A Potential Elicitor of Green Alga (*Ulva lactuca*) and Commercial Algae Products against Late Blight Disease of *Solanum tuberosum L.*

Soad. M. Ahmed[1*], Saad R. El-Zemity[1],Rasha E. Selim[2] and Fahmy A. Kassem[1]

[1] Pesticide Chemistry Department, Faculty of Agriculture (Elshatby), Alexandria University Alexandria, Egypt,

[2] Central Agric.Pestic. Lab. Agric Research Center. Egypt

[*]*Corresponding author's email: soad2005eg [AT] yahoo.com*

ABRSTRACT---- ***The phenomenon of systemic acquired resistance has attracted attention as a new strategy for controlling plant disease. The resistance was evident as a reduction in disease incidence compared with infected control. The objective of this research was to determine the efficacy of green alga (Ulva lactuca) extract and some Commercial algae products for management of late blight disease of two varieties of potatoes (Lady pulford and Burren) under field conditions. Also, we investigated the ability of these algae to elicit induced resistance in potato against late blight disease caused by Phytophthora infestans, through, their effects on biochemical changes which lead to induction of plant defense against the pathogen. A negative correlation between the disease severity and the content of total soluble polyphenols , this correlation reached to 50% in Burren variety while it was 67% in the variety Lady pulford. Negative correlation between the disease severity index and total protein was found also and the correlation reached to 80% in the variety Lady pulford. The same trend was also noticed in PPO and RNase. So, Lady Pulford is more tolerant to the disease than Burren. Finally, we could say the use of algae extract might help to overcome the pathogen infection by increasing levels of defense-related enzymes and phenolic and protein substances.***

Keywords – Algae, Potato, Late blight, Elicitor

1. INTRODUCTION

Potato plant (*Solanum tuberosum* L.) considered one of the most important vegetable crops overall the world. It has the sixth largest crop with production reaching a record of 365 million tons in 2012 [1]. Potato is one of the most important vegetables in Egypt. Approximately 178,000 hectares are cultivated to potatoes in 2013 with an average yield of 27 tons/ha [2]. Also, it gained a considerable importance as an export crop to European markets and one of the national income resources [3], [4].

Due to its widespread occurrence and genetic diversity, the fungus-like
Organism *Phytophthora infestans* is among the most important potato pathogen [5], [6], [7].This disease is known as late blight (LB). Worldwide average losses on unprotected fields in tuber yield due to late blight disease vary enormously from 25–100% [8], [9], [10].

Chemicals largely used as pesticides in crop protection could be environmentalpollutants and have undesirable biological effects on animals and human beings.The toxic effect of synthetic chemicals can be overcome, only by persistent search for new and safer pesticides accompanied by wide use of pest controlmethods, which are ecofriendly and effective [11]. One source of potential new pesticides is natural products produced by algae.Marine algae represent a great source of a vast variety of complex natural products and could be a promising source of a novel bioactive compound that can help plant survival by offering protection against stress imposed by pathogens[12], [13]. Extracts derived from seaweedsare biodegradable, non-toxic, non-polluting and non-hazardous to humans, animals and birds [14]. One of the most popular types of algae used in this field is the genus *Ulva* (*Phylum Chlorophyta*, Class *Ulvophyceae*, Order *Ulvales*, Family *Ulvaceae*) which was first identified by Linnaeus in 1753 [15].

Systemic acquired resistance (SAR) or induction of resistance to pathogen is a promising approach for controlling potato late blight disease. Exogenous or endogenous factors could substantially affect host physiology, leading to rapid and coordinated defense-gene activation in plants normally expressing susceptibility to pathogen infection [16]. This phenomenon, that resistance of plant to pathogens can be enhanced by the application of various biotic and biotic agents, called induce systemic resistance in plants [17], [18], [19]. Elicitors are molecules known to trigger plant defense responses against pathogens. Marine algae could be an interesting source of active molecules since numerous algal

