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Nutritional Optimization of Camptothecin Biosynthesis by *Aspergillus terreus* and *Penicillum sclerotigenum*.

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Abstract: Camptothecin (CPT) is a cytotoxic quinoline alkaloid that inhibits the DNA enzyme topoisomerase I (topo I). Endophytic and filamentus fungal isolates recovered from various medicinal plants were screened for their CPT biosynthetic potency. Among these isolates, *Aspergillus terreus* and *Penicillum sclerotigenum* recovered from *Cinnamomum camphora* and *Ficus elastica* respectively were reported as potent camptothecin producers (89.4 mg/l and 80.2mg/l). The chemical identity of *A. terreus* and *P. sclerotigenum* extracted CPT was verified by Thin Layer Chromatography (TLC) and Ultra Violet Spectroscopy Analyses (UV). Upon using the Blackett-Burman design to optimize their nutritional requirements by the selected fungal isolates, their yield of camptothecin was increased by approximately 7 folds, revealing the essentiality of some carbon, nitrogen sources, and growth elicitors for the biosynthesis of camptothecin.

keywords: Camptothecin, *Cinnamomum camphora*, *Aspergillus terreus*, TLC, UV.

Introduction

A pentacyclic pyrroloquinoline alkaloid known as camptothecin (CPT) was first discovered and identified in the Chinese tree *Camptotheca acuminata* (happy tree) [1]. It naturally appears in southern China and India [2]. Plant species *Merrilliodendron*, *Nothapodytes*, *Ervatamia*, *Mostuea* and *Ophiorrhiza* have also been identified as possible producers of camptothecin [3]. Furthermore, CPT is a potent anticancer drug with a special affinity for DNA topoisomerase-I (Topo 1) [4], which causes protein-DNA breaks in a variety of tumour cell types [5]. The DNA supercoiling is relaxed by the topoisomerase-I by establishing an ester bond with the 3' end of nicked DNA through its catalytic tyrosine [6], this releases supercoiled product as a result of excessive tumor cell replication. CPT is a member of the monoterpenoid indole alkaloids and is produced when the monoterpenoid "secologanin" and the indole tryptamine are combined by the enzyme strictosidine synthase to yield the widespread precursor strictosidine (STR) [7, 8]. Tryptophan decarboxylase (TDC) converts tryptophan into the decarboxylated form of tryptamine, whereas secologanin is produced through the mevalonate route from terpenes [9]. The bulk of bioactive secondary metabolites, including Taxol and Camptothecin, are synthesised via the mevalonate pathway, which is a universal 5-carbon precursor (isopentenyl pyrophosphate and dimethylallyl pyrophosphate) pathway [10]. Several rate-limiting enzymes, including geraniol synthase (GES), secologanin synthetase (SLS), strictosidine synthase (STR), strictosidine -glucosidase (SGD), and tryptophan decarboxylase, control the committed phases of CPT biosynthesis. (TDC). The likely process of camptothecin production in fungi is shown. After Taxol and Vincristine, CPT is the third-largest anticancer drug in terms of revenue [6]. Nevertheless, there are significant obstacles that prevent this chemical from being used in clinical applications as the core camptothecin component's gastrointestinal sensitivity and low water solubility [3, 10]. Interestingly, this restriction has been overcomed by the creation of novel chemical compounds of camptothecin with increased

solubility in water, as 10-hydroxy-camptothecin, irinotecan and topotecan [6], which has received FDA approval as a wide range anticancer medication. Due to the high demand and poor camptothecin yield from their original sources (*C. acuminate*), these plants were destructively harvested in China and India, which had a significant impact on the ecosystems of those countries. Typically, the bioactive chemicals found in plants seem to be limited, difficult to extract, complicated satirically with many chiral centers, bulky, and aromatically varied [11, 12], so the current challenge is to find alternative methods with better camptothecin output. Untapped reservoirs of highly physiologically active substances including Taxol [13, 14, 15], Hypericin [16], and Azadirachtin [17] are thought to exist in fungal entophytes. The feasibility of metabolic manipulation, their rapid growth on culture media, their ability to produce large quantities of biomass, and their independence from environmental conditions, support the viability of prospective endophytic fungi applications for the synthesis of beneficial secondary metabolites [13, 14, 15, 18]. CPT was firstly isolated from *Entrophospora infrequens* and *Nothapodytes foetida*" [19, 20, 21]. *Fusarium solani* "endophyte of *Apodytes dimidiate*" [22], *Aspergillus* spp, and *Trichoderma atroviride* "endophytes of *C. acuminata*" [23], *Alternaria alternate*, *Fomitopsis* sp and *Phomopsis* sp "endophytes of *Miquelia dentata*" were described as methoxy camptothecin and hydroxy-camptothecin producers [24]. Nevertheless, the largest barrier preventing the further use of the fungal approach for industrial application is the lower yield of fungal CPT, which comes in addition to the loss of CPT productivity and attenuating the expression of their biosynthetic machinery with the fungal storage and repetitive sub-culturing [3,23,24]. So the ultimate goal is to find new endophytic fungal isolates that possess stable and long-lasting CPT molecular biosynthetic machinery and to maximize its yield.

