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Comparative evaluation of insecticidal properties of essential oils of some selected botanicals as bio-pesticides against Cowpea bruchid, *Callosobruchus maculatus* (Fabricius) [Coleoptera: Chrysomelidae]



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Abstract

Background: The insecticidal activities of essential oils from seeds of five botanicals *Tetrapleura tetraptera*, *Annona muricata*, and *Aframomum melegueta* and leaves of *Eucalyptus globulus* and *Ficus exasperata* were evaluated as biopesticides against the storage pest of cowpea *Callosobruchus maculatus* (Fabricius). The oils were applied at the concentration of 1 ml/kg, 3 ml/kg, and 5 ml/kg of cowpea seeds mixed evenly. The experiment was laid out in a completely randomized design. The parameters assessed include the percentage of mortality, oviposition, adult emergence, seeds damaged, weight loss, and germinability capacity of the protected seeds.

Results: The results from the study showed that the effectiveness of the essential oils was dependent on application rates and time of exposure. In the treated cowpea seeds, *A. melegueta* essential oil was the most effective with 50% lethal concentration (LC50) value of 2.42 ml/kg and 95% lethal concentration (LC95) value of 4.86 ml/kg when compared to the other treatments as indicated by the application rate which caused 50% and 95% mortality of cowpea beetles. *A. melegueta* essential oil at the concentration of 5 ml/kg achieved 100% mortality of *C. maculatus* at 48 h of application. Cowpea seeds treated with 5 ml/kg of *E. globulus* essential oil significantly reduced (P < 0.05) oviposition compared to the other treatments. Cowpea seeds treated with 5 ml/kg of *A. muricata* essential oil reduced adult emergence (3.67) and weight loss (0.59). The cowpea seeds treated with essential oils at different concentrations had no significant (P > 0.05) adverse effect on seed germination after 120 days of storage.

Conclusion: Based on the findings of this study, the essential oils could be explored as an alternative bio-pesticide to synthetic insecticide in the protection of stored cowpea against *C. maculatus*.

Keywords: Botanicals, Essential oils, *Callosobruchus maculatus*, Mortality, Adult emergence

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Background

Cowpea (Vigna unguiculata (L.)) is a major staple food in sub-Saharan Africa; it is among the major sources of protein for human and livestock (Ileke et al. 2013). The high protein content of the seed makes it extremely valuable in tropical Africa where meat and fish are expensive for the teeming population (Olakojo et al. 2007) thus serving as an alternative to expensive sources of protein (Ileke et al. 2013). There are several reasons for the storage of agricultural produce in time of surplus in the tropical and sub-tropical regions in order to ensure food security. Among these agents militating against safe storage are arthropods which may include coleopterans and lepidopterans insect pest. A wide range of insect pests from the order Coleoptera attacks field crops up to storage (Udo 2011). Callosobruchus maculatus had been reported to cause 100% loss to cowpea seed during storage with a loss of 30 million US dollar in Nigeria (Udo and Epidi 2009). Cowpea bruchid, C. maculatus, is also known to cause substantial losses in terms of quality (nutritional loss) and quantity such as loss in market values, weight loss, and viability loss (Ofuya 2003; Ofuya et al. 2010). Approaches aimed at controlling attack by insect pests have relied heavily on the use of synthetic insecticides (Isman 2006). These synthetic chemicals are associated with shortcomings such as the emergence of resistant strains of pests, elimination of natural enemies and non-target species, environmental health hazard, and contamination of foods with chemicals (Ileke et al. 2013). Currently, research efforts are being focused on the use of plant biopesticides such as plant extracts which are tolerable than synthetic insecticides (Tamo 2012). Plant-based insecticides have been used as an alternative to synthetic chemical insecticides for pest management because botanicals pose no threat to the environment or to human health (Isman 2006; Maia and Moore 2011). Plant extracts and essential oils have traditionally been used to kill or repel stored product insects, more so many of them are believed to contain myriads of chemicals that could be insecticidal (Ileke and Adesina 2018). Hence, the current study was designed to evaluate the insecticidal activity of essential oils from seeds of Tetrapleura tetraptera, Annona muricata, and Aframomum melegueta and leaves of Ficus exasperata and Eucalyptus globulus for the control of C. maculatus on cowpea seeds.

