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ARTICLE

The Influence of Induced Drought Stress on Germination of *Cenchrus ciliaris* L. and *Cenchrus setigerus* Vahl.: Implications for Rangeland Restoration in the Arid Desert Environment of Kuwait

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ABSTRACT

Drought impacts in arid desert ecosystems can result in decreased ecosystem productivity and biodiversity. Implementation of restoration projects in arid desert environments is largely dependent on water availability and soil moisture condition. This study investigated the influence of induced drought stress by using polyethylene glycol (PEG-6000) solution on germination viz. *Cenchrus ciliaris* and *Cenchrus setigerus* as the important rangeland species. The water stress potential treatments were 0 (control), –0.5 MPa, –1.0 MPa, –1.5 MPa, and –2.0 MPa. The extent of seed germination was severely affected by decreased water stress potential. As drought increased, the percentage of germination decreased in both *Cenchrus'* species. The water deficit at –0.5 MPa showed a significant ($P < 0.001$) reduction in the final germination percentage in the case of *C. setigerus* and *C. ciliaris* by 65% and 42.5%, respectively. At –1.0 MPa to –1.5 MPa, changes in intermediate germination were observed in *C. ciliaris* (from 35% to 17.5%, respectively) and *C. setigerus* (from 22.5% to 11.25% respectively). Higher levels of water stress (–2.0 MPa) prevented the survival of both species. Understanding the germination strategies of native desert plant species associated with drought stress and identifying favorable conditions during the germination process can be useful for restoration practices and rangeland management actions to improve desert ecosystems and maintain biodiversity.

Keywords: Arid ecosystems; Desert biodiversity; Drought stress; Desert restoration; Water stress potential; Seeds germination ecophysiology; *Cenchrus ciliaris* and *Cenchrus setigerus*; Polyethylene glycol (PEG-6000)

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1. Introduction

Among all complex environmental stresses, drought stress is considered the prime limitation affecting the ecological function and biodiversity of arid desert terrestrial ecosystems in that it negatively influences plant survival, reproduction, performance and net productivity [1]. The levels of drought severity and frequency can seriously alter the biodiversity of plant communities' composition and structure. Water availability and the amount of rainfall are directly responsible for multiple biological processes in arid desert ecosystems [2,3]. In view of the climate change crisis, it is postulated that the fluctuation in terms of rainfall and water scarcity will increase considerably worldwide [4], resulting in more severe consequences including changes in biodiversity, the extinction of endangered species, and the redistribution of natural biota. Therefore, understanding the plant adaptation strategies and eco-physiological responses to environmental stresses, specifically in the case of drought stress tolerance at the seeds germination level and seedlings stage, has become one of the major research emphases in determining not only the influence of climate change on ecological function, but also with regard to the restoration and re-vegetation efforts of disturbed arid desert ecosystems [5-7].

Seed germination and seedling establishment are the most sensitive stages in the development of biodiversity and natural plant communities' structure in arid desert ecosystems [8]. The development of native desert plants and their seed germination depends completely on the interaction between seasonal rainfall, soil moisture condition and seed vitality [8]. The seeds of native desert plants near the surface of the soil are highly susceptible to exposure to the robust environment of an arid desert system. In typical ecological settings, low soil moisture conditions and extreme temperatures typically found in desert ecosystems are the major factors in determining the success or failure of native vegetation establishment [9]. Higher levels of salinity however may also disturb seeds germination by producing an external osmotic potential preventing water absorption due to the impacts of sodium and chloride (NaCl) on the seeds' germination [10]. Alam [11] indicated that salinity in terms of NaCl has less influence than induced water stress (PEG) on germinated seeds rate due to the decrease in osmotic potential. Almansouri [12] suggested that seed germination is capable of eventually tolerating salinity stress but not drought stress.

