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Carbofuran Relevance and their Cyanobacterial Degradation in Rice Fields

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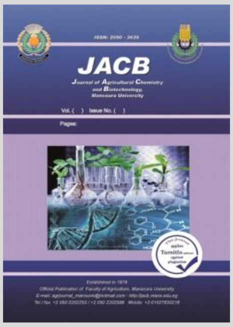

ABSTRACT

Carbofuran is widely used for controlling pests on rice plant. These pesticide pollute the environment. Cyanobacteria can be used to get rid of pesticides such as carbofuran able to remove from agricultural fields. Therefore, the main objective of this research is to assess the biodegradation of the carbofuran by *Anabaena oryzae* and *Nostoc muscorum* these are cyanobacterial strains in the rice fields which polluted by this insecticide. For determination of biodegradation carbofuran, nitrogen fixation rate, dry weight formation, total count of cyanobacteria, absorbance measurements by spectrophotometer in addition gas chromatography–mass spectrometry (GC-MS) used to perform carbofuran, residues. The data showed the ability of cyanobacteria to degrade all components of carbofuran by cyanobacteria and the mixture of *Nostoc muscorum* and *Anabaena oryzae* had improved in dry weight and increased the nitrogen fixation in environment. Furthermore, the growth and yield for rice plant after inoculation with the mixture treatment from cyanobacterial strains were increased compared to the control. In addition, it highlights the need for cyanobacteria application in the contaminated area is a better candidate for biological decomposition of insecticides.

Keywords: *Anabaena oryzae*, *Nostoc muscorum*, carbofuran, rice plant

INTRODUCTION

The pesticides are consider 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). Currently, the use of cyanobacteria are the best way to remove pesticides and chemicals that pollute soil. Because cyanobacteria have the ability to degrade pesticides at a faster rate (Verma *et al*, 2014). Carbofuran is applied on a large scale in rice fields as remains biologically active in soil (Lakshmi *et al*., 2008). These remains is due to degradation of carbofuran in soil as affected by its initial concentration, soil moisture, temperature, and pH (Racke *et al.*, 1994; Awasthi and Prakash, 1997). Carbofuran is one of the most toxic broad-spectrum and systemic *n* methyl carbamate pesticide, which is extensively applied as insecticide for agricultural, domestic and industrial purposes (Mishra *et al*., 2020). Microbial degradation technology has emerged as the most effective and potent remediation strategy for the removal of carbofuran contamination from the environment (Zulpa *et al*., 2003; Singh *et al*., 2016; Sammauria *et al*., 2020). Therefore, investigation of the ecotoxicological effects of pesticides on the structure and function of the tropical paddy field associated cyanobacteria is urgent and need to estimate (Singh *et al*., 2018). Lately, great attention has been paid to the beneficial effects of cyanobacteria in rice fields (Castenholz 2015; Abou Elatta, 2018; Abou Elatta *et al*., 2019). Little of reports are also available for pesticides degradation by cyanobacteria (Lee *et al*., 2003; Barton *et al.*, 2004; El-bestawy *et al.*, 2007; Cáceres *et al*., 2008). Photoautotrophic microorganisms, such as cyanobacteria, are used for wastewater treatment to remove nitrogen and phosphorus (Ibrahim 2011). They have potential to remove various pollutants, such as dyes (Rangabhashiyam, *et al*., 2014), heavy metals (Ibrahim 2011) and pesticides (Ibrahim *et al*., 2014). Cyanobacteria are a diverse group of oxygenic photosynthetic prokaryotes with uniquephysiology, broad ecological valence (Seckbach, 2007). Rice is the important food of over half the world's population (Yadav *et al*., 2010). However, where population pressure is high, there is no option except to produce more food. It is necessary to increase productivity (Swaminathan, 2000). Therefore, the efficiency of the cyanobacterial strains were increased growth, rice yield and its components (Afify *et al*., 2018). Thus, the aim of this study was to evaluate the survival of cyanobacterial strains *Anabaena oryzae* and *Nostoc muscorum* in biodegradation of carbofuran. Moreover, effect of inoculation with cyanobacterial strains combined with carbofuran on growth and yield of rice plant.

MATERIALS AND METHODS

Cyanobacterial strains:

Cyanobacterial strains were isolated from soil sample polluted with carbofuran and identified as *Anabaena oryzae* and *Nostoc muscorum* according to Afify, *et al.* (2023b).

