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Allelopathic effect of *Calotropis procera* and *Leptadenia pyrotechnica* on seed germination and seedling growth of *Acacia senegal*: a laboratory investigation

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ABSTRACT

The objective of this study is to investigate the allelopathic effect of water extract from different parts of *Calotropis procera* and *Leptadenia pyrotechnica* on seed germination and seedling growth of *Acacia senegal*. Water extracts from *C. procera* and *L. pyrotechnica* parts i.e. leaves, stem, root, leaves/stem, leaves/root, stem/root and leaves/stem/root were prepared at four

concentrations (1, 2.5, 5 and 10 %) in addition to distilled water used as control. Effects of extract on germination percentage, speed of germination, shoot length, root length and seedling vigor were investigated in the lab. Results showed that water extract from different *C. procera* parts has no effect on germination percentage, however, leaves water extract (10%) significantly ($P < 0.05$) delayed germination rate to 2.55 seed/day. Whereas, water extract from *L. pyrotechnica* parts, showed no differences on germination percentage and significantly enhanced germination except leaves/root at 10% (2.25 seed/day) compared to control (2.55 seed/day). Seedling vigor index (VI) was also reduced significantly in response to all *C. procera* extracts. The results concluded that *C. procera* might play an important role in the formation of its natural habitats as it contains allelochemical compounds that enable the plant to retard germination and seedling growth of *A. senegal*. Whereas *L. pyrotechnica* contains allelochemical compounds may enhanced germination and seedling growth of *A. senegal*. The varying degree of inhibition and stimulation observed in *A. senegal* seeds/seedlings used in this work highlights the differential responses to different water extracts and the need to evaluate the allelopathic compatibility of *A. senegal* with *C. procera* and *L. pyrotechnica* shrubs before their introduction into the agroforestry systems.

Keywords: *Acacia senegal*; germination inhibition; germination speed; phytotoxic properties; seedling vigor index (SVI).

1. INTRODUCTION

Plants and their reproductive parts have been purposively and/or accidently transported over the world by human activities for various reasons (Rich, 2004). Sometimes introduction of a certain species in the new area may destroy or even extinct the native species (Pisula and Meiners, 2010; Rich, 2004). Several mechanisms for successful invasion have been recognized. Among them is allelopathy, which determines the dynamics of plant species and governing their activities in different ecosystems (Rich, 2004). A plant that is not evidently allelopathic in its home range may turn out to a new region (Rich, 2004). One of the ways that non-native plants successfully invade and flourish in communities of a new region is through allelopathy. Allelopathic plants release organic chemicals, termed allelochemicals, into the soil which interfere with the growth, survival or reproduction of other plants. An allelochemical may be released directly through the roots, or indirectly through leaching of litter or runoff from leaves. Because a non-native plant did not evolve with the invaded community, its allelochemicals may be particularly effective in suppressing the survival, growth or reproduction of the native flora (Callaway and Aschehoug, 2000).

