ISSN: 0974-5823 Vol. 7 No. 2 February, 2022

International Journal of Mechanical Engineering

Study of the analysis of microbial dusting pesticides caused by Pseudomonas bacteria and Trichoderma fungi

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Abstract - The experiment was carried out during the fall season 2020 using a completely randomized CRD design, with the aim of studying the possibility of microbial decomposition of fumigants under different incubation conditions, as it included three factors, the first factor: bacterial and fungal (P.flurences, T.harzanium, mixture of bacteria and fungi, comparison), The second factor: fumigation pesticides (Comparative, Raxil, Dividend) and the third factor: the incubation period (7, 14, 21, 28) days, The results showed that the treatment of the mixture of bacteria and fungi with the presence of the pesticides, Dividend and Raxil, recorded the highest values for the increase in the numbers of Pseudomonas bacteria in the soil, reaching (52.3, 66.93) × 10^6 cfu gm^{-1} for each of Raxil and Dividend, respectively, and the highest values for the numbers of Trichoderma in the soil It reached (26.0, 29.0) × 106 cfu g^{-1}, and the treatment of the mixture of bacteria, mushrooms and mushrooms alone recorded the lowest value for the residues of the pesticides Raxil and Dividend in the soil. It reached 0 parts per million for Raxil and Dividend for the experiment, respectively, and the triple interaction treatment between the mixture of bacteria, fungi and Dividend was recorded, and the incubation period was 21 days on the highest liberated amount of Co_2, 385.0 mg 100 which gm^{-1} of soil.

Index Terms - About four, alphabetical order, key words or phrases, separated by commas (e.g., Camera-ready, FIE format, Preparation of papers, Two-column format).

INTRODUCION

Pesticide residues are the quantities or concentrations of pesticides that remain in the soil and on the surface or inside agricultural and food products after being treated with pesticides, including derivatives resulting from decomposition or conversion or the results of the interaction of these materials or their toxins. These concentrations vary according to the type of crop or type of pesticide and are often associated with The chemical and physical properties of the soil, And it is an important source of soil, water, air and food pollution, as the issue of chemical pesticides and their analysis and knowledge of the minimum and upper limits of their residues is of importance in the global food trade and to avoid strict legislation set by developed countries in developing many methods of analysis of pesticide residues to achieve high levels of accuracy.

As the study of these residues is one of the important topics in developing countries, including Iraq, because most farmers do not adhere to the recommended concentrations or the post-treatment period to ensure the eligibility of the product for consumption (Al-Ghazi et al., 2011). Raxil and Dividend, two of the systemic fungicides, are used to control wheat and barley diseases for a long time, as they are used as seed sterilizers to combat pathogens inside the seed and on its outer surface to ensure high quality of the crop (Bayer, 2021; Syngenta, 2021). By farmers and farmers to protect agricultural crops and sterilize the soil from fungal diseases.

The bacteria Pseudomonas, spp has the ability to degrade pesticides by several types of enzymes that they secrete and use them as a source of energy and carbon (Al-Saeedi et al., 2015). Also, the fungus Trichoderma spp has a good ability to analyze chemical pesticides of a wide range, as it has proven its efficiency in decomposition due to the presence of an enzyme system outside the cell that stimulates reactions that can degrade toxic compounds, and its role as a natural decomposer is due to its ability to accelerate growth and nutrient uptake (Cao et al., 2011). In order to know the residues of barley seed dusting pesticides (Raxil and Devidind) and the possibility of biological treatment using Pseudomonas bacteria and Trichoderma fungi, the study aimed to: Study the activity of soil organisms by releasing CO_2, by analyzing the dusting pesticides microbially using Pseudomonas spp bacteria and Trichoderma fungi for different incubation periods and measuring the remaining ones.

