

Activity of antioxidants on various crops by the inoculation of Mycorrhiza under drought stress: A review

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ABSTRACT

The drought stress in plants persuades some biochemical reactions in the plants. The plants themselves produce antioxidants which act as a defense system in plants. This review states the study of biochemical parameters on the crops with the response of mycorrhiza under the influence of drought. Drought tolerance is the mechanism in plants where the plant tolerates stress by producing antioxidants and develops some morphological and physiological changes. The plants were subjected to drought conditions and various biochemical parameters such as abscisic acid, super oxidase dismutase (SOD), and catalase (CAT) were figured out. The elevated concentration of antioxidants was spotted in the crop in the water scare situation which shows that plants can tolerate water stress and this change brings positive results in stress condition. The antioxidant's activity such as CAT and SOD were shown high content whereas ascorbate acid content showed positive results with the symbiosis of Arbuscular mycorrhiza fungi (AMF) in the stress condition. The present review investigates the activity of antioxidants with the inoculation of AMF in drought and well-water conditions.

1. INTRODUCTION

The antioxidants participate as a prime substance in the plants [1]. The building of reactive oxygen species (ROS) in bulk amounts can trigger plant metabolism [1]. This situation arises in drought stress [1]. However, at the same time, the plants also produce compounds that stop the oxidation process and acquire themselves as a defense system to drop the level of free radicals such as O₂⁻, OH⁻, and H₂O₂; the improvements of water content in tissue are done by major osmolytes such as proline, glycine, betaine, and sugars [2]. Drought stress produces a negative effect on the plants. It knocks the development of the plant, growth, and upgrade the richness of the soil [1]. Water stress activates the loss of water and decreases the water potential which will lead a reduction in turgidity of the cell. The production of abscisic acid (ABA) on the plants leads to reduction of loss of transpirational water by closing of stomata in water-deficient condition [2]. Intensity of drought will increase due to the long spell of drought. The osmotic adjustment leads to lower the root-shoot ratio, modification of cell wall. Apart from this, the activation of antioxidant system also occurs [3]. The plants when

performed the photosynthesis like the diffusion of gas barrier which can deplete the intercellular CO₂ concentration. When the reduction of CO₂ takes place, the activation of ribulose -1-5-biphosphate oxygenate undertake place. This leads the proffering of H₂O₂ (hydrogen peroxidase) in peroxisomes [4]. Mycorrhiza also plays a role in the sheltering the host plants in moisture stress.

It takes part in water absorption by extra radical mycelium, improvement of nutrient acquisition, defense system of plants. However, Arbuscular mycorrhiza fungi (AMF) plants also adapt the host plants morphologically and improve the structure of soil through the spread of hyphae. Furthermore, molecular changes in plants are also observed. The levels of ROS are dynamic during colonization of fungus. The generation of hydrogen peroxidase in mycorrhiza-contained cortical cell ensures the colonization of AM fungus in roots, but the production of H₂O₂ is transient and eliminates by building of super oxidase dismutase (SOD), CAT, and carotenoids. The study on *Medicago truncatula*, the accumulation of hydrogen peroxidase in arbuscular containing root of plants has observed but its roots were less branched. When hydrogen peroxide is scattered the hyphal wall of AMF branches, the fungus started senescence. The AMF inoculation raises the level of antioxidant in plants by 16% [5]. When sesame plants are come under drought stress, *Funneliformis mosseae* and *Rhizophagus irregularis* both increased the activity of ascorbate peroxidase (APX), phenylalanine ammonia-lyase (PAL). Apart from this, the content of total soluble sugar also high in plant [6]. In addition

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to this [7] in plant chicory, the level of antioxidant like SOD increases and reduced the accumulation of hydrogen peroxidase. Similarly in lettuce, symbiosis of AMF improved the concentration of carotenoids and anthocyanins in drought stress condition [8].

When there is more gathering of antioxidants enzymes, it alleviates the toxicity on lipids and DNA [9]. Therefore, AMF plants have more endurance than non-inoculated plants.