elicitors have been identified, most notably polysaccharide. Extracts of the green alga *Ulva* were shown to contain new elicitor-active compounds called ulvans, [20-25]. Ulvans are complex sulphatedheteropolysaccharides mainly composed of rhamnose, xylose and uronic acids [25-29]. More recently, a crude extract prepared from the green macroalga *Ulva armoricana* was found to protect plants against fungal diseases [30-32]. In addition, ulvans induced protection against a broad range of pathogens in plants by stimulating defense responses and activate SAR [30], [33], [34]. The extract from the seaweed showed enhanced activities of various defense-related enzymes including chitinase, B-1,3-glucanase, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, and lipoxygenase. [35-38]. In addition, there are many biochemical changes in SAR-protected plants that then become infected. Large increase in phenolic synthesis in plants after attack by plant pathogens was recorded by [39], [40], [10]. Also, there are some evidences indicating that the activation of polyphenol oxidase plays a crucial role in resistance of plant to pathogenic attack [10], [41-44]. RNases may have diverse functions in plants, including protection against pathogens' attack and the extent of the resistance correlated with the level of ribonuclease activity. A decrease in the ribonuclease activity was accompanied by an increase in the susceptibility to infection [45-47].

The objective of this research was to determine the efficacy of green alga (*Ulva lactuca*) extract and some Commercial algae products for management of late blight disease of two varieties of potatoes (Lady pulford and Burren) under field conditions. Also,we investigated the ability of these algae to elicit induced resistance in potato against late blight disease caused by *Phytophthora infestans, besides,* their effects on biochemical changes which lead to induction of plant defense against the pathogen.

2. MATERIALS AND METHODS

2.1.Fungicidal activity of *Ulva lactuca* (water extract) and commercial products of macro algae on *phytopthora infestnas,*the causal agent of potato late blight disease.

2.1.1. Collection and identification of algal material

Samples of *Ulva lactuca* (green alga) were collected monthly from September 2012 to September 2013 from Abu-Qir and El-Kalaa of Alexandria. Samples were air dried under shade for two weeks and ground. The alga was identified according to [48].

a. Water extract

100g of powdered seaweed was mixed with distilled water in the ratio of 1:10 ratio and autoclaved at 150 lb pressure for 1hour. Then the extract was concentrated by rotary evaporator at 60ºc, and taken as 100% seaweed concentrate (SWC) as described by [49].

b. Commercial products

Algifol is concentrated from brown algae, *Ascophyllum nodosum* (Chema Industries products made in Egypt), **Cytokan-s** is concentrated from algae, *Ascophyllum nodosum* and *Fhylum Phaophyta*. Cytokan-S (Salquisa made in Spain), **Start-S** It's an extract of *Ascophyllum nodosum*. (Spainsh Salquisa Company).

2.1.2. Antifungal activity technique

The antifungal activity of alga extracts and commercial algae products on *phytopthora infestnas*were tested using the radial growth technique method [50]. Appropriate volumes of the stock solutions of the algae extracts in distilled water were added to molten nutrient agar (potato dextrose agar Medium, PDA) to obtain range of concentrations (100, 200, 250, 500, 1000, 1500, 2000, 4000 and 8000 µg ml^{-1}) immediately before pouring into the Petri dishes (9.0 cm in diameter) at 40-45ºC. Each concentration was tested in triplicate. Parallel control was maintained. The discs of mycelial felt (0.5 cm diameter) of the plant pathogenic fungi, taken from 8-day-old cultures on PDA plates, were transferred aseptically to the centre of Petri dishes. The treatments were incubated at 25ºC in the dark. Colony growth diameter was measured after the fungal growth in the control treatments had completely covered the Petri dishes. Percentage of mycelial growth inhibition was calculated from the formula: Mycelial growth inhibition = [(DC-DT) /DC] × 100 [51], where DC and DT are average diameters of fungal colony of control and treatment, respectively. The concentration of extract that inhibits the fungi mycelial growth by 50% (EC_{50}) was determined by a linear regression method [52].

2.2..Efficacy of green algae extract and commercial algae products on Disease Severity Index (DSI) and foliage protection percentage (FPP) against potato late blight.

