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Coldengue: A novel surveillance and control tool for *Aedes aegypti*

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Abstract

In view of the high epidemiological and economic impact of urban arboviruses associated with *A. aegyptii* (A-ae) in tropical countries, WHO within the 2030 agenda for Sustainable Development, highlights the need for new alternatives to control and eliminate those diseases. In response to this call, we proposed the Coldengue as a new tool of surveillance and vector control for A-ae. It is a tool operated manually and by sweeping in breeding sites. This tool was calibrated in laboratory showing effectiveness rate between 41.0 - 62.9% for larvae removal and 62.9 - 91.3% for pupae. Later Coldengue was applied in houses of Villavicencio, a hyperendemic city for dengue in Colombia. During six and eight interventions in two different neighborhoods, trials with intervals of 7 to 10 days; 10,522 larvae and 1,950 pupae of A-ae were eliminated. Coldengue in addition to responding to the traditional aedic index too allows determining the productivity of the breeding sites and the spatial-temporal dynamics for the pest mosquito. The tool presented acceptability and community adhesion above 90.0%, is fiscally sustainable (<3 USD unit), has a useful life until 6 months (with weekly use); is eco-friendly, can be used by all members of the community and is a resilient climate change strategy. Therefore, we suggest that Coldengue can be incorporated as a regular action of the health surveillance and vector control programs for urban arboviruses in Colombia and other countries of the world where domestic artificial water containers are productive for *A. aegypti*.

Keywords: Surveillance; Control; Dengue; Chikungunya; Zika; Arboviruses;

1. Introduction

The tropics is considered endemic for dengue, chikungunya, Zika and it has as a vector the mosquito species *Aedes aegypti* and *Aedes albopictus* [1,2]. Since 2014, there have been large epidemic outbreaks of the three main urban arboviruses mentioned, and they prevent economic development by requiring expenses in medical care and indirect expenses in reducing productivity and tourism in the affected countries [3]. Only dengue represents about 390 million infections every year [4] and demanded around 8.9 billion dollars worldwide in 2013 [5].

In the absence of an efficient vaccine to control these arboviruses, the fight against these diseases is mainly based on vector control [6]. Globally several surveillance and entomological control strategies have been developed, from the traditional larval aedic index to the use of insecticides to reduce and eradicate *A. aegypti* populations[7]. The aedic index measure the presence or absence of immature mosquitoes in artificial water containers. In addition to the underreporting of cryptic breeding site, these indices are being reported in municipality or city scales, losing the sense of targeting vector risk and its association with epidemiological indicators (prevalence and incidence of cases) [8].

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Since the 1990s some novel surveillance and control strategies have been applied as a cone-shaped trap or sweeping net to combat *A. aegypti* from domestic breeding [9–12]. However, no evidence has been found on the evaluation or application of these strategies in the countries of the America.

On the other hand, recently, the World Health Organization (WHO) invites us to continue looking for new alternatives to control and eliminate infections associated with *A. aegypti*, grouping all the needs into four pillars: **i.** reinforces intersectoral and intrasectoral actions, **ii.** Mobilize communities, **iii.** Improve surveillance and monitoring, and **iv.** expand and integrate vector control interventions as a basic element of national health strategies and plans to implement the “2030 Agenda for Sustainable Development” [13]. In response the call of WHO and all the limitations showed in this paper, *we evaluated* ColDengue in an area hiperendemic for arboviruses and we propose it as a new surveillance and control tool for *A. aegypti*.

2. Material and methods

2.1. Study area

The city of Villavicencio is located at the geographical coordinate's 4°08'31" north latitude and 73°37'35" west longitude, at an altitude above sea level at 441m. It is located in the foothills of the eastern mountain range, northwest of the department of Meta, Colombia [14] (Fig. 1).



Figure 1 Geographical locations of the department of Meta in Colombia (A), the municipality of Villavicencio divided into Communes (B) and neighborhoods studied (C)

2.1.1. Eco-epidemiological data

The city has a total annual average rainfall of 4,383mm. During the year it presents two dry seasons and two rainy seasons. The average temperature is 25.5°C (maximum daily peaks between 28°C and 32°C), it has a relative humidity between 67.0% and 83.0% [14].

