



Effects of Various Soil Tillage and Herbicide Applications on Yield and Weeds in Chickpea Production

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Abstract: The effects of various tillage methods and herbicide applications on crop yield and broadleaf weeds in chickpea production were investigated. For this reason, a 3-year (2020-21, 2021-22, and 2022-23 chickpea growing season) field experiment was conducted in Diyarbakır region, Türkiye. The experiments were laid out in split-plot design with three replications, in which tillage methods [moldboard plow+cultivator (MP+C), disc harrow+cultivator (DH+C) and cultivator (C)] were main plots, and herbicide applications [pre-emergence (Pre), post-emergence (Post), pre-emergence+post-emergence (Pre+Post), Weed-free check, weedy check] were sub-plots. The higher grain yield was observed under MP+C than DH+C and C treatments although the difference among tillage treatments was not significant in the 2020-21 and 2022-23 production seasons. Among the herbicide applications, the highest grain yield was found at the pre-emergence herbicide treatment following the weed-free check plots. When the treatments were evaluated in terms of their effects on weed observations, it was observed that tillage with moldboard plow and pre-emergence herbicide application were more effective in controlling weeds.

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1. Introduction

Chickpea is a legume that has been cultivated and consumed by humans since ancient times. It has played a great role in the nutrition of people for centuries because it is very rich in protein, mineral substances, and vitamins (Güler, 2011). Also, it is known that chickpea is an important crop rotation plant because it improve the physical, chemical, and biological properties of the soil and increase the organic matter content of the soil due to the nodosity bacteria in their roots (Kayan, 2005; Güler, 2011). According to the FAO 2021 datum, India has the highest chickpea sowing area with 10.9 million ha, accounting for approximately 73% of the total chickpea cultivation area worldwide, Türkiye ranked the fourth country with an area of 482 thousand ha and constituted 3.2% of the world's total chickpea

cultivation area. In Türkiye, chickpea sowing ranks first with 50.5% of the total legume cultivation area. However, the chickpea sowing area reduced by about 11.2% in the last five years. Although chickpea can be grown in all regions, it is intensively cultivated especially in Central Anatolia, Southeastern Anatolia, and the northern part of the Mediterranean Region. Diyarbakır has 2579 ha of chickpea cultivated area in Türkiye (Burucu, 2023).

Since chickpea is largely grown under rainfed in Türkiye, significant yield reduction occurs in dry years. Several biotic and abiotic factors affect chickpea growth and grain yield (Richards et al., 2022). Among these biotic and abiotic factors, weeds significantly limit chickpea cultivation and cause yield losses (Ratnam et al., 2011). Chickpea is very sensitive to weed competition due to its slow growth and small leaf area in the early growing period. If weed control is not done in time, yield loss increases by up to 75% (Chaudhary et al., 2005).

Various control methods in chickpea production, as in many cultivated plants, are applied to minimize the damage of weeds. These weed control methods can be listed as cultural, mechanical, and chemical weed control methods. Cultural weed control includes crop management practices such as variety selection, crop rotation, soil tillage, mulches, harvest, and postharvest processing. Soil tillage is known as one of the most important practices in weed control due to its great effect on the germination and development of weed seeds (Lutman et al., 2002). In recent years, increasing environmental awareness and sensitivities towards chemical use around the world further increases the importance of soil tillage in weed control (Buhler et al., 2000). Tillage changes soil physical properties that affect the emergence and growth of different types of weeds. Also, the effects of different soil tillage tools and machines and the soil tillage methods resulting from the periodic use of these tools and machines on weed control are different due to their different effects on the distribution, density, and germination abilities of weed seeds in the soil (Barberi and Cascio, 2001; Reuss et al., 2001; Lutman et al., 2002). Therefore, the change in tillage method affects not only the growth of the cultivated plant but also weed growth.

