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Impact of Transplanting Date and Animal Manure Fertilizer on Yield and Yield Components of Yarrow (*Achillea millefolium* L.)

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ABSTRACT

Finding the appropriate fertilizer level and the best transplanting dates of yarrow as one of the most important medicinal plants can help farmers achieve better cultivation results. Therefore, this study aimed to determine yarrow optimal transplanting dates and animal manure level and to investigate the effect of these two factors on yield and seed yield components. A split plate experiment was carried out based on a randomized complete block design in three replications during 2021-2022. The experimental treatments consisted of animal manure fertilizer as the main factor (0, 10, 20, 30 ton ha^{-1}) and transplanting dates (April 4, April 24, May 14) as the sub-factor. The studied traits included essential oil content, yield, and seed yield components. The results showed that the application of animal manure has a positive effect on the numbers of inflorescences (0.77 m^{-2}), florets (216.3 m^{-2}), flowering stems (6.3 m^{-2}), seeds per inflorescence (78840 m^{-2}), and thousand seed weight (3.6 g). April 4 was the best transplanting date in terms of the evaluated traits. The highest essential oil yield (2.2 g m^{-2}) was observed for 30 ton ha^{-1} animal manure treatment on April 4. In general, based on the results of this study, applying 30 ton ha^{-1} of animal manure and choosing April 4 as the transplanting date can promote essential oil content, yield, and seed yield components. Therefore, it is possible to consider the use of animal manure as an organic fertilizer to improve the yield of medicinal plants, including yarrow.

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1. Introduction

Yarrow (*Achillea millefolium* L.) is a perennial herbaceous plant belonging to the Asteraceae family (Radušienė *et al*., 2023). Yarrow does not require specific conditions for growth and can grow in any climate. It is a diurnal plant, and the most suitable temperature range for its growth is between 18-26°C (Salehi Sardoei, 2022a). Therefore, it grows better in warm and sunny regions, producing more flowers (Tkhaganov *et al*., 2022). Besides being renowned as a medicinal plant worldwide, yarrow is also used as an ornamental plant in green spaces (Pornaro *et al*., 2023).

Animal manure contains a wide range of nutrients compared to chemical fertilizers, as it contains many of the nutrients present in plants that are consumed by animals (Azizi *et al*., 2019). Additionally, animal manure plays an effective role in nutrient cycling and organic matter turnover in agroecosystems, contributing to increased productivity and improved ecosystem stability. Some of the side effects of chemical fertilizers include water pollution, reduced biodiversity, decreased soil fertility, and a decline in the quality of agricultural products. Therefore, using animal manure instead of chemical fertilizers can play a crucial role in the health of agroecosystems and the enhancement of crop quality (Jalilian *et al*., 2021).

Studying the effect of animal manure on chamomile (*Matricaria chamomilla* L.) showed that promoting the application of animal manure can increase chamomile yield and essential oil content (Ahmadian *et al*., 2010). Rezvani Moghaddam et al. (2014) stated that animal manure has a significant effect on the yield and yield components of safflower (*Carthamus tinctorius* L.), improving soil structure and nutrient retention

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capacity. Increase in the number of seeds per plant, weight of 1000 seeds, seed yield, dry weight of aerial parts and percentage of essential oil due to increasing the level of manure in cumin (*Cuminum cyminum* L.) (Khoramdel *et al*., 2017), sesame (*Sesamum indicum*) (Jalilian *et al*., 2021), basil (*Ocimum basilicum*) (Mohammadzadeh Tootoonchi, 2020), fennel (*Foeniculum vulgare* Mill.) (Nada *et al*., 2022) and peas (*Cicer arietinum*) (Khan *et al*., 2022) have also been reported.

