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Biological Control of Faba Bean Pathogenic Fungi by Three Cyanobacterial Filtrates

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Abstract: The aim of the present study is to evaluate the biological control aptitude of the cyanobacteria, *Anabaena subcylindrica*, *Nostoc muscorum* and *Oscillatoria angusta* filtrates on the growth of the isolated pathogenic fungi from the different organs of Faba bean. Three caynobacterial (*Anabaena subcylindrica*, *Nostoc muscorum* and *Oscillatoria angusta*) filtrates were prepared in different concentrations and their effects on the isolated pathogenic fungi from leaves, stems and roots of Faba bean were studied. The study revealed high efficiency of the three algal filtrates on the control of the isolated pathogenic fungi from the three organs of Faba bean plants. The reduction in fungal mat growth diameter was greater than in that of the fungal dry weight showing inhibited fungal spread by greater rate. The reduction in the fungal dry weight was mostly linear and significantly correlated with the algal filtrate concentrations. The Efficient Algal Filtrate Concentration (EAFC) ranged between 104 and 461% for the three algal filtrates on the studied fungi dry weight. Complete control of the isolated fungi could be achieved by of a mixture of two algal filtrates in their EAFC and that of *Nostoc muscorum* + *Oscillatoria angusta* filtrates with an EAFC 368 and 194% were the best and economic mixture.

Key words: Faba bean, cyanobateria, algal filtrate, biological control, EAFC (efficient algal filtrate concentration)

INTRODUCTION

Fungicides have been used for a long period in control of many plant diseases (El-Helaly, 1950; Mansour and Kamel, 1975; Nassib and Tadross, 1976; Hanounik, 1981; Bernier *et al*., 1993). However, these chemical compounds may be phytotoxic to the plant. Therefore, use of these fungicides have to be scheduled precisely to attain the highest disease control and avoid or at least decrease the amount of the chemicals in the plant seeds which may affect human health (El-Morsy *et al*., 1996). On the other hand, fungicides pollute the environment, cause harmful effects to all living organisms and reduce the population of the useful micro-organisms in soil (Elad *et al*., 1980; Abdel-Moity and Shatla, 1981; Papavizas and Lewis, 1989; Khalifa, 1991; Khalifa *et al*., 1995; Lewis *et al*., 1996). Many investigators believed that fungicides of plant source are usually cheep, non-phytotoxic and easily biodegradable in the environment (Ibrahim *et al*., 1994; Sukul, 1994).

There have been many of successful uses of biological control agents (Henis *et al*., 1978; Rahhal, 1994; Xiaoqiang *et al*., 1997; Nassar *et al*., 1999). Algae, is one of the chief biological agents that have been studied for the control of plant pathogens, particularly soil borne fungi (Papavizas and Lumseden, 1980; Svian, 1987; Abdel-Kader, 1997; Hewedy *et al*., 2000). This due mainly to a number of cyanobacteria and eukaryotic algae, particularly macroalgae, produce various biologically active compounds, those could operate in biological control of plant pathogens (Kulik, 1995; Schlegel *et al*., 1998). These biologically active compounds includes antibiotics and toxins (de Caire *et al*., 1987, 1990; Bonjouklian *et al*., 1991; Carmichael, 1992; Frankmolle *et al*., 1992a, b; Kiviranta *et al*., 2006).

Although the cyanobacteria, which constitute the largest, most diverse and most widely distributed group of photosynthetic prokaryotes (Stanier and Cohen-Bazire, 1977), together with the eukaryotic algae make up of the world's biomass (Cannell, 1993), they have received little attention as potential bio-control agents of plant diseases. Yanni and Osman (1990) found that *Anabaena cylindrica*, *Anabaena oryzae*, *Nostoc muscorum* and *Tolypothrix tenuis* soil application reduced the incidence and severity of leaf and neck infection by *Pyricularia oryzae*, De Caire *et al*. (1990) reported that extra-cellular products from *Nostoc muscorum* are promising as a biological control of soybean seedlings damping off. Hagmann and Juttner (1996) showed that Fischerellin A, an active allelochemical compound produced by *Fischerella muscicola* caused a total inhibition of *Uromyces appendiculatus* on *Phasealus vulgaris* and *Erysiphe*

