RESEARCH ARTICLE Agricultural Science Digest, Volume 45 Issue 4: 665-671 (August 2025)

Laboratory Methodology for Screening of Cruciferous Host for Egg Laying Preference of Diamondback Moth, *Plutella xylostella* (L.)

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10.18805/ag.D-6167

ABSTRACT

Background: Diamondback moth (DBM), *Plutella xylostella* (L.), significantly damages Cole crops worldwide, causing major yield losses and management costs. Efficient control measures, including host plant resistance are imperative. While molecular methods offer insights into resistance mechanisms, conventional screening methods remain advantageous. Here, we present a novel laboratory-based oviposition preference screening method for DBM, significantly shortening screening time in resistance breeding.

Methods: We studied DBM's oviposition preferences among cruciferous hosts and plant tissues. Extract was prepared from leaves and heads of dark green and light green cabbage varieties, as well as cauliflower. Aluminum egg-laying sheets were treated with these extracts and introduced into mating boxes with insects. Egg-laying was monitored over seven days, with the number of eggs recorded daily.

Result: DBM adults preferred light green cabbage for egg laying (1238±10.54 eggs), with no significant difference between leaf and head tissues. Dark green cabbage was the second choice (514.5±15.78 eggs), while cauliflower was the least preferred (217±37.2 eggs). Interestingly, DBM exhibited varying preferences between leaf and head tissues, with cauliflower heads being favored over leaves. Additionally, we observed distinct day-wise egg laying trends, providing insights into DBM's oviposition behavior over time. This study demonstrates the efficiency of lab-based screening in identifying resistant crop varieties, aiding sustainable pest management and crop production and highlights the importance of considering host plant selection and tissue specificity in screening of genotype for resistance breeding programs.

Key words: Diamondback moth (DBM), Host plant resistance, Host preference, Oviposition, Resistance breeding, Screening, Sustainable pest management.

INTRODUCTION

The diamondback moth, *Plutella xylostella* (L.), presents a formidable challenge to worldwide cole crop production, particularly affecting cruciferous vegetables such as cabbage, broccoli and cauliflower (Furlong *et al*., 2013). Its voracious larvae can consume 62-78% of host leaves, resulting in substantial yield losses (Gangurde and Wankhede, 2009). With documented resistance to various insecticide classes and limited efficacy of biological agents like entomopathogenic fungi, the annual management costs for this pest have soared to an estimated USD 4~5 billion (Wang *et al*., 2012; Soth *et al*., 2022). Given these challenges, the urgency of developing alternative control measures, such as host plant resistance, is evident (Sharma *et al*., 2017). Cole crops, crucial for global food security and economic stability, necessitate effective management of diamondback moth infestations to sustain agricultural productivity (Smith and Brown, 2020). Host plant preference significantly influences the dynamics of *P. xylostella* populations within Cole crop systems, emphasizing the need for understanding the mechanisms driving host selection to devise targeted management approaches, especially in resistance breeding programs (Dias *et al*., 2018).

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How to cite this article: Zirwal, Y.N., Dongarjal, S.B., Polineni, V., Vishal, V., Polack, G.W. and Calderon-Urrea, A. (2025). Laboratory Methodology for Screening of Cruciferous Host for Egg Laying Preference of Diamondback Moth, *Plutella xylostella* (L). *Agricultural Science Digest*. **45(4):** 665-671. doi: 10.18805/ag.D-6167.

Submitted: 26-08-2024 **Accepted:** 11-11-2024 **Online:** 26-12-2024

Recent advancements in molecular-based methods offer promising avenues for pest management research, yet conventional screening methodologies remain advantageous for screening host preferences of *P. xylostella*. Oviposition preference serves as an early indicator of a host plant's resistance to pests, with susceptible or less resistant plants often being preferred by pests (Kogan and Ortman, 1978).

Assessing the oviposition preference of diamondback moth (DBM) in resistance variety screening is crucial for identifying crop varieties less favored for egg-laying, thereby indicating host plant resistance. This trait aids in selecting resistant cultivars, reducing pesticide dependency and promoting sustainable pest management. Typically, DBM's oviposition preference has been evaluated on various hosts under conventional *in vivo* conditions (Navnatha and Srivastava, 2006; Henniges-Janssen *et al*., 2011; Reddy *et al*., 2004).

