

Research Article

Doses and Timing of 2, 4-D Application for Broadleaf Weed Control, Botanical Compositions, Productivity, and Nutritive Value of Natural Pasture

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2,4-Dichlorophenoxyacetic acid (2,4-D) is among the most common and inexpensive herbicides used to control broadleaf weeds in natural pasture. However, different studies have pointed out the risk of forage injury. Consequently, no research data on the productivity and nutritive value of natural pasture in response to different rates and times of 2,4-D application exists in Ethiopia. Therefore, this study was conducted to investigate natural pasture yield and nutritive response to 2,4-D application at different rates (1, 1.5, and 2 L·ha⁻¹) and time (mid-July, early, and mid-August) with control. The experiment was designed as a factorial randomized complete block design with three replicates for two years. Grasses and legume proportions in the pasture were significantly influenced by the rate and time ($P < 0.05$) of 2, 4-D application and their interaction ($P < 0.001$). Interaction of rate and time of 2, 4-D application had a significant ($P < 0.05$) influence on dry matter yield of legumes and non-significant ($P > 0.05$) effect on forage portions (grasses + legumes) and grasses. Crude protein and in vitro dry matter digestible yield of pasture forage portions were not significantly ($P > 0.05$) influenced by the rate and time of 2, 4-D application and their interaction. Weed dry matter yield was significantly affected by the interaction of rate and time of 2, 4-D application and mid-July application at 1.5 and 2 L·ha⁻¹ gave the lowest yield. Therefore, to produce optimum quality and quantity of forage from natural pasture, the application of 2, 4-D in mid-July at 1.5 L·ha⁻¹ is recommended.

1. Introduction

Natural pasture hay productivity and nutritional quality are influenced by a number of biophysical factors, including soil, vegetation type and growth stage, plant parts, climate, and pasture management practices like fertilizer application [1]. Indeed, broadleaf and shrub weeds in Ethiopia's highlands reduce the feed value of natural pasture, contributing to low quality and quantity.

Weeds can reduce the number and lifespan of attractive forage plants in pastures and hayfields. These undesirable plants compete with existing or desired forage species for light, water, and nutrients, and they are often more aggressive. This leads to growing weeds, which costs a lot of money, because weeds can reduce the quality and

palatability of available grass for livestock to graze, and some weed species are poisonous to grazing animals. As a result, weed management strategies that reduce the impact of weeds on feed production could be beneficial [2].

Correct soil pH and nutrient levels, combined with cultural control, such as appropriate grazing management, effective crop rotation, topping, and alternating silage and grazing, are some methods for reducing weed problems. Herbicides can also help and provide satisfactory short-term control. However, if not combined with good husbandry and cultural control, weeds will reappear. Herbicides, tillage, crop competition, crop rotation, mowing, and fire are examples of alternative weed management strategies that can be used alone or in combination. Available time, labor, equipment, and other costs, as well as the types of weeds and

infected areas, must all be considered when planning a weed control program [3]. Herbicide is one of the most efficient ways to control or eliminate weed infestations [4].

Herbicides such as 2, 4-D, dicamba, picloram, aminopyralid, fluroxypyr, and triclopyr mimic natural plant auxins that are transported through the xylem and phloem [5]. These herbicides cause stem twisting and malformed leaves by interfering with normal plant growth. Auxinic herbicides (growth regulators) such as phenoxy or benzoic acid herbicides are not tolerated by legumes in pastures or rangelands [6]. The white clover has demonstrated some tolerance to 2, 4-D. [4]. In warm, moist soil, the average persistence is 1–4 weeks, and the average half-life is 10 days [7]. Clovers are susceptible to herbicides used in pastures to control broadleaf weeds, and no pasture herbicides that are not susceptible to clover are currently available [8].

2, 4-D is one of the most widely used and inexpensive herbicides for controlling broadleaf weeds [9]. In the United States, the most commonly used herbicide for rangeland weed control is 2, 4-D [10], which is a popular choice because it controls a wide range of broadleaf weeds at a low chemical cost [11]. Although 2, 4-D is effective against a wide range of broadleaf weeds, drift can cause harm to nontarget species. Non-2, 4-D-containing herbicides have become popular in pastures as a result of this problem [12]. White clover has been shown to be resistant to 2, 4-D [4]. GrazonNext® [5], a Dow Agro Sciences herbicide labeled for use on pastures and rangeland, contains 2, 4-D as one of its active ingredients. It is recommended that 2, 4-D LV4® be applied at a rate of 1120 g/ha, but it should not be used on any susceptible broadleaf crop, including forage legumes [13].

For many producers, the most difficult aspect of chemical control of broadleaf weeds is determining how and when to apply herbicides without harming pasture legumes [14]. The level of weed control is likely to be influenced by the timing of herbicide application [15]. It is difficult to know when to apply herbicides to common pasture weeds, especially when there are multiple weed species in a single pasture or hayfield [4]. Indeed, no research into the effects of the rate and timing of 2,4-D applications on the degree of weed control in natural pasture has been done in Ethiopia. Therefore, the objectives of this research were to determine the best rate and timing for applying 2,4-D for invasive weed control while also improving natural pasture productivity and nutritive quality.