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graminis on barley at 250 and 1000 ppm, respectively. Also, 80% inhibition occurred with *Phytophthora infestans* on tomatoes and *Pericularia oryzae* on rice at 1000 ppm. Frankmolle *et al.* (1992 a and b) reported that crude ethanolic extracts from *Anabaena laxa* Rabenh inhibited the growth of *Aspergillus oryzae* and *Penicillum notatum*. He isolated and purified the fungicidal compounds and named them Laxaphycins A, B, C, D and E. Kulik (1995) mentioned that a greater chance of success might be attained with formulations of culture filtrates or cell extracts from cyanobacteria and algae applied to seeds as protectants against damping-off fungi such as *Fusarium* sp., *Pythium* sp. and *Rhizoctonia solani* or sprayed on leaves to protect them from pathogenic bacteria and fungi. Also, he reported that although cyanobacteria and algae are capable of producing substances *in vitro*, it still remains to be proven that these substances are produced in nature. Even if it is shown that cyanobacteria and algae do not produce antibacterial and antifungal substances *in vivo*, the substances produced by them *in vitro* may prove useful in controlling bacterial and fungal plant pathogens. Since the majority of species of cyanobacteria and algae are obligate photoautotrophs, they will not be able to grow below the surface of the soil in the vicinity of germinating seeds or plant roots. With additional research, it should be possible to develop thin film formulations of bactericidal and fungicidal cyanobacterial and algal products that would confer protection against soil borne pathogens that attack seeds and seedlings (Biondi *et al*., 2004; Khan *et al*., 2007).

MATERIALS AND METHODS

Experimental organisms:

Cyanobacteria: *Anabaena subcylindrica*, *Nostoc muscorum* and *Oscillatoria angusta* were obtained from the laboratory of Phycology, Department of Botany, Faculty of Science, Tanta University, Egypt.

Faba bean pathogenic fungi: The fungal flora which may be present in the internal tissues of leaves, stems and roots of faba bean were isolated from the collected diseased parts of the plant samples from the fields. These samples were thoroughly washed with tap water, dried between two sterilized filter papers, cut into small pieces, surface sterilized by dipping in 0.1% mercuric chloride solution for 5 min and then washed thoroughly with sterile tap water. Four pieces were plated in perti-dishes containing different media (PDA or LEFA medium). Plates were incubated at 28±2°C for 3-5 days. The purification of isolated fungi was by using either the single hyphal-tip method or the single spore isolation technique (Deverall, 1969) and the isolates were kept in slants for further studies. In order to prevent the probable bacterial contamination the antibiotic containing streptomycin (1 g LG^{1}) was incorporated in the medium, during purification. The colour and structure of the conidia and conidiophores of the purified isolates were examined by the use of the slide culture technique as outlined by Deverall (1969). Identification of fungal genera and species was according to Booth (1977), Domsch *et al.* (1980) and Sinclair and Backmann (1989).

Allen medium used for cyanobacteria: This medium consists of solution I and solution II. Solution I contains (g LG^{1}) 1.5 Na NO_3, 0.039 K_2HPO_4, 0.075 $MgSO_4.7H_2O$, 0.027 $CaCl_2.2H_2O$, 0.02 Na_2CO_3, 0.058 $Na_2SiO_3.9H_2O$, 0.006 ferric citrate, 0.006 citric acid and 0.001 EDTA. Solution II contains (g LG^{1}), 2.86 H_3PO_3, 1.81 $MnCl_2.4H_2O$, 0.222 $ZnSO_4.7H_2O$, 0.391 Na_2MoO_4. $2H_2O$, 0.019 $CuSO_4$. $5H_2O$ and 0.049 Co $(NO_3)_2$. $6H_2O$. The medium was prepared by adding 1 mL of solution II to 1 L of solution I and autoclaved at 15 pound per square inch, for 20 min. Twenty grams of agar were added to a liter of medium to become solid (Allen and Stanier, 1968).

Media used for fungi: 1) Potato Dextrose Agar Medium (PDA): It consists of 250 g of peeled potato and 20 g of dextrose then the volume was completed to 1 L with water. Twenty grams of agar were added to the medium for solidification. The medium was autoclaved at 15 pound per square inch for 20 min.

Leaves Extract of Faba bean Agar medium (LEFA): This medium was described by Omar *et al.* (1993) and consists of *Broad bean* leaves (250 g extracted with boiled water), 15 g sucrose, 30 g NaCl and 15 g agar and was made up to a liter and autoclaved at 15 pound per square inch for 20 min. This medium was used to isolate only *Botrytis fabae*.

Fungal growth measurements: 1- Linear growth method: The filtrates of the three cyanobacterial-isolates (*Anabaena subcylindeica*, *Nostoc muscorum* and *Oscillatoria angusta*) were taken under sterilized conditions by 0.2 µm filter (Syborn, Nalgen Co., Rochester, N Y). The filtrates were added to autoclaved PDA and LEFA media to give 0, 25, 50, 75% (v/v) algal filtrate concentrations. Three Petri dishes (9 cm) containing 15 mL of each algal filtrate for every treatment were prepared. The cultures were inoculated with 2 mm. disc from 7 days-old PDA and LEFA fungal cultures. Plates containing PDA and LEFA media without algal

filtrates were used as control. The plates were incubated at 25±2°C for 3 days. The growth of fungi was measured by determining the mean of colony growth diameters (Cobb *et al*., 1968).

