

Effects of burning crop residues and drenched the soil with water on the populations of the bacteria, *Thiobacillus* spp. and the fungus, *Trichoderma* spp. in the open fields

Abstract

Burning crop residues and/or drenched fields with water is one of the most common methods for controlling weeds and plant diseases in Iraq that farmers use after the wheat and barley harvest season every year. It is known that the soil contains many microorganisms that coexist with the roots of plants, such as fungi and symbiotic bacteria. This study aimed to show the effect of soil burning harvest residues or drenched with water on the population density of *Trichodermaspp.* fungus and *Thiobacillus* spp. bacteria. The results of the experiment showed that the burning process negatively affects the population density of microorganisms in the soil, which live at a level of 10 cm below the soil surface. The results indicated that there was a clear and significant difference between the treatments, like the burning of harvest residues or drenched of the soil, which reduced the population density of the tested microorganisms in the experiments.

Keywords: Burning crop residues, soil drenched, *Trichodermaspp.*, *Thiobacillus* spp.

2. Introduction

The Iraqi farmer uses some tools and methods to get rid of weeds and plant diseases. For that matter, he burns the leftovers from the wheat and barley harvest by setting fire to the field or drenched it with water. This makes the field ready to grow other crops in the summer.

This widespread use of incineration has an important role in the ecosystem in the fields treated with incineration. Burning is also used to reduce vegetative cover after harvest and to reduce plant residues, which are characterized as being of a toxic nature to microorganisms in the soil, as well as to plants grown in the same area (Rayn, 2000).

The process of burning plant residues can also lead to an increase in production in the following season, as Al-Taie and Al-Taie (2004) indicated that most

of the studied characteristics of the plants grown for wheat and barley crops in the unburned plant residues were low compared to those grown in the burned residues, and the loss of these values indicate the presence of phytotoxins in wheat residues and their effect on the vegetative growth characteristics of plants growing in them. The phytotoxins released from wheat residues as phenolic acids (Coumaric acid, P-acid zoichydroxyben, and Varillic acid). These toxins can stay in the soil for more than 6 weeks, and Al-Ta'i and Al-Ta'i (2004) found that burning the plant remains of wheat plants gets rid of most of the plant toxins that were in those remains.

On the other hand, the burning process may negatively affect the density and growth of various microorganisms in the soil. Fire can affect soil microbes directly through heat and indirectly by modifying the properties of the soil. It seems that the intensity of burning has the most effect on soil microbes. This is because fire intensity, duration, and soil properties affect the number of beneficial microbes in the soil, especially fungi, which are more sensitive to heat than bacteria and actinomycetes (Mataix-Solera et al., 2009).

The fungus *Trichoderma* spp. is one of the microorganisms that is characterized by its high ability to help plants obtain some basic elements from the soil, which leads to the improvement of plant growth and also contributes to stimulating growth by secreting some growth regulators. Which is found in various organic matters and in soil. Some species prefer dry and temperate places, and other cool, humid places (Abdul Wahid et al., 2007), which increase the building of the organic mass of the plant and stimulate the development of lateral roots (Al-Samarrai, 2002) and (Baland Suryya., 2008), mentioned that *Trichoderma* is affected by the burning process, as he indicated the effect of burning on the population density of the fungus. In addition *Trichoderma* in a burned oak forest in northern India. The most isolates were found five months after the forest was burned (Mabuhay et al, 2006).

Thiobacillus is one of the most important types of soil-endemic aerobic bacteria because of its important role in the oxidation of sulfur. It is a gram-negative bacterium in the form of rods with round ends or in the form of single cells, or sometimes in pairs, but rarely in triplets, with an average diameter of about 0.5 μm in length and 1 μm or less of mobility due to their terminal flagella, as described as colorless oxidizing to sulfur, and sulfur does not accumulate inside or outside its very

small cells (Boden, et al. 2017). The study aimed to show the effect of burning plant residues or drenched the field on the population density of *Trichoderma* spp. fungus and *Thiobacillus* bacteria after harvesting operations.

3. Materials and Methods

3.1- Study location: The study was conducted in the Microbiology Laboratory / Department of Field Crops / College of Agriculture / Wasit University.

3.2. Sample collection: Samples were collected from the soil of the wheat fields at the end of the harvest season two days before burning of the crop residues or drenched with water were carried out on 10 May 2022, and collected again two days after the burning of the crop residues or drenched, which were taken from the different soil for a distance of 1-5 cm after cleaning the soil layer wheat soil weighing 500g. The fields of SayedShate village, South-East of Kut city, and the field of Al-Djele village South of kut city, Iraq. The samples were kept in clean bags, then transferred to the Microbiology laboratory, sifted to get rid of impurities, and then the method of isolating fungus on PDA culture media was performed on them (Widden, 1986), for *Thiobacillus* spp. bacterial used 9k selective culture medium.

