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ARTICLE

Investigation the Effect of Exposure *Aspergillus niger* Isolate to the UV Radiation on Its Superphosphate Fertilizer Dissolving Efficiency in Calcareous Soil

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ABSTRACT

A laboratory experiment was conducted to demonstrate the importance of improving the efficiency of six isolates of *Aspergillus niger* fungi. Four isolates were exposed to UV-rays radiation at a distance of 30 cm, a wavelength of 254–255 nm during M_{15}, M_{30}, and M_{45} minutes. Exposure periods, both of wild (no UV-rays exposure) and no *A. niger* (C) as controls, all six isolates were identified molecularly by polymerase chain reaction technique extracted DNA of *A. niger* was analyzed to ensure gene completion through multiple sequence alignment by bioinformatic programs to study the improvement of dissolving efficiency of mutant and wild *A. niger*. They were incubated after the addition of superphosphate fertilizer (47% P_2O_5) at 90 mg P kg^{-1} applied to soil with controls (no *A. niger* or no superphosphate) for I, II, III, and IV weeks of incubation periods at 28 ± 1 °C. In addition, the DNA extraction and purification by NanoDrop of Thermo Scientific-200 A280/A260 ratio was 2.01 and confirmed that the sequences of nitrogenous bases by the method of multiple sequence alignment (MSA) as compared to the reference sequence of *A. niger* recorded in the gene bank under the accession number LC632396. Results proved that the M_{30} minutes exposure UV-rays radiation period was the superior dose when mutant *Aspergillus niger* obtained the highest amount of dissolved phosphate, reducing soil-pH with maximum biomass of *A. niger* during the M_{30} isolate during the third week (III).

Keywords: *A. niger*; Mutation; PCR; UV-Rays Radiation

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1. Introduction

Phosphorus is one of the main essential major nutrients for plant life, as it is involved in the composition of amino acids for proteins, enzymatic reactions for DNA and RNA, and react energy reactions such as respiration, photosynthesis when phosphoric compounds like ATP and ADP are manufactured for optimal plant growth [1]. It also contributes to the formation nuclear proteins and stimulates the growth of fibrous and lateral roots [2]. In soil, phosphate compounds face serious problems due to adsorption or sedimentation processes by involving either Ca^{2+} carbonate or bicarbonates. The availability of phosphorus decreases in these soils, especially in the soils of central and southern Iraq, and it converts into compounds that are insoluble in soil solution and thus not available. Deficiency sympotoms in plants appear, especially when insoluble forms increase during the addition of more P-level (as P-fertilizers), which leads to high costs for farmers and environmental pollution with heavy metals like cadmium, affecting human health and leading to additional financial costs for farmers [3]. Recently, farmers and researchers have used phosphate biofertilizers by adding microbial inoculates, such as bacteria, fungi, and algae, which live in the rhizosphere zone and can support plant growth, such as *Aspergillus niger* fungi, which dissolve insoluble phosphate forms into soluble ones, either initially or when added to soil as fertilizers through several mechanisms such as the secretion of organic acids as lactic, citric, oxalic, etc., or inorganic acids like nitric and sulfuric [4]. The two processes of metabolism and mineralization carried out by microorganisms in the phosphorus cycle in nature are of particular importance, as the continuity of these two processes largely controls the amount of dissolved phosphorus dissolved. One of the main vital processes of microbes in the soil is the dissolution of various forms of insoluble mineral phosphate compounds, such as Al-P, Fe-P, and Ca-P [5, 6]. Phosphate-dissolving fungi like *Aspergillus niger* are present in all soils, and their numbers depend on the physical and chemical properties of the soil and organic matter content [7]. Environmental factors also affect their ability to dissolve phosphate, which occurs when *A.niger* can simulate alkaline or acidic phosphatase enzymes depending on the soil pH in rhizosphere [8–12]. Therefore, the use of inoculants like *Aspergillus niger* fungi as a supplement to chemical fertilizers is considered a common method in most countries, such as Russia, America, and India [9] . On this basis, modern and advanced studies have relied on what is known as biotechnology, the most important objectives of which are to improve and develop the performance of living soil organisms by causing some changes in the genetic structure of nucleotides on the DNA strand, which is known as genetic mutations. These aim to stimulate the secretion of decomposing enzymes, hormones, and organic acids [13]. Genetically, methods improve the work of microorganisms in soil by making changes in the sequences nitrogen of bases on DNA when microbes (such as fungi) are exposed to limited doses of mutation, which stimulates enzymes, hormones, and organic acids. For example, *Pseudomonas* aeruginosa produces alginate (a product of alginic acid) after exposure to ultraviolet rays at doses of 2, 4, 6, 8 and 10 J/m, indicating that physical mutation with ultraviolet rays (as one of the physical mutagenic factors) leads to an increase in their ability to produce alginate by obtaining continuous microbials mutations in its high productivity of alginate compared to wild isolates [13]. Mutation can be divided into spontaneous mutations that happen in nature daily or can occure by inducing techniques using physical or chemical factors [14]. UV-ray (ultraviolet) radiation creates mutants for microorganisms that are more efficient than wild microbes [15] also indicated the improvement of the production of indole acetic acid from two mutated isolates of *Trichoderma harzianum.* More studies confirmed that directing ultraviolet rays at different exposure periods exposure has led to the development of the efficiency of living organisms, as these mutagenic microorganisms have taken a distinguished position [16]. One of the most widespread aspects of biotechnology and its relationship to biological science is the science of molecular genetics, which is closely linked to the development of modern tools and techniques such as instruments that can save time and cost when applied for the identification and arrangement of huge amounts of DNA sequencing of microorganisms by polymerase chain reaction (PCR) technique [17]. As a result of massive modernization and development of biological and informatic tools, which are gene regulation by polymerase chain reaction, as well as a focus on studying DNA sequences, as [18] noted, the control of a number of genetic sites known as quantitative trait sites can be achieved by identifying these and identifying the markers associated with them. The phenotypic variation of the traits to be improved can be predicted early,

