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PHARMACOLOGICAL PROPERTIES OF SEROTONIN SYSTEM INVOLVED IN FEEDING BEHAVIOR OF *Nilaparvata lugens* STÅL (HEMIPTERA: DELPHACIDAE)

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A monoamine neurotransmitter, 5-hydroxytrptamine (5-HT) or Serotonin is an important regulator of many behavioral and physiological processes in both plants and animals. The involvement of 5-HT in the feeding behavior of different insect species has been reported; however, the effect of this neurotransmitter on feeding behavior of *Nilaparvata lugens* is still not investigated. Five distinct waveforms were observed by the feeding of *N. lugens* on sucrose diet using electropenetrography. Present study proved that 5-HT significantly increased the time of first insertion of stylet into the diet at both 100 mg L^{-1} (26.97 min) and 1000 mg L^{-1} (89.90 min); however, no significant difference was observed in average and total time of ingestion by *N. lugens* feeding on 5-HT. Ketanserin is the antagonist of 5-HT receptor which showed significant increase in average and total duration of non-probing period at 25 and 100 mg L^{-1}. The mean percentage of ingestion significantly decreased while insect feeding on 5-HT receptor antagonists mianserin hydrochloride and yohimbine hydrochloride. Methyldopa is the inhibitor of aromatic L-amino acid decarboxylase enzyme which helps in synthesis of serotonin. It significantly increased the total non-probing duration (218.45 min) but decreased (106.70 min) the total ingestion duration of *N. lugens* when feeding at 200 mg L^{-1} concentration. 5-HT receptor antagonists reduced the feeding ability of *N. lugens*.
Keywords: Brown plant hopper, serotonin, electrical penetration graph, receptor antagonists, feeding behavior.

INTRODUCTION

Hemipterans insect pests have been proved difficult to control despite of changes in farming practices, integrated pest management (IPM) strategies and synthesis of improved insecticides (Heong and Hardy, 2009). The brown plant hopper (BPH), *Nilaparvata lugens* Stål (Hemiptera: Delphacidae), is one of the most destructive pests among the herbivorous rice insects. Direct damage by BPH includes sucking the cell sap resulting in yellowing, drying of leaves, wilting of tillers and drying of the whole crop which is known as "hopper-bun" (Hao *et al*., 2008) caused due to severe infestation. Indirect damage includes vectoring of ragged stunt virus and grassy stunt virus diseases that cause economic loss (Cabauatan *et al*., 2009).

High levels of resistance against different insecticides (Whitten and Oakeshott, 1991), resistant-breaking biotypes against resistance rice varieties (Pathak and Khush, 1979) and development of pest resurgence (Hadfield, 1993) magnifies the problem for the management of this pest. This difficulty is protracted into the genetic engineering approach due to the lack of effective Bt-toxins *(Bacillus thuringiensis*).

N. lugens feeding depends upon the phloem chemistry and specific feeding stimulant (Cook and Denno, 1994). Limited amount of information is available on the constitutive and adaptive responses of plants by phloem feeding insects. However, characterization of the different interactions that exist between plant and phloem feeding insect is a rapidly expanding field of research (Gatehouse, 2002). Knowledge of these plant-insect interactions will be crucial for the development of successful new control strategies. 5-HT plays a critical role in different biological process of human and its disruption causes many disorders (Jones and Blackburn, 2002). As in vertebrates, serotonergic pathways also modulate feeding behavior of many insect species (Dacks *et al*., 2003; Kaufmann *et al*., 2004; Neckameyer, 2010; Falibene *et al*., 2012).

Serotonin neurons and their receptors have been involved in controlling feeding activities of several insects and have been reported to be found in the foregut of several insect species including ants (C*amponotus mus*) (Falibene *et al*., 2012), fruit flies *(Drosophila melanogaster*) (Budnik *et al*., 1989; Neckameyer, 2010), stable flies (*Stomoxys calcitrans*) (Liu *et al*., 2011), locusts (*Locusta migratoria*) (Molaei and Lange, 2003) and mosquitos (*Aedes aegypti*) (Moffett and Moffett, 2005; Pietrantonio *et al*., 2001). In flesh fly *Neobelliera bullata*, serotonin reduces the feeding and decreases sucrose consumption in blowfly (Long and Murdock, 1983; Dacks *et al*., 2003).