2. Materials and methods

1- Plant Samples Collection:

Seven medicinal plants *Cinnamomum camphora, Ficus elastica, Cynancum acutum, Callisteman lancealatus, Hibiscus rosa, Lantana camera and catharanthus roseus* were chosen as sources of endophytic fungi. Fresh parts were removed from the leaves, twigs, and inner barks of the entire plants and transferred to the lab in clean, dry plastic sheets. (Mycology Lab, Faculty of Science, Mansoura University). The plant parts were cut into 1 × 1 cm pieces, surface sterilised with 2.5% sodium hypochlorite for 2 minutes and 70% ethanol for 1 minute, and then cautiously washed with sterile distilled water to get rid of any epiphytes. The fungus's mature hyphal ends were harvested, filtered on PDA (potato extract 200 ml, glucose 20 g, and agar 20 g in 1L d.H2O), and kept at 4 °C as slope cultures for additional research [23].

2-Media Used for Isolation and Identification of Fungi:

1-Potato Dextrose Agar Medium (PDA) [25].

2- Czapek's- Dox medium [26].

3- Malt extract agar medium (MEA) [27].

3- Isolation and Culturing of theEndophytic Fungi:

The plant's segments were put on Czapek's-Dox medium with ampicillin (1g/mL) (an antibacterial agent) and potato dextrose agar media (PDA) [26]. Moreover, the plate medium lacking plant components served as a control to ensure that the work-space was sterile. Furthermore, a plate medium containing unsterilized plant pieces was employed as a control to examine the endophytic fungus ecology. The fungal hyphal tips were harvested after the incubation time (5 days) at 30°C, purified by sub-culturing on the same medium, and stored as slope cultures at 4°C for further screening investigations. According to morphological characteristics, the recovered fungal isolates were recognized using the keys of fungal identification [26, 28, 29].

Morphological Identification of the Recovered Endophytic Fungi:

Morphological features of all fungal isolates (colony diameter, extracellular exudates, pigmentation, mycelium colour, conidial heads, fruiting bodies, and sporulation) were used to evaluate the developed fungal colonies. Based on morphological criteria, the fungal isolates were classified according to the following keys:

[26] for *Aspergillus* species, [28] for *Fusarium* species, [29] for *Penicillium* species, and [30] for *Dematiaceous* fungus.

5- Screening for CPT Productivity by the Collected Fungi:

The endophytic fungal isolates from the various medicinal plants were evaluated for their ability to biosynthesize camptothecin by growing on potato dextrose broth (PDB) [15, 23]. A culture (6th days old PDA) was inoculated into 50 mL PDB medium/250 mL Erlenmeyer flask (2 agar plugs of 5 mm) for each fungal isolate and cultured at 30^0C for twenty days on a rotatory shaker incubator at 140 rpm, each fungal isolate undergoes three biological replicates. As a negative control, three flasks of broth medium without growth were employed. By using sterile cheesecloth, the cultures were filtered and any particles were then removed by centrifuging the filtrates at 5000 rpm. $CHCl_3$: MeOH (4:1) was employed to extract CPT from the supernatant [31]. A rotary evaporator was used to concentrate the organic phase, producing a crude oily extract.

6- Purification and Characterization of CPT:

6.1- Thin Layer Chromatography Analysis:

The extract was separated by TLC using a chloroform: methanol (9:1, v/v) (developing solvent solution) and Merck 1 mm (20 x 20 cm) pre-coated silica gel plates (TLC Silica gel 60 F254, Merck KGaA, Darmstadt, Germany) [32].After development, the putative CPT spots exhibited the same shade of blue color when the plates were viewed by UV illumination at 254 nm (MinUVIS, Heid., Germany), while also taking into account the relative mobility of the authentic one. The Image J software evaluated the putative spots' intensities in relation to the authentic sample known concentration.

