Effect of Foliar Sprays of Sulfuric Acid and Thiourea on Herbage and Seed Yield in Berseem (*Trifolium Alexandra* L.)

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**ABSTRACT**

An abiotic stress such as low temperature or frost poses serious challenge for proper growth and development of the crop. The study was carried out for two consecutive years to investigate the effect of mixed cropping (berseem + oat vs pure berseem) and two bio-regulators (H\(_4\)SO\(_4\) and Thiourea) applied at different concentrations (0.05 and 0.1%) during low temperature conditions on growth, fodder yield and seed yield of berseem. Berseem + oat cropping recorded significantly higher green fodder yield, dry matter yield and crude protein yield by 7.0%, 6.1% and 3.4% respectively over pure stand. Bio-regulators sprays viz., H\(_4\)SO\(_4\) and Thiourea after first cut recorded 5.2-11.2% increase in green fodder yield. Berseem seed yield and harvest index were significantly superior in pure stand than intercrops. An increase of 21.4% in berseem seed yield was recorded with foliar sprays of 0.05% TU over control.

**Key words:** Berseem, Bio-regulators, Fodder and seed yield, Foliar spray, Mixed cropping, Quality.

**INTRODUCTION**

Berseem (*Trifolium alexandrinum* L.) is an important legume fodder crop grown for livestock in Asia, Africa, USA and Australia. In India berseem is grown in *Rabi* (winter) season and it occupies 2 million ha area (Kumar et al., 2013). Berseem is generally grown as pure stand by the farmers for feeding to the livestock. During severe winter months periods from December to February, berseem growth is slow due to low minimum air temperature or cold wave or frost occurrence which affects fodder availability to the livestock. To increase growth of berseem either in pure stand or in mixture, farmers generally use excessive urea which not only affects nodule development in roots of legumes but also pollute the environment (Dogra and Dudeja, 1993). Mixture of berseem clover with cereal crop enhances total dry matter yield, improves fodder quality, reduces fertilizer use and also increases subsequent crop yield (Ross et al., 2004). Farmer harvests 3-4 cuttings of berseem up to late mid April as fodder for livestock and then leaves the berseem crop for seed production. After 3-4 cuts as green fodder, berseem plants retain less foliage, poor flowering and finally low seed production (Kumar et al., 2013). It is very essential that berseem crop attains sufficient height and tillering capacity during initial cuts for obtaining high fodder yield and later on crop left for seed production produces good quantity and quality of seed. Abiotic stress factors cause extensive loss to agriculture production worldwide (Anitha et al., 2006; Kumar et al., 2013; Wahid et al., 2017). Among abiotic stress factors, low temperature or frost causes considerable reduction in growth and development of berseem leading to reduced availability of green fodder. Measures for alleviating cold injury in crops so far are confined to the application of irrigation at large (Rao and Sahu, 1991). But in berseem and its intercrops, frequent irrigation impede root growth and causes diseases in crop due to more damp conditions and also make difficult to harvest fodder. Chemical means of assuage cold or low temperature injury, therefore, appears necessary for optimizing berseem and its intercrops productivity during low temperature condition. Thiourea (TU) is an organic compound having molecular formula Cs(NH\(_4\))\(_2\) has been identified as an effective bio-regulator to improve growth of plants under stress condition (Pandey et al., 2013; Amin et al., 2014; Singh, 2015). Foliar spray of TU improves vegetative growth and dry matter production in legume (Dayanad et al., 2013; Amin et al., 2014), enhances carbohydrate and nitrogen metabolism (Singh, 2015), improve photosynthetic efficiency (Pandey et al., 2013), promote synthesis of auxin and cytokinin for the enhancement of cell division and chlorophyll formation under stress conditions (Amin et al., 2014). Sulphur from H\(_4\)SO\(_4\) promote oil synthesis and enhances seed protein, amino acids, haematin enzymes (such as catalase and peroxidase) and improve chlorophyll formation (Singh, 2015). In the present study, the selected chemicals have different characteristics, easily available and economical which make them suitable choice for mitigating the damage induced by low temperature stress. So, keeping this objective

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in view, a field experiment was designed to know the effect of different bio-regulators sprays during early cuts on the growth of berseem grown as pure stand and in mixture against very low minimum air temperature/frost injury and seed production of berseem under different sowing conditions.

