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THE PROMOTIVE EFFECT OF CYANOBACTERIA ON RICE SEEDS GERMINATION AND SEEDLINGS GROWTH

Herzalla, Nagat, A.; M.M. Abd EL-Monium and K. A. Abd EL- Kawi
Agric. Microbiol. Dept., Soils, Water and Environ. Res. Institute, Giza, Egypt

ABSTRACT

Beside their ability to fix atmospheric nitrogen, cyanobacteria are known to secrete into the surrounding medium a variety of compounds that could ameliorate or enhance seed germination and growth of rice plants. In an attempt to expound how far the tested cyanobacterial candidates could contribute to rice development, 24-48 hrs and 4 days laboratory incubation experiments in the dark were carried out where three rice cultivars (Giza 172, Giza 176 and Sakha 102) were determined for seed germination and seedling growth when treated with cell-free extracts (filtrates) of the cyanobacterial strains viz. *Anabaena sp., Nostoc muscorum, Tolypothrix tenuis* and *Aulosira fertilissima*, each separately. Results indicate that cyanobacterial culture extracts (filtrates) had stimulated roots and shoots growth of rice seedlings besides increasing seed germination for the tested rice cultivars. *N. muscorum* had recorded the highest percentages germination of 98, 92 and 90 for rice seeds cultivars Giza 176, Sakha 102 and Giza 172 after 48 h incubation in the dark, respectively. Extending the incubation period to 7 days, again *N. muscorum* supported greater growth pattern of rice shoots and roots than other cyanobacterial candidates.

INTRODUCTION

The importance of nitrogen-fixing cyanobacteria as a source of combined nitrogen to paddy crop, has been appreciated ever since De (1939) had demonstrated the capacity of certain cyanobacteria to fix atmospheric nitrogen in rice fields. Many workers have paid attention to the study of the cyanobacteria flora of rice fields and their effect on rice plants (Singh, 1961). He reported that rice plants fertilized with *Aulosira fertilissima*, gave rice yield three times greater than the control. Gupta and Lata (1965), while working on cyanobacteria of paddy fields, has observed that *Fischerella*, *Scytonema* and *Nostoc* sp. accelerate the germination of seeds.

Besides nitrogenous compounds, cyanobacteria have other biologically active constituents like vitamin B_{12} and auxins may also contribute appreciably to their nitrogen fixing fertilizing activity (Singh and Trehan, 1973). Various intracellular amino acids of *Cylindrospermum muscicola*, cystine, tyrosine and phenylalanine seem to be available to rice plants. Gupta and Shukla 1967) have reported that rice seedlings, treated with an extract of *phormidium* sp., showed a marked stimulation of root and shoot growth.

In a cumulative review, Roger and Kulasooriya (1980) reported that besides increasing nitrogen fertility, blue-green algae BGA have been said to benefit rice plants by producing growth-promoting substances. More direct evidence for hormonal effects has come primarily from treatments of rice seedling with algal culture or their extracts. Presoaking of rice seeds in cyanobacteria cultures or extracts decreased losses from sulphate – reducing

processes and this has been attributed to the enhancement of germination and a faster seedling growth due to algal exudates. On the other hand, extracts of *Cylindrospermum muscicola* that have given a positive effect on root growth of rice seedlings had an action similar to that produced by vitamin B_{12} which was found to be present in the cyanobacteria cells (1.5 µg g^{-1}).

Pathak and Jha, (1995) documented that when maize, wheat, mustard (*Brassica juncea*) and lady's finger (*Abelmoschus esculentus)* seeds were inoculated with *Synechocystis aquatilis, Microcystis elabens, Anabaena doliolum* or *Nostoc linckia,* seed germination in wheat and mustard was highest with *S. aquatilis.* In maize and lady's finger, it was highest with *M. elabens.* Maize and wheat yields were highest when inoculated with *A. doliolum*, while the yields of mustard and lady's finger were highest when inoculated with *N. linckia.*

Mule *et al.*, (1999) observed that the inoculation of rice plants either with *Nostoc muscorum* or *Tolypothrix tenuis* each alone or combined with urea gave higher seedlings dry weight and shoot length than the control treatment without cyanobacteria inoculation. Shoots were longest with *N. muscorum* combined with urea indicating that this treatment more effective than each cyanobacteria alone.