Dry weight method: Potato dextrose agar medium (PDA) was used for studying the effect of algal filtrates on the dry weight of different isolates of fungi, except for *Botrytis fabae* leaves extract of Broad bean (LEFA) was used. Concentrations 0, 25, 50, 75 and 90% of each *Anabaena subcylindeica*, *Nostoc muscorum* and *Oscillatoria angusta* filtrates were prepared and 100 mL of each concentration in 250 mL Erlenmeyer flask was inoculated with 5 mm discs from 7 days old fungi cultures. Three flasks were prepared for each treatment or control. The flasks were incubated at 25±2°C for 15 days. The mycelial mats were dried at 60±3°C to constant weight and their dry weights were recorded.

Statistical analysis: The obtained data were statistically analyzed using the randomized and factorial analysis and the correlation and regression coefficient (Snedecor and Cochran, 1967). Averages were compared with 0.05 and 0.01 levels of probability (Fisher, 1948) and Standard Deviations (SD) were calculated.

RESULTS

Isolation experiments: In the present study seven fungal-flora were isolated from the internal tissues of roots, stems and leaves of faba bean plant, as identified according to Booth (1977), Domsch *et al*. (1980) and Sinclair and Backmann (1989). The isolated fungi belong to six different families and most of them are Ascomycetes. They cause some serious diseases for faba bean as recorded in Table 1.

Fungal linear (diameter) growth (cm): The linear growth (cm) of the isolated faba bean pathogenic fungi under the effect of different filtrate concentrations of *Anabaena subcylindrica*, *Nostoc muscorum* and *Oscillatoria angusta* was recorded Fig. 1. Statistical analysis indicated that the obtained differences in fungal mat diameters due to the study treatments of algal filtrate concentrations, type of treated fungi and their interactions were highly significant ($p<0.01$) as recorded in Table 2.

The data revealed a progressive decrease in growth diameter of all tested fungi with increasing algal filtrate concentration, except for *Macrophomina phaseolina* where the filtrate of *Anabaena subcylindrica* seemed with no effect on its growth particularly at low and moderate filtrate concentrations. The highest filtrate concentration of *Anabaena subcylindrica* (75%) caused a dramatic reduction in diameter of all tested fungi more pronounced in those of *B. fabae* and *A. alternata*, where 78 and 60%

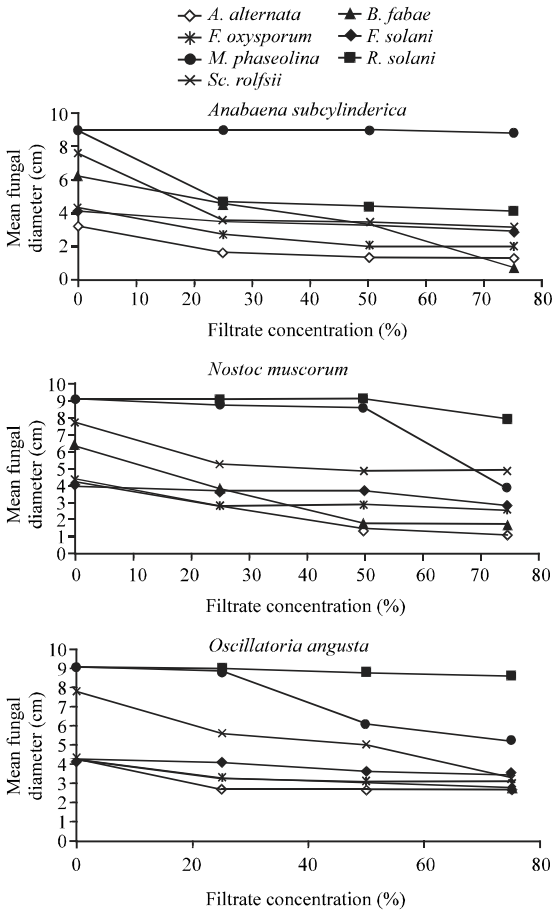

Fig. 1: Fungal growth in diameter (cm) under the effect of concentrations of three algal filtrates

Table 1: The isolated fungi from the different organs of faba bean

Isolation fungi	Family	Organ	Disease	Disease severity
Alternaria alternate	Pleoporaceae	Leaf	Alternaria leaf spots	Sever
Botrytis fabae	Moniliaceae		Chocolate leaf spots	High sever
Fusarium oxysporum	Hypocreaceae	Stem and leaf	Root-rot daping-off and wilt	Sever
Fusarium solani	Hypocreaceae			Sever
Macrophomina phaseolina	Incertae sedis			Sever
Rhizooctonia solani	Ceratobasidiaceae			High sever
Sclerotium rolfsii	Atheliaceae			Sever