**MATERIALS AND METHODS**

Field experiments were conducted during two consecutive years at the Guru Angad Dev Veterinary & Animal Sciences University, Ludhiana (30°56’ N, 75°52’ E, 247 m above sea level), Punjab, India. The soil of the experimental field was loamy sand in texture having pH 7.8, with 0.4% Walkley and Black carbon, 15.0 kg P ha⁻¹ Olsen P and 347 kg K ha⁻¹ NH₄OAc-extractable K. The climatic parameters were recorded at the meteorological observatory of Punjab Agricultural University, Ludhiana. Total amount of rainfall received during the crop seasons from October to June were 117.5 mm and 164.4 mm in 2009-10 and 2010-11, respectively. During the year 2009-10, mean minimum air temperature during the month of December, January and February were 6.5°C, 6.6°C and 9.9°C whereas during the year 2010-11 corresponding figures were 5.6°C, 5.2°C and 8.8°C respectively. Number of days with <5°C minimum air temperature during the months of December, January and February were 11, 6 and 1 while during the year 2010-11 number of days were 12, 20 and 0 days, respectively. Minimum air temperature was <3°C for 3 days in the month of December during 2009-10 and for 5 days during the month of December and January in the year 2010-11. Frost occurred for one day in the month of December during 2009-10 while for 11 and 15 days in the month of December and January during the year 2010-11. Mean monthly maximum and minimum air temperatures recorded were in May i.e. 40.2°C and 24.4°C and 39.4°C and 25.0°C during the year 2009-10 and 2010-11, respectively.

The treatments included combinations of two mixture (berseem pure stand and berseem + oat mixture) and 2 combinations of bio-regulators and their concentrations, namely H₂SO₄, & Thiourea (0.05% and 0.1%). An additional untreated control (water spray) was also included in the study. The experiment was laid out in a factorial randomized complete block design with three replications. Two foliar sprays of the Thiourea and diluted H₂SO₄ were applied at one week interval 10 days after first and second cut. The cultivar BL 42 of berseem and Kent of oat was used in this study. The crop was sown as per treatment in plot area of 10.5 m² on October 20 and October 16 during the year 2009-10 and 2010-11, respectively. Sole crop of berseem was sown evenly broadcasting the 25 kg seed ha⁻¹ on well prepared seed bed under dry soil conditions. Similarly in mixed cropping, oat seed @ 30 kg ha⁻¹ was drilled with hand plough in the soil keeping line to line spacing of 22 cm apart and then leveled the plot. Afterwards, berseem seed @ 25 kg ha⁻¹ was broadcasted and raked in dry condition. In both treatment plots (sole berseem and berseem + oat mixture), light irrigation was provided on the same day after sowing. A basal dose of 25 kg N (as urea) and 32.8 kg P ha⁻¹ was applied at sowing time. In sole and mixed crop (berseem and oat), 25 kg N was applied after each cut. Four cuts of green fodder were taken from each plot up to mid April and then crop was left for seed production. As green fodder, 1st, 2nd, 3rd and 4th cuts were taken in 4th week of December, 1st week of February, 2nd week of March and 2nd week of April during both the years of study.

Observations were recorded on plant height, tillers plant⁻¹, green fodder yield (GFY), dry matter yield (DMY), crude protein content and crude protein yield. After taking four cuts during both the year’s, crop was allowed to grow for seed production when the seeds in pods turned yellow. Different growth parameters such as plant height and tillers plant⁻¹ and yield attributes like heads m⁻², seeds head⁻¹, 1000 grain weight and seed yield were measured at harvest. The data were analyzed using analysis of variance (ANOVA) using IRRISTAT version 92 (IRRI, 1992). The crop data were analyzed keeping mixtures as factor A and foliar treatments as factor B in factorial randomized block design. The data presented is averaged over the years and comparison of treatment means was made by the least significant difference (LSD) at p=0.05.

**RESULTS AND DISCUSSION**

**Plant height and tillers plant⁻¹:** Plant height at different cuts of berseem pure and in mixture with oat is presented in Table 1. Data pertaining to berseem plant height and tillers plant⁻¹ under different treatments was recorded at 2nd, 3rd, 4th and at harvest while oat plant height and tillers were recorded at 2nd and 3rd cuts only owing its cessation after that. No significant difference in plant height of berseem was recorded in berseem pure and berseem+oat mixture during the 2nd, 4th cuts and at harvest. During 3rd cut, berseem plants in pure stand gained significantly more height than in mixture (Table 1) owing to no competition from other crop. Similarly significantly more number of tillers plant⁻¹ in berseem were recorded in pure stand than in mixture during 3rd, 4th cuts and at harvest except at 1st cut. Higher numbers of tillers plant⁻¹ in pure stand might be due to better availability of growth resources to the plants. As oat was present only in mixture (berseem + oat) data related to its plant height and tillers plant⁻¹ is not presented in Table 1.

