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Inhibitory effect of cyanobacteria byproducts on *Bean yellow mosaic virus* on faba bean plant

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ABSTRACT

The inhibitory effect of culture filtrates and fresh biomass of three cyanobacteria isolates i.e., *Nostoc muscorum*, *Spirulina platensis* and *Oscillatoria* sp. was studied, individually or in a mixture, against *Bean yellow mosaic virus* (BYMV) infection *on Vicia faba* L. Giza 716 and *Chenopodium amaranticolor* Cost and Reyn. The non-inoculated algal synthetic media were used as control. Findings revealed that the mixture of the three algal filtrates gave the highest percentages of inhibition. Lower inhibition effect was produced by culture filtrate of *N. muscorum* and *S. platensis*, respectively in particular in plants treated with algal filtrates by mixed with virus inoculum immediately before inoculation. Spraying plants with the algal filtrates 24 hrs. before inoculation produced a higher inhibitory effect against BYMV than that obtained by spraying 24 hrs. after inoculation. The same trend of inhibition effect that was detected with algal biomass was lower than with filtrates. The indirect ELISA was carried out to confirm the identity of the virus isolate and the obtained results of this study. Spraying faba bean plants with either algal filtrates or biomass also increased the plant growth parameters, i.e. plant stem and root length as well as plant fresh and dry weight. Algal proteins were identified by the protein profile pattern using SDS-PAGE method. The contents of alkaloids, phenols and terpenoids in both algal filtrates and biomass were determined.

Key words: Cyanobacteria, Culture filtrates, Biomass, Secondary metabolites, *Bean yellow mosaic virus*.

INTRODUCTION

Faba bean is considered the world`s fifth food legume after dry bean , dry pea, chickpea and lentil (Adak *et al*.,1998).This is due to its high nutritive value in both energy and protein contents. Therefore, attempts are made to increase faba bean yield as well as to protect the crop against pests and diseases, which cause losses in seed quality and quantity. Among faba bean viruses, BYMV consider as one of the most devastating viruses affecting faba bean plants in Egypt (Radwan *et al*., 2008). The use of biological control in plant diseases became promising target especially with plant virus diseases because once a plant is infected it cannot be cured. Antiphytovirals are substances exist that can affect the development of virus diseases in plants. Algae are swiftly proving to be an extremely important source of biologically active secondary metabolites (Gademann and Portmann, 2008) that could be used for the biological control of plant pathogens (Hewedy *et al*.,2000).Cyanobacteria and eukaryotic algae, particulary macroalgae, are known to produce intracellular and extracellular biologically active metabolites such as antifungal, antibacterial and antiviral activity (Jimenez *et al*., 2011). These biologically active compounds include antibiotics and toxins (Kiviranta *et al*., 2006). Abd-El-Baky *et al.* (2008) mentioned that antiviral compounds are grouped as alkaloids, terpenoids, flavonoids, peptides, fatty acids, phenolic compounds and specific proteins. Antiviral proteins are widely distributed in higher plants, mushrooms and algae (Barron *et al.*, 2007). These compounds hold promise for agricultural and pharmaceutical applications (Mehta and Boston, 1998). Pardee (2001) recorded that out of thirty one species of methanolic

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algal extracts screened, *Fucus gardenri* contained strong bioactivity of dramatically reduced the number of local lesions induced by *Tobacco mosaic virus* (TMV) on *C. quinoa*. In addition, Sano (1998) reported that alginate extracted from marine algae *Laminaria hyperborean* had antiviral activity against *Tobacco mosaic virus*. Also, Piero *et al*. (2000) found that the culture filtrates and intracellular contents from both *Nostoc* sp. and *Synechococcus lepoliensis* reduced at least 50% of local lesion number caused by TMV. However, Pardee *et al* .(2004) revealed that out of six algal methanolic extracts studied i.e., *Fucus gardenri*, *Alaria marginata*, *Ralfsia* sp., *Codium fragile*, *Fragilaria oceanica* and *Egregia menziesii*, the higher efficiency was detected on *C. quinoa* with *Fucus gardenri* which inhibited *Potato virus x* infectivity by 100% followed by *Alaria nana* (98.9 %). Similarly, Jimenez *et al*. (2011) found that aqueous and ethanolic extracts from the brown alga *Durvillaea antarctica* were able to reduce the damage symptoms in tobacco such as the number and the size of necrotic lesions produced by *Tobacco mosaic virus*. Moreover, many investigators (Barron *et al*., 2007) documented that many antiviral proteins isolated from algae inactivate human immunodeficiency virus (HIV). Therefore, This work aims to screen three cyanobacterial culture filtrates and fresh biomass as bioagents to control *Bean yellow mosaic virus* (BYMV).

MATERIALS AND METHODS

Virus source

BYMV was isolated from naturally infected faba bean plants collected from Agricultural Research Station, Giza, ARC. Samples were tested using I. ELISA (Koeing, 1981). Against four faba bean viruses (*Alfalfa mosaic virus*, *Broad bean stain virus*, *Broad bean wilt virus* and *Bean yellow mosaic virus* using specific antiseram produced in Virus and Phytoplasma Research.Department.

