Split nitrogen sources effects on nitrogen use efficiency, yield and seed quality of safflower (Carthamus tinctorius L.)

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Abstract
The effects of nitrogen (N) on crop yields have historically been assessed with field trials, but selection and use of the best sources and optimal timing N applications have a significant role in realizing the maximum potential of oilseeds quality and quantity. This study was conducted to determine the combine effects of N sources [ammonium nitrate (AN), ammonium sulphate (AS), sulphur coated urea (SCU), and urea (U)] and split N fertilisation [(1/4,3/4,0), (1/3,1/3,1/3), (1/2,1/2,0), and (1/3,2/3,0)] on safflower (Carthamus tinctorius L.) some growth characters, yield and seed quality, and N use efficiency based on a split plot design with three replications at the experimental research station, Shiraz University in 2015 and 2016. The highest safflower dry matter (5140.93 kg ha⁻¹), seed yield (3303.52 kg ha⁻¹) and protein yield (694.95 kg ha⁻¹) were achieved with the application of AN fertiliser in a split pattern of 1/2,1/2,0 (applying half of the N at sowing time and the rest at stem elongation), while the highest oil yield (753.09 kg ha⁻¹) was observed by U fertiliser and similar split pattern. Applying AN fertiliser and split patterns of 1/3,2/3,0 (applying one third of the N at sowing and two thirds of the N at stem elongation) and 1/4,3/4,0 (applying one quarter of the N at sowing and three quarters at stem elongation) maximised safflower N uptake efficiency (NUpE) (0.78 kg kg⁻¹). However, the highest N utilisation efficiency (NUfE) (43.70 kg kg⁻¹) was obtained when AN fertiliser in a split pattern of 1/3,2/3,0 was applied. On the contrary, applying AS and SCU fertilisers was less effective on safflower performance by all split patterns. It is concluded that applying AN fertiliser in a split pattern of 1/3,2/3,0 and or U fertiliser in a split pattern of 1/3,2/3,0 not only enhanced safflower growth, yield and seed quality improved, but also increased the N use efficiency of safflower.

Introduction
Oilseeds are of great value in nutritional demands of mankind, animal feeding, and medicine. Among them, safflower (Carthamus tinctorius L.) is cultivated for many purposes: its achenes are commonly used as birdseeds and for the extraction of edible oil; corollas can be used for dyeing fabrics as food colourings and cosmetics and in painting or for producing medicines as pointed out by Danieli et al. (2011) and also as forage crop as reported by Cazzato et al. (2011). An important characteristic of this crop is its adaptation to semi-arid growing conditions owing to a deep root system (2-3 m in depth) enabling it to obtain moisture from unavailable levels for most crops (Ashkani et al., 2007; Yeilaghi et al., 2012). Quoted by Siadat et al. (2011) and Cazzato et al. (2013), the efficient use of fertilisers is one of the most important factors in maximizing crop yield and sustainability. Rathke et al. (2005) have underlined the significance of higher soil nutrient and particularly N availability in determining the yield quantity and quality of oilseeds. Moradi-Telavat et al. (2008) reported that nitrogen (N) increased rapeseed yield through increased silique number and seed weight. But a significant negative effect of N on oil content has been observed (Malhi and Gill, 2004; Siadat et al., 2011). Fismes et al. (2000) and Li et al. (2007) noted that the response of crop to N fertiliser is influenced by the formulation, fertiliser management, soil properties, and seasonal trends. Muhammad et al. (2007) reported that the highest seed protein was obtained when rapeseed (Brassica napus L.) treated by calcium ammonium nitrate. Similarly, Ozturk (2010) found that ammonium sulphate (AS) and urea (U) fertilisers gave higher crop yield than ammonium nitrate (AN) fertiliser, while Osman et al. (2014) showed that AN increased growth and yield parameters of rapeseed compared to other N fertiliser sources.
Because N availability is an important factor in determining crop productivity, managing fertiliser rate and application timing can be a suitable strategy to improve crop growth and yield when crops need it or when water is available to enhance nutrient uptake (Barlo’g and Grzebisz, 2004; Corbellini et al., 2006). Some studies have shown that, depending on the level of fertility of the initial soil, split applications of N fertiliser result in higher rates of plant recovery and higher seed yields than under single applica-
Materials and methods

