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Antiphytoviral Potentialities of Algal Extracts Against Cucumber Mosaic Virus

Abdel-Moneim M. Galal[1] and Idris M. Al-Turk[2]

[1]Department of Botany, Faculty of Science, Zagazig University, Zagazig, Egypt.
[2]Department of Biology, Faculty of Science, Taibah University,
Al-Madinah Al-Monawarah, Kingdom of Saudi Arabia

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Twelve algal species belonging to Chlorophyta (6), Halophyte (1) and Rhodophyta(4) were screened for their antiphytoviral activity against cucumber mosaic virus (CMV) infected cucumber plants. They collected from the red sea at Yanbua shore, Kingdom of Saudi Arabia. Ethyl alcohol extracts or Lectin preparations of *Ulva linza, Enteromorpha intestinalis, Cladophora glomerata, Caulerpa verticillatta, Chara blatic Nitella cernua (Chlorophyta), Ectocarpus globifer, Dictyota dichotoma, Fucus vesiculosus, Fucus serratus, Sargassum vulgare (Phaeophyta), Polysiphonia sp. (Rhodophyta)* were tested *in vitro* and *in vivo* against the infectivity of CMV. Induction of resistance in cucumber plants were recorded as treatment were done before virus inoculation at different periods. Also acquired resistance against CMV was observed as the cucumber seeds were soaked in algal extracts at different periods. The chlorophyll contents of the treated cucumber plants were more or less similar with that recorded in healthy plants.

Key words: Marine algae, ethanolic extracts, lectin preparation, antiphytoviral, pigments.

Algae are rich sources for antiphytoviral-containing substances as well as anti animal viruses or antihuman viruses. Lipid extracts of 13 algal species were screened against tobacco mosaic virus (TMV) and found that extracts of both *Zanardinia prototypus* and *Cystoseira balirico* represented the best antiviral source among the tested species[1]. A new antiviral agent (UF 131) from the green alga *Ulva fasciata*[2]. It exhibited antiviral activity against semliki forest virus and other viruses. The structure of UF131 was established as 2-N-plamitoyl-4,5-dihydro-I,3,4,5-tetra-hydroxysphingosine. Carrageenan, a polysaccharide extracted from red algae, was shown to inhibit DNA and RNA-containing viruses and also inhibited a specific retroviral enzyme (reverse transcriptase). Furthermore, sulphated polysaccharides from red algae had a broad-spectrum antiviral activity with low cost[3]. A protein-containing polysaccharide having antiviral activity was obtained from algae belonging to the genera *Nemacystus, Kjellmaniella, Laminaria, Undaria, Hizikia, Porphyra, Gelidium, Gloiopeltis, Gracilaria, Hemi-neura, Ulva, Spirogyra, Codium* and *Acetabularia*[4].

Seventy three aqueous ethanolic extracts from 18 marine algal species belonging to

* To whom all correspondence should be addressed.
E-mail: dr_glal2003@yahoo.com.au

Chlorophyta, Phaeophyta, and Rhodophyta, and 3 sea grasses were tested *in vitro* against several viral diseases[5]. More than half the extracts were active against at least one virus. On the same line, 71 species of marine macrophytes from the central Mediterranean have been screened for the production of anti-bacterial, antifungal and antiviral compounds. From the tested plants, 12% showed antiviral activity[6]. Ethanolic extracts of 31 species belonging to Rhodophyceae, Phaeophyceae, and Chlorophyceae were tested against TMV, from which 17 species produced antiviral activity[7]. The antiviral activity observed in *Codium elongatum* and the two species of Hypnea was attributed to the polysaccharides. The palmitolyl ester amide of dihydroxy sphingosine was found to be the antiviral principle of *Viva fasciata.* Similarly, Glucan preparation was obtained by transformation of laminaran from the alga *Laminaria cichoriodes* with endo-β-1,3-g1ucanase from marine mollusc. This preparation, like those of other sources, had an inhibitory effect on TMV infection of detached leaves of local and systemic host. tobacco plants[8].

The effect of 21 algal species belonging different families were invistigated on the infectivity of tobacco mosaic virus and tomato mosaic virus[9]. They recorded that the most species extracts as well as the lectin preparation led to high inhibitory effects on either tested viruses.

