

IMPACT OF SOME TREATMENTS ON SEED GERMINATION AND SEEDLING VIGOUR OF KANGAR (*GUNDELIA* SP. L.)

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Abstract. This research was conducted at the Horticulture Department, College of Agricultural Engineering Sciences, University of Sulaimani, Kurdistan region-Iraq to study the effect of various treatments such as removing fruit layers, soaking seeds in distilled water, GA₃ with 100 and 200 ppm, *Moringa oleifera* leaf extract with various concentrations (0.5, 1.0, 1.5 and 2.0 g/L) for 24 h and subjecting the seeds to freezing temperature at -2 °C for 24 and 48 h on Kangar (*Gundelia* sp.) seed germination and growth. Removing the seed coat and submerging seeds in 200 and 100 ppm recorded the highest germination percentages of 50, 46.67 and 33.33%, respectively. Removing the seed coats enhanced leaf proliferation (1.67). Moreover, maximum lateral root number (23.34), leaf length (7.42 cm) and leaf width (1.70 cm) were achieved in soaking seeds in 100 ppm GA₃. Whereas, the highest taproot length was observed after soaking the seeds with 200 ppm GA₃, which reached at 16.6 cm. In addition, the seeds that were treated with moringa leaf extract exhibited the highest chlorophyll and carotenoid concentrations, at 1.5 g/L. In most aspects, removing the seed coat and GA₃ were noticeably more effective for promoting *Gundelia* seed germination.

Keywords: seed treatment, GA₃, freezing, seed coat removal, plant extract

Introduction

Kangar (*Gundelia* sp. L.), belongs the sunflower family Asteraceae, is a spiny and thistle-like flowering plant and disseminated widely in the semi-dried areas of Lebanon, Syria, Palestine, Israel, Jordan, Iraq, Iran, Azerbaijan, Armenia, and Anatolia (Ayoubi and Baradari, 2015). The only distinguished species of this genus is *Gundelia tournefortii*, L. (Firat, 2017), however, recently, many new species of this genus were recorded, such as *G. Anatolica*, *G. Asperrima*, *G. Cilicica*, *G. Colemerikensis*, *G. Dersim*, *G. Glabra*, *G. Komagenensis*, *G. Mesopotamica*, *G. Munzuriensis*, *G. Rosea*, *G. Tournefortii*, *G. Vitekii* (Genç and Firat, 2019). *Gundelia* L. found in Mediterranean regions and was used as food, especially *Gundelia tournefortii* L. (Owies et al., 2004). The youngest stem and head of it are used for food, and many nutritive values (proteins, fibers and minerals) were found in it (Oweis, 2003). Overharvesting, land cultivation and overgrazing threaten of *Gundelia* L. for extinction (Shibli et al., 2009; Yazdanshenas et al., 2016). In this regard, some efforts have been exerted to propagate the important wild species of this genus through seed or *in vitro*.

Seed propagation is the easiest and cheapest method for propagation of plants, at the same time a large number of new plants can be achieved through seeds (Hartmann et al., 2011). The seed of many plant species required specialized treatment to improve

germination due to seed dormancy phenomenon. It was found that removing the seed coat enhanced seed germination because the seed coat acts as a physical barrier for water and oxygen uptake, a mechanical barrier for embryo emergence, or prevents leaching of germinating inhibitors from the embryo (Hopkins and Hüner, 2008). Additionally, soaking seeds in water for a period increases germination percentage of seeds as well, Hossain (2005) found that seed germination of *the Terminalia chebula* (Retz.) was raised by soaking the seeds in water for 48 h. Soaking seed in an optimal concentration of certain phytohormones is also beneficial for germination seeds of some species (Afzal et al., 2005). Stratification of seeds is the common method used to break dormancy in the dormant seeds of many species, Vaisi et al. (2018) reported that stratification improved seed germination in *Gundelia tournefortii* seeds in the combination of scarification and GA₃ treatment. On the other hand, plant extracts, which are safer for human and environment, are used as an alternative for synthetic seed germinating promoters, Phiri and Mbewe (2010) referred that moringa (*Moringa oleifera*) leaf extracts increased germination percentage of cowpea by 4%. The objective of the current study is to study the effect of some treatments on seed germination of *Gundelia* L.

Materials and methods

Plant materials and preparation

This research was conducted at Horticulture Department, College of Agricultural Engineering Sciences, University of Sulaimani, Kurdistan region-Iraq to study effects of some treatments on germination of *Gundelia* seeds and seedling vigorous. The seeds were collected from the wild *Gundelia* plants in middle July 2019, then the seeds placed in the refrigerator at 5 °C until the time of using. The intact and healthy seeds selected on October 23, 2019, 23/10/2019 for the germination experiment. The dry seeds were soaked in distilled water (WS), gibberellin (GA₃) with 100 and 200 ppm (GA₃-100 and GA₃-200), and *Moringa oleifera* leaf extract with concentrations: 0.5 (MO-0.5), 1.0 (MO-1.0), 1.5 (MO-1.5) and 2 g/L (MO-2.0) for 24 h. Moringa leaf extract solutions were prepared by placing moringa leaf in the water bath at 40 °C for 3 h, after that placed in the refrigerator for 24 h, then, in the following day, they were filtered through filter paper and used for the seed treating. The other treatments were applied with the *Gundelia* sp. seeds by subjecting the seeds to freezing at -2 °C for 24 (FR-24 h) and 48 h (FR-48 h), removing the seed coat (DE), while, the untreated seeds were directly sown as control seeds (CO). Seeds coat-removal were achieved manually, after seeds soaking in distilled water for 2 h at 25 °C to simplify removal of the seed coat. Finally, the treated and control seeds were sown in peat moss medium with three replications for each replication 10 seeds were sown. The experiment was performed in a plastic high tunnel, and it was laid out in Complete Randomized Design (CRD). The maximum and minimum temperature of inside the high tunnel was weekly calculated in *Figure 1*, from 23/10/2019, which was the date of starting the experiment, to 31/12/2019. After this period, the measured traits were: germination percentage (GP), number of lateral roots (NLR), taproot length (TPL), number of leaves (NL), leaf length (LL) and leaf width (LW).

