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# Influence of *Trichoderma harzianum*- seed Coating on the Biochemical Characteristics of Wheat (*Triticum aestivum* L.) Under Salt Stress

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**Abstract**

Salt stress is one of the main limitations to *Triticum aestivum* productivity all around the world. An experiment was conducted to assess the effect of *Trichoderma harzianum* seed coating on germination and seedling development of wheat under salt stress (60 and 120 mM NaCl). The seeds of six wheat cultivars, namely Shafaq-06, Punjab-11, Millet, Seher, Pirsabik, and Aari were seed coated with *Trichoderma (*taken from NARC Pakistan) at the rate of 2 x $10^7$ CFU using PelGel for 24 h. After air-drying at room temperature for 12 h, the coated seeds were sown in small pots. Experimentation was laid out in a completely randomized design with three replications. The data for various biochemical attributes were collected after 30 d of germination to test the seed and seedling vigor, respectively. *Trichoderma harzianum* seed coating reduced the amount of hydrogen peroxide, catalase, Malondialdehyde, and increased protein content, Ascorbate Peroidase, and total phenolics under salt stress advocating that its use is effective in the cultivation of crops in saline areas because it inhibits oxidative damage by triggering various phenolic compounds and scavenging proteins.

***Keywords:*** Wheat, Seed coating, Salt stress, *Trichoderma*

## Introduction

All around the globe, more than twenty percent of the cultivable land is harmed by salt stress. Due to climate change and anthropogenic activities, the salt affected area is tended to increase day by day [1]. The problem of salt stress remains prevailing and drastically affects morphological, physiological, and growth attributes of various crops in both arid as well as semi arid regions [2-5]. A high rate of surface evaporation, poor cultural practices, and low precipitation rate are the most prominent factors that lead to increased salinity [6]. Increased salinity level influences plants at all levels, such as the death of the whole plant and a decrease in productivity [7]. pH or electrical conductivity (EC) value of soil exceeding 8.5 $dSm^{-1}$ harms crop to such an extent that it is not feasible to cultivate without soil amendments [8].

Among different crops of commercial importance, wheat is also being victimized by salt stress [9]. Wheat is well known as a staple food since about 35% of the total world's population depends on it for its nutrition purpose, and this demand is going to increase with a rapid increase in the human population [10]. In this scenario of increasing requirement of the wheat crop, it's utmost necessary to prevent this crop from damages

of salt stress. This can be attained by physiological, genetic, as well as agronomic interventions, crop modeling, and new initiatives of climate monitoring [11]. The latest farming approaches have one side effect of causing pollution and disturbing human health [12]. In this case, the use of antagonistic micro-organisms for diseases and pests management can be a viable alternative method. Out of all fungal antagonistics, *Trichoderma* species have been well known since the 1930s. They are considered effective means of different types of air borne and soil borne diseases, as well as eradicating biotic and abiotic stresses. These fungal species are also considered to enhance overall plant growth and development. *Trichoderma* is soil borne fungus belonging to the ascomycetes group and generates green colored spores. Currently, 1100 strains have been determined from seventy five families of this fungus [13]. The latest four new species have been identified, *T.odoratum, T. aesterinium, T. pseudobritdaniae,* and *T.henanense* [14].

*Trichoderma gamsii* has been proved to be very effective in combating the Fusarium Head Blight disease of wheat [15]. *T. harzianum* has been reported to combat widespread rust disease of wheat [16]. To control many other biotic disorders such as wheat blast, spot blotch, cereal cyst nematode, aphids, *Trichoderma* strains have been found environment safe, feasible, and economical tool [17]. Along with biotic diseases, abiotic stresses have also been reported to combat by various species of *Trichoderma*. Crops grown nutrient deficient soil showed appropriate growth because of the addition of *Trichoderma* as a biofertilizer [18]. Rani-Th-14 has shown maximum drought tolerance in treated seeds as compared to control [19].

In relation to the above discussed potential benefits of different *Trichoderma* species to combat various biotic as well as abiotic stresses, the current study has been planned to evaluate the efficacy of *Trichoderma harzianum* seed coating of six wheat cultivars to eradicate salt stress.

