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### FEEDING TOXICITY OF INSECTICIDES AGAINST RED PALM WEEVIL, *RHYNCHOPHORUS FERRUGINEUS*, (COLEOPTERA: DRYOPHTHORIDAE) IN A CONTROLLED ENVIRONMENT

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#### ABSTRACT

*Rhynchophorus ferrugineus* (Olivier, 1790), often known as the Asian palm weevil or red palm weevil (RPW) (Coleoptera: Curculionidae), had long been considered disruptive to many palm trees (Arecaceae), especially date palms. Observing adults of RPW inside the trunk of palm trees was challenging. Date palm trees provided shelter from bad weather for the larvae that lived and fed within. Finding the red palm weevils required dissecting the palm. Damage could occur to almost every commercially planted date palm variety in the nation, including Aseel, Hillawi, Karbalain, Mozawati, Kecheh, and Dhaki. All year round, adult red palm weevils could be found inside palm trees, although they were most active in the summer. The primary method of managing RPW was the frequent use of synthetic pesticides, which were used both preventatively and therapeutically in an effort to lessen RPW damage and spread. In the present study, the soft stem dip method was used to evaluate the toxicity of three different insecticides (clothianidin, trichlorophon (systemic), and fipronil). The results showed that LC50 values with 95% CI ( $\mu\text{g}/\text{ml}$ ) after 72 hours were recorded as 4.096  $\mu\text{g}/\text{ml}$  in the case of clothianidin + trichlorfon, representing the highest toxicity. Trichlorophon against 3<sup>rd</sup> instar larvae of the red palm weevil was more toxic compared to clothianidin and fipronil. The study, therefore, suggested that these insecticides need to be properly included in IPM plans to combat this pest. To test and enhance the efficacy of such pesticides in field settings, more work was necessary.

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#### INTRODUCTION

One of the most significant and widespread fruit crops in the Middle East and North Africa is the date palm, *Phoenix dactylifera* L., which is high in carbohydrates (Abbas, 2013). Approximately 7.5 million metric tons of

date fruit are produced annually, with more than 100 million date palms planted on one million hectares of land worldwide (El Hadrami and Al-Khayri, 2012). With an annual production of 0.47 million tons, Pakistan ranks sixth globally in terms of date palm production,

behind Saudi Arabia, Iraq, Egypt, Iran, and Algeria (FAO, 2018). Date fruits are a great source of several nutrients and are regarded as a complete food. Dates are rich in dietary fiber, carbohydrates, and trace levels of proteins, lipids, and several minerals such as calcium, iron, magnesium, phosphorus, potassium, zinc, sulfur, and cobalt, among others (Maqsood et al., 2020). Date fruit has 70% carbohydrates, and although date proteins are abundant in amino acids (Mortazavi et al., 2015). The most destructive pest of several palm species is the red palm weevil, *Rhynchophorus ferrugineus* (RPW) (Olivier) (Coleoptera: Curculionidae) (Rabel and Solaiman, 2011; Dhoubi et al., 2017). It targets almost all palm species worldwide (Faleiro, 2006a,b). Since the United Arab Emirates' initial invasion of the Gulf region in the mid-1980s, it has grown to be a serious danger. After that, in 1987, it was found in Saudi Arabia, and in 1993, it was first noted in Egypt (Cox, 1993). The RPW is said to be indigenous to Pakistan and other subcontinental nations (Al-Saoud et al., 2010); however, it made its introduction in Pakistan sometime in the early 1980s (Baloch et al., 1992). Today, it has spread to practically every region in the nation that grows date palms (Manzoor et al., 2020). Nearly all of the commercial date palm varieties grown in Pakistan are susceptible to damage, with certain kinds such as Aseel, Hillawi, Karbalain, Mozawati, Kechnanr, Dhaki, etc. (Shar et al., 2012; Manzoor et al., 2020) being the most vulnerable, with reports of losses in date output reaching 20% (Baloch et al., 1992). Up to 200 eggs can be laid by the mature red palm weevil female at the base of newly sprouting leaves as well as in wounds hidden in the host stem. Before pupating, the young grubs pierce through the soft fibers and enter the tissues of the terminal buds, growing to a size of roughly 5 cm (Longo, 2006). The soft, tender tissues of different varieties of date palm are what the larvae eat. The fully developed larvae approach the interior tissues shortly before pupation, digging tunnels and building cocoons out of dry threads. Larval tunneling promotes secondary pests and diseases (e.g., fungal pathogens) infestation (Downer et al., 2009; Dembilio and Jaques, 2015; Kontodimas et al., 2017). It is quite difficult to identify the first infection since the larvae consume the palm's internal tissues. Usually, the infection becomes apparent after the palm tree is significantly damaged (Blumberg, 2008). The signs of infected palms include pupal cases and adults in the leaf axils, falling pupal cases close to the base of the date tree, chewed-up fibers surrounding

