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THE EFFECT OF SALT STRESS ON THE GERMINATION AND SEEDLING GROWTH PARAMETERS IN BIRDSFOOT TREFOIL (*Lotus Corniculatus* L.)

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Abstract: This study was carried out to determine the response of birdsfoot trefoil to salt stress during germination and seedling growth. The seeds of birdsfoot trefoil (Sarıyıldız variety) was used as material. In the study; 6 different doses of NaCl (pure water (control), 250 ppm, 750 ppm, 2500 ppm, 5000 ppm, 10000 ppm) was applied to birdsfoot trefoil seeds during germination and emergence. Germination experiment was carried out in darkness (20±2 °C) in the petri dishes according to the completely randomized design with 4 replications. Observations were made every day at the same time, seeds with radicle length exceeding 2 mm were considered germinated. According to the results of the research, salt concentrations in many features in terms of germination and seedling development were statistically significant. Increased salt concentrations negatively affected germination and seedling growth. In terms of all the properties examined the lowest values were obtained in 10000 ppm application.

Keywords: Salt tolerance, Birdsfoot trefoil, Forage crops

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1. Introduction

The growth and devolopment of plants in the environment they live in can be adversely affected by biotic or abiotic elements which are considered stress factors. Salinity, which is one of the leading abiotic stress factors, is defined as the accumulation of salt on the soil surface and near the surface as a result of the rising of the salts, which are washed into the groundwater by washing, especially in regions with arid and semi-arid climate characteristics, to the soil surface through capillarity with high ground water and the separation of water from the soil by evaporation (Kwiatowsky, 1998; Kara, 2002). Salinity stress causes significant losses in plant yield and quality, especially by changing the soil structure. Salinity, which is one of the limiting factors in increasing crop productivity and yield potential in the areas agricultural production, is a problem such as low precipitation, high evapotranspiration, high ground water, salty irrigation water and incorrect irrigation practices in arid and semi-arid regions where the amount of precipitation is low and the temperature is high. This situation emerges as one of the most important problems threatening food security for societies whose livelihood is plant and animal production (Cowie et al., 2018; Tietel et al., 2019). In our country salinity is a problem which is seen in 1,518,746 ha of the land and 867,405 ha of the agricultural lands moreover on a worldwide basis this ratio follows as 12,781 ha of agricultural land (Karaoğlu and Yalçın, 2018; Dursun and Mikailsoy, 2020). It is also said that the area of saline soils in the world today continues to increase steadily (Athar et al., 2009). It is recommended that the germination period is the most sensitive period of the life of plants to salt stress (Ahmad et al., 2013), and in the salinity studies, when the development periods of the plant are compared, germination and seedling development periods should be emphasized more and these developmental stages should be taken into consideration more in determining the salt responses of the species (Van Hoorn 1991; Ghoulam and Fares 2001). The response of plants to salt stress varies according to plant species, as well as differences within the same species. As a result of many studies conducted to date, it has been revealed that salinity significantly reduces germination and even completely prevents germination, and it has been reported that this effect varies depending on the plant type, variety and salt dose (Acar et al., 2011; Şentürk and Sivritepe, 2015; Önal Aşcı and Üney, 2016; Uslu and Gedik, 2020, Kızılşimşek and Süren, 2020). In the case of salt in the soil where most cultivated forage crops are grown, which will limit their growth and development, their yields decrease greatly. Especially in arid and semi-arid regions, saline soils limit yields in areas where forage crops are grown (Ateş and Tekeli, 2007). It has been reported that if the amount of salt in the soil is more than 1.5% of the dry soil weight, most forage crops cannot be grown (Gökkuş, 2009). On the other hand, it has been reported that high salt concentrations such as 200 mM NaCl cause very significant decreases in germination and seedling development of *Trifolium incarnatum*, *Trifolium repens* (Gravandi, 2013), *Trifolium resupinatum* (Ates and Tekeli, 2007), *Trifolium fragiferum* (Can et al., 2013), *Trifolium repens, Trifolium alexandrinum* (Saberi et al., 2013), *Medicago sativa* (Zhanwu et al., 2011; Kaplan et al., 2015), *Trifolium pratense* (Tolan et al., 2017), *Melilotus officinalis* (Ghaderi-Far et al., 2010), *Onobrychis sativa* (Majidi et al., 2010), *Pisum sativum* (Demirkol et al., 2019), *Vicia* spp. seeds (Uslu and Gedik, 2020). Since the reclamation of salty soils is a difficult and costly process, it is more appropriate to grow salinity-resistant plants to increase crop production in these areas (Turhan and Şeniz, 2010; Önal Aşcı, 2011). Therefore, in recent years, studies have focused on the salinity resistance of plant species and varieties. Determination of plant species and varieties that are tolerant to high soil salinity and that can produce economically have been expressed as the primary biotic approach in bringing the areas with salinity problems into cultivation (Ashraf and Harris, 2004). Birdsfoot trefoil (*Lotus corniculatus* L.) is a medium-perennial, perennial, easily self-renewing special legume widely found in different parts of the world. With a high protein content (15-28%), birdsfoot trefoil can be grown mostly for grass, silage and cover plant, or in rangeland pure or mixed. It is also considered a valuable forege crops that improves the performance of ruminants (Hannaway and Myers, 2004; Waghorn, 2008; Anonymous, 2014). Birdsfoot trefoil is a rangeland plant that is resistant to drought and cold, adapts to a very different soil structure in terms of acidity and moisture, is palatable, has no swelling feature, can easily renew itself in pastures due to its seed pouring feature, forms a good mixture with kentucky bluegrass, smooth broom, cocksfoot and timothy for grazing purposes (Açıkgöz, 2001). Birdsfoot trefoil is an N-stabilizing legume, an important part of sustainable agriculture and organic production (Tomic et al., 2007). It has the property of tolerating wet acidic soil (pH = 4.5), soil salinity and some drought conditions (Karadağ et al., 2017). For this reason, it is very important to determine the salt resistance of legume forage crop species and varieties that can grow efficiently with grasses in salty areas (Rogers, 1997). Although birdsfoot trefoil species are seen in natural vegetation in almost every region of our country, the fact that there are not enough studies on these species is considered to be a great deficiency for our country. The current state of rangelands and cultivation of forage crops, and the Mediterranean climate zone, in which our country is a part, reveal the need to give priority to this plant at least as much as alfalfa and Trifolium spp. or even more so according to the situation (Uzun et al., 2008). In this respect, it is necessary to evaluate the types of birdsfoot trefoil. Therefore, in this research, the resistance of birdsfoot trefoil, which is a productive and high quality forage crop, to salt stress during germination and seedling period was investigated.

