Plant Archives Vol. 20, Supplement 2, 2020 pp. 1050-1055 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF SPRAYING WITH ORGANIC AND BIO-FERTILIZERS ON SOME GROWTH CHARACTERISTICS OF SUNFLOWER PLANT (*HELIANTHUS ANNUUS* L.)

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Abstract

A field experiment was carried out in the fields of the College of Agricultural Engineering Sciences, Baghdad University in the spring season 2019 according to the Randomized Complete Block Design RCBD in factorial experiments to identify the role of organic and biological fertilizers in some growth characteristics of the sunflower Akmar variety. The experiment included two factors, the first included spraying the organic matter (Bilirubin) in three concentrations 1, 2 and 3 ml L^{-1} , as well as the comparison treatment (without spraying), while the second factor included spraying the dry yeast powder with three concentrations 1, 2 and 3 g L^{-1} as well as the comparison treatment (without spraying). The two substances (Bilirubin and yeast) were sprayed in two stages, the first in the stage of four real leaves (75% of plants) and the second the beginning of the appearance of flowering buds (75% of the plants). The results of the study showed the superiority of spray plants with a concentration of 2 ml L^{-1} of bilirubin in all studied growth characteristics except the number of leaf characteristics. As for yeast, the growth characteristics increased with increasing concentrations of spraying and reached their maximum average when spraying with a concentration of 3 g L^{-1} of yeast except for the leaf chlorophyll content, sprayed plants were superior at a concentration of 2 g L^{-1} over all other concentrations.

Keywords: bilirubin, yeast, growth characteristics, sunflower

Introduction

The *Helianthus annuus* L. sunflower ranked the second after soybeans in the most popular oil health crops in the global commercial markets, where the oil percentage in its seeds reached 55%, which is one of the important oils because it contains unsaturated fatty acids as well as antioxidants. However, its productivity is still below the required level compared to world countries, and this comes from several reasons, including not adopting modern technologies in the field of crop management. The nutrient management is one of the most important fields of management for farmers, as the intensive use of mineral fertilizers and various methods it reduced soil fertility, deteriorating its physical and chemical properties and exacerbating pollution problems. As well as, the coloration of water and food with the remains of these fertilizers and the resulting harmful effects on human and animal health.

Therefore, researchers have recently focused on the use of safe alternatives and environmentally friendly that increase production and reduce these losses and are economically feasible, including the use of organic and biomaterials. Furthermore, the concept of organic agriculture is receiving increased attention in many countries because of increased food and health awareness, and the organic food market is expanding rapidly and this expansion makes it possible for farmers to sell their products at different prices due to increased consumer demand (Gopinath *et al.*, 2008; Homedan, 2006). Organic matter has been used for a long time to improve soil health and provide the plant with nutrients and used various sources of organic waste, including farm fertilizer, poultry fertilizer, sheep and cow waste, city waste and industrial waste such as sugar, cotton, etc. (Ibrahim *et al.*, 2008). However, some materials are not used, including the Bilirubin, which is an organic substance from animal sources similar to the dye of Phytocobilin and the dye of Phytochrome, which are sensitive photoreceptors found in many flowering plants that enter in the regulation of many vital processes, including flowering date, seed germination. In addition to seedling elongation, size, shape, a number of leaves, and formation of chlorophyll, as well as being Bilepigments that consider as one of a group of compounds called antioxidants (wlllows *et al.*, 2004; Baranano *et al.*, 2002; Sedlak *et al.*, 2009). Yeast is a vital fertilizer that supports the directions of organic agriculture and a source of many growth materials including, vitamins (B1, B2, B3, B6, and B12) and a source of Auxins, Gibberellic, and Cytokinines, as well as enzymes, proteins and mineral nutrients (Wanas 2006; Sacakli *et al.*, 2013). Consequently, it promotes plant growth and thereby increases yield and quality (Pu-Guixin *et al.*, 2008; Vessey 2003; Shevanande, 2008). Moreover, its role as a biological fertilizer in reducing the severity of using chemical fertilizers and one of the alternatives for clean agricultural production (Agamy *et al.*, 2013). For the reasons mentioned above and the lack of studies on using the Bilirubin on crops and the absence of a study on a sunflower that involves spraying this substance or yeast powder. Thus, this study was carried out to identify the response of some growth characteristics of the sunflower plant, Akmar variety through the spraying of Bilirubin and yeast powder, and determining the best concentration of both substances and their interaction.

