



ISSN: 2455-9377

# Germination of alfalfa (*Medicago sativa* L.) seeds in Desert soil after fertilization with raw and digested animal manure

Jamal Abubaker<sup>1,4\*</sup>, Abdelsalam Abobaker<sup>2</sup>, Mohamed Essalem<sup>3,4</sup>,  
Najia Z. Bertata<sup>1</sup>

<sup>1</sup>Department of Microbiology, Faculty of Science, Sebha University, P. O. Box 19631, Sebha, Libya, <sup>2</sup>Department of Horticulture, Faculty of Agriculture, Sebha University, P. O. Box 18758, Sebha, Libya, <sup>3</sup>Department of Crops, Faculty of Agriculture, Sebha University, P. O. Box 18758, Sebha, Libya, <sup>4</sup>Research and Consulting Center, Sebha University, P. O. Box 18758, Sebha, Libya

## ABSTRACT

Animal manure has been approved as an appropriate soil fertilizer. However, the effect on seed germination, growth, and yield of crops still needs more evaluation across various agricultural ecosystems. A germination experiment was conducted to evaluate the performance of raw and digested sheep and poultry manure on alfalfa (*Medicago sativa* L.) seed germination in desert soil. Four sowing dates were evaluated in the experiment, i.e. directly, 10, 20, and 30 days after soil fertilization. The fertilizers were applied at rates corresponding to 50 and 100 kg Tot N ha<sup>-1</sup>. In addition, the response of germination to seed inoculation method was also assessed (Arabic gum as an adhesive solution and sawdust as a seed coating material). The germination was evaluated by determining seed germination time (SGT), time to reach maximum germination (TMG), germination index (GI), and final germination percentage (F<sub>CP</sub>). The results showed that sown seeds directly after fertilization with raw/digested sheep or poultry manure reduced and delayed seed germination. This was confirmed by all germination indices, long SGT, long TMG, low GI values, and a reduction in F<sub>CP</sub>. Moreover, when the seeds were sown 10 days after fertilization, all germination attributes were significantly (p<0.05) improved. Furthermore, the results revealed that the inoculation method used in the study had a positive effect on seed germination. To achieve better germination when using animal manure (raw or digested) as soil fertilizer, it is recommended to sow alfalfa seed 10 days after soil fertilization. Moreover, inoculating the seeds using Arabic gum as an adhesive solution and sawdust as a seed coating enhances germination in fertilized soil.

**KEYWORDS:** Alfalfa seeds germination, Germination index, Poultry manure, Seed germination time, Sheep manure

Received: December 24, 2023  
Revised: May 09, 2024  
Accepted: May 14, 2024  
Published: May 22, 2024

\*Corresponding author:  
Jamal Abubaker  
E-mail: jam.abubaker@  
sebhau.edu.ly

## INTRODUCTION

Plants require essential nutrients like nitrogen, phosphorus, and potassium to maintain growth and yield. A deficiency in these nutrients can impair plant growth and soil productivity. In southern Libya, the predominant soil is desert soil (Saad *et al.*, 2011), which has a sandy texture that is lacking organic matter and essential nutrients for plant growth (Zurqani *et al.*, 2019). All of the above-mentioned features of soils make reclamation and cultivation of this soil difficult. Therefore, to improve the soil's fertility and sustain crop production, fertilizer application is necessary.

Chemical fertilizers are commonly used in agriculture to provide plants with essential nutrients in order to increase crop

yield (Hillel, 2008). While many farmers consider chemical chemical fertilizers as a necessary practice, there are several issues associated with their use. One such issue is the increased production cost (Zheng *et al.*, 2022), environmental pollution (Savci, 2012) and inability to improve soil properties (Hati & Bandyopadhyay, 2011; Jia *et al.*, 2022). Therefore, it is essential to consider alternative techniques that can boost plant growth and productivity without relying on chemical fertilizers.

Many studies have revealed that organic fertilizers and bio-fertilizers, like animal manure and effective microbial strains, can enhance soil fertility and productivity (Ye *et al.*, 2020; Du *et al.*, 2022; Esmaeilian *et al.*, 2022; Zhao *et al.*, 2022). This means that using organic fertilizers can be a great alternative to chemical fertilizers. However, it is important to use organic

Copyright: © The authors. This article is open access and licensed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>) which permits unrestricted, use, distribution and reproduction in any medium, or format for any purpose, even commercially provided the work is properly cited. Attribution — You must give appropriate credit, provide a link to the license, and indicate if changes were made.

fertilizers correctly to ensure that plants get sufficient nutrients to achieve optimum productivity. For example, adding organic fertilizers to the soil several weeks or months before planting crops can be beneficial (Ahmad *et al.*, 2016). This approach will give enough time for soil microorganisms to decompose the organic compounds in the fertilizers and release the nutrients.

It is widely recognized that desert soils typically have low levels of organic matter and soil microbial activity (Buyanovsky *et al.*, 1982; Dwevedi *et al.*, 2017), which can hinder the efficient decomposition of organic fertilizers. One possible solution to address the problem of insufficient nutrient uptake by plants is to consider treating organic fertilizers before application. Composting or anaerobic digestion are two methods that can be used to enhance the availability of nutrients to plants (Lin *et al.*, 2018; Song *et al.*, 2021). By doing so, the plants can have a better chance of receiving the essential nutrients they need to grow.

When organic waste undergoes anaerobic digestion, it produces a residue called digestate that is abundant in nutrients that benefit plant growth (Ezemagu *et al.*, 2021; Song *et al.*, 2021). Studies have shown that digestate generated from anaerobic digestion contains a high percentage of nutrients in a form easily absorbed by plants, making it comparable to chemical fertilizers, which positively impact crop growth and production (Lošák *et al.*, 2011; Ezemagu *et al.*, 2021; Li *et al.*, 2023a).

However, it was proven that the application of animal manure as fertilizer for desert soil, whether digested or undigested, reduces the germination of wheat and alfalfa seeds when sowing is made directly after fertilization (El-Zeadani *et al.*, 2018; Abubaker *et al.*, 2020, 2022a, b). Furthermore, it has been reported that the adverse effect of these fertilizers on seed germination was reduced when wheat seeds were sown after 20 to 30 days from fertilization (El-Zeadani *et al.*, 2018; Abubaker *et al.*, 2020). This study aims to (1) investigate the effect of raw and anaerobically digested sheep and poultry manures on the germination of alfalfa seeds; (2) evaluate the germination at different time intervals between sowing and the application of raw and digested manure; and (3) investigate the effect of fertilization rate on germination.

