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Response of agave coyote (*Agave* spp.) to the application of slow-release fertilizers under field conditions.

Respuesta de agave coyote (*Agave* spp.) a la aplicación de fertilizantes de liberación lenta en condiciones de campo.

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ABSTRACT

The agave coyote (Agave spp.) is a wild species used for its great potential for mezcal production in Oaxaca, Mexico. The present study aimed to evaluate the growth, nutritional and total soluble solids (TSS, sugars) response of agave coyote to slow-release fertilizers (SRFs) application under field conditions. Three treatments: control (no fertilization), Osmocote plus® (15-09-12), and Multicote Agri® (18-06-12) were evaluated in a completely randomized block design. Each treatment included 20 plants and four replicates, with the number of plants per treatment in each replicate equal to 5. After 10 months, plant height (PH), number of unfolded leaves (UL), stem circumference, root volume, root density, fresh leaf weight (FLW), fresh stem weight (FSW), fresh roots weight (FRW), dry leaves weight (DLW), dry stem weight (DSW), dry root weight (DRW), TSS and leaf contents of Ca2+, Na+, NO₃ and K⁺ were measured. Compared to the control plants, Osmocote increased PH by 21.2 %, UL by 28.4 %, FLW by 77.0 %, FSW by 62.8 %, DLW by 177.0 %, DSW by 53.1 % and DRW by 39.1 %. Multicote increased PH by 15.3 % and leaf content of K⁺ by 25.6 %, NO₃⁻ by 26.2 %, and Na⁺ by 29.8 %. Under field conditions, applying SRFs promoted growth and nutrition but not TSS.

KEY WORDS: Wild agave, Plant growth, Multicote, Plant nutrition, Osmocote.

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RESUMEN

Agave coyote (*Agave* spp.) es una especie silvestre utilizada por su gran potencial para la elaboración de mezcal en Oaxaca, México. En este estudio se evaluó la respuesta en el crecimiento, nutrición y contenido de sólidos solubles totales (TSS, azúcares) de agave coyote a la aplicación de fertilizantes de liberación lenta (SRFs) en condiciones de campo. Bajo un diseño de bloques completamente al azar se evaluaron tres tratamientos: control (sin fertilización), Osmocote plus® (15-09-12) y Multicote Agri® (18-06-12). Cada tratamiento incluyó 20 plantas y cuatro repeticiones, el número de plantas por tratamiento en cada repetición fue igual a 5. Después de 10 meses se midió altura de planta (PH); número de hojas desplegadas (UL); circunferencia de tallo; volumen radicular; densidad radicular; peso fresco de hojas (FLW), tallo (FSW) y raíz (FRW); peso seco de hojas (DLW), tallo (DSW) y raíz (DRW); TSS y contenido foliar de Ca²+, Na+, NO₃- y K+. Con respecto a las plantas control, la PH incrementó 21.2 %, UL 28.4 %, FLW 77.0 %, FSW 62.8 %, DLW 177.0 %, DSW 53.1 % y DRW 39.1 % con la aplicación de Osmocote. Multicote aumentó 15.3 % la PH, así como el contenido foliar de K+ en 25.6 %, el de NO₃- en 26.2 % y el de Na+ en 29.8 %. En condiciones de campo, la aplicación de SRFs promovió el crecimiento y la nutrición de agave coyote, pero no el contenido de TSS.

PALABRAS CLAVE: Agave silvestre, Crecimiento vegetal, Multicote, Nutrición vegetal, Osmocote.

Introduction

There are 211 species of *Agave* spp. in the Americas, of which 75 % are distributed in Mexico, with 57 % of endemic species, mainly in two subgenera: *Agave* and *Littaea* (García-Mendoza *et al.*, 2019). Cactaceae and Agavaceae families represent one of the most important natural resources with a strong socioeconomic and sociocultural impact in Mexico (Cortés-Zárraga & Basurto-Peña, 2021; Gutiérrez-Rojas *et al.*, 2022). Twenty-two categories of uses have been reported for agave, also known as maguey (Colunga-GarcíaMarín *et al.*, 2017). These uses include obtaining food, beverages, biofuels, hard fibers extracted from the leaves (ixtle), fertilizers, housing construction, and the elaboration of agricultural tools, among others (Aguilar *et al.*, 2014). Agave plants can also be good carbon fixers, according to García-Moya *et al.* (2010).