2. DROUGHT STRESS IN PLANTS

Drought stress is the momentous phase where diverse changes included the morphological, physiological, and biochemical responses are observed in the plants [Figure 1]. The stress in the plants leads the reduction of growth. It decreases the root length as well as shoots length [10,11]. It also reduces biomass in plants. Flowering as well as fruiting of plants is also affected. The physiological changes in plants are also observed such as the photosynthesis rate will decrease, the osmotic balance gets disrupted. Biochemically, the plants are also affected by the level of osmolytes like content of proline which can change according to the condition. The defense system like antioxidants which shows changes in the concentration as compared to the normal plants which cannot face any stress condition [12,13].

2.1. Drought Tolerance: Insight Mechanism

The plants adapt itself in stress condition and cope up the effect of stress. Moisture stress changes the plant by physiologically and biochemically as well as morphologically. Drought avoidance is the mechanism by which plants can adapt in environment [14,15]. The deep and thick roots are the characteristics feature of the drought avoidance. The biomasses of roots as well as its length are increases which help the crop to avoid itself from drought [16]. Talking about drought tolerance in plants glaucousness also called waxy loom is the main characteristics for drought tolerance. These desirable traits help the plants to maintain the potential of water in tissues [17]. In wheat, this trait helps to increase the efficiency of water. This characteristic does not affect the harvest index of plants as well as total water use of plants. Glaucous leaves give more advantages to plants as compared to non-glaucous leaves. The glaucous leaves are cooler than normal leaves. The temperature of this waxy leaves is 0.7° cooler than non-waxy leaves. The other advantages of these leaves are that the rates of senescence of leaves are less which is higher in non-waxy leaves. The

mechanism where plants lower the temperature by 0.5° the period of grain filling will increase up to 3 days or more. However, the yields in this condition are not increases. The stoma plays a crucial role for transpiration of plants [18]. The holes that are present in the surface of leaf became the main trail for entry of carbon dioxide. When the water stress occurs, the plant follows a mechanism of closure of stomata. This closing of stomata helps to decrease the water potential of leaf. This closure also lowers down the oxidative damage and assimilation of carbon. This feature increases the temperature of canopy [19]. Other traits like hairy leaves which decrease the leaf temperature of leaves and also the transpiration [20]. The hairiness of leaves decreases the water loss and increases the reflectance of light. Other characteristic like leaf pubescence helps the plants from heat. This is xeromorphic trait found when drought stress occurs. These types of mechanism which are establish in the plants assist the plants to prevail the moisture stress.

3. BIOCHEMICAL REACTION IN PLANTS UNDER DROUGHT STRESS

When plants are affected with drought, the various biochemical changes are occur. The plants result in accumulation of proline, solutes, free radicals, and hydrogen peroxides which result in the destruction of its cell metabolic functions [21,22]. The antioxidants include SOD and catalase (CAT) which also active in plants to protect from stress which makes various positive changes in crops [22]. The function of antioxidants not only the defense mechanism but also maintains redox balance during oxidative stress [23]. The moisture stress in plants causes the ROS production. This ROS cause the cell to destroy their machinery. ROS contains singlet oxygen, hydrogen peroxide, superoxide, and hydroxyl peroxide [24]. As hydrogen peroxide is the signal molecule which is produced during abiotic stress like drought. The removal of H_2O_2 is done by the ascorbic acid which is very useful for the protection of the plants. This ascorbic acid is useful for lower down the level of ROS in plants. The antioxidant mechanism is not much as simple because it requires the enzymes called as APX which is catalyzed to proceed the reaction used for elimination of H_2O_2 in cell. This APX known as APX mainly found in chloroplast, mitochondria. In oxidative stress, the molecules of hydrogen peroxide are produce. Hence, the plants face the drought conditions, the more content of hydrogen peroxide has been observed [25].

