



# Effect of ferrous sulfate supplementation on physiological and quality traits of *Brassica campestris* under artificially salinized soil

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## General Note



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## ABSTRACT

An experiment to see the effect of FeSO<sub>4</sub> supplementation (0, 5, 10 and 15 mg k.g<sup>-1</sup> FeSO<sub>4</sub>) along with NPK basal dose (20:25:10 mg kg<sup>-1</sup>) on growth and ionic concentration of *Brassica campestris* (Var. Sultan Raya) under various levels (0, 5 and 10 dS m<sup>-1</sup>) of NaCl salinity during 2017-18 was conducted at National Agricultural Research Centre, Islamabad. Brassica seeds were sown and grown to

maturity and data on plant height, pods/plant<sup>-1</sup>, seeds.pod<sup>-1</sup>, 100s-seeds weight (g), seed yield and total biomass were recorded at harvesting of the plant. A considerable diminution in growth of plants and seed yield (35 and 41 % respectively) at higher salinity (10 d.S m<sup>-1</sup>) compared with non-saline medium was observed while FeSO<sub>4</sub> supplementation considerably improved plant growth and seeds yield under all salinity levels. Overall, 12 % increase in plant growth and 11 to 14 % gain in seed yield was examined due to application of 15 mg kg<sup>-1</sup> FeSO<sub>4</sub> as compared to control receiving no FeSO<sub>4</sub> under 10 dS m<sup>-1</sup> NaCl stress. However, a slight improvement in growth of Brassica was noted with higher level of FeSO<sub>4</sub> (15 mg kg<sup>-1</sup>) but was statistically non-significant when compared with lower rate (10 mg kg<sup>-1</sup> FeSO<sub>4</sub>). Similar inclination was seen in total biomass production. Sodium (Na<sup>+</sup>) concentration in plant tissues increased with increasing NaCl salinity and decreased K<sup>+</sup> concentration significantly. Although, the FeSO<sub>4</sub> supplementation effect on concentration of Ca<sup>2+</sup> and Mg<sup>2+</sup> in plant tissues was non-significant, however, a decreasing trend in accumulation of Ca<sup>2+</sup> and Mg<sup>2+</sup> was noted with increasing NaCl salinity in the root medium.

**Keywords:** *Brassica campestris*; FeSO<sub>4</sub> supplementation; NaCl salt stress; Growth and seed yield; Ions accumulation.

## 1. INTRODUCTION

Brassica is very important oil seed crop having seed production is 251 x 10<sup>3</sup> tons and grown on an area of 305 x 10<sup>3</sup> hectares in Pakistan [1]. Its contribution is 21% of edible oil consumed in the country. In the last five years huge amount of foreign exchange almost 10 billion rupees have been spent and it has increased to Rs. 42 billion up to date [2]. With growing population and increasing demand of edible oil, there is a dire need to enhance oil seed production in Pakistan. Globally, almost 20% irrigated lands are salt affected causing decline in crop yields decline [3]. The germination and seedling stages of plant are more vulnerable to salinity than adult stage [4]. Germination, development, photosynthesis, protein synthesis, lipid metabolism, leaf chlorosis, and senescence may also be hampered by salinity and sodicity [5, 6, 7, 8]. Soil salinity pose serious threat to sustainable agriculture production, about 6.62 mha is salt-affected in Pakistan [1]. High salt concentration may affect badly some species of Brassica. Some brassica cultivars may thrive successfully but their growth and development is stunted because of salinity. Rapeseeds and mustards are also significant source of edible oil [9, 10, 11] yet farmers do not focus on these crops. Though, the brassica is grown in all the provinces of Pakistan however, Punjab comprises 2/3<sup>rd</sup> area [4]. There is declining trend in its production has been mainly due to salinity and absence of certified seeds of improved varieties [1].