Indigenous desert rangeland grasses including *Cenchrus ciliaris* L. and *Cenchrus setigerus* Vahl. are important perennial grass species in that they can grow vigorously on sandy, nutrient-poor and saline soils, whereas others will not survive. Both *Cenchrus* species are remarkably hardy, tolerating temperatures as high as 50 °C [13], low and infrequent rainfall, prolonged dry seasons, and the strong winds of arid desert ecosystems. In many areas around the desert rangeland of Kuwait, these species provide the sole forage for livestock during the grazing season and they can actively grow back even when heavily grazed. Nevertheless, a large number of the Kuwait native flora including both of these species are currently susceptible to extinction due to uncontrolled overgrazing, prolonged drought episodes, and the progression of desertification, all of which have reduced their availability in the desert rangeland. Expand current knowledge of the physiological germination requirements of the species by exploring seed responsiveness to changes in water availability during germination. The objective of this study was to investigate the influence of induced drought stress on physiological germination responses over time and the final germination of *Cenchrus ciliaris* and *Cenchrus setigerus* by using polyethylene glycol (PEG-6000) solution. Applying polyethylene glycol (PEG-6000) in terms of inducing drought stress on seed germination appears to be an optimum indicator when it comes to evaluating drought tolerance potential [14-17]. Understanding the germination strategies and seed responsiveness of native desert plant species in the face of drought stress and identifying the favorable conditions during the germination stage can be supportive to promote these species throughout the restoration programs, the re-vegetation of degraded rangelands, and the forage production system in the country.

2. Material and methods

2.1 Habitat location

The seed fascicles of *Cenchrus ciliaris* and *Cenchrus setigerus* were collected from the Al-Nuwaiseeb district in the Ahmadi Governorate in the south of the State of Kuwait (28.572°N 48.383°E) in June 2017 (**Figure 1**). The experimental site was about 50 hectares and the plant community of this location is dominated by several perennial desert types of grass including *Cenchrus* species, *Panicum turgidum* and *Pennisetum divisum*. The landscape is a flat desert plain with gentle undulations in hilly areas with a 1 to 3 percent slope. The soils are Typic Torripsamments, slightly calcareous (3%-8%), non-saline (ECe < 2 dS/m), slightly alkaline (pH 8.2-8.3), and with a sand content of over 90% [18]. The climate is that of a typical hyper-arid desert environment with two distinct seasons: Long, dry and hot summers and short-term winters (**Figure 2**). The highest temperatures can reach up to 50 °C during the summer with no precipitation. The rainfall occurs only during the winter months with the average annual rainfall varying from 110 mm/year to 150 mm/year [19].

Figure 1. Map showing the location of the seed harvesting area from the Al-Nuwaiseeb district in the Ahmadi Governorate south of the State of Kuwait.

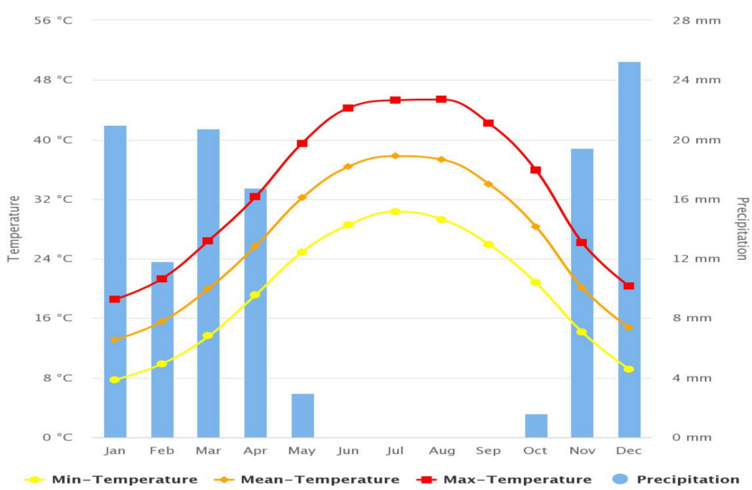

Figure 2. Mean monthly rainfall and temperature for the State of Kuwait from 1991-2020. Bars diagram: monthly rainfall. Lines: monthly minimum temperature, mean-temperature and maximum temperature.

Source: Climate Change Knowledge Portal (CCKP) [19].

