Influence of pH, pesticide and radiation interactions on the chemical composition of *Chlorella vulgaris* algae

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Abstract

The objective of this study was to stimulate the chemical content (protein, lipids, carbohydrates, gross energy, fatty acid, chlorophyll) of *Chlorella vulgaris* algae by pH manipulation, acetamiprid, and radiation exposure of *Chlorella vulgaris* algae. The results showed that the pH, acetamipride and radiation period could negatively affect the biochemical composition of *Chlorella vulgaris* algae.

Keywords: *Chlorella vulgaris*, protein, lipids, carbohydrates, fatty acid, radiation, acetamiprid

Introduction

Algae, which is a group of autotrophic that do not possess roots, stems or leaves, as well as not having fruits and flowers, as chlorophyll a is the main pigments in the photosynthesis process, and algae also possess several auxiliary pigments, including carotenoids, and xanthophyll (Al-Husseinawy, 2015). The study of chemical content is one of the methods of studying the nutritional value of microalgae (Fedekar et al., 2012).

Through the chemical composition data, can know the nutritional value of algae, especially since algae cultivation is characterized by its exposure to different effects that change the components of the single cell and thus change some components biomass, and therefore the nutritional value of it varies widely according to the conditions of cultivation such as pH, light, temperature, and the composition of the cultured medium (Lavens and Sargeloos, 1996).

Through the information about the chemical composition of algae, it is possible to know which of them has a higher nutritional value and thus benefit from it as a food source. It is known that there may be some kind of interaction between two or more factors, and that this interaction may affect, negatively or positively, many physiological activities of algae, in addition to its effect on the chemical content. They exist in environments under the influence of more than one factor, drawing attention to the interfering effects.

Pesticides are chemicals that eliminate unwanted living organisms and include many types depending on the target that works to eliminate them, the most prominent of which are insecticides that are used in the fight against harmful insects (EPA, 2011). In addition, it has a negative impact on the chemical content of the target and non-target organisms that are in contact with it due to the importance of algae in the food field as one of the important food sources, so the issue of water pollution with various types of pollutants, including pesticides,
has an impact on aquatic organisms, including algae, among the important topics that many countries have taken care of, and many laws have been put in place to limit and prevent the further pollution of water bodies. Pesticides are among the most prominent water pollutants due to lack of awareness and their poor disposal in different environments (Peterson et al., 1997).

There are many rays within the visible spectrum, some of them dangerous and others less dangerous, and the most prominent of which is the ultraviolet radiation (UV). It is an invisible electromagnetic ray that has different wavelengths and is emitted with the sun's rays. The effect of UV radiation on the biological and chemical side has become an important issue during the past three decades, especially after the changes in the ozone layer, as this layer covers the atmosphere and protects the neighborhoods from harmful UV rays, especially UV-B (Lesser et al., 2002). There are many effects of UV rays, including beneficial and harmful rays. Many researchers have studied the effect of these rays on neighborhoods and algae has occupied an important aspect of these studies because of its importance in the aquatic environment. Most research and studies have focused on the effect of UV-B rays being rays The most accessible to the earth’s surface, especially when there is damage to the ozone layer (Herrmann et al., 1996).

The pH was considered one of the important and influencing factors in many aspects, including its effect on the growth of neighborhoods and in particular on the growth of algae. As acidic and basic conditions may lead to the decomposition of some cellular compounds or the breakdown of some enzymes (Atlas and Bartha, 1986), and thus its effect on some chemical compounds, carbohydrates and fats, and some of the important processes that affect the chemical content and nutritional value of algae. The pH is also an important factor in the characteristics of the aquatic environment societies and the distribution of neighborhoods in it. It was observed that algae are present in environments with a pH close to the equivalent between (7.5-7). For example, it has been observed that the Spirulina prefers to grow in environments of a basic pH (8.5-9) in some of the world's lakes. In a study conducted by (Taraldsvik and Myklestad 2000), showed that the optimum pH for better growth and higher productivity in certain species of algae such as (Chlorella and Scenedesmus) was 8, while there was a decrease in the growth rate and lack of productivity at the pH 9.4. The present study aims to studying the effect of the interaction of studied factors (the combined effect), which are (pH and acetamiprid and then radiation period and acetamiprid) on the biochemical composition of Chlorella vulgaris algae.

