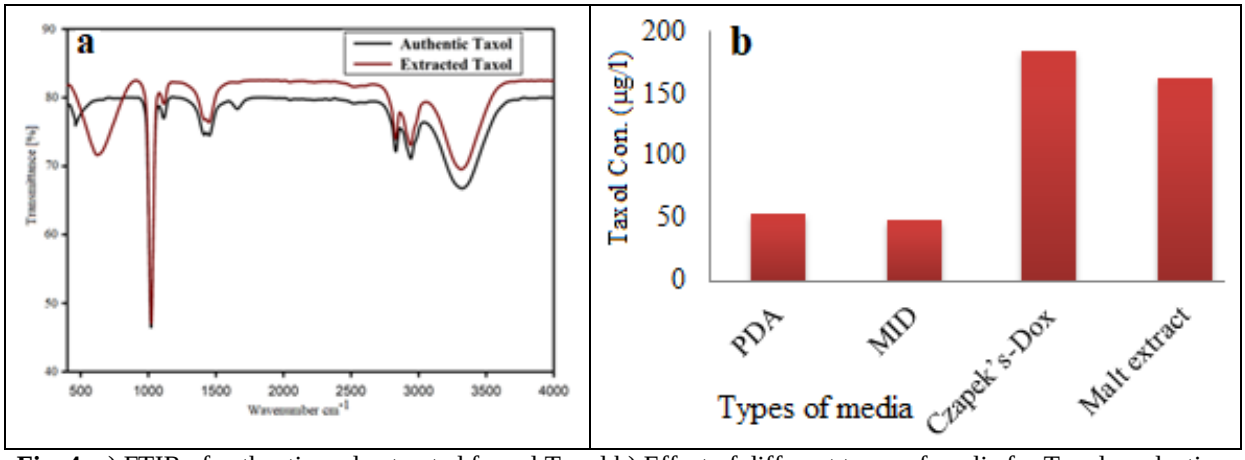

Fig. 4: a) FTIR of authentic and extracted fungal Taxol b) Effect of different types of media for Taxol production

Morphological description and Taxol crop:

The morphological characteristics of the great three fungal isolates (*Penicillium* sp_5, *A. niger*$_{10}$ and *Fusarium* sp_8.) producing Taxol has been presented in (Figure 2a; 2b) these fungal isolates were identified and agree with the universal morphological keys[21]. Endophytes isolates are specially *Penicillium* sp. developing optimally at 30°C at Czapek's-Dox agar, blue green conidia beside a granular colony surface were produced, with exudate droplets. The conidiophores are terverticillate, lightly roughened on Czapek's with special phialides and metula, with smooth-walled and globose conidia[19]. The colony convert to cream yellow color pigment dispersed into the agar medium. Morphological identification of *A. niger*$_{10}$ showed that colonies on PDA grow rapidly at 30°C with filamentous, white, basal mycelium. After a few days, the colonies started to sporulate with black, velvety conidia. The *Fusarium* sp. was also reported in our study as endophytic isolates which produced white villous colonies and produced purple pigment after seven days on PDA at 30°C. The morphological and microscopical features of this isolate ideally follow the explanation of *Penicillium* $sp._5$, *A. niger*$_{10}$ *and Fusarium* $sp._8$ as displayed in (Figure 2). Taxol Production by *Penicillium* $sp._5$ has been chosen for further experiments.

Enhancement Taxol *Penicillium* sp_5 by the various type of medium:

The influence of different types of media towards the yield of Taxol by *Penicillium* $sp._5$ has been investigated by cultivating the endophytes isolate on MID, PDB, Czapek's-Dox and Malt extract. After incubation for isolates; Taxol was separated and determined as mentioned above. The results in (Figure 4b), the maximum Taxol crops by *Penicillium* $sp._5$ has been recovered by cultivating on Czapek's-Dox medium (184.3µg/l), followed by Malt Extract media (162.8 µg/l) however, average crop of Taxol was recovered upon emerging the isolate on PDB and MID media (54.42 and 49.6µg/l) respectively Thus, upon nutritional optimization bioprocessing, Taxol production with *Penicillium* $sp._5$ has been enhanced from (54.42µg/l) to (184.3µg/l).