and selection programs can be developed based on these markers, which may be functional mutations in genes affecting production [19, 20]. This was shown to have a significant impact on scientific progress in this field due to the great development in biological tools, multiple sequence alignment, phylogenetic and BLAST analysis because these techniques can determine the genetic structure [20]. Therefore, this study aims to identify the molecular characteristics of *Aspergillus niger* fungi isolated by PCR technique and to examine the role of physical mutation with UV-ray radiation on phosphate dissolving efficiency compared to wild *Aspergillus niger* fungi in calcareous soil enriched with super phosphate fertilizer.

2. Materials and Methods

2.1. Rhizosphere Soil Samples Collection

Many samples from various soil rhizosphere regions were collected in southern sites of Basra province. The samples were kept in a refrigerator for isolation experiments [21, 22].

Isolation and Morphology Diagnosed of Phosphate Dissolving Fungi

In the present study fungus were isolated from soil after microscopic confirmation the fungus were grown on potato dextrose agar at 25 °C for 48–72 hour, The pure cultures of *A. niger* were maintained on potato dextrose agar slants, about one gram of soil sample was added in 9 ml distilled water to make 10^{-1} to 10^{-6} of diluted suspensions, only series 10^{-4}, 10^{-5} and 10^{-6} at petri dishes contained distilled rose – bengel Agar media enriched with 5 ml 10% K_2HPO_4 and 10 ml 10% $CaCl_2$ to convert insoluble phosphate as reaction below:

$$CaCl_2 + K_2HPO_4 \longrightarrow CaHPO_4 + 2KCl$$

isolates were incubated at 28 ± 1 °C for 3–4 days conduct capable of dissolved phosphate by fungi in petri dishes, clear halos zones around colonies as indicator to dissolve insoluble phosphate to soluble form in media. Isolates fungi were belonged to *Aspergillus niger* depending on morphological characteristics according to plant protection department, agriculture college Basra University.

