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Biodegradation of Residual Oxamyl Compound by Algae: Description and Traits of Root-knot Nematode Control

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ABSTRACT

Accelerated biodegradation of the residual oxamyl (a systematic nematicide widely used for the control of soil pathogenic-nematodes), utilized at the recommended dose in soil cultured by banana plants and artificially infested with root-knot nematode (RKN, *Meloidogyne incognita*), was observed using algal bioassay studies. Algae such as *Chlorella vulgaris*, *Scenedesmus obliquus*, *Anabaena oryza* and *Nostoc muscorum* were used to determine the degradability enhancement of oxamyl by an accelerated biodegradation process. All oxamyl-degrading species were highly effective to enhance biodegradation of oxamyl compound. Moreover, algal species were effective for controlling RKN, *M. incognita* because of their enhanced defensive power gained from the oxamyl compound supplemented to the soil. Also, this was improved by the force of the integrated algal suspension to inhibit parasitic nematode. The incorporated application of alga, *S. obliquus* was the most successful one for oxamyl degradation in plants (75%) and soil (100%) by using HPLC analysis, and had an active promoting effect on banana health. Unlike, the alga, *C. vulgaris* was the most successful action in diminishing the nematode juveniles (J2s) count in soil (57.55%) and galls count on roots (52.87%).

Keywords: Oxamyl; biodegradation; RKN, *Meloidogyne incognita*; banana; algae.

INTRODUCTION

Root-knot nematodes (RKNs) and root-lesion nematodes (RLNs) are of great economic effect worldwide (Koenning et al., 1999; Decraemer and Hunt, 2006; Jones et al., 2013). Large-scale also, rehashed utilization of similar substance pesticides for various years without any crops or pesticides rotation has occasionally resulted in unexpected failures to control the target pests (Singh et al., 2005). Specially, nematicides are mainly worldwide used for agriculture purposes, and play an important role in controlling PPNs in modern cultivation (Evans and Haydock, 1999; Georgis et al., 2006). The wide use of chemical nematicides in conventional crop production causes environmental pollution (Cornejo et al., 2000; Robacer et al., 2016), whereas the residues of pesticides widespread in surface water (De Geronimo et al., 2014; Wee and Airs, 2017). They are immobile and persist on the top of soil

granules and become injurious to microorganisms, plants, animals and people (Radivojevic et al., 2008).

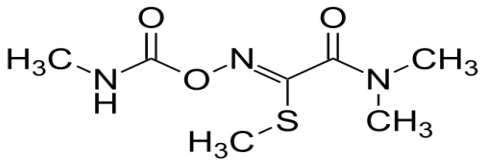
Oxamyl (Vydate SL 24% a.i.) is a carbamate component widely applied in intensive agriculture and it is incorporated into the 10 to 15cm of the soil surface (Tomlin, 2002), and also, it is a systemic formulation as a pesticide (Tomlin, 2005). Vydate has a high solvability in water (280 g L^{1}) and a great toxic synthesis to mammals (LD_{50} = 3.1 mg/kg^{-1} oral dose for male rats) (Tomlin, 2002). Taking these into consideration, finding methods to degrade nematicides to keep away from hazards is a necessity.

Hence, the use of microalgae for controlling RKN, *Meloidogyne incognita* and to act as a bio-fertilizer to enhance plant growth is suggested (Khan et al., 2005; El-Ansary and Hamouda, 2014; Hamouda and El-Ansary, 2017). Because of some amino acids like glycine, arginine and glutamine act as a nitrogen (N_2) source in blue green algae, cyanobacteria where, arginine amino acid needs for a conformational turn in *nif* gene to adjust N_2 fixation (Flores and Herrero, 1994). Subsequently, Cyanobacteria have the ability to fix N_2 under aerobic conditions and releasing oxygen (O_2) together (Kulasooriya and Magana-Arachchi, 2016).