Field experiment

A 2-year field experiment was conducted at the experimental research station (Badjgah), Shiraz University (52° 46' E, 29° 50' N and 1810 m), Iran in 2015 and 2016. The soil was silty clay loam and the rheological parameters of wheat. Sheibani and Ghadiri (2012) observed that the application of U at a rate of 304 kg ha⁻¹, half at the sowing time and the other half at the end of rosette stage of rapeseed, enhanced seed yield up to 35%. Timing of N fertiliser also increases the efficiency of N use efficiency, with a higher plant recovery of this mineral and an increase in seed yield and quality, compared to a single application (Lopez-Bellido et al., 2012). Tedone et al. (2014) found that N application efficiency and N recovery efficiency in wheat crops increased when N fertiliser was applied at the stem elongation phase, whereas high amounts of N at sowing time and tillering, resulted in poor efficiency.

Therefore, it is important to develop strategies to enhance efficiency in uptake and utilisation of mineral nutrients by crops. Although N fertilisation has been effectively used in agricultural ecosystems' management, the effect of split N sources on oil crop production is unclear. In order to achieve the maximum potential of the safflower yield quantity and quality, this study was conducted to determine the combined effects of N sources and splitting N fertilisation on growth, yield and seed quality of safflower yield, and changes in N use efficiency.

Table 1. Some monthly weather parameters during the 30-year period from 1986-2016 (the years 2015 and 2016 have been presented separately).

| Months   | Average temperature (°C) | Precipitation (mm) | Average relative humidity (%) |
|----------|--------------------------|--------------------|-------------------------------|
| 2015     | 2016                     | 30-Year            | 2015 | 2016 | 30-Year | 2015 | 2016 | 30-Year |
| April    | 13.90                    | 10.20              | 11.23 | 39.50 | 33.50   | 45.82 | 43.10 | 43.16   | 51.85   |
| May      | 17.60                    | 17.30              | 16.15 | 10.00 | 0.50    | 11.70 | 34.56 | 33.81   | 48.41   |
| June     | 23.00                    | 20.30              | 20.49 | 0.00  | 0.00    | 0.76  | 24.65 | 27.29   | 39.47   |
| July     | 26.00                    | 25.29              | 25.43 | 0.00  | 0.00    | 0.30  | 24.48 | 25.31   | 37.49   |
| August   | 24.00                    | 23.04              | 24.23 | 0.00  | 0.00    | 0.27  | 26.37 | 27.05   | 37.96   |
| Average/Total | 20.90 | 19.62              | 19.33 | 9.90  | 6.80    | 11.77 | 30.63 | 31.32   | 43.04   |

(NUE) = \frac{\text{Seed yield}}{\text{Total N uptake}}

(NUPE) = \frac{\text{Total N uptake}}{\text{Pure N requirement of crop}}

(HI) = \frac{\text{Seed yield}}{\text{Biological yield}}
were estimated according to Rathke et al. (2006). Uptake efficiency is the ability of the plant to remove N from the soil as nitrate and ammonium ions, while the utilisation efficiency is the ability to use N to produce seed yield.

**Statistical analysis**

Differences between means were tested using SAS 9.1 software (SAS Institute, 2003). Statistical tests included one-way ANOVA (general linear model) followed by least significant difference (LSD) test at 5% probability level (Petersen, 1994), assuming a normal distribution of the dependent variable data and homogeneity of variances. The effect of year and interaction between year and all treatments were not significant, so the combined data were reported.

