This was reflected by no change in ranking order of Medicago sativa due to relatively uniform growing conditions (rainfall, temperature etc.) during the experimental year. Environmental factors such as soil characteristics, moisture and temperature over years and locations have an impact on growth performance. There is strong influence of environmental factors during various stages of crop growth (Bull *et al*., 1992), thus genotypes differ widely in their response to environments. Some genotypes exhibit highly specific response to a particular environment (soil, rainfall and temperature), others are uniform in performance over a range of environments. Diriba *et al* (2014) has reported that, cultivar has no significant effect on the stand height and he also suggested that, a cooler climate encourages more dormant alfalfa growth. However, Meyer and Nudell (2008) indicated that companion crops compete with under seeded alfalfa, which can affect the stand that can be established.

### *3.2.6. Number of leaves per plant*

The mean of *Medicago sativa* forage at varies harvesting stage, treatment of phosphorus fertilizer and interaction statistically had not a significant effect (P > 0.05) on number of leaves per plant as mentioned in (Table 3). The number of leaves plays a vital role in the growth and development of plants. The increase or decrease in the number of leaves per plant directly affects the yield of forage crops. Closely similar results were obtained for number of leaves per plant for Medicago sativa by (Ali, 2000).

### *3.2.7. Leaf length per plant*

The leaf to stem ratio is determined by separately collecting the central part of two adjacent middle rows with a sampling area of ​​(50 cm × 50 cm) and dividing the collected biomass into leaf and stem parts. Harvesting period, phosphorus fertilizer application rates and the interaction of harvesting period by phosphorus were statistically not significant effect (P > 0.05) at the level of leaf length per plant (Table 3). Whereas, numerically slightly variation with harvesting period and also phosphorus fertilizer application rates.

### *3.2.8. Number of branches per plant*

Harvesting stage of *Medicgo sativa* were statistically a significant effect (P < 0.05) at the level of number of branches per plant (Table 3). Number of branches per plant with harvesting stage of full-flowering stage or FF (47.53) was significantly more than the other harvesting stage. Another hand, at pre-flowering stage or PF showed less number of branches per plant (30.86). However, there was no statistically significant (P > 0.05) effect at the level of phosphorus fertilizer application rates and their interaction on the number of branches per plant.

### *3.2.9. Leaf to stem ratio*

Leaf to stem ratio was determined by separately harvesting a central section of two adjacent middle rows with a sampling area of ​​(50 cm × 50 cm) and by partitioning the harvested biomass in to leaf and stem fractions. Leaf to stem ratio of the current study had no significant differences (P > 0.05) effects are shown in Table 3, this was comparable with the report of Diriba et al. (2014) and Afsharamanesh (2009) unlike, Gashaw et al. (2015) for the same alfalfa caltivars. While, the evaluated value of Medicago sativa in the present study ranged from 0.74 to 0.785 and it was inferior as compared to the value reported by Diriba et al. (2014) ranging from 0.95 to 1.21 for the same cultivars. This might have occurred due to the difference of soil type, management and climatic condition. Similarly, Katic et al. (2006) reported that the proportion of leaves and stems in alfalfa can vary greatly, depending on maturity at harvest, cultivars, handling, and rain damage.

Leaf to stem ratio is an important trait in the selection of appropriate forage cultivar as it is strongly related to forage quality (Sheaffer et al., 2000). Alfalfa leaves have significantly higher nutritive value than stems, so to advance forage quality has been to develop cultivars which possess a greater proportion of leaves than steam (Ray et al., 1999a). Because, leaves have a stable protein content that is much higher than that of the stems. Stem develops at the expense of leaves and their cell walls and lignin content increases with maturity (Veronesi et al., 2010). The proportion of leaves at harvest is the main factor that determines the quality of the forage (Jung, 2005). The percentage of leaves is expected to be as high as possible because the crude protein content in the leaves is better than in the stems (Mihai et al., 2012).

**Table 3**: PHT, NLPP, LLPP, NBPP and LSR at different HS and P fertilizer application

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Harvesting stage** | **Parameter** | | | | |
| **PHT (cm)** | **NLPP** | **LLPP(cm)** | **NBPP** | **LSR** |
| 10 | 66.67 | 256.73 | 1.99b | 30.86b | 0.78 |
| 50 | 74.38 | 261.07 | 2.19ab | 41.46ab | 0.77 |
| 100 | 83.31 | 270.8 | 2.29a | 47.53a | 0.75 |
| LSD | 23.88 | 82.3 | 0.26 | 13.12 | 0.04 |
| *P value* | NS | NS | NS | 0.04 | NS |
| DAP fertilizer rate (kg/ha) |  | | | | |
| 0 | 69.74 | 256.44 | 2.12 | 38 | 0.785 |
| 10 | 73.68 | 258.33 | 2.13 | 38.66 | 0.77 |
| 20 | 78.99 | 265.89 | 2.17 | 41.11 | 0.78 |
| 30 | 75.76 | 257.44 | 2.17 | 37.88 | 0.76 |
| 40 | 75.78 | 276.22 | 2.18 | 44.11 | 0.74 |
| LSD | 30.83 | 106.27 | 0.34 | 16.94 | 0.05 |
| CV (%) | 42.82 | 41.99 | 16.55 | 44.06 | 7.65 |
| *P value* | NS | NS | NS | NS | NS |
| Mean | 74.78 | 262.8 | 2.15 | 39.95 | 0.77 |
| SEM | ±32.03 | ±110.37 | ±0.35 | ±17.6 | ±0.05 |
| HS\*DAP (*P value*) | NS | NS | NS | NS | NS |