the tunnel holes, and a hole in the stem of the tree that releases a yellow liquid. When there is significant damage, a deformed crown is a common indicator of a red palm weevil infestation and can be clearly distinguished from other weevil infestation symptoms (Soroker et al., 2005). Red palm weevils are typically found throughout the palm, from the ground surface of the trunk to the apical bud (Alkhalil et al., 2009). Whereas 80-90% of the infestation in *Phoenix canariensis* was discovered in the apical section of the tree, around 70% of the infestation in date palms was detected from the ground up to 1-1.5 meters (Pugliese et al., 2017).

The most popular method of controlling infestations is chemical control, which involves applying pesticides repeatedly as both preventative and curative treatments to stop the infestation from spreading. Although there are not many written records, applying fumigants and insecticides on date palms in Pakistan has a long history of preventing *R. ferrugineus*. The primary method of managing RPW is the frequent use of synthetic pesticides, which are used both preventatively and therapeutically in an effort to reduce RPW damage and spread (El-Mergawy and Al-Ajlan, 2011). Carbamate, organophosphate (chlorpyrifos, trichlorfon), and phenylpyrazole (fipronil) pesticides are employed for both relieving and preventive measures (Dembilio and Jaques, 2015; Al-Dosary et al., 2016; Faleiro et al., 2016). During field testing, ten different insecticides were tested against *R. ferrugineus*; the results indicated that the most effective ones were methidathion, spirotetramat, fipronil, and chlorpyrifos (Shar et al., 2012). Another study suggested that distinct modes of action of spinosad and lambda-cyhalothrin (Wakil et al., 2018) could be employed in insecticide rotation or to help phase out the usage of older pesticides against red palm weevil (Abraham et al., 1975). Seven insecticides have been shown to be effective in controlling *R. ferrugineus*, and fipronil is an effective pesticide for managing various immature stages of *R. ferrugineus* (Abdul-Salam et al., 2001). Although clothianidin acts more quickly, it is generally less successful in controlling the adult stages (Di Ilio et al., 2018).

Date palm growers in Pakistan have questioned the efficacy of insecticides that are used commercially. Considering the importance of selecting pesticides based on the mode of action, this study reveals the effects of clothianidin (systemic), trichlorfon, and fipronil

(systemic) against the 3<sup>rd</sup> instars of *R. ferrugineus* under controlled conditions.

## MATERIALS AND METHODS

### Collection of weevils

In the date palm orchard located at the Horticultural Research Station in Bahawalpur, Punjab, Pakistan, various stages of RPW larvae were collected. Date palm trees infected with RPW were inspected for collection of RPW. The laboratory population of red palm weevils was collected, and the population was placed in a plastic container (10 cm in length, 7 cm in breadth, and 7 cm in height). Soft palm pieces were used as a food source, with the immature red palm weevils feeding inside the date palm's inner and outer trunk. Laboratory conditions were maintained at  $25 \pm 2^\circ\text{C}$  with 65-70% humidity and a 24-hour dark cycle, considering the external environment since RPW larvae feed inside date palm trunks. A camel hair brush was used to separate red palm weevils in their third instar. Using feeding bioassays, sixty third-instar larvae were employed for a single concentration.

### Insecticides

Clothianidin (Telsta; 20SC, FMC), trichlorphon (Diptrex®; 80SP, Bayer Crop Sciences), and fipronil (Regent 80WG, Bayer Crop Science) were purchased from the market with multinational brands. Concentrations of each insecticide were prepared based on the toxicity mentioned in previous literature.