Determination dry weight and total count of cyanobacteria:

To determine dry weight of cyanobacterial strains (El-Ayouty and Ayyad, 1972), calculated the differences in weights of growth gave the dry weight of the cyanobacteria biomass according to Taha, (2000). By Most probable Number (MPN) method total cyanobacterial strains were counted (*Anabaena oryzae* and *Nostoc muscorum*) according to Cochran (1950).

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Total nitrogen determination:

Total nitrogen in both cyanobacterial strains was determined by using micro-kjeldahl according to Jackson (1958).

Determination of carbofuran residues:

The Gas chromatography–mass spectrometry (GC-MS) system (Agilent Technologies) was equipped at Central Laboratories Network, National Research Centre, Cairo, Egypt. Analyses were stored in Wiley and NIST Mass Spectral Library data (Rasekhi *et al.* 2014).

Rice grains:

Oryza sativa cv. Sakha 108 grains were kindly obtained from Rice Research Dept., Field Crops Res. Institute, Agricultural Research Center (ARC), Giza, Egypt.

Nitrogen fertilizer:

Amount of nitrogen added is 75% of the recommended dose in the form of urea (46.5% N) was used in this investigation.

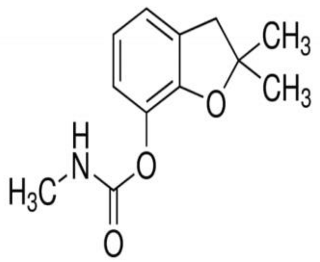
Insecticide used:

Commercial grade carbofuran (Feurdan 10%) - Sigma- Co., USA was used in this study. Carbofuran **is** N-Methyl carbamate**.** In Table (1) is presented chemical characteristics of carbofuran according to **Chowdhury *et al.* (2014**). Carbofuran degradation were calculated by the following equation:

$$X\% = \frac{C_{ck} - C_x}{C_{ck}} \times 100$$

Where, X is carbofuran degradation; C_x the concentration of chlorpyrifos (mg l^{-1}) in the medium that carbofuran degradation microbial strains; C_{CK} the concentration of carbofuran (mg l^{-1}) in the medium that does not contain carbofuran degradation strains.

Table 1. Chemical characteristics of carbofuran insecticide

Compound	Chemical name	Formula	Structure
Carbofuran	N-Methyl carbamate	$C_{12}H_{15}NO_3$	

Analysis of soil:

Physical and chemical properties of soil sample was clay loam soil (Abou Elatta *et al.,* 2023) determined according to Piper (1950) and Jackson (1958).

Field experiment:

A field experiment with clay loam soil was conducted during summer season of 2020 to evaluate the biodegradation of carbofuran in rice field inoculated with liquid cultures of the most efficient cyanobacterial strains (*Anabaena oryzae* and *Nostoc muscorum).* Rice plant seedlings were transplanted after month from planting in the field. After one week from transplanting the cyanobacterial inoculants were applied. While, the carbofuran was added at 15 days after transplanting the rice at a rate of one liter /fed. and injected with irrigation water. Inorganic phosphorus fertilizer as recommended was added before planting and after irrigation.

Field experiment treatments as:

1- *Nostoc muscorum* + Carbofuran
2- *Anabaena oryzae* + Carbofuran
3- Mixture of *Nostoc muscorum* +*Anabaena oryzae* + Carbofuran
4- Carbofuran
5- Control.

The growth parameters of plant:

Plant height (cm): Plant height (cm) was estimated from the soil surface up to the panicle top.

Number of panicle in hill: Panicles number was calculated as an average of five hills at maturity stage.

Fertility percentage: Fertility percentage was determined by account full grains divide by all spikelet number for panicle.

Weight of 1000-grain (g): To measure the 1000-grain weight, sample was taken from threshed dry rice grains

Rice yield (ton/ha): Inner five square meters were identified in each plot, and harvested for grains and straw yields determination.

Harvest index in rice crop: The harvest index in rice crop was determined according to the following equation of (Yoshida, 1981)

$$\text{Harvest index in rice crop} = \frac{\text{Grain yield}}{\text{Grain yield} + \text{Straw yield}} \times 100$$

