Effect of Fungicides on Enzymatic Activity and Oxidative Stress Index for Three Species of Brassicaceae Plants

Dhafir A. Jameel

Iraq, University of Al-Qadisiyah, College of Education, Department of Biology
Dhafir.altaweel@qu.edu.iq

Abstract:
A pots experiment was conducted under the conditions of Al-Diwaniyah Governorate climate in a special nursery was specially constructed for the experiment during the autumn season (2017 - 2018) in order to determine the effect of various fungicides concentrations spraying on enzymatic activity and growth indicators for three species of Brassicaceae plants. The design of the experiment was randomized complete blocks (RCBD) in a factorial arrangement consisting of two factors with three replicates (3 plant species × 11 fungicides concentrations × 3 replicates); the first factor of three species of Brassicaceae plants: Arugula (Eruca sativa Mill.), Radish (Raphanus sativus L.) and Garden cress (Lepidium sativum L.), while the second factor included eleven concentrations of fungicides (Bayfidan, Tapsen 50 and Sigal). The mean of the treatments was compared with a significant difference in the use of less significant difference (LSD) when referred to significant effect at $P \leq 0.05$ probability level, the results showed the superiority of radish plant on arugula and garden cress by statistically significant in enzymatic activity of α-amylase and protease. While the increase of enzymatic activity of polyphenol oxidase and concentration of MDA for arugula plant compared to radish or garden cress, and the latter had the most catalase activity and significant in terms of arugula and radish. The significant effect of triple mixture of fungicide treatment (Bayfidan + Tapsen 50 + Sigal) in increasing the enzymatic activity of the majority of enzymes and MDA, except for the enzymatic effect of catalase which was reduced by the treatment of different fungicides concentrations. Significant interference between plants and fungicides returned with positive statistical results by recording the treatment with the triple mixture of fungicides (Bayfidan + Tapsen 50 + Sigal) achieved the highest enzymatic activity of polyphenol oxidase and protease, as well as MDA concentration in arugula plant.

Keywords: Fungicides; Enzymatic activity; Oxidative Stress; Brassicaceae.

1. Introduction:
Medicinal plants are a natural source of safe and possible medicines, and are of great importance in the treatment and prevention of various diseases, they provide plant nutrition for the first level of health care systems in rural areas and remote mountain areas; more than 70% of the population relies on the traditional medicine system (Marwat et al., 2008), due to their high planting efficiency, low damage, very low adverse outcomes, and high cost of therapeutic drugs (Marwat et al., 2009).

The cruciferous vegetables contain a number of nutrients and phytochemicals with chemical properties that protect against cancer, including folic acid, fibers, carotenoids and chlorophyll, however, cruciferous vegetables are unique because they are rich sources of glucosinolates and sulfur compounds responsible for their pungent aromas and spicy taste or bitter (Drewnowski and Gomez-Carneros, 2000). The hydrolysis of glucosinolates by the myrosinase enzyme results in the formation of biologically active compounds including indoles and isothiocyanates (Holst and Williamson, 2004). More than 100 compounds of glucosinolate have been identified with unique hydrolysis products in plants, radish is a good source of glucocaraphanin and glucosinolate precursors of sulforaphane (SFN), glucobrassicin, and indole-3-carbinol (I3C) (Zhang, 2004). In contrast, watercress is a rich source of gluconasturtinin and phenethyl isothiocyanate (PEITC) (Higdon et al., 2007). Garden cress contains
glucotropoeoline, 4-methoxyglucobrassicin, sinapine, sinapic acid, calmodulin, sinapoyglucose, ester of caffeic, coumaric, ferulic, quinic acids, protein, minerals, vitamins and 5 4-dihydroxy-7,8,3,5-tetramethoxyflavones, 5-3-dihydroxy-7,8,4trimethoxyflavones and 5-3-dihydroxy-6,7,4trimethoxyflavones (Ku et al., 2016).

