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Research Article

Comparison of commercial ethoprophos sources in symphylids control and nematode proliferation in pineapple (*Ananas comosus* MD-2)

Agustín Rodríguez[1], **Juan Delgado**[2], **Eduardo Salas**[3], **César Guillén**[4] and **Mario Araya**[5*]

1. AMVAC-Costa Rica.
2. LIFE-RID-AMVAC-Costa Rica.
3. Catedrático Escuela Ciencias Agrarias, Universidad Nacional-Costa Rica.
4. Entomologist University of Costa Rica.
5. AMVAC Chemical Corporation.

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Corresponding Author
Mario Araya
E-mail: marioa@amvac.com
Tel: +506 8915 0083.

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Abstract

In a randomized complete block design with five repetitions an experiment was conducted to compare the effectiveness of ethoprophos sources at the rate indicated by the manufacturer in the product label, for symphylids control and nematode proliferation. The treatments consisted of ethoprophos (Mocap® 72EC-AMVAC) at 8 L ha^{-1}, ethoprophos generic-1 at 13.6 L ha^{-1}, ethoprophos generic-2 at 12 L ha^{-1}, Nemacur® 40EC (fenamiphos-AMVAC) at 8 L ha^{-1}, each one in 2000 L of water by hectare plus the untreated control. The pre-treatment number of symphylids was similar ($P = 0.8391$) among experimental plots, varying between 3.52 to 4.56 per plant. When comparing the symphylids per plant at pre-treatment against the average of the evaluations at 15, 35 and 70 days after application in each treatment, ethoprophos generic-1 reduced ($P < 0.0001$) the population in 86%, ethoprophos generic-2 ($P < 0.0001$) by 91%, Mocap® ($P < 0.0001$) in 83% and Nemacur® ($P < 0.0001$) in 78%, while in the untreated plants, the population did not change ($P = 0.1621$). Compared to the untreated plants, the same applied treatments prevented the infection of *Helicotylenchus* spp. ($P = 0.0126$) between 86 and 93%, *Pratylenchus* spp. ($P = 0.0369$) between 58 and 63% and total nematodes ($P = 0.0212$) between 61 and 65%. Although all the products tested were statistically equal in the control of symphylids and the prevention of nematode infection, in the ethoprophos generic-1 and ethoprophos generic-2, the rates tested, which were those registered on the label, were 70 and 50% higher than that of Mocap®, which resulted in higher chemical load. In addition, depending on the ethoprophos generic price, the pest control cost, may be higher with the generic products.

1. Introduction

Pineapples (*Ananas comosus*) are cultivated in Costa Rica for export markets. Usually, it is the second most important crop, accounting for 2019, before the COVID-19 pandemic, for almost 8.3% (US $930 million) of the Costa Rican total exportations, representing 31% of the agricultural gross national product. Besides the constraints of the pineapple market requirements and demands, there are other factors limiting production. Among the important abiotic factors constraining pineapple yield, it is a shallow soil water table level and edaphic conditions, mainly due to clay texture and poor structure. These constraints differ between farms and not all happen in a specific farm. Plantations are found in flat areas with no more than 4% of slope, with the planted area between 70 and 600 masl.

Within the biotic factors, symphylids (*Scutigerella* spp. and *Hanseniella* spp.) and nematodes (*Pratylenchus* spp., *Helicotylenchus* spp., *Meloidogyne* spp., *Rotylenchulus reniformis*) are important soil pest in pineapple plantations in many pineapples producing countries like Australia [1], Colombia [2], México [3, 4], Ivory Coast [5], Brazil [6], Costa Rica [7-9]. In local pineapple plantations, symphylids and nematodes [7, 8, 10] are a common soil pest, which can affect the crop from planting up to harvest [11]. Symphylids are frequently associated with crop debris and eating organic matter [1, 4, 12], but in the presence of the crop, they prefer to feed on the plant, chewing off the root hairs [1, 12-14] and preventing development of a healthy root system. Agredo et al. [2] found that symphylids caused a pineapple root loss in an average of 66%. Also, they can tunnel into the roots and stems leading to stunting and plant loss. Infested pineapple plants often produce a mass of numerous, fine roots in areas that have been chewed. Clusters of symphylids have been found at the base of stunted, unhealthy plants where the rootlets and root hairs had been removed restricting water and nutrients uptake. In Colombia yield losses due to symphylids damage are estimated over 40% [2].