The involvement of serotonergic system in feeding mechanisms of fruit fly, *D. melanogaster* (Neckameyer, 2010; Gasque *et al*., 2013), flesh fly, *Neobellieri abullata*,

Honey bee, *Apis melifera* (French *et al*., 2014), yellow fever mosquito, *Aedes aegypti* (Novak *et al*., 1995), *Calliphora vicina*, cockroach *Periplaneta americana* (Kaufmann *et al*., 2004; Walz *et al*., 2006), aphid, *Myzus persicae* (Kaufmann *et al*., 2004) has been documented; however, studies regarding the mediatory role of serotonin in feeding behavior of *N. lugens* is largely lacking.

The objective of this study was to find the effect of serotonin (5-HT) - a neurotransmitter and its receptor antagonists on the feeding behavior of *N. lugens* using Electropenetrography.

MATERIALS AND METHODS

Insect culture: Adults of *N. lugens* were collected from the rice field of Wuhan, Hubei, China and were reared continuously on rice seedlings of the TN1, a rice variety susceptible to *N. lugens* under laboratory (Hubei Insect Resources Utilization and Sustainable Pest Management Key Laboratory) controlled conditions at 25 ± 2°C, 60±5% RH and 14:10h (L: D) photoperiod. One or two days old adult females were used in all the experiments because of their larger size than male.

Chemicals: Chemicals which were used in this study shown in Table 1. Stock solution of chemicals was made in DMSO and diluted with distilled water to obtain desired concentrations (Table 1).

***Electrical recording of feeding behavior of N. lugens*:** To record the feeding behavior of N. lugens, Giga-4 DC EPG amplifier with a 10 Ω input resistance and an input bias current of less than 1pA (Wageningen Agricultural University, Wageningen, The Netherlands) developed by Tjallingii (1978) was used. Females were starved for 1 hour prior to the experiments. After being immobilized with CO_2 for approximately 10s, one end of a golden wire (20µm diameter ×10 cm length) was attached to the dorsal thorax of the insect with water-soluble silver conductive glue. The other end of the gold wire attached by the silver glue to the copper wire attached to the brass pin which was inserted into the input connector of the amplifier. Females carefully placed on the container containing diet (1% of the desired concentration of treatment mixes with 10% sucrose solution) covered by stretched parafilm membrane. Insects feed on sucrose solution in control. The gain of the amplifier was set at 50 x, the voltage of the diet solution was adjusted to obtain an output voltage of between -5 and +5 V. All EPG recordings were conducted at temperature of 25 ± 2°C, and 60±5% RH with continuous light conditions in a Faraday cage, to prevent the amount of ambient electronic noise entering the system. Each insect feeding was recorded for 6 h continuously.

The EPG signals were analyzed using PROBE 3.0 software (Wageningen Agricultural University, Wageningen, Netherlands). When insect feeds on sucrose solution diet, five waveforms were characterized as np, non-probing; P, penetration initiation; I, ingestion of diet; M, stylet movement in the diet; X, unknown activity of insect. EPG waveforms were analyzed using the method of Sarria *et al.* (2009) and He *et al*. (2011).

The number of occurrences of each waveform represents the number of times that a waveform occurred during the recording time. The total duration of each EPG waveform represents the sum of duration of all the occurrences of the waveform within the recording time. The average duration of each EPG waveform was the mean duration of each occurrence of a waveform within the observation time.

Additional parameters of EPG wave were also analyzed. These variables included the transition period from the start of the experiment to the first probe (P), the number of probes before the first ingestion I, duration from the beginning to the first Stable ingestion I, the duration of first I, the total duration on non-ingestion waveforms (including P, M, X waveforms), the longest duration of ingestion I, time from the beginning to the first X waveform occurred, the interval from first probing (P) to the first ingestion (I), the interval between the first and second probing P, the interval between from first ingestion to second ingestion I. Furthermore, the percentage of insects feeding in each concentration and in control was also calculated.