Decision on the appropriate sowing date and transplanting date for a crop is of great importance and one of the key factors for achieving maximum yield in plants (Dorri *et al*., 2015). Moosavi et al. (2020) stated that one of the major factors for achieving high yields in medicinal plants is selecting an appropriate sowing date and optimal plant nutrition. The sowing date is the first important point in crop production management decisions, it seems necessary for the proper establishment of the plant in the field due to its effect on seed germination power. This is especially important in regions with environmental limitations such as early or late frosts at the beginning and end of the growing season, or extreme heat in mid-summer (Cann *et al*., 2020). Understanding the appropriate sowing date for each crop provides the condition for better use of timing, light, temperature, rainfall, and other environmental factors. In early-sowing date conditions, low soil temperatures and frostbite injuries can result in poor plant establishment in spring (Hashemian *et al*., 2022). In Plants ajwain (*Ammi copticum*) (Boroumand Rezazadeh *et al*., 2010), milk thistle (*Silybum mavianum*) (Dorri *et al*., 2015), black mustard (*Brassica nigara*) (Asgarnezhad *et al*., 2015), ziziphora (*Ziziphora clinopodioides*) (Amin Gafori *et al*., 2015), common purslane (*Portulaca oleracea*) (Javadi *et al*., 2017) and viper's-bugloss (*Echium vulgare*) (Hasanvand *et al*., 2018), planting at the beginning of the season increased its growth traits, yield and yield components, as well as essential oils compared to other cultivation dates. A review of the current situation of medicinal plants in the country shows that we have not yet achieved the primary role of medicinal plants in the economy despite paying particular attention to the issue of medicinal plants and trying to develop cultivation and processing and placing medicinal plants as well as their products in people's food basket (Salehi Sardoei, 2022b). Therefore, according to the studies done this study aimed to determine the optimal level of animal manure and transplanting date for the medicinal plant yarrow. It assessed the effects of different transplanting dates and fertilizer levels on the essential oil content, yield, and yield components, as well.

2. Materials and methods

This study was conducted in Organic Agriculture Research and Education Farm, Campus Agriculture and Natural Resources, Razi University, Kermanshah, Iran during 2021-2022. A split plots experiment was carried out based on a randomized complete block design with three replications. The experimental treatments consisted of four levels of animal manure as the main factor (0, 10, 20, 30 ton ha^{-1}) and three transplanting dates (April 4, April 24, May 14) as the sub-factor (Salehi Shanjani *et al*., 2019).

Land preparation operations including semi-deep plowing, disking, leveling and terracing were carried out in March 2021. Approximately two weeks before sowing, animal manure was added to the soil based on the desired treatments and mixed with the soil to a depth of 30 cm. The characteristics of the applied animal manure are presented in Table 1. Before conducting the experiment, samples were taken from the field soil up to a depth of 30 cm to analyze the soil and determine its physical and chemical characteristics. The test results are presented in Table 2.

Table 1. Characteristics of animal manure used in the experimental farm.

K (mg kg^{-1})	P (mg kg^{-1})	N (%)	EC (ds m^{-1})	pH
17500	8600	0.71	0.54	7.76

Table 2. Characteristics of experiment soil at 0-30 cm depth.

K (mg kg^{-1})	P (mg Kg^{-1})	N (%)	EC (ds m^{-1})	pH	Texture
280	13	0.11	0.87	7.7	Silt loam

Each sub-plot consisted of 4 rows. The spacing between sowing rows was set at 50 cm, and the final plant density in the field was 10 plants m^{-2}. The required seeds were obtained from Pakan Bazr Company, Isfahan, Iran, with suitable germination power. The required seeding rate was 2.5 to 3 kg ha^{-1}. The seeds were planted in the greenhouse, and the seedlings were transplanted to the main field at the 4 to 6-leaf stage. The transplanting was done manually at a suitable depth of 5 to 8 cm, considering the transplanting dates. After transplanting, irrigation was

carried out using pressurized sprinkler irrigation, with intervals of 7 to 10 days. Hand weeding was performed during the growing period to control weeds.

For sampling, after the physiological maturity of the plants, the bushes were harvested from an area of one m^{-2}, including marginal effects, in order to measure traits such as the number of inflorescences per plant, the number of seeds per inflorescence, the number of florets, the number of flowering stems, and the thousand seed weight for seed yield assessment (Dorri *et al*., 2015).

In order to extract the essential oil, flowering branches were taken from one m^{-2} and after shade drying, 30 g of each treatment were ground. The essential oil was extracted using the water distillation method and a Clevenger apparatus, and the oil yield was measured (Farhodi and Mehrnia, 2015). The essential oil percentage yield was calculated using the following formula (Taheri Bookani and Najafzadeh, 2019):

$$\text{Essential oil percentage yield} = (\text{Essential oil yield} / \text{Plant dry weight}) \times 100 \quad (1)$$

Finally, data analysis was carried out using the SAS software version 9.1. Duncan's multiple range test was used to compare the means of the data. $P \leq 0.05$ was considered as statistically significant.

