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EFFECT OF DIFFERENT LEVELS OF CHARCOAL AND NITROGEN ON GROWTH AND YIELD TRAITS OF BROCCOLI

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soil conditioner when applied to agricultural fields. When used in combination with nitrogen fertilizers, it has a synergistic effect that boosts plant growth. However, charcoal application alone or in combination with nitrogen fertilizer on vegetable crops is not fully understood. Therefore, a pots experiment was carried out to investigate the effect of charcoal and nitrogen levels on the growth and yield of broccoli. The variety of broccoli used was Centauro. The experiment was laid out in a two-factor Completely Randomized Design with five replications during the winter season of 2019–2020 at Sundarbazar, Lamjung, Nepal. Treatments consisted of four levels of charcoal (0%, 2.5%, 5%, and 7.5% per weight of soil) and three levels of nitrogen (0, 187.5 and 375 kg N ha⁻¹). Results revealed that increasing nitrogen levels from 0 to 375 kg N ha⁻¹ significantly increased the number of leaves, leaf area, head diameter, head weight and aboveground biomass. The maximum head weight per plant (258.77 g) was found by applying a nitrogen level of 375 kg N ha⁻¹ and the lowest value at 0 kg N ha-1. The application of increasing levels of charcoal significantly improved root length, leaf area and head diameter. It was concluded from the results that the optimum nitrogen level for broccoli production could be 375 kg N ha⁻¹.

ABSTRACT. Charcoal is a carbon-rich organic matter, which serves as a

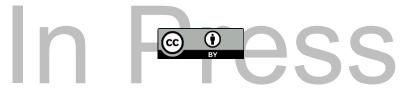
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Introduction

Broccoli (*Brassica oleracea*, L. var. *italica*) is a green-leaf Cole vegetable that belongs to the mustard family (Brassicaceae). This plant is native to the Mediterranean region but can be successfully grown in our country. It is a fast-growing; cool-season crop that is resistant to mild frost and has an optimum temperature in the range of 18–24 °C. It is an annual plant that may reach a height of 50 cm or more and is mainly

grown for its edible flower buds and stalks. Broccoli is more nutritious than any other vegetable of the same 30 genera (Al-Taey *et al.*, 2019). It is extremely nutritive due to its high content of vitamin A and C, thiamine, niacin, riboflavin, calcium, and carotenoids that have a plausible effect on maintaining good human health and have potent anti-diabetic, anticarcinogenic, antiinflammatory, and antioxidant properties (Hamza, Al-Taey, 2020; Moreira-Rodríguez *et al.*, 2017).





The term "charcoal" refers to various carbonized materials with varying combustion qualities (Wood, Baldwin, 1985). It is a fine-grained, light-weighted, porous solid obtained by partially burning or heating carbonaceous materials at a higher temperature with limited oxygen access by the method known as pyrolysis. Black carbon, elemental carbon, pyrogenic carbon, and biochar are used interchangeably to describe charcoal. Charcoal is chemically and microbially stable, and it has existed in the environment for millennia because of its polycyclic aromatic composition (Glaser et al., 2001). Because of its long-lasting nature, charcoal can be used as a long-term carbon sink for CO₂ (Glaser et al., 2002). Charcoal in agriculture has recently received attention as a useful technique for raising crop yield, improving soil fertility, increasing fertilizer use efficiency, and as a carbon sequestration option (Ogawa, Okimori, 2010). When applied to agricultural soil, it improves the soil's physical, chemical, and biological properties (Laird et al., 2010). The effects of charcoal on plant growth and yield vary greatly depending on the experimental setting, soil qualities, charcoal supply, crop nature, and climatic conditions (Mukherjee, Lal, 2014). Charcoal treatment on various crops resulted in a statistically significant 10% increase in mean yield in an experiment using statistical meta-analysis (Jeffery et al., 2015). Treatment of lettuce and Chinese cabbage plants in pots with rice husk charcoal enhanced the final biomass, root biomass, plant height, and leaf number (Carter et al., 2013). According to Vaccari et al. (2015), adding charcoal to tomato cultivation improved soil quality and fertility and promoted plant growth, however, yield increase was not obtained due to the tomato's indeterminate habit. McDonald et al. (2019) reported a similar outcome, in which charcoal treatment had no effect on cabbage yield but had a beneficial impact on soil quality. Overall, the beneficial response to the charcoal application on crop growth and production is obtained in a tropical environment where the soil is deficient in nutrients (Crane-Droesch et al., 2013) and in acid soils (Liu et al., 2013), which is mainly due to liming property of alkaline charcoal. However, in a temperate climate with alkaline soil, it has a short-term effect on crops and plants (Rousk et al., 2013; Borchard et al., 2014; Tammeorg et al., 2014; Wei et al., 2021).