The experiment was designed to study the efficacy of algae extracts either water extracts of green alga with three concentrations or commercial alga products besides Diathane M-45 as fungicide reference with their recommended rates (Table A) on Late blight disease caused by *Phytophthora infestans* in potato (*Solanum tuberosum, L.*). Field experiments were conducted at the Research station of Faculty of Agriculture Alexandria University, during successive growing season of winter 2014/2015 on potato. Potato tubers were planted in October 15th 2014. Field soil texture was sandy clay. Within the variety trial, two varieties were chosen: Burren (B) and Lady Pulford (LP). These varieties have been recently introduced to Egypt as promising varieties for potato production. These varieties are all considered to be suitable for manufacturing. A split-plot design with four replications was used. Plots of four rows and 6m long (24m²) with four replications in complete randomized block design were used. Seven treatments were used. Algae extracts solutions were prepared by dissolving definite amount of the chemicals in definite quantity of plain water. Spray was initiated just after

the detection of late blight symptoms in the experimental area and repeated four times at an interval of 7-14 days. Care was taken during sprayboth the upper and lower surface of leaves and stems was covered by algae extracts solution. Spray tank was thoroughly washed before filling algae extracts solution materials. All plots received traditional agricultural practices such as irrigation and fertilization.

Table (A): names, active ingredients and application rate examined treatments.

Treatments	Active ingredient(s)	Rate/100 L water
Control	-	-
Diathane M 45	*Mancozeb*	250gm
Cytokan-s	*Ascophyllum nodosum and Fhylum phaophyta*	100ml
Start-s	*Ascophyllum nodosum*	100gm
Algifol	*Ascophyllum nodosum*	100ml
Algae extract(1)	*Ulva lactuca (1000PPM)*	100gm
Algae extract(2)	*Ulva lactuca (2000PPM)*	200gm
Algae extract(3)	*Ulva lactuca (4000PPM)*	400gm

Disease severity Index (DSI) and foliage protection percentage (FPP).

Disease severity index (DSI) was recorded by estimating the leaf lesions on a scale from 0 to 4 suggested by [53], **DSI = ∑ ((n*c)/N*df)** Where DSI. = Disease Intensity, n = Number of infected leaves per category, c = Category number and N = Total number of leaves and df= degree of freedom. Foliage protection percentage (FPP) was calculated {**FPP (%) = 100 (1- x/y)}** Where, x and y are disease severity values for treated and control plants, respectively, as also described by [54]. Each value of DSI or FPP calculated as a mean from four sprays during the growing season.

2.3.Effect on some parameters related to plant defense against late blight disease.

There are many biochemical parameters which lead to induction of plant defense against the pathogen, total soluble phenol content, protein content, poly phenol oxidase activity and ribonuclease activity are established that they had a great role in the induction plant defense against plant disease.

2.3.1 Total Soluble Phenol Content: Total soluble phenol content of potato leaves, was extracted with methanol 80% according to [55]. **µg tannic acid /gm fresh weight = (((A/K)*(20/0.2)/1)),** Where: A= absorbance at 765 nm, K= the extension coefficient =0.016898 µg-¹

2.3.2. Total protein assay: Total protein was determined according to method described by [56], with slight modification proposed by [57].**mg protein/gm fresh weight = ((O/K)*100)/0.25),** K (BSA protein) = 0.029 mg/ml, O = The absorption at595nm.

2.3.3.Polyphenol oxidase (PPO) activity: Polyphenol oxidase activity was determined according to [58]. **% Activity = ((A1/A2)*100),** A1, A2: Absorbance of the treatment and control samples at 575 nm

2.3.4. Ribonuclase activity (RNase): Ribonuclase (RNase) was extracted anddetermined according to [59]. **% Activity= ((A1/A2)*100),** A1, A2 : Absorbance of the treatment and control samples at 260 nm.

All the results were submitted to statistical processing using an analysis ofvariance (SPSS 14 software Package) and differences between meanswere compared by the Duncan's test at p = 0.05, [60]

3. RESULTS DISCUSSION

3.1.Fungicidal activity of *Ulva lactuca* (water extract) and commercial products of macro algae on *phytopthora infestnas,* the causal agent of potato late blight disease.

