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In vitro and *in vivo* antifungal activity of microalgal treatments against *Alternaria brassicicola*

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Abstract

Commercial preparations of three algal species (*Arthrospira platensis, Chlorella vulgaris,* and *C. pyrenoidosa)* were evaluated according to *in vitro* (paper disc method), and *in vivo* (seed, foliar, seed+foliar treatments) assays to determine the antifungal activity against *Alternia brassicicola.* For *in vitro* assays, the extracts of *A. platensis, C. pyrenoidosa,* and *C. vulgaris* showed the highest antifungal activity, inhibiting the growth of *A. brassicicola* at a concentration of 50 mg/ml with inhibition zones of 4.9, 4.2, and 3.9 cm, respectively. For *in vivo* assays, the microalgal suspensions were observed to have remarkable antifungal activity at increasing concentrations against *A. brassicicola* compared with control treatments. Particularly, the seed+foliar treatment of a mixture of the microalgal suspension (*A. platensis*+ *C.vulgaris*+ *C. pyrenoidosa*) at a concentration of 15 g/l demonstrated the highest antifungal activity with an inhibition rate above 98% in cabbage (*Brassica oleracea*) and mustard (*Brassica juncea*). The present study confirmed that the microalgae treatments had a significant potential, as an applicable and eco-friendly tool against *A. brassicicola,* to reduce exposure and risks of chemical pesticides.

Keywords: *Alternaria brassiciola,* cabbage, mustard, microalgae, antifungal activity.

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1. Introduction

The diseases caused by plant pathogenic bacteria and fungi give rise to remarkable losses in crop yield and unavoidable product damages worldwide (Avelino *et al.*, 2015). *Brassica* spp. are broadly cultivated major vegetable crops, which include many cultivars distributed throughout the world. The production of Brassica vegetables is affected by the presence of various plant pathogens which cause especially foliar diseases and huge economic losses. Alternaria black leaf spot caused by *Alternaria brassicicola* is the most destructive disease of *Brassica* spp. worldwide and widespread in many countries (Meena *et al.*, 2016; Kumar *et al.*, 2014; Reis and Boiteux, 2010). Typical disease symptoms include black necrotic lesions encircled by chlorotic areas on leaves, seedlings, stems, and siliquae causing a serious decrease in yield quantity and the production of high-quality seeds (Ahmad and Ashraf, 2016; Saharan *et al.*, 2016; Iacomi-Vasilescu *et al.* 2004). Brassica vegetables could be affected at all developmental stages. The pathogen could remain alive for several years in crop residues and could be disseminated from the sources of incoculum to neighbor fields or long distances (Yadav *et al.*, 2014; Kohl *et al.*, 2010). Recently, use of chemical pesticides against plant diseases is considered as the most effective method. The control of *A. brassicicola* is suppressed using several different families of fungicides including benzimidazoles, carbamates, dicarboximides, and triazoles as seed or foliar treatments in many countries. However, chemical pesticides, which are used to control *A. brassicicola,* can be easily absorbed by soil, causing pollution of food crops and a toxic effect on non-target populations (Satapute *et al.*, 2019; Fox *et al.*, 2007;). Besides, emergence of resistant strains of the pathogen as a result of long-term use of pesticides is a serious problem and another disadvantage. So, there is a rising demand to accelerate and improve new management strategies to ensure better disease control. Studies of the use of natural origin preparations on pest control have carried a great importance because of unconscious and overuse of chemical pesticides. In this manner, the microalgae-based products including *Arthrospira* spp. and *Chlorella* spp. can be a feasible alternative in plant disease management (Ronga *et al.*, 2019). The antimicrobial activity of microalgae has been associated with bioactive compounds belonging to different chemical groups such as terpenes, phytohormones, phenols, fatty acids, *etc*. (Singh *et al.*, 2017). Previous studies indicated that *Chlorella pyrenoidosa* (Abd Elhafiz *et al.*, 2015), *Chlorella vulgaris* (Dineshkumar *et al.*, 2019; Özdemir *et al.*, 2016), and *Arthrospira platensis* (Dineshkumar *et al.*, 2019; Anitha *et al.*, 2016) include a broad range of bioactive compounds, which could improve the growth and yield potential of crop plants in overcoming pathogenic attack and could activate plant defense mechanism that is characterized as induced resistance (IR) in a wide range of cultivated plants (Calvo *et al.* 2014; Khan *et al.* 2009). Although many commercial products obtained from microalgae were reported against several phytopathogenic fungi such as *Aspergillus niger*, *Aspergillus flavus*, *Cercospora beticola*, *Sclerotinia sclerotiorum*, *S. minor*, *Sclerotium rolfsii*, *Fusarium oxysporum*, *F. roseum*, *F. solani*, *F. verticillioides*, *Penicillium expansum*, *Rhizoctonia solani*, *Alternaria dauci*, *A. solani, Verticillium albo-atrum*, *etc*. (Vehapi *et al.*, 2020; Al-ghanayem, 2017; Abdel-Kader and El-Mougy, 2013), they have received little attention as potential agents for plant diseases. The present study aimed to evaluate the antifungal activity of *Arthrospira platensis, Chlorella vulgaris,* and *Chlorella pyrenoidosa*, applied at certain concentrations, against *Alternaria brassicicola* in cabbage and mustard plants.

