Evaluation the efficiency of algae and nano-algae extracts and some biological factors in resistance to *Fusarium culmorum* infection

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Abstract

The results of using the algae alone and nano-algae extracts and Streptomyces sp. that all treatments resulted in a significant reduction in the percentage and severity of infection in comparison to Fusarium culmorum treatment only, which were (87 and 46%) respectively. The treatment (ST+F.c) with commercial Streptomyces sp. achieved the highest percentage decrease in the percentage and severity of infection (2, 1%) respectively, which was not significantly different from the treatment (N+SB+ST+F.c); this treatment included nano-algae, local and commercial bacteria compared to the fungi treatment alone, where the percentage and severity of infection for the fungus treatment only was (87%) and (46%) respectively. The study revealed that nano-algae (N) treatments also reduced the percentage and severity of infection with F.c.

Furthermore, the results indicated a significant differences in the biochemical indicators to stimulate induce systemic resistance (ISR) in the wheat, and also the plant's nutrient content. The efficiency of the studied treatments proved their effect on the peroxidase enzyme activity, as the treatment (N+A+ SB +ST +F.c) was with high efficacy to elevate the enzyme activity; the enzyme activity was reached (0.74), followed by (A+SB+ST+ F.c) treatment with (0.69). However, each treatment alone achieved an elevation in the activity of peroxidase enzyme.

All the studied treatments caused a significant increase in the content of phenols and proteins in the wheat plant compared to Fusarium culmorum treatment; however the treatments were differed in the content of phenols and proteins after 7, 14 and 21 days of treatment.

All treatments significantly increased the chlorophyll content in the wheat after 30 days of fungus treatment in comparison to the treatment of pathogenic fungus alone; where the combination treatment (N+A+SB +ST) achieved the highest percentage of chlorophyll content at (18.1), followed by (N+A+SB+ST+Fc) at (16.4) and algae alone (A) at (14.3) which did not differ significantly from nano-algea treatment (N) (13.2).

Keywords: Fusarium culmorum, Streptomyces sp., nano-algae, Peroxidase

1. INTRODUCTION

Countries worldwide strive to achieve food security that guarantees all the world's population will obtain what they need from food, especially cereal production, as the most important source of food energy. The crop of wheat, *Triticum aestivum* L., in Iraq is one of the most important cereal crops in terms of cultivated area and production, as it is one of the strategic crops that are cultivated in large areas in most of the Iraqi provinces (Mason (2007),

Interestingly, there are many problems facing the wheat plant agriculture process that cause a significant reduction in its production and quality, including the pathogens that affect its growth in all stages and attack its various parts causing various diseases that lead to the deterioration of its production in quantity and quality and the occurrence of economic losses, especially when the appropriate conditions are available. Among these diseases is wheat root rot disease, and *Fusarium culmorum* was one of the most important fungi that cause root rot in wheat (Winter et al., 2019) in addition to the mycotoxins produced by this fungus associated with pollution of crops and grains, which negatively affect human and animal health (Miller, 2008; Zain, 2011)

Although, the using of chemical pesticides in controlling plant diseases, including wheat root rot disease, has proven perceptible efficiency in general, but it is faulted due to having negative effects on the various environmental elements and the biomass, which may lead to imbalance in the natural balance and inhibit the activities of beneficial microorganisms that are endemic to soil as well as the possibility of its direct impact on human and animal health if used incorrectly (Jouany, 2007; AL-Musawi, 2012).

Streptomycets is used as a bio fertilizer for many crops due to its ability to enhance the plant growth and biological control of fungi and pathogenic bacteria in the plant; the reason is due to the metabolism of antibiotics and the production of organic compounds in the soil (Vurukonda et al., 2018). Recently, nanotechnology technique expands to include many fields in our daily life, where there is a need for environmentally friendly and energy-saving methods for the nanoparticles biosynthesis; algae have been discovered to reduce metal ions and therefor it is possible for using in the biosynthesis of nanoparticles.

Furthermore, the biosynthesis of nanoparticles by algae is an environmentally friendly, economical, high-yielding, fast and energy-saving method, and also due to the unique physical, chemical and electrical properties of nano-algae n(N), thus many applications in various fields were found (Fawcett et al., 2017). Several studies confirmed that the biosynthesis of nanoparticles such as silver nanoparticles and magnesium oxide nanoparticles using algae increases the efficiency of the latter in inhibiting diseases and reducing the negative side and harmful effects of silver and magnesium oxide nanoparticles (Dahoumane et al., 2017; Rahman et al., 2020).

2. MATERIALS & METHODS

The experiment was carried out for the purpose of studying the effect of algae and nano-algae extract and some biological factors in controlling *F. culmorum* growth. The loam soil was brought to the laboratory and then the soil was prepared for agriculture through removing some of the impurities present in it. This experiment was conducted in the greenhouse

affiliated to the Plant Pathology Laboratory/Department of Plant Protection/College of Agricultural Engineering Sciences/University of Baghdad.