***Statistical analysis*:** EPG data were statistically analyzed using SPSS 20. The Duncan's multiple range test (DMRT) was conducted for multiple comparisons of the EPG variables between control (CK) and treatments at different concentrations. Statistical significance was set at $P < 0.05$.

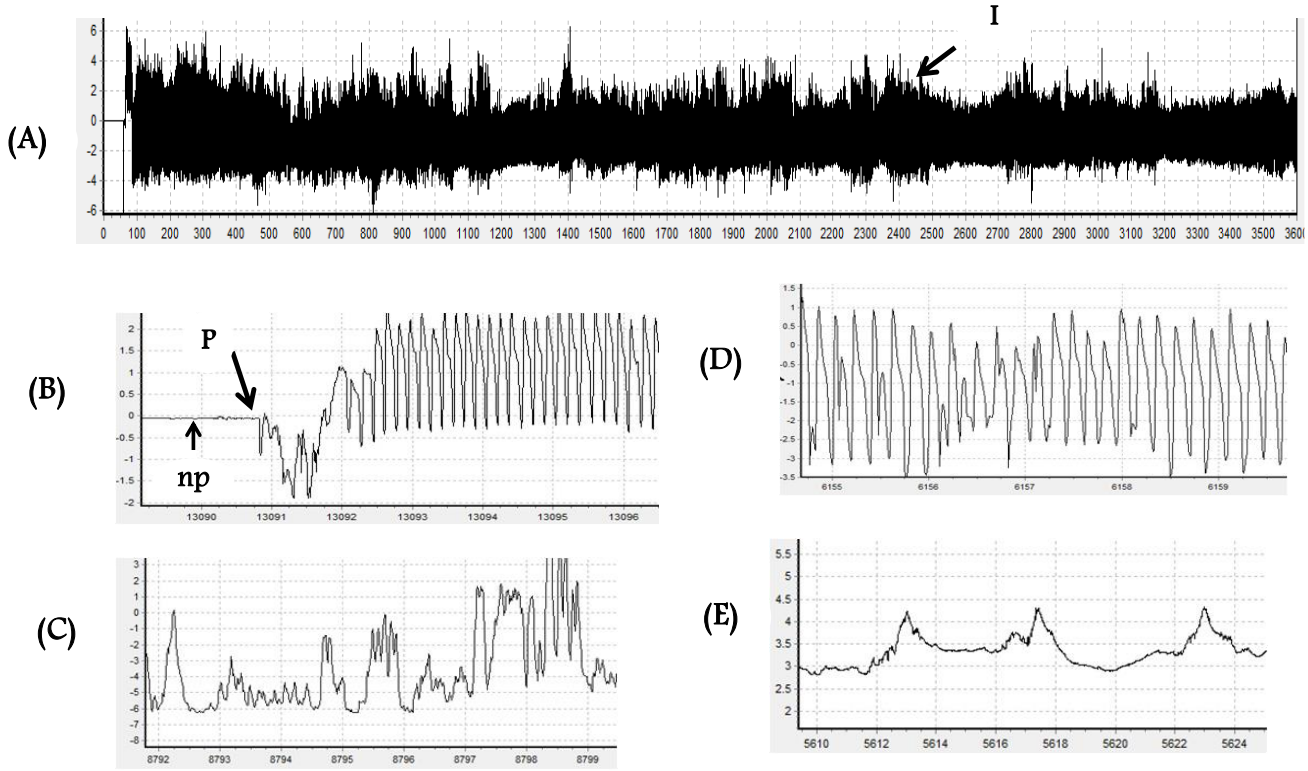
RESULTS

Characterization of the EPG waveforms: Feeding behavior includes all the activities performed. Feeding starts when an insect inserts its stylet into the artificial diet.

The first waveform np, stands for non-probing, in which insect stabilizes and has not inserted its stylet in the sucrose

Table 1. List of chemicals used in the present study.