Apart from salinity micronutrient deficiencies is another impediment for viable growth of plants. Since CaCO<sub>3</sub> is predominantly available that fixes micronutrients; moreover alkaline not only pH reduces their solubility but marginal organic matter retards the availability of these trace elements [12]. Since micronutrient deficiencies adversely affect crop productivity and human health consequently. Iron (Fe) is essential for plant vigor and production of food. Fe as the ferrous (Fe<sup>2+</sup>) plays significant role of many enzymes associated with energy transfer, nitrogen fixation, and lignin formation. Trace element Fe is an essential micronutrient required for optimum crop growth [13]. Alkaline calcareous soils register Fe deficiency [14, 15, 16, 17]. Alkaline soil pH, soil calcareousness, low soil organic matter, exposed subsoil (eroded or leveled), sandy texture, Fe-free fertilizers are factors which induce Fe deficiency. The soil of Pakistan is deficient in Fe or its availability causing reduced crop yield productivity and quality. Iron (Fe) mining by high yielding varieties, and/or flooding induce electro-chemical changes. Despite Fe deficiency farmers never bother about the application of trace elements. Brassica has great potential to thrive on marginal lands with balanced fertilization including micronutrient. Micronutrients play an important role to enhance yield of brassica through N fixing by symbiotic process. Excessive removal of Fe is very pronounced under multiple cropping systems [4, 18, 19]. Plants emerged from seeds with low concentrations of Fe could be highly sensitive to biotic and abiotic stresses [20].

Successful production of oilseed crops on moderately salt-affected soils having good quality edible oil, demands the balanced fertilization including trace elements especially Fe, appropriate selection of variety, especially with superior ability to tolerate the salt stress [3, 21, 22, 23, 24]. Obviously, the edible oil seed production from saline soils could be increased by two means, either by adequate balanced nutrition or growing tolerant varieties against salinity. In the present situation, consequently, we must search for adequate fertilizers application to brassica crop to meet the edible oil requirements of mounting population. Under the current circumstances, there is very low yield production per hectare of this crop than that potential yields. Therefore, the objective of this study was to investigate the effect of FeSO<sub>4</sub> supplementation for brassica crop production with better yield and quality from salt-affected lands.

## 2. MATERIALS AND METHOD

A pot study was carried out to investigate the response of *Brassica campestris* regarding its yield and quality to FeSO<sub>4</sub> supplementation under three NaCl salinity levels during 2017-18 at National Agricultural Research Centre, Islamabad. Normal sandy

clay loam soils was collected from the field and prepared for filling in pots of eight kilogram capacity. Analysis regarding Physico-chemicals properties of the soil under study was carried out from a composite sample collected before initiation of experiment (Table 1). The pots were filled with prepared soil and Brassica seeds were sown. Required salinity (5 sand 10 d.S m<sup>-1</sup>) was developed artificially in respective pots after seedling establishment in three splits using NaCl salt in the respective pots. The various treatments planned for this study were as follow:

T1 = 0 mg FeSO<sub>4</sub> kg<sup>-1</sup> soil (Control)

T2 = 5 mg FeSO<sub>4</sub> kg<sup>-1</sup> soil

T3 = 10 mg FeSO<sub>4</sub> kg<sup>-1</sup> soil

T4 = 15 mg FeSO<sub>4</sub> kg<sup>-1</sup> soil

The study was carried out following completely randomized designs (CRD) within three replications. A Basal dose of N, P and K @ 20, 25 and 10 mg kg<sup>-1</sup> ash urea, Single Super Phosphate (SSP) and Sulphate of Potash (SOP) were applied at sowing time. The crop was allowed to stand till maturity. Canal irrigation water was applied whenever required throughout the growth season. Data on plant growth as well as yield contributing parameters in terms of plant height, branch numbers per plant, pods per plant, seeds per pod and seeds yield were collected at maturity of crop. Samples for chemical analysis were collected at the time of harvest and dried in oven at a constant temperature of 60 °C and ground to 40-mesh screen in a Wiley Mills. Grounded samples of plants were processed to digest (di-acid mixture of nitric acid and perchloric acid in 2:1) for estimation of Fe, Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>+2</sup> concentrations to in seeds by the atomic absorption spectroscopy. Crude protein in seeds was determined by Kjeldahl method and crude oil percentage in seeds by Soxhlet apparatus (AOAC, 1990). The soil sample collected before application of treatment was air-dried and sieved with a 5 mm sieve and their chemical and physical properties were determined. The samples were analyzed for soil textural class by hydrometer method [26]. Soil organic matter by oxidation with potassium dichromate in sulfuric acid medium under standardized conditions by Walkley and Black procedure [27]. Soils pHs was determined in 1:1 ratio of water and soil. Electrical conductivity (ECe) was determined with conductivity meters. Concentration of P, K and Zn were measured following Ammonium Bicarb-DTPA method [28]. The results were analyzed statistically with the help of MSTAT-C package on computer. [29].