2. Materials and Methods

2.1. Site Description. A field experiment was conducted for two consecutive years during 2019–2020 on a natural pasture at Holetta Agricultural Research Center, Ethiopia. Holetta Agricultural Research Center is located at an altitude of 2400 m.a.s.l at 9°00'N latitude and 38°30'E longitude. The predominant soil type in the area is red nitosol. The long-term (30 years) average annual rainfall and temperature of the study area are 997 mm and 14.6 °C, respectively. While the soil characteristics and monthly temperature and rainfall are presented in Tables 1 and 2.

2.2. Experimental Design, Treatments, and Herbicide Applications. The treatments evaluated are listed in Table 3. The treatments are laid out in a factorial RCBD format (3 rates and 3 times of application with control). The experimental plot was mowed at the start of the main rainy season (mid-June) to allow the plant to grow. Mid-July (30–35 days after clearing), early (45–50 days after clearing), and mid-August (60–65 days after clearing) were the application times for each of the 2,4-D. The application rates were 1, 1.5, and 2 L·ha⁻¹, respectively. A backpack sprayer was used for all applications.

2.3. Sampling Procedures. During a predetermined sampling period, weed, grass, and legume samples from each treatment were collected using a 0.25 m² (0.5 m × 0.5 m) quadrant at three points. The sample was harvested with a sickle at a height of 5 cm above the ground. The quadrant was thrown three times at random per plot, and the average weight of three harvests per plot was used to determine pasture yield and quality. Following harvest, forage samples from each plot were weighed, labeled, and air-dried in the shade before being stored in separate perforated bags for chemical analysis.

To estimate species diversity, a 0.5 m × 0.5 m quadrant was randomly placed in three different locations in each plot. The herbaceous vegetation in the quadrant was classified as grasses, legumes, and weeds, and the biomass and dry weight of each were determined after drying it in an oven to constant weight. By relating the weights of each group to the weight of the entire sample, the botanical composition about the relative proportion of grasses, legumes, and other herbage in the treatment plots on a weight basis was determined. The dry weight rank procedure [20], which involves cutting and sorting by hand, was used to calculate the percentage proportion of each forage type.

2.4. Nutritive Value Analysis. For the determination of partial DM, 500-gm fresh herbage samples were weighed and dried in a forced draft oven at 60°C for 72 hours [21]. The partially dried herbage sample was weighed and ground in a Willey mill to pass through a 1-mm screen before being stored in airtight individual plastic bags until analysis. Representative samples from each plastic bag were taken and analyzed for DM, Ash, CP, NDF, ADF, ADL, and in vitro digestibility. Three grams of each sample were scanned by NIRS at 1108–2492 nm with an 8-nm step [22].

2.5. Statistical Analysis. For the factorial experiment, the treatment effect on botanical composition, productivity, and nutritional quality of natural pasture was analyzed using SAS 9.4 software. LSD was used to separate the means. The following was the design model:

$$Y_{ijk} = \mu + T_i + R_j + TR_{ij} + R_k + E_{ijkR}, \quad (1)$$

where Y_{ijk} = observation in the j th harvesting stage and i th fertilizer application (response variable). μ = overall mean. T_i = i th timing effect (mid-July, early, and mid-August).

TABLE 1: Properties of soils in the study area.

Parameter	Values	Method of analysis
pH (1 : 2.5 H ₂ O)	4.94	Potentiometric method
Organic carbon (%)	1.79	Dichromate oxidation method [16]
Total nitrogen (%)	0.20	Kjeldahl method [17]
Available P (ppm)	5.60	Olsen method [18]
CEC (meq/100 g)	18.24	NH ₄ OAc method (pH = 7)
Na ⁺ (meq/100 g)	0.16	NH ₄ OAc method [19]
K ⁺ (meq/100 g)	5.03	NH ₄ OAc method [19]
Ca ²⁺ (meq/100 g)	29.50	NH ₄ OAc method [19]
Mg ²⁺ (meq/100 g)	13.75	NH ₄ OAc method [19]
P (mg·kg ⁻¹)	5.6	NH ₄ OAc method [19]
Texture		
Sand (%)	18	Boycouos hydrometric method
Silt (%)	15	Boycouos hydrometric method
Clay (%)	67	Boycouos hydrometric method

Source; Holetta Agricultural Research Center meteorological data report.

TABLE 2: Monthly total rainfall and monthly mean temperature of the study area.

Month	Rainfall (mm)		Temperature (°C)	
	2019	2020	2019	2020
January	80.1	0.0	17.6	14.9
February	2.6	0.0	16.3	15.4
March	53.4	73.2	16.8	16.9
April	80.1	92.4	17.8	17.1
May	109.2	100.6	17.2	16.9
June	187.4	126.1	16.2	16.1
July	249.0	280.3	16.9	15.6
August	356.1	334.2	15.1	15.2
September	187.0	216.2	14.3	14.9
October	7.8	31.6	13.2	14.3
November	28.2	8.0	14.3	13.2
December	3.6	3.2	13.9	12.5
Total/aver	1344.5	1265.8	15.8	15.3

Source; Holetta Agricultural Research Center meteorological data.

R_j = the effect of j th rates (1, 1.5, and 2 L·ha⁻¹). R_k = the effect of k th replication. TR_{ij} = the effect of ij th interaction between timing and rate. $EijkR$ = random error (residuals).

