



# Mating Behaviour of Brown Planthopper *Nilaparvata lugens* Stål (Homoptera: Delphacidea) Under Certain Biological and Environmental Factors

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## ABSTRACT

To date, there persists a significant gap in the mating behavioural study of brown planthopper (BPH) for almost two decades. Therefore, in this study mating response of brown planthopper (BPH), a serious pest of rice was observed under the influence of different factors such as insect age (male 1-7 days and female 1-9 days), plant age as a food source (25-30 days, 55-60 days and 85-90 days), temporal effects of the day (0800- 1100, 1200-1500 and 1800-2100), temperature (22±2°C, 27±2°C and 32±2°C) and wind velocity (0.00-1.00 m/sec, 1.00-1.50 m/sec and 1.50-2.00 m/sec). The variation in mating response induced by these factors were measured based on several mating parameters such as female abdominal vibration, male dancing, male arrival, successful mating and mating duration. Observations revealed that five days old insect pairing that had been reared on 25-30 days old rice plants displayed the highest response in all mating parameters during the evening hours when temperature and wind velocity was observed to be low or at an optimum level. Understating the influence of these factors on the mating behaviour of BPH is essential as it serves as baseline for recording acoustic courtship signals for future studies.

## Article Information

Received 6 May 2015

Revised 18 August 2015

Accepted 25 August 2015

Available online 1 January 2016

## Authors' Contributions

RM, ZO, IV, DL and MQ conceived, designed and supervised the project. AMA did the experimental work and analyzed the data. The manuscript was written by AMA and MG and critically reviewed by RM, ZO, IV and DL.

## Key words

Brown planthopper, insect age, plant age, temporal phase, wind velocity, female abdominal vibration, male dancing, male arrival, successful mating and mating duration.

## INTRODUCTION

**B**rown planthopper (BPH), *Nilaparvata lugens* (Stål) is a serious pest of rice that has caused significant yield losses over the years especially in Asian countries (Heong, 2009; Win *et al.*, 2011). Both nymphs and adults cause hopper burn in rice by sucking phloem sap and they also transmit plant viral diseases such as rice ragged stunt virus (RRSV) and rice grassy stunt virus (RSGV) (Hibino, 1996). Various control approaches involving pesticide application as well as the growth of resistant plant varieties have been applied in field, however the success of this techniques has not been durable as incidence of resurgence often arise. Generally a successful pest management procedure is achieved by suppressing the pest population level (Naeemullah *et al.*, 2004) and this can be achieved by disrupting the mating behaviour of the pest. There have been a number of studies on BPH, however only a few studies in the past

have focused on their mating response (Esaki and Hashimoto, 1937; Oyama, 1972; Takeda, 1974; Ichikawa and Ishii, 1974; Claridge *et al.*, 1984). In most of the studies, a thorough examination on the influence of physical and physiological factors on the mating behaviour which in turn contributes to reproductive efficiency has not been specifically observed.

The mating success of BPH relies on plant-borne vibrational signals which are intensely predisposed to the physical characteristics of their acoustic environment, which coincides with the environment of their host plant. As a result, signaling and signals are enhanced according to the physical properties of the substrate and the environment (Polajnar *et al.*, 2014). As mating is the most important and fundamental process of selecting the best partner to produce progeny (Sakai and Ishida, 2001), often abiotic and biotic conditions such as temperature, daylight, wind, rainfall, insect age, and predator population plays a critical role that results in the development of specialized niche conditions.

The mating in insects often constitutes multiple steps (Alexander *et al.*, 1997) and in BPH, sexually receptive female inserts her stylet into a rice plant stem and vibrates her abdomen (female abdominal vibration)

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in a dorso-ventral direction without tapping the rice plant directly (Ichikawa, 1979; Claridge *et al.*, 1984). Meanwhile, the male performs a male dance and proceeds to move towards the vibrating female and finally positions himself at a location that ensures successful mating. In this sequence of events, various crucial factors contribute further to the success of mating. For example, the ages of insect are an important physiological feature that has been observed to influence successful mating as portrayed by the male treehopper during female seeking in which the success of mating varies with early and increasing-age (De Luca and Cocroft, 2011). In addition, sufficient food supplies (host plants) influences the sexual maturity of BPH (Kumar and Saxena, 1978). Temperature also influences the reproductive behaviour, population distribution, development and survival of insects (Bale *et al.*, 2002; Long *et al.*, 2012) whereby, FAV and mate-location behaviour of BPH have been reported to be affected at variable temperatures (Long *et al.*, 2012). Mating in insects has also been observed to occur at a particular time of the day (Liu *et al.*, 2004; de Carvalho *et al.*, 2012). A narrow window of the day, which is called “circadian gate”, is often established in reproductive behaviours including mate searching, courtship, and copulation (Pittendrigh and Skopik, 1970). This temporal regulation of mating is related to the circadian rhythm of the sexual ability or receptiveness of one or both sexes of insects (de Carvalho *et al.*, 2012).

To establish any future successful pest management strategy that targets pest population suppression via mating disruption, it is extremely important to achieve a complete understanding on the interaction and influences of environmental and biological factors on an individual's mating behaviour. Because, small insects like hemiptera and homoptera do not rely on pheromones for mating communication; substrate borne vibrational communication is the only effective way to communicate within same species. Hence, this study was carried out to obtain comprehensive information about the relationship between reproductive behaviour of *N. lugens* (Stål) and certain physical as well as physiological factors. Based on these findings, it could be possible to develop a future control strategy that manipulates both the physical and physiological factors thus resulting in mating disruption that further reduces the BPH population below significant level.

## MATERIALS AND METHODS

### *Collection and rearing of BPH*

Insects were collected randomly using a sweep net

from the rice fields of Tanjung Karang, Selangor, Malaysia and with an aspirator from Field-10, Universiti Putra Malaysia. The collected specimens were brought to the glass house at Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia and reared in two wooden cages (90 x 42 x 42 cm) covered with mesh for proper aeration at  $28\pm 2^{\circ}\text{C}$ , 70% R/H and photoperiod of 12D:12L. This produced the main culture for the two insect populations. Both temperature and relative humidity were recorded on simple graph paper using a hygrothermograph (Mechel -15+45°C, Italy) placed inside the glass house. Throughout the study, insects were reared on the rice variety of MR-219.