2. Materials and Methods

This study was carried out in Muş Alparslan University Central Laboratory to determine the effects of salt concentrations applied to the birdsfoot trefoil on germination and seedling growth characteristics. Seeds are subjected to germination testing to obtain information about the viability of the seed, calculate the amount of seed to be sown and compare different seeds about biological value. In order for plants to develop in a healthy way, the seed, which is the first stage of production, must germinate in a uniform way. Before starting to work for this, the seeds of the Sarıyıldız variety of the birsfoot trefoil, which was first procured from the Central Black Sea Transition Zone Agricultural Research Institute Directorate, were subjected to germination testing with four repetitions and 50 seeds per repeat. As a result of the germination tests, the germination rate of the birdsfoot trefoil seeds was determined as 65% on average. Among the reasons for the low germination rates of birdsfoot trefoil, it has been reported by studies conducted in different locations that there is a significant hardseededness in the seeds of birdsfoot trefoil and that it is affected by climatic events (Gençkan, 1992; Hatipoğlu and Avcıoğlu, 2009). In another study, it was reported that there were 92.3% hard seeds in birsfoot trefoil seeds, which adversely affected the germination rate and average germination time. From this point of view, before the seeds are exposed to salt stress, the seed pods are eroded with the help of sandpaper in order to increase germination rates and to obtain more accurate results. The sanded seeds were subjected to germination testing with four repetitions and 50 seeds per repeat before the trial. As a result of the test, the average germination rate was determined as 95% and thus the seeds whose hardseededness properties were broken were prepared by sanding again for the experiment. Before the germination trial, the seeds were kept in a 1% sodium hypochlorite solution for five minutes for surface sterilization, then rinsed three times with pure water (Özkurt ve ark., 2018). The rinsed seeds were dried in air on filter paper and placed in petri dishes with a diameter of 9 mm in filter paper so that there were 50 seeds each. It is prepared so that the salt concentrations to be applied to the seeds are 0, 250, 750, 2500, 5000, 10000 ppm (Uslu ve Gedik, 2020). The petri dishes with added concentrations were followed in the dark at a temperature of 20±1 °C for a 15-day germination period (Azarafshan and Abbaspour, 2014), and the seeds that produced 2 mm of radicle were considered germinated. The germination rate was calculated by dividing the germinated seeds by the total number of seeds and then multiplying them by 100 (Maquire, 1962). For seedling lengths, radicle and plumule lengths were measured separately, and then the seedling length was determined by adding both lengths (ISTA, 1984). The radicle and plumule were weighed as fresh and the fresh weights of the seedlings were determined. The dry weight of the seedlings dried by soaking at 70 °C for 48 hours in the oven was determined (ISTA, 1984). The Vigor index value was calculated by multiplying the seedling length by the germination rate (Abdul-Baki and Anderson, 1973; ISTA, 1983). To determine the difference between doses in terms of the tolerance of birdsfoot trefoil to salinity, the salt tolerance index (%TTI) as a function of seedling fresh weight was calculated with the following formula (Bağcı et al., 2003): Salt tolerant indeks = (total fresh weight in salt concentration / total fresh weight in control application) * 100.