Methanolic extracts from 30 species of marine algae were assayed for antiviral activity against potatovirus (PVX) in local lesion assays using *Chenopodium quinoa* L. as host[10]. They added that extracts from six algal species (*Fucus gardneri Silva, alfsia* sp. (Berkelry), *Codium fragile (Suringer Alaria marginata Postels* and *Ruprecht, R) Hariot, Fragilaria oceanica Cleve,* and *Egregia menziesii* (Turner) J.E. (Areschoug) inhibited PVX infectivity by more than 80%.

The aim of the present work was the detection of antiphytoviral effects of different algal extracts collected from Yanbua shore, KSA as well as the lectin preparations of these genera on the infectivity of CMV on cucumber plants .Induction of resistance against CMV and the changes of chlorophyll contents in either healthy cucumber plants, inoculated plants with CMV or inoculated and treated plants were also studied.

MATERIAL AND METHODS

Virus

Cucumber mosaic virus (CMV), naturally infected cucumber plants were used for this investigation. Reinoculation of healthy cucumber plants was done and the original symptoms with mosaic and yellowing features was recorded.

Algal species

Twelve algal species belonging to Chlorophyta (six genera), Pheophyta (five genera) and Rhodophyta (one genus) were collected from the red sea of Yanbua shore, Kingdom of Saudi Arabia. The samples were thoroughly washed with bicarbonate buffer (1 mg VI) and spread on cheesecloth under a dry air stream 50°C till constant weight and then ground. They were identified according to[11] and presented in table 1.

Table 1. Algal species used in the study of antiviral activity

Algal Species	Family
Ulva linza, *Enteromorpha intestinalis* *Cladophora glomerata* *Caulerpa verticillatta* *Chara blaticNitella* *Nitella cernu*	Chlorophyceae
Ectocarpus globifer *Dictyota dichotoma* *Fucus vesiculosus* *Fucus serratus*	Rhodophyceae
Sargassum vulgare *Polysiphonia sp*	Pheophyceae

Extraction methods

Ethanolic extraction

Ethanolic extractions of 50g of each fresh algal species were made using ethyl alcohol (70%, v/v, by distilled water) in a soxhlet apparatus. The obtained extracts were concentrated until dryness using a rotary evaporator at 40°C. The residue was redissolved in water before application.

Lectin preparation

Lectin preparation was done according to the method given by Stripe *et al.*[12]. Air dried

algal tissues were used for lectin extraction using saline phosphate buffer (pH 7.2), the homogenate was left overnight at 4°c, then strained with cheese cloth and centrifuged at 3000 r.p.m. for 30 minutes. The supernatant was collected and concentrated to 1/10 of the initial volume.

Methods of applications

***In vitro* application**

50 µL of CMV inoculum was mixed with 50µlL of either alcoholic extract, or lectin preparation on the cotyledonary leaves of cucumber plants. After dusting the leaves with with carborundum (400 mesh) and the inoculated leaves were then washed with distilled water. The developed mosaic symptoms were observed and recorded after 21 days of inoculation The percentage of inhibition for each treatment was calculated .The control plants were supplied with CMV only and the percentage of inhibition was calculated. Healthy control plants was also done.

***In vivo* application**

Pre-inoculation

Treatment of inoculated cucumber plants with CMV by either alcoholic or lectin algal extracts were done after 2, 6, 12 and 24 hours of inoculation. The percentages of induction of resistance were calculated .

Post –inoculation

Treatment of inoculated cucumber plants with CMV by either alcoholic or lectin algal extracts were done before 2, 6, 12 and 24 hours of inoculation. The percentages of induction of resistance were calculated.

Seed soaking

Fifty cucumber seeds were soaked in each algal alcoholic extract for 2, 4, and 6 hours, then grown in pots. The growing seedlings were inoculated with CMV and the developed mosaic symptoms was recorded. The percentage of induction of resistance due to each extract was calculated.

Estimation of chlorophyll pigments

Plant chlorophyll pigments a, b and a+b were estimated in either healthy, inoculated plants with CMV or in plants inoculated with the mixture of viral sap and each algal extract according to[13] by acetone extraction of cucumber leaves. The pigments were calculated by using spectrophotometer at wave lengths of 649 and 665 for chlorophyll a and b, respectively.