Nitrogen(N) being an essential element for successful plant growth, nitrogen fertilization for many decades has been a powerful tool in improving yield, productivity, and quality of food crops and vegetables (Leghari *et al.*, 2016; Wang *et al.*, 2008). It is a major indispensable constituent of proteins, nucleic acids, phytohormones, and other cellular components in plants, and contributes 40–50% of crop yield (Marschner, 2012). Its use has an immediate effect on plant cell division and cell enlargement, resulting in better crop growth and establishment (Xin *et al.*, 2014). According to Yoldas *et al.* (2008), nitrogen application resulted in increased yield, the average weight of main and secondary heads, and diameter in broccoli when compared to the control. As a

result, adding extra N to the soil reduces the overall risk of successful broccoli production while increasing broccoli growth and yield (Bika *et al.*, 2018; Dhakal *et al.*, 2016; Tripathi *et al.*, 2020).

Charcoal is usually used as a soil conditioner rather than a fertilizer because of its low nutrient concentrations. As a result, combining charcoal with chemical fertilizers has a synergistic impact, resulting in increased plant growth responses (Gathorne-Hardy *et al.*, 2009; Saarnio *et al.*, 2013). Because nitrogen (N) is such an important component for plant growth, combining it with charcoal has resulted in large yield increases when compared to control, ranging from 266 % in radish to modest gains (between 12–64 %) in rice (Ali *et al.*, 2020; Piash *et al.*, 2019). However, other studies have found that using charcoal and nitrogen fertilizers together does not affect plant growth and yield (Mavi *et al.*, 2018; Silitonga *et al.*, 2018), and some have even found yield declines in particular conditions (Gaskin *et al.*, 2010).

The interactions between charcoal and fertilizers employed and between charcoal and crops necessitate more research and understanding before the charcoal application is adopted as a regular agricultural practice by farmers. Furthermore, because most charcoal trials are conducted on cereal crops, there is little information on the dose-dependent effect of charcoal and nitrogen on vegetable production. In addition, the impact of charcoal on broccoli production is unknown. Therefore, this experiment was conducted to determine the individual effect of charcoal and nitrogen on broccoli's growth and yield and assess their combined impact on broccoli production.

Material and Methods

Experimental site

A pots experiment was conducted at the Horticulture Farm of the Institute of Agriculture and Animal Science (IAAS), Lamjung, Nepal from September 2019 to February 2020. The farm is located at 28°7' North latitude and 84° 25' East longitude, with an altitude of 632 m above sea level having a sub-tropical climate. The climatic data during the experiment is given in Table 1.

 Table 1. Climate data of the experimental location in 2019–2020

Months	Temp	o., ℃	Rainfall,	Relative humidity,		
Wollins	max. min.		mm	%		
2019						
September	28	18	266.55	78		
October	26	12	70.36	65		
November	25	6	11.76	58		
December	20	3	0.00	50		
2020						
January	18	3	32.73	46		
February	22	4	69.96	46		

Description of experimental materials

"Centauro" variety of Broccoli was grown in this experiment. This variety can be cultivated in the Lamjung district of Nepal. The seeds were obtained from Dawadi Agrovet, Narayangarh, Chitwan, Nepal. The source of the nitrogen fertilizer was urea (46% N). The urea, single superphosphate (SSP) and muriate of potash (MOP) were obtained from the same Agrovet. Charcoal particles were produced at the farm by the pyrolysis method, collecting dry woods from nearby forests following the procedures as explained by Artiola and Wardell (2017).