The loam and peat moss soil at a ratio of 1: 2 were sterilized using autoclave at 121° C and a pressure of 1.5 kg/cm^2 twice, then distributed in plastic pots of 15 cm in diameter at a rate of 1 kg/pot. Wheat seeds were cultivate at a rate of 15 seeds per pot that sterilized superficially with sodium hypochlorate (1% free chlorine) for one minute, and then washed with sterile distilled water several times, and subsequently placed on sterile filter paper to remove the free water from them and soaked for 30 minutes in algae and nano-algae extracts at a concentration of 1.5%. separately, 1.5 mL of algae and nano-algae extracts was added to 100 mL of sterile deionized water to obtain a concentration of 1.5%.; the seeds also soaked in *Streptomyces* sp. suspension at a concentration of 70x10^5 (colony forming unit / ml) for local isolate and 80x10^5 (CFU/ml) for commercial isolate (CFU/ml) for 30 minutes, and after 15 days of seeds cultivation, and growth of *F. culmorum* was detected on PDA culture medium after 7 days. Then, each 6-day-old pot was inoculated with *F.culmorum* at three quarters ratio for each one, as cracks were made in the pot and the soil contaminated near the seedlings' roots.

The experiment was carried out according to Completely Randomized Design (CRD) by three replicates for each treatment with the comparison group (control) without fungi treatment, and the experiment was followed up and the results were recorded as estimating peroxidase enzyme level and total phenol and protein content after 7, 14 and 21 days; also the incidence and severity percentage of infection were calculated. Additionally, Wet and dry weight, the length and high of the shoot and root systems and chlorophyll content after 30 days of the addition of   *F. culmorum* inoculation was estimated.

$$\text{The infection percentage (\%)} = \frac{\text{No. of infected plants}}{\text{Total No. of examined samples}} \times 100$$

The severity of the infection was estimated on the shoot and root systems using the 5-degrees of pathology evidence, as follow:

0 = Uninfected white root system and normal growth of green shoot system.

1 = Coloring more than 0-25% of the root system with light brown color and yellowing of the shoot system at 25%.

2 = Coloring more than 26-50% of the root system with dark brown color and yellowing of the shoot system at 50%.

3 = Coloring more than 51-75% of the root system with dark brown color and yellowing of the shoot system at 75%.

4 = Coloring more than 76-100% of the root system with dark brown color and yellowing of the shoot system more than 75% and then death of the plant.

$$\text{Percentage of infection severity} = \frac{\text{No. of plants at degree 0x0+.....+ No. of plants at degree 4x4}}{} \times 100$$

Total No. of examined plants x highest degree

The experiment included the following treatments with three replicates for each one:

No.	Treatment
1	Control(co)
2	*F.culmorum*(F.c)
3	Normal algae (A)
4	Nano-algae (N)
5	Local *Streptomyces* sp (SB)
6	Commercial *Streptomyces* sp (ST)
7	A+F.c
8	N+F.c
9	SB+F.c
10	ST+F.c
11	A+SB
12	A+ST
13	N+SB
14	N+ST
15	A+SB+ST
16	N+SB+ST
17	A+SB+ST+F.c
18	N+SB+ST+F.c
19	N+A+SB+ST
20	N+A+SB+ST+F.c

* *F. culmorum* (F.c), Algea (A), N = nano-algae, Local *Streptomyces* sp. = (SB), Commercial *Streptomyces* sp. = (ST).

Peroxidase activity was estimated in the wheat after 7, 14 and 21 days of treatment with *F. culmorum* according to Nezih et al. (1985) method; 1 mL of Hydrogen Peroxide (0.1%) was mixed with 1 mL of Guaiacol which prepared by mixing 1.36 mL of Guaiacol in a flask, and the volume complete up to 250 ml with distilled water. The effectiveness was estimated by adding 2 ml of the reaction mixture to the cell of Spectrophotometer, and also 100 μl of sample extract was added and the absorption record at a wavelength of 420 nm using spectrophotometer every 30 seconds for three minutes, assessed on the basis of the change rate in the optical density/minute/g of wet weight; the absorbance change was recorded according to the following equation: -

The absorbance change $= \dfrac{\frac{\Delta A}{\Delta t}}{wet\ weight(g)}$

While ΔA= The absorbance change (changing in the reading)

    Δt = Time chang/minute

Estimation of the Protein level in the pots experiment

The total concentration of protein in the plant was estimated after 7, 14 and 21 days of adding of fungus using following method:

Preparation of Protein dye reagent

Protein dye reagent was prepared by dissolving 0.1g of Comassie brilliant blue (G-250) (Chemical Ltd BDH) in 50 mL of 95% ethanol, then 100 mL of 85% phosphoric acid were added and the volume was completed up to 1 liter with cold distilled water; the reagent kept in dark vial at 4° C, until using for standard curve of protein that used for protein concentration estimation.

Preparation of Bovine Serum Albumin (BSA)

BSA solution was prepare at a concentration of 1 mg/mL by dissolving 10 mg of bovine serum albumin (supplied by Pharmacia Fine Chemical Uppsale-Sweden) in 10 mL of distilled water.