In recent years, conservation tillage systems have increased interest due to reducing soil erosion and runoff, and preventing soil moisture loss (Hussain et al., 2021; Surek et al., 2022). However, it is stated that direct sowing and surface cultivation in stubble increases the number of weed seeds remaining on the surface of the soil (Carter et al., 2002; Carter and Ivany, 2006; Giller et al., 2015; Nichols et al., 2015; Yankov and Drumeva, 2021). While many researchers (Carter et al., 2002; Cardina et al., 2002; Adeux et al., 2019) observed that conservation tillage systems resulted in increased weed pressure, Samarajeewa et al. (2006) stated that stubble on the field surface makes a significant contribution to preventing the amount of weeds. These inconsistent results of tillage systems on weed control and yield may be due to differences in climate, soil conditions, crop patterns, and applied agronomic processes.

Herbicides are mostly used to control the weeds in chickpea production. Hosseini et al. (1997) determined that the use of herbicides in chickpea cultivation resulted in a 50% yield increase by significantly reducing weed growth when compared to untreated fields. However, due to the phytotoxic effect of herbicides on plants, the appropriate herbicide selection and application method must be chosen by taking into account soil, plant, and climate conditions. Poonia and Pithia (2013), and Singh and Sharma (2013) stated that there was an urgent need to identify more effective herbicides with a broad spectrum of weed control and wider adaptability in chickpea production. Therefore, evaluating the performance of herbicides used in chickpea cultivation under different soil tillage systems will make a significant contribution to the control of weeds in these soil tillage systems.

The objective of this study is to seek the effects of various soil tillage systems and herbicide applications on broad-spectrum weeds and grain yield in chickpea production.

2. Material and Methods

The field experiments were implemented at Dicle University's field crop production area (37°53'25"N, 40°16'29"E) in Diyarbakır located in South Eastern Anatolian region of Türkiye during the 2020-2023 chickpea growing season. The climate in this area is characterized by a semi-arid climate (humid winters and dry summers). Monthly rainfalls, average temperature, and relative humidity records during the experimental years (2020-2023 chickpea growing seasons) and over the long term (2003–2023) are shown in Table 1. In the first year of the experiment, the average temperature (4.9 °C) in January was higher than the average temperature of the long-term years (2.9 °C). While the average

temperatures of March, April, and May were higher than the average temperature of the long-term years, monthly rainfall in March, April, and May of the same year (59.2, 9.4, and 6.4 mm, respectively) was lower than the average of long years (70.9, 71.9 and 51.7 mm respectively). Monthly temperature averages of 2022 relatively was lower than both the previous year and the average of long years. The monthly rainfall of March, April, and May (56.8, 9.8, and 44.0 mm, respectively) was found to be lower than the average of long years (70.9, 71.9, and 51.7 mm respectively). The average temperature (7.0 °C) of December in the 2022-23 crop growing period was much higher than both the previous year (3.8 °C) and the long-term average (4.3 °C). Monthly total rainfall in December of 2022-23 (7.4 mm) was much lower than the previous year (38.0 mm) and the long-term average (82.1 mm).

Table 1. Monthly rainfall, average temperature, relative humidity during the experimental year, and long years average

