Journal of Agricultural Chemistry and Biotechnology

Journal homepage & Available online at: www.jacb.journals.ekb.eg

Factors Affecting Biodegradation of the Insecticides by Cyanobacterial Strains, Isolated from Soil Contaminated with Pesticides

Aida H. Afify^{1*}; F. I. A. Hauka¹; H. A. H. El- Zawawy² and A. E. A. Abou Elatta¹

¹Microbiol. Dept., Fac. Agric., Mansoura Univ., Mansoura, Egypt. ²Botany Dept. (Microbiology), Fac. of Agric., Al-Azhar Univ., Cairo, Egypt.





This research aim to study the effect of some factors (concentration of insecticide, temperature and pH levels) affecting biodegradation of insecticides by cyanobacteria. The superior isolates out of twelve cyanobacterial isolates, which isolated from soil contaminated with insecticides were Nostoc muscorum and Anabaena oryzae. Results indicated that an increase in dry weight and N₂ fixation was recorded with increasing chlorpyrifos concentration from 0 up to150 ppm. These results were noted with Anabaena oryzae. On the other hand, there was an increase in the average of dry weight and N_2 fixation with increasing the concentration of carbofuran from zero up to 80 ppm. The highest nitrogen content was recorded in Nostoc muscorum. The biodegradation of the pesticide (chlorpyrifos and carbofuran in liquid cultures) by cyanobacterial isolates was evidenced by complete discoloration of cyanobacterial growth at 12 days of incubation. The fixed nitrogen and dry weight of Anabaena oryzae and Nostoc muscorum grown at different concentrations of chlorpyrifos (0, 50, 100, 150 and 200) and different temperature degrees (20,25,30 and 35°C) as well as different pH levels (5, 6, 7 and 8) were determined. Results indicated that the maximum uptake of chlorpyrifos by Anabaena oryzae and Nostoc muscorum was recorded at 30°C and pH 7.0. Moreover, the maximum removal of carbofuran by Anabaena oryzae and Nostoc muscorum at different concentrations (0, 40, 80 and 120) was recorded at 25°C and pH 7.0

Keywords: cyanobacteria, insecticide, temperature, pH

INTRODUCTION

The pesticides are considering the most important factor in agrochemical when the need for controlling of agricultural pests. This leads to protect food production from agricultural pests while, these pesticides contaminate the environment. Biological ways for several beneficial microorganisms, including cyanobacteria, is involved in decreasing the chemical remains (Subashchanhrabose et al., 2013). The different periods for degradation of chlorpyrifos in soil are affected by many factors such as initial concentration, soil moisture, temperature, and pH (Racke et al., 1994; Awasthi and Prakash, 1997). The important factors of chlorpyrifos degradation are microbial degradation and other chemical factors on soil (Getzin 1981; Racke et al., 1988). The degradation of carbamate nematicides can develop rapidly in soils with a pH higher than 6.5. If the microbial populations have established themselves in the soil, they might last for up to two years in soils with a low pH and for longer than five years in soils with a high pH (Arbeli and Fuentes 2007). The cyanobacterial removal ability increases with an increase in the optimal temperature or initial temperature or carbofuran concentration (Roriz et al. 2009). The ellipse plots showed that the degradation potential of carbofuran was greatly affected by changes in pH or temperature. The reduction in carbon forane increases with increasing temperature. After reaching the optimum temperature, biodegradability decreases with increasing temperature. The degradation efficiency of carbofuran is up to 95% as follows: carbofuran concentration 92.50 mg/L,

temperature 27.50°C and pH 7. The biodegradation of chlorpyrifos by Ps. desmolyticum NCIM 2112, the optimum pH and temperature for degradation were found to be 7.0 and 30 °C, respectively. Ps. desmolyticum NCIM 2112 reduces chlorpyrifos to non-toxic metabolites such as 2-pyridinol and and Mali 2013). phosphorothioate (Rokade The bioremediation of chlorpyrifos in soil using the Bacillus cereus Ct3 strain isolated from cotton plant soil. Bacillus cereus Ct3 tolerates chlorpyrifos up to 125 mg/L and reaches 88% of chlorpyrifos in 8 days at pH 8. Bacillus cereus Ct3 tolerates temperatures of around 30-40 °C (Farhan et al. 2021). Cyanobacteria can remove many contaminants from water. Cyanobacterial biodegradation processes can occur extracellularly; intracellular; or a combination of both, where initial degradation occurs extracellularly and fragments are subsequently degraded intracellularly (Touliabah et al. 2022). Therefore, the aim of this study was to evaluate insecticides concentration, temperature and pH affecting biodegradation of the insecticides (chlorpyrifos and carbofuran) by cyanobacterial strains, isolated from soil contaminated with pesticides.

Cross Mark

MATERIALS AND METHODS

Source of soil samples

Soil samples were collected from different locations at Kafr Elsheikh Governorate cultivated with rice and contaminated with insecticides. The collected soil samples were used as a source of cyanobacterial isolates. Some chemicals and physicals analyses of soil (Piper 1950 and

^{*} Corresponding author.

Jackson 1958) were previously presented by Abou Elatta *et al.* (2023).

Preparation of cyanobacterial inoculum

Liquid cultures of cyanobacterial isolates were prepared using Modified Watanabe liquid medium with incubation at 28-30°C under continuous illumination (2500 lux) for 21 days.

Total nitrogen determination

Using the micro-kjeldahl method according to Jackson (1958), total nitrogen was determined in 100 ml broth culture from cyanobacteria.