In 2016, North Kordofan State, Sudan initiated the Gum Arabic Belt Rehabilitation Project as a key for sustainable rural development. The target is to plant thirty million *Acacia senegal* trees (locally *Hashab*) by 2020, covering an area represents 25% of total state area. The objectives of the 5-year plan (2016-2020) of the project are to develop and increase per capita income and increase production and productivity of cash crops through protecting *Hashab* trees by the so-called protective-productive community. The afforestation program includes degraded and abandon areas already covered by shrubs of low economic value such as *Calotropis procera* and *Leptadenia pyrotechnica* in some parts of the state. It also includes rehabilitation of *Hashab* gardens and community forests. Enrichment planting of *Hashab* trees in areas covered by *L. pyrotechnica* and/or *C. procera* may affect the establishment of *Hashab* since the two species possess allelopathic effect interaction under field conditions (Moustafa *et al.*, 2007; Srivastava *et al.*, 2012). Allelopathy seems to be more intense in poor soils (Inderjit and Callaway, 2003; Belz and Hurle, 2004; Suding *et al.*, 2004). A number of secondary metabolites or allelochemical were isolated from *L. pyrotechnica* and *C. procera* (Moustafa *et al.*, 2007; Srivastava *et al.*, 2012). Studies on the allelopathic influence of the emergent trees and shrubs on gum Arabic degraded lands are rare. Although field studies are necessary to see if allelopathic effects extend into complex field environments (Inderjit and Nielsen, 2003). In this context, simple laboratory germination assays can be used as a first step to examine potential allelopathic effects of a plant species (Alexa *et al.*, 2008). Laboratory studies also have the benefit of allowing the evaluation of direct impacts of allelochemical (Cipollini *et al.*, 2012). Moreover, laboratory bioassays further provide a simple tool to examine comparative allelopathic effects among several species growing in the same area (Pisula and Meiners, 2010). There is a need to screen the inhibitory and stimulatory influence of a wide range of water extracts, from trees and shrubs in Sudan, on seed germination and seedling growth of *A. senegal*. The objective of this study is to investigate allelopathic potential of water extracts from different parts of *L. pyrotechnica* and *C. procera* on seed germination and seedling growth of *A. sengal*.

2. MATERIALS AND METHODS

This study was carried out at Soba Tissue Culture Laboratory, Agricultural Research Corporation (ARC), Forests and Gum Arabic Research Center, Khartoum, Sudan. Two experiments were conducted, in March, 2017, to examine whether there is an inhibitory or stimulatory effect of different *C. procera and L. pyrotechnica* parts on seed germination and seedlings growth of *Acacia senegal*.

Experiment layout

Seeds of *Acacia senegal* were collected from *Umkraidem* area, West Bara Locality, North Kordofan, Sudan. Healthy uniform seeds were selected manually as test planting material. The seeds were surface sterilized according to Caboun and John (2015) with 70% Ethanol for 7 minutes and thoroughly washed in sterilized distilled water several times to avoid the effects of fungal contamination. The seeds were then placed under the laminar flow cabinet to dry. Fresh insect/disease-free parts (leaves, stems and roots) of *C. procera* and *L. pyrotechnica* were collected from abandoned lands near *Umgulgi* village, North Kordofan. After collection, plant parts were separated, washed thoroughly with distilled water to remove the adherent dust particles and then air-dried at room temperature for two months. The dried samples were chopped into small pieces, kept in an oven at 65 °C for 48 hours and then crushed by millet grinder to make powder. One hundred grams of each part were separately soaked in one liter sterilized distilled water (1:10 w/v, weigh/volume) and kept in shaker for 2 hours to get aqueous extracts. After shaking, extracts were placed at room temperature for 24 hours (Jadhav and Gaynar, 1995). The aqueous extracts were filtered through the sterilized muslin cloth followed by Whatman filter paper No. 1 and then centrifuged at 3000 rpm for 30 minutes (Kuo *et al.,* 1983). Separate extracts of stem, leaves, root, stem/root, stem/leaves, leaves/root and stem/leaves/root were prepared. Extracts were diluted with distilled water to obtain the concentrations of 1%, 2.5%, 5% and 10 % (on the basis of volume), in addition to distilled water used as control. Extracts were stored at 4ºC in the refrigerator until used to limit degradation of the allelochemicals (Richardson and Williamson, 1988).

A total of 116 glass Petri dishes, of 9 cm diameter, lined with a single layer of filter paper were covered with aluminum foil and then autoclaved at 120 °C for 30 minutes before use. All metal tools used were sterilized with 70% ethanol solution and flaming. Ten seeds of *A. senegal* were placed in each Petri dish and 5 ml aliquots of the different concentrates were then introduced, when needed, while distilled water was used as a control. Petri dishes were incubated at 27±C temperature with a 14/10h light/dark cycle for 12 and 15 days for *C. procera* and *L. pyrotechnica*, respectively.