2.2. Mutation Selected *A. niger* Fungi

Four selected isolates *A. niger* were exposed to UV-rays radiation at 30 cm distance from UV-radiation for at 15, 30 and 45 (M_{15}, M_{30} and M_{45}) as exposure time wavelength isolates of *A. niger* were put in petri dishes with nutrient agar (leave no UV-rays as control) then incubated for 3–4 days at 30 ºC, harvested them as a suspensions for each either wild or mutant *A. niger* fungi in 10 ml of distilled salty solution for using in bioassay experiment.

2.3. Molecular Identification of Wild and Mutant *A. niger* with PCR Technique

By First Base Laboratories Selangor, Malaysia. to conduct the sequences genetic and degree the convergence of *A. niger* isolates which extracted DNA according to [23] by using kit produced from Gene aid company, Kore then estimated quantities and purity at DNA at NanoDrop Thermo Scientific-200 as indicator A260/ A280 ratio.

2.4. DNA Sequencing

After obtaining the PCR amplification product of the samples and purifying and drying them, samples were sent to a laboratory in Malaysia (First BASE Laboratories Malaysia 2016) to perform the analysis to find out the sequence of nitrogenous bases after receiving the results of all sent samples, to know the amino acid content of nitrogenous base sequences and phylogenetic tree BLAST by the programs were used bioinformatics software geneious software,

2.5. Bioassay Experiment in Studied Calcareous Soil

An experiment was conducted in calcareous soil added supper phosphate fertilizer (47% P_2O_5) applied as 90 mg p kg^{-1} soil and left controls (no *A. niger* or no superphosphate), soil samples were analyzed and determined some of chemical-physical and biological properties as according to [24–27] as **Table 1** soil samples were inoculated with either wild or mutant isolates of *A. niger* (W, M_{15}, M_{30} and M_{45}) then incubated at different periods incubation (I, II, III and IV weeks) to study effect of *A. niger* on phosphate fertilizer dissolving in calcareous soil.

2.6. Statistical Analysis

The experiment was statistically designed with three replications as a factorial experiment by using complete ran-

Table 1. Chemical-physical and biological properties of calcareous studied soil.

Properties		Unite	Value
pH (1:1)		———————	7.80
E Ce		Ds m^{-1}	10.0
Total Ca-carbonate		G m^{-1}	229.00
Cation exchange capacity		c mol(+) kg^{-1}	14.34
Organic matter		g kg^{-1}	4.35
Total nitrogen		g kg^{-1}	0.46
Available phosphour		mg kg^{-1}	11.34
Soluble cation and anion	Cu^{2+}	mmole L^{-1}	40.50
	Mg^{2+}		25.60
	Na^{+}		20.16
	K^{+}		10.30
	Cl^{-}		80.19
	SO_4^{2-}		13.24
	CO_3^{2-}		0.00
	HCO^{3-}		2.87
Soil texture			Clay silt
Clay		g kg^{-1}	480.20
Silt			399.20
Sand			68.44
Total biomas bacteria		CFU g^{-1} soil	3.86
Fungi			2.70
Aspergillus niger			0.60

domized design (C.R.D). Analyzing the data was by using the statistical program as SPSS , the averages of the coefficients was compared by using the test of the least significant difference rate (R.L.S.D.) at a probability level of ($P < 0.01$) [28, 29].

3. Results and Discussions

3.1. Polymerase Chain Reaction (PCR)

By using Go Taq® Green master mix from promega company, USA, **Table 2** which prepared with 100 µm tube then add sample to 25 µ as ingredients quantities (ml) from (DNA = 3, GoTaq® Green master mix = 12.5, both of forward and Reverse primer = 1, nuclease free water = 7.5 with total volume 25 µl) put tubes in centrifugation during 30 sec for 10 times then translate to thermocycler meter according to My Genie-96-384 gradient thermal Bioneer, Korea which adjust with program of meter according to used primer, **Table 3** that referred to PCR primer detection then adjust temperature for annealing connect primer with DNA.