Methods

Study site

The study was conducted in the Entomology Laboratory of the Department of Crop, Soil and Pest Management, The Federal University of Technology, Akure (FUTA), under ambient laboratory conditions of 28 ± 3 °C temperature and $65 \pm 10\%$ relative humidity.

Culturing of C. maculatus

The cowpea storage beetle *C. maculatus* was cultured on cowpea seeds "Ife Brown" a famous susceptible variety in Nigeria. The seeds were first cleaned of any impurities before disinfested in a deep freezer for 2 weeks. After removal from the freezer, the seeds were left to equilibrate at room temperature for 12–24 h. About 50 pairs of *C. maculatus* adults were introduced into a glass jar containing 200 g of clean Ife Brown cowpea seeds. Thereafter, the setup culture was kept in an insect cage for the emergence of progeny. Insect cultures were recycled once a month to facilitate an adequate supply of insects during the study at mean temperature and relative humidity of 29.4 °C and 75.7%, respectively.

Source and identification of botanical materials

Seeds of *T. tetraptera*, *A. muricata*, and *A. melegueta* and leaves of *F. exasperata* and *E. globulus* were obtained from the Teaching and Research Farm of the Federal University of Technology, Akure. The characteristics of the plant used are shown in Table 1. The identity of the botanicals was confirmed at the herbarium of the Crop, Soil and Pest Management Department of the Federal University of Technology, Akure. The seeds and the leaves were air-dried for 2 weeks in a well-ventilated shaded area from the sun to minimize the degradation of volatile compounds. The air-dried plant materials were later milled into fine powder using Philips blender DC7104 model. Thereafter, the powders are stored separately in an air-tight polythene bag for a period of 2 days before extraction of the essential oils.

Extraction of essential oils from botanicals

The essential oils were extracted from the seeds of Tetrapleura tetraptera, Annona muricata, and Aframomum melegueta and leaves of Eucalyptus globulus and Ficus exasperata separately following the method of extraction described by Shaaya et al. (1977). The essential oils were extracted using volatile essential oil steam distillation apparatus which is made of 2000 ml capacity distilling flask with a thick round condenser and graduated measuring tube with a collecting tap at the end. In carrying out the steam distillation process, 120 g each of the powders of the five different botanicals was weighed separately into a distilling flask and 300 ml of water added; the apparatus was set up using a clamp on a heating mantle and heated for a period of 4 h. The essential oil deposited on the water was then collected through the attached graduated measuring tube by opening the tap. The extraction was carried out throughout the period of the experiment to facilitate a steady supply of essential oil for the study. The essential oils extracted were collected separately inside a bottle with a tight-fitted cover, and each of the bottles was labeled with the names of each essential oil. Thereafter, the bottles containing the oils were stored in a cool refrigerator at a temperature of 4 °C until use.

Table 1 Plant essential oils evaluated for insecticidal properties against Callosobruchus maculatus

Scientific name	Common name	Family	Plant part used
Tetrapleura tetraptera	Aridan	Mimosaceae	Seed
Annona muricata	Soursop	Annonaceae	Seed
Aframomum melegueta	Alligator pepper	Zingiberaceae	Seed
Ficus exasperate	Sandpaper tree	Moraceae	Leaf
Eucalyptus globulus	Southern blue gum	Myrtaceae	Leaf

Bioassay

Essential oils extracted from the seeds of *T. tetraptera*, *A.* muricata, and A. melegueta and leaves of F. exasperata and E. globulus were evaluated for the control of C. maculatus at the rate of 1 ml/kg, 3 ml/kg, and 5 ml/kg of cowpea seeds which form the bulk seeds adopting the procedure described by Lale (1995). Ten freshly emerged adults (5 males and 5 females) of C. maculatus (0-48 h old) were used to infest 20 g of cowpea seeds weighed from the treated bulk seeds, and a control was also set up containing seeds that were not treated with essential oils. The experiment was replicated three times. Insect mortality was monitored at 12, 24, and 48 h for C. maculatus. Thereafter, the number of eggs laid on cowpea seeds were counted and recorded, seeds were examined for adult emergence at 21 days after oviposition, and the number of adults that emerged were counted and recorded. Ten days after adult emergence, the cowpea seeds were reweighed to obtain the seed weight loss as a result of larva consumption. Seed germination test was carried out on the seeds. Ten seeds from each treatment were separately and randomly selected. The seeds were placed in Petri dishes containing moistened filter paper (Whatman no. 1) and arranged in the laboratory shelf for emergence. From 3 to 7 days, the number of germinated seedlings from each Petri dish was counted and recorded.