### Dip bioassay

In our study, 5 concentrations were prepared in  $\mu\text{g}/\text{ml}$  for each insecticide (clothianidin, trichlorphon, and fipronil) with one control. Firstly, 5 different concentrations of clothianidin (500, 250, 125, 63, and 31  $\mu\text{g}/\text{ml}$ , micrograms per milliliter), and control (0.00  $\mu\text{g}/\text{ml}$ ) were made in five different beakers (500 ml beaker) using 100 ml of distilled water in each beaker to make enough solution for dipping the soft inner stem of the date palm. Subsequently, beakers were labeled with their respective concentrations (1-5), where 1 denoted the highest concentration, 5 denoted the lowest concentration, and 6 denoted the control. Complete Randomized Design (CRD) was used in the dip bioassay. The experiment consisted of 4 treatments (clothianidin, trichlorphon, fipronil, and clothianidin + fipronil), with each pesticide designated as a treatment. Before the experiment, soft inner stem pieces of the date palm were dried at room temperature and placed into a container

in which ten 3<sup>rd</sup> instar larvae of RPW were placed (five replicates were made; total: 50 RPW) for each concentration. The same procedure was done for each insecticide.

### Data recording

Dead 3<sup>rd</sup> instar RPW larvae were counted to evaluate the mortality (%) data. After 24, 48, and 72 h, the third instar mortality percentage of RPW was recorded.

### Statistical analysis

Using the "Probit" software, the LC50 values were calculated for each insecticide using the methodology described by Finney (1971). The mortality (%) was calculated in MS Excel 2019.

## RESULTS

### Mortality of red palm weevil by insecticides

Mortality was directly correlated with treatment concentrations and exposure time for all treatments. Only the percentage of deaths at the highest concentrations of each dataset was shown and LC50 values for each treatment for the 24, 48, and 72-h recording intervals were provided.

For clothianidin, the highest concentration (500 ppm) caused maximum mortality, with rates of 62%, 76%, and 86% observed after 24, 48, and 72 h respectively (Figure 1). The highest concentration (500 ppm) of trichlorphon resulted in extreme mortality rates of 68%, 80%, and 92% after 24, 48, and 72 h, respectively (Figure 2). Fipronil caused mortality rates of 48%, 54%, and 66% after 24, 48, and 72 h at the highest concentration (500 ppm) (Figure 3). In the case of combined treatments, the highest concentration (20 ppm) of clothianidin (Systemic) + fipronil (systemic) resulted in the highest mortality rates of 68%, 84%, and 96% after 24, 48, and 72 h respectively (Figure 4).

### Toxicity of insecticides against red palm weevil

The LC50 values for clothianidin were recorded as 150.468, 48.120, and 24.790  $\mu\text{g}/\text{ml}$  after 24, 48, and 72 h of feeding, respectively (Table 1). Trichlorphon caused toxicity in *R. ferrugineus* with LC50 values of 92.248, 36.092, and 11.757  $\mu\text{g}/\text{ml}$  after 24, 48, and 72 h. LC50 values of fipronil were observed as 671.800, 332.182, and 77.337  $\mu\text{g}/\text{ml}$  after 24, 48, and 72 h respectively. In the case of combined insecticides, clothianidin + fipronil showed LC50 values of 92.274, 3.139, and 4.096  $\mu\text{g}/\text{ml}$  after 24, 48, and 72 h of feeding, respectively. Overall, the highest LC50 value with 95% CI ( $\mu\text{g}/\text{ml}$ ) after 24 h of exposure was

recorded as 671.800 for fipronil. The lowest LC50 values with 95% CI ( $\mu\text{g/ml}$ ) after 72 h were recorded as 4.096 in the case of clothianidin + trichlorfon, indicating the highest toxicity.

