

Organic Amendments with Chemical Fertilizers Improve Soil Fertility and Microbial Biomass in Rice-Rice-Rice Triple Crops Cropping Systems

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Abstract

Medium-term changes in the labile nutrient pool of microbial biomass carbon (MBC) and nitrogen (MBN) resulting from organic manure application in rice (Oryza sativa L.)-based triple cropping systems have been poorly studied. Therefore, the effects of organic materials on the soil physico-chemical properties and microbial biomass in rice fields were investigated at Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh, from April 2010 to December 2012. Five treatments (control, cow dung, poultry manure, rice straw, and chemical fertilizer) were laid out in a randomized complete block design with four replications. The organic residues (2 t C ha⁻¹) were applied 7 days before transplanting and were combined with inorganic fertilizers, following integrated plant nutrition systems. This paper presents the results from the last of the five consecutive rice growing seasons. All of the organic residues increased the pH, and organic C, N, P, and K contents of the soil. However, poultry manure was more efficient in increasing soil fertility than cow dung and rice straw, resulting in a significant increase in P from 22 mg·kg⁻¹ to 63 mg·kg⁻¹ at crop harvest. All of the organic residues also increased the soil water holding capacity and decreased bulk density. Furthermore, poultry manure resulted in significantly higher microbial biomass C (432 mg·kg⁻¹; P < 0.05) and N (31.60 mg·kg⁻¹; P < 0.05) levels in the soil at crop harvest, followed by cow dung and rice straw. These findings indicate that the regular application of organic residues and manures will help to enhance soil fertility and production sustainability.

Keywords

Integrated Plant Nutrition Systems (IPNS), Poultry Manure, Rice Straw,

Water Holding Capacity

1. Background

The soil microbial biomass (SMB) indicates the ecological and biological quality and fertility of the soil, and is affected by organic/inorganic fertilizer application [1]. The SMB is involved in soil biogeochemical processes in soil-water-plant systems [1] [2], not only driving the nutrient cycle in agro-ecosystems, but also playing a vital role in soil nutrient transformations and acting as a labile nutrient pool that is available to plants [3] [4]. Several studies have emphasized the effects of organic manures such as soil organic carbon (SOC) on soil physicochemical properties. However, SOC may take a very long time to increase [5] [6] and observation of SOC changes alone may not explain the effect of organic matter on nutrient availability via microbiological activities. Alternatively, soil microbial biomass, which measures changes in both SOC and total soil nitrogen (TSN), can be used as a management indicator for soil fertility and crop productivity [7]. However, the effects of the various influencing factors, such as climatic and edaphic factors, fertilizer use, and crop establishment practices, on SMB are difficult to quantify due to their interactions; and estimation of the spatial and temporal distribution of SMB on the basis of limited field observations are even more difficult [4] [8].

Conventional agriculture on the Indo-Gangetic Plains is in a fragile state due to the overexploitation of land and water resources, and the indiscriminate use of agrochemicals [9] which reduce the diversity and quantity of microbes in the soil [10]. The use of different organic and inorganic fertilizers improves crop yields and also has direct or indirect effects on the physicochemical and biological properties of the soil. These changes are believed to have a significant effect on the quality and productive capacity of the soil on a long-term basis [11]. However, existing information on this issue is scarce and the long-term effects of organic inputs in combination with synthetic fertilizers on SMB have not been duly addressed.

Rice (Oryza sativa L.)-based cropping systems in the Indo-Gangetic Plains are very resource consumptive and input intensive [12] [13]. Though farmers use chemical fertilizers, the rates and methods of application are not justified according to either the integrated nutrient management system (INMS) or the integrated plant nutrition system (IPNS), and so most of the applied fertilizers are lost through various channels. Determination of microbial biomass carbon (MBC) and nitrogen (MBN) levels is crucial for the determination of C and N dynamics in rice systems, which regulate the SOC and N availability and loss, particularly in high-input-dependent cropping systems [14]. However, little research has investigated the effects of organic amendments on MBC and MBN in rice cropping systems, particularly in the eastern part of the Gangetic Plains, limiting our ability to generalize about soil C and N dynamics in subtropical cli-



matic areas. Therefore, there is an urgent need to study how soil fertility and microbial biomass change with organic amendments under intensive rice-based cropping systems.