## MATERIALS AND METHODS

### The Soil

The soil used in the experiment was loamy sand taken from land located in the Libyan Desert, 10 km west of Sabha City (Fezzan region), a southern part of Libya (22°30' N and 30°00' E and between the meridians of 10° E and 18° E). The soil used in the experiment was not cultivated or fertilized before and at sampling, it was totally dry. After collection, the soil was transported to the lab and stored at room temperature (27 ± 3 °C) until use. Before the experiment started, the soil was cleaned, sieved (4-mm screen), thoroughly mixed, and analyzed for its physical and chemical properties. The methods used in analysing the soil are described in Abubaker *et al.* (2017). The physical and chemical properties of the soil are shown in Table 1.

### Animal Manure

Dry sheep and poultry manure were collected from one farm that is located north of Sabha City, weighing around 40 kg from each. The sheep and poultry in the farm are mainly fed with clover and grinded grain, respectively. After collection, the manures were transported to the lab where the cleaning of the stones was done and stored at room temperature (27 ± 3 °C) until use. Portions from both sheep and poultry manure were anaerobically digested using the same method described by Abubaker *et al.* (2017). The physical and chemical properties of both raw and digested manures were analyzed and the methods used in the analysis are described in Abubaker *et al.* (2017). The results of physical and chemical analysis are given in Table 2.

### Experiment Setup

The experiment was conducted in the research and consulting centre of Sebha University, Libya, using plastic pots (10 cm diam. x 12 cm height). The experiment had 20 treatments shown in Table 3 with 4 replicates of each. Four sowing dates were performed i.e. directly after soil fertilization, and 10, 20, and 30 days from fertilization. At each date, 64 pots were sown which represented 16 treatments i.e. from 5 to 20, as shown in Table 3. Besides the control treatment (i.e. untreated seeds sown in unfertilized soil), extra treatments were included to assess the effect of the inoculation method on seed germination, which were sown only one time in unfertilized soil. These treatments

**Table 1: Physical and chemical properties of the soil used in the experiment**

Parameters	Values
Physical properties	
Sand (%)	69
Silt (%)	22
Clay (%)	9
Water-holding capacity (%)	24.7
Chemical properties	
pH	8.2
OM (%)	0.3
Total N (%)	0.08
Total C (%)	0.9
P (g kg <sup>-1</sup> dw)	0.2
K (g kg <sup>-1</sup> dw)	0.06
Na (g kg <sup>-1</sup> dw)	0.3
Ca (g kg <sup>-1</sup> dw)	0.7

**Table 2: Physical and chemical characteristics of raw and digested manures. Reported values are the average ± standard deviation (n=3)**

Parameters	Raw		Digested	
	Poultry manure	Sheep manure	Poultry manure	Sheep manure
DM (%)	91.2 ± 0.1	91.7 ± 0.1	19.2 ± 0.9	19.0 ± 0.2
pH	7.9 ± 0.2	8.3 ± 0.4	7.8 ± 0.5	7.4 ± 0.7
Tot N (g kg <sup>-1</sup> dw)	36.1 ± 2	16.2 ± 0.6	32.4 ± 1.5	11.3 ± 0.4
Tot C (g kg <sup>-1</sup> dw)	364 ± 13	355 ± 6	340 ± 1	378 ± 1
C/N	10	22	11	34
Tot P (g kg <sup>-1</sup> dw)	15.8 ± 0.1	2.12 ± 0.02	21.4 ± 0.2	2.59 ± 0.2
Tot K (g kg <sup>-1</sup> dw)	17.6 ± 0.4	9.4 ± 0.06	18.8 ± 0.1	13.8 ± 0.8

**Table 3: The treatments used in the germination experiment**

S. No.	Treatments	Abbreviations
1	Control (untreated seeds)–unfertilized soil	C
2	Treated seeds with Arabic gum–unfertilized soil	S_AG
3	Treated seeds with sawdust–unfertilized soil	S_S
4	Treated seeds with Arabic gum and sawdust–unfertilized soil	S_AGS
5	Raw sheep manure (50 kg N ha <sup>-1</sup> )+Untreated seeds	SM50
6	Raw sheep manure (50 kg N ha <sup>-1</sup> )+Treated seeds (Arabic gum+sawdust)	SM50+AGS
7	Raw sheep manure (100 kg N ha <sup>-1</sup> )+Untreated seeds	SM100
8	Raw sheep manure (100 kg N ha <sup>-1</sup> )+Treated seeds (Arabic gum+sawdust)	SM100+AGS
9	Digested sheep manure (50 kg N ha <sup>-1</sup> )+Untreated seeds	DSM50
10	Digested sheep manure (50 kg N ha <sup>-1</sup> )+Treated seeds (Arabic gum+sawdust)	DSM50+AGS
11	Digested sheep manure (100 kg N ha <sup>-1</sup> )+Untreated seeds	DSM100
12	Digested sheep manure (100 kg N ha <sup>-1</sup> )+Treated seeds (Arabic gum+sawdust)	DSM100+AGS
13	Raw poultry manure (50 kg N ha <sup>-1</sup> )+Untreated seeds	PM50
14	Raw poultry manure (50 kg N ha <sup>-1</sup> )+Treated seeds (Arabic gum+sawdust)	PM50+AGS
15	Raw poultry manure (100 kg N ha <sup>-1</sup> )+Untreated seeds	PM100
16	Raw poultry manure (100 kg N ha <sup>-1</sup> )+Treated seeds (Arabic gum+sawdust)	PM100+AGS
17	Digested poultry manure (50 kg N ha <sup>-1</sup> )+Untreated seeds	DPM50
18	Digested poultry manure (50 kg N ha <sup>-1</sup> )+Treated seeds (Arabic gum+sawdust)	DPM50+AGS
19	Digested poultry manure (100 kg N ha <sup>-1</sup> )+Untreated seeds	DPM100
20	Digested poultry manure (100 kg N ha <sup>-1</sup> )+Treated seeds (Arabic gum+sawdust)	DPM100+AGS

are represented by treatment number (2) treating seeds with only Arabic gum, treatment number (3) treating seeds with only sawdust and treatment number (4) treating seeds with Arabic gum and sawdust. The fertilizers used in the experiment were sheep and poultry manure in two conditions, raw (non-digested) and digested applied to the soil at two rates corresponding to 50 and 100 kg Tot N ha<sup>-1</sup>. At the start, all pots were filled with 800 g of pure soil and amended with the target fertilizer rate and then irrigated with 150 mL of water.