Insecticides used

Chlorpyrifos (Bestban 48%) and carbofuran (Feurdan 10%) were used in this investigation. Chlorpyrifos is O,Odiethyl O-3,5,6- trichloro-2- pyridyl phosphorothi oate (TCP). Carbofuran is N-Methyl carbamate. Some chemical characteristics of chlorpyrifos according to Venkta Mohan *et al.*, (2004). Also, chemical characteristics of carbofuran according to Chowdhury *et al.* (2014) are presented in Abou Elatta *et al.*, (2023) and Afify *et al.*, (2023b). These insecticides were obtained from Sigma - Co., USA.

Statistical analysis

Treatments differences modified L.S.D. compared with 5% and Duncans , follow the procedure outlined by Steel and Torrie (1980).

RESULTS AND DISCUSSION

Twelve cyanobacterial isolates were obtained from a soil sample contaminated with insecticides in Kafr Elsheikh

Governorate. These twelve isolates were found to be belonging to four genera (*Anabaena*, *Nostoc*, *Oscillatoria* and *Chroococcus*). Scientific names are presented in Afify, *et al.* (2023a).

Effect concentrations of insecticides

1. Determination of cyanobacterial growth

Biodegradation ability of pesticides by cyanobacteria can be evidenced by measuring the growth and nitrogen fixation of cyanobacteria. The results presented in Table (1) show great variation in the biomass production of different cyanobacterial species. During incubation period (7, 14, 21 days), the highest amount of biomass produced by cvanobacteria was recorded for Anabaena oryzae followed Nostoc muscorum. On the other hand, as the chlorpyrifos concentration increased from 0 to 150 ppm in the same incubation period, the biomass values increased and then decreased at 200 ppm. Anabaena oryzae reached the highest significant dry weight (Sarnaik et al., 2006 and John et al., 2014). A similar study was conducted by Yang et al. (2005) they found that 76.2% degradation of chlorpyrifos (100 mgl-¹) by Alcaligenes faecalis DSP3 was achieved after 18 days of culture. Bacillus pumilus C2A1 degraded 89% of 1000 mgl chlorpyrifos in 15 days. Moreover, the obtained data indicate that there are mostly gradual increases in cyanobacterial biomass (dry weight) with increasing incubation period, where the highest cyanobacteria dry weights were recorded with all strains between 14-21 days of incubation period.

Table 1. Effect of different chlorpyrifos concentrations on dry weight (g/100 ml culture) of cyanobacterial strains at different incubation periods.

Concentration of chlorpyrifos (ppm)		Contro	bl	50			100				150		200			
Cyanobacterial	Incubation Time(days)			Incubation Time(days)			Incubation Time(days)			Incubation Time (days)			Incubation Time (days)			
strains	7	14	21	7	14	21	7	14	21	7	14	21	7	14	21	
Nostoc muscorum	48n	65h	91b	55k	80c	95b	60n	68j	98b	64jk	70f	105b	41b	32d	23ij	
Nostoc paludosum	31t	57k	65h	39p	65i	74f	50r	580	81e	58lm	63k	88c	35c	23ij	21kl	
Nostoc entophytum	42q	551	62i	45n	62j	73f	49rs	66k	79f	510	68g	84d	31d	22jk	19mn	
Nostoc pruniforme	24x	40r	79c	31s	46n	76e	52q	64l	85c	54n	67gh	89c	27ef	18n	150	
Nostoc viride	33s	62i	72f	38p	69h	81c	44t	72i	85c	47p	64jk	88c	24hi	19mn	12q	
Nostoc verrucosum	28u	460	60j	35q	49m	70gh	42u	76g	81e	45q	66hi	85d	27ef	21kl	201m	
Nostoc rivulare	26v	40r	58k	28t	46n	61j	32z	48s	65kl	37r	55n	66hi	28e	13pq	10r	
Anabaena oryzae	52m	74e	98a	55k	80c	99a	62m	85c	102a	65ij	89c	108a	50a	35c	25gh	
Anabaena qelatinicola	30t	45op	76d	32rs	48m	78d	42u	49rs	83d	45q	55n	85d	22jk	150	12q	
Anabaena variabilis	27uv	44p	65h	36q	521	71g	41u	56p	82de	44q	57m	85d	26fg	14op	13pq	
Chroococcus minor	26v	51m	65h	33r	531	69h	35x	62m	74h	38r	70f	76e	23ij	12q	6t	
Oscillatoria brevis	23x	34s	67g	29t	43o	81c	38v	580	83d	48p	591	89c	150	9rs	8s	

Means followed by different letter(s) during 21 days of incubation time and concentration of chlorpyrifos are significantly different

The results in Table (2) show that dry weight of cyanobacterial isolates increased with increasing the incubation time. On the other hand, during the same incubation period, dry cell weight increased as the carbofuran concentration increased from 0 to 80 ppm and then decreased at a concentration of 120 ppm. *Nostoc muscorum* had highest dry cell weight with all carbofuran concentrations at all incubation times. Significant differences in dry weight were recorded depending upon the pesticide type and the applied dose as well as the type of cyanobacteria. For example, *Anabaena variabilis* tolerates arosine, alachlor, butachlor and 2,4-dichlorophenoxyacetate insecticides, but with increasing the concentration of these insecticides, the growth of cyanobacteria (*Nostoc punctiforme, Nostoc calcicola*,

Anabaena variabilis, Gloeocapsa sp. and Aphanocapsa sp.) decreased and arosine exhibited the highest toxicity (Singh and Datta, 2006).