Data analysis

Data on percentage mortality, seed weight loss, and percentage seed germinations were arcsine transformed while data on the number of eggs laid were square-root transformed before subjected to analysis of variance (ANOVA). Where the analysis of variance test indicated significant differences between treatments, Tukey's test was used to separate means at 5% level of probability. Data on mortality was subjected to probit analysis to determine the fiducial limit of the essential oil that can cause 50% and 90% mortality.

Results

Mortality of adult *C. maculatus* in treated cowpea seeds with plant essential oils

The percentage mortality of *C. maculatus* on cowpea seeds treated with different concentrations of the essential oils from botanicals at 12, 24, and 48 h is presented in Table 2. At 12 h, adult mortality of *C. maculatus* was

significantly higher (P < 0.05) on cowpea seeds treated with essential oils than the control treatment. Mortality of adult C. maculatus increased with the increase in the rate of the essential oil and time of exposure. However, the result showed that there were no significant differences in adult mortality of C. maculatus on cowpea seeds treated with the essential oils of the five botanicals. Mortality was highest (26.67%) on cowpea seeds treated with 5 ml/kg of A. melegueta essential oil and the lowest (6.67%) mortality was observed on seeds treated with 1 ml/kg of T. tetraptera essential oil. At 24 h, the percentage mortality of C. maculatus on treated seeds was significantly higher than control. It was similarly observed that the mortality of adult C. maculatus increased with the increase in the concentration of the essential oils. At 5 ml/kg concentration of *F. exasperate* essential oil, adult mortality of C. maculatus was significantly higher compared to other treatments. At 48 h of application, the result shows that mortality of adult C. maculatus was significantly higher (P < 0.05) on the treated seeds than the control treatment. Also, adult mortality of C. maculatus on cowpea seeds treated with different concentrations of the essential oil differs significantly. At 3 ml/kg concentration of the essential oil, adult mortality was 50% and above on cowpea seeds treated with A. melegueta (76.67%), A. muricata (50%), F. exasperata (60%), and T. tetraptera (66.67%) except for E. globulus where mortality was 40%. Adult mortality of 100% was recorded on seeds treated with A. melegueta and 90% with T. tetraptera at 5 ml/kg concentration.

Effect of plant essential oil treatment on oviposition, adult emergence, and weight loss

The mean number of eggs laid, the percentage of emerged adults, and weight loss caused by C. maculatus on cowpea seeds treated with essential oils and the control differ significantly (Table 3). Irrespective of the essential oil treatment, eggs laid by female C. maculatus on treated cowpea seeds were significantly lower (P < 0.05) compared to the control. The lowest value of eggs laid was recorded on cowpea seeds treated with $5 \, \text{ml/kg}$ of essential oil, while the highest number of eggs laid was observed on cowpea seeds treated with $1 \, \text{ml/kg}$ of essential oil. Also, in Table 3, the results showed that cowpea seeds treated with essential oils significantly

Table 2 Percentage mortality of *C. maculatus* on cowpea seeds treated with different concentrations of essential oil from botanicals at different time intervals

Conc. (ml)	Aframomum melegueta	Annona muricata	Eucalyptus globules	Ficus exasperate	Tetrapleura tetraptera
12 h					
0	0.00 ^c				
1	13.33 ^{ab}	13.33 ^{ab}	13.33 ^{ab}	16.67 ^{ab}	6.67 ^{ab}
3	20.00 ^a	16.67 ^a	16.67 ^a	20.00 ^a	16.67 ^a
5	26.67 ^a	16.67 ^a	20.00 ^a	20.00 ^a	20.00 ^a
24 h					
0	0.00 ^a	0.00 ^c	0.00 ^c	0.00 ^c	0.00 ^c
1	16.67 ^b	20.00 ^b	20.00 ^b	23.33 ^{ab}	16.67 ^b
3	33.33 ^a	23.33 ^{ab}	23.33 ^{ab}	30.00 ^a	23.33 ^{ab}
5	40.00 ^a	33.33 ^a	40.00 ^a	43.33 ^a	36.67 ^a
48 h					
0	3.33 ^c	0.00 ^c	3.33 ^c	3.33 ^c	0.00 ^c
1	26.67 ^b	30.00 ^b	33.33 ^b	40.00 ^b	30.00 ^b
3	76.67 ^{ab}	54.00 ^{ab}	52.00 ^{ab}	60.00 ^{ab}	66.67 ^{ab}
5	100.00 ^a	80.00 ^a	73.33 ^a	70.00 ^a	90.00 ^a