### *Species confirmation (Nilaparvata lugens)*

Twenty gravid female planthoppers were randomly selected from the main culture populations (10 from each location) and species identification was performed according to the taxonomic key given by Mochida and Okada (1979). Here, the characteristic of the female genitalia was observed as a key guide to identify the exact species of BPH under the Dino Lite digital microscope (AnMo Electronics Corp, Taiwan) which was connected to a laptop. Gravid females that were confirmed to be *N. lugens* were then released onto rice plants for oviposition and development; hence a stock culture of BPH was established. All subsequent studies will be carried out using individuals from the stock culture that has been confirmed to be *N. lugens*.

### *General experimental design*

This section provides detailed explanation on the experimental setup and observed parameters which are similar for the following experiments thus avoiding unnecessary repetition throughout. To observe the mating response, two 30-40 days old rice plants in separate small pots were connected at the leaf blades and placed under 100 watt flood lamp. Male and female BPH were then released onto the plants and their mating responses were observed (Fig. 1). The experiment was conducted in a controlled condition; however treatments and conditions differed according to the respective studies. In all experiments, five different mating parameters were recorded in percentage; female abdominal vibration (FAV), male dancing (MD), male arrival (MA), successful mating (SM) and mating duration (MTD) in seconds. The experiments were based on a completely randomized design (CRD). All experiments were conducted using 15 replications per treatment at 20 min observation per replicate except for temporal studies whereby 12 replications at 15 min observation per replicate were observed.

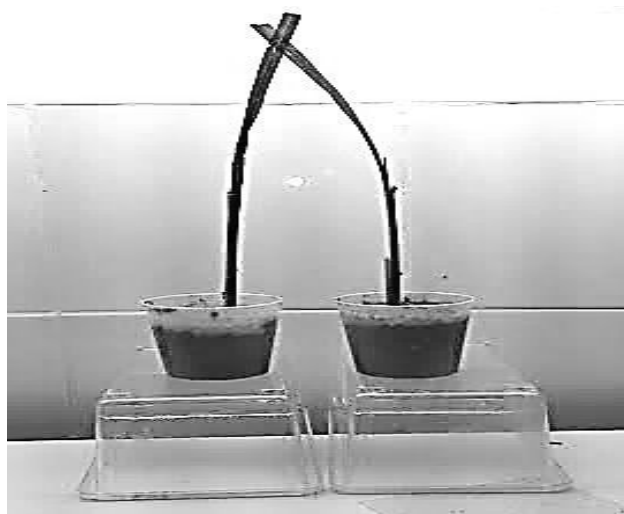


Fig. 1. Experimental setup of connected leaves of rice plant on which both sexes were individually introduced to observe the mating behaviour of *Nilaparvata lugens* (Stål).

#### *Influence of different age pairing of BPH on mating*

The effects of age on the mating response were studied by pairing males and females of different age combination. The age range of virgin males was one to seven days, whereas virgin females ranged between one to nine days. Brachypteris virgin females and macropteris virgin males were used during all experiments. To obtain virgin individuals of the exact age, fifth BPH instars from the stock culture were removed and reared on separate rice seedlings until last post-eclosion. Within 24 h of emergence, the adults were separated according to sex and released on rice seedlings in separate cages. Hereafter, individuals were maintained in their respective cages and were further subjected to the general experimental design. This study was carried out in an insectary at  $28 \pm 1^\circ\text{C}$  in the evening at 1700 to 2000.

#### *Influence of rice plant age on BPH mating*

In this study, the third BPH instars were taken from the stock culture and reared on rice plants of three different ages; 25-30 days, 55-60 days and 85-90 days (days after transplanting). At emergence, males and females were separated and rearing was continued on the respective plants of the specifically chosen age. Insects were reared until the age of five days and they were subsequently subjected to the general experimental design. Five days old BPH pairs were chosen for this study and all the following experiments based on the results of age influence studies which highlighted the highest efficient mating response occurred in five days old BPH pairs.

#### *Temporal effects of day on BPH mating*

Here, the effect of three different phases of the day, Phase-I (0800-1100), Phase-II (1200-1500) and Phase-III (1800-2100) on BPH mating response was studied in a glass house. Each phase was further divided into three periods; whereby each period constituted of an hour. Using five days old BPH pairs, the general experimental design was carried out accordingly at the different phases.

#### *Temperature effects on BPH mating*

This experiment was conducted in a controlled condition in an enclosed small insectary chamber where three different temperatures were maintained;  $22 \pm 2^\circ\text{C}$ ,  $27 \pm 2^\circ\text{C}$  and  $32 \pm 2^\circ\text{C}$ , respectively. The maintained temperatures were monitored using a digital thermometer (Model=BT-3, Japan). Using five day old BPH pairs, the general experimental procedure was carried out accordingly at the different maintained temperatures.

#### *Wind effects on BPH mating*

In this study, the effect of three variable wind velocities ranging between 0.00-1.00 m/sec, 1.00-1.50 m/sec and 1.50-2.00 m/sec on BPH mating response was observed. Experimental design of McNett *et al.* (2010) was followed with modifications. The selected wind velocity were produced using an electronic fan (Model=TF-121, KHinD, Malaysia) and measured with a portable digital anemometer (MS6252-MASTECH, China). This study was conducted in an enclosed small room at  $27 \pm 1^\circ\text{C}$ . Using five days old BPH pairs, the general experimental procedure was carried out accordingly at the three different wind velocity ranges.

#### *Statistically analyses*

Collected data were first square root transformed and analysed using one-way analysis of variance (ANOVA) except in temporal studies in which two way-analysis of variance was used. Whereas least significant difference (LSD) at 0.05 probabilities was used to separate means with significant difference. All the analysis were done using Statistical Analysis Software version 9.3 (SAS Institute Inc. 2009).

## RESULTS

#### *Species confirmation*

The top portion of the lateral lobes (Valvifer-VIII) of all collected gravid brachypteris females from both rice locations were convex (raised face) and the genitalia were not bifurcated apically (Fig.2) thus conforming them to be *N. lugens*.