Fungicides are one of the most effective and integrated methods for controlling pathogenic fungi of phytopathogenic fungi in agriculture, however, the toxicity and contamination of fungicides cannot be neglected, it depends on the toxic effect of a particular pesticide on its distribution, persistence and, metabolism and active form as well as concentration (Zhang et al., 2015). Some pesticides interfere with the metabolic pathways of plants and some interfere specifically with photosynthesis with still unknown work patterns; many results suggest that some fungicides may affect photosynthesis, altering the pathway of photosynthesis by closing the stomata, while others, such as Azoles, stimulate photosynthesis; triadimefon and hexaconazole can be used as a potential tool for manipulating carbohydrate metabolism (Jaleel et al., 2007).

Since the bioaccumulation of fungicides is a complex physiological process, the bioaccumulation mechanism of fungicides is becoming suspicious of its effect on the biological pathways within the plant. Although there are no scientific studies to explain the biochemical impact of pesticides after their transfer to the plants, the aim of the study was to demonstrate the effect of fungicides on the enzymatic activity of a number of enzymes as well as the oxidative stress index (MDA) in the vegetative part of plants.

2. Materials and methods:
A- Experimental design and procedure:
Randomized Complete Block Design (RCBD) was adopted for a factorial experiment consisting of two factors with three replicates (3 plant species x 11 fungicides concentrations x 3 replicates), the first factor consist of three types of Brassicaceae plants (Arugula, radish and garden cress) while the second factor of eleven concentrations of fungicides:
1- Bayfidan/ Produced by Bayer company - Turkey: A fungicide that is toxic to the active ingredient of Triadimenol (250 g. L⁻¹).
2- Tapsen 50/ Produced by Green River Company - China: A protective and therapeutic fungicide containing of Thiphanate - Methyl (50%).
3- Sigal/ Produced by Greenfields company - Australia: A protective and therapeutic fungicide containing of Propamacard HCl (722 g. L⁻¹).

Two concentrations of each pesticide (recommended and double recommended) were used together with the recommended concentration (three double treatments and one triple treatment) in accordance with the fungicide guidance manual, as well as a comparison treatment that was limited to sprinkling with irrigation water only (no fungicide were used), resulting in 11 treatments (Table 1) and three replicates per treatment, randomly distributed in each block. The total experimental units became 99 experimental units (3 x 11 x 3 = 99).

The experiment was conducted in the autumn season (2017 - 2018), corresponding to 15/10/2017, in order to determine the effect of fungicide spraying on the total vegetation of three types of Brassicaceae (arugula, radish and garden cress), and their interference in enzymatic activity and oxidative stress index of plants. Local seeds were obtained from plants and fungicides from the approved agricultural offices within Al-Diwaniyah Governorate. The experiment was carried out through the cultivation of seeds (5 seeds in each pot and was reduced after emergence to 4) for plant species in pots with dimensions (20 cm x 15 cm diameter) and 5 kg sand soil.

Table 1: Different concentrations treatments of fungicides used

| No. | Treatment symbol | Treatment name |
|-----|------------------|----------------|
|     |                  |                |
The random distribution of treatments was also ensured. According to the data below, the experiment and agriculture were implemented:
1. The date of planting was 15/10/2017, and the date of the emergence of seedlings fully was 1/11/2017.
2. Continuation of irrigation operations at a rate of three days between irrigation and other, in addition to continuing the weeding operations throughout the period of cultivation.
3. Fertilize the plants with the NPK balanced fertilizer once before treatment with fungicides.
4. The date of the first treatment with fungicides was carried out on 1/12/2017 by leaf spraying with the known concentrations of each fungicide and by a weekly frequency of one month.