While molecular approaches provide insights into genetic resistance mechanisms, they often demand extensive resources and expertise, limiting their scalability (Zhao *et al*., 2019; Narvaez *et al*., 2018). But contemplating the conventional data in addition to this are still sought by most of researchers. In contrast, with conventional green house and field screening laboratory-based oviposition preference screening enables cost-effective and time saving evaluation of multiple host genotypes or recombinant inbred lines (RILs), complementing molecular analyses and expediting the identification of resistant candidates (Sharma *et al*., 2017; Smith and Brown, 2020) especially at early stages of breeding program. By replacing time-consuming conventional screening with short-scale lab screening, breeders can efficiently triage genotypes for molecular characterization, facilitating the development of resistant crop varieties suited to combat diamondback moth infestations. Laboratory-based methodologies offer a controlled setting for investigating the egg laying preferences of *P. xylostella* across a range of cruciferous hosts, enabling the efficient screening of host genotypes or recombinant inbred lines (RILs) to identify promising candidates for resistance breeding (Sharma *et al*., 2017).

By elucidating the factors governing host preference in *P. xylostella* within Cole crop systems, this research aims to provide crucial insights into pest behavior, ultimately facilitating the development of more resilient and sustainable Cole crop varieties. The researcher can carry out experiments in both choice and non-choice manner and can also generate biochemical/molecular data to support the finding. This experiment is carried out in a non-choice manner.

MATERIALS AND METHODS

All experiments were carried out at the Telluris Biotech R and D facility in Genome Valley, Hyderabad, during the winter seasons of 2022-2023 and 2023-2024.

Insect culture and mass rearing of DBM

The nucleus culture of *P. xylostella,* the larvae and egg masses, were collected from cabbage fields at Hyderabad. The initial culture of larvae was reared on cabbage leaves till their pupation in laboratory conditions of 24±1°C, RH 50±5 and 16:8 L:D. The adults that emerged were separated based on sex, male and females were released in matting jars for oviposition. A 10% honey solution fortified with vitamin E (Evion™) was provided as diet for adults. Newly hatched F1 generation larvae were reared as described above until pupation. F1 generation adults that emerged from the pupae were used in the experiment.

Plant material and extract preparation

Two varieties of cabbage: dark green (variety Golden acre (Urja seeds), light green (Seedlings collected from a local field, source not confirmed) and cauliflower (variety Snow crown (Takii seeds) were locally available and hence selected for the study. Samples for preparation of extract were obtained at two different stages of plant growth and sample drawn from leaf as well as head respectively. At active growth stage (after seedling establishment), leaf samples were drawn and at reproductive stage, head samples were drawn for extract preparation. The extract was prepared by blending 65 gm fresh leaf/head and 500 ml distilled water. The prepared extract was added to a 750 ml conical flask (Borosil) and autoclaved for 21 min. The cooled extract was sieved through a muslin cloth and placed in a tray for sheet treatment.

Preparation of Egg sheet and mating boxes

Egg laying sheets (dimension of 11.5×30 cm) were made of aluminum (11 µm thickness) (Fig 1B). Aluminum sheets cut in designated size are gently immersed in extract and kept for draining in upright manner loosely folding it for 30 hrs. The treated sheets with extract can be stored at 4°C for future use. Egg sheets were ragged with a sterile comb to make grooves for better egg laying. Mating boxes were prepared using plastic jars (30 cm height and 15 cm diameter). 50 freshly emerged adults were placed in a mating chamber in 1:1 ratio of males and females closing it with a double layered black cloth. Egg laying sheets were inserted and hanged in the middle of the container (Fig 1 A). Oviposition strips were collected and changed daily and the number of eggs were counted and recorded after every 24 hrs. up to 7 days. 10% honey solution fortified with vitamin E (Evion™) was provided as diet for adults.

For each treatment of no choice experiments (egg laying sheet emersed in T1: Light green cabbage leaf extract, T2: Light green cabbage head extract, T3: Dark green cabbage leaf extract, T4: Dark green cabbage head extract, T5: Cauliflower leaf extract, T6: Cauliflower head extract) 3 replications were maintained and randomized completely. The number of eggs were counted and recorded on every sheet after every 24 hrs. up to 7 days.