Isolation and propagation

Faba bean plants were mechanically inoculated with sap from naturally infected plants showing the characteristic symptoms of BYMV. Plants reacted only with positive reaction against BYMV-specific antiserum were kept in an insect-proof greenhouse (20-25°C) for recording symptom development. The virus isolate was biologically purified using single local lesion technique (Noordam, 1973). *Chenopodium amaranticolor* plants were used as local lesion host and faba bean cv. Giza1 was used as source plant in the subsequent experiments.

Algal species and culture conditions

Three cyanobacteria strains namely, *Nostoc muscorum*, *Oscillatoria* sp and *Spirulina platensis* were kindly supplied from Department of Microbiology, Soils, Water and Environment Research Institute (SWERI), *Agricultural Research Center* (ARC), *Giza*, *Egypt*. *Nostoc muscorum* and *Oscillatoria* sp were maintained on N-free BG-11_0 and BG-11 media, respectively (Rippka *et al.*, 1979) while, *Spirulina platensis* was grown on Zarrouk medium (Zarrouk, 1966). Cultures were incubated in a growth chamber under continuous shaking (150 rpm) and illumination (2000 Lux) at 25±1°C for 30 days. Table (1) shows the characterization i.e., pH, Optical density at 560 (nm), total chlorophyll (mgl^{-1}) and algal cells dry weight (gl-1) of the algal inoculums APHA (1998).

Preparation of algal filtrates and biomass

Cyanobacteria cultures were homogenized and centrifuged at 3000 rpm for 20 min then filtrates (the supernatant) were sterilized through 0.45 µm filter. Cyanobacterial pellets were repeatedly washed with distilled water to remove salts and the biomass was then frozen in -4°C.

Table (1) Characterization of cyanobacteria cultures

Parameters	Cultures growth parameters		
	Nostoc muscorum	*Oscillatoria* sp.	*Spirulina platensis*
pH	7.75	6.85	10.59
Optical density at 560 (nm)	1.23	0.30	2.61
Total chlorophyll (mg l^{-1})	4.55	5.1	24.50
Dry weight (g l^{-1})	0.79	0.192	1.67

Measuring the effect of algal fresh biomass and their culture filtrates on BYMV infectivity

Two experiments were carried out in the greenhouse belongs Virus and Phytoplasma Res. Dep., Plant pathology Res. Institute, (ARC), Giza, Egypt during two successive winter seasons(2013-2014 & 2014-2015) using plastic pots (25cm in diameter) each was packed with 3 Kg of clay:peat moss: vermicolliet (1:1:1) to study the effect of algal culture filtrates and fresh biomass on BYMV infectivity. The non-algal inoculated synthetic media were used as a control treatment. *Ch. amaranticolor* (40 days old) and faba bean (20 days old) seedlings divided into three groups and treated with cell free cultures filtrate (2 ml/5 plants) and fresh biomass (2 gm fresh weight after freezing, thawing and homogenizing in a mixer with 2 ml sterilized distilled water /5 plants) of three cyanobacteria strains individually and in mixture as follows:

a- Plants in the first group were sprayed with the tested materials 24 hrs. before virus inoculation.
b- Plants in the second group were treated with the mixture of both virus inoculum and the tested materials 1:1(v/v) immediately after mixing.
c- Plants in the third group were sprayed with the tested materials 24 hrs. after inoculation.

Twenty faba bean seedlings and ten leaves of *Ch. amaranticolor* were used as a replicates in each trial. The same number of faba bean seedlings Giza1 and *Ch. amaranticolor* leaves were inoculated with the virus and sprayed with distilled water to serve as a control. Tested plants were observed daily for the developing of local lesions on *Ch. amaranticolor* leaves or appearance of systemic symptoms on faba bean plants.

Inhibitory effect of the tested materials on virus infectivity on local lesion host was determined as described by Devi *et al*. (2004) using the following equation:

$$\textbf{Inhibition\%} = (A\text{-}B/A) \times 100$$

Where: (A) the number of lesions on control leaves and (B) the number of lesions on treated leaves.

The same equation was used in the case of systemic host where, (A) the number of infected plants in control (untreated) and (B) the number of infected plants as result of treatment.

A scale of 1-5 categories was used to asses severity: 1-no symptoms; 2-mild chlorotic patterns and slight distortion of leaves; 3-mosaic patterns on all leaves ,leaf distortion; 4-mosaic patterns on all leaves ,leaf distortion, and general reduction in leaf size; 5-severe mosaic on all leaves and stunting of whole plant. Disease severity (DS) percentage was calculated according to Wydra and Verdier (2002) using the following equation:

$$\textbf{DS (\%)} = \sum(n \times V)/5N \times 100$$

Where, n: number of infected leaves in each category, N: total number of the leaves inspected and V: numerical value of the categories (1-5).

Measuring the effect of algal filtrates and biomass on the vegetative characters of faba bean plants

The vegetative growth parameters, i.e. root length (cm), stem length(cm), vegetative parts fresh weight(g) and dry weight(g) were measured to detect the significance of these differences. Dry weight was measured after drying the

vegetative parts in hot air oven at 70 °C until constant weight.