### Sowing and Germination Indices

The pots were divided into four sets, each set consisting of 64 pots and sown either directly or after 10, 20, and 30 days from soil fertilization. After fertilization 15 alfalfa seeds were sown in each pot, i.e. corresponding to 35 kg seeds ha<sup>-1</sup>. The sown seeds were selected carefully using a magnifying glass to avoid deformed seeds, such as broken ones. The seed's vitality was tested according to Ellis *et al.* (1985) and was 88±4%. After sowing, all pots all pots were kept under field condition (14±4 h day, 10±3 h night, temperature 28±3 °C and air humidity 31%) and checked daily for moisture and germination. The counts of germinated seeds were started after observing the first germination, which was continued for 30 days. In addition, seed germination time (SGT), time to reach maximum germination (TMG), germination index (GI), and final germination percentage (F<sub>CP</sub>) were assessed. GI and F<sub>CP</sub> were calculated according to the formulas described by Farooq *et al.* (2005) and Dastanpoor *et al.* (2013). To measure the SGT, we recorded the number of days it took for the seeds to germinate under different treatments. We also recorded the number of days it took for the seeds to reach F<sub>CP</sub>, indicating the time it took to reach maximum germination (TMG).

### Statistical Analysis

The data was analysed using SPSS (WIN. Version 17) where General Linear Model of multiple variables was used with Tukey (HSD) multiple comparison tests for repeated testing of paired

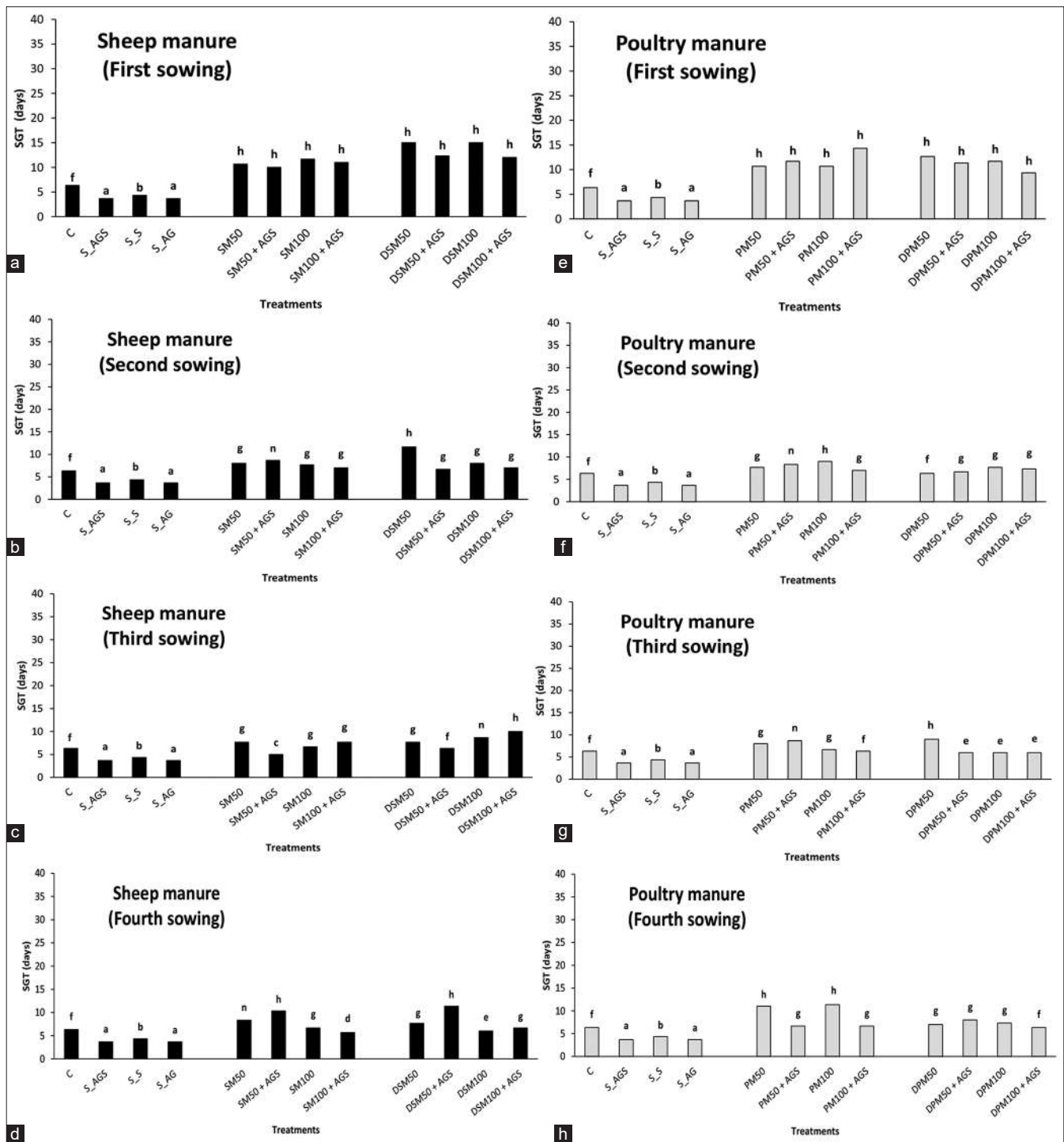
differences between treatments for SGT, TMG, GI, and F<sub>CP</sub>. In the analysis, the inoculation method, the time of sowing, fertilizer type, application rate, and their interactions were considered as fixed factors. The presented results are averages based on four samples (n=4). Results with different letters are significantly different (p<0.05).

## RESULTS

### Seed Germination Time

Figure 1 shows the number of days required for the seeds to germinate in each treatment, which is known as seed germination time (SGT). According to the analysis of variance, there was a significant effect (p<0.05) on SGT caused by the method of inoculation, date of sowing, and type of fertilizer. However, the rate of fertilization did not have any significant effect on SGT. The results of the first sowing showed that sown alfalfa seeds in the soil directly after fertilization with raw and digested manure led to a significant (p<0.05) delay in seed germination compared with unfertilized soil (Figure 1a & e). The required time for the seeds to germinate in the first sowing ranged from 9 to 15 days, whereas in the control it took 6 days. At the second sown, the time taken by the seeds to germinate in all treatments was reduced significantly (p<0.05), ranging from 7 to 12 days compared to the first sown (Figure 1b & f), with the exception of DSM50 and PM100 in the second sown where they were not significantly different from the first sown.