Estimation of protein concentration

The protein concentration in the crude extract of the enzyme was estimated by adding 0.1 mL of the enzymatic solution into a test tube and 1 ml of protein reagent was added to it and mixed well using vortex and left for 5 minutes at room temperature; the absorbance was measured at the wavelength of 595 nm. Protein concentration was measured according to the standard curve Fig. (1). The control solution blank was prepared by following similar previous steps, with the exception of using distilled water instead of a solution (Bradford, 1976).

$$y = 3.9014x + 0.003$$
$$R^2 = 0.9991$$

Optical density (595 nm) vs Protein concentrattion (mg.ml)
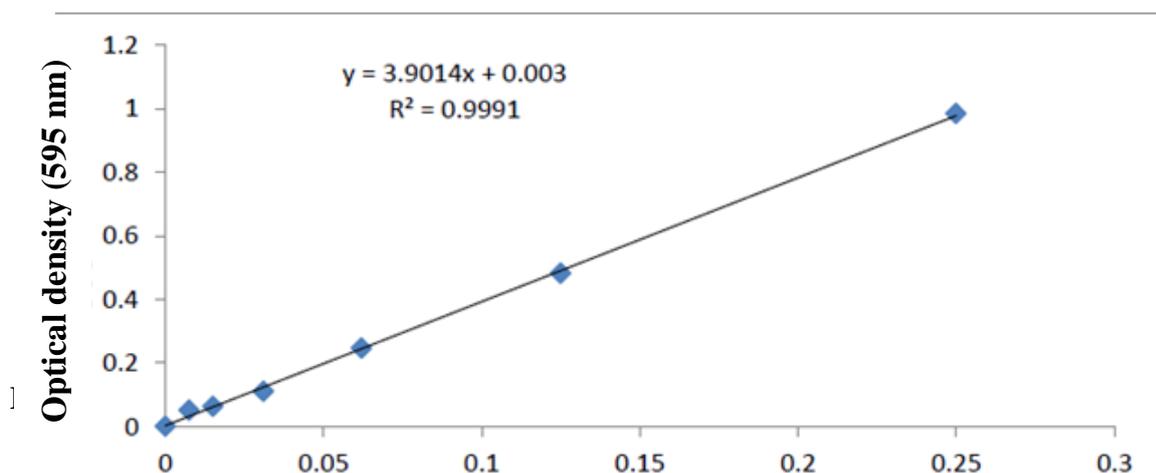
Estimation of total phenol

The phenol content was estimated after 7, 14 and 21 of the treatment with studied fungus; the phenol content was calculated on the basis of milligram of phenols per each gram of fresh vegetative tissue and catechol was used a standard substance (Rishi et al., 2008) as following:

A 1 gm of fresh plant leaves was crushed with 10 ml of 80% methanol, then put it in the water bath at a temperature of 70° C with constant stirring for 15 minutes, the mixture was filtered using gauze and 1 ml of the filtrate was mix with 5 ml of sterile distilled water and 2.5 ml of the folin reagent in a sterile glass tube; the solution was incubated at a temperature

of 25° C for 30 minutes. An appropriate amount of the solution was taken and placed in spectrophotometer cell and the absorbance was measured at a wavelength of 725 nm.

Estimation of chlorophyll content in the wheat
The total chlorophyll content in the fresh leaves of the randomly selected wheat plant was estimated at a rate of 3 leaves per repetition, and the chlorophyll content was estimated using acetone solvent (80%). A 1 gm of the fresh leaves was taken randomly and crushed with a crushing mortar (jar) by using 5 ml of acetone (80%); the suspension was filtrated using filter papers in Buchner funnel and placed in opaque and sealed glass bottles, the absorbance of the sample was read using spectrophotometer at the wavelengths 663 and 645 nm according to the chlorophyll content and to Goodwin (1976) method and according to the following equation:
Total Chlorophyll (mg.L^{-1}) $=(20.2D(645) + 8.02D(663)$

3. RESULTS AND DISCUSSION

As shown in Table (1), the results revealed a significant differences between the treatments and that all of them caused a significant reduction in the percentage and severity of infection compared to the treatment of the fungus only, which was (87 and 46%) respectively. These results are consistent with observations of Cepni et al. (2012) and Hajihassani et al. (2013) that found the fungus invades the roots and crown area, and also causes rotting of roots, leaf tissues and crown (Burgess et al. 2001; Smiley et al. 2009); moreover it may also invade the inner layer and thus prevent the continuity of water and nutrient transport across the roots, crown and stem area (Marley et al., 1989; Chekali et al., 2011). The reason is due to the high efficiency of *F. culmorum* in causing root and crown rot and also vascular occlusion; this is may possibly because the fungus produces two different types of toxins in various types, which are (DON) toxin and nivalenol (NIV) toxin which has a toxicity of 10 times than DON (Minervini et al., 2004; Alexander et al., 2011). *F. culmorum* is one of the most common fungi that infects wheat and causes a high rate and severity of infection (Tunalı et al., 2006; Kammoun et al., 2010); also the increasing in toxins accumulate in this fungus will increased its pathogenicity under appropriate environmental factors (Xu et al. 2008, Chelkowski et al., 2012).