Experimental layout, design and cultural practices

A pots experiment was conducted in a two-factorial Completely Randomized Design with 12 treatments and 5 replications. Charcoal was one factor with four levels at the rate of 0, 125, 250 and 375 g charcoal pot⁻¹ (=0, 2.5, 5 and 7.5% by soil weight). Similarly, nitrogen was another factor with three different levels at the rate of 0, 11 and 22 g N pot⁻¹ (=0, 187 and 375 kg N ha⁻¹). A single pot (top diameter of 22.5 cm, base diameter of 16.5 cm and height of 18 cm) with a single plant in it was considered as an experimental unit. Five kg of soil, farmyard manure (FYM) at the rate of 10 tons ha⁻¹, charcoal and nutrient as per the treatments were mixed and filled in the pot. Altogether, there were 60 experimental pots.

Seeds were sown in the nursery bed of 1×1 m on September 21, 2019. One-month-old, robust and healthy seedlings were transplanted into the pots during evening time and light irrigation was done immediately. The standard agronomical practices such as irrigation, intercultural operations and insecticide application were then adopted for healthy crop growth as per requirement.

Phosphorus and potassium to the plants were supplemented at the rate of 175:80 kg PK ha⁻¹ (MOALD, 2020). Half of the required dose of nitrogen (as per treatment) as urea with the full dose of phosphorus as Single Superphosphate (SSP) and potash as Muriate of Potash (MOP) was applied as basal dose. Half of the remaining nitrogen was applied in two split doses at 30 DAT (Days after Transplanting) and 45 DAT (Table 3). Micronutrient, AgrolivTM at the rate of 2.5 mL per litre of water was sprayed with a hand sprayer at 20, 35 and 50 DAT. Harvesting was done when the head of broccoli was at the green tight stage.

 Table 2. Details of factors used in treatments in the experiment

N1: C1: $0 \text{ kg N ha}^{-1} = 0 \text{ g N pot}^{-1}$ 0% by soil weight = 0 g charcoal pot^{-1}
$0 \text{ kg N ha}^{-1} = 0 \text{ g N pot}^{-1}$ 0 % by soil weight = 0 g charcoal pot ⁻¹
N2: C2:
$187.5 \text{ kg N} \text{ ha}^{-1} = 11 \text{ g N} \text{ pot}^{-1} 2.5 \% \text{ by soil weight} = 125 \text{ g charcoal pot}^{-1}$
N3: C3:
$375 \text{ kg N} \text{ha}^{-1} = 22 \text{ g N} \text{pot}^{-1}$ 5 % by soil weight = 250 g charcoal pot^{-1}
C4:
7.5 % by soil weight = 375 g charcoal pot ^{-1}

Where soil weight=5 kg

 Table 3. NPK application at basal and split-dose for pot experiment on broccoli

Fertilizers	Basal dose (g pot ⁻¹)	Split dose (g pot ⁻¹)
Urea	N1: 0, N2: 5.5 and	At 30 DAT (N1: 0, N2: 2.75
	N3: 11	and N3: 5.5).
		At 45 DAT (N1: 0, N2: 2.75
		and N3: 5.5)
SSP	30 g	
MOD	20	

MOP 3.6 g

SSP – single superphosphate and MOP – muriate of potash, N1, N2 and N3 – nitrogen levels (treatments)

Data observation

The uprooted plants were taken to the laboratory, where the root was removed. The number of leaves, leaf area (cm^2), root length (cm), head diameter (cm), head weight (g) and aboveground biomass (g) were then recorded on the same day of harvesting using a measuring scale and electric weighing balance.

Data analysis

Data were recorded and entered into MS Excel 2016. Data were analyzed using the F-test in R-Studio 1.1.463 with R 3.5.2. Completely randomized design two-way ANOVA was used to analyze data. Treatment means were compared with Duncan's multiple range tests ($P \le 0.05$).