In the third sown, when seeds were sown (past participle) 20 days after fertilization the SGT was enhanced in all treatments and ranged from 5 to 10 days (Figure 1c & g), where they germinated faster in the treatments of digested poultry manure compared with the other treatments. The fourth sown (seeds were sown (past participle) 30 days after soil fertilization) displayed a different pattern compared to the third sown which means SGT was reduced in most treatments (ranging from 6 to 12 days) (Figure 1d & h). Furthermore, treating alfalfa seeds with Arabic



**Figure 1:** Seed germination time (SGT) after sowing at four different dates in the soil amended with raw and digested manures, i.e. sheep manure (a, b, c and d) and poultry manure (e, f, g and h), at two rates corresponding to 50 and 100 kg N ha<sup>-1</sup>. Bars represent means (n = 4) and the same letter appears on the bars across the figures indicates there are no significant differences (according to the Tukey test with p=0.05)

gum and straw before sowing to inoculate them with rhizobium bacteria (treatments of S\_AG, S\_S and S\_ASGT) enhanced SGT significantly ( $p < 0.05$ ) compared with non-treated seeds (c). However, the effect of Arabic gum and sawdust on SGT in the fertilized soil with raw and digested sheep and poultry manure was unclear, which means the effect was positive in some treatments but insignificant compared with untreated seeds. The results

showed no clear effect of fertilizer rate on the SGT whether in treatments fertilized with sheep or poultry manure.

### Final Germination Percentage

The analysis of variance showed that the inoculation method, sowing date, fertilizer type, and fertilization rate had a

significant effect ( $p < 0.05$ ) on  $F_{CP}$ . In the first sowing,  $F_{CP}$  was significantly ( $p < 0.05$ ) low in all fertilized treatments where it ranging from 19 to 58% whereas in the control was 88% (Table 4). Furthermore, low application rate (50 kg N ha<sup>-1</sup>) of raw poultry manure resulted in high  $F_{CP}$  compared to the other treatments. At the second sowing,  $F_{CP}$  increased significantly ( $p < 0.05$ ) in most treatments (ranging from 18 to 73%), and dropped down in the raw poultry manure treatments (Table 4). In the third sowing,  $F_{CP}$  decreased slightly in most fertilized treatments (where it ranged from 17 to 62%) while in the fourth sowing increased in digested sheep manure treatments at the application rate of 100 kg Tot N ha<sup>-1</sup>.

Additionally, the use of Arabic gum and sawdust in as inoculation method had a positive effect on the  $F_{CP}$  in fertilized treatments compared to untreated seeds. However, in unfertilized soil, sowing treated seeds (S\_ASGT, S\_S, and S\_AG) resulted in less  $F_{CP}$  compared to the control which was sown with untreated seeds (Table 4).

### Germination Index

Germination index (GI) revealed seed germination speed, where higher values indicate faster germination and lower values indicate slower germination (Table 5). The analysis of variances displayed a significant ( $p < 0.05$ ) effect of inoculation method, sowing date and fertilizer type on GI, while fertilization rate had no significant effect. In the first sowing, the GI ranged from 1 to 4.2 in all fertilized treatments. However, unfertilized soil (C) had a significantly higher GI (6.9). Unfertilized treatments sown with seeds treated with Arabic gum and sawdust had a higher GI compared to fertilized treatments, ranging from

3.6 to 4.7 (see treatments S\_ASG, S\_S and S\_AG in Table 5). Furthermore, there was indication that raw manure (sheep and poultry) enhanced GI compared to digested manure. In the second sowing, GI increased significantly ( $p < 0.05$ ) in most fertilized treatments compared with the GI of the first sowing. Raw manure treatments resulted in higher GI compared to digested manure treatments, especially the treatments sowed with seeds treated with Arabic gum and sawdust. In comparison with the second sowing, GI decreased in most treatments and increased in some treatments during the third and fourth sowings, respectively.

### Time to Reach Maximum Germination

Figure 2 displays the TMG in each treatment, indicating the required time for the seeds to reach their maximum germination percentage (final germination percentage). Analysis of variance revealed that inoculation method, sowing date, fertilization rate, and fertilizer type had a significant ( $p < 0.05$ ) effect on TMG.

In the first sowing, the fertilized treatments had  $F_{CP}$  ranging between 19% and 58%, and TMG ranged between 15 and 19 days (Figure 2a & e). This was a significantly ( $p < 0.05$ ) longer period compared to the TMG of unfertilized treatments (C, S\_ASGT, S\_S, and S\_AG), where the  $F_{CP}$  ranging from 60% to 88% and their TMG ranged from 6 to 9 days.