The antifungal activities of Water extract of *Ulva lactuca*, Algaefol, Strat S, and Cytokan in terms of radial growth inhibition are summarized in Table(1). Water extract of *Ulva lactuca* exhibited the strongest antifungal effect against *Phytophthora infestan* with EC50 value 1382.1 µg ml^{-1}. While Strat S and Algifol had a moderate antifungal activity with EC_{50} values of 1565 and 2113.8µg ml^{-1}, respectively. Cytokan ranked last in the antifungal activity against *Phytophthora infestan* (the causal agent of potato late blight disease) with EC_{50} values of 4863.4µg ml^{-1}. Our results in this point were supported by the results of [25], [38] and [61].

Table (1) Fungicidal activity of water green alga extract and commercial products of macroalgae against *Phytopthora infestnas*.

Treatments	EC_{50} (µg ml^{-1})	95% confidence limits		Slope
		Upper	Lower	
Ulva water extract	1144.3	1670.5	1382.1	1.6
Algifol	1831.0	2440.4	2113.8	1.9
Start –S	1311.6	1867.4	1565.0	1.4
Cytocan-S	4272.4	5536.9	4863.4	2.8

3.2.Efficacy of green algae extract and commercial algae products on Disease Severity index (DSI)and foliage protection percentage (FPP) against potato late blight disease.

Effect of the used seven treatments and untreated control on the late blight disease as disease severity index (DSI) and foliage protection percentage (FPP) of the two varieties of potatoes were shown in tables (2&3). It was clear that all the treatments, Cytokan, Start-S, Algifol, Ulva Extract and Diathane M-45 treatments significantly decreased the DSI of the late blight disease and the most effective one was Algifol with a mean value of disease severity index (DSI) 0.82 with no significant values with other treatments. It is notable that the fungicide treatment was less influential in terms of reduction in DSI with a value of 1.42. Given the varieties, it was obviously clear that there was not a significant difference in the response of them towards the disease. Concerning to the sprays, continued reduction moral clear and obvious until the third spray fell this effect in the fourth one.

The foliage protection percentage in burren ranged from 62.2 to 76% while it ranged from 48.2 to 76.4% for Lady Pulford..

Our results were compatible with [62] since they found that The commercial algae products Bio-Algeen S 90 and Kelpak SL decreased the late blight severity on potato plants as compared with the infection rates noted in the control treatment; [31], [32] who found that the green macroalga *Ulva armoricana* protected bean, grapevine and cucumber against powdery mildew reductions in disease severity control up to 90%; [20], [22] since brown algae (Phaeophyta) have shown to be effectiveness in controlling grapevine plant from fungus *Plasmopara viticola* diseases.

Table (2): Efficacy of green algae extract and commercial algae products on Disease Severity Index (DSI) of potato varieties.

Treatments	Disease severity index (DSI)						
	Burren	Lady	1st	2nd	3rd	4th spray	Mean
Control	3.41	3.48	1.64	1.7	2.49	7.94	3.44^{A}
Cytokan-S	1.17	1.11	0.10	0.00	0.00	4.47	1.14^{C}
Start-S	1.18	1.46	0.36	0.00	0.23	4.69	1.31^{BC}
Algifol	0.82	0.82	0.58	0.00	0.31	2.39	0.82^{BC}
Ulva Extract 1000ppm	1.18	1.02	0.50	0.00	0.00	3.90	1.09^{BC}
Ulva Extract 2000ppm	0.96	1.13	0.41	0.00	0.16	3.615	1.05^{BC}
Ulva Extract 4000ppm	1.29	0.98	0.61	0.00	0.155	3.78	1.14^{BC}
Diathane M-45	1.04	1.80	0.16	0.125	0.575	4.83	1.42^{B}
Mean	1.38^{A}	1.47^{A}	0.61^{B}	0.26^{B}	0.55^{B}	5.01^{A}	
						LSD (general) = 0.74	

Different letters indicate significant differences among treatments according to least significant difference test (P=0.05).

Table (3): Efficacy of green algae extract and commercial algae productson Foliage Protection Percentage (FPP) of potato varieties.