Data analysis

Number of eggs laid on the sheet (Fig 1C) were counted daily. Data tested for normality and homogeneity of variance, one way ANOVA conducted for testing hypothesis and mean were separated using post hoc DMRT test. All these tests were performed on IBM SPSS v.29 and significance level was set at 0.05.

RESULTS AND DISCUSSION

Oviposition preference

Egg laying sheets immersed in light green cabbage extract (untraced source) both leaf and head tissue were most preferred by DBM adults. The mean number of eggs laid on these sheets were 1241±12.12 and 1235±20 on the leaf and head extract treated sheet, respectively. Statistically, these are at par with each other (p= 0.720). The combined Mean of eggs laid on leaf and head extract treated sheet was 1238±10.54 and is highest among all tested host dark green cabbage (variety Golden acre (Urja seeds) 514.5 ± 5.78 and cauliflower 217±37.2 has a significant difference among all (F=474.18, Df=15 and p=<0.001) (Table 1 and 2). In the dark green cabbage case, the number of eggs laid on leaf extract treated sheet (549±5.03) was significantly higher than head extract treated sheet (480±5.5) (p=<0.001). But in the case of cauliflower altered trend was observed, where the number of eggs laid on leaf extract treated sheet (135±8.71) was significantly lower than head extract treated sheet (299±10.96) (p=<0.001).

Day wise egg laying trend

Egg sheets immersed in light green cabbage extract and cauliflower extracts received egg laying from day 1 and continued up until day 5. But egg laying was higher in the former than the latter. The daily average number of eggs laid on sheets treated with light green cabbage leaf extract and its head tissue extract was 248±20.34 and 247±18.35, respectively. However, on sheets treated with cauliflower leaf extract, the daily average number of eggs laid was 27 ±6.76 for up to 5 days. On sheets treated with cauliflower head tissue extract, the daily average egg laying was 74± 10.33, but egg laying categorically ceased after 4 days. On dark green cabbage leaf extract treated sheets, egg laying started on 3rd day and ceased on the 7th day with an average of eggs laid per day being 109±23.68. On egg laying sheets treated with head extract of dark green cabbage egg laying (96±14.81) was observed from day 1 to 5 (Fig 2).

The main purpose of this study was to demonstrate short, quick and resource saving methodology for screening of genotype for DBM oviposition preference. This can be