Name	Action	Concentration
5-HT(5-hydroxytrptamine) (99%w/w)	Neurotransmitter	100 mg L^{-1}, 1000 mg L^{-1}
Ketanserin (98% w/w)	Receptor antagonist	1.5625 mg L^{-1}, 25 mg L^{-1}, 100 mg L^{-1}
Mianserin hydrochloride (100% w/w)	Receptor antagonist	100 mg L^{-1}, 200 mg L^{-1}, 400 mg L^{-1}
Yohimbine Hydrochloride (99% w/w)	Receptor antagonist	100 mg L^{-1}, 200 mg L^{-1}, 400 mg L^{-1}
Methyldopa (98% w/w)	Inhibitor of 5-HT synthesis enzyme (aromatic L-amino acid decarboxylase)	25 mg L^{-1}, 100 mg L^{-1}, 200 mg L^{-1}



X: time (seconds); Y: volt (V)

Figure 1. Typical EPG waveforms identified from *Nilaparvata lugens* when feeding on sucrose diet, showing general picture of 1h of recording (A); np, non-probing; P, probing (B); M, Movement of stylet in the diet (C); I, ingestion (D); X, Unknown activity of insect (E).

diet. A base line with some short and small signals frequently occurred. These signals may cause by the movement of insect on the surface. When insect inserted its stylet, there was sudden increase or decrease in the voltage, this waveform named as "P (Probing)" lasted for only few seconds. A smooth signal of same amplitude was shown called "I (ingestion)" (Fig. 1-C) waveforms always followed by P and M waveform, lasted from few minutes to several hours; repetitive multiple rapid peaks were formed as insect feeds on diet. Stylet movement in the diet resulted into irregular peaks labeled as M waveforms. X waveforms were occasional appeared and contained rather noisy signals and activity of the insect during these signals were still unknown. Irregular peaks at some interval were formed (Fig. 1-D). If comparison, np and I waveforms were the most appeared waveforms in all treatments (Fig. 3-7), X waveforms appear in all chemicals.

By the help of EPG technique, serotonin system involvement in feeding of *N. lugens* was identified. 5-HT and its receptor antagonists at different doses were selected for monitoring the feeding behavior. All variables were studied and analyzed to evaluate the role of neurotransmitter and its antagonists in feeding of *N. lugens*.

***Comparison between the feeding behavior of N. lugens on 5-HT, its receptor antagonists and its inhibition enzyme (Methyldopa)*:** 10-15 useable recordings were used to compare the behavior of *N. lugens* on sucrose diet and different doses of chemicals. Those recording were used in which insect reached the ingestion phase. By compare all the number of insects used for feeding behavior, we observed significance decreased in percentage of insects reaching ingestion phase on different chemicals. 5-HT at 1000 mg L^{-1}, Mianserin at 400 mg $L^{-1,}$ Yohimbine at 200 and 400 mg L^{-1} and Methyldopa at all doses showed significant reduction in feeding of test insects, 78% insects did not reach ingestion waveform. Ketanserin 1.5625 mg L^{-1} showed no difference (Fig. 2).

Two doses of 5-HT, 100 mg L^{-1} and 1000 mg L^{-1} were tested against adult females of *N. lugens*. Comparison between the percentages duration of all the waveforms within the observation time showed that significant difference was observed only in non-ingestion (nI) period. No significant differences were observed in percentage of I, np, M and X waveforms (Fig. 3).

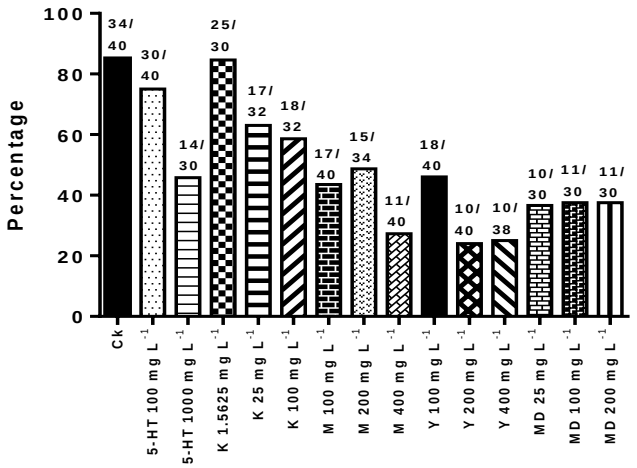


Figure 2. Percent feeding of *N. lugens* on different concentrations of chemicals having probe and ingestion waveforms used in present study. 5-HT: 5-hydroxytrptamine, K: Ketanserin, M: Mianserin hydrochloride, Y: Yohimbine hydrochloride, MD: Methyldopa. **5**-HT