6.2- Ultra -Violet Analysis:

The Camptothecin putative spots were detached from the silica plate, dissolved in methanol, and then detected by UV-vis at a wavelength of 200-500 nm (RIGOL, Ultra-3000 Series Spectrophotometer). Using methanol as a baseline blank, different concentrations of authentic CPT were measured at 360 nm [33], and the concentration of the corresponding putative CPT sample was detected.

7- Screenning of the Tested Factors to Optimize of *A. terrus* CPT Biosynthesis by the Factorial Plackett-Burman Design:

The impact of various carbon and nitrogen sources, elicitors, incubation times and pH were investigated to determine the best nutritional needs for the chosen fungal isolate to optimize the CPT yield. To increase its yield from the selected fungus, various physicochemical parameters (physical-chemical) including malt extract, calcium chloride, incubation time, peptone, sucrose, fructose, glucose, salicylic acid, pH, cysteine, yeast, sodium citrate, sodium acetate, ferric chloride, glycine sodium chloride, starch, copper sulphate, and potassium di-hydrogenphosphate were optimized by Plackett-Burman design to increase the target fungus's camptothecin output [34, 35]. According to the First order response, the two variables of the Plackett-Burman design, each representing high (+ 1) and low (1) values were used to screen the 19 parameters. Statistical nutritional optimization of the potent camptothecin-producing fungal isolate may represent an acceptable strategy to assess the interactions of the independent factors and their effects on the final response, or "CPT output," with a reduction in the production cost and time as much as possible [36, 37]. Because interaction effects between various parameters are not taken into account in the standard optimization technique (one factor at a time), it may be an inaccurate strategy [38]. Thus, the significance of investigated parameters resulting in the maximum output of CPT by the potent fungal isolates was examined using the fractional factorial approach (Plackett-Burman design). In this part, 24 runs were used to screen 19 components at two levels (high and low). To determine the average yield of CPT (µg/l), all experiments were run in triplicate in 250 ml flasks with 50 ml medium. By doing a typical regression analysis and determining the P-value (0.05) and confidence intervals, the significant factors were determined.

3. Results

1- Fungal Isolation and Identification

Seven plants were chosen as the source for the tested endophytic fungus. (Fig.1). These

plant parts were surface sterilized, cut into segments and placed in 90 mm Petri dishes containing 20 ml sterilized Potato Dextrose Agar (PDA), Czapek's-Dox and malt extract agar. After incubation for 5 days at 30°C, the developed hyphal tips of the fungal colonies were purified by sub-culturing on the corresponding media and incubated at standard conditions. Thirty-one fungal isolates were recovered and identified to the species level based on their morphological and physiological features (Fig. 2).

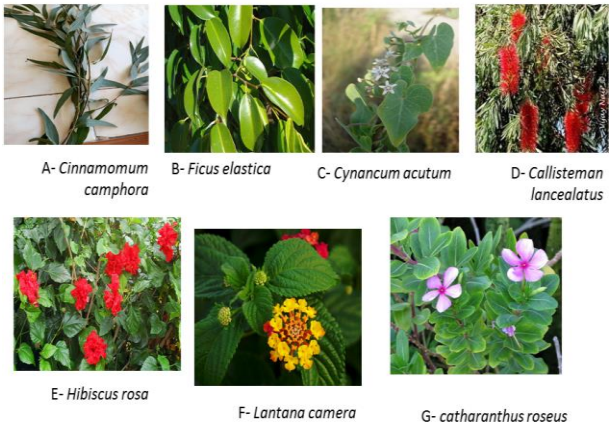


Figure (1): Morphological view of medicinal plants that are used in this study.

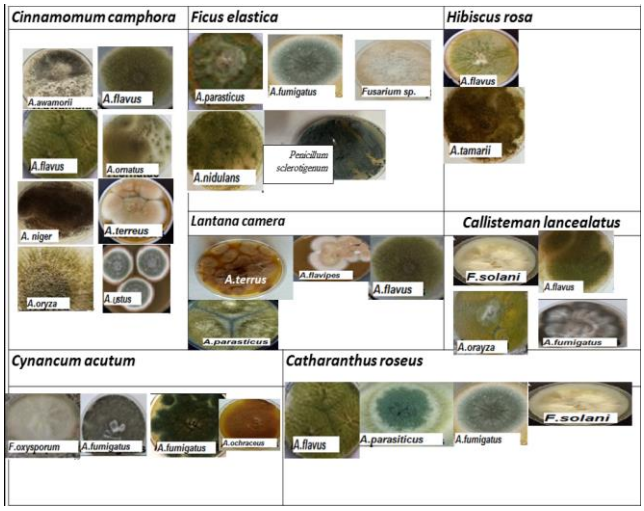


Figure (2): Purified fungal endophytes isolated from medicinal plants.