Materials and Methods

A field experiment was carried out in the fields of the College of Agricultural Engineering Sciences, Baghdad University in the spring season 2019 according to the Randomized Complete Block Design (RCBD) in factorial experiments to identify the role of organic and biological fertilizers in some growth characteristics of the sunflower variety Akmar. The experiment land was plowed by two perpendicular plows using the moldboard plow and harrowed with Rotovater plows then leveled and divided into three replicates by 16 experimental units per replicate, where the number of experimental units reached 48 units with an area of 9 m^2 (3 * 3 m). The experimental unit included five lines with a distance of 75 cm and between one plant and another 20 cm to obtain a plant density of 66,666 $plants.ha^{-1}$. Furthermore, the experiment included two factors, the first included spraying the Bilirubin organic matter (sourced from cows) with three concentrations 1, 2 and 3 ml L^{-1} , as well as the comparison treatment (without spraying), while the second factor included spraying dry yeast powder with three concentrations 1, 2 and 3 g L^{-1} , as well as the comparison treatment (without spraying). The two substances (Bilirubin and yeast) were sprayed in two stages, the first in the stage of four real leaves (75% of plants) and the second at the beginning of the appearance the flowering buds (75% of the plants) and was sprayed at night using a knapsack sprayer with liquid soap as a surfactant. Besides, the experiment land was planted on 25/29/2019 by placing 3-5 seeds in one hole at a depth of 4-5 cm, and the defoliation was carried out to one plant two weeks after emergence. The Nitrogen fertilizer was added in the form of urea (N% 46) at a rate of 360 kg N ha in two batches, the first was in the stage of four real leaf emergence and the second batch in the stage of beginning appearance of flower buds (Al-Rawi, 2001). Finally, the cutworm (*Agrotis ipsilon*) was controlled with Morisban4 at a rate of 50 ml per 50 liters of water, and all crop service operations were carried out according to the plant need.

Studied growth Characteristics

Upon completion of flowering, 5 plants were randomly selected from the three center lines of each experimental unit to study the following vegetative growth characteristics:

- **Average plant height (cm):** The plant height was calculated from the soil surface to the base of the disk as an average of five plants.
- **Stem diameter (mm):** The stem circumference was calculated from the center of the stem using the Vernier device.
- **The number of leaves:** The total number of leaves per plant was calculated starting from the first green leaf at the surface of the soil to the last leaf on the plant.
- **The leaf area (cm^2):** It was calculated by measuring the maximum width of the plant leaves, then the total width squares were multiplying by 0.65 (El-Sahooki and El-Dabas 1982).
- **Leaf area index:** It is calculated from dividing the leaf area by the area occupied by the plant (Hunt, 1982).
- **Chlorophyll content (mg/g fresh weight):** The total chlorophyll was estimated by the Goodwin method (1976).