**Results**

**Growth and yield response**

Safflower growth and yield were significantly influenced by split N fertiliser sources (Table 2). Results showed that the highest plant height (121 cm) and total dry matter (5140.93 kg ha–1) were obtained by AN fertiliser and split pattern of 1/3,2/3,0. Applying AN fertiliser and split pattern of 1/2,1/2,0 increased stem and leaf dry matter approximately 18 and 12%, respectively compared to U fertiliser in a similar split pattern. Likewise, the highest capitulums number (101 per m2), seeds number (88), 1000-seed weight (39.25 g) (Table 3), seed yield (3303.52 kg ha–1), protein yield (694.95 kg ha–1), biological yield (8443.60 kg ha–1), and harvest index (39.14%) were obtained by AN fertiliser and split pattern of 1/3,2/3,0, while the highest oil yield (753.09 kg ha–1) was obtained when U fertiliser was applied in a split pattern of 1/2,1/2,0 (Table 4). Applying AN fertiliser and split pattern of 1/3,2/3,0 increased seed and protein yields (approximately 22 and 63%, respectively) compared to U fertiliser in a similar split pattern. On the contrary, applying U fertiliser in a split pattern of 1/2,1/2,0 increased oil yield (approximately 5%) compared to AN fertiliser in a similar split pattern. On the contrary, applying AS and SCU fertilisers increased safflower growth and yield to a lower extent compared to other N sources (Tables 3 and 4).

**Nitrogen use efficiency changes**

The effects of N sources and split patterns were significant on safflower N use efficiency (Table 2). AN fertiliser and split patterns of 1/3,2/3,0 and 1/2,1/2,0 maximised safflower NUE (0.78 kg kg–1), however the highest safflower NUE (43.70 kg kg–1) was obtained when AN fertiliser was applied in a split pattern of 1/2,1/2,0. In contrast, applying U fertiliser increased both of them N efficiencies, when split pattern of 1/2,1/2,0 was applied. The lowest NUE (0.40 kg kg–1) and NUE (12.31 kg kg–1) was observed.

**Table 2. The summary of the source of variation and the mean square of plant height, total, stem and leaf dry matters, capitulums number, seeds number, 1000-seed weight, seed, oil, protein and biological yields, harvest index, NUEp, and NUE as affected by split N fertilisers application.**

| Source of variation | df | Plant height | Total dry matter | Stem dry matter | Leaf dry matter | Capitulums number | Seeds number | 1000-seed weight |
|---------------------|----|--------------|------------------|-----------------|-----------------|-------------------|--------------|-----------------|
| Year                | 1  | 176.04**     | 93,543.86**      | 47,530.27**     | 7714.90**       | 13.50**           | 68.34**      | 22.98**         |
| Error (a)           | 4  | 107.71**     | 50,675.80**      | 27,490.88**     | 3200.01**       | 3.33**            | 121.34**     | 9.63**          |
| Nitrogen source (N) | 3  | 828.25**     | 5,876,466.65**   | 4,006,696.02**  | 185,611.31**    | 175,34**          | 840.28**     | 531.67**        |
| Year * N            | 3  | 126.56**     | 48,661.31**      | 25,458.35**     | 730.41**        | 31.80**           | 26.17**      | 4.95**          |
| Error (b)           | 12 | 22.68**      | 313,362.60**     | 16,818.60**     | 224.66**        | 37.93**           | 175.94**     | 15.82**         |
| Nitrogen split (S)  | 3  | 665.36**     | 802,940.88**     | 433,629.22**    | 56,428.16**     | 324.06**          | 2434.64**    | 73.41**         |
| N * S               | 9  | 221.13**     | 279,727.07**     | 151,058.04**    | 1972.97**       | 210.41**          | 261.02**     | 24.25**         |
| Year * S            | 3  | 2.79**       | 1002.38**        | 536.38**        | 72.30**         | 2.41**            | 8.81**       | 2.91**          |
| Year * N * S        | 9  | 18.02**      | 336.83**         | 175.93**        | 25.98**         | 5.09**            | 20.90**      | 3.66**          |
| Error (c)           | 48 | 54.57        | 58,399.23        | 39,858.85       | 4314.73         | 59.82             | 105.18       | 11.04           |