Foliar sprays of different bio-regulators recorded significant effect on plant height and tillers plant⁻¹ of berseem and oat (Table 2). Foliar sprays of thiourea @ 0.05% recorded taller plants and more tillers plant⁻¹ in berseem and oat at different cuts compared to corresponding untreated control. Foliar application of 0.1% H₂SO₄ also recoded
significant increase in plant height and tillers plant\(^{-1}\) of berseem and oat over control. The increase in growth parameters of berseem and oat with exogenous application of thiourea might possibly be due to increased translocation of sucrose metabolites from source to sink, improved plant growth potential due to enhanced photosynthetic efficiency, enhancement of cell division and chlorophyll formation (Amin et al., 2014; Singh, 2015; Anitha et al., 2006). Many researchers had also reported positive effect of exogenous application of thiourea in chickpea (Cicer arietinum), faba bean (Vicia faba), wheat (Triticum aestivum L.) and maize (Zea mays L.) crops (Duyanand et al., 2013; Amin et al., 2014; Pandey et al., 2013; Singh, 2015). Exogenously applied thiourea also imparts stress tolerance and has been reported to increase the growth and development of plants when the conditions are not favorable (Pandey et al., 2013) hence a favorable effect of its application on growth and development of berseem and oat had been observed in our study during low temperature condition. Sharma and Jain (2003) and Jat et al. (2014) also recorded positive effect of foliar application of sulfuric acid (0.1%) on growth attributes of Indian mustard (Brassica juncea) and chick pea under low temperature injury in arid irrigated conditions.

**Green fodder yield (GFY) and dry matter yield (DMY):** Production of greater forage and dry matter yield per hectare is very important for the producers. Data manifested in Table 3 shows that intercrop mixtures and application of different chemicals as foliar sprays had significant effect on the green fodder yield (GFY) and dry matter yield (DMY) during various cuts. Noticeable increase in green fodder and dry matter yield was obtained in 1\(^{st}\), 2\(^{nd}\) and 3\(^{rd}\) cuts in berseem + oat intercrop over berseem pure stand where as in the 4\(^{th}\) cut both these parameters were at par with each other. Highest green fodder and dry matter in 3\(^{rd}\) cut was due to favorable temperature for growth of the crops where as lower temperature affected its growth in previous cuts. In respect to total green fodder and dry matter yield, berseem + oat mixture recorded significantly higher respective values by 7.0% and 6.1% than pure stand of berseem (Table 3). The benefits of intercropping berseem clover with cereal crops, include increased total green fodder and dry matter yield, improved forage quality (Ross et al., 2004), reduced fertilizer requirements and increased subsequent crop yield (Dogra and Dudeja, 1993). Mixture also leads to more efficient absorption of nutrients from the soil, more interception of light energy at different layers and finally more photosynthetic rate, better translocation of photosynthates from source to sink leading to higher green fodder and dry matter yield.

**Table 1:** Plant height (cm) and tillers plant\(^{-1}\) of berseem during different cuts under different foliar chemical sprays (average of two years).

| Treatments | 2\(^{nd}\) Cut | 3\(^{rd}\) cut | 4\(^{th}\) cut | At harvest |
|------------|----------------|----------------|----------------|------------|
|            | Plant height (cm) | Tillers plant\(^{-1}\) | Plant height (cm) | Tillers plant\(^{-1}\) | Plant height (cm) | Tillers plant\(^{-1}\) | Plant height (cm) | Tillers plant\(^{-1}\) |
| Mixture    |                |                |                |            |
| Berseem Pure | 53.8           | 7.21           | 65.7           | 8.32       | 65.0           | 9.50           | 55.0           | 10.1       |
| Berseem + oat mixture | 52.6           | 6.82           | 62.5           | 8.00       | 66.3           | 8.75           | 56.0           | 9.2        |
| LSD (p=0.05) | NS             | NS             | 2.2            | 0.31       | NS             | 0.62           | NS             | 0.8        |
| Bio-regulators |          |                |                |            |
| Control   | 49.5           | 6.31           | 60.3           | 7.30       | 62.7           | 8.11           | 52.7           | 8.1        |
| H\(_2\)SO\(_4\) 0.05% | 52.2           | 6.81           | 62.6           | 8.00       | 65.6           | 8.73           | 54.5           | 9.3        |
| H\(_2\)SO\(_4\) 0.1% | 53.7           | 6.93           | 64.2           | 8.21       | 66.6           | 8.97           | 55.2           | 9.7        |
| TU 0.05% | 56.6           | 7.81           | 67.9           | 8.91       | 67.5           | 10.21          | 58.0           | 11.0       |
| TU 0.1% | 54.0           | 7.23           | 65.6           | 8.40       | 65.5           | 9.60           | 57.6           | 10.3       |
| LSD (p=0.05) | 1.9            | 0.62           | 3.5            | 0.49       | 2.0            | 0.98           | 2.6            | 1.2        |