2. Morphological Identification of Endophytic Fungi from Medicinal Plants

According to the universal keys, fungal isolates were identified morphologically established on their microscopic characteristics; these endophytes predominantly belonged to the three genera *Aspergillus*, *Penicillum*, and *Fusarium*. Eighty-eight percent of the plants used in the experiment belonged to the *Aspergillus* genus, while eleven percent belonged to *Fusarium* and one percent to *Penicillum*. The species of *Aspergilli* were belonging to 12 groups as follows; (*A. terreus*), (*A. flavus*), (*A. flavipes*), (*A. fumigatus*), (*A. ornatus*), (*A. ochraceus*), (*A. nidulans*), (*A. ustus*), (*A. oryzae*), (*A. tamari*), (*A. parasiticus)*, and (*A. niger*), the *Fusarium* genus was represented by three species, *F. solani*, *F. oxysporum*, *Fusarium. sp*. and the genus *Penicillum* was represented by one specie (*Penicillum sclerotigenum*) (Table. 1).

3- Screening for the Producing Potency of Camptothecin

By cultivating on PDB media and incubating under the recommended conditions, the yield of Camptothecin by the obtained edophyttic fungal isolates was evaluated. Three biological duplicates from each fungal isolate were prepared**.** The fungal isolates recovered from medicinal plants were selected for the screening of CPT productivity. Eight fungal endophytes were recovered from *Cinnamomum camphora*, five fungal endophytes were recovered from *Ficus elastica*, two fungal endophytes were recovered from *Hibiscus rosa*, four fungal endophytes were recovered from *Callisteman lancealatus*, four isolates were recovered from *Lantana camera*, three isolates were recovered from *Cynancum acutum*, four isolates were recovered from *catharanthus roseus.* The screening profile revealed that *Aspergillus terreus* (1A) endophytes of (*Cinnamomum camphora*) (89.4 µg/l) and *Penicillum sclerotigenum* (2B) entophytes of (*Ficus elastic*) (80.2 µg/l) had the highest CPT yield (Table 1).

4- Thin Layer Chromatography Analysis

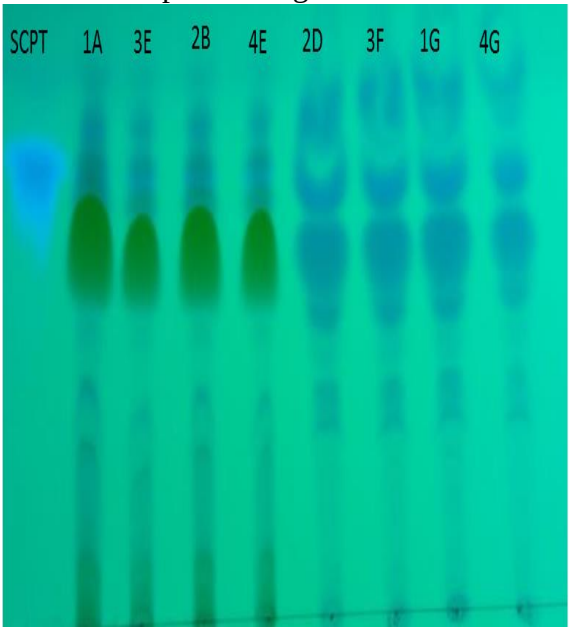
Seventeen fungal showed potent production of CPT on PDB media, where all of them showed CPT band under UV illumination at 260-290 nm with R.f value 0.65 which was identical to standard CPT. Also, the CPT spots showed a bluish spot when reacted with vanillin/sulfuric acid reagent similar to that of authentic CPT (Fig. 3). The screening profile showed that the endophytes of *Cinnamomum camphora* and *Ficus elastica*, *Aspergillus terreus* (1A) (89.4 g/l) and *Penicillum sclerotigenum* (2B) (80.2 g/ml), respectively, reported the highest camptothecin output when cultivated on PDB media. While, the remaining

fungal isolates showed a little / invisible amounts of CPT.

Table (1): Screening for camptothecin productivity from fungal isolates inhabiting the selected medicinal plants by Thin Layer Chromatography.