The mean values within the column followed by the same letters are not significantly different at the 5% probability level. PHT = plant height; NLPP = number of leaves per plant; LLPP = leaf length per plant; NBPP = number of branches per plant; LSR = leaf-to-stem ratio; LSD = least significant difference; CV (%) = coefficient variation; SEM = standard error; HS = harvesting stage; DAP = di-ammonium fertilizer; NS = none significant; 10 = % of the pre-flowering stage; 50 = % of the half-flowering stage; 100 = % of the full-flowering stage; HS\*DAP = interaction effect of harvesting stage and DAP fertilizer rates.

### *3.2.10. Total fresh biomass yield*

There were no significant differences (P > 0.05) between harvesting stages, treatment of phosphorus fertilizer and their interaction in terms of forage fresh yield (ton/ha-1) of *Medicago sativa* as shown in Table 4. The green forage yield is one of the most important traits and the ultimate goal of forage production is to obtain a high biomass with a reasonable quality. However, according to *Lamb et al. (2003)*, green herbage yield in *Medicago sativa* is affected by environment, plant density and genotype, but disagree with this research and also not consistent with the results obtained by Breazeal et al. (2000) who found that the high population density of *Medicago sativa* did not increase grass yield. Increasing the number of plants per unit area will reduce the volume of air and soil that a single plant can use, thereby increasing the competition between plants for soil nutrients, carbon dioxide and light. Elkaouri (1977) reported the opposite result. I have found that the fresh forage yield obtained from this research is superior to the results of Cevheri and Avcioglu (1998), Eginlioglu and others (Nineteen ninety six). The results of Sengul (2002) on the increase in the number of *Medicago sativa* stalks due to aging confirm the current research.

### *3.2.11. Dry matter yield (ton/ha-1)*

Mean values of dry matter yield (DMY) of *Medicago sativa* result showed non-significant effect (P>0.05) at forage harvesting stage, treatment of phosphorus fertilizer rates and interaction on dry matter yield Medicago sativa are presented in Table 4. Then, an increasing trend of dry yield was observed until the third cut, and then began to decrease. This is consistent with Lloveras (2001), who reported an increase in dry Medicago sativa forage production from the first cut (2.77 ton/ha-1) to the second cut (3.52 ton/ ha-1). The previous economic traits, namely plant height, number of leaves per plant, leaf length per plant, number of branches per plant, and dry matter yield, indicate that the high dry matter yield can be achieved by treating 30 kg/ha-1 of phosphorus due to these plant characteristics. Therefore, choosing a trait will lead to the improvement of all the ideal agronomic traits of *Medicago sativa* (Lodge, 1986). Medicago sativa DMY results are consistent with previous studies (Avci et al., 2007; Kavut and Avcioglu, 2015). The current DMY results regarding harvest stag, fertilization rate and their interaction, show no significant difference between the control and control results; this leads to disagreement (Lodge, 1986). This was reflected by no change in ranking order of *Medicago sativa* due to relatively uniform growing conditions (rainfall, temperature etc.) during the experimental year. The yielding ability of Medicago sativa is the result of its interaction with the environment. Environmental factors such as soil characteristics, moisture and temperature over year and locations have an impact on yield performance. There is strong influence of environmental factors during various stages of crop growth (Bull *et al*., 1992), thus *Medicago sativa* differ widely in their response to environments.

### *3.2.12.* *Digestible dry matter yield*

The stage of harvesting, level of phosphorus fertilizer and their interaction had not significant effect (P > 0.05) on DDMY (ton/ha-1) as seen in Table 4. The digestible dry matter yield (DDMY)is considered one of the most important criteria in evaluating forage productivity; since it takes into account both DM yield and digestibility of the DM. As stage of harvesting increased in age, the mean value of DDMY decreased, and increased level of P fertilizer rate forced to increase DDMY; might be due to the function of total DMY and DDM concentration in the plant tissue. This finding is agreeing with Aschalew et al. (1996), Berhanu (2004) and Berihun and Terefe (2017), who reported an increasing trend in DDMY of Medicago sativa with late stage of harvesting. On the other hand, the inconsistency of phosphorus fertilizer influence on result of DDMY, might be due to the response of *Medicago sativa* on phosphorus fertilizer, that Medicago sativa response well phosphorus fertilizer after basic nitrogen application during sowing Valenzeula and Smith (2002). Digestible DMY is inversely related with digestible dry matter because younger *Medicago sativa* had higher digestible rate than older Medicago sativa this is the fact that in older *Medicago sativa* had higher content of dry matter but lower in intake and digestible by animal due to higher content of NDF and ADF, respectively.

### *3.2.13. Crude protein yield*

The analysis of variance showed that the effect of harvesting stage, phosphorus fertilization and the interaction of harvesting stage by phosphorus fertilization was not significant (P > 0.05) on crude protein yields(ton/ha-1) are shown in Table 4. Thus, the average effects of the harvesting stage and phosphorus fertilization were presented separately. Though harvesting stage effect was not significant as presented in Table 5, at 10 % (PF) early bloom had relatively the highest crude protein yields, recorded as (0.95 ton/ha-1) while at 100 % (FF) exhibited lowest crude protein yields (0.74 ton/ha-1).