All isolates with control were genotypically characterized by cloning and sequencing the 16SrRNA. Briefly, the genomic DNA from a pure culture of each isolate was extracted and purified for PCR amplification of the 16S rRNA (**Figure 1**) notes bundle of DNA extraction from *A. niger* fungi as dissolving phosphate which confirm purity by NanoDrop at percentage A280/A260 for six isolates, it was 2.01 as a guid to the best purity, and bundle amplification of PCR was a molecular weight 308 bp.

Table 2. Steps of PCR for *A. niger* isolates.

PCR Step	Temperature	Time	Repeat Cycle
Initial denaturation	95 °C	5 min	1
Denaturation	95 °C	1 min	
Annealing	52 °C	1 min	
Extension	72 °C	2 min	
Final extension	72 °C	10 min	1

3.2. Molecular Detection of Nitrogenous

results of the detection of nitrogenous base sequences by multiple sequence alignment method showed that by MSA (**Figure 2**) comparison with the reference sequence of *A. niger* which registered in GenBank under Accession number LC632396, six different genetic isolates were obtained,

these isolates differed from each other due to a number of silent mutations (not resulting from the occurrence of a new amino acid) or tangible mutations (resulting from a change in the amino acid) occurred in addition to a number of these mutations occurred in some samples and not others, while some mutations occurred in more than one sample in addition to the genetic sequence of these isolates was obtained then classified based on the DNA genetic sequence using the UPGMA phylogenetic tree (**Figure 2**) classification method, evolutionary analyses were performed according to sequence controller program as **Table 4**.

Table 3. PCR primer detection of *A. niger*.

Sequence (5'->3')	Template Strand	Length	Start	Stop	Tm	GC%	Self Complementarity	Self 3' Complementarity
Forward primer	ATCTCTTGGTTCCG-GCATCG	Plus	20	268	287	60.18	55	4
Reverse primer	TTCAGCGGGTATCCC-TACCT	Minus	20	610	591	59.74	55	4

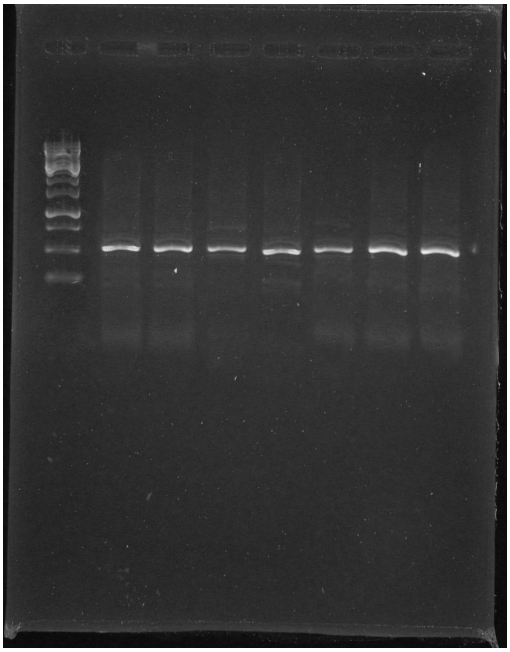

Figure 1. Amplification products of the DNA of the segment 308 bp of *A. niger* electrophoresis product PCR product.

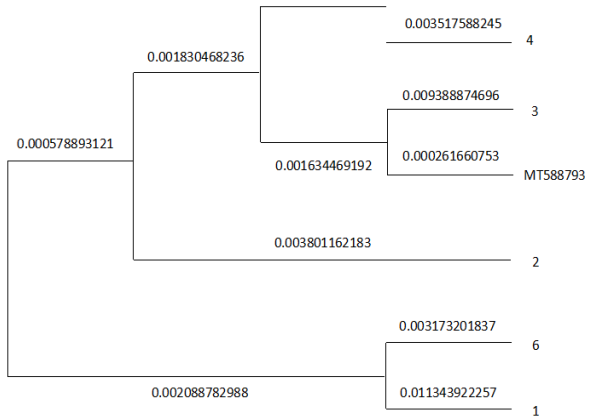

Figure 2. Phylogenetic tree of wild and UV-mutant *A. niger* fungi.