**Materials and Method**

*Chlorella vulgaris* (Isolated by Kufa Agency of Scientific Training Services, 2020) liquid cultures were inoculated in BG11 medium (Rippka and Herdman, 1992) and incubated with shaking at 25°C under constant illumination light of 40 µmol photons m-2s-1 measured at the light incubator for 6 days. Two groups of glass containers were filled with 1L of BG11 and cultured with 100 ml of Chlorella vulgaris then one group was pH and acetamiprid manipulated as: pH=8 without acetamiprid (control), pH= 6.5 with 0.5ppm of acetamiprid (T1), pH=9 with 15ppm of acetamiprid (T2), and pH=10 with 25ppm of acetamiprid (T3)(n=3 for each treatment and control); the second treatment was to test the effect of Effect of UV radiation interaction with acetamiprid as: no UV-radiation without acetamiprid (Control, 0.5ppm of acetamiprid with wm -21 UV-radiation (T4), 15ppm of acetamiprid with wm-22 UV-radiation (T5), and 25ppm of acetamiprid with wm-23 UV-radiation (T6) (n=3 for each treatment and control).


According to Hadson and Hay (1989), a volume of 10ml was taken from the algal culture, centrifuged on 3000 rpm for 15 minutes then precipitate was taken and completed to 10ml then measured on two wavelengths (280 and 260) nm and the following equation was applied.

\[
\text{Protein content} = (1.55 \times A_{280}) - (0.77 \times A_{260})
\]

\(A\) : is the absorbance at the specified wavelength

The rate of algal protein digestion was estimated in vitro according to the method (Akeson and Slahmann, 1964) described in (Ventakaraman and Bercker, 1985), in which the algae powder was incubated with (pepsin) at a temperature of (37 °C) for a period of (3) hours then, he dealt with (pancreatine) for a period of (24) hours, and the measurements were made every (4) hours as the undigested protein was deposited by TCA (10%) and the undigested protein content was estimated and then the digestion rate. The total amount of carbohydrates in algae samples was estimated using the Phenol-Sulpheric acid method, according to Herbert et al. (1971). As for the fat content, Harborne (1984) method was used to extract the total lipids using the soxhlet and the solvent used was hexane. The chlorophyll concentration was calculated depends on Lorenzen formula (Vollenweider, 1974).

The results were statistically analyzed by using an analysis of variance (ANOVA test) to find out the significance of the effect of different treatments using the Minitab program and the significance of the differences of the averages was tested by using the (R .L. S. D) Revised Least Significant Differences Test (AL-Rawwi and Khalaf Allah, 1980).

**Results**

**A-Effect of interaction of pH with insecticide factors on the biochemical composition of Chlorella vulgaris algae:**

**Proteins:** From Fig.1 (A), it appears that the highest value of protein content in this study was recorded in samples exposed to (T2) which amounted to 40.13% dry weight compared to the control sample in which 64.20% dry weight and less were recorded. A value for protein was recorded in samples exposed to (T3), which amounted to 11.2% dry weight. As for the rate of digestion of proteins, the highest value 71.35% recorded a dry weight of the sample proteins presented to (pH9 + 15 mg / L pesticide), while the lowest value was recorded for the digestion of proteins in the proteins of samples with values of (T3).

**Carbohydrate:** It is noted from the results of Fig.1 (c) that the highest concentration of carbohydrates appeared at low values (T1) and 10.31% was dry weight, while the lowest carbohydrate content 2.7% was recorded dry weight at values (T3) compared to the control sample in which the highest concentration of carbohydrates 14.3% was recorded dry.