Microalgae also are considered more powerful than other organisms (e.g., microfungi, mycorrhiza, bacteria, protozoa and yeasts) in biosorption and degrading pesticides (Gao et al., 2011). Biosorption involves a number of processes which by turn includes: absorption, ion exchange, adsorption, precipitation, and surface complexation (Ata et al., 2012), while biodegradation is defined as the method by which chemical materials are destroyed and converted into smaller ingredients by living microorganisms (Marinescu et al., 2009). For instance, some fresh algae: *Chlorella vulgaris*, *Scenedesmus platydiscus*, *S. quadricauda* and *S. capricornutum* are capable of degrading some toxic compounds like, polycyclic aromatic hydrocarbons (Wang and Zhao, 2007). Microalgae called mixotrophic, have the ability to use organic carbon and light concurrently as ~~a~~ sources of energy (Hamouda et al., 2016), and they are capable of absorbing as well as metabolizing organic compounds containing synthetic pesticides (Priyadarshani et al., 2011). That give microalgae an essential competitive advantage over fungi and bacteria to degrade organic pollutants (Chekroun et al., 2014). Yet, natural biodegrading components, like exogenous polyamines from microorganisms, give the treated plants the ability to tolerate concentricity of pollutants 500 times higher than control (Maheshwari et al., 2014).

Several mechanisms of algal degradation have been proposed to explain pesticide metabolization by using microalgae, *C. vulgaris* and cyanobacteria, *Nostoc muscorum* as they degraded organophosphate and used the pesticide as a phosphorus source (Megharaj et al., 1994). Notable, within four treatment days, *Chlorella* sp. changed or degrade more than 99% of the pesticide, whereas, it showed powerful effect in detoxification of (Nemacur EC 40% a.i.) fenamiphos (Caceres et al., 2008; Subashchandrabose et al., 2013). With regard to composition of most algae cell wall determined by three space network of macromolecules (Arnold et al., 2015) these algae produce complex sulfated polysaccharides, arabino galactan proteins (AGPs) extension and lignin existent in embryophyte walls (Domozych et al., 2012), whereas the metal ions embedded in water are commonly in cationic structure, then imbibed to

cell wall of alga under acidic media (pH 3-5) (Ata et al., 2012). Accordingly, there are many factors which can change metabolism, and also affect biodegradation (Alexander, 1999; Lu et al., 2018). Physicochemical characteristics of the environmental factors like: soil type, temperature, humidity, air condition, pH and substrate usability, would affect the biodegradation success (Kumar et al., 2017). For example, a higher degree of moisture in soil affects the carbofuran biodegradation lessening (Shelton and Parkin, 1991). The present study focuses on the efficacy of the accelerated algal degradation of the nematicide-oxamyl in sandy soil and in reducing its residues in plant by using different algal models, besides their roles as a biocontrol agent and biofertilizer. Fig. 1 shows the chemical structure of oxamyl.



Oxamyl

Fig. 1. Chemical structure of oxamyl {2-(dimethylamino)-N-[[(methylamino) carbonyl] oxy]-2-oxoethanimidothioate}

MATERIALS AND METHODS

1. Algal suspension preparation

Ten ml of green algae: *C. vulgaris* and *S. obliquus*; and blue green algae: *N. muscorum* and *A. oryza* were inoculated into 200ml Erlenmeyer flasks, each flask contained 90ml of sterilized BG11 medium (Stanier et al., 1971) (Table 1) and then incubated for 14 days (stationary phases of growth) at 25±1°C under constant light 80µ mol^1.

Table 1. BG11 medium constitute (according to Stanier et al., 1971).

Constituent A	
Stock solution	g/L
$NaNo_3$	1.5
K_2HPO_4 $3H_2O$	0.04
$MgSO_4$ $7H_2O$	0.075
$CaCL_2$ H_2O	0.036
Na_2CO_3	0.02
Constituent B	
EDTA (disodium magnesium salt)	0.001 g/L
Constituent C	mg/L
Trace elements	2.8
H_3BO_3	1.81
$MnCl_2$ $4H_2O$	0.222
$ZnSO_4$ $7H_2O$	0.079
$CuSO_4$ H_2O	0.39
Na MoO_4 $2H_2O$	0.0494

2. The physical and chemical traits of the used soil

The structure of the artificial soil was prepared in laboratory conditions (containing 4.5 Kg/pot) of sterilized soil mixture (clay / sand 1:2) with pH 7.26. It included sand (25.33%), silt (35.30%), and clay (39.37%). The other physical and chemical characteristics of the soil texture are recorded in Table (2). Micro and macroelements were measured by inductively coupled plasma-atomic emission spectroscopy (ICP-AES, Thermo Scientific)