3. Results

3.1. Year, Doses, and Time of 2, 4-D application and Their Interaction Effects on the Productivity and Nutritive Value of Natural Pasture. Year, dose, and time interaction of doses and year, time, and year had a significant ($P < 0.05$) effect on the dry matter yield (DMY) of the grasses plant family. Alike, the plant height of grasses was significantly ($P < 0.001$) influenced by the interaction of dose and time of 2, 4-D applied. The composition percentage of grasses plant families in the natural pasture was also significantly influenced by dose, time ($P < 0.05$), dose and time interaction, and year, dose, and time interaction ($P < 0.01$) (Table 4).

The DMY and plant height of the legumes was significantly influenced by year ($P < 0.001$). Alike dose and year interaction ($P < 0.05$), the interaction of dose and time, time and year, and year, time, and dose interaction ($P < 0.01$) had

TABLE 3: Treatment arrangement.

Treatments	2, 4-D	
	Rate of application (L·ha ⁻¹)	Time of application
T1	—	—
T2	1	Mid-July
T3	1	Early-August
T4	1	Mid-August
T5	1.5	Mid-July
T6	1.5	Early-August
T7	1.5	Mid-August
T8	2	Mid-July
T9	2	Early-August
T10	2	Mid-August

a significant effect on the DMY of legumes. Consequently, dose, time ($P < 0.05$), the interaction of dose and time ($P < 0.001$), and year, dose, and time interaction had significant ($P < 0.001$) effects on the composition percentage of legume plant families in the natural pasture.

Dry matter yield of forage portions (grasses + legumes) of the natural pasture was significantly influenced by the interaction of dose and time, doses, and year ($P < 0.05$). The plant height of the forage portion of natural pasture was significantly influenced by year ($P < 0.001$) and the interaction of dose and time ($P < 0.05$). Botanical composition and percentage of the forage portions of natural pasture were significantly influenced by dose, time ($P < 0.001$), year, the interaction of dose and time ($P < 0.01$), and dose, time, and year interaction ($P < 0.05$).

Crude protein yield of the forage portions of natural pasture was significantly ($P < 0.05$) influenced by year. Interaction of dose and year ($P < 0.05$), doses, and time ($P < 0.01$), of 2, 4-D had a significant effect on in vitro dry matter yield of the forage portion of natural pasture.

3.2. Botanical Composition. The dominant grasses, legumes, and weed species in the treated natural pasture are indicated in Table 5. The percentage contribution of grasses and legumes to the pasture's DMY is indicated in Table 6. The

TABLE 4: Effect of year, doses, and time of application of 2, 4-D herbicides and their interaction on plant height (cm), dry matter, and crude protein yield (t·ha⁻¹) of pasture land plant species.

Plant species	Parameters	Year	Doses (R)	Time (T)	R * T	R * Y	T * Y	Y * R * T	Mean	CV
Grasses	Dry matter yield	ns	ns	ns	ns	*	*	*	3.52	25.17
	Plant height	ns	ns	ns	***	ns	ns	ns	88.45	13.67
	Grass composition (%)	ns	*	*	**	ns	ns	**	66.54	25.71
Legumes	Dry matter yield	***	ns	ns	**	*	**	**	1.20	30.68
	Plant height	***	ns	ns	ns	ns	ns	ns	66.73	11.03
	Legume composition (%)	ns	*	*	***	ns	ns	**	18.51	21.64
Weeds	Dry matter yield	*	*	**	*	ns	ns	ns	0.50	32.55
	Plant height	***	ns	ns	ns	ns	ns	ns	51.63	19.59
Forage portion (grasses + legumes)	Dry matter yield	ns	ns	ns	ns	*	ns	ns	4.71	23.62
	Plant height	***	ns	ns	*	ns	ns	ns	77.59	9.16
	Botanical composition (%)	**	***	***	**	ns	ns	*	85.06	9.42
	Crude protein yield (t·ha ⁻¹)	*	ns	ns	ns	ns	ns	ns	0.33	30.28
	IVDMDY	ns	ns	ns	ns	*	ns	ns	2.15	22.66

Y = year, R = doses of application, T = time of application, R * T = doses and time interaction, R * Y = Doses and year interaction, T * Y = time and year interaction, Y * R * T = year, doses and time interaction, ns = nonsignificant ($P > 0.05$), * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, CV = coefficient variation; forage portion = the average result of grass and legume.

TABLE 5: Dominant grasses, legumes, and weed species in the natural pasture.

Grasses	Legumes	Weeds
<i>Andropogon</i>	<i>Oxalis</i>	<i>Bidens pachyluma</i>
<i>Cynodon dactylon</i>	<i>Trifolium quartimianum</i>	<i>Comomolima</i>
<i>Cyprus</i>	<i>Trifolium ruppelliam</i>	<i>Forbs</i>
<i>Eleusine</i>	<i>Vicia</i>	<i>Galium spurium</i>
<i>Hypernia</i>		<i>Ocimum</i>
<i>Pennisetum glabrum</i>		<i>Rumex bacalti</i>
<i>Pennisetum schimperi</i>		
<i>Pennisetum villosum</i>		
<i>Sporobolus</i>		

TABLE 6: Botanical composition (%) / density percentage per·m² / of legumes and grasses in natural pasture under different doses and time of 2, 4-D herbicide application.