Chlorophyll and carotenoid contents

Chlorophylls (CHa and CHb) and carotenoids (CA) were quantified according to Sumanta et al. (2014). The leaf samples were ground in liquid nitrogen, then 0.5 g of

the sample was homogenized in 10 mL of 80% Acetone, after Centrifuging for 5000 rpm for 30 min at 4 °C, 0.5 mL of the supernatant were remixed with 4.5 mL of 80% Acetone. The solution of the mixture was analyzed spectrophotometrically at various wavelengths 470, 646.8 and 663.2 nm for chlorophyll a (CHa), chlorophyll b (CHb), and carotenoids (CA). The following equations were used for the quantifications.

$$\text{CHa } (\mu\text{g/mL}) = 12.25 \times A_{663.2} - 2.79 \times A_{646.8} \quad (\text{Eq.1})$$

$$\text{CHb } (\mu\text{g/mL}) = 21.50 \times A_{646.8} - 5.10 \times A_{663.2} \quad (\text{Eq.2})$$

$$\text{CA } (\mu\text{g/mL}) = (1000 \times A_{470} - 1.82 \text{ CH a} - 85.02 \text{ CH b})/198 \quad (\text{Eq.3})$$

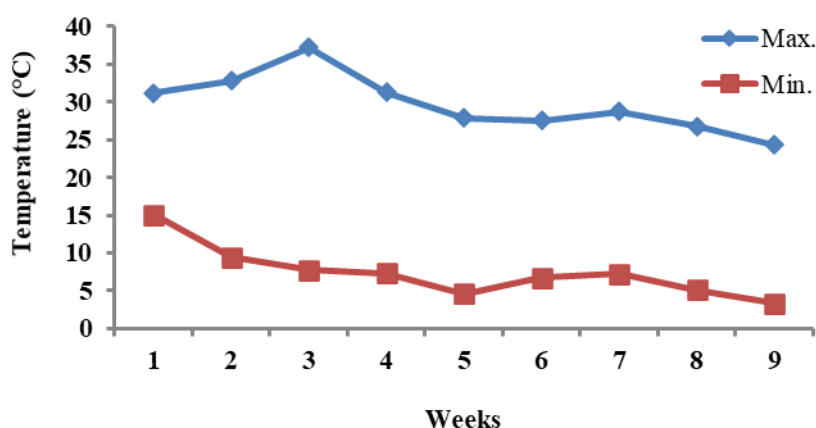


Figure 1. Weekly maximum and minimum temperatures inside the plastic high tunnel from 23/10/2019 to 31/12/2019

Statistical analysis

After 9 weeks the experiment was terminated, the parameters of germination percentage and seedling lateral root number, seedling taproot length, seedling leaf number, seedling leaf length, seedling leaf width and pigments concentration (CHa, CHb and CA) were measured. Using XLSTAT software version 2019.2.2, one-way ANOVA-CRD, Duncan's new multiple-range test and principal component analysis (PCA) has been used to determine significant differences between different treatments and relationships between different variables ($p \leq 0.05$). Correlation analysis was conducted by Displayr software.

Results and discussion

Impact of different treatments on germination percentage

The effect of different treatments on seed germination showed that there were no significant differences between all the treatments and the control (*Table 1; Fig. 2*), while the germination percentage (GP) significantly varied among the treatments themselves. Decoated seeds played a significant role in accelerating germination percentage, which it recorded the highest germination percentage (50%), followed by

the seeds soaked in 100 and 200 ppm GA₃ (33.33 and 46.67%, respectively). The lowest germination percentage (3.33 and 6.67%) was achieved from the seeds that soaked in 0.5 and 1 g/L moringa extracts, respectively. A non-significant variation was observed between the decoated seeds and seed treated by GA₃.