Results and Discussion

Plant Height

The data in Table 1 indicated that there was a significant difference in the characteristic of plant height with the effect of spraying Bilirubin and yeast and the interaction between them. As the sprayed plants exceeded with a concentration of 2 ml L^{-1} of Bilirubin by giving the highest average plant height reached 240.7 cm compared to concentrations 0 and 1 and 3 ml / L, which gave averages reached 226.4, 238.3 and 212.0, respectively. The two concentrations of 2 and 1 ml L^{-1} did not differ significantly between them, while it can be observed that when the Bilirubin concentration increased to 3 ml L^{-1}, the plant height decreased significantly and reached a minimum average of 212.0 cm. Perhaps, the reason for the plant height increasing was this substance is organic and it is a natural antioxidant similar to the phytocobilin and Photochrome pigments, which are sensitive receptors present in many flowering plants that regulate many processes, including seedling elongation (Wlllows *et al.*, 2004; Cary *et al.*, 2009). Especially, since the spraying took place in two stages which are at 4 real leaves and the beginning of forming flower buds. The first stage is the beginning of vegetative growth (stem and leaves), while the second stage where the stem still in the growth stage in the sense that spraying in these stages coincided with the vegetative growth of the plant (stem). In addition, it may have led to increase its elongation, these results are consistent with (Nasser allah *et al.*, 2018; Zeboon, 2019; Baqir and Zeboon, 2020) findings. From the same Table, it can be observed that the maximum plant height was achieved when spraying the plants by yeast at a concentration of 3 g/L by 232.3 cm with an increase of 4.92% compared to the control treatment 0 g L^{-1} that gave the lowest average for this characteristic by 221.4 cm. The reason for this increase may be attributed to the effect of spraying yeast because it can produce growth-stimulating materials such as Auxins, Gibberellic acid, Cytokinines and some vitamins (EL-Kholy *et al.*, 2007), which by their physiological action are stimulated the elongation and cell division. Besides, they contain amino acids and some mineral elements such as nitrogen and other mineral elements that stimulating growth, this result is consistent with what (Ahmed *et al.*, 2011; Hammad and Ali, 2014; Baqir, 2018) reached, that indicated to the vital role of yeast in increasing plant height for different plants. As for the interaction, it was significant among the two factors and it can observe from the same Table the response of this characteristic was different from increasing concentrations of spraying yeast when increasing concentrations of Bilirubin itself. As the response of this characteristic was similar at concentration 0 and 3 g L^{-1} of yeast, where the plant height increased with increasing the Bilirubin concentration from 0 to 1 ml/L. Then, there was a decrease in this characteristic at the concentration of 2 ml L^{-1} but it was not significant, while it reached to the significance level when increasing the concentration to 3 ml L^{-1} from spraying the Bilirubin. While at the two concentrations of 1 and 2 g L^{-1} of yeast this characteristic exhibited behavior different from its behavior at concentrations 0 and 3 g L^{-1}. The plant height increased a direct increase by increases the concentrations of spraying Bilirubin to concentration 2 ml L^{-1}, and then there was a significant decrease in this characteristic at concentration 3 ml L^{-1}

Table 1 : Effect of Bilirubin spraying and yeast concentrations and their interaction on plant height (cm) for the spring season 2018-2019.

Bilirubin concentrations ml L^{-1}	Yeast concentrations g L^{-1}				Average
	0	1	2	3	
0	206.0	227.4	230.3	242.0	226.4
1	232.6	236.8	238.0	245.7	238.3
2	229.7	241.6	246.7	244.9	240.7
3	217.1	222.7	211.5	196.7	212.0
L.S.D	12.39				6.20
Average	221.4	232.1	231.6	232.3	
L.S.D	6.20				

Stem diameter

The results in Table 2 indicated that the diameter of the stem was significantly affected when spraying both Bilirubin and yeast, while the interaction between them was not significant in this characteristic. This Table also showed that the highest average diameter of the stem was 3.65 cm, recorded at the plants treated by the concentration of 2 ml L^{-1} of Bilirubin with significant differences from the other concentrations, while the lowest average for this characteristic was recorded by 3.38 cm at control treatment. This may be due to the fact that these bili pigment or Bilirubin are one of a group of compounds called anti-oxidants that inhibiting damage to the cellular genetic system (DNA) (Liuxt, 2002; Baranano *et al.*, 2002; Wangner *et al.*, 2007; Sedlak *et al.*, 2009). Furthermore, in terms of biochemistry, it is a protein (Cary *et al.*, 2009) and perhaps, it spraying on plant parts improves the biological activities taking place in it and organizing them, which are reflected in increasing the cell division and expansion, and then its growth (stem diameter). Maybe the concentration of 2 ml L^{-1} was sufficient for doing this increase, the stem diameter increased gradually and exponentially with the concentrations increasing of spraying yeast powder. It reached a maximum average at a concentration of 3 g L^{-1} amounted to 3.59 cm without significant difference with the concentrations 1 and 2 g L^{-1}, which gave an average of 3.50 cm and 3.55 cm respectively. Whereas, the lowest average for this characteristic was recorded at 3.30 cm without spraying yeast powder (comparison treatment). The reason for increasing this characteristic when spraying yeast powder maybe because it contains plant hormones (Auxins, Gibberellic, Cytokinines), which play an important role in the growth and differentiation of plant tissue. As well as many enzymes such as Sucrase, Maltase, Lactse, Reductase, Carboxylase, Hexosephosphatse and metabolic derivatives like fats, proteins. In addition to amino acids such as Glycine, Valine and others that promote metabolism (Dinkha and AL-Khazragii, 1990; EL-Tohamy *et al.*, 2008; Sacakli *et al.*, 2013; Abbas 2013). Moreover, mineral elements such as iron, potassium, magnesium, calcium, phosphorus, zinc, manganese and some nucleic acids such as Adenine, Guanine, and vitamins (Vitamin B group) (AL-Khfagi, 1990 and Wanas, 2006) which they have a role in stimulating division and cell expansion and increasing its thickness (stem diameter). This result was consistent with (AL-Samaraee *et al.*, 2011), they concluded that the use of yeast with several concentrations increased the diameter of the main stem of the marigold plant without significant differences between the concentrations.