The data obtained in the study were subjected to analysis of variance in accordance with the randomized design with four replication using JMP-13.0 statistics package program to compare significant differences among treatments. Duncan's multiple range test was applied to compare the means if there were any significant differences.

3. Results and Discussion

The effects of salt doses on germination rate were given in Figure 1 and it was determined that there was a statistical difference between salt doses in terms of germination rate (P<0.01). Salt stress often reduced germination relative to control, but the increase in salt dose did not always reduce germination rate linearly (Figure 1). Therefore, the dose of 250 ppm salt was statistically included in the same group as the control. In the study, the germination rate ranged between 13.00-98.00%, and the lowest germination rate (13.00%) was realized at the highest salt dose (10000 ppm). While there was no significant reduction in the germination rate up to 5000 ppm application, the obtained values were found to be lower than the control. Salinity has been reported to adversely affect germination by creating osmotic stress in plants (Doğan and Çarpıcı, 2016). As the salt concentration in the germination environment increases, the osmotic pressure increases and therefore the seed in the environment does not receive enough water for germination. As a matter of fact, osmotic stress due to salinity is clearly seen at the dose of 10000 ppm, where the lowest germination occurs. Findings that germination rate decreases as salt concentration increases in many plants under salt stress support the study results (Tolan et al., 2017; Özkurt et al., 2018; Demirkol et al., 2019; Okcu 2020; Uslu and Gedik, 2020).

The effect of salt doses on the mean germination time is given in Figure 2 and it was determined that there was a statistical difference between the salt doses in terms of the average germination time (P<0.01). Salt stress often increased the mean germination time compared to the control, but the increase in salt dose did not always increase the mean germination time linearly.

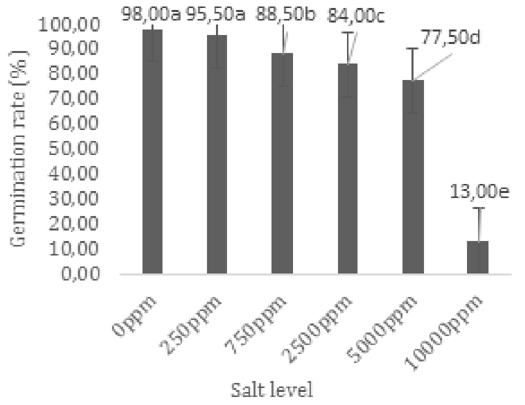

Figure 1. Effect of salt level on germination rate.

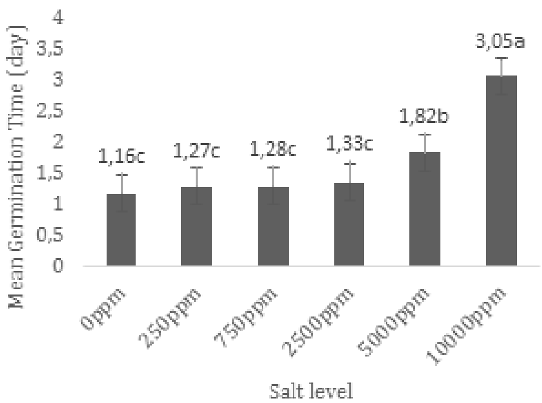

Figure 2. Effect of salt level on mean germination time.