MATERIALS AND METHODS

A factorial experiment was carried out in the Soil Microbiology Laboratory at the Department of Soil Sciences and Water Resources - College of Agriculture/University of Diyala using a fully randomized design (CRD) to study the activity of microorganisms added when fogging pesticides were added, and the possibility of their decomposition by measuring their residues. The experiment included 48 treatments that resulted from Three factors overlap : Bacterial and fungal inoculating agent (P.fluorscens, T.harzianum, mixture of P.fluorscens and T.harzianum, in addition to comparison), fumigating agent (no added, Raxil, Dividend) and incubating agent (7, 14, 21, 28) days and with three replications, bringing the number of experimental units to 144. As 100 gm of soil (field experiment soil) was placed in glass bottles for each treatment after preparing the soil, as the stones and bushes were removed, crushed and sifted using a sieve with a diameter of 2 mm, and 0.14 mg and 0.14 ml were added for every 100 gm of soil of the pesticides Raxil and Dividend on For the pesticide-treated treatments, 1 ml of P. fluorscens inoculum and 1.6 mg of T. harzianum in suspension were added per 100 gm of soil for bacterial and fungal inoculation treatments. The soil was moistened with a moisture content of (55-66)% of the field capacity, and 15 ml of sodium hydroxide (1) p was added to the container for that and placed inside the glass bottle containing the soil, taking care not to pour it on the soil for the purpose of To estimate the CO_2 released, the glass bottles containing soil and NaOH were tightly closed.

Studied measurements

- *Amount of CO_2: A sodium hydroxide solution is taken after each incubation period and crushed with 1p hydrochloric acid, as carbon dioxide gas reacts with sodium hydroxide and produces turbid sodium carbonate, which is precipitated by adding 2 ml of barium chloride, as in the following equation:*

$$2NaOH+CO_2 \rightarrow Na_2CO_3+H_2O$$

$$Na_2CO_3+BaCl_2 \rightarrow 2NaCl+BaCO_3$$

It is crushed against 1 standard of hydrochloric acid, using the phenonphthalein dye to indicate the end point of the reaction, and the number of milligrams of carbon liberated in the form of carbon dioxide from each soil, each treatment, and for each incubation period, is calculated by calculating the volume of the known standard hydrochloric acid used for the hydroxide equation Excess sodium according to

Janzen, (1987) and according to the following equation:

$$CO_2 mg .100gm\ soil = (B - V)NE$$

Since:

B = volume of acid consumed (ml) in comparison.

V = volume of acid consumed (ml) in the sample.

N = standard of hydrochloric acid HCl.

E = the equivalent weight of gas (CO2) and is equal to 22 .

- Numbers of Pseudomonas bacteria: The numbers of Pseudomonas bacteria were estimated after the incubation period of 28 days by dilution and plate counting using KingB medium for them, according to what was men .
- Trichoderma numbers: The numbers of Trichoderma were calculated after the incubation period, 28 days. The population units ofTrichoderma were estimated by dilution and counting by plateusing the medium(PDA) tioned in Black (1965a).
- Pesticide residues: The residues of the dusting pesticides, Raxil and Dividend, were measured for each repeater after the end of the incubation period by a HPLC device, where the samples were preserved by freezing below the degree of -4 until the analysis is performed, as explained in the paragraph extraction, purification and analysis of pesticide residues.

SOIL EXTRACTION METHOD

The soil preserved in the bag of polyethylene and preserved in freezing is stirred to obtain a homogeneous sample, and then it is air-dried to get rid of the existing moisture. 10 g of soil is taken and 10 ml of acetonitrile is added to it. Mixing is done at a rate of 500 cycles / minute. The QUCHERS method presented by Anastassiade et al. was used. (2003) The method can be summarized as:

The sample is taken and placed in a 50ml plastic vial, and 4 gm of anhydrous magnesium sulfate (MgSo4) and 1 gm of sodium chloride (Nacl) are added. Shake manually and then with a magnaticstirrer device for a minute.