The micro-organismal growth and activities are affected by different factors depending on soil type and management practices [15]. Soil microbes are active drivers of soil nutrient cycling, being associated with the decomposition of organic matter, and the transformation and cycling of nutrients, which help to maintain crop productivity and the physical and chemical quality of the soil [16]. Thus, an improved understanding and quantification of the effect of organic residue applications on MBC and MBN, and soil properties will enhance our ability to manipulate nutrient cycling, to maintain and improve soil quality and production sustainability.

2. Objectives

Therefore, the study investigated the changes of MBC and MBN over the rice growing season; and effect of application of various organic residues in addition to chemical fertilizer on other soil characteristics after growing five consecutive rice crops.

3. Materials and Methods

This study was conducted at the experimental farm of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh, as part of an ongoing research project entitled "Carbon sequestration in soils of Bangladesh," which ran from April 2010 to July 2012. This period covered five consecutive seasons of transplanted aman (T. aman) and boro rice, starting with T. aman in 2010. Aman rice is grown as a rainfed crop during the monsoon season, when maximum rainfall occurs (300 - 650 mm; supplemental irrigation is occasionally required when there is insufficient rainfall), while boro rice is grown after T. aman had been harvested under fully irrigated conditions during the dry season (0 - 55 mm rainfall). This report covers the last of the five consecutive growing seasons which was T. aman season rice.

3.1. Experimental Site and Climate

The study site belongs to the Salna series and has been classified as Shallow Red-Brown Terrace soil (Inceptisol) under the US Department of Agriculture classification system [17]. The area is part of the Madhupur Tract agro-ecological zone (AEZ No. 28), in which the soils are acidic in nature and characterized by clay loam within 50 cm of the surface. The site is located at 24.09°N latitude and 90.25°E longitude, with an elevation of 8.2 meters above sea level. The experimental area experiences a subtropical climate, and is characterized by comparatively high levels of rainfall, high humidities, high temperatures, and relatively long days from April to September, and minimum levels of rainfall, low humidities, low temperatures, and short days from October to March (Figure 1).

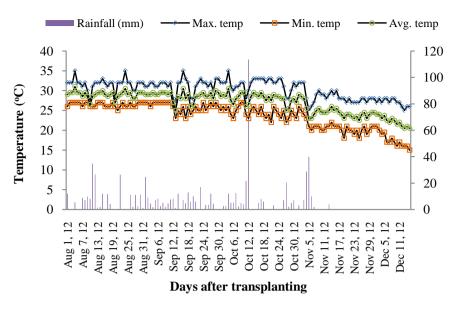


Figure 1. The maximum, minimum and average temperature (°C) with line graphs (unit on the primary axis); the bars show the precipitation (mm) in the area over the growing season (unit on the secondary axis).

3.2. Experimental Treatments

The experimental crop was rice and variety BRRI dhan39 was procured from Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh and used as the experimental crop. This variety is non-photosensitive, short-duration. The grains are long slender and kernels are white. This variety takes about 130 to 145 days to mature. It attains a plant height of 95 - 100 cm and at maturity the flag leaf remains green and erect. The experiment comprised five treatments (T_1 = Control [no fertilizer]; T_2 = cow dung; T_3 = poultry manure; T_4 = rice straw; and T_5 = soil test based chemical fertilizer [STB]), which were laid out in a randomized complete block design with four replications per treatment. IPNS-based chemical fertilizers were applied in combination with treatments T_2 , T_3 , and T_4 .The organic materials were applied 7 days before transplanting at 2 t C ha⁻¹ during the *Aman* seasons.

On August 5, 2012, 30-day-old rice seedlings were transplanted into the experimental plots at 20 cm × 20 cm spacing. The recommended doses of chemical fertilizers were calculated based on soil test results. Urea was applied in three equal parts, whereby one-third of the urea and the entire amount of triple superphosphate (TSP), murate of potash (MOP), and gypsum were applied during final land preparation; one-third of the urea was applied at the maximum vegetative growth stage (30 days after transplanting [DAT]); and one-third of the urea was applied before the panicle initiation stage of boro season rice (45 DAT).