B- Estimation of enzymatic activity:
1. Estimation of $\alpha$-amylase activity: according to the method of Fischer and Stein (1961), and calculated according to the following equation:
   
   \[
   \text{Enzyme activity} = \text{Reading from the standard curve} \times \text{Absorption} \times \text{Mitigation factor}
   \]

   Where:
   - $\Delta \text{Abs}$: the difference between the absorption (first – second).
   - $\text{min}$: reaction time.
   - $\text{Reaction volume} = 2.4 \text{ml}$.
   - 0.001: constant of the definition of unit of activity of the enzyme (Unit): the amount of enzyme that causes an increase in absorption by 0.001 units per minute under the conditions of estimation.

2. Estimation of catalase activity: according to the method of Aebi (1984), and calculated according to the following equation:

   \[
   \text{Catalase activity (Unit. ml}^{-1}) = \frac{\Delta \text{abc min} \times \text{Reaction volume}}{0.001}
   \]

   Where:
   - \(\Delta \text{abc min}\): reaction time.
   - $\text{Reaction volume} = 2.4 \text{ml}$.
   - 0.001: constant of the definition of unit of activity of the enzyme (Unit): the amount of enzyme that causes an increase in absorption by 0.001 units per minute under the conditions of estimation.

3. Estimation of polyphenol oxidase activity: according to the method of Wong et al. (1971), and calculated according to the following equation (Fujita et al., 1995):

   \[
   \text{Enzyme activity (Unit. ml}^{-1}) = \frac{(AF_{\text{blank}} - AI_{\text{blank}}) - (AF_{\text{sample}} - AI_{\text{sample}})}{0.001 \times t}
   \]

   Where:
   - $AI_{\text{sample}}$: the initial absorption of the sample.
   - $AF_{\text{sample}}$: the final absorption of the sample.
   - $AI_{\text{blank}}$: Primary absorption of the comparison sample.
4. Estimation of protease activity: according to the method of Hayash (1975), and calculated according to the following equation:

\[
\text{Enzyme activity (Unit. ml}^{-1}\text{)} = \frac{\text{Absorbance at 280 nm}}{0.001 \times 20 \times 0.2}
\]

Where:
- 0.001: constant of the definition of unit of activity of the enzyme (Unit): the amount of enzyme that causes an increase in absorption by 0.001 units per minute under the conditions of estimation.
- 20: reaction time (min.).
- 0.2: volume of the added enzyme solution (ml).

C- Estimation of malondialdehyde (MDA) concentration: according to the method of Zacheo et al. (2000), and calculated according to Beer-Lambert equation:

\[
C = A \times B \times E
\]

Where:
- C: MDA concentration.
- A: Absorption at 532 nm.
- B: Immersion ratio = 1 cm.
- E: Degradation coefficient = 153 mmol. cm. L\textsuperscript{-1}.

D- Statistical analysis: Randomized Complete Block Design (RCBD) used according to the experience of factorial experiment composed of two factor (3 \times 11) with three replicates. The results were statistically analyzed using the analysis of variance, and the mean of the coefficients was measured when the differences were significant by using the Least Significant Difference (LSD) at the probability level P \leq 0.05 (Steel et al., 1997).

3. Results:

1. α-amylase activity (Unit. ml\textsuperscript{-1})

The data presented in table (2) showed the moral effect of the study and their interference in α-amylase activity, the radish plant recording the highest of 21.94 unit. ml\textsuperscript{-1}, which did not differ significantly with the arugula plant has the effectiveness of 21.92 unit. ml\textsuperscript{-1}, but they were significantly higher than the less effective garden cress plant 15.85 unit. ml\textsuperscript{-1}. The majority of fungicides showed a positive effect on increased enzymatic activity compared to the mean plants of 16.11 unit. ml\textsuperscript{-1}.

The significant interference between plant species and fungicides, it was observed that the triple mixture of fungicides had the highest enzymatic activity of arugula and garden cress of 24.67 and 18.64 unit. ml\textsuperscript{-1}, respectively, against the registration of the Tapsen 50 treatment with a double concentration of the highest enzymatic activity of radish plants of 26.15 unit. ml\textsuperscript{-1}.