Table 2. The physiochemical properties of the tested soil

Parameter	Value
pH	7.26
EC (dS m1)	580.3
Total dissolved salts (ppm)	383
Chloride (ppm)	167
Carbonate (ppm)	271
Sodium adsorption ratio (SAR)	167
Micro and macro elements (ppm)	
Na^{+}	40.05
Mg^{2+}	19.43
K^{+}	27.65
Ca^{2+}	2.67
Mn^{+2}	13.39
Fe^{+2}	0.43
Zn^{+2}	13.39
Cu^{+2}	3.28
Cd^{+2}	1.53
Co^{+2}	1.71

3. Nematicidal assessment

3.1. Nematode culture

Extraction of *M. incognita* eggs from tomato roots (*Solanum lycopersicum* cv. Castle Rock), with 1% sodium hypochlorite (NaOCl) solution was done according to Hussey and Barker (1973). Egg masses were isolated from the infected roots and were hatched in water for three days to obtain J2s at 28±2°C. Active juveniles (J2s) of nematode were collected from hatched eggs daily and were stored at 15°C. The used nematode J2s were less than 5 days old.

3.2. Plants and nematicides

Banana plants (*Musa* spp.) were obtained from laboratories of Tissue Culture, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City, Minoufiya, Egypt. Vydate (Oxamyl SL 24% a.i), was obtained from a

local manufacturer, "Shoura Chemicals Company", situated at Kilo 28 – Cairo - Alexandria Desert Road, Egypt.

3.3. Experimental design

Eight -week-old of banana plantlets (cv. Grande-Naine) were planted in 30 cm diameter pots to study the management of RKN by applications of oxamyl, followed by four algal species to degrade the nematicide oxamyl. Seedlings were planted in pots containing 4.5 kg of sterilized of the artificial soil mixture. The soil temperature was 28±2^{o} C for most time. Twenty four pots were infested with 3,000 J2s/ pot at the cultivating time. The second stage juveniles (J2s) were distributed in the soil at the middle of the pots for about 10-cm depth around the root. Six days later, 16 pots were treated with 0.1ml oxamyl /Kg soil oxamyl in 10 ml distilled water in three holes made in soil. Three days later, at the previous pots, 200 ml algal culture of *C. vulgaris, S. obliquus, A. oryza* and *N. muscorum* in stationary phase was added. Four infected pots were managed with oxamyl only (0.45ml/pot). The remaining four infested pots served as nematode only (infected control). One treatment kept without application or nematode, check (uninfected control). Distribution of algae and oxamyl into the soil was made by uniform injection in three connected holes around the plant.

3.4. Data collection

All applications practically distributed using completely random design in a greenhouse with four replicates for each treatment. Banana plants were examined for residues after 45 days of infestation. The roots of plants were washed by water to separate soil from the fresh roots. Then, these were stained with Phloxine B (3.5g in 750 ml distilled water+250 ml acetic acid 5%) solution for 5 minutes to facilitate the calculating of nematode phases by dissection under binuclear using forceps, i.e., females and females with egg masses. Also, egg masses were counted on the external surface of the roots. Reproduction parameters of nematode were done through galls count, egg masses/ root. Nematode J2s were extracted according to Cobb's method (Cobb, 1918) from 250g soil using accumulated sieves (60 mesh and 325 mesh) to facilitate counting of nematode in the water suspension under light microscope using Hawkesly counting slide which contain 1ml of nematode suspension. The resulting number was used to calculate the total number in the whole suspension. The banana growth criteria; shoot length and weight, root length and weight, and finally corm weight were also listed.

3.5. Extraction technique of oxamyl residues

Fifty grams of soil were taken after 14 days of oxamyl's application. The soil was soaked in 200 ml methanol and shook for 28 hours at room temperature. The mixture was filtered according to McGarvey (1993) under vacuum. About two grams of banana leaves were extracted three times with 50ml of methanol by shaking for one hour (Krause, 1979). The methanol extract was combined, concentrated and determined by HPLC analysis for estimating the oxamyl residues in banana plants.

4. High performance liquid chromatography (HPLC) analysis

Chromatographic conditions for oxamyl analysis by HPLC are used according to Keith et al. (1983) HPLC Agilent, 1200 Series consists of a quaternary pump, 20µl sample loop, a UV/Vis detector and Chemstation Software 15. Column supelcosil

containing octadecylsilyl (ODS) group for packing material of stationary phase (C18, 5µm, 250 mm*4.6 mm). Mobile phase was mixture between acetonitrile and water with fixed ratio (80:20 v/v). Flow rate was 1ml/min. The UV detector wavelength was 220 nm and the sample injection volume was 20µl.

