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Effect of Some Herbicide (Metribuzin, Pendimethalin and Fluazifop-p-Butyl) on *Bacillus cereus* and *Pseudomonas putida*

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Abstract: Beneficial bacteria are used as plant growth promoters in agriculture. The off-target effects of herbicides on beneficial bacteria are needed to be investigated. This study was carried out to determine the positive or negative effects of active compounds of herbicides on the reproduction of beneficial bacteria, *Bacillus cereus* and *Pseudomonas putida*. The herbicides used in this study contained active compounds such as Fluazifop-p-buthyl, Pendimethalin and Metribuzin. Effects of the active compounds were evaluated on the bacteria under *in vitro*, greenhouse and field conditions. Three doses of each active compound were used: the recommended dose (N), twice the recommended dose (2N) and three folds (3N) the recommended dose. It was observed that the increased dose of Pendimethalin, Fluazifop-p-buthyl, Metribuzin had significant effects on *B. cereus* and *P. putida* under *in vitro* conditions. Also, the increased dose of Pendimethalin, Fluazifop-p-buthyl significantly reduced the density of both bacteria. On the other hand, increasing doses of Pendimethalin decreased the density of *P. putida*, but did not affect that of *B. cereus*. Under greenhouse conditions, the density of *B. cereus* was not affected with increased doses of Fluazifop-p-buthyl and Metribuzin while the bacteria concentration were increased with doses of pendimethalin. As the doses of active substances increased, *P. putida* bacterial density also increased in greenhouse. Under field conditions, the recommended doses of Pendimethalin, Metribuzin did not inhibit *B. cereus* density, while the dose of Fluazifop-p-buthyl increased the bacterial concentration. Additionally, under similar conditions, Fluazifop-p-buthyl and Metribuzin did not affect *P. putida*, while Pendimethalin decreased concentration of *P putida*. In the present study results showed that increased active substances of Pendimethalin, Fluazifop-p-buthyl and Metribuzin are decreasing or increasing the densities of *P. putida* and *B. cereus* with dependent on experimental conditions. All these active substances are not eradicating the beneficial bacterial population in soil. It would be appropriate to give some quantitative values of the obtained results in the abstract.

Keywords: *Bacillus cereus*, density of bacteria, *Pseudomonas putida*, recommended dose, soil

Bazı Herbisitlerin (Metribuzin, Pendimethalin ve Fluazifop-p-Butil) *Bacillus cereus* ve *Pseudomonas putida* Üzerine Etkisi

Öz: Birçok faydası olan yararlı bakteriler tarımda bitki gelişimini destekleyici olarak kullanılmaktadır. Tarımsal alanlarda kullanılan herbisitlerin faydalı bakteriler üzerindeki hedef dışı etkilerinin belirlenmesi gerekmektedir. Bu çalışma, herbisitlerin faydalı bakteriler *Bacillus cereus* ve *Pseudomonas putida* 'nın çoğalmasına olumlu veya olumsuz etkilerini belirlemek amacıyla gerçekleştirilmiştir. Test edilen herbisit aktif maddeleri Fluazifop-p-buthyl, Pendimethalin ve Metribuzin'dir. Aktif maddelerin etkileri *in vitro*, sera saksı deneyleri ve tarla denemelerinde değerlendirilmiştir. Her herbisit için aktif maddenin üç dozu kullanılmıştır: önerilen doz (N), önerilen dozun iki katı (2N) ve önerilen dozun üç katı (3N). Artan Pendimethalin ve Fluazifop-p-buthyl dozları her iki bakterinin de yoğunluğunu azaltmaktadır. Öte yandan, artan Pendimethalin dozları *P. putida*'nın yoğunluğunu azaltırken, *B. cereus*'u etkilememiştir. Serada, *B. cereus*'un yoğunluğu Fluazifop-p-buthyl ve Metribuzin'in artan dozlarından etkilenmezken, pendimethalin ile artmıştır. Aktif maddelerin dozları arttıkça, serada *P. putida* bakteri yoğunluğu da artmıştır. Tarla denemelerinde, Pendimethalin ve Metribuzin'in önerilen dozları *B. cereus* yoğunluğunu engellemezken, Fluazifop-p-buthyl bakteri yoğunluğunu artırmıştır. Fluazifop-p-buthyl ve Metribuzin *P. putida* yoğunluğunu etkilemezken, Pendimethalin *P. putida* yoğunluğunu azaltmıştır. Bu çalışmada, sonuçlar herbisit etken maddeleri olan Pendimethalin, Fluazifop-p-buthyl ve Metribuzin'in *P. putida* ve *B. cereus*'un yoğunluklarını deneysel koşullara bağlı olarak artırdığını veya azalttığını, ancak topraktaki popülasyonlarını tamamen ortadan kaldırmadığını açıkça göstermiştir. Elde edilen sonuçlara ait bazı kantitatif değerlerin de özet kısmında verilmesi uygun olacaktır.