Means followed by the same letter along the column were not significantly different at 5% using Tukey's test

Table 3 Effects of essential oils on the insect biology and seed weight loss by Callosobruchus maculatus

Botanicals	Dose (ml)	Oviposition	Adult emergence	Weight loss (%)
Aframomum melegueta	0	180.33 ^a	62.67 ^a	7.73 ^a
	1	125.00 ^{ab}	37.33 ^{ab}	4.32 ^b
	3	91.00 ^b	24.33 ^b	2.98 ^b
	5	92.67 ^b	14.33 ^c	1.91 ^c
Ficus exasperate	0	168.00 ^a	68.67 ^a	9.40 ^a
	1	155.67 ^{ab}	38.67 ^{ab}	4.89 ^b
	3	140.33 ^b	29.33 ^b	3.40 ^b
	5	129.33 ^c	18.00c	2.48 ^b
Tetrapleura tetraptera	0	165.67 ^a	68.67 ^a	7.62 ^a
	1	103.67 ^b	38.67 ^b	3.98 ^b
	3	105.67 ^b	29.33 ^b	2.33 ^b
	5	105.67 ^b	18.00 ^b	1.08 ^c
Annona muricata	0	152.33a	61.00 ^a	7.55 ^a
	1	143.67 ^{ab}	34.67 ^{ab}	4.53 ^{ab}
	3	120.67 ^b	22.00 ^b	2.93 ^b
	5	117.67 ^b	3.67 ^c	0.59 ^c
Eucalyptus globules	0	159.33 ^a	62.33 ^a	8.69 ^a
	1	107.33 ^{ab}	32.67 ^b	4.05 ^{ab}
	3	115.33 ^{ab}	23.67 ^{bc}	2.30 ^b
	5	85.67 ^b	15.33 ^c	2.13 ^b

Means followed by the same letter along the column were not significantly different at 5% using Tukey's test

reduced (P < 0.05) emergence of adults compared to the untreated control. Similarly, cowpea seeds treated with 5 ml/kg of essential oils recorded the lowest adult emergence, while cowpea seeds treated with 1 ml/kg of the essential oils recorded the highest adult emergence compared to other treatments involving 3 ml/kg and 5 ml/kg. Generally, it was observed that the number of eggs laid and adult emergence decreased significantly with the increase in rates of the essential oil and exposure period. The percentage weight loss of cowpea seeds treated with essential oils was significantly lower (P < 0.05) when compared to untreated control. Irrespective of the plant materials, cowpea seeds treated with 5 ml/kg of essential oil achieved the lowest seed weight loss, while cowpea seeds treated with 1 ml/kg of essential oils had the highest seed weight loss and were significantly different (P < 0.05) compared to other treatments involving 3 ml/kg and 5 ml/kg.

Lethal dose (LD) of plant essential oils against *C. maculatus*

The result of lethal concentrations of essential oils (LC $_{50}$ and LC $_{95}$) on *C. maculatus* at 48 h of exposure is presented in Table 4. The essential oil of *A. melegueta* fiducial limit 1.80–3.11 and 3.93–5.12 was the most effective in causing adult mortality with LC $_{50}$ value of 2.42 ml/kg and LC $_{95}$ value of 4.86 ml/kg when compared to other treatment as indicated by the application rate which caused 50% and 95% mortality of cowpea beetles, while *E. globulus* with fiducial limit 2.82–4.96 and 5.34–8.32 appeared to be least effective in causing adult mortality with LC $_{50}$ value of 3.91 ml/kg and LC $_{95}$ value of 6.01 ml/kg compared to other treatments.