5. Statistical analysis

Statistical analysis was carried out using ANOVA, analysis of variance (version 19) to compare between treatments means according to Sokal and Rohlf (1995). Significant differences between means of parameters were determined by using Duncan's multiple range tests with probability ≤ 0.05. All the previously mentioned measurable investigations were directed by utilizing SPSS software.

RESULTS

1. Oxamyl degradation by algae

The results shown in Table (3) indicated that, the concentration of oxamyl residues was calculated from regression coefficient at the same chromatographic condition of standard and samples through HPLC analysis. In addition oxamyl reference standard at 2.6min retention times through 10 min of total run time was recorded (Fig.2).. The results obtained by HPLC designated the alga, *S. obliquus* as the most potential to biodegrade oxamyl in banana (75%) followed by *C. vulgaris* (69.61%) relative to oxamyl only. Accordingly, the treatment *S. obliquus* was the most effective in reducing oxamyl residues in plant (25%).

In contrast, the algae: *A. oryza* and *N. muscorum* achieved less potential to biodegrade oxamyl in plant (64.8%) and (54.58%), respectively. The oxamyl residues in the soil were noticed only in the soil samples without incorporated application of the algae (Table 3 & Fig.2).

Table 3. The degradation (%) of the oxamyl (SL 24% a.i) in banana plants and soil samples after microalgae application.

Treatments	Oxamyl conc. µg/g		Oxamyl	
	Soil	Absorbed by plant	Residue absorbed by plant (%)	Degradation (%)
Control, Oxamyl only	9.57	14.32	59.30	-
C. vulgaris	-	7.29	30.38	69.61
S. obliquus	-	6.0	25	75
A. oryza	-	8.44	35.19	64.8
N. muscorum	-	10.90	45.42	54.58