Irrigation was used to keep the paddy field well flooded until the rice plants reached maturity. This was applied at 1, 5, 10, 20, 30, 40, 48, 54, 63, and 78 DAT, with a total of 45 cm of irrigation water being applied during rice growth. Weeding was performed at 25 DAT. Precautionary measures were taken at every stage of crop production using natural methods of pest and disease management, such as light traps, the placement of sticks for birds to stand on, and the removal of disease-infected plants at an early stage of infection, removing any need for chemical and herbicide use.

3.3. Soil Sample Collection and Processing

Soil samples were collected from depths of 0 - 5, 5 - 10 and 10 - 15 cm separately at 0, 30, 60, 90, and 120 DAT, and were immediately placed in separate labeled ice bags to minimize microbial activity. The samples were then taken to the BSMRAU Soil Science Laboratory for analysis as quickly as possible to preserve their field properties. In the laboratory, they were spread out for partial air drying following removal of the roots, insects, worms, stones, and some small pieces of organic matter. This procedure was necessary to reduce the excess soil moisture and to allow the samples to be passed through a 2-mm mesh sieve. After sieving, part of each sample was kept in a polythene bag and incubated aerobically for 3 weeks at 25°C to remove the effect of handling on soil respiration. The remainder of each sample was fully air dried, ground, passed through a 2-mm sieve, and then stored in a clean polythene bag for physical and chemical analysis.

The soil samples were analyzed for bulk density, pH, SOC, total N, available N (mineral N), available P, exchangeable K, MBC, and MBN. The maximum water holding capacity was also determined. Initial soil samples were also collected at 0 - 15 cm depth and analyzed for pH, SOC, total N, available P, and exchangeable K.

3.4. Methods of Soil Analysis

The soil bulk density was determined using the core sampling method, as outlined by Karim *et al.* [18]. Soil pH was measured with a glass electrode pH meter (Horiba pH meter M-8L) using a soil water suspension ratio of 1:2.5, as described by Jackson [19].

Maximum water holding capacity (MWHC) and adjustment of soil moisture at 60% MWHC were performed as suggested by Haider *et al.* [20]. Maximum water holding capacity (MWHC) of the soil was estimated by placing a quantity of soil (25 g) in a glass funnel lined with filter paper. Distilled water was then poured into the funnel and allowed to drain out for at least 1 hour. Following this, a small amount of soil was removed and placed in a pre-weighed aluminum can, and the combined weight was noted. The soil was then dried in an oven at 105°C for at least 24 hours until it attained a constant weight. The ratio of the weight of soil water to the weight of oven-dried soil was considered the MWHC of the soil. To adjust the soil samples to 60% MWHC, the difference between the initial moisture content of the soil and 60% MWHC was calculated, and the measured amount of water was then added to the samples.

Soil organic carbon (SOC) was estimated using Walkly and Black's wet oxidation method, as described by Jackson [19]. Total nitrogen was determined using the micro-Kjeldahl method [21], available P was measured using Olsen's method [22], and exchangeable K was estimated using the ammonium acetate extraction method [23].

3.5. Measurements of Microbial Biomass C and N

The chloroform fumigation extraction method was used to estimate the amount of microbial biomass C in the soil samples, according to the method described by Vance *et al.* [24]. Biomass N was determined according to the method described by Brookes *et al.* [25] [26].

3.6. Statistical Analysis

SPSS statistical software was used to analyze the data using one-way analysis of variance (ANOVA) and univariate analysis. Means were separated using the least significant difference (LSD) test at the 95% confidence level.

4. Results

4.1. Soil Chemical Properties

The initial soil samples indicated that the soil in the study area was slightly acidic, and contained medium SOC, very low N, optimum P, and low K contents (**Table 1**), reflecting a low to medium overall fertility. After five consecutive rice growing seasons, the soil chemical properties significantly varied among treatments (P < 0.05), with soil pH, SOC, and available P significantly increasing following application of the different organic residues (**Table 1**).

 Table 1. Soil properties of the experimental field under different organic residues and chemical fertilizer management at harvest.