2. Catalase activity (Unit. ml\textsuperscript{-1})

Table (3) by its data showed that the catalase activity of garden cress plant was higher than that of radish and arugula, with a significant increase of 9.24, 8.81 and 8.82 unit. ml\textsuperscript{-1}, respectively.

The treatment with fungicides showed a decrease in enzymatic activity of all treatments from the control treatment of 20.70 unit. ml\textsuperscript{-1} and in reverse with increased concentration of fungicides. In the same context, the significant interference between the two study showed a decrease in the enzymatic
effectiveness of plants when treated with all fungicides under study in terms of recording the highest enzymatic activity in control plants 21.19 unit. ml⁻¹, as shown in the same table.

3. Polyphenol oxidase activity (Unit. ml⁻¹)

The data of table (4) showed higher activity of the polyphenol oxidase at arugula, then radish and garden cress respectively, reaching 16.32, 15.26 and 13.60 units. ml⁻¹, and the other hand, the enzymatic activity increased steadily with the fungicides concentration increased or its mixtures increased to reach the highest activity in the triple mixture of fungicides, reaching 28.44 units. ml⁻¹.

Interference of plants and fungicides gave the same effect to the individual factors. The highest enzymatic activity was obtained by the effect of the triple mixture of arugula, radish and garden cress respectively, with a significant difference between them, as well as the significant differences between other treatments on the comparatively treatments with lower enzymatic activity.

4. Protease activity (Unit. ml⁻¹)

The results of the statistical analysis of the data in table (5) showed that the effectiveness of the protease in the radish plant was significantly higher than that of the arugula plant, which also surpassed the garden cress plant at 8.06, 7.94 and 6.95 units. ml⁻¹, respectively.

Treatment with different fungicides showed a significant positive effect in increasing the enzymatic activity of protease, which reached a maximum of 8.83 units. ml⁻¹ when treated with a triple fungicides mixture. The same applies to the interference between the study factors in the arugula and garden cress which had the highest enzyme activity of 9.56 and 7.98 units. ml⁻¹, respectively, when treated with the triple mixture combination of fungicides, while the highest enzymatic activity of protease in radish was recorded when treated with a double concentration of Tapsen 50, which was 9.10 units. ml⁻¹.

5. MDA concentration (µmol. L⁻¹)

The data of table (6) showed a significant increase in the concentration of the oxidative stress index (MDA) in leaves of the arugula plant (52.08 µmol. L⁻¹) compared with radish and garden cress with a significantly lower concentration of 38.23 and 32.44 µmol. L⁻¹, respectively. All fungicides treatment showed a significant increase in the MDA concentration of plants to a maximum concentration of 71.56 µmol. L⁻¹ when treated with the triple mixture of fungicides (Bayfidan + Tapsen 50 + Sigal).

The interference between plants and fungicides was observed to have the same effect on individual factors; the highest concentration of the studied effect was on the effect of the triple mixture of fungicides (Bayfidan + Tapsen 50 + Sigal) on the arugula, radish, and garden cress plants 85.51, 66.24 and 62.93 µmol. L⁻¹ respectively, with a significant difference between them, as well as the significant differences between the other factors for the comparison treatments with the lowest content 13.77, 10.64 and 6.72 µmol. L⁻¹ respectively, as shown in the same table.