2. Determination nitrogen fixation by cyanobacteria

The results presented in Table (3) show that all tested cyanobacterial strains gradually increase nitrogen fixation (mg N/100 ml culture) with increasing the incubation time, reaching much high fixed nitrogen at 21^{st} day. Moreover, it was noticed that highest amount of fixed nitrogen was recorded for *Anabaena oryzae*. Otherwise, over the same incubation period, the average N₂ fixation increases as the chlorpyrifos concentration increases from 0 to 150 ppm and then decreases at a concentration of 200 ppm (Sarnaik *et al.*, 2006; John and Shaike 2015). *Anabaena oryzae* was found to

J. of Agricultural Chemistry and Biotechnology, Mansoura Univ., Vol. 14 (11), November, 2023

have the highest significant nitrogen content. This increase is due to the microbial catabolic ability to break down chlorpyrifos (John *et al.* 2014; Singh *et al.* 2004). Therefore, it is important to determine the effectiveness at which cyanobacterial isolates fix atmospheric nitrogen, as previous studies of the effects of many pesticides on cyanobacterial growth and activity have shown that the effects are dosedependent. While low doses increased synthetic pigments, high doses decreased cyanobacterial growth, photosynthetic pigments and enzyme activities and increased oxidative stress in *Nostoc* and *Anabaena* cyanobacterial species (Kumar *et al.*, 2008 and Kumar *et al.*, 2013).

Table 2. Effect of different carbofuran concentrations on dry weight (g/100 ml culture) of cyanobacterial strains at different incubation periods.

Concentration of carbofuran (ppm)	Control				40			80		120			
Cyanobacterial	Incub	ation Tin	ne(days)	Incubation Time(days)			Incuba	ation Tin	ne(days)	Incubation Time(days)			
strains	7	14	21	7	14	21	7	14	21	7	14	21	
Nostoc muscorum	58j	67e	93a	65k	82d	95a	68lm	92d	105a	43a	32d	23gh	
Nostoc paludosum	33q	55k	65fg	39r	75g	84c	56q	83h	95c	35c	23gh	21ij	
Nostoc entophytum	46n	511	62i	55m	66k	74gh	59p	78i	94c	31d	22hi	19kl	
Nostoc pruniforme	22u	40p	71d	36t	49o	71i	50s	610	91d	27ef	181	15m	
Nostoc viride	31r	63hi	75c	39r	68j	82d	47t	74j	92d	24g	19kl	12no	
Nostoc verrucosum	28s	42o	58j	37st	47p	73h	46t	65n	87fg	27ef	21ij	20jk	
Nostoc rivulare	26t	32qr	48m	38rs	48op	621	47t	56q	691	28e	13n	10p	
Anabaena oryzae	521	64gh	88b	55m	80e	89b	65n	89e	103b	40b	31d	22hi	
Anabaena qelatinicola	31r	45n	66ef	42q	68j	78f	55qr	64n	89e	22hi	15m	12no	
Anabaena variabilis	28s	41op	55k	37st	621	73h	54r	67m	86g	26f	16m	13n	
Chroococcus minor	26t	521	62i	33u	53n	65k	38u	60op	71k	23gh	11op	8q	
Oscillatoria brevis	22u	33q	57j	30v	41q	611	46t	691	88ef	15m	10p	7q	

Means followed by different letter(s) during 21 days of incubation time and concentration of carofuran are significantly different

Table 3. Mean amounts of fixed-nitrogen (mg N/100 ml-culture) by cyanobacterial strains with different concentrations of chlorpyriphos

						Concent	tration o	f chlorp	yriphos	ppm					
Cyanobacterial		Zero			50			100			150			200	
strains	Incuba	tion Tim	e(days)	Incubat	tion Tim	e(days)	Incuba	tion Tim	e(days)	Incubat	tion Tin	ne(days)	Incubat	ionTim	e(days)
	7	14	21	7	14	21	7	14	21	7	14	21	7	14	21
Nostoc muscorum	2.33k-p	5.25c-h	9.71a	2.46j-m	5.54d-g	9.89a	2.871-n	6.54d-h	10.16a	4.97j-n	7.34e-h	10.46ab	1.87cd	1.21d	1.06d
Nostoc paludosum	2.251-p	4.14d-l	5.55c-f	2.32k-m	4.41g-i	9.65ab	2.911-n	4.63h-m	9.88ab	4.011-o	5.43h-l	9.91a-c	1.71cd	1.16d	1.02d
Nostoc entophytum	2.11m-p	3.45g-n	5.06c-i	2.33k-m	4.51g-i	9.35ab	2.33n	6.03e-i	9.45ab	3.43m-o	7.01f-i	9.55a-d	1.33cd	1.14d	0.35d
Nostoc pruniforme	2.24l-p	3.58g-n	5.98b-d	2.39k-m	4.64g-i	8.14a-c	2.49n	4.74g-l	8.55a-c	3.39no	6.23g-k	9.34a-d	1.39cd	1.12d	0.14d
Nostoc viride	2.31k-p	4.16d-l	5.81b-e	2.44j-m	4.36g-j	6.78с-е	2.64n	5.02g-k	6.98c-f	4.211-o	5.86h-l	7.88d-g	1.24cd	1.16d	0.98d
Nostoc verrucosum	2.12m-p	3.35h-o	4.35d-j	2.35k-m	4.21g-k	5.22e-h	2.55n	5.11f-k	6.42d-h	3.35no	5.61h-l	6.52f-j	1.35cd	1.11d	0.42d
Nostoc rivulare	1.41op	2.261-p	4.27d-k	2.12lm	3.38h-l	5.61d-g	2.26n	5.24f-k	6.31e-h	4.11l-o	6.22g-k	7.81d-g	1.12d	1.08d	0.77d
Anabaena oryzae	3.25і-р	5.35c-g	9.81a	3.31h-m	5.45d-g	9.92a	3.37k-n	6.58d-g	10.31a	4.24l-o	8.89b-e	10.81a	3.16bc	4.35b	6.54a
Anabaena qelatinicola	2.46ј-р	3.89e-m	7.42b	3.11i-m	5.25e-h	7.96bc	3.53k-n	6.01e-i	8.56a-c	4.11l-o	6.45f-j	9.06а-е	1.11d	1d	0.26d
Anabaena variabilis	1.35p	3.61f-n	6.46bc	1.41m	3.96g-l	7.27cd	2.41n	4.01j-n	8.26b-d	2.680	5.06j-n	8.27c-f	1.41cd	1.26cd	1.07d
Chroococcus minor	1.91m-p	2.83j-p	4.18d-l	2.41klm	3.25ijklm	4.85f-i	2.81mn	4.14i-n	5.15f-k	3.41m-o	4.35k-o	5.35i-m	1.36cd	1.15d	0.25d
Oscillatoria brevis	1.87n-p	2.84ј-р	4.34d-j	3.25ijklm	5.25efgh	6.61c-f	3.64k-n	5.72e-j	7.31с-е	4.251-o	6.85f-j	7.81d-g	1.25cd	1.05d	0.54d