## Materials and Methods

The current experimentation was performed at the Department of Botany Nusrat Jahan College, Rabwah Chenab Nagar Pakistan, to evaluate *Trichoderma harzianum* seed coating's effect on inducing tolerance against salt stress in wheat. Seeds of six wheat cultivars, namely, Shafq-06, Punjab-11, Millet, Seher, Pirsabik, and Aari obtained from NARC, Islamabad, Pakistan, were used for present experimentation. All seeds were surface sterilized using mercuric chloride and then seed coated with *Trichoderma harzianum* at the rate of 2 x $10^7$ CFU using PelGel (bio priming binder) for 24 h. After seed coating, seeds were air dried for 12 h at room temperature. Then seeds were sown in small pots of sand. Salt stress (60 and 120 mM NaCl) was applied after one week of sowing. The control set of seeds was not given salt stress but was seed coated, while the "non *Trichoderma"* set was without coating, and salt stress was applied and "stress set" contained seed coated seeds with salt stress. Seeds germination was monitored for thirty days daily and was provided essential nutrients at routine intervals as per requirement. After 30 days, germinated plants were harvested.

### *Preservation and Centrifugation*

After rinsing, plants (roots and shoots) were separately preserved and ground in 50 mM potassium phosphate buffer. After preservation, roots and shoots samples were centrifuged for 15 min at 14000 rpm. Then following biochemical tests were conducted.

### *Total Soluble Proteins*

The concentration of total soluble proteins was examined using the method [20] with few amendments. The 1 mL supernatant was reacted with 2 mL Bradford Reagent and incubated for 15-20 min, then reading was measured at 595 nm. Total soluble proteins were computed using a standard graph. Bovine Serum Albumin was used as standard.

### *Catalase Activity*

Catalase working was observed according to the method [21] with few amendments. 3 mL CAT reaction solution consisted of 50 mM phosphate buffer (pH 7.8), 5.9 mM hydrogen peroxide and 0.1 mL enzyme extract. The reaction was triggered by adding hydrogen peroxide to the reaction solution. CAT working was examined for 3 min, after every 30 sec at 240 nm with a spectrophotometer. One unit of Peroxidase (POD) activity was defined as an absorbance change of 0.01 U $min^{-1}$.

### *Ascorbate Peroxidase (APX) Activity*

The APX working was measured using the reported method [22]. The reaction solution (1600 µL) comprised 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM $H_2O_2$ and 400 µL of enzyme extract. The absorbance was taken at 290 nm against the blank, and the enzyme activity was represented in $Umg^{-1}$ protein (U=change in 0.1 absorbance $min^{-1}$ $mg^{-1}$ protein).

### *Malondialdehyde Contents*

Malondialdehyde (MDA) was determined in accordance to the method proposed by [23]. In the 2 mL TCA, added 2 mL of 0.6% thiobarbituric acid. It was heated at 100 $^oC$ for 20 min in a water bath. After heating, immediately cooled for 20 min and then centrifuged at 10000 rpm for 10 min. The resulting color was taken at 450 nm, 532 nm, 600 nm on a spectrophotometer.

### *Hydrogen Peroxide Determination*

$H_2O_2$ concentration was determined according to the protocol [24]. 0.1 mL of supernatant was added to 0.1 mL of 10 Mm potassium phosphate buffer (PH 7.0) and 1M IKI. The absorbance was taken at 390 nm. The contents of $H_2O_2$ in the tissue were given a standard curve constructed using a series (0, 20, 40, 60.80, and 100 µm) of analytic reagent grade $H_2O_2$.

### *Total Phenolics Content*

Total phenolics were evaluated with the help of Folin-Ciocalteu protocol [25] with few amendments. Samples were mixed with 5 mL Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 mL (75 g/L) sodium carbonate. The tubes were shaken for fifteen sec and were permitted to stand for 30 min at 40 ℃ so that the color develops. Then absorbance was taken at 765 nm on a spectrophotometer. Total phenolic content was represented as mg/g tannic acid equivalent using the following equation based on the calibration curve: $y = 0.1216x$, $r^2 = 0.9365$, where x was the absorbance and y the tannic acid equivalent (mg/g).