**Table 2:** Plant height (cm) and tillers plant\(^{-1}\) of oat during second and third cut under different foliar chemical sprays (average of two years).

| Bio-regulators | 2\(^{nd}\) cut | 3\(^{rd}\) cut |
|---------------|----------------|----------------|
|               | Plant height (cm) | Tillers plant\(^{-1}\) | Plant height(cm) | Tillers plant\(^{-1}\) |
| Control       | 37.5           | 4.3            | 32.5           | 3.0        |
| H\(_2\)SO\(_4\) 0.05% | 40.7           | 4.6            | 35.1           | 3.2        |
| H\(_2\)SO\(_4\) 0.1% | 41.8           | 4.7            | 37.4           | 3.4        |
| TU 0.05%      | 44.3           | 5.1            | 40.8           | 4.2        |
| TU 0.1%       | 43.8           | 4.7            | 38.9           | 3.9        |
| LSD (p=0.05)  | 4.1            | 0.3            | 3.0            | 0.4        |
Foliar sprays of different bio-regulators on crop significantly affected green fodder and dry matter yield during (Table 3). Among different chemical foliar treatments, foliar sprays of thiourea and H$_2$SO$_4$ recorded significantly higher green fodder and dry matter yield than control. Foliar spray of 0.05% thiourea on crop recorded 11.2%, whereas dilute sulfuric acid at 0.05 and 0.1% led to 5.2 and 8.0% increase in green fodder yield over control. The increase in green fodder yield with foliar application of 0.1% TU was less compared to 0.05% TU but was significantly better than control. Similarly, highest total dry matter yield with foliar sprays of 0.05% TU was significantly better than foliar sprays of 0.05, 0.1% H$_2$SO$_4$ and control. The increase in total DMY with foliar sprays of 0.05% TU was to the tune of 11.4, 5.5 and 2.8% with respect to control, foliar sprays of 0.05% and 0.1% H$_2$SO$_4$, respectively. The increase in GFY and DMY in 2$^{nd}$, 3$^{rd}$ and 4$^{th}$ cut as well as total with foliar sprays of different bio-regulators may be attributed to enhanced growth of crop in subsequent cuts after 1$^{st}$ cut due to maintenance of redox state of the cell under stress conditions (Sharma and Jain, 2003; Anitha et al., 2006; Kumar et al., 2013). Singh (2015) and Sharma and Jain (2003) also reported significant improvement in the potato and mustard crop yield with foliar application of 0.1% TU and concentrated H$_2$SO$_4$ (1.0 ml/L water) over control due to crop protection from the low temperature. Further, sulphydryl compound play role in improving dry matter partitioning and thus improve the productivity. Garg et al. (2006) reported that thiourea (TU) as foliar spray on the plant significantly improved plant height, leaf area, dry matter production, net photosynthetic rate and concentration of total chlorophyll and starch in the leaves compared to control. The slight reduction in green fodder and dry matter yield at higher concentration of TU might be due to reduction in soluble protein in plants as higher concentration stresses the plant as reported by Mani et al. (2013).