Table 2: Effect of fungicides on the enzymatic activity of α-amylase (unit. ml⁻¹) for three species of Brassicaceae plants

| Plants    | A  | B  | C  | D  | E  | F  | G  | H  | I  | J  | K  | Mean     |
|-----------|----|----|----|----|----|----|----|----|----|----|----|----------|
| **E. sativa** | 17.9 | 19.8 | 21.6 | 22.0 | 23.0 | 20.2 | 21.2 | 22.9 | 23.5 | 23.9 | 24.6 | 21.92    |
| **R. sativus** | 16.3 | 20.7 | 22.4 | 23.8 | 26.1 | 19.9 | 21.2 | 22.0 | 22.5 | 22.8 | 23.1 | 21.94    |
| **L. sativum** | 13.9 | 14.7 | 15.2 | 15.5 | 16.3 | 13.9 | 14.7 | 16.6 | 16.9 | 17.6 | 18.6 | 15.85    |
### Table 3: Effect of fungicides on the enzymatic activity of catalase (unit. ml\(^{-1}\)) for three species of Brassicaceae plants

| Plants     | Fungicides | Mean plant effect |
|------------|------------|-------------------|
|            | A          | B                 | C       | D       | E       | F       | G       | H       | I       | J       | K       |
| *E. sativa*| 21.1       | 15.7             | 10.7    | 6.28    | 4.61    | 6.92    | 5.42    | 7.44    | 6.92    | 6.31    | 5.42    | 8.82    |
| *R. sativus*| 22.2      | 16.0             | 10.0    | 7.88    | 5.34    | 6.50    | 5.83    | 6.78    | 5.91    | 5.58    | 4.79    | 8.81    |
| *L. sativum*| 18.6      | 13.9             | 12.6    | 10.9    | 9.86    | 7.80    | 6.04    | 6.44    | 6.13    | 5.39    | 3.83    | 9.24    |

LSD \((P \leq 0.05)\) Plant = 0.03  Fungicide = 0.07  Interference = 0.11

### Table 4: Effect of fungicides on the enzymatic activity of polyphenol oxidase (unit. ml\(^{-1}\)) for three species of Brassicaceae plants

| Plants    | Fungicides | Mean plant effect |
|-----------|------------|-------------------|
|           | A          | B                 | C  | D | E | F  | G | H  | I | J | K |
| *E. sativa*| 5.65       | 9.26             | 10.2 | 12.0 | 13.6 | 8.16 | 11.8 | 21.0 | 25.7 | 29.1 | 32.4 | 16.32 |
| *R. sativus*| 6.31      | 7.73             | 9.16 | 11.8 | 12.6 | 11.3 | 15.7 | 5 | 19.2 | 20.8 | 24.7 | 28.2 | 15.26 |
| *L. sativum*| 4.47      | 7.05             | 8.64 | 10.2 | 11.8 | 11.0 | 14.0 | 16.1 | 4 | 19.5 | 22.0 | 25.3 | 28.4 | 13.60 |

LSD \((P \leq 0.05)\) Plant = 0.05  Fungicide = 0.10  Interference = 0.17

### Table 5: Effect of fungicides on the enzymatic activity of protease (unit. ml\(^{-1}\)) for three species of Brassicaceae plants

| Plants  | Fungicides | Mean plant effect |
|---------|------------|-------------------|
|         | A          | B     | C | D | E | F  | G | H  | I | J | K |
| *E. sativa*| 6.82       | 7.01 | 7.52 | 7.60 | 8.04 | 6.43 | 7.23 | 8.80 | 9.03 | 9.29 | 9.56 | 7.94 |
| *R. sativus*| 6.76      | 7.71 | 8.43 | 8.80 | 9.10 | 7.05 | 7.60 | 7.86 | 8.05 | 8.39 | 8.95 | 8.06 |
| *L. sativum*| 5.89      | 6.15 | 6.25 | 6.45 | 6.59 | 6.83 | 7.27 | 7.35 | 7.81 | 7.89 | 7.98 | 6.95 |