In the case of nematodes, usually only polyspecific nematode communities occur, consisting mainly of a mixture of *Helicotylenchus* spp., and *Pratylenchus* spp. with very low populations of *Meloidogyne* spp., and very rarely *Rotylenchulus reniformis* [8-10, 15-19]. Nematodes increase the time for pineapple leaf emergence, reduce plant growth, fruit weight, and increase the crop cycle duration [12, 13, 20, 21-25]. The inflicted injury that both pests cause to the roots may also provide entrance to wound pathogens, which can destroy the roots. Then, more time is required to reach the appropriate plant weight for flower induction which increases the crop cycle duration and reduces fruit weight, its quality, and yield.

Before planting, a good soil preparation will reduce symphylids and nematode infestation at the time of plant establishment. After planting, to avoid or reduce symphylids and nematode damage, the only management strategy currently available is the application of synthetic pesticides, which growers know that it is economically feasible. From the recommended products, ethoprophos [3, 6, 12, 13, 26] and fenamiphos [27, 28] are available as liquid formulations. Then, the objective of this study was to compare the effectiveness of these active ingredients (ethoprophos sources and fenamiphos) for pineapple symphylids control and nematode proliferation.

2. Materials and methods

2.1. *Plant material and growth conditions*

The field experiment was carried out within a commercial pineapple plantation located in Upala County, Costa Rica. The plant crop of an area of a fifth planting was used for the experiment. The soil was clean and free of plant residues and weeds, and then ripped to 90 cm depth and later cross ploughed to a depth of 40-50 cm and finished with a disc harrow and beds conformation using a tractor, two months prior to planting. The soil was of clay texture (36% sand, 11% silt, 53% clay), classified as Inceptisol with 2.3% organic matter content and a pH of 5.9. Manual planting was done with suckers of *Ananas comosus* at a plant density of 65000 plants ha^{-1}. The average rainfall during the year of the experiment was 2071.5 mm evenly distributed. January and April were the driest months with 120 and 50 mm, respectively. During the time of the experiment, the rainfall was 266 mm with a

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maximum of 39.4 mm in one day. A system of primary, secondary, and tertiary drains was provided to disperse excess rainfall and prevent water logging during heavy rains. Mean daily maximum/minimum temperatures were 33.1/22.4 ^{0}C with a mean average of 26.3 ^{0}C.

Fertilizer, hydro-complex 12-11-18-3-0.015-8 (N-P_2O_5-K_2O-MgO-B-S) at 3-4 g per sucker was applied 45 days after planting and then every 15 days a mix of urea 38 kg ha^{-1} + magnesium sulphate 21 kg ha^{-1} + potassium chloride 20 kg ha^{-1} + iron sulphate 2 kg ha^{-1} + boric acid 2 kg ha^{-1} + zinc sulphate 1.5 kg ha^{-1} in 1800 L of water were foliar applied with spray boom. Foliage diseases were controlled with alternate application every 15 to 30 days of either methalaxyl, benzimidazole, carbendazine, tebuconazole, propiconazole, or Fosetyl-aluminum alone or in mixture in a water solution uniformly applied with spray boom. Weeds were controlled pre-planting with oxyfluorfen and post planting with a mixture of ametryn with clethodim or galoksifop-R-metil.

2.2. *Treatment and experimental design*

The treatments evaluated were commercial sources of ethoprophos and fenamiphos at the recommended rate on the label consisting of Mocap® 72EC (ethoprophos, AMVAC original commercial product) at 8 L ha^{-1}, ethoprophos generic-1 at 13.6 L ha^{-1}, ethoprophos generic-2 at 12 L ha^{-1}, Nemacur® 40EC (fenamiphos-AMVAC) at 8 L ha^{-1}, plus the untreated control. The rectangular plots (10 beds wide by 10-15 m long) consisted of 800-1160 plants with 40 plants in the centre of the plot as experimental units. Plots were arranged in a randomized complete block design with five replicates. The rates of the products were applied once, 65 days after planting. The different rates were applied in a water solution volume of 2000 L ha^{-1} with a spray boom equipped with conical nozzles XRC-Teejet 80005, adapted to the New Holland T6020 tractor at 1500 rpm, running at 1.6 km by hour with a pressure of 3 bar.