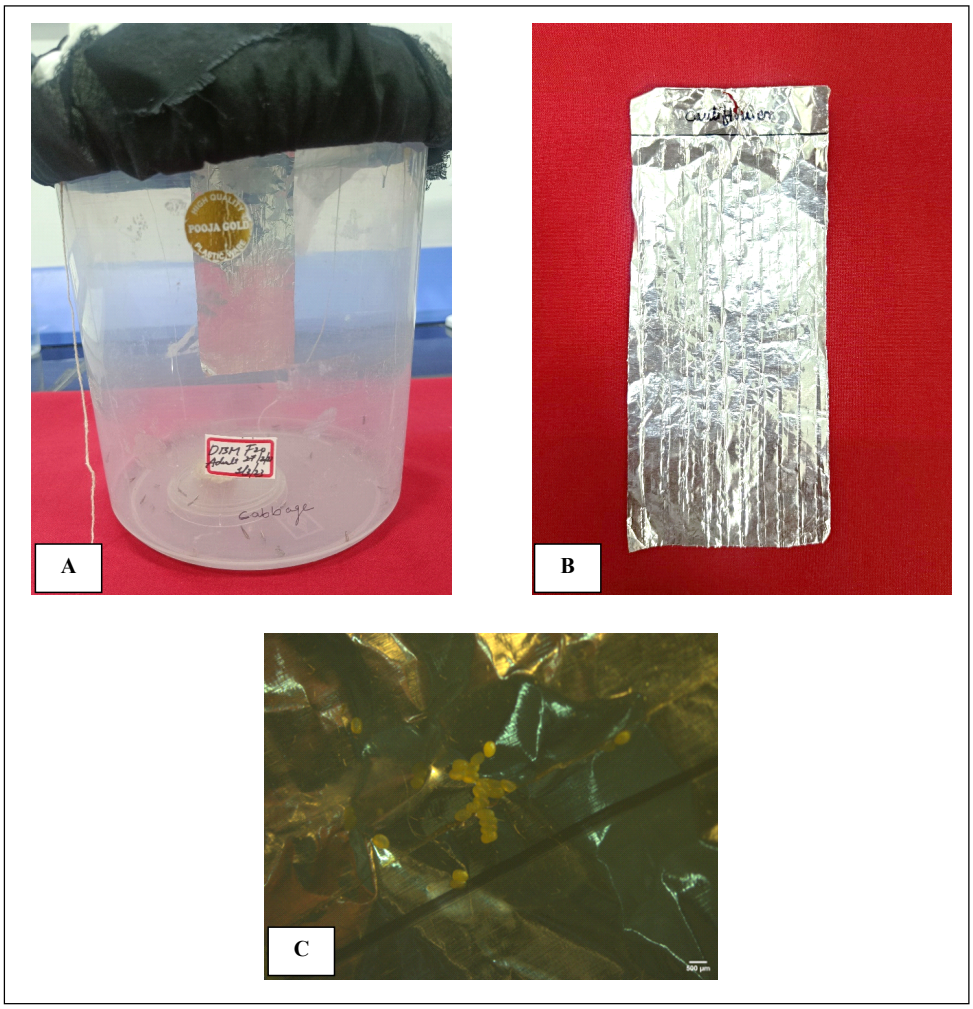


Fig 1: A) Mating cage having DBM adults hanged with egg laying sheet in the middle. B) Egg laying sheet-ragged with comb and emersed in the extract. C) Egg laid by DBM female moth (scale 500 µm).

extensively employed in the early-stage resistance breeding program where a large number of genotype/RIL population need to be evaluated. The data generated using this quick methodology will help in validating molecular and biochemical marker assisted outcomes. We selected the oviposition preference variable of DBM, because it is a critical factor influencing DBM population dynamics and the extent of damage, they cause to cruciferous crops during the crop cycle and this is highly influenced by host plant chemical cues (Vet and Dicke, 1992). Adult oviposition preference and larval performance of DBM were positively co-related (Zhang *et al*., 2012). This might be because DBM can rank hosts and select the most suitable host for their offspring, which supports the optimal oviposition

Table 1: Stage and tissue specific egg laying preference of DBM adults.

Treatment details	No. of egg laid (Mean ± SE)/ Sheet	95% Confidence interval for mean	
		Lower bound	Upper bound
Light green cabbage leaf	1241±12.12 e	1188.83	1293.16
Light green cabbage head	1235±20 e	1148.94	1321.05
Dark green cabbage leaf	549±5.03 d	527.34	570.65
Dark green cabbage head	480±5.5 c	456.3	503.69
Cauliflower leaf	135±8.71 a	97.49	172.5
Cauliflower head	299±10.96 b	251.8	346.19
F value	1680.26		
Df	5, 12		
P value (0.05)	<0.001		

Mean ± SE (Std, Error) (3 replications), Same letter in besides mean value indicate no significant difference in mean as determined by post hoc DMRT test (p=0.05).

Table 2: Host specific oviposition by DBM.

Treatment details	No. of egg laid (Mean ± SE)/ Sheet	95% Confidence interval for mean	
		Lower bound	Upper bound
T1: Light green cabbage	1238±10.54 c	1210.89	1265.1
T2: Dark green cabbage	514.5±15.78 b	473.92	555.07
T3: Cauliflower	217±37.2 a	121.36	312.63
F value	474.18		
Df	2, 15		
P value (0.05)	<0.001		

Mean ± SE (Std, Error) (3 replications each having 25 females), Same letter in besides mean value indicate no significant difference in mean as determined by post hoc DMRT test (p=0.05).