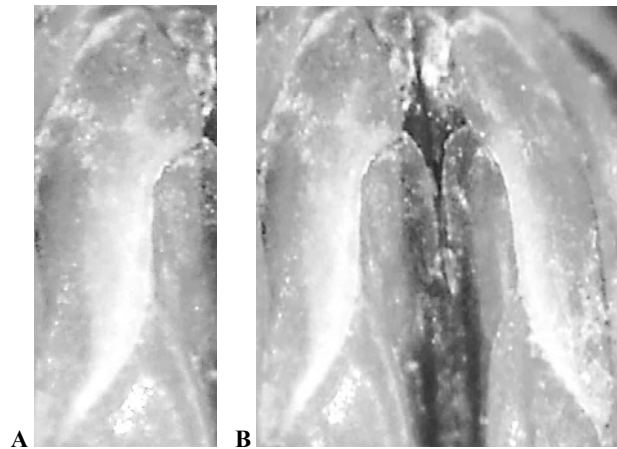


Fig. 2. Female genitalia observed under digital microscope showing non-bifurcated apically convex Valvifer-VIII, which is main identification of *lugens* species in brown planthopper. A, Female genitalia (lateral lobes); B, Ventral view of female genitalia.

#### Influence of different age pairing of BPH on mating

The mating parameters of BPH observed under the influence of different age pairings are given in Table I, whereby significantly highest percentage (100%) of successful mating ( $F=10.7$ ,  $dF=19$ ,  $280$ ;  $P<0.05$ ) with maximum mean mating duration of  $92.5\pm5.4$  sec. was observed when both pairs of BPH were five days old. At this age pairing, all other mating parameters (FAV, MD, MA and MTD) were recorded highest (100%) at significant difference. Pairing between five days and five and seven day old individuals also produced similar results with non-significant difference. Meanwhile, the lowest SM percentage of  $13.3\pm9.1$  was observed when one day male was paired with three and five day old female. Overall, percentage of mating was low with non-significant difference whenever young (one day) and old (seven days) males were paired with females of all age group. The FAV was observed occasionally on rice plant and was maximum (100%) with significant difference ( $F=10.1$ ,  $dF=19$ ,  $280$ ;  $P<0.05$ ) in five and seven days' old females that were paired with five days old males. The percentage of FAV was observed to decline from  $100.0\pm0.0$  to  $73.3\pm11.8$  with increasing age of female even when she was paired with five days old male (which is to be considered sexually fit male).

In response to FAV, the percentage of MD efficiency was observed significantly different in selected age pairings ( $F=9.1$ ,  $dF=19$ ,  $280$ ;  $P<0.05$ ). The highest male dancing of 100% was performed by five days old male when paired with both five and seven days old virgin females. However, the lowest MD percentage of

$53.3\pm13.3$  was observed in one day and seven days old virgin males. Therefore, based on these observations and results it was concluded that both sexes were sexually mature and became fit at the age of five days with the capacity to display maximum responses (FAV and MD).

One day old male unlike female, mated when paired with different aged female but percentage of their MA ability was low at  $40.0\pm13.1$  to  $46.7\pm13.3$  and such results were not significantly different from seven days old male. The overall mean mating duration was significantly different in different age groups ( $F=11.8$ ,  $dF=19$ ,  $280$ ;  $P<0.05$ ) with the longest at 148 sec. being observed in five days old pairing and the shortest at 46 sec. being displayed between one and nine days old pairing. The overall shortest mean mating duration of  $21.3\pm8.2$  to  $50.8\pm11.2$  sec was observed in nine days old female when paired with different aged group of male.

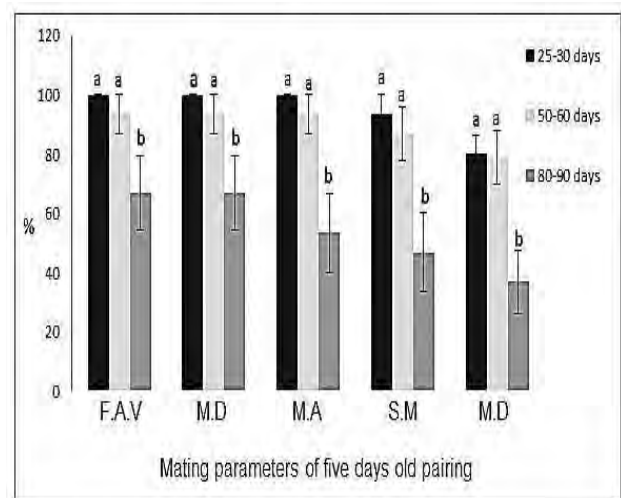


Fig. 3. Effect of host plant age as a food source on the mating behaviour of *Nilaparvata lugens* (Stål). Five days old virgin male and female pairing were used. Mating parameters were FAV, female abdominal vibration; MD, male dancing; MA, male arrival; SM, successful Mating and MTD, mating duration. Means followed by different letters within the same bar gram are significantly different ( $p\leq0.05$ ).

#### Influence of different age of rice plant

Results from this study (Fig. 3) highlighted that the age of rice plants as a food source for BPH had a significantly different influence in BPH successful mating percentage ( $F=6.2$ ,  $dF=2$ ,  $42$ ;  $p<0.05$ ). Rice plants of 25-30 days old (tillering stage) served as the best food source in eliciting timely maturity of BPH. Overall no significant difference was found in all mating parameters