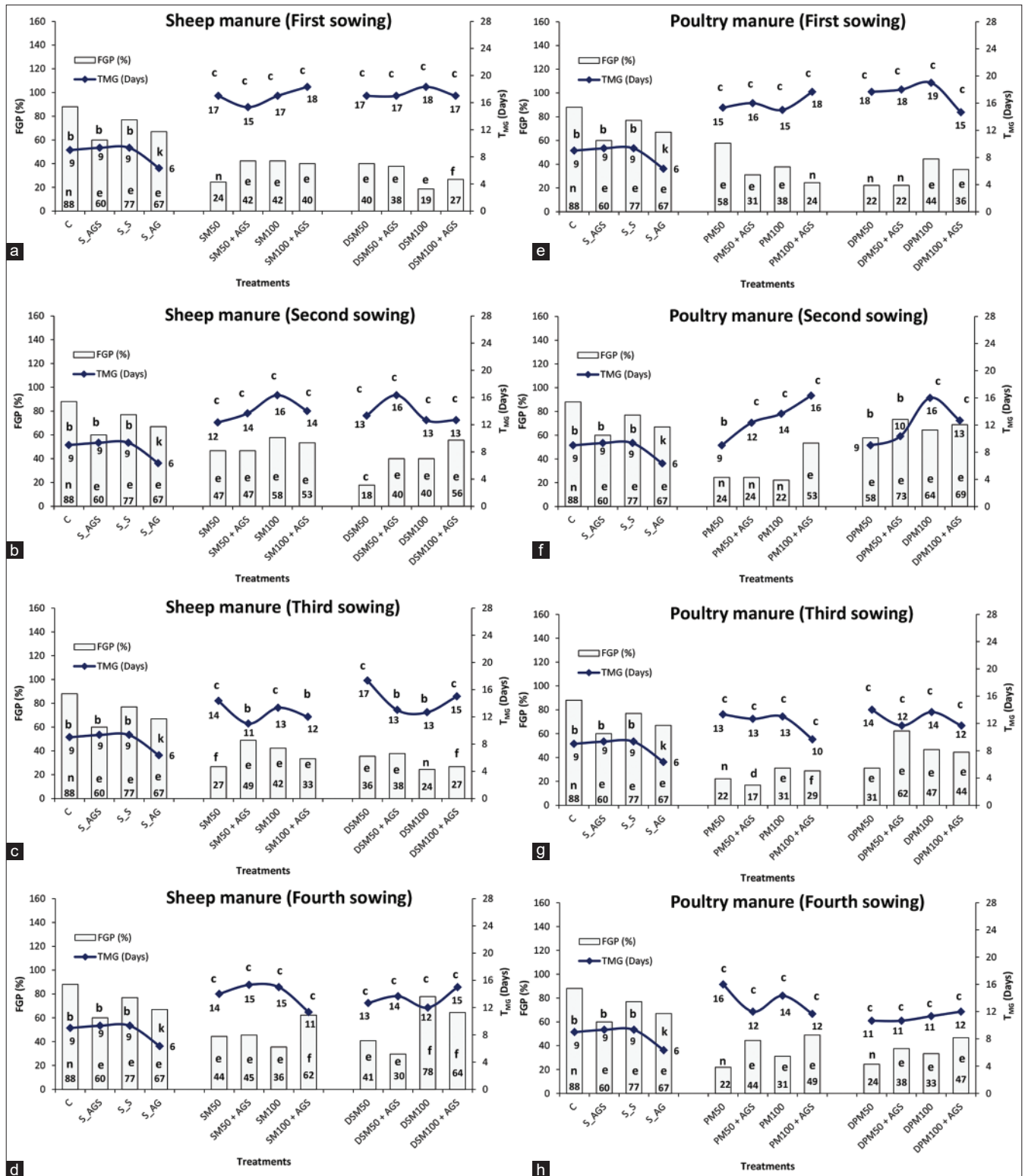
In the second sowing, there was a significant decrease ( $p < 0.05$ ) in TMG in certain poultry manure treatments, while in sheep manure treatments the decrease was not significant. This decrease occurred within a range of 9 to 16 days, as illustrated in Figure 2b and f.

**Table 4: Final germination percentage ( $F_{GP}$ ) 20 days after sowing at four different dates in different treatments amended with raw and digested sheep and poultry manure. Values represent the means (n=4) and if the same letters appear among the columns, it indicates that there are no significant differences (according to the Tukey test with  $p=0.05$ )**

Treatments	First sowing	Second sowing	Third sowing	Fourth sowing
C	88 <sup>a</sup>			
S_AGS	60 <sup>e</sup>			
S_S	77 <sup>e</sup>			
S_AG	67 <sup>e</sup>			
SM50	24 <sup>n</sup>	47 <sup>e</sup>	27 <sup>f</sup>	44 <sup>e</sup>
SM50+AGS	42 <sup>e</sup>	47 <sup>e</sup>	49 <sup>e</sup>	45 <sup>e</sup>
SM100	42 <sup>e</sup>	58 <sup>e</sup>	42 <sup>e</sup>	36 <sup>e</sup>
SM100+AGS	40 <sup>e</sup>	53 <sup>e</sup>	33 <sup>e</sup>	62 <sup>e</sup>
DSM50	40 <sup>e</sup>	18 <sup>c</sup>	36 <sup>e</sup>	41 <sup>e</sup>
DSM50+AGS	38 <sup>e</sup>	40 <sup>e</sup>	38 <sup>e</sup>	30 <sup>f</sup>
DSM100	19 <sup>c</sup>	40 <sup>e</sup>	24 <sup>n</sup>	78 <sup>e</sup>
DSM100+AGS	27 <sup>f</sup>	56 <sup>e</sup>	27 <sup>f</sup>	64 <sup>e</sup>
PM50	58 <sup>e</sup>	24 <sup>n</sup>	22 <sup>n</sup>	22 <sup>n</sup>
PM50+AGS	31 <sup>e</sup>	24 <sup>n</sup>	17 <sup>d</sup>	44 <sup>e</sup>
PM100	38 <sup>e</sup>	22 <sup>n</sup>	31 <sup>e</sup>	31 <sup>e</sup>
PM100+AGS	24 <sup>n</sup>	53 <sup>e</sup>	29 <sup>f</sup>	49 <sup>e</sup>
DPM50	22 <sup>n</sup>	58 <sup>e</sup>	31 <sup>e</sup>	24 <sup>n</sup>
DPM50+AGS	22 <sup>n</sup>	73 <sup>e</sup>	62 <sup>e</sup>	38 <sup>e</sup>
DPM100	44 <sup>e</sup>	64 <sup>e</sup>	47 <sup>e</sup>	33 <sup>e</sup>
DPM100+AGS	36 <sup>e</sup>	69 <sup>e</sup>	44 <sup>e</sup>	47 <sup>e</sup>

**Table 5: Germination index (GI) after sowing in the soil at different dates after amendment with raw and digested sheep and poultry manure. Values represent the means (n=4) and if the same letters appear among the columns, it indicates that there are no significant differences (according to the Tukey test with  $p=0.05$ )**

Treatments	First sowing	Second sowing	Third sowing	Fourth sowing
C	6.9 <sup>z</sup>			
S_AGS	3.6 <sup>k</sup>			
S_S	4.3 <sup>u</sup>			
S_AG	4.7 <sup>z</sup>			
SM50	2.2 <sup>f</sup>	5.0 <sup>z</sup>	2.3 <sup>f</sup>	3.8 <sup>k</sup>
SM50+AGS	3.2 <sup>m</sup>	4.3 <sup>u</sup>	5.5 <sup>z</sup>	3.1 <sup>m</sup>
SM100	2.6 <sup>m</sup>	5.0 <sup>z</sup>	3.9 <sup>k</sup>	2.3 <sup>f</sup>
SM100+AGS	2.6 <sup>m</sup>	5.6 <sup>z</sup>	1.4 <sup>c</sup>	6.5 <sup>z</sup>
DSM50	2.6 <sup>m</sup>	1.6 <sup>d</sup>	2.8 <sup>m</sup>	3.4 <sup>k</sup>
DSM50+AGS	2.5 <sup>n</sup>	4.5 <sup>z</sup>	3.5 <sup>k</sup>	1.9 <sup>e</sup>
DSM100	1.0 <sup>c</sup>	4.2 <sup>u</sup>	1.0 <sup>b</sup>	8.4 <sup>z</sup>
DSM100+AGS	2.0 <sup>e</sup>	5.5 <sup>z</sup>	2.4 <sup>f</sup>	4.2 <sup>u</sup>
PM50	4.2 <sup>u</sup>	2.6 <sup>m</sup>	1.9 <sup>e</sup>	1.4 <sup>c</sup>
PM50+AGS	1.9 <sup>e</sup>	2.8 <sup>m</sup>	1.5 <sup>d</sup>	4.4 <sup>u</sup>
PM100	2.3 <sup>f</sup>	6.4 <sup>z</sup>	2.3 <sup>f</sup>	2.5 <sup>n</sup>
PM100+AGS	1.6 <sup>d</sup>	8.0 <sup>z</sup>	3.1 <sup>m</sup>	4.0 <sup>u</sup>
DPM50	1.7 <sup>e</sup>	1.8 <sup>e</sup>	0.9 <sup>a</sup>	2.1 <sup>e</sup>
DPM50+AGS	1.4 <sup>c</sup>	4.9 <sup>z</sup>	6.5 <sup>z</sup>	4.6 <sup>z</sup>
DPM100	2.8 <sup>m</sup>	6.2 <sup>z</sup>	4.4 <sup>z</sup>	2.9 <sup>m</sup>
DPM100+AGS	2.8 <sup>m</sup>	7.4 <sup>z</sup>	5.6 <sup>z</sup>	2.7 <sup>m</sup>



