

EFFICACY OF CERTAIN ALGAE EXTRACTS TO CONTROL DRY ROT OF POTATO

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ABSTRACT

Two tested acetone algae extracts of *Spirulina platensis* and *Chlorella vulgaris* in the present study significantly decreased radial growth (colony diameter) of the potato dry rot fungus, *Fusarium sambucinum* (isolate, F.s. 2), grown on PDA amended with the tested algae extracts, 7 days after inoculation and incubation at 25 °C. This effect increased with increasing the concentration where 160 mg/ml of any of the tested extracts completely (100%) inhibited the fungus growth. Meanwhile, treatments with the two tested acetone algae extracts (as dipping or coating in gelatinous substance) significantly decreased dry rot disease severity on the inoculated potato with F.s. 2 under cold storage conditions ($7\pm 2^{\circ}\text{C}$) compared to the untreated inoculated control and the effect increased with increasing the concentration from 160mg/ml to 200 mg/ml. Also, coating tubers with the tested algae extracts was even more effective to decrease dry rot disease severity during storage. *Spirulina* extract treatments consistently decreased the developed dry rot severity compared to *Chlorella* treatments. This effect of algae extracts may be explained in view that the algae extracts treatments were accompanied with significant

increase in total phenols as well as polyphenol oxidase and peroxidase activity in the treated tubers and also, this effect was accompanied with significant control for the tuber weight loss (%). This study clearly demonstrated that blue green algae such *Spirulina platensis* and *Chlorella vulgaris* can be a potential source of antifungal compounds useful in agriculture and plant diseases control.

Keywords: Potato Dry rot, *Fusarium sambucinum*, algae extracts, *Spirulina platensis*, *Chlorella vulgaris*

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INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important economic crops worldwide, which plays an important role in the Egyptian agriculture (Abo-El-Seoud *et al.*, 2010; Awad *et al.*, 2020; Tiwari *et al.*, 2020). Egypt has been considered one of the largest producers and exporters of potato in Africa (Abd-Elgawad and Youssef 2008). Currently, potato cultivation and consumption are increasing and spreading in many parts of the world, especially in the developing countries. Potato is the fourth most important crop after wheat, rice and maize in the global economy (Marie *et al.*, 2012; Cunnington, 2008). Unfortunately, potato cultivation in Egypt is subjected to several plant diseases where *Fusarium* dry rot is considered a major problem in the field and during storage.

Potato dry rot is a devastating fungal disease caused by *Fusarium* in Egypt and worldwide. Soil and seed-borne inoculum can affect the plants in the field but the main damage occurs during storage. Dry rot affects the crop stand by inhibiting the development of potato sprouts and cause losses up to 25% with infection as high as 60% during storage.

Worldwide, more than 13 *Fusarium* species are causing dry rot disease in potato. *Fusarium sambucinum* is considered the most aggressive pathogen for this disease in major parts of Europe, China and North America. Sometimes, *Fusarium oxysporum* is the most common fungus causing dry rot while *F. sambucinum* is the most aggressive. In Egypt, *Fusarium* dry rot is one of the most important diseases of potato (*Solanum tuberosum* L.), affecting the tubers in storage and the seed pieces after planting. *Fusarium sambucinum* and *F. solani* are common pathogens causing dry rot of stored tubers in Egypt and temperate areas, heavily infected tubers become shriveled and mummified (Wale *et al.*, 2008; Abo-El-Seoud *et al.*, 2010; Awad *et al.*, 2020; Tiwari *et al.*, 2020).

Several methods were proposed to control dry rot during storage where potato tubers were badly affected even under fridge conditions. The usage of algae and their extracts with their broad range of biological activities, including antifungal and antibacterial were found to provide alternative ecofriendly alternative methods for plant diseases control that could be used as a natural fungicide due to its strong antagonistic effects against multiple pathogens (Oranday *et al.*, 2004; Yang *et al.*, 2006; Latique *et al.*, 2014; Al-ghanayem 2017; El-Sheekh *et al.*, 2020; Vehapi *et al.*, 2020; Pourakbar *et al.*, 2021)

Therefore, aim of the present work was to study the efficacy of certain algae extracts (*Chlorella vulgaris*, *Spirulina platensis*) as alternative ecofriendly strategies for controlling potato post-harvest dry rot disease as pre-storage tuber treatments.