In the state of Oaxaca, Mexico, about nine species of agave are used primarily for the production of distilled alcoholic beverages such as mezcal, of which *Agave angustifolia* Haw. colloquially known as "agave espadín" is the most sought-after and the only one that is significantly cultivated in semi-arid soils (Bautista-Cruz & Martínez-Gallegos, 2020). Another eight agave species, including the one commonly known as "coyote" (*Agave* spp.), are collected from wild



or semi-cultivated populations, mainly in live fences, with little or no agronomic management (Bautista-Cruz & Martínez-Gallegos, 2020). According to Palma *et al.* (2016), the number of agave coyote plants in the state of Oaxaca in 2014 was only 153, a very low number compared to the 26,172,984 agave espadín plants cultivated in the same year. Although the agave coyote is not a widely cultivated species, it has great potential for mezcal production; in this regard, Martínez-Jiménez *et al.* (2019) pointed out that 10.6 kg of the stem or "piña" can yield 1 liter of mezcal, a yield similar to that of agave espadín (9.16 kg of piña yields 1 liter of mezcal) and higher than *A. potatorum*, commonly known as "maguey tobalá" or "maguey papalomé" (16.57 kg of piña yields 1 liter of mezcal). In addition, agave coyote is earlier (5.33 years to maturity) compared to agave espadín (7.0 years to maturity) and maguey tobalá (6.33 years to maturity).

For the production of beverages such as mezcal, tequila, pulque, and agave honey, mature agave plants are harvested in the reproductive stage (once inflorescences are formed). This results in the total loss of pollen and seeds (Arrazola-Cárdenas *et al.*, 2020). This continuous harvesting of reproductive agaves causes their populations to decline and alters their genetic distribution (Sebbenn *et al.*, 2008).

In general, soils, where species of the genus *Agave* grow, are poor in organic matter, N and P (Bautista-Cruz *et al.*, 2007). Under these conditions, improving current yields in agricultural production systems despite the effects of climate change is a major challenge (Zúñiga-Estrada *et al.*, 2018). However, previous studies have shown positive effects of fertilizers on agave growth (Enríquez del Valle *et al.*, 2018; García-Martínez *et al.*, 2020; Sánchez-Mendoza *et al.*, 2020; Zúñiga-Estrada *et al.*, 2018).

Slow-release fertilizers (SRFs) can be a viable alternative for plant nutrition, as they provide nutrient availability to the plant over a longer period of time, which promotes greater efficiency of use and thus less negative impact on the environment and human health (Kiplangat *et al.*, 2019). The main disadvantage of SRFs is their high cost (Vásquez-Cisneros *et al.*, 2018), however, these products have the potential to increase fertilization efficiency (Soti *et al.*, 2015), in addition to requiring fewer applications to the crop (Aguilera-Rodríguez *et al.*, 2016), generating savings in time and labor.

For all these reasons, the aim of this work was to evaluate the response of growth, nutrition, and total soluble solids content of agave coyote to the application of SRFs under field conditions.

Material and Methods

The experiment was established under rainfed conditions in San Jacinto Chilateca (16°50′28″ N, 96°41′03″ W), Ocotlán de Morelos, Oaxaca (Mexico), at an average altitude of 1,533 masl and a semi-warm, temperate subhumid climate (Secretaría de Desarrollo Social, 2021). Some of the physical and chemical properties of the soil where the experiment was established are shown in Table 1.

Rhizomatous tillers of agave coyote were obtained from 3-year-old mother plants at 7 to 8 months of age; these tillers had an average height of 33.5 cm and were visually inspected for evidence of lesions, decay, or insect attack. The roots were cut to generate a new root system



that would promote their development at the time of transplantation in the field. They were then disinfected by immersion in a 0.624 % sodium hypochlorite solution for 5 minutes (Sánchez-Mendoza *et al.*, 2020).

Table 1. Physical and chemical properties of the soil in which the experiment was conducted.