| Months | Average temperature (°C) | | | | Rainfall (mm) | | | | Relative humidity (%) | | | |
|----------|--------------------------|-----------|-----------|-----------|---------------|-----------|-----------|-------------|-----------------------|-----------|-----------|-----------|
| | 2020-2021 | 2021-2022 | 2022-2023 | Long-term | 2020-2021 | 2021-2022 | 2022-2023 | Uzun yıllar | 2020-2021 | 2021-2022 | 2022-2023 | Long-term |
| November | 8.4 | 12.9 | 10.7 | 9.5 | 0.6 | 20.8 | 87.6 | 42.2 | 67.9 | 69.8 | 77.5 | 67.4 |
| December | 4.9 | 3.8 | 7.0 | 4.3 | 29.8 | 38.0 | 7.4 | 82.1 | 82.2 | 80.5 | 84.6 | 82.0 |
| January | 4.2 | 1.8 | 3.5 | 2.9 | 42.8 | 25.8 | 13.8 | 64.7 | 72.3 | 75.8 | 75.0 | 83.8 |
| February | 6.9 | 6.7 | 3.8 | 4.8 | 34.2 | 35.8 | 34.8 | 60.5 | 69.0 | 68.1 | 66.1 | 76.8 |
| March | 8.0 | 5.4 | 11.7 | 9.0 | 59.2 | 56.8 | 89.2 | 70.9 | 70.3 | 75.4 | 69.8 | 72.4 |
| April | 15.6 | 16.5 | 14.1 | 13.6 | 9.4 | 9.8 | 61.6 | 71.9 | 58.6 | 47.7 | 71.5 | 71.6 |
| May | 23.0 | 18.1 | 18.8 | 19.0 | 6.4 | 44.0 | 12.0 | 51.7 | 35.7 | 57.6 | 56.7 | 63.1 |
| June | 27.4 | 26.9 | 26.2 | 26.3 | 0.0 | 10.8 | 0.0 | 7.5 | 26.1 | 34.0 | 37.5 | 36.1 |

The previous crop, which was wheat in the 2020-21 and 2022-23 growing seasons and barley in the 2021-22 growing season in the experiment area, was harvested by a combine with a chaff collector and the residue amount at the experimental field was 78.18, 229.59 and 199.40 g m⁻² at 2020-21, 2021-22 and 2022-23 growing seasons, respectively. The soil at the 0-20 cm depth of the experimental field was clay soil (56 g kg⁻¹ clay, 24 g kg⁻¹ silt, and 14 g kg⁻¹ sand, by weight) with 0.79 g kg⁻¹ of organic matter, 7.99 of pH, 1.014 of total salt, and 47.5 kg ha⁻¹ of P₂O₅. The experiments were conducted in a split-plot design with three replications. The treatments in the main plots were tillage treatments [moldboard plow+cultivator (MP+C), disc harrow+cultivator (DH+C) and cultivator (C)] and the sub-plots included five weed control treatments [pre-emergence (Pre), post-emergence (Post), pre-emergence+ post-emergence (Pre+Post), weed-free check, weedy check]. Total number of the plots in the experiment were 45. The width and length of each sub-plot were 2 m and 5 m, respectively.

In, the tillage methods, the MP+C treatment included tilling with mouldboard at 25-30 cm depth in July and cultivator at 5–10 cm depth 1-2 days before seeding; the DH+C treatment had disc harrowing at 15-20 cm depth in July and tilling with cultivator at 5–10 cm depth 1-2 days before seeding; the plots in the C treatment is tilled only by cultivator at 5–10 cm depth 1-2 days before seeding. The chickpea Azkan variety was sown on the 8th, the 3rd, and the 12th of December in the 2020-21, 2021-22, and 2022-23 seasons by a universal grain drill, respectively. The seeding rate was 145 kg ha⁻¹ and seeding depth was approximately 6 cm. The space between the rows was 28 cm. The 130 kg ha⁻¹ of diammonium phosphate (18% N and 46% P₂O₅) was applied to the plots during planting.

In the experiment, herbicide applications against broad-leaf weeds were applied before emergence, after seed emergence, and both before and after emergence. In the pre-emergence herbicide application, Flurochloridone (2.5 L a.i. ha⁻¹) was applied just after seeding. In the post-emergence herbicide application, Aclonifen (1.25 L a.i. ha⁻¹) was applied at 95, 123, and 117 days after seeding at 2020-21, 2021-22, and 2022-23 growing seasons, respectively, when weeds were at the stage of 3-4 true leaves. In the weed-free plots, weeds were removed by hand every week until 82-90 days after seeding. In completely weedy plots, any weed control was not applied. In the 2021-22 growing seasons, wild oats, one of the narrow-leaf weeds, were very dense in the experimental area. Therefore, Clethodim was applied to all plots of the experimental area. Both pre-emergence and post-emergence herbicide applications were made with the fan beam nozzle of the 16-liter capacity mechanical back sprayer.