When the seed germinates, *F. culmorum* penetrates the primary roots and then the plumule, or it enters through the stomata in the leaf sheath located at the base of the stem, after that it causes cell death between the epidermis and the cortex, then the fungus continues to grow in a symplast, a continuous network of protoplasts of interconnected plant cells. Subsequently, the growth of the fungus may continue along the stalk; moreover discoloration may occur at the base of the stem before the presence of the fungus in these parts as a result of the plant's response to infection (Beccari et al. 2011; Covarelli et al. 2012). Thus, the fungus will affect the length and height of the plant as well as the wet and dry weight of the shoot and root system of the plant (Eslahi, 2012; Scherm et al., 2013; Motallebi et al., 2015).

The results found that (ST+F.c) treatment achieved the highest percentage decrease in the percentage and severity of infection (2 and 1%) respectively, which did not differ significantly from (N+SB+ST+F.c) treatment compared to treatment of algae alone which was 87 and 46% (Table 1). These results are in agreement with URSAN et al. (2018) and

Piante et al. (2018) studies which revealed that *Streptomyces* sp. has inhibitory effect of *F. culmorum* by 100%; the reason for the fungus inhibition by bacteria may be due to the ability of bacteria to produce a variety of secondary biologically active compounds, such as anti-fungi, anti-bacterial and antiviral agents (Liu et al., 2013; de Lima Procópio et al., 2012). Additionally, previous studies showed that *Streptomyces* sp. has a large number of hydrolysis enzymes and has a role to interact with other organisms in the environment (Chater et al., 2010). This abundance of enzymes, antibiotics and secondary compounds makes them compete fiercely with other organisms in natural environments (Chater, 2016). Furthermore, *Streptomyces* sp. also produces high levels of chitinase, β-glucanase, and antipyretics agents, and it is resistant to up to 70% of disease reduction under greenhouse conditions (Al-Askar et al., 2015; Wang et al., 2016; Rashad et al., 2017).

Ziedan et al. (2010) found that *Streptomyces* sp. filaments are wrapped around the fungal hyphae and dissolve its cytoplasmic membrane, thus the bacteria support the host plants by increasing the growth and the weight of the shoot and root system resulting in protecting the plant from pathogens (Bakker et al. 2007; Lugtenberg and Kamilova, 2009; Babalola, 2010). The bacteria also increased the production of plant hormones, such as auxins and gibberellins (Goudjal et al., 2013), which stimulate the growth of wheat seedlings as well as protect wheat from *F. culmorum*. Toumatia et al. (2016) determined that the seeds coated with IA1strain of *Streptomyces* sp. can reduce the incidence of disease with *F. culmorum* by 64.7%, reduces the infection severity by (79.6%) and protects wheat seedlings and stimulate their growth.

As shown in Table (1), the treatments with the nano-algae (N) also reduced the percentage and severity of infection with *F. culmorum*. The reason may be due to the high surface area of the nanoparticles to be in greater contact with microorganisms and also to their small size that enables them to easily penetrate the cell wall, which leads to the deformation of the fungal filaments (hyphae) and their swelling and thus their decomposition, leading to cytoplasm flow outside the cells and the death of the fungus (Yehia and Ahmed, 2013; Tang and Bin-Feng, 2014; Ahmed et al., 2016).

Previous studies have been indicated the ability of *A. nodosum* to reduce the infection percentage with many pathogens by activating the role of plant defenses against these pathogens (Jayaraman et al., 2010). Moreover, several studies have observed that the use of nanoparticles in controlling pathogens is an effective and successful alternative to traditional methods; these results are in agreement with many studies represented by the use of nanoparticles to increase the average of root or shoot length, dry and wet weight, flowering characteristics and also increase the production through controlling plant pests and diseases (Chen and Yada, 2011; Prasad et al., 2014, Saurabh et al., 2015).

Carbonic substances have been used to enhance plant growth by increasing water absorption (Khodakovsky et al., 2000; Patel et al., 2014). Interestingly, after examining the fungal hyphae microscopically, the nucleotides are exposed to severe damage when exposed to nanomaterial (Min et al. 2009).

The algae extract had a significant effect in increasing the height and weight of the shoot and root system and the yield of the wheat plant; the reason may be due to the fact that it contains more than one group of growth-promoting substances such as oxins, gibberellins, cytokinins, and some major and minor elements (Strick et al., 2003), and adding them to the soil it improves its physical, chemical, and biological properties, increases its ability to retain

moisture, increases the activity of microorganisms (Kuwada et al., 2006), increases the growth of the root and shoot system, yield and resists biotic and abiotic stresses especially salt stress (Abd El-Baky et al., 2008), and also increases the efficiency of mineral absorption, increase the chlorophyll content of the leaves, increase the processes of photosynthesis and respiration (Spinelli et al., 2009). It is also act as antioxidants agents through their role in increasing the activity of some enzymes (Ayad, 1998).