| Source of variation | df | Seed yield | Oil yield | Protein yield | Biological yield | Harvest index | NUEp | NUE |
|---------------------|----|------------|-----------|---------------|------------------|---------------|------|-----|
| Year                | 1  | 129.49**   | 17,943.40**| 6352.06**     | 4449.12**        | 24.65**       | 0.01**| 2.71**|
| Error (a)           | 4  | 969.54**   | 3337.43**  | 156.02**      | 49,700.00**      | 9.69**        | 0.008**| 21.18**|
| Nitrogen source (N) | 3  | 18,053,031.56** | 1,339,872.29**| 606,560.46** | 44,455,591.0** | 1817.71**     | 0.43**| 1874.95**|
| Year * N            | 3  | 12,888.68**| 4312.89**  | 474.63**      | 97,302.3**       | 2.44**        | 0.0004**| 11.50**|
| Error (b)           | 12 | 284,342.42**| 39,851.10** | 8476.76**     | 326,065.8**      | 39.78**       | 0.005**| 96.19**|
| Nitrogen split (S)  | 3  | 3,741,045.90**| 139,661.31** | 196,570.70**  | 7,382,675.9**    | 370.91**      | 0.11**| 526.31**|
| N * S               | 9  | 708,925.42**| 35,517.14** | 57,518.75**   | 1,618,282.8**    | 44.43**       | 0.06**| 161.72**|
| Year * S            | 3  | 3673.19**   | 588.51**   | 434.90**      | 584.05**         | 0.46**        | 0.002**| 6.39**|
| Year * N * S        | 9  | 20,200.43** | 2236.57**  | 1099.99**     | 18,866.3**       | 2.79**        | 0.002**| 8.62**|
| Error (c)           | 48 | 173,045.92  | 14,342.54  | 3980.01       | 274,979.1        | 19.60         | 0.005  | 44.33 |

*Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ns not significant.
when AS fertiliser was applied in split patterns of 1/2,1/2,0 and 1/3,2/3,0, respectively. Additionally, applying SCU fertiliser and split patterns of 1/3,1/3,1/3 and 1/3,2/3,0 enhanced NUpE up to 0.50 kg kg⁻¹, while NUE increased up to 21 kg kg⁻¹ when SCU fertiliser was applied in a split pattern of 1/3,2/3,0 (Table 4).

### Yield, yield components, and nitrogen efficiency correlation

Correlation results showed that increasing yield components tended to enhance safflower yield and seed yield changes are closely related to the capitulums number (0.86**), seeds number (0.80**), total plant height (0.82**), and 1000-seed weight (0.80**).

### Table 3. Effects of split N fertilisers application on plant height, total, stem, and leaf dry matters, capitulums number, seeds number, and 1000-seed weight.

| Nitrogen sources | Split pattern | Plant height (cm) | Total dry matter (kg ha⁻¹) | Stem dry matter (kg ha⁻¹) | Leaf dry matter (kg ha⁻¹) | Capitulums number per m² | Seeds number per capitulum | 1000-seed weight (g) |
|------------------|---------------|-------------------|-----------------------------|---------------------------|--------------------------|---------------------------|----------------------------|--------------------------|
| Ammonium sulfate (AS) | 1/3 1/3 1/3 | 3421.10±104.62 | 1046.37±30.45 | 289.62±8.78 | 65.35±2.41 | 3.27±0.08 | 80.00±7.50 | 27.62±4.35 |
| Urea (U) | 1/3 1/3 1/3 | 3223.54±96.32 | 96.23±2.78 | 26.52±0.83 | 5.76±0.31 | 2.90±0.24 | 73.66±3.10 | 25.36±3.84 |

### Table 4. Effects of split N fertilisers application on seed, oil, protein, and biological yields, harvest index, NUpE, and NUtE.

| Nitrogen sources | Split pattern | Seed yield (kg ha⁻¹) | Oil yield (kg ha⁻¹) | Protein yield (kg ha⁻¹) | Biological yield (kg ha⁻¹) | Harvest index (%) | NUpE (kg ha⁻¹) | NUtE (kg ha⁻¹) |
|------------------|---------------|---------------------|---------------------|------------------------|--------------------------|-----------------|---------------|---------------|
| Ammonium sulfate (AS) | 1/4 1/4 1/4 | 222.45±6.54 | 414.83±12.65 | 96.23±2.78 | 26.52±0.83 | 5.76±0.31 | 2.90±0.24 | 73.66±3.10 | 25.36±3.84 |
| Urea (U) | 1/4 1/4 1/4 | 271.15±7.81 | 529.64±20.91 | 125.89±3.45 | 31.75±0.95 | 1.67±0.13 | 25.00±1.59 | 10.00±1.25 | 2.35±0.34 |

LSD (5%) = Standard deviation with indication of significant differences.
that the highest yield response of safflower was obtained by AN fertiliser sources (especially AN fertiliser) in over production of protein yield and a reduction in oil content is consistent with the findings of Tuncturk and Yildirim (2004) and Farahbakhsh et al. (2006) that increasing N uptake by plants resulted in production of amino acid and other nitrogenous compounds during peptide bands. When N sources (especially AN fertiliser) in over production of protein yield and a reduction in oil content is consistent with the findings of Tuncturk and Yildirim (2004) and Farahbakhsh et al. (2006) that increasing N uptake by plants resulted in production of amino acid and other nitrogenous compounds during peptide bands. When amino acid and other compounds increased, the percentage of fatty acids decreased.