Table 1. Analysis of variance of germination percentage (GP), number of lateral root (NLR) and taproot length (TPL) of Kangar

GP					
Source	DF	Sum of squares	Mean squares	F	Pr > F
Treatment	10	6854.55	685.45	2.79*	0.02
Error	22	5400.00	245.45		
Corrected total	32	12254.55			
NLR					
Source	DF	Sum of squares	Mean squares	F	Pr > F
Treatment	10	1406.16	140.62	2.87**	0.01
Error	22	1075.28	48.88		
Corrected total	32	2481.44			
TPL					
Source	DF	Sum of squares	Mean squares	F	Pr > F
Treatment	10	574.40	57.44	3.11**	0.01
Error	22	406.01	18.46		
Corrected total	32	980.42			

*: significant difference among treatments, **: high significant difference among treatments

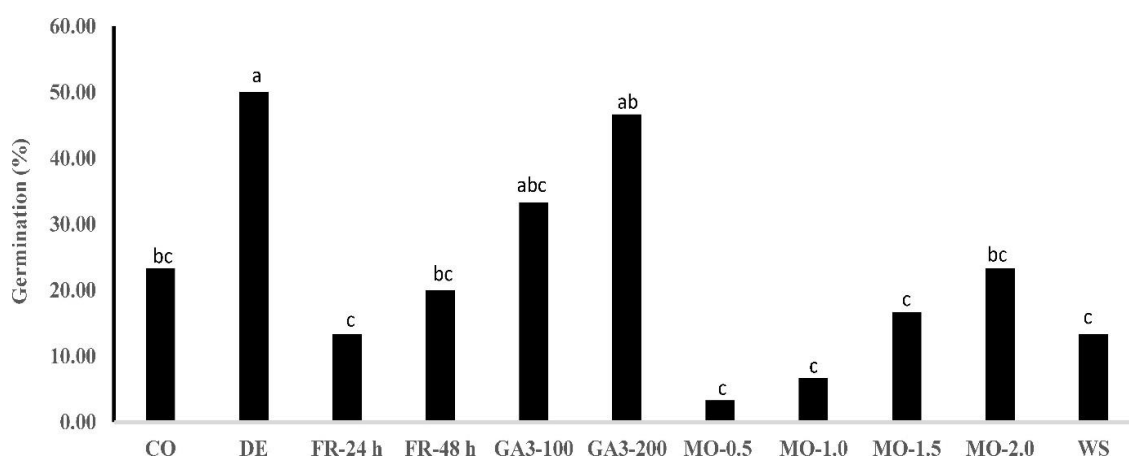


Figure 2. Effect of the different treatments on germination percentage of Kangar. Each value represents the mean of three replicates. Means sharing a specific letter are not significantly different according to Multiple Duncan Test at $p \leq 0.05$. CO: Control, DE: decoated seeds, FR-24 h: treatment of seeds by freezing for 24 h, FR-48 h: treatment of seeds by freezing for 48 h, GA₃-100: treatment of seeds by gibberellin (GA₃) at concentration of 100 ppm, GA₃-200: treatment of seeds by gibberellin (GA₃) at concentration of 200 ppm, MO-0.5: treatment of seeds by moringa leaf extract at concentration of 0.5 g/L, MO-1.0: treatment of seeds by moringa leaf extract at concentration of 1.0 g/L, MO-1.5: treatment of seeds by moringa leaf extract at concentration of 1.5 g/L, MO-2.0: treatment of seeds by moringa leaf extract at concentration of 2.0 g/L, and WS: treatment of seeds by distilled water

Similarly, AbuQaoud and Alkony (1995) found *Gundelia tournefortii* germination seeds were increased by removing the seed coat. Improving seed germination by removing the seed coat could be interpreted as an embryo rescue from the restriction of seed coat, which acts as a mechanical barrier for embryo emergence, and it helps the embryo to reach water and gas easily and quickly; despite these seed the coat prevents leaching of germination inhibitors from the seed, or supplying them to an embryo (Hartmann et al., 2011). Besides, Shibli et al. (2009) reported that soaking *Gundelia tournefortii* seeds in 250 ppm GA₃ gave the highest germination percentage in the greenhouse experiment. Gibberellin stimulates germination via enzymatic weakening of the covering effects of endosperm and seed coat around radicle, storage, food mobilization and embryo cell expansion (Finch-Savage and Leubner-Metzger, 2006). Generally, in this study, moringa leaf extracts did not enhance seed germination compared to control. In this regard, Tahir et al. (2018 and 2020) found that reduction of seed germination as a result of application moringa extracts may due to that moringa extracts contained chemical fractions [eicosane, gamma-sitosterol, 1-(+)-ascorbic acid, 2,6-dihexadecanoate, octadecanoic acid, methyl 11,14,17-eicosatrienoate, and octadec-9-enoic acid], which were matched positively with the inhibition of seed germination of wild mustard. In the foregoing experiment, also, they further explained that the above allelopathic compounds minimized hydrolytic enzymes during germination.

Influence of various treatments on root growth

As shown in *Table 1* and *Figure 3*, the results showed significant variations in the number of lateral roots and taproot length among treatments. The highest number of lateral root numbers (23.24 and 20.04) in seeds soaked in GA₃-100 and GA₃-200, respectively, was significantly induced on taproot; afterwards, the decoated seeds gave (18.74) root number, whereas, the lowest root number (2.33) was observed in seeds soaked in MO-1.0 extract (*Fig. 3A*). Moreover, TPL reached the highest value (16.60 cm) in seeds, were soaked in GA₃-200 and to a lesser extent (16.33 cm) in seeds soaked in MO-1.5 extract, however, they were significantly different with control seeds by (8.47 cm), whereas soaking seeds in MO-0.5 and MO-1.0 extracts showed the shortest TPL (3.33 and 4.33 cm, respectively) (*Fig. 3B*). A non-significant difference was stated among the treatments: DE, GA₃-100 and GA₃-200. The elongation taproot in treated seeds with GA₃ may be by reason of that GA₃ induces cell elongation at the sub-apical region of the roots (Parab et al., 2017).