Although the variety of crude protein content is not significant, this may be due to the difference in fresh yield depending on climatic conditions and soil characteristics. Many researchers have also reported that crude protein production increases with increasing dry matter production (Kir and Soya, 2008). In this research, the crude protein yield was lower than the 1219-2976 kg/ha-1 given by Inal (2015). However, Sengul et al., (2003) and Yilmaz and Albayrak (2016) reported higher crude protein yields, for example in the range of 2700-3250, 2464-3213 and 3174-3838 kg/ha-1, respectively. Growth rate, decreased crude protein content, and decreased digestibility are affected by many different external factors, such as environmental factors (temperature, solar radiation, and water), phosphorous supply, and fertilization. The amount of phosphorus fertilizer and the fertilization time have a significant effect on the crude protein content. As the amount of available phosphorus in the soil decreases, the absorption rate of plants cannot keep up with their growth rate. This leads to the dilution of the available protein over a higher yield of dry matter, which leads to a decrease in protein, as pointed out by Geleti, (2014).

**Table 4**: Mean of TFY, DMY, DDMY and CPY at different HS and P fertilizer application.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Harvesting stage** | **Parameter, ton/ha-1** | | | |
| **TFY** | **DMY** | **DDMY** | **CPY** |
| 10 | 16.74 | 3 | 1.49 | 0.95 |
| 50 | 16.7 | 3.42 | 1.75 | 0.84 |
| 100 | 16.35 | 3.75 | 1.95 | 0.74 |
| LSD | 2.93 | 0.96 | 0.49 | 0.28 |
| *P value* | NS | NS | NS | NS |
| DAP fertilizer rates (kg/ha) |  | | | |  |  |  |
| 0 | 15.62 | 2.98 | 1.47 | 0.67 |
| 10 | 15.6 | 3.21 | 1.51 | 0.78 |
| 20 | 16.18 | 3.34 | 1.71 | 0.84 |
| 30 | 18 | 3.8 | 2.11 | 0.96 |
| 40 | 17.5 | 3.61 | 1.84 | 0.97 |
| LSD | 3.78 | 1.24 | 0.64 | 0.37 |
| CV (%) | 23.66 | 38.06 | 38.54 | 45.6 |
| *P value* | NS | NS | NS | NS |
| Mean | 16.6 | 3.39 | 1.72 | 0.84 |
| SEM | ±3.93 | ±1.29 | ±0.66 | ±0.38 |
| HS\*DAP (*P value*) | NS | NS | NS | NS |

The mean values within columns followed by different letters are significantly different at the 5% probability level. TFY = total fresh yield; DMY = dry matter yield; CPY = crude protein yield; DDMY = digestible dry matter yield; LSD = least significant difference; CV (%) = coefficient variation; SEM = standard error of means; HS = harvesting stage; DAP = di-ammonium phosphate; NS = none significant; 10 = % of the pre-flowering stage; 50 = % of the half-flowering stage; 100 = % of the full-flowering stage; HS\*P = interaction effect of harvesting stage and DAP fertilizer rates.

## 3.3. Nutritional Value of Medicago sativa

### *3.3.1. Dry matter content (DM %)*

Dry matter content concentration was significantly affected harvesting stage (P < 0.03), but not affected by P fertilizer application rates and their interaction (Table 5). The relatively highest mean result was recorded at 100 % (full-flowering stage) of harvesting and the lowest mean was recorded at 10 % (pre-flowering stage); 21.7 % and 17.7 %, respectively. As harvesting stage increases, the dry matter content also increases. The DM content of the current study was comparable with Gashew et al. (2015), while higher DM content was indicated as compared to Geleti et al. (2014) for the same Medicago sativa species. The DM (%) content was comparable with the report of Walie et al. (2016), but the current study had more DM (%) content of *Medicago sativa* as compared to the same author. According to Diriba et al. (2014) the DM content of legume forage increased linearly with an advance in age of the forage. This is because of characterized by an increase in structural components and a decline in leaf to stem ratio.

### *3.3.2. Crude protein content*

The crude protein content of Medicago sativa showed slightly significant effect (P < 0.01) P fertilizer application rates at non-fertilized, 10 kg/ha-1 and 20 kg/ha-1 as numerically 16.73 %, 16.64 % and 17.18 %; respectively (Table 5). The highest overall average crude protein content was recorded at 10 % of the harvest (pre-flowering stage), and the lowest was recorded at 100 % of the harvest (full-flowering stage), 18.27 % and 16.9 %, respectively. From the point of view of the amount of phosphorus application, the CP content was the highest in the 40 kg/ha-1 treatment and the lowest in the 10 kg/ha-1 treatment. The average crude protein content was 19.05 % and 16.64 %, respectively. The results showed that as the amount of phosphorus fertilizer increased, the crude protein content also increased, but with the increase of plant age, the crude protein content decreased significantly in descending order, while the fertilizer rate P was the opposite.