Table 2: Statistical ANOVA and LSD for the obtained results of faba bean isolated fungi under the effect of different concentrations of three cyanobacterial filtrates

			Effect of		
Test	Algae	LSD at	Type of fungi	Filtrate concentration	Interaction
Fungal diameter	*Anabaena subcylinderica*	5%	0.33*	0.25*	0.65*
		1%	0.43**	0.33**	0.87**
	Nostoc muscorum	5%	0.22*	0.17*	0.45**
		1%	0.30**	0.22**	0.59**
	Oscillatoria angusta	5%	0.27*	0.21*	0.54*
		1%	0.36**	0.27**	0.72**
Fungal dry weight	*Anabaena subcylinderica*	5%	0.07*	0.06*	ns
		1%	0.09**	0.08**	ns
	Nostoc muscorum	5%	0.09*	0.07*	0.19*
		1%	0.11**	0.09**	ns
	Oscillatoria angusta	5%	0.07*	0.06*	ns
		1%	0.09**	0.08**	ns

*Significant, **Highly significant, ns = Non-significant

growth reductions were reached respectively as compared to control. Also, *Anabaena subcylindrica* filtrate succeeded to inhibit the growth of *Rhizoctonia solani* and *Fusarium oxysporum*, the most serious pathogenic fungi to Faba bean plant, by about 54%. *Anabaena subcylindrica* filtrate caused up to 30% reduction in case of *F. solni*.

Nostoc muscorum filtrate remarkably lowered the diameter growth of the isolated pathogenic fungi, more prominently at the higher concentration of algal filtrate, except that of *R. solani* where the filtrate seemed without clear effect particularly at the low and moderate concentrations, but a slight decrease (13%) was responded to 75% algal concentration. The results showed the high efficiency of *Nostoc muscorum* filtrate to suppress the fungal growth of most pathogens even at low dose (25%). *Nostoc muscorum* filtrate resembled *Anabaena subcylindrica* filtrate and caused the highest reduction of both *A. alternate* and *B. fabae* growth.

The filtrate of *Oscillatoria angusta* affected the growth of the recorded faba bean pathogenic fungi (Fig. 1). The data revealed that the addition of *Oscillatoria angusta* filtrate to the fungal media caused appreciable reduction in the growth of most tested fungi. The highest reduction was cleared with using the high concentration of filtrate. The results further showed that although a high efficiency of *Oscillatoria angusta* filtrate to inhibit the fungal growth of most tested fungi was recorded even at low filtrate concentration, *R. solani* was not affected by its low concentration (25%) whereas exhibited a slight decrease (3.0 and 4.9%) in response to 50 and 75% concentrations, respectively. Again, the lowest concentration (25%) of *Oscillatoria angusta* induced a slight inhibition on *F. solani* and *M. phaseolina* growth whereas the highest concentration of algal filtrate caused attenuate reduction in the growth of the two fungi.

Fungal mat dry weight (g): The different concentrations of *Anabaena subcylindrica*, *Nostoc muscorum* and *Oscillatoria angusta* filtrates led to significant variations ($p<0.01$) in the mat dry weights of the isolated fungi from the different organs of faba bean (Table 2 and Fig. 2). The data revealed a progressive decrease in the fungal mat dry weight of all studied fungi with increasing concentration of the applied algal filtrates. The remarkable reduction by *Anabaena subcylindrica* filtrate was recorded in fungal mat dry weight of *R. solani*, where 74.29% reduction was found by 90% algal filtrate. On contrast, 25 and 50% concentration of *Anabaena subcylindrica* filtrate had no effect on *M. phaseolina* mat dry weights, while its higher concentrations promoted a slight reduction in comparison with the same treatment on the growth of the other corresponding fungi.

The different concentrations of *Nostoc muscorum* filtrate caused accelerated decrease in the fungi mat dry weight of faba bean pathogenic fungi with increasing algal filtrate concentration. The maximum reduction occurred in *M. phaseolina* and *F. solani* fungal mat dry weight by using all the concentrations of *Nostoc muscorum* filtrate compared to the other corresponding treated fungi. Decreases reached to 69.3 and 34.9% for the two fungi, respectively with the 90% algal filtrate treatment. Also, this highest algal filtrate treatment (90%) reduced the fungal mat dry weight of *F. oxysporum* and *R. solani* by about 33.3 and 26.7%.