**Table 1** Physicochemical analysis of the soils collected for the study

Parameters	Unit	Values
pH	–	8.49
ECe	dS m <sup>-1</sup>	0.37
CaCO <sub>3</sub>	%	3.51
OM	%	0.34
NO <sub>3</sub> -N	mg kg <sup>-1</sup>	2.35
Extractable P (AB-DTPA)	mg kg <sup>-1</sup>	2.68
Extractable K (AB-DTPA)	mg kg <sup>-1</sup>	49.54
Fe (ABDTPA)	mg kg <sup>-1</sup>	2.98
Sand	%	31.72
Silt	%	28.27
Clay	%	40.11
Textural Class	–	Sandy clay loam

### 3. RESULTS AND DISCUSSION

#### Plant Growth and Seed Yield

A considerable reduction in plants growth and seed yields (35 and 41 %, respectively) was calculated at higher salinity (10 d.S/m<sup>-1</sup>) compared with non saline medium while FeSO<sub>4</sub> supplementation considerably improved plant growth and seed yield under all salinity levels (Table 2 and 3). The reason might be that the most of oilseed crops are sensitive to high soil salinity due to which the plants showed stunted growth and hence reduced yield [30-33]. Negative consequences of higher salinity on plants growth and yield had also been reported by Aslam *et al.* [34]. Grattan and Grieve [35] reported that nutritional imbalance and uptake of toxic ions under stressed conditions cause huge reduction in crop yields. Similar results have also been discussed by [3, 22, 24].

**Table 2** Growth parameters of *Brassica campestris* influenced by FeSO<sub>4</sub> supplementation under NaCl soil salinity levels (Average of three Repeats)

Treatments	Plant Height (cm)				No. of Branches (Plant <sup>-1</sup> )				No. of Pods (Plant <sup>-1</sup> )			
	0 dS m <sup>-1</sup>	5 dS m <sup>-1</sup>	10 dS m <sup>-1</sup>	Mean	0 dS m <sup>-1</sup>	5 dS m <sup>-1</sup>	10 dS m <sup>-1</sup>	Mean	0 dS m <sup>-1</sup>	5 dS m <sup>-1</sup>	10 dS m <sup>-1</sup>	Mean
0 mg kg <sup>-1</sup> FeSO <sub>4</sub>	146 b	122 d	108 e	125 B	13.18 c	10.07 f	8.62 h	10.69 D	111.07 d	88.66 e	69.33	89.69 C
5 mg kg <sup>-1</sup> FeSO <sub>4</sub>	147 a	129 c	111 de	129 A	13.33 c	11.67 e	8.89 h	11.30 C	128.83 b	110.78 d	78.66	106.09 B
10 mg kg <sup>-1</sup> FeSO <sub>4</sub>	149 a	131 c	111 d	130 A	14.17 b	13.24 d	9.48 g	12.30 B	134.37 a	117.82 c	92.54	105.18 B
15 mg kg <sup>-1</sup> FeSO <sub>4</sub>	151 a	132 c	112 d	132 A	15.94 a	13.96 d	9.87 g	13.26 A	134.48 a	119.36 c	93.72	115.85 A
Mean	148 A	129 B	111 C		14.16 A	12.24 B	9.22 C		124.79 A	109.16 B	83.56 C	
L.S.D.	4.1147				0.6875				3.1468			

Means bearing same letter(s) in each column are statistically similar at  $p \leq 0.05$

<sup>NS</sup> Means in each column are non-significant

**Table 3** Yield and yield components of *Brassica campestris* influenced by FeSO<sub>4</sub> supplementation under NaCl soil salinity levels (Average of three Repeats)