**Crude protein (CP) content and crude protein yield (CPY):** Crude protein content and crude protein yield are important parameters for determining the quality of the fodder produced. Mixtures had significant effect on the

### Table 3: Green fodder (GFY) and dry matter yield (DMY) of berseem pure and berseem + oat mixture under different foliar chemical sprays during different cuts (average of two years).

| Treatments             | 1$^{st}$ Cut | 2$^{nd}$ cut | 3$^{rd}$ cut | 4$^{th}$ cut | Total GFY | Total DMY |
|------------------------|--------------|--------------|--------------|--------------|-----------|-----------|
|                        | GFY (q ha$^{-1}$) | DMY (q ha$^{-1}$) | GFY (q ha$^{-1}$) | DMY (q ha$^{-1}$) | GFY (q ha$^{-1}$) | DMY (q ha$^{-1}$) |
| Berseem Pure           | 81.5         | 6.9          | 125.3        | 12.2         | 207.9      | 21.8       | 203.7      | 26.5         | 618.4      | 67.4      |
| Berseem + oat mixture  | 94.7         | 8.1          | 142.8        | 14.0         | 221.8      | 23.3       | 201.8      | 26.2         | 661.1      | 71.5      |
| LSD (p=0.05)           | 3.3          | 0.3          | 5.1          | 0.5          | 7.3        | 0.8        | NS         | NS           | 10.8       | 1.2       |

### Table 4: Crude protein content and crude protein yield (CPY) of berseem pure and berseem + oat mixture under different foliar chemical sprays (average of two years).

| Treatments             | 1$^{st}$ Cut | 2$^{nd}$ cut | 3$^{rd}$ cut | 4$^{th}$ cut | Average | Total CPY |
|------------------------|--------------|--------------|--------------|--------------|---------|-----------|
|                        | CP (%)       | CPY (q ha$^{-1}$) | CP (%)       | CPY (q ha$^{-1}$) | CP (%)   | CPY (q ha$^{-1}$) |
| Berseem Pure           | 21.3         | 1.48         | 21.2         | 2.59         | 21.0     | 4.59       | 23.3       | 6.20         | 21.8       | 14.9      |
| Berseem + oat mixture  | 18.4         | 1.50         | 19.8         | 2.75         | 21.2     | 4.95       | 23.5       | 6.18         | 20.7       | 15.4      |
| LSD (p=0.05)           | 0.3          | NS           | 0.5          | 0.14         | NS       | 0.16       | NS         | NS           | 0.60       | 0.3       |

### Bio-regulators

|                        | Control | H$_2$SO$_4$ 0.05% | H$_2$SO$_4$ 0.1% | TU 0.05% | TU 0.1% | LSD (p=0.05) |
|------------------------|---------|-------------------|------------------|----------|---------|--------------|
|                        | 19.9    | 20.0              | 20.0             | 20.0     | 20.0    | 0.3          |
| CP (%)                 | 1.46    | 20.0              | 2.56             | 2.72     | 2.84    | NS           |
| CPY (q ha$^{-1}$)      | 19.6    | 21.1              | 21.3             | 21.9     | 20.4    | NS           |
| Average CPY (q ha$^{-1}$) | 21.3  | 21.1              | 21.3             | 21.9     | 20.4    | NS           |
protein content and crude protein yield of the forage produced (Table 4). During 1st and 2nd cut pure berseem crop recorded significantly higher protein content than berseem + oat mixture intercrop. However, 3rd and 4th cut registered non significant differences in respect to crude protein content which might be due to low or no presence of oat crop in these cuts. Average crude protein content in berseem pure crop was 5.3% higher than berseem + oat mixed crop. With respect to crude protein yield (CPY), berseem + oat mixture in 2nd and 3rd cut recorded significantly more crude protein yield possibly due increase in green fodder and dry matter yield (Table 3). Berseem + oat mixture registered significant increase in total crude protein yield by 3.4% over berseem pure stand. Zhang et al. (2015) also advocated that forage intercropping (alfalfa+ rye grass) system exhibited higher DM and CP yields than the sole alfalfa forage crop.

Foliar sprays of bio-regulators had also significant effect on crude protein content and crude protein yield of green fodder produced during different cuts (Table 4). Significant improvement in crude protein content of fodder was recorded with foliar sprays of 0.05% TU over foliar application of 0.05% H$_2$SO$_4$ and control during 2nd, 3rd and 4th cut. Average crude protein content was improved by 6.3% over control with foliar application of 0.05% TU. Similarly crude protein yield (CPY) was also influenced significantly with application of bio-regulator. Highest CPY was obtained with foliar sprays of 0.05% TU during 2nd, 3rd and 4th cut and was found statistically better than 0.05 and 0.1% H$_2$SO$_4$ and control. With the 0.05% TU, 0.05 and 0.1% H$_2$SO$_4$ application, increment in total CPY was 19.1%, 9.2% and 6.6% over control. Pandey et al. (2013) also recorded 2.5% and 19% increase in protein and oil content of TU treated mustard plant over control due to increased total uptake of N P and K from soil. Higher concentration of TU (0.1%) significantly reduced crude protein content during 2nd and 3rd cut while significant reduction in CPY with higher dose of TU was recorded in 3rd cut only (Table 3). Mani et al. (2013) also reported significant reduction in soluble protein in potato plants treated with 1000 nM TU over 250 nM suggesting higher concentration of TU stresses the plant due to low transfer of photosynthates in sink organ. However, in our study average crude protein content and total CPY were found to be statistically similar with foliar sprays of 0.05% and 0.1% TU.