Treatments (2, 4-D herbicide doses and application time)	Grasses			Legumes		
	1 st year	2 nd year	Mean	1 st year	2 nd year	Mean
Control (no treatment)	68.53	55.37 ^{bcd}	61.95 ^{bcd}	20.48 ^{bc}	22.31 ^{abc}	21.39 ^{ab}
1 L·ha ⁻¹ at early (mid-July)	68.89	49.33 ^{cd}	59.11 ^{cd}	17.91 ^{bc}	25.33 ^{ab}	21.62 ^{ab}
1 L·ha ⁻¹ at mid (early-August)	74.49	44.94 ^d	59.72 ^{cd}	13.33 ^c	27.53 ^a	20.43 ^{abc}
1 L·ha ⁻¹ at late (mid-August)	54.48	52.83 ^{cd}	53.66 ^d	31.06 ^a	23.59 ^{ab}	27.32 ^a
1.5 L·ha ⁻¹ at early (mid-July)	72.75	85.76 ^{abc}	79.26 ^{abc}	19.65 ^{bc}	7.12 ^{bcd}	13.39 ^{bcd}
1.5 L·ha ⁻¹ at mid (early-August)	69.22	61.54 ^{abcd}	65.38 ^{abcd}	17.49 ^{bc}	19.23 ^{abcd}	18.36 ^{cd}
1.5 L·ha ⁻¹ at late (mid-August)	64.27	62.88 ^{abcd}	63.57 ^{abcd}	23.18 ^{ab}	18.56 ^{abcd}	20.87 ^{abc}
2 L·ha ⁻¹ at early (mid-July)	79.62	91.28 ^{ab}	85.45 ^a	11.48 ^c	4.36 ^{cd}	7.92 ^d
2 L·ha ⁻¹ at mid (early-August)	68.71	97.07 ^a	82.89 ^{ab}	17.34 ^{bc}	1.46 ^d	9.40 ^{cd}
2 L·ha ⁻¹ at late (mid-August)	69.24	26.10 ^d	51.98 ^d	18.09 ^{bc}	36.96 ^a	25.64 ^a
Mean	69.02	63.97	66.54	19.00	18.01	18.51
CV	13.39	29.39	25.91	29.74	32.20	28.47
P value	0.2062	0.0067	0.0092	0.0296	0.0067	0.0056

1st year = 2019, 2nd year = 2020, CV = coefficient variation. Means with different letters are significantly different.

percentage of grasses was significantly ($P < 0.01$) affected by the doses and time of 2, 4-D herbicide application in the second year and combined analysis. The higher grass

percentage was recorded for the application of 2, 4-D herbicide in mid-July at 2 L·ha⁻¹ followed by application in early-August at 2 L·ha⁻¹ doses. The doses and time of 2, 4-D

herbicide application had a significant effect on the proportion of legumes in the dry matter yield of pasture in the first ($P < 0.05$) and second year and combined analysis ($P < 0.01$).

3.3. Plant Height of Grasses, Legumes, and Weeds. The mean plant height of forage harvesting of grasses and legumes, and forage portion in the natural pasture is indicated in Tables 7 and 8, respectively. The results of the first and second year and combined analysis revealed that the doses, time, and their interaction of 2, 4-D herbicide application did not have a significant ($P > 0.05$) effect on the plant height of grasses and legumes in the natural pasture.

3.4. Dry Matter Yield of Grasses, Legumes, and Weeds. The dry matter yield of grasses and legumes in the natural pasture under application of 2, 4-D at different doses and times is indicated in Table 9. In the second year, the dry matter yield of grasses in the natural pasture was significantly influenced by the dose and timing of 2, 4-D application.

In the first ($P < 0.05$) and second year ($P < 0.001$), and combined analysis ($P < 0.05$), dry matter yield of the legumes from the natural pasture was significantly influenced by the doses and timing of 2, 4-D application. In the first year, application of 2, 4-D in a natural pasture in mid-August at 1 Lha^{-1} gave the highest ($P < 0.05$) DMY of legumes followed by application in mid-August at 1.5 Lha^{-1} .

The effect of 2, 4-D application at different doses and timing interaction on weed plant family of natural pasture is indicated in Table 10. The results of the second year and combined analysis showed that the application of 2, 4-D in different dose and time interactions had a significant ($P < 0.05$) effect on weed DMY. In the second year, the application of 2, 4-D in mid-July had a lower DMY. The results of the second year and combined analysis showed that the application of 2, 4-D in early and mid-August results in a higher weed dry matter yield ($P < 0.01$).

The effect of 2, 4-D application at different doses, time, and their interaction on forage (grasses + legumes) DMY of natural pasture is indicated in Table 11. The result revealed that DMY was not significantly ($P > 0.05$) influenced by doses and time of 2, 4-D application.