Villavicencio has a population of 516,802 people (DANE 2018). It is considered a hyperendemic city for dengue, with more than 3,000 (1,500 to 6,000) cases confirmed annually in the last 4 years [16]. It is an endemic territory for chikungunya and Zika; between 2015 and 2016, 14,000 and 2,000 cases were confirmed for the respective pathologies, including 14 cases of microcephaly in human neonates associated with the Zika virus [16].

Data from March-April 2017 and September-October 2018 showed that in Villavicencio between 15.0 and 20.0% of homes are located with residential containers breeding for *A. aegypti* mosquito (unpublished data).

2.2. Method

2.2.1. ColDengue description

ColDengue is a tool consisting of a homemade plastic strainer (for domestic use) 20cm in diameter, 7cm deep, with nylon mesh approximately 0.5mm in diameter eye mesh, with 20cm grip handle. Depending on the dimensions of the breeding site, a wooden stand 30, 50, 100 or 150cm long x 2.5cm in diameter was adapted, with the help of plastic straps (Fig. 2).

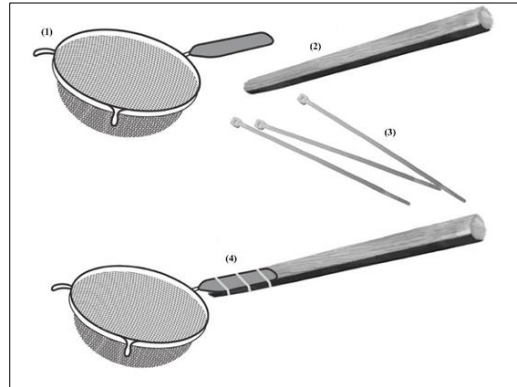


Figure 2 Characteristics of ColDengue as a new tool surveillance and control for *A. aegypti*

ColDengue was initially evaluated under laboratory conditions to determine the removal rate of larvae and pupae of *A. aegypti*. Three tanks with a capacity for 200 liters were used for this purpose. Tests with a volume of 50 and 100 liters of water with three replicas were made and for each test 50 larvae (development stage ≥ 3) were used and subsequently in a separate test with 50 pupae in each tank. In five repetitions in a row for 3 minutes for each tank the scan was made with ColDengue and the number of removed immatures was counted.

2.2.2. Selection of neighborhoods and homes for intervention with ColDengue

The Popular and La Madrid neighborhoods of commune 4 and 8, respectively, urban area of Villavicencio (Fig. 1), were selected according to data by Secretaria de Salud de Villavicencio (Villavicencio Health Department). In each of the neighborhoods, prior consultation with the Junta de Accion Comunal-JAC (Community Action Board) was carried out and then the house visit was made to prioritize the houses to be intervened.

Before initiating the home intervention, we present to them an informed consent about the scope, objectives, that there was no economic remuneration for any of the parties, that a weekly follow-up would be carried out, and that it is a low-risk strategy and the participation or refusal to the intervention was voluntary. Following the authorization with the signature, the water deposits were inspected, the entomological survey was carried out, following the indicators of the traditional aedic index [17].

The inclusion of the home corresponded in the following order and with the fulfillment of all the criteria: 1. Allow the visit, 2. Have water deposits and 3. Authorize the participation and weekly monitoring. Even in the third follow-up, the inclusion of homes to the study was allowed.

In each intervened house, the number of larvae and pupae of *A. aegypti* captured in approximate 3 minutes with ColDengue was recorded in physical form. Random samples were collected for diagnostic confirmation in the laboratory using taxonomic guides [18].

2.2.3. Data processing

Descriptive statistical analysis was performed to show dispersion measures and central tendency, and analytical statistical analysis to find significant statistical differences through ANOVA (Generalized Lineal Model-GLM). For this purpose, the number of larvae and pupae was established as a dependent variable and independent variables number of controls in each neighborhood. A significance level of 0.05 was established and the analyses were performed separately for each neighborhood, since there were different sampling times and numbers of controls. All analyses were conducted in the statistical program SPSS V.25 [19].

Next, the probabilistic density of immatures (larvae and pupae) was determined through the Kernel algorithm [20], using the 50m radius as the minimum dispersion area of an adult of *A. aegypti* [21] to identify distribution patterns of immature mosquitoes in the free software [22].