2.2 Seed collection and preparation

Both *Cenchrus* species i.e. *C. ciliaris* and *C. setigerus* were identified separately. This is essential since they are relatively identical and usually distinguished by the color of the inflorescent and the hard bristles on the seeds heads (**Figure 3**). The seed heads were harvested by handpicking from healthy and vigorous wild plants. For each species, only mature and ripened seed heads were selected to ensure good quality and development of the seed germination. After collection, damaged and predated seed heads were separated, removed and discarded. Seed heads were pre-cleaned and allowed to air-dry by spreading them on a laboratory bench at ambient room temperature (25 °C) for three to four months. A long period of drying was used to increase the germination rate by eradicating germination inhibitors in the involucres. The seed fascicles of both species were carefully extracted and removed from the seed heads to obtain clean and sound seeds for experimental use. Seeds were desiccated (using silica gel) to remove excess moisture, labeled according to Kuwait Institute for Scientific Research (KISR) Seed bank registration number and placed in an airtight container.

(A) *C. ciliaris* (B) *C. setigerus*

Figure 3. The difference between mature inflorescence (seed head) of (A) *Cenchrus ciliaris* and (B) *Cenchrus setigerus* with several fascicles (seed units).

2.3 Seed germination and drought stress stimulated by PEG-6000

A drought-induced (i.e., decreased osmotic potentials) experiment was conducted in KISR laboratory to evaluate the water stress potential on seeds germination of *C. ciliaris* and *C. setigerus* using polyethylene glycol (PEG-6000) solution. One hundred dry healthy seeds from each species were used for the experiment. Seeds were germinated in 9 mm glass Petri dishes on a double layer of Whatman No. 3 filter paper and were moistened with 10 mL of solution with five different osmotic potentials. Twenty seeds of each species were placed in each Petri dishes and a distilled water (0 MPa) control treatment or polyethylene glycol solution was added to the Petri dishes. The PEG-6000 solutions were made up of distilled water to lower the water potential to one of the following water stress potentials: –0.5, –1.0, –1.5, and –2.0 MPa. The water stress potential solution was established using a PEG-6000 solution and was prepared as identified by Michel and Kaufman [20]. Petri dishes were hermetically sealed and then put in an incubator at 25 °C with a 12 h light/dark cycle to prevent evaporation. The germination rate was evaluated on the second day after the initiation of a 28 days trial. The number of seeds germinated was counted every 2 days and the final germination percentage was calculated. Seeds were considered to be germinated when a 1-2 mm long radical had emerged.

2.4 Experimental design and statistical analysis

The induced drought experiment involved a completely randomized design with four replicates of 20 seeds of each species, with five different osmotic potential concentration treatments (including a control). The data were statistically analyzed separately for each species using one-way ANOVA to determine differences among treatments. Significant differences between the means in terms of treatments were calculated to examine differences at $p \leq 0.05$. All the statistical analyses were performed using Genstat® software, version 22 (VSN International, 2022) [21].

3. Results

The time course of cumulative germination curves shows that both species decreased significantly ($P < 0.001$, d.f. = 19) with the decrease in osmotic potential (**Figure 4**). The highest germination percentage of *C. ciliaris* and *C. setigerus* was achieved in the control treatment (0 MPa) with 95% and 87.5%, respectively. The germination percentage was lower in various degrees of negative water potentials and did not attain complete germination. Overall, the maximum germination percentage observed in the control treatment represents the viability and non-dormant caryopsis per species used in the experiment. The germination of both *Cenchrus* species was observed to be intensive in the first 4 to 12 days following the initial start of the treatment. Across all water stress potential treatments, the probability of new germination was at a minimum by day 16 (**Figure 4**).