2. Materials and methods

2.1 Material

In the present study, three algal species in the form of a dry powder (*Arthrospira platensis,*

Chlorella vulgaris, and *Chlorella pyrenoidosa*) were purchased as 100% pure and assured commercial preparations. *A. brassicicola* was isolated from the infected samples showing black spot symptoms on leaves in commercial fields. Potato dextrose agar (PDA) medium was used for isolation and cultivation of the pathogen. The seeds of the cabbage (*Brassica oleracea*) and mustard (*Brassica juncea*) were purchased and used to determine antifungal activity of the microalgae treatments against *A. brassicicola*. The study was maintained *in vitro* and *in vivo* assays (foliar, seed, seed + foliar treatments) at 5 different concentrations. *In vivo* experiments were performed using 30 samples of the susceptible cabbage and mustard seeds per polyethylene pot (25 × 20 cm diameter) containing 150 g sterile peat.

2.2 Isolation, identification and pathogenicity of the fungal isolates

The seed samples were disinfected by 1% sodium hypochlorite solution (SHS) for 5 min and rinsed 3 times with sterile distilled water (SDW). After drying process on sterile filter paper, the seeds were placed in PDA plates and incubated at 25°C for 7 days. After incubation period, the fungal isolates were purified by a single spore technique and kept at -10°C throughout the study. The purified isolates were identified according to colony appearance, conidial morphology, microscopic and pathological properties (Aneja *et al.*, 2014; Bessadat *et al.*, 2014). The pathogenicity of 3 isolates of *A. brassicicola* was pre-assessed on the basis of diseased leaf area on cabbage and mustard cotyledons. The fungal isolates were applied to cotyledons 1 week after sowing; the plants were kept moist for 48 h and grown under plant growth room conditions (at 25 ºC, 16 hrs of photoperiod) for 4-5 days and then disease symptoms were observed to detect the most pathogenic fungal isolate for *in vitro* and *in vivo* experiments (Kubota *et al.*, 2006).

2.3 Determination of the antifungal activity of the microalgae extracts *in vitro*

After the dried microalgae powders were extracted overnight in methanol (Starr *et al.*, 1962), they were tested using standard paper disc method. Six mm diameter discs were prepared using sterile Whatman No.1 filter paper. The discs were saturated with 20 µl of microalgae extracts (*Arthrospira platensis, Chlorella vulgaris*, and *Chlorella pyrenoidosa*) at different concentrations (10 mg/ml, 20 mg/ml, 30 mg/ml, 40 mg/ml, and 50 mg/ml) to evaluate their antifungal activity against *A. brassicicola*. The disks were placed in the center of the agar surface containing a pathogen inoculum at a concentration of 1×10^5 conidia/ml. After an incubation period at 25°C for 7 days, the diameter of inhibition zones formed around the paper disc was measured to determine the antifungal activity as a result of the average of 5 independent replicates. The microalgae-free PDA medium, containing only SDW and a culture disk of the pathogen, was used as a control.