Finally, a quantitative assessment of community satisfaction was carried out as for the ColDengue strategy. For this, a semi-open questionnaire with seven questions: **i.** Have you used ColDengue? (Yes / No), **ii.** Who used it? (Children, Adults, both), **iii.** Frequency of use (Weekly / Biweekly / When did you see larvae or jumping), **iv.** What benefits has ColDengue brought you? (Free answer), **v.** What damage has ColDengue brought you? (Free answer), **vi.** From 1 to 5, where 1 is completely unsatisfied and 5 completely satisfied, how satisfied are you with the ColDengue strategy to eliminate mosquitoes from your water deposits? And, **vii.** Do you have any recommendations and / or suggestions for the strategy implemented? (Free answer). Descriptive analysis were performed and word maps were generated in the free word cloud program (<https://www.nubedepalabras.es/>).

3. Results

3.1. Acceptability and community adherence indicators to ColDengue

It was found that 61.5% (123/200) and 63.0% (126/200) of the selected homes in the Popular and La Madrid neighborhoods respectively, accepted the initial visit of the field staff. The 9.0% (18/200) and 31.0% (62/200) corresponding to the order of the previous neighborhoods were initially reluctant. The rest corresponds to inhabited houses and/or there were minors. Until the third week, other families were linked to the project, achieving the goal of 88.5% (177/200) in the popular neighborhood and 109.0% (218/200) in La Madrid.

Until the last visit, more than 90.0% of community adhered to the ColDengue; this means that the 10.1% (20/182) in Popular and 5.5% (12/218) in La Madrid neighborhood desisted from weekly visits.

Of the 100 satisfaction surveys, in 78.0% adults have used ColDengue, 12.0% children and in 10.00% both of them. The frequency of the use is weekly (57.0%), biweekly (3.0%), when they saw larvae (23.0%) and in 17.0% this question was not asked.

Within the scale of 1 to 5 (3 to 5 was considered approval), 83.0% (83/100) of the people indicated that the use of ColDengue brought benefit related to the reduction of mosquitoes, saving and stored water for a longer time in the containers and reduces the dirtiness of the deposits. There was no report of damages. However, in addition to following the community recommendations and following the strategy, they requested fumigation (insecticide).

3.2. Entomological indicators

The laboratory results showed data that met the parametric statistical route (normality and homogeneity of variance; $P_v > 0.05$). During the proof of the tool, no statistically significant difference was found in the rate of immature removal amongst the original ColDengue with an extra net layer and the adaptation without the mesh, whereas the difference was found between biological stages and the volume of water (Fig. 3A and B). The larval removal rate is between 44.0% and 55.3% (IC_{95%}: 41.0-62.9) and for pupae between 67.7% and 79.3% (IC_{95%}: 62.9 -91.3). Independent of the biological stage, the elimination rate is more efficient at lower volume water (Fig. 3C).

According to the traditional aedic index, of every 100 homes in each neighborhood studied 33.4% and 24.7% respectively maintain water containers. Between 23.7 and 15.5% of them, were found with immature mosquitoes (Table 1A). A total of 10,522 larvae and 1,950 pupae were eliminated (average of 9 larvae and 2 pupae per breeding sites) (Table 1B).

Of the 227 samples (821 individuals) were taxonomically identified as *A. aegypti*, 2.5% was not possible (pupae or bad condition) and 0.5% as individuals of *Culex quinquefasciatus*.

The GLM analysis did not identify significant statistical differences between the number of larvae and pupae for any of the neighborhoods between the control moments. However, the patterns of spatial-temporal distribution of residential breeding sites of *A. aegypti* were identified (Fig. 4).