3.5. Crude Protein and In Vitro Digestible Dry Matter Yield of Natural Pasture. The crude protein yield (CPY) and in vitro digestible dry matter yield (IVDDMY) of natural pasture (mixture of grasses and legume pasture) under different doses and timing application of 2, 4-D herbicide are indicated in Table 12. The results of application in the combined first and second years showed that the CPY and the IVDDMY of natural pasture (grasses and legume pasture) were not influenced significantly ($P > 0.05$) by the interaction of dose and timing of 2, 4-D application. Likewise, the results of the first, second, and combined analysis showed that the doses and time of 2, 4-D application did not

significantly ($P > 0.05$) influence CPY and IVDDMY of natural pasture (grasses and legume pasture).

3.6. Nutritive Values of Natural Pasture. The effect of the doses and timing application of 2, 4-D herbicides on the nutritive value of natural pasture (mixture of grasses and legume pasture) is indicated in Table 13. The results of the analysis showed that the nutritive values (DM, Ash, CP, NDF, ADF, ADL, and IVDMD) of natural pasture were not significantly ($P > 0.05$) influenced by the interaction of dose and timing application of 2, 4-D herbicide. And the doses and time of 2, 4-D application were also not significantly ($P > 0.05$) influenced by the nutritive value (DM, Ash, CP, NDF, ADF, ADL, and IVDMD) of foraged natural pasture.

4. Discussion

The forage dry matter yield of pasture was significantly influenced by the year of study, and this result was consistent with the fact that the general trend in the growth of tropical grasses about a physiological, biochemical, and anatomical adaptation of tropical grasses was affected by temperature and solar radiation in the tropical environment [21, 23]. Hence, there was a temperature change between the first (15.8°C) and the second year (15.3°C) of this experiment. The dry matter yield of the grass plant family was significantly affected by year, dose, and time interaction of doses with year, and time with the year, and this result suggests that the doses and time of 2, 4-D applied did not show a consistent response to control the invasive weeds over the two years of the experiment. This could be attributed to the significant variation of weeds with year, dose, and time of 2, 4-D applied. This is because the yield trend of weeds and grasses in the natural pasture is antagonistic.

Dry matter yield of forage portions (grasses + legumes) of the natural pasture was significantly influenced by the interaction of dose and time, and dose and year, and this result might be influenced by the result observed for grasses; hence, most portions of the natural pasture were grasses. This result is also supported by the findings of Bourdôt et al. [24], which reported that 2, 4-D selectively controlled many broad-leaved plants without harming grasses.

The higher grass percentage was recorded for the application of 2, 4-D herbicide in mid-July followed by early-August at $2 \text{ L}\cdot\text{ha}^{-1}$ doses, and this might be because the weed control is effective where the herbicides were applied at the early stage of growth [25, 26]. Reverse to the grasses, a higher proportion of legumes in the dry matter yield of pasture was obtained from the application of 2, 4-D in mid-August at the doses of 1 and $2 \text{ L}\cdot\text{ha}^{-1}$. This increase in the proportion of legumes for mid-August applications is likely related to the stage of maturity. Hence, legumes are susceptible to the chemicals applied for weed control in pasture, and at the time of the mid-August application, the legume is semi-dormant and not as actively growing as during the mid-July application timing. The age and size of the plant can also determine the herbicide rate and its potential effectiveness, and newly seeded forage grasses or legumes can be injured if herbicides are applied before or soon after a new seeding or

TABLE 7: Effect of dose and time of 2, 4-D application on plant height (cm) of natural pasture.

Factors (2, 4-D herbicide doses and time of application)	Grasses			Legumes		
	1 st year	2 nd year	Mean	1 st year	2 nd year	Mean
Doses						
0 Lha ⁻¹ (control)	69.33	100.90	85.12	58.00	70.00	64.00
1 Lha ⁻¹	73.78	99.63	86.71	57.44	77.59	67.52
1.5 Lha ⁻¹	80.22	100.37	90.29	57.44	75.73	66.59
2 Lha ⁻¹	83.11	96.70	89.51	59.00	76.04	67.02
P-value	0.45	0.81	0.87	0.96	0.63	0.94
Time						
Control	69.33	100.90	85.12	58.00	70.00	64.00
Mid-July	81.33	96.31	88.82	57.11	80.00	68.56
Early August	73.33	99.82	86.58	57.22	76.10	66.66
Mid-August	82.44	101.05	91.20	59.56	72.91	65.84
P-value	0.43	0.68	0.82	0.88	0.25	0.85
R * T	ns	ns	ns	ns	ns	ns
Mean	78.07	99.18	88.45	57.97	75.77	66.87
CV	19.93	8.70	19.12	12.32	11.33	18.37

1st year = 2019, 2nd year = 2020, CV = coefficient variation, R * T = dose and time interaction.

TABLE 8: Effect of dose and time of application of 2, 4-D herbicide on plant height (cm) of natural pasture forage portions and weeds.