MATERIALS AND METHODS

The tested potato dry rot fungus

Culture of the tested isolate, in the present study, is a highly pathogenic dry rot isolate of the causal fungus, *Fusarium sambucinum*

(isolate, F.s.2), was obtained from the Plant Pathology Dept. culture, Faculty of Agriculture, Damanhour University.

The tested algae extracts

Two algae, *i.e.* *Spirulina platensis* and *Chlorella vulgaris*, were obtained as powder from Algal Biotechnology Unit, Fertilization Technology Department, National Research Centre, Cairo, Egypt and their acetone extracts were prepared according to **Hlima et al. (2019)** by stirring 100 g of freeze-dried alga with 300 mL of acetone for 48h at 30°C in the dark, then filtration through sterile Whatman paper N1, and the extract was concentrated in a rotary evaporator until drying. All dry residues were quantified and separately dissolved into 1 mL of Dimethyl sulfoxide (DMSO) and kept at +4 °C until use.

1. In vitro effects of algae extracts to control dry rot fungus

Three concentrations (*i.e.*, 40,80 and 160 mg/ml) according to previous study (**Ammar et al., 2017**) for each alga extract solutions were tested for their efficacy in inhibiting mycelial growth of *Fusarium sambucinum* where algae acetone extracts were added to PDA medium in petri-dishes before solidification. Inoculation was carried out using fungal 5mm-diameter discs, three replicates were used for each tested concentration. Petri-dishes were kept at 20±2 °C. Sterilized water free was served as control. When mycelial growth covered the entire surface in control plates, radial growth was determined for each concentration according to **Mostafa et al. (2022)** by the following formula:

$$\% \text{ inhibition} = (dc - dt) / dc \times 100,$$

where dc is the average increase in control hyphal growth, and dt is the average increase in growth in treated mycelium.

2-Effect of algae acetone extracts on dry rot disease severity

According to **Ammar et al. (2017)**, good looking potato tubers were surface disinfested with 70% ethyl alcohol, wounded (3 - mm depth) using a sterile cork bore, inoculated with 100 μ L of 10^7 conidia /ml (in sterile distilled water, harvested from 7-day-old cultures), and left for six hours under room temperature . Then, the inoculated tubers were treated with acetone algae extracts (*Spirulina platensis* or *Chlorella vulgaris*) by dipping the tubers in different concentrations (160 mg/ml & 200 mg/ml), or coating tubers in gelatinous substance containing the acetone algae extract in the previous concentrations , while control tubers were inoculated only with the pathogen.

Tuber coating was prepared according to **Mabrouk et al. (2019)** by dissolving 2 g pectin in 100 ml of distilled water, and glycerol (0.5 g/g of pectin) and calcium chloride (0.01g/g of pectin) were added to the suspension. The suspension was then heated to 70°C under stirring, until all the solids were dissolved and a homogeneous suspension was achieved. The suspension was cooled to 40°C, then the alga extract was added to the tested concentrations.

All potato tubers were incubated for 60 days at $7\pm 2^\circ\text{C}$ and RH = 90% in a fridge. At the end of the incubation period, tubers were rated with the developed dry rot according to **De Cal et al. (1995)** as follows: 1= no dry rot symptoms, 2= dry rot covers $\leq 25\%$ of the tuber diameter, 3= dry rot covers $>25\% - \leq 50\%$ of the tuber diameter, 4= dry rot covers $> 50\% \leq 75\%$ of the tuber diameter, and 5= dry rot covers $> 75\%$ of the tuber diameter. Then, Fusarium dry rot disease severity index (DSI) was determined according to **De Cal et al. (1995)** using the following formula:

$$\text{DSI} = \frac{\sum (\text{rating number} \times \text{number of plants in the rating})}{(\text{Total number of plants} \times \text{highest rating})} \times 100$$

3. Chemical changes in potato tubers inoculated with *F. sambucinum*, treated with algae extracts, and stored under cold conditions

At the end of the storage experiment, 60 days after inoculation and algae treatments, 5g sample of each treated tuber, adjacent to the developed rot was take for the determination of total phenols, and the oxidative enzymes activities (peroxidase and polyphenol oxidase).