Elham (2001) reported that soaking rice seeds cv. Sakha 102 in *Nostoc sp.* filtrate stimulated the seeds germination percentage to reach 90 % as compared with water soaked seed. She also added that this stimulation effect occurred with *Nostoc sp.* had appeared with both *Nostoc muscorum* and *Anabaena sp.* in respective to percentage increases of 86.8 and 83.3 %.

This work is to evaluate the effect of cyanobacteria filtrate on the seed germination and the growth of three different rice cultivars (Giza 172, Giza 176 and Sakha 102).

MATERIALS AND METHODS

Four cyanobacterial strains (*Anabaena sp., Nostoc muscorum, Tolypothrix tenuis* and *Aulosira fertilissima*) are kindly supplied by Agricultural Microbiology Department, Soils, Water and Environment Research Institute, Giza, Egypt. These cyanobacteria strains were grown on Watanabe medium (EL-Nawawy *et al;* 1958) under continuous illumination (2500 Lux) at temperature of 28–32^{0}C up to their appropriate logarithmic phase, each of them was then filtered and their filtrates were used to evaluate their extract influence on the germination and the growth of rice seeds cultivars Giza 172, Giza 176 and Sakha 102 supplied by Rice Research Institute, Sakha Research Station, Agricultural Research Center, Giza. Egypt.

The surface sterilized rice seeds were soaked in a saturated sodium hypochlorite solution (2 % v/v) for 2 h, with periodical agitation, and then washed thoroughly with sterile distilled water. The seeds were allowed to germinate in a petri – dish of 10 cm diameter containing 25 ml watery agar (1.0 g agar 100 ml^{-1} distilled water). Each petri – dish was supplied with 10 seeds and supplemented with 2 ml crude filtrate of cyanobacteria.

The plates were incubated for 24, 48 h and 7days old at 28 –30^{0}C in the dark. As sterility check plates without seeds were also incubated. In the control Petri – dishes cyanobacterial filtrate was substituted with distilled water and/or Watanabe medium for comparison, each treatment was repeated in triplicates and arranged in complete randomized design. The percentage of germination of rice seeds were calculated as follows:

$$\text{Germination \%} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

After 7 days of incubation, rice seedlings were measured for shoots and roots lengths in comparison with those of the control treatments.

RESULTS AND DISCUSSION

Data in Tables (1& 2) and illustrated by Figures (1, 2 &3) indicate the promotion effect of the cyanobacteria filtrates on rice seeds germination after 24 and 48 hours incubation in the dark as well as the growth pattern of rice seedlings growth (shoots and roots) after 7 days incubation period.
Within 24 hrs of water moistening, 10 rice cv. Giza 172 seeds out of 100 were germinated increased to 60 after 48 hrs with distilled water treatment.

Table(1) : Effect of cyanobacteria filtrate on rice seed germination after 24 and 48 hrs.

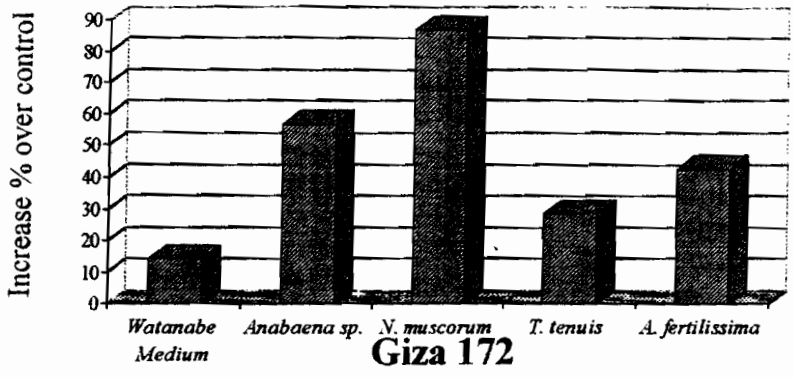
Rice cultivars	Additives	Germination % of total seeds	
		24 hrs.	48 hrs.
Giza 172	(Distilled water control)	10.00	60.00
	Watanabe medium	15.00	66.00
	Filtrate of :		
	Anabaena sp.	20.00	80.00
	Nostoc muscorum	30.00	90.00
	Tolypothrix tenuis	47.00	87.00
	Aulosira fertilissima	30.00	70.00
Giza 176	(Distilled water control)	15.00	64.00
	Watanabe medium	20.00	80.00
	Filtrate of :		
	Anabaena sp.	40.00	86.00
	Nostoc muscorum	35.00	98.00
	Tolypothrix tenuis	45.00	88.00
	Aulosira fertilissima	37.00	79.00
Sakha 102	(Distilled water control)	13.00	72.00
	Watanabe medium	18.00	80.00
	Filtrate of :		
	Anabaena sp.	45.00	85.00
	Nostoc muscorum	29.00	92.00
	Tolypothrix tenuis	48.00	89.00
	Aulosira fertilissima	34.00	74.00