Means followed by different letter(s) during 21 days of incubation time and concentration of chlorpyrifos significantly different

The results in Table (4) show that nitrogen fixation by cyanobacteria with different carbofuran concentrations gradually increased as the experiment progressed, with the significant highest nitrogen fixation by cyanobacteria being recorded during 14-21 days of incubation. It was determined that nitrogen fixation and excretion capacity of all different strains were recorded the highest nitrogen fixation value with *Nostoc muscorum* culture after 21 days. This increase is due to

microbial degradation of carbofuran when organisms such as cyanobacteria, bacteria and fungi use pesticides as source of caron and energy. Microbial degradation occurs rapidly in soil conditions suitable for microbial activity. These factors include temperature, pH, humidity, aeration (oxygen source) and fertility. The degree of absorption also affects microbial degradation, as most pesticides must be in solution to be absorbed and metabolized by bacteria. Frequent use of pesticides is

Aida H. Afify et al.

also an important factor affecting this deterioration (Harrison, 1990).

Nostoc muscorum and *Anabaena oryzae* were found to be the superior cyanobacterial isolates, which isolated from

soil contaminated with insecticides. Both isolates were tested for their ability to degrade insecticides under different temperature degrees and pH levels. The obtained results are presented below:

Table 4. Effect of different carbofuran concentrations on nitrogen fixation (mg N/100 ml culture) of cyanobacterial strains.

	Concentration of carbofuran ppm												
Cyanobacterial		Control			40			80			120		
strains	Incubat	ion Time(days)	Incubat	ion Time	e(days)	Incuba	ation Time	e(days)	Incubation Time(days)			
-	7	14	21	7	14	21	7	14	21	7	14	21	
Nostoc muscorum	3.25h-o	5.35d-f	9.81a	4.31f-j	6.45с-е	9.92a	5.24k-o	8.89b-e	10.91a	3.16а-с	4.35a	3.54ab	
Nostoc paludosum	2.51j-p	4.14e-l	5.53de	3.32h-k	4.45f-i	8.65ab	4.61k-p	6.43g-k	9.81-c	2.11b-d	1.16d	1.02d	
Nostoc entophytum	2.13m-p	3.35g-n	5.32d-f	3.33h-k	4.53f-i	8.35ab	3.53n-p	5.01k-p	9.5a-d	1.31cd	1.14d	0.35d	
Nostoc pruniforme	2.251-p	3.48f-m	5.67de	3.39h-k	4.61e-i	8.14а-с	3.49op	6.13h-k	9.34a-d	1.49cd	1.12d	0.19d	
Nostoc viride	2.49j-p	4.26d-k	5.82с-е	3.44h-k	4.39fg-j	7.78b-d	4.81k-p	5.46j-n	7.88d-h	1.28cd	1.06d	0.91d	
Nostoc verrucosum	2.33k-p	3.36gh-n	4.32d-j	2.95i-k	4.23g-j	6.22d-f	3.35op	5.62j-m	6.56fg-k	1.35cd	1.1d	0.41d	
Nostoc rivulare	1.45n-p	2.26l-p	4.22d-l	2.62i-k	3.38h-k	5.61e-g	4.111-p	6.24g-k	7.83d-h	1.12d	1.0d	0.76d	
Anabaena oryzae	3.13h-p	5.25d-g	8.81ab	3.46h-k	5.54e-g	9.89a	4.97k-p	7.34e-j	10.46ab	1.87b-d	1.21d	1.06d	
Anabaena qelatinicola	2.46j-p	4.89d-h	7.52bc	3.11h-k	4.25g-j	8.96ab	4.71k-p	6.15h-k	8.06cd-g	1.61cd	1.3cd	0.86d	
Anabaena variabilis	1.85m-p	4.61d-i	6.16cd	2.41jk	4.96e-h	7.77b-d	3.68m-p	5.16k-p	8.37c-f	1.49cd	1.16d	1.07d	
Chroococcus minor	1.21p	2.73i-p	4.18e-l	2.41jk	4.25g-j	4.15g-k	3.48op	4.15l-p	5.25kl-o	1.36cd	1.1d	0.55d	
Oscillatoria brevis	1.37op	2.14m-p	4.14e-l	2.25k	4.21g-k	5.61e-g	3.25p	5.85i-l	7.71d-i	1.25cd	1.05d	0.53d	
Moone followed by differe	ont lattor(c) d	luring 21 do	ve of incu	botion tim	a and con	contration	of corofur	on oro cian	ificantly dif	Foront			

Means followed by different letter(s) during 21 days of incubation time and concentration of carofuran are significantly different

Effect pH on the growth of cyanobacterial strains in presence of different concentrations of chlorpyrifos and carbofuran.