3.1. Total phenols content determination.

This was determined by the method of **Fu *et al.* (2010)** and **Burgos *et al.* (2013)**. An aliquot of 0.2 ml was taken and mixed with 1.0 ml of the Folin-Ciocalteu reagent 1:10 and 0.8 ml of 7.5% NaCO₃, followed by stirring. After 30 mins of rest in the dark, the spectrophotometer reading at 760 nm was performed, using gallic acid as standard and results were expressed as µg gallic acid / g fresh tissue.

3.2. Determination of activity of the oxidative enzymes

Tuber samples were used for Peroxidase and PPO activities determination by extraction method performed according to **Tan (2015)**, then, enzymes activity were determined in the obtained extract.

3.2.1 Polyphenol oxidase activity determination

Polyphenol oxidase (PPO) activity was determined based on the intensity of dark-coloured polymeric compounds formed by oxidation of catechol. The PPO reaction mixture contained 4 ml of 0.5% catechol solution and 200 µl of enzymatic extract. Absorbance was read at 400 nm, 60s and 180 s after the addition of extracts. The activity was calculated and expressed as the absorbance min/g fw according to **Trabelsi *et al.* (2020)**.

3.2.2. Peroxidase activity determination

Peroxidase (POX) activity was recorded by following the appearance of the brown coloration resulting from guaiacol oxidation in the presence of hydrogen peroxide. The POX reaction mixture contained 2 ml of 0.1 M (PPB) at pH 6.5, 50 μ l of 0.02 M guaiacol and 200 μ l of 30% H₂O₂. Absorbance increase was noted with a spectrophotometer at 470 nm, 120 s after the addition of extracts. Peroxidase activity was then expressed as absorbance min./g fw according to **Trabelsi *et al.* (2020)**.

4. Effect on tuber loss of weight

Losses in potato tubers fresh weight (LW) percentage were estimated in the inoculated and non-inoculated potato tubers for all treatments according to (Naffa, 2012) by the following formula:

$LW (\%) = \frac{\text{Initial weight} - \text{weight of potato tubers at sampling date}}{\text{Initial weight of potato tubers}} \times 100$

RESULTS

1-The *in vitro* effect of algae extracts to inhibit potato dry rot fungus

It is evident from Table (1) and Fig. (1) that tested algae extracts (*Spirulina* and *Chlorella*) efficiently decreased radial growth (colony diameter) of potato dry rot fungus, *F. sambucinum* (isolate, F.s.2), grown on PDA amended with the tested algae extracts, 7 days after inoculation and incubation at 25 °C. This effect, meanwhile, increased with increasing the concentration where 160 mg/ml of any of the tested extracts completely (100%) inhibited the fungus growth (Table 1).

Table1: The *in vitro* percentage of inhibition of acetone algae extracts (*Spirulina platensis* and *Chlorella vulgaris*) at different concentrations on radial hyphal growth (colony diameter) of *F. sambucinum* (isolate, F.s. 2) grown on amended PDA medium, 7 days after inoculation and incubation at 25 ±2°C.

Conc. (mg/ml)	Spirulina extract		Chlorella extract		Mean	
	R.G. (cm)	Inh.%	R.G. (cm)	Inh.%	R.G. (cm)	Inh.%
40	2.81 ^b	68.67 ^c	3.26 ^b	63.67 ^c	3.04 ^B	66.17 ^c
80	0.39 ^c	95.67 ^b	0.78 ^c	91.33 ^b	0.59 ^C	89.00 ^B
160	0.00 ^d	100.00 ^a	0.00 ^d	100.00 ^a	0.00 ^D	100.00 ^A
control	9.00 ^a	0.00 ^d	9.00 ^a	0.00 ^d	9.00 ^A	0.00 ^D
Mean		66.08 ^A		63.75 ^B		

Values or means followed by different letter for each single parameter are significantly different at p= 0.05. , R.G. = Radial growth, Inh.% = Inhibition %.