Table (2): Effect of cyanobacteria filtrate on rice seedlings growth after 7 days incubation.

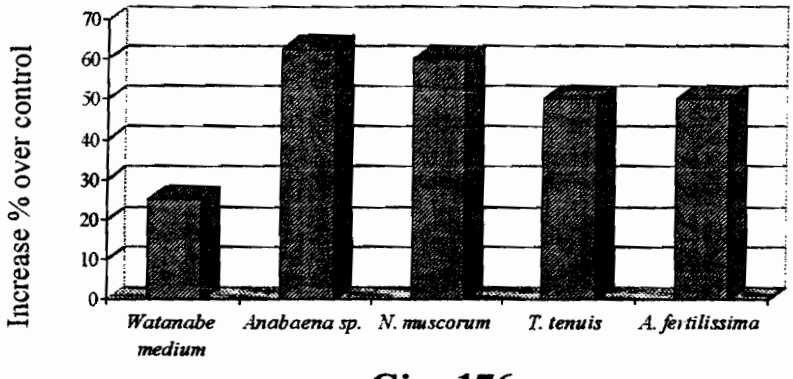
Rice cultivars	Additives	Seven days old seedlings	
		Shoot length cm	Root length cm
Giza 172	(Distilled water control)	0.70	0.20
	Watanabe medium	0.80	0.40
	Filtrate of :		
	Anabaena sp.	1.10	1.90
	Nostoc muscorum	1.30	2.00
	Tolypothrix tenuis	0.90	0.80
	Aulosira fertilissima	1.00	1.60
Giza 176	(Distilled water control)	0.80	0.20
	Watanabe medium	1.00	0.40
	Filtrate of :		
	Anabaena sp.	1.30	0.50
	Nostoc muscorum	1.52	1.10
	Tolypothrix tenuis	1.20	0.40
	Aulosira fertilissima	1.20	0.60
Sakha 102	(Distilled water control)	0.40	0.20
	Watanabe medium	0.60	0.80
	Filtrate of :		
	Anabaena sp.	0.72	1.20
	Nostoc muscorum	0.76	1.90
	Tolypothrix tenuis	0.70	1.40
	Aulosira fertilissima	0.66	1.30

Germination percentages of 15 and 64, 13 and 72 were obtained for cv. Giza 176 and cv. Sakha 102 also with distilled water treatment after 24 hrs, respectively. Seeding on Watanabe medium supported better germination rate of the tested rice cultivars with respective percentages of 15-66 (Giza 172), 20-80 (Giza 176) and 18-80 (Sakha 102) corresponding to 24 and 48 hrs incubation periods. Cyanobacterial filtrates promoted rice seed germination in rates differed from one strains to another. *N. muscorum* appeared to be the superior stimulant resulting in germination of 98, 92 and 90% of rice seeds cultivars Giza 176, Sakha 102 and Giza 172 after 48 hrs, respectively followed by *T. tenuis, Anabaena sp.* and *A. fertilissima* with respective germination percentages of (87, 88 and 89), (80, 86 and 85) and (70, 79 and 74) corresponding to rice cultivars Giza 172, Giza 176 and Sakha 102.

The early growth rates of rice seedlings (shoots and roots) and the percentage increases due to cyanobacterial culture filtrates are demonstrated in Table (2) and Figures (2 &3). Soaking rice seeds cultivars in Watanabe medium for 7 days resulted in increasing both shoots and roots compared with those soaked in distilled water. The corresponding increases percentages were 14.3, 25.0 and 50.0 (shoot) and 100, 100 and 300 (root) for Giza 172, Giza 176 and Sakha 102, respectively. Similarly, seeds of all rice cultivars responded positively to cyanobacterial culture filtrates and gave different increases for both shoots and roots compared with those soaked in distilled water.