Biochemical analysis

Protein content and profile

Protein content of algal pellets and filtrates was determined by the micro Kjeldahl method (AOAC, 1997). Samples for protein profile were prepared from cyanobacterial pellets after filtration and were washed with distilled water then treated with extraction buffer 0.062 M Tris-HCl, pH 6.8. Cyanobacterial pellets were homogenized using a pre-chilled mortar and pestle in the presence of powder (~0.5mm) adding ice-cold extraction buffer. The samples were centrifuged at 15000 rpm for 15 min and the process was repeated twice to obtain clear supernatants (Kumar Saha *et al*., 2003). Sodium dodecylsulfate-polyacylamide gel electrophoresis (SDS-PAGE) was carried out according to method of (Bollag and Edelstein, 1993) using 15% sodium dodecyl sulfate polyacrylamide gel and stained with silver nitrate according to Sammons *et al*. (1981). Obtained gels were scanned for band Rf using gel documintation system. Different molecular weights (MW) of bands were determined against protein marker 66, 25 and 11kDa.

Phytochemical analysis of algal secondary metabolites

Secondary metabolites (Total phenols, total alkaloids and total terpenoides) in water extracts of algal biomass and algal culture filtrate were determined. Total phenolic contents of algae were extracted according to the method described by De Marco *et al*. (2007) and estimated by the Folin Ciocalteu method using catechol as a standard and the absorbance measured at 750 nm (Meda *et al.*,2005). Total alkaloids content were determined according to Sabri *et al*. (1973). Total terpenoides were determined by freshly prepared vanillin reagent measured using spectrophotometer at 473nm (Ebrahimzadeh and Niknam, 1998).

Experimental layout and statistical analysis

The treatments were arranged in three replicates with five pots in each experimental unit and the layout was split plot design. The application treatments (algal filtrate and algal Biomass) were the main plots while the algal strains were the sub-plots. Obtained results were subjected to statistical analysis according to Snedecor and Cochran (1980) and the treatments were compared by L.S.D at 0.05 level of probability.

RESULTS

Serological detection

Both naturally infected and mechanically inoculated plants were indexed for virus detection by indirect ELISA. Indirect – ELISA technique was carried out for either confirm the identity of the virus isolate or demonstrate the obtained results.

Effect of algal filtrates or biomass on BYMV infectivity

The obtained results revealed that the use of either algal filtrates or biomass caused a great inhibition of BYMV infection.

Effect of treatments on local lesion infection

Data presented in Table (2) revealed that algal filtrates caused significant inhibitory effects on number of local lesion produced on *Ch. amaranticolor* leaves inoculated with BYMV. The higher inhibitory effects was recorded when algal filtrates were mixed with virus inoculum immediately before inoculation. The inhibitory effect of the filtrate of each alga individually was recorded. The highest inhibitory effect was recorded by *N. muscorum* followed by *S. platensis* while *Oscillatoria* sp. gave the lowest record. The same trend of results with lower inhibitory effect was obtained when algal

biomass were tested. Regarding the application method, the immediate mixing of the algal filtrates or biomass with virus inoculums has the highest inhibition. The effectiveness of the algal filtrate was superior with the mixture of cyanobacteria culture filtrates treatment immediately with virus inoculums. In all cases, the non-algal inoculated synthetic media had no effect on BYMV infectivity.

Table (2) Effect of filtrates or biomass of cyanobacteria on the inhibition percentage of local lesion number produced by *Bean yellow mosaic virus* on *Chenopodium amaranticolor* (Mean value of two experiments conducted in 2013-2014& 2014-2015)

Application method (A)	Treatments (B)	Pre-inoculation		Post inoculation		Immediately Mix	
		Local lesion number	Inhibition %	Local lesion number	Inhibition %	Local lesion number	Inhibition %
Algal Filtrate	Control	14.00	0.00	14.00	0.00	14.00	0.00
	N. muscorum	1.70	87.80	2.50	82.10	1.50	89.20
	Oscillatoria sp.	2.20	84.30	3.00	78.50	1.90	86.40
	S. platensis	1.90	86.40	2.60	81.40	1.50	89.20
	Mixed	1.10	92.00	1.70	87.80	0.90	93.50
Algal Biomass	Control	14.00	0.00	14.00	0.00	14.00	0.00
	N. muscorum	2.00	85.70	2.60	81.40	1.70	87.80
	Oscillatoria sp.	2.30	83.50	3.30	76.40	1.90	86.40
	S. platensis	2.60	81.40	3.30	76.40	2.20	84.30
	Mixed	2.00	85.70	2.50	82.10	1.50	89.20
LSD 0.05%	(A)	0.418	0.706	0.368	0.562	0.464	0.569
	(B)	0.540	0.916	0.475	0.726	0.599	0.735
	(A×B)	0.763	1.289	0.672	1.027	0.847	1.039

Effect of treatments on systemic infection

Data given in Table (3) showed that mixing algal filtrates gave the highest inhibitory effect on BYMV- systemicly infected faba bean seedlings. This effect can be arranged descendingly with *N. muscorum*, *S. platensis* and *Oscillatoria* sp. The inhibitory effect was less pronounced when algal filtrates were sprayed on the tested plants 24 hrs. before virus inoculation. The same trend of results was obtained with lower inhibitory effect by algal fresh biomass. The inhibitory effect of either algal filtrates or biomass was also noticed on disease severity of BYMV infection. The highest inhibitory effect of the algal filtrates or biomass gave the lowest disease severity on faba bean plants. In all cases, algal-free synthetic media had no effect on BYMV infectivity. The inhibitory effect of either algal filtrates or biomass was a reflection to disease severity of BYMV infection as shown in the obtained data in Fig. (1).