3. Results and discussion

3.1. Seed yield

Variance analysis results showed that animal manure level, transplanting date, and their interaction have a significant effect (1% probability level) on seed yield (Table 3). The highest seed yield was observed in the treatment with 30 ton ha^{-1} of animal manure and the transplanting date of April 4, while the lowest yield was recorded in the treatments with 10 and 20 ton ha^{-1} of animal manure and the transplanting date of May 14 (Table 4). Increasing the animal manure level increased seed yield by 11.76%. Increasing animal manure levels and choosing a suitable transplanting date (early in the season) improved plant yield and, consequently, higher seed yield, due to their positive effects on the absorption of water and nutrients, such as nitrogen, phosphorus, and potassium (Mohammadzadeh Totounchi, 2020). Animal manure level has shown a positive effect on safflower yield (Saeedi *et al*., 2022). Similarly, increasing animal manure levels resulted in higher seed yield in sesame and rice plants (Iqbal *et al*., 2020; Jalilian *et al*., 2021). By providing macro and micronutrients and also creating suitable physical conditions for micro-organisms in the soil, the manure will increase their activity and increase the yield. The application of animal manure has also promoted the yield and yield components in crops such as melon (Aryafar *et al*., 2022), sunflower (*Helianthus annuus*) (Aryafar *et al*., 2022), and tea (*Camellia sinensis*) (Ye *et al*., 2023). Research has shown that the favorable effects of animal manure are due to the change in physical, chemical and microbial and biological conditions of the cultivation environment, pH regulation, significant increase in the water storage capacity in the cultivation environment, etc. The delay in the sowing date (November 21) resulted in a decrease in the seed yield of wheat (Khosravi *et al*., 2023; Moghaddam *et al*., 2023). In another study, the appropriate sowing date, along with optimal conditions for wheat cultivars, provided favorable physiological conditions for the plants, including photosynthesis, ultimately leading to increased seed yield (Khosravi *et al*., 2022). For lentil (*Lens culinaris* Medikus.), the highest yield was obtained by sowing early in the season (Maphosa *et al*., 2023).

3.2. The number of inflorescence

The analysis of variance revealed that the transplanting date, animal manure, and their interaction had a significant effect in the 1% probability level on the number of inflorescences (Table 3). The highest number of inflorescence was observed in the treatment with 30 ton ha^{-1} of animal manure and the transplanting date of April 4, while the lowest number was recorded in the absence of animal manure and the transplanting date of May 14 (Table 4). The use of animal manure and an appropriate transplanting date increased the number of inflorescences by 36.4%. The increase in microbial activity results from the increase in soil organic matter (due to the use of animal manure) and the decrease in soil pH. The metabolites produced by microbial activity form unstable complexes with nutrients, preventing nutrient fixation in the soil and making them available to plants. As a result, the plants' nutrient uptake capacity improves, leading to increased photosynthetic rate and dry matter production, and consequently, promoting vegetative growth, plant

height, number of branches per plant, and the number of inflorescences per plant (Jalilian *et al*., 2021). Another study reported that the application of animal manure increases the number of inflorescences in chamomile plants (Abdou *et al*., 2015).

3.3. The number of seeds per inflorescence

The results of the variance analysis showed that animal manure, transplanting date, and their interaction have a significant effect (1% probability level) on the number of seeds per inflorescence (Table 3). The highest number of seeds per inflorescence was observed in the treatment with 30 ton ha^{-1} of animal manure and the transplanting date of April 4, while the lowest number was recorded in the control treatment without animal manure and the sowing date of 24 May 14, showing no significant difference with 10 ton ha^{-1} of animal manure treatment at the transplanting date (Table 4). Suitable animal manure and early-season transplanting date resulted in a 21.5% increase in the number of seeds per inflorescence. The number of seeds determines the plant's capacity, and a larger reservoir allows for assimilation, leading to an increase in the number of seeds. Sufficient nutrients and water are required for seed production, and their deficiency may result in incomplete seed formation or inadequate filling. The use of animal manure provides the necessary nutrients, and increasing its application enhances the number of seeds per inflorescence and seed filling (Jalilian *et al*., 2021). The application of animal manure and increasing its amount also promoted the number of seeds per inflorescence in chamomile plants (Abdou *et al*., 2015).

3.4. Thousand seed weight

The results of the variance analysis showed that animal manure, transplanting date, and their interaction have a significant effect in 1% probability level on the thousand seed weight (Table 3). The highest thousand seed weight was observed in the treatment with 30 ton ha^{-1} of animal manure and the transplanting date of April 4, while the lowest number was recorded in the absence of animal manure and the transplanting date of May 14 (Table 4). This amount of animal manure and an appropriate sowing date resulted in a 9.8% increase in the thousand seed weight. It seems that with the increase in animal manure level, the amount of soil elements such as potassium increases, as well. Moreover, the increase in photosynthesis rate and carbon dioxide absorption is associated with the improvement in the transfer of photosynthesis compounds, the facilitation of amino acids transfer, and the increase in thousand seed weight (Jalilian *et al*., 2018). In another study, it was found that the use of animal manure increases thousand seed weight in yarrow (Ferraz *et al*., 2014). The application of animal manure increased the thousand seed weight and grain yield as compared to the control for wheat (Moradi *et al*., 2016).