2.3. *Variables evaluated*

One day before, the product application and thereafter at 15, 35 and 70 days, 10 plants were torn off with the help of a shovel and their root system and adhered soil were examined to count the number of symphylids. From the population, 20 symphylids were identified at the genus and species level, when possible, based on the morphological characteristics under a light microscope, following the description of Salazar *et al*. [29] and the key of Dominguez [30]. The roots from these plants were removed from the stem with a knife, placed in labelled plastic bags identified with the treatment and repetition and delivered to CORBANA Nematology laboratory in insulated chests. In the laboratory, root samples were registered and processed, and when necessary, stored in a refrigerator at 6-8°C until processed. Roots were rinsed free of soil, cut into pieces of 1-2 cm long and randomly mixed. Nematode extraction was conducted using the maceration method [31]. Root samples consisting of 25 g tissue were processed and nematodes recovered on a 0.025-mm mesh sieve were identified to genus and species level, when possible, based on morphological characteristics under a light microscope following the key of Siddiqi [32]. Population densities of all plant-parasitic nematodes present were determined, and values were converted to numbers per 100 g of fresh roots. Total nematodes correspond to the sum of the plant-parasitic nematodes detected.

2.4. *Statistical analysis*

The average number of symphylids by repetition (10 plants) and evaluation was analyzed with the model of generalized estimating equation in Proc Genmod of SAS and submitted to ANOVA and mean separation by LSD-test. Then, a repeated measurements analysis was done including all evaluations with Proc Mixed of SAS. In this model, a covariance structure of heterogeneous compound symmetry with an additional treatment group effect was used to account for

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the heterogeneity of variances among repeated measures and treatments. Within each treatment, the number of symphylids pre-treatment applications was compared to the average of the evaluations done at 15, 35 and 70 days after application by orthogonal contrasts. The effectiveness per plot, treatment, and evaluation time post application was determined following the Abbott [33] formula (untreated-treated/ untreated * 100) and submitted to ANOVA and mean separation by LSD. Since in the first root sampling, most of the samples were negative (without parasitic nematodes) the analysis was run with the data of the three samplings post product application. The composition of the nematode population was determined for the average of the three samplings post application. The number of nematodes was analyzed with generalized linear models, using the log transformation as a link function and negative binomial distribution of the errors for the average of the three nematode samplings after product application and then submitted to ANOVA and mean separation by LSD.

3. Results

3.1. *Symphylids*

The number of symphylids (*Scutigerella* spp. and *Hanseniella* spp.) pre-treatment application was similar among the experimental plots, varying between 3.52 and 4.56 (P= 0.8391) by plant (Fig 1A). When comparing evaluations within each treatment, always there was a difference (P< 0.0001), including the untreated plots, only that the change in symphylids number was smaller in the untreated plants, varying between 2.2 and 4.36 by plant, while in the treated plants, the population decreased as much as 0.12 by the plant (Fig 1A). The application of the products reduced the number of symphylids by plant as follows: ethoprophos generic-1 by 86% (P< 0.0001), ethoprophos generic-2 by 91% (P< 0.0001), Mocap® by 83% (P< 0.0001), and Nemacur® by 78% (P< 0.0001; Fig

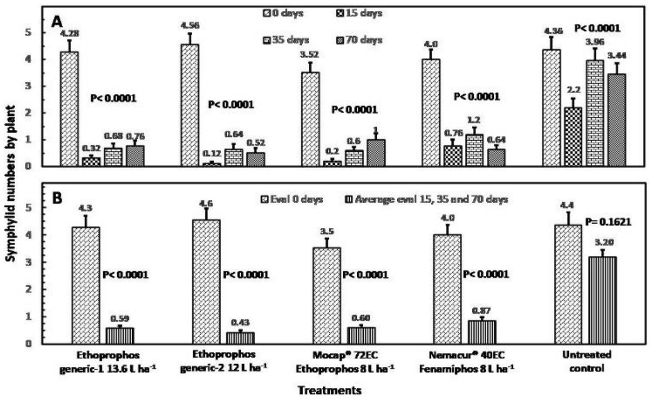

Figure 1. A) Symphylids (*Scutigerella* spp. and *Hanseniella* spp.) number by treatment at four evaluation times. Each bar is the mean ± standard error of 5 repetitions. B) comparison of symphylid numbers by plant in each treatment before application (0 days) vs the average of the evaluation at 15, 35 and 70 days, post application.