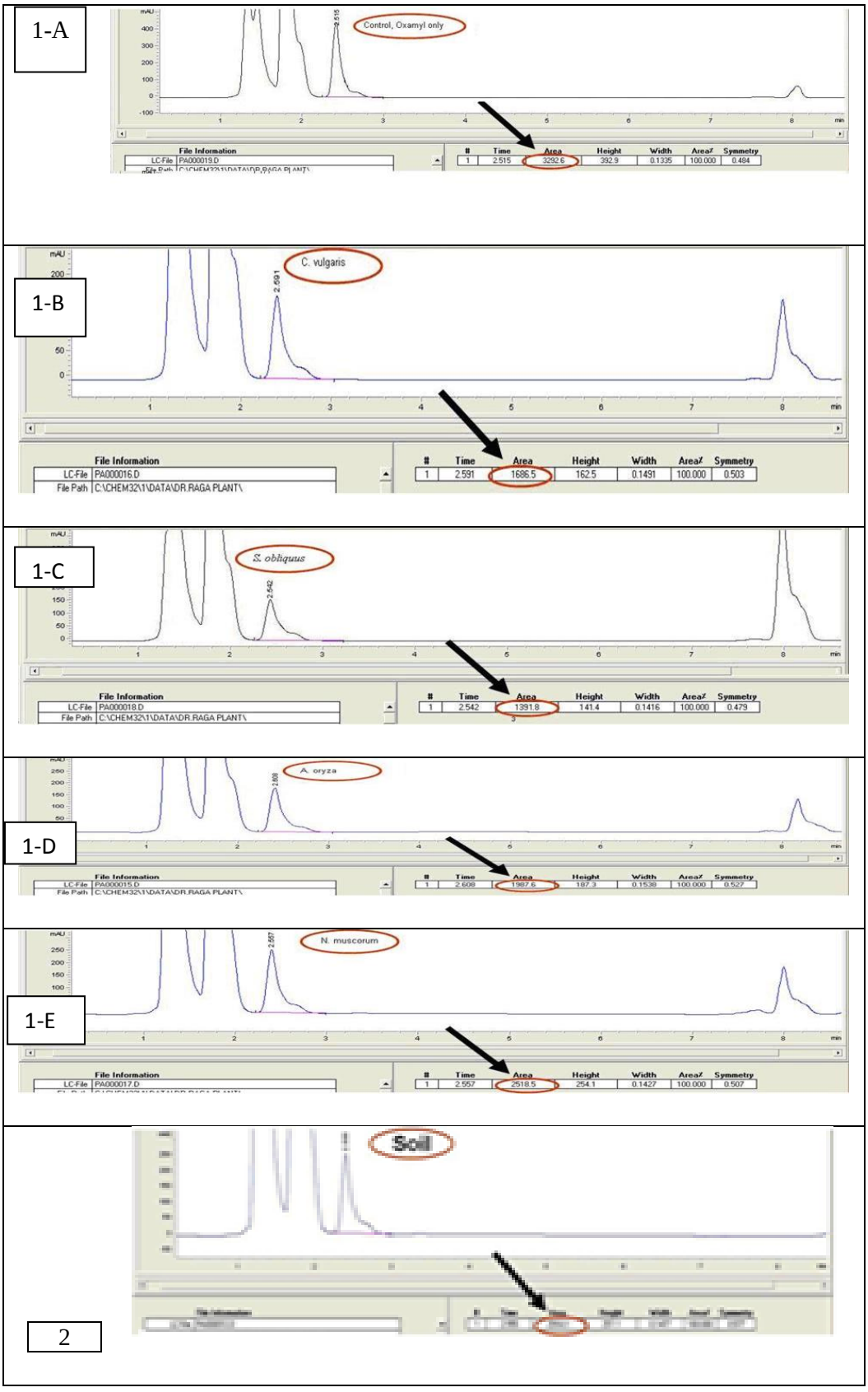


Fig. 2. Oxamyl biodegradation in plants and soil using microalgae (HPLC analysis)

1-Oxamyl residues in plant: **1-A** (*C. vulgaris*), **1-B** (*S. obliquus*), **1-C** (*A. oryza*), **1-D** (*N. muscorum*), **1-E** (Control, oxamyl only)**; 2-** Oxamyl residues in soil without algal application

2.Behavior of oxamyl biodegradation by algae and management of *M. incognita* in banana

All selected oxamyl-degrading species, i.e. *C. vulgaris*; *S. obliquus*; *A. oryza*; *N. muscorum* were compared with oxamyl only (control) and nematode only (infected control). All the tested applications significantly ($P \leq 0.05$) reduced the number of J2s in soil compared with nematode only (infected control). Similar findings were observed for the number of galls, females and females with egg masses per rhizome system (Table 4).

Table 4. Effect of algal species after oxamyl application on root-knot nematode, *Meloidogyne incognita* infecting banana.

Treatments	Number/ root			Juveniles/ 250g soil
	Galls	Females	Females with egg masses	
C. vulgaris	105.74c	196.61bc	147.29bc	1624b
S. obliquus	130.58bc	230.79b	165.11b	1695.25b
A. oryza	130.12bc	235.58b	173.5b	1867.5b
N. muscorum	143.66b	222.43b	184.98b	1664b
Oxamyl only (Control)	125.49bc	157.15c	112.21c	1240.5b
Nematode only (infected control)	224.37a	312.69a	250.34a	3826a

Means connected with the same letter(s) within a column are not significantly different ($P \leq 0.05$) according to Duncan's Multiple Range tests.

However, *C. vulgaris* after oxamyl application was the most successful treatment in decreasing nematode soil population of J2s (57.55%). The same outcome was noticed for oxamyl only (control, 67.58%). Also, the alga, *C. vulgaris* decreased the counts of females in roots (37.12%) next to oxamyl only (control, 49.74%), as depicted in Fig. 3.

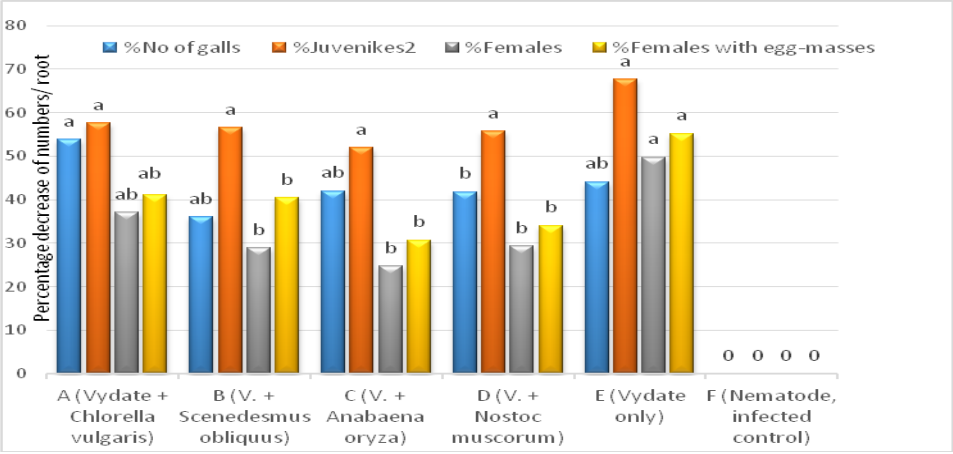


Fig. 3. Effect of algal species after oxamyl application on drop (%) of nematode counts in banana roots and soil samples.