Therefore, salt doses of 0 (1.16 days), 250 (1.27 days), 750 (1.28 days), 2500 (1.33 days) ppm were statistically in the same group in terms of mean germination time, that is, germination showed germination for similar periods, and germination in the longest time (3.05 days) was realized in 10000 ppm salt application. The observed increase in mean germination times due to increased salt doses coincides with the findings of various researchers (Önal Aşçı and Üney, 2016; Tolan et al., 2017; Şimşek Soysal et al., 2018; Şimşek Soysal et al., 2021).

The effects of salt doses on germination index were given in Figure 3 and it was determined that there was a statistical difference between salt doses in terms of germination index (P<0.01).

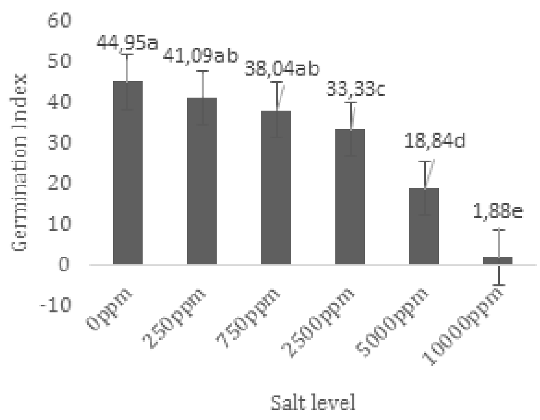

Figure 3. Effect of salt level on germination index.

As a result of the salt doses applied to the birdsfoot trefoil seeds, the germination index varied in the range of 1.88-44.95, and the highest values were obtained from the control application and the lowest values were obtained from the highest salt concentration of 10000 ppm. It has been stated that the high germination index is an indicator of high seed strength (Maquire, 1962). In other words, as the salt dose increases, it has been observed that the decrease in the germination index also reduces the seed strength. In similar studies, it has been reported that the germination index decreases as salt stress increases (Bilgili et al., 2018; Ertekin et al., 2017; Beyazçiçek and Yılmaz, 2020).

The effects of salt doses on germination energy were given in Figure 4 and it was determined that there was a statistical difference between salt doses in terms of germination energy (P<0.01). As a result of the salt doses applied to the birdsfoot trefoil seeds, the germination energy ranged from 0.0-21.37 and the highest values were obtained from the highest values control application and the lowest values were obtained from the highest salt concentration of 10000 ppm. Germination energy is one of the most important parameters in which the strength and quality of the seed is evaluated, and high germination energy indicates that the quality and strength of the seed is high. Although salt stress up to 2500 ppm can be tolerated, the values obtained after 5000 ppm have caused the strength of the seed to decrease greatly.

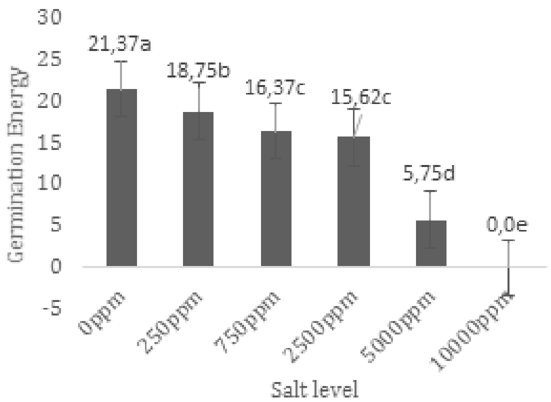

Figure 4. Effect of salt level on mean germination energy.

In the study, plumule length, radicle length, seedling length, seedling length, seedling fresh and dry weights were examined to determine the effects of salt doses on seedling development. The effect of salt doses on the development of plumule and radicle of birdsfoot trefoil seedlings was found to be statistically significant (P<0.01). Plumule and radicle lengths are important parameters in terms of salt stress. From these parameters, the radicle is taken to the plant by direct contact with soil and water and contributes to plumule development (Haileselasie and Gselasie 2012). Therefore, radicle and plumule lengths give the first information about the level of salt exposure of plants.