Results and Discussion

Mean square values and probabilities of growth and yield traits of broccoli are given in Table 4. Charcoal levels significantly affected only three traits; leaf area, root length and head diameter but did not significantly affect the rest of the traits. Nitrogen significantly affected all traits but its interaction with charcoal levels did not significantly affect the traits (Table 4). The number of leaves per plant did not vary statistically with various levels of charcoal and their combination with nitrogen (Table 5 and 7). However, the number of leaves varied from 10 to 14 with the mean value of 12 with the application of nitrogen levels (Table 6). The highest number of leaves (14) was observed at 375 kg N ha⁻¹ and was followed by 187.5 kg N ha⁻¹. The lowest number of leaves (10) was at 0 kg N ha⁻¹ (Table 6). Application of charcoal and nitrogen significantly (<0.05) improved leaf area, however, there was no interactive effect (Table 5, 6 and 7). In the case of charcoal, the treatment that received 7.5% produced a plant with a maximum leaf area (482.83 cm^2) that was at par with treatment that received 5%. The minimum leaf area (396.01 cm²) was produced in the plant receiving 2.5% (Table 5). Likewise, in the case of nitrogen, the best average of leaf area (632.09 cm^2) was produced when broccoli received the highest nitrogen dose of 375 kg N ha⁻¹ that was followed by broccoli receiving 187.5 kg N ha⁻¹. The minimum leaf area (130.50 cm²) in broccoli was produced in control and the average value obtained was 431.75 cm^2 (Table 6). Root length significantly (<0.05) differed with various levels of charcoal and nitrogen but had no significant interactive effect (Table 5, 6 and 7). An increasing level of charcoal increased root length in broccoli with the longest root length (28.19 cm) at 7.5% that was at par with 5% and the shortest root length (20.77 cm) at control (Table 5). Similarly, 187.5 kg N ha⁻¹ produced the longest root length (28.03 cm) in broccoli, which was at par with 375 kg N ha-1, and shortest root length (19.28 cm) at control. The average root length was 24.48 cm (Table 6).

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Table 4. Mean square values and p	robabilities of growth and	d yield traits of broccoli	i at Lamjung, Nep	al during the winter	season
of 2019–2020	-	-		-	

	Degree of freedom (df)			Mean square values			Probabilities		
Traits	Nitrogen	Charcoal	Nitrogen ×	Nitrogen levels	Charcoal	Nitrogen ×	Nitrogen	Charcoal	Nitrogen ×
	levels	levels	charcoal	-	levels	charcoal	levels	levels	charcoal
No. of leaves	2	3	6	116.217	0.378	1.794	< 0.001	0.815	0.200
Leaf area, cm ²	2	3	6	14 10 676	21 265	3 528	< 0.001	0.024	0.752
Root length, cm	2	3	6	423.65	175.15	39.73	< 0.001	0.001	0.245
Head diameter, cm	2	3	6	506.589	4.054	0.898	< 0.001	0.025	0.607
Head weight, g	2	3	6	281 135	1 087	1 076	< 0.001	0.380	0.413
Aboveground biomass, g	2	3	6	2 486 375	19 164	30 750	< 0.001	0.299	0.081

Table 5. Effect of different levels of charcoal on No. of leaves, leaf area, root length, head diameter, head weight and aboveground biomass of broccoli at Lamjung, Nepal during the winter season of 2019–2020

Charcoal levels	No. of leaves	Leaf area,	Root length,	Head diameter,	Head weight,	Aboveground biomass,
(%/soil weight)		cm ²	cm	cm	g	g
C1 (0 g charcoal pot ⁻¹)	12	412.93b	20.77c	12.62b	149.91	483.19
C2 (125 g charcoal pot ^{-1})	12	396.01b	22.55bc	12.79b	146.93	433.51
C3 (250 g charcoal pot ^{-1})	12	435.22ab	26.42ab	13.34ab	164.17	488.89
C4 (375 g charcoal pot ^{-1})	12	482.83a	28.19a	13.75a	161.64	519.87
Mean	12	431.75	24.48	13.13	155.67	481.36
SEM	0.28	20.3	1.38	0.28	23.65	31.9
F test	NS	*	**	*	NS	NS
LSD (0.05)	0.804	57.774	3.950	0.799	23.651	90.616

NS – not significant, * – significant at 0.05 level of probability, ** – significant at 0.01 level of probability, SEM – standard error of mean, LSD – least significant difference. Means followed by the same letters in the same column are not significantly different at the 0.05 level of probability.