Soil properties	
Bulk density	1.23 g cm ⁻³
Organic matter	2.4 %
pH 1:2	8.39
Electrical conductivity (EC 1:2)	0.31 dS m ⁻¹
N-NO ₃ -	4.85 mg kg ⁻¹
Available phosphorus Exchangeable bases	24.5 mg kg ⁻¹
Ca ²⁺	6354 cmol (+) kg ⁻¹
Mg ²⁺	241 cmol (+) kg ⁻¹
Na⁺	24.5 cmol (+) kg ⁻¹
K⁺	264 cmol (+) kg ⁻¹
Micronutrients	
Cu	0.81 mg kg ⁻¹
Mn	1.65 mg kg ⁻¹
Fe	2.82 mg kg ⁻¹
Zn	0.15 mg kg ⁻¹
В	0.45 mg kg ⁻¹

Transplanting was performed on October 22, 2020, with a plant spacing of 1.5 m and a row spacing of 3 m, resulting in a planting density of 2,178 plants ha⁻¹ (Figure 1). Fertilization was carried out two months after transplanting. The fertilizers used were 1) Osmocote plus® (15-09-12) brand eveRRRIS ILC Fertilizer Company, Dublin, OH, United States (15 % N, 9 % P_2O_5 , 12 % K_2O , 6.0 % SO_4 , 0.02 % B, 0.05 % Cu, 0.46 % Fe, 0.06 % Mn, 0.02 % Mo, and 0.05 % Zn) with a release period of 5-6 months and 2) Multicote Agri® (18-06-12) brand Haifa Chemicals Ltd. Haifa, Israel (18 %N, 6 % P_2O_5 , 12 % K_2O , 2 % CaO, 3.5 % MgO, and 2.1 % Si) with an 8-month release time. According to Sanchez-Mendoza *et al.* (2020), 100 g of SRFs were manually applied around each plant at a depth of 5 cm and 5 cm from the stem. The experiment was set up in a completely randomized block design. Three treatments with four replications were evaluated and 20 plants were included in each treatment (the number of plants per treatment in each replication was equal to 5). The treatments evaluated were T1) control (no fertilization), T2) Osmocote plus® and T3) Multicote Agri®. The evaluation period was 10 months.



At the end of the experiment, plant height (PH) the and number of unfolded leaves (UL) were determined by visual counting of all plants per treatment. Half of the plants in each treatment were randomly harvested i.e. 10 plants. Stem circumference (SC) was determined with a tape measure; root volume (RV) was determined in a 1000 ml test tube with a known volume of water, the roots were introduced and the volume of displaced water was measured; root density (RD) was determined by the mass-volume ratio; the fresh leaves weight (FLW), stem (FSW) and roots (FRW); the dry leaves weight (DLW), stem (DSW) and root (DRW), for which the plant material was dehydrated in a solar dryer until constant weight. Sugar content (total soluble solids in the stem, TSS) was determined with a portable refractometer RHB-32 ATC. Quantification of Ca²+, Na+, NO₃-, and K+ content was made on leaves, and a sample composed of sap from the central part of all leaves was used. The elements were then determined in this composite sample using a LAQUAtwin ion meter. During the experiment, the percentage of soil moisture and temperature in the rhizosphere of agave plants were determined *in situ*. For this purpose, a digital instrument from Nennimber Gmbh was used. The average soil temperature was 31 °C and the average soil moisture was 61.1 %.





Figure 1. Agave plants in the experimental plot beginning (left) and ending (right) the study.



Statistical Analysis

Data for PH and UL were analyzed for normality using the Kolmogorov-Smirnov test; normality analysis for the remaining variables was performed using the Shapiro-Wilk test. Variables that did not meet the normality assumptions were transformed to log10 (x) or square root. The homogeneity of variance was tested using Bartlett's test. An ANOVA and a mean comparison test were then performed using Duncan's method ($p \le 0.05$). SAS v. 9.1 software (SAS Institute, 2004) was used for all statistical procedures.

Results and Discussion

Of the 16 variables evaluated, 10 responded significantly to SRFs application. Compared to the control plants, Osmocote application increased PH by 21.2 %, UL by 28.4 %, FLW by 77.0 %, FSW by 62.8 %, DLW by 177.0 %, DSW by 53.1 %, DRW by 39.1 % and leaf Na⁺ content by 29.8 % (Tables 2, 3 and 4). Multicote fertilization also increased PH by 15.3 % (Table 2), leaf K⁺ content by 25.6 %, NO₃⁻ by 26.2 %, and Na⁺ by 29.8 % (Table 4). Variables such as SC, RV, FRW (Tables 2 and 3) and TSS content in the stem of agave plants (Table 2) did not respond significantly to the application of Osmocote or Multicote. In contrast, RD and leaf Ca²⁺ content were negatively affected by SRFs (Tables 2 and 4), as their values were higher in control plants.