LSD \((P \leq 0.05)\) Plant = 0.01  Fungicide = 0.02  Interference = 0.03

### Table 6: Effect of fungicides on the MDA concentration (µmol. L\(^{-1}\)) for three species of Brassicaceae plants

| Plants | Fungicides | Mean plant effect |
|--------|------------|-------------------|
|        | A          | B     | C | D | E | F  | G | H  | I | J | K |
| *E. sativa*| 6.16       | 7.01 | 7.52 | 7.60 | 8.04 | 6.43 | 7.23 | 8.80 | 9.03 | 9.29 | 9.56 | 7.94 |
| *R. sativus*| 6.76      | 7.71 | 8.43 | 8.80 | 9.10 | 7.05 | 7.60 | 7.86 | 8.05 | 8.39 | 8.95 | 8.06 |
| *L. sativum*| 5.89      | 6.15 | 6.25 | 6.45 | 6.59 | 6.83 | 7.27 | 7.35 | 7.81 | 7.89 | 7.98 | 6.95 |

LSD \((P \leq 0.05)\) Plant = 0.01  Fungicide = 0.02  Interference = 0.03
### Plants

| Plants       | Mean plant effect |
|--------------|------------------|
| **E. sativa** | 52.08            |
| **R. sativus** | 38.23            |
| **L. sativum** | 32.44            |

#### LSD (P ≤ 0.05)

- **Plant = 0.21**
- **Fungicide = 0.41**
- **Interference = 0.71**

## 4. Discussion

In recent decades, the use of fungicides in agriculture to control fungal diseases has become crucial. Fungicide researches have produced a variety of products with new methods of work. However, the intensive use of these compounds in the agricultural system raises general concern about the harmful potential of these substances in the environment and on human health. In addition, the toxic effects of some plant fungicides are not known, in addition to the lack of information on the effect of these compounds on the photosynthetic system and the metabolism of plants in terms of metabolizing the compounds in the plant and converting them from toxic to non-toxic and their effects on plant physiology both quantitatively and qualitatively, indicating the biotic assimilation of pesticides by plants on the one hand, and on the other hand, this biological assimilation may be returned with negative results on the validity of plant consumption in terms of nutrition explained by the results under study in terms of increasing the effectiveness of alpha-amylase activity in radish plant (Table 2) significantly compared to other plants, the reason for this is related to the percentage of total carbohydrates, and the role of enzyme in starch analysis, which resulted from the result in the current study was the highest in the radish compared to arugula and garden cress. The same thing was shown with increased protease in the radish plant (Table 5), which was associated with the degradation of proteins that increased in radish as well. On the other hand, increasing the enzymatic activity of polyphenol oxidase in arugula compared to other plants (Table 4) is directly related to the increase in oxidative stress, which gave the same in table (6) of MDA.

The combined effect of fungicides (Bayfidan + Tapsen 50 + Sigal) in increasing the enzymatic activity of enzymes under study (except catalase) and MDA can be explained on the basis of the chemical content of each fungicide. The majority of these compounds consist of volatile aromatic and hydrocarbon compounds for Bayfidan fungicide, while the majority of the constituents of the Tapsen 50 fungicide are phenolic compounds, as well as Sigal fungicide is majority consist of compounds are phenolic, and few of them are sulfuric and aromatic hydrocarbons, or the regulated action of fungicide may be attributed to the secondary role of each fungicide, regardless of its toxicity and rapid permeability within the cells of organisms (Milenkovski et al., 2010), as follows:

1. Bayfidan fungicide contains the triazoles group (Triadimenol), which play a vital role in the synthesis of sterols biosynthesis.
2. Tapsen 50 fungicide contains thiophanates group (Thiphaneate-methyl), which play a vital role in cytoskeleton and motor protein.
3. Sigal fungicide contains carbamates group (Propamocarb) with a vital role in lipid synthesis and transfer / membrane integrity or function.