2.4 Determination of *in vivo* antifungal activity of the microalgae extracts on Alternaria black leaf spot

To determine the antifungal activity of microalgae treatments against *A. brassicicola*, the suspensions of *A. platensis, C. vulgaris, C. pyrenoidosa,* and a mixture suspension of the microalgae (*A. platensis + C. vulgaris + C. pyrenoidosa*) were examined as the foliar, seed, and seed + foliar treatments at concentrations of 3 g/l, 6 g/l, 9 g/l, 12 g/l, and 15 g/l. The plant seeds were disinfected with a 1% SHS for 5 min and rinsed three times with SDW before the microalgae treatments. After

disinfection process, inoculated seeds of susceptible cabbage and mustard plants at a concentration of 1×10^5 conidia/ml were sown in experimental pots (22 × 15 cm diameter) containing a sterile peat and were grown in the plant growth room conditions. When plants reached 7-day-old after sowing, the microalgal suspensions were sprayed as a foliar treatment separately to upper and lower surfaces of cabbage and mustard cotyledons with a dose-adjusted spray. After microalgae treatments were allowed to dry on cotyledons (approximately 1-2 h), the treated plants were kept under polyethylene bags for 48 h and then moved to a plant growth room (Sabry *et al.*, 2015). To determine the antifungal activity of the seed treatments against Alternaria black leaf spot in cabbage and mustard plants, the seeds were immersed for 10 min in microalgal suspensions at different concentrations and allowed to dry overnight. The following day, the pathogen inoculum at a concentration of 1×10^5 conidia/ml was applied to the seeds before sowing in pots. The pots were watered and placed in a growth room (Amein *et al.*, 2011). In addition to that, seed + foliar treatment of microalgae suspensions was examined to evaluate the antifungal activity according to the same procedure as seed and foliar treatments. The cabbage and mustard seeds were immersed for 10 min in microalgal suspensions at different concentrations and allowed to dry overnight. Afterwards, the pathogen inoculum was applied to the seeds before sowing in pots. When the seedlings reached 7-day-old after sowing, the microalgal suspensions were sprayed onto cotyledons as a foliar treatment. After allowing to dry on cotyledons for 1-2 h, the treated plants were incubated under growth room conditions. The experiment was conducted in a plant growth room under a 16 h photoperiod cycle at 25 ºC with an average of 5 independent replicates. The microalgae-free pots, containing only SDW and a spore suspension of the pathogen (1×10^5 conidia/ml), were used as a control. The disease severity on cabbage and mustard plants was determined after 3 weeks following inoculation process. The disease severity on seedlings was rated and assessed according to percentage of diseased cotyledons using 0 to 5 scale (0 = no disease, 1 = 1-10%; 2 = 11-25%; 3 = 26-50%; 4 = 51-75%; 5 = > 76%) (Verma and Saharan, 1994). The disease severity (DS) was calculated by using the formula given by El-Morsi *et al.* (2009):

$$D.S\ (\%) = (\Sigma\ (n \times v)/\ N \times V) \times 100$$

Where: DS=Disease severity, n = Number of diseased cotyledons in each category, v = Numerical value of each category, N = Total number of assessed cotyledons, V = Maximum numerical value.

The effect of the microalgae treatments on *Alternaria brassicicola* was evaluated on the basis of disease severity using the following formula (Topps and Wain, 1957):

$$I\ \%= [(C-T/C)] \times 100$$

Where: I % = Inhibition rate, C = Disease severity in control treatment, T = Disease severity after microalgae treatments.

2.5 Statistical analysis

The obtained data were statistically analyzed by ANOVA (one-way analysis of variance). Significant differences ($p<0.05$) between the means were evaluated by using the Duncan's Multiple Range Test (DMRT) for the diameter of inhibition zone of the microalgae extracts and for disease severity of the pathogenic isolate after *in vivo* assays.

3. Results

The extracts of *Arthrospira platensis, Chlorella pyrenoidosa,* and *Chlorella vulgaris* demonstrated the highest antifungal activity, inhibiting the growth of *A. brassicicola* at a concentration of 50 mg/ml with inhibition zones of 4.9, 4.2 and 3.9 cm, respectively. The lowest antifungal activity was observed at a concentration of 10 mg/ml against pathogen with inhibition zones of 1.1, 1.6 and 2.2 cm for *C. vulgaris*, C. *pyrenoidosa,* and *A. platensis,* respectively under *in vitro* conditions (Figure 1). Namely, the increment of the inhibition zones was observed at increasing concentrations compared to lower concentrations (Table1).