Effect of algal filtrates or biomass on the morphological characters of faba bean plants

Data presented in Table (4) revealed that all morphological parameters of faba bean plants increased significantly ($p = 0.5$) because of spraying with either algal filtrates or algal biomass. The mixed filtrates or biomass gave the highest effect followed by *N. muscorum* and *S. platensis* while, *Oscillatoria* sp. gave the lowest effect.

Table (3) Effect of filtrates or biomass of cyanobacteria on BYMV systemically infected faba bean seedlings (Mean value of two experiments conducted in 2013-2014& 2014-2015)

Application method (A)	Treatments (B)	Pre-inoculation			Post inoculation			Immediately Mix		
		No. Infected plants	Inhibition %	D.S. %	No. Infected plants	Inhibition %	D.S. %	No. Infected plants	Inhibition %	D.S. %
Algal Filterate	Control	20.00	0.00	100.00	20.00	0.00	100.00	20.00	0.00	100.00
	N. muscorum	6.00	70.00	6.50	8.00	60.00	9.20	3.00	85.00	2.30
	Oscillatoria sp.	8.00	60.00	14.30	11.00	45.00	18.50	7.00	65.00	8.50
	S. platensis	7.00	65.00	7.80	10.00	50.00	8.80	4.00	80.00	5.80
	Mixed	5.00	75.00	5.70	7.00	65.00	8.80	2.00	90.00	2.10
Algal Biomass	Control	20.00	0.00	100.00	20.00	0.00	100.00	20.00	0.00	100.00
	N. muscorum	7.00	65.00	11.30	12.00	40.00	14.50	6.00	70.00	5.20
	Oscillatoria sp.	10.00	50.00	12.20	16.00	20.00	15.30	10.00	50.00	6.10
	S. platensis	8.00	60.00	12.20	16.00	20.00	15.40	7.00	65.00	5.80
	Mixed	7.00	65.00	8.10	8.00	60.00	9.80	5.00	75.00	5.70
LSD 0.05%	(A)	1.172	2.320	1.590	0.603	1.860	1.307	0.971	2.299	0.812
	(B)	1.603	2.990	2.093	0.833	2.490	1.726	1.360	3.061	1.193
	(A×B)	2.266	4.235	2.960	1.177	3.526	2.441	1.924	4.329	1.687

Infected control | **Mixed of algal culture filtrates** | **Culture filtrate of *N. muscorum*** | **Culture filtrate of *S. platensis*** | **Culture filtrate of *Oscillatoria* sp.**

Fig. (1). Faba bean plants showing the effect of BYMV infection before and after treated with different cyanobacteria culture filtrates.

Biochemical analysis

Phytochemical analysis of algal secondary metabolites

Table (5) illustrated the algal culture biomass and filtrates contained the major secondary metabolites (Total phenos, terpenoids and alkaloids) contents. Generally, the extracellular contained higher contents comparing with the intracellular metabolites in the three tested strains. Extra- and intracellular phenols, terpenoids and alkaloids contents of the three algae could be ranked in order of: *Nostoc muscourum*> Spirulina platensis> *Oscillatoria* sp.

Nostoc muscourum recorded the greatest total contents of the three intra- and extra-cellular metabolites followed by *Spirulina platensis*, while *Oscillatoria* sp. showed the least records as shown in Table (5).

Table (4) Effect of algal filtrates or biomass on the morphological characters of faba bean plants (Mean value of two experiments conducted in 2013-2014& 2014-2015)