In order to determine the effects of tillage and herbicide applications on the weed density and the weed dry biomass, two quadrats of 100 cm×100 cm were randomly located in each plot. Weed density observations were taken 10-15 days before harvest in 2020-21, 2021-22, and 2022-23 growing

season. Weed emergence and growth status were taken as the basis for determining the observation time of weed density. Total weed biomass was determined just before crop harvest. The total weed biomass in each plot was laid on newspaper papers and left to dry for a month to get their dry weights. After the weeds dried, their dry weights were weighed.

The crop, which was manually harvested in an 8 m² harvest area of each plot, was threshed by a thresher. Grain yield was calculated as kg ha⁻¹ from grain weight in each plot.

Analysis of variance was performed by using the JMP statistical software (SAS Institute Inc., 2002) to examine the effects of treatments, and LSD's multiple-range tests were used to identify significantly different means within dependent variables at $P \leq 0.05$. All data were tested for normality distribution to determine if transformation was necessary. After the normality test, weed density and weed dry biomass weight data were square-root transformed ($\sqrt{x + 0.5}$). The main effects of the tillage methods and the herbicide applications were presented when the interaction effects were not significant; otherwise, the simple effects of herbicide applications for the tillage methods as well as the main effects were examined and presented

3. Results and Discussion

Common weed species in the experimental plots are presented in Table 2. The most dominant weeds in the experiment area in each growing season were *Triticum* spp, *Avena sterilis*, and *Galium tricornutum*.

Table 2. Common weed species in the experimental plots

| Common name | Scientific name | The ratio of the species to total species (%) | | |
|------------------------|---|---|---------|---------|
| | | 2020-21 | 2021-22 | 2022-23 |
| Grass | | | | |
| Volunteer wheat | <i>Triticum</i> spp. | 22.27 | 8.50 | 0 |
| Wild oat | <i>Avena sterilis</i> L. | 12.48 | 13.79 | 5.14 |
| Johnsongrass | <i>Sorghum halepense</i> (L.) Pers. | 2.83 | 0.39 | 0 |
| Quackgrass | <i>Agropyron repens</i> (L.) P.Beauv. | 0.82 | 2.06 | 0 |
| Volunteer barley | <i>Hordeum vulgare</i> L. | 7.11 | 7.35 | 23.7 |
| Broadleaf | | | | |
| Wild Mustard | <i>Sinapis arvensis</i> L. | 1.53 | 4.38 | 1.01 |
| Cocklebur | <i>Xanthium strumarium</i> L. | 24.79 | 8.44 | 2.51 |
| Rough bedstraw | <i>Galium tricornutum</i> Dandy | 3.20 | 10.95 | 4.71 |
| Field bindweed | <i>Convolvulus arvensis</i> L. | 3.71 | 2.56 | 2.91 |
| Spurge | <i>Euphorbia fistulose</i> M. S. Khan | 0.32 | 1.61 | 14.84 |
| Scarlet pimpernel | <i>Anagallis arvensis</i> L. | 0.23 | 0.29 | 2.91 |
| Oriental larkspur | <i>Consolida oliveriana</i> (DC.) Schrod. | 1.30 | 5.61 | 0 |
| Broadleaf false carrot | <i>Turgenia latifolia</i> (L.) Hoffm. | 0.32 | 0 | 0 |
| Galactic Bindweed | <i>Convolvulus galaticus</i> Rost. ex Choisy | 2.78 | 0 | 0 |
| Knapweed | <i>Centaurea balsamita</i> Lam. | 0.33 | 0.39 | 16.15 |
| Birds eye-cress | <i>Myagrurn perfoliatum</i> L. | 1.16 | 0.32 | 2.60 |
| Chicory | <i>Cichorium intybus</i> L. | 1.44 | 9.41 | 2.57 |
| Cow parsnip | <i>Tordylium trachycarpum</i> (Boiss.) Jury & Al-Eisawi | 0,05 | 0 | 0 |
| Common vetch | <i>Vicia</i> sp. | 0.18 | 0.064 | 1.2 |
| Corn Buttercup | <i>Ranunculus arvensis</i> L. | 0.69 | 0.32 | 0 |
| Narbon vetch | <i>Vicia narbonensis</i> var. <i>narbonensis</i> L. | 0.09 | 0 | 0 |
| European knotweed | <i>Polygonum arenarium</i> Waldst. Et Kit. | 9.10 | 8.11 | 0.5 |