Measurement of germination indices and growth parameters

Seeds germination or emergency were daily recorded up to 12 and 15 days for *C. procera and L. pyrotechnica* extracts, respectively. The emergence of a radical (approximately 2 mm in diameter) was considered as an index of germination. Length of shoot and root were measured in mm.

Calculations and analysis

The following germination indices were calculated using formulas as mentioned by Anjum and Rukhsana (2005):

Total germination (TG)

$$\mathrm{TG} = \frac{[\mathrm{T} \times 100]}{N}$$

Where:

T: proportion of germinated seeds in each treatment for the final measurement; N: Number of seeds used in bioassay.

Speed of germination (S)

$$S = (N_1 \times 1) + (N_2 - N_1) \times \frac{1}{2} + (N_3 - N_2) \times \frac{1}{3} + \cdots + (N_n - N_{n-1}) \times \frac{1}{n}$$

Where: N_1, N_2, N_3, N_n and N_{n-1} = proportion of germinated seeds observed at first, second, third, n and n –1 days after the bioassay beginning.

Seedling vigor index (SVI)

Seedling vigor index was calculated according to formula described by Orchard (1977):

$$SVI = L \times TG$$

Where:

SVI = Seedling vigor index

L = Seedling length

GT = Total germination

Data were subjected to UNIVARIATE analysis using the statistical program SPSS version 20 and a POST-HOC test based on Scheffe test was performed if significant differences ($p < 0.05$) between means of the measurements detected.

3. RESULTS AND DISCUSSION

Effect of water extracts on *A. senegal* seeds germination and speed

UNIVARIATE analysis showed insignificant ($P < 0.05$) differences in germination percentage among different water extract concentrations of *C. procera* and *L. pyrotechnica*. However, there was slight tendency of reduction from leaves and stem/root extracts reached to 82% at high (10%) concentration (Table 1). Across all *C. procera* water extract treatments; leaves, leaves/root and leaves/stem showed significant delay 2.55, 2.56 and 2.97 seed/day respectively, at concentration 10%. However leaves/stem/root showed slight tendency of delay 3.75 seed/day. Leaves water extract was the slowest reached 2.55 seed/day at 10%. In contrast, stem was the fastest reached 4.72 seed/day at 5%. On the other hand, across all *L. pyrotechnica* water extract treatments; there were significant acceleration compared to control 2.55 seed/day, except Leaves/Root which showed significant delay with 2.25 seed/day at 10% concentration. The fastest germination was shown by Leave/Stem reached 4.67 seed/day at concentration 5% (Table 2). Taking into consideration all mentioned indices (Table 1 and Table 2) depicting germination behavior it becomes clear that water extract of *C. procera* leaves significantly delayed the seed germination of *A. senegal* as the concentration increases.

Table 1: Effect of water extract of different parts of Calotropis *procera* and *Leptadenia pyrotechnica* on seed germination percentage of *Acacia senegal*

Part	*Calotropis procera*					*Leptadenia pyrotechnica*				
	Water extracts Concentrates (%)									
	0	1	2.5	5	10	0	1	2.5	5	10
	Germination (%)									
Leaves	96.7	95.0	100.0	90.0	82.5	85.0	92.5	90.0	80.0	90.0
Stem	96.7	95.0	97.5	95.0	97.5	85.0	90.0	87.5	85.0	97.5
Root	96.7	92.5	95.0	100.0	90.0	85.0	90.0	92.5	92.5	85.0
Leaves/stem	96.7	97.5	95.0	90.0	90.0	85.0	92.5	85.0	92.5	87.5
Leaves/root	96.7	90.0	92.5	97.5	95.0	85.0	87.5	95.0	85.0	85.0
Stem/root	96.7	95.0	97.5	95.0	92.5	85.0	85.0	95.0	85.0	82.5
Leaves/stem/root	96.7	95.0	92.5	95.0	90.0	85.0	95.0	97.5	97.5	95.0

Table 2: Effect of water extract of different parts of *Calotropis procera* and *Leptadenia pyrotechnica* on germination speed of *Acacia senegal* seeds.