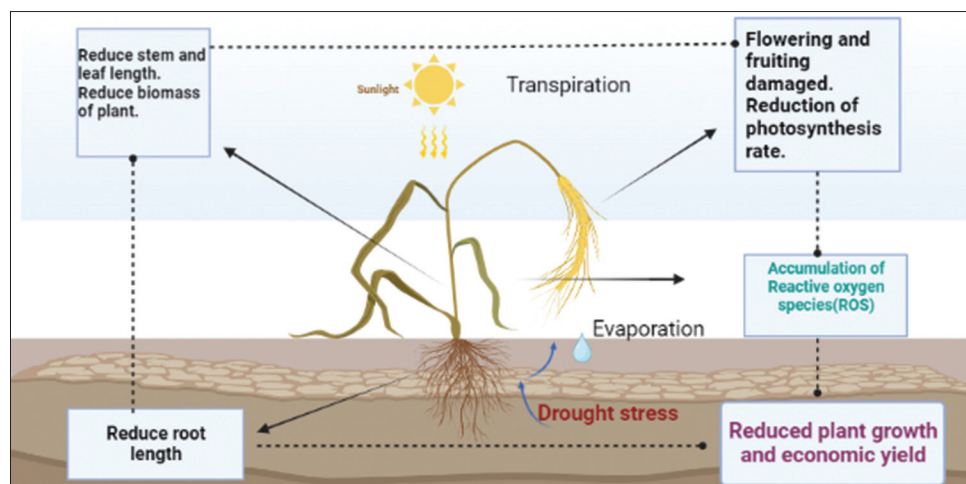


Figure 1: Illustration of drought stress in plants.

4. AMF-INOCULATED PLANTS IN DROUGHT STRESS

Mycorrhiza permits a mutual relationship between higher plants and the roots of fungi. Arbuscular mycorrhiza is also known AMF or Vesicular arbuscular mycorrhiza. There are many species of mycorrhiza which make the contribution [26]. The endomycorrhiza which is one of the type of AMF can penetrate in the cortical cell of plants and form structure. The structure is known as Arbuscular which is branch like structure and vesicles which is the storage organ [27]. The soil structures and stability are also improved by mycorrhiza. Apart from this, mycorrhiza can provide the plant nutrients like nitrogen and phosphorus to host plants. Mycorrhiza produces the antibodies and hormones which decrease the prevalence of disease and increases the production of plants [28]. The mycorrhiza gives protection against stress like drought and salt and it increases growth and productivity of the crops [29] [Figure 2]. From the point of morphological, physiological, and biochemical, the plants show better response as compared to normal plants [30]. Biochemically, the concentration of proline gets affected [31]. The antioxidants defense system is also affected by the mycorrhiza association. In rice plants, the mycorrhiza also shows better result in drought stress condition. The researches have shown that if plants are affected in drought stress, the morphologically physiologically [32] and biochemically [33] shows better result as compared to non-AMF plants in drought stress conditions. As different researches are conducted by scientists on the rice with several species of mycorrhiza, in one research where the scientist conduct a research with one species known as *Glomus intraradices* and observes the result at 70 days after transplanting (DAT) which seems to be positive. After 70 DAT, the AMF colonization increases. Similarly, the increment seen as the days passes. As the colonization increases, the effect of AMF in plants which are positive is also increases [34].

The rice with AMF has higher photosynthesis efficiency than without AMF plants. When the rice plants are imposed to drought stress, the photosynthetic activity has been affected. As the study suggested that when the plant in drought condition is more vulnerable to injury as compared to AMF plants. Growth system is affected and efficiency of CO₂ in drought condition is also increasing in AMF plants. This damage causes the production of harmful chemicals in plant cell [35]. In this process, colonization by AMF makes the improvement in establishment of diverse network of hyphae and also secrete the glomalin. This secretion provides assistance in uptake of water and

nutrient and therefore increases the structure of soil [36]. Various studies have been there which suggested that AMF itself develops drought adaptive strategies by extra radical hyphae. This impacted the plant mechanism which effects other rate of photosynthesis, hydraulic conductivity of root, and architecture of root [37].

The response of AMF composes the multifaceted mechanism of drought-responsive genes, along with activation of different metabolism pathways [38]. The amelioration of drought by AM symbioses which are engage in up and down-regulation of various biochemical and physiological pathways. The amendment of regulation of water by AMF has occurred in host plant by generation of hormonal signal and prompting of osmolytes. The enhancement of maize growth has been studied by the symbiosis of AMF. This was reported in drought-sensitive varieties in maize. This positive impact of symbiosis by AMF was correlated with better efficiency of photosystem II, stability of membrane, and lipid peroxidation [39-41].

