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Research Article

EVALUATION OF BIOLOGICAL TRAITS, MORPHOMETRIC CHARACTERISTICS, FEEDING PREFERENCES, AND INFESTATION POTENTIAL OF PULSE BEETLE (*CALLOSOBRUCHUS MACULATUS* FAB.) ON VARIOUS PULSE CROPS

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ABSTRACT

The pulse beetle, *Callosobruchus maculatus* (Fab.), is a major invasive pest of stored legume seeds in Pakistan. This study aimed to investigate the biology, morphometrics, feeding potential, and seed damage caused by the beetle on different pulses under laboratory conditions during 2023. The insect laid the highest number of eggs on *Vigna unguiculata* (8.53±0.95), followed by *V. radiata* (7.55±0.78), *V. mungo* (6.66±0.75), *Phaseolus vulgaris* (6.61±0.63), and *Cicer arietinum* (5.02±0.37). The mean egg incubation periods were recorded as follows: black gram (5.60±0.54), cowpeas (5.50±0.21), chickpea (4.00±0.72), green gram (4.00±0.37), and kidney bean (0.00±0.00) days, respectively. The egg hatching rates recorded were (39.30%) green gram, (36.10%) cowpeas, (19.50%) chickpea, (5.10%) black gram, and (0.00%) for kidney beans. Notably, while (23.30%) of the eggs were laid on kidney beans, none hatched, and no adults emerged due to the smooth surface of the seed coat. The larvae and pupae development period lasted (24-27) days on black gram, (23-25) days on white gram, (19-21) days on cowpeas, and (18-20) days on green gram. Adult females survived for (14-15) days on cowpeas, (10-15) days on green gram, (12-14) days on black gram, and (10-11) days on chickpea. The mean fecundity was recorded at (6.12±3.17) eggs when two males fertilized one female and (8.73±4.22) eggs when one male fertilized two females. Cowpea seeds were identified as the preferred food source for *C. maculatus* compared to other pulses. Therefore, effective control of this pest is crucial to protect legume crops.

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INTRODUCTION

Pulses are remarkable plants that can thrive in different climatic conditions and contribute significantly to soil fertility (Singh et al., 2015). Often referred to as the “poor man’s meat”, pulses are rich in proteins, amino acids, minerals, and vitamins, playing a vital role in human nutrition (Hossain et al., 2014). Approximately 840 million people worldwide suffer from malnutrition due to inadequate nutrient intake (Susmita, 2016). Recognizing of their contributions, the United Nations declared the International Year of Pulses during its 68th General Assembly (GOI, 2016). India is the largest producer and consumer of pulses, accounting for 29% of the global production area and 19% of total production (Singh et al., 2015). In Pakistan, gram is the predominant pulse crop, covering 76% of the total pulse area and contributing 74% to the country's total pulse production, followed by green grams, which occupy 13% of the area and contribute 16% to production (Anonymous, 2010). However, Pakistan imports substantial quantities of pulses to bridge the gap between domestic production and consumption (Pakistan Bureau of Statistics, 2020-21).

Pulse beetles are the most significant limiting factors in pulse production, causing severe yield losses in storage and field conditions. *Callosobruchus analis*, *C. chinensis*, and *C. maculatus* are common pest species frequently found in stored pulse seeds (Arora, 2016). These beetles multiply rapidly, leading to substantial losses during storage (Singhal, 1986). Insect pest species cause heavy significant economic losses to fruits and seeds of the cultivars such as *Batrachedra amydraula* infest to unripe date fruits (Mangrio et al., 2024a; Jatoi et al., 2021). Similarly, another a notorious tissue-boring insect, red palm weevil, severely damages date palm trees, and exacerbating great yield losses (Kubar et al., 2017). In India, five species of bruchids have been reported across 24 states, with *C. maculatus* being the most abundant and dominant species (Aidbhavi et al., 2023). The cowpea beetle (*Callosobruchus maculatus*) is a particularly destructive pest of *Vigna unguiculata*, causing over 50% losses during storage (Caswell, 1981). The larvae of insect pests are voracious feeders, further aggravating the damage (Jatoi et al., 2020; Mangrio et al., 2020). After hatching, the larvae of these pests directly penetrate maturing pods, enter the seeds and fruits, and continue consuming the internal contents (Ali et al., 2004; Kousar et al., 2020).