Table 2 : Effect of spraying Bilirubin and yeast concentrations and their interaction on stem diameter (cm) for the spring season 2018-2019.

Bilirubin concentrations ml L^{-1}	Yeast concentrations g L^{-1}				Average
	0	1	2	3	
0	3.21	3.37	3.44	3.48	3.38
1	3.28	3.40	3.55	3.59	3.46
2	3.42	3.65	3.75	3.79	3.65
3	3.28	3.57	3.47	3.49	3.45
L.S.D	N.S				0.179
Average	3.30	3.50	3.55	3.59	
L.S.D	0.179				

Number of leaves

The results in Table 3 indicate that there were no significant differences in the number of leaves with the effect of spraying Bilirubin and yeast powder, as well as the interaction between them, and the reason for this may be because the number of leaves is a genetic trait and rarely affected by environmental factors.

Table 3 : Effect of spraying Bilirubin and yeast concentrations and their interaction on the number of leaves (leaf $plant^{-1}$) for the spring season 2018-2019.

Bilirubin concentrations ml L^{-1}	Yeast concentrations g L^{-1}				Average
	0	1	2	3	
0	22.97	24.60	25.00	24.27	24.21
1	25.17	26.50	24.13	25.33	25.28
2	23.18	24.70	23.91	25.35	24.28
3	22.87	25.13	23.13	23.07	23.55
L.S.D	N.S				N.S
Average	23.54	25.23	24.04	24.50	
L.S.D	N.S				