Treatments	Pod Length (cm)				No. of Seeds (Pod <sup>-1</sup> )				Seed Yield (kg ha <sup>-1</sup> )			
	0 dS m <sup>-1</sup>	5 dS m <sup>-1</sup>	10 dS m <sup>-1</sup>	Mean	0 dS m <sup>-1</sup>	5 dS m <sup>-1</sup>	10 dS m <sup>-1</sup>	Mean	0 dS m <sup>-1</sup>	5 dS m <sup>-1</sup>	10 dS m <sup>-1</sup>	Mean
0 mg kg <sup>-1</sup> FeSO <sub>4</sub>	99.11 e	87.52	68.78 g	85.14 C	12.02 b	9.56 c	7.83 d	9.80 C	1096 g	814 i	789 j	900 D
5 mg kg <sup>-1</sup> FeSO <sub>4</sub>	117.58 c	98.49 e	86.53 f	100.87 B	14.98 a	12.87 b	9.91 c	12.59 B	1338 b	1187 e	971 h	1165 C
10 mg kg <sup>-1</sup> FeSO <sub>4</sub>	131.37 b	104.45 d	97.43 e	111.08 A	16.54 a	14.26 a	10.32 bc	13.71 AB	1429 a	1243 c	1084 g	1252 A
15 mg kg <sup>-1</sup> FeSO <sub>4</sub>	139.45 a	105.67 d	99.28 e	114.80 A	16.98 a	14.95 a	11.59 b	14.51 A	1436 a	1248 c	1097 g	1260 A
Mean	121.88 A	99.03 B	88.01 C		15.13 A	12.91 B	9.91 C		1325 A	1123 B	985 C	
L.S.D.	4.1850				1.8877				14.0013			

Means bearing same letter(s) in each column are statistically similar at  $p \leq 0.05$

<sup>NS</sup> Means in each column are non-significant

Application of FeSO<sub>4</sub> along with basal dose of N, P and K (20, 25 and 10 mg kg<sup>-1</sup>) substantially improved growth and yield of brassica which might have caused constructive influence on plant establishment under salinity stress. Plant height, number of branches and pods plant<sup>-1</sup> were improved significantly with successive supplementation of FeSO<sub>4</sub> up to 10 mg kg<sup>-1</sup>, which was statistically similar with higher rates of FeSO<sub>4</sub> (15 mg kg<sup>-1</sup>). Although the treatment values collected with 10 mg kg<sup>-1</sup> supplementation were statistically similar with FeSO<sub>4</sub> application @ 15 mg kg<sup>-1</sup>. However, a close observation mentions that a slight augmentation in growth and yield contributing parameters were noted with FeSO<sub>4</sub> application @ 15 mg kg<sup>-1</sup>. The results of our investigation are in close agreements with those of reported by [36, 37, 38, 39, 40, 41] who have also reported a significant increase in growth attributes and seed yields of brassica due to application of sulphur @ 40 to 60 kg ha<sup>-1</sup>.

#### Oil and Protein contents

There were significant differences in oils and proteins content of brassica (Table 4) under varying salt-affected soil conditions. Maximum percentage of oil content (40.14 %) was observed in the seeds of Sultan Raya with 12 mg kg<sup>-1</sup> FeSO<sub>4</sub> supplementation

followed by  $\text{FeSO}_4$  ( $10 \text{ mg kg}^{-1}$ ) under normal soil while it decreased significantly (16 and 24 %) with increasing salinity to 5 and 10  $\text{dS m}^{-1}$  respectively. Similarly a considerable improvement in protein contents of brassica seeds were significantly higher with  $\text{FeSO}_4$  under non saline soil than that of non supplemented  $\text{FeSO}_4$  treatments as well as harvested from saline soils. Overall, a considerable reduction in protein (34 %) contents in brassica seeds was observed due to increased salinity ( $10 \text{ dS m}^{-1}$ ) level which was significantly improved  $\text{FeSO}_4$  supplementation even with lower rate of  $\text{FeSO}_4$  ( $5 \text{ mg kg}^{-1}$ ). Comparatively less oil and protein contents in brassica seeds harvested from salinized soils might probably be as a result of unnecessary assimilation of noxious elements that caused reduced plant growth and hence yields with poor quality as well. Depressed uptake of nutrients due to nutritional imbalance in root medium, transport in shoot, breakdown of chlorophyll and impaired distribution of mineral ions in plant tissues presumably have caused less percentage of oil contents in seeds. Interpreting earlier results of various researchers, it was confirmed that application of iron sulphate provided excellent results [16, 20]. Since, deficiency of iron cause directly chlorophyll degradation and chlorosis as well which effect protein synthesis leading to reduced growth of plant [17]. Iron is also involved in electron transport during the process of photosystem which cause probably chlorophyll content reduction due to iron deficiency. Similar discussion has also been made by [15, 16]. A comprehensive similar discussion had been reported by many earlier researchers that how salinity disturb the mechanism of plants through accumulation of toxic saline ions [4, 6, 12, 42, 43]. Resembling interpretations have also been reported by Flowers and Yeo [22], Gorham *et al.* [44], Yeo and Flowers [45], Akhtar *et al.* [46], Ali *et al.* [4], Ahmed *et al.* [33] and Mahmood *et al.* [12].