The CP content reported in the present study was slightly higher when compared with others research findings (Diriba et al., 2014; Mekuanint et al., 2015). High quality Medicago sativa was reported to contain >19% CP (Redfearn and Zhang, 2011). On the other hand, Medicago sativa forage quality values at full bloom stage contain CP >16 % (Dunham, 1998). In this study, the tested *Medicago sativa* had CP content greater than the threshold value > 19% indicated by other researchers (Redfearn and Zhang, 2011). *Medicago sativa* nutritive value is identified with protein content which depends on the share of leaves in dry matter yield which in its turn is positively correlated with protein content (Julier et al., 2001*;* Katic et al., 2005). Protein content in *Medicago sativa* dry matter varies from18 to 25% depending on the growth stage, cultivar and storage method (Katic et al., 2006). According to (Katic et al., 2003) harvesting at earlier development stages produces more crude protein and less crude cellulose. A wider range of values observed in the literature for CP and fiber fractions of Medicago sativa can be attributed to various factors such as species, climatic and agronomic management practices and/or their interactions (Diriba et al., 2014). The result showed that Medicago sativa produces more protein per hectare than other legume and grasses; therefore, it is widely used for hay production and as pasture for livestock, especially to ruminants (Monteros and Bouton, 2009). Indeed, all the genotypes had CP values of above 15 %, a level suggested for a protein source supplement to be considered optimal to support lactation and growth in dairy cattle (Nsahlai et al., 1996).

### *3.3.3. Neutral detergent fiber (NDF %)*

Significant differences (P < 0.001) were determined at the harvesting stage of *Medicago sativa* for NDF content (Table 5). On the other hand, according to Mekuanint *et al*. (2015), the NDF contents of *Medicago sativa* were not significantly affected by treatment with varying amounts of phosphorus fertilizers. Numerically, the result revealed that the highest mean of NDF contents recorded was at 100% of the full-flowering stage; however, relatively, the lowest mean values of NDF content recorded at 10% of the pre-flowering stage were recorded at 47.07% and 41.57%, respectively.

High-quality *Medicago sativa* was reported to contain NDF <400 g/kg DM (Ball et al., 1997; Redfearn and Zhang, 2011; Kazemi et al., 2012). Low NDF values are desirable and are associated with increased animal intake. On the other hand, *Medicago sativa* forage quality values at full bloom stage contain NDF < 530 g/kg DM, reported as better quality (Dunham, 1998). The fact that the fact that the NDF content of all the genotypes was below the critical level (530 g/kg DM) reported (Dunham, 1998) could indicate that it has better digestibility. Canbolat et al. (2006) found that the NDF content of alfalfa was 44.7% and 54.2% at the stages of flowering and late flowering, respectively, which were slightly similar to our data. However, the NDF values reported in the present study were closer to the threshold level (<400 g/kg DM) reported for high-quality Medicago sativa in the literature (Ball *et al*., 1997; Redfearn and Zhang, 2011; Kazemi et al., 2012). In the previous research (Avci *et al.,* 2007, 2010; Spandel and Hesterman, 1997), NDF concentrations of alfalfa were found to be 46.4–47.3%, 45.0-48.7%, and 39.4–47.8%, depending on cultivars and years. Significant differences were registered in the content of NDF that were caused by genetic factors (Katić et al., 2008). Furthermore, Sheaffer et al.(1998) obtained significant differences in the contents of NDF between low-, medium-, and high-quality alfalfa cultivars. In general, a wider range of values observed for fibre fractions of alfalfa in the literature can be attributed to factors such as cultivar, climatic factors, and agronomic management practices or their interactions.

### *3.3.4. Acid detergent fiber*

The mean ADF content of *Medicago sativa* showed a highly significant effect (P < 0.001) on the on the harvesting stage and fertiliser application rates (P < 0.002), as shown in Table 5. Numerically, the highest mean ADF content was recorded at 100% (FF) full-flowering stage of harvesting and the lowest at 10% (PF) pre-flowering stage of harvesting (36.34% and 31.18%), respectively, and was highly significant at the lowest result obtained. According to DAP fertiliser application, the highest mean recorded at 10 kg/ha, while the lowest mean ADF content was recorded at 30 kg/ha, was obtained at 35.75 % and 32.47 %, respectively; however, there was a great variation, especially at the maximum level of fertiliser application rates (20 kg/ha, 30 kg/ha, and 40 kg/ha), recorded numerically, but low results were recorded at 32.75 %, 38.8%, and 35.3%, respectively.

Generally, high-yielding *Medicago sativa* rates have low values in terms of forage quality. Putnam et al*.* (2005) stated that yield and quality are inversely related, and the highest in yield was found to be the to be the lowest in quality (highest in ADF), and the *Medicago sativa* lowest in yield was typically the highest quality (lowest in ADF). When considered from this perspective, the numerically lowest (pre-flowering stage (31.88%) and 30 kg/ha (p 32.47%) came to the forefront as high-quality forage. In previous research, ADF concentrations for *Medicago sativa* were found by Putnam et al. (2005), Avci et al. (2007) and (2010), and Spandel and Hesterman (1997), respectively. In general, it is known that forage quality is strongly related to plant maturity differences at harvests within a year, cultivars, location, etc. Mekuanint et al. (2015) and Diriba et al. (2014) also reported non-significant differences in the ADF contents of Medicago sativa, which disagrees with this study. On the other hand, *Medicago sativa* forage quality values at full bloom stage contain NDF < 530 g/kg DM and ADF < 410 g/kg DM reported as better quality (Dunham, 1998). Significant differences were also registered in the contents of ADF that were caused by genetic factors (Katić et al., 2008).