Effect of different treatments on leaf growth

The results of *Table 2* and *Figure 4* explained the significant difference among various treatments in terms of NL, LL and LW traits. DE and GA₃ (both concentrations), MO-1.5, FR-24 and FR-48 h, produced the highest leaf numbers compared to control treatment. The maximum leaf number (1.67) was recorded in the decoated seeds followed by the seeds treated with GA₃ and freezing. The seeds soaked with MO-0.5 and M0-1.0 extracts gave the lowest leaf number (0.33). Additionally, the different treatments resulted in different LL significantly, the longest leaf (7.42 cm) was observed in the seeds soaked in GA₃-100, by contrast, the shortest leaf (0.45 cm) was achieved from soaked seeds in MO-1.0 extract. The seeds soaked in GA₃-100 gave also the broadest leaves (1.70 cm), but the narrowest leaves (0.30 cm) observed from the seeds were soaked in MO-1.0 moringa extract. Removing the seed coat and GA₃

treatments in this study gave the best leaf traits, that is maybe due to the early initiated germination, which gives more time to the seedlings to produce the higher number of leaves, accelerate the exposure of leaves to sunlight to do photosynthesis, develop more root number for nutrient uptake, and consequently improving leaf growth in term of leaf length and width. Muralidhara et al. (2016) reported that decoated seeds and treatment of seeds with GA₃ led to the improvement of seed germination in mango varieties.

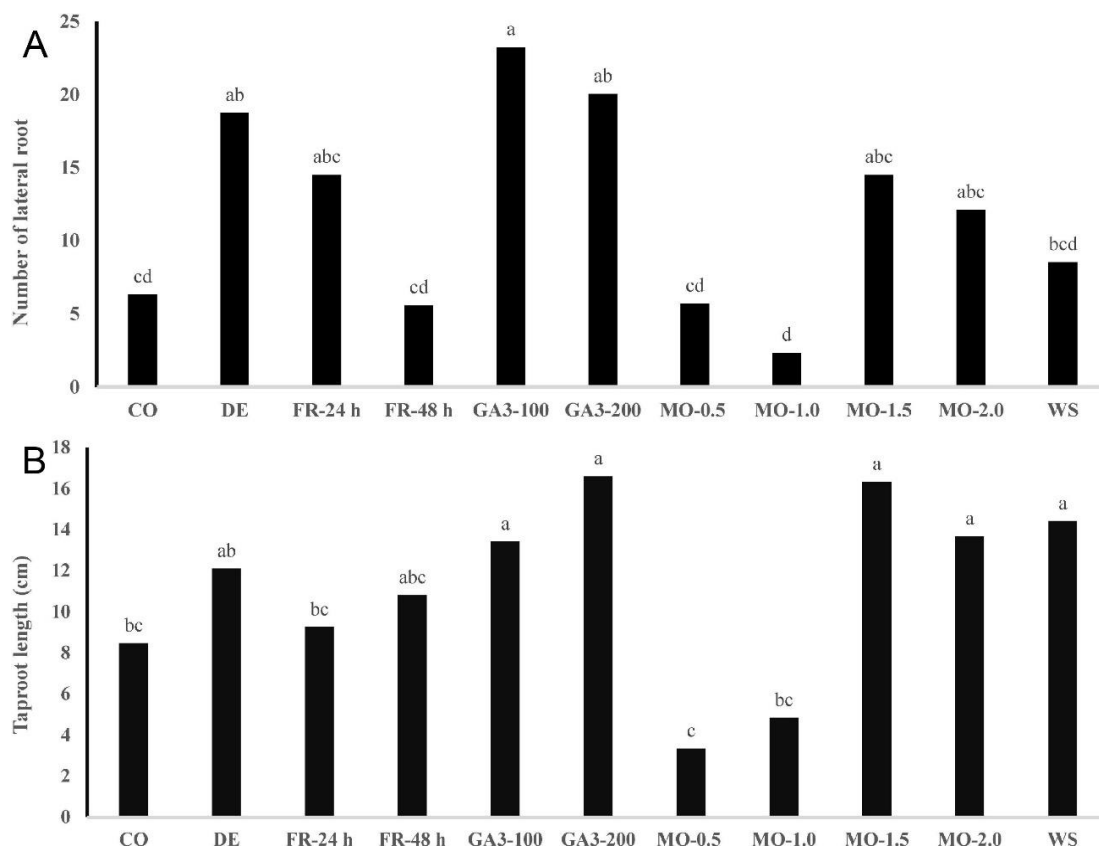


Figure 3. Impact of the various treatments on the number of lateral roots (A) and taproot length (B) of *Gundelia*. Each value signifies the mean of three replicates. Means sharing a specific letter are not significantly different according to Multiple Duncan Test at $p \leq 0.05$. CO: Control, DE: decoated seeds, FR-24 h: treatment of seeds by freezing for 24 h, FR-48 h: treatment of seeds by freezing for 48 h, GA₃-100: treatment of seeds by gibberellin (GA₃) at concentration of 100 ppm, GA₃-200: treatment of seeds by gibberellin (GA₃) at concentration of 200 ppm, MO-0.5: treatment of seeds by moringa leaf extract at concentration of 0.5 g/L, MO-1.0: treatment of seeds by moringa leaf extract at concentration of 1.0 g/L, MO-1.5: treatment of seeds by moringa leaf extract at concentration of 1.5 g/L, MO-2.0: treatment of seeds by moringa leaf extract at concentration of 2.0 g/L, and WS: treatment of seeds by distilled water