Overall, comparatively high percentage of oils and proteins content in seeds of brassica cultivar Sultan Raya treated with  $\text{FeSO}_4$  even at lower rate ( $5 \text{ mg kg}^{-1}$ ) might be probably due to more N uptake which improved its growth and yield eventually improving the quality. Since, salt stress is considered for the elevation of ionic concentration in plant root and shoots which ultimately play an important role by activating enzymes known to be related with excessive utilization of mineral nutrients. Perhaps a similar situation has prevailed that increased protein contents under stress condition. This is supported by the investigation of Ahmeds *et al.* [33], Mahmood *et al.* [12] and Ali *et al.* [4].

**Table 4** Oil and Protein content in seeds of Brassica influenced by  $\text{FeSO}_4$  supplementation under NaCl soil salinity (Average of three Repeats)

Treatments	Oil content (%)				Protein (%)			
	0 $\text{dS m}^{-1}$	5 $\text{dS m}^{-1}$	10 $\text{dS m}^{-1}$	Mean	0 $\text{dS m}^{-1}$	5 $\text{dS m}^{-1}$	10 $\text{dS m}^{-1}$	Mean
0 $\text{mg kg}^{-1}$ $\text{FeSO}_4$	27.34 e	21.28 f	18.77 g	22.46 D	12.79 ab	8.81 c	7.05 d	9.55 C
5 $\text{mg kg}^{-1}$ $\text{FeSO}_4$	36.81 b	27.14 e	22.13 f	28.69 C	13.54 a	11.74 b	9.92 c	11.73 AB
10 $\text{mg kg}^{-1}$ $\text{FeSO}_4$	39.72 a	31.35 d	28.16 e	33.08 B	14.25 a	13.25 ab	11.23 b	12.91 A
15 $\text{mg kg}^{-1}$ $\text{FeSO}_4$	40.14 a	34.57 c	32.35 d	35.69 A	14.87 a	13.98 a	11.98 b	13.61 A
Mean	36.01 A	28.59 B	25.35 C		13.86 A	11.95 B	10.05 C	
L.S.D.	1.2417				1.3354			

Means bearing same letter(s) in each column are statistically similar at  $p \leq 0.05$

<sup>NS</sup> Means in each column are non-significant

### Ionic Concentration

The influence of  $\text{FeSO}_4$  supplementation on ionic concentration in brassica seeds are shown in Table 5. Iron (Fe) concentration in brassica seed increased significantly as a result of  $\text{FeSO}_4$  applications even at lower rate ( $5 \text{ mg kg}^{-1}$  soil). Although, NaCl salinity drastically reduced Fe concentration in brassica seeds, however, the application of  $\text{FeSO}_4$  through soil increased appreciably total Fe content in seeds. Generally, on an average, Fe concentration decreased significantly with increasing salinity level, but its concentration appreciably augmented in seeds from  $10.22 \text{ mg kg}^{-1}$  in control (0  $\text{FeSO}_4$ ) to  $51.06 \text{ mg kg}^{-1}$  in higher dose of  $\text{FeSO}_4$  ( $15 \text{ mg kg}^{-1}$  Soil) followed by  $10 \text{ mg kg}^{-1}$   $\text{FeSO}_4$  application which might be attributed to maximum availability (Adiloglu, 2006; Hu-lin *et al.*, 2007). However, Fe concentration in seed of brassica significantly differed under various salinity levels probably due to toxic ions in root medium which affected its utilization.