The tube is placed in the centrifuge for 5 minutes at a rate of 3600 revolutions / minute. The upper layers consist of a clear solution representing the pesticide with the solvent, the middle layersrepresenting the soil and the lower layers representing insoluble salts.

2 ml of the resulting liquid in the upper layer is taken and transferred to a 10 ml plastic tube, and 0.15 g of anhydrous magnesium sulfate (MgSo4) is added to it.

Placed in the centrifuge at a rate of 3600 revolutions / min 1 for five minutes.

After that, the mixture is filtered using a 0.45 mm milipore filter.

The output is transferred to a small tube (vial tube) in preparation for injection into the HPLC device.

RESULTS AND DISCUSSION

The results showed in Table (1) that the addition of P.flurences and T.harzanium bacteria singly or in pairs led to a significant increase in the amount of CO2 liberated from the soil compared to the control treatment, as it was recorded (97.77, 146.86, 180.55,) mg 100 g-1. Bacteria and fungi alone and the mixture of bacteria and fungi respectively compared to the control treatment Which recorded 52.55 mg 100 g-1 soil with a significant increase of (86.05, 179.46,243.57)%, respectively. The reason for the increase may be due to the relative increase in the numbers of microorganisms, as noted in Table (3,4), and as a result of the decomposition of other organic materials present in the soil Which led to an increase in its activity.

The addition of Dusting and Dividend pesticides led to a significant increase in the amount of CO2 released from the soil compared to the control treatment, as it gave (163.11,161.11) mg/100gm soil to Dividend and Dividend respectively compared to the control treatment which was 33.05 mg/100g-1 soil, with a significantincreaseof(389.22,393.52)%. reason for the increase in the presence of pesticides may be due to microorganisms adapting to these pesticides, and began analyzing them, reducing their toxicity, and using them as sources of energy and food, as these pesticides contain carbon and nitrogen in their chemical composition, and thus increased emissions of carbon dioxide (Cycon, 2004).

Increasing the incubation period from (7-28) days led to a significant increase in the amount of CO_2 released from the soil, as it was recorded (79.63, 164.12, 155.23, 78.75) mg 100 g^{-1} soil, the amount of CO_2 released has increased significantly from (7- 14) days, then it decreased significantly at (21,28) day as the highest value was recorded at the incubation period of 14 days, reaching (164.12) mg 100 g^{-1} soil compared to the incubation period 7 days, which was (79.63) mg 100 g^{-1} soil. And with a significant increase of (106.10)% as the neighborhoods have adapted, printed, printed and analyzed these pesticides and benefited from them as a food source. As for the reason for the decrease, it may be explained by the depletion of pesticides, and then the depletion of nutrients resulting from the decomposition of pesticides and the accumulation of waste(Al-Issa, 2009).

The interaction between the addition of Pseudomonas bacteria and Trichoderma fungi, singly or in combination, and when adding a dusting pesticide, had a significant effect on the amount of CO_2 liberated from the soil, as the amount of CO_2 liberated increased significantly when the bacterial and fungal vaccines were added with the presence of the fogging pesticides compared to not adding the vaccine, and the treatment of the mixture of bacteria and mushrooms was recorded. When adding the pesticide Raxil and Dividend, the highest amount of CO_2 liberated, as it gave (251.33, 249.33) mg $100gm^{-1}$ of soil, as these organisms have the ability to metabolize the pesticide, as these pesticides support the growth of microbes and are used as a source of energy, carbon and nitrogen, which led to an increase in CO_2 (Al Hammadi, 2015) The increase in the release of CO_2 in the presence of the pesticide Raxil may be due to its presence in the solid state compared to the liquid Dividend, in addition to the fact that the Raxil contains one molecule of chlorine in its chemical composition, while Dividend contains two molecules, and therefore it has more toxicity than the Dividend, as it was found by Katayama et al., (2010). Biology works to decompose fungicides mainly through their chemical composition, and these organisms have the ability to change the pesticide molecules and turn them into inactive molecules through the mineralization process.