Anahtar kelimeleri: *Bacillus cereus*, bakteri yoğunluğu, *Pseudomonas putida*, tavsiye edilen doz, toprak

1. Introduction

The world population is increasing at an unprecedented rate and the ability to meet the dietary requirement is one of the goals of crop scientists. In developing countries, most part of their economy is based on agriculture. To increase the yield obtained from land area, it is necessary to adopt sustainable agricultural control methods to protect crops from pests, weeds and invasive plants (Digrak et al., 1998). Herbicides provide a powerful and chemical control option in the control of weed. However, active ingredients in herbicides may pose a threat to beneficial organisms in the soil. Therefore, it is necessary to determine whether newly developed herbicides have a negative impact on beneficial organisms in the soil before its application.

Although chemical pesticides contribute greatly to the increase in crop yield, they also tend to poison beneficial microorganisms. The effectiveness, low-cost and easy applicability of synthetic herbicides make them highly preferred by farmers (Gulec et al., 2015; Yavuz et al., 2017). Herbicides are one of the pesticides widely used in agricultural production and their use is increasing day by day (FAO, 2023; Kitis et al., 2016). While herbicides aid in the control of weeds, they also affect fungal pathogens and bacteria in the soil (Akbulut, 2008). The application of herbicides to the field soils can potentially affect soil beneficial bacteria and their activities (Madhuri et al., 2012). This can lead to stimulation, reduction or alteration of biological processes in the soil (Vig et al., 2008). There are many studies on the effects of herbicides on soil microflora. These literatures should be mentioned more in the introduction.

Beneficial bacteria in the soil improves soil fertility, soil aeration, and overcomes environmental problems such as pollution and soil degradation. *Pseudomonas putida* and *Bacillus cereus* are soil-borne bacteria that promote plant growth (Aktan and Soylu, 2020; Cakmakci, 2004; Gupta et al., 2015). Herbicides used in modern agricultural systems are preferred for their efficacy, while their potential harmful effects are often ignored. The effects of active substances-Metribuzin, Pendimethalin and Fluazifop-p-butyl- on *P. putida* and *B. cereus* are not known. Since soil beneficial bacteria may be affected by chemical pesticides, it is necessary to investigate the effects of common active substances on these beneficial organisms.

There are several beneficial bacteria species in soil microbiota. *Pseudomonas* and *Bacillus* are most prominent since they have good antagonistic properties against pathogens (Altin and Bora, 2005; Torun, 2015). There is no sufficient information about the off-target effects of herbicides (Karaaslan and Gur, 2009) on *Pseudomonas* and *Bacillus* bacteria. In the present study, the effects of herbicide active substances such as metribuzin, pendimethalin and fluazifop-p-butyl on growth and reproduction of *Pseudomonas* and *Bacillus* bacteria are investigated.

2. Material and Methods

2.1. Material

In the study, Metribuzin, Pendimethalin and Fluazifop-p-butyl, which are widely preferred by farmers for weed control, were used (Table 1). Isolates of *P. putida* and *B. cereus* were obtained from pepper production areas in a previous study conducted by Kayaaslan (2021).