Statistical analysis

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

RESULTS AND DISCCUION

Data in Table (2) showed that in cultures from cyanobacterial strains, the dry weight and fixed nitrogen increased with increasing the incubation period as well as with increasing carbofuran concentration from 0 until 80 ppm, while at the concentration of 120 ppm declined. With *Nostoc muscorum* was noted the highest total nitrogen (Sarnaik *et al.,* 2006; John and Shaike 2015). Fix atmospheric nitrogen and to survive in polluted environments makes them the suitable for biodegradation by photoautotrophic bacteria (Sorkhoh *et al.,* 1995 and Kumar and Kumar 1998). The use of cyanobacteria to remove pollutants is inexpensive method since they have few requirements for growth (Chungjatupornchai and Fa-Aroonsawat, 2008). Therefore, they are an important microorganism in both terrestrial and aquatic ecosystems (Palanisami *et al.,* 2009). Nitrogen-fixing cyanobacteria as bio-fertilizers are commonly seen in paddy fields (Kuritz, 2010). Cyanobacterial strains are able to accumulate high concentrations of pesticide (Chen *et al.*, 2007 and Vijayakumar, 2012).

The results in Table(2) showed that by dry weight and nitrogen fixation of cyanobacteria strains compared with different concentrations of carbofuran 0, 40, 80 and 120 ppm therefore with *Nostoc muscorum* and *Anabaena oryzae* at concentrations from zero to 80 ppm the highest dry weight and total nitrogen fixation were observed. This increase was attributed to the ability of microbes to degrade carbofuran either catabolic (Singh *et al.* 2004) ; (Mishra *et al*., 2019); or co-metabolic by mineralization and use as a source of carbon and energy (karpouzas and Singh 2006).

Table 2. Effect of different concentrations of carbofuran on dry weight (mg/100ml-culture) and fixed nitrogen (mg N/100 ml - culture) in cyanobacterial strains cultures

Cyanobacteria strains + Concentration of Carbofuran	Dry weight (mg/100ml-culture) Incubation Period (Days)			Fixed nitrogen (mg N/100 ml-culture) Incubation Period (Days)		
	7	14	21	7	14	21
Anabaena oryzae	52l	64gh	88b	3.13h-p	5.25d-g	8.81ab
Anabaena oryzae +40 ppm Carbofuran	55m	80e	89b	3.46h-k	5.54e-g	9.89a
Anabaena oryzae +80 ppm Carbofuran	65n	89e	103b	4.97k-p	7.34e-j	10.46ab
Anabaena oryzae +120 ppm Carbofuran	40b	31d	22hi	1.87b-d	1.21d	1.06d
Nostoc muscorum	58j	67e	93a	3.25h-o	5.35d-f	9.81a
Nostoc muscorum +40 ppm Carbofuran	65k	82d	95a	4.31f-j	6.45c-e	9.92a
Nostoc muscorum +80 ppm Carbofuran	68lm	92d	105a	5.24k-o	8.89b-e	10.91a
Nostoc muscorum +120 ppm Carbofuran	43a	32d	23gh	3.16a-c	4.35a	3.54ab

Means followed by different letter(s) in the three columns of incubation time are significantly different

The data represent in Table (3) showed that the decomposition of carbofuran gave several types of compounds. The remaining carbofuran score is 0 out of 80 ppm. *Nostoc muscorum* culture, has different percentage of the bioactive compounds. The decrease of carbofuran in *Nostoc muscorum* culture may be due to its ability to use carbofuran as a source of carbon and energy sources (Mishra *et al*., 2020) by intracellular degradation of carbofuran (Zulpa *et al.,* 2003). Ortiz *et al*., (2011) and Singh *et al.,* (2016) showed that environment unaffected by pesticides has the potential to use carbofuran's only source of carbon and energy. This pre-exposure of microorganisms makes them well-suited to break down pollutants through growth, development and metabolism, thus maximizing the rate at which pesticides are removed from the soil (Park *et al*., 2006). The biodegradation insecticide by bacteria are depend on enzymes and also on the environmental factors (Tien *et al*., 2017).

Table 3. Degradation compounds of carbofuran insecticide by *Nostoc muscorum* culture through GC-Mass method.