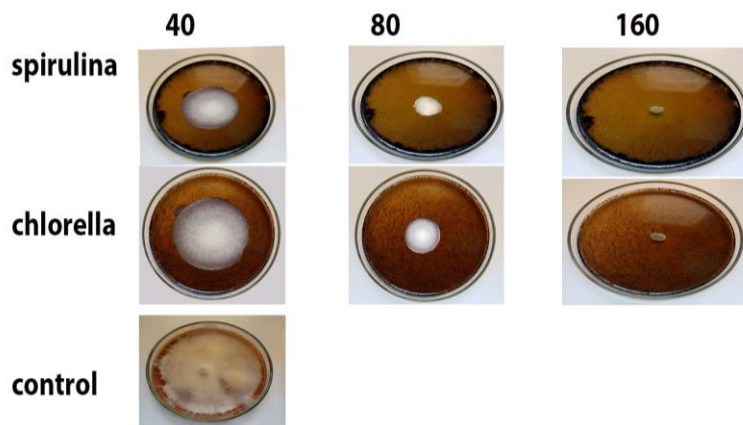


Fig.1: The *in vitro* inhibition effect of acetone algae extracts (*Spirulina platensis* and *Chlorella vulgaris*) at different concentrations (40, 80 and 160 mg/ml) on radial hyphal growth (colony diameter) of *F. sambucinum* (isolate, F.s. 2)

grown on amended PDA medium, 7 days after inoculation and incubation at 25 ±2°C.

2-Effect of algae extracts on dry rot disease severity

Healthy mature potato tubers of the susceptible cv. Cara were inoculated with conidial suspension of aggressive *F. sambucinum* (isolate, F.s.2) then, were singly dipped or coated in the *in vitro* most effective concentration of the above tested algae extracts (160mg/ml and 200 mg/ml) and stored for 60days at 7±2°C in darkness. It is evident from Table (2) and Fig. (2) that the tested algae extract obviously decreased dry rot developed on the inoculated tubers during storage. Meanwhile, data in Table (2) exhibited that the tow tested algae extracts significantly decreased dry rot disease severity on the inoculated potato under storage conditions compared to the untreated inoculated control and the effect increased with increasing the concentration from 160mg/ml to 200 mg/ml. Meanwhile, coating tubers with the tested algae extracts was even more effective to decrease dry rot disease severity during storage. Also, Spirulina extract treatments consistently decreased the developed dry rot severity compared to Chlorella treatments.

Table 2: Disease severity (%) of dry rot on potato, cv .Cara, inoculated with *Fusarium sambucinum*, isolate F.s. 2, and then treated with acetone algae extracts (*Spirulina platensis* and *Chlorella vulgaris*) and stored for 60 days at 7±2°C in darkness.

Treatment		160 mg/ml	200 mg/ml	Overall mean
Spirulina	Dipping	16.67 ^c	4.67 ^{bc}	9.5 B
	Coating	14.67 ^d	2.00 ^d	
Chlorella	Dipping	19.33 ^b	5.33 ^b	11.33 A
	Coating	17.33 ^{bc}	3.33 ^{cd}	
Control (inoculated & untreated)		99.33 ^a		99.33 A

Values or means followed by different letters for each single parameter are significantly different at p= 0.05.