Development of Taxol *Penicillium sp.*$_5$ by Gamma irradiation:

Data presented in (Figure 5a) indicated that the mycelium growth of *Penicillium* $sp._5$ was irradiated by gamma rays at several doses (0.0, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0 and 3.0 kGy) and cultivated on the optimized Czapek's-Dox medium. The endophytes isolates were incubated under suitable conditions and Taxol was purified and determined by UV-Vis. spectrum. In the recent study, strain improvement via exposure to gamma radiation. From (Figure 5a) low dose of radiation has lower results on Taxol crop (135.4µg/l to 239.5µg/l) at three irradiation dose (0.50, 0.75 and 1.0 kGy. The obtained data indicated the effective dose (1.25 kGy) for production of Taxol (274.6µg/l).

Table 4: Effect of different doses of Gamma irradiation for Taxol yield by *Penicillium* sp.$_5$

Fungal Isolate	Doses (kGy)	Taxol Concentration (µg/ ml)
Penicillium sp_5	0	135.4
	0.5	198.6
	0.75	216.2
	1.0	239.5
	1.25	274.6
	1.50	188.2
	2.0	120.2
	3.0	93.4

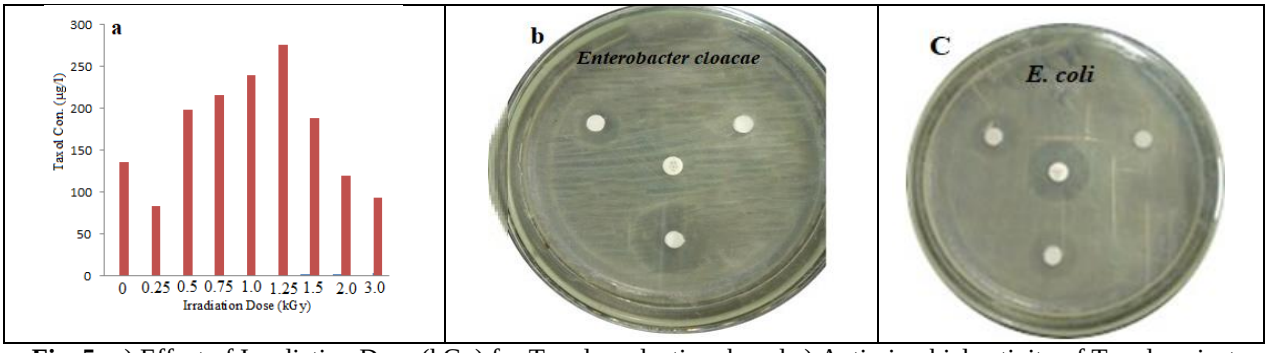

Fig. 5: a) Effect of Irradiation Dose (kGy) for Taxol production, b and c) Antimicrobial activity of Taxol against different microorganism

Antimicrobial activity Taxol against pathogenic bacteria:

The antimicrobial action of the separated Taxol from *Penicillium* sp_5 has been mentioned against different multidrug resistant bacteria as *Staphylococcus aureus* and *Staphylococcus epidermidis* a model of Gram-positive also *Enterobacter cloacae* and *Escherichia coli* as a model of Gram-negative by the disc diffusion methods. Authentic Taxol and AMC were employed as positive controls. From our results (Figure 5b,c) and table (3) the antimicrobial action of extracted Taxol had a greater significant toward *E. coli* (15.0mm ZOI) and *E. cloacae* (22.0mm ZOI). Whereas Taxol mild activity towards *S. epidermidis* and *S. aureus* (13.0mm-12.0mm ZOI) respectively. Taxol performed important antimicrobial activity toward Gram negative bacteria more than Gram positive bacteria.

Table 3: Antimicrobial activity of Taxol against different pathogenic microorganisms represented by diameter of inhibition zone (mm):

	Authentic Taxol	AMC	Methanol	*Penicillium* $sp._5$ Taxol
Staphylococcus epidermidis	8.0	25.0	6.0	13.6
Staphylococcus aureus	9.0	24.0	6.0	12.0
Escherichia coli	12.0	23.0	6.0	15.0
Enterobacter cloacae	14.0	6.0	6.0	22.0