At 0 days, each bar is the mean ± standard error of 5 repetitions and at the average (15-35-70 days), each bar is the mean ± standard error of 15 repetitions. In each replicate, 10 *pineapples* (*Ananas comosus*) plants were evaluated.

1B). Although in the untreated plants, a drop of 27% was found, the difference was not large enough to be significant (P= 0.1621). The efficacy of symphylids control at 15 days of the application varied between 65.4 and 94.5% (P= 0.1022) at 35 days oscillated between 69.7 and 84.8% (P= 0.3840) and at 70 days it varied between 70.9 and 84.9% (P= 0,6807) without difference among the products (Table 1). When the treatment effect (average of the evaluations 15, 35 and 70 days) was compared, all the products differed (P< 0.0001) from the untreated control.

3.2. *Nematodes*

The nematode population through the three samplings was composed mainly of *Pratylenchus* spp. with 97% and *Helicotylenchus* spp. with 3% (data not shown). When comparing the average of the three samplings post application, a difference was found for the population of *Helicotylenchus* spp. (P= 0.0126), *Pratylenchus* spp. (P= 0.0369) and total nematodes (P= 0.0212) among the treatments (Figure 2A-C).

At the three sampling times 15, 35, and 70 days

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Table 1. Percentage of efficacy according to Abbott (1925) formula (untreated - treated / untreated * 100) on symphylids (*Scutigerella* spp. and *Hanseniella* spp.) control at 15, 35 and 70 days after product application with different commercial ethoprophos sources or fenamiphos, all rates in 2000 L ha^{-1} of water solution on pineapples (*Ananas comosus* MD-2).

Treatment	Evaluation time		
	15 days	35 days	70 days
Ethoprop generic-1	85.4	82.8	77.9
Ethoprop generic-2	94.5	83.8	84.9
Mocap® 72EC	91.0	84.8	70.9
Nemacur® 40EC	65.4	69.7	81.4
Probability	P= 0.1022	P= 0.3840	P= 0.6807

Each value is the mean of 5 replicates. In each repetition 10 plants were evaluated.

post product application, always the higher nematode population was found in the untreated plants. When comparing the average of nematodes of the three samplings of the products against that of the untreated plants, the ethoprophos generic-1 prevented the infection of *Helicotylenchus* spp. in 86% of *Pratylenchus* spp. in 63% and total nematodes in 65%, the ethoprophos generic-2, in 86, 58 and 61%, Mocap® 72EC (ethoprophos-original) in 93, 61 and 64% and fenamiphos in 89, 59 and 63%, respectively.

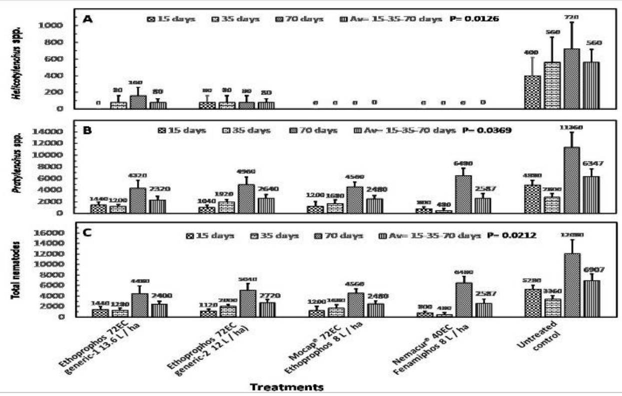

Figure 2. Nematode numbers per 100 g of pineapple (*Ananas comosus* MD-2) roots by plant in each sampling time in each treatment. At 15, 35 and 70 days, each bar is the mean ± standard error of 5 repetitions. Av= 15-35-70 days, each bar is the mean ± standard error of 15 repetitions with the probability comparing the treatments. In each replicate, nematodes were extracted from a composite sample of 5 pineapple plant roots.