Impact of various treatments on pigments content

The obtained results in Table 3 and Figure 5 showed that the concentration of chlorophylls and carotenoids significantly impacted by the different treatments. The prominent chlorophyll (CHa and CHb) and carotenoid contents (CA) were measured at MO-1.5 and MO-2.0 extracts, and they were over the control (CO) and other treatments significantly. The highest CHa (1.79 µg/mL) and CA (0.37 µg/mL) contents were stated from the treatment of MO-1.5 moringa extract. Inversely, the seeds were soaked in GA₃-

100 resulted in the lowest concentrations of CHa (0.98 µg/mL) and CA (0.08 µg/mL). CHb displayed no significant variation among different treatments. Chlorophyll raising in seeds soaked with moringa extracts may be due to the presence of compounds in moringa leaf extract like cytokinin, zeatin, and zeatin, which have the role in the production of chlorophyll (Hala et al., 2017). Otherwise, it was reported that exogenous application of GA₃ increased leaf area, but it affected inversely on the concentration of chlorophyll per leaf unit area (Leite et al., 2003; El-Shraiy and Hegazi, 2009).

Table 2. Analysis of variance of number of leaves (NL), leaf length (LL) and leaf width (LW) of Kangar

NL					
Source	DF	Sum of squares	Mean squares	F	Pr > F
Treatment	10	5.40	0.54	2.10ns	0.06
Error	22	5.64	0.26		
Corrected total	32	11.04			
LL					
Source	DF	Sum of squares	Mean squares	F	Pr > F
Treatment	10	149.58	14.96	3.85**	0.00
Error	22	85.31	3.88		
Corrected total	32	234.89			
LW					
Source	DF	Sum of squares	Mean squares	F	Pr > F
Treatment	10	6.73	0.67	2.63*	0.02
Error	22	5.63	0.26		
Corrected total	32	12.36			

*: significant difference among treatments, **: high significant difference among treatments, Ns: non-significant difference among treatments

Table 3. Analysis of variance of different pigments in Kangar leaf

CHa					
Source	DF	Sum of squares	Mean squares	F	Pr > F
Treatment	10	0.96	0.10	38.21**	< 0.0001
Error	11	0.03	0.003		
Corrected total	21	0.99			
CHb					
Source	DF	Sum of squares	Mean squares	F	Pr > F
Treatment	10	0.07	0.01	0.54ns	0.83
Error	11	0.13	0.01		
Corrected total	21	0.20			
CA					
Source	DF	Sum of squares	Mean squares	F	Pr > F
Treatment	10	0.12	0.01	4.83**	0.01
Error	11	0.03	0.003		
Corrected total	21	0.15			