Factors (2, 4-D herbicide doses and time of application)	Grasses + legumes			Weeds		
	1 st year	2 nd year	Mean	1 st year	2 nd year	Mean
Doses						
0 Lha ⁻¹ (control)	63.67	85.45	74.56	40.00	66.63	53.32
1 Lha ⁻¹	65.61	88.61	77.11	31.00	76.86	53.93
1.5 Lha ⁻¹	68.83	88.05	78.44	28.56	72.60	50.58
2 Lha ⁻¹	71.06	86.37	78.26	31.67	70.00	49.71
P-value	0.57	0.78	0.92	0.08	0.64	0.95
Time						
Control	63.67	85.45	74.56	40.00	66.63	53.32
Mid-July	69.22	88.16	78.69	28.67	75.00	51.83
Early August	65.28	87.96	76.62	30.33	73.71	52.02
Mid-August	71.00	86.98	78.52	32.22	70.84	50.39
P-value	0.53	0.89	0.89	0.07	0.79	0.99
R * T	ns	ns	ns	ns	ns	ns
Mean	68.02	87.49	77.82	31.37	72.59	51.63
CV	14.46	6.57	16.79	19.68	18.83	47.94

1st year = 2019, 2nd year = 2020, CV = coefficient variation, R * T = dose and time interaction, R * T = dose and time interaction; grass + legume = the average result of grass and legume.

TABLE 9: Effect of dose and time of application of 2, 4-D herbicide on dry matter yield (t·ha⁻¹) of natural pasture forage species.

Treatments (2, 4-D herbicide doses and application time)	Grasses			Legumes		
	1 st year	2 nd year	Mean	1 st year	2 nd year	Mean
Control (no treatment)	3.80	2.21 ^d	3.01	1.17 ^{ab}	1.76 ^{abc}	1.46 ^{ab}
1 L·ha ⁻¹ at early (mid-July)	3.16	4.57 ^{abc}	3.86	0.82 ^{bc}	0.90 ^{de}	0.86 ^{bc}
1 L·ha ⁻¹ at mid (early-August)	3.50	4.70 ^{abc}	4.10	0.63 ^c	1.20 ^{dc}	0.92 ^{abc}
1 L·ha ⁻¹ at late (mid-August)	2.77	2.62 ^{cd}	2.69	1.53 ^a	1.53 ^{bcd}	1.53 ^a
1.5 L·ha ⁻¹ at early (mid-July)	3.46	3.09 ^{bcd}	3.28	0.92 ^{bc}	1.07 ^{cde}	0.99 ^{abc}
1.5 L·ha ⁻¹ at mid (early-August)	3.61	2.77 ^{cd}	3.19	0.91 ^{bc}	1.96 ^{ab}	1.43 ^{ab}
1.5 L·ha ⁻¹ at late (mid-August)	3.14	3.92 ^{abcd}	3.53	1.11 ^{ab}	1.16 ^{cde}	1.14 ^{abc}
2 L·ha ⁻¹ at early (mid-July)	3.97	3.25 ^{abcd}	3.61	0.57 ^c	2.11 ^{ab}	1.34 ^{ab}
2 L·ha ⁻¹ at mid (early-August)	3.20	4.97 ^{ab}	4.08	0.80 ^{bc}	2.27 ^a	1.54 ^a
2 L·ha ⁻¹ at late (mid-August)	2.97	5.27 ^a	3.89	0.77 ^{bc}	0.44 ^e	0.61 ^c
Mean	3.36	3.68	3.52	0.92	1.44	1.18
CV	19.97	28.95	29.73	30.50	29.76	46.23
P-value	0.507	0.024	0.303	0.019	0.0007	0.0428

1st year = 2019, 2nd year = 2020, CV = coefficient variation. Means with different letters are significantly different.

TABLE 10: Effect of the interaction of dose and time of 2, 4-D application on dry matter yield ($t\text{-ha}^{-1}$) of weeds.

Treatments	1 st year	2 nd year	Mean
Control (no treatment)	0.65	0.70 ^a	0.68 ^a
1 L·ha ⁻¹ at early (mid-July)	0.61	0.44 ^{ab}	0.53 ^{ab}
1 L·ha ⁻¹ at mid (early-August)	0.58	0.64 ^a	0.61 ^a
1 L·ha ⁻¹ at late (mid-August)	0.71	0.70 ^a	0.71 ^a
1.5 L·ha ⁻¹ at early (mid-July)	0.35	0.12 ^{bc}	0.24 ^b
1.5 L·ha ⁻¹ at mid (early-August)	0.69	0.61 ^a	0.65 ^a
1.5 L·ha ⁻¹ at late (mid-August)	0.61	0.45 ^{ab}	0.53 ^{ab}
2 L·ha ⁻¹ at early (mid-July)	0.42	0.02 ^c	0.22 ^b
2 L·ha ⁻¹ at mid (early-August)	0.64	0.05 ^{bc}	0.35 ^{ab}
2 L·ha ⁻¹ at late (mid-August)	0.54	0.60 ^a	0.56 ^{ab}
Mean	0.58	0.43	0.50
CV	22.21	21.24	34.38
P-value	0.8996	0.0027	0.0188

1st year = 2019, 2nd year = 2020. Means in each column with different letters have a significant difference.

TABLE 11: Effect of doses, time, and their interaction of application of 2, 4-D herbicide on dry matter yield ($t\text{-ha}^{-1}$) of natural pasture forage portions.