Infested seeds are also susceptible to fungal attacks, which produce mycotoxins, leading to biochemical changes that render the seeds unfit for human consumption and diminish their market value (Augustine and Balikai, 2019). Given the critical importance of pulses and the losses caused by *C. maculatus*, this study was conducted to investigate various parameters related to this insect pest. There is an urgent need to enhance stable, eco-friendly Integrated Pest Management (IPM) techniques, adopt suitable measures, and strengthen biological, cultural, and structural remedies to protect valuable cultivars (Mangrio et al., 2022).

MATERIALS AND METHODS

Preparation of pulse beetle stock culture

The infested seeds containing *Callosobruchus maculatus* were purchased from grocery stores in Karachi, brought to the laboratory, and placed in plastic jars for further multiplication. The jars containing the infested seeds were covered with muslin cloth and secured with rubber bands to prevent the escape of adult beetles. The beetle populations were cultured at an average room temperature of 25.68±0.35°C and 49±1.02% relative humidity. The cultured stock was observed on daily basis, and from the stock, 20 new pairs of beetles were transferred into separate jars, each containing 500g of disinfested cowpea seeds. The cowpea seeds were disinfested by placing them in an oven at 50°C for one hour. The newly emerged adults were then used for laboratory examinations.

Biological and morphological attributes of pulse beetles on different host pulses

To study the biological and morphometric parameters of *C. maculatus* on different host pulses, a freshly emerged virgin pair (male and female) was released into Petri dishes with a diameter of 12 cm. In each Petri dish, 10 disinfested seeds of each host pulse were provided for egg laying. The experiment was designed using a randomized complete block design (RCBD) with three replications. Data were collected on various parameters, including egg incubation period, fecundity, fertility, oviposition, post-oviposition, adult longevity, larval and pupae period, mating behavior, and total lifespan from egg to adult. Egg laying was recorded on daily basis for all replications on each host pulse seed. The seeds containing eggs were replaced with new ones daily in each replication. The oviposition preference and egg hatching percentage were calculated using the following formula:

$$\text{Oviposition preference (\%)} = \frac{\text{No. of eggs laid on specific pulse}}{\text{Total no. of eggs laid by single female on pulse cultivars}} \times 100$$

$$\text{Eggs hatched (\%)} = \frac{\text{No. of eggs hatched}}{\text{Total no. of eggs kept on different pulse cultivars}} \times 100$$

Oviposition preference of pulse beetle on different pulses

Free choice test

In the free choice test, 20 disinfested seeds of each host pulse, such as green gram, black gram, chickpea, cowpea, and kidney bean, were placed in separate Petri dishes, each with a diameter of 12 cm. A newly emerged pair of male and female *Callosobruchus maculatus* was released from the culture into each Petri dish for egg-laying. Each Petri dish was arranged and kept in a laboratory at an average room temperature of 25.68°C±0.35°C and a relative humidity of 49±1.02%. In all three replications, host pulse seeds containing the egg population were replaced with fresh seeds on daily basis.

No choice test

In the no choice test, 20 disinfested seeds of a single pulse variety were placed in separate Petri dishes, each measuring 12 cm in diameter. A newly emerged pair of *C. maculatus* was released into each dish for egg-laying. Three replicates were performed for each treatment individually, including cowpea, green gram, chickpea, and black gram. In all replications, after counting the eggs on the host pulse seeds, the seeds with eggs were replaced with fresh seeds until the adult beetles died.

Weight loss percentage in different pulses

To assess the percentage of weight loss in different pulses, 100g of disinfested seeds of green gram, black gram, chickpea, and cowpea were placed separately in plastic jars. In each jar, five newly emerged pairs of *C. maculatus* were released for egg-laying. The jars were covered with muslin cloth, and secured with rubber bands to prevent adult beetles from escaping. The jars were kept in a laboratory at an average temperature of 25.68°C±0.35°C and a relative humidity of 49±1.02% for one and a half months. After this period, the adults were removed from each treatment, and the weight of each jar containing infested seeds was measured to determine the percentage of weight loss. The weight loss percentage was calculated using the following formula:

$$\text{Weight loss (\%)} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

Statistical analysis

All insect pest data were subjected to analysis using the SXW 8.1 statistical software (USA), and significant differences among homogeneous groups were calculated at (*P*< 0.05).