Table 2. *In vitro* Effect of ethanolic extract (E) and lectin preparation (L) of algal species on the infectivity of *CMV* and chlorophyll contents of cucumber plants

Algal species	%I		Chlorophyll		
	E	L	a	b	a+b
Ulva linza,	85	92	4.59	5.50	8.27
Enteromorpha intestinalis	42	58	6.13	6.77	9.30
Cladophora glomerata	18	60	5.62	6.92	12.01
Caulerpa verticillatta	22	46	6.61	8.09	14.01
Chara blaticNitella	23	62	5.62	6.98	12.22
Nitella cernu	20	21	5.96	7.01	11.89
Ectocarpus globifer	0	24	6.22	7.13	13.21
Dictyota dichotoma	0	42	6.58	7.79	13.22
Fucus vesiculosus	80	96	5.62	6.83	11.89
Fucus serratus	0	08	5.90	7.06	12.00
Sargassum vulgare	20	40	6.32	7.66	13.24
Polysiphonia sp	94	100	5.64	6.53	12.34
Control plants	-	-	5.13	6.59	11.27

Where: % I = Percentage of inhibition of virus = $\frac{\text{control virus - teatment}}{\text{control virus}} \times 100$

E = Ethanolic extract L = Lectin preparation

RESULTS AND DISCUSSION

Twelve ethanolic extracts (E) or lectin preparations (L) were in vitro tested on cucumber plants after mixed with equal volume of CMV sap (table 2).The data showed that the tested species led to inhibitory effects on CMV with different values. Complete inhibition of viral symptoms was reached with the treatment by lectin of *Polysiphonia sp*. Otherwise, 94% inhibition was reached with the ethanolic extract of the same species . Ethanolic extract or lectin preparation of either *Ulva linza or Fucus vesiculosus* showed highly activity on CMV infectivity [(85 %,92 %) and (80 %,96 %)] , respectively. These results may be explained on the basis that the lectin preparation contains Mn^{2}+ and Ca^{2}+ ions and both are essential for the carbohydrate binding process and agglutinating activities[14,15]. Also, algal lectins are protein, or of glyco-protein nature of diverse molecular weight, having the property of binding carbo-hydrate-containing molecules. Plant lectin preparations showed a broad antiviral spectrum effect[16]. Such an inhibitory nature was explained by their effect on the viral transmission or viral replication. In addition, lectins enhanced the resistance of host pre-infected with TNV. The use of such a type of application was recommended because it is easy to obtain and not expensive.

Furthermore, *Ectocarpus globifer* or *Dictyota dichotoma* extract had no effect on the virus but it's lectins preparation gave 24% and 42% of inhibition of viral symptoms respectively.These results depend mainly on the algal species, type of virus or the method of extraction. These findings in agreement with[16-17] who reported thatapplication of crude lectin preparations of ten plants gave antiviral activities against Tobcco Necrosis Virus and Cucumber Mosaic Virus. Also,[5] screened the aqueous ethanolic extracts from 18 sea weed species belonging to Chlorophyta,Phaeophyta and Rhodophyta against several viral diseases *in vitro.* With respect to chlorophyll pigments, the treatment of cucumber plants with either ethanolic extracts or lectin preparations led to recovery of mosaic symptoms regarding the stimulatory effects of these treatments on chlorophyll pigments of a, b, and a+b which were more or less similar of that reached with healthy cucumber plants. These results were similar to that found with[16-17].

In vivo application of algal extracts (E) before or after CMV inoculation were represented in tables 3 and 4. However, the highest antiviral activities were reached with *Dictyota dichotoma* (96%) when applied 24 hours after viral

Table 3. *In vivo* effect of alcoholic extracts (E) of algal species on the infectivity of CMV pre-inoculated of cucumber plants at different periods (2, 6, 12 and 24 hours)

Species	%I at (hours)			
	2	6	12	24
Ulva linza	58	58	60	64
Enteromorpha intestinalis	80	84	84	88
Cladophora glomerata	44	48	52	54
Caulerpa verticillatta	84	80	86	92
Chara blatic Nitella	70	76	80	80
Nitella cernu	52	56	56	64
Ectocarpus globifer	68	68	76	76
Dictyota dichotoma	84	88	88	96
Fucus vesiculosus	12	16	16	18
Fucus serratus	32	36	38	38
Sargassum vulgare	66	70	76	76
Polysiphonia sp	36	34	40	42