Table 1. Herbicides used and their recommended doses.
Çizelge 1. *Kullanılan herbisitler ve önerilen dozlar*

Active substances	Mode of action	Recommended dose
Metribuzin	Broadleaf weeds	50-75 g/da
Pendimethalin	Annual grasses and broadleaf weed	500 ml/da
Fluazifop-p-butyl	Narrow leaf weeds	100 ml/da

2.2. Methods

2.2.1. *In vitro* tests

Three different doses of Metribuzin, Pendimethalin and Fluazifop-p-butyl were applied: the recommended dose (N), twice of the recommended dose (2N) and thrice of the recommended dose (3N) were added into nutrient agar (NA) medium. The NA, a 1000 ml solution was containing 20 g nutrient agar ((Merck, Germany), was used as medium in the study (Lelliot and Stead, 1987). The NA medium was autoclaved at 121 °C for 15 min and kept at 40 °C. The herbicides were added into the media at the determined concentrations (herbicides were calculated according to the liquid ratio for 20 ml of PDA to a petri dish) for each dose and mixed with a magnetic stirrer and poured into 90 mm diameter Petri dishes with a volume of 20 ml. Distilled water was used as a negative control. Bacterial isolates of *P. putida* and *B. cereus* which in stock culture at -20 °C, were cultivated on NA medium and incubated at 25±2 °C for 24 hours. Then, a bacterial suspension was prepared in saline buffer (0.85 g NaCl per liter) and adjusted to an absorbance value of 0.3 (A_{600}: 0.3) at 600 nm wavelength in a spectrophotometer (Madison WI 53711, USA). Bacteria density in suspension were $1x10^8$ cfu ml^{-1} and 100 µl of the suspensions were transferred to the NA medium containing the herbicides doses and incubated at 25±2 °C for 24 hours. At the end of the incubation, bacterial colonies were collected, and densities were measured in a spectrophotometer (A600:0.3) (Belguzar et al., 2019). The experiments were conducted using the randomized block research design with five replications and the experiment was repeated twice.

2.2.2. Greenhouse tests

The effects of herbicides on *P. putida* and *B. cereus* were investigated in a pot experiment under greenhouse conditions. Each of pots were filled with 1 kg of sterile mixture of soil, peat and perlite (2 volume: 1 v: 1 v). A suspension of *B. cereus* and *P. putida* cultures was prepared at a density of $1x10^8$ cfu ml^{-1} (200 ml per pot). The prepared suspension was mixed with autoclaved soil and filled into pots (Belguzar et al., 2018). The N, 2N and 3N doses of Metribuzin, Pendimethalin, Fluazifop-p-butyl herbicides were applied according to the surface area of the pots (160x145 mm). The pots in the control group were filled with sterile soil inoculated with bacteria but no herbicides treatment. The bacteria containing pots were applied with N, 2N and 3N doses of herbicides individually. Soil samples were taken at 15-day intervals for 60 days to determine the bacterial density. Soil samples were taken from 10 cm depth, the samples were dried for a day under laboratory conditions and then were sieved through a 2 mm mesh sieve. Then, 10 g of the sieved soil samples was added to 90 ml nutrient broth medium and they were shaken at room temperature at 100 rpm for two hours on a shaker (IKA HS 501, Germany). The suspension (1 ml) was added to a tube containing 9 ml of physiological saline buffer (saline buffer - 0.85% NaCl) and this process was repeated 6 times (Schaad et al., 2001). In this way, six times (6) dilution series were prepared. Afterwards, 100 µl of the -4, -5 and -6 dilutions of the prepared series were s transferred on Nutrient Agar medium in 2 replicates and incubated at 25 ± 2 °C for 24 hours. Bacterial colonies in petri dishes were counted according to the plate counting technique (Klement et al., 1990).

2.2.3. Field study

The field studies were conducted in the experimental field of Agricultural Research and Application Center field at Tokat Gaziosmanpaşa University. 18l cans were filled with 15 kg of soil from the field and buried in the soil. A suspension of *B. cereus* and *P. putida* cultures was prepared at a density of $1x10^8$ cfu ml^{-1} (2 liter per can). The prepared suspension was mixed with field soil and filled into cans (Belguzar et al., 2018). The N dose of Metribuzin, Pendimethalin, Fluazifop-p-butyl herbicides was applied according to the surface area of the cans (236 x 236 mm). Only irrigation water was applied to the control cans. The field trials were established using a randomized block design with three replications and repeated twice. Four samples were taken from each treatment. Soil samples were taken from 20 cm depth and were dried for one day under laboratory conditions and sieved through a 2 mm sieve. Bacterial density in the field soil was determined as described in the greenhouse tests.