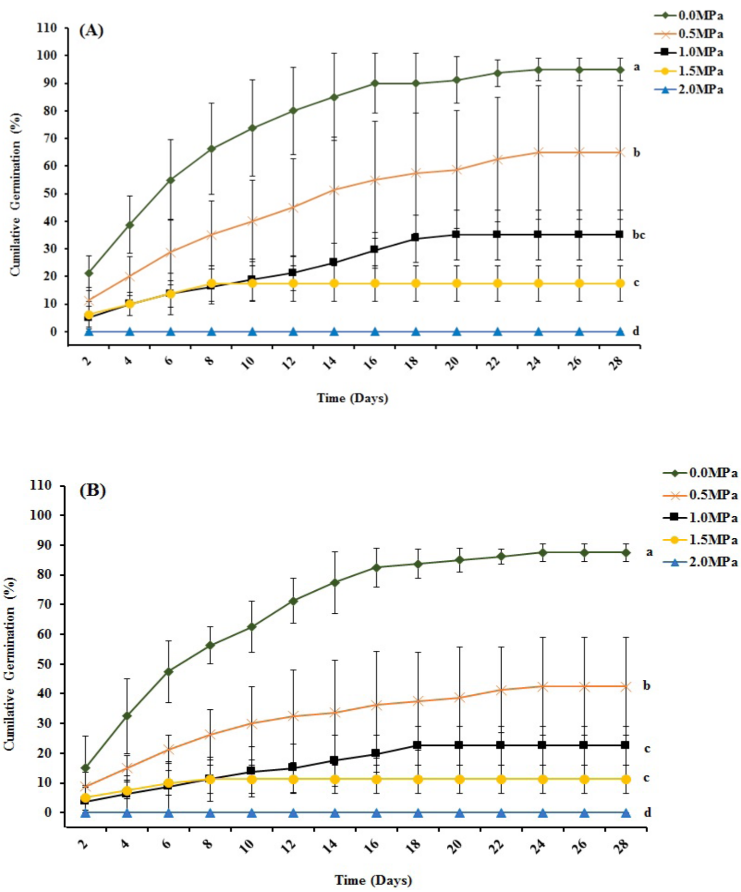

Figure 4. Cumulative mean percentage germination curves of (A) *C. ciliaris* and (B) *C. setigerus* against time and different osmotic potential treatments: 0, –0.5, –1.0, –1.5, and –2.0 MPa. Data are mean values (n= 4) for each species. Vertical bars (I) represent ± SD of the mean. Different letters indicate significance at $P < 0.001$. d.f. = 19.

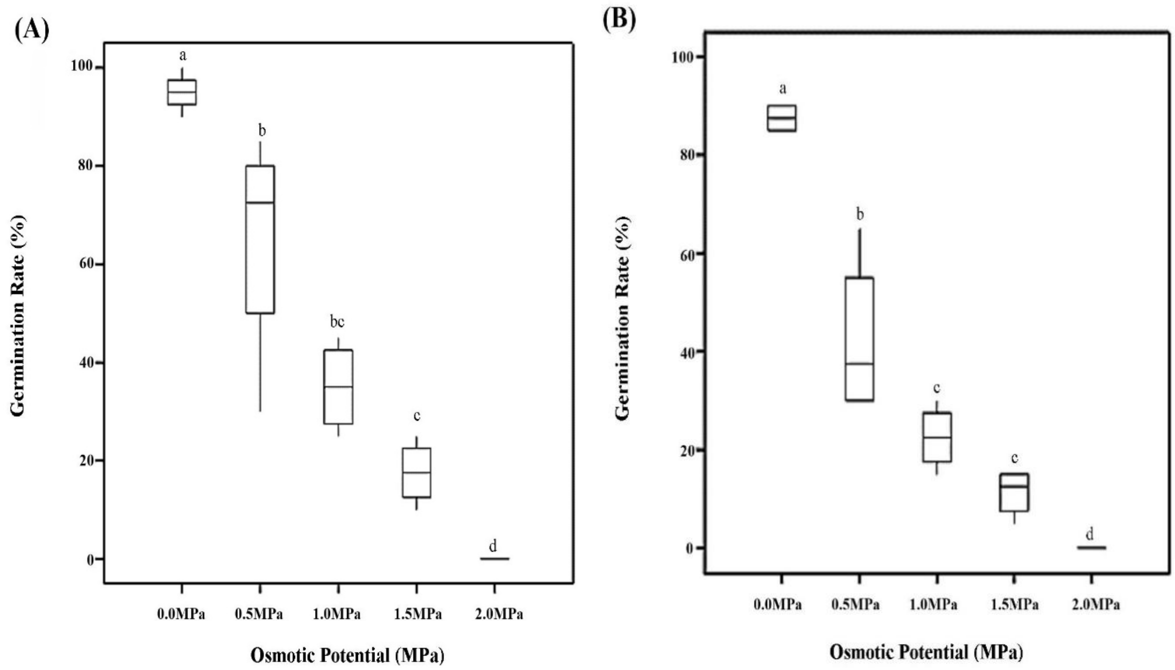

Figure 5. Box-plot diagrams showing effects of different osmotic potential treatments: 0, –0.5, –1.0, –1.5, and –2.0 MPa on final germination of (A) *C. ciliaris* and (B) *C. setigerus*. Data are mean values (n= 4) for each species. Vertical bars (I) represent ± SD of the mean. Different letters indicate significance at $P < 0.001$. d.f. = 19.