3.5. The number of florets

The results of the variance analysis showed that animal manure, transplanting date, and their interaction have a significant effect (1% probability level) on the number of florets (Table 3). While the lowest number of florets was recorded in the absence of animal manure treatment and the transplanting date of May 14 the highest number of florets was observed in the treatment with 30 ton ha^{-1} of animal manure and the transplanting date of April 4 (Table 4). Application of animal manure and an appropriate sowing date promoted the number of florets by 37.3%. It seems that suitable animal manure and an extended growth period contribute to an increase in the number of flowering stems and, consequently, the number of florets in this medicinal plant. The application of animal manure has shown a positive effect on the number of flowers In saffron (*Crocus sativus* L.), accompanied by an increase in plant yield (Aalizadeh *et al*., 2021).

3.6. The number of flowering stems

The results of the variance analysis showed that animal manure, transplanting date, (1% probability level) and their interaction (5% probability level) have a significant impact on the number of flowering stems (Table 3). The lowest number of flowering stems was observed in the absence of animal manure treatment and the transplanting date of May 14, whereas the highest number was observed in the treatment with 30 ton ha^{-1} of animal manure and a transplanting date of April 4 showing no significant with 20 ton ha^{-1} of animal manure per hectare treatment at the same transplanting date. The application of animal manure and a suitable transplanting date at the beginning of the season resulted in a 22.4% increase in the number of flowering stems in yarrow plants.

Table 3. Analysis of variance (Mean of squared) of the effect of animal manure and transplanting date on the yield and yield components of Yarrow.

Source of variation	df	Mean of squared					
		Seed yield	Number of inflorescences	Number of seeds per inflorescence	Thousand seed weight	Number of florets	Number of flowering stems
Fertilizer (F)	3	174246.35**	1560.990**	17295008**	6.352**	9407.92**	15.841**
Block	2	288.58^{ns}	4.527^{ns}	20846.58^{ns}	0.172^{ns}	230.58^{ns}	2.827**
Error a	6	1947.28	7.82	27525.81	0.022	95.62	0.110
Transplanting date (T)	2	159386.37**	1270.027**	183442.58**	3.027**	10987**	8.540**
F×T	6	669452.93**	46.435**	242044.47**	0.442**	684.59**	1.230*
Error b	16	1543.53	9.04	13464.4	0.011	117.73	0.365
CV (%)	-	12.98	7.31	9.51	8.52	9.32	17.05

* and ** are significant at 1% and 5%, respectively and ns is non-significant differences.

Table 4. Mean comparison of manure and sowing dates on the yield and yield components of yarrow.

Fertilizer level (ton h^{-1})	Transplanting date (month)	Seed yield (g m^{-2})	Number of inflorescences (m^{-2})	Number of seeds per inflorescence (m^{-2})	Thousand seed weight (g)	Number of florets (m^{-2})	Number of flowering stems (plant)
0	April 4	0.4^{d}	39.66^{f}	2256.7^{f}	0.88^{ef}	116.33^{cde}	2.12^{fg}
	April 24	0.27^{d}	30^{g}	1959^{hg}	0.58^{g}	108.32^{e}	2.27^{efg}
	May 14	0.20^{d}	28^{g}	1694^{i}	0.35^{h}	80.66^{g}	1.41^{g}
10	April 4	0.5^{d}	57.32^{cd}	3661.7^{d}	1.07^{e}	135^{c}	3.22^{def}
	April 24	0.4^{d}	49.66^{e}	2840^{e}	0.92^{ef}	117.33^{cde}	3.43^{def}
	May 14	0.34^{d}	39.66^{f}	1753.3^{hi}	0.78^{fg}	86.33^{fg}	2.40^{efg}
20	April 4	1.1^{b}	60^{bc}	4848.7^{b}	2.35^{b}	176.33^{b}	5.8^{ab}
	April 24	0.81^{d}	42^{f}	2799.7^{e}	1.73^{d}	113.33^{de}	3.85^{cd}
	May 14	0.47^{d}	31.66^{g}	2093.3^{fg}	1.03^{e}	104.66^{ef}	3.33^{def}
30	April 4	1.7^{a}	77^{a}	7884^{a}	3.56^{a}	216.33^{a}	6.29^{a}
	April 24	1.04^{a}	63^{b}	4308.7^{c}	2.13^{c}	179^{b}	4.81^{bc}
	May 14	0.8^{d}	53.33^{cd}	3472^{d}	1.76^{d}	103.33^{dc}	3.56^{cde}

According to Duncan's test, the means with similar letters in each column are not significantly different at the probability level of 5%.