## Results and Discussion

### *Total Protein Content of Root and Shoot*

In the case of total protein content in shoots of all wheat cultivars under study as well as roots, an increase was obtained under salt stress at both levels (60 mM and 120 mM) as compared to non *Trichoderma* set and control (Fig. 1), indicating that *Trichoderma harzianum* seed coating has effectively

reduced stress condition and seedlings have grown in a healthy manner with the increase in protein quantity, irrespective of salt stress application.

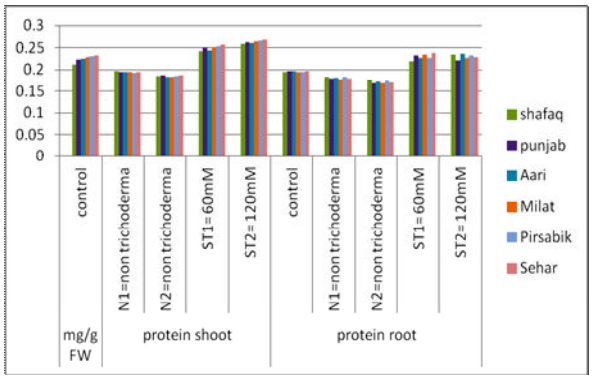


***Figure 1.*** **Increased protein content (mg/g FW) under stress in shoots and roots**

### *Catalase activity in roots and shoots*

According to tables (Table 1 and 2), it is apparent that due to *Trichoderma* seed coating, catalase activity was reduced under salt stress in comparison to non *Trichoderma* coated set and control, which in otherwise case would have increased because, under stress conditions, hydrogen peroxide concentration is enhanced. Resultantly catalase enzyme production is also increased so as to protect cells from the harms of overproduction of hydrogen peroxide. However, under examination, wheat cultivars have responded differently to *Trichoderma* at both applied salt concentrations. This difference in response may be due to some genotypic variations.

***Table 1.*** **Decrease in catalase activity (U/g FW) under salt stress in shoots.**

| CAT | Units & Time | Treatmnt | Shafaq | Punjab | Aari | Millat | Sehar | Pirsabik |
|---|---|---|---|---|---|---|---|---|
| Shoot | U/gFW | Control | 2.432 | 2.487 | 2.451 | 2.711 | 2.637 | 2.550 |
| | | N1:Non Trich | 2.581 | 2.576 | 2.566 | 2.897 | 2.777 | 2.655 |
| | | N2:Non Trich | 2.580 | 2.572 | 2.562 | 2.893 | 2.772 | 2.651 |
| | 0 min | ST1:60 mM | 2.429 | 2.485 | 2.455 | 2.711 | 2.321 | 2.496 |
| | | ST2:120 mM | 2.423 | 2.483 | 2.451 | 2.707 | 2.313 | 2.491 |
| | 1 min | Control | 2.423 | 2.475 | 2.450 | 2.711 | 2.312 | 2.482 |
| | | N1:Non Trich | 2.579 | 2.570 | 2.565 | 2.891 | 2.765 | 2.649 |
| | | N2:Non Trich | 2.575 | 2.568 | 2.563 | 2.879 | 2.762 | 2.647 |
| | | ST1:60 mM | 2.422 | 2.470 | 2.450 | 2.706 | 2.283 | 2.477 |
| | | ST2:120 mM | 2.421 | 2.464 | 2.450 | 2.702 | 2.250 | 2.471 |
| | 2 min | Control | 2.421 | 2.461 | 2.448 | 2.710 | 2.247 | 2.468 |
| | | N1:Non Trich | 2.576 | 2.564 | 2.556 | 2.870 | 2.759 | 2.641 |
| | | N2:Non Trich | 2.572 | 2.561 | 2.550 | 2.868 | 2.754 | 2.639 |
| | | ST1:60 mM | 2.419 | 2.455 | 2.442 | 2.706 | 2.245 | 2.463 |
| | | ST2:120 mM | 2.417 | 2.451 | 2.440 | 2.701 | 2.241 | 2.458 |
| | 3 min | Control | 2.420 | 2.447 | 2.456 | 2.709 | 2.239 | 2.456 |
| | | N1:Non Trich | 2.567 | 2.558 | 2.549 | 2.861 | 2.751 | 2.637 |
| | | N2:Non Trich | 2.562 | 2.520 | 2.545 | 2.859 | 2.749 | 2.632 |
| | | ST1:60 mM | 2.418 | 2.443 | 2.438 | 2.699 | 2.235 | 2.451 |
| | | ST2:120 mM | 2.417 | 2.435 | 2.435 | 2.697 | 2.231 | 2.447 |