Treatment	pН	Organic carbon (%)	Total nitrogen (%)	Available phosphorus (μg·g⁻¹)	Exchangeable potassium (meq 100 g ⁻¹ soil)
Initial soil properties	5.85	1.13	0.09	22.0	0.13
Rice straw [§]	5.88	1.81	0.16	27.2	0.26
Poultry manure [§]	7.17	1.83	0.19	63.3	0.23
Cow dung [§]	5.89	1.45	0.19	36.0	0.22
Soil test based fertilizer	5.82	1.25	0.15	28.3	0.17
Control	5.59	1.17	0.13	18.2	0.15
SE (±)	0.52	0.10	0.82	1.22	0.83
LSD _{0.05}	0.16	0.32	NS	3.76	NS
CV (%)	1.70	1.70	13.6	7.9	8.5

Legends: SE (\pm) means standard error of means; LSD_{0.05} means least significant differences at 5% level of significance and CV (%) are coefficient of variations. [§]-nutrient content in the organic amendment was deducted from recommended fertilizer dose.



Soil pH was significantly higher in the poultry manure treated plots (pH 7.17) than in the control plots (pH 5.59) and the initial soil samples (pH 5.85) (P < 0.05). This reflected a 23% increase compared with the initial soil pH.

Plots that were treated with a combination of organic residues and chemical fertilizers also had significantly higher SOC contents than the control plots (P < 0.05). Soil organic C concentrations in the rice straw (1.81%) and poultry manure (1.83%) treated plots were statistically similar (P > 0.05), but significantly higher than the cow dung treated plots (1.45%; P < 0.05). By contrast, the plots that were only treated with chemical fertilizer had a significantly lower SOC (1.25%) than all of the organic residue treated plots.

Available P was greatly enhanced in the poultry manure treated plots (P < 0.05; Table 1), increasing from an initial level of 22 mg P kg⁻¹ soil to 63 mg·kg⁻¹. The cow dung treated plots also had 14 mg·kg⁻¹ more available P than the initial soil, whereas the control plots experienced a 3.78 mg·kg⁻¹ depletion in available P.

4.2. Soil Biomass Carbon

The MBC content of the soil varied between treatments at 0, 30, 60, 90, and 120 DAT (**Table 2**). The significant difference on transplanting day (day 0; P < 0.05) was due to the experiment starting back in 2010. This was observed not only on transplanting day, but also found in 30, 60, 90 and 120 days after transplanting (**Table 2**). Irrespective of residues or for all treatments, the soil MBC contents increased with advancement of the rice growing period, *i.e.*, maximum MBC was found at crop harvest and followed the order 0 < 30 < 60 < 90 < 120 DAT. Soil MBC contents were significantly higher in plots that were treated with organic residues than in the control plots (P < 0.05), with poultry manure having the greatest effect and rice straw having the least effect. Thus, the maximum MBC was found in the poultry manure treated plots at harvest (432 mg·kg⁻¹), while the

Table 2. Microbial biomass carbon (mg·kg⁻¹) in soil under different organic residues and chemical fertilizer management practices at different days after transplanting (DAT).

Treatment	Biomass carbon (mg·kg ⁻¹) at different days after transplanting				
Treatment	0	30	60	90	120
Rice straw [§]	32.5	47.5	67.5	182	305
Poultry manure [§]	101	115	121	301	432
Cow dung [§]	47.3	71.3	91.6	178	380
Soil test based fertilizer	73.4	141	111	167	329
Control	17.0	35.5	49.3	118	200
SE (±)	0.78	12.4	4.58	9.42	14.7
LSD(0.05)	2.41	38.2	14.1	29.0	45.5
CV (%)	2.90	30.3	10.4	10.0	9.00

Legends: SE (\pm) means standard error of means; LSD_{0.05} means least significant differences at 5% level of significance and CV (%) are coefficient of variations. [§]-nutrient content in the organic amendment was deducted from recommended fertilizer dose.

lowest level was found in the control plots on transplanting day (17 mg·kg⁻¹).

4.3. Soil Biomass Nitrogen

The treatments also affected the MBN content of the soil at different days after transplanting the rice (**Table 3**) and significantly differed between each other on transplanting day (P < 0.05).

As observed for MBC, the maximum MBN was recorded at crop harvest and followed the order 0 < 30 < 60 < 90 < 120 DAT. Soil MBN contents were significantly higher in plots that were treated with organic residues than the control plots (*P* < 0.05), and poultry manure again had the greatest effect, followed by cow dung and then rice straw. Thus, at harvest, the maximum MBN was found in the poultry manure treated plots (32 mg·kg⁻¹), while the lowest was found in the control plots (9.88 mg·kg⁻¹).