Isolate Source	Isolate No	Fungal Isolate	Putative CPT yield on TLC (ug/l) PDB medium
Cinnamomum camphora	1	*Aspergillus terrus* 1A	89.4
	2	*Aspergillus ustus* 2A	3.2
	3	*Aspergillus flavus* 3A	3.3
	4	*Aspergillus niger* 4A	-
	5	*Aspergillus oryzae* 5A	-
	6	*Aspergillus flavus* 6A	4.3
	7	*Aspergillus ornatus* 7A	-
	8	*Aspergillus awamorii* 8A	4.2
Ficus elastica	1	*Aspergillus fumigatus* 1B	-
	2	*Penicillium sclerotigenum* 2B	80.2
	3	*Aspergillus parasiticus* 3B	-
	4	*Fusarium sp* 4B	-
	5	*Aspergillus nidulans* 5B	3.5
Hibiscus rosa	1	*Aspergillus tamari* 1C	2.3
	2	*Aspergillus flavus* 2C	2.4
Callisteman lancealatus	1	*Fusarium solani* 1D	0.9
	2	*Aspergillus fumigatus* 2D	0.8
	3	*Aspergillus oryzae* 3D	-
	4	*Aspergillus flavus* 4D	-
Lantana camera	1	*Aspergillus parasiticus* 1E	0.2
	2	*Aspergillus flavipes* 2E	0.2
	3	*Aspergillus terreus* 3E	20.6
	4	*Aspergillus flavus* 4E	25.6
Cynancum acutum	1	*Aspergillus fumigatus* 1F	0.2
	2	*Fusarium oxysporum* 2F	-
	3	*Aspergillus ochraceus* 3F	-
catharanthus roseus	1	*Aspergillus fumigatus* 1G	-
	2	*Aspergillus flavus* 2G	3.2
	3	*Aspergillus parasiticus* 3G	-
	4	*Fusarium solani* 4G	-

Figure (3): TLC chromatogram of extracted CPT from the potent fungal isolates



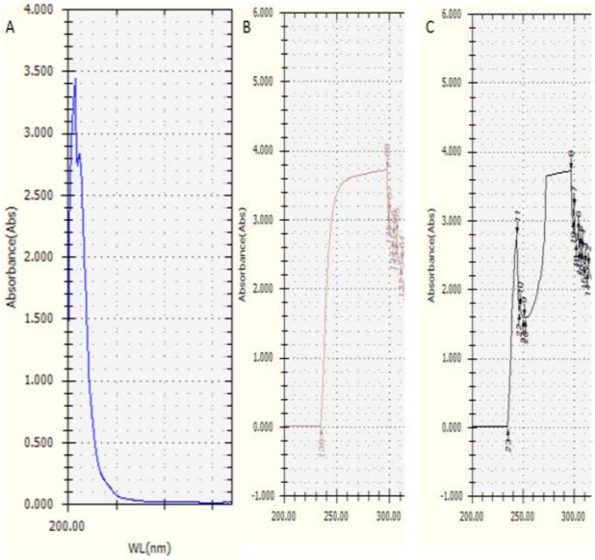


Figure (4): UV- spectrum of authentic CPT (A), UV- spectrum of CPT sample from *A. terreus* (B) and UV- spectrum of CPT sample from *P. sclerotigenum* (C) were scanned from 200-800 nm.

5 -Ultra-Violet Spectroscopic Analysis

The camptothecin samples purified from *Aspergillus terreus* and *Penicillum sclerotigenum* fungal isolates were dissolved in methanol and scanned from 200-800 nm, (methanol was used as a baseline). From the UV- analysis, *Aspergillus terreus* and *Penicillum sclerotigenum* camptothecin samples provided the same absorption pattern

(an authentic CPT) at 290 nm under the same conditions (Fig .4).

Morphological Identification of the Potent Fungal Isolates Producing CPT

The macro and microscopic features of the selected isolate were observed on PDA agar media. After inoculation of the fungal inoculum to the center of the agar media, the macroscopic features such as the color of mycelium, pigmentation and surface exudates were observed internally. In parallel, the microscopic features as type of a sterigmata, conidial heads, conidiophores anastomosis, conidial ontogeny, and fruiting bodies on medium, were investigated. Depending on morphological criteria, Colonies on PDA plate of isolate (1A), appeared suede- like cinnamon-buff to sand brown colonies, reverse with yellow to a deep dirty brown, conidial heads are compact, columnar and biserrate sterigmata. Conidiophore is hyaline and Smooth walled.

