Original Research Article

The effect of burning crop residues and Soils drowned in water on the population of *Thiobacillus* spp. bactrial and *Trichoderma spp.* fungus in fields

Abstract

Burning crop residues or flooding fields with water is one of the most common methods of controlling weeds and plant diseases in Iraq that the farmer uses 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 burning harvest residues or flooding fields on the population density of *Trichoderma* spp. fungus and *Thiobacillus* spp bacterial. The results of the experiment showed that the burning process negatively affects the population density of beneficial microorganisms in the soil, which live at a level of 10 cm from the soil surface. The results indicated that there was a clear significant difference between the treatments, as burning of harvest residues or flooding of the soil reduced the population density with the tested microorganisms in the experiments.

Keywords: Burning crop residues**,** flooding fields**,** *Trichoderma* spp, *Thiobacillus* spp**.**

2. Introduction

The Iraqi farmer uses some means and methods of combating weeds and plant pathogens according to the habits of burning wheat and barley harvest residues by setting fire to the field or using water to flood the field and preparing the field for cultivation with other crops for the summer season.

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 Tai and Al Tai (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 it. Whereas, Zoein (1996) was able to identify the phytotoxins released from wheat residues, which are phenolic acids (Coumaric acid, P-acid zoic hydroxyben and Varillic acid). These toxins have the ability to remain in the soil for a period of more than 6 weeks, and burning the plant residues of wheat plants leads to the disposal of most of the plant toxins contained in those residues, Al Tai and Al Tai (2004).

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 most important factor affecting soil microbes is the intensity of burning. , which are controlled by factors such as fire intensity and duration and soil properties that usually lead to a decrease in the number of beneficial microbes in the soil, including fungi that are more sensitive to heating 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. It is found in various organic matter and soil. Some species prefer dry and temperate places. And other cool humid places (Abdul Wahid et al., 2007), which increases the building of the organic mass of the plant and stimulates the development of lateral roots (Al-Samarrai, 2002) and (Bal et al., 2008), and Trichoderma is affected by the burning process, as he indicated the effect of burning on the population density of the fungus. Trichoderma in a burnt oak forest in northern India and the maximum number of isolates appeared after four months of burning.

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 About 0.5 µm in length and 1 µm or less have 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 flooding the field on the population density of *Trichoderma* spp. and *Thiobacillus* bacteria after harvesting operations.

3. Methods and Materials

3.1- Samples: samples of wheat soil weighing (500) g were taken from two locations, the fields of Sayed Shate village, South-East of Kut city and the field of Al-Djele village South of kut city.

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

3.3- Sample collection: Samples were collected from the soil of the wheat fields on which the burning of the crop residues was carried out in November of the year 2021, as well as in the month of May of 2022, the end of the harvest season, and were taken from the soil for a distance of (5-10 cm) after cleaning the soil layer. The samples were kept in clean bags, then transferred to the laboratory, sifted to get rid of impurities, and then the method of isolating fungi on PDA culture media was performed on them (Widden, 1986).

3.4- Isolation of fungi:

3.4.1: 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 (1/200) 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 as After the culture medium cooled to 45 ° C, potato 200 gm, dextrose 20 gm, (PDA, distill water 1000 ml, agar15 gm), (to compare the growth of fungi on them) by five replicates for each sample and then stir in a circular motion to mix well and leave to solidify and then incubate in the incubator at 28°C for 5-7 days, after which the fungi were diagnosed.

3.4.2: Examination and diagnosis of isolated fungi:

Morphological characteristics:

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

3.4.2.2: Microstructure Characteristics:

It included the presence of spores, their shape and the number of cells. By transferring a small part of the mushroom 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, x40 to observe the microscopic characteristics of the mycelium.

3.5- Examination and diagnosis of *Thiobacillus* sp

Isolation, purification and growth of bacteria from soil on suitable nutrient medium for study as in paragraph 4-1. The selective culture medium consists of the following materials at pH 2.0:

List 1: The selective culture medium

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.

3.6- Bacterial identification

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.

3.7- Pots Experiment

Pots with a size of 4 kg were used in which wheat seeds were planted in formalin sterilized soil for 4 days and distributed according to the following treatments:

1 - Control transaction (without addition)

2 - Treatment of adding *Trichoderma* spp. fungus

3 - Treatment of adding the bacteria *Thiobacillus*

4 - Treatment of adding the fungus *Trichoderma* + burning the soil

5 - Treatment of adding *Trichoderma* spp. fungus + flooding the soil

- 6 Treatment of adding *Thiobacillus* + burning the soil

7 - Treatment of adding *Thiobacillus* + flooding the soil

After two months of planting, samples were taken from the soil for the purpose of calculating the numbers of *Trichoderma* fungus and the bacteria *Thiobacillus*.

3.8- Calculating Frequency Percentage:

The percentage frequency of fungi isolated from the soil of plants was calculated according to the following equation:

$$F = \frac{\text{The number of isolates of fungi or bacteria per unit}}{\text{The number of total isolates in the area}} \times 100$$

where F = percentage frequency. (1978, Krebs).

3.9- Statistical analysis :

The randomized block design (RCD) was used in the distribution of transactions for both laboratory and pot experiments, with three replications for all treatments. Results data were subjected to analysis of variance using GenStat software, and the means between treatments and comparison treatment were compared using least significant difference tests ($P < 0.05$) (1984, Gomez and Gomez).