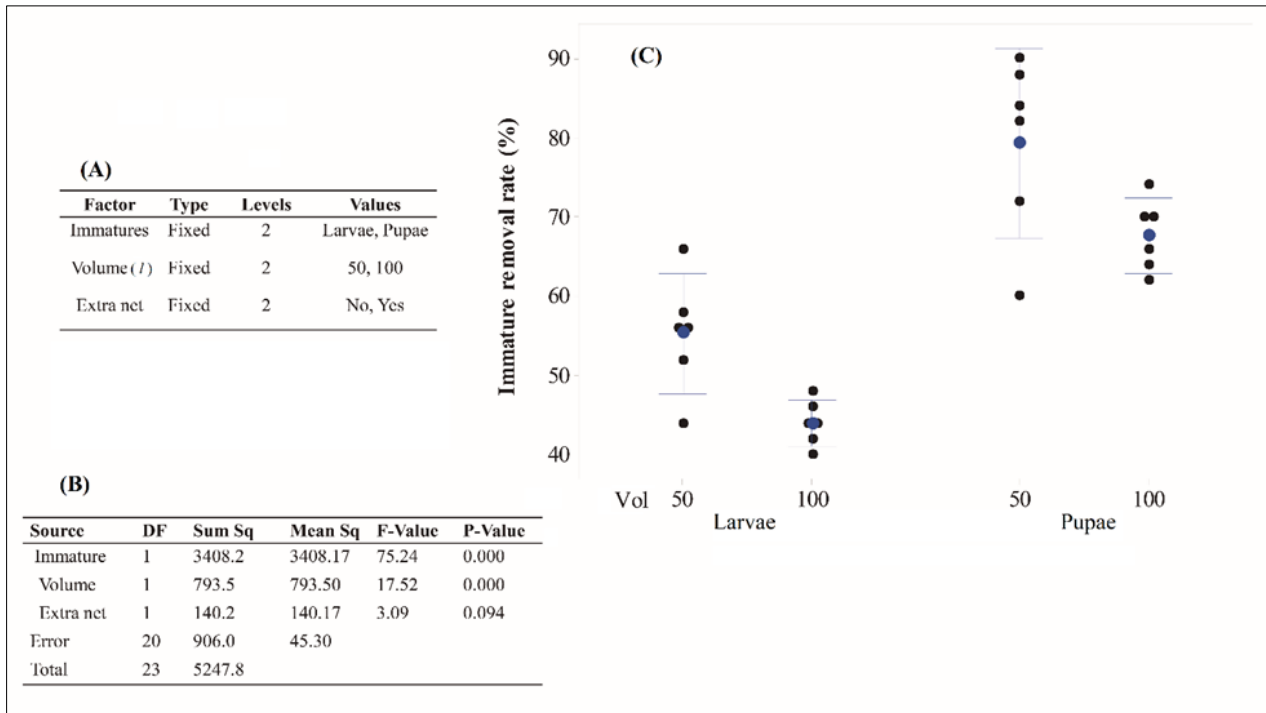


Figure 3 Laboratory test results with ColDengue: characteristics of the tested variables (A), ANOVA test result (B) and removal rate of immature *A. aegypti* with 95% IC (C)

Table 1A Entomological indicators for immatures *A. aegypti* through the traditional method aedic index in two Villavicencio neighborhood, Colombia

Controls	Popular							La Madrid						
	Visited house	Positives house	Inspected containers	Positives containers	HI (%)	CI (%)	BI (%)	Visited house	Positives house	Inspected containers	Positives containers	HI (%)	CI (%)	BI (%)
1	45	20	89	20	44.4	22.5	44.4	40	6	88	6	15	6.8	15.0
2	43	17	89	18	39.5	20.2	41.9	155	54	315	56	35	17.8	36.1
3	52	23	48	23	44.2	47.9	44.2	102	31	140	35	30	25.0	34.3
4	81	23	166	29	28.4	17.5	35.8	118	26	130	27	22	20.8	22.9
5	137	44	239	44	32.1	18.4	32.1	114	26	247	26	23	10.5	22.8
6	84	21	116	23	25.0	19.8	27.4	137	32	269	32	23	11.9	23.4
7	62	15	113	16	24.2	14.2	25.8	-	-	-	-	-	-	-
8	104	40	158	56	38.5	35.4	53.8	-	-	-	-	-	-	-
9	112	27	183	31	24.1	16.9	27.7	-	-	-	-	-	-	-
Average	80.0	25.6	133.4	28.9	33.4	23.7	37.0	111	29.2	198	30.3	24.7	15.5	25.7

HI: House Index; CI: Containers Index, and BI: Breteau Index

Table 1B Entomological indicators for *A. aegypti* through the tool ColDengue in two Villavicencio neighborhood, Colombia

