



RESEARCH ARTICLE

Biophysical and biochemical bases of resistance to *Maruca vitrata* F. in selected vegetable cowpea genotypes

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Abstract

Host plant resistance is a crucial factor in mitigating the damages caused by *Maruca vitrata*, the most devastating pest of vegetable cowpea, commonly known as the legume pod borer. Understanding the biophysical and biochemical aspects of resistance is essential for developing methods to breed insect pest resistance in germplasm. To identify genotypes with diverse traits associated with resistance to this pest, we investigated the biophysical and biochemical traits implicated in conferring resistance to *M. vitrata* across ten accessions of vegetable cowpea, alongside resistant and susceptible check. Trichomes on pods emerged as a pivotal aspects of resistance in these genotypes, exhibiting a significant negative correlation with the percentage of seed damage inflicted by *M. vitrata*. In our study, genotypes with higher pod trichome density showed a reduction in seed damage caused by the pest. Additionally, a high phenol concentration and low levels of total sugars and proteins were associated with resistance to *M. vitrata*. Vegetable cowpea genotypes characterized by abundant pod trichomes and elevated phenol level hold promise for breeding efforts aimed at developing cultivars resistant to *M. vitrata*.

Keywords

Vegetable cowpea; *Maruca vitrata*; phenol content; total sugars; protein content; host plant resistance

Introduction

Vegetable cowpea, or *Vigna unguiculata* (L.) Walp., boasts significant nutritional value. This crop thrives in semi-arid tropical regions across Asia, Africa and other nations. It demonstrated remarkable adaptability to various agroecosystems, particularly those found in tropical and sub-tropical regions (1) and is capable of thriving in soils with diverse pH levels, including sandy soils (2). Recognized for its high protein content, low fat content and favourable amino acid profile that complements cereal grains (3). Studies indicate that cowpea seeds and leaves contain crude protein levels ranging from 22.0 to 30.0 % (4). Furthermore, as a vegetable, cowpea is abundant in vitamins, minerals and bioactive compounds like phenolic compounds, which are linked to various health benefits, including the prevention of diseases such as diabetes, cancer and cardiovascular conditions (5). Identified as a crop that can enhance food security and improve nutritional status, particularly in regions vulnerable to climate challenges, cowpea plays a crucial role in promoting human health and well-being (6).

However, the economic output of vegetable cowpea often fails to reach its full potential due to infestation by insect pests. The extent of infestation, severity of incidents and resulting damage in cowpea fields vary between regions and depend on the plant growth stage. Among these insect pests, *Maruca vitrata* poses a significant threat to vegetable cowpea. A researcher first documented this pest on beans in Indonesia (7). In regions where legumes are cultivated extensively, the polyphagous larva of this moth can cause damage to almost all varieties of legumes under various environmental conditions (8). Given that its damage typically surpasses the economic threshold level, researchers worldwide have shown considerable interest in studying this pest (9).

To date, the predominant strategy for controlling this insect pest in vegetable cowpea remains the use of synthetic pesticides. However, efforts have been made to explore environmentally friendly alternates, including cultural practices, biological control, utilisation of biopesticides and resistant varieties. These initiatives are in response to the potential hazards associated with chemical pesticides to humans, animals and the environment as well as the emergence of insecticide resistance among insect pests. Leveraging varietal resistance stands out as the most cost-effective and environmentally friendly approach to mitigating the impact of insect pests on the cultivation and post-harvest technology of edible cowpeas (10, 11).

Indeed, plant defence mechanisms, including biochemical and biophysical traits, play a crucial role in pest control by influencing the physiology and feeding behaviour of pests or by acting as deterrents or anti-feedants. Researchers have revealed that biophysical traits such as trichome length and density on cowpea pods are associated with resistance against *M. vitrata* (12-14).

While significant progress has been made in understanding pigeonpea and other closely related legume species, further research is necessary to explore the trichomes on the pods of different genotypes of vegetable cowpea and their correlation with insect resistance. Along with their biophysical traits, vegetable cowpea pods also harbor phenols, total sugars and proteins that influence the feeding behaviour and preferences of *M. vitrata* (15-17) in cowpea. Thus, the present studies aimed to investigate the relationship between the expressions of resistance to *M. vitrata* and various biophysical characters (trichome length and density) as well as biochemical parameters, viz., total sugars, protein content and phenol content, in the pods of selected vegetable cowpea genotypes.