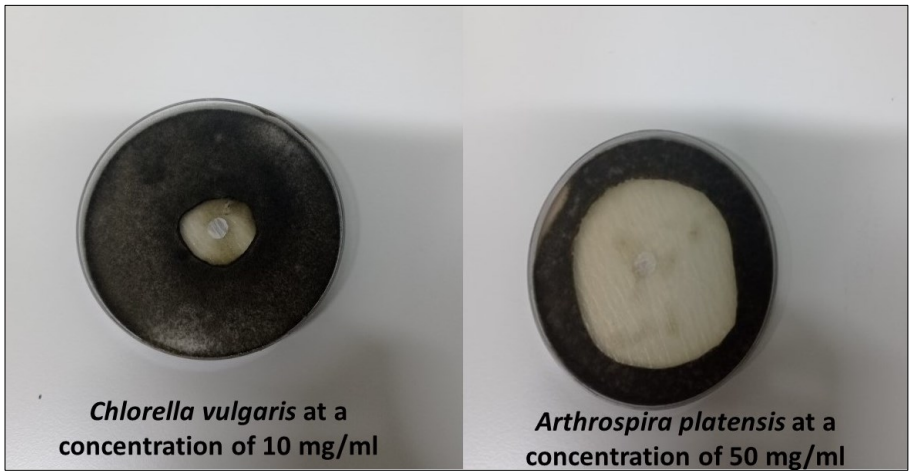

Figure 1: Effect of different concentrations of the microalgae extracts against *A. brassicicola* after *in vitro* treatments.

Table 1: Effect of different concentrations of the microalgae extracts (diameter of the inhibition zone) against *A. brassicicola*.

Concentrations (mg/ml)	Inhibition zone in diameter (cm)		
	A. platensis	*C. vulgaris*	*C. pyrenoidosa*
10	$2.2\pm1.21^{b*}$	$1.1\pm1.10^{a*}$	$1.6\pm0.93^{ab*}$
20	$2.7\pm1.03b^{c}$	1.8 ± 0.82^{ab}	2.1 ± 0.58^{abc}
30	3.6 ± 0.71^{c}	2.6 ± 0.63^{b}	2.9 ± 0.77^{b}
40	4.0 ± 0.50^{cd}	3.1 ± 0.56^{bc}	3.4 ± 0.41^{bc}
50	4.9 ± 0.36^{d}	3.9 ± 0.49^{c}	4.2 ± 0.28^{c}
Control	0.8 ± 1.60^{a}	0.8 ± 1.60^{a}	0.8 ± 1.60^{a}

*The concentration results are averaged on five replicates. Values given separately for *in vitro* assays within each column followed by different letters are significantly different at $p<0.05$.

Taking into account in vivo assays (seed, foliar, and seed + foliar treatments), the microalgal suspensions were generally found to have considerable antifungal activity at increasing concentrations against *A. brassicicola* compared with control treatments. For both cabbage and mustard, the seed + foliar treatment of *Arthrospira platensis*, *Chlorella pyrenoidosa*, and *Chlorella vulgaris* suspensions at a concentration of 15 g/l was found to have a strong antifungal activity with an inhibition rate above 89% (Table 2, 3 and 4). In particular, the seed + foliar treatment of a mixture of the microalgal suspension (*A. platensis* + *C. vulgaris* + *C. pyrenoidosa*) at a concentration of 15 g/l exhibited the highest antifungal activity,

suppressing growth of the pathogen with an inhibition rate above 98%, while foliar treatment of *C. vulgaris* at a concentration of 3 g/l was determined to have the lowest antifungal activity with an inhibition rate under 40.4% in cabbage and mustard plants (Table 3 and 5).

Table 2: Effect of different concentrations of *A. platensis* on Alternaria black leaf spot disease caused by *Alternaria brassicicola* in cabbage and mustard seedlings.