2.2.4. Statistical Analysis

For statistical evaluations of the results Mstat-C package program was used. The LSD test was used and differences between significant means were grouped according to 5% significance level. Doses and days were compared among themselves, and averages were taken. Herbicides and bacteria treatments were not compared among themselves.

3. Results

3.1. *In vitro* test

Pendimethalin, Metribuzin and Fluazifop-p-butyl showed different effects on *P. putida* and *B. cereus* under *in vitro* conditions (Table 2). N dose of metribuzin significantly reduced *B. cereus* bacteria compared to the control. The 2N and 3N doses reduced bacterial density more than the N dose, but the 2N dose was not statistically different from the 3N dose. In contrast to observations made on *B. cereus*, there was a direct relationship between Metribuzin doses and the density of *P. putida*.Thus as the doses of Metribuzin increased, an increase in the density of *P. putida* was observed .

Similarly, fluazifop-the increased doses of p-butyl decreased the density of *B. cereus,* while the inverse was observed for density of *P. putida* density. Compared to the control, the difference between them was not significant as increasing Pendimethalin doses increased. Pendimethalin herbicide treatment did not have any decreasing or increasing effect on *B. cereus* population irrespective of the various doses' treatment. However, it did have an increasing effect on *P. putida* density.

3.2. Pots experiments in greenhouse

Bacterial densities in pots were measured for 60 days at 15-day intervals and the effect of herbicides on the density of bacteria is summarized in Table 3 and Table 4. On the 15th day, isolations of *B. cereus* from pot soils treated with fluazifop-p-butyl revealed a decrease in the density of the bacteria. In contrast, the density of *P. putida* did not differ at N and 2N doses of the chemical but increase was observed at 3N dose treatment. Also, on the 30th day the density of *B. cereus* increased, whilst the density of *P. putida* was found to have decreased after isolation. On the 45th day, both bacterial densities were lower than control pots in all doses, but they were not significantly differences were observed. On the 60th day, it was determined that density of *B. cereus* decreased in all doses, while *P. putida* bacterial density increased at 2N dose, but did not differ in other doses. In general, the effect of fluazifop-p-butyl on *B. cereus* bacterial density was not significant at all doses compared to the control, while *P. putida* bacterial density increased at 2N and 3N doses.

Table 2. Metribuzin, Fluazifop-p-butyl and Pendimethalin effect on *Pseudomonas putida* and *Bacillus cereus* density (cfu/ml)

Çizelge 2. *Metribuzin, Fluazifop-p-butil ve Pendimethalinin Pseudomonas putida ve Bacillus cereus yoğunluğu üzerine etkisi*

Active substances	*Dose*	*Bacillus cereus*	*Pseudomonas putida*
Metribuzin	Control	2.044^{a}	1.713^{b}
	N	1.682^{b}	1.800^{ab}
	2N	0.398^{c}	1.919^{a}
	3N	0.445^{c}	1.856^{a}
Fluazifop-p-butyl	Control	2.044^{a}	1.713^{c}
	N	1.699^{b}	1.725^{c}
	2N	1.608^{b}	1.851^{b}
	3N	1.755^{b}	2.028 ^{a}
Pendimethalin	Control	2.044^{ns}	1.713^{b}
	N	2.084	2.271^{a}
	2N	2.058	2.196^{a}
	3N	2.115	2.218^{a}

^{NS}: not significant. Means followed by a different letter are in the same column significantly different at an alpha level of 0.05 according to LSD test

Table 3. Effect of different doses of herbicides on *Bacillus cereus* growth in pots (cfu/ml)

Çizelge 3. *Farklı dozlardaki herbisitlerin saksılarda Bacillus cereus büyümesi üzerine etkisi*