Materials and Methods

Experimental materials

The plant materials used in this study were sourced from the NBPGR Regional Station, Hyderabad, ICAR-IIHR, Bengaluru and KAU, Vellayani. Three resistant vegetable cowpea genotypes (IC 202796, IC 206240, IC 214751) and 7 moderately resistant genotypes (EC 738122, IC 259063,

EC 367692, EC 390225, EC 367694, EC 724471, IC257449), along with a resistant check (Arka Suman) and a susceptible check (Bhagyalakshmi), were subjected to resistance testing against *Maruca vitrata* across 2 seasons (summer, 2021 & kharif, 2021) in field screenings (unpublished). These materials were employed to validate their resistance levels against *M. vitrata* and investigate the underlying biophysical and biochemical mechanisms of resistance in this study.

Experimental location and design

The plant materials were screened to re-validate their response to *M. vitrata*. Plants were cultivated using the pot-culture technique. Mud pots, each measuring 12 inches in diameter and 15 inches in length and filled with approximately 2.2 kg of soil, were employed. The soil mixture comprised red soil, sand and farmyard manure in a ratio of 3:1:1. The experiment took place in the screen house of the Department of Entomology, College of Agriculture and OUAT, Bhubaneswar (20°15'55.8" N, 85°48'41.8" E and Alt 45 m above sea level). Ten genotypes served as treatments in the experiments, which followed a completely randomised design (CRD) with 12 treatments, including a resistant and a susceptible check, across 3 replications. Each replication involved one container with one plant.

Cultural practices

To prepare the soil mixture for planting, it was moistened and left for 24 h. Seeds were then planted in each pot at a depth of 3 cm, resulting in the growth of seedlings. At 15 days after planting (DAP), when the seedlings had produced at least one fully developed true leaf, they were thinned to one per pot. Additionally, 1 g of NPK (19:19:19) fertilizer was applied per pot at 7 DAP. Hand weeding was performed to remove any weeds, and irrigation was carried out daily until the conclusion of the trial.

Trichome length and density

Trichome length was measured using 10 evenly developed pods per replication. Pods from the test genotype were divided into 9 mm² (3 mm × 3 mm) segments. The epidermal layer of the pods was then examined under a scanning electron microscope to determine trichome length and quantity per unit area.

Estimation of biochemical components of pods

The pods at the immature stage of each genotype were freeze-dried in a lyophilizer and were subsequently ground into a powder to measure the levels of total soluble sugars, proteins and phenols. Quantities of phenols, proteins and total sugars were calculated for each accession in three replicates. Total sugars were estimated by Anthrone method (18), while the protein content was determined following the Lowry's method (19). Phenols in the pods were measured by Folin-Ciocalteu reagent method (20).

Statistical analysis

To assess any significant differences among the genotypes, the data underwent analysis of variance (ANOVA) using the statistical package GRAPES 1.1.0 (21). Treatment means were compared using the least significant differ-

ence test, with a significance level set at $p < 0.05$. Pearson correlation analysis was conducted to establish associations, with data analyzed using the 'sjPlot' package (22) to generate correlation matrix tables and the 'corrplot' package (23) to generate correlograms in R software, version 4.3.2. This analysis focused on examining the relationships between biophysical and biochemical components and the % of seed damage and developmental attributes of *Maruca* larvae reared on selected vegetable cowpea genotypes.

Results

Length and density of trichomes on cowpea pods

The study examined 2 biophysical parameters, trichome length and trichome density on the pod, to determine whether these morphological characteristics are associated with resistance or directly influence the extent of damage caused by *Maruca* infestation.

Trichome length ranged from 51.53 μm to 84.76 μm across the genotypes. Among them, genotype IC 214751 exhibited the longest trichomes at 84.76 μm , followed closely by IC 206240 (82.82 μm) and IC 202796 (81.51 μm) (Table 1 and Fig. 1). In contrast, Bhagyalakshmi had the shortest trichomes, measuring 51.53 μm , with IC 257449 following at 59.86 μm .

Table 1. Biophysical basis of resistance against *Maruca vitrata* in selected vegetable cowpea genotypes.