** : high significant difference among treatments, Ns: non-significant difference among treatments

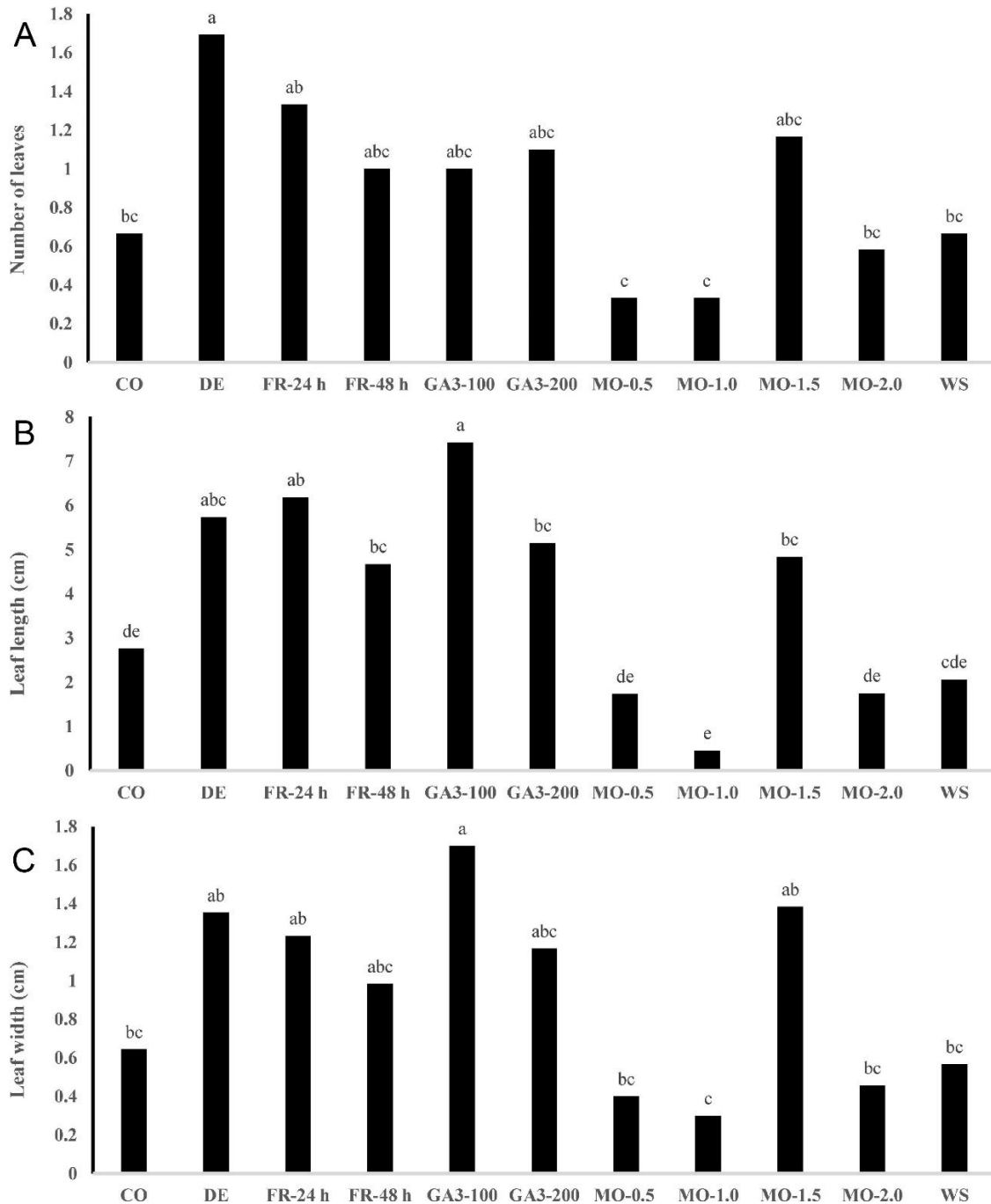


Figure 4. The influence of the various treatments on the number of leaves (A), leaf length (B) and leaf width (C) of *Gundelia*. Each value denotes the mean of three replicates. Means sharing a common letter are not significantly different according to Multiple Duncan Test at $p \leq 0.05$. CO: Control, DE: decoated seeds, FR-24 h: treatment of seeds by freezing for 24 h, FR-48 h: treatment of seeds by freezing for 48 h, GA₃-100: treatment of seeds by gibberellin (GA₃) at concentration of 100 ppm, GA₃-200: treatment of seeds by gibberellin (GA₃) at concentration of 200 ppm, MO-0.5: treatment of seeds by moringa leaf extract at concentration of 0.5 g/L, MO-1.0: treatment of seeds by moringa leaf extract at concentration of 1.0 g/L, MO-1.5: treatment of seeds by moringa leaf extract at concentration of 1.5 g/L, MO-2.0: treatment of seeds by moringa leaf extract at concentration of 2.0 g/L, and WS: treatment of seeds by distilled water