Table (1): The efficiency of algae and nano-algae and some biological factors in resisting *Fusarium culmorum* in wheat plant

Treatments	Incidence of infection (%)	Severity of infection (%)	The plant length (cm)	The plant heigh (cm)	The wet weight (gm)	The wet dry (gm)
Control	0.000	0.000	40.333	15.000	1.467	0.467
F.c.	87.333	46.333	23.000	11.000	0.533	0.167
A	16.333	13.167	43.000	17.333	1.800	0.533
N	10.167	4.867	43.333	18.667	1.867	0.467
SB	9.000	4.067	41.667	16.333	1.600	0.467
ST	7.667	4.300	40.333	15.667	1.633	0.467
A+F.c	19.200	10.333	43.000	15.333	1.767	0.600
N+F.c	6.667	4.333	40.667	15.333	1.933	0.833
SB+F.c	6.667	3.867	42.333	16.667	1.933	0.667
ST+F.c	2.000	1.000	42.000	16.333	1.967	0.667
A+SB	14.933	7.067	37.667	16.000	1.633	0.600
A+ST	15.767	8.200	41.667	18.333	1.867	0.667
N+SB	6.000	3.533	39.333	16.667	1.567	0.433
N+ST	4.000	1.000	40.000	15.333	1.633	0.500
A+SB+ST	14.167	6.967	42.333	20.333	1.833	0.600
N+SB+ST	8.333	5.300	41.333	15.667	1.667	0.567
A+SB+ST+F.c	11.200	5.133	40.667	16.667	2.067	0.767
N+SB+ST+F.c	2.000	1.067	39.667	14.667	0.833	0.433
N+A+SB+ST	10.100	5.267	46.333	19.000	2.500	0.767
N+A+SB+ST+F.c	9.867	4.400	43.667	15.333	1.833	0.600
LSD(5%)	2.397**	2.397**	1.808**	1.296**	0.194**	0.104**

* *F. culmorum* (F.c), Algea (A), N = nano-algae, Local *Streptomyces* sp. = (SB), Commercial *Streptomyces* sp. = (ST).

Estimation of some induction factors and some nutrients in the wheat plant.

The results indicated that there were significant differences in the biochemical indicators of stimulating the induced systemic resistance (ISR) in the wheat and also in the plant's content of nutrients, as the efficiency of the studied treatments was proved on the peroxidase activity

(Figure 2); after 7 days of treatment with *F. culmorum*, the biochemical indicators increased, as the treatment (N+A+ S+ST+Fc) exceeded the enzyme activity (0.74), followed by the treatment (A + SB + ST + Fc) with (0.69), and each treatment separately was elevated in the activity of peroxidase. While after 14 days, the enzyme activity was decreased in (N+A+SB+ST+Fc) treatment with (0.65), and also the result was similar to (A + SB + ST + Fc) treatment, where the enzyme activity decreased to (0.45), and the decreasing in the enzyme activity continued up to 21 days, where the activity was (0.38), whereas the treatment (N+A+SB+ST+Fc) increased the enzyme activity to (0.76) and this is a good indication that the survival of nano-agae *A. nodosum* extract for a longer period in the plant or soil increases the induction factors and does not affect the growth parameters of the plant and does not have a negative effect on *Streptomyces* sp. Additionally, this study found that the treatment with the fungus alone increased the enzyme activity after 7 days by 0.49, and its highest concentration reached after 14 days by 0.54.
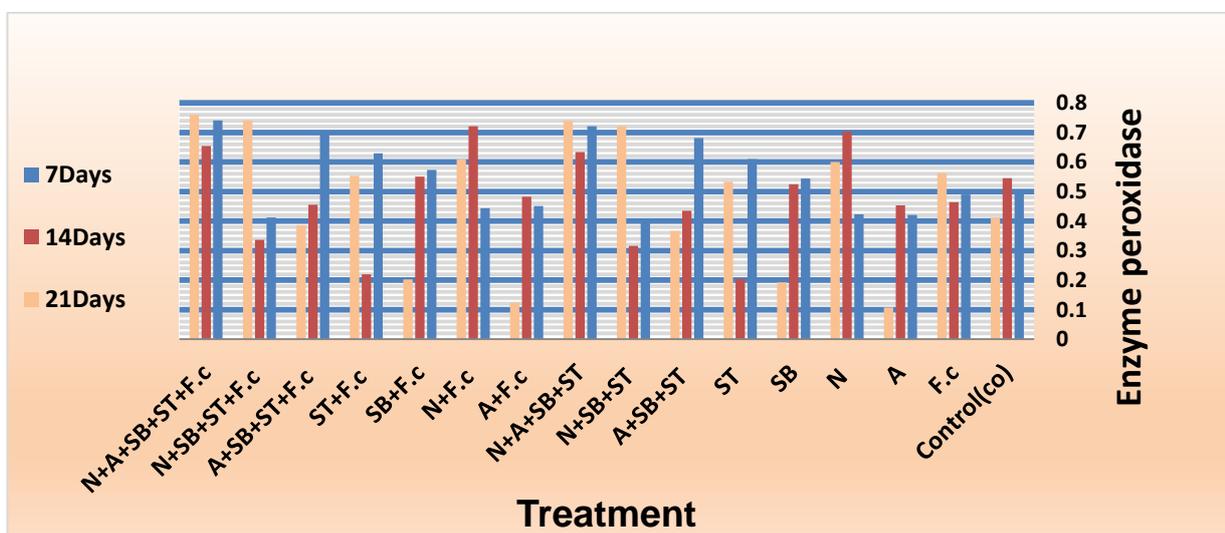
* each column represent the average of three replicates