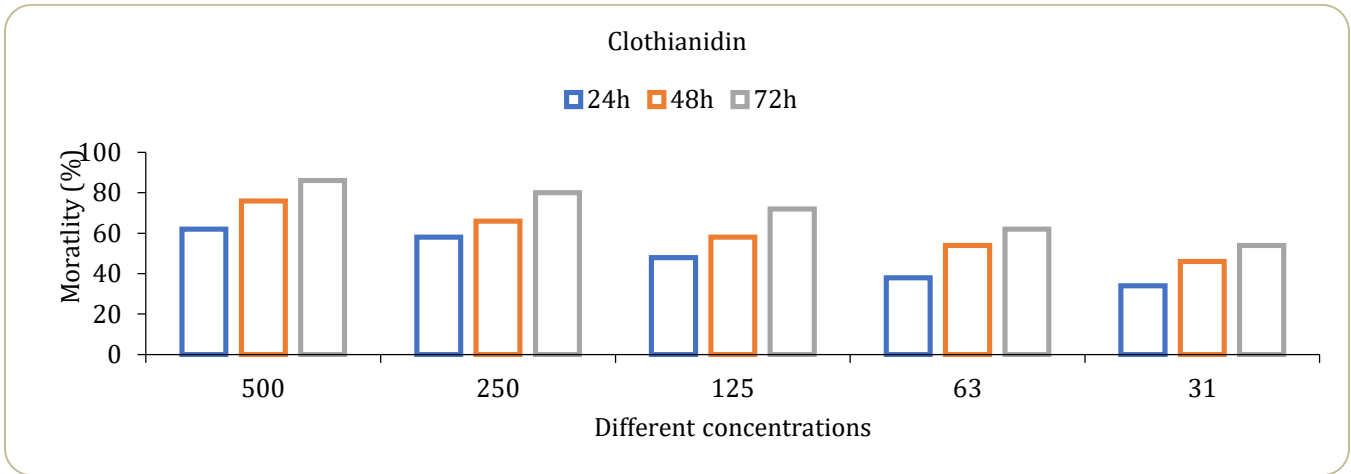


Figure 1. Mortality (%) of *R. ferrugineus* after 24, 48, and 72 hrs when treated with clothianidin.

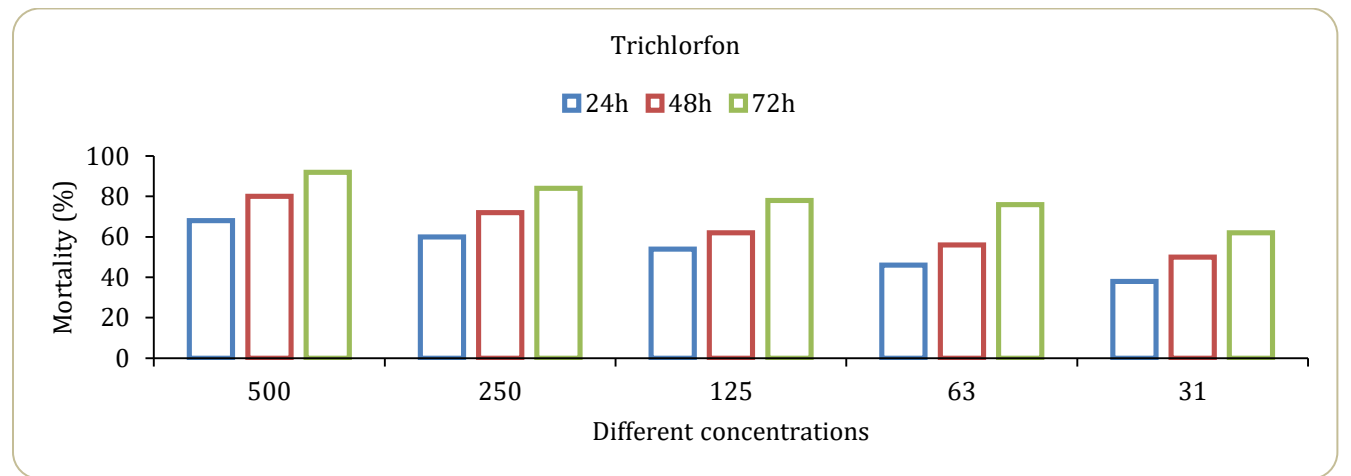


Figure 2. Mortality (%) of *R. ferrugineus* after 24, 48, and 72 hrs when treated with trichlorfon.