The fixed nitrogen and dry weight of cyanobacterial strains grown at different concentrations of chlorpyrifos are presented in Table (5). Among the isolated cyanobacteria *Anabaena oryzae* and *Nostoc muscorum* were found to have the highest dry weight at 150 ppm chlorpyrifos concentration. This increase was attributed to the ability of microbes to degrade chlorpyrifos (John *et al.* 2014; Singh *et al.* 2004). Strains were selected and grown in nutrient medium with

different pH levels, and the most suitable pH for all cyanobacterial isolates tested was found to be pH 7.0. The results for the removal of chlorpyrifos in the acidic and alkaline conditions showed maximum removal at pH 7.0. Our observations are based on the maximum removal (mg/L) of chlorpyrifos pH 7.0 (Fang *et al.*, 2008). The significant maximum biodegradation of chlorpyrifos by bacteria has been reported in the neutral pH or alkaline range (Singh *et al.* 2003; Yang *et al.* 2005; Xu *et al.* 2007; Fang *et al.* 2008; Thengodkar and Sivakami 2010). Rokade and Mali (2013) reported that the optimum pH value for degradation is 7.0.

 Table 5. Effect different pH values on dry weight (mg/100ml-culture) of cyanobacterial strains at different concentrations of chlorpyrifos

Incu	bation T	ime(7 d	lays)	Incubation Time(21 days)							
					ŀ	oH valu	es				
5	6	7	8	5	6	7	8	5	6	7	8
5uv	28n	42f	40g	55k-m	65g	74cd	68f	80kl	91f	98de	92f
11st	31lm	45de	36ij	46no	57jk	70e	58ij	73no	85ij	99d	97e
13s	321	52c	38h	54lm	65g	75c	71e	77lm	82k	102c	87h
22pq	34k	65a	46d	69ef	80b	89a	73d	93f	98de	108a	99d
8tu	23p	38h	36j	41p	52m	55lm	53m	58s	62r	75mn	63r
бu	18r	28n	21q	29t	36r	45o	39pq	37u	50t	71o	65q
10t	260	45de	35jk	38q	460	60h	54m	65q	69p	75mn	710
17r	35jk	40g	37hi	450	54m	68f	58j	70op	84j	91f	89g
18r	40g	54b	44e	56kl	65g	70e	59hi	74n	86hi	105b	101c
3v	21q	30m	260	33s	41p	52m	49n	58s	65q	791	71o
	5 5uv 11st 13s 22pq 8tu 6u 10t 17r 18r	5 6 5uv 28n 11st 31lm 13s 321 22pq 34k 8tu 23p 6u 18r 10t 26o 17r 35jk 18r 40g	5 6 7 5uv 28n 42f 11st 31lm 45de 13s 32l 52c 22pq 34k 65a 8tu 23p 38h 6u 18r 28n 10t 26o 45de 17r 35jk 40g 18r 40g 54b	5uv 28n 42f 40g 11st 31lm 45de 36ij 13s 32l 52c 38h 22pq 34k 65a 46d 8tu 23p 38h 36j 6u 18r 28n 21q 10t 26o 45de 35jk 17r 35jk 40g 37hi 18r 40g 54b 44e	5 6 7 8 5 5uv 28n 42f 40g 55k-m 11st 31lm 45de 36ij 46no 13s 32l 52c 38h 54lm 22pq 34k 65a 46d 69ef 8tu 23p 38h 36j 41p 6u 18r 28n 21q 29t 10t 26o 45de 35jk 38q 17r 35jk 40g 37hi 45o 18r 40g 54b 44e 56kl	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $

Means followed by different letter(s) in the same incubation time are significantly different

Results in Table (6) showed the effect of different pH levels on dry weight of cyanobacterial strains growing on different concentrations of carbofuran 0,40,80 and120 ppm. The highest dry weight was observed with *Nostoc muscorum* and *Anabaena oryzae* at concentrations from zero to 80 ppm. Therefore, the efficiency of cyanobacteria strains in nitrogen fixation and dry weight was evidence by the biodegradation of carbofuran. *Nostoc muscorum* and *Anabaena oryzae* were selected and grown in nutrient media containing different pH, and the maximum removal of carbofuran was recorded at pH 7.0. The significant high maximum

biodegradation of carbofuran by bacteria has been reported in the neutral pH or alkaline range (Singh *et al.* 2018).

Effect temperature on the growth of cyanobacterial strains with different concentrations of chlorpyrifos and carbofuran

The efficiency of *Anabaena oryzae* and *Nostoc muscorum* in biodegradation of chlorpyrifos insecticide was tested at different temperature degrees (*i.e.* 20, 25, 30 and 35°C). Data in Table (7) indicated that the significant maximum uptake of chlorpyrifos was recorded at 30°C. After estimating the average dry weight of cyanobacterial

J. of Agricultural Chemistry and Biotechnology, Mansoura Univ., Vol. 14 (11), November, 2023

strains added to different concentrations of chlorpyrifos (0, 50, 100, 150 and 200 ppm), the highest dry weight was recorded for *Anabaena oryzae* and *Nostoc muscorum*. This increase is due to the ability of cyanobacteria to degrade chlorpyrifos. Therefore, the efficiency of the cyanobacterial isolates concerning dry weight was evidence by the biodegradation of chlorpyrifos. The strains

were selected and cultivated in a nutrient medium with different temperatures to find out the most suitable degree for the biocracking process, and the best temperature was 30°C. Rokade and Mali (2013) described the biodegradation of chlorpyrifos by *Ps. desmolyticum* NCIM 2112 and the best temperature for degradation was 30°C.