Giza 172



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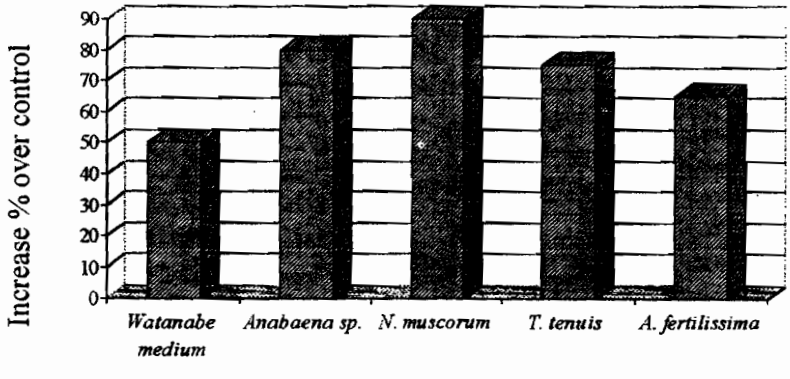
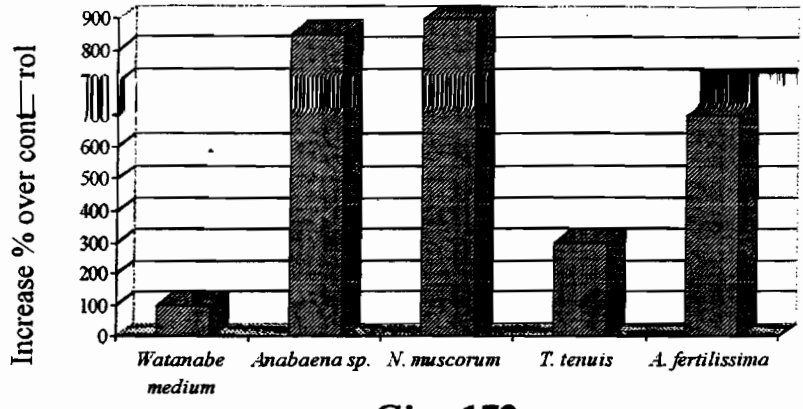
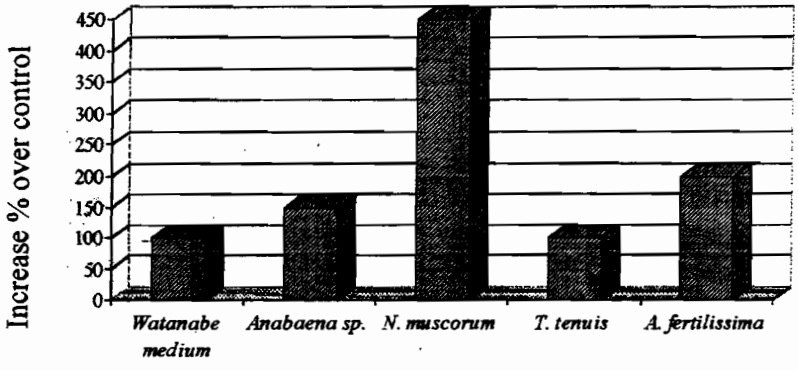


Sakha 102

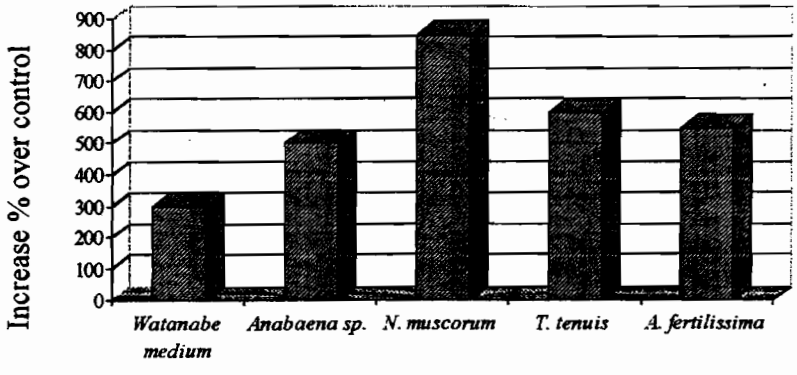
Fig. (1) : Effect of cyanobacterial filtrates on rice shoot seedlings growth after 7 days incubation.