The effect of the interaction between the addition of Pseudomonas bacteria and Trichoderma fungi singly or double and the incubation period significantly on the amount of CO_2 released from the soil, as the quantity increased when adding bacteria and mushrooms and at all incubation periods compared to the control treatment, and that the increase in the incubation period from (7-28) days led to Significant increase in the amount of CO_2 released in all treatments of bacterial and fungal inoculum, and the treatment of the mixture of bacteria and mushrooms recorded the highest amount released during the incubation period (21) days , as it reached (88 ,263) mg 100 gm^{-1} of soil for each of Raxil and Dividend, respectively, as the increase may be due to the activity of the added microorganisms that carry out the decomposition process as a result of restoring their biological activity in the soil by decomposing easily assimilated materials (Al-Essa, 2009) and passing In the logarithmic phase, the rate of growth is maximum according to the growth curve.

As for the bilateral interaction between the addition of fogging pesticides and the duration of incubation, it had a significant effect on the amount of CO_2 released from the soil, as the amount of CO_2 released increased when adding the pesticides Raxil and Dividend, and at all incubation periods compared to the comparison treatment. Increasing the incubation period from 7-28 days led to Significant increase in the amount of CO_2 released when adding the pesticides Raxil and Dividend, and the treatment of adding Dividend at the 14-day incubation period gave the highest amount of CO_2 released, as it reached 232.45 mg per 100 gm^{-1} of soil, as Cycon and Kaczynska, (2004) indicated that some pesticides have good proportions of carbon rings and some elements included in the molecular structure of the pesticide such as nitrogen, which is one of the most important substrates on which some bacteria and fungi depend to sustain their vital processes. Which leads to the destruction of the pesticide molecules and the degree of their dissolution depends on the chemical composition and the type of bonds present in the pesticide, as it was observed that CO_2 gas is liberated from the Dividend pesticide is higher than the Raxil because of its high degree of decomposition by the action of microorganisms, as the survival period of the Dividend pesticide is (53 days).), which is less than the shelf life of the exterminator, which ranges between (49-610) days (Fitriyani et al., 2017).

The triple interaction between the addition of bacteria, mushrooms, fumigants and incubation periods had a significant effect on the amount of CO_2 liberated from the soil, as the amount of liberated CO_2 increased significantly when adding bacteria and mushrooms singly or double, and when adding a fumigant to all incubation periods, the incubation period increased from 7-14 Day led to a significant increase in the amount of CO_2 released in all treatments of adding bacterial and fungal inoculums and when adding Raxil and Dividend fumigants, and the treatment of the mixture of bacteria and fungi when Dividend was added and during the incubation period of 21 days recorded the highest amount of CO_2 liberated. It reached 385.0 $mg/100gm^{-1}$ of soil. The increase may be due to an increase in the activity of Pseudomonas bacteria and Trichoderma fungi according to the growth curve, as it went through the lag phase in which the growth rate is zero and then the logarithmic growth phase, in which the growth rate is maximum and thus the decomposition of these pesticides and utilization of them as a food source, as important elements such as C and N are available as a result of the decomposition of these pesticides, And that the death of some of them would be a source of food for others, and this in turn led to its rise and then its decline again, as Lo, (2010) found that some fungicides stimulate the growth of microorganisms, as the results of many studies showed the ability of bacteria and fungi to consume a wide range of pesticides In most cases, one or more compounds are consumed and used as a source of carbon and energy (Albrusci et al., 2011).