5. ACTIVITY OF ANTIOXIDANTS IN MOISTURE STRESS

Drought stress is emerging problem due to less water availability. When there is a stress-like condition, it occurs the ROS active in plants and its accumulation leads to the damaging to the cell. When this ROS is produced, the production of antioxidants takes place which results in a lower level of ROS in plants. The antioxidants such as SOD, hydrogen peroxidase, and CAT these all antioxidants reduced the level of harmful ROS and mitigate the damage which are caused by lipid peroxidation [Table 1]. Therefore, the antioxidants shield the structure of the cell membrane and also save from moisture stress [42]. Stress-responsive genes have a role in osmotic regulation and aquaporin's which are manipulated in drought stress and intensify the tolerance of drought in plants [43,44]. An antioxidant like SOD is also called as a metal enzyme. This enzyme can eliminate the superoxide anion free radicals. As a result, the hydrogen peroxide is converted into the water by the activity of the CAT enzyme [45]. The plant in water-deficient conditions closes its stomata and reduces the loss of transpiration. When the ROS produced, the plants are also producing antioxidants which are very useful for plants in condition like drought stress [46].

The antioxidants such as CAT, SOD, and APX are generally produced. According to different species of plants the concentrations of enzymes are varies. Taking to the nature of CAT this enzyme is heme contains enzymes which are used to catalyze the H₂O₂ into oxygen and

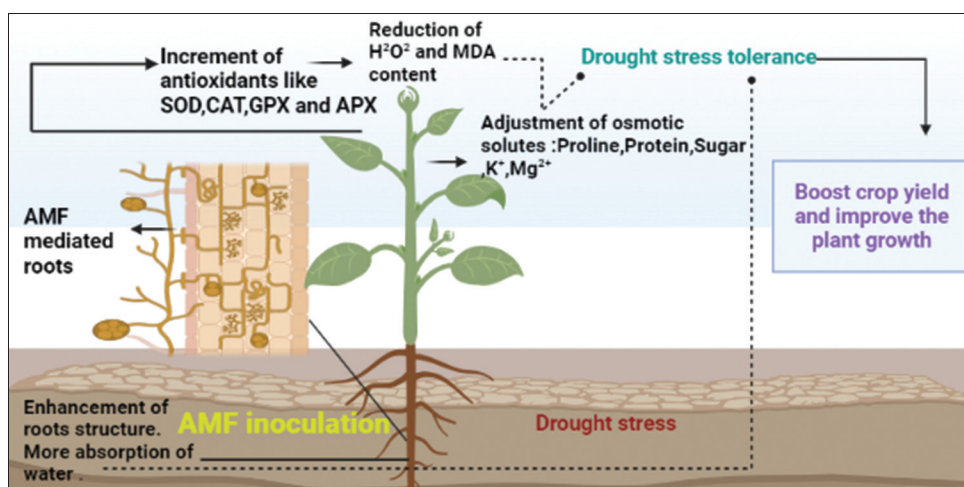


Figure 2: Mechanism of drought tolerance with Arbuscular mycorrhiza fungi in plants.

Table 1: Antioxidants activity in various crops with response to AMF under water deficient conditions.

Crops	Drought stress	Antioxidant activity	References
Wheat	At 50% field capacity	Reduction of Reactive oxygen species and improvement of CAT, SOD, Ascorbate peroxidase (APX), Glucocorticoid protein (GR)	[75]
Tobacco	At 30% field capacity	Mycorrhiza reduced the level of MDA (Malondialdehyde) and improved APX, CAT, SOD activities	[68]
Soybean	Drought stress was given at pod and seed development stage by skipping irrigation	AMF reduced the level of MDA and improved APX, CAT and SOD activities. Enhancement of P5CDH (Δ^1 Pyroline-5-carboxylatedehydrogenase)	[76]
Sorghum	Skipping of irrigation was done to create moisture stress	AMF decrease the level of ROS and raise the level of CAT, POD (peroxidase) activity	[77]
White clover	At 55% field capacity	Mycorrhiza decreases the gathering of MDA and raise the level of CAT, SOD activities	[78]
Date palm	At 25% field capacity	AMF ameliorate the stability of membrane and high the content of CAT and SOD activities	[79]
Orange	At 55% field capacity	Reduction of MDA and improvement of CAT and SOD activities by AMF	[80]
Ryegrass	At 25% field capacity	AMF lower the assembling of MDA and improvement of CAT and SOD activities	[81]
French bean	Drought stress was inflict by retaining the irrigation for 5 days	Mycorrhiza boost the activities of CAT, SOD and GSH (Glutathione synthetase)	[82]
Tea	At 55% field capacity	AMF lower the level of MDA content	[83]