Treatment	Concentrations (g/l)	Cabbage Seedlings		Mustard Seedlings	
		Disease severity (%)	Inhibition rate (%)	Disease severity (%)	Inhibition rate (%)
Foliar treatment	3	47.4±2.31^{cd*}	50.8	53.2±1.99^{bc*}	46.4
	6	42.6±1.81^{c-f}	55.8	48.2±2.05^{b-e}	51.4
	9	29.6±0.71^{d}	69.2	46.8±1.52^{c}	52.8
	12	24.8±1.11^{d-f}	74.2	43.7±1.03^{c-f}	55.9
	15	22.5±0.63^{d-g}	76.6	41.8±1.44^{c-h}	57.9
Seed treatment	3	27.9±1.53^{de}	71.0	38.0±0.70^{d}	61.7
	6	24.2±0.66^{d-f}	74.8	34.6±0.53^{de}	65.1
	9	22.4±1.92^{d-g}	76.7	23.0±0.82^{d-g}	76.8
	12	19.9±0.80^{d-g}	79.3	20.4±1.67^{d-h}	79.4
	15	17.7±1.34^{e}	81.6	18.6±0.49^{e}	81.2
Seed + foliar treatment	3	26.0±2.51^{de}	73.0	20.1±0.81^{d-h}	79.7
	6	23.9±2.33^{d-f}	75.2	17.6±0.59^{ef}	82.2
	9	19.6±0.92^{d-g}	79.6	15.6±0.94^{e-g}	84.2
	12	16.0±1.20^{ef}	83.4	9.3±0.31^{f}	90.6
	15	9.3±0.45^{e-g}	90.3	7.2±0.23^{fg}	92.7
Control	-	96.4±3.22^{a}	-	99.3±3.90^{a}	-

*The concentration results are averaged on five replicates. Values given separately for *in vivo* assays within each column followed by different letters are significantly different at $p<0.05$.

Table 3: Effect of different concentrations of *C. vulgaris* on Alternaria black leaf spot disease caused by *Alternaria brassicicola* in cabbage and mustard seedlings.

Treatment	Concentrations (g/l)	Cabbage Seedlings		Mustard Seedlings	
		Disease severity (%)	Inhibition rate (%)	Disease severity (%)	Inhibition rate (%)
Foliar treatment	3	58.1±2.98^{b*}	39.7	59.2±3.12^{b*}	40.3
	6	53.5±2.01^{b-d}	44.5	57.2±2.35^{b}	42.3
	9	50.7±1.61^{b-e}	47.4	53.3±2.78^{bc}	46.3
	12	47.4±1.05^{cd}	50.8	51.9±1.98^{b-d}	47.7
	15	42.7±0.82^{c-f}	55.7	45.9±1.22^{cd}	53.7
Seed treatment	3	47.8±1.25^{cd}	50.4	52.1±2.05^{b-d}	47.5
	6	43.4±0.97^{c-f}	54.9	46.1±1.62^{c}	53.5
	9	41.0±2.25^{c-g}	57.4	44.2±1.20^{c-e}	55.4
	12	29.0±0.51^{d}	69.9	42.5±0.81^{c-g}	57.2
	15	27.2±0.64^{de}	71.7	29.6±0.76^{d-f}	70.1
Seed + foliar treatment	3	24.2±1.72^{d-f}	74.8	26.2±1.04^{d-f}	73.6
	6	21.3±0.91^{d-g}	77.9	23.6±1.57^{d-g}	76.2
	9	18.8±0.44^{e}	80.4	20.0±0.85^{d-h}	79.8
	12	10.1±0.68^{e-g}	89.5	16.3±0.99^{e-g}	83.5
	15	8.2±0.38^{e-g}	91.4	9.3±0.24^{f}	90.6
Control	-	96.4±3.22^{a}	-	99.3±3.90^{a}	-

*The concentration results are averaged on five replicates. Values given separately for *in vivo* assays within each column followed by different letters are significantly different at $p<0.05$.

Table 4: Effect of different concentrations of *C. pyrenoidosa* on Alternaria black leaf spot disease caused by *Alternaria brassicicola* in cabbage and mustard seedlings.