The ADF values reported in the present study were closer to those reported by Dunham (1998) and reported in the literature (Ball et al., 1997; Redfearn and Zhang, 2011; Kazemi et al., 2012). In general, a wider range of values observed for fibre fractions of *Medicago sativa* in the literature can be attributed to factors such as harvesting stage, P fertiliser application rates, climatic factors, and agronomic management practices or their interactions. The present study agreed with the findings the findings reported by Mekuanint et al. (2015), Diriba et al. (2014), and Katić et al. (2008), who stated that the ADF content of *Panicum maximum* was significantly affected by DAP fertilisation and increased DAP fertilisation significantly decreased ADF content at the same stage of harvesting; however, the authors described that the increase in the level of fertiliser application on the pasture at different stages of growth had no significant role in maintaining the nutritive value of the pasture forage regarding fiber fractions, but in this study there are significant differences in the stages of growth had no significant role in maintaining the nutritive value of the pasture forage regarding fiber fractions, but in this study there are significant differences in the stage of harvesting, DAP fertilizer application rates, and their interaction.

***3.3.5. Acid detergent lignin***

The ADL content showed variability and a highly significant effect (P < 0.002) on the on the harvesting stage of *Medicago sativa*. This is in discord with the results reported by Diriba et al. (2014) and Mekuanint et al. (2015). On the other hand, lignification degree was not affected by DAP fertilizer application rates, and there were no significant differences compared to control data (Table 6). The relatively highest mean content of ADL was recorded at 100% (full-flowering stage) of harvesting stage (9.64%), while, relatively almost the same, the lowest mean content of ADL was recorded at 10% (pre-flowering stage) of harvesting stage (8.18%).

Lignification of the forages appeared to occur almost constantly with a continuous increment of lignification in the increasing stage of harvesting.And also, the lignin component contributes erective strength and resistance to plant tissue, thereby limiting the ability of rumen microbes to digest cell wall polysaccharides such as cellulose and hemicellulose (Reed et al., 1988). Hence, *Medicago sativa* forage with lower lignin content should have better digestibility. High lignin content in *Medicago sativa* plants increases their resistance to lodging; however, lignin is a major factor that limits cell wall digestibility because it inhibits the digestibility of polysaccharides (Katic et al., 2008).The ADL content was higher than that reported by Markovic et al. (2007) (4.64%), but much lower than that reported by Yu et al. (2003) (19.9%). A wider range of values observed in the literature for CP and fibre fractions of *Medicago sativa* can be attributed to various factors. This study is consistent with the results of Markovic et al. (2007) and Yu et al. (2003), indicated that the lignin content increases with the age of the plant. The current results indicate that the amount of DAP fertilizer has an effect on the content of ADL; this may be due to the variability of soil type, soil moisture content, climate, and agronomic management practices and/or their interactions (Munns et al., 2000; Cramer, 2000). They point out that during periods of high evapotranspiration related to soil water scarcity, the hydrated state of the plant will limit the rate of leaf expansion.

**Table 5**: Mean of DM, CP, NDF, ADF and ADL at different stage of harvesting and phosphorous fertilizer application.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Harvesting stage** | **Parameter, %** | | | | |
| **DM** | **CP** | **NDF** | **ADF** | **ADL** |
| 10 | 17.72b | 18.27a | 41.57b | 31.18c | 8.18c |
| 50 | 20.01ab | 17.28ab | 44.76a | 33.25b | 8.98b |
| 100 | 21.71a | 16.9b | 47.07a | 36.34a | 9.64a |
| LSD | 2.96 | 1.16 | 2.72 | 1.47 | 0.6 |
| *P value* | 0.03 | NS | 0.001 | <0.0001 | 0.0002 |
| DAP fertilizer rates (kg) |  | | | | |
| 0 | 18.59 | 16.73b | 44.18 | 34.98a | 9.14 |
| 10 | 19.77 | 16.64b | 44.05 | 34.13ab | 8.95 |
| 20 | 19.62 | 17.18b | 43.12 | 32.75b | 8.68 |
| 30 | 20.79 | 17.82ab | 45.78 | 30.8c | 9.02 |
| 40 | 20.3 | 19.05a | 45.2 | 35.3a | 8.87 |
| LSD | 3.82 | 1.5 | 3.51 | 1.9 | 0.78 |
| CV (%) | 20.03 | 8.93 | 8.21 | 5.9 | 9.13 |
| *P value* | NS | 0.01 | NS | 0.0002 | NS |
| Mean | 19.81 | 17.48 | 44.46 | 33.59 | 8.93 |
| SEM | ±3.97 | 1.56 | ±3.65 | ±1.98 | ±0.81 |
| HS\*DAP (*P value*) | NS | NS | NS | NS | NS |

The mean values within columns followed by different letters are significantly different at the 5% probability level. DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; LSD = least significant difference; CV (%) = coefficient variation; SEM = standard error of means; HS = harvesting stage; DAP = di-ammonium phosphate; NS = none significant; 10 = % of the preflowering stage; 50 = % of the half flowering stage; 100 = % of the full flowering stage; HS\*DAP = interaction effect of harvesting stage and P fertilizer rates.