**Figure 2:** Shows the time required for the seeds to reach maximum germination (represented by a smooth line curve on top of the bars) and the final germination percentage (represented by the bars) after being sown on different dates and fertilized with either raw or digested sheep or poultry manure. The letters a, b, c, and d correspond to sheep manure treatments, while e, f, g, and h correspond to poultry manure treatments. Values next the smooth lines curves or next to the bars represent means (n=4). The same letters among bars or lines across figures indicate no significant differences (Tukey p=0.05)

During the third and fourth sowings, the time taken for reaching maximum germination ranged from 10 to 15 days, and the final

germination percentage was between 17% and 78% (Figure 2c, d, g & h). It is notable that the TMG decreased during the

third and fourth sowings when compared to the first sowing. In addition, there was no clear pattern of the effect of Arabic gum and sawdust on the TMG in fertilized treatments compared to treatments sown with untreated seeds.

## DISCUSSION

To achieve a high alfalfa yield, it is essential to ensure a quick and high seed germination rate. When cultivating alfalfa in virgin land, especially in poor soil such as desert soil, manure can support the growth by providing essential macro and micronutrients (Abubaker *et al.*, 2022). However, it is crucial to assess the germination rate of alfalfa seeds when using organic fertilizer. In this study, the germination of alfalfa seeds was evaluated by sowing them at different intervals after fertilizing desert soil with either raw or digested animal manure.

Based on the findings, the germination of alfalfa seeds was notably delayed and significantly reduced when sown directly after fertilization with either raw or digested sheep or poultry manure. This delay was evident by the growth indicators examined in the study, long seed germination time and high values of the germination index (Kader, 2005). Additionally, the final germination percentage was also affected by fertilization and sowing time as observed in the first sowing where final germination percentage was reduced compared to the control. Moreover, the results displayed that the required time for the seeds to reach the final germination percentage (maximum germination) was significantly longer in the first sown compared to the control. In the second sown, the required time for the seeds to reach the final germination percentage has been reduced.

These findings are consistent with the results reported by Abubaker *et al.* (2022), who found that sowing alfalfa seeds two days after fertilizing the soil with non-digested or digested cattle manure showed a reduction in the germination of alfalfa seeds compared with unfertilized treatment. Abubaker *et al.* (2022) stated that raw and digested cattle manure decreased the final germination percentage and germination index of alfalfa seeds in comparison with the control. A similar trend of results was observed in other studies by El-Zeadani *et al.* (2018) and Abubaker *et al.* (2020) who showed that the wheat seed varieties are sown directly after soil fertilization. This adverse effect on seed germination has been indicated in several studies. Gupta and Gupta (2011) found that treating okra seeds with anaerobically digested poultry manure for 6 to 18 hours reduced seed germination significantly compared to diluted digested manure in water. This has been explained by Bacilio *et al.* (2003) and Šerá and Novák (2011) who have shown that humic acids present in digested manure which are formed during anaerobic digestion can delay and reduce seed germination. Additionally, research by Kaparaju *et al.* (2012) found that anaerobically digested generated from orange waste can be toxic to Chinese cabbage seed and prevent germination due to the presence of ammonia and organic acids in the digestate. The concentration of ammonia in digested manure is higher compared to undigested manure, as noted by García-González *et al.* (2016) and Li *et al.* (2023b). A high concentration of

ammonia has been found to have negative effects on wheat seed germination and root hair formation, according to Bremner and Krogmeier (1989) and Wan *et al.* (2016). Additionally, de Tunes *et al.* (2012) reported that the presence of organic acids resulting from the anaerobic decomposition of organic material can significantly reduce wheat cultivar germination, radicle length, and dry weight. These studies suggest that the presence of humic acid in the digestate or soil after manure application may have affected alfalfa seed germination.

The results of the second sowing - where the seeds were planted in the soil ten days following fertilization - demonstrated a marked improvement in germination indicators as compared to the first sowing. This improvement included seed germination time being reduced, a decrease GI, an increase in  $F_{CP}$ , and a decrease in the time required to reach  $F_{CP}$ . These results are constant with what has been reported in previous studies. El-Zeadani *et al.* (2018) and Abubaker *et al.* (2020) noted that delaying the sowing of wheat seeds by 10 to 20 days after fertilization can reduce the negative impact on seed germination. This improvement can be attributed to the breakdown of fertilizer components by soil microorganisms (Powlson *et al.*, 2001).

In addition, the inoculation method used to inoculate alfalfa seeds with effective rhizobium bacteria to enhance growth and yield was also evaluated on seed germination in both fertilized and unfertilized soil. The study showed that treating the seeds with Arabic gum and sawdust before sowing positively impacted germination, compared to untreated seed. Another study found that using sawdust as a coating material for alfalfa seeds, along with Arabic gum, resulted in significantly higher germination rates (Abubaker *et al.*, 2022). The positive effect was explained by that coating materials can affect seed imbibition by altering water availability and gaseous exchange in the seedbed, which stimulates germination (Halmer, 2008).