Table 4. *In vivo* effect of alcoholic extracts (E) of algal species on the infectivity of CMV post-inoculated of cucumber plants at different periods (2, 6, 12 and 24 hours)

Species	%I at (hours)			
	2	6	12	24
Ulva linza	36	40	22	22
Enteromorpha intestinalis	12	18	18	20
Cladophora glomerata	26	28	28	36
Caulerpa verticillatta	08	14	20	16
Chara blatic Nitella	36	44	44	28
Nitella cernu	24	24	32	32
Ectocarpus globifer	04	12	12	18
Dictyota dichotoma	82	84	84	84
Fucus vesiculosus	82	84	84	88
Fucus serratus	62	62	64	68
Sargassum vulgare	24	28	30	34
Polysiphonia sp	58	60	66	64

Table 5. Effect of cucumber seeds soaked in algal alcoholic extracts (E) on the infectivity of CMV inoculated at different periods of soaking (2, 4 and 6 hours).

Species	%I at (hours)		
	2	4	6
Ulva linza	36	40	22
Enteromorpha intestinalis	12	18	18
Cladophora glomerata	26	28	28
Caulerpa verticillatta	08	14	20
Chara blaticNitella	36	44	44
Nitella cernu	24	24	32
Ectocarpus globifer	04	12	12
Dictyota dichotoma	82	84	84
Fucus vesiculosus	82	84	84
Fucus serratus	62	62	64
Sargassum vulgare	24	28	30
Polysiphonia sp	58	60	66

inoculation followed by *Caulerpa verticillatta* (92% after 24 houras), *Enteromorpha intestinalis* (88% after 24 hours). It's obvious that antiviral activities were enhanced with all treatments by algal extracts at different times of applications with variable values.

Regarding the effects of ethanolic extracts, it was indicated that the cucumber soaked seeds in extracts of either *Dictyota dichotoma* or *Fucus vesiculosus* had the maximum rate of inhibition against CMV (82-88%), at 2, 4 or 6 hours of seed soaking as well as the maximum increase in host resistance as compared with the other treatments. It was obvious that many algae contain substances which can be extracted either by distilled water, buffers, or by organic solvents. The antiviral effects of these extracts differ greatly from one species to another in the method of application, the type of virus, and the host plants[8,21]. The induction of resistance to virus diseases occurs as a result of a primary inoculation or chemical treatment. The resistance may be due to the localization of the protecting virus around the infection sites and induced resistance in uninfected tissue[22-26].

REFERENCES

1. Caccomes S., Azzolina R., Furnai G., Cormaci M. and Grasso S., Antimicrobial and antiviral activities of some marine algae from eastern Sicily, Italy. *Botanica Marina*, 1981; **24**: 365-368.
2. Pandey M., Gary H.S., Bhakuni D.S., and Dhar M.M., An antiviral agent from Ulua fasciata. Proc. Symp. "Bioactive compounds from marine organisms with emphasis on the Indian Ocean", New Delhi, 1-3 October 1991 reds: Thomson M.F., Sarojini R. and Nagabh u.R.). 1991; 283-287.
3. Neushul ,M., Antiviral carbohydrates from marine red algae. Bioactive compound from marine organisms with emphasis on the Indian Ocean. An Indian-United State Symposium. Thomson, M. F.:Sarogjini, R. and Nagabhushanan,R. (EDS.) 1991; 275-281.
4. Muto S., Numura K., Oahara M., Ogachi Y, Matsunaga K. and Hiroses K., Poly-saccharides and antiviral drugs containing the same as active gradient. *Biotechnology,* Abstract No 2752818 1992.
5. Premnathan M., Chandra K., Bajpai S. and Kathiresan K., A survey of some Indian marine plants for antiviral activity. *Botanica Marina,* 1992; **35**: 21-324.
6. Ballesteros E., Martin D., and Uriz M.J., Biological activity of extracts from some Mediterranean macrophytes. *Botanica Marina,* 1992; **35**: 481-485 .
7. Kamat S.Y, Wahidulla S., Souza L., Naik C.G:, Bhakuni D.S., Goel A.K. and Srimal R.C., Bioactivity of marine organisms. 6. Antiviral evaluation of marine algal extracts from the Indian Cost. *Botanica Marina,* 1992; **35**: 161-164
8. Reunov A.V, Lapshhia L.A., Nagorskaya V.P. and Elyakova L.A., Effect of 1,3; 1,6-b-D-glucan on infection of detached tobacco leaves with tobacco mosaic virus. *Phytopathology*, 1996; **144**: 247-249.
9. Galal A.M.,El-Ayouty,Y.M., Hashem,S.A. and El-Sabbahy,G., Screening of different algal extracts for their efficiency against mechanical transmission of two phytoviruses.*Acta Hydrobiol*., 1999; **41**(2):155-
10. Pardee K.I., P., Ellis, M., Bouthillier, G.H.N., Towers, and C.J.French., Plant virus inhibitors from marine algae. *Can.J. Bot.,* 2004; **82**(3): 304-309.