3.3. Bioassay Experiment of Wild and Mutant *A. niger* in Studied Soil

3.3.1. Soil pH

As **Table 5** results showed that significant ($p < 0.05$) of wild *A. niger* when soil pH reduced from 7.95 at control (without *A. niger*) to 6.76, 4.77, 4.55 and 6.71 at M_{15}, M_{30} and M_{45} of exposure periods respectively , but *A. niger* mutant at M_{15}, M_{30} and M_{45} have more capable superior than wild so both wild and mutant of *A. niger* may be decreased soil pH as according one of mechanisms like secretion organic acids in studied soil or through excretion of mineral acids [8, 30], in addition to results at **Table 5** showed that significant ($p < 0.05$) effect of incubation periods when soil-pH reduced from 6.37 at I week to 6.32, 5.66 and 6.39 during II, III and IV week in addition to Interaction between UV-rays exposure time and incubation periods results showed that the optima treatment at M_{30} with exposure time during III week when soil pH was 3.0 as compared to control treatment (no exposure time during I week incubation time), so the exposure time with periods incubation on *A.niger* dissolving efficiency when may be help to create optima condition growth to *A. niger* in addition to decrease soil pH, then increase nutrients availability like phosphate [15].

3.3.2. Electrical Conductivity

At **Table 6** results showed that significant effect of incubation periods of *A.niger* on soil- electrical conductivity when increase from 7.9 ds m^{-1} at 7 days (I week) to 8.0 and 8.1 ds m^{-1} during II and III weeks incubation periods respectively while reached to 7.9 ds m^{-1} at IV week, the interaction

Table 4. DNA Code table according to sequence controller program.

Amino Acids Biochemic			
Non polar	polar	basic	acidic

1st Base	2nd Base T		2nd Base C		2nd Base A		2nd Base G		3rd Base
T	TTT	(Phe/F)	TCT	(Ser/S)	TAT	(Tyr/Y)	TGT	(Cys/C)	T
	TTC	phenyl alanine	TCC	Serine	TAC	Tyrosine	TGC	Cysteine	C
	TTA		TCA		TAA	Stop (Ochre)	TGA	Stop (Onal)	A
	TTG		TCG		TAG	(Amber)	TGG	(W) Trvnte	G
C	CTT	(Leu/L)	CCT	(Pro/P)	CAT	(His/H)	CGT	(Arg/R)	T
	CTC	Leucine	CCC	Proline	CAC	Histidine	CGC	Arginine	C
	CTA		CCA		CAA	(Gln/Q)	CGA		A
	CTG		CCG		CAG	Glutamine	CGG		G
A	ATT	(IIe/I)	ACT	(Thr/T)	AAT	(Asn/N)	AGT	(Ser/S)	T
	ATC	Isoleucine	ACC	Threonine	AAC	Aspara	AGC	Serine	C
	ATA		ACA		AAA	(Lys/K)	AGA	(Arg/R)	A
	ATG	(M) Methin	ACG		AAG	Lysine	AGG	Arginine	G
G	GTT		GCT		GAT	(Asp/D)	GGT		T
	GTC	(Val/V)	GCC	(Ala/A)	GAC	Asparti	GGC	(Gly/G)	C
	GTA	Valine	GCA	Alanine	GAA	(Glu/E)	GGA	Glycine	A
	GTG		GCG		GAG	Glutamic acid	GGG		G

effect between UV-rays doses and incubation periods on *A. niger* which was significant when was 6.2 ds m^{-1} at M_{15} UV-rays dose during II week incubation as compared to 8.9 ds m^{-1} during M_{45} UV-ray dose and II week incubation period so that at M_{15} dose during II week conduct to the role of exposure UV-ray dose the best dose which *A. niger* capable to reduce amounts of salts in studied soil as [30] who was noticed significant effect of optima UV-rays dose to encourage dissolved salts in soil solution.

Table 5. Effect of UV-rays doses exposure and incubation periods of *A. niger* on soil pH in calcareous soil.