## CONCLUSION

In the present study, the germination of alfalfa seeds was evaluated in virgin desert soil fertilized with raw and digested animal manure. The results showed that seed germination was reduced and delayed when seeds were sown directly after fertilization. Moreover, when sowing was done between 10 and 30 days after soil fertilization the germination improved. Application rates used in the study have only a minor effect on seed germination. The study also found that inoculating the seeds using Arabic gum and sawdust enhanced seed germination. Nonetheless, further investigation is required on a field scale to fully assess the effectiveness of raw and digested animal manure, along with the optimal sowing date after soil fertilization, in supporting alfalfa growth and yield.

## ACKNOWLEDGMENTS

The authors expressed their appreciation to the research center's laboratory for providing the necessary equipment and chemicals that were instrumental in conducting the research.

## REFERENCES

- Abubaker, J., Alaswd, A., Mohammed, N. S., El-Zeadani, H., & Khalifa, M. (2022a). Alfalfa (*Medicago sativa* L.) growth and yield in desert soil fertilized with raw and anaerobically digested cattle manure. *Journal of Plant Nutrition*, 45(7), 992-1003. <https://doi.org/10.1080/01904167.2021.1994605>
- Abubaker, J., Elnesairy, N., & Ahmed, S. (2017). Effects of non-digested and anaerobically digested farmyard manures on wheat crop cultivated in desert soil. *Journal of Aridland Agriculture*, 3, 1-10. <https://doi.org/10.19071/jaa.2017.v3.3127>
- Abubaker, J., Essalem, M., El-Zeadani, H., & Alghali, A. (2020). Effect of time interval between sowing and application of nondigested/digested cattle manure on germination and seedling growth of several wheat cultivars. *Agriculture Research and Technology*, 24(1), 556252. <https://doi.org/10.19080/ARTOAJ.2020.22.556252>
- Abubaker, J., Mohammed, N. S., Essalem, M., Abobaker, A., & Khalifa, M. (2022b). Effect of seed inoculation method with Rhizobium species on the germination of alfalfa seeds (*Medicago sativa* L.). *Sebha University Journal of Pure & Applied Sciences*, 21(2), 135-140. <https://doi.org/10.51984/jopas.v21i2.1876>
- Ahmad, A. A., Radovich, T. J. K., Nguyen, H. V., Uyeda, J., Arakaki, A., Cadby, J., Paull, P., Sugano, S., & Teves, G. (2016). Use of organic fertilizers to enhance soil fertility, plant growth, and yield in a tropical environment. In L. L. Marcelo & S. Sonia (Eds.), *Organic Fertilizers* London, UK: IntechOpen Limited. <https://doi.org/10.5772/62529>
- Bacilio, M., Vazquez, P., & Bashan, Y. (2003). Alleviation of noxious effects of cattle ranch composts on wheat seed germination by inoculation with *Azospirillum* spp. *Biology and Fertility of Soils*, 38, 261-266. <https://doi.org/10.1007/s00374-003-0650-1>
- Bremner, J. M., & Krogmeier, M. J. (1989). Evidence that the adverse effect of urea fertilizer on seed germination in soil is due to ammonia formed through hydrolysis of urea by soil urease. *Proceedings of the National Academy of Sciences of the United States of America*, 86(21), 8185-8188. <https://doi.org/10.1073/pnas.86.21.8185>
- Buyanovsky, G., Dicke, M., & Berwick, P. (1982). Soil environment and activity of soil microflora in the Negev desert. *Journal of Arid Environments*, 5(1), 13-28. [https://doi.org/10.1016/S0140-1963\(18\)31459-9](https://doi.org/10.1016/S0140-1963(18)31459-9)
- Dastanpoor, N., Fahimi, H., Shariati, M., Davazdahemami, S., & Hashemi, S. M. M. (2013). Effects of hydropriming on seed germination and seedling growth in sage (*Salvia officinalis* L.). *African Journal of Biotechnology*, 12(11), 1223-1228.
- de Tunes, L. M., Avelar, S. A. G., Barros, A. C. S. A., Pedroso, D. C., Muniz, M. F. B., & de Menezes, N. L. (2012). Critical levels of organic acids on seed germination and seedling growth of wheat. *Revista Brasileira de Sementes*, 34(3), 366-372.
- Du, T.-Y., He, H.-Y., Zhang, Q., Lu, L., Mao, W.-J., Zhai, M.-Z. (2022). Positive effects of organic fertilizers and biofertilizers on soil microbial community composition and walnut yield. *Applied Soil Ecology*, 175, 104457. <https://doi.org/10.1016/j.apsoil.2022.104457>
- Dwevedi, A., Kumar, P., Kumar, P., Kumar, Y., Sharma, Y. K., & Kayastha, A. M. (2017). Soil sensors: detailed insight into research updates, significance, and future prospects. In A. M. Grumezescu (Eds.), *New Pesticides and Soil Sensors* (pp. 561-594) Cambridge, US: Academic Press. <https://doi.org/10.1016/B978-0-12-804299-1.00016-3>
- Ellis, R. H., Hong, T. D., & Roberts, E. H., (1985). *Handbook of seed technology for genebanks. Volume I. Principles and methodology*. Rome: International Board for Plant Genetic Resources.
- El-Zeadani, H., Abubaker, J., Essalem, M., Alghali, A. (2018). Germination of several wheat cultivars in desert soil after amendment with raw and digested poultry manure with and without combination with mineral fertilizer. *International Journal of Recycling of Organic Waste in Agriculture*, 7, 335-343. <https://doi.org/10.1007/s40093-018-0219-5>
- Esmailian, Y., Amiri, M. B., Tavassoli, A., Caballero-Calvo, A., & Rodrigo-Comino, J. (2022). Replacing chemical fertilizers with organic and biological ones in transition to organic farming systems in saffron (*Crocus sativus*) cultivation. *Chemosphere*, 307, 135537. <https://doi.org/10.1016/j.chemosphere.2022.135537>
- Ezemagu, I. G., Ejimofor, M. I., Menkiti, M. C., & Diyoike, C. (2021). Biofertilizer production via composting of digestate obtained from anaerobic digestion of post biocoagulation sludge blended with saw dust: Physicochemical characterization and kinetic study. *Environmental Challenges*, 5, 100288. <https://doi.org/10.1016/j.envc.2021.100288>
- Farooq, M., Basra, S. M. A., Ahmad, N., & Hafeez, K. (2005). Thermal hardening: A new seed vigor enhancement tool in rice. *Journal of Integrative Plant Biology*, 47(2), 187-193. <https://doi.org/10.1111/j.1744-7909.2005.00031.x>
- García-González, M. C., Vanotti, M. B., & Szogi, A. A. (2016). Recovery of ammonia from anaerobically digested manure using gas-permeable membranes. *Scientia Agricola*, 73(5), 434-438. <https://doi.org/10.1590/0103-9016-2015-0159>
- Gupta, N., & Gupta, U. (2011). Effect of anaerobically digested slurry of cowdung and kitchen waste on the seed quality in Okra (*Abelmoschus Esculentus* L.). *Journal of Advanced Laboratory Research in Biology*, 2(4), 158-160.
- Halmer, P. (2008). Seed technology and seed enhancement. *ISH Acta Horticulturae*, 771, 17-26. <https://doi.org/10.17660/ActaHortic.2008.771.1>
- Hati, K., & Bandyopadhyay, K. (2011). Fertilizers (mineral, organic), effect on soil physical properties. In J. Gliński, J. Horabik & J. Lipiec (Eds.), *Encyclopedia of Agrophysics* (pp. 296-299) Netherlands, Dordrecht: Springer. [https://doi.org/10.1007/978-90-481-3585-1\\_201](https://doi.org/10.1007/978-90-481-3585-1_201)
- Hillel, D. (2008). Soil fertility and plant nutrition. In D. Hillel (Eds.), *Soil in the Environment* (pp. 151-162) San Diego, California: Academic Press.
- Jia, S., Yuan, D., Li, W., He, W., Raza, S., Kuz'yakov, Y., Zamanian, K., & Zhao, X. (2022). Soil chemical properties depending on fertilization and management in China: A Meta-analysis. *Agronomy*, 12(10), 2501. <https://doi.org/10.3390/agronomy12102501>
- Kader, M. A. (2005). A comparison of seed germination calculation formulae and the associated interpretation of resulting data. *Journal & Proceedings of the Royal Society of New South Wales*, 138, 65-75.
- Kaparaju, P., Rintala, J., & Oikari, A. (2012). Agricultural potential of anaerobically digested industrial orange waste with and without aerobic post-treatment. *Environmental Technology*, 33(1), 85-94. <https://doi.org/10.1080/09593330.2011.551839>
- Li, F., Yuan, Y., Shimizu, N., Magaña, J., Gong, P., & Na, R. (2023a). Impact of organic fertilization by the digestate from by-product on growth, yield and fruit quality of tomato (*Solanum lycopersicon*) and soil properties under greenhouse and field conditions. *Chemical and Biological Technologies in Agriculture*, 10, 70. <https://doi.org/10.1186/s40538-023-00448-x>
- Li, Y., Zhu, J., Tang, Y., Shi, X., Anwar, S., Wang, J., Gao, L., & Zhang, J. (2023b). Impact of varying mass concentrations of ammonia nitrogen on biogas production and system stability of anaerobic fermentation. *Agriculture*, 13(8), 1645. <https://doi.org/10.3390/agriculture13081645>
- Lin, L., Xu, F., Ge, X., & Li, Y. (2018). Improving the sustainability of organic waste management practices in the food-energy-water nexus: A comparative review of anaerobic digestion and composting. *Renewable and Sustainable Energy Reviews*, 89, 151-167. <https://doi.org/10.1016/j.rser.2018.03.025>
- Lošák, T., Zatloukalová, A., Szostková, M., Hlušek, J., Fryč, J., & Vítěz, T. (2011). Comparison of the effectiveness of digestate and mineral fertilisers on yields and quality of kohlrabi (*Brassica oleracea*, L.). *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 59(3), 117-122. <https://doi.org/10.11118/actaun201159030117>
- Powlson, D. S., Hirsch, P. R., & Brookes, P. C. (2001). The role of soil microorganisms in soil organic matter conservation in the tropics. *Nutrient Cycling in Agroecosystems*, 61, 41-51. <https://doi.org/10.1023/a:1013338028454>
- Saad, A. M. A., Shariff, N. M., & Gairola, S. (2011). Nature and causes of land degradation and desertification in Libya: Need for sustainable land management. *African Journal of Biotechnology*, 10(63), 13680-13687. <https://doi.org/10.5897/AJB11.1235>
- Savci, S. (2012). Investigation of effect of chemical fertilizers on environment. *APCBEE Procedia*, 1, 287-292. <https://doi.org/10.1016/j.apcbee.2012.03.047>
- Šerá, B., Novák, F. (2011). The effect of humic substances on germination and early growth of Lamb's Quarters (*Chenopodium album* agg.). *Biologia*, 66(3), 470-476. <https://doi.org/10.2478/s11756-011-0037-y>
- Song, S., Lim, J. W., Lee, J. T. E., Cheong, J. C., Hoy, S. H., Hu, Q., Tan, J. K. N., Chiam, Z., Arora, S., Lum, T. Q. H., Lim, E. Y., Wang, C.-H., Tan, H. T. W., & Tong, Y. W. (2021). Food-waste anaerobic digestate as a fertilizer: The agronomic properties of untreated digestate and biochar-filtered digestate residue. *Waste Management*, 136, 143-152. <https://doi.org/10.1016/j.wasman.2021.10.011>
- Wan, X., Wu, W., Li, C., Liu, Y., Wen, X., & Liao, Y. (2016). Soil ammonia