AMF: Arbuscular mycorrhiza fungi, CAT: Catalase, SOD: Super oxidase dismutase.

hydrogen in the ROS production. The CAT acts as a ROS scavenger and detoxifies the effect of ROS in plants. The research had done to check the activity of CAT on the crops such as wheat, beans tobacco, rice, and cotton. The rice in drought stress also produces the ROS and plant itself produced the CAT. The research had done by four varieties of rice Xiangnou no.1 and Zimano as drought-sensitive varieties and Xianghongxian no.2 and IR 50 as drought tolerant varieties. The result can change according to the types of cultivars. The concentration of CAT in rice sensitive variety was more as compared to rice tolerate variety [47].

The stress condition where AMF is colonized in plants, the production of antioxidant takes place. This enzymatic action acts as a defensive system which is mediated by AMF [48-51]. AMF plants steeply high the antioxidant content in plants by 16%. The study of sesame showed that the mycorrhiza species such as *F. mosseae* and *R. irregularis* increase the percentage of APX, polyphenol oxidase, CAT, PAL activities, and total soluble sugar content. The same study was observed by [7] who explain that the more activity of enzymes in chicory plants by the same species of mycorrhiza [8]. It was further observed that the more antioxidants in lettuce like carotenoids and anthocyanin cause more improvement in water-deficient conditions than well-watered conditions. The high content of antioxidant in mycorrhizal plants at the influence of moisture stress alleviate the toxicity effect on lipids, proteins and DNA and it causes high outcome in the functioning of plants under stress [9]. Despite of this fact, the mycorrhizal plants are more adaptive to tolerance than non-AMF plants. It also activates the secondary metabolites such as anthocyanins, flavonoids, and total phenol compounds in contrast to control plants which unveil to water deficit condition in the soil [52]. As per the report suggestion in *Zea mays*, the total phenol content was increased by *Glomus versiforme* in water-deficient condition which shows that the AMF mediating phenol acts as an active scavenger of ROS and this will protect the cellular structure of host plant [53]. Various study has shown that AMF plants increase the carotenoids level in *Aloe barbadensis*, *Citrus aurantifolia*, and *Ricinus communis* in water less condition [54-56]. When the

changes in ROS occur by AMF, plants produce enzymatic and non-enzymatic antioxidant activities in drought stress condition.

This will protect the organism from the damage which is caused by oxidative stress. It was observed that inoculation of AMF with *Funneli formismossea* made high hydrogen peroxide effluxes in plant root, it was found in the root meristem zone in orange under water shortage conditions. In addition, extra radical hyphae of mycorrhiza take part in the efflux of H_2O_2 , it is due to hyphae of mycorrhiza consist of aquaporins, which are used to pass the hydrogen and hydrogen peroxidase [57]. However, colonization by mycorrhiza enhanced the net Ca^{2+} influxes which are found in the elongation zone of the root in trifoliolate orange under the water stress. These influxes of Ca^{2+} act as a downstream component in the H_2O_2 signaling pathway. The invasion of calcium is generally related to root efflux of hydrogen peroxidase, which suggests that plant having AMF influx of Ca^{2+} cause less H_2O_2 production. In another study of [58], colonization by AMF concentrated more levels of calmodulin (CaM). This level of CaM may participate in the regulation of superoxide dismutase activity along with CaM binding protein as well as the activity of CAT. It was also shown that the activity of Cu/Zn –SOD and Mn–SOD which was increased by mycorrhiza in drought stress. On the other hand, it showed the opposite result in well moisture conditions. This study was concluded that the stress of water raises the activity of AM which can activate the over-expression of SOD isozymes, and lead a less concentration of ROS in the host plant. If the plants faces drought stress and the same plants are associated with mycorrhiza, the levels of SOD are higher in treated plants. The generation of superoxide also begins as the plants face drought stress. The crops such as lettuce, alfalfa, bean, coffee, maize, tobacco, and rice have undergone the change when plants face drought stress. The levels of SOD can change according to the nature of the variety of crops. The concentration increases when plants are inoculated with mycorrhiza and it was decreased in plants without the mycorrhiza. The research found that 93% content of SOD was increased in plants that had mycorrhiza [59].