Application method (A)	Treatments (B)	Pre-inoculation				Post inoculation				Immediately Mix			
		Root length (cm)	Stem length (cm	Plant fresh weight (gm)	Plant dry weight (gm)	Root length (cm)	Stem length (cm)	Plant fresh weight (gm)	Plant dry weight (gm)	Root length (cm)	Stem length (cm	Plant fresh weight (gm)	Plant dry weight (gm)
Algal Filterate	Control	14.00	58.50	20.10	1.90	14.00	58.50	20.10	1.90	14.00	58.50	20.10	1.90
	N. muscorum	17.50	65.10	25.10	2.50	16.60	64.80	24.50	2.40	17.80	66.80	25.30	2.50
	Oscillatoria sp.	16.50	63.20	24.20	2.20	15.10	61.10	21.50	2.30	14.10	61.90	23.10	2.30
	S. platensis	17.10	64.60	24.90	2.50	16.50	63.30	23.30	2.20	15.90	63.20	24.90	2.50
	Mixed	18.90	67.10	26.50	2.70	18.20	68.30	25.50	2.50	19.20	68.90	27.90	2.80
Algal Biomass	Control	14.00	58.50	20.10	1.90	14.00	58.50	20.10	1.90	14.00	58.50	20.10	
	N. muscorum	18.00	68.30	26.10	2.50	17.60	66.90	25.20	2.40	18.10	69.50	26.30	1.90
	Oscillatoria sp.	16.30	63.60	22.40	2.40	15.90	64.50	22.80	2.30	16.50	65.60	23.00	2.60
	S. platensis	17.50	68.40	25.50	2.50	17.30	65.50	24.10	2.40	17.80	68.30	26.00	2.20
	Mixed	19.10	71.40	28.50	2.90	19.50	70.50	27.50	2.80	19.90	72.50	28.90	2.50
LSD 0.05%	(A)	0.776	1.162	1.298	0.112	0.926	2.752	0.553	0.211	1.090	0.915	0.855	0.116
	(B)	1.073	1.591	1.689	0.145	1.224	3.573	0.742	0.319	1.494	1.241	1.170	0.179
	(A×B)	1.517	2.250	2.388	0.205	1.731	5.052	1.050	0.451	2.113	1.755	1.654	0.253

Table (5) Secondary metabolites as mg/g in culture filtrates (extracellular) and algal biomass (intracellular) of the cyanobacteria strains (Mean value of two experiments conducted in 2013-2014& 2014-2015).

Treatments (A)	Strains (B)	Phenols	Terpenoids	Alkaloids
Extracellular	*N. muscorum*	0.960	0.530	2.600
	S. platensis	0.680	0.470	2.320
	Oscillatoria sp.	0.290	0.380	1.610
Mean (A)		0.640	0.460	2.180
Intracellular	*N. muscorum*	0.300	0.340	1.500
	S. platensis	0.140	0.210	1.210
	Oscillatoria sp.	0.100	0.130	0.980
Mean (A)		0.180	0.230	1.230
Mean (B)	*N. muscorum*	0.630	0.430	2.050
	S. platensis	0.410	0.340	1.770
	Oscillatoria sp.	0.200	0.260	1.300
LSD 0.05%	(A)	0.072	0.061	0.050
	(B)	0.078	0.065	0.056
	(A×B)	0.110	0.086	0.079

Protein pattern profile

Electrophoretic analysis for protein pattern of the three algae are clearly shown in (Fig,2). Most of the expected bands lied between 11, 19 and 29Kda. No protein bands of high molecular weights were recorded, a 11 kDa protein band was detected in both *N.muscorum* and *S.platensis.* Furthermore, a 17, 19 and 29KDa protein bands were only visualized in *S.platensis*, one other protein band of 13KDa was found in *Oscillatoria sp.*

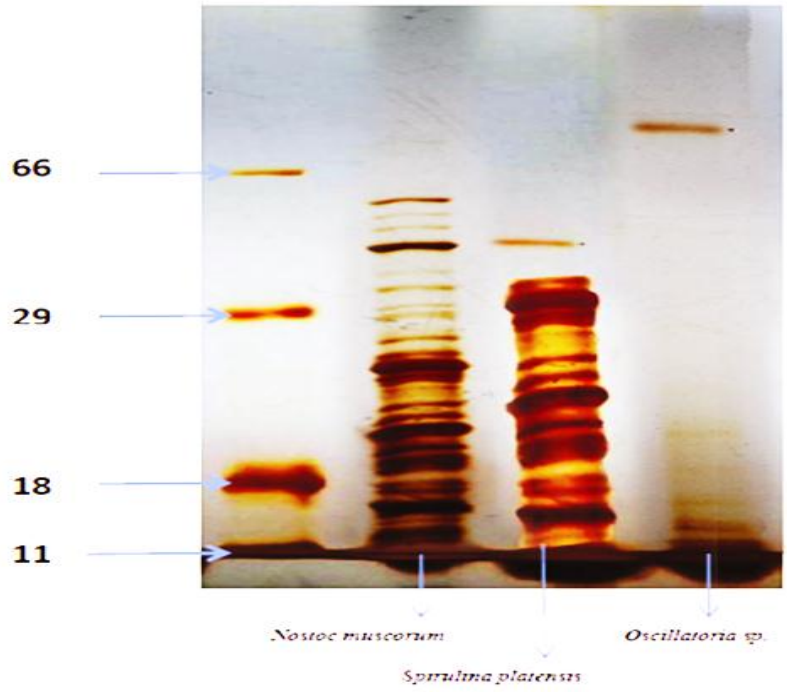

Fig. (2). Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS –PAGE) analysis of total protein extracted from *N. muscorum, S. platensis* and *Oscillatoria sp.* (M=protein marker, Lane 1=*N. muscorum,* Lane 2=*S. platensis,* Lane 3=*Oscillatoria* sp.)

DISCUSSION

The present work was designed to study the inhibitory effect of some algae i.e. *N. muscorum, S. platensis* and *Oscillatoria* sp. on the infectivity of BYMV. Faba bean and *Ch. amaranticolor* were used as a systemic and local lesion hosts, respectively. The culture filtrates and biomass of the three algae were used.