**Lipids content:** The behavior of Lipids was similar to the behavior of carbohydrates Fig. 2 as it was observed that the highest value of the concentration of Lipids at T1 was 5.9% dry weight where the difference compared to the control sample was few 6.4% dry weight, while The deficiency was severe when the algae was exposed to a high acetamiprid concentration (T3) as it recorded 2.1% dry weight .By observing the concentration of both proteins, carbohydrates and lipids , it appears that the highest total energy value was 312 kilocalories / kg at T2 while the lowest total energy value was recorded at (T3) and reached 95 kilograms price / kg.
**Chlorophyll content:** The results of Fig.1 (B) show that the highest concentration of chlorophyll was 1.1 mg/100ml at T1, while the lowest concentration was recorded at T3 and it was 0.2 mg/100ml it is a very small value when compared to the control sample (1.8 mg/100ml).

**Content of fatty acids:** Fatty acids of both saturated and saturated types recorded their highest value (T1, Fig 2). For example, palmitic fatty acid recorded its highest concentration at the mentioned values and reached (25.1%) total lipids, which is a value close to what appeared in the control sample, as its concentration (27.9%) reached total fat. As for the linoleic fatty acid, its highest concentration (17.1%) was total fat as well at the same previous values. A clear decrease was recorded compared to the control control as its concentration reached (22.3%) total lipids. Whereas, all fatty acids were observed at concentration, high pesticide values and pH (T3).

**Fig. 1:** the effect of pH interaction with acetamiprid on Protein (A), chlorophyll (B), Protein degradation rates (C), Carbohydrate (D), Lipids (E), and Total energy (F) contents of *Chlorella vulgaris* algae.
Fig. 2: the effect of pH interaction with acetamiprid on fatty acid contents of *C. vulgaris* (A: Palmatic, B: Stearic, C: Palmitoleic, D: Oleic, E: Linoleic, and F: Linolenic).

**B-Effect of acetamiprid and the radiation period interaction on the biochemical composition of Chlorella vulgaris algae**

**Protein content:** The effect of the acetamiprid and radiation factors has a clear effect on all chemical components of the studied algae Fig.3 (A, B). The highest concentration of proteins was recorded at T4 which reached (30.71%) dry weight and it was less than what appeared in the control sample (64.20%) dry weight and with an increase in the concentration to T5, the disappearance of proteins was observed in the studied sample. The highest rate of digestion of proteins was recorded at the same conditions T4 in which the highest concentration of proteins was recorded as it reached (50.27%) dry weight, but no value was recorded for the digestion of proteins at high concentration ns. acetamiprid and length of irradiation time (T6).

**Carbohydrate content:** The highest concentration of carbohydrates obtained was (10.51%) dry weight at T4. Then it was also observed that the carbohydrates disappear with an increase in the concentration of the acetamiprid and the exposure period (T6, Fig 3: C).
**Lipids content:** Results showed an estimate of the concentration of lipids in *Chlorella vulgaris* algae, Fig. 3(D). The highest fat concentration was at T4 (2.53%), which is less than what was recorded in the control sample (6.40%) dry weight and by increasing the concentration of the acetamiprid and the length of the radiation exposure period to (T5) no lipids was obtained under these conditions. According to what was mentioned, the highest total energy value was (242.05) kilocalories/kg, and no total energy value was recorded at T6 treatment.

**Content of fatty acids:** Variation of the effect of acetamiprid and radiation factors on the fat content Fig.4 of the fatty acids recorded their highest value at low concentrations and the short exposure period (T4) such as palmitic and linoleic acid which reached (5.2% and 3.1% respectively total fat, while the disappearance of other fatty acids was observed at these same concentrations, but with T5 and T6 we observed that all saturated and unsaturated fatty acids were disappeared significantly.