The results of Table (2) showed that the addition of Pseudomonas bacteria and Trichoderma, singly or in double, led to a significant increase in the number of Pseudomonas bacteria in the soil compared to the control treatment. dry compared to the control treatment which amounted to 10.66×105 cfu g^{-1} soil, with a significant increase of 386.49 %, And these results show the success of the process of adding bacterial and fungal inoculums individually or in combination, especially the treatment containing a mixture of Pseudomonas bacteria and Trichoderma fungi, evidence of their good growth, and the reason may be attributed to the compatibility and the presence of synergy between the metabolites of Pseudomonas bacteria and Trichoderma, where a positive interaction was found. It encouraged an increase in the number of Pseudomonas bacteria (Rajeswari, 2019).

The addition of the pesticide Dividend and Raxil led to a significant increase in the numbers of Pseudomonas bacteria compared to the control treatment, which was (29.57, 34.56, 105) cfu g^{-1} dry soil for the pesticides, Dividend, respectively compared to the control treatment, which amounted to 17.81×105 cfu g^{-1} dry soil with a percentage of An increase of (66.03, 94.04) %,The reason for the increase in the numbers of Pseudomonas bacteria in the presence of the two pesticides is evidence of their decomposition by bacteria, and their use as a source of food and energy, i.e. the metabolism of the pesticides, and this was confirmed by the results of many studies and research in the ability of microorganisms (bacteria and fungi) to consume a wide range of pesticides, In most cases, its ability to consume one or more compounds as a source of energy and carbon (Albrusci et al., 2011), and the reason for the increase in the numbers of Pseudomonas bacteria in the presence of Dividend compared to the Raxil pesticide may be due to the faster decomposition of the pesticide as a result of its presence in the recommended quantity, which encourages the growth of these bacteria by The way to decompose it and provide a source of energy and carbon from the decomposition products. Satapute (2016) was found. Pseudomonas bacteria have the ability to degrade the fungicide propiconazole and use it as the sole carbon source. The decomposition was through the secretion of three metabolites, 1,2,4<Triazol, 2,4, dibenzoic acid and 1-chlorobenzene.

The effect of the interaction between biological treatments and pesticides significantly on increasing the numbers of Pseudomonas bacteria, as the addition of bacteria and mushrooms individually or in combination led to a significant increase in the numbers of Pseudomonas bacteria, and when adding and not adding a fumigation pesticide, except for the addition of Trichoderma fungus, and when not adding a fogging pesticide, no significant increase was recorded. In the numbers of bacteria, and the addition of dusting pesticides led to a significant increase in the numbers of bacteria compared to the comparison (no addition) and in all biological treatments, the treatment of the mixture of bacteria and mushrooms recorded the highest value for the numbers of Pseudomonas bacteria, which amounted to (52.33,66.93) x 10^5 cfu gm^{-1} dry soil for the Raxil and Dividend,respectively Trichoderma fungus may encourage the growth apositive of Pseudomonas bacteria, as was found betwee interactio these organisms, and we did not find an inhibitory role, as there is a synergy between the products of these organisms (Harshita and others, 2018), as well as the role of these organisms collectively on the decomposition of fungicides and benefit from their decomposition products, Thus, providing a good environment equipped with nutrients that lead to the revival of Pseudomonas bacteria, as it was found by Bacmaga et al. (2021) that the addition of the pesticide Raxil (tebuconazole) led to stimulating the proliferation of organotrophic bacteria and its population increased by 64.46% compared to the comparison treatment. The reason was attributed to the presence of conditions that facilitate its growth and reproduction, Including the use of the pesticide as a source of carbon in addition to the fact that bacteria are more tolerant of tebuconazole due to the specific structure of its cell wall, and Sharma, (2016) .

Table 1: Effect of adding Pseudomonas bacteria, Trichoderma fungi, fungicides and interactions Between them on the release of carbon dioxide (CO_2) mg 100 g $^{-1}$ soil.