RESULTS

Eggs of pulse beetle

The eggs of *C. maculatus* were observed small, oval-shaped, and firmly attached to the pulse grains. Freshly laid eggs were translucent, shiny, and smooth on the 1st day, turned yellow-greenish on 2nd day, after 4th and 5th day, a black spot and developing grub became visible. After 6th and 7th days, the eggs were filled with frass and larval feces, resulting from the feeding activity of the grub inside the egg. Females typically laid eggs singly and about 80% on the seeds; however, when seeds were scarce, they my deposited 8 to 12 eggs per seed, as shown in Figure 1.

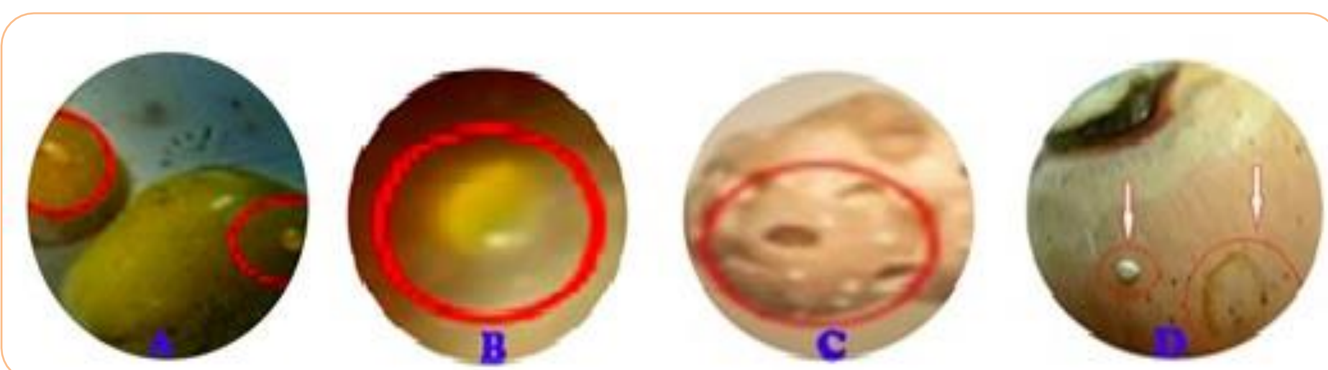

Figure 1. a; 1st day fresh eggs, b; 2nd day yellowish egg, c; Eggs per seed, d; Frass filled eggs.

Grubs (1st, 2nd, 3rd and 4th instars) of pulse beetle

The beetle grubs were observed to be yellow-white, c-shaped, with a small black head. Upon completing incubation, the grub entered the seed directly without emerging from the eggshell. A small hole on the surface of the grain was seen when the egg was removed, indicating where the larvae penetrated the seed. The grub underwent a series of molts, passing through four instar stages (Figure 2). The duration of the grub stage ranges from 8 to 9 days. Accurately recording the duration of each instar was challenging, as the entire developmental process occurred inside the seed. However, different instars were identified based on the damage observed in cowpea seeds.

Figure 2, e; 1st stage, f; 2nd stage, g; 3rd stage, h; 4th stage larvae.

Pupae stages of pulse beetle

The pupae stage involves a series of developmental changes before the insect emerges as an adult. 1st day, wing rudiments were observed and 2nd day, the antennae, proboscis, and leg-like appendages were visible. 3rd day, the mouthparts, eyes, hind wings, forewings, and developed legs 4th day, almost all body parts were fully formed and 5th day, the forewings darkened to a brown color with black spots. The entire pupae period lasts between 5 and 6 days (Figure 3).

Figure 3. i; pre-pupae, j; Pupae, k; Post-pupae.

Adult male and female pulse beetle

The adults emerge by chewing a round hole in the seed. Both adult males and females have a black head and a body that is brown to reddish-brown with black patches. After emergence, they are capable of flight. Males and females were distinguished by a plate at the end of the abdomen. In case of females, the plate was large and oval, with black on both sides and a white longitudinal stripe in the middle, while in males, the plate was smaller and lacks stripes. Males were more rounded and smaller than females. In *C. maculatus*, both male and female adults had serrate antennae. After pupation, both males and females remained inside the seed for about 24-36^h until they reached full maturity. Then exit the seed by chewing through a circular piece of the seed coat. After emerging, males chased females until they were ready to copulate, which lasted approximately 6-8 min (Figure 4).