Treatment	Concentrations (g/l)	Cabbage Seedlings		Mustard Seedlings	
		Disease severity (%)	Inhibition rate (%)	Disease severity (%)	Inhibition rate (%)
Foliar treatment	3	56.0±2.25^{bc*}	41.9	45.8±3.01^{cd*}	53.8
	6	53.2±3.01^{b-d}	44.8	40.5±2.56^{c-h}	59.2
	9	48.2±2.19^{c}	50.0	29.8±0.74^{d-f}	69.9
	12	45.8±1.34^{c-e}	52.4	26.8±1.80^{d-f}	73.0
	15	42.7±1.77^{c-f}	55.7	24.1±0.45^{d-g}	75.7
Seed treatment	3	43.4±1.26^{c-f}	54.9	30.0±2.17^{d-f}	69.7
	6	40.6±0.94^{c-g}	57.8	27.2±2.02^{d-f}	72.6
	9	28.4±0.72^{d}	70.5	24.4±0.85^{d-g}	75.4
	12	26.1±0.55^{de}	72.9	21.8±1.43^{d-h}	78.0
	15	24.6±1.10^{d-f}	74.4	19.5±0.24^{d-h}	80.3
Seed + foliar treatment	3	23.2±0.97^{d-f}	75.9	28.0±1.03^{d-f}	71.8
	6	19.9±0.65^{d-g}	79.3	24.3±0.93^{d-g}	75.5
	9	16.4±0.99^{ef}	82.9	20.0±0.51^{d-h}	79.8
	12	9.4±0.26^{e-g}	90.2	17.2±1.19^{ef}	82.6
	15	7.7±0.18^{e-g}	92.0	10.0±0.73^{f}	89.9
Control	-	96.4±3.22^{a}	-	99.3±3.90^{a}	-

*The concentration results are averaged on five replicates. Values given separately for *in vivo* assays within each column followed by different letters are significantly different at $p<0.05$.

Table 5: Effect of different concentrations of *Arthrospira platensis* + *Chlorella vulgaris*+ *Chlorella pyrenoidosa* on Alternaria black leaf spot disease caused by *Alternaria brassicicola* in cabbage and mustard seedlings.

Treatment	Concentrations (g/l)	Cabbage Seedlings		Mustard Seedlings	
		Disease severity (%)	Inhibition rate (%)	Disease severity (%)	Inhibition rate (%)
Foliar treatment	3	29.6±1.69^{d*}	69.2	30.0±2.28^{d-f*}	69.7
	6	26.9±1.26^{de}	72.0	28.8±1.02^{d-f}	70.9
	9	24.4±0.97^{d-f}	74.6	26.4±1.21^{d-f}	73.4
	12	22.6±0.38^{d-g}	76.5	23.1±0.77^{d-g}	76.7
	15	19.6±0.24^{d-g}	79.6	26.6±0.94^{d-f}	73.2
Seed treatment	3	23.9±0.70^{d-f}	75.2	20.2±0.43^{d-h}	79.6
	6	20.8±1.88^{d-g}	78.4	23.9±0.52^{d-g}	75.9
	9	18.2±0.84^{e}	81.1	19.3±1.05^{d-h}	80.5
	12	10.1±1.06^{e-g}	89.5	16.0±0.90^{e-g}	83.8
	15	8.1±0.31^{e-g}	91.5	8.5±0.63^{fg}	91.4
Seed + foliar treatment	3	8.2±0.42^{e-g}	91.4	9.1±0.35^{f}	90.8
	6	6.6±0.79^{f}	93.1	7.9±0.44^{fg}	92.0
	9	4.5±0.56^{fg}	95.3	5.6±0.21^{g}	94.3
	12	1.7±0.11^{g}	98.2	1.9±0.10^{h}	98.0
	15	1.3±0.19^{g}	98.6	1.4±0.17^{h}	98.5
Control	-	96.4±3.22^{a}	-	99.3±3.90^{a}	-

*The concentration results are averaged on five replicates. Values given separately for *in vivo* assays within each column followed by different letters are significantly different at $p<0.05$.