**Table I.- Effects of age on mating behaviour of brown planthopper, *Nilaparvata lugens* (Mean±S.E).**

Age (days)	M-F	% FAV	% MD	% MA	% SM	Mean MTD (Sec.)
1-1		0.0±0.0 c	0.0±0.0 c	0.0±0.0 e	0.0±0.0 g	0.0±0.0 i
1-3		60.0±13.1 b	53.3±13.3 b	46.7±13.3 cd	13.3±9.1 gf	10.5±7.1 hi
1-5		66.7±12.6 b	53.3±13.3 b	40.0±13.1 d	13.3±9.1 gf	11.1±7.5 hi
1-7		60.0±13.1b	53.3±13.3 b	40.0±13.1 d	20.0±10.7 efg	16.3±8.7 ghi
1-9		53.3±13.3 b	53.3±13.3 b	40.0±13.1 d	33.3±12.5 def	21.3±8.2 fghi
3-1		0.0±0.0 c	0.0±0.0 c	0.0±0.0 e	0.0±0.0 g	0.0±0.0 i
3-3		73.3±11.8 ab	66.7±12.5 b	66.7±12.5bcd	46.7±13.3 cde	38.6±11 def
3-5		80.0±10.7 ab	80.0±10.7 ab	73.3±11.8 abc	60.0±13.1 bcd	50.8±11.2 cde
3-7		80.0±10.7 ab	80.0±10.7 ab	80.0±10.7 ab	73.3±11.8 abc	53.1±8.8 cd
3-9		60.0±13.1 b	53.3±13.3 b	46.7±13.3 cd	46.7±13.3 cde	31.1±9.1 efg
5-1		0.0±0.0 c	0.0±0.0 c	0.0±0.0 e	0.0±0.0 g	0.0±0.0 i
5-3		80.0±10.7 ab	80.0±10.7 ab	80.0±10.7 ab	80.0±10.7 ab	68.3±9.2 bc
5-5		100.0±0.0 a	100.0±0.0 a	100.0±0.0 a	100.0±0.0 a	92.5±5.4 a
5-7		100.0±0.0 a	100.0±0.0 a	100.0±0.0 a	100.0±0.0 a	81.8±3.5 ab
5-9		73.3±11.8 ab	73.3±11.8 ab	73.3±11.8 abc	73.3±11.8 abc	50.1±8.5 cde
7-1		0.0±0.0 c	0.0±0.0 c	0.0±0.0 e	0.0±0.0 g	0.0±0.0 i
7-3		60.0±13.1 b	53.3±13.3 b	40.0±13.1 d	20.0±10.7 efg	17.0±9.1 fghi
7-5		80.0±10.7 ab	73.3±11.8 ab	53.3±13.3 bcd	33.3±12.6 def	25.5±9.6 fgh
7-7		73.3±11.8 ab	66.7±12.6 b	53.3±13.3 bcd	46.7±13.3 cde	32.7±9.5 defg
7-9		66.7±12.6 b	53.3±13.3 b	40.0±13.1 d	40.0±13.1 def	25.3±8.4 fgh

Means followed by different letters within the same column are significantly different ( $p \leq 0.05$ ). M, male (1-7 days old); F, female (1-9 days old). For Abbreviations see Figure 3.

when nymphs and adult insects were reared prior to mating on 25-30 days (tillering) and 55-60 days (booting) old rice plants. The maximum percentage of highest successful mating of  $93.3 \pm 6.7$  was observed in BPH pairs that were reared on 25-30 days old rice followed by  $86.6 \pm 9.1$  on 55-60 days old rice plants and lowest of  $46.7 \pm 13.3$  when reared on 85-90 days old rice plants, respectively.

When insects were reared on old plants, the insect could not display the maximum response as their maturity was affected thus resulting in the lowest FAV percentage of  $66.7 \pm 12.5$ , MD of  $66.7 \pm 12.5\%$  and MA of  $53.3 \pm 13.3\%$ , respectively. Meanwhile, females that were reared on young aged rice plants were found to be timely matured on the fifth day, thus permitting them to successfully display the highest (100) percentage of FAV with significant difference ( $F=4.5$ ,  $dF=2$ ,  $42$ ;  $P<0.05$ ). Accordingly, maximum mating response was observed in each and every mating parameters of BPH when reared on 25-30 days old rice plant. In addition, the lowest mean mating duration of  $36.8 \pm 10.7$  sec. was observed in insects reared on old rice plant (85-90 days) and the highest of  $80.8 \pm 6.4$  sec. occurred in BPH pairs reared on young 25-30 days old rice plants.

#### Temporal effects

The effect of varying temporal phases on the

mating response in BPH (Table II) showed significant differences in successful mating percentage ( $F=3.1$ ;  $dF=10$ ,  $25$ ;  $P<0.05$ ). Overall the highest successful mating percentage of  $83.3 \pm 11.2$  was observed in Phase-III with 100% SM within period-II of that phase. Meanwhile, the lowest percentage of  $25.0 \pm 13.1$  was observed in Phase-II, whereby no pair mated within period-I of the same phase. The frequency of female abdominal vibration fluctuated throughout the day during experimental period from morning to evening and similarly the male efficiency to locate mate wavered. The percentage of FAV was found non-significantly different between Phase-I and Phase-II and between Phase-I and Phase-III but it was significantly different ( $F=1.7$ ;  $dF=10$ ,  $25$ ;  $P<0.05$ ) between Phases-II and Phase-III, respectively. Further, it was also observed that in the last period of Phase-I, FAV % started to decrease from  $100.0 \pm 0.0$  to  $75.0 \pm 2.5$ . The percentage of FAV was low and fluctuated during all periods of Phase-II but eventually it reached a maximum of 100 % in all periods of Phase-III.

The MD which is a response of male towards FAV was observed to be significantly different in all phases throughout the day ( $F=3.6$ ;  $dF=10$ ,  $25$ ;  $P<0.05$ ). All males showed 100% response by performing MD in period-I of Phase-I which then started to decline and fluctuated from  $25.0 \pm 25.0$  % to  $75.0 \pm 13.1$  % in all

periods of Phase-I and Phase-II. But as time passed, it eventually increased and maintained at the highest percentage of MD (100%) in all periods of Phase-III, respectively. However, the response of MD was not significantly different in Phase-I and Phase-II but it was lowest at  $25.0 \pm 25.0$  % in period-I of Phase-II. The searching ability of male was also affected with significant difference ( $F=3.1$ ;  $dF=10, 25$ ;  $P<0.05$ ) after spontaneous male dance in all phases and periods throughout the day. The arrival percentage of male was low at  $25.0 \pm 13.1$  in Phase-II and especially during period-I in which the percentage was zero. Meanwhile, the overall highest male arrival percentage of  $83.3 \pm 11.2$  was observed in Phase-III with 100% of male successfully locating their mate during period-II. In addition, the lowest mean mating duration of  $21.9 \pm 11.5$  sec. was observed in Phase-II and the highest at  $78.7 \pm 11.2$  sec. occurred during Phase-III in period-II with significant difference ( $F=4.3$ ,  $dF=10, 25$ ;  $P<0.05$ ).