% *R*= percent reduction compared with nematode only (infected control)

Table 5. Impact of algal species on growth measurements of banana infected with *Meloidogyne incognita* after oxamyl application.

Treatments	Shoot		Root		Corm Weight (g)
	Length (cm)	Weight (g)	Length (cm)	Weight (g)	
C. vulgaris	38.5a	55.93b	42b	30.65b	19.95a
S. obliquus	40.25a	62.39b	57.75b	43.53b	19.79a
A. oryza	35.5a	48.86b	74.75c	34.69b	19.75a
N. muscorum	39.75a	56.25b	57.75b	35.28b	20.99a
Oxamyl only (control)	39.5a	42.99ab	47b	35.06b	18.41a
Nematode only (infected control)	35.5a	24.47a	18.5a	13.65a	19.41a
Check (uninfected control)	41.25a	46.35b	52.5b	31.1b	22.64a

Means connected with the same letter(s) within a column are not significantly different ($P \leq 0.05$) according to Duncan's Multiple Range tests**.**

Banana growth criteria, e.g. weight and length of shoots and roots as well as weight of corms, are recorded in Table (5). Most of the algal applications on banana plants significantly ($P \leq 0.05$) increased the growth indicators compared with nematode only (infected control). The noticed banana health had the greatest potential in the alga *S. obliquus* compared with check (uninfected control).

DISCUSSION

In the present study, accelerated biodegradation of oxamyl was investigated in soil and plants using different models of algae. Also, the effect of the current applications on depressing RNK, *M. incognita* and promoting banana growth was noticed on the plants. However, RKN is well known parasite of banana roots. Chemical control of PPN which is widely used in agriculture (Evans and Haydock, 1999), and traditional crops production cause high environmental toxicity (Wang et al., 2018). So, our research establishes only the additional report concerning the effectuation of oxamyl-degrading algae (Megharaj et al., 1999). *In vivo* estimation of four algal species: *C. vulgaris*, *S. obliquus*, *A. oryza* and *N. muscorum* proved their beneficial power for RKN management and promoting plant health.

It also confirmed their vital role in biodegradation of oxamyl residues in soil and plants. Inhibition of J2s number in soil and count of nematode in root represented vigorous nematicidal action for algal applications. Hence, all the current applications became more effective to RKN control because of their defensive potent from adding the oxamyl compound followed by the algal treatment. This agrees with the utilization of microalgae for controlling of RNK, *M. incognita* (Singh et al., 2005; Holajjer et al., 2013), and acting as bio-fertilizer to enhance plant growth (El-Ansary and Hamouda, 2014; Hamouda and El-Ansary, 2017). Moreover, they are considered to be very effective organisms in biodegrading of residual pesticides (Gao et al., 2011). This case demonstrated successful inhibition of J2s in soil. In addition all tested applications as compared to the oxamyl only (control) caused an exceptional

increment in banana health than the nematode only (infected control). Especially the alga *S. obliquus* was the most effective one for promoting effect on plant growth.

Generally, such advantages of these algal treatments can be attributed in part to that they contain all macro, microelements and several growth regulators like auxins, gibberellins and betaine (Thirumaran et al., 2009). Overall, the soil and banana plants were analyzed by HPLC after fourteen days of soil treated with oxamyl and followed by algae. Notable, the alga, *S. obliquus* had the most potential to biodegrade nematicides (75%) in the plants compared to control (oxamyl only). It has also been reported that microalgae are capable of biosorption and metabolizing organic compounds including pesticides (Priyadarshani et al., 2011), and these algae have affinity for hydrophobic non-polar compounds (Casserly et al., 1983). There are several mechanisms of algae to degrade the oxamyl. For example, algae may break bonds via producing enzymes that hydrolyze oxamyl to oxamyl oxime (Ou and Rao, 1986). Genetically, no reports on the hydrolysis pathway and the genes involved in the algal degradation of carbamate oxamyl were provided. However, the disadvantage of some degradation products is more hazardous than the parent component (Gadd, 2009). In addition, algae grow under heterotrophic condition and use oxamyl as a sole carbon source (El-Sheekh et al., 2013). Microgreen and blue green algae have the ability to develop under heterotrophic condition via crude oil as sole carbon source (El-Sheekh and Hamouda, 2014). Some algae also degrade an organophosphate and utilized pesticide as a source of phosphorus during the pesticides metabolization (Megharaj et al., 1994).