Retention time	Name	Degradation component	Area Sum %
3.161	Decane, 2-methyl-	$C_{11}H_{24}$	2.37
3.196	Nonane, 4,5-dimethyl-	$C_{11}H_{24}$	3.93
3.356	Nonane, 4-ethyl-5-methyl-	$C_{12}H_{26}$	3.1
3.402	Decane, 2,3,5,8-tetramethyl-	$C_{14}H_{30}$	5.11
3.47	Octane, 4,5-diethyl-	$C_{12}H_{26}$	1.63
3.774	Tetradecane	$C_{14}H_{30}$	2.84
3.814	pentadecane	$C_{15}H_{32}$	1.72
3.848	Hexadecane	$C_{16}H_{34}$	1.06
3.957	Nonadecane	$C_{19}H_{40}$	1.39
3.991	Heptadecane, 2,6,10,14-tetramethyl-	$C_{21}H_{44}$	3.86
4.1	Dodecane, 2,6,10-trimethyl-	$C_{15}H_{32}$	2.69
4.123	Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$	6.28
4.214	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	40.31
4.781	Methoxyacetic acid, 3-tetradecyl ester	$C_{17}H_{34}O_3$	1.68
5.164	Eicosane	$C_{20}H_{42}$	2.8
5.221	Octadecane, 2-methyl-	$C_{19}H_{40}$	4.95
5.507	1-Decanol, 2-hexyl-	$C_{16}H_{34}O$	2.39
6.206	Heneicosane	$C_{21}H_{44}$	1.67
6.846	Nonadecane, 2-methyl-	$C_{20}H_{42}$	4.23
7.224	Octacosane	$C_{28}H_{58}$	1.08
8.117	1-Ethynylcyclopentanol	C_7H_{100}	0.51
8.517	Heptadecane, 2-methyl-	$C_{18}H_{38}$	1.22
8.866	Eicosane, 2-methyl-	$C_{21}H_{44}$	1.56
9.301	2-Methyltetracosane	$C_{25}H_{52}$	1.58

Changes in the total count of the *Nostoc muscorum* and *Anabaena oryzae* strains at zero, 30 and 60 days after applied in soil (Table 4).

Results showed that the total count of cyanobacteria was increased with increase the days after transplanting. It was noted that the highest total count was found at 60 days under all orders and reached to $0.250x10^4$ with mixture of *Nostoc* sp.+*Anabaena* sp. + Carbofuran. EL-Zawawy, *et al.* (2021) observed that cyanobacteria total count increased at all stages of rice plant growth. Inoculation with mixture of *Nostoc* sp. + *Anabaena* sp. + Carbofuran. Yielded increasing by the tested cyanobacterial species, and this persistent increase is evidence of cyanobacterial strains ability to biodegrade carbofuran (Mishra *et al*., 2019). . However, inoculation with a mixture of *Anabaena oryzae* + *Nostoc muscorum* + Carbofuran gave a higher number than inoculation with any of them alone. These results are confirmed with those reported by (Ghazal *et al*. 2011) the inoculation with different types of blue-green algae resulted in large numbers of blue-green algae and large amounts of soil organic matter and nutrients, including nitrogen, leading to improved plant breeding. Cyanobacteria play an important role in the functioning ecosystem in arid environments and are seen as soil crusts forming an important component of the soil (Afify *et al.* 2023a).

Table 4. Total cyanobacteria strains count (10^4cfu/g dry soil) in soil cultivated with rice plant

Treatments	DAT (Days)		
	0	30	60
Nostoc muscorum + Carbofuran	0.081a	0.185a	0.235a
Anabaena oryzae + Carbofuran	0.075a	0.165a	0.220a
Mixture + Carbofuran	0.087a	0.191a	0.250a
Carbofuran	0.035a	0.094a	0.128a
Control	0.021a	0.078a	0.095a

Mixture: *A. oryzae* + *N.muscorum* + Carbofuran DAT: Days after transplanting

Effect of cyanobacteria on growth of rice plant:

The data in Table (5) showed that the use of cyanobacteria and carbofuran in the farm caused an increase in plant height (cm), spike length (cm), sibling number/plant and spike weight (g). This improvement is caused by cyanobacteria breaking down carbofuran, which acts as plant growth regulators and fixes nitrogen, so that rice can extract nutrients from the soil, etc. Bacteria spreading into the environment can reduce and use most of the organic compounds found in carbofuran as carbon (Mishra *et al*. 2019). Whereas, the characteristic of plant height with applied of cyanobacteria combined carbofuran insecticide was increased compare with control. In addition to panicle length (cm) was increased by applied of cyanobacteria with carbofuran than control values. Therefore, the highest values number of tillers/plant were obtained from applied mixture of *Nostoc* and *Anabaena* with carbofuran and lowest value was achieved with control. Concern number of panicles/plant the superior values was achieved with *Nostoc muscorum* + Carbofuran while, the decline values was recorded with control. For panicle weight (g) the applied of cyanobacteria increased but not significantly compare with control whereas the weightiest value of panicle was confirmed with mixture treatment (EL-Zawawy, *et al*. 2021) and also due to ability of *Anabaena oryzae* and *Nostoc muscorum* for producing of ammonia and oxygen when fix nitrogen and carbon dioxide (Phathka *et al*. 2018; Abou Elatta *et al*. 2019 and Godlewska *et al*. 2019).