Table 6. Effect of different levels of nitrogen on number of leaves, leaf area, root length, head diameter, head weight and aboveground biomass of broccoli at Lamjung, Nepal during the winter season of 2019–2020

Nitrogen levels	No. of leaves	Leaf area, cm ²	Root length, cm	Head diameter, cm	Head weight, g	Above-ground biomass, g
N1 (0 g N pot ⁻¹)	10c	130.50c	19.28b	7.40c	26.11c	94.27c
N2 (11 g N pot ⁻¹)	13b	532.65b	28.03a	15.15b	182.11b	565.65b
N3 (22 kg N pot ⁻¹)	14a	632.09a	26.13a	16.84a	258.77a	784.17a
Mean	12	431.75	24.48	13.13	155.67	481.36
SEM	0.24	17.6	1.20	0.69	7.20	27.6
F test	***	***	***	***	***	***
LSD (0.05)	0.696	50.033	3.421	0.692	20.483	78.476

*** - significant at 0.001 level of probability. SEM - standard error of mean. LSD - least significant difference. Means followed by the same letters in the same column are not significantly different at the 0.05 level of probability

Application of charcoal and nitrogen significantly (<0.05) improved head diameter, however, there was no interactive effect (Table 5, 6 and 7). In the case of charcoal, a level of 7.5 % produced the head of greatest diameter (13.75 cm) that was at par with the charcoal concentration of 5% and control produced the head of the smallest diameter (12.62 cm) (Table 5). Similarly, increasing the application of nitrogen also increased the head diameter of broccoli. The highest head diameter (16.84 cm) was recorded at the highest nitrogen level of 375 kg N ha⁻¹ that was followed by a nitrogen application of 187.5 kg N ha-1, while the lowest head diameter (7.40 cm) was found at 0 kg N ha⁻¹ (Table 6). The application of charcoal and its combination with nitrogen did not bring a significant difference (<0.05) in the head weight (Table 5 and 7). However, the application of different doses of nitrogen did affect head weight (Table 6). Head weight increased with elevation of nitrogen doses ranging from 26.11 to 258.77 g, with the highest head weight (258.77 g) at the highest nitrogen dose of 375 kg N ha⁻¹ and lowest head weight at control (Table 6). Aboveground biomass production did not differ significantly (<0.05) with various charcoal levels and their interaction with nitrogen (Table 5 and 7). In contrast, it did vary significantly with various nitrogen levels, with the maximum value of biomass (784.17 g) produced at 375 kg N ha⁻¹, followed by 187.5 kg N ha⁻¹. The minimum aboveground biomass (94.27 g) was produced at 0 kg N ha⁻¹ (Table 6).

The charcoal application did not bring significant changes in the most of growth and yield parameters of broccoli. The non-significant effect coincided with several other findings where charcoal amendment did not improve crop productivity of some temperate vegetables and root crops (Boersma et al., 2017; Hammond et al., 2014). The reasons behind the nonefficacy of charcoal could be several interacting factors such as charcoal ageing and nutrient content of used charcoal. In this experiment, the charcoal used was pure, uninoculated, and inactivated. Charcoal if incorporated without activation, its high adsorption capacity (AC) and cation exchange capacity (CEC) could lead to absorption and fixing of available nutrients and water in the soil (Schmidt, 2008). As a result, until it has been charged or aged organically, plant development may be impeded or not helped by its administration, at least in the beginning (several months to a year). The "ageing" of charcoal is linked to

the notion of expanding the reactive surface on a burned product to improve nutrient retention, bioavailability, and transport in the soil (Mia et al., 2017). Another possible explanation for the lack of significance is the low nutrient content of the charcoal employed in the experiment, which was insufficient to cause beneficial changes in broccoli growth and yield. However, charcoal alone significantly affected root length and had some statistical effect on leaf area and head diameter of broccoli. Higher charcoal levels resulted in longer root length (Xiang et al., 2017), most likely as a result of direct interactions between charcoal and root particles, as indicated by visual observation of charcoal particles grouped and bound around the crop's root and root hairs (Lehmann et al., 2011; Prendergast-Miller et al., 2014). Furthermore, charcoal's ability to improve physical and chemical properties of a given soil, such as nutrient or water availability and retention, porosity, aeration, PH, CEC etc., is likely to improve root length and other morphological characteristics of broccoli, such as leaf area and head diameter (Glaser et al., 2002; Zoghi et al., 2019).