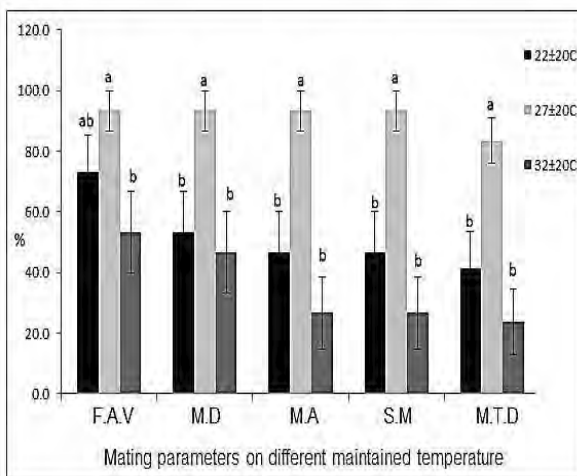


Fig. 4. Effect of different variable temperatures on mating behaviour of *Nilaparvata lugens* (Stål). Five days old virgin male and female pairing were used. For abbreviations of mating parameters See legend of Figure 3.

#### Temperature effects

The results of this study (Fig. 4) showed significant difference ( $F=9.7$ ;  $dF=2, 42$ ;  $P<0.05$ ) in successful mating among selected pairings at variable temperatures. The highest percentage of successful mating of  $93.3 \pm 6.6$  with a mean mating duration of  $83.4 \pm 7.4$  sec. was observed at  $27 \pm 2^\circ\text{C}$  followed by  $46.6 \pm 13.3$  with a MTD of  $41.4 \pm 12.0$  sec. at  $22 \pm 2^\circ\text{C}$  and lowest at  $26.6 \pm 11.8$  with MTD of  $24.0 \pm 10.8$  sec. at  $32 \pm 2^\circ\text{C}$ . The female abdominal vibration in *N. lugens*

was also significantly different at variable maintained temperatures ( $F=3.3$ ;  $dF=2, 42$ ;  $P<0.05$ ). The highest percentage FAV observed was  $93.3 \pm 6.6$  at  $27 \pm 2^\circ\text{C}$  followed by  $73.3 \pm 11.8$  at  $22 \pm 2^\circ\text{C}$  and the lowest was  $53.3 \pm 13.3$  at  $32 \pm 2^\circ\text{C}$ . The FAV was not significantly different at  $32^\circ\text{C}$  and  $27^\circ\text{C}$ ; however, the differences were prominent at  $22^\circ\text{C}$  and  $27^\circ\text{C}$ , respectively. Macropteris males of BPH responded after detecting the female abdominal vibration on rice plants, and but the percentage of their response (MD) was significantly different at low and high temperature as compared to optimum temperature ( $F=4.7$ ;  $dF=2, 42$ ;  $P<0.05$ ). The highest percentage of male dance ( $93.3 \pm 6.6$ ) occurred at  $27 \pm 2^\circ\text{C}$ . The overall fluctuation in percentage of male response to FAV was observed  $73.3 \pm 11.8$  to  $53.3 \pm 13.3$  at  $22^\circ\text{C}$  and  $53.3 \pm 3.3$  to  $46.6 \pm 13.3$  at  $32^\circ\text{C}$ . The male arrival efficiency also showed a significant difference ( $F=9.7$ ;  $dF=2, 42$ ;  $P<0.05$ ) at the variable temperatures studied. At a temperature of  $22^\circ\text{C}$  and  $32^\circ\text{C}$ , the percentage of mate location by male was low at  $46.6 \pm 13.3$  and  $26.6 \pm 11.8$  respectively. However, the maximum percentage in male locating female occurred at  $27 \pm 2^\circ\text{C}$  with a value of  $93.3 \pm 6.6$ .

#### Wind velocity effects

The results of the effect of variation in wind velocity on the mating response of BPH are shown in Table III. Highly significant effect of wind on the successful mating response of *N. lugens* occurred on all mating parameters ( $F=56.0$ ;  $dF=2, 42$ ;  $P<0.05$ ). Among, the three selected ranges of wind velocities, mating was only actively observed in BPH at  $0.0-1.0$  m/sec, at a successful mating percentage of  $80.0 \pm 10.0$  and a mean mating duration of  $72.6 \pm 11.4$  sec. At this velocity, female actively performed FAV with highly significant difference ( $F=91.0$ ,  $dF=2, 42$ ;  $P<0.05$ ) at a maximum percentage of  $86.6 \pm 9.0$  and similarly males responded by doing male dance with significant difference ( $F=91.0$ ;  $dF=2, 42$ ;  $P<0.05$ ). A total of  $80.0 \pm 10.0$  % male located vibrating females on adjacent rice plant at this velocity ( $F=56.0$ ,  $dF=2, 42$ ;  $P<0.05$ ). Meanwhile, continuous blowing of wind by the electronic fan at a velocity of more than 1 m/sec completely hindered BPH from performing any mating activity. In fact insects were observed to be engaged in adjusting their balance on the plant rather than producing abdominal vibration or male dance.

## DISCUSSION

To study the mating behavior of insects, species recognition is important because mating behaviour displayed by each individual is species specific; thus any

**Table II.- Temporal effects of different phases of day on mating behaviour *N. lugens* (Means±S.E).**

Phases	Periods	FAV %	MD %	MA %	SM %	MTD (sec.)
Phase-I	Pr-I	100.0±0.0 a	100.0±0.0 a	75.0±25.0 ab	75.0±25.0 ab	62.0±20.9 abc
	Pr-II	100.0±0.0 a	75.0±25.0 ab	50.0±28.8 abc	50.0±28.8 abc	41.7±24.1 bcd
	Pr-III	75.0±25.0 ab	50.0±28.8 bc	50.0±28.8 abc	50.0±28.8 abc	40.7±23.7 bcd
	Total	91.6± 8.3ab	75.0±13.1ab	58.3±14.9ab	58.3±14.9ab	48.0±12.3b
Phase-II	Pr-I	50.0±28.8 b	25.0±25.0 c	0.0±0.0 c	0.0±0.0 c	0.0±0.0 d
	Pr-II	75.0±25.0 ab	50.0±28.8 bc	25.0±25.0 bc	25.0±25.0 bc	19.2±19.2 cd
	Pr-III	75.0±25.0 ab	75.0±25.0 ab	50.0±28.8 abc	50.0±28.8 abc	46.5±26.8 bcd
	Total	66.6±14.2b	50.0±15.1b	25.0±13.1b	25.0±13.1b	21.9±11.5b
Phase-III	Pr-I	100.0±0.0 a	100.0±0.0 a	75.0±25.0 ab	75.0±25.0 ab	72.5±25.0 ab
	Pr-II	100.0±0.0 a	100.0±0.0 a	100.0±0.0 a	100.0±0.0 a	99.0±8.4 a
	Pr-III	100.0±0.0 a	100.0±0.0 a	75.0±25.0 ab	75.0±25.0 ab	64.7±21.7 abc
	Total	100.0±0.0a	100.0±0.0a	83.3±11.2a	83.3±11.2a	78.7±11.2 a

Means followed by different letters within the same column are significantly different ( $p \leq 0.05$ ). For other abbreviations see Figure 3. Phase-I= Morning (0800 to 1100 hours), Phase-II= Afternoon (1200 to 1500) and Phase-III= Evening (1800 to 2100).