Sl. No.	Genotype	Trichome length (μm)	Trichome density (9 mm^2)
1	IC 202796	81.51 \pm 1.96 ^{ab}	124.33 \pm 2.6 ^a
2	EC 738122	66.71 \pm 1.49 ^e	92.33 \pm 0.88 ^d
3	IC 259063	73.08 \pm 2.18 ^d	88.33 \pm 0.88 ^{de}
4	IC 206240	82.82 \pm 1.63 ^a	120.33 \pm 1.45 ^{ab}
5	EC 367692	73.97 \pm 0.75 ^d	77.33 \pm 1.45 ^f
6	EC 390225	75.12 \pm 1.63 ^d	57.67 \pm 1.86 ^e
7	IC 214751	84.76 \pm 1.06 ^a	123.33 \pm 2.6 ^a
8	EC 367694	75.95 \pm 2.09 ^{cd}	98 \pm 1.73 ^c
9	EC 724471	77.7 \pm 0.33 ^{bcd}	84.67 \pm 1.45 ^e
10	IC 257449	59.86 \pm 1.78 ^f	73 \pm 2.08 ^f
11	Arka Suman (RC)	80.13 \pm 1.37 ^{abc}	118 \pm 2.08 ^b
12	Bhagyalakshmi (SC)	51.53 \pm 1.06 ^e	16.33 \pm 0.88 ^h
	SE (m) \pm	1.561	1.632
	CD (5%)	4.578	4.786

Data are presented as the average of two replicates \pm standard error of mean (SEM); Numbers followed by the same letter are not significantly different ($p = 0.05$) by DMRT.

Trichome density on the pod was measured, revealing a significant variation ($p < 0.05$) among the selected vegetable cowpea genotypes. The highest density (124.33 / 9 mm^2) was observed on IC 202796, which was comparable to IC 214751 (123.33 / 9 mm^2) and IC 206240 (120.33 / 9 mm^2). Conversely, the lowest density (16.33 / 9 mm^2) was found on Bhagyalakshmi, followed by EC 390225 (57.67 / 9 mm^2), IC 257449 (73.00 / 9 mm^2) and EC 367692 (77.33 / 9 mm^2) (Table 1 and Fig. 1).

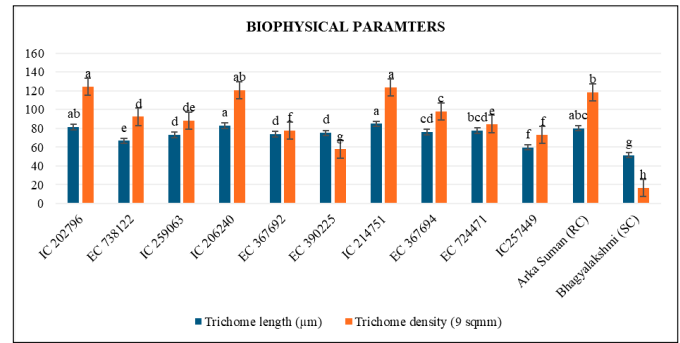


Fig. 1. Trichome length and trichome density quantified on pods of selected vegetable cowpea genotypes.

Estimation of biochemical constituents in pods of selected vegetable cowpea genotypes

The study examined the relationship between various biochemical components of the pods, including total sugar, protein and phenol content, as these factors are known to influence the nutritional value of the host plant for insects. Understanding these relationships is crucial for determining the plant's vulnerability to insect attack. The study's findings shed light on how the presence of these components affects pest proliferation. Results revealed that pods of resistant genotypes exhibited higher levels of phenols and lower levels of total sugars and proteins. Table 2 summarizes the findings of the study estimating the total phenol content, protein and total sugars, depicted in Fig. 2.

Table 2. Biochemical constituents of selected vegetable cowpea genotypes.