Treatments	Foliage protection percentage (FPP) %						
	Burren	Lady	1st spray	2nd	3rd	4th	Mean
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cytokan-S	65.6	68.0	94.2	100.0	100.0	43.7	66.8
Start-S	65.5	58.0	78.4	100.0	90.7	41.0	61.7
Algifol	76.0	76.4	64.8	100.0	87.6	69.9	76.2
Ulva Extract 1000ppm	65.5	70.6	69.7	100.0	100.0	50.9	68.1
Ulva Extract 2000ppm	71.8	67.6	75.1	100.0	93.8	54.5	69.7
Ulva Extract 4000ppm	62.2	71.7	63.2	100.0	93.8	52.4	67.0
Diathane M-45	69.5	48.2	90.6	92.6	76.9	39.2	58.9
Mean	59.6	58.04	67.00	86.58	80.35	43.95	

Different letters indicate significant differences among treatments according to least significant difference test (P=0.05).

3.3.Effect on some parameters related to plant defense against potato late blight disease.

3.3.1. Effect of on total soluble phenol of potato leaves.

Many plant phenolic compounds are known to be antimicrobial, function as precursors to structural polymers such as lignin, or serve as signal molecules [63]. Significant increase in phenolic content was positively proportional to the degree of plant resistance against the pathogens, [64]. Folair Application of green alga extractand commercial products of algae stimulated the formation of total soluble phenols to not only reach but also exceed their content in healthy control. Results in Table (4) showed that total soluble phenols markedly increased in leaves of all treated potato plants, especially the treatment of Ulva extract which gave a content of total soluble phenol 2044.1 ug / gm fresh weight comparing to the control treatment which gave 1589.5ug.

It was obviously that the Lady Pulford variety had high content of polyphenol of the potato leaves as a response of all treatments more than the variety Burren. It was not surprise that the variety lady pulford had high content of polyphenols than Burrren variety, since it was consistent with being the most tolerant to disease.

Our results were in agreement with [38], [65-67] who demonstrated that seaweed treatments caused higher levels and early accumulation of soluble and bound phenols in plant leaves.

Table (4): Effect of green alga (*Ulva lactuca*) extracts and commercial algae products on Total Soluble Phenol Contentas µg tannic acid /gm fresh weight of potato leaves.

	Total Soluble Phenol (µg tannic acid /gm fr.wt.)						
Treatments	Burren	Lady	1st spray	2nd spray	3rd spray	4th spray	Mean
Control	1430.47	1748.5	1052.27	1473.37	2166.67	1665.68	1589.5^{C}
Cytokan-S	1485.21	1990.6	1125.25	1532.54	2169.63	2124.26	1737.9^{BC}
Start-S	1698.22	2270.7	1335.31	2157.79	2262.33	2182.45	1984.5^{A}
Algifol	1616.86	2255.4	1146.94	2031.56	2268.24	2297.83	1936.1^{AB}
Ulva Extract 1000ppm	1646.94	2198.2	1770.22	1843.20	2099.61	1977.32	1922.6^{AB}
Ulva Extract 2000ppm	1674.56	2413.71	1415.19	2041.42	2461.54	2258.38	2044.1^{A}
Ulva Extract 4000ppm	1799.80	2045.36	1013.81	2533.53	2050.30	2092.70	1922.6^{AB}
Diathane M-45	2055.23	2236.69	1068.05	2152.86	2264.30	3098.62	2145.9^{A}
Mean	1675.9^{B}	2145^{A}	1240.9^{C}	1970.8^{B}	2217.8^{A}	2212^{A}	

Different letters indicate significant differences among treatments according to least significant difference test (P=0.05).

3.3.2. Effect on Protein content of potato leaves.

The effect of different treatments of the tested green alga extract and commercial algae products on total soluble protein content in potato leaves was determined after 7days from the treatment. The results were recorded in table (5). It was represented as mg protein /gm fresh weight of potato leaves.