4. Discussion

No difference in the symphylids population among the plots was observed before treatment application, which means that any difference detected later should be attributed to the treatment effect. The initial population varied between 3.52 and 4.56 symphylids per plant, but no foliage symptoms were developed or observed. These populations were above the economic threshold, since its control is recommended when 2 or more symphylids are found per plant [3, 4]. The lack of foliage symptoms in the plants of this experiment, contrasts with the observation of Agredo et al., [2], who reported pineapple foliage symptoms in 67% of plants with an infestation of 3 symphylids and with that of Nurfadhilah et al., [34], who found symptomatic plants with an average of 1.9 symphylids in its roots.

With the ethoprophos rates tested the symphylids population was under the economic threshold up to 70 days, the period that the experiment lasted, but it is known that ethoprophos has a soil half-life of 98 [35] and up to 120 days [36], then a longer control would be expected. The control observed agrees with that reported from Australia in Pineapple News [37] where Mocap® EC at 10 L ha^{-1} applied twice was the optimum treatment for symphylids control. Also, this is in parallel with that found by Reyes et al., [14] in México who recommended the application of Mocap® (ethoprophos) 15G, a granular formulation at the rate of 50-100 kg ha^{-1} and with Pinto da Cunha et al., [6], Petty et al. [12] and Py et al. [13], suggestion of applied ethoprophos for its control, either as pre-plant or post plant treatment.

In the case of Fenamiphos is an insecticide-nematicide used for the control of mealybugs [28] and nematodes [27, 38-40] on pineapples, which gave similar symphylids control as the ethoprophos sources. Fenamiphos has a soil half-life of 120 [41] or up to 190 days [36], so a longer

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control would be expected if the evaluation had been made later after its application.

In Australia, when potted pineapple plants were infested with 12, 24 or 48 symphylids per plant, roots were reduced, in 9 weeks by 47,7%, 61.7% and 92.8%, respectively [42]. In Martinique, Lacoeuilhe, 1977 cited by Py et al. [13] found with the control of symphylids an increase of the fruit mean weight from 0.72 to 1.27 kg and of the number of suckers per plant at harvest from 0.1 to 0.63; and in Ivory Coast, Kéhé 1979 also cited by Py et al. [13] reported with its control 22% increase in the fruit mean weight with a significant reduction in the number of small fruits.

In the case of nematodes, in the first root sampling, 65 days after planting, most of the samples were negative, which is reasonable since the propagule used as planting material was free of nematodes, since they were suckers without roots, which become infected in the soil when root emission begins after planting. In the other root samplings, the two nematode genera detected are consistent with those previously reported in Costa Rica [8-10, 15-19] and are widely reported pests in pineapple roots around the world [12, 13, 20, 21, 25, 43-47]. The high *Pratylenchus* spp. population was favored by the pineapple monoculture and the affinity of this nematode with the crop and coincides with local studies and with studies from Australia [48] and Ivory Coast [49, 50].

There is scientific information on the damage caused by nematodes in pineapple. In Peru, Julca and Carbonell [45] found that *Pratylenchus*, *Helicotylenchus* and *Meloidogyne* diminished the D leaf length and weight, reduced the fruit weight and its diameter, and dropped the fruit brix %. Guerout [51] inoculating *Pratylenchus brachyurus* in pineapple plants, found a 26% decline in the area of the D leaf, 64% drop in root mass and 35% reduction in fruit weight compared to control plants. Later, the same Guerout [20] reported a 35-40% reduction in plant growth, leaf emission and leaf weight. In Colombia, *Pratylenchus* neglectus reduced the fresh plant weight by 54% and, reduced the root mass and the thickness and size of the leaves [52]. In Mexico, pineapple losses by nematode ranged between 15 and 45% [3] and more recently between 15 and 60% [4]. Román [38] in Puerto Rico, found in soils infested with nematodes a reduction in fruit weight of 47.5%. Hutton [21] in Jamaica, comparing the control of *Helicotylenchus multicinctus* and *Pratylenchus* spp. before and after planting with untreated plants, reported an increase in fruit weight of 79% for Red Spanish, 146% for Smooth Cayenne, and 94% for Sugarloaf.