11. Cormaci M. and Furnari G., Flora of marine algae. Rhodophyceae, Phaeophyceae, Chloro-phyceae. *Inf. Bot. Ital.,* 1979; **11**: 221-250
12. Stripe F., Olsens S. and Pihl A., Gelonin, a new inhibitor of protein synthesis, non toxic to intact cell. Isolation, characterization and preparation of cytotoxic conjugates with concanavalin. *A. J. BioI. Chern.,* 1980; **255**: 6947-6955.
13. Selly, G.R., The chlorophylls . Academic press, NewYork and London. 1966; 211-242.
14. Sharon N. and Lis H., Lectins: cell-agglutinating and sugar-specific proteins. *Science*, 1972; **177**: 949-959.
15. Andrews A.T., Nevy {Haricot}-bean (Phaseolus vulgaris) lectin. Isolation and characterisation of two components from a toxic agglutinating extract. *Biochem. J.*, 1974; **135**: 307-314.
16. Jaffe W.G., Algal lectin, nature and properties. In: Liener I.E. (ed.) Toxic constituents of plant food stuffs. New York 1969.
17. Galal A.M., Antivirals against local and systemic viral infections of Phaseolus vulgaris. *PhD thesis*, Faculty of Science, Zagazig University, 1990 ; 80.
18. Hori, K.; Miyazawa, K. and Ito,K., Prelaminary characterization of agglutinins from seven marine algal species. *Bull. Jpn. Sci. Soc.* 1986; **52**: 323-331.
19. Bear, J. S. and Ratual, H. S., Leaf crinkle virus induced biochemical changes in mash bean, *Vigna munyo* (L.) and its effect on Aphis craccivora, *koch.J. INSECT Sci.* 1990; **3**(1): 62-66.
20. Yordanova A., Kaprarov A., Stoimenova K. and Starcheva M., Antiphytoviral activity of 1-morpholinomethyl-tetiahydro-2(1H)-PyrimidinoneCDD13}. *Plant Pathology,* 1996; **45**: 547-551 .
21. Fraser R.S.S., Mechanisms of induced resistance to virus disease. *Physiol. Plant Pathol.,* 1985; 373-397.
22. Ghaly, M.F., AWNY N.M., Galal; A.M., and Askora A., :Characterization and action of antiphytoviral agent produced by certain Streptomycetes species against zucchini yellow mosaic virus. *Egypt. J. Biotechnol*. 2005; 19,
23. Zawahry, Y.A.; Galal , A.M Abou El-Hawa, .; M.E., El. Sayed, A.E. ;.El-hirbiny, E.A and Abd El-Shafi , S., Indyction of systemic resistance in cucumber plants against Zucchini yellow mosaic virus using bacterial biotic inducers. *N. Egypt. Microbiol.* 2006; **13,** 124-130.
24. Galal A.M., Induction of systemic acquired resistance in cucumber plant against cucumber mosaic cucumovirus by local *Streptomyces* strains. *Plant Pathol. J.,* 2006; **5**(3): 343-349.
25. Abou El-Hawa , M.E.; -Shirbiny , E.A.El ; El-Zawahry , Y.A. ; El- Sayed, A.El- Sayed; Galal, A.M. and Abd El-Shafi, S., Extraction, purification and characterizationof two antiphytoviral substance (s) produced by zucchini yellow mosaic virus. *Int. J. Agri.Biol.,* 2006; **8**(6).