**Table III.- Effects of variable wind velocities on mating behaviour of *Nilaparvata lugens* (Means±S.E).**

Wind velocity (m/sec)	FAV %	MD %	MA %	SM %	MTD (sec.)
1.5-2.0	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b
1.0-1.5	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b
0.0-1.0	86.6±9.1 a	86.6±9.1 a	80.0±10.0 a	80.0±10.0 a	72.6±11.4 a

Means followed by different letters within the same column are significantly different ( $p \leq 0.05$ ). For abbreviations see Figure 3.

form of error in species identification will interfere in the subsequent studies. In insects the genitalia are the most important characters that distinguish the species, especially aedeagi for male and styles lateral lobes for female adults (Mochida and Okada, 1979). In this study, prior to the establishment of stock culture, the collected females from both locations were identified for *lugens* species. Species we confirmed by observing the top portion of the lateral lobes which is also known as Valvifer-VIII. A convex (raised face) and not apically bifurcated genitalia were the distinct feature of *N. lugens* (Mochida and Okada, 1979). Similar descriptions of the genitalia has also been given by Wilson and Claridge (1984) who described the distinct features between BPH species; in which Valvifer-VIII is rounded in *lugens* and hooked at the base in *bakeri* and *muri* species of *Nilaparvata* genus. Further, Heady and Wilson (1990) has also provided reliable features for female identification through genital examination in planthoppers.

Variation in age plays an important role in the display of mating responses in BPH. In this study the ages of both sexes were selected based on previous mating behavioural studies on brown planthopper

(Ichikawa and Ishii, 1974; Takeda, 1974), cotton leafhopper, *Amrasca devantans* (Dist.) (Kumar and Saxena, 1986) and Nearctic leafhopper, *Scaphoideus titanus* (Ball). According to these studies the most crucial age in plant and leafhopper for displaying mating response is between one to ten days. In our selected pairings, females did not vibrate her abdomen until she reached the age of three days. This highlighted that sexual maturity occurred at a later stage in female BPH as opposed to male which responded on the first day after emergence. Similarly, it has been also observed in *Heiroglyphus* spp. regarding early maturity of male compared to female (Riffat and Wagan, 2008). Further, FAV was synchronised with the maturity of female (the age having maximum mature eggs) with a peak at fifth day after last eclosion. Female behaviour is usually more complex than that of males because she invests more in offspring than males (Ayasse *et al.*, 2001) thus her behaviour changes in accordance with the activity of her corpora allata. Female abdominal vibration was observed strongly synchronized to the mating willingness of BPH (Ichikawa and Ishii, 1974) which indirectly reflect her maturity. Claridge *et al.* (1984) reported that BPH females do not vibrate spontaneously like male and her

vibration is occasional on rice plant during courtship. Often, virgin females displayed vibrational response at the beginning of her maturity on third day (Butlin, 1993) and it often peaks on fifth and seventh day after emergence (Ichikawa and Ishii, 1974) which continued throughout the entire lives of virgin females at a varying rate. However, such responses vary in winged and wingless insects and it also depend on food supplies. In addition, the age of both partners and stimulation by male activity present on the same rice plants does also influence the FAV (Ichikawa, 1979). Kumar and Saxena (1978) reported that with the increase in age of female *Amrasca devastans* (Dist.) there was a decline in their sexual response and this was possibly due to an increase of the mature oocytes in their ovarioles. Such physiological conditions could be the reason for the decline in FAV in older BPH female as observed in this study; low response (FAV) was observed in aged female (nine days old). However older females were observed to allow males to mate with minimum rejection. This helps the aging female who already has a shortened preference window for the selection of best males to increase her reproductive potential.

In mating response of BPH, it was observed that male does not often refuse copulation with vibrating female after locating her (Claridge *et al.*, 1984) but his response could be affected by physiological factors like age or changes in environmental condition. Therefore some young and old males did not succeed in producing a maximum percentage of stimulation when paired with sexually mature five days old female; and even if they responded, all of those males did not successfully arrive beside the vibrating female within experimental time. Often, an aggressive display of MD in response to FAV among males peaked at five days of age and declined with the increase in male age. Similar aggressive mating response has also been observed in cotton leafhopper, *Amrasca devastans* (Dist.) Kumar and Saxena (1986) and in previous studies of BPH by (Ichikawa, 1979; Takeda, 1974) and male Caribbean fruit flies, *Anastrepha suspense* (Loew) (Teal *et al.*, 2000). This again highlights that too young and old males are not at the peak of mating efficiency.

In response to FAV, males crossed the connected leaf blades, went down the stem and oriented themselves parallel with female but slightly to her rear and initiated mating efforts. Prior to copulation, the receptive female lifted her abdomen and male raised his wings vertically above his body and turned as to make genitalic contact (Fig. 5) with female, thus ensuring successful mating (Claridge *et al.*, 1984). Here, FAV is also seen as a guide for male to locate the female depending upon the vibrating frequency (Ichikawa, 1979). Even on successful

arrival of male, only a few were accepted by virgin females for mating. The maximum