The increase in *Oscillatoria angusta* filtrate concentrations led to a significant correlated decrease in all studied fungi mat dry weights. The highest effect appeared in case of *Sc. rolfsii* followed by *R. solani* with 78.9 and 58.1% reduction in mat dry weight, respectively, while the least effect cleared with *A. alternata*, where the reduction was only by 23.0%. However these highest reduction effects on all studied fungi appeared at the highest algal concentration (90%).

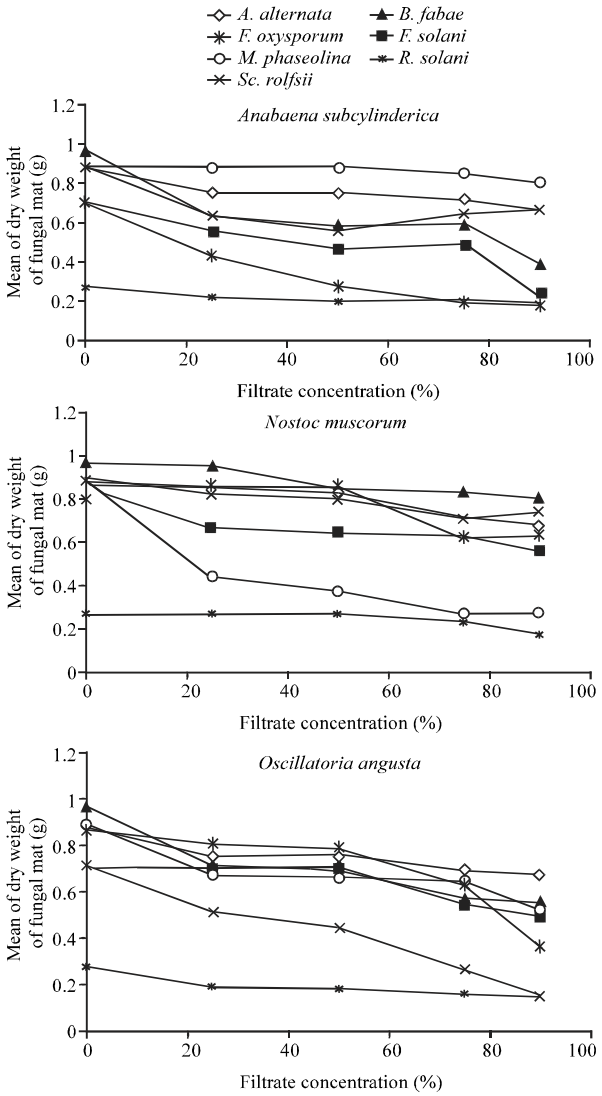

Fig. 2: Effect of algal filtrate concentrations (%) on dry weight of fungal mat (g)

Fungal mat density: The mean fungal mat density of all isolated fungi exhibited an increasing with the increase in *Anabaena subcylindrica*, *Nostoc muscorum* and *Oscillatoria angusta* filtrate concentrations, except 75% concentration of *Oscillatoria angusta* that caused a similar mean mat density to control (Table 3). The mean fungal mat density under the highest concentration (75%) of each *Anabaena subcylindrica* and *Nostoc muscorum* filtrate was nearly double the control value. The mat density of each *A. alternata*, *B. fabae*, *F. oxysporum* and *Sc. rolfsii* increased in response to the increase in the applied filtrates concentration of *A. subcylindrica* and *Nostoc muscorum.* The mat density of the other isolated fungi did not show a clear response to the increase of the two algal filtrates concentration. On the other hand, the increase in *Oscillatoria angusta* filtrate concentration mostly increased the mat density of *A. alternata*, *F. solani* and *M. phaseolina* but decreased those of *F. oxysporum* and *R. solani*. In general, *A. alternata* acquired the highest mat density under the control, while *F. oxysporum* acquired the lowest. Also, *A. alternata* exhibited the highest fungal mat density under the highest filtrate concentration (75%) of each *Anabaena subcylindrica*, *Nostoc muscorum* and *Oscillatoria angusta*, while the lowest value was recorded for *R. solani*, *M. phaseolina* and *F. oxysporum* under the filtrate of the three algae, respectively.