Factors (2, 4-D herbicide doses and time of application)	1 st year	2 nd year	Mean
Doses			
0 L·ha ⁻¹ (control)	4.97	3.97	4.47
1 L·ha ⁻¹	4.13	5.17	4.65
1.5 L·ha ⁻¹	4.38	4.65	4.52
2 L·ha ⁻¹	4.10	5.94	5.02
P-value	0.14	0.13	0.63
Time			
Control	4.97	3.97	4.47
Mid-July	4.30	5.00	4.65
Early August	4.22	5.95	5.09
Mid-August	4.10	4.82	4.46
P-value	0.18	0.15	0.46
R * T	ns	ns	ns
Mean	4.28	5.16	4.71
CV	14.88	28.19	27.23

1st year = 2019, 2nd year = 2020, CV = coefficient variation, ns = nonsignificant, R * T = dose and time interaction, R * T = dose and time interaction.

TABLE 12: Effect of dose and time of application of 2, 4-D herbicide on crude protein and in vitro dry matter digestibility yield ($t\text{-ha}^{-1}$) of forage portion (grasses + legumes).

Factors (2, 4-D herbicide doses and time of application)	CPY			IVDDMY		
	1 st year	2 nd year	Mean	1 st year	2 nd year	Mean
Doses						
0 L·ha ⁻¹ (control)	0.34	0.28	0.31	2.31	1.85	2.08
1 L·ha ⁻¹	0.30	0.37	0.34	1.90	2.36	2.13
1.5 L·ha ⁻¹	0.32	0.34	0.33	2.02	2.10	2.06
2 L·ha ⁻¹	0.28	0.42	0.34	1.88	2.78	2.30
P-value	0.53	0.35	0.93	0.20	0.06	0.60
Time						
Control	0.34	0.28	0.31	2.31	1.85	2.08
Mid-July	0.33	0.39	0.36	2.02	2.33	2.18
Early August	0.28	0.38	0.33	1.89	2.64	2.27
Mid-August	0.30	0.35	0.32	1.88	2.21	2.04
P-value	0.40	0.57	0.70	0.20	0.20	0.65
R * T	ns	ns	ns	ns	ns	ns
Mean	0.31	0.36	0.33	1.97	2.34	2.15
CV	23.99	33.88	31.09	17.43	25.62	25.92

1st year = 2019, 2nd year = 2020, CPY = crude protein yield, IVDDMY = in vitro digestible dry matter yield, R * T = dose and time interaction, R * T = dose and time interaction.

TABLE 13: Effect of doses, time, and their interaction of 2, 4-D application on the nutritional quality of natural pasture.

Factors (2, 4-D herbicide doses and time of application)	DM	Ash	CP	NDF	ADF	ADL	IVDMD
Doses							
0 L·ha ⁻¹ (control)	89.27	14.12	6.93	67.70	44.40	6.91	46.56
1 L·ha ⁻¹	89.34	14.10	7.32	67.77	43.93	6.82	46.00
1.5 L·ha ⁻¹	89.50	13.67	7.35	69.14	44.14	6.75	46.05
2 L·ha ⁻¹	89.58	13.29	6.69	69.74	44.51	6.87	45.34
<i>P</i> -value	0.70	0.25	0.69	0.43	0.95	0.93	0.96
Time							
Control	89.27	14.12	6.93	67.70	44.40	6.91	46.56
Mid-July	89.32	14.21	7.66	67.81	44.90	7.06	46.86
Early August	89.58	13.13	6.51	70.14	44.57	6.84	44.84
Mid-August	89.51	13.77	7.24	68.56	42.94	6.50	45.73
<i>P</i> -value	0.64	0.08	0.32	0.30	0.30	0.07	0.69
<i>R * T</i>	ns	ns	ns	ns	ns	Ns	ns
Mean	89.46	13.74	7.12	68.66	44.09	6.81	46.01
CV	0.52	6.52	16.95	3.97	4.96	5.41	8.29
<i>P</i> -value	0.462	0.302	0.346	0.462	0.498	0.075	0.945

DMY = Dry matter, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, ADL = acid detergent fiber, IVDMD = In vitro dry matter digestibility, CV = coefficient variation, ns = nonsignificant ($P > 0.05$), $R * T$ = interaction of doses and time of 2, 4-D application.

pasture renovation [2]. Indeed, the major legumes observed in the experimental pasture were clover species, and concurrent to this result, Refs. [4, 27] pointed out that clovers can be susceptible to herbicides applied in pastures to control broadleaf weeds.

The increment of grasses in the botanical composition was consistent with the decrement of legumes. Concurrent to the result of this study, Refs. [28, 29] and [30] reported that 2, 4-D applications increased grasses with the decrease of other family plants.

The mean proportion of grasses in the dry matter yield of pasture obtained in this study (66.54%) was higher than the value (44.53%) reported by Seyoum Bediye et al. [31] for proportions of grasses in the dry matter yield of pasture under mechanical weed control at Holetta. This result suggests that the application of 2, 4-D herbicides to natural pasture can increase the proportion of grasses by 22.01%. Consequently, the mean legume (18.51%) proportion observed in this study was higher than the value reported by Bediye et al. [31] for legumes proportions at Holetta. This implies the advantage of 2, 4-D herbicide application over mechanical weed control. However, the variations in the results might be also attributed to the general effect of climate (rainfall, temperature), management, and harvesting stage.