Due to the absence of studies and research to explain the mechanisms of the work of fungicide in the plant, whether infected or not infected; similar studies have been found on microorganisms,
including the study of Radzuhn et al. (1984), which showed that the membranes of fungus cells treated with etridiazole are sensitive to the solar radiation of the linoleic acid, which is a common lipid in the membrane due to the hydrolysis of fungicide to lipid in the cell membrane to free fatty acids and lysofosvadyl, leading to the degradation of cellular membranes in fungi. Rodgers (1986) added that the etridiazole fungicide reduced the nitrification rate of the oxidizing bacteria of ammonium in the soil. The study of Bosca et al. (1998) showed that the negative effects of the fungicide on the microorganisms membrane were altered by changing the structure and function of microorganisms in the soil; the structure of lipids and the basic components of cell membranes were modified by fungicides of the aromatic hydrocarbons (AH), affecting the functions of microbial systems, such as dicloran (2,6 dichloro-4-nitroaniline), a fungicide of the aromatic hydrocarbons group (AH) registered in North America, Europe and South Africa since the year 1975 to control of the fungi: Basidiomycetes, Deuteromycetes and Rhizopus, which cause photosynthesis. De Ouveira et al. (2009) added that fungicides have side effects on other microorganisms in the soil, dicloran can cause a mutation in Salmonella typhimurium by stressful of hydrophobic interferences inside the membrane. Cycon et al. (2010) reported that the fungicide of dimethomorph (EZ) -4- [3- (4-chlorophenyl) -3- (3,4-dimethoxyphenyl acryloyl) mororpholine can affect the effectiveness of the bacteria involved in the nitrogen cycle, with effect on nitrification and ammonification through its differential effect on different bacterial environmental types and changes in the structure of the bacterial community.

Linked to the above and through the results of enzymatic activity (tables 2, 4, and 5) as well as the results of the oxidative stress index (MDA) (Table 6) for plant samples shows that the mechanism of plant biogeography was better in radish and garden cress plants in terms of dietary consumption and metabolism of chemical compounds than arugula plant which increased enzymatic activity and oxidative stress due to treatment with different fungicides under study, but the final indication and according to the available results, that it is not suitable for food consumption after treatment with different fungicides.

5. Conclusion:

Brassicaceae plants showed the significant superiority of each species in certain traits, which distinguished it from its counterpart in other traits depending on the genetic factor and the specific conditions of each plant. While the significant effect of fungicides concentrations was evident in increasing enzymatic activity steadily with increased concentrations of fungicides. The interference between plant species and fungicides increased enzymatic activity and oxidative stress index (MDA) than in single-effect.

6. References:

[1] Aebi, H. (1984). Catalase in vitro. Methods in Enzymology (Vol. 105, pp: 121-126). Academic Press.

[2] Bosca, F.; Miranda, M.A.; Serrano, G. and Vargas, F. (1998). Photochemistry and photobiological properties of dicloran, a postharvest fungicide with photosensitizing side effects. Photochemistry and Photobiology, 67(5): 532-537.

[3] Cycon, M.; Piotrowska-Seeget, Z. and Kozdroj, J. (2010). Responses of indigenous microorganisms to a fungicidal mixture of mancozeb and dimethomorph added to sandy soils. Int. Biodeterioration and Biodegradation, 64(4): 316-323.

[4] De Ouveira, D.P.; Sakagami, M.; Warren, S.; Kummrow, F. and DeUmbuglzeiro, G.A. (2009). Evaluation of dicloran's contribution to the mutagenic activity of Cristais river, Brazil, water samples,” Environ. Toxicol. Chem., 28(9): 1881-1884.