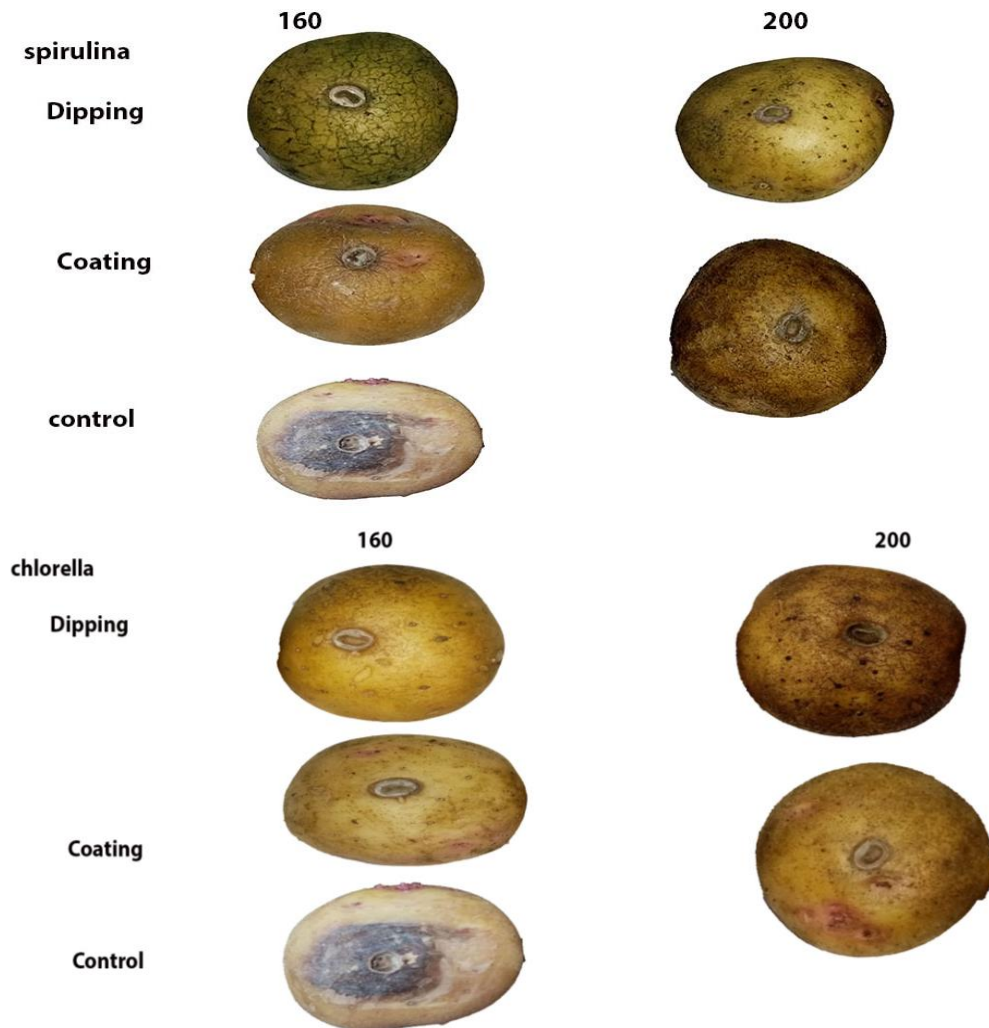


Fig.2: Disease severity of dry rot disease on potato, cv .Cara, inoculated with *Fusarium sambucinum* , isolate F.s. 2, and then treated with acetone algae extracts (*Spirulina platensis* and *Chlorella vulgaris*) 160 and 200 mg/ml and stored for 60 days at $7\pm 2^{\circ}\text{C}$ in darkness.

3- Chemical changes in potato tubers inoculated with *F. sambucinum*, treated with algae extracts, and stored under cold conditions

Data in Table (3) showed that treatment of the inoculated (*Fusarium sambucinum*, isolate F.s. 2) potato tubers (cv. Cara) with the tested algae extracts (200 mg/ml) significantly increased total phenols as well as polyphenol oxidase and peroxidase activity compared to the untreated inoculated control for both the dipping and the coating treatments. Meanwhile, Spirulina treatments consistently showed values higher than that of Chlorella treatments.