**Yield attributes and berseem seed yield:** In our study, fodder mixture and bio-regulators influenced berseem yield attributes, biological yield and seed yield significantly (Table 5). Considerably higher numbers of pod plant m$^{-2}$, 1000-grain weight, biological yield, seed yield and harvest index were recorded in berseem pure stand than berseem + oat intercrop mixture. Increase in pods m$^{-2}$ was by 4.4% while 1000-grain weight was improved by 7.2% in berseem pure crop over mixture. Pure stand of berseem recorded 7.9% higher biological yield than mixed berseem and oat. Seed yield is important criterion for realizing better return in any crop. In our study, seed yield of berseem was significantly improved in berseem pure stand than berseem + oat mixture crop (Table 5) possibly due to better plant stand. The results were in line with Shoaib et al. (2013) who reported that higher yield production of Egyptian clover at each growth stage was due to sufficient berseem plant size at first cut that ensured more reserves stored in lower part of the plant that helped high re-growth as compared to Egyptian clover in mixture. Harvest index was significantly superior in berseem pure stand than berseem + oat mixture (Table 5) possibly due to higher biological and seed yield because of better growth of plants.

Different foliar treatments of thiourea and dilute sulfuric acid recorded significantly more berseem pods m$^{-2}$, seeds pod$^{-1}$, 1000-grain weight, biological yield and seed yield over control (Table 5). Application of H$_2$SO$_4$ and TU increased the mean head density by 7.7-14.4% over the control treatment. The highest heads m$^{-2}$ were recorded with foliar application of 0.05% TU which was statistically not different from H$_2$SO$_4$ and TU (0.1%) but significantly better than H$_2$SO$_4$ 0.05% and control. Jat et al. (2014) also reported significant improvement in pods plant$^{-1}$ with foliar sprays of H$_2$SO$_4$ on chickpea during low temperature conditions. All

**Table 5:** Yield attributes and seed yield of berseem under different mixture and foliar chemical sprays (average of two years).

| Treatments | Pods m$^{-2}$ | Seeds pod$^{-1}$ | 1000-grain weight (gm) | Biological yield (q ha$^{-1}$) | Seed yield (q ha$^{-1}$) | Harvest index |
|------------|---------------|-----------------|------------------------|-------------------------------|------------------------|---------------|
| **Mixture** |               |                 |                        |                               |                        |               |
| Berseem Pure | 331.4         | 47.2            | 2.68                   | 60.0                          | 8.91                   | 14.9          |
| Berseem + oat | 317.2         | 45.7            | 2.50                   | 55.6                          | 7.92                   | 14.3          |
| LSD (p=0.05) | 9.6           | NS              | 0.08                   | 2.1                           | 0.4                    | 0.4           |
| **Bio-regulators** |           |                 |                        |                               |                        |               |
| Control | 298.8         | 42.1            | 2.51                   | 50.0                          | 7.45                   | 14.9          |
| H$_2$SO$_4$ 0.05% | 321.8         | 45.6            | 2.54                   | 55.9                          | 8.17                   | 14.7          |
| H$_2$SO$_4$ 0.1% | 328.0         | 47.1            | 2.60                   | 58.6                          | 8.71                   | 14.9          |
| Thiourea 0.05% | 341.9         | 50.1            | 2.70                   | 64.0                          | 9.05                   | 14.2          |
| Thiourea 0.1% | 331.0         | 47.4            | 2.63                   | 61.0                          | 8.73                   | 14.3          |
| LSD (p=0.05) | 15.2          | 4.7             | 0.12                   | 3.4                           | 0.6                    | NS            |
Table 6: Growth parameters, fodder yield, crude protein content, yield attributes and berseem seed yield during different years (data pooled across intercrops mixture and foliar treatments).