Conidia are globose to subglobose, hyaline to slightly yellow and Smooth walled without sclerotia. From the morphological features**,** the isolate is very close to *A. terreus* (Fig. 5A). Depending on the morphological criteria of isolate (2B), mycelium has powdery masses of dark blue-green spores on the upper surface and yellowish on the lower surface. Hyphae are thread-like branching, septate and hyaline. The conidiophores are rough (Stone like) and colorless. Phialides are both uniseriate (arranged in one row) and biseriate and the ascospores develop within sclerotia (Fig. 5B). The morphological features of this fungal isolate typically follow the anamorphic state of *Penicillum sclerotigenum.*

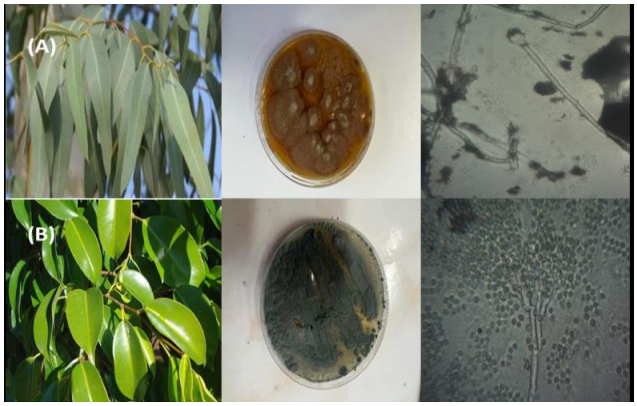


Figure (5): Culture characteristics of CPT potent fungal endophytes. (A); Morphological view of *C.camphora*, *Aspergillus terreus* growing on the surface of PDA medium, the conidial heads microscopic view at 1000× magnification (Light Microscope). (B): Morphological view of *F.elastic*, *P. sclerotigenum* growing in PDA medium, the microscopic view of the conidial heads at 1000× magnification (Light Microscope).

Bioprocess optimization of Camptothecin production by *A.terreus* by Plackett–Burman Design

Aspergillus terreus has been selected for further nutritional optimization to maximize their yield of camptothecin, since the identity of medium chemical components and their interactions are pivotal for the synthesis of secondary metabolites by fungi. The nutritional requirements for camptothecin production by *A. terreus* were optimized by Plackett–Burman design as " the 1st order model equation" to determine the significant factors affecting camptothecin productivity. Nineteen factors comprising the different carbon, nitrogen sources as precursors of CPT, and other physical factors as incubation time and pH were studied, with their higher and lower values (+ 1, − 1) related to camptothecin production. The levels of the tested factors were summarized in the Plackett– Burman design (Table 2).

Table (2): The coded and actual values for the tested variables.

Codes	Factors	Levels	
		-1	1
X1	PH	2	7
X2	Incubation Time	7	16
X3	Peptone	0.5	10
X4	Glucose	2	10
X5	Calcium Chloride	0.1	0.4
X6	Cystine	4	8
X7	Malt Extract	4	10
X8	Fructose	4	10
X9	Sucrose	4	10
X10	Salicylic acid	2	4
X11	Sodium Chloride	0.1	0.4
X12	Yeast	2	8
X13	Potassium Di-hydrogen-phosphate	0.1	0.4
X14	Sodium Citrate	1	4
X15	Sodium Acetate	1	4
X16	Ferric Chloride	0.1	0.4
X17	Glycine	1	4
X18	Starch	2	15
X19	Copper Sulphate	0.1	0.4

The experimental design's analysis of variance (ANOVA) was computed, and the confidence intervals, the coefficients, t Stat and p-value were stated. The chosen independent variables were held responsible for the variation in the response (98.87%). The F-value (9.87), p-value (0.0007), and adjusted determination coefficient (Adj. R2 = 0.92) all confirmed the model's significance. There are six distinct independent variables, that are determined by the normal probability and the main effects of the components under study; pH, incubation time, salicylic acid, peptone, glucose, and glycine that have an optimistic impact on the productivity of CPT, while there are three independent factors that have a negative effect on camptothecin production; ferric chloride, calcium chloride and malt extract and the other independent variables have no effect (Table 3).

The highest camptothecin yield (127.6µg/L) was achieved in run 4, while the lowest yield (16.8µg/L) was assessed in run 22. From the analysis of variance (ANOVA), the created model was greatly significant, like shown by the Fisher's F-test values (9.87) and the probability of p-value (0.0007). The actual

Camptothecin productivity by *Aspergillus terreus* varied substantially from 0.42 to 3.19 mg/L and this confirms the validity of the tested factors on CPT productivity. The Plackett–Burman design effectiveness and the determination values coefficient (R2 = 0.98) demonstrate the goodness-offit for the linear regression models measuring (Table 4).

Table (3): Plackett-Burman experimental design matrix.