**Mean values of catalase activity for different treatments at regular time intervals**

***Table 2*. Decrease in catalase activity (U/gFW) under salt stress in roots.**

| CAT | Units & Time | Treatmnt | Shafaq | Punjab | Aari | Millat | Sehar | Pirsabik |
|---|---|---|---|---|---|---|---|---|
| Root | U/gFW | Control | 2.462 | 2.491 | 2.461 | 2.712 | 2.640 | 2.510 |
| | | N1:Non Trich | 2.587 | 2.576 | 2.576 | 2.898 | 2.777 | 2.655 |
| | | N2:Non Trich | 2.585 | 2.572 | 2.572 | 2.894 | 2.772 | 2.651 |
| | 0 min | ST1:60 mM | 2.459 | 2.487 | 2.458 | 2.710 | 2.321 | 2.496 |
| | | ST2:120 mM | 2.453 | 2.483 | 2.455 | 2.707 | 2.320 | 2.490 |
| | 1 min | Control | 2.450 | 2.475 | 2.459 | 2.711 | 2.310 | 2.482 |
| | | N1:Non Trich | 2.583 | 2.570 | 2.569 | 2.891 | 2.765 | 2.649 |
| | | N2:Non Trich | 2.580 | 2.568 | 2.567 | 2.879 | 2.762 | 2.647 |
| | | ST1:60 mM | 2.448 | 2.471 | 2.453 | 2.706 | 2.280 | 2.477 |
| | | ST2:120 mM | 2.442 | 2.466 | 2.450 | 2.702 | 2.250 | 2.471 |
| | 2 min | Control | 2.440 | 2.461 | 2.458 | 2.710 | 2.247 | 2.468 |
| | | N1:Non Trich | 2.576 | 2.564 | 2.556 | 2.870 | 2.759 | 2.641 |
| | | N2:Non Trich | 2.572 | 2.561 | 2.550 | 2.868 | 2.754 | 2.639 |
| | | ST1:60 mM | 2.437 | 2.457 | 2.452 | 2.706 | 2.245 | 2.463 |
| | | ST2:120 mM | 2.431 | 2.451 | 2.448 | 2.701 | 2.241 | 2.458 |
| | 3 min | Control | 2.427 | 2.447 | 2.456 | 2.709 | 2.239 | 2.456 |
| | | N1:Non Trich | 2.567 | 2.558 | 2.549 | 2.861 | 2.751 | 2.637 |
| | | N2:Non Trich | 2.562 | 2.520 | 2.545 | 2.859 | 2.749 | 2.632 |
| | | ST1:60 mM | 2.421 | 2.443 | 2.450 | 2.699 | 2.235 | 2.451 |
| | | ST2:120 mM | 2.417 | 2.435 | 2.447 | 2.697 | 2.231 | 2.447 |

**Mean values of catalase activity for different treatments at regular time intervals**

## *APX activity in roots and shoots*

Both in roots and shoots, it has been seen that *Trichoderma* application has increased ascorbate peroxidase (APX) activity under salt stress conditions, while non *Trichoderma* set at both salt concentrations showed a decrease in APX activity, the control group also showed a decline (Fig. 2). However, the response of wheat cultivars at both salt concentrations is slightly different from each other to applied fungal strain.