Periods / *A. niger*	Incubation Periods (Week) I	II	III	IV	Average
C	8.85	7.70	7.05	8.20	7.95
W	7.00	6.80	7.12	6.10	6.76
M_{15}	5.06	4.60	5.12	4.30	4.77
M_{30}	5.00	4.90	3.00	5.30	4.55
M_{45}	6.00	7.60	6.03	7.56	6.79
average	6.37	6.32	5.66	6.39	
$RLSD_{0.01}$					
Periods	0.34				
A. niger	0.07				
p × *A. niger*	0.054				

Note: C: control; W: wild; M: mutant.

3.3.3. Dissolved Phosphate in Studied Soil

At **Table 7** results showed that significant effect ($P < 0.005$) of wild *A. niger* on dissolved phosphate during incubation periods I, II, III and IV weeks when dissolved phosphate increased from 5.8 , 3.3 , 2.7 and 2.8 mg p kg^{-1} dried soil at control treatment (only soil) to 27.1 , 39.24 , 22.63 and 13.0 mg p kg^{-1} dried soil during incubation periods respectively, so *A. niger* may be dissolved insoluble soil phosphate as added superphosphate (47% P_2O_5) for some of known mechanisms as alkaline phosphate enzyme, especial bicarbonate Ca^{+2} in calcareous soil or may be *A.niger* may be released organic acids like maleic, ascorbic ….e.g. [31], moreover reduction P-soluble amounts at last incubation week (VI week) may be referred to consumption of *A.niger* to metabolism for building its bodies or limiting capable of wild *A. niger* with progress time. In addition to results at **Table 7** referred to significant effect at M_{15} mints of exposure time UV-rays along incubation periods (I, II, III and VI weeks) as compared to wild *A. niger* when dissolved phosphate increased from 27.1, 39.2, 22.6 and 13.0 mg p kg^{-1} dried soil to 53.01, 46.73, 86.6 and 66.5 mg p kg^{-1} dried soil during incubation periods respectively at increased percent at 73.9% compared to first week of incubation periods. Results at **Table 7** showed that significant effect of exposure time of UV-rays at M_{30} as compared to no UV-rays (no exposure) on phosphate dissolved in incubated calcareous soil when increased from 27.1, 39.2, 22.6 and 13.0 mg p kg^{-1} dried soil to 36.71, 52.39, 76.07 and 86.8 mg p kg^{-1} dried soil throughout incubation period I, II, III and VI weeks respectively, so that referred to positive role of UV-rays exposure time at M_{30} mints to get more *A. niger* stimulation for more either enzymatically activity or may be by double counts increased then need more energy source to build new cells of *A. niger* when exposed to more

UV-rays exposure time as[32] who noticed to positive role of UV-rays exposure on both of *B. polymyxa* when significant effect on growth parameters as more counts then inducing indole acetic acid (IAA) as regulatory growth. In addition to results at **Table 7** results conducted that significant negative effect of UV-rays exposure time especial at M_{45} when dissolved phosphate amounts reduced from 58.5 mg p kg^{-1} dried soil during III week to 36.92 mg p kg^{-1} dried soil at VI week which refers to damage effect of -rays on *A. niger* activity, because high rays irradiation may be inhibited *A. niger* cells then its capable to dissolve superphosphate as a fertilizer added to calcareous studied soil.

Table 6. Effect of UV-rays doses exposure and incubation periods of *A. niger* on electrical conductivity (ds m^{-1}) in calcareous studied soil.

Periods	Incubation Periods (Week)				Average
A. niger	I	II	III	IV	
C	5.4	6.4	7.7	7.4	8.6
W	7.3	8.8	8.8	7.0	8.8
M_{15}	8.8	6.11	7.0	8.17	7.0
M_{30}	5.4	6.46	6.87	8.67	7.6
M_{45}	9.76	9.22	8.77	8.34	9.2
average	7.33	7.39	7.81	7.92	
$RLSD_{0.01}$					
Periods	0.67				
A. niger	0.023				
p × *A. niger*	0.021				

Note: C: control; W: wild; M: mutant.

Table 7. Effect of UV-rays doses exposure and incubation periods of *A. niger* on dissolved phosphate (mg p kg^{-1} dried soil) in calcareous soil.