The results of variance analysis indicated that while total weed density 10-15 days before harvest was not influenced by tillage ($p < 0.05$), herbicide application methods had a very significant effect on total weed density ($p < 0.01$) in each growing season. The interaction effects between tillage methods and herbicide applications were not significant. However, while tillage methods had no significant effect on the total dry biomass weight of weeds in the 2020-21 and 2021-22 chickpea growing seasons, the effect of tillage methods on total weed dry biomass was significant at the 0.05 probability for 2022-23 growing

season. Herbicide application affected total weed dry biomass at the $p < 0.01$ probability level in each growing season. No interaction effects between tillage and herbicide application were significant (Table 3).

Table 3. Significance of ANOVA for the total weed density and weed dry biomass affected by tillage system and herbicide applications in chickpea production

| Sources of variance | df | Mean squares | | | | | |
|------------------------------|----|--------------------|-----------|----------|------------------------|-----------|-----------|
| | | Total weed density | | | Total weed dry biomass | | |
| | | 2020-21 | 2021-22 | 2022-23 | 2020-21 | 2021-22 | 2022-23 |
| Tillage (T) | 2 | 5.322ns | 2.734ns | 8.364ns | 8.493ns | 6.688ns | 133.921* |
| Error 1 | 4 | 0.812 | 0.448 | 1.387 | 6.329 ns | 6.891 | 29.273 |
| Herbicide (H) | 4 | 89.847** | 123.429** | 42.148** | 180.01 ** | 216.84 ** | 484.674** |
| T x H | 8 | 0.989 ns | 0.633 ns | 1.330 ns | 6.115 ns | 9.65938 | 13.385 ns |
| Error | 24 | 1.580 | 1.013 | 0.760 | 3.693 | 5.633 | 7.948 |
| Coefficient of variation (%) | | 20.12 | 20.95 | 20.71 | 22.70 | 28.73 | 22.19 |

df, degree of freedom; *, significant at 5% level; **, significant at 1% level; ns, not significant.

The effects of the tillage methods and the herbicide applications on the total weed density in the 2020-21, 2021-22, and 2022-23 chickpea growing seasons are presented in Figure 1. Comparisons among tillage methods indicated that while total weed density was the lowest under the MP+C treatment during the 2020-21 and 2022-23 growing seasons, it was lower under the C treatment during the 2021-22 growing season although the difference among the treatments was not statistically significant. In general, tillage with the MP+C was found to reduce weed density in all years. When the effects of herbicide applications on weed density were compared, the effects of pre-emergence, post-emergence, and both pre+post-emergence herbicide applications during the 2020-21 growing season were found to be statistically similar and no difference was found among these herbicide applications. It was observed that the highest weed density was found under the weedy check treatment. A comparison of herbicide applications at 2021-22 and 2022-23 growing seasons showed that pre-emergence herbicide (Flurochloridone) application has been more effective than post-emergence herbicide (Aclonifen) application in controlling weeds. The difference between pre-emergence and pre+post emergence herbicide applications was not statistically significant.