In South Africa, where *Meloidogyne* and *Helicotylenchus* are serious pests of pineapple, pre-seeding dipping in a solution with systemic nematicide followed by post-seeding treatment at monthly intervals for 12 months, increased crop yield by 916 boxes (12 kg) per hectare [23]. In Puerto Rico, Ayala and Sequeira [53] found an increase in yield of 1350 boxes per hectare and Roman [38] up to 2166 boxes per hectare when controlling pineapple nematodes. Hutton [21] found a yield improvement of up to 1058 boxes per hectare when controlling nematodes in Jamaica. Costa Rica reported increases in fruit weight up to 205 g [15], which multiplied by 55000 fruits (85% of marketable fruits) per hectare would result in 11.2 Tm (939 boxes) more per hectare with the control of nematodes. In Hawaii, Apt and Caswell [27], and Apt [54] found increases in yield of 36.9 tm (3075 boxes) and 50 tm (4166 boxes) more per hectare, respectively, with the control of nematodes in pineapple. Then, the prevention of the nematode population to build up, it is highly recommended. To accomplish this, the nematode population needs to be monitored periodically (pre-planting and vegetative growth) to make timely decisions about their control options.

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Even though, measurements of the root system were not considered in the experiment, the symphylids and nematode control should prevent the loss of roots, which is the goal, since pineapples roots do not regenerate or produce again if damaged by pests and diseases [12, 43, 55].

The three ethoprophos sources as well as fenamiphos reduced statistically equal the symphylids population, with similar efficacy on its control and as well all prevented the nematode proliferation. However, in the ethoprophos generic-1 and ethoprophos generic-2 the rate was 70 and 50% higher than that of the original ethoprophos (Mocap®), which means that exists difference in the biological efficacy of ethoprophos sources. Such differences may come either from the ethoprophos formulated commercial product or from the active ingredient technical grade used in the formulated products. In the market, there are available different active ingredients of ethoprophos technical grade as well as different formulations of ethoprophos commercial products. The no difference found in symphylids and in their effectiveness in the control as well as in the prevention of nematode infection means that the lowest rate of ethoprophos (Mocap® original product) of 8 L ha^{-1} will end in a lower chemical load. Additionally, depending on the Ethoprophos generic price, the pest control cost, may be higher with the generic products.

Even though, it is known that Mocap® penetrated the roots [56, 57] after soil application, it is not systemic (57, 58, 59]. Then the liquid application in spray boom needs to be done soon after planting, up to 5 months, when the plant has little foliage, to allow more of the solution to reach the soil.

Ethoprophos and fenamiphos are insecticide-nematicides that belong to the organophosphate chemical group, with a mode of action based upon the inhibition of the enzyme acetyl-cholinesterase-nerve actions [60, 61]. This means that any organism that belongs to the animal kingdom, such as the symphylids and nematodes, which are multicellular organisms with a nervous system may be threatened by the presence of the product. Meanwhile, the soil microflora that is composed mainly of bacteria, actinomycetes, fungi and microalgae [62]), which are single-celled organisms, without a nervous system [63] would not be affected as have been found [64, 65]. Even more, free-living nematodes have not been reduced after a nematicide application [66, 67].

5. Conclusions

All the products tested were statistically equal in the control of symphylids and the prevention of nematode infection. However, in the ethoprophos generic-1 and ethoprophos generic-2, the rates tested, which were those registered on the label, were 70 and 50% higher than that of Mocap® original product, which resulted in higher chemical load. In addition, depending on the ethoprophos generic price, the pest control cost, may be higher with the generic products.

Authors' contributions

Performed the research work, A.R. and M.A.; Data analysis, E.S. and M.A.; Manuscript drafting, M.A., J.D. and C.G.; Figures drawn, E.S. and C.G.; Critically revised the work, A.R., E.S., J.D., C.G. and M.A.

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Availability of data and materials

Data supporting this study are included in the article.

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Conflicts of interest

The authors declare no conflict of interest.

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