The plumule lengths of birdsfoot trefoil varied in the range of 0.7525-4.5675 mm. While 250 ppm salt administration was statistically in the same group as the control, salt doses of 750 ppm and above adversely affected plumule development. The radicle lengths of the birdsfoot trefoil ranged from 0.4325 to 2.32 mm. The highest radicle length was obtained from the control group and the lowest was obtained from the salt dose of 10000 ppm. Salt doses of 250 ppm and above adversely affected the development of the radicle. The development of the plant to the radicle is important in terms of salt resistance. It is known that salt has an inhibitory effect on the development of radicle during germination. This study reveals that salinity has a negative effect on radicle development, and studies by different researchers support this (Ahmed et al., 2017; Bose et al., 2018). The seedling length of the birdsfoot trefoil varied in the range of 1.1850-6.8875 mm, while the application of 250 ppm salt was statistically in the same group as the control, salt doses of 750 ppm and above adversely affected the length of the seedlings. The fact that the roots that first come into contact with the salt concentration under salt stress conditions are adversely affected by this situation also adversely affects the intake of water and other nutrients to the plant. The development problem that starts from the radicle component prevents the plant from feeding enough and this situation adversely affects plumule development. It is thought that the reason for the decrease in plumule, radicle and seedling length compared to increasing salt concentrations is due to the inhibition of cell division and elongation due to salt stress and the toxic effect of these salts (Van Horn 1991; Delgado and Sanchez-Raya 2007).

Various studies investigating the effects of salt stress on the birdsfoot trefoil plant also confirm that salt stress has a negative effect on plumule, radicle and seedling development (Teakle et al., 2006; Galloway et al., 2010; Azarafshan and Abbaspour, 2014).

Fresh weights and dry weights of birdsfoot trefoil are 5.22-18.32 mg/seedling, respectively; it varied in the range of 0.74-1.17 mg/seedling. In terms of seedling age weights, 250 ppm salt application was statistically in the same group as the control group, while salt doses of 750 ppm and above adversely affected the fresh seedling weight. In terms of dry seedling weights, salt applications after 250 ppm adversely affected the seedling dry weight compared to the control application, and dry seedling weight exhibited the lowest values in 10000 ppm salt applications. Salinity causes physiological drought and as a result, plants do not get enough water (Goertz and Coons, 1989). When the water lost by transpiration cannot be met, the turgor pressure in the cells decreases and plant growth is limited (Ashraf, 1994). As a result of the inability of plants to get water, a decrease in seedling weight occurs. It has been reported that salt stress as an abiotic factor causes inhibition of the development of root and above-ground organs in plants and reduces the dry weight of roots and stems (Epstein, 1985). In the current study, as salt concentrations increased, the seedling fresh and dry weights of birdsfoot trefoil decreased. Many plants under salt stress have been reported by different researchers to have significant reductions in seedling fresh and dry weight (Saboraa et al., 2006; Karakullukçu and Adak, 2008; Benlioğlu and Özkan, 2015; Akçay and Tan, 2018).

The vigor index is a value that indicates the vitality and performance level of seeds during germination and development of seedlings (Uslu and Gedik, 2020).

The effect of salt doses on vigor index in birdsfoot trefoil is seen in Figure 5. The vigor index values of the birdsfoot trefoil against the applied salt doses varied between 17.23-674.97, and the vigor index value decreased as a result of the increased salt applications and this decrease was found to be statistically significant. In similar studies, similar to the current study, it has been found that the vigor index decreases with the increase in salt concentrations (Özkurt et al., 2018; Uslu and Gedik, 2020).

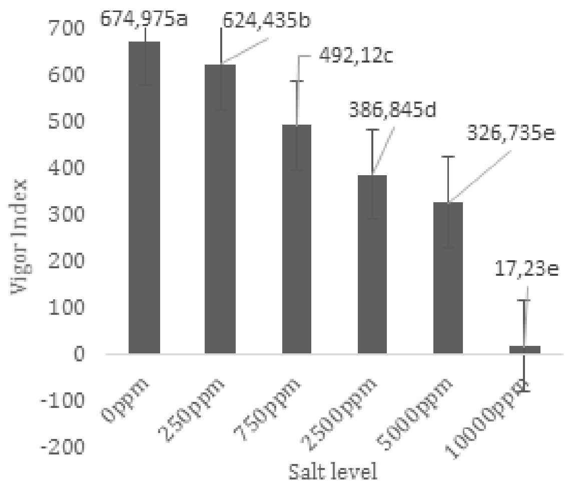

Figure 5. Effect of salt level on vigor index.