3.3- Isolation of fungi:

3.3.1: The method of isolating fungi: A soil sample of 5.0 g was taken and dissolved in 100 ml of distilled water at a ratio of (1/100) g of soil/distilled water, and after shaking for one minute, 5.0 ml of the suspension was withdrawn into a sterile Petri dish and added to it after the culture medium cooled to 45 °C, the PDA (Potato infusion 200 gm, Dextrose 20 gm and agar 20 gm for distilled water 1000 ml) was divided into three replicates for each sample. They were then stirred in a circular motion to mix well, left to harden, and then put in the incubator at 30 °C for 5–7 days before the fungi were identified.

3.3.2: Examination and diagnosis of isolated fungi:

3.3.2.1. Morphological characteristics: It included the colony's shape, colour, texture, and the pigment it produces from the back of the plate.

3.3.2.2: Microstructure Characteristics:

It included the presence of spores, their shape, and the number of cells. By transferring a small part of the fungi colony using a sterile inoculation needle to a drop of lactophenol dye on a clean glass slide, the slide was heated after placing the slide cover by passing it slightly over the flame of a Bunsen lamp. Then it was examined under a microscope at a power of x4, x10, and x40 to observe the microscopic characteristics of the mycelium.

3.4- Examination and diagnosis of *Thiobacillus* sp

Isolation, purification, and growth of bacteria from the soil on a suitable nutrient medium for study as in paragraphs 3.2. The selective culture medium consists of the following materials at pH 2.0:

| Materials | Quantity |
|-------------------------|----------|
| Ammonium sulphate | 0.400 g |
| Monopotassium phosphate | 4.000 g |
| Ferrous sulphate | 0.010 g |
| Calcium chloride | 0.250 g |
| Magnesium sulphate | 0.500 g |
| Sodium thiosulphate | 5.000 g |
| Agar | 12.500 g |
| distilled water | 1000 mL |

Samples containing nutrient medium were incubated at 30°C for 48 hours.

3.5- Bacterial identification

A microscopic examination was used, where a swab of each bacterial culture was taken, mixed with a drop of distilled water, placed on a glass slide, and stained with Gram stain, then examined under a light microscope using an oil lens with a final magnification of X100 to observe the response of cells to the dye, their shape, and arrangement.

3.6- Calculating *Thiobacillus* and *Trichoderma* colony forming unit CFU:

For calculate CFU/g soil, 10 g of soil was placed into 100 mL sterile distilled water. The solution was serial diluted 10x (1/10). 0.1 mL of the dilution was spread onto the agar plate. Using an equation that includes multiplying the number of growing colonies by the reciprocal of the dilution, the number of bacteria = the number of colonies * the reciprocal of the dilution.

3.7- Statistical analysis :

The randomized complete design (RCD) was used in the distribution of transactions experiments, with three replications for all treatments. The GenStat software was used to do an analysis of variance in the results, and the least significant difference (LSD) tests ($P < 0.05$) were used to compare the means between treatments and comparison treatments (Gomez and Gomez, 1984,).

4. Results and Discussion

4.1. The effect of burning crop residues and soils drenched with water on the population of *Thiobacillus* spp. bacteria and *Trichoderma* spp. fungus in the SayedShate village field

The results showed that the bacteria *Thiobacillus* was reached 6.40×10^6 CFU/g-1 in control treatment before the soil was burned or drowned in water, and reached 0 and 7.99×10^2 CFU/g-1 when the crop residues were treated with burning and drowned in water, respectively.

The population density of *Trichoderma* fungus reached 6.76×10^5 , 402 and 4.33×10^3 CFU/g-1 (Table 1) (Figure 1). It is noticed from the results that there is a clear and significant difference between the treatments.



Figure 1: The burning crop residues field

Table (1): The effect of burning crop residues and soil drenched with water on the population of *Thiobacillus* spp. and *Trichoderma* spp. in fields of the SayedShate village.

| Treatments | <i>Thiobacillus</i> g ⁻¹ | <i>Trichoderma</i> g ⁻¹ |
|-----------------------|---------------------------------------|------------------------------------|
| Control | 6.40*±2 × 10 ^{7b} | 6.76±2.7 × 10 ^{5a} |
| Burning crop residues | 0.00±0.00 (Not detected) ^c | 402±37 ^c |
| Drenched with water | 7.99±2 × 10 ^{2a} | 4.33±1.5 × 10 ^{3b} |
| L.S.D. | 290** | 215 |

*The number is an average of three replicates

**Means in a column followed with the same letter are significantly different (P<0.05)

4.2. The effect of burning crop residues and drenched fields on the population of *Thiobacillus* spp. and *Trichoderma* spp. fungus in the Dajele fields

The results showed that the bacteria *Thiobacillus* spp. and *Trichoderma* spp. fungus in the Dajele fields (Table 2) were reached at 3.40×10^6 CFU/g-1 in control treatment before the soil was treated with burning or drowning in water, where it reached 0 and 2.69×10^2 CFU/g-1 when the crop residues were treated with burning and drowning in water, respectively.