BYMV was isolated and identified using indirect-ELISA assay. ELISA proved to be reliable and sensitive method for detecting and identify of BYMV in infected faba bean plants (Mahdy *et al*., 2007).

Results reported here in clearly demonstrated the effectiveness of algal filtrates and biomass in reducing the number of local lesion formed on *Ch. amaranticolor* leaves particulary those used by mixing either culture filtrates or biomass of the three algae. All tested algal treatments were significantly reduced local lesion number but the largest inhibition was obtained by mixing culture filtrates of *N. muscorum* or *S. platensis* individually with the virus inoculum for 30 min. or applied 24hrs.before virus inoculation. These results are in harmony with Piero *et al*. (2000) who found that the culture filtrates and intracellular contents from either *Nostoc* sp. or *Synechococcus lepoliensis* reduced the number of local lesions caused by *Tobacco mosaic virus* on tobacco by at least 50% when mixed with the virus inoculum or applied 48h before inoculation. The inhibitory effect of the filtrate of each alga individually was recorded with *N. muscorum* followed by *S. platensis*. All treatments under study gave the significant percentage of inhibition of BYMV at remote sited (systemic infection) and lowest disease severity on faba bean plants. These results are in agreement with those reported by Jimenez *et al*. (2011) who found that the inhibitory effect of aqueous and ethanolic extracts from the brown algae (*Durvillaea antarctica)* reduced the damage symptoms in tobacco leaves produced following TMV challenge. The protecting effect presented by both extracts led to a reduction of the number and the size of

necrotic lesions.The three algae under investigation contain considerable values of phenolic compounds, terpenoids and alkaloids. The inhibitory effect of such components on virus infectivity was recorded by several authors. Piero *et al*. (2000) demonstrated that the variance in activity in the tested algal filtrates of *Nostoc* sp. and *Synechococcus leopoliensis* could be due to the phenolic contents in various concentrations of each filtrate. Maclas *et al*. (2007) documented that microalgae produce a remarkable diversity of biologically active metabolites include a lot of chemical class of natural products, ranging from fatty acids to alkaloids, as well as many peptides and amino acids. The action of antiviral agents has been reviewed by Hirai (1977) who reported that the antiviral agents may be divided into two categories: (1) inhibitors against virus infection, and (2) inhibitors against virus multiplication. Some of the inhibitors against virus infection are induced from plant extract, from microorganisms, oxidized phenolic compounds; whereas inhibitors against virus multiplication are the antimetabolites. Sastry and Zitter (2014) tested the effect of phenolic compound ribavirin against *Tobacco mosaic virus* multiplication as sprayer injected into tobacco plant and found that ribavirin reduced symptoms drastically and may eliminate the virus from the tested host. Among the algal treatments, the significant increase in morphological characters of faba bean plants i.e. root length, stem length, plant fresh and dry weight were achieved by mixing culture filtrates of the three algae followed by *N. musorum* and *S. platensis*, respectively. These results was concured with Gupta and Lata (1964) who found that cyanobacteria promoted seedling growth. Ordog (1999) documented that the suspension of extract of cyanobacteria and microalgae contain a special set of biologically active compounds including plant growth regulators, increase leaf chlorophyll, protein content, root and shoot development. In addition, the increase in growth characters, yield and its attributes by foliar fertilization may be due to that the sprayed solution of nutrients is readly absorbed by the leaves and not lost through fixation, decomposition or leaching (Abd El- Mohsen and Ahmed, 2015). Cyanobacteria currently seem to be offering a potentially environmental friendly alternative to the use of chemical fertilizers, they can enhance the plant growth directly and/or indirectly. The direct ways include the production of various plant growth promoting biologically active substances including phytohormones, such as auxin, gibberellins and cytokinins. Meanwhile, the indirect promotion of plant growth occurs when cyanobacteria prevent or counter deleterious effect of phytopathogenic microorganisms (Rai, 2006).

Recently, algae have been found to contain compounds inhibitory to plant viruses (Galal *et al*., 1999). Vera *et al*.(2011) stated that spraying tobacco leaves with oligo-alginate poly-Ma isolated from marine algae induced a sustained increase in Phenylalanine ammonia-lyase activity and induced an effective protection against (TMV)in tobacco plants. Moreover, Sano (1998) reported that the antiviral activity of alginate on infectivity of *Tobacco mosaic virus* (TMV) may be caused blocking the decapsulation process of TMV protein on the cell membrane surface. In this study results of SDS –PAGE confirmed the presence of the antiviral proteins in algal biomass of the three algae. Four protein bands with molecular weights of 11, 17, 19 and 29 KDa were found in *S. platensis*. Also, one protein band of 11KDa was found in *N. muscorum* and another protein band of 13KDa was found in *Oscillatoria* sp. These results are in harmony with those by Barron *et al.* (2007). They isolated antiviral protein cyanovirin (CV-N) with molecular weight of 11KDa from *Nostoc* sp. and documented that many