Table 2. Mean value ± standard error of growth variables and total soluble solids content in stem (TSS) of agave coyote (*Agave* spp.) plants in response to the application of slow-release fertilizers under field conditions.

Tuestanoute	UL	PH	sc	RV	RD	TSS
Treatments		cm		cm³	g cm ⁻³	°Brix
Control	21.50±1.0b	50.44±2.0b	40.47±2.9a	40.00±5.7a	2.15±0.2 ^a	8.40±0.8a
Osmocote plus®	27.60±1.1a	61.15±2.4a	47.98±1.1a	54.00±3.0a	1.47±0.1b	10.30±0.7a
Multicote agri®	24.20±1.5ab	58.16±2.3a	45.77±6.5a	50.00±8.9a	1.61±0.1b	9.90±0.7a

UL: unfolded leaves number; PH: plant height; SC: stem circumference; RV: root volume; RD: root density. Mean values with different letters in each column are statistically different according to the Duncan test $(p \le 0.05)$.



Growth promotion of agave coyote plants with Osmocote may be due to the fact that this fertilizer has a shorter release period (5-6 months) compared to Multicote (8 months). It is likely that by increasing the levels of these nutrients in the soil over a shorter period of time with the addition of Osmocote, the plants were able to increase the efficiency of their metabolic processes, resulting in better growth.

Table 3. Mean value ± standard error of plant biomass accumulation in agave coyote (*Agave* spp.) in response to the application of slow-release fertilizers under field conditions.

Treatments	FLW	FSW	FRW	DLW	DSW	DRW
Control	2606.6±637.7b	1110.10±213.9b	76.69±7.8a	476.70±110.6b	271.99±68.1b	28.14±4.5b
Osmocote plus®	4613.7±445.1a	1807.70±122.4a	80.05±8.0a	1320.50±143.6a	416.36±37.9a	39.14±3.4a
Multicote agri®	3654.9±788.6ab	1358.50±321.9ab	74.01±10.8a	1040.90±208.1a	286.96±51.3ab	36.92±4.3ab

FLW: fresh leaves weight; FSW: fresh stem weight; FRW: fresh roots weight; DLW: dry leaves weight; DSW: dry stem weight; DRW: dry roots weight. Mean values with different letters in each column are statistically different according to the Duncan test ($p \le 0.05$).

Table 4. Mean value ± standard error of leaf nutrient content in agave coyote (*Agave* spp.) plants in response to the application of slow-release fertilizers under field conditions.

Treatments	Ca ²⁺	K⁺ mg I	NO ₃ -	Na ⁺
Control	476.00±35.4a	2730.00±109.5b	309.00±12.2b	26.20±1.1b
Osmocote plus®	391.00±18.5b	2730.00±86.9b	268.00±9.7b	34.00±1.2ª
Multicote agri®	293.00±21.6c	3430.00±26.5a	388.00±17.5a	33.70±0.8a

Mean values with different letters in each column are statistically different according to the Duncan test $(p \le 0.05)$.



Since the study was conducted under rainfed conditions, it is important to consider soil moisture and temperature, which could condition the efficiency of SRFs and the biological response of the plant. During the experiment, the average soil temperature was 31 °C and the average soil moisture was 61.1 %. In this sense, an SRF should ideally release a number of nutrients that match the requirement of the plant, even under fluctuating environmental conditions. Unfortunately, no SRF meets this requirement due to the fact that nutrient release is affected by temperature more than any other extrinsic factor. However, there is no consensus in related literature about the effect of temperature on the nutrient release rate of SRFs (Adams *et al.*, 2013). According to Adams *et al.* (2013), SRFs nutrients most affected by temperature were N, K, B, Cu, and Zn, while the least affected were P, Mg, and Fe. These authors also reported that Osmocote nutrients were released faster than indicated at both high and low temperatures. This suggests that agave plants may have greater nutrient availability when fertilized with Osmocote than with Multicote, which promoted their growth.