Effect of plant essential oil treatment on the percentage of germination

Table 5 shows the germination percentage for cowpea seeds treated with different rates of essential oils. Cowpea seed treated with essential oils at different rates had no significant adverse effect on seed germination after 120 days of storage. Though 100% seed germination was almost achieved for all the treatment concentration, however, from the result, it was observed that 1 ml/kg and 3 ml/kg produced 100% seed germination, but the

percentage of seeds that germinated starts to decrease at 5 ml/kg treatment rate.

Discussion

Plant-based insecticides have been used as a substitute to synthetic chemical insecticides that pose environmental health hazard for insect pest control because phytopesticides comes with no threat to the environment and human health (Isman 2006; Maia and Moore 2011). Ileke and Adesina (2018) reported that botanical oils contained numerous phytochemicals that could be insecticidal in nature.

The result of this study has unequivocally showed that essential oils from seeds of A. melegueta, A. muricata, and T. tetraptera and leaves of E. globulus and F. exasperata possessed insecticidal properties at varying degrees. Generally, the effects of essential oils to cause mortality of adult C. maculatus increased with the increase in treatment rates and time of exposure. A. melegueta essential oil was the most effective with LC50 value of $2.42\,\text{ml/kg}$ and LC_{95} value of $4.86\,\text{ml/kg}$ when compared to other treatments as indicated by the application rate which caused 50% and 95% mortality of cowpea beetles. A. melegueta essential oil caused above 50% insect mortality at the rates of 3 ml/kg (76.67%) and also at the rates of 5 ml/kg achieved complete (100%) mortality at 48 h. A similar observation has been reported by Adesina et al. (2015) that A. melegueta seed extract exerted 85% mortality at 120 h post-infestation against C. maculatus and can be used in formulating ecofriendly herbal insecticide. The high mortality caused by essential oils may be due to the choking smell of the essential oils which must have disrupted the normal respiratory process of the insects. Keita et al. (2000) reported similar results while working with various essentials oils on C. maculatus; Raja and John (2008) also reported an increase in the mortality of adult C. maculatus with increased rates of different volatile oils as the time of exposure increased.

Essential oils played an important role in reducing oviposition by female beetles, adult emergence, and percentage weight loss. The lowest value of eggs laid was recorded on cowpea seeds treated with 5 ml/kg of *E. globulus* essential oil compared to other treatments. This

Table 4 Lethal concentrations (LC₅₀ and LC ₉₅) of essential oils on *C. maculatus* at 48 h of exposure

Botanicals	Slope ± SE	Intercept ± SE	χ^2	LC ₅₀	95% FL	LC ₉₅	95% FL	P value
Aframomum melegueta	0.96 ± 0.13	-0.40 ± 0.10	5.14	2.42	1.80-3.11	4.86	3.93-5.12	0.00
Annona muricata	0.81 ± 0.11	-0.32 ± 0.12	4.01	3.00	2.16-3.76	5.52	4.81-6.24	0.01
Eucalyptus globulus	0.32 ± 0.10	-0.61 ± 0.11	8.13	3.91	2.82-4.96	6.01	5.34-8.32	0.00
Ficus exasperate	0.48 ± 0.11	-0.42 ± 0.14	3.11	2.78	1.95-3.24	5.93	5.05-7.14	0.00
T. tetraptera	0.71 ± 0.12	-0.28 ± 0.12	6.16	2.51	1.86-3.18	5.16	4.12-6.85	0.01

 $[\]chi^2$ chi-square, SE Standard error, LC Lethal concentration, FL Fiducial limits

Table 5 Effect of essential oils on the germination of cowpea seeds after 120 days

Botanicals	Concentration (ml)					
	0	1	3	5		
Aframomum melegueta	80ª	100 ^a	100 ^a	90ª		
Annona muricata	85ª	100 ^a	100 ^a	90 ^a		
Eucalyptus globulus	85 ^a	100 ^a	100 ^a	100 ^a		
Ficus exasperate	80 ^a	100 ^a	100 ^a	90 ^a		
Tetrapleura tetraptera	85 ^a	100 ^a	100 ^a	100 ^a		