Controls	Popular									La Madrid								
	HI (%)	CI (%)	BI (%)	Removed total		Removed average		SEM		HI (%)	CI (%)	BI (%)	Removed total		Removed average		SEM	
	I	I	I	L	P	L	P	L	P	I	I	I	L	P	L	P	L	P
1	44.4	22.5	44.4	220	19	5	0	1.4	0.2	15	6.8	15.0	244	0	6	0	3.1	0
2	39.5	20.2	41.9	271	76	6	2	2.5	1.5	34.8	17.8	36.1	1111	111	7	1	1.5	0.2
3	44.2	47.9	44.2	730	46	23	1	15.5	0.7	30.4	25.0	34.3	391	84	4	1	0.8	0.3
4	28.4	17.5	35.8	492	198	6	2	1.9	2.1	22	20.8	22.9	225	32	12	0	6.5	0
5	32.1	18.4	32.1	470	110	3	1	1	0.3	22.8	10.5	22.8	879	0	8	0	2.5	0.2
6	25.0	19.8	27.4	756	78	9	1	2.8	0.6	23.4	11.9	23.4	1080	475	8	3	3.9	2.3
7	24.2	14.2	25.8	315	43	5	1	2.9	0.5	-	-	-	-	-	-	-	-	-
8	38.5	35.4	53.8	1396	225	13	2	3.3	0.8	-	-	-	-	-	-	-	-	-
9	24.1	16.9	27.7	1942	441	17	4	10.9	2.7	-	-	-	-	-	-	-	-	-
Average	33.4	23.7	37.0	732	137	10	2	5	1.04	24.7	15.5	25.7	655	117	8	1	3	1