Table 6. Effect different pH values on the dry weight (mg/100ml-culture) of cyanobacteria strains growing on different concentrations of carbofuran

Cyanobacterial strains	Incu	Incubation Time(7 days)					Time(14	l days)	Incubation Time(21 days)			
and concentration							pН					
of carbofuran (ppm)	5	6	7	8	5	6	7	8	5	6	7	8
Anabaena oryzae	7n	25k	41e	30no	45j	55h	74gh	67k	81e	89hi	98ef	92h
Anabaena oryza + 40	211	33g	44d	36m	49i	57g	71hi	68jk	74gh	81k	96fg	87i
Anabaena oryzae + 80	32gh	44d	66b	45j	69d	80b	89c	85d	93b	98ef	108b	99e
Anabaena oryzae + 120	8n	221	31h	260	391	42k	591	53m	68jk	72lm	79k	741
Nostoc muscorum	9n	28j	38f	31n	46j	66e	75g	69ij	77f	80k	91h	85j
Nostoc muscorum + 40	10n	29ij	45d	34m	48i	69d	80e	74gh	85d	89i	95g	91h
Nostoc muscorum + 80	38f	57c	74a	64f	76c	85a	95a	89c	94ab	103d	115a	106c
Nostoc muscorum + 120	13m	211	30hi	260	23p	31n	45o	39p	48n	550	69n	71m
Means followed by different letter(s) in the same inc	ubation	time are	significa	ntly diff	erent						

 Table 7. Effect of different temperature degree on dry weight (mg/100ml-culture) of cyanobacteria strains added to different concentrations of chlorpyrifos

Temperature degree					25°C			30°C		35°C		
Cyanobacterial strains and	Incubat	ion Tin	ne(days)	Incubation Time(days)			Incubat	ion Time	(days)	Incubation Time(days)		
concentration of chlorpyrifos (ppm)	7	14	21	7	14	21	7	14	21	7	14	21
Anabaena oryzae	55p	691	84f	58mn	75i	89e	78op	105f	120d	53p	82j	97d
Anabaena oryzae +50ppm	63mn	75i	95c	69jk	85g	104c	86l-n	110e	125c	62o	85i	99c
Anabaena oryzae +100ppm	66m	81g	98b	75i	86g	108b	88k-m	120d	130b	71lm	92f	105b
Anabaena oryzae +150ppm	72jk	90d	110a	86fg	89e	111a	90jk	125c	135a	77k	98cd	108a
Anabaena oryzae +200ppm	31s	33s	55p	32r	48p	62m	37s	74p	82o	24t	39r	49p
Nostoc muscorum	45q	590	74ij	48p	651	79h	68q	85n	91j	630	72lm	87h
Nostoc muscorum +50ppm	56p	62n	78h	58n	68k	72j	77p	86mn	99h	68n	731	90g
Nostoc muscorum +100ppm	60no	691	88e	651	80h	99d	85n	89kl	103g	70mn	81j	92f
Nostoc muscorum +150ppm	71k	85f	96c	72j	88ef	108b	88k-m	94i	109e	72lm	85i	95e
Nostoc muscorum +200ppm	21t	43r	590	37q	52o	71j	44r	68q	76p	36s	46q	630
Means followed by different letter(s) in the same incubation time are significantly different												

The data in Table (8) indicated that insecticide (carbofuran) removal by *Anabaena oryzae* and *Nostoc muscorum* was studied at temperatures (20, 25, 30 and 35°C). The results showed that significant maximum degradation of carbofuran was observed at 25°C. After estimating dry weight of cyanobacterial strains added to different concentrations of carbofuran (0, 40, 80 and 120) the highest dry weight was achieved by *Nostoc muscorum* and *Anabaena oryzae*. This increase is due to the ability of cyanobacteria to degrade carbofuran. Temperature influences both algal biomass composition and productivity.

The cyanobacterial strains were cultivated in a nutrient medium with different temperatures to screen the suitable degree and the best temperature was 25°C. These results are in agreement with those achieved by Umar Mustapha, *et al.* (2020). An optimization method using surface response method was used to analyze the effects of four different factors (pH, nitrogen source, temperature and initial carbofuran concentration) and their effects on carbofuran degradation. Carbofuran is effectively degraded up to 95% as follows: carbofuran concentration 92.50 mg/L, temperature of 27.50 °C.

 Table 8. Effect of different temperature degrees on dry weight (mg/100ml-culture) of cyanobacteria strains added to different concentrations of carbofuran

	Temperature													
Cyanobacterial strains and concentration		20°C			25°C			30°C		35°C				
of carbofuran (ppm)	Incubation Time(days)			Incuba	tion Time	e(days)	Incuba	tion Tin	ne(days)	Incubation Time(days)				
or carborur an (ppm)	7	14	21	7	14	21	7	14	21	7	14	21		
Anabaena oryzae	45n	591	74g	68r	85k	95i	580	80j	92f	53k	82h	91f		
Anabaena oryzae +40ppm	53m	65j	85e	76op	100g	98h	69m	78kl	94e	62j	85g	93e		
Anabaena oryzae +80ppm	62k	80f	100b	80mn	115d	125b	76l	89g	105b	77i	96d	101b		
Anabaena oryzae +120ppm	20s	23r	350	37t	64s	72q	27s	48p	41q	22p	330	41m		
Nostoc muscorum	56m	69i	85e	78no	104f	120c	68m	85h	99d	53k	82h	96d		
Nostoc muscorum +40ppm	62k	75g	95c	831	110e	125b	79jk	99d	101c	62j	85g	99c		
Nostoc muscorum +80ppm	72h	90d	110a	90j	125b	135a	82i	91f	111a	77i	98c	108a		
Nostoc muscorum +120ppm	31q	33p	55m	37t	74p	82lm	32r	48p	62n	24p	39n	491		

Means followed by different letter(s) in the same incubation time are significantly different