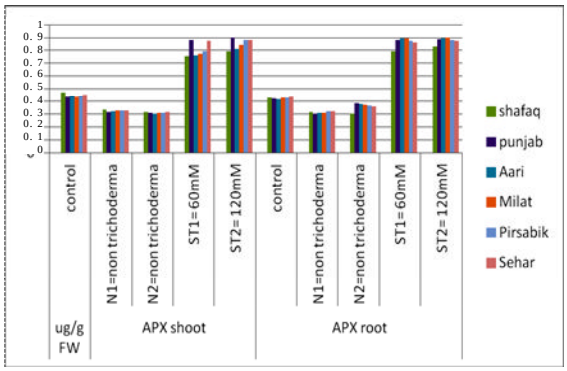


***Figure 2*. Increase in APX activity(ug/g FW) under both salt concentrations in shoots and roots**

***Table 3.*** **Decreased MDA content (µmol/g FW) in shoots and roots under salt stress as control.**

| Unit: µmol/g FW | SEHAR | SHAFAQ | PUNJAB | AARI | MILAT | PIRSABIK |
|---|---|---|---|---|---|---|
| Control shoot | 0.130 | 0.132 | 0.127 | 0.130 | 0.122 | 0.121 |
| N1= non trichoderma | 0.141 | 0.142 | 0.167 | 0.149 | 0.155 | 0.158 |
| N2= non trichoderma | 0.153 | 0.165 | 0.172 | 0.155 | 0.146 | 0.151 |
| ST1= 60 mM | 0.127 | 0.125 | 0.124 | 0.124 | 0.117 | 0.119 |
| ST2= 120 mM | 0.116 | 0.119 | 0.106 | 0.113 | 0.104 | 0.103 |
| Control root | 0.112 | 0.104 | 0.121 | 0.128 | 0.130 | 0.120 |
| N1= non trichoderma | 0.138 | 0.139 | 0.147 | 0.143 | 0.142 | 0.143 |
| N2= non trichoderma | 0.149 | 0.147 | 0.159 | 0.156 | 0.152 | 0.149 |
| ST1= 60 mM | 0.100 | 0.109 | 0.112 | 0.119 | 0.115 | 0.116 |
| ST2= 120 mM | 0.089 | 0.096 | 0.103 | 0.104 | 0.104 | 0.102 |

### *MDA content in roots and shoots*

Malondialdehyde content in roots and shoots was reduced in Trichoderma coated seeds under both levels of salt stress compared to control conditions and non fungal sets. Table 3 illustrates this decrease in fungal coated seeds under stress conditions.

### *Hydrogen peroxide quantity in roots and shoots*

Results depict a pronounced decrease in hydrogen peroxide quantity in treated seedlings at both salt concentrations compared to non coated seeds and control in all wheat cultivars (Fig. 3). Results suggest that *Trichoderma* has been proved an effective agent against salt stress by reducing hydrogen peroxide production and consequently protecting membrane instabilities and rupturing.

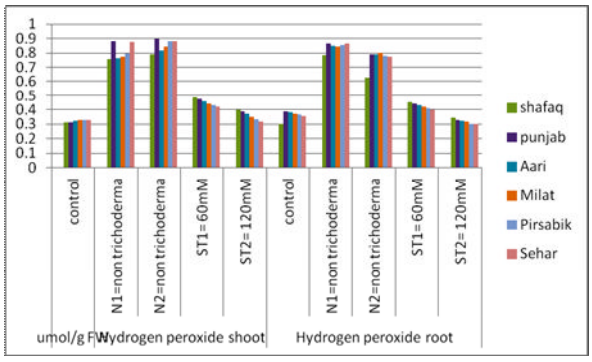


***Figure 3.*** **Decline in hydrogen peroxide (µmol/g FW) concentration in shoots and roots at both salt levels**

### *Total phenolics roots and shoots*

A prominent increase in total phenolic content was examined in fungal treated seedlings compared to non coated seedlings and controls both in root and shoot zones (Fig. 4). However, in the shoot region, maximum phenolics were reported at 120 mM concentration in Punjab cultivar, while in roots, maximum phenolics were observed in Aari at 120 mM NaCl concentration.

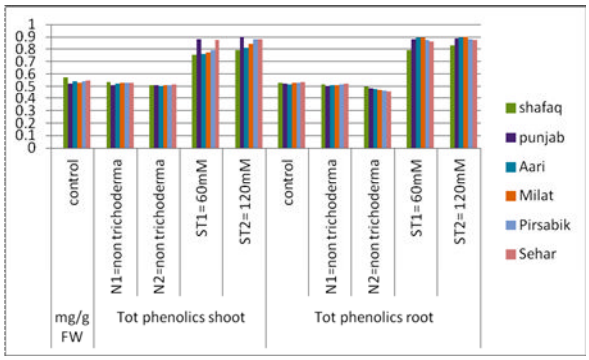


***Figure 4.*** **An increase in total phenolic (mg/g FW) content in stress in shoots and roots**

The main aim of conducting current research was to determine whether *Trichoderma harzianum* seed coating effectively eradicates salt stress and can be used as the most beneficial bioagent to cultivate healthy wheat crops under saline conditions without crop being affected by the harms of increased salt levels. It is well established that salinity causes toxic and osmotic harm to plants grown in such areas.