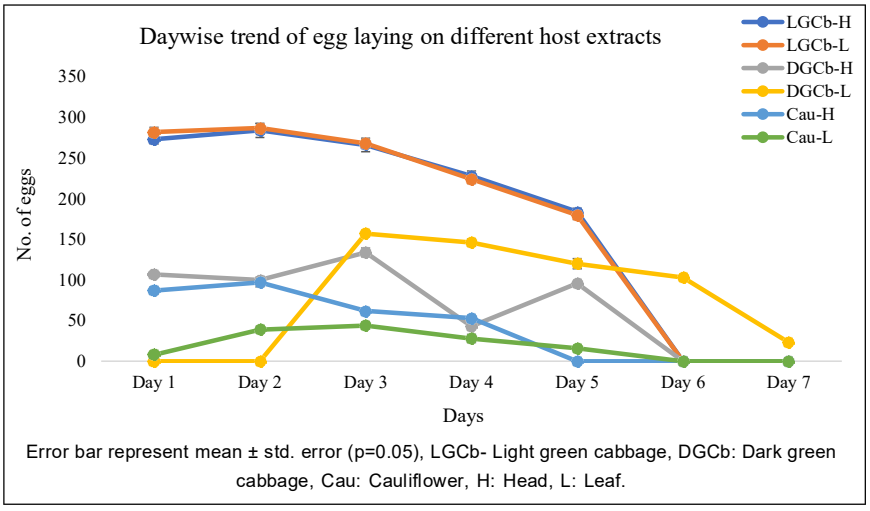


Fig 2: Day wise egg laying trend of DBM adults (Average of 3 replications each having 25 females).

theory (Jaenike, 1978). Hence, this study investigated the oviposition preferences of DBM indicatively on different cabbage varieties and cauliflower, with a particular focus on the influence of cues of leaf and head tissue for egg laying preference. Also, in early-stage breeding programs it can save resources and time compared to conventional time-consuming screening.

Host preference

Herbivorous insects, when given options, may select a host plant for feeding or egg-laying, or they may not choose at all. Their specialization in host plant selection determines which plants are suitable. Within acceptable plants, factors like nutrition, egg-laying sites, competition, or protection from predators may differ. Thus, an insect's capability to evaluate and pick the optimal plant benefits it (Whitham, 1978). Insect choice of host plants is also influenced by the future food potential of the plant (Cunningham, 2011). Theoretically, insect oviposition preference should align with food preference, as the emerging larvae rely on the plant they hatch on (Cunningham, 2011). Depending on their mobility, larvae may later access other plant species. To maximize offspring success, a female should select oviposition sites that double as good food sources (Cunningham, 2011), a concept known as the mother-knows-best hypothesis (Valladares and Lawton, 1991; Vencl *et al*., 2013), linked to the preference-performance hypothesis (Balagawi *et al*., 2013). Thompson and Pellmyr (1991) reviewed lepidopteran oviposition preference, finding that females show varied preferences toward plants, typically favoring those that offer the best larval food sources. For instance, adults of the fern moth (*Herpetogramma theseusalis*) (Lepidoptera: Crambidae) prefer marsh fern (*Thelypteris palustris*) over sensitive fern (*Onoclea sensibilis*). Since *T. palustris* is more nutritious for larvae, this preference suggests an optimal choice for offspring (Bar-Yam and Morse, 2011).

Our results highlight intriguing patterns of oviposition preference and provide insights into the factors influencing DBM's choices. This study found that DBM adults exhibited a strong preference for egg laying on leaf and head tissue of light green cabbage. Preference was evident in the remarkably high mean number of eggs laid on these sheets, which were statistically comparable. Interestingly, when examining the overall oviposition preferences across different host plants, light green cabbage emerged as the more preferred host, with a significantly higher mean number of eggs laid compared to dark green cabbage and cauliflower. This discrepancy in preference could be attributed to various factors, including plant chemistry, morphology and volatile organic compounds (Thompson and Pellmyr 1991; Henniges-Janssen *et al*., 2011; Liu *et al*., 2012). These findings are also in line with previous studies indicating that DBM often exhibits a preference for cabbage over cauliflower and other cruciferous hosts (Reddy *et al*., 2004, Jones *et al*., 2018; Smith and Doe, 2020).

Stage/Tissue preference

In our study, we investigated the oviposition preference of the DBM's (*P. xylostella*) two different cruciferous hosts, focusing on the leaf and head stages of cabbage and cauliflower. Notably, we observed distinct differences in oviposition behavior between these stages and plant types. Firstly, our results indicate that both leaf and head tissues of both cultivars of cabbage were highly preferred by DBM adults for egg laying, as evidenced by the significant number of eggs deposited on sheets immersed in light green cabbage extract. This preference was consistent across both leaf and head stages, with no statistically significant difference (but numerically higher in leaves than head tissues) observed between the mean number of eggs laid on leaf and head extract-treated sheets. In a previous study, preference of DBM for egg laying was observed more in true leaf stages of kale Chinese plant (Zhu *et al*., 2021) validating our finding regarding plant stage specific difference in oviposition.