### *3.3.8. Total ash*

The mean total ash contents of *Medicago sativa* are given in Table 6. There were highly significant differences in terms mean of total ash at harvesting stage (P < 0.001). Ash is the remaining portion of unburnt dry matter and is commonly used as an indicator of mineral contents (Gençtan, 1998). Minerals play a significant role in various processes in animals (hormone synthesis, enzyme activity) and they should be taken externally since they were not synthesized in animal body (Ülger and Kaplan, 2016). Total ash contents ranged between 8.32 and 9.38 %. Gungor et al. (2008) and Kavut and Avcioglu (2015) found 7.48-11.27 % and 8.82-9.43 % total ash contents, respectively. Our results were greater than that of their results. Manga (1978), Acar (2002) and Geleti et al. (2014) found 12.85-15.56 %, 11.37-12.02 % and 10.03-10.69 % total ash contents in their studies, respectively and their means were similar to our results. Tongel and Ayan (2010) reported that the total ash contents ranged between 7.50-8.45 % in the second harvest. Acikgoz (2001) stated that the variation of total ash content of the varieties depend on soil and climatic factors.

The present mean of total ash varied between 12.22 to 16.12 % and values were greater than that of Basbag et al. (2009), Davodi et al. (2011) and Kiraz (2011). Such differences were attributed the differences in plant genetics, soil and climate conditions. The total ash content decreased as stage of harvesting advanced, in other hand, as P fertilizer application increased, total ash content also increased and decreased. The result agrees with studies of (McDonald etal.,1995 and Diriba, 2000) who reported that decreased trend of total ash content age of grass advanced and other author concluded that as the photosynthetic area increases, DM production go far ahead of mineral uptake, resulting in a decline in mineral concentration (Fleming,1973).

The ash content reported in the present study was higher when compared with others research reports (Diriba et al., 2014; Mekuanint et al., 2015). The ash content was highest at free-flowering stage and was least at full-flowering stage, 16.12 % and 12.22 %, respectively; and the present study was in agreement with other findings (Manga, 1978). The mineral content is affected by the stage of maturity and the leaf to stem ratio, since alfalfa leaves contain more P, Ca, Mg, Cu, Zn, Fe and Mn while stems contain more K (Markovic et al., 2009). Since the concentration of minerals in forages are affected by stage of maturity, climatic and seasonal changes (Minson, 1990), regular analysis has been recommended for formulating appropriate mineral supplementation schedules (Spears, 1994). Other studies also indicated that concentration of minerals in forage varies due to factors like plant developmental stage, morphological fractions, climatic conditions, soil characteristics and fertilization regime (McDowell and Valle, 2000; Jukenvicius and Sabiene, 2007). Differences in both proportion and composition of the different morphological fractions could explain varietal differences in ash content. Alfalfa is a highly valued animal feed. It is a rich source of proteins, fibers, minerals and vitamins used in the diet of livestock, especially ruminants. The content of minerals in Medicago sativa fully meets the livestock requirements while the content of fats is low (averaging 3.8 g/kg), and it varies slightly among cultivars (Katić et al., 2009).

### *3.3.9. Phosphorus*

The analysis of variance showed that the effect of harvesting stage, phosphorus fertilization and the interaction of harvesting stage by phosphorus fertilization was not significant effect (P > 0.05) on phosphorus (g/kg) are shown in Table 6. Relatively decreasing order of phosphorus value indicated that forages advance in age and lower level of P fertilizer application affects phosphorus value in general. In present study the effect of harvesting stage advanced in age the phosphorus value slightly decreasing down ward and as P fertilizer level increasing the amount of phosphorus also increases upward.

### *3.3.10. Calcium (g/kg)*

The mean calcium of *Medicago sativa* showed that the effect of harvesting stage, phosphorus fertilization and the interaction of harvesting stage by DAP fertilization was not significant (P > 0.05) shown in Table 6. The concentrations of calcium value decreases, in contrast, as P fertilizer rates increase, calcium value decreased. This was probably due to an age effect, as reported by Ibrahim (1981), that Calcium concentrations decrease with advancing maturity, which is reflected on the calcium value that decline in a similar pattern to that of total ash due to a natural dilution process and translocation of mineral to seeds and root (Mcdonald et al., 2002; Minson, 1980). According to McDowell (1985) dietary calcium requirement of cattle is about 0.4%. Hence, the calcium value of present finding is quite lower than that stated by McDowell (1985) for growth, which requires calcium supplementation for Animals grazing on *Medicago sativa* under rain fed condition. The Calcium concentrations reported in this study at the harvesting stage and P application rates, are disagree with those reported by Dougall and Bogdan (1966), Van Rensburg (1968) and Everist (1969), because the present study result was very low. An inadequate intake of Calcium may cause weakened bones, slow growth and low milk production.