Table 5. Effect of cyanobacterial strains and carbofuran insecticide on growth and yield characteristics of rice plant

Treatment	Plant height (cm)	Panicle length (cm)	Number of tillers/plant	Number of panicles/ plant	Panicle weight (g)
Nostoc muscorum + Carbofuran	99.8ab	24.4ab	25.45ab	25.41a	4.72a
Anabaena oryzae + Carbofuran	98.3bc	23.6ab	24.50ab	22.22b	4.63a
Mixture + Carbofuran	101.6a	25.3a	26.60a	22.25b	4.98a
Carbofuran	96.6c	22.2b	23.40b	21.53b	4.30a
Control	96.0c	22.0b	18.00c	17.00c	4.18a

Means followed by different letter(s) in the column are significantly different

The information in Table (6) clearly shows the difference in the effect of cyanobacteria treated with carbofuran insecticide on fertility percentage, thousand grain weight, rice yield t/ha and harvest index. The results showed that the combined fertility, thousand-grain weight, yield and harvest index all reached the highest values of *Nostoc* sp. and *Anabaena* sp. combined with carbofuran treatment. The *Nostoc muscorum* and *Anabaena oryzae* enhances plant biomass (Mishra *et al*. 2019 and Yanni *et al*. 2020). Also, the supply of nitrogen by *Nostoc muscorum* and *Anabaena oryzae* stimulate the growth substances which increasing the uptake of nutrients N, P and K (EL-Zawawy *et al*. 2021). It means that cyanobacteria inoculation is economic practice (Nayak and Adhikary 2004; Singh *et al*. 2004; (Chittapun *et al*. 2018). Cyanobacteria treatment has been shown to significantly increase crop yields compared to other treatments. This increases rice production/fed. The addition of blue-green algae may result from an increase in fertility percentage, thousand grain weight, grains yield, and harvest index expressed as percent grains + straw yield. These data are consistent with Jan *et al*. (2018).

Table 6. The application of cyanobacterial strains and carbofuran insecticide on characteristics of rice plant

Treatment	Fertility Percentage	1000-Grain weight (g)	Grains yield t/ha	Harvest index
Nostoc muscorum+ Carbofuran	97ab	29.08ab	10.96a	52.69b
Anabaena oryzae+ Carbofuran	96a-c	29.01ab	10.78a	48.39c
Mixture +Carbofuran	98a	29.76a	11.29a	55.59a
Carbofuran	95bc	27.28b	9.92a	42.98d
Control	93c	26.09b	9.21a	41.74d

Means followed by different letter(s) in the column are significantly different

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كاربوفيوران وتكسيره بالسيانوبكتيريا فى حقول الأرز

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الملخص

إن إستخدام الكاربوفيوران كأحد المبيدات الحشرية ذات التأثير الكبير لمقاومة الآفات التى تصيب نبات الأرز . وبالتالى يؤدى إستخدامه إلى مشاكل بيئية ويشكل تهديدًا كبيرًا للكائنات الحية الدقيقة المفيدة. وقد أجريت هذه التجربه لدراسة تحلل المبيد الحشري كاربوفيوران حيويا بواسطة سلالات من السيانوبكتيريا *Nostoc muscorum* و *Anabaena oryzae* وذلك من خلال قياس معدل تثبيت النيتروجين وكمية الوزن الجاف والعدد الكلى للسيانوبكتيريا وقياسات الإمتصاص بواسطة مقياس الكروماتوجرافيا (GC-MS) الذى يستخدم لتحديد المكونات المتبقية. وقد سجلت النتائج أن يمكن تحليل الكاربوفيوران بواسطة سلالات السيانوبكتيريا وقد أدت المعامله بواسطة خليط سلالات السيانوبكتيريا والمبيد الحشرى الكاربوفيوران إلى زيادة كمية الوزن الجاف للحبوب وكذلك تثبيت النيتروجين فى البيئة بالإضافة إلى الزيادة في النمو والإنتاجية والمساهمة في زيادة محصول الأرز مقارنة بالكنترول (بدون التلقيح بالسيانوبكتيريا وعدم إضافة مبيد الكاربوفيوران). وبالتالى يمكن القول أن السيانوبكتيريا تعتبر من العوامل الفعاله والصديقه للبيئه عند تحليل المبيدات .