Leaf area

The leaf area differed significantly with the effect of spraying different concentrations of Bilirubin and yeast and the interaction between them as shown in Table 4. As plants sprayed with a concentration of 2 ml L^{-1} of Bilirubin gave, the highest average of leaf area of 1.107 m^2 with an increasing percentage of 20.85%, 14.71%, and 0.81% compared to plants sprayed with concentrations 0, 1 and 3 ml L^{-1} of Bilirubin, and the concentrations of 2 and 3 ml L^{-1} did not differ significantly between them. This result may be attributed to the role of these pigments (Bilipigment), which is similar to the Phytocobilin pigment and Phytochrome pigment that are sensitive photoreceptors present in most of the leaves. Furthermore, they are involved in regulating many vital processes, including the size and shape of the leaves, the formation of chlorophyll. In addition to being one of the antioxidant compounds that it works to remove the free radicals formed from the process of carbon fixation, then increase the efficiency of this process and increase its products, which are used to increase growth (leaf area). These results are consistent with the (Nasserallah *et al.*, 2018; Zeboon, 2019; Baqir and Zeboon, 2020) findings, which they indicated the role of this substance in increasing the leaf area of wheat and maize. As for the yeast powder, it can be observed from the same Table that there was a clear response to this trait by increasing concentrations of spraying yeast. The sprayed plants exceeded at a concentration of 3 g L^{-1} significantly by giving the highest average reached 1.138 m^2 compared to plants sprayed with concentrations 0, 1 and 2 g L^{-1}, which recorded averages of 0.808, 1.056, and 1.084m^2, respectively. The concentrations of 2, 3 g L^{-1} and 2, 1 g L^{-1} did not differ significantly between them, and the increase in the leaf area due to the effect of spraying with yeast powder may be due to its containing stimulants for growth such as Thiamin, Riboflavin, Niacin. As well as, Folic Acid, mineral elements, and Enzymes involved in carbon fixation processes (Nagoda 1990). This may lead to an increase in the effectiveness of this process and the increase of its products, which contribute to increased plant growth (leaf area), this result is consistent with (Al Shammary *et al.*, 2017; Al-Ani and Al-Obeidi 2017), when increased the leaf area during using yeast for potatoes and maize respectively. The behavior of this characteristic differed with the different concentrations of yeast powder and when increasing concentrations of spraying the Bilirubin, as it can be observed that at the concentration of 2 g L^{-1} of the yeast powder, the leaf area increased exponentially with increasing concentrations of spraying the Bilirubin. However, at concentrations 0 and 1 g L^{-1}, the leaf area increased until the concentration 2 ml L^{-1} then this characteristic decreased significantly during increasing the concentration of Bilirubin to 3 ml L^{-1} without spraying yeast, and was not significant at concentration 1 g L^{-1}. While at concentration 3 g L^{-1} of the yeast, the characteristic decreased when the concentration of Bilirubin increased, but it was not significant.

Table 4 : Effect of spraying Bilirubin and yeast concentrations and their interaction on leaf area (m^2) for the spring season 2018-2019

Bilirubin concentrations ml L^{-1}	**Yeast concentrations g L^{-1}**				**Average**
	0	**1**	**2**	**3**	
0	0.613	0.868	0.959	1.226	0.916
1	0.795	0.926	0.969	1.171	0.965
2	0.996	1.254	1.089	1.090	1.107
3	0.827	1.178	1.321	1.065	1.098
L.S.D	0.1497				0.0749
Average	0.808	1.056	1.084	1.138	
L.S.D	0.0749				

Leaf area index

The leaf area index was significantly affected at spraying Bilirubin and yeast, and the interaction between them as shown in Table 5. The plants sprayed with a concentration of 2 ml L^{-1} of Bilirubin gave the highest average of the leaf area index of 7.38 compared to concentrations 0, 1 and 3 ml L^{-1} that recorded averages reached 6.11, 6.44, and 7.32 respectively. As well as, the concentrations 2 and 3 ml L^{-1} did not differ significantly between them; the significant increase in this characteristic was due to the superiority of the plants sprayed with this substance and by the same concentration 2 ml L^{-1} significantly in increasing the leaf area as in Table 4, which reflected in increasing its index. As for the yeast, the same Table showed that the plants sprayed with the yeast powder by a concentration of 3 g L^{-1} were significant superior by giving the highest average of 7.59 compared to the concentrations 0, 1 and 2 g L^{-1} be because spraying yeast with a concentration of 3 g L^{-1} resulted in an increase in the leaf area and then this led to an increase in its index. As for the interaction between the two factors, which was significant in this characteristic. It can be observed from the same Table the behavior of this characteristic differs with the different spraying yeast concentrations when increasing concentrations of spraying the Bilirubin, as this characteristic exhibited similar behavior at without spraying yeast by a concentration of 1 g L^{-1}. The leaf area index increased by increasing concentrations of spraying Bilirubin from 0 to 2 ml L^{-1} exponentially until the concentration 2 ml L^{-1} then this characteristic decreased when increasing the concentration to 3 ml L^{-1} of the same substance, which was significant without spraying yeast, and not significant at concentration 1 g L^{-1}. While the behavior of characteristic was different when increasing spraying yeast concentrations to 2 and 3 g L^{-1}, as the leaf area index increased exponentially and continuously by increasing concentrations of spraying Bilirubin from 0 to 3 ml L^{-1} at concentration 2 g L^{-1} of yeast. Whereas, it decreased gradually when the yeast concentration increased to 3 g L^{-1} with increased concentrations of Bilirubin.