4. Discussion

Although the microalgae such as *Arthrospira* spp. and *Chlorella* spp. are important providers of a wide array of various bioactive compounds, they have received little attention as potential antifungal agents against plant diseases. When taking into account microalgae treatments considerably reduced disease severity of *A. brassicicola* at elevated concentrations, the findings were in agreement with data obtained by a previous study, which indicated that blue-green algal commercial compounds had the potential for suppression of soil-borne fungi (*Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani*, *Alternaria*

solani, *Sclerotium rolfsii*, *Sclerotinia sclerotiorum*, and *S. minor*) and expanded the antagonistic ability of fungal, bacterial, and yeast bio-agents with increasing concentrations (Abdel-Kader and El-Mougy, 2013). Moreover, various species of *Chlorella* were reported to demonstrate a high antifungal activity against *Aspergillus niger*, *Alternaria alternata*, and *Penicillium expansum.* Particularly, *C. vulgaris* showed a strong antifungal activity against *A. alternata* and *P. expansum* with a decrease in mycelial growth rate at increasing concentrations (Vehapi *et al.*, 2020). In another study, a pre-treatment with *Chlorella fusca* suspension reduced anthracnose disease severity caused by *Colletotrichum orbiculare* and it was supposed that it could induce systemic acquired resistance (SAR) by activating defense responses of host cells in cucumber plants (Kim *et al.*, 2018). Lee *et al.* (2016) revealed that the conidia of *C. orbiculare* were banded by *C. fusca* cells, suppressing appressorium formation on cucumber plants. In addition to that, the disease severity of gray mold disease caused by *Botrytis squamosa* was reduced in Chinese chives by more than 24.2% with a treatment of *C. fusca*. Hence, it was suggested that *Chlorella* species might take a significant role in reducing disease severity of *A. brassicicola* by inhibiting appressorium formation or activating induced resistance mechanism as an elicitor on cabbage and mustard plants. Nevertheless, little information is available in literature about antifungal activity of *Spirulina* (*Arthrospira*) *platensis* against phytopathogenic fungi. In the present study, the results regarding to the antifungal activity of *Arthrospira platensis* are consistent with previous reports, informing that it suppressed mycelium growth or spore production of *Cercospora beticola, Fusarium oxysporum*, *Fusarium roseum*, *Botrytis cinerea*, *Aspergillus niger*, *A. flavus*, *Alternaria dauci*, A. *alternata*, and *Penicillium expansum* at increasing concentrations (Al-ghanayem, 2017; Cosoveanu *et al.*, 2010; Hussien *et al.*, 2009). It was considered that *Arthrospira platensis* might have an antifungal effect to disrupt the living structures of *A. brassicicola* or have an elicitor acvitiy to trigger plant defense responses, due to the presence of some bioactive compounds in its composition. Furthermore, the present study is the first report, remarking on that the seed+foliar treatment of a mixture of the microalgal suspension (*A. platensis* + *C. vulgaris* + *C. pyrenoidosa*) has maximum antifungal activity at a concentration of 15 g/l against *A. brassicicola*. In this regard, synergistic effect of the mixture suspension could lead to degrade the fungal cell wall and penetrate into the pathogen cell. Hence, it might cause the metabolic breakdown by preventing the synthesis of glucan, ergosterol, chitin, glucosamine, and proteins in pathogenic organism (Marino *et al.*, 2001). Besides, the mixture suspension could act as a plant growth stimulator by producing phytohormones like gibberellin, jasmonic acid, ethylene, auxin, cytokinin, and abscisic acid to promote plant defense, in addition to activating induced resistance mechanism as an elicitor. As a result of this study, it was concluded that the inhibitory activity of *A. platensis* and *Chlorella* spp. could be related to the amount and presence of bioactive compounds (e.g., phenolic compounds, phytohormones, fatty acids, terpenoids, saponins, alkoloids, sterols, sulfur-containing heterocyclic compounds, and carbohydrates etc.) in their chemical composition. We hope that microalgae-derived preparations will be applicable and environmentally friendly means that can be used instead of synthetic chemicals to control the black leaf spot caused by *A. brassicicola.*

5. Conclusion

Microalgal suspensions were found to be effective and applicable at high concentrations against *A. brassicicola* for *in vitro* and *in vivo* assays. It was considered that the microalgal suspensions suppressed the pathogen growth due to bioactive compounds in their composition. In spite of the fact that the obtained results are sufficient and promising, algal compounds having antifungal properties should be investigated to expand antifungal spectrum and optimize antifungal activity for efficient and satisfactory formulations. In this respect, the present study will encourage the use of algal preparations compared to other low-efficiency, toxic or destructive methods for the control of black leaf spot disease caused by *Alternaria brassicicola*.

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