* L.S.D. at (5%) 0.0042**

* *F. culmorum* (F.c), Algea (A), nano-algae (N), Local *Streptomyces* sp. (SB), Commercial *Streptomyces* sp. = (ST).

Figure 2: The effectiveness of normal algae and nano-algae and some biological factors on peroxidase activity (change in absorption/min/g wet weight) in the wheat plant.

Peroxidase is induced in plant tissues upon exposure to pathogens or inducers (promoters), and has a critical role in determining the level of host resistance, and is a key enzyme in lignin biosynthesis, suberin precipitation, and cell wall biosynthesis i.e. increased structural defenses (Almagro et al., 2009; Thakker et al., 2013; Jogaiah et al., 2013). Moreover, it converts the extensins secreted in the Apoplast from soluble monomers into an insoluble network, depending on H_2O_2, which in turn increases the plant defense by making the cell wall more complex and resistant to mechanical stress during penetration of the fungus by the adhesion organ (Shah et al. 2004; Fry et al. 2004) ; Thakker et al., 2013). These findings are in agreement with Pritsch et al. (2000) studt where they demonstrated that PR-5 and

peroxidase were induced in both resistant and susceptible cultivars after inoculation with F.c., as these proteins were detected as early as 6-12 hours after inoculation and peaked after 36-48 hours. Higher amounts of 1-β, 3-glucanase and chitinase were also detected in resistant cultivars and after treatment with F.c. (Kang et al., 2007).

As shown in Figures (3, 4) that the treatment with F.c. only caused a significant decrease in the total of phenolate and protein content after 7, 14 and 21 days, and a lowest percentage in the phenols content was after 21 days by (351) mg and the lowest protein content was recorded after 7 days by 0.320. These results are in agreement with Al-Abdalall (2010) who reported that F.c. decreased the content of protein, carbohydrates and lipids of the wheat plant; the reason for the decrease in the protein content and other nutrients is that the fungi consume sugar during growth and energy production, and this led to a decrease in the content of nutrients and a weakness of shoot system (Wildermuth et al., 1992; Al-Abdalall, 1998), or the decreasing in protein content may explained due to using protein by fungi in their enzymatic system (Smiley and Uddin, 1993; Smiley et al., 2005b).

   It was found that all the treatments significantly increased the concentration of phenols in the wheat compared to the fungus treatment only (Figure 3); the treatments differed in the content of phenols among them after 7, 14 and 21 days, since there were treatments recorded the highest content of phenols after 7 days which decreased to lowest content after 21 days. The treatments with algae alone and algae with F.c (A and A + Fc) increased content of phenol at high rate within 7 days, as it reached (933 and 950) respectively, while this content decrease until it reached the lowest rate after 21 days, it was 371 and 408 respectively. On the other hand, the treatments with nano-algaealson or in combination with F.c (N and N + Fc) recorded the lowest phenolate content after 7 days (710 and 730) and it increased until to the highest content after 21 days, it was (1755,1730) respectively.
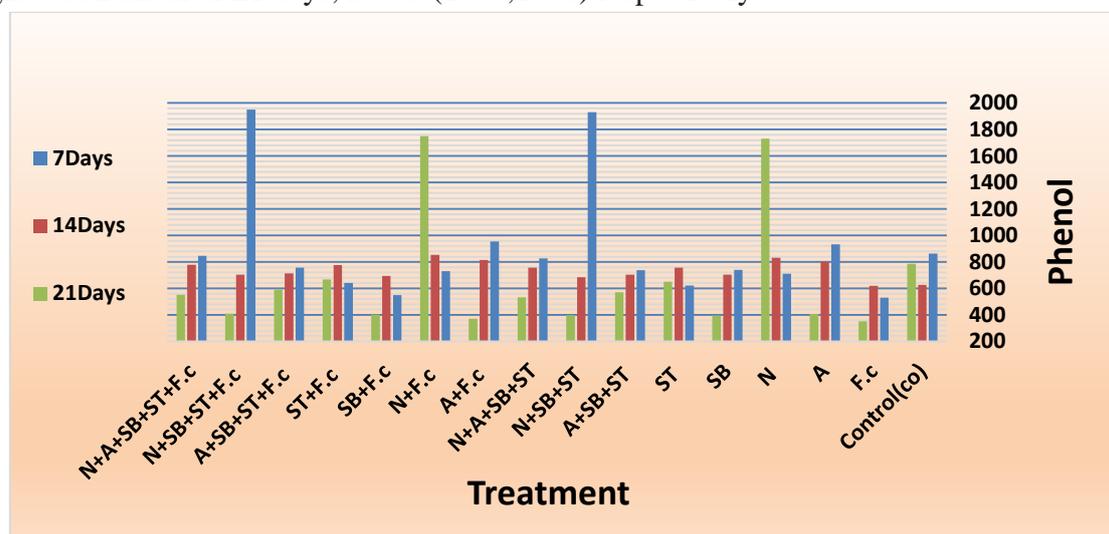
* each column represent the average of three replicates
* L.S.D. at (5%) 1.096**
* *F. culmorum* (F.c), Algea (A), nano-algae (N), Local *Streptomyces* sp. (SB), Commercial *Streptomyces* sp. = (ST).