4.4. Soil Water Holding Capacity

Organic amended soils had a significantly higher water holding capacity than the STB fertilizer and control treatments (**Figure 2**) after five consecutive rice cultivating seasons and repeated additions of the organic manures. The lowest water holding capacity was found in soil in the control plots (53%). There was no significant difference in the water holding capacity of soil between the three organic residue treatments, but cow dung treated plots had the highest level while poultry manure treated plots had the lowest.

4.5. Soil Bulk Density

The soil bulk density varied significantly following the application of different organic residues in the surface soil layer (**Table 4**). However, the highest bulk density was found in the control plots, while the lowest was found in the cow dung treated plots at all soil depths. Thus, the bulk density varied from 1.29 to

 Table 3. Microbial biomass nitrogen (mg·kg⁻¹) in soil under organic residues and chemical fertilizer management practices at different days after transplanting (DAT).

Treatment	Biomass nitrogen (mg·kg $^{-1}$) at different days after transplanting					
Treatment	0DAT	30DAT	60DAT	90DAT	120DAT	
Rice straw [§]	4.65	6.81	8.41	15.5	18.2	
Poultry manure [§]	5.01	13.4	13.8	18.7	31.6	
Cow dung [§]	4.70	6.99	11.6	14.4	27.2	
Soil test based fertilizer	5.79	11.8	10.9	18.2	20.1	
Control	3.25	6.43	6.01	11.5	9.88	
SE (±)	0.17	0.65	0.53	1.48	1.30	
LSD(0.05)	0.53	2.02	1.63	4.56	4.01	
CV (%)	7.40	14.5	10.6	18.9	12.2	

Legends: SE (±) means standard error of means; LSD_{0.05} means least significant differences at 5% level of significance and CV (%) are coefficient of variations. $^{\$}$ -nutrient content in the organic amendment was deducted from recommended fertilizer rate.



Treatment —	Bulk density (g·cm ⁻¹) at different soil depth				
Treatment	0 - 5 cm	5 - 10 cm	10 - 15 cm		
Rice straw [§]	1.36	1.31	1.45		
Poultry manure [§]	1.34	1.41	1.38		
Cow dung [§]	1.29	1.17	1.20		
Soil test based fertilizer	1.38	1.47	1.44		
Control	1.48	1.54	1.46		
$SE(\pm)$	0.14	0.24	0.27		
LSD _{0.05}	0.08	ns	ns		
CV (%)	2.20	3.60	4.00		

 Table 4. Bulk density of soil under different organic residues and chemical fertilizer management practices at different depths.

Legends: SE (\pm) means standard error of means; LSD_{0.05} means least significant differences at 5% level of significance and CV (%) are coefficient of variations. [§]nutrient content in the organic amendment was deducted from recommended fertilizer rate.

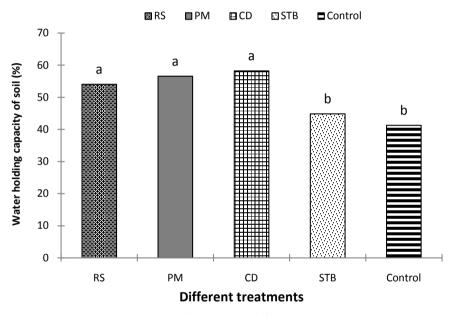


Figure 2. Water holding capacity of soil under different organic residues and chemical fertilizer management practices. RS-Rice straw + IPNS based chemical fertilizer, PM-poultry manure + IPNS based chemical fertilizer, CD-cow dung+ IPNS based chemical fertilizer, STB-soil test based chemical fertilizer and control. Vertical bars represent standard error of means.

 $1.43 \text{ g} \cdot \text{cm}^{-3}$ in the top 0 - 5 cm of soil, from 1.17 to 1.47 g $\cdot \text{cm}^{-3}$ at a depth of 5 - 10 cm, and 1.20 to 1.44 g $\cdot \text{cm}^{-3}$ at a depth of 10 - 15 cm for the control and cow dung treated plots, respectively. Microbial biomass C and MBN are inversely related to the soil bulk density.