To sum up, the testing strategy showed that alga, *C. vulgaris* (after oxamyl treatment), had the greatest antagonistic action on the *M. incognita* population and reproduction in banana. Unlike, the alga *S. obliquus* which extremely increased seedlings growth than other treatments, it would decrease the environmental pollution, improve workers' safety and accelerate the nematicide, oxamyl biodegradation phenomenon. Subashchandrabose et al., (2011) detected that the alga, *Chlorella* sp. was capable of biodegrading the organophosphate during 4 days, and metabolize more than 99% of the pesticide, whereas it has potent effect on detoxification of fenamiphos (Nemacur EC 40% a.i.). Finally, using microalgae as bio-control agents, bio-fertilizer and pesticides biodegradation will stop the extreme cost operation in cultivation, leading to safety food production for the dense growing population of the world.

CONCLUSIONS

We studied the degradation of four oxamyl-degrading species of algae on banana plants infected with RKN, *M. incognita*. All algal applications, specially the alga, *S. obliquus* was the most successful one for oxamyl degradation and had an active promoting effect on banana growth. With regard to the alga, *C. vulgaris* (after oxamyl treatment), it was the most successful treatment in diminishing of both count of J2s in soil and females number in roots. Likewise, the mechanisms of algae to degrade the oxamyl may be related to the capability of algae to grow under heterotrophic conditions and using oxamyl as a sole carbon source, whereas they produce enzymes that break the bonds in the nematicide molecules to oxamyl oxime. At the molecular level, the mechanism of oxamyl degradation by microalgae needs supplemental research.

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الملخص العربى

التحلل الحيوى لمتبقيات مركب الاكساميل بواسطة الطحالب: وصف و مميزات مكافحة نيماتودا تعقد الجذور

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يعتبر الامان البيئى بما فيه صحة الانسان من اهم العوامل الهامة فى برامج انتاج المحاصيل. و جدير بالذكر الى ان المبيدات النيماتودية الكيميائية بما فيها المبيد الجهازى النيماتودى (الاكساميل)، هو من المركبات الشائعة الاستخدام فى مكافحة النيماتودا الممرضة للنبات بالتربة. لذلك فانه يمكن التخلص من متبقيات مركب الاكساميل باستخدام الطحالب بالجرعات الموصى بها فى التربة و المنزرعة بالموز المصاب بنيماتودا تعقد الجذور من نوع *Meloidogyne incognita*، و فى هذا البحث يمكن استخدام الطحالب التالية: *Chlorella vulgaris* و *Scenedesmus obliquus* و *Nostoc muscoru* و*Anabaena oryza* فى زيادة عملية التكسير. اثبتت النتائج ان كل انواع الطحالب المستخدمة كشفت عن تاثيرها الايجابى فى التحلل الحيوى للاكساميل. وان الطحالب المختبرة اكثر فعالية فى مكافحة النيماتودا و نتيجة الى القوة التكافلية بين فعالية مركب الاكساميل المحقون فى التربة بالاضافة الى فعالية الطحالب فى تثبيط النيماتودا المتطفلة. كما اشارت النتائج المتحصل عليها من جهاز HPLC ان الطحلب *S. obliquus* من افضل الطحالب فعالية فى عملية التحلل الحيوى لمركب الاكساميل فى النبات بنسبة 75% و التربة بنسبة 100%، اضافة الى انه من اكثر المعاملات فى تحسين نمو النبات. كما سجل الطحلب *C. vulgaris* و المحقون فى التربة بعد مركب الاكساميل هى من افضل المعاملات المستخدمة فى تثبيط يرقات النيماتودا فى التربة بنسبة 57,55% و كذلك اعداد العقد المتكونة على الجذور بنسبة 52,87%.