Ransom *et al.* (2020) indicated that soil moisture does not appear to be a limiting factor for nutrient release when SRF granules are in direct contact with the soil, as was the case in this study where SRF was placed at a depth of 5 cm. In contrast, Du *et al.* (2006) concluded that nutrient release from Multicote in pure water was faster than the release in saturated sand and significantly faster than in sand at field capacity. However, there is no theoretical basis for these differences as the authors did not consider the possible chemical effects of sand in their measurements.

According to Ransom *et al.* (2020), manufacturer-estimated SRFs nutrient release times rarely match release times under field conditions. This is because, in the laboratory, SRF granules are placed in a flask that is periodically shaken during the test at a constant temperature. However, these conditions are far from those experienced in the field.

Studies evaluating the effect of SRFs on plants of the genus Agave are scarce. Sánchez-Mendoza et al. (2020) found no significant differences for PH and UL compared to the control in agave espadín plants under field conditions fertilized with the SRFs Multigro 6® (21-14-10 NPK + 2 MgO), Multigro 3[®] (24-05-13 NPK + 2 MgO) and Turf Builder[®] (27-03-04 NPK). Although the same authors reported an increase in root length, FLW, FSW, and stem diameter in agave espadín plants fertilized with SRF Multigro 6®, with the exception of those obtained for the variables root length and stem diameter, these results coincide with those reported in this work. Sánchez-Mendoza & Bautista-Cruz (2022) evaluated the effect of SRFs Osmocote plus® and Basacote plus® on the growth and stem sugar content of agave espadín plants under nursery conditions and found that both SRFs promoted the growth of this agave. However, the greatest increase in most growth variables was obtained with Osmocote plus®. Compared to the control plants, Osmocote plus® increased UL by 10.1 %, PH by 10.4 %, stem diameter by 10.2 %, FLW by 28.4 %, and FSW by 33.1 %. Basacote plus® increased PH by 6.4 %, plant stem diameter by 5.8 %, FSW by 16.1 %, and FRW by 42.1 %. These authors also found no increase in TSS with the addition of SRFs. These results are consistent with those reported in this study where the plant growth variables evaluated also responded positively to the application of Osmocote.

Aguilera-Rodríguez *et al.* (2016) evaluated the effect of individual and combined applications of 8 g L⁻¹ of the SRFs Multicote[®] (18-6-12, one with an 8-month release period and



another with a 4-month release period) and Osmocote plus® (15-9-12, with an 8-9-month and a 5-6-month release period) mixed with two substrates: 1) pine sawdust (60 %), composted pine bark (15 %), peat moss (15 %), and vermiculite (10 %) and; 2) pine sawdust (70 %), composted pine bark (15 %), peat moss (15 %), and vermiculite (10 %). The authors found that pine sawdust substrates combined with Multicote® or Osmocote plus® with a release period of 8 to 9 months were sufficient to increase PH, plant stem diameter, aerial dry weight, and DRW in *Pinus pseudostrobus* plants. This study is also consistent with a positive response of plant growth variables to the Osmocote application.

Reyes-Castro *et al.* (2020) evaluated the effect of the application of triple 17 fertilizer (17N-17P-17K) at doses of 3.3 (low), 6.6 (medium), and 10 kg m⁻³ (high), and Osmocote[®] fertilizer (15N-9P-12K) at doses of 10 (low), 20 (medium) and 30 kg m⁻³ (high) on the growth of jagua (*Genipa americana* L.) plants during the nursery stage. Like the results of this work, these authors obtained the highest PH, collar diameter, root length, and aerial and root biomass with the application of Osmocote[®] at the three evaluated doses.

Previous research has reported that some agaves have also responded positively to conventional fertilization. For example, like this study, Cruz-Vasconcelos *et al.* (2020) found that PH increased by 51.1 % in *A. salmiana* plants that received conventional fertilization with triple 17 (17-17-17), urea (46-00-00) and Yara Star (21-17-3) compared to control plants. In contrast, Martínez-Ramírez *et al.* (2013) also reported that UL increased by 15.5 % in maguey tobalá and 18.9 % in agave espadín with high (90-60-45 kg ha⁻¹) and medium (60-40-30 kg ha⁻¹) doses of conventional fertilization (triple superphosphate, potassium sulfate, and ammonium sulfate).