Meanwhile, Fig. (3) showed strong negative correlations between the chemical changes. *i.e.* total phenols, polyphenol oxidase activity and peroxidase activity (induced by the acetone algae extract treatments) and dry rot disease severity (%) on potato tubers after cold storage conditions for 60 days. Correlation values were $r = -0.821$, $r = -0.707$, and $r = -0.774$, for the previous chemical parameters, respectively.

Table3: chemical changes in potato tubers (cv.Cara) inoculated with *Fusarium sambucinum* (isolate F.s. 2) and then treated with acetone algae extracts (*Spirulina platensis* and *Chlorella vulgaris*) and stored for 60 days at $7\pm 2^{\circ}\text{C}$ in darkness.

Treatment		Total Phenols (mg/g/fw)	Polyphenol oxidase (Abs./min ₂ /g fw)	Peroxidase (Abs./min ₂ /g fw)
Spirulina	Dipping	13.60 b	0.50 b	0.35 b
	Coating	15.30 a	0.63 a	0.47 a
Chlorella	Dipping	11.00 d	0.23 d	0.22 c
	Coating	12.67 c	0.33 cd	0.27 bc
Control (inoculated untreated)		6.50 e	0.02 e	0.00 d

Values or means followed by different letters for each single parameter are significantly different at $p= 0.05$.

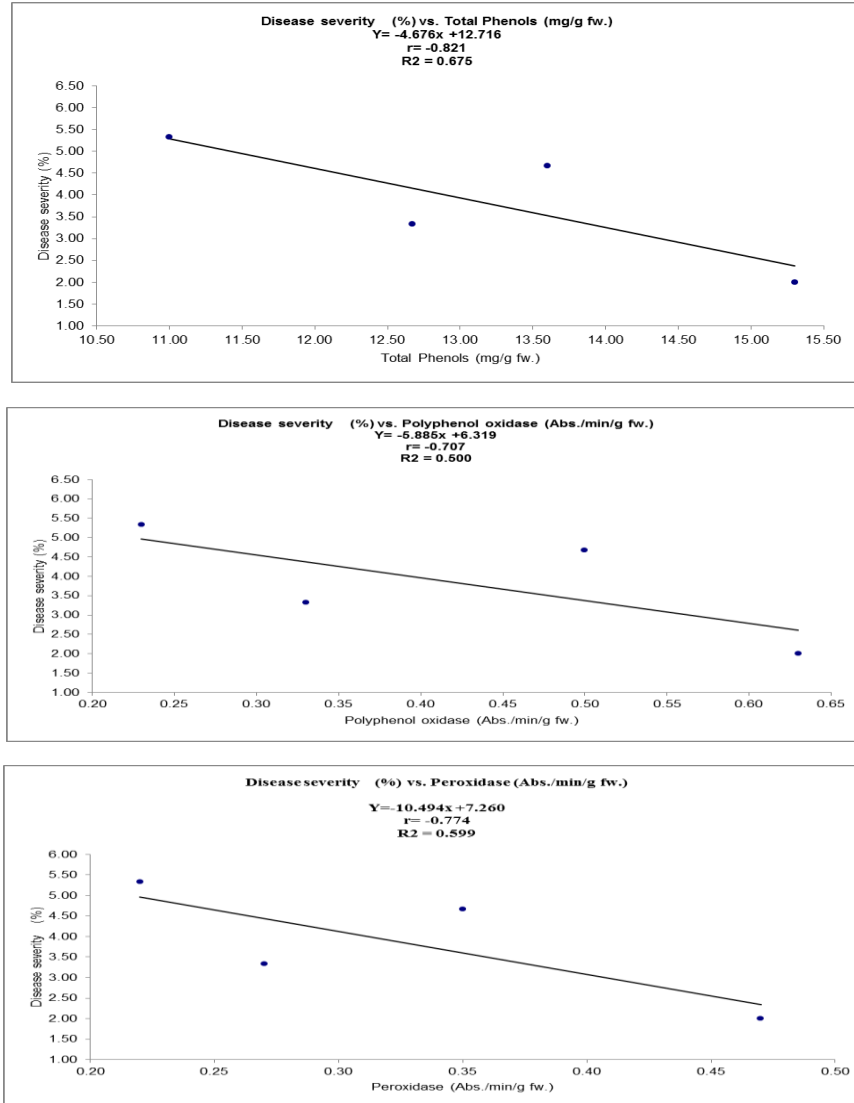


Fig. 3: Correlation between disease severity of dry rot on potato and total phenol content, peroxidase activity, and polyphenol oxidase activity in potato