4. Results and Discussion

4.1. The effect of burning crop residues and Soils drowned in water on the population of *Thiobacillus* spp. and *Trichoderma spp.* fungus in the Sayed Shate village field

The results showed that bacteria *Thiobacillus* were reached 6.40×10^7 cfu/ g^{-1} in control treatment before the soil was treated with burn or drown in water where reached 0 and 7.99×10^2 cfu/ g^{-1} when the crop residues treated with burning and drown in water respectively.

As for the population density of *Trichoderma* fungus where 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 significant difference between the treatments.

Figure 1: The burning crop residues field

Table (1): The effect of burning crop residues and Soils drowned in water on the population of *Thiobacillus* spp. and *Trichoderma spp.* fungus in fields of the Sayed Shate village.

Treatments	*Thiobacillus* g^{-1}	*Trichoderma* g^{-1}
control	6.40×10^7	6.76×10^5
Burning crop residues	Not detected	402
Soils drowned in water	7.99×10^2	4.33×10^3
L.S.D.	0.837	2.15

***The number is an average of three replicates**

4.2. The effect of burning crop residues and Soils drowned in water on the population of *Thiobacillus* spp. and *Trichoderma spp.* fungus in the Dejeale fields

The results effect of burning crop residues and Soils drowned in water on the population of *Thiobacillus* spp. and *Trichoderma spp.* fungus in the Dejeale fields (Table 2) were showed that bacteria *Thiobacillus* were reached 3.40 × 10^6 cfu/ g^{-1} in control treatment before the soil was treated with burn or drown in water where reached 0 and 2.69× 10^2 cfu/ g^{-1} when the crop residues treated with burning and drown in water respectively.

As for the population density of *Trichoderma* fungus where reached 7.20× 10^6 , 0 and 970 cfu/ g^{-1}. It is noticed from the results that there is a clear significant difference between the treatments (Figure 2).

Figure 2: The crop residues field treated with drowned in water

Table (2): The effect of burning crop residues and Soils drowned in water on the population of *Thiobacillus* spp. and *Trichoderma spp.* fungus in fields of the Dejeale

Treatments	*Thiobacillus* g^{-1}	*Trichoderma* g^{-1}
control	3.40 × 10^5	7.20× 10^6
Burning crop residues	Not detected	Not detected
Soils drowned in water	2.69× 10^2	970
L.S.D.	0.131	8.61

***The number is an average of three replicates**

4.3. The effect of burning crop residues and Soils drowned in water on the population of *Thiobacillus* spp. and *Trichoderma spp.* fungus in the pots

The results effect of burning crop residues and Soils drowned in water on the population of *Thiobacillus* spp. and *Trichoderma spp.* fungus in pots in field of Agriculture College of Wasit University were showed that bacteria *Thiobacillus* were reached 8.66 × 10^6 cfu/ g^{-1} in control treatment before the soil was treated with burn

or drown in water where reached 9.45× 10^2 and 3.40× 10^3 cfu/ g^{-1} when the crop residues treated with burning and drown in water respectively (Table 3).
As for the population density of *Trichoderma* fungus where reached 5.70× 10^6, 7.12× 10^2 and 4.66× 10^4 cfu/ g^{-1} (Table 3). It is noticed from the results that there is a clear significant difference between the treatments.

Table 3: Effect of burning crop residues and Soils drowned in water on the population of *Thiobacillus* spp. and *Trichoderma spp*. fungus in the pots

Treatments	*Thiobacillus* g^{-1}	*Trichoderma* g^{-1}
control	8.66 × 10^6	5.70× 10^6
Burning crop residues	9.45× 10^2	7.12× 10^2
Soils drowned in water	3.40× 10^3	4.66× 10^4
L.S.D.	1.73	1.21

***The number is an average of three replicates**

The results indicated that there was a clear significant difference between the treatments, as burning of harvest residues or flooding of the soil reduced the population density with the tested microorganisms in the experiments.

Crop residue burning caused 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 (Lin, M, and Beghoa, 2022).

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 (Jain et al., 2014). This subsequently impacts the monetary cost involved in recovering the soil fertility, as well as the potential for further pollution through the increased use of fertilizer.

Burning of rice residue results in a loss of almost all C, leading to a drop in C sequestration (Singh and Singh., 2020), a loss of 90% of N, 60% of S and 20–25% of P and K as well as other micro-nutrients (Dobermann and Fairhurst, 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, and a combination of burned and retained residue in respect to their ability to improve soil organic matter and carbon and nitrogen availability. The results showed that residue retention significantly increased the amounts of mineralizable C and N compared to the alternatives, and soil organic matter, total nitrogen, carbon/nitrogen ratios were affected by the long-term burning of crop residues.

Water is not only an essential transport medium for substrates, 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 (about 104 times) than in air which will reduce the activity of aerobic microorganisms (Kozlowski, 1984; Skopp, Jawson, & Doran, 1990), but could increase the activities of anaerobes. Lack of water reduces microbial activity and growth (Bottner, 1985), C and N mineralization (Sleutel et al., 2008) and shifts microbial community structure (Sorensen etal, 2013).

Fierer and Schimel (2003) were indicted to the concentration of available substrate and microbial activity peak in the first 24 h after 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 etal, 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 increasing number of dry and rewetting cycles (Mikha etal, 2005).

6. Conclusion and recommendations

This study proved that agricultural operations used to control pathogens or weeds after harvest, such as crop residue burning or soil flooding, may lead to a decrease in the number of microorganisms that live symbiotic with plants or provide them with nutrients, especially those microorganisms that are aerobic . Thus, we recommend that other studies be conducted to find out the possibility of the return of these organisms and their spread in the soil after a period of agricultural operations**.**

7. References

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