Algal filtrate utilization efficiency: The correlation and linear regression coefficients were applied for the relationships between the concentration of the three algal filtrates and the obtained dry weight of the studied fungi under these concentrations. The correlation coefficient r-values were negative and mostly significant signifying the progressive decrease of the fungal dry weight with increasing algal filtrates of the three algae (Table 4). The correlation coefficients also, indicated that there were no significant effects for *Anabaena subcylindrica* filtrate on each *F. oxysporum*, *M. phaseolina* and *Sc. rolfsii*, for *Nostoc muscorum* filtrate on each *F. oxysporum* and *R. solani* and for *Oscillatoria angusta* filtrate on *F. solani* It could be extracted from these significant correlations that *Oscillatoria angusta* filtrate had the highest efficiency as it acquired significant inhibiting effect on the dry weight of six of the seven isolated fungi followed by *Nostoc muscorum* filtrate (5 fungi) and *Anabaena subcylindrica* filtrate (4 fungi). Also, *Oscillatoria angusta* filtrate was the only one acquiring a significant reducing effect on the growth of *F. oxysporum.*

The regression equation of the significant relationships between the algal filtrate concentration and fungal dry weight is:

Algal filtrate concentration (%) = a + bx fungal dry weight

Where:
(a) is the intercept of (y) axes
(b) is the slope of the linear relationship.

From this equation, the intercept (a) represented in Table 4 equivalent to the maximum filtrate concentration causing complete fungal death or fungal dry weight reaches zero. This intercept value was considered as the Efficient Algal Filtrate Concentration (EAFC). The data of the EAFC showed that it ranged between a minimum concentration percentage of 104% for *Anabaena subcylindrica* filtrate on *R. solani* and a maximum

Table 3: The fungal mate density (g cmG^{1}) for the studied fungi under the effect of different concentrations of three algal filtrates

	Fungal mate density, weight/diameter (g cmG^{1})							
Filtrate conc.	*A. alternate*	*B. fabae*	*F. oxysporum*	*F. solani*	*M. phaseolina*	*R. solani*	*Sc. rolfsii*	Mean
Anabaena subcylinderica								
0%	0.272	0.155	0.063	0.171	0.098	0.078	0.116	0.136
25%	0.469	0.139	0.082	0.159	0.098	0.091	0.177	0.174
50%	0.551	0.173	0.100	0.138	0.098	0.062	0.162	0.183
75%	0.555	0.843	0.107	0.171	0.097	0.046	0.203	0.289
Nostoc muscorum								
0%	0.207	0.152	0.063	0.215	0.098	0.096	0.116	0.135
25%	0.311	0.255	0.069	0.182	0.049	0.094	0.158	0.164
50%	0.586	0.486	0.098	0.173	0.045	0.094	0.166	0.235
75%	0.670	0.509	0.095	0.227	0.070	0.079	0.149	0.257
Oscillatoria angusta								
0%	0.207	0.222	0.063	0.167	0.098	0.096	0.092	0.135
25%	0.275	0.220	0.058	0.169	0.076	0.089	0.091	0.140
50%	0.281	0.228	0.059	0.193	0.108	0.089	0.088	0.150
75%	0.253	0.207	0.053	0.156	0.123	0.072	0.080	0.135

Table 4: Correlation coefficient and regression line parameters for the studied fungi dry weight with different algal filtrates

Parameters	*A. alternate*	*B. fabae*	*F. oxysporum*	*F. solani*	*M. phaseolina*	*R. solani*	*Sc. rolfsii*
Anabaena subcylinderica							
R-value	-0.939	-0.899	-0.873	-0.900	-0.818	-0.952	-0.800
Slope	-425.900	-158.900	-1023.200	-183.500	-854.500	-159.500	-180.700
Intercept	365.700	148.100	271.100	136.800	781.200	104.500	169.800
Nostoc muscorum							
R-value	-0.955	-0.952	-0.804	-0.890	-0.888	-0.872	-0.944
Slope	-407.200	-471.500	-750.000	-288.700	-128.500	-254.100	-513.900
Intercept	368.100	461.000	232.500	241.200	105.300	241.700	454.000
Oscillatoria angusta							
R-value	-0.938	-0.943	-0.971	-0.875	-0.907	-0.892	-0.991
Slope	-437.900	-210.400	-705.600	-310.900	-254.900	-161.400	-165.600
Intercept	375.600	194.500	182.100	242.400	219.800	158.400	116.500

percentage of 461% for *Nostoc muscorum* filtrate on *B. fabae* of the used filtrate concentration in this study. The EAFC on *A. alternata* did not differ greatly for the three algal filtrates. The best EAFC was for *Anabaena subcylindrica* on *R. solani* (104%). It was for *Nostoc muscorum* on *M. phaseolina* (105%) and for *Oscillatoria angusta* on *Sc. rolfsii* (116%) in comparison with the other algae, those reached to their EAFC on the other fungi with higher filtrate concentrations.