Part	*Calotropis procera*					*Leptadenia pyrotechnica*				
	Water extracts Concentrates (%)									
	0	1	2.5	5	10	0	1	2.5	5	10
	Germination speed (germinated seed/day)									
Leaves	4.40±0.26	4.10±0.49a	4.52±0.58a	4.15±0.51a	2.55±0.34b	2.55±0.81	3.52±1.07	4.27±0.48	3.70±0.52	4.00±0.29
Stem	4.40±0.26	3.97±0.81a	4.32±0.25a	4.72±0.37a	4.32±0.94a	2.55±0.81	3.87±0.63	4.05±0.75	4.17±0.39	3.67±0.42
Root	4.40±0.26	4.77±0.26a	4.20±0.56a	3.97±0.60a	4.20±0.92a	2.55±0.81	3.60±0.79	4.45±0.65	4.10±0.35	3.90±0.67
Leaves/stem	4.40±0.26	4.32±0.53a	4.35± 0.31a	4.22±0.56a	2.97±0.38b	2.55±0.81	3.72±1.30	4.20±0.40	4.67±0.47	4.45±0.80
Leaves/root	4.40±0.26	3.65±1.08a	4.47±0.33a	4.62±0.56a	2.65±0.05b	2.55±0.81	4.15±1.06	4.32±0.53	3.62±0.96	2.25±0.77
Stem/root	4.40±0.26	4.30±0.90a	4.62±0.09a	4.60±0.33a	4.47±0.55a	2.55±0.81	3.97±0.61	4.05±0.66	3.87±0.66	4.10±0.50
Leaves/stem/root	4.40±0.26	3.70±0.42a	4.37±0.42a	4.17±0.71a	3.75±0.68a	2.55±0.81	4.07±1.03	4.10±0.62	4.00±0.91	3.52±1.16

In this experiment, *A. senegal* seems to be more sensitive to leaves extracts. The dual mixture extract that contains leaves i.e. leaves/root or leaves/stem significantly delayed seed germination. While, the triple mixture i.e. leaves/stem/root showed insignificantly with slight tendency of reduction. These findings are consistent with previous studies (Tukey and Morgan, 1994; June, 2006; Sale, 2013; Rice, 1984; DelMoral and Muller, 1998, 1999) that reported *eucalyptus* leaves produced allelochemicals which might be inhibited seed germination of other plants. In contrast, *L. pyrotechnica* water extracts significantly accelerated seed germination of *A. senegal* except leaves/root/stem at the highest concentration compared to control. This acceleration in seed germination indicates a presence of water soluble stimulants in *L. pyrotechnica* extract. These findings are in line with Tukey (1966) who reported that growth of seedling immediately after germination, not all allelochemical substances are inhibitors they can be stimulants, like mineral nutrients, organic acids, carbohydrates and growth regulators. The results of insignificant effect on germination percentage of *C. procera* and *L. pyrotechnica* are not consistent with the findings of Yassin (2012); Al-Zahrani and Al-Robai (2007) who reported a general reduced crops performance in response to application of *C. procera* extracts. Seeds in this study were pretreated by ethanol and distilled water to prevent fungi contamination. The insignificant effect may be due to this pretreatment (soaking) as permeability of seed coat by ethanol may affect seed germination. On the other hand the low concentrations of water extracts parts may have no effect especially with suppressed feature which seemed in high concentration (10%).