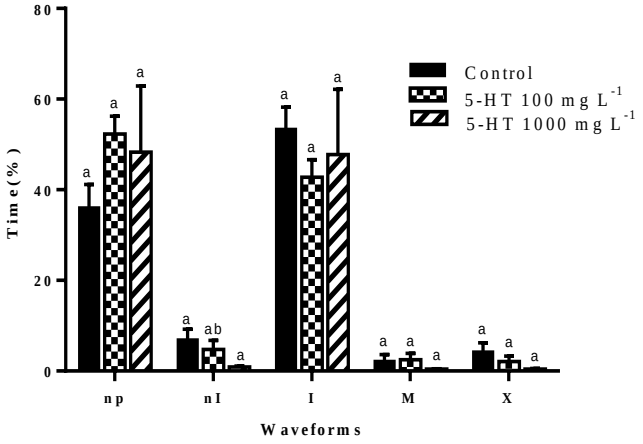


Figure 3. Mean percentage of time *N. lugens* spent in each waveform on different doses of 5-HT during 6-h recording. Np: non-probing, nI: non-ingestion (P+M+X), I: ingestion, M: Movement of stylet, X: Unknown activity of stylet.

When feeding on 100 mg L^{-1}, no significant differences was found in first time of insertion of stylet (CK= 15.36±4.16 min; 5-HT 100 mg L^{-1}= 26.97±6.07 min) but significantly increases the time duration in 1000 mg L^{-1} concentration (89.90±28.97min) (Table 2).

The time from first probe to the second and from first ingestion to the second was found non-significant at the 100 mg L^{-1} dose but it showed significant results when compared to 1000 mg L^{-1} (time from first to second probe= 0 min; time from first to second ingestion =26.74±10.68min).

Between concentrations of 5-HT, total duration of non-probing, longest ingestion duration, time from first probe to first ingestion showed no significant change. Similar results were observed in total and average mean duration of M and X waveforms. Number of occurrences of "np" (2±0.6) and "I" (1.54±0.46) in 5-HT 1000 mg L^{-1} (Table 2) were found significant. Highest concentration i.e. 1000 mg L^{-1} delayed the first time of probing about 70 min as compared to control. No significant differences were observed in total duration of ingestion and average duration of ingestion as insects recovered from it after 1-2 h and start feeding normally.

5-HT receptor antagonists: Mianserin hydrochloride, Ketanserin and Yohimbine hydrochloride are receptor antagonists used to examine the feeding behavior of N. lugens. Comparison between 5-HT and its receptor antagonists showed more effectiveness in inhibiting the feeding of N. lugens**.**

Mianserin hydrochloride: In 200 mg L^{-1} and 400 mg L^{-1} concentration percentage of np waveform significantly increased and I waveforms were significantly decreased (Fig. 4). Ingestion was suppressing as a result of feeding on mianserin hydrochloride different doses.

Total duration of np increases and ingestion decreases with the increase in concentrations of mianserin hydrochloride (Table 3). Likewise the mean total duration of ingestion was significantly decreased with the increase in the concentration of mianserin hydrochloride. But there were no significance difference in mean average duration of ingestion in different concentrations of mianserin hydrochloride. Except X waveforms, significance differences were showed in mean number of occurrences on non-probing, probing, ingestion and stylet movement.