A water deficit at –0.5 MPa showed a significant ($P < 0.001$, d.f. = 19) reduction in the germination rate in both species, although the reduction was higher in the case of *C. setigerus* than *C. ciliaris* with germination percentages of 42.5% and 65%, respectively, compared to the control treatment. From –1.0 MPa to –1.5 MPa, intermediate germination rates were observed in both *C. ciliaris* (from 35% to 17.5%, respectively) and decreased even more in the case of *C. setigerus* than (from 22.5% to 11.25% respectively), while significant differences ($P < 0.001$, d.f. = 19) were found in both species at the two levels of water deficit treatments (**Figure 5**). At the lowest osmotic potential of –2.0 MPa no germination occurred in *C. ciliaris* and *C. setigerus* indicating that both *Cenchrus* species were completely intolerant to simulated drought stress (**Figure 5**).

4. Discussion

This investigation quantified the germination response of *C. ciliaris* and *C. setigerus* to induced drought stress by different levels of decreased osmotic potentials. The seed germination responses of both *Cenchrus* species were adversely affected by increased water stress. It is probable that water stress dehydrated the seeds and affected the germination percentage including varying patterns of seed response to water availability among various osmotic potential treatments [22]. The percentage of seed germination in both *Cenchrus* species strongly decreased at lower water potentials from –1.5 MPa to 2.0 MPa, suggesting the negative effect of water absorption by the seeds. These changing levels led to a decline in the vitality of the seed germination process. Drought influences seed germination, seedling survival, and the growth and reproduction of plants at different stages depending on the frequency and persistence of the drought stress [23]. Braga [24] indicated that decreased osmotic potentials may lead to several negative effects causing all parameters to decline (germination percentage, size and seedling weight), in both *Cenchrus* species seeds in that there was a decrease both were submitted to lower water potentials and there was a reduction in the germination percentage. Although the reduction in germination rates was significant at all water deficit levels compared to the control treatment, both *Cenchrus* species demonstrated a greater ability to tolerate and survive drought stress at –0.5 MPa, –1.0 MPa and even at –1.5 MPa. The capacity of these species to germinate at low water potential is commonly associated with adaptation to dry environments [25,26]. Native perennial grasses from arid desert ecosystems including these *Cenchrus* species are probably highly adapted to arid environments with low water availability ascertained by their capability to germinate at low water potential.

Previous studies have shown that seeds of the *Cenchrus ciliaris* were able to germinate in a wide range of osmotic potentials ranging from 0 to –1.6 MPa [27]. Other studies reported that the *Cenchrus* species was capable of germinating with regard to minimum osmotic potentials of –1.2 MPa to –1.5 MPa [28,29]. This study demonstrates that the seeds of both *Cenchrus* species collected from their natural population also had a broad array of tolerance to drought stress. The higher percentage of seed germination in both *Cenchrus* species and higher germination speed (3 days to 26 days) achieved in the control treatment (0 MPa) can be associated with the timing of the seed collection during the summer month (June) and the long period of drying at room temperature, suggesting that the seeds of these species may require the accumulation of more thermal time to stimulate germination. Both thermal and hydric conditions are more restrictive for germination in highly fluctuating environments of arid and semiarid regions [22,30]. An earlier investigation (Madouh) [31], on the summer matured seeds of *Cenchrus ciliaris*, *Cenchrus setigerus*, *Lasiurus sindicus*, *Pennisetum divisum* showed germination rates of 80% to 100% when seeds were fully irrigated with an interval of 3 days over a period of two weeks. The germination study was conducted under greenhouse conditions (25 °C ±2 and 70% relative humidity). It was also observed that the best time to collect healthy matured seeds of the above-mentioned species is during May-June. It is likely that the development of mature seeds of native desert plant species during the hot summer months of the desert environment, and their immediate response and fast germination followed by low precipitation levels of the winter months, can be used to procure sustainable longevity and persistence by using the limited resources of the desert ecosystem. Further investigation is suggested to associate the eco-physiological responses and adaptation strategies to drought, heat and salinity stresses of various native plant species important for arid desert restoration.