Active substances	**Doses**	**Days**				Mean
		15	**30**	**45**	**60**	
Fluazifop-p-buthyl	Control	13.70 ^{a}	4.00 ^{b}	6.00^{NS}	5.76 ^{a}	7.37^{NS}
	N	5.76 ^{b}	10.76 ^{a}	3.26	2.76 ^{c}	5.64
	2N	5.50 ^{b}	6.76 ^{ab}	5.76	4.00 ^{b}	5.51
	3N	7.00 ^{b}	11.00 ^{a}	3.00	2.50 ^{c}	5.88
Metribuzin	Control	13.76^{NS}	4.00^{NS}	6.00 ^{a}	5.76 ^{a}	7.38^{NS}
	N	10.26	5.50	6.76 ^{a}	5.50 ^{a}	7.01
	2N	12.00	6.00	3.76 ^{b}	2.50 ^{b}	6.07
	3N	27.60	6.26	4.26 ^{b}	4.76 ^{ab}	10.77
Pendimethalin	Control	13.70 ^{c}	4.00 ^{b}	6.00^{NS}	5.76^{NS}	7.38 ^{b}
	N	10.50 ^{c}	5.76 ^{b}	7.76	2.76	6.70 ^{b}
	2N	64.50 ^{a}	8.50 ^{b}	7.00	2.00	20.50 ^{a}
	3N	46.26 ^{b}	22.00 ^{a}	7.00	8.26	20.88 ^{a}

^{NS}: not significant. Means followed by a different letter in the same column are significantly different at an alpha level of 0.05 according to LSD test.

Compared to the control, there was no effect of N and 2N dose of metribuzin on the density of *B. cereus* at day 15, while density of *P. putida* increased only at 3N dose but they were not significant differences between them. The N dose of metribuzin had no effect on *B. cereus* density on the 45th and 60th days, while the 2N and 3N doses decreased the density *B. cereus*. At 45 and 60 days, the effect of metribuzin on density of *P. putida* was not statistically significant. In general, the effect of all three doses of metribuzin on the density of *B. cereus* bacteria was not statistically significant compared to the control. In *P. putida*, the N dose of the herbicide decreased the bacterial density compared to the control, while the difference between the other doses was not significant.

While the N dose of pendimethalin did not affect the bacterial density on day 15, 2N and 3N doses increased the density of *B. cereus* compared to control. There was an increasing effect of pendimethalin on density *P. putida* at all three doses. The N and 2N doses of pendimethalin were compared to the control on day 30, the differences were not significant for density of *B. cereus*, while at 3N dose, the density increased. On day 30, the N dose of pendimethalin increased the density of *P. putida*. The 2N dose of pendimethalin was not different compared to the control, while the 3N dose had a decreasing effect on the bacterial density. On days 45 and 60, the effect of pendimethalin did not have a significant effect on the density of *B. cereus* at all doses. Also, on day 45, 2N dose of pendimethalin increased *P. putida* bacterial density, while there was no difference among other doses and control. On the 60th day, the effect of pendimethalin was not significant, although it reduced density of *P. putida*. In general, N dose of pendimethalin was not different from control, but the density of *B. cereus* increased at 2N and 3N doses. The N and 2N doses of the pendimethalin increased the density of *P. putida* compared to the control, while there was a significant difference between the 3N dose and control.

Table 4. Effect of different doses of herbicides on *Pseudomonas putida* growth in pots (cfu/ml)
Çizelge 4. *Farklı dozlardaki herbisitlerin saksılarda Pseudomonas putida büyümesi üzerine etkisi (cfu/ml)*

Active substances	Doses	Days 15	30	45	60	Mean
Fluazifop-p-buthyl	Control	1.75 b	3.00 a	4.25NS	2.00 bc	2.75 b
	N	2.75 b	1.25 c	4.00	0.75 c	2.19 b
	2N	0.75 b	2.25 b	6.25	7.50 a	4.19 a
	3N	8.00 a	1.75 bc	5.25	3.25 b	4.56 a
Metribuzin	Control	1.75ab	3.00NS	4.25NS	2.00NS	2.75 a
	N	0.75b	1.25	3.75	1.50	1.81 b
	2N	0.50b	2.00	2.75	3.00	2.06 ab
	3N	2.75a	1.75	4.50	1.50	2.63 ab
Pendimethalin	Control	1.75NS	3.00b	4.25b	2.00NS	2.75 c
	N	3.75	4.00a	7.00b	0.75	3.88 b
	2N	4.50	2.75b	12.50a	0.75	5.13 a
	3N	4.50	1.75c	6.00b	1.75	3.50 bc

NS: not significant. Means followed by a different letter in the same column are significantly different at an alpha level of 0.05 according to LSD test.