Figure 4. l; Adult female, m; Adult male, n and o; Male and female matting behavior.

Egg-laying capacity of *C. maculatus* on different pulse varieties

Adult fertilized females of *C. maculatus* began laying eggs within 24h after mating. The highest number of eggs was laid on the first day after mating, with counts as follows: cowpea (14.66±5.24), green gram (12.33±5.18), kidney bean (11.25±4.22), black gram (9.66±4.12), and white gram (8.33±3.85). By the 10th day after mating, the egg population decreased to the following levels: cowpea (3.85±3.45), green gram (3.35±3.75), kidney bean (2.33±3.25), black gram (2.15±3.27), and white gram (2.12±3.14). Cowpea seeds were the preferred substrate for oviposition, with females laying the highest total number of eggs: cowpea (8.53±0.95), green gram (7.55±0.78), kidney bean (6.61±0.63), black gram (6.66±0.57), and white gram (5.02±0.37). The beetles laid the maximum number of eggs (8-14) per day during the first five days on all observed pulse varieties. After 5th day, the egg-laying capacity gradually decreased and ceased by the 11th day. In no-choice tests, the number of eggs laid differed significantly ($P < 0.05$) among the different pulse varieties (Table 1).

Table 1: Day wise egg laying behavior of *Callosobruchus maculatus* in no choice test.

Days	Cowpeas	Green gram	Kidney bean	Black gram	White gram
1st	14.66±5.24	12.33±5.18	11.25±4.22	9.66±4.12	8.33±3.85
2nd	12.85±3.45	11.45±3.34	9.81±3.25	8.76±2.78	7.75±2.56
3rd	12.66±4.77	11.33±3.56	9.33±2.85	7.66±2.85	6.55±2.21
4th	11.85±4.35	10.33±3.56	9.33±2.65	7.33±2.35	6.33±2.15
5th	8.35±2.95	7.45±2.35	6.55±1.95	5.66±1.55	5.33±1.46
6th	6.35±1.57	6.12±1.22	5.77±1.36	5.15±1.25	4.44±1.24
7th	5.75±1.87	5.15±1.47	4.85±1.43	4.15±1.45	3.75±1.35
8th	4.66±1.78	4.35±1.22	3.66±1.14	3.15±1.09	2.85±1.03
9th	4.33±2.95	3.66±2.85	3.25±2.78	2.95±2.22	2.75±2.15
10th	3.85±3.45	3.35±3.75	2.33±3.25	2.15±3.27	2.12±3.14
Total	8.53±0.95	7.55±0.78	6.61±0.63	6.66±0.57	5.02±0.37

Means in columns with similar letters are non-significantly different ($P < 0.05$) from each other.
Values are Means±SD.

Egg incubation and different developmental stages of *C. maculatus*

The maximum mean incubation periods were observed as follows: black gram (5.60±0.547 days), cowpeas (5.50±0.21), chickpeas (4.00±0.72), and green grams (4.00±0.37) days, with a temperature of 25.68±0.35°C and relative humidity of 49±1.02%. The oviposition preference of *C. maculatus* varied significantly ($P < 0.05$) among different host pulses in a free-choice test. The percentage of oviposition preference was recorded as cowpea (34.90%), green gram (24.40%), kidney bean (23.30%), white gram (15.50%), and black gram

(1.90%). There was a significant difference in the number of eggs that hatched on different pulse cultivars (*P*< 0.05) in the free-choice test. The highest percentage of egg hatching was observed on green gram (39.30%), followed by cowpea (36.10%), chickpea (19.50%), black gram (5.10%), and no hatching on kidney beans (0.00%). Notably, although beetles laid eggs on kidney beans, the eggs did not hatch, possibly due to the hard seed coat. The oviposition period ranged from 9th to 10th days for chickpea, black gram, cowpea, and kidney bean, whereas it ranged from 7th to 10th days for green gram. The post-oviposition period lasted a maximum of 3rd to 4th days and a minimum of 1st to 3rd days. The larvae consumed the pulse seeds for a maximum of 25th to 27th days and a minimum of 18th to 20th days. The adult female pulse beetle lived for a maximum of 14th to 15th days and a minimum of 10th to 11th days on the stored pulse grains (Table 2).