Discussion

It seems that applying AN and U fertiliser sources are able to stimulate safflower growth by means of an enlarged leaf canopy and a greater rate of leaf expansion, which increased light interception and enhances photosynthesis (Ozturk, 2010). Additionally, a high availability of N in the form of nitrate (NO₃⁻) and ammonia (NH₄⁺) increased cations uptake such as potassium (K), calcium (Ca) and magnesium (Mg) (Rathke et al., 2005; Narits, 2010). It seems that increasing safflower growth plays a vital role in enhancing crop yield (Ozturk, 2010). These results are in agreement with those Osman et al. (2014), who reported that in oilseed rape, yield is closely related to the capitulums number and the physiological restrictions to capitulum formation are related to poor crop growth and limited leaf expansion. Bagawan and Ravikumar (2001) found a positive correlation between capitulums number and safflower seed yield. Tuncturk and Yildirim (2004) observed that the highest yield response of safflower was obtained by AN and U fertilisers application.

Furthermore, we found that the highest growth and yield responses obtained when safflower was treated by split pattern of 1/3 at sowing time, 1/3 at stem elongation. This high crop growth and performance are the result of high application rate of N in early growing season as it helps plants to avoid competing for N sources (especially AN fertiliser) in over production of protein yield and a reduction in oil content is consistent with the findings of Tuncturk and Yildirim (2004) and Farahbakhsh et al. (2006) that increasing N uptake by plants resulted in production of amino acid and other nitrogenous compounds during peptide bands. When amino acid and other compounds increased, the percentage of fatty acids decreased.

Conclusions

To enhance the productivity of safflower, different sources and application timing of N adapted to site conditions are remarkable growing strategies, which could help to realise the maximum potential of safflower quality and quantity. Our results showed that timing of N fertiliser application has the potential to change safflower agronomical parameters, alter productivity and quality of safflower yield, and influence safflower N uptake and utilisation efficiency. Overall, our findings suggested that applying AN fertiliser in a split pattern of 1/3,2/3,0 and /or U fertiliser in a split pattern of 1/3,2/3,0 not only improves safflower growth, yield and seed quality, but also enhances the N use efficiency of safflower.

Table 5. Correlation between yield, yield components, and N efficiency of safflower.

| Seed yield | No. Capitulums | No. Seeds | 1000-seed weight | Oil yield | Protein yield | Harvest index | NUpE | NUtE |
|------------|----------------|-----------|------------------|-----------|--------------|---------------|------|------|
| 1.00       | 0.86**         | 1.00      |                  |           |              |               |      |      |
| No. Capitulums | 0.95**         | 0.72**    | 1.00             |           |              |               |      |      |
| No. Seeds   | 0.92**         | 0.86**    | 0.82**           | 1.00      |              |               |      |      |
| 1000-seed weight | 0.64*          | 0.48*     | 0.54*            | 0.44*     | 1.00         |               |      |      |
| Oil yield   | 0.86**         | 0.74**    | 0.79**           | 0.69*     | –0.65*       | 1.00          |      |      |
| Protein yield | 0.93**         | 0.84**    | 0.97**           | 0.91**    | 0.58*        | 0.81**        | 1.00 |      |
| Harvest index | 0.89**         | 0.90**    | 0.88*            | 0.85**    | –0.55*       | 0.79**        | 0.92**| 1.00 |
| NUpE        | 0.98**         | 0.86**    | 0.92**           | 0.90**    | –0.65*       | 0.82**        | 0.96**| 0.81**|
| NUtE        |                |           |                  |           |              |               |      |      |

*Significant at the 0.05 probability level; **Significant at the 0.01 probability level.
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