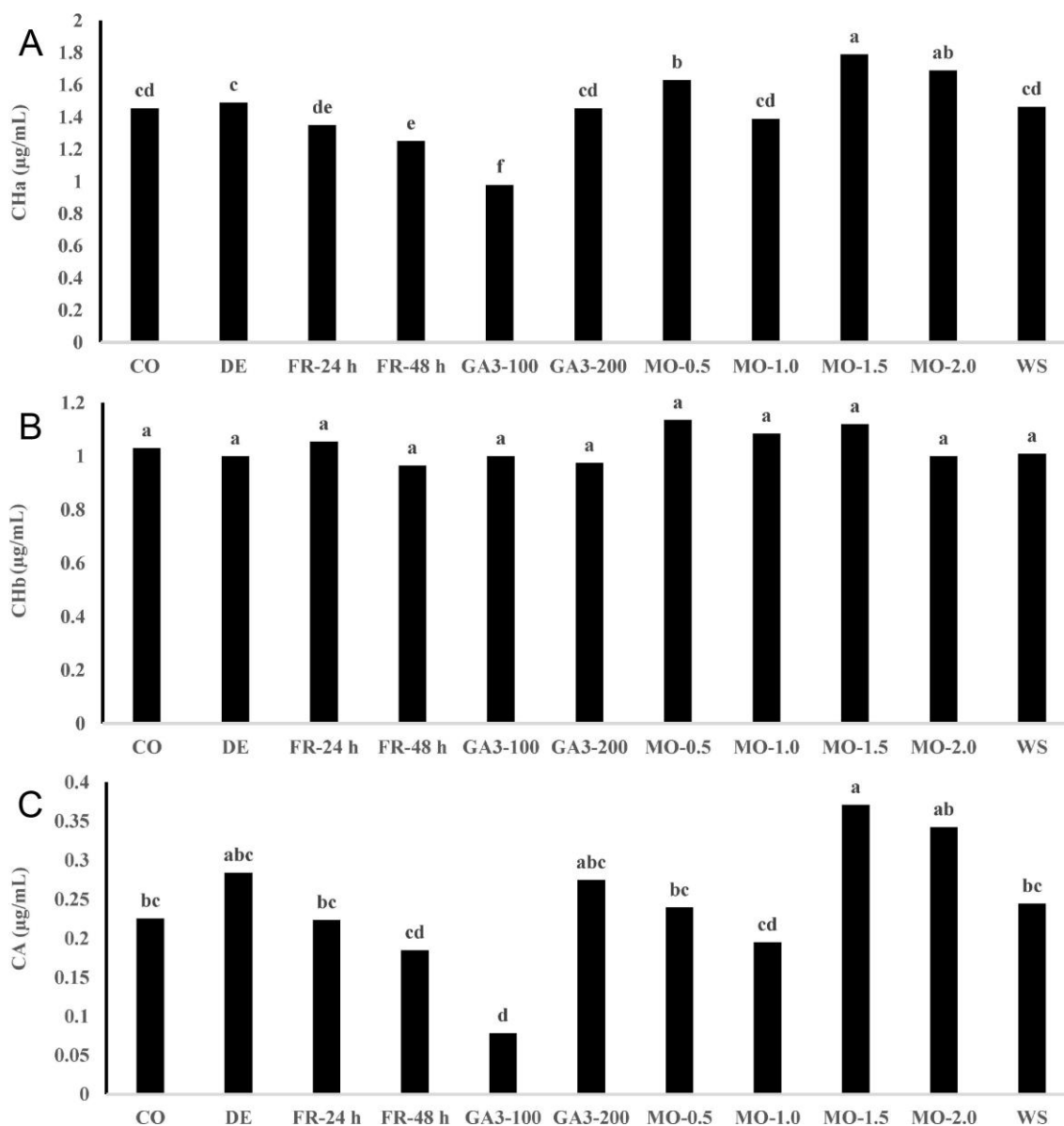


Figure 5. Effect of the treatments on leaf chlorophylls (CHa and CHb) and carotenoids (CA). Values are the average of three measurements. Average separation within a trait followed by the common letters does not differ significantly ($P \leq 0.05$) according to Duncan's Multiple Range Test. CO: Control, DE: decoated seeds, FR-24 h: treatment of seeds by freezing for 24 h, FR-48 h: treatment of seeds by freezing for 48 h, GA₃-100: treatment of seeds by gibberellin (GA₃) at concentration of 100 ppm, GA₃-200: treatment of seeds by gibberellin (GA₃) at concentration of 200 ppm, MO-0.5: treatment of seeds by moringa leaf extract at concentration of 0.5 g/L, MO-1.0: treatment of seeds by moringa leaf extract at concentration of 1.0 g/L, MO-1.5: treatment of seeds by moringa leaf extract at concentration of 1.5 g/L, MO-2.0: treatment of seeds by moringa leaf extract at concentration of 2.0 g/L, and WS: treatment of seeds by distilled water

Relationship between different treatments and traits

The principal component analysis was conducted to determine the relationship between the variables (treatments and traits) that account for the observed variance in

the data. The differential influence of the variables in each main component is calculated by the association between each variable and the main component. The first two main components (PC1 and PC2) clarified together 78.51% of the observed variance and were thus depicted in a two - dimensional space (*Fig. 6*). PC1 plotted on the horizontal axis, clarified the highest proportion of the variance (54.74%), while PC2, plotted on the vertical axis, accounted for a further 23.77% of the total variation. *Figure 6* shows the PCA plot distribution of the treatments and studied characters on two first components. DE and GA₃-200 treatments seemed to be positively correlated with TPL, NL, NLR, GP, LW and LL, positioning themselves on the positive side of the horizontal axis representing PC1. As seen in *Figure 7*, GP was positively and significantly associated with NLR ($r = 0.78$, p -value = 0.007 and NL ($r = 0.68$, p -value = 0.021), while negatively linked to CHb ($r = -0.67$, p -value = 0.024). It also shows that NLR is well correlated with TPL ($r = 0.67$, p -value = 0.024), NL ($r = 0.70$, p -value = 0.016), LW ($r = 0.82$, p -value = 0.002) and LW ($r = 0.84$, p -value = 0.001). A strong, positive and significant association was observed between LL and LW ($r = 0.97$, p -value = 0.000) and between Cha and CA. ($r = 0.94$, p -value = 0.000). On the other hand, CHb, which are very close to zero, shows the negative correlations with MO-0.5, WS and CO, placing themselves on the negative side of the horizontal axis reflecting PC1. PC2 was positively correlated with CA and MO-1.5, while negatively associated with FR-48 h, positioning themselves on the positive and negative sides of the vertical axis of PC2, respectively. As appeared in figure, we can conclude that DE and GA₃-200 had a positive impact on the following traits: TPL, NL, NLR, GP, LW and LL.