García-Martínez *et al.* (2020) found that the addition of 43.5 mg kg⁻¹ P increased PH by 13.2 %, FLW by 34.9 %, FSW by 36.1 % and stem diameter by 21.5 % in maguey tobalá plants. The same authors indicated that in agave coyote, the dose of 29.0 mg kg⁻¹ P increased the PH by 16.4 % and the FSW by 44.4 %. These results are consistent with those obtained in this study.

Sánchez-Mendoza *et al.* (2020), like this work, also found no significant effect of SRFs on the TSS content of the stem or "pineapple" of agave espadín. Zúñiga-Estrada *et al.* (2018) reported that *A. tequilana* plants receiving basic fertilization (162-150-250 kg ha⁻¹ of N, P, and K) + fertigation (315.3 g N; 179.9 g P_2O_5 ; 353.4 g K_2O ; 111 g CaO, and 89.1 g MgO) also showed no increase in TSS content. Possibly, there was no increase in TSS content in agave coyote plants with SRFs application because each agave species responds differently to fertilization, depending on its phenotypic and genotypic characteristics, as well as its nutritional requirements according to its phenological stages.

Nitrogen is an essential macronutrient and one of the most important components of molecules such as amino acids, proteins, chlorophyll, and nucleic acids required for agave plant nutrition. The assimilable forms of nitrogen for the plant are NO₃- and NH₄+, the deficiency of this nutrient element is manifested with initial chlorosis in the oldest leaves and progressively towards the youngest ones (Miguel-Zarate *et al.*, 2021). In the agave plant, it has been observed that when the N supply is limited, growth decreases and the foliage takes on a green color instead of the characteristic blue (Zúñiga-Estrada, 2013). In addition to macronutrients such



as N and K, the SRFs studied contain P, S, Mg, and various micronutrients, which together may promote the growth of agave plants. In addition, SRFs can slow down the conversion of N to ammonium, reduce the N release rate of fertilizers, synchronize N supply with plant N demand, and maintain a sustained and stable supply of nutrients during the growing season, thus improving dry matter synthesis capacity of plants (Tian *et al.*, 2018; Zhang *et al.*, 2020). Dry matter is the accumulation of photosynthetic substances and absorbed nutrients in plants, which directly affects crop yield (Wang *et al.*, 2021).

The highest leaf N content, in the form of N-NO₃, was found in agave plants fertilized with Multicote, which is consistent with the percentage content of this nutrient in the SRF, since Multicote has a higher amount of N (18 %) than Osmocote (15 %). These results are in agreement with those reported by Aguilera-Rodríguez *et al.* (2016), who evaluated the application of Basacote, Multicote, and Osmocote on *Pinus montezumae* Lamb. plants under nursery conditions and found that the highest concentration of N in the foliage was presented in treatments with Multicote.

K⁺ was the nutrient with the highest content in the leaves of agave coyote plants. In this regard, Zúñiga-Estrada (2013) reported that the K⁺ extraction rate was 11.9 g month⁻¹ in *A. tequilana* plants after 41 months of development. In contrast, the maximum monthly N extraction was 5.9 g after 53 months of agave plant development. These data seem to indicate that agave plants extract more K⁺ from the soil than N. Accumulated nutrients in leaves of agave plants are important as they can be recycled in the soil and be available for new plantings, since generally during the "jima" (harvesting of agave) agave leaves are deposited and left in the soil to initiate the process of microbial degradation of the plant material and gradually reintegrate nutrients into the soil.

Conclusions

Under field conditions, SRFs application promoted the growth and nutrition of agave coyote, but not TSS levels. SRF Osmocote plus® increased FLW, DLW, FSW, DSW, and DRW. Fertilization with Multicote Agri® increased PH, as well as leaf K⁺, NO₃⁻, and Na⁺ content. However, since the agave plant has a long growing cycle, about 5 to 7 years, depending on the conditions of the production system, it is necessary to carry out a greater number of evaluations to verify the response of agave coyote to the application of SRFs under field conditions.

Author contribution

Methodology development, C.C.R. Conceptualization of the work, acquisition of funds, methodology development, writing and preparation of the manuscript, A.B.C. Methodology development, analysis of results, writing and preparation of the manuscript, S.S.M. Revision and editing of the manuscript, E.E.Q.A. All authors of this manuscript have read and approved the published version of the manuscript.



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Conflicts of interest

The authors declare that there are no conflicts of interest.

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