Application of 2, 4-D at 2 L·ha⁻¹ doses in mid-August resulted in greater grasses dry matter production than control alike applications at 1 and 1.5 L·ha⁻¹ doses in mid-August, mid-July, and early-August. This result might be due to the newly seeded forage grasses or legumes can be injured if herbicides are applied before or soon after a new seeding or pasture renovation [1]. Cinar et al. [27] also reported that the application of herbicides such as 2, 4-D, paraquat, and glyphosate affected not only weeds but also valuable pasture plants. Concurrent to the results of this study, Twidwell and Strahan [12] also pointed out 2, 4-D is very effective on many broadleaf weeds, but it may also damage nontarget species through drift.

In the first year, the application of 2, 4-D in a natural pasture in mid-August at 1 L·ha⁻¹ gave the highest dry

matter yield of legumes followed by application in mid-August at 1.5 L·ha⁻¹, and this might be because at the time of the mid-August applications, the legume is semidormant and not as actively growing as during the mid-July and early-August application timing. Moreover, the most available legumes in the experimental pasture were clover and the result was concurrent with the report; clovers can be susceptible to herbicides applied in pastures to control broadleaf weeds, and there are currently no pasture herbicides available where clover would not be susceptible [13]. Reverse to the first year, in the second year, the highest ($P < 0.001$) legume dry matter yield was obtained from the application in early-August followed by no-treated control, and this might be due to the environmental conditions (rainfall and temperature) variation. Proper timing of herbicide application should be based on the stage of weed growth, the potential risk to nearby sensitive crops, and environmental conditions, such as air temperatures and humidity [1].

In the second year, a lower dry matter yield of weeds was recorded with the application of 2, 4-D in mid-July. This result suggests that the effectiveness of 2, 4-D application for invasive weed control and adverse effects on natural pasture productivity is highly influenced by the timing of herbicide application. This implies that to tackle the problem of weeds through chemical control, a precarious focus should be given the timing rather than the amount of application [32]. The highest weed dry matter yield was obtained in the first year than in the second year, and this result suggests the non-resistance of weeds to the 2, 4-D herbicide over two years of application. This implies that ragwort (*Bidens pachyluma*) species weeds were nonresistance to 2, 4-D application for two years, due to the major weeds in the experimental pasture that was belonging to ragwort (*Bidens pachyluma*) [33].

The application of 2, 4-D in early and mid-August was more weed yielded ($P < 0.01$), and this might be due to its difficulty to control invasive weeds when target plants have deep vegetative reproductive structures. The result is in line with the fact that the best management of pasture weeds is to

recognize potential weed problems and early control weeds before they reproduce and spread. In support of the results of this study, Honore et al. [34] also reported that 2, 4-D is not effective in controlling very mature weeds and perennials.

The forage (grasses + legumes) proportion in the botanical composition increased with the decrease in weeds, and this might be because weeds compete with pasture grasses and legumes for resources such as water, light, and mineral nutrients [35]. In agreement with the results of this study, Tozer et al. [36] pointed out that the competitive interaction between weeds and pasture species can lead to a reduction in the survivorship, growth, and reproduction of the pasture species, which is dominated by the weed species. Bourdot et al. [24] also reported that pasture plants in pastures can be replaced by weed species through competition.

The mean crude protein yield value ($0.33 \text{ t}\cdot\text{ha}^{-1}$) of natural pasture (mixture of grasses and legume pasture) obtained in this study was slightly in line with the value ($0.40 \text{ t}\cdot\text{ha}^{-1}$) reported by Bediye et al. [31] for natural pasture at Holetta, while the mean value ($2.15 \text{ t}\cdot\text{ha}^{-1}$) of in vitro digestible dry matter yield recorded for a mixture of grasses and legume pastured in this study was slightly in line with the value ($2.53 \text{ t}\cdot\text{ha}^{-1}$) reported by Bediye et al. [31] for a mixture of grasses and legumes pasture at Holetta.

The crude protein content value (7.12%) obtained for natural pasture in this study was in line with the value (7.50%) reported by Bediye et al. [31] for natural pasture under different fertilizer treatments at Holetta. The mean values of IVDMD (46.01%) and NDF (68.66%) observed in this study were slightly higher than the values (52.70 and 62.00%) reported by Bediye et al. [31] for natural pasture under different fertilizer treatments at Holetta. This variation could be due to the difference in climatic conditions (rainfall) among the experimental years, management and treatment of 2, 4-D herbicide, harvesting stage, and fertilizers.

5. Conclusion

This research result demonstrated that the application of 2, 4-D in mid-July at 1.5 and $2 \text{ L}\cdot\text{ha}^{-1}$ decreased the infestation of broadleaf weeds in natural pasture according to the averages of two years of data. Application of 2, 4-D herbicide in mid-July has negatively affected the proportions and biomass yield of weeds in the natural pasture without significant influence on the dry matter yield and quality of forage pasture. Consequently, to control the broadleaf weeds in natural pasture effectively and to obtain quality and quantity forage simultaneously, the application of 2, 4-D in mid-July at $1.5 \text{ L}\cdot\text{ha}^{-1}$ is recommended.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Fekede Feyissa and Mulisa Faji conceived and designed the study. Mulisa Faji, Gezahagn Kebede, and Kedir Mohammed conducted experiments and analyzed the data. Mulisa Faji wrote the manuscript, with comments and revisions by Gezahagn Mengistu. All authors read and approved the manuscript.

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