From these results, it was clear that levels of total soluble protein significantly increased in potato plants treated with green alga extract and commercial algae products, especially the treatment of green alga extract at the concentration 4000, 1000 ppm and the commercial alga Cytokan-S since values of protein contents were 1995.98, 1819.54 and 1695.98 mg / gm potato leaves, respectively. It is notable that these treatments increased the protein content of potato leaves even more than the standard fungicide Diathane M-45 which gave 1256.90 mg protein/gm fresh weight of potato leaves. For the varieties we found that the variety Burren outweigh the other variety Lady Pulford in the content of protein and increased excellence in all treatments where the overall average protein content was 2232 in the first one and 990.7 mg for the second one. Our results were agreement with [68] who found that positive relation between foliar application of brown seaweed and total protein content. The importance of total soluble proteins to plant defense has been related to: (a) their rapid and early accumulation often associated with incompatibility, (b) their antimicrobial activity and (c) their ability to reduce symptoms development [69], [70]. So constitutive expression of genes encoding PRPs is one of the strategies proposed to obtain a broad and durable level of resistance against different phytopathogenic fungi [71].

Table (5): Effect of green alga (*Ulva lactuca*) extracts and commercial algae products on protein content as mg protein/gm fresh weight of potato leaves.

	protein content as mg protein/gm fresh weight potato leaves						
Treatments	Burren	Lady	1st spray	2nd spray	3rd spray	4th spray	Mean
Control	1739.08	737.93	1459.77	1781.61	1011.49	701.15	1238.51^{C}
Cytokan-S	2365.52	1026.44	1728.74	2549.43	1278.16	1227.59	1695.98^{A}
Start-S	2251.72	997.70	1560.92	2114.94	1393.10	1429.89	1624.71^{ABC}
Algifol	2139.08	1212.64	2340.23	2133.33	1174.71	1055.17	1675.86^{AB}
Ulva Extract 1000ppm	2441.38	1197.70	2050.57	2583.91	1457.47	1186.21	1819.54^{A}
Ulva Extract 2000ppm	2236.78	929.89	1503.45	2236.78	1560.92	1032.18	1583.33^{ABC}
Ulva Extract 4000ppm	2985.06	1006.90	1774.71	2388.51	1365.52	2455.17	1995.98^{A}
Diathane M-45	1697.70	816.09	1793.10	1255.17	1314.94	664.37	1256.90^{B}
Mean	2232^{A}	990.7^{B}	1776.4^{B}	2130.5^{A}	1319.5^{C}	1219.0^{C}	1611.35
				LSD (General) = 430.5			

Different letters indicate significant differences among treatments according to least significant difference test (P=0.05).

3.3.3. Effect on Polyphenol oxidase activity (PPO) of potato leaves.

Polyphenol oxidases are involved in the oxidation of polyphenols into quinones (antimicrobial compounds) and lignification of plant cells during the microbial invasion. There are some evidences indicating that the activation of polyphenol oxidase plays a crucial role in resistance of plant to pathogenic attack [41], [42], [43], [44], [72], [73].

Data of table (6) showed that all the treatments of green alga extract and commercial algae products significantly increased the activity of polyphenol oxidase (PPO) in the two varieties of potato leaves compared with the control treatment during the four sparys, the highest percentage of activation reached to nearly 40% and it was the share of the treatment of Cytokan-S and Strat-S in the Burren variety as a mean of the four sprays. The effect of Ulva extract was clearly in lady pulford variety. Within the varieties, there is significant difference in the response of PPO activity in lady pulford and Burren. The Burren variety was higher activation of PPO than lady pulford variety. Our results were agreement with [36], [38] who found that the extract from the brown seaweed *Ascophyllum nodosum* enhanced activities of various defense-related enzymes especially peroxidase, polyphenol oxidase. PPOs have frequently been suggested to participate in plant defense against pests and pathogens [74].

Table (6): Effect of green alga (*Ulva lactuca*) extracts and commercial algae products on Polyphenol oxidase activity of potato leaves.

	PPO Activity %						
Treatments	Burren	Lady	1st spray	2nd spray	3rd spray	4th spray	Mean
Control	100.00	100.00	100.00	100.00	100.00	100.00	100.00^{B}
Cytokan-S	142.30	119.47	137.77	129.96	137.82	118.00	130.89^{A}
Start-S	139.61	121.56	143.21	125.81	131.93	121.40	130.59^{A}
Algifol	133.94	119.32	126.52	129.83	129.24	120.94	126.63^{A}
Ulva Extract 1000ppm	130.10	130.24	129.88	140.51	132.67	117.63	130.17^{A}
Ulva Extract 2000ppm	127.84	137.23	135.71	138.30	139.32	116.81	132.53^{A}
Ulva Extract 4000ppm	132.54	130.78	136.48	121.72	145.68	122.76	131.66^{A}
Diathane M-45	150.32	106.64	116.51	139.83	129.79	127.79	128.48^{A}
Mean	132.08^{A}	120.65^{B}	128.259^{A}	128.245^{A}	130.80^{A}	118.167^{B}	
				LSD (General) = 13.9			

Different letters indicate significant differences among treatments according to least significant difference test (P=0.05).