antiviral proteins were isolated from *S. platensis* with molecular weight of 29 KDa. The mechanism of its antiviral activity was due to a potent anti HIV activity, presumably acting by direct binding to the glucans that are abundantly present on the HIV1 gp120. Murugan and Radhamadhavan (2011) reported that antiviral protein C-phycocyanin isolated from *S. platensis* with a molecular weight of 17&19KDa (α & β) phycocyanin was shown to have antiviral activity against *Hepatitis-A virus* and *Poliovirus*. Nuhu (2013) documented that a recent study on the antiviral activity of *S.platensis* has resulted in the isolation of cyanovirin-N (CV-N), a novel cyanobacterial carbohydrate binding protein. Huskens and Schols (2012) reported that cyanovirin-N and *O. agardhii* agglutinin homolog (OAAH) antiviral proteins not only inhibit cells - to- cell movement but also efficiently prevent virus transmission from infected cells to uninfected ones. Finally, Yang *et al*. (2012) mentioned that the inhibitory effect of *Spinacia oleracea* against TMV on *Nicotiana glutinosa* was due to the antiviral proteins with molecular weight 19,26,34 and 50KDa. In short, cyanobacteria can be recommended as a mean in controlling BYMV infected faba bean plants.

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REFERENCES

Abd El-Baky, H. H.; El Baz, F. K. and El-Baroty, G. S. (2008). Evaluation of marine alga *Ulva lactca* L. as a source of natural preservative ingredient. Am. Eurasian J. Agric. Environ. Sci. 3(3):434-444.

Abd El- Mohsen, A.A. and Ahmed, M.A. (2015).The effect of applying different fertilizer regimes on productivity and profitability of Egyptian cotton under middle Egypt conditions. Adv.Agric.Biol.4 (1): 31-38.

Adak, M. S.; Vlukan, H. and Guler, M.(1998). Determination of some agronomic traits in Turkish faba bean (*Vicia faba* L.) Lines. FABIS, News Letter 42:29-31.

APHA (1998). Standard methods for the examination of water and wastewater, 20th ed., American Public Health Association Washington, DC.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS - AOAC. Official methods of analysis, 16th ed. Gaithersburg: AOAC, Gaithersburg, 1997. 202p.

Barron, B. L.; Torre,s j. M.; Chamarro, V. G. and Estrad,a A. Z. (2007). *Spirulina* as an antiviral agent. In: *Spirulina* Human Nutrition and Health. In A. Belay and M. E. Gershwin(eds.), Taylor&Francis Group.Broken sound Parkway.Boca Raton,CRC Press.pp.312.

Bollag, M. D. and Edelstein, S. J.(1993). Protein Methods. John Wiley& Sons, Inc., New York.230pp.

De Marco, E.; Savaresa, M.; Paduano, A. and Sacchi, R.(2007). Characterization and fractionation of phenolic compounds. Food Chemistry 104:858-867.

Devi, P.R.; Doraiswamy, S.; Nakkeeran, S.; Rabindran, R.; Ganapathy, T.; Ramiah, M. and Mathiyazhagan, S.(2004). Antiviral action of *Harpulia cupanioides* and *Mirabilis jalapa* against tomato spotted wilt virus (TSWV) infecting tomato. Archives of Phytopathology and Plant Protection 37 (4): 245-259.

Ebrahimzadeh, H. and Niknam, V.(1998). A revised spectrophotometric method for determination of triterpenoid saponins. Indian drugs 32 (6): 379-381.

Gademann, K.and Portmann, C.(2008). Secondary metabolites from cyanobacteria: complex structure and powerful bioactivities.Curr. Org. Chem. 12: 326-341.

Galal, A. M.; El-Ayouty, Y. M.; Hashem, S. A.; and El-Sabbahy, G.(1999). Screening of different algal extracts for their efficacy against mechanical transmission of two phytoviruses. Acta Hydrobiol. 41:155-163.

Gupta, A.B.and Lata, K.(1964). Effect of algal growth hormones on the germination of paddy seeds. Hidrobiologia 24(1-3): 430 -434.

Hewedy, M. A.; Rahhal, A. M. and Ismail, I. A.(2000). Pathological studies on soybean damping-off disease.Egypt. J. Applied Sci. 15:88-102.

Hirai, T.(1977). Action of antiviral agents,In: Plant Dis. An Advanced Treatise.J.G.Horsball and E.B.Cowling (eds.)Vol.1, Academic Press 285-306.

Huskens, D. and Schols, D.(2012). Algal lectins potential HIV microbicide candidates. Mar. Drugs 10:1476-1497.

Jimenez, E.; Dorta, F.; Medina, C.; Ramirez, A. and Cortes, H. P.(2011). Anti-phytopathogenic activities of macro-algae extracts. Drugs 3(9):739-756.

Kiviranta, J.; Abdel-Hamid, A.; Sivonen, K.; Niemela, S.I.and Carlberg, G.(2006). Toxicity of cyanobacteria to mosquito larvae-screening of active compounds. Environ. Toxicol. Water Qual. 8: 63-71.

Koeing, R.C.(1981). Indirect ELISA methods for broad specifity detection of plant viruses. J. of Gen.Virol. 55: 53-62.