From the economic point of view, when the two or the three types of filtrate acquire significant reducing effect on the pathogenic fungi the low coast one, or mixture of more than one, is preferable. However, the data in the present study revealed that a mixture of *Anabaena subcylindrica* + *Oscillatoria angusta* and *Nostoc muscorum* + *Oscillatoria angusta* led in their EAFC to complete control of all isolated fungi from the different organs of Faba bean plant. The data of growth curves of the three utilized algae showed that the dry weight production per unit time at the stationary phase was of *Nostoc muscorum* > of *Oscillatoria angusta* > of *Anabaena subcylindrica* (Fig. 3). This appreciated the utilization of *Nostoc muscorum* + *Oscillatoria angusta* mixture as the best for controlling the recorded pathogenic fungi of faba bean.

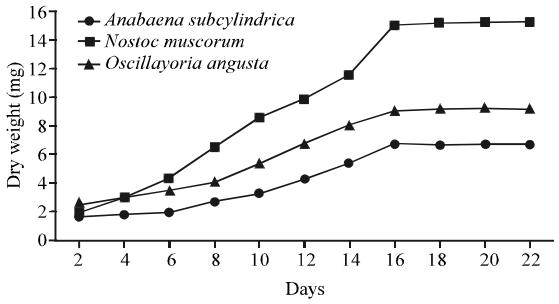

Fig. 3: The standard growth curves of *Anabaena subcylinderica*, *Nostoc muscorum* and *Oscillatoria angusta*

DISCUSSION

The filtrates of *Anabaena subcylindrica*, *Oscillatoria angusta* and *Nostoc muscorum* contain a wide variety of compounds with biological activities such as antibiotics and toxins (Metting and Pyne, 1986; Carmichael, 1992). However, the growth of the isolated fungi from the Faba bean roots, stems and leaves, in general, was seriously affected when subjected to the three algal filtrates. The reductions in the growth of the studied fungi by the increased concentration of the three

algal filtrates were significant and in most cases, the relationships between the pathogenic fungi dry weights and the algal filtrates concentrations were linear and significant. It is also important to note that the isolated fungi belongs to six families which indicated that the study algal filtrate could be generalized as an important control for a great number of pathogenic fungi.

Generally, it was clear that, fungal growth in diameter was decreased progressively by the increase of the three algal filtrate concentrations. *Anabaena subcylindrica* filtrate caused the supreme inhibition of *B. fabae*, *A. alternata*, *Sc. rolfsii*, *F. oxysporum* and *R. solani* particularly at its 75% concentration. Kellam *et al*. (1988) found similar results where an extract from *Anabaena variabilis* severely inhibited the growth of the cellulolytic fungus *Chaetomium globosum.* Also, Frankmolle *et al.* (1992a and b) reported that crud ethanolic extracts from *Anabaena laxa* inhibited the growth of *Aspergillus oryzae*, *Candida albicans*, *Penicillium notatum*, *Saccharomyces cerevisiae* and *Trichophyton mentagrophytes.*

Using *Nostoc muscorum* filtrate reduced the mat diameter of *A. alternata* and *B. fabae* with a greater extent than the other isolated fungi followed by *M. phasoelina.* These results are in agreement with de Cano *et al.* (1990) who found a significant inhibition in *Candida albicans* growth and due this growth reduction to the phenolic compounds extracted from *Nostoc muscorum* cells. In similar technique, de Mule *et al*. (1977) found that culture filtrates of *Nostoc muscorum* inhibited the mycelia development of the saprophyte *Cunninghamella blakesleeana* in liquid culture.

The filtrate of *Oscillatoria angusta* minimized the mat diameters of the isolated fungi and to a great extent of *Sc. rolfsii*, *M. phaseolina* and *B. fabae.* Moon and Martin (1992) and Smitka *et al.* (1992) isolated from *Calothrix fusea*, *Fischerella ambigua*, *Hapalosiphon hibernicus* and *Westie llopsis prolifica,* six hapalindole type alkaloids with a fungicidal activity which could due the inhibited growth in the fungal mat diameter to the alkaloids content of the studied algal filtrates.

Various concentrations of each *Anabaena subcylindrica*, *Nostoc muscorum* and *Oscillatoria angusta* filtrate had reduced the fungal mat dry weights with a enormous reduction in *Sc. rolfsii* grown in medium contain 90% *Oscillatria angusta* filtrate followed by *R. solani* and *M. phasoelina* in the 90% *Anabaena subcylindrica* or *Nostoc muscorum* filtrates, respectively. Also, de Caire *et al*. (1987) and de Caire *et al.* (1990) found that the growth of the plant pathogens, *S. sclerotiorum* and *R. solani*, was inhibited by cells extract or extra cellular products of *N. muscorum.* They reported an inhibited average growth of *R. solani* by extra cellular products to 77% whereas by cell extract to 14% compared to control. Generally, the inhibitory effect may be due to the algal products have biologically active compounds (Kulik, 1995).