Incubation period (day)						
Interaction of vaccines with pesticides	**28**	**21**	**14**	**7**	**Pesticides**	Treatmeant
26.08 L	17.66 b*	35.00 xy	28.66 z	23.00 a*	**Control**	**Control**
63.95 H	71.00 t	80.50 r	69.66 t	34.66 xy	**Raxil**	
67.62 G	75.00 s	87.66 q	70.50 t	37.33 xw	**Dividend**	
32.66 K	21.00 ba	38.00 xw	37.00 xw	34.66 xy	**Control**	**P.fluorescens**
132.00 E	95.33 p	122.33 m	243 h	70.00 t	**Raxil**	
128.00 F	93.00 p	114.66 n	239.33 h	65.00 u	**Dividend**	
34.25 J	21.00 ba	39.00 w	39.00 w	38.00 xw	**Control**	***T.harzanium***
198.83 D	136.33 l	275.66 f	269.0 g	114.33 n	**Raxil**	
207.50 C	145.66 k	278.33 f	285.66 e	120.33 m	**Dividend**	
41.00 I	32.33 zy	43.66 v	43.00 v	45.00 v	**Control**	***P.fluorescens* + *T.harzanium***
251.33 A	133.00 l	363.00 b	310.33 d	199.00 i	**Raxil**	
249.33 B	103.66 o	385.0 a	334.33 c	174.33 j	**Dividend**	
Interference of vaccinations with incubation period						

Biological Vaccines		28	21	14	7	Treatmeant
52.55 D		54.55 l	67.72 j	56.27 lk	31.66 m	**Control**
97.77 C		69.77 i	91.66 g	173.11 d	56.55 k	***P.fluorescens***
146.86 B		101.0 f	197.66 c	197.88 c	90.88 hg	***T.harzanium***
180.55 A		89.66 h	263.88 a	229.22 b	139.44 e	***P.fluorescens + T.harzanium***
Pesticide interaction with incubation period						
The effect of the type of pesticide		28	21	14	**7**	**Treatmeant**
33.05 C		23.0 k	38.91 h	36.91 i	35.16 j	**Control**
161.69 B		108.91 e	210.37 d	223.0 b	104.50 f	**Raxil**
163.11 A		104.33 f	216.41 c	232.45 a	99.25 g	**Dividend**
		78.75 D	155.23 B	164.12 A	79.63 E	**Effect of incubation time**

Table 2: Effect of adding Pseudomonas bacteria, Trichoderma fungi and fungicides and the interaction between them on the numbers of Pseudomonas bacteria x 105 cfu g-1 dry soil.

Mean	Divid end	Raxil	control	Treatmeant
10.66 D	14.16 g	12.3 h	5.5 i	Control
33.61 B	39.66 c	38.83 c	22.33 e	*P.fluorescens*
13.14 C	17.5 f	14.83 g	7.1 i	*T.harzanium*
51.86 A	66.93 a	52.33 b	36.33 d	*P.fluorescens& T.harzanium*
	34.56 A	29.57 B	17.81 C	Mean

Table 3: Effect of adding Pseudomonas bacteria, Trichoderma fungi and fungicid And the interaction between them on the preparation of Trichoderma× 10^5 cfu gm^{-1} dry soil.

Mean	Divider	Raxil	control	Treatmeant
2.86 C	3.5 gf	2.83 gf	2.25 g	Control
3.30 C	4.15 f	3.5 gf	2.25 g	*P.fluorescen*
15.04 E	18 c	17.53 c	9.6 e	*T.harzanium*
22.50 A	29.0 a	26.0 b	12.5 d	*fluorescens & T.harzanium*
	13.66 A	12.46 B	6.65 C	Mean

Table 4: Effect of adding Pseudomonas, Trichoderma and fungicides and their interaction on the residues of Raxil and Dividend (ppm) in thesoil.

Mean	Dividend	Raxil	Treatmeant
0.0012 A	0.0015 b	0.0021 a	Control
0.00034 B	0.0010 c	0 d	*P.fluorescen*
0 C	0 d	0 d	*T.harzaniun*
0 C	0 d	0 d	*fluorescens + T.harzaniun*
	0.00064 A	0.00053 A	Mean

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