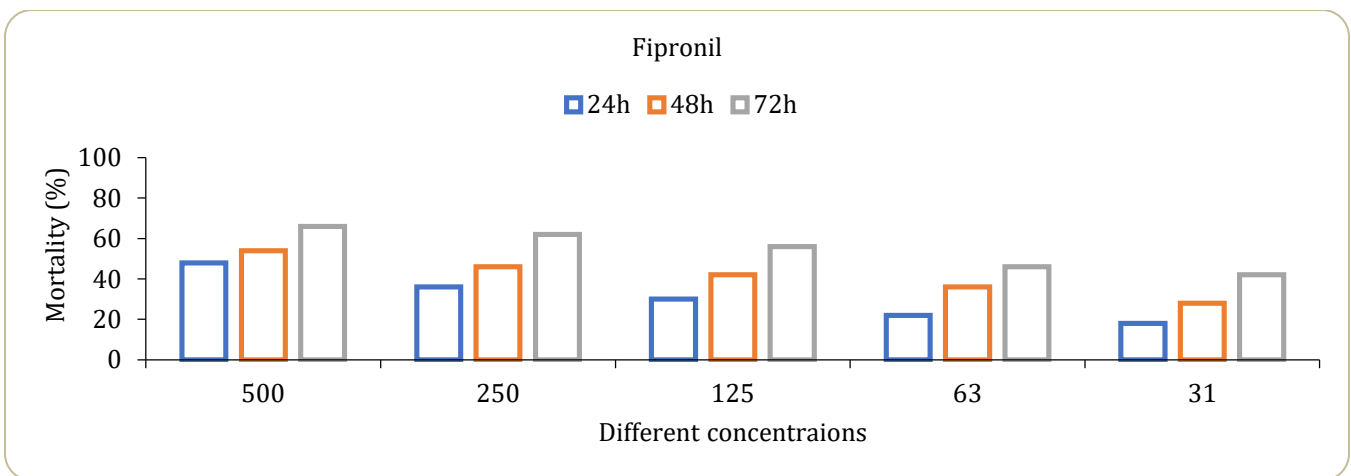


Figure 3. Mortality (%) of *R. ferrugineus* after 24, 48, and 72 hrs when treated with fipronil.

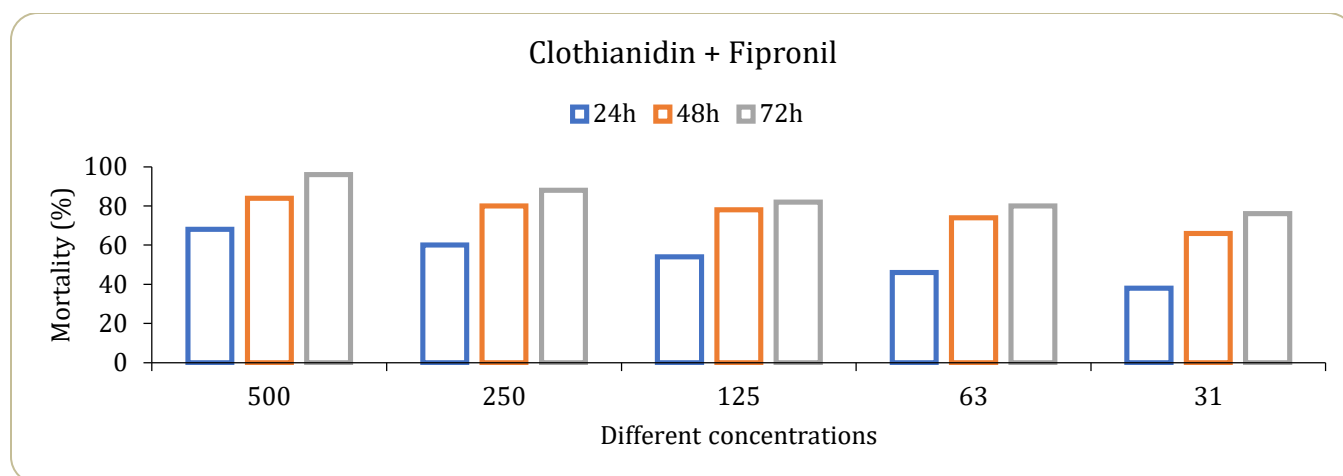


Figure 4. Mortality (%) of *R. ferrugineus* after 24, 48, and 72 hrs when treated with chlothianidin + fipronil.

Table 1. Feeding toxicity of three different systemic pesticides against 3rd instar of red palm weevil.