Giza 172

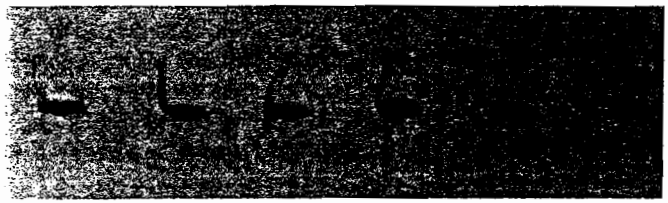


Giza 176



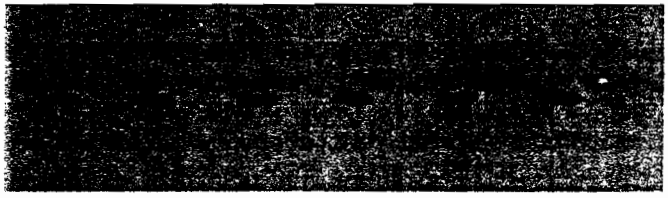
Sakha 102

Fig. (2) : Effect of cyanobacterial filtrates on rice root seedlings growth after 7 days incubation.



Rice variety Giza 172

Rice variety Giza 176



1 2 3 4 5 6

Rice variety Sakha 102

Fig.3 : Pattern growth of rice seeds cultivars under the influence of different cyanobacteria filtrates.

The treatments from left to right:

1- Control (distilled water).
2- Watanabe medium filtrate.
3- *Anabaena sp.* filtrate.
4- *Nostoc muscorum* filtrate.
5- *Tolypothrix tenuis* filtrate.
6- *Aulosira fertilissima* filtrate

Treatment of rice seed cultivars with *Anabaena sp.* filtrates supported percentage increases of 57.2, 62.5 and 80.0 in respective to rice cultivars Giza 172, Giza 176 and Sakha 102 in comparison with those recorded by distilled water treatment. The corresponding roots increases percentages were 850, 150 and 500. In respect to other tested cyanobacterial strains, *N. muscorum* recorded percentage increases for either shoot or root as 85.7, 90.5 and 90 for shoots against 900, 450 and 850 corresponding to Giza 172, Giza 176 and Sakha 102 rice cultivars, respectively. *T. tenuis* gave such increases percentages as 28.6, 50.0 and 75.0 (shoots) and 300, 100 and 600 (roots) in respective to Giza 172, Giza 176 and Sakha 102, consequently.

The positive response of tested rice cultivar to the treatment with *A. fertilissima* is expressed by the shoot increases were found to be 42.9 % (Giza 172), 50 % (Giza 176) and 65% (Sakha 102). The respective increases in root lengths were 700 %, 200 % and 550 %. Culture filtrates of *N. muscorum* supported greater growth pattern of rice shoots and roots than other cyanobacterial candidates (Table, 2 and Figs. 2 &3). Same trend was observed when compared with those presoaked with Watanabe medium only. For instance, rice seeds treated with *N. muscorum* filtrate had given percentage increases over those presoaked in Watanabe medium being 62.5, 52 and 26.7 (shoots) and 400, 175 and 137.5 (roots) for rice cultivars Giza 172, Giza 176 and Sakha 102, respectively.