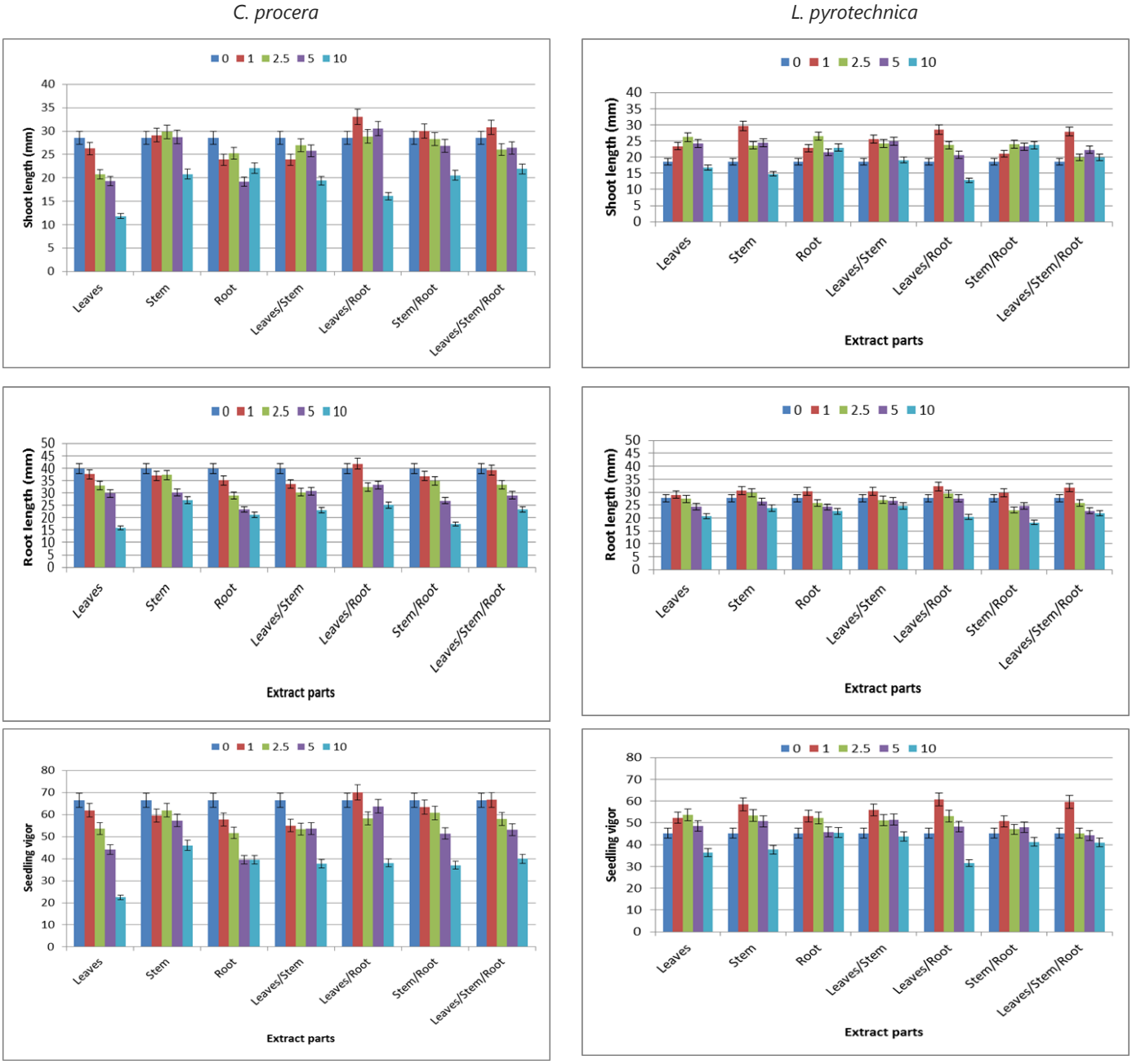

Fig.1: Effect of different *C. procera* and *L. pyrotechnica* water extract concentrations on growth and vigor of *A. senegal* seedlings.