**Chlorophyll content:** The chlorophyll was of a similar pattern to the rest of the chemical components. Its highest content was recorded at low concentrations associated with short periods of radiation (T4). A concentration of (0.2) mg / 100 cm ³ was recorded, but with the concentration of the pesticide raised. To T5 treatment, the chlorophyll disappeared, and Fig. 3(F) was not observed.
Fig. 3: the effect of UV-radiation period interaction with acetamiprid on Protein (A), Protein degradation rates (B), Carbohydrate (C), Lipids (D), Total energy (E), and Chlorophyll (F) contents of *Chlorella vulgaris* algae.
Fig. 4: The effect of acetamiprid concentration and the radiation period factors on fatty acid contents of C. vulgaris (A: Palmatic, B: Stearic, C: Palmitoleic, D: Oleic, E: Linoleic, and F: Linolenic).

**DISCUSSION**

**A-Effect of interaction of pH and acetamiprid factors on the biochemical composition of Chlorella vulgaris algae**

Interference between factors in the environment or in the agricultural media may increase or reduce their effect on living organisms, and among these effects is their effect on the chemical content, especially of algae, where this interference appears in different forms depending on the interfering factors and their concentrations. It may appear in the form of an antagonistic effect either one reduces the harmful effect of the other cooperative (positive), meaning that the effect of the interfering factors increases in comparison with the effect of them individually. In lesser cases, the effect is equal, that is, it is equivalent to the sum of its effects individually. The results of the current study showed that the studied algae content of proteins after exposing them to the influence of pH and pesticide factors together, that there was a decrease in low values and concentrations (pH6.5 + 50 mg / L acetamiprid), and then there was an increase in the protein content at (pH9 + 15 mg / L acetamiprid) and after that there was a sharp decrease in the content of proteins when raising the pH values and concentrations of the acetamiprid. The reason may be mainly due to the effect of these two factors on the...
content of the nitrogen component, which also recorded a decrease in low values and concentrations, which was reflected in the decrease in the protein content being the primary component in building amino acids and proteins. When there was an increase in the content of this element, an increase in the content of proteins was observed, and after that a decrease in proteins occurred due to a decrease in the concentration of this element. Among those who support this view (Thompson and Calvert, 1994) if they noticed in a study that they had a strong correlation between the algal cell content of proteins and their nitrogen content. It was found that there is a close relationship between them. What caused the change resulting from these two factors, which caused a disturbance in the internal structure of the cells, then the cells started trying to adapt to the pH and the pesticide and started to recycle the proteins, which led to an increase in their content, but due to the failure of the cells and their inability to resist, especially with the increased exposure period that accompanied it. An increase in pH values and acetamiprid concentrations resulted in a clear decrease in protein content. Because the rate of digestion of proteins reflects the algae content of proteins. The highest protein digestion rate was recorded at the same values and concentrations in which the highest protein content was recorded (pH9 + 15 mg / L acetamiprid), as the protein digestion rate gives a good indication of the algae content of proteins, especially with the ease of extracting proteins due to the lack of cell wall and increased cell and container membrane destruction. On fats, which gives values close to what is inside the algal cell of the genus *Chlorella vulgaris* which increases the nutritional value of this algae.

The highest carbohydrate content was recorded in the current study at lower pH and pesticide concentrations (pH 6.5 + 0.5 mg / L acetamiprid), and then the carbohydrate content gradually decreased with increasing pH values and pesticide concentrations. The reason may be the impact of these two factors on the process of photosynthesis. If a decrease in the rate of this process is observed when algae is grown in a pH medium that is far from optimal and appropriate values accompanied by the harmful effect of the acetamiprid on the photosynthesis system and consequently a decrease in the carbohydrate content of the cell with an increase in the values and concentrations used, or it may be that the reason is the effect of these two factors on the enzymes responsible for the production process of carbohydrates (photosynthesis), which in turn is reflected in the content of carbohydrates. This is consistent with the note (Goldman et al., 1982) who confirmed damage to photosynthetic enzymes when exposing algae to certain concentrations of acetamiprid and the lack of appropriate conditions for the cultivation process, including the pH value, while the reason may also be due to a decrease in biomass. Algae is caused by a decrease in its growth rate due to the lack of appropriate conditions for the cultivation process, including the pH and the presence of toxic substances such as acetamiprid that reduce the effectiveness of the algae, which is reflected in the algae content of the chemical compounds, including carbohydrates.