Bioactive	Time interval (h)	LC50 ( $\mu\text{g}/\text{ml}$ )	Slope $\pm$ SE	$\chi^2$	df
Clothianidin	24	150.468	$0.647 \pm 0.191$	0.272	3
	48	48.120	$0.634 \pm 0.193$	0.305	3
	72	24.790	$0.828 \pm 0.207$	0.035	3
Trichlorfon	24	92.248	$0.630 \pm 0.190$	0.024	3
	48	36.092	$0.694 \pm 0.197$	0.282	3
	72	11.757	$0.811 \pm 0.221$	0.736	3
Fipronil	24	671.800	$0.719 \pm 0.202$	0.198	3
	48	332.183	$0.538 \pm 0.191$	0.117	3
	72	77.337	$0.544 \pm 0.190$	0.178	3
C+T	24	92.274	$0.631 \pm 0.191$	0.026	3
	48	3.139	$0.458 \pm 0.208$	0.188	3
	72	4.096	$0.712 \pm 0.241$	1.471	3

Cl: Confidence limit,  $\chi^2$ : Pearson's chi-squared goodness-of fit test. df: Degree of freedom. C+T: Clothianidin + Trichlorfon.

## DISCUSSION

In the past and present, the use of pesticides has been the primary method of controlling agricultural pests in fields and orchards. In date palm orchards, the RPW poses a significant challenge that requires appropriate design of control programs and the selection of the most appropriate pesticide. This study is the first to explain the potency of systemic and non-systemic insecticides against *R. ferrugineus* in South Punjab, Pakistan.

The results of the current investigation demonstrate a positive link between the percentage of mortality and the concentrations employed. This could be because sensitive larvae were the only ones affected by sub-lethal concentrations, and some of them were tolerant to

the insecticide. However, both sensitive and tolerant individuals were more affected by high concentrations, leading to increased mortality as the concentration increased, which is similar to the study by Mahyoub and Alghamdi (2020).

Another reason might be a longer exposure period, which allowed the active ingredients of the insecticide to attach to the target sites inside the weevil's body and produced a toxic effect. Conversely, a shorter exposure period could be one of the reasons the insecticide does not reach and attach to the intended sites, aligning with previous studies (Mahyoub and Alghamdi, 2020).

According to our laboratory tests, the combination of clothianidin with trichlorfon is effective in control

approaches against adult RPW, which is consistent with the results of Di Ilio et al. (2018) who stated that thiamethoxam and clothianidin were both successful in control approaches against adult RPW (Mahyoub and Alghamdi, 2020). In our study, fipronil was the least effective against immature RPW when compared to trichlorfon and clothianidin, possibly due to *R. ferrugineus* developing resistance to fipronil. However, in another study, pirimiphos-methyl was the most effective compared to other new chemistry pesticides in controlling immature stages of *R. ferrugineus*, with a mortality percentage in treated larvae reaching 100% (Ajlan et al., 2000).

Another study showed that different organophosphorus pesticides were best against RPW. Neither the genetics of resistance in this species nor any cross-resistance patterns are understood. Nonetheless, other species in Pakistan have shown diverse resistances to both novel and conventional chemical insecticides, with some instances of cross-resistance within insecticide classes (AlJabr et al., 2017). It is common in Pakistan to combine traditional and modern pesticides to manage insect infestations in commercially significant crops; thus, deciding on the existence of cross-resistance between these substances would be premature.

Spinosad and lambda-cyhalothrin have demonstrated strong efficacy against the harmful larval stage of *R. ferrugineus* (Wakil et al., 2018). Additionally, our recent study shows that the combination of clothianidin and trichlorfon was the most effective against RPW.

## CONCLUSION

Our study concluded that the combination of clothianidin and trichlorfon was best against red palm weevil immature stages. Trichlorfon is most effective against 3<sup>rd</sup> instar larvae of red palm weevil.

## RECOMMENDATION

This study recommends that clothianidin and trichlorfon should be considered in integrated pest management strategies against red palm weevil. To test and enhance the efficacy of such insecticides in fields/orchards, more work is needed.

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## AUTHORS' CONTRIBUTIONS

MI, WJ, and AN conceived and designed the experiments; MI, WJ, AN and BA performed the experiments; MI, WJ, MBS, RA, and BA analyzed the data; MBS, RA, MAA, BA, MFAK, LA, KS, MAB contributed materials, analysis, and tools; MI, WJ and AN wrote the paper. Furthermore, MI and WJ are equally contributed authors.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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