REFERENCES

- Abou Elatta, A.E.A; El-Zawawy, H.A.H.; Afify, Aida H. and Hauka, F.I.A. (2023). Degradation of chlorpyrifos by cyanobacteria strains in rice fields. J. Agric. Chem. and Biotech., Mansoura Univ., Vol. 14(5): 43-49.
- Afify, Aida H.; Hauka, F.I.A.; El-Zawawy, H.A.H. and Abou Elatta, A.E.A. (2023a). Characterization of cyanobacterial strains isolated from soils polluted with insecticides. J. Agric. Chem. and Biotechn., Mansoura Univ., Vol. 14(6): 73-78.
- Afify, Aida H.; Hauka, F.I.A.; El-Zawawy, H.A.H. and Abou Elatta, A.E.A. (2023b). Carbofuran relevance and their cyanobacterial degradation in rice fields. J. Agric. Chem. and Biotechn., Mansoura Univ., Vol. 14(8): 93-98.
- Arbeli, Z. and Fuentes, C.L. (2007). Accelerated biodegradation of pesticides: An overview of the phenomenon, its basis and possible solutions; and a discussion on the tropical dimension. Crop Prot. 26, 1733-1746.
- Awasthi, M.D. and Prakash, N.B. (1997). Persistence of chlorpyrifos in soils under different moisture regimes. Pest. Sci., 50: 1–4.
- Chowdhury, M. A. Z.; Jahan, I.; Karim, N.; Alam, M. K.; Rahman, M. A.; Moniruzzaman, M.; Gan, S. H. and Fakhruddin, A. N. M. (2014). Determination of carbamate and organophosphorus pesticides in vegetable samples and the efficiency of gammaradiation in their removal. Hindawi Publishing Corporation BioMed Res. Intern. Article ID 145159, 9 pages.
- Fang, H.; Xiang, Y.Q.; Hao, Y.J.; Chu, X.Q.; Pan, X.D.; Yu, J.Q. and Yu, Y.L. (2008). Fungal degradation of chlorpyrifos by *Verticillium* sp. DSP in pure cultures and its use in bioremediation of contaminated soil and pakchoi. Int. Biodeterior. Biodegrad. 61:294– 303.
- Farhan, M.; Ahmad, M.; Kanwal, A.; Ali Butt, Z.A.; Khan, Q.F.; Raza, S.A.; Qayyum, H. and Wahid, A. (2021). Biodegradation of chlorpyrifos using isolates from contaminated agricultural soil, its kinetic studies. Scientific Reports 11:10320.
- Getzin, L.W. (1981). Degradation of chlorpyrifos in soil: influence of autoclaving, soil moisture and temperature. J. Econ. Entomol., 74:158–162.
- Harrison, S.A., (1990). The fate of pesticides in the environment. Adapted from the fate of pesticides in the environment and groundwater protection, by Brown, C. L. and Hock, W.K. Agrochemical Factsheet ;7: 286.
- Jackson, M.L. (1958). "Soil Chemical Analysis, Constable and CO₂". Agric. Exp. Mad. Wisconsin., pp. 183-187.
- John, M. E.; Rebello, S. and Jisha, M. S. (2014). Chlorpyrifos degradation using bacterial consortium obtained from soil. Institute of Res. Eng. and Doctors, 1-4.
- John, E.M. and Shaike, J.M. (2015). Chlorpyrifos: Pollution and Remediation. J. of Environ. Chem. Letters, 13: 269-291.

- Kumar, C. P.; Yashoda, P.; Dinesh, P. and Shashwa, N. (2013). Study of soil cyanobacteria to evaluate metabolite production during various incubations in their culture filtrate. Sch. Acad. J. Biosci., 1:154-158.
- Kumar, S.; Habib K. and Fatma, T. (2008). Endosulfan induced biochemical changes in nitrogen-fixing cyanobacteria. Sci. Total Environ., 403:130–138.
- Piper, C. S. (1950). "Soil and Plant Analysis". Inter. Sci. Publisher, Inc. New York, USA.
- Racke, K.D.; Coats, T. R. and Titus, K. R. (1988). Degradation of chlorpyrifos and its hydrolysis products, 3, 5, 6-trichloro-2-pyridinol, in soil. J. Environ. Sci. Health, 23:527–539.
- Racke, K. D.; Fontaine, D. D.; Yoder, R. N. and Miller, J. R. (1994). Chlorpyrifos degradation in soil at termiticidal application rates. Pest. Sci., 42:43–51.
- Rokade, B. K. and Mali, V. G. (2013). Biodegradation of chlorpyrifos by *Pseudomonas desmolyticum ncim* 2112. Intern. J. of Pharma and Bio. Sci., 4 (2): 609 – 616.
- Roriz, M.S.; Osma, J.F.; Teixeira, J.A. and Rodriguez, Couto S. (2009). Application of response surface methodological approach to optimise reactive black 5 decolouration by crude laccase from *Trametes pubescens*. J. Hazard Mater.;169: 691-6.
- Sarnaik, S.S.; Kanekar, P.P.; Raut, V.M.; Taware, S.P.; Chavan, K.S. and Bhadbhade, B.J. (2006). Effect of application of different pesticides to soybean on the soil microflora. J. of Environ. Biol., Vol. 27(2): 423-426.
- Singh, A. K.; Singh, P. P.; Tripathi, V.; Verma, H.; Singh, S. K.; Srivastava, A. K. and Kumar, A. (2018). Distribution of cyanobacteria and their interactions with pesticides in paddy field: a comprehensive review. J. of Environ. Management, 224: 361-375.
- Singh, B. K.; Walker, A.; Morgan, J.A.W. and Wright, D. J. (2003). Effects of soil pH on the biodegradation of chlorpyrifos and isolation of a chlorpyrifos degrading bacterium. Appl. Environ. Microbiol., 69:5198–5206.
- Singh, B. K.; Walker, A.; Morgan, A. W. J. and Wright, J. D. (2004). Biodegradation of Chlorpyrifos by *Enterobacter* strain B-14 and its use in bioremediation of contaminated soils. Appl. and Environ. Microbiol., 70 (8): 4855-4863.
- Singh, S. and Datta, P. (2006). Screening and selection of most potent diazotrophic cyanobacterial isolate exhibiting natural tolerance to rice field herbicides for exploration as biofertilizer. J. Basic. Microbiol. 46:219–225.
- Steel, R. G. and Torrie, J. H. (1980). "Principles and procedures of statistics. Ambometrical approach" MC Grow whill, New York.
- Subashchanhrabose, S. R.; Balasubramanian, R.; Mallavarapu, M.; Kadiyala, V. and Ravi, N. (2013). Mixotrophic cyanobacteria and microlgae as distinctive biological agents for organic pollutant degradation. Environ. Intern. 51: 59-72.