**Table 6:** Mean of HEM, CEL, TA, PHOS and Ca at different HS and phosphorous fertilizer application

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Harvesting stage** |  | **Parameter** | | | | |
|  | **HEM** | **CEL (%)** | **TA (%)** | **PHOS (g/kg)** | **Ca (g/kg)** |
| 10 | 10.38 | | 25.04c | 16.12a | 0.156 | 0.109 |
| 50 | 11.5 | | 27.99b | 14.15b | 0.155 | 0.109 |
| 100 | 10.73 | | 30.35a | 12.22c | 0.156 | 0.107 |
| LSD | 2.17 | | 1.98 | 1.29 | 0.021 | 0.009 |
| *P-value* | NS | | <.0001 | <.0001 | NS | NS |
| DAP fertilizer rates (kg/ha) |  | | | | | |  |  |  |  |
| 0 | 9.19b | | 28.93a | 14.75 | 0.154 | 0.108 |
| 10 | 9.92b | | 29.43a | 13.53 | 0.153 | 0.113 |
| 20 | 10.36b | | 27.42a | 14.1 | 0.155 | 0.107 |
| 30 | 14.9a | | 23.63b | 14.01 | 0.154 | 0.105 |
| 40 | 9.9b | | 29.55a | 14.41 | 0.161 | 0.107 |
| LSD | 2.8 | | 2.56 | 1.66 | 0.027 | 0.012 |
| CV (%) | 26.83 | | 9.58 | 12.24 | 18.15 | 11.6 |
| *P-value* | 0.001 | | 0.0002 | NS | NS | NS |
| Mean | 10.87 | | 27.79 | 14.16 | 0.155 | 0.11 |
| SEM | ±2.91 | | ±2.66 | ±1.73 | ±0.02 | ±0.01 |
| HS\*DAP (*P value*) | NS | | NS | NS | NS | NS |

The mean values within columns followed by different letters are significantly different at the 5% probability level. HEM = hemicellulose; CEL = cellulose; TA = total ash; PHOS = phosphorus in sample; Ca = calcium; LSD = least significant difference; CV (%) = coefficient variation; SEM = standard error; HS = harvesting stage; DAP = di-ammonium phosphate; NS = none significant; 10 = % of the pre-flowering stage; 50 = % of the half-flowering stage; 100 = % of the full-flowering stage; HS\*DAP = interaction effect of harvesting stage and phosphorous fertilizer rates.

### *3.3.11. In vitro dry matter digestibility (IVDMD %)*

The present value of ME decreases with an increasing harvest period and increases with an increasing application of phosphate fertilizers. This may be due to the fact that the fertilizer promotes the germination of the leaves of the plants, which contain higher energy, and because a higher concentration of cellulose is also reflected in the lower metabolizable energy value of *Medicago sativa* (McDonald et al., 2010). According to ILRI (2013), as the growth age of tropical grasses increases, the increase in the structural components of the cell wall leads to a significant decrease in the relationship between leaf and stem and other important agronomic parameters.

The IVDMD, which ranged from a mean of 58.17% to 63.19% of the Medicago sativa, has a significant effect (P < 0.01) at P fertilizer application, as presented in Table 7. The result revealed that the highest IVDMD value was recorded at a treatment of 40 kg/ha (63.19%), while non-fertilized traits had the lowest value. The highest and lowest values recorded, respectively, in the present study were in agreement with other findings (Mekuanint et al., 2015). The significant difference (P < 0.01) among the tested genotypes was in agreement with Diriba *et al*. (2014) and in disparity with other findings (Mekuanint et al., 2015). In vitro dry matter digestibility (IVDMD) values vary greatly between treatments, which are consistent with widely reported. For example, Volenec and Cherney (1990) reported significant differences in IVDMD between cultivars and Medicago sativa germplasm, and these differences are related to changes in Tremblay stem digestibility (2002). Similarly, Walie et al. (2016) found that the previously recorded results are roughly similar to the values recorded in this study and are consistent with their reports. The choice to improve forage quality also successfully increased the IVDMD of *Medicago sativa* (Monirifar, 2011).

Volenec and Cherney (1990) reported significant differences in IVDMD among *Medicago sativa* forages, and these differences were indicated to be associated with variation in digestibility of the stem fraction (Tremblay et al., 2002). A significant difference among 14 Medicago sativa varieties was also reported for IVDMD, with values ranging from 59 to 66% (Kamalak *et al*., 2005), which indeed were a little bit greater than those recorded in the present work. Previous research has demonstrated variability among *Medicago sativa* germplasm for the ruminal degradation of total crude protein (Tremblay *et al*., 2003). The digestibility of Medicago sativa organic matter depends on the contents of cellulose and lignin. As lignin is virtually indigestible, intensive lignification of the cell wall in the late stages of *Medicago sativa* development tends to reduce the coefficient of digestibility. Organic matter digestibility ranges from 55% to 77% and depends on growth stage, leaf-to-stem ratio, cutting frequency, harvesting conditions, and processing (INRA, 2007). The digestibility of *Medicago sativa* decreases with maturity as a result of increased concentrations of cell wall material in stems, decreased stem digestibility, and a decreased leaf weight ratio (Albrecht et al., 1987). Soil fertility, cultivar, climatic conditions, harvesting stage, and preservation method are some of the factors affecting *Medicago sativa* hay quality (Stancheva et al., 2008).