Table 2: Different life stages of *C. maculatus* on pulse varieties.

Host pulses	Egg incubation	Egg laying	% Oviposition preference	%Egg hatching	Oviposition (Range)	Post-oviposition (Range)	larvae and Pupae period (Range)	Female life (Range)
Chickpea	4.00±0.72	5.02±0.37[a]	15.50	19.50[b]	9-10	2-3	23-25	10-11
Black gram	5.60±0.54	6.66±0.57[ab]	1.90	5.10[c]	9-10	1-3	24-27	12-14
Cow peas	5.50±0.21	8.53±0.95[cd]	34.90	36.10[a]	9-10	3-4	19-21	14-15
Green gram	4.00±0.37	7.55±0.78[bc]	24.40	39.30[a]	7-10	2-3	18-20	10-15
Kidney bean	0.00±0.00	6.61±0.63[ab]	23.30	0.00	9-10	1-3	0.00	0.00

Means in columns with similar letters are non-significantly different (*P*< 0.05) from each other.

Fecundity of adult female *C. maculatus* fertilized by one or two males on different days on cowpea seeds

When two virgin males and one virgin female of *C. maculatus* were released onto cowpea seeds, the single female exhibited the highest egg fecundity on the 1st day (16.33±3.45) eggs and the lowest on the 10th day (1.33±0.85) eggs. This suggests that the egg fecundity of *C. maculatus* is highest in the initial days but decreases over time. For females fertilized by two virgin males, the overall egg fecundity was recorded as (6.66±3.21) eggs.

Similarly, when one virgin male and two virgin females were released on cowpea seeds, both females laid the maximum number of eggs on the 1st day (22.00±5.22) eggs, with a gradual decline observed by the 10th day (1.66±0.95) eggs. When two females were fertilized by one male, the egg fecundity was (9.14±4.37) eggs.

This data confirmed that egg fecundity was highest during the initial days and gradually decreased. ANOVA analysis of the overall mean egg fecundity revealed a significant difference between the treatments of one female fertilized by two males and two females fertilized by one male (*P*< 0.05), as shown in Table 3.

Table 3. Mean fecundity of two ♂ with one ♀ and one ♂ with two ♀ in different days.

Days	Two male and one female	One male and two female
1	16.33±3.45	22.00±5.22
2	15.33±2.33	9.33±2.95
3	8.00±2.85	9.00±3.25
4	5.33±2.15	9.00±3.12
5	5.33±3.85	8.66±3.27
6	3.00±1.85	8.66±3.22
7	3.00±1.78	7.33±2.45
8	2.33±1.35	6.66±3.21
9	1.33±0.85	1.66±0.95
10	1.22±0.55	1.48±0.63
Mean	6.12±3.17	8.37±4.22

Means in rows with similar letters are non-significantly different (*P*< 0.05) from each other.
The values are represented as Mean±SD.

Assessment of weight loss over one and a half months in different pulses

The weight loss caused by five pairs of male and female pulse beetles was highest in disinfested cowpea seeds (3.7%), followed by green gram (1.8%), chickpea (1%), and black gram (0.2%) over a one-and-a-half-month storage period. No weight loss was observed in kidney beans, as the grubs were unable to penetrate the hard seed coats. Analysis of variance revealed significant differences in weight loss percentages among the different pulse seeds ($P< 0.05$), as shown in Table 4.

Table 4. Comparison of weight loss percentage in different pulses.

Host pulses	Initial weight (g)	Final weight (g)	Weight loss (%)
Chickpea	100	99.0	1.00%
Black gram	100	99.8	0.20%
Cowpea	100	96.3	3.70%
Green gram	100	98.2	1.80%
Kidney bean	100	100	0.00%

The % in weight loss columns are non-significantly different ($P< 0.05$) from each other.