| Year     | First | Second | LSD (p=0.05) |
|----------|-------|--------|--------------|
| Height  (cm) | 55.6  | 53.5   | 53.5         |
| Fodder Yield (q ha⁻¹) | 69.5  | 9.20   | 9.20         |
| Protein content (%) | 61.7  | 2.2    | 2.2          |
| Total Crude Protein (q ha⁻¹) | 640.0 | 7.2     | 7.2          |
| Pod harvest | 55.6  | 9.68   | 9.68         |
| Green plant tillers plant⁻¹ | 53.5  | 9.20   | 9.20         |
| Total Fodder Matter (gm) | 614.0 | 2.2    | 2.2          |

Yield is expression of various morphological, physiological and growth parameters in crop. Foliar sprays of dilute sulfuric acid and TU at different concentrations led to improvement in the seed yield of berseem over control. Maximum increase in berseem seed yield was observed under the foliar sprays of 0.05% TU which was 21.4% more than control followed by foliar sprays of 0.1% TU (17.1%). Foliar sprays of H₂SO₄ (0.05 and 0.1%) helped to increase the seed yield ranging from 9.6-16.9% over control. Singh (2015) also recorded that foliar application of concentrated H₂SO₄ @ 1.0 ml/L of water and 0.1% TU not only protected the potato crop from frost injury but also led to 11.1 and 15.8% increase in the tuber yield. Increase in seed yield of berseem in our study might be due to enhanced growth and tillering of the crops after the foliar application these bio-regulators (Table 1). Higher growth and development in terms of height and tillering under TU treatment at 2nd cut might had resulted in more food reserves leading to better growth during subsequent cuts and finally in seed yield of berseem. TU can save cells from harm and maintain their structure during stress condition which in turn can lead to more absorption
and translocation of minerals from the soil to the plants (Anitha et al., 2006; Pandey et al., 2013; Kumar et al., 2013). Accumulation of more nutrients and reserves in the plant might be responsible for good and healthy plants in later cuts resulting more seeds yield. Higher concentration TU (0.1%) recorded slight decrease in seed yield of berseem than lower concentration (0.05%) possibly due to suppressing of water and mineral absorption by the roots (Mani et al., 2013). Foliar application of different bio-regulators did not have any significant effect on the harvest index.

Year’s effect: Berseem plant height and tillers differed significantly during the year 2009-10 and 2010-11 (Table 6). Significantly taller and with more tillering capacity berseem plants were recorded during first year study than second. Yadav (2010) also reviewed that cold conditions generally results in stunted growth and reduced tillering in the crops. Similarly, more green fodder yield and dry matter yield was recorded during the year 2009-10 over 2010-11 possibly due to suitable climatic conditions during the first year. As frost occurred for 26 days during the 2010-11, might impeded the growth of plant as evidenced by height and tillers of berseem at harvest resulted in lower generation of photosynthates causing low green fodder and dry matter yield. Average crude protein content and total crude protein yield was also significantly low during second year of study. Yadav (2010) also advocated that exposure of the plant to the cold stress causes reduction and impairing of photosynthesis, reduction in protein synthesis and general metabolic process. Significantly higher numbers of pods m⁻² were recorded in 2009-10 probably due to more growth and tillering of plants due to normal weather conditions. Seeds pod⁻¹ and 1000 grain weight was statistically similar during the both years of study. Biological yield and seed yield recorded 3.9% and 6.7% increase during first year of study than the year 2010-11 possibly due to lesser occurrence of frost days during that year. Kumar et al. (2013) also reported that temperature and rainfall can influence deposition of photosynthates in the seeds resulting variation in berseem seed production. Harvest index was significantly more during first year of study due to production of more biomass by the crop.

CONCLUSION

The results obtained in the present study showed that mixture of berseem and oat resulted in higher green fodder yield and dry matter yield than berseem pure stand. During harsh winter months when very low minimum air temperature or frost injury occurs, two foliar sprays of thiourea at 0.05% at weekly interval 10 days after 1st and 2nd cut is promising in enhancing green fodder and seed yield of berseem grown in pure stand and in mixture with oat. Foliar application of TU resulted in 11.2% and 21.4% increase in green fodder yield and seed yield of berseem. The use of TU as bio-regulatory compounds thus opens up a new avenue for increasing green fodder and seed yield improving the fodder availability for the livestock around the world.

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