Table 7. Interactive effect of different levels of nitrogen and charcoal on No. of leaves, leaf area, root length, head diameter, head weight and above-ground biomass of broccoli at Lamjung, Nepal during the winter season of 2019–2020

Nitrogen ×	No. of	Leaf	Root	Head	Head	Above-
Charcoal	leaves	area,	length,	diameter,	weight,	ground
levels		cm ²	cm	cm	g	biomass,
						g
N1C1	9	125.08	13.76	7.25	24.20	89.75
N1C2	10	125.80	16.22	6.99	26.57	91.13
N1C3	10	118.30	21.30	7.55	26.29	88.67
N1C4	10	152.83	25.84	7.80	27.39	107.52
N2C1	13	509.01	22.84	14.60	164.32	530.60
N2C2	13	486.62	25.70	15.25	164.65	464.80
N2C3	13	526.28	32.64	15.31	190.04	540.00
N2C4	14	608.69	30.94	15.43	209.42	727.20
N3C1	14	604.70	25.70	16.00	261.21	829.20
N3C2	15	575.60	25.72	16.14	249.57	744.60
N3C3	15	661.07	25.32	17.17	276.19	838.00
N4C4	13	686.97	27.78	18.03	248.09	724.88
Mean	12	431.75	24.48	13.13	155.66	481.36
F test	NS	NS	NS	NS	NS	NS
LSD	1.393	100.064	6.843	1.387	40.969	156.953

NS - not significant at 0.05 level of probability

Nitrogen levels were found to have a substantial impact on broccoli growth and yield traits in the study. Almost all of the evaluated growth and yield-related parameters were significantly higher in the crop fertilized with a higher level of nitrogen, 375 kg N ha⁻¹. The improved performance of most of the traits studied must be linked to improved plant metabolism because of higher nitrogen application levels. Because nitrogen is a significant component of proteins, nucleic acids, phytohormones, and chlorophyll in plants, its application may have accelerated plant growth, resulting in increased leaf number, leaf area, and root length in broccoli plants (Dhakal et al., 2016; Marschner, 2012). According to Ghoneim (2005), a rise in the number of leaves could be related to the positive effect of nitrogen, which boosts meristemic activity and so increases the number of tissues and organs (leaves). Nitrogen stimulates the production of cytokinin in plant roots, resulting in increased cytokinin transfer to the leaves and, as a result, increased cell division. As a result, leaf area and leaf expansion increase noticeably. Hence, as the nitrogen dose increases, so does the leaf area (Kaur, Sharma, 2020). These promotive effects of nitrogen on vegetative growth ultimately led to more photosynthetic activities and buildup of photosynthates and enhanced head diameter, head weight and aboveground biomass in broccoli (Bika *et al.*, 2018; Kaur, Sharma, 2020).

The combined effect of charcoal and nitrogen fertilizer on broccoli was non-significant, contrary to the findings of several previous studies that revealed a synergistic relationship between the two and showed the greatest plant growth responses when they were applied together (Steiner et al., 2007). However, Mavi et al. (2018) found that using charcoal with or without N fertilizer did not affect plant yield. When applied to high-input vegetable cropping systems, Boersma et al. (2017) also found that wood-based charcoal had no meaningful effect. The reason behind this could be decreased mineralization of nitrogen due to the high C/N ratio present in wood charcoal (Deenik et al., 2010; Demspter et al., 2012). Charcoal although serves as a long-term nutrient source, its application in soil temporarily decreases N availability to crops (Santalla et al., 2011).

Conclusion

Nitrogen applications can increase the growth and productivity of broccoli. Applying charcoal to the broccoli plant improved only the leaf area, root length, and head diameter, with no significant changes in the majority of the traits studied. In addition, combining charcoal and nitrogen levels did not affect the plant's growth or yield. The findings of this experiment suggest that the use of 375 kg N ha⁻¹ has a positive and promoting effect on the growth and yield of broccoli.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Author contributions

PB –Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

BL, PS, SB – Performed experiment and data analysis; Wrote the paper.

AK – Conceived and designed the experiment, supervision of the experiment, revision of the manuscript.

 $\mathbf{JS}-\mathbf{Critical}$ revision of manuscript, data analysis and wrote manuscript.

All the authors read and approved the final manuscript.

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