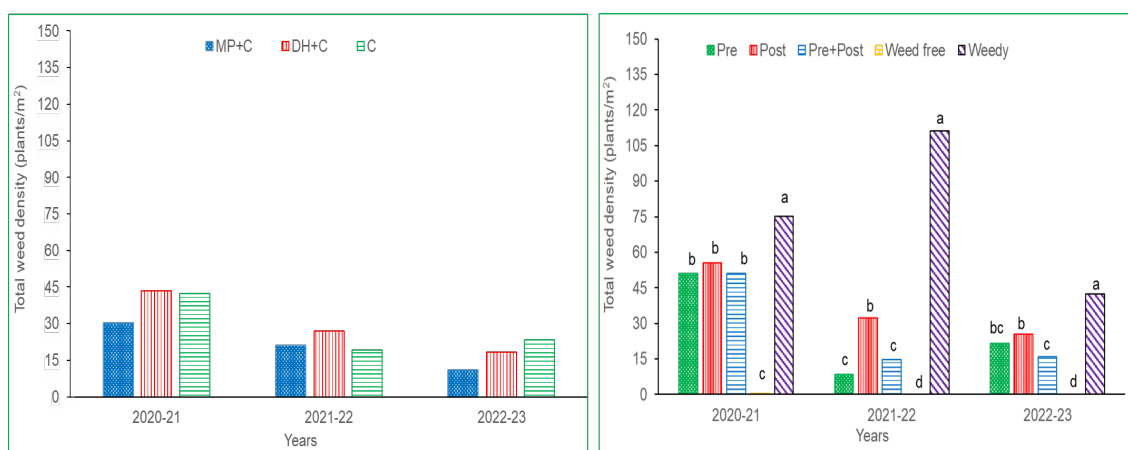


Figure 1. The effect of the tillage methods and the herbicide applications on total weed density 10-15 days before harvest. The treatments followed by different letters are significantly different at the 5% level by LSD's multiple range test.

When the effects of tillage methods on the total dry weed biomass were compared, the difference among tillage methods was not significant in the 2020-21 and 2021-22 growing seasons although the total weed dry biomass was lower under the MP+C and the C treatments than under the DH+C. During the 2022-23 growing season, the MP+C treatment significantly reduced the total weed dry biomass (Figure 2). Changes in tillage practices influence the vertical distribution of weed seeds in the soil; for

example, mouldboard plow can reduce the germination of weed seeds by burying them underground. Whereas, conservation tillage tools such as disc harrows and cultivator leaves the weed seeds on or close to the soil surface after crop planting which may promote greater emergence of weed species. However, the effects of tillage methods on weed density and biomass vary according to weed seed burial, residue on the soil surface, and micro-environmental attributes of the soil (Chauhan et al., 2006; Singh et al., 2015). Similarly, Buhler (1995) reported that the effect of tillage systems on weed dynamics appears to be complex and controlled by interacting factors (soil type, weed species, quality and type of residue, allelopathy, and environmental conditions). Several researchers (e.g. Sharma et al., 2004; Khattak et al., 2005; Chhokar et al., 2008; Ozpinar and Ozpinar, 2011) noted that weed density was lower under conventional tillage systems than under reduced and conservation tillage systems and the most suitable tillage tool to control weeds was moldboard plow.

A comparison of herbicide applications showed that the highest dry weed biomass weight was obtained under post-emergence herbicide (Aclonifen) application after a weedy check. The difference between pre-emergence and pre+post emergence herbicide applications was not statistically significant.