3.3. Effects of herbicides on *Pseudomonas putida* and *Bacillus cereus* under field conditions

In the field trials, the effects of herbicides on bacteria were determined using the density of bacteria for 60 days at 15 days intervals. Bacteria were isolated from soils treated with herbicides at the recommended dose (Table 5). The active substances Pendimethalin and Fluazifop-p-butyl increased the density of *B. cereus* on day 15, while Metribuzin was not affected. The effect of herbicides on *P. putida* was not significant on day 15 compared to the control. The effects of Metribuzin, Fluazifop-p-butyl and Pendimethalin on density of *B. cereus* were not different from control. On day 30, the effect of active substances on *P. putida* was not significant. Active substances did not affect the density of *B. cereus* on day 45, while they decreased the density of *P. putida* under field conditions. When the effect of the recommended doses (N) of the herbicides on *B. cereus* were compared with the control, the difference between them was not significant. Only Metribuzin increased the density of *P. putida* bacteria, while Pendimethalin and Fluazifop-p-buthyl were not affected.

Table 5. Effect of herbicides on *Bacillus cereus* and *Pseudomonas putida* growth in field soil (cfu/ml)
Çizelge 5. *Herbisitlerin tarla toprağında Bacillus cereus ve Pseudomonas putida büyümesi üzerine etkisi (cfu/ml)*

	Herbicides/days	15	30	45	60	Mean
B. cereus	Control	4.75 b	5.00 ab	2.50 b	4.75NS	4.25 bc
	Pendimethalin	11.00 a	8.25 a	2.50 b	6.00	6.94 ab
	Metribuzin	3.25 b	2.25 b	2.00 b	3.25	2.69 c
	Fluazifop-P-Buthyl	13.00 a	2.25 b	13.75 a	3.75	8.19 a
P. putida	Control	4.50 NS	4.50 NS	5.00 a	2.00 b	4.00 a
	Pendimethalin	5.25	3.50	1.25 b	1.50 b	2.88 b
	Metribuzin	4.50	5.25	1.50 b	3.25 a	3.63ab
	Fluazifop-P-Buthyl	4.25	4.00	2.00 b	1.75 b	3.00 ab

NS: not significant. Means followed by a different letter in the same column are significantly different at an alpha level of 0.05 according to LSD test.

In general, the density of *B. cereus* bacteria in soils treated with Fluazifop-p-buthyl was higher than the control, while the density of bacteria in soils treated with Metribuzin and pendimethalin was like the control.

When the effect of Metribuzin and Fluazifop-p-buthylon *P. putida* bacteria were compared with the control, the difference was not significant, while Pendimethalin decreased the density of *P. putida* bacteria. Finally, it was discovered that both bacteria were still viable in soil samples taken on day 60.

4. Discussion

In modern agriculture, pesticides (herbicides, fungicides, insecticides, etc.) are commonly used to increase crop yields and control various pests (weeds, fungal pathogens, and insects) (Thiour-Mauprivez et al., 2019). Pesticides are applied to prevent pests and not to adversely affect other living organisms (Guven and Koc, 2020). Irrespective of the importance of pesticides in agriculture, some active ingredients of herbicides may have adverse effects on growth and reproduction of non-target organisms (Madhuri et al., 2012). Therefore, the positive and negative effects of herbicide on the density of *Pseudomonas putida* and *Bacillus cereus* were investigated.

In vitro application of metribuzin decreased the density of *B. cereus* bacteria at all three doses. However, there was no effect of metribuzin doses on the density of *B. cereus* under greenhouse and field conditions. The negative effects of metribuzin active substances in vitro may be due to the fact that bacteria are only exposed to the active substance outside the nutrient agar. Whereas, in greenhouse and in the field, the ineffectiveness of metribuzin on bacterial growth may be due to organic matter or other microorganisms in the field soil that promote bacterial growth. At the recommended dose of Metribuzin, *P. putida* density under in vitro conditions was not affected, but density of *P. putida* increased at increasing doses. In greenhouse conditions, density of *P. putida* decreased at N dose of Metribuzin, but there is no effect at other doses. In the field, recommended dose of Metribuzin had no effect on density of *P. putida*. Kotan and Tozlu (2021) tested the bactericidal effects of herbicides with seven active ingredients against nine bacterial isolates using recommended doses under in vitro conditions and it was determined that metribuzin did not show bactericidal effect. Erguven (2019) investigated the influence of metribuzin on *Bacillus subtilis* in field trial, and emphasized that *B. subtilis* degraded Metribuzin, therefore *Bacillus subtilis* was not affected by this herbicide. Zaid et al. (2014) applied pre-emergence herbicides to pea fields and investigated their effects on soil microflora and nitrogen fixing bacteria. From their study, it was reported that metribuzin increased the soil bacterial density.