3.3.4. Effect on Ribonuclase activity (RNase) of potato leaves.

RNases may have diverse functions inplants, including protection against pathogens' attack and the extent of the resistance correlated with the level of ribonuclease activity, a decrease in the ribonuclease activity was accompanied by an increase in the susceptibility to infection [45], [46], [47], [75].

Results of the effect of the foliar application of the treatment on Ribonuclase activity of potato leaves were recorded in table (7) and showed that all the treatments of green alga extract and commercial algae products significantly increased the activity RNase of potato leaves. Ulva extract treatment was more effective in increasing the activity of the enzyme especially in the variety Burren with activation rate 43.71% followed Aglifol, Cytokan-S and Strat-S with activation rate 36.13, 24.32 and11.11% . concerning to the variety lady pulford the activation of RNase was increased significantly also in all treatments and Ulva extract still ranked the first with activation rate 35.92%. In general, the activaty of RNase of potato leaves was increased in all treatments and the order of this activity as mean of two varieties during the four sparys was the ulva extract, algifol ,cytokan-s and strat-s with rate 28.19, 21.73 , 19.29 and 12%.

Table (7): Effect of green alga (*Ulva lactuca*) extracts and commercial algae products on Ribonuclase activity (RNase) of potato leaves.

	RNase Activity %						
Treatments	Burren	Lady	1st spray	2nd spray	3rd spray	4th spray	Mean
Control	100.00	100.00	100.00	100.00	100.00	100.00	100.00^{D}
Cytokan-S	124.32	122.47	111.92	122.47	118.57	124.19	119.29^{BC}
Start-S	111.19	117.55	106.44	117.55	102.87	121.12	112.00^{C}
Algifol	136.13	120.74	114.35	120.74	135.12	116.69	121.73^{AB}
Ulva Extract 1000ppm	138.81	135.92	127.02	135.92	112.57	117.37	123.22^{AB}
Ulva Extract 2000ppm	143.71	129.29	126.60	129.29	132.18	124.68	128.19^{A}
Ulva Extract 4000ppm	125.72	121.13	116.61	121.13	123.95	120.41	120.53^{AB}
Diathane M-45	137.21	125.76	117.27	125.76	127.11	109.84	120.00^{ABC}
Mean	127.134^{A}	121.6^{A}	115.02^{B}	121.6^{A}	119.04^{AB}	116.78^{AB}	
LSD	LSD (General) = 23.03						

Different letters indicate significant differences among treatments according to least significant difference test (P=0.05).

It could be seen from the correlation relationship between the disease severity index and the four physiological parameters of the two potatoes varieties of the subject of this study as a result of the used seven treatments (table B). The values of correlation factor (r) confirmed the results obtained previously since we noticed a negative correlation between the disease severity and the content of total soluble polyphenols , this correlation reached to 50% in Burren variety while it was 67% in the variety Lady pulford. Negative correlation between the disease severity index and total protein was found also and the correlation reached to 80% in the variety Lady pulford. The same trend was also noticed in PPO and RNase. So, Lady Pulford is more tolerant to the disease than Burren. Finally, we could say the use of algae extract might help to overcome the pathogen infection by increasing levels of defense-related enzymes and phenolic and protein substances.

Table (B): correlation factor of Disease Severity index and the tested physiological parameters.

Parameters	Correlation Factor		
	Burren	Lady Pulford	Mean
Total Soluble Phenols	-0.50	-0.67	-0.67
Total Protein	-0.38	-0.80	-0.65
PPO	-0.85	-0.79	-0.95
RNase	-0.78	-0.73	-0.89

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