Kumar Saha, S.; Uma, L. and Subrmsanian, G.(2003). Nitrogen stress induced changes in the marine cyanobacterium *Oscillatoria willei* BDU 130511. FEMS Microbiology Ecology 45: 263-272.

Maclas, F. A.; Galindo, j. L. G.; Diaz, G.; Diaz, G. M. D. and Galindo, J. C. G.(2007). Allelopathic agents from aquatic ecosystems: potential biopesticides models. Phytochem. Rev. 7:155-178.

Mahdy, A.M.M.; Fawzy, R.N.; Hafez, M.A.; Mohamed, H.A.N. and Shahwan, E.S.M.(2007). Egyptian J. Virol. 4:223-241.

Meda, A.; Lamien, C.; Romito, M.; Millogo, J.Nacoulma, O. G.(2005). Determination of the total phenolic,flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chemistry 91: 571-577.

Mehta, A. D. and Boston, R. S.(1998). Ribosome- inactivating proteins. In: A lock beyond transcription:Mechanisms determining mRNA stability and translation in plants. In Baiely-Serres J and D.R. Gallie (eds.), American Society of Plant Physiologists.Rockville:145-152.

Murugan, T. and Radhamadhavan, M.(2011). Screening for antifungal and antiviral activity of C-phycocyanin from *Spirulina platensis*. Journal of Pharmacy Research 4(11): 4161-4169.

Noordam, D.(1973). Identification of plant viruses.Method and experiments. Center for Agric. Pub. and Document. Wageningen, Netherlands,207 pp.

Nuhu, A. A.(2013). *Spirulina* (*Arthrospira*): An important source of nutritional and medicinal compounds. J. of Marine Biology,vol.2013,Article ID 325636, 8pp.

Ordog, V. (1999). Beneficial effects of microalgae and cyanobacteria in plant soil system,with special regard to their auxin and cytokinin –like activity. International workshop and training course on microalgal biology and biotechnology. Mosonmagyorovar,Hungary,pp.13-26.

Pardee, K.(2001).Plant virus inhibitors from marine algae.M.Sc.,University

of British Columbia, Vancouver,Canada,119pp.

Pardee, K. I.; Ellis, P.; Bouthillier, M.; Towers, G.H.N. and French, C. J. (2004).Plant virus inhibitors from marine algae.Can.J.Bot. 82:304-309.

Piero, R. M. D. I.; Pascholati, S. F. and Rezende, J. A. M.(2000). Effect of the cyanobacteria *Synechococcus leopoliensis* and and *Nostoc* sp.on the infectivity of tobacco mosaic virus (TMV).Summa Phytopathologica 26(2):215-220.

Radwan, D. E. M.; Lug, F. K. A.; Mahmoud, S. Y. and Hamad, A.(2008). Protective action of salicylic acid against bean yellow mosaic virus in *Vicia faba* leaves. Journal of Plant Physiology165:845-857.

Rai, M. K.(2006). Handbook of microbial biofertilizers. Haworth Press. New York. 543 pp.

Rippka, R.; Deruelles, J.; Waterburg, J. B.; Herdman, M. and Stanier, R. Y. (1979): Generic assignments, strain histories and properties of pure cultures of cyanobacteira. J. Gen. Microbiol. 111: 1-16.

Sabri, N. N., El-Masry, S. and Khafagy, S. M.(1973). Phytochemical investigation of *Hyoscyamus deseroru*.Planta Med. 23(1): 4-9.

Sammons, D. W., Adams, L. D. and Nishizawa, E. E.(1981). Ultra-sensitive silver based colour staining of polypeptides in piolyacrylamide gels. Electrophoresis 2:135-140.

Sano, Y.(1998). Antiviral activity of alginate against infection by tobacco mosaic virus. Carbohydrate Polymers 38(2):183-186.

Sastry, K.S.; and Zitter, A. (2014): Plant virus and viroid diseases in the tropics. Volume 2: Epidemiology and Management. Springer, New York.

Snedecor G.W; Cochran W. G. Statistical Methods. 7th ed. Iowa State Univ. Press. Ames. Iowa, USA, 1980:507.

Vera, j.; Castro, J.; Gonzale,z A; Barrientos, H.; Matsuhiro, B.; Arce, P.; Zuniqa, G. and Moenne, A. (2011). Long-term protection against tobacco mosaic virus induced by the marine alga oligo-sulphated galactan poly-Ga in tobacco plants.Mol. Plant Pathol. 12(5): 437-474.

Wydra, K. and Verdier, V. (2002). Occurrence of cassava diseases in relation to environmental,agronomic and plant characteristics. Agriculture Ecosystem& Environment :211-226.

Yang, J.; Jin, G. H.; Luo, Z. p.; Yin, Q. S.; Gin, L. F. and lin, F. C.(2012). Spinacia oleracea proteins with antiviral activity against tobacco mosaic virus .African Journal of Biotechnology 11(26): 6802-6808.

Zarrouk, C. (1966): Contribution á l'étude d'une cyanophycée. Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthése de Spirulina maxima (Setch. Et Gardner) Geitler. Ph.D. Thesis, University of Paris, France.