L: Larvae; P: Pupae and SEM: Standard error of the mean

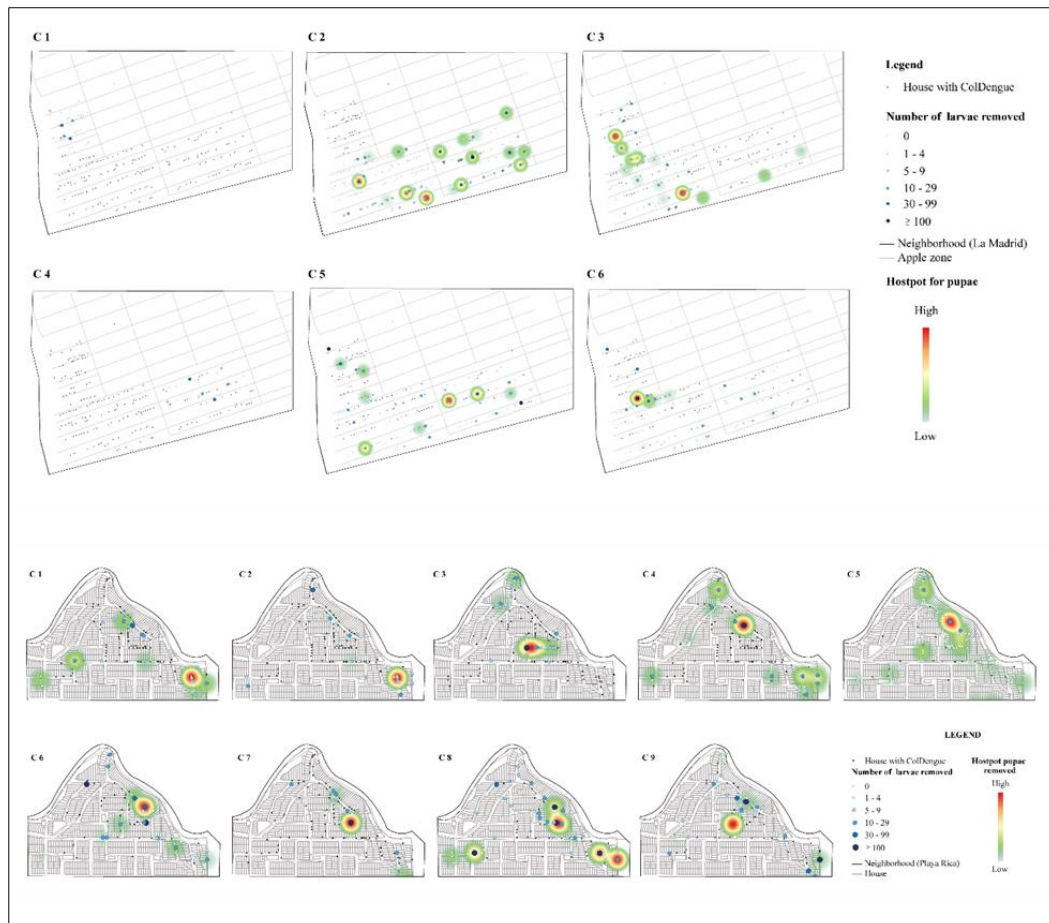


Figure 4 Identification spatial of hotspot for *A. aegypti* invertebrates with the implementation of ColDengue in two Villavicencio neighborhood, Colombia

4. Discussion

This pilot study demonstrates that, in addition to responding to aedic index, ColDengue is more informative and at the same time it works as a vector control strategy from the community level or as a regular action of health programs. Given the results of lab proof, it is evident that the tool's effectiveness rate is higher for pupae removal than for larvae (Fig. 3C). Among the strengths is that for larvae and pupae the impact of the strategy is measured *in situ* and immediately, something that does not happen with the majority of strategies for immature in artificial containers: larvicides, covered, washed and brushed, biological controls, etc. The other advantage is that it fights pupae, considered the best entomological indicator and for quantification of epidemiological risk [23,24]. Among the limitations we found that, stage 1 and 2 larvae easily pass through the sieve. Therefore, new tests with other sieve adaptations are required.

On the other hand, in most of the temporary controls for both neighborhoods, it becomes clear that the aedic indices are inversely proportional to the productivity of the breeding site (Table 1B), which demonstrates the qualitative and quantitative limitations of those indices [8,23–25]. Another indicator provided by ColDengue is the proportion of larvae and pupae in positive containers, for every 9 larvae 2 pupae were found (Table 1B). This information, together with the hotspot map productivity for *A. aegypti* (Fig. 4), is useful for focusing control actions. Inasmuch as ColDengue showed a community acceptability and adhesion that exceeds 90.0% in both neighborhoods, we also recommend the use of this tool in times of epidemic outbreaks of any arboviruses. We remember that in parallel form, behavior of the community should be reinforced, given that they accept and adhere to the strategy, but they keep the insecticide rooted as the first line of combat to the mosquito.

ColDengue, in comparison with various physical control (tank washing), chemical (Insecticides) and biological control strategies (larvicides, fish, and arthropods) has the following advantages:

- Fiscal Sustainability: The tool does not exceed the value of \$ 3.0 USD per unit and, at least, it was functional for six months with weekly use, making the method cost-effective from the first moment of use, compared to the cost of insecticides from state and household level [26].
- Environmental sustainability: ColDengue has no negative impacts on water, animals and the man who handles it. It is a tool that must be explored as a climate-resilient strategy, avoid continuous washing of deposits and allows water reserves to be maintained for a longer period of time;
- Operation: ColDengue is easy to handle, no technical knowledge is required and easy to operate by children and adults.

In spite of the mentioned advantages, future studies in greater time, standardization of the number of houses and breeding sites, indicators of sensitivity and efficiency; as they have been done with similar techniques in other countries [9–12], are necessary and will allow the best indicators for ColDengue to be defined.

5. Conclusion

ColDengue is a strategy that in addition to responding to the traditional aedic index, provides quantitative indicators. The productivity of breeding for larvae and pupae, allows discovering hotspot (house) and spatiotemporal reproduction dynamics for *A. aegypti*. The tool presented acceptability and community adhesion above 90,0%, is fiscally sustainable (<3 USD unit) and can be used by all members of the community and is a resilient climate change strategy. Compliance with ethical standards

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have the exclusive responsibility for the accuracy and correctness of the contents of the article submitted and declare that they have no conflicts of interest.

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Author contributions

D. Montenegro, L. Barbosa and J. Daza were contributed to concept and design of the manuscript, DM, L. Berbeu and C. Ramirez were responsible for the collection of information in the field and laboratory tests and trials. All authors contributed to critically revising the manuscript for important intellectual content and final approval of the version to be published. All authors agree to be accountable for all aspects of the work and in ensuring that questions related to the accuracy or integrity of any part of the work have been appropriately investigated and resolved.

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