The effects of salt doses applied to birdsfoot trefoil on salt tolerance index are given in Figure 6 and it is determined that salt tolerance index decreases as salt dose increases and this decrease is statistically significant.

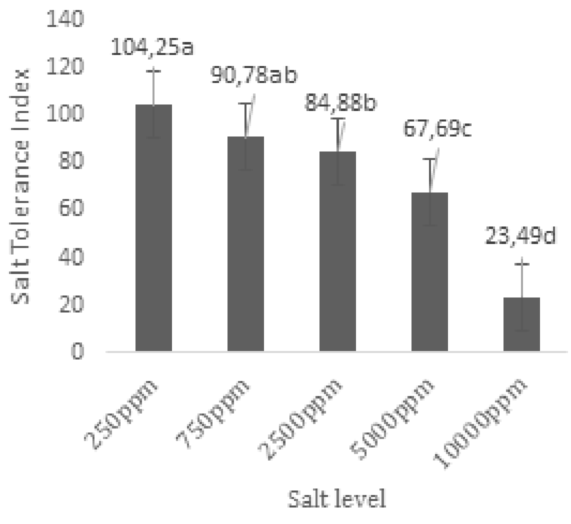

Figure 6. Effect of salt level on salt tolerance index.

While the highest level of salt tolerance birdsfoot trefoil was determined to be at a dose of 750 ppm (90.78) and 2500 ppm (84.88) salt, which are in the same statistical group with the highest 250 ppm (104.25), tolerance to salt at a dose of 10000 ppm was considerably reduced. It has been stated that when plants are grown in salty environments, the Na^+ and Cl^- ions they absorb accumulate in the roots, stems and leaves, and the accumulation of Na^+ and Cl^- in various organs of the plants slows down the development and seriously affects their tolerance to salt (Salisbury and Ross, 1992). Similar studies have also reported that increased salt concentrations reduce the salt tolerance index (Kökten et al., 2010; Avcı, 2019; Uslu and Gedik, 2020; Güleç Şen, 2021).

4. Conclusion

In this study, the effect of different salt concentrations on germination and seedling development parameters in birdsfoot trefoil was investigated. It has been determined that increasing salt concentrations significantly reduce the germination rate, germination index, germination energy, plumule length, radicle length, seedling length, seedling fresh and dry weights, vigor index, salt tolerance index (0 ppm) of the birdsfoot trefoil compared to control applications and increase the average germination time. When the findings are evaluated in their entirety, they show that the germination and seedling parameters considered together with the increase in salt concentration are also adversely affected, and this negative effect increases as the salt concentration increases. It has been observed that the germination rate of birdsfoot trefoil, especially at 10000 ppm, which is the highest salt concentration, is significantly reduced. The first negative effect of salt stress in terms of germination rate was seen after 5000 ppm, but it is thought that when plants complete germination and have poor development in their first development cycle, they may not maintain their subsequent vegetative development well and may cause a decrease in their yield. As a matter of fact, salt stress causes the sensitivity of plants to biotic stressors that they will encounter in their future development periods to increase. As a result of the study, although the germination rate in the birdsfoot trefoil can be tolerated up to 5000 ppm, it was seen that other parameters decreased as the salt dose increased according to the control application. It has also been determined that birdsfoot trefoil can tolerate salt doses of 250 ppm, 750 ppm, 2500 ppm. At the same time, it is thought that in the areas where the salinity problem of the studied variety is experienced, it will be important to determine the yield and quality performance in field conditions in terms of evaluating the salty areas. However, in order to make healthier recommendations, it will be useful to carry out new studies carried out in pot and field conditions of this study.

Author Contributions

The percentage of the author(s) contributions is present below. All authors reviewed and approved final version of the manuscript.

	S.B.	M.Y.	A.N.K.
C			100
D			100
S			100
DCP	25	25	50
DAI	25	25	50
L			100
W			100
CR			100
SR			100
PM			100
FA			100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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