Sl. No	Genotype	Total sugars (%)	Protein content (mg/g)	Phenol content (mg/g)
1	IC 202796	2.19 \pm 0.08 ^f	14.19 \pm 0.64 ^e	2.69 \pm 0.09 ^{ab}
2	EC 738122	3.87 \pm 0.17 ^{de}	19.78 \pm 0.9 ^{bcd}	0.87 \pm 0.01 ^{cd}
3	IC 259063	4.42 \pm 0.09 ^c	20.7 \pm 1.02 ^{bc}	0.79 \pm 0.04 ^d
4	IC 206240	2.24 \pm 0.07 ^f	14.83 \pm 0.84 ^e	2.87 \pm 0.08 ^a
5	EC 367692	4.86 \pm 0.11 ^b	20.87 \pm 1.23 ^b	0.91 \pm 0.04 ^{cd}
6	EC 390225	3.8 \pm 0.06 ^e	20.11 \pm 1.01 ^{bc}	0.83 \pm 0.02 ^{cd}
7	IC 214751	2.44 \pm 0.06 ^f	14.95 \pm 0.55 ^e	2.71 \pm 0.12 ^{ab}
8	EC 367694	4.21 \pm 0.15 ^{cd}	18.32 \pm 0.92 ^d	1.02 \pm 0.02 ^c
9	EC 724471	3.85 \pm 0.11 ^{de}	18.97 \pm 0.43 ^{cd}	0.92 \pm 0.02 ^{cd}
10	IC 257449	4.18 \pm 0.08 ^{cd}	20.23 \pm 0.47 ^{bc}	0.81 \pm 0.04 ^d
11	Arka Suman (RC)	2.27 \pm 0.11 ^f	13.39 \pm 0.16 ^e	2.55 \pm 0.06 ^b
12	Bhagyalakshmi (SC)	8.72 \pm 0.16 ^a	22.76 \pm 0.39 ^a	0.59 \pm 0.04 ^e
	SE (m) \pm	0.114	0.594	0.061
	CD (5%)	0.334	1.743	0.178

Data are presented as the average of two replicates \pm standard error of mean (SEM); Different letters in the same line means statistical difference ($p < 0.05$) by Duncan test.

Total sugars

The highest total sugar content was observed in Bhagyalakshmi (8.72 %), while the lowest total sugars were recorded in IC 202796 (2.19 %), which was comparable to IC 206240 (2.24 %), IC 214751 (2.44 %) and Arka Suman (2.27 %).

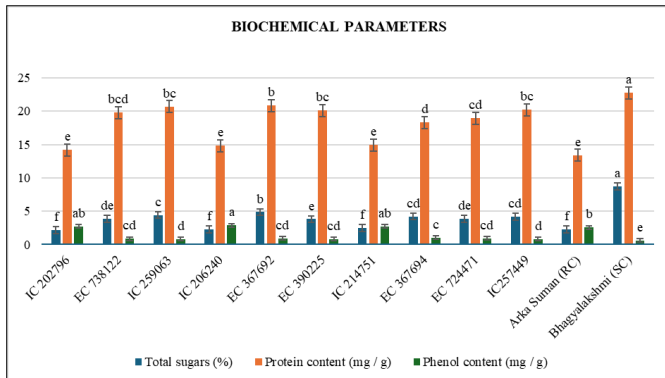


Fig. 2. Total sugars, protein content and phenol content quantified in pods of selected vegetable cowpea genotypes.

Protein content

The genotype Bhagyalakshmi exhibited the highest protein content (22.76 mg/g), while the lowest protein content was observed in IC 202796 (14.19 mg/g), which was similar to IC 206240 (14.83 mg/g) and IC 214751 (14.95 mg/g).

Notably, in this study, resistant genotypes displayed lower protein levels compared to susceptible genotypes, suggesting that they may provide more favourable conditions for the growth and development of *Maruca* larvae.

Phenol content

The genotype IC 206240 exhibited the highest phenol content (2.87 mg/g), which was comparable to IC 202796 (2.69 mg/g) and IC 214751 (2.71 mg/g), while Bhagyalakshmi had the lowest phenol content (0.59 mg/g).

Correlation between bio-physical, bio-chemical characters and seed damage %

The correlation analysis between the developmental attributes of *M. vitrata* larvae reared on pods of the studied vegetable cowpea genotypes and biophysical and biochemical parameters, along with seed-damage %, revealed significant negative correlations (at the 1 % level of significance) with trichome length, trichome density and phenol content. Conversely, there was a significant positive correlation (at the 1 % level of significance) with protein content and total sugar content. (Table 3. and Fig 3.)

Table 3. Correlation between bio-physical, bio-chemical characters and seed damage % of selected vegetable cowpea genotypes.