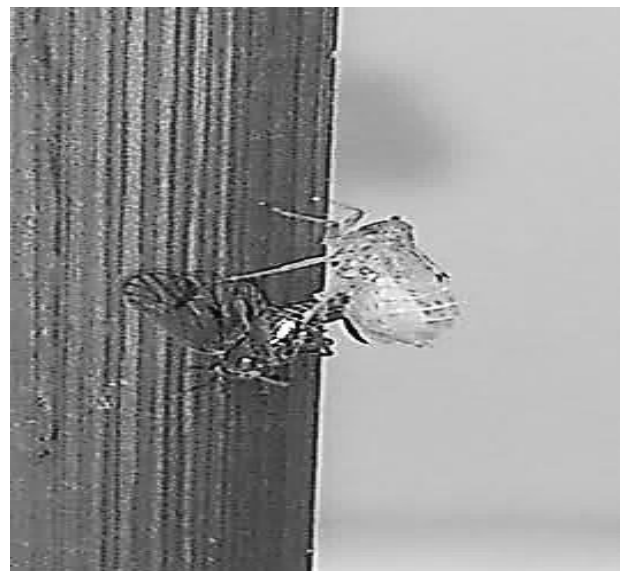


Fig. 5. Macropteris male (dark brown color) and brachypteris female (pale yellow color) showing mating in which male raised his wings vertically above his body and turned downward as to make genitalic contact with female for ensuring successful copulation on rice plant

percentage of receptiveness was mostly observed in five to seven days old female and her willingness often correlated with male fitness (age). In BPH, females mate for limited number of times, whereby two to three times of mating is quite sufficient for their entire life to fertilize their eggs (Mochida and Okada, 1979; Heong and Hardy, 2009). Therefore, they were found to be selective in choosing their partner; often scrutinizing either male size or male call to ensure the fittest male sires the progeny. Unlike females, males showed post copulatory mating response because they often can mate with a maximum of nine females within 24 h (Mochida and Okada, 1979; Heong and Hardy, 2009). Often, aged female of nine days old showed less interest in long mating duration and was observed kicking and repelling the male to dislodge immediately after a short mating duration when paired with different aged group of male.

Generally, male insects invest energy in locating mates and producing sperm and they often copulate with what appears to be any available and receptive female. However, their success is often at peak during their prime age which is roughly at five days old which coincides with their sexual maturity and fitness. Meanwhile,



females mate choice is subjected to a variety of influencing factors such as size, colour, fighting or courting activities including male calls. These conditions do further interact with the sexual maturity age of females which begins in three days old female. However, flexibility in mate selection has been observed in older females possibly to increase reproductive potential (Butlin, 1993).

In this study, it was found that the source of food ingested by BPH does also play an important role in the mating behaviours displayed. It was observed that the mortality percentage was higher in third instar nymphs that were reared on old age rice plants. Further, adults of both sexes that emerged from older plants could not display aggressive and successful mating response as compared to the other BPH pairs which were reared on young rice plants. This situation could be due to the lack of succulent nutritious parts in old plants which is essential in providing sufficient energy for the physiological process of maturity and metabolic function within the body of insects. Previously, Chen and Cheng (1978) and Win *et al.* (2011) observed that BPH growth and rice damage level was found higher at the tillering and booting stage and least at milking stage of rice plant. This clearly indicates the preference of insects towards a specific age stage in plants that can ultimately support their physiological and metabolic development. Often, insufficient food supplies affect the maturity of ovaries in female BPH which results in delayed mating behaviour in BPH females (Kumar and Saxena, 1978). Denno *et al.* (1991) and also reported that planthoppers survive better moult and fecund if they develop on nitrogen rich host plants. Similarly observation from this study highlights that all mating parameter of BPH were less aggressive or less responsive in BPH pairs that were reared on old rice plants.

When observing the effect of varying temporal phases on the mating response of BPH, it was found that both sexes can mate anytime throughout the day with certain fluctuation and the possible reasons of such fluctuation could be variable environmental factors such as temperature, wind and light which need to be further identified thoroughly. Here, we observed that insects have set certain periodical window for mating. The maximum mating response of BPH was observed in evening and morning time and based on such information their mating behaviour could be considered as crepuscular.

Sakai and Ishida (2001) reported that insects show specific preference window for mating because the physiological and behavioural activities of many insects are often restricted to specific times of the day. Such as *Heiroglyphus* spp., mate round the clock but peak was at the day time (Riffat and Wagan, 2008) that showed

mating preference in particular time of the day. The changes in mating response under the influence physiological and environmental conditions have been reported in a number of insects (Engelmann, 1970; Haskell, 1974; Kawada and Kitamura, 1983; Kumar and Saxena, 1986). Ichikawa (1979) had reported that both sexes of the different species of plant and leafhoppers, [*Nilaparvata lugens*, *Laodelphax striatellus* (Fallén), *Sogatella furcifera* (Horváth), *Nephotettix cincticeps* (Uhler)] displayed mating behaviour irrespective of the time of the day on rice seedling under laboratory conditions. However, Suenaga (1963) observed the copulation of *N. lugens* frequently from 1800-2000 in a greenhouse condition and similarly Arai (1977) observed the copulation of leafhopper, *Hishimonus sellatus* (Uhler) from evening to night in field conditions. In addition, the active flight behaviour in *N. lugens* and *L. striatellus* is often recorded in the early morning and from evening to night (Kisimoto, 1968; Ohkubo and Kisimoto, 1971; Macquillan, 1975). It is probable that the insect mated more frequently during those hours as they are capable of moving efficiently to locate mate (Ichikawa, 1979). Therefore, under such findings it could be interpreted that movement of insects in such particular time is related with mating behaviour and similar results were observed in this study.

Under the influence of unfavourable environmental condition as in Phase-II which was affected by the high temperature of the day, males required more time to locate female. In mate searching efficiency, environmental condition is one of the most essential influencing factor that have been realized in different phases of the day because temperature would have the potential to affect the moving and flying activities of insects which in turn influences their timely search for their mates (Kondoh and Ide, 2003). The mating phases of insects are also influenced by the presence of predators, thus the interconnected relationship between predatory activity and circadian rhythm and could affect the choice of the period of mating (Ashe and Timm, 1987). Similarly, study on reproductive strategy of the Nearctic leafhopper, *Scaphoideus titanus* (Ball) by Mazzoni *et al.* (2009) observed that peak mating behaviour were in the early evening period from 1800–2000 within 24 h. Similarly, crepuscular flight activity has been reported for leafhoppers of the genus *Dalbulus* (Taylor *et al.*, 1993).