As for the population density of *Trichoderma* fungus, it reached 7.20×10^5 , 0 and 970 CFU/ g-1. It is noticed from the results that there is a clear and significant difference between the treatments (Figure 2).



Figure 2: The crop residues field treated soildrenchedwith water

Table (2): The effect of burning crop residues and drenched fieldson the population of *Thiobacillus* spp. bacteria and *Trichoderma* spp. fungus in fields of the Dajele

| Treatments | <i>Thiobacillus</i>⁻¹ | <i>Trichodermag</i>⁻¹ |
|---|---|---|
| Control before Burning or Drenched | 3.40*±1.4 × 10^{5a} | 7.20±3.2 × 10^{6a} |
| Burning crop residues | 0.00±0.00 (Not detected)^c | 0.00±0.00 (Not detected)^c |
| Drenched with water | 2.69±1.2 × 10^{2b} | 970±72^b |
| L.S.D. | 131** | 861 |

*The number is an average of three replicates

**Means in a column followed with the same letter are significantly different (P<0.05)

The findings revealed a significant difference between treatments, as burning harvest residues or drenched the soil reduced the population density of the tested microorganisms in the experiments.

Crop residue burning causes atmospheric pollutants, with seasonal crop burning being a major contributor. The burning of crop residue is reported to degrade

the soil, increase the risk of erosion, and increase the soil temperature, consequently decimating soil microorganisms. This impacts the monetary cost involved in recovering soil fertility and the potential for further pollution through the increased use of fertilizer (Linand Beghoa, 2022).

Jain et al. (2014) report that the burning of crop residue increases the soil temperature to about 42 °C, consequently decimating soil microorganisms up to a depth of about 2.5 cm. This has an effect on how much it will cost to make the soil fertile again, and it could also cause more pollution by causing more fertilizer to be used.

Burning of rice residue results in a loss of almost all carbon, leading to a drop in carbon sequestration (Singh and Singh, 2020), a loss of about 90% of N, loss of about 60% of S, and a loss of about 20–25% of P, and K as well as other micro-nutrients (Dobermann, 2002). In India, the burning of rice straws, wheat, and sugarcane stubble results in a loss of about 0.45 Mt, 0.144 Mt, and 0.84 Mt of NPK annually, respectively (Jain et al., 2014). The burning of crop residues degrades the soil structure and increases the risk of erosion (Sarkar et al., 2020). Gupta et al. (1994) assessed soils with residues burned, retained, or a combination of burned and retained residues with respect to their ability to improve soil organic matter and carbon and nitrogen availability. However, the results emphasized that keeping the crop residues increased the amount of mineralizable C and N much more than the other options. Long-term burning of crop residues also changed the soil's organic matter, total nitrogen, and carbon/nitrogen ratios.

Water is not only an essential transport medium for substrates, but it is also an important participant in hydrolysis processes. Therefore, soil water content controls microbial activity and is a major factor that determines the rates of mineralization (Paul et al., 2003). However, excess soil water content results in limited O₂ diffusion because O₂ diffusion in water is much lower than in air (about 104 times), which will reduce the activity of aerobic microorganisms (Kozłowski, 1984; Skopp et al, 1990), but could increase the activity of anaerobes. Lack of water reduces microbial activity and growth (Bottner, 1985), C and N mineralization (Sleutel et al., 2008), and microbial community structure (Sorensen et al, 2013).

According to Fierer and Schimel (2003), the concentration of available substrate and microbial activity peak within the first 24 hours of rewetting. This is because, upon rewetting, cells of sensitive microbes lyse, whilst other microbial genotypes release the organic solutes they accumulated during the dry phase (Halverson and Firestone, 2000). Furthermore, soil aggregates break down and their previously protected organic matter is exposed and can then be decomposed. Microbial biomass, activity, and nitrification decrease with the increasing number of dry and rewetting cycles (Mikhaet al, 2005).

6. Conclusion and recommendations

This study proved that the agricultural process used to control pathogens or weeds after harvests, such as burning crop residue or soil drenched in water, may decrease the number of microorganisms that live symbiotic with plants or provide them with nutrients, especially those microorganisms that are aerobic. Thus, we recommend that more other studies should be conducted to find out the possible ways or methods that helps of the return or keeping a considerable population of these organisms and their distribution in the soil after the period of agricultural process.

7. References

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