Contrastingly, cauliflower exhibited a divergent pattern in oviposition preference between its leaf and head stages. While both stages received egg laying, the number of eggs deposited on leaf extract-treated sheets was significantly lower compared to head extract-treated sheets. This unexpected preference for cauliflower head over leaves suggests potential differences in chemical cues or physiological attributes influencing DBM oviposition behavior between these plant parts. All Brassicaceae species produce glucosinolates, chemicals that attract and stimulate feeding in DBM (Freeman and Beattie, 2008). Also, previous research indicates that glucosinolates are oviposition stimulants for DBM and show the positive correlation egg laying (Renwick *et al*., 2006; Sun *et al*., 2009; Badenes-Pérez *et al*., 2020). The oviposition preference might have decreased or influenced by reduction of glucosinolates as Lin *et al*. (2022) evidently reported the negative correlation of glucosinolate content and age of broccoli seedling.

Within the context of dark green cabbage, the study further elucidated DBM's oviposition behavior. It was found that DBM preferred to lay eggs on leaf extract-treated sheets as opposed to head extract-treated sheets. This preference for leaf tissue may be due to factors such as the nutritional quality of cabbage leaves or the suitability of leaf tissue for egg development. This result is consistent with the general understanding that DBM females often select specific plant parts for oviposition to optimize offspring survival (Jaenike, 1978).

In contrast, the study observed an altered trend in cauliflower. DBM exhibited a preference for head extract-treated sheets over leaf extract-treated sheets. This deviation in preference suggests that cauliflower may have unique attributes that attract DBM to its heads rather than its leaves. We couldn't correlate these findings with earlier studies but understanding these subtle differences in

oviposition behavior is crucial for tailoring pest management strategies to specific crops and plant tissues.

Day wise egg laying trend

The study found that DBM began laying eggs on sheets treated with light green cabbage extract and cauliflower extractss from day 1 and continued to do so up until day 5. However, it is noteworthy that the daily egg-laying rate on light green cabbage extract-treated sheets was consistently higher than on cauliflower extract-treated sheets. Interestingly, in dark green cabbage case, egg laying on leaf extract-treated sheets began on day 3 and continued until day 7. But egg laying in head tissue extract treated started from day 1. This indicates that DBM preferred to lay eggs on leaf tissue of dark green cabbage and the egg-laying period was extended compared to light green cabbage and cauliflower.

CONCLUSION

In conclusion, this research sheds light on the intricate oviposition preferences of DBM and their responsiveness to light green cabbage extract-treated sheets. These findings underscore the importance of considering both host plant selection and plant tissue specificity in laboratory screening of genotypes for resistance breeding programs. The findings underscored the importance of considering both plant type and stage in understanding DBM oviposition behavior. While light green cabbage emerged as the most preferred host overall, intriguing differences were observed between leaf and head tissues, especially in cauliflower. These nuanced preferences suggest a complex interplay of factors influencing DBM's choice of oviposition sites, including plant nutritional quality, chemical cues and physiological attributes.

The study of egg-laying preferences in Diamondback Moth (DBM), *P. xylostella* presents a valuable opportunity to develop early-stage screening methods for genotype in resistance breeding programs aimed at DBM resistance. Understanding the oviposition preferences of this destructive pest with additional biochemical or molecular data is essential for identifying and selecting resistant plant genotypes, which can significantly contribute to sustainable pest management in cruciferous crops.

ACKNOWLEDGEMENT

Authors acknowledges the Telluris Biotech India Pvt Ltd., Hyderabad for financial support.

The authors also acknowledge Shylesh Muthu, for supporting the execution of this research and R. Rajesh, Alluri Usharani, G. Vamshi for their help in conducting the experiments.

Author contribution statement

Yogiraj Namdeo Zirwal conceptualized and designed the research work, analyzed the data, prepared and improvised the draft of the manuscript. Sagar Bhagwan Dongarjal executed the research work and compiled data. Vivek Vishal reviewed and corrected the manuscript. Venu Polineni, Glenda W. Polack and Alejandro Calderon-Urrea reviewed and approved the manuscript. All authors agreed on the final draft of the manuscript.

Disclaimers

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Informed consent

No studies on animal have conducted that require approval of Committee of Experimental Animal Care.

Conflict of interest

Authors declares no conflict of interest.

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