Figure 3: The effect of normal algae and nano-algae and some biological factors on the total phenol concentration (mg/kg)) in the wheat plant.

The results also showed that all the treatments caused a significant increase in the protein content of the wheat compared to the fungus treatment only (Figure 4), however they differed in their effect on the protein content, as there were the highest protein content after 14 days, as the highest content of protein content after 14 days found in the treatment (N+F.c) by (0.945), then decreased after 21 days, while other treatments exert the highest protein content after 21 days, and the highest rate content was after 21 days in the treatment (N+A+SB+ST+F.c) by (0.923). .
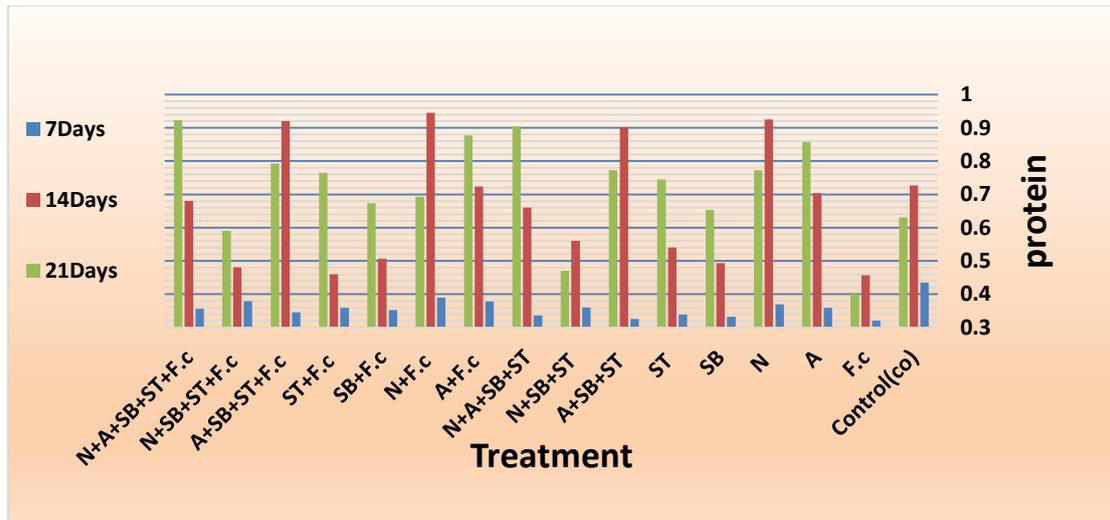
* each column represent the average of three replicates
* L.S.D. at (5%) 0.0904**
* *F. culmorum* (F.c), Algea (A), nano-algae (N), Local *Streptomyces* sp. (SB), Commercial *Streptomyces* sp. = (ST).
Figure 4: The effects of normal algae and nano-algae and some biological factors on the total protein content in the wheat plant.

Additionally, the results showed that all the treatments achieved a significant increase in the chlorophyll content of the wheat measured by Spad unit after 30 days of inoculation with fungus in comparison to the funfus treatment alone; the combination treatment (N+A+SB+ST) achieved the highest percentage of chlorophyll content by (18.1) followed by the treatment (N+A+SB+ST+F.c) by (16.4), and then algae treatment alone where the chlorophyll content was (14.3), which did not differ significantly from the nano-algae treatment (N) only which was (13.2) .
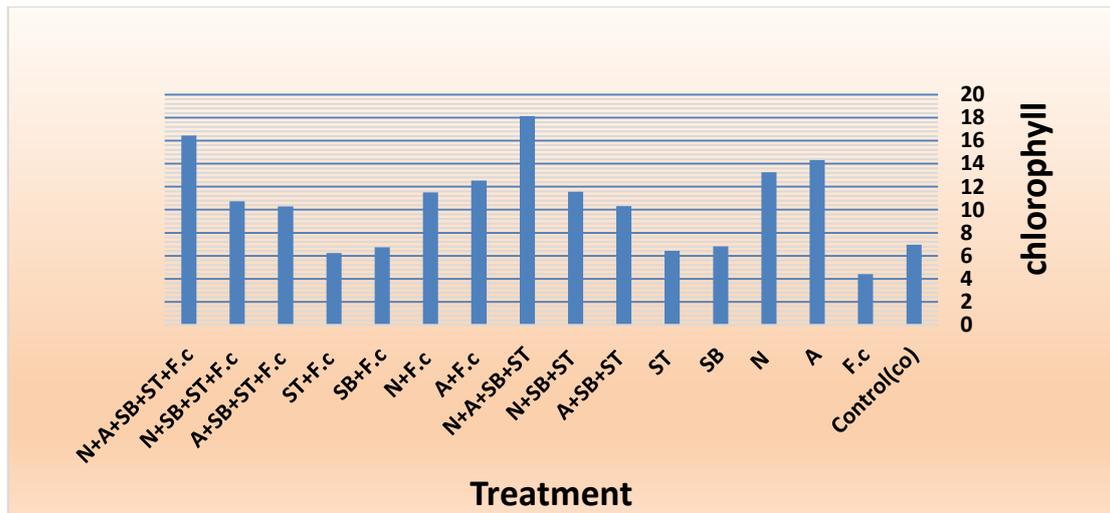
* each column represent the average of three replicates