	Trichome length	Trichome density	Total sugars	Protein content	Phenol content	Seed damage %
Trichome length	1.000					
Trichome density	0.848***	1.000				
Total sugars	-0.856***	-0.938***	1.000			
Protein content	-0.785**	-0.820**	0.839***	1.000		
Phenol content	0.719**	0.718**	-0.733**	-0.950***	1.000	
Seed damage %	-0.690*	-0.838***	0.735**	0.719**	-0.754**	1.000

***: Correlation is significant at the 0.001 level (2-tailed); **: Correlation is significant at the 0.01 level (2-tailed); *: Correlation is significant at the 0.05 level (2-tailed).

Discussion

Length and density of trichomes on cowpea pods

Trichomes, found on both leaves and pods of plants, play a crucial role in plant defense mechanisms against insect pests such as *Maruca vitrata*. Previous studies have high-

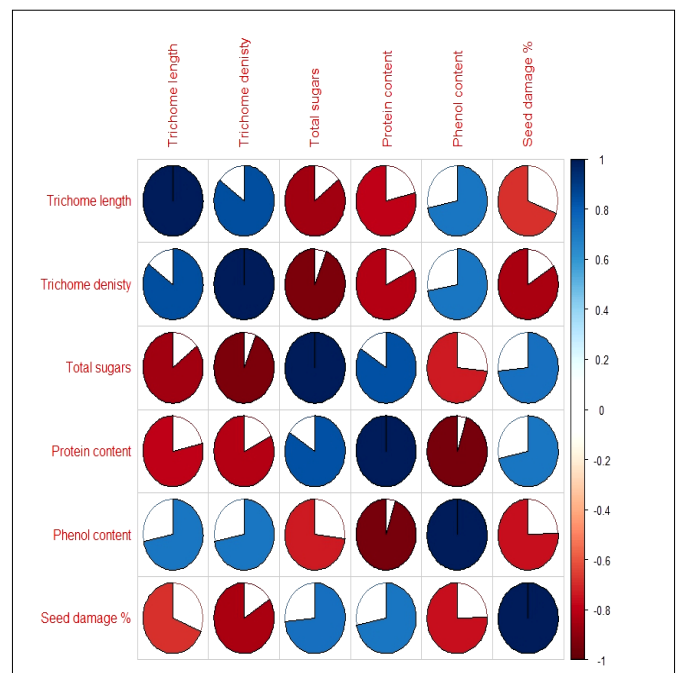


Fig. 3. Correlogram showing association of biophysical and biochemical traits with seed damage % in selected vegetable cowpea genotypes.

lighted the association between trichome density and length with resistance to this pest. For instance, a study demonstrated a positive correlation between trichome length and density on leaves and pods and resistance to *M. vitrata* in pigeonpea genotypes (12). Similarly, a research noted significant variations in trichome length on leaves and pods among pigeonpea varieties, with longer trichomes associated with resistance (13). A study also reported that trichome density positively influenced pest resistance in cowpea genotypes (24). The present findings further underscore the importance of trichome distribution pattern and length on pods in determining the pest's impact in vegetable cowpea.

A research observed that the susceptible cultivar had the lowest number of trichomes, while the resistant cultivar exhibited the highest number of trichomes, thereby conferring resistance against *M. vitrata* in cowpea (14). Similarly, researchers found in pigeonpea that trichome

density was a key factor in imparting resistance against the legume pod borer (12). The findings also align with the results of the present study (13). Moreover, the current results are consistent with the study (25), which demonstrated that higher pod trichome density correlated with reduced damage caused by pod borers in chickpea.

Total sugars

Sugar is a primary nutrient essential for the development of insects, supporting their survival and growth. A plant's susceptibility to insect infestation often correlates with its total sugar content. Susceptible genotypes with higher levels of carbohydrates in their tissues tend to facilitate better insect growth, unlike resistant genotypes where lower sugar levels may not support normal insect development and reproduction (26). Sugar is one of the main ingredients in insects' diets (27). Therefore, in the present study, susceptible genotypes exhibited elevated total sugar levels, potentially contributing to their vulnerability to pod borer damage on the pod wall.

Protein content

Protein constitutes a major dietary component for insects, playing a crucial role in their development, survival and reproduction. The amount of protein consumed significantly influences insect growth and development, with insects thriving on a high-protein diet (27).

In the present study, resistant genotypes exhibited lower protein content compared to susceptible genotypes. This characteristic makes resistant genotypes more favourable hosts for the growth and development of *Maruca* larvae.