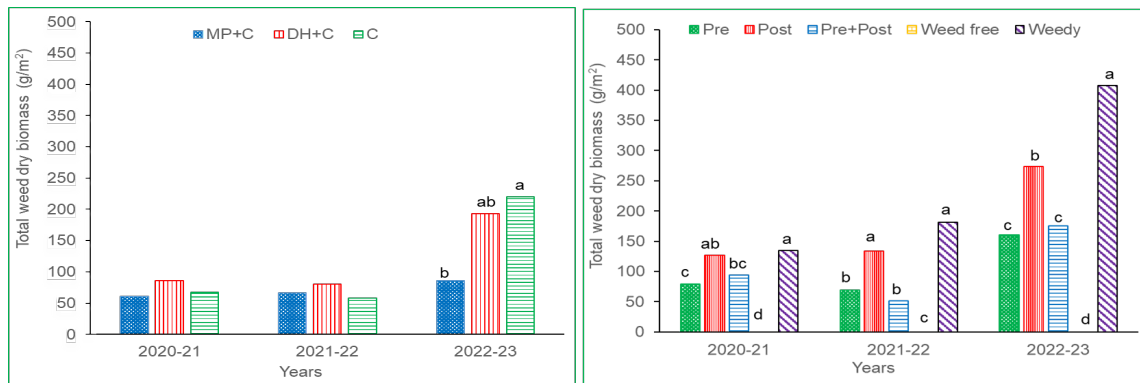


Figure 2. The effect of the tillage methods and the herbicide applications on total weed dry biomass before harvest. The treatments followed by different letters are significantly different at the 5% level by LSD's multiple range test.

The ANOVA showed that while the effect of tillage methods on the grain yield of chickpea was not significant in each growing season, the herbicide application methods had a significant effect ($p < 0.01$) on the grain yield of chickpea at all growing seasons. Tillage x herbicide interaction was only significant at the 2021-22 growing season (Table 4). This significant interaction indicates that the effect of herbicide application on the grain yield of chickpea changed according to tillage systems.

Table 4. Significance of ANOVA for grain yield affected by tillage system and herbicide application in chickpea production

| Sources of variance | Df | Mean squares | | |
|------------------------------|----|--------------|-----------|------------|
| | | Grain yield | | |
| | | 2020-21 | 2021-22 | 2022-23 |
| Tillage (T) | 2 | 466.022 ns | 3270.07ns | 2992.96 ns |
| Error 1 | 4 | 186.62 | 541.23 | 1156.42 |
| Herbicide (H) | 4 | 2109.87 ** | 9954.92** | 18967.80** |
| T x H | 8 | 90.13 ns | 409.87** | 1077.09 ns |
| Error | 24 | 76.661 | 127.06 | 736.65 |
| Coefficient of variation (%) | | 39.25 | 17.59 | 24.22 |

df, degree of freedom; *, significant at the 5% level; **, significant at the 1% level; ns, not significant.

Figure 3 indicates the effects of the tillage methods and the herbicide applications on the grain yield of chickpea in the 2020-21, 2021-22, and 2022-23 chickpea growing seasons. Among tillage treatments, the MP+C treatment produced the highest grain yield of chickpea although the difference between tillage methods was not significant at each chickpea growing season. The lower weed population under the MP+C treatment may result in higher yield. Also, The higher yield might be due

to favorable soil microclimate for crop growth and establishment under the MP+C treatment as reported by Khan et al. (2011). In previous studies, there have been inconsistent results on the effect of tillage systems on crop yield performance. While Pala et al. (2000) found that shallow tillage (ducks-foot cultivator) or zero-till were the best systems for legumes, Camara et al. (2003) found that yield was significantly greater under a moldboard plow than under a subsurface sweep and offset disk. Sandoval-Avila et al. (1994) found no significant effect of tillage on bean yield. In this study, moldboard plowing resulted in higher yield, probably due to higher efficiency of weed control.

A comparison of herbicide applications showed the pre-emergence herbicide application produced the highest grain yield followed by weed-free check treatment, while weedy check plots gave the lowest grain yield. The weedy control plots with the lowest yield were followed by post-emergence herbicide (Aclonifen) application and pre+post emergence herbicide (Flurochloridone+Aclonifen) applications. The lower grain yield in the pre+post emergence herbicide (Flurochloridone+Aclonifen) application than in the pre-emergence herbicide (Flurochloridone) was perhaps due to its toxic effect on the plant. The lowest grain yield in the weedy check treatment was probably due to more weed competition.