Table 5 : Effect of spraying Bilirubin and yeast concentrations and their interaction on leaf area index for the spring season 2018-2019.

Bilirubin concentrations ml L^{-1}	**Yeast concentrations g L^{-1}**				**Average**
	0	**1**	**2**	**3**	
0	4.09	5.79	6.39	8.17	6.11
1	5.30	6.17	6.46	7.81	6.44
2	6.64	8.36	7.26	7.26	7.38
3	5.52	7.85	8.81	7.10	7.32
L.S.D	1.00				0.50
Average	5.39	7.04	7.23	7.59	
L.S.D	0.50				

Leaf chlorophyll content

From the results shown in Table 6, it can be observed that the leaf content of total chlorophyll was significantly affected by spraying Bilirubin and yeast powder and their interaction between them. As the increasing percentage in the total leaves content of chlorophyll reached to 17.12% when spraying Bilirubin at a concentration of 2 ml L^{-1} compared to without spraying treatment which recorded the lowest average for this characteristic was 39.94 mg g fresh weight, and the concentration 2 and 3 ml L^{-1} did not differ significantly between them. The increase in the chlorophyll content during the spraying of Bilirubin may be attributed because the added organic matter (Bilirubin) works to improve the plant condition when it performs vital metabolic processes. It is an antioxidant that prevents damage because it is similar to the chlorophyll pigment in terms of composition, as it consists of a series of four rings of Pyrrole as in chlorophyll (Baranano, 2002 and Sedlak *et al.*, 2009), spraying with this substance may have increased the leaf content of chlorophyll. As for the yeast powder, the plants sprayed with yeast powder by a concentration of 2 g L^{-1} was superior by giving the highest average reached 47.73 mg / g fresh weight compared to the other concentrations 0, 1 and 3 g L^{-1}, which recorded an average of 42.64, 44.94 and 42.88 mg/g fresh weight respectively. The increase in the chlorophyll content when spraying yeast powder is because the yeast contains the hormones that stimulate the formation of chlorophyll, and from these hormones are Cytokinin, which is necessary for the emergence of the chloroplast, and is the cornerstone for the formation of chlorophyll. As well as, it containing nitrogen, which is involved in the formation of chlorophyll, and this may have led to an increase in the plant content of Chlorophyll, these results are consistent with (EzzEL-Din and Hendawy 2010; Baqer, 2018) results. The interaction between the two factors in this characteristic was significant, and it can be observed from the same Table the behavior of this characteristic differs at without spraying yeast from the other concentrations and when increase the Bilirubin concentrations. As the increase was exponentially and continuous with increasing the Bilirubin concentrations, while this increase was at concentration 2 and 3 g L^{-1} of yeast up to a concentration of 2 ml L^{-1} of Bilirubin. Then this characteristic decreased when the concentration increased to 3 ml L^{-1}, whereas the chlorophyll content fluctuated between the increase and decrease at the concentration of 1 g L^{-1} of yeast with increasing concentrations of spraying Bilirubin.

Table 6 : Effect of spraying Bilirubin and yeast concentrations and their interaction on chlorophyll content (mg g fresh weight) for the spring season 2018-2019

Bilirubin concentrations ml L^{-1}	**Yeast concentrations g L^{-1}**				**Average**
	0	**1**	**2**	**3**	
0	36.13	40.47	44.10	39.07	39.94
1	40.26	46.00	49.74	44.01	45.00
2	44.81	45.40	50.86	46.04	46.78
3	49.36	47.90	46.21	42.40	46.46
L.S.D	2.872				1.436
Average	42.64	44.94	47.73	42.88	
L.S.D	1.436				

Conclusions

it can be concluded from this study the possibility of spraying sunflower plant with bilirubin by a concentration of 2 ml L^{-1} and yeast by a concentration of 3 g L^{-1}, because they enhanced the all growth characteristics in addition to the fact that the two are natural and safe substances for the plant and the environment and economically feasible.

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