[5] Drewnowski, A. and Gomez-Carneros, C. (2000). Bitter taste, phytonutrients, and the consumer: a review. Am. J. Clin. Nutr., 72: 1424-1435.
[6] Fischer, E.H. and Stein, E.A. (1961). Use of Dinitrosalicylic Acid. Biochemical Preparations. Vol., 8: p. 27.
[7] Ahmed Sabah Al-Jasimee et al 2020 J. Phys.: Conf. Ser. 1664 012141.
[8] Fujita, S.; Saari, N.B.; Maegawa, M.; Tetsuka, T.; Hayashi, N. and Tono, T. (1995). Purification and properties of polyphenol oxidase from cabbage (Brassica oleracea L.). J. Agric. Food Chem., 43(5): 1138-1142.
[9] Hayash, H. (1975). The Intracellular Neutral SH-Dependent Protease Associated with Inflammatory Reactions. International Review of Cytology (Vol. 40, pp. 101-151). Academic Press.
[10] Higdon, J.V.; Delage, B.; Williams, D.E. and Dashwood, R.H. (2007). Cruciferous vegetables and human cancer risk: epidemiologic evidence and mechanistic basis. Pharmacol. Res., 55(3): 224-236.
[11] Holst, B. and Williamson G.A. (2004). Critical review of the bioavailability of glucosinolates and related compounds. Nat. Prod. Rep., 21: 425-447.
[12] Jaleel, C.A.; Kishorekumar, A.; Manivannan, P.; Sankar, B.; Gomathinayagam, M.; Gopi, R. and Panneerselvam, R. (2007). Alterations in carbohydrate metabolism and enhancement in tuber production in white yam (Dioscorea rotundata Poir.) under triadimefon and hexaconazole applications. Plant Growth Regulation, 53(1): 7-16.
[13] Ku, K.M.; Kim, M.J.; Jeffery, E.H.; Kang, Y.H. and Juvik, J.A. (2016). Profiles of glucosinolates, their hydrolysis products, and quinone reductase inducing activity from 39 arugula (Eruca sativa Mill.) accessions. J. Agric. Food Chem., 64(34): 6524-6532.
[14] Marwat, S.K.; Khan, M.A.; Ahmad, M.; Zafar, M. and Fazal-ur-Rehman, A. (2008). Ethnophytomedicines for treatment of various diseases in D.I Khan District, Sarhad. J. Agric., 24(2): 305-316.
[15] Marwat, S.K.; Khan, M.A.; Khan, M.A.; Fazal-ur-Rehman, A.; Ahmad, M.; Zafar, M. and Sultana, S. (2009). Plant species mentioned in the holy Qura’n and A hadithand their ethnomedicinal importance American-Eurasian. J. Agric. Environ. Sci., 5(2): 284-295.
[16] Milenkovski, S.; Baath, E.; Lindgren, P.E. and Berglund, O. (2010). Toxicity of fungicides to natural bacterial communities in wetland water and sediment measured using leucine incorporation and potential denitrification. Ecotoxicol., 19(2): 285-294.
[17] Radzuhn, B. and Lyr, H. (1984). On the mode of action of the fungicide etridiazole. Pesticide Biochem. Physiol., 22(1): 14-23.
[18] Rodgers, G.A. (1986). Potency of nitrification inhibitors following their repeated application to soil. Biol. Fertil. Soils, 2(2): 105-108.
[19] Steel, R.G.D.; Torrie, J.H. and Dickey, D.A. (1997). Principles and Procedures of Statistics: A Biometrical Approach, 3rd Ed. McGraw Hill Book Co. Inc., New York, USA.
[20] Wong, T.C.; Luh, B.S. and Whitaker, J.R. (1971). Isolation and characterization of polyphenol oxidase isozymes of clingstone peach. Plant Physiol., 48(1): 19-23.
[21] Zacheo, G.; Cappello, M.S.; Gallo, A.; Santino, A. and Cappelo, A.R. (2000). Changes associated with post-harvest ageing in Almond seeds. Lebensm-Wiss U. Technol., 33: 415-423.
[22] Zhang, Z.; Jiang, W.; Jian, Q.; Song, W.; Zheng, Z.; Wang, D. and Liu, X. (2015). Residues and dissipation kinetics of triazole fungicides difenoconazole and propiconazole in wheat and soil in Chinese fields. Food Chem., 168: 396-403.
[23] Dakhil Shyaa , F., & Mohammed Ali Hadi, I. (2019). Strongly t-continuous and Strongly t-semisimple Modules. Al-Qadisiyah Journal Of Pure Science, 24(1), 37-44.