- volatilization following urea application suppresses root hair formation and reduces seed germination in six wheat varieties. *Environmental and Experimental Botany*, 132, 130-139. <https://doi.org/10.1016/j.envexpbot.2016.08.010>
- Ye, L., Zhao, X., Bao, E., Li, J., Zou, Z., & Cao, K. (2020). Bio-organic fertilizer with reduced rates of chemical fertilization improves soil fertility and enhances tomato yield and quality. *Scientific Reports*, 10, 177. <https://doi.org/10.1038/s41598-019-56954-2>
- Zhao, Y., Lu, G., Jin, X., Wang, Y., Ma, K., Zhang, H., Yan, H., & Zhou, X. (2022). Effects of microbial fertilizer on soil fertility and alfalfa rhizosphere microbiota in alpine grassland. *Agronomy*, 12(7), 1722. <https://doi.org/10.3390/agronomy12071722>
- Zheng, S., Yin, K., & Yu, L. (2022). Factors influencing the farmer's chemical fertilizer reduction behavior from the perspective of farmer differentiation. *Heliyon*, 8(12), e11918. <https://doi.org/10.1016/j.heliyon.2022.e11918>
- Zurqani, H. A., Mikhailova, E. A., Post, C. J., Schlautman, M. A., & Elhaweij, A. R. (2019). A review of Libyan soil databases for use within an ecosystem services framework. *Land*, 8(5), 82. <https://doi.org/10.3390/land8050082>