Periods	Incubation Periods (Week)				Average
A. niger	I	II	III	IV	
C	5.8	3.3	2.8	2.7	3.65
W	27.12	39.24	22.63	13.0	25.49
M_{15}	53.01	46.73	86.60	66.50	63.22
M_{30}	36.71	52.39	76.07	86.84	63.00
M_{45}	47.59	53.59	58.53	36.92	49.15
average	34.00	39.04	49.32	44.30	
$RLSD_{0.01}$					
Periods =	1.34				
A. niger	1.64				
p × *A. niger* =	0.78				

Note: C: control; W: wild; M: mutant.

So, that effect of UV-rays exposure doses (10, 15, 30 and 45 mints) which may be caused encourage *A. niger* activity to stimulate amounts of organic acids then dissolved soil salts either carbonate or bicarbonate Ca^{+2} to dissolve its manner.

3.4. Biomass *A. niger* in Soil

Result at **Figure 3** referred to significant interaction between UV-rays exposure periods (C, M_{15}, M_{30} and M_{45} mints) and periods time of incubation of either wild or mutant of A.niger in study of calcareous soil, when logarithm of numbers biomass increased from lower (0-0-1-1)*103 cfu gm^{-1} soil at control (no exposure) at periods I, II, III and IV weeks respectively to higher total biomass at M_{30} (mutant *A. niger* at M_{30}) when were 4, 5, 6 and 5 cfu gm^{-1} soil during I, II, III and IV respectively that noticed by[15, 32–34] in addition to results at **Figure 3** conducted that negative affect of UV-rays exposure time at M_{45} mints. When reduced biomass to 2, 3, 2 and 2 during Incubation periods I, II, III and IV respectively , results showed that significant interaction between UV-rays exposure periods and Incubation periods *A.niger* when higher total biomass were 6*103 cfu gm^{-1} soil at $M_{30during}$III week and lower at 1*103 cfu gm^{-1} soil during either III or IV weeks Incubation periods may be curtained capable of *A.niger to increase its* biomass although UV-rays exposure, which positive reflected on decrease soil-pH or increase dissolved phosphate in studied soil.

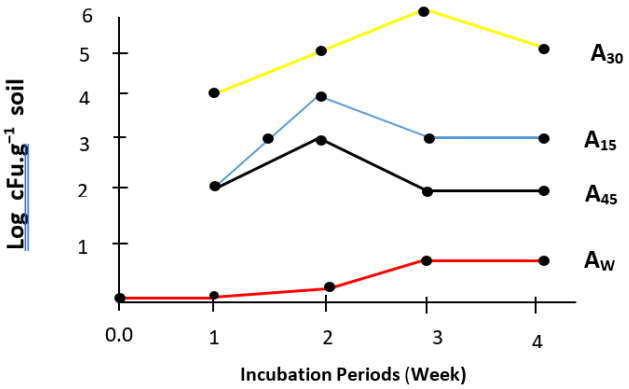

Figure 3. Effect of UV-rays doses exposure and incubation periods of *A. niger* on total biomass in calcareous soil.

4. Conclusions

This study successfully identified wild and mutant strains of *Aspergillus niger* and demonstrated that UV exposure, particularly at 254 nm for 30 minutes, significantly enhanced the phosphate-dissolving efficiency of the mutants in calcareous soil. Molecular analysis confirmed genetic vari-

ations that contributed to improved solubilization of superphosphate, leading to lower soil pH and increased biomass. These findings suggest that UV treatment can be an effective strategy for developing biofertilizers, potentially improving soil fertility and agricultural productivity. Further research is recommended to explore the practical applications of these enhanced strains in real-world agricultural settings.

Author Contributions

Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing—original draft preparation, writing—review, editing, visualization and supervision, Z.K.H.; Conceptualization, methodology, investigation, resources, data curation, writing—original draft preparation, N.A.; investigation, resources, data curation, writing—original draft preparation, writing—review, editing, visualization and supervision, H.N.H.; All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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