From observing the results of the current study, it appears that the studied algae content of lipids has gradually decreased with increasing pH values and acetamiprid concentrations used. This may be explained by the same reason that led to a decrease in the carbohydrate content, which is the biomass and the growth rate of algae when using these two factors, which led to a shortage of organic compounds, including fatty substances or it may explain the deficiency based on the decrease in the content of fatty acids. That lipids is formed due to the effect of these two factors, especially fat-soluble acetamiprid and their interaction with the calculated values of fats, or perhaps the reason is due to the occurrence of a type of chemical reaction between the acetamiprid compounds and the solvents used in the extraction process, which affects the cell content of the fatty substances (Abubakar et al., 2012). It has been shown that the highest total energy value obtained was at (pH9 + 15 mg / L acetamiprid) and the reason...
may be due to the high content of proteins at these values, which was reflected in the total energy calculation compared to the content of both fats and carbohydrates.

The behavior of chlorophyll was similar to that of most chemical compounds within the studied algae body. The results showed that the chlorophyll content decreased with increasing pH values and acetamiprid concentrations. The reason may be due to a shortage of magnesium, which enters into the synthesis of chlorophyll. Or perhaps the reason for the deficiency is the harmful effect of these two factors on the chlorophyll tincture, which leads to its gradual decomposition, whether with increasing the exposure period for the workers or by increasing the values and concentrations used. This leads to the joint effect of these two factors on the growth of algae and its chemical compounds. As there was a lack of most of the chemical structures inside the moss's body, including the chlorophyll content. This is what he referred to (Atlas and Bartha, 1986). While the reason may be the occurrence of inhibition or damage to the enzymes responsible for the manufacture of this dye and thus a gradual decrease in their content. By observing the results, we note that the effect of the interaction of the pH and acetamiprid factors together had an effect on the chemical content of algae.

The results from the current study indicate a decrease in the studied algal content of fatty acids by increasing the values used for the studied workers (pH and pesticide). The reason was attributed to the inadequate conditions of the agricultural milieu for the construction and formation of fatty acids, which led to a gradual decrease in their content until it was observed that most of the saturated and unsaturated fatty acids disappeared with increasing pH values and uv radiation concentrations, or the reason for the deficiency may be the effect of these two factors on Inhibition of the work of manufacturing enzymes as a result of damage to the composition of these enzymes (which are of a protein nature), which was reflected on the content of fatty acids and among the most prominent who supported this view (Dong et al., 2002).

B-Effect of acetamiprid and the radiation period interaction on the biochemical composition of Chlorella vulgaris algae

Due to the exposure of the studied algae to the effect of two types of pollutants, which are the pesticide and the radiation, it has a negative effect on most of the chemical components of the algae cells, including the content of proteins whose highest concentration was recorded at (0.5 mg /L acetamiprid + wm-2 l radiation). The algae is unable to tolerate the high concentrations of the acetamiprid with the rays for long periods. The reason may be due to a failure in the mechanisms of making proteins due to the damage of most of the organisms and enzymes responsible for this process, which is reflected in the protein content, or perhaps the reason is due to the algae's inability to build organic compounds and thus its death gradually as a result of its intolerance to inappropriate conditions and severe pollution. Consistent with what he indicated (Franklin and Forster, 1997) who confirmed that most algae, could not resist the toxic effect resulting from the impact of two types of strong pollutants, which are acetamiprid and radiation . Obviously, the highest rate of digestion of proteins was recorded at the same values in which the highest protein content was recorded, as the results indicate that there are no values for digestion at other concentrations and high periods of radiation, and this reinforces the opinion that the rate of digestion of proteins is an indication of the extent of protein availability and its ratio in Moss body or protein damage due to the effect of radiation (Sheath and Hambrock, 1990).