Std. Order	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16	X17	X18	X19	CPT Yield (µg/l)
1	7	7	0.5	2	0.1	4	10	4	10	2	0.4	8	0.1	1	4	0.4	1	15	0.1	22.4
2	7	16	0.5	2	0.1	4	4	10	4	4	0.1	8	0.4	1	1	0.4	4	2	0.4	96.8
3	7	16	0.5	10	0.4	4	4	4	10	2	0.1	2	0.4	4	1	0.1	4	15	0.1	87.2
4	7	16	10	10	0.1	4	4	4	4	4	0.4	2	0.1	4	4	0.1	1	15	0.4	127.6
5	7	16	10	10	0.4	8	4	4	4	2	0.1	8	0.1	1	4	0.4	1	2	0.4	72.4
6	2	16	10	10	0.1	8	10	4	4	2	0.4	2	0.4	1	1	0.4	4	2	0.1	45.6
7	7	7	10	10	0.1	8	10	10	4	2	0.1	8	0.1	4	1	0.1	4	15	0.1	90
8	2	16	10	2	0.1	8	10	10	10	2	0.1	2	0.4	1	4	0.1	1	15	0.4	25.2
9	7	7	0.5	10	0.1	8	10	10	10	4	0.1	2	0.1	4	1	0.4	1	2	0.4	73.6
10	7	16	10	2	0.4	4	10	10	10	4	0.1	2	0.1	1	4	0.1	4	2	0.1	87.2
11	2	16	0.5	10	0.4	8	4	10	10	4	0.4	2	0.1	1	1	0.4	1	15	0.1	36.4
12	2	7	10	10	0.4	4	10	4	10	4	0.4	8	0.1	1	1	0.1	4	2	0.4	44.8
13	7	7	10	2	0.4	8	4	10	4	4	0.4	8	0.4	1	1	0.1	1	15	0.1	85.6
14	7	16	0.5	2	0.4	8	10	4	10	2	0.4	8	0.4	4	1	0.1	1	2	0.4	45.6
15	2	16	0.5	10	0.1	4	10	10	4	4	0.4	8	0.4	4	4	0.1	1	2	0.1	36.4
16	2	7	10	10	0.4	4	4	10	10	2	0.1	8	0.4	4	4	0.4	1	2	0.1	20.4
17	7	7	10	2	0.1	8	4	4	10	4	0.4	2	0.4	4	4	0.4	4	2	0.1	90
18	2	16	0.5	2	0.4	8	10	4	4	4	0.1	8	0.1	4	4	0.4	4	15	0.1	36.4
19	7	7	0.5	10	0.4	4	10	10	4	2	0.4	2	0.4	1	4	0.4	4	15	0.4	45.6
20	2	16	10	2	0.1	4	4	10	10	2	0.4	8	0.1	4	1	0.4	4	15	0.4	44.8
21	2	7	0.5	10	0.1	8	4	4	10	4	0.1	8	0.4	1	4	0.1	4	15	0.4	44.8
22	2	7	10	2	0.4	4	10	4	4	4	0.1	2	0.4	4	1	0.4	1	15	0.4	16.8
23	2	7	0.5	2	0.4	8	4	10	4	2	0.4	2	0.1	4	4	0.1	4	2	0.4	22.4
24	2	7	0.5	2	0.1	4	4	4	4	2	0.1	2	0.1	1	1	0.1	1	2	0.1	20.4

Table (4): Placket-Burman design (Analysis of variance (ANOVA) and regression statistics).

Source	DF	Sum of square	Mean Square	F-Value	P-value
Intercept	20	13.3828	0.66914	16.36	0.022
X1	1	7.3041	7.30407	178.54	0.001
X2	1	0.7073	0.70727	17.29	0.025
X3	1	0.8664	0.086640	21.18	0.019
X4	1	0.4483	0.44827	10.96	0.045
X5	1	0.3553	0.355327	8.68	0.060
X6	1	0.0081	0.00807	0.20	0.687
X7	1	0.0363	0.83627	20.44	0.020
X8	1	0.0028	0.00262	0.07	0.810
X9	1	0.1411	0.1417	3.45	0.160
X10	1	1.4308	1.43082	43.9	0.01
X11	1	0.0150	0.01500	0.73	0.558
X12	1	0.0368	0.03682	0.90	0.413
X13	1	0.0384	0.03840	0.94	0.404
X14	1	0.1067	0.10667	2.61	0.205
X15	1	0.0840	0.08402	2.05	0.247
X16	1	0.3504	0.35042	8.57	0.061
X17	1	0.6080	0.60802	14.86	0.031
X18	1	.0.0013	0.00135	0.03	0.867
X19	1	0.0001	0.00015	0.00	0.956