DISCUSSIONS

In the present study, the highest number of eggs was significantly recorded on cowpeas, followed by green gram, black gram, kidney beans, and chickpeas. These findings align with the research of Radha and Susheela (2014), who reported that *C. maculatus* preferred to lay eggs on cowpeas, followed by green gram, chickpeas, black gram, and red gram. Similarly, Ahmad et al. (2018) reported that *C. chinensis* laid the maximum number of eggs on chickpeas and the minimum on black gram. In terms of egg hatching, green gram exhibited the highest rate among the pulses, which is consistent with Radha and Susheela (2014), who reported a higher percentage (98%) of egg hatching in cowpeas, followed by green gram (97.25%). Jehajo and Memon (2020) documented a significantly higher susceptibility index in cowpeas and green gram and lower in black gram and chickpeas. Cowpea seeds were observed as a preferred food, resulting in a greater percentage of seed weight loss compared to green gram, chickpeas, black gram, and kidney beans over a storage period of one and a half months. Babu et al. (2021) also reported higher infestation rates in cowpeas, green gram, and chickpeas, with lower rates in black gram and soybeans after 60 days of infestation. Faizan et al. (2023) reported the maximum weight loss percentage in mung beans and cowpeas, and the minimum in chickpea cultivars.

Interestingly, pulse beetles prefer to lay eggs on kidney beans but are unable to penetrate due to the smoothness of the seed coat. Girish et al. (1974) reported that the smoothness of the seed coat and the size of the seed might play a role in the egg-laying preference of *C. maculatus*. Bhaduria and Jakhmola (2006) corroborated that bruchids prefer to lay eggs on seeds with smooth seed coats. Ajayi and Lale (2001) also emphasized that the texture of the seed coat is a key factor in egg-laying and development. Chakraborty and Mondal (2016) reported that *C. maculatus* caused the highest pest infestation percentage in cowpeas, followed by green gram and black gram, with the lowest in kidney beans. In this study, a significantly higher percentage of egg hatching was recorded on green gram, followed by cowpeas and chickpeas, with significantly lower percentages recorded on black gram and zero percent on kidney beans in the free choice test. Mannan and Bhuiyah (1996) similarly reported a higher percentage of egg hatching in cowpeas and less in black gram.

In the present study, the incubation period was recorded as 4-6 days on different pulses in the free choice test. This finding is in great conformity with Beck and Blumer (2014), who reported an incubation period of *C. maculatus* ranging from 3 to 4.5 days on green gram, black gram, cowpeas, horse gram, and green peas. The maximum development occurred on black gram and the minimum on green gram. Karthik et al. (2023) reported maximum development in chickpeas, black gram, and soybeans, with minimum development in green gram, cowpeas, and peas. Faizan et al. (2023) documented maximum development in chickpeas and minimum in black gram cultivars. Sindh province is regarded as a well-established agricultural zone for pulse cultivars, but it is crucial for the government and other organizations to invest significantly in this sector to ensure and earn profit (Mangrio et al., 2024b).

CONCLUSION AND RECOMMENDATIONS

In conclusion, bruchids cause significant quantitative and qualitative losses, particularly under post-harvest storage conditions. It has been well-established that cowpea, green gram, and chickpea are highly suitable host cultivars for *Callosobruchus maculatus*. This biological and morphological study of the pulse beetle reveals that this insect species causes the greatest percentage of weight loss by laying a large number of eggs on these host cultivars. This study exhibited the highest susceptibility to infestation on cowpea, followed by green gram and chickpea. Interestingly, the smooth surface of seeds plays a crucial role in preventing insect pest damage. The beetle caused minimal damage to black gram and no damage to kidney beans. Therefore, it is strongly recommended to exercise utmost care when storing cowpeas, green gram, and chickpea in warehouses, homes, and grocery shops. These pulses, being the most susceptible, should be stored separately from others to prevent cross-infestation.

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AUTHORS CONTRIBUTION

BM purchased the pulse seeds, designed and conducted the experiments, and contributed to the conceptualization of the study; WMM collected data on the various life stages of the pest and drafted the manuscript; HAS performed the statistical analysis of the pest data and provided the morphological descriptions; GHJ critically reviewed the manuscript, providing detailed feedback; AAS and AHM coordinated the procurement of tools and materials necessary for the research; MAC, KAP, and FIS provided support at various stages of the scientific work, ensuring the successful completion of the study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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