C. ciliaris and *C. setigerus* are perennial grass species highly adapted to arid desert ecosystems. They are an important native forage plant species specifically in the case of Kuwait and the Arabian Peninsula [32], where drought and high temperatures are the major critical factors influencing the natural desert biodiversity. Both of these *Cenchrus* species are exceptionally drought tolerant, and resistant to heavy grazing with fast recovery. Albeit, visual observation indicates that both of these species are likely to be susceptible to cold stress of the winter desert months by restricting their growth and reproduction. Cold stress can cause biomass reduction and the leaf blades and inflorescences to turn purple in *C. ciliaris* and *C. setigerus,* yet when cold stress is alleviated, the matured inflorescences changed to pale straw color or completely white in the case of the former specie. Parera et al. [33], reported that *Cenchrus* species are highly affected by low temperatures at all stages of their life cycle. Nonetheless, they are considered highly palatable and nutritious forages for all types of grazing animals [34] and highly digestible when green [35] and remain palatable at maturity [36]. Because of these physiognomy traits, these species are valuable native forage plants and have desirable qualities for use on degraded rangelands of these desert regions. In contrast, both species and particularly *C. ciliaris* have been introduced to different desert regions such as Western Australia [37], northwestern Mexico and the southwestern United States [27,38] as forage plants and for their fodder value. However, it has been reported that *C. ciliaris* has spread from forage grasslands to adjacent natural desert habitats and invaded native plant communities [39-41]. This fast spread of *C. ciliaris* to other habitats disturbs the ecosystem function and could be largely attributed to improved water availability and favorable environmental conditions. Ward et al. [42], indicated that insufficient soil moisture may not prevent the germination of buffelgrass (*Pennisetum ciliare*) which can respond to the adequate water potential of desert soil to as low as 6.3 mm. It appears that the germination physiological responses of these perennial desert grasses might be associated with specific environmental variables and local adaptation to regulate the seed germination process.

The results of this study strongly indicate that these *Cenchrus* species have the potential to germinate under lower osmotic potential conditions. Water stress may reduce the probability of seed germination, seedling survival and development because of the inadequate water availability and soil moisture content. Despite their inability to germinate under higher water stress treatment (i.e. –2.0 MPa), the seeds of both species are able to germinate and tolerate reasonable drought stress as low as –1.5 MPa of osmotic potential. In light of this, it is likely that the seed germination and seedling establishment of native desert plant species, particularly perennial grasses, can be successful at low soil moisture levels under field conditions providing that there is sufficient moisture at lower levels in the soil for growth establishment and development [43]. Overall, the data of this study provided evidence that both of these *Cenchrus* species demonstrated a wide range of tolerance to lower water potential in the case of arid desert soils, and this wide tolerance could be a beneficial mechanism for the restoration and establishment of degraded rangeland ecosystems and disturbed desert habitats. Understanding the influence of drought stress on the germination of native desert plant species and their eco-physiological responsiveness to various environmental stresses can be helpful when it comes to identifying the possibility of resistance mechanisms and adaptation strategies at the species-specific level in order to assist in the degradation, damaged and recovery management of an ecosystem.

5. Conclusions

The seeds of native desert plants tend to be tolerant of a wide range of drought stress. *C. ciliaris* and *C. setigerus* have demonstrated their flexibility when it comes to germinating in low water stress potential conditions, implying a rapid response to light rainfall events. Under adequate moisture conditions, the germination of both *Cenchrus* species' seeds can be vigorously enhanced, indicating that such seeds respond positively to water availability and can be synchronized with the alleviation of the drought stress period of the hot summer months of the desert environment. Consequently, knowing the seed germination strategies of native desert plant species associated with the face of drought stress, and identifying the favorable conditions during the crucial life stage of the germination process can be useful for restoration practices and for rangeland re-vegetation management actions. These may generate great benefits with regard to improving overall arid desert ecosystems and maintaining their natural biodiversity. Nonetheless, it is highly probable that these species may have invasive characteristics and can actively compete with other plant species over water availability and soil nutrients particularly when introduced to regions with more adequate environmental conditions.

Author Contributions

Dr. Tareq A. Madouh formulated the idea, experimental design, data analysis and writing of the manuscript. The author carried out the execution of the experiment, data recording and observations.

Conflict of Interest

The author declares that he has no conflict of interest.

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