3.7. Essential oil content

The results of the variance analysis showed that animal manure, transplanting date, and their interaction have a significant impact on the essential oil content (Table 5). The lowest essential oil content was recorded in the treatment with 30 ton ha^{-1} of animal manure and a transplanting date of April 4, whereas the highest essential oil content was observed in the absence of animal manure treatment and the transplanting date of May 14. The application of animal manure led to a 44.3% reduction in the essential oil content compared to the control treatment (Table 6). In another study reported that the use of animal manure in the cultivation of yarrow and an increase in its amount diminishes the essential oil content of this medicinal plant (Abdou *et al*., 2015).

3.8. Essential oil yield

The results of the variance analysis showed that animal manure, transplanting date, and their interaction have a significant impact on the essential oil yield (Table 5). The lowest essential oil yield was observed in the absence of animal manure treatment and the transplanting date of May 14, whereas the highest essential oil yield was observed in the treatment with 30 ton ha^{-1} of animal manure and the transplanting date of April 4 (Table 6). The increase in animal manure resulted in a 33.6% increase in the essential oil yield of yarrow plants. The application of animal manure and the elongation of the growth period had a positive effect on plant biochemical processes, leading to an increase in the essential oil yield. The correct use of nutrients during the growth stages of medicinal plants not only plays a fundamental role in increasing their yield but also affects the quantity and quality of the active ingredient of the produced product (Fatahi Siahkamari *et al*., 2020).In a study on the effect of animal manure on yarrow plants, it was indicated that animal manure has a significant impact on yarrow essential oil content, and an increase in animal manure promotes the essential oil yield of this plant (Ferraz *et al*., 2014). The application of animal manure in comparison to chemical fertilizer has improved the quantitative and qualitative characteristics of essential oil in Peppermint (*Mentha* × *piperita*) plants (Asadi *et al*., 2023).

Table 5. Analysis of variance (Mean of squared) of the effect of animal manure and sowing date on the essential oil percent and essential oil yield of Yarrow.

Source of variation	df	Mean of squared	
		Essential oil percent	Essential oil yield
Fertilizer (F)	3	0.011^{**}	0.699^{**}
Block	2	0.027^{ns}	0.175^{ns}
Error a	6	0.07	0.0035
Transplanting date (T)	2	0.05^{**}	0.495^{**}
F×T	6	0.025^{**}	0.016^{**}
Error b	16	0.03	0.010
CV (%)	-	11.09	8.77

** and ns are significant at 1% and non-significant differences, respectively.

Table 6. Mean comparison of manure and sowing dates on the essential oil percent and essential oil yield of Yarrow.

Fertilizer level (ton h^{-1})	Transplanting date (month)	Essential oil percent	Essential oil yield (g m^{-2})
0	April 4	0.054^{b}	1.33^{d}
	April 24	0.048^{c}	0.99^{fg}
	May 14	0.07^{a}	0.73^{h}
10	April 4	0.034^{e}	1.57^{c}
	April 24	0.037^{d}	1.18^{de}
	May 14	0.048^{c}	0.93^{g}
20	April 4	0.029^{fgh}	1.79^{b}
	April 24	0.030^{fg}	1.32^{d}
	May 14	0.031^{f}	1.13^{ef}
30	April 4	0.021^{k}	2.17^{a}
	April 24	0.025^{j}	1.56^{c}
	May 14	0.028^{ghi}	1.28^{de}

According to Duncan's test, the means with similar letters in each column are not significantly different at the probability level of 5%.

4. Conclusion

In conclusion, animal manures can serve as suitable alternatives to chemical fertilizers in ecological systems with low pharmaceutical input to improve soil fertility and produce healthy medicinal products due to their impact on soil structure enhancement, root development, nutrient availability for plants, soil temperature adjustment, microbial populations promotion, and nutrient uptake by plants. Based on the results of this study, the highest yield for all the assessed traits was observed in the treatment with 30 ton ha^{-1} of animal manure and the transplanting date of April 4. Suitable transplanting dates also resulted in the development of plants with proper vegetative growth and consequently better yield.

Conflict of Interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No human or animals were used in the present research.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

Informed Consent

The authors declare not to use any patients in this research.

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