Variation in temperature also has an impact on the mating response of BPH. Insects were found to be most responsive at a temperature of 27°C; however temperature below or above this limit results in changes to their mating response. Long *et al.* (2012) previously observed similar findings whereby temperatures of 20°C

and 32°C inhibited the production of FAV and to some extent reduced male response to FAV in BPH. Our results are also consistent with Liu *et al.* (2004) who reported that the proportion of female abdominal vibration in *N. lugens* reduced by 7.8% and 16.3% at 32°C and 18°C, respectively as compared to 28°C.

The abdominal vibration in *N. lugens* is controlled by a pair of dorsal longitudinal muscle (Mitomi *et al.*, 1984) and the performance of these muscles may depend on the thoracic temperature, which in turn depends on the ambient thermal conditions. Variations in environmental temperature often affect the metabolic rate of insect and this in turn influences the insect's daily activities (Prosser and Nelson, 1981; Hazel, 1995; Krebs and Holbrook, 2001). As temperature decreases, metabolism slows as the kinetic energy imparted to chemical reactions decreases, resulting in reduced activity. Often at thermal extremes, the functions of nervous systems are often first reduced as compared to other essential major functions and hence behavioural responses may be impacted before protein denaturation occurs (Prosser and Nelson, 1981). Thus, insect movement will stop when temperature decreases (Hazell *et al.*, 2010). The low temperature at which insects cease their movement (CT min) is however, normally above their lower lethal threshold and does not change protein structure but in contrast reduces their potential effectiveness (Piyaphongkul *et al.*, 2012). The effect of temperature on the physiological behaviour such as induction of diapause has also been studied in detail by Saunders (2014).

According to Ichikawa (1979) the efficiency of male in finding vibrating female is synchronized by FAV which in turn could be altered by changes in the body temperature (Tb) or ambient temperature (Ta). Any changes may result in receiver not responding correctly, thus resulting in communication breakdown (Drosopoulos and Claridge, 2005). Suitable temperature is essential to facilitate the development, survival, and reproduction of *N. lugens* (Li, 1984; Chen *et al.*, 1986; Zhu *et al.*, 1994). A temperature of 25°C to 28°C often promotes, whereas low temperature of 18°C to 20°C and high temperature of 32°C to 35°C reduces the mating in *N. lugens* (Yu and Wu, 1991; Liu *et al.*, 2004). Based on this study, we postulate that temperatures of 22±2°C and 32±2°C negatively affected the physiology of the muscle controlling the vibration behaviour of both sexes and thus inhibited FAV and reduced the ability of male to locate female.

As BPH communication relies on vibrational signals that are transmitted through plants, any form of external vibration will interrupt the mating signals and eventually hinder the communication of BPH. Previous studies on plant-dwelling arthropods showed that

behaviourally relevant abiotic noise arises predominantly from wind (Barth *et al.*, 1988; Casas *et al.*, 1998; Cocroft and Rodriguez, 2005; Tishechkin, 2007; McNett *et al.*, 2010). Such noises have primarily low dominant frequency (5-30 Hz) but when wind is strong enough to cause colliding of leaves, the frequency band can extend up to 200 Hz (Barth *et al.*, 1988). Therefore, indirectly altering or disturbing the mating response of insects present on these plants. Similarly, in this study BPH subjected to a wind velocity more than 1 m/sec did not exhibit any mating response. All selected pairs did not show any physical mating response when wind was high because their communication through substrate borne vibrational signal prior to mating was disturbed (Ichikawa and Ishii, 1974; Michelsen *et al.*, 1982; Claridge *et al.*, 1984; Butlin, 1993).

Another important characteristic of noise generated by wind is that it occurs on two different time scales. At a given location, it may show predictable variation over the course of the day because wind speed is lower in the morning and in the evening. Meanwhile unpredictable variation can occur on a scale of seconds or minutes (Cocroft and Rodriguez, 2005; Tishechkin, 2007; McNett *et al.*, 2010). Based on the characteristic of wind flow, insects often adapt to the situation. For example, treehopper *Enchenopa binotata* (Say) who communicates in the similar manner as BPH was observed to communicate during the morning and evening when wind speed is lowest. Male response was also low during wind bursts as compared to wind-free gaps. Males also avoided producing advertisement signals during wind gusts. Female failed to respond to male when wind was at 1.5 m/sec however in the absence of wind-induced noise at 0.7 m/sec females of tree hopper responded more consistently (McNett *et al.*, 2010). Therefore, it could be understood that an optimum wind velocity is essential for proper communication between both sexes of BPH.

The relationships between all of these factors are also important to know. Based on the personal observations during this study, it could be understood that the relationship between wind velocity and temperature was negative during day and night. Whereas, there were neither any influence of wind velocity on insect age nor host plant age thus it was not important to noted. However, based on our preliminary understanding on the level and influence of the studied factor on the BPH mating behaviour, this data can be further utilized in future to study any possible simultaneous influence of these factors in field conditions.

## CONCLUSION

The influence of insect age, plant age, temporal

phase, environmental temperature and velocity has been studied in detail on the mating behavior of brown plant hopper. Both sexes of BPH achieve sexually maturity and showed maximum mating responses on the fifth day after last eclosion. In eliciting timely maturity of both sexes on the fifth day after emergence, 25-30 d old rice plant served as an excellent food source. Further, BPH could mate any time when mate is available but the best time for mating was observed during the early morning and evening (Phase-III) because at that time the speed of wind velocity with optimum temperature favors the condition for initiating mating activities. Similarly, low and high temperature inhibited mating response of both sexes whereby insects were found most comfortable to mate at  $27\pm 2^{\circ}\text{C}$ , and highly sensitive at high  $32\pm 2^{\circ}\text{C}$  and low  $22\pm 2^{\circ}\text{C}$  temperature ranges. In addition, BPH only exhibited active mating behavior at 0-0.1.0 m/sec and at any other wind velocity no mating response was observed. The relationship between mating response with these physical and physiological factors has not been previously studied in detail on BPH. Therefore, such basic information will be helpful to execute certain control measures to manage pest population below significant levels. Further, such studies will be supportive to observe the mating behavior of BPH by using high definition transducer for our future studies.

### ACKNOWLEDGEMENTS

This work was supported by the Fundamental Research Grant Scheme (FRGS) in Malaysia. We also like to thank Sindh Agriculture University, Tandojam, Sindh, Pakistan for the financial support granted to the first author.

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