Regression Statistics		
R Square	Adjusted R Square	Predicted R Square
99.09%	93.03%	41.84%

The statistical design's validity was indicated by the observed values having the greatest correlation. The regression model showed that 127.6 µg/L of camptothecin was the greatest actual yield produced by *A. terreus*. The Plackett- Burman design indicates that the ideal components of medium for *A. terreus*'s CPT yield comprise peptone (10g/L), glucose (10g/L), calcium chloride (0.1 g/L), cysteine (4 g/L), malt extract (4 g/L), salicylic acid (2 g/L), fructose (4 g/L), glucose (4 g/L), ferric chloride (0.1g/L) and starch (15 g/L), after incubation time (16 days) at pH 7. By comparing the data obtained, it is obviously clear that run 4 is to be optima for CPT production by *A.terrus* as the maximum production of CPT was obtained at it achieving 127.6µg/ml while the minimum production was obtained at the run number (22) achieving 16.8µg/ml.

4. Discussion

Camptothecin is a potent anticancer plant-based alkaloid because of its specific potential to interact with DNA topoisomerase [39], a vital enzyme for controlling the shape of DNA strands during replication, RNA transcription and chromatin assembly [40]. The most often provided anticancer medications worldwide are the semi-synthetic water-soluble analogs of CPT (Topotecan and Irinotecan); however, the main challenge is the reduced yield of this precursor [16]. As compared to plant sources, the capacity of endophytic fungi to synthesize CPT increases the probability that this compound will be produced commercially [16, 19, 22]. This is due to the fact that fungi grow quickly, are accessible to large amounts of biomass, and are not affected by environmental conditions. Tested plants produced thirty-one fungal isolates, and the CPT productivity of these isolates was evaluated. Comparing these isolates to other closely similar morphological isolates from other hosts, *A. terreus* (89.4 µg/l) and *P. sclerotigenum* (80.2 µg/l) endophytes of *C. camphora* and *F. elastica* showed the highest CPT yield. The yield of CPT by the current *A. terreus* isolate inhabiting *C. camphora* was similar to those by *A. terreus* an endophyte of *C. parqui* (~110 µg/l). Coincidently, isolates of *Aspergillus terreus*, endophytes of *F. elastica*, *Cestrum parqui*, and *Astragalus fruticosus* were recognized as CPT producers confirming the possessing of a distinct CPT biosynthetic machinery by this fungal species independent on their plant hosts. [3]. Practically, several morphologically closely related isolates of *A. terreus* were recovered from the different parts of *L. camera*, however, their CPT productivity were very tiny, revealing the implantation of specific molecular signal from this part on triggering the expression of the biosynthetic machinery of CPT, unlike to the absence of these signals from other plant hosts. Thus, the physiological identity of plant could have a significant contribution not only to the identity of their endogenous microbiome but also to the pattern of metabolic and physiological behavior of these microbiomes [14]. The dependence of the biosynthetic machinery of CPT by the tested fungal isolates on the communicating/ expression signals from the plant host has been obviously detected for the host *F. elastica*, *Delonix regia* and *Hibiscus rosa*. Spectroscopic and chromatographic analyses verified the camptothecin's chemical identification after being isolated from *A. terreus* and *P. sclerotigenum*. The chemical identification of the extracted compound was determined to be camptothecin [23, 41, 42]. The fungus is known to produce CPT in PDB media, the optimum biosynthesis of CPT in a profitable manner can be achieved by using various techniques. Hence, further exploration is required to enhance extraction through a variety of approaches [43, 44]. The optimization for dependent factors is proportionate to medium diverse parameters such as carbon supply, nitrogen source, pH, and incubation time. Response Surface Methodology (RSM) is now inferred with great accuracy [45]. In order to boost the CPT production yield, we modified the growth conditions of *A. terreus* which was isolated from *C. camphora*. The effect of elicitors, adsorbent resins, incubation period and different carbon and nitrogen sources were conducted. The highest camptothecin yield by *Aspergillus terreus* was informed using PDB medium at an initial pH of 7.0, which is comparable to that reported for *T. atroviride* and this at the 16th day of incubation [23]. Among various carbon sources, glucose was shown to be the most effective inducer (10g/l

for *A. terreus* generating CPT). Other carbon sources had a negative impact on production, which coincided with CPT from *Fusarium solani* [46]. The largest CPT production was produced when peptone was added, followed by yeast extract, with the remaining nitrogen sources having no appreciable stimulating effects on the fungus' ability to produce CPT.

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