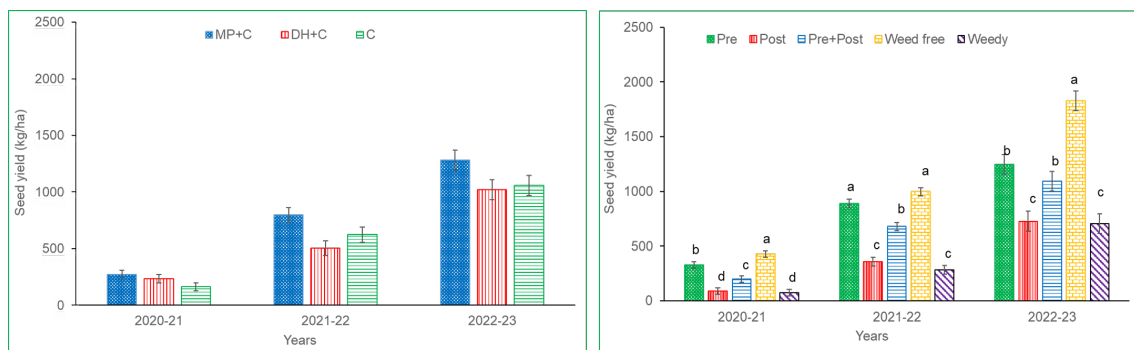


Figure 3. The effect of the tillage methods and the herbicide applications on grain yield of chickpea. The treatments followed by different letters are different at the 5% level by LSD's multiple range test.

There was a significant tillage x herbicide interaction during the 2021-22 growing season which indicates that the effect of herbicide application on the grain yield of chickpea changed according to tillage systems. While there was no significant difference between the post-emergence herbicide application and the pre+post emergence herbicide application under the MP+C method, the lower grain yield of chickpea was obtained at the post-emergence herbicide application than the pre+post emergence herbicide application under the DH+C and the C tillage methods (Figure 4).

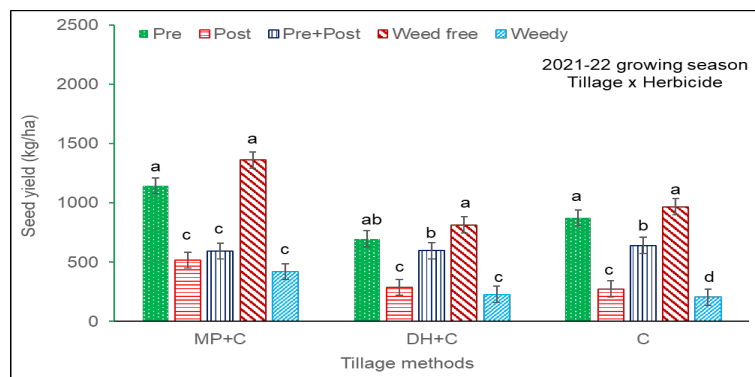


Figure 4. The effect of tillage x herbicide application interaction on the grain yield of chickpea at 2021-22 growing season. The treatments followed by different letters are significantly different at the 5% level by LSD's multiple range test.

Conclusion

In this study, the effects of various tillage methods and herbicide application on weed control and grain yield in chickpea production were evaluated. The results of the study showed that MP+C increased the grain yield of chickpea due to probably reducing total weed density and biomass. Amongst the herbicide application treatments, the pre-emergence herbicide (Flurochloridone) was found to be the most effective in controlling weeds and increasing crop yield. The post-emergence herbicide (Aclonifen) application was less effective in controlling weeds than the pre-emergence herbicide (Flurochloridone) application. The post-emergence herbicide (Aclonifen) application reduced the grain yield due to probably its toxic effect on the chickpea plant.

Ethical Statement

Ethical approval is not required for this study.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Author Contributions

Leyla BAYRAM designed the research, conducted the experiments, analyzed data and wrote the manuscript. Songül GÜRSOY and Cumali ÖZASLAN assisted in performing the study, analyzing data, writing and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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