Phenol content

Studies have demonstrated the pivotal role of phenolic compounds in determining the resistance or susceptibility of a host to insect pests. Phenols act as deterrents, inhibiting insect feeding and growth. Resistant varieties typically exhibit higher levels of phenols compared to susceptible ones. Due to their direct toxicity, phenolics can deter insect pests from establishing themselves (28) and negatively affect the oviposition behavior of adult insects (29).

The current findings indicate that resistant genotypes exhibit higher levels of phenols compared to susceptible ones. The elevated phenol content in resistant genotypes likely contributes to plant defense mechanisms against insect pests by acting as antifeedants and repellents (30) and anti-nutritional factors (31). Similarly, it was observed that increased phenolic compound levels in the cowpea genotype TVu 946, enhancing its resistance to *Maruca* infestation (32). These results are in line with those who reported heightened phenol concentrations in the flowers and pods of the cultivar ICPL 98003, attributing its resistance to *M. vitrata* to this factor (12). Additionally, it was observed that resistant genotypes contained higher phenol content compared to susceptible ones (33).

Correlation between bio-physical, bio-chemical characters and seed damage %

The strong negative correlation observed between the numbers of trichomes on the pods suggests that this factor contributes significantly to the resistance of vegetable cowpea against the legume pod borer. Numerous researchers have highlighted the role of trichomes in plant pest resistance (34-36). For instance, pod wall trichomes have been implicated in the resistance of wild cowpea (*Vigna vexillata*) to pests such as *Clavigralla tomentosicollis* and *Maruca testulalis* (37, 38).

According to correlation analysis, genotypes exhibiting higher trichome densities and greater phenol contents displayed reduced damage compared to other genotypes. These findings concerning the correlation with % seed damage are consistent with the research on trichome density, which revealed a highly significant inverse relationship between trichome density and pod damage % (12). Similarly, an inverse correlation between trichome abundance and pod damage was observed in the studied genotypes (39). It was also noted a positive correlation between increased pubescence and damage by *M. vitrata*, underscoring the importance of trichome characteristics in influencing pest interactions (40).

In a study, researchers uncovered a significant inverse relationship between the density of trichomes on leaves and pods and both larval weight increase and the percentage of pod damage (13). They also noted a similar negative correlations between the length of trichomes on leaves and pods and both larval weight gain and the % of pod damage. Another study reported similar findings in cowpea (14).

The current study revealed a negative correlation between legume pod borer infestation and phenol levels in pods. However, both total sugar and protein content showed a significant positive correlation with the % seed damage. These findings align with another study who reported a significant positive association between pod damage and the sugar and protein content of pods (12). Another study observed that an increase in total sugar content enhances pest incidence and made similar observations (2). Phenol concentration in pods was inversely related to pod infestation by *Maruca*, whereas total sugars and proteins showed significant positive correlations in mung bean (15), urd bean (16) and in cowpea (18).

Based on the findings of the present investigation, 3 genotypes viz., IC 206240, IC 214751 and IC 202796 were found to be resistant. Employing an integrated pest control strategy, these genotypes can be considered suitable for cultivation in areas where the legume pod borer is endemic. These genotypes, exhibiting a relatively high degree of resistance, can be utilised in breeding programmes for vegetable cowpea to develop resistance to the spotted pod borer.

Conclusion

The genotypes IC 206240, IC 214751 and IC 202796 were identified as sources of resistance against *Maruca vitrata*, indicating their potential utility in breeding programs and integrated pest management strategies. This conclusion is drawn from the comprehensive investigation, which highlights the significance of both biophysical and biochemical traits in conferring resistance. While resistant varieties offer a safe, affordable and sustainable alternative for pest control, their efficacy can be enhanced by combining them with other pest management approaches. Further research is warranted to transfer resistant genes to susceptible but high-yielding varieties through hybridization and subsequent testing of the resulting F1 generation. Addi-

tionally, there is a need to identify specific plant volatiles released by vegetable cowpea germplasm to understand legume pod borer preferences.

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Authors' contributions

All authors, including MS, participated in the conception and design of the analysis. MS was responsible for data collection, experiment execution, data analysis and article writing. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors assert that they have no conflict of interest in this research.

Ethical issues: None.

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