tubers, cv . Cara, inoculated with *Fusarium sambucinum* , isolate F.s. 2, treated with acetone algae extracts (*Spirulina platensis* and *Chlorella vulgaris*) at 200 mg/ml, and stored for 60 days at 7±2°C in darkness.

4- Effect on tuber loss of weight

Data tabulated in Table (4) exhibited that tuber treatments with the two tested algae extracts significantly controlled tuber weight loss (%) compared to the inoculated untreated control. The overall weight loss with *Spirulina* and *Chlorella* were as low as 5.20%, and 6.86% compared to 23.67% for the inoculated untreated control over the two types of treatments as dipping or coating extract treatments.

Table 4: Effect of algae extracts (*Spirulina platensis* and *Chlorella vulgaris*) at two concentrations on physical characteristics (% loss of weight) of potato tubers (cv. Cara) inoculated with *F. sambucinum* (isolate, 2), treated, and stored for 60 days at 7±2°C in darkness.

Treatment		160 mg/ml	200 mg/ml	Overall mean
Spirulina	Dipping	7.38 ^b	4.58 ^b	5.20 B
	Coating	5.37 ^b	3.50 ^b	
Chlorella	Dipping	8.00 ^b	7.23 ^b	6.86 B
	Coating	6.80 ^b	5.43 ^b	
Control (inoculated untreated)		23.67 ^a		23.67 A

Values or means followed by different letters for each single parameter are significantly different at p= 0.05.

DISCUSSION

Furasium dry rot is one of the most important postharvest diseases of potato tubers occurring worldwide. It infects tubers in storage, as well as tuber pieces underground after planting. Losses attributed to dry rot in storage ranged from 6 to 25% with up to 60% of tubers affected in some cases (**Abo El-Seoud et al., 2010**).

In the present study the two tested acetone algae extracts (*Spirulina platensis* and *Chlorella vulgaris*) significantly decreased radial growth (colony diameter) of potato dry rot fungus, *F. sambucinum* (isolate, F.s.2), grown on PDA amended with the tested algae extracts, 7 days after inoculation and incubation at 25 °C. This effect, meanwhile, increased with increasing the concentration where 160 mg/ml of any of the tested extracts completely (100%) inhibited the fungus growth.

Meanwhile, the two tested algae extracts significantly decreased dry rot disease severity on the inoculated potato stored under cold $7\pm 2^{\circ}\text{C}$ conditions compared to the untreated inoculated control and the effect increased with increasing the concentration from 160 mg/ml to 200 mg/ml. Also, coating tubers with the tested algae extracts was even more effective to decreased dry rot disease severity during storage. *Spirulina* extract treatments consistently decreased the developed dry rot severity compared to *Chlorella* treatments. These results are in agreement with the findings of **Trabelsi and Chérif (2009)** who exhibited that production and accumulation of phenolic compounds were detected at higher amounts in potato tubers treated with some abiotic agents and these phenols seem to be responsible to the reduction of fungal growth and dry rot development.

Also, **Ahmed et al. (2016)** showed that all the treatments of green alga extract and commercial algae products against late blight disease significantly increased the activity of polyphenol oxidase (PPO) in the

two varieties of potato leaves compared with the control treatment. This effect of algae extracts may be explained in view that the algae extracts treatments, in the present study, were accompanied with significant increase in total phenols as well as polyphenol oxidase and peroxidase activity in the treated tubers compared to the untreated inoculated control for both the dipping and the coating treatments. There are some evidence indicating that the activation of polyphenol oxidase plays an important role in resistance of plant to pathogenic attack (**Cherif *et al.*, 2007; Hassan *et al.*, 2007; Ahmed, 2010**) by involving in the oxidation of polyphenols into quinones, lignification of plant cells during the microbial invasion (**Sheze *et al.*, 2008**) or serving as signal molecules (**Hammerschmidt *et al.*, 2005**). Also, high negative correlations ($r = -0.821$, $r = -0.707$ and $r = -0.774$) were revealed between diseases severity and total phenol content, peroxidase activity and polyphenol oxidase activity, respectively, in the treated potato tubers.