The transcriptomic analysis also has done where the level of SOD concentration had increased. The high level of antioxidants will expand the photosynthetic activity in plants. This will alter the plant biomass as plants are associated with mycorrhiza [60].

5.1. APX Content in Mycorrhizal Plants

The ascorbate concentrations in plants are increasing when the plants are in drought stress but in rice plants, the result was the opposite as the study suggested. As the ascorbate is used to remove the hydrogen peroxide and it acts as an electron donor in the removal of hydrogen [61].

5.2. ABA in AMF Plants under Drought Stress

In drought stress, mycorrhiza plants prompt the biosynthesis of ABA and as a result, the levels of ABA in plants are increases. This causes the stomata to close to reduce the loss of water by transpiration [62,63]. In contrast to this, a decrease the level of ABA production, or a change in the signaling pathway of ABA has been noticed in the roots of *Glycyrrhiza uralensis* [64] when the roots are not under water-deficient conditions. It is evident that a low level of ABA indicates tolerance against moisture stress in mycorrhizal plants. This tolerant level upgrade the performance and metabolite function of the plant. Another non-enzyme is glutathione which is very important in the removal of ROS [65]. The antioxidant glutathione content is also high in plants that face drought stress [66].

5.3. Effect of H₂O₂ in Drought Stress with Mycorrhiza

The plants which are associated with mycorrhiza are less prone to oxidative damage and they also produced less amount of H₂O₂ [67]. The major finding suggested the difference in the level of hydrogen peroxide in plants with mycorrhiza and plants without mycorrhiza in drought stress conditions. In the plants which were having AMF, the concentration of hydrogen peroxides had decreased while in other plants, it has been increased.

5.4. Role of Phenols in Plants under the Influence of Mycorrhiza in Drought Stress

Phenols also act as major antioxidants in plants and perform a major role in the defense mechanism in drought stress. When the plants are subjected to drought stress with the association of AMF, the gathering of the phenolic compound increases. As a result, the tolerance of drought is improved and leads to strengthening antioxidant fortification [68]. Polyphenol compounds are the scavengers that attack on free radicals. It also safeguards the cell from damage and makes the cell to function properly. It was reported that plants that have an inoculation of AMF have more assemblage of 50–60% of phenolic compound. This lodgment of phenol makes the plant more tolerant to the stress of water. The phenols and flavonoids in AMF plants adapt to help the plants to adapt in drought stress and turn on the cellular signaling of the plant [69,70]. The enzyme PAL is there in plant and plays a major role in the metabolism of the plant. It also protects the plant from biotic and abiotic stress [71]. By the inoculation of AMF in plants, there is an improvement of PAL enzyme. The upregulation of PAL boosts the growth of plant and makes better protection in drought stress [72,73].

Furthermore, AMF is also high level of total glomalin-related soil protein (T-GRSP), easily extractable GRSP (EE-GRSP), and difficultly extractable globin protein (DE-GRSP) in moisture stress. This protein which has different carbohydrates, protein, and other aliphatic compound worked as a connector to bind the particles of the soil. The increment

of T-GRSP, EE-GRSP and DE-GRSP in drought stress increases the chances of uptake of water and tolerance of plant in drought stress [74].

6. CONCLUSION

The AMF is also called VAM and is associated with various functions, but the review stated that the antioxidants engage an essential part in diminishing moisture stress. The antioxidants such as CAT and superoxide dismutase activity are shown high content with the inoculation of AMF in stress conditions in any type of crop. On the other hand, ascorbate acid content showed more positive results with the symbiosis of AMF in the stress condition. In stress conditions, hydrogen peroxides content is less accumulated in the plants which state that AMF brings positive changes. Therefore, it is concluded that the inoculation of AMF in plants which are effective in the changes such as biochemical and physiological. The plant with AMF protects the plants and produces positive changes which help the plants to cope the water stress and this change is remarkable in the production of more yield under the drought stress condition. The evidence and studies suggested that AMF can bring changes not only physiologically but also biochemically too. The antioxidants bring desirable changes because the antioxidants have the ability to cope up the stress. Further researches are going on to bring constructive result.

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All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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The authors declare that there are no conflicts of interest.

11. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

12. DATA AVAILABILITY

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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