Cyanobacterial culture extracts have enhanced root and shoot growth of rice seedlings besides increasing seed germination. This might possibly due to the production of growth promoting substances by the microorganisms Kaptiyeva and Tantsiurkenko (1971) found that water soluble product from 8 *Calothrix Spp.*, *Anabaena Sp.* and *Nostoc sp.* had rhizogenous effect and stimulated rice growth. Presoaking of rice seedlings in extracts of *phormidium* had been shown to accelerate seed germination (Gupta and Lata, 1965), promote the vegetative growth of rice plants and increase the weight and protein content of grains (Gupta and Shukla, 1967). The growth pattern of rice seedling treated with filterates of *Aulosira fertilisima* resembled seedlings treated with gibbrellic acid (Singh and Trehan, 1973). It has also been demonstrated that amino acids and vitamin B_{12} obtained from algal extracts had a rhizogenous influence in rice (Venkataraman and Neelkantan, 1987). Soaking seeds of cucumber and pumpkin with an extract of *Westiellopsis prolifica* a N_2-fixing cyanobacterium promoted and enhanced germination and their subsequent growth and development of seedlings. An extract of *Lyngbya sp.* a non-N_2-fixing cyanobacterium had no significant effect (Nanda *et al.,* 1991). The germination of seeds of some crop plants treated with either live inoculum, algal filtrate (exogenous) or boiled algal extract (endogenous) of the nitrogen fixing cyanobacterium *Nostoc muscorum* was significantly increased due to the nitrogenous compounds as well as nitrate reductase activities and peptides produced in the algal filtrate and or other compounds that stimulate growth of crop plants (Adam, 1999).

Soaking rice seeds cv. Sakha 102 in *Nostoc sp.* filtrate had stimulated the seeds germination percentage to reach 90 % as compared with water soaked seed (Aref, 2001). She also added that this stimulation

effect occurred with *Nostoc sp.* had appeared with both *Nostoc muscorum* and *Anabaena sp.* in respective to percentage increases of 86.8 and 83.3%. Tantawy and Mussa (2001) have soaked the seeds of some crops cultivars (wheat, soybean and clover) in the cyanobacterial extract of *Nostoc muscorum* and \ or *Anabaena flos aquae* and found that they had positively stimulated and improved both the germination and the plant growth for all tested crops.

However, the production by cyanobacteria of substances that have growth promoting like effect on rice plants is more or less well established, but what is the nature of these substances, this is the question which still needs an answer.

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التأثير المنشط للسيانوبكتريا على انبات بذور الأرز وعلى نمو البادرات

نجاة عبد العزيز حرزالله –ممدوح محمد عبد المنعم – خالد عبد الفتاح عبد القوى
قسم الميكروبيولوجيا الزراعية ــ معهد بحوث الأراضى والمياة والبيئة – الجيزة– مصر

فى هذه الدراسة أجريت تجربة في المعمل باستخدام أربعة سلالات من السيانوبكتريا كل على حدة وكانت هذه السلالات هي:

Aulosira fertilissima, Tolypothrix tenuis, Nostoc muscorum, and Anabaena sp.

حيث حضنت هذة السلالات النامية على بيئة *Watanabe* عند درجة حرارة مــن ٢٨ –٣٢°م تحت الإضاءة المستمرة لمدة ٢١ يوم للحصول على طور النمو اللوغاريتمى لها.بعد ذلــك رشحت نموات هذة السلالات للحصول على الراشح وذلك لدراسة أثره على انبات ثلاثة أصنــاف من الأرز هى جيزة ١٧٢، ١٧٦، سخا ١٠٢ وذلك بعد التحضين لحبوب هذة الأصنــاف النامية على آجار مائى فى الظلام لمدة ٢٤و ٤٨ ساعة وكذلك قياس أطوال سيقان وجذور البادرات بعــد سبعة أيام من التحضين ، ولقد أوضحت النتائج مايلى:–

١– لقد شجع راشح سلالات السيانوبكتريا إنبات تقاوي الأرز لأصناف جيزة ١٧٢، ١٧٦، ســخا ١٠٢ كما أدى الراشح إلى زيادة طول كل من جذور بادرات الأرز وكذا سيقانها.

٢– حققت ألسلالة *Nostoc muscorum* أعلى نسبة انبات لأصناف الأرز تحت الدراسة بعــد ٤٨ ساعة تحضين فى الظلام ، حيث كانت هذه النسب هى٩٨ % (جيزة ١٧٦) و ٩٢ % لكــل من (سخا ١٠٢) و(جيزة ١٧٢)، وذلك بالمقارنة مع السلالات الأخرى.

٣– كذلك حققت ألسلالة *Nostoc muscorum* أعلى أطوال لسيقان النباتات وكذلــك الجــذور لجميع أصناف الأرز تحت الدراسة وذلك بعد تحضينه لمدة سبعة أيام فى الظلام مع راشح هــذة السلالة وذلك بالمقارنة مع ماحققتة السلالات الأخرى .