Pendimethalin did not affect the density of *B. cereus* under both in vitro and field conditions. In greenhouse conditions, pendimethalin increased the density of *B. cereus* bacteria at increasing doses. Oyeleke et al. (2011) reported that the recommended dose of pendimethalin decreased the microbial population and increasing doses of pendimethalin decreased the microbial population in the soil, supporting the results in the study. Pendimethalin increased the density of *P. putida* bacteria in both laboratory and greenhouse conditions. In the field, Pendimethalin caused a decrease in density of *P. putida*. Singh et al. (2021) reported that pendimethalin had the lowest inhibition on *P. fluorescens* compared to other herbicides under in vitro conditions. Raghavendra et al. (2017) observed that pendimethalin applied at the recommended dose reduced the density of Azotobacter, Rhizobium and phosphorus degrading bacteria compared to control, indicating similar results to the present study in field trials with pendimethalin. et al., 2011). Guven and Koc (2020) reported that pendimethalin caused a decrease in the number of bacteria in soil. On the other hand, Maheswari et al. (2016) observed minimum inhibition in Pendimethalin (0.3 ml/100 ml).

Fluazifop-p-buthyl decreased the density of *B. cereus* at all three doses in vitro, but there was no effect was observed under greenhouse condition. Fluazifop-p-buthyl increased the density of *B. cereus* in soil under field conditions. Fluazifop-p-buthyl did not affect *P. putida* at the recommended doses in vitro and greenhouse, while density *P. putida* increased at increasing dose. Under field conditions, there is no effect of Fluazifop-p-buthyl to *P. putida* density. Darine et al. (2015) determined that Fluazifop-p-buthyl increased the bacterial density in soil. Erguven and Nuhoglu (2020) reported that bacteria such as *Brevibacterium macrolides, Bacillus macrolides, Microbacterium chocolatum, Bacillus subtilis, Ochrobactrum thiophenivorans, Sphingomonas meloni s*and *Sphingomonas aquatilis* degraded the Fluazifop-p-buthyl in the soil, but the value was never zero.

Results showed that herbicides with the active substances such as Pendimethalin, Fluazifop-p-buthyl, Metribuzin increased the presence of *P. putida* and *B. cereus* beneficial bacteria in some conditions and decreased in some conditions but did not eliminate them. Lo (2010) reported that the pesticides stimulated the growth of some microorganisms in the soil, reduced the growth of others and even had no effect on some microorganisms. The density of *P. putida* and *B. cereus* bacteria initially increased after herbicide application.

Similarly, Oyeleke et al. (2011) reported also an increase in the density of bacteria in the soil at the beginning of herbicide application and reached a maximum of the density in the next few weeks. This may be due to soil microflora that can temporarily mineralize and use the herbicide as an energy source (Kunc et al., 1985).

5. Conclusion

In this study the herbicides' active substances resulted different effects on soil beneficial bacteria *in vitro*, greenhouse and in field conditions. However, in general, the herbicide active compounds either increased or did not significantly affect the growth of beneficial bacteria. The obvious negative effects of active substances in the *in vitro* may be since the bacteria were only exposed to the active substance and medium contents. In greenhouse and field, the lack of effect of the active compounds on bacterial growth, or even an increase, may be due to organic matters or other microorganisms in the field soil which promote bacterial growth or soil chemical and physical features. It may also be that the active substances are degraded by beneficial bacteria and, thereby their effect could be reduced. Finally, Pendimethalin, Fluazifop-p-buthyl and Metribuzin can be safely used at recommended doses for weed control as they did not harm *P. putida* and *B. cereus* bacteria under greenhouse and field conditions.

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