### *3.3.12. Digestible dry matter (DDM %)*

Highly significant differences (P < 0.0001) were observed among *Medicago sativa* for DDM% effects of harvesting stage, and also significant differences (P < 0.001) on the controlling group of P fertilizer application rates obtained as shown in Table 7. Based on the average mean of the harvesting stage, the highest DDM% was recorded at the early free-flowering stage, whereas the lowest DDM% was recorded at the late full-flowering stage numerically (54.4%) and 47.43%, respectively.

The results of the current treatment show that as the amount of phosphate fertiliser increases, the value of the DDM% content also increases slightly. However, the amount of phosphorus fertiliser above 20 kg/hashows an increasing trend, and it is possible that a greater amount of fertiliser is required to overcome structural properties such as cellulose, carbohydrates, and lignin. The highest and lowest DDM% content of Medicago sativa is mainly due to its highest and lowest leaf/vapour ratio, respectively. As mentioned above, compared to stems, *Medicago sativa* leaves have a higher nutritional value and higher intake potential. Julier and Huyghe (1997) also pointed out that *Medicago sativa* varieties show differences in digestibility depending on their leaf/stem ratio. The DDM% content of Medicago sativa determined in this study is similar to the previous reports by Avci et al. (2007) and Camborat et al. (2006).

### *3.3.13. Metabolizable energy (MJ/kg/ha-1)*

The metabolizable energy(ME) values were also directly related to the legume content in the forage. Significant differences (P<0.01) were observed among *Medicago sativa* for ME effects on harvesting stage (Table 7). At the stage of harvesting, the greatest result was recorded at the pre-flowering stage; on the other hand, the lowest result was obtained at the full-flowering stage, which was recorded at 8.68 and 8.09, respectively.

The present value of ME decreases with an increasing harvest period and increases with an increasing application of phosphorus fertilizers. This may be due to the fact that fertilisers accelerate the sprouts of plant leaves, which contain higher energy, and because the higher concentration of cellulose is also reflected in the lower metabolizable energy value of *Medicago sativa* (McDonald et al., 2002). According to ILRI (2013), as the growth age of tropical grasses increases, the increase in the structural components of the cell wall leads to a significant decrease in different important agronomic parameters, such as the leaf-stem relationship. Generally, the results of the current study indicate that different fodder plant species have different chemical compositions. The main difference is most probably because of the difference in species type, as also indicated in Taylor (2015).

**Table 7**: Mean value of IVDMD, DDM and ME content affected by HS and P fertilizer application rates.

|  |  |  |  |
| --- | --- | --- | --- |
| **Harvesting stage** | **Parameter** | | |
| **IVDMD %** | **DDM %** | **ME (MJ/kg/ha-1)** |
| 10 | 61.27 | 54.4a | 8.68a |
| 50 | 59.8 | 50.06b | 8.31ab |
| 100 | 61.11 | 47.43c | 8.09b |
| LSD | 2.23 | 2.13 | 0.38 |
| *P-value* | NS | <.0001 | 0.01 |
| DAP fertilizer rate (kg/ha) |  | | |
| 0 | 58.17c | 49.35bc | 8.1b |
| 10 | 60.86abc | 48.19c | 8.34ab |
| 20 | 59.89bc | 51.3b | 8.18b |
| 30 | 61.51ab | 54.2a | 8.45ab |
| 40 | 63.19a | 50.11bc | 8.73a |
| LSD | 2.88 | 2.75 | 0.5 |
| CV (%) | 4.93 | 5.65 | 6.24 |
| *P-value* | 0.01 | 0.001 | NS |
| Mean | 60.73 | 50.63 | 8.36 |
| SEM | ±2.99 | ±2.86 | ±0.52 |
| HS\*DAP (*P value*) | NS | NS | NS |

The mean values within the column followed by the same letters are not significantly different at the 5% probability level. *IVDMD* = *in vitro* dry matter digestibility; DDM = digestible dry matter; ME = metabolizable energy; LSD = least significant difference; CV (%) = coefficient variation; SEM = standard error; HS = harvesting stage; DAP = di-ammonium phosphate; 10 = % of pre-flowering stage; 50 = % of half-flowering stage; 100 = % of full-flowering stage; HS\*DAP = interaction effect of harvesting stage and DAP fertilizer rates.

Conclusion

Compared with other surveys and papers, almost all results have a higher chemical composition: crude protein content was slightly greater than 16%, ash content was greater than 11%, dry matter yield was greater than 90%, IVDMD was greater than 60%, and metabolizable energy was greater than 8%. On the other hand, compared with other researchers, calcium and phosphorus were lower than 0.12% and 0.16%, respectively. The result showed that the levels of phosphorus and calcium recorded at the harvesting stage and the treatment level overall are close to each other. In conclusion, this study has highlighted the benefits of growing Medicago sativa using different levels of phosphorus fertilization to maximize yield and quality in forage production. As observed in this study, the planting of Medicago sativa has not only enhanced total DM production but also raised the quality of the forage produced, in particular the CP concentration and increased total energy in the fodder produced. Based on the current work, the following recommendations and future. More forage crop extension work should be done on the sustainable production and utilization of Medicago sativa. Further, fertilized Medicago sativa (above 20 kg/ha) is given priority for dissemination to the livestock farming community because it has a higher nutritional value than the rest. More studies need to be undertaken in varying environments, and harvesting stages are needed to determine biomass yield, nutritional quality, and management practices.