J. of Agricultural Chemistry and Biotechnology, Mansoura Univ., Vol. 14 (11), November, 2023

- Thengodkar, R.R.M. and Sivakami, B. (2010). Degradation of chlorpyrifos by alkaline phosphatase from the cyanobacterium *Spirulina platensis*. Biodegrad. 21:637–644.
- Touliabah, H. E. S.; El-Sheekh, M. M.; Ismail, M. M. and El-Kassas, H. A. (2022). Review of microalgae and cyanobacteria-based biodegradation of organic pollutants. Molecules, 27: 1141.
- Umar Mustapha; Halimoon, M. N.; Johari, W.L.W. and Yunus, Abd Shokur M. (2020). Optimization of carbofuran insecticide degradation by *Enterobacter* sp. J. King Saud University: 1037.
- Venkta, Mohan S.; Sirisha, K.; Rao, N.C.; Sarma, P.N. and Reddy, S.J. (2004). Degradation of chlorpyrifos contaminated soil by bioslurry reactor operated in sequencing batch mode: bioprocess monitoring. J. Hazard Mater., 116: 39.
- Xu, G.; Li, Y.; Zheng, W.; Peng, X.; Li, W. and Yan, Y. (2007). Mineralization of chlorpyrifos by co- culture of *Serratia* and *Trichosporon* spp. Biotechnol. Lett. 29:1469–1473.
- Yang, L.; Zhao, Y.H.; Zhang, B.X.; Yang, C.H. and Zhang, X. (2005). Isolation and characterization of a chlorpyrifos and 3,5,6-trichloro-2- pyridinol degrading bacterium. FEMS Microbiol. Lett. 251(1):67–73. doi:10.1016/j. femsle. 2005. 07.031.

العوامل المؤثره على التكسير الحيوى لمبيدات الآفات بواسطة سلالات السيانوبكتيريا المعزوله من الأراضي الملوثه بمبيدات الآفات

عايده حافظ عفيفي1 ، فتحى إسماعيل على حوقه1، حسن أحمد حسن الزواوى2 و أحمد السيد عبد الرحمن أبو العطا1

¹ قسم الميكروبيولوجي – كلية الزراعة – جامعة المنصورة – المنصورة – مصر 2قسم النبات (ميكروبيولوجي) – كلية الزراعة – جامعة الأز هر – القاهرة – مصر

الملخص

يهدف هذا البحث إلى در اسة تقييم العوامل المؤثر، على قدرة السيانوبكتيريا المحلله لمبيدات الأفات حيث تم الحصول على إثنى عشر، عزله من السيانوبكتيريا من الأراضى الملوثه بمبيدات الأفات من محلفظة كفر الشيخ وعند تقدير تأثير بعض العوامل وخاصة تركيزات مختلفه من مبيدين من مبيدات الأفات (الكلوروبيروفوس والكاربوفيرران) بعد ذلك در اسة تأثير درجة الحراره ودرجة الحموضه على أكفاً عزلتين من السيانوبكتيريا في تحليل المبيدات عند تركيزات مختلفه من مبيدين من مبيدات الأفات (الكلوروبيروفوس والكاربوفيرران) بعد ذلك در اسة تأثير درجة الحراره ودرجة الحموضه على أكفاً عزلتين من السيانوبكتيريا في تحليل المبيدات عند تركيزات مختلفه من مبيدات الأفات (الكلوروبيروفوس والكاربوفيرران) بعد ذلك در اسة Anabaena oryzae وقد أظهرت النتائج زياده في الوزن الجاف وكذلك النتروجين المثبت عند زيادة فترة التحضين وذلك بزيادة تركيز المبيد الكلوروبيروفوس والكاربوفيران مع سلالة Anoyzae من يلدي الميدان الجاف وكذلك النتروجين المثبت عند زيادة فترة التحضين وذلك بزيادة تركيز المبيد الكوروبيروفوس 150 جزء في المليون مع سلالة Anoyzae من ناحية أخرى زيادة الوزن الجاف ولنتروجين المثبت عند تركيز مبيد الكاربوفيروزان 80 جزء في المليون عمد سلالة الماليون من ناحية أخرى زيادة الوزن الجاف والنتروجين المثبت عند تركيز مريد الان 80 جزء في المليون مع سلالة السيانوبكتيريا المين من 80 جزء في المليون عمد سجلة من المليون التكسير الحيوى لكلا الميدين حقى المثبت عند تركيز مبيد الكاربوفيوران 80 جزء في المليون مع سلالة السيانوبكتيريا المين 30 من عمد سجلت السلالتين التكسير الحيوى لكلا الميدين حقى اليوم الثانى عشر من التحضين. وبالنسبه لتأثير درجة الحراره ودرجة الحراره 30 م ودرجة حموضه 7 مع المبيد الكلوروفوس ولكن درجة حدراره 20 م ودجة حموضه 7 مع المبيد الكاربوفيوران على التوالي .