Our study has taken into account various biochemical parameters that are most often drastically affected by salt stress to examine the working of *Trichoderma harzianum* on these parameters under saline conditions. This present study reports an increase in protein content of *Trichoderma* treated wheat shoots and roots. The possible reason for the increase in protein amount can be the production of certain phytohormones such as gibberellins and cytokinins, which cause an increase in biomass along with managing stress conditions. Hence it can be said that *Trichoderma* application triggers the production of various growth regulating hormones. These results of protein enhancement in treated seedlings under saline conditions are in accordance with the finding [26], who have found that plant growth factors and biomasses are increased under salt stress when treated with *Trichoderma* fungus. Hydrogen peroxide production is a very important parameter. Its concentration increases with an increase in salt stress, and so because of its increased production and accumulation, membrane permeability is imbalanced, resulting in solute leakage. In our research *Trichoderma* showed a decline in hydrogen peroxide amount in salt stress exposed seedlings. Resultantly less solute leakage and membrane instability were examined. This reduction in solute seepage could be because Trichoderma has induced such antioxidant mechanisms that prevent the plant from the harm of oxidative damages. Our findings regarding the low production of hydrogen peroxide during salt stress conditions support the work done by [27], who primed rice seeds with *Trichoderma* and found a decrease in hydrogen peroxide concentration under salinity. Since hydrogen peroxide is produced in a lesser amount in our work, we have seen that our results present a decline in the catalase enzyme activity. As the function of this enzyme is to degrade hydrogen peroxide, the more the concentration of hydrogen peroxide more it will amount to this enzyme. But because *Trichoderma* has reduced the concentration of hydrogen peroxide in our study, the catalase amount was also decreased. According to [28], under abiotic stress of any type, MDA content increases because polyunsaturated fatty acids present in membranes get oxidized with free oxygen radicals. Thus more the amount of MDA content more will be oxidative damage. Results of our research with respect to MDA content are similar to work reported earlier [29]. We found that *Trichoderma* seed coating has reduced the MDA content of seedlings exposed to salt stress. This reduction may be due to induction of increased triggering of stress-related proteins such as glutathione S-transferase (GST), glutathione-dependent formaldehyde dehydrogenase (FALDH), and peroxidase by *Trichoderma*. These proteins work as scavengers, and whenever in stress conditions, free radicals are boosted in stress conditions, these proteins deteriorate them and prevent oxidative damage. These reactive oxygen species (ROS) scavenging proteins are triggered due to enhanced APX activity. Shoresh and Harman (2008) also found the same impact of this fungus in maize crops [28]. Total phenolics appeared to increase in *Trichoderma* treated seeds under both salt stress levels. The phenolic compounds are well known for combating salt stress and various other stresses by scavenging reactive oxygen species produced in response to stress. Results of this study are supported by earlier findings, which reported that root colonization by *T. harzianum* results in amplified levels of plant enzymes, such as chitinases, and the consequential variations in plant metabolism could cause the gathering of compounds like phytoalexins and phenolics [29]. Thus, the present research confirms the potential use of *Trichoderma harzainum* in crops cultivation in saline areas by examining the overall positive results in combating salt stress by activating phenolic compounds and scavenging proteins.

This research also provides a working platform for genetics to examine the ground realities of *Trichoderma* application at the gene level.

## Conclusion

Since globaly a large area of land is saline. In order to fulfill the food demand of large population there is need to extend agriculture. By adapting practical approaches saline areas can be utilized tor agricultural purposes. Current study eplored that use of *Trichoderma* is one of the solution to resolve the salinity prolem. By the uses of Trichoderma not only salt stress tolerance enhanced but also growth and yield attributes were improved. Hence it is concluded that seed priming with *Trichoderma harzianum* is an effective approach in combating salinity harms on crops. As this treatment is cost effective and environment friendly, it should be promoted in saline areas to grow healthy crops. Furthermore, challenge of food scarcity can be reduced.

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## Conflict of Interest

There is no conflict of interest with any commodity or organization. All research data presented in this document is authors own research work.

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