Ionic concentration ( $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Ca}^{+2}$ ) in seeds of different brassica varied significantly under different salinity levels (Table 5). Minimum  $\text{Na}^+$  and maximum  $\text{K}^+$  and  $\text{Ca}^{+2}$  concentrations were observed in seeds of brassica even at higher ( $10 \text{ dS m}^{-1}$ ) salinity level with the application of  $\text{FeSO}_4$  but under control where no  $\text{FeSO}_4$  was applied, a significant decrease in  $\text{K}^+$  and increase in  $\text{Na}^+$  and  $\text{Ca}^{+2}$  concentrations were observed presumably due to injurious effect of salinity. However, comparatively better performance of Sultan Raya in maintaining higher level of  $\text{K}^+$  and less percentage of  $\text{Na}^+$  content in seeds might be because of its genetic ability for

better salt tolerance. These findings could be supported by the results of Mahmood *et al.* [23], Flowers [24], Shirazi *et al.* [50], Nawaz *et al.* [12] and Ali, *et al.* [4].

**Table 5** Ionic concentration in Brassica seeds influenced by FeSO<sub>4</sub> supplementation under NaCl soil salinity (Average of three Repeats)

Treatments	Fe ( $\mu\text{g g}^{-1}$ )				K (%)			
	0 dS m <sup>-1</sup>	5 dS m <sup>-1</sup>	10 dS m <sup>-1</sup>	Mean	0 dS m <sup>-1</sup>	5 dS m <sup>-1</sup>	10 dS m <sup>-1</sup>	Mean
0 mg kg <sup>-1</sup> FeSO <sub>4</sub>	13.32 g	9.79 h	7.56 i	10.22 D	5.47 a	3.78 c	2.03 d	3.76 B
5 mg kg <sup>-1</sup> FeSO <sub>4</sub>	49.87 c	42.36 e	39.25 f	43.83 C	6.48 a	4.32 b	3.36 c	4.92 A
10 mg kg <sup>-1</sup> FeSO <sub>4</sub>	56.22 b	47.85 d	43.58 e	49.22 B	6.56 a	4.63 b	3.53 c	5.03 A
15 mg kg <sup>-1</sup> FeSO <sub>4</sub>	59.38 a	49.58 c	44.21 e	51.06 A	6.59 a	4.86 b	3.64 c	5.06 A
Mean	44.70 A	37.40 B	33.65 C		6.28 A	4.32 B	3.14 C	
L.S.D.	1.9107				1.1418			
	Na (%)				Ca (%)			
0 mg kg <sup>-1</sup> FeSO <sub>4</sub>	3.93 f	4.66 c	5.32 a	4.64 A	2.16 c	1.66 d	1.33 de	1.72 C
5 mg kg <sup>-1</sup> FeSO <sub>4</sub>	3.48 g	4.33 d	4.99 b	4.27 B	2.24 c	1.99 cd	1.66 d	1.96 C
10 mg kg <sup>-1</sup> FeSO <sub>4</sub>	3.46 g	3.97 f	4.18 e	3.87 C	3.51 a	2.84 b	2.42 bc	2.92 B
15 mg kg <sup>-1</sup> FeSO <sub>4</sub>	3.41 g	3.76 g	4.13 e	3.77 C	3.58 a	2.97 b	2.44 bc	3.01 A
Mean	3.57 C	4.18 B	4.66 A		2.87 A	2.37 B	1.96 C	
L.S.D.	0.1435				0.4311			

Means bearing same letter(s) in each column are statistically similar at  $p \leq 0.05$

<sup>NS</sup> Means in each column are non-significant

#### 4. CONCLUSION

Satisfactory brassica seed yield with better quality could be produced from salt-affected lands through FeSO<sub>4</sub> supplementation. Among all treatments under study, 10 mg FeSO<sub>4</sub> kg<sup>-1</sup> soil showed better results which were statistically at par with higher rate (15 mg FeSO<sub>4</sub>) even under higher salinity (10 dS m<sup>-1</sup>) level. These results lead to conclude that 10 mg FeSO<sub>4</sub> kg<sup>-1</sup> soil should be applied along with recommended NPK fertilizers to brassica genotype Sultan Raya to get optimum yield from salt-affected lands.

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#### Conflict of Interest:

The authors declare that there are no conflicts of interests.

#### Peer-review:

External peer-review was done through double-blind method.

#### Data and materials availability:

All data associated with this study are present in the paper.

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