The effect of the algae extracts can be explained in view that algae considerably contain a wide mix of bioactive compounds with synergetic effect which are considered to enhance plant performance and resilience that demonstrate a successful application of microalgae as biostimulant and biocontrol agent that could being used as not only biopesticide but also biostimulant to enhance the natural defense response in plants (**Chanda *et al.*, 2019; Kim *et al.*, 2020**). Also, this effect was accompanied with significant control for the tuber weight loss (%) as the overall weight loss with *Spirolina* and *Chlorella* were as low as 5.20%, and 6.86% compared to 23.67% for the inoculated untreated control over the two types of treatments as dipping or coating extract treatments. These findings are in harmony with several investigators (**Stevenson *et al.*, 2001; Desjardins, 2006; El-Hassan *et al.*, 2007; Leslie and Summerell,**

2008; Ammar *et al.*, 2017; Al-Nazwani *et al.*, 2021; Perveen *et al.*, 2022).

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الملخص العربي

فعالية بعض مستخلصات الطحالب في مكافحة العفن الجاف في البطاطس

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تم دراسة مدى فاعلية إثنين من المستخلصات الأسيوتونية لطحلي سبيرولينا بلانتيسيس , كلوريلافولجارس بتركيزات 40, 80, 160 مجم/مل على فطر فيوزاريوم سامبسنيوم (عزله فيوزاريوم 2) المسببه للعفن الجاف لدرنات البطاطس. أظهرت النتائج إنخفاض ملحوظ في النمو الشعاعي (قطر المستعمره) للفطر على بيئة آجار البطاطس المضاف إليها مستخلص أى من الطحليين وذلك بعد سبعة أيام من التلقيح بالفطر والتحصين على درجة 25 درجة مئوية. كما لوحظ زيادة التأثير التثبيطي مع زيادة تركيز المستخلص حيث أدى تركيز 160 مجم/مل لكلا الطحليين إلى تثبيط كامل لنمو الفطر. كما أدت معاملة درنات البطاطس صنف كارا بالمستخلصات غمرا أو تغليفا بماده جيلاتينية والسابق تلقيحها بالفطر إلى خفض معنوى لشدة الإصابة بالعفن الجاف تحت ظروف التخزين البارد (7 ± 2 م °) مقارنة بمعاملة الكنترول المعده وغير المعاملة وكان التأثير أكثر وضوحا مع تركيز 200 مجم/مل عن 160 مجم/مل. أظهرت النتائج أيضا أن طريقة تغليف الدرنات بمستخلص أى من الطحليين أكثر فاعلية عن طريقة غمر الدرنات في تقليل شدة مرض العفن الجاف أثناء التخزين. كما أن معاملات مستخلص طحلب سبيرولينا أكثر فاعلية من مستخلص كلوريلافولجارس. أظهرت النتائج زيادة معنوية في محتوى الدرنات المعاملة من الفينولات الكلية ونشاط إنزيمي بولى فينول أوكسيديز و بيروكسيديز. كما لوحظت علاقة إيجابية معنوية بين المعاملة بأى من المستخلصين المختبرين وإنخفاض في نقص وزن الدرنات (%), بصورة عامة أظهرت الدراسة أن إستخدام مستخلصات هذين الطحليين ممكن أن تكون مصدرا جيدا لتقليل الأثر الضار لفطر فيوزاريوم سامبسنيوم المسبب للعفن الجاف لدرنات البطاطس وكذلك تحسين الإنتاجية كما ونوعا.