Fungal mat density progressively increased due to the unequal reduction in fungal mat diameter and dry weight in response to the increased *Anabaena subcylindrica*, *Nostoc muscorum* and *Oscillatoria angusta* filtrates concentration, except 75% concentration of *Oscillatoria angusta*. The mean mat density of all pathogenic was nearly doubled by the highest filtrate concentration of each *Anabaena subcylindrica* and *Nostoc muscorum.* The increased mat density was due mainly to over reduction of fungal mat diameter compared to mat dry weight. This may indicate that the studied algal filtrates diminished fungal spread on the diseased area with greater extent than fungal mat dry weight. This get a great importance if the disease was in the plant leaves. The remarkable increase in mat density was of *A. alternata*, *B. fabae*, *F. oxysporum* and *Sc. rolfsii* in response to the increase in filtrate concentration of *Anabaena subcylindrica* and *Nostoc muscorum.* The decrease in each fungal mat diameter and dry weight was with similar rates in the other studied fungi by the two algal filtrates, which reflected more or less similar mat density. The filtrate of *Oscillatoria angusta* mostly increased mat densities of *A. alternata*, *F. solani* and *M. phaseolina*, but decreased those of *F. oxysporum* and *R. solani*. In general, *A. alternata* acquired the highest mat density under the control, while *F. oxysporum* acquired the lowest. *Anabaena subcylindrica*, *Nostoc muscorum* and *Oscillatoria angusta* 75% filtrate concentration raised *A. alternata* fungal mat density to its maximum, while decreased those *R. solani*, *M. phaseolina* and *F. oxysporum* to their lowest under the filtrates of the three algae, respectively.

The used algal filtrate concentrations relationship with dry weights of the studied fungi signified a progressive and linear decrease of all fungal dry weights with an exception of *Anabaena subcylindrica* on each *F. oxysporum*, *M. phaseolina* and *S. rolfsii*, for *Nostoc muscorum* on each *F. oxysporum* and *R. solani* and for *Oscillatoria angusta* filtrate on *F. solani.* The relationship also, confirmed *Oscillatoria angusta* filtrate as the most efficient as it acquired significant control on six of the seven isolated fungi, in one hand and it was the only one acquiring a significant control on *F. oxysporum*, on the other. *Oscillatoria angusta* filtrate was followed by *Nostoc muscorum* filtrate (5 fungi) and *Anabaena subcylindrica* filtrate (4 fungi). However, the results suggested a filtrate mixture of the three algae because no

one of them could be used singly for complete control of all isolated fungi and two algal filtrates, *Oscillatoria angusta* filtrate must be one of them, could be enough for this purpose.

The highest Efficient Algal Filtrate Concentration (EAFC) could be obtained by algal filtrates concentration ranged 104 to 461% of the used filtrate concentration in this study for *Anabaena subcylindrica* filtrate on *R. solani* and for *Nostoc muscorum* filtrate on *B. fabae*. The EAFC of the three algal filtrates on *A. alternata* did not differ greatly indicating that the filtrate of the three algae acquired more or less similar inhibitory effect on the different isolated fungi. However, least coast one could be used if the plant infected with *A. alternata.* The best EAFC was the lowest and for *Anabaena subcylindrica* on *R. solani* (104%), for *Nostoc muscorum* on *M. phaseolina* (105%) and for *Oscillatoria angusta* on *Sc. rolfsii* (116%). The mixtures of *Anabaena subcylindrica* + *Oscillatoria angusta* and *Nostoc muscorum* + *Oscillatoria angusta* in their EAFC could acquire completely control on the isolated fungi from the different organs of Faba bean plant. Dry weight production of *Nostoc muscorum* was greater than that of *Oscillatoria angusta* than that of *Anabaena subcylindrica*. This appreciated utilization of *Nostoc muscorum* + *Oscillatoria angusta* filtrate mixture with an EAFC 368 and 194% for the complete control of isolated pathogenic fungi of Faba bean economically.

In conclusion, the study revealed high efficiency of the three algae filtrates on the control of the isolated pathogenic fungi from the roots, stems and leaves of Faba bean plants. The reduction in fungal mat diameter was greater than in that of the fungal dry weight showing inhibited fungal spread by greater rate, which is important in decreasing the damage in the photosynthetic capacity of the leaf infected plants. The reduction in the fungal dry weight was mostly linear and significantly correlated with the algal filtrate concentrations. These significant relationships calculated an EAFC ranged between 104 and 461% for the three algae filtrate concentrations on the studied fungi dry weight. For complete control of the isolated fungi the study recommended the utilization of a mixture of two algae filtrates in their EAFC and that of *Nostoc muscorum* + *Oscillatoria angusta* filtrates with an EAFC 368 and 194% was the best and economic mixture.

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