Means followed by the same letter along the column were not significantly different at 5% using Tukey's test

present study also shows that E. globulus significantly reduced oviposition as the rate of the essential oils applied to cowpea seeds increased. Thus, the oils from the leaves of E. globulus excerted oviposition deterrent effect on C. maculatus. Also, it was visually observed that the few eggs laid by C. maculatus were unable to glue to the surface of cowpea seeds due to the obstruction effects of the oil. This rendered the cowpea seeds unacceptable to the beetle as they showed oviposition preference for untreated cowpeas. Idoko (2016) opined that the commercial use of plant products as anti-ovipositant against C. maculatus is undoubtedly an alternative insecticide. In addition, cowpea seeds treated with 5 ml/kg of A. muricata essential oil reduced adult emergence (3.67). Thang et al. (2013) reported that A. muricuta volatile oil consists of active compounds and acids with contact and growth inhibition properties. This growing body of experimental proof supports the idea that A. muricata shows insecticidal activity against assorted types of insects. Adeoye and Ewete (2010) reported that A. muricata seed extract significantly decreased the oviposition and emerged adults of *C. maculatus* and appeared to be a promising protectant against the respective insect in stored cowpea.

The number of emerged adults was significantly lower on cowpea seeds treated with essential oils at all rates, because of adult mortality and reduced oviposition which consequently reduced adult's emergence at all rates applied. Ketoh et al. (2005) observed that essential oil extracted from Cymbopogon schoenanthus was effective in lowering the population of *C. maculatus*; the reduction was high enough to prevent the emergence of larva stage. The reproductive potential of C. maculatus, which were exposed to essential oils, reduced significantly. A similar observation was reported by Manzoomi et al. (2010) that various essential oils were highly effective in causing adult mortality, reducing oviposition, adult emergence, and reduced weight loss caused by C. maculatus. Weight loss was significantly reduced in cowpea seeds treated with 5 ml/kg of A. muricata essential oil (0.59); this could be as a result of inability of the beetle to oviposit on the seeds, or few eggs laid were unable to hatched into larva or adult stages thereby prevented the holing of the protected seeds (Janzen 1977). The effect of essential oils on insect development has been caused by phytochemical and insecticidal properties of the plant extract (Nivea et al. 2013).

The results on germination on seeds treated with the essential oils have further substantiated the report of many other workers (Kritzinger et al. 2002; Chaubey 2013; Keita et al. 2002) that essential oil has no adverse effect on germination of treated seeds. The five plant essential oils examined for treatment effect on seed germination achieved 100% seed germination at treatment concentration of 1 ml/kg and 3 ml/kg. Consistent with the report of this study, Dinesh and Deepshikha (2012) examined four plant oils for treatment effect on cowpea seed germination and reported that different oils at 1 ml/kg and 3 ml/kg seed had no significant adverse effect on seed germination at all concentrations. However, it was also observed in this study that 5 ml/kg treatment concentrations of essential oil reduced the germination of the seed thereby preventing 100% germination in most of the plant materials used. Similar to the observation in this study, Qi and Burkholder (1981) reported diminishing germination capabilities in wheat when 5 ml and 10 ml of vegetable oil were used in treated wheat grain. From the result of this study, it is opined that the essential oils can be used at lower concentration in preserving cowpea seeds intended for planting.

Conclusions

Based on the findings from these results, the essential oils used in this study could be explored as an alternative bio-pesticide to synthetic insecticide in the protection of stored cowpea against *C. maculatus*. The essential oils from *A. melegueta* seed were highly effective in causing high mortality of *C. maculatus*, while *E. globulus* leaf essential oil reduced oviposition and *A. muricata* seed essential oil reduced adult emergence and weight loss of cowpea seeds by *C. maculatus*. *F. exasperata* showed to be the least effective in controlling *C. maculatus* in all the parameters assessed. Also, in order to avoid diminishing germination of cowpea seed, the application of the oils at a rate of 5 ml/kg or more should be discouraged in storing seeds intended for planting.

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Authors' contributions

JEI conceived and designed the study and reviewed the manuscript. JEI and KDI collected data on insect bioassay experiments. KDI analyzed all data

collected, search references, and manuscript draft. All authors read and approved the final manuscript.

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Availability of data and materials

Data collected and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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