5. Discussion

At all sampling dates, MBC and MBN were significantly higher in the poultry manure treated plots than in the other treatment plots. This may have been due

to the higher amounts of N that were present in the poultry manure resulting in a lower or optimum C:N ratio, favoring faster microbial colonization. These findings agree with those of several other studies [27] [28]. In addition, a higher amount of MBN was found in the STB fertilizer treatment than in the rice straw and cow dung treatments. This was due to the presence of higher amounts of available nutrients, particularly N, in the fertilizer treatment, which favored rapid microbial activity, i.e., mineralization in the soils. These findings agree with those of Akmal et al. [29] who attributed higher microbial biomass to higher available C substrates and nutrient contents in soils. Thus, the availability of organic residues in the soils coupled with high moisture contents favored microbial activities, which ultimately contributed to the higher MBC and MBN under these treatments [30] [31].

The use of organic amendments in combination with chemical fertilizers improved the pH, and SOC, N, P, and K contents of the soils after seven consecutive rice growing seasons. The use of poultry manure led to the greatest improvement in these initially low to medium fertility soils. Organic C was applied at a rate of 2 t C ha⁻¹ in all plots, irrespective of its source. However, poultry manure contains a higher amount of the stable form of C, which explains the greater level of C enrichment in these plots. The higher pH in the poultry manure treated plots can be attributed to the presence of Ca in poultry manure due to the calcium-rich diet of poultry. The accumulation of Ca in the soil will have neutralized the soil acidity and ultimately increased the soil pH to around neutral levels. Finally, it is known that poultry manure usually contains a higher concentration of P, with a previous study showing that at 30% - 35% moisture level, poultry manure, cow dung, and rice straw contained 0.70%, 0.30%, and 0.10% P, respectively [32]. Since the rate of C application in the present study was determined on an oven dry basis, the P accumulation will have been higher than these reported values. Therefore, the application of different organic residues improved soil fertility, with poultry manure being the most efficient organic amendment.

The soil water holding capacity also increased following the application of different organic residues (Figure 2), with rice straw, cow dung, and poultry manure having similar effects. The greatest improvement was recorded with poultry manure, which resulted in 38% and 30% higher levels than the control and STB fertilizer treated soils, respectively. Once again, this can be explained by the availability of organic residues in these soils coupled with their high moisture contents favoring microbial activities. These findings agree with those of Pavlova et al. [31] and Efremov et al. [30], who reported that the total pool of microbial biomass was dependent on the soil moisture content, which predetermined the water permeability of soils.

At the soil surface (top 0 - 5 cm), organic amendment combined with chemical fertilizers significantly decreased the soil bulk density, and similar trends were observed at 5 - 10 cm and 10 - 15 cm. This resulted from the organic residues favoring microbial biomass growth [33] and matches the findings of Glover



et al. [34], who confirmed that the addition of organic residues with chemical N resulted in lower bulk densities and higher microbial biomass.

6. Conclusion

Poultry manure amendment in combination with chemical fertilizers under rice growing conditions resulted in the most biologically active soils with higher levels of MBC and MBN. Thus, poultry manure is a more efficient organic amendment than cow dung and rice straw for increasing soil fertility. The application of organic residues also increased the pH, and SOC, N, P, and K contents of the soil, further improving soil fertility. Compared with the initial soil, the poultry manure treatment increased the soil pH from an acidic (5.85) to neutral (7.17) state, SOC by 62%, available P by 187%, total N by 112%, and exchangeable K by 77%, though the latter two were similar to other organic amendments. The soil water holding capacity and bulk density increases that were observed following the application of organic residues reflected the microbial biomass improvements as a result of the same treatments, with poultry manure having the greatest effect and rice straw the least effect. The maximum MBC and MBN were found in the poultry manure treated plots at harvest (432 and 31.6 mg·kg⁻¹, respectively). Furthermore, irrespective of treatment, the biomass carbon contents increased with advancement of the rice growing period, *i.e.*, 0 < 30 < 60 < 90 < 0120 DAT, with a maximum level at crop harvest. These findings show that the regular application of organic materials to soils using whatever sources available will increase the microbial dynamics and nutrient pools in the soil, enhancing soil fertility and productivity.

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Declaration of Conflict of Interest

The Authors of the manuscript are hereby declared that there will not take place any conflicts of interest for any reasons, such as personal and/or financial relationships, academic competition, and intellectual passion. No gender issue is also involved in publishing the manuscript.

Authors' Contributions

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in The Journal of Agricultural Science and Technology.

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