* L.S.D. at (5%) 0.0.006403**

* *F. culmorum* (F.c), Algea (A), nano-algae (N), Local *Streptomyces* sp. (SB), Commercial *Streptomyces* sp. = (ST).

Figure 4: The effects of normal algae and nano-algae and some biological factors on the total chlorophyll content in the wheat plant**.**

These results are close to the study conducted by Al-Khlifawi (2017), which showed the effectiveness of different concentrations of nano-iron at 1, 2, 3, and 4 g.L^{-1}, which caused a significant increase in most studied shoot system growth parameters as well as achieving the highest percentage of nitrogen, protein, phosphorus, potassium, calcium, iron and carbohydrates. In addition, the activity of Catalyase and peroxidase increased in different proportions according to the concentration used.

Moreover, El-Argawy et al. (2017) confirmed the efficiency of 100 ppm concentration of magnesium oxide nanoparticles (MgO NPs), titanium dioxide nanoparticles (TiO2 NPs) and zinc oxide nanoparticles (ZnO NPs) in controlling the sugar beet (*Beta vulgaris*) root rot disease caused by *F. oxysporum*, f.sp betae, *Sclerotium rolfsii* and *Rhizoctonia solani*, which resulted in an increase in sucrose and total soluble solids (TSS) as well as total phenol content and the activity of the defense-related enzyme, polyphenol oxidase.

Whereas Alhamiri (2013) indicated the efficiency of using *Enterobacter cloacae*, *P. putida*, the biological suspension EM1 and the seaweed Sb29 extract in reducing the incidence and severity of infection with *F. oxysporum* f.sp. *lycopersici* under greenhouse and field conditions, and also improvement of the growth and production parameters of the crop, the effectiveness of biological control agents in inducing systemic resistance in tomato plant, increased the activity of peroxidase and PAL enzyme, plant content of total protein, phenols and chlorophyll. Furthermore, the study observed an increase in the effectiveness of defensive enzymes such as peroxidase (PO) and polyphenol oxidase (PPO) and increased nutrient absorption and chlorophyll content (Babu et al., 2015).

The efficacy of *Streptomyces* sp. may be due to the fact that it produces antibiotics, nutrients, and hydrolytic enzymes, and induces resistance and nitrous oxide production (Cohen et al.,

2005; Cohen and Mazzola 2006; Mahmoudi et al., 2011). In addition, *Streptomyces* sp. it may increase the inducible resistance of plant after infection with plant diseases and induces defense-related enzymes such as Peroxidase, Polyphenol oxidase and Phenylalanine ammonia-lyase as well as the accumulation of Phenolics and Flavonoids; it is also induces the plant to produce ethylene, jasmonic acid and Salicylic acid (Connerse et al., 2008; Pieterse et al., 2014), and significantly elevated the activities of peroxidase, phenylalanine, ammonia-lyase,-1,3-glucanase and chlorophyll levels in cucumber leaves (Zhao et al., 2012).

Seaweed extracts is one of the organic sources used in agricultural production, and is a supplement to fertilizers but not a substitute for it (Verkleij, 1992). These extracts are non-fertilizers that stimulate plant growth in low concentrations and contain macro and micro nutrients and have more than one group of growth-promoting substances such as cytokinins, auxins, vitamins, amino acids, organic acids and compounds similar to auxins (Spinelli et al., 2009) and polysaccharides such as laminaran, fucoidan, and alginate, which have a wide range of influence on vital activities in plants (Osman et al., 2010); in addition it is work to increase the chlorophyll content of leaves and also increase the processes of photosynthesis and respiration (Santana et al., 2006; (Basak, 2008, 2008) because it contains betaine, which has an important role in preventing the degradation of chlorophyll (Kuwada et al., 2006).

Algae extracts contain compounds that may act as an antioxidant for leaves due to the presence of alpha-tocopherol, betacarotene, niacin, thymine, and ascorbic acid, and through their role in increasing the activity of the enzymes superoxide dismutase, glutathione reductase and ascorbate peroxidase (A.O.A.C, 1970).

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