Effect of water extracts on seedling growth of *A. senegal*

C. procera and *L. pyrotechnica* water extract concentrations showed significant effect on *A. senegal* seedling shoot length. Leaves water extract at concentration 10% resulted in the lowest shoot length value 11.84 mm while leaves/root at 1% resulted in the highest shoot length value 33.03 mm. Regard to *L. pyrotechnica*, leaves/root at 10% resulted shortest length by 12.87 mm and stem at 1% caused the tallest shoot seedling length by 29.67 mm. The effect of extract concentrations varied with extract parts (Fig.1). In *C. procera* the shortest length 15.83 mm was observed in leaves at 10%, in contrast the tallest length 41.83 mm was observed in leaves/root at 1%. In this context, a remarkable decrease in root length was observed as level of concentration increases. Concerning *L. pyrotechnica*, the tallest length observed in Leaves/root 32.2 mm at 1% concentration and the shortest with 18.3 mm observed in leaves/root at 10%. Generally there was noticeable increase in root length in response to lowest level of concentration and then decreased (Fig.1). Results obtain from shoot length, root length and seedling vigor revealed that all *C. procera* extracts were found to be phytotoxic and decrease seedling growth of *A. senegal* compared to control. This result agrees with previous studies (El-Khatib and Abd-Elaah, 1998; Al-Zahrani and Al-Robai, 2007; Tanveer *et al.,* 2010; Chandra and Mali, 2012; Pukclai and Kato-Noguchi, 2012). While *L. pyrotechnica* extracts, stimulated firstly by the lowest extract concentration and then reduced. This indicates that the effect

might have been the result of water soluble allelochemical in the extracts and their inhibitory or stimulatory on the measured parameters. This finding confirms with previous studies (Rice, 1984; Sang-Uk *et al*. 2000; Peng *et al*., 2004) that reported biological response of receiver plants to allelochemicals concentration. The shoot length showed significant reduction to *C. procera* extracts of leaves and root as well as leaves/stem and leaves/root just at the highest concentration compared to control. However, *C. procera* extracts of leaves/stem/root, stem/root and stem showed insignificant, though they had slight tendency of reduction compared to control. While, the root growth reduced significantly in response to all *C. procera* extracts. On the other hand, shoot length showed significant reduction at the highest concentration of both *L. pyrotechnica* extracts leaves/root and stem. While in root length, all *L. pyrotechnica* extracts except leaves/stem had significant reduction at the highest concentration. These conclusions in both *C. procera* and *L. pyrotechnica* water extracts agreed with the finding of Abdel-Farid and Syed (2013) who stated that root growth of *C. procera* is more sensitive to extracts than shoot growth as roots are first to emerge and encounter allelochemicals effect.

Effect of water extracts on seedling vigor of *A. senegal*

The results showed significant effect ($P < 0.05$) of water extracts concentrations of *C. procera* and *L. pyrotechnica* on seedling vigor (Fig.1). In general, there was a reduction in seedling vigor with increasing extract concentration. Results from seedling vigor showed that *C. procera and L. pyrotechnica* water extract part varied in their allelopathic effect activity against *A. senegal* seedling this may be due to differed allelochemical compounds leached from parts of *C. procera* and *L. pyrotechnica*. This result is in lined with past studies conducted by Turk and Tawaha (2002); Peng *et al.* (2004); Sisodia and Siddiqui (2010) who stated that the variance of allelopathy among plant parts may referred to the profile of allelochemical present in different parts of the donor plant.

4. CONCLUSION

Generally, it can be concluded that *C. procera* plays an important role in the formation of its natural habitats as it contains allelochemical compounds that enable the plant to retard seed germination and seedling growth of *A. senegal*. In contrast, *L. pyrotechnica* demonstrates stimulatory effect on *A. senegal* including enhanced germination and stimulated seedling vigor except at 10% concentration which possesses suppression indication. The varying degree of inhibition of *A. senegal* seeds germination and seedlings growth due to water extracts necessitates the evaluation of allelopathic effect of *C. procera* and *L. pyrotechnica* shrubs on *A. senegal* in the field.

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The authors declare that there are no conflicts of interests.

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Data and materials availability: All data associated with this study are present in the paper.

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