Carbohydrates showed more tolerance to when exposed to both pesticide and radiation factors, as results indicate a decrease in carbohydrate content by increasing pesticide
concentrations and irradiation periods until carbohydrate disappearance is observed at (25 mg / L acetamiprid + wm-2 3 radiation). In the tolerance of carbohydrates to the algae cells forming carbohydrate materials as a kind of adaptation to the new conditions, but with increasing concentrations, most of the enzymes responsible for the manufacture of carbohydrates are damaged, which ultimately leads to their disappearance or the reason may be that the photosynthesis process showed more tolerance to the inappropriate conditions but with increased concentrations of acetamiprid and radiation, complete damage to the photovoltaic systems occurs, which leads to stopping this process, or it could be that the moss is unable to continue to grow and build its cells due to its exposure to high concentrations of toxic substances or heavy metals caused by acetamiprid in addition to the effect of radiation that works to break down most of the internal chemical components, which in turn is reflected on the content of algae and its inability to communicate and then gradually die (Becker, 2007).

The behavior of fats was similar to the behavior of the rest of the components, as a decrease in the fat content appeared at (0.5 mg / L acetamiprid + 1 wm-2 radiation), and then the disappearance of fatty substances at concentrations and high periods. One of the most prominent reasons is what has been mentioned, which is the failure of cells to continue building most The organic components necessary for their continuation leading to the death of algae, or perhaps the reason is the absence of any type of fatty acid at these concentrations and values, which leads to a deficiency in one of the most important materials needed for the manufacture of fats, or the reason may be the breakdown of most enzymes and the destruction of all structures Necessary for the process of manufacturing fatty substances while the most acceptable reasons were the dissolution of most insecticides (especially those that are soluble in fats) in the fats present in the studied algae, which affects the percentage and calculations of the percentage of fatty substances due to the change in their composition, in addition to the negative effect of radiation, especially with Increasing the length of exposure, which leads to a lack of fatty substances as well as fatty acids (this is what has been observed through the results of the current study) and is a prominent supporter of this view (Rajagopal et al., 1998), and based on the results show that it is normal for the total energy value obtained from exposing Chlorella vulgaris algae to acetamiprid and radiation workers to be very few, as it reached (242.051) kilocalories / kg at low acetamiprid concentrations and the short radiation period due to the limited algae content of its basic organic compounds (Murray, 2003).

It is clear from what has been reached from the results that the chlorophyll content has also suffered from the influence of the acetamiprid and radiation factors, which led to a decrease in its content with increased pesticide concentrations and irradiation period, and the reason may be due, as previously mentioned, to the failure of algal cells to continue to perform their vital activities among them is the manufacture of chlorophyll or perhaps the reason is a damage to the enzymes responsible for making this dye, or it could be due to a shortage of raw materials needed to build chlorophyll, including a magnesium component, which leads to a decrease in the amount of raw materials and thus a lack of the dye produced, has been confirmed. Lesser et al. (2002). It is that in the event of a shortage of magnesium due to the effect of radiation, it leads to a decrease in the amount of chlorophyll produced (Rajagopal et al. 1998). The opinion that explains that most of the compositions due to the effect resulting from the treatment of algae by the radiation factor in addition to the toxic effect due to acetamiprid leads to the weakening of cells and thus their death.

It is shown by observing the current results of a decrease in the content of fatty acids at the low concentrations of the acetamiprid and the short radiation period after which it was observed that all the fatty acids disappeared with an increase in the concentrations of the pesticide and long radiation periods. The reason may be due to damage to the internal
structure of the algae and its inability to manufacture any of the chemical components, or perhaps the reason for this is the inhibition of these two factors for all enzymes responsible for the biosynthesis processes of organic components and compounds, or the reason may be the algae inability to resist the effect. The harmful effects of the interference of these two factors. The results of the current study showed an increase in the toxic effect of the acetamiprid and radiation factors together, as their effect was cooperative (positive), as it was observed to increase the damage when the algal cells were exposed to these two factors combined as a result of the increased toxicity of these agents throughout the exposure period.

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