The concentration of authentic Taxol and *Penicillium* sp_5 Taxol was is 20 µM

DISCUSSION

Endophytic isolates with Taxol giving potency advanced the support for a mass crop of Taxol due to their speedy growth, cost-effective fermentation process, independence on climatic changes, resistance to shearing and utility of genetic manipulation[10,7]**.** Most of the extracted Taxol producing by isolates was recovered from *Taxus* sp and *Podocarpus* sp which are belonging to family *Taxaceae*[24]**.** Medicinal plants act as a patent war zone as highly valuable commodities through modern technologies. Several advanced countries were actively engaged with medicinal plants for therapeutically precious and biologically effective Phytochemical[20]. This study also showed such a trend of the variety of fungal isolates which was apparent with the twigs, barks, leaves and buds parts collected from *Moringa* and *Hibiscus* plant. Out of twenty seven fungal isolates screened, the predominant isolated fungal genus was *Aspergillus*, *Penicillium*, *Cladosporium* and *Fusarium.* This study coincides with the study of[2]**.** Between these isolates, the prevalence of genus *Aspergillus* (70.3%) while *Fusarium* remark (14.8%); also *Penicillium* reported (11.1%) and finally *Cladosporium* (3.7%). In a similar study including biodiversity studies on fungal endophytes from *Calotrophis gigantea* belongs to the species *Aspergillus*, *Phoma* and *Penicillium*[12]**.** Three species of *Penicillium* were recovered, one species of *Cladosporium* and four species of *Fusarium.* Our results agree with a similar study[11] the authors found Endophytic fungi produce the most broadly used antibiotic and anticancer drugs.

In our study, Out of eight *Aspergillus* isolates*;* three of them were recovered from Barks and two isolates from leaves, twigs and buds. Whereas two isolates belonged to the genera *Fusarium sp.* and finally one isolate belonged to genera *Penicillium sp.* and *Cladosporium sp.* similar screening study for Taxol crop has been described for endophytes from *Podocarpus gracilior*[5] and other plants. Production of Taxol by *Penicillium sp* which agree with *P. polonicum* which has been represented as endophytes from *Ginko biloba*[1], *A. flavipes* and *A. terreus* an fungal isolates of *Podocarpus gracilior*, moreover for fungal isolates of *Taxus spp* as *Fusarium solani*, *A. niger*[28] and *A. fumigatus*[25]. Results of TLC, UV- absorption and HPLC analysis the maximum yield of Taxol has been recorded by *Penicillium* sp_5. (54.42µg/l), followed by *Aspergillus* $niger_{10}$ (43.95µg/l), *Fusarium* sp_8 (26.8µg/l), *Aspergillus* $fumigatus_6$ (17.62µg/l). Different concentration of Taxol (10.34 - 14.25 µg/l) were recorded by *Aspergillus* $niger_9$, *Aspergillus* $terreus_4$, *Fusarium* spp_8 and *Aspergillus* $flavus_6$ a similar screening model for Taxol crop was represented with fungal endophytes from *Ginko biloba*[1] and other plants[5,7]. The Taxol yield by *Penicillium* $sp._5$ has been improved by adaptation of the chemical components of Czapek's-Dox as general growing medium. Taxol separated by *Penicillium* $sp._5$ has been enhanced from (54.42µg/l) to (184.3µg/l).

Gamma irradiation plays an important role in enrichment of Taxol crops by exposure of the culture to gamma rays and the fungal cultures were cultivated on the adjusted Czapek's-Dox medium, Taxol was separated and quantified. The Taxol crop by *Penicillium* $sp._5$ was improved from (135.4µg/l to 274.6µg/l) at the optimum dose 1.25 kGy. Similar results have been described for yield of Taxol by *P. polonicum* and *Fusarium maire* that improved by

Gamma irradiation[1,28]. The purified Taxol revealed inhibitory activity towards Gram negative bacteria (*E. coli* 15.0mm and *E. cloacae* (22.0mm.) more than Gram positive bacteria. This study coincides with the study of Abdel-Fatah et al.[1].

CONCLUSION

The variety of fungal endophytes which was apparent with the twigs, barks, leaves and buds parts collected from *Moringa* and *Hibiscus* plant were observed and this isolates were classified into species according to their morphological features. The isolate *Penicillium* sp.$_5$ was recovered from barks of *Moringa* plant and accepted as a great Taxol yielder. Taxol production by *Penicillium* sp.$_5$ was improved by using nutritional requirements of the isolate by cultivated on the Czapek's-Dox medium. Taxol production was enhanced by using different dose of gamma rays from (135.4µg/l to 274.6µg/l) at 1.25 kGy.

Ethical statement: This article does not contain any studies with human participants or animals.

- The authors declare that they have no financial or non-financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher

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