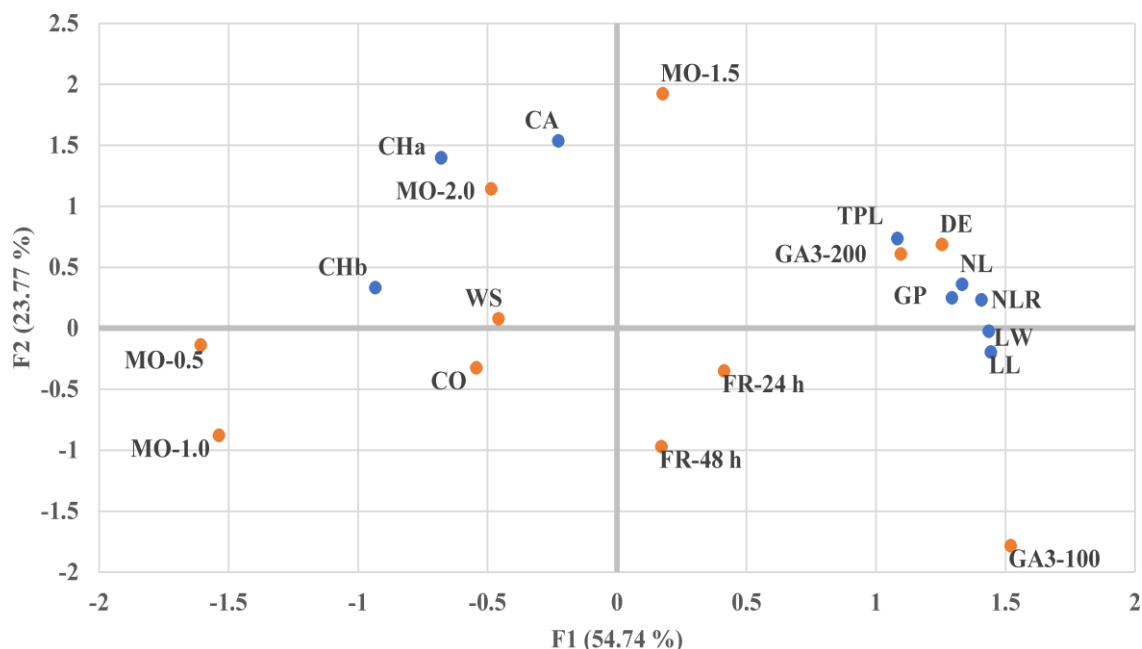


Figure 6. PCA plot showing the distribution and relationship of different treatments and traits. CO: Control, DE: decoated seeds, FR-24 h: treatment of seeds by freezing for 24 h, FR-48 h: treatment of seeds by freezing for 48 h, GA₃-100: treatment of seeds by gibberellin (GA₃) at concentration of 100 ppm, GA₃-200: treatment of seeds by gibberellin (GA₃) at concentration of 200 ppm, MO-0.5: treatment of seeds by moringa leaf extract at concentration of 0.5 g/L, MO-1.0: treatment of seeds by moringa leaf extract at concentration of 1.0 g/L, MO-1.5: treatment of seeds by moringa leaf extract at concentration of 1.5 g/L, MO-2.0: treatment of seeds by moringa leaf extract at concentration of 2.0 g/L, and WS: treatment of seeds by distilled water

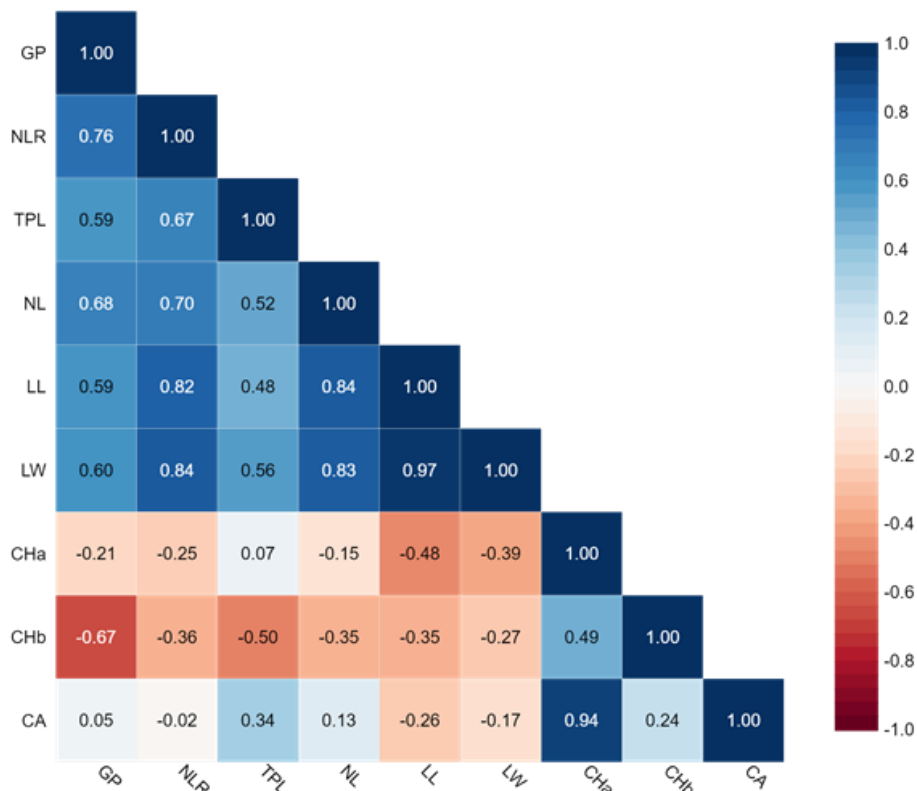


Figure 7. Pearson correlation analysis of nine studied characters. GP: germination percentage, NLR: number of lateral roots, TPL: taproot length, NL: number of leaves, LL: leaf length, LW: leaf width, CHa: chlorophyll a content, CHb: chlorophyll b content, and CA: carotenoids content.

Conclusion

Results of this research indicate that removing the *Gundelia* seed coat has improved its germination level and the number of roots. Though, GA₃ has been successful in growing the number of lateral roots, taproot length, leaf length and leaf width. In addition, the moringa extracts increased chlorophyll and carotenoid concentrations. Further analysis of the use of some plant extracts, such as *Glycyrrhiza glabra* and some germination enhancement agent, such as PEG at low concentrations, is advised.

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