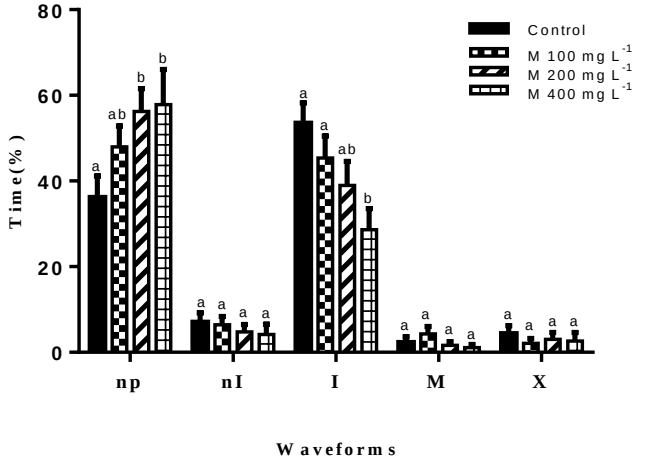


Figure 4. Percentage of time insect spent in each waveform on different doses of Mianserin hydrochloride during 6-h recording. Np: non-probing, nI: non-ingestion (P+M+X), I: ingestion, M: Movement of stylet, X: Unknown activity of stylet.

Table 2. Comparison of 6-h EPG waveforms (responses) of different concentrations of 5-HT on feeding behavior of *N. lugens* adults.

	5-hydroxytryptamine		
Variables	**Control**	**5-HT 100 mg L^{-1}**	**5-HT 1000 mg L^{-1}**
Time to first probe	15.36±4.16 b	26.97±6.07 b	89.90±28.97 a
No. of np occurrences	3.8± 0.3 a	4.0±0.4 a	2±0.01 b
Total np duration	130.21±17.87 a	167.48±11.17 a	173.92±30.60 a
Duration of first probe	108.55±28.72 ab	94.02±14.45 b	175.04±31.89 a
No. of probes occurrences	2.9±0.30 a	3.0±0.46 a	1±0.30 b
Average I Duration	89.20±21.42a	68.88±14.90a	127.75±28.09a
Total I Duration	192.48±17.22 a	170.64±13.38 a	171.92±30.92 a
Longest I duration	134.19±22.94 a	94.51±14.23 a	145.47±28.25 a
No. of I waveform occurrences	3.53±0.06 a	3.83±0.85 a	1.54±0.46 b
No. of M waveforms occurrences	3.26±0.63 a	3.0±1.14 a	0.81±0.24 a
No. of X waveform occurrences	0.7±0.30 a	0.91±0.48 a	0.09±0.02 a
Time from 1st P to 2nd P	126.27±28.99 a	141.37±26.38 a	0 b
Time from 1st I to 2nd I	101.28±24.39 a	132.14±27.29 a	26.74±10.68 b

The values in this table is Mean± SE, the unit of duration variable is min. Mean followed by same letter in rows are not significantly different at *P=0.05* among the different concentration.

Table 3. Comparison of 6-h EPG waveforms (responses) of different doses of Mianserin hydrochloride on feeding behavior of *N. lugens* adults.

		Mianserin Hydrochloride		
Variables	**Control**	**100 mg L^{-1}**	**200 mg L^{-1}**	**400 mg L^{-1}**
Time to first probe	15.36±4.16 c	20.98±3.49 c	72.18±13.15 b	161.84±36.58 a
No. of np occurrences	3.8±0.32 a	3.58±0.61 ab	2.3±0.18 c	2.4±.37 bc
Total np duration	130.21±17.87 b	172.61±2.87 ab	202.50±19.11 a	218.55±29.26 a
Duration of first probe	108.55±28.72 a	99.78±1.66 a	130.65±21.51 a	98.93±31.47 a
No. of probes occurrences	2.9±0.30 a	2.5±0.61 ab	1.3±0.18 c	1.6±0.4 bc
Average I duration	89.20±21.42 a	58.30±0.97 a	100.51±22.31 a	103.05±30.74 a
Total I duration	192.48±17.22 a	163.36±2.72 ab	140.21±20.22 ab	116.23±28.43 b
Longest I duration	134.19±22.94 a	88.66±1.47 a	125.53±19.83 a	108.00±29.54 a
No. of I waveform occurrences	3.5±0.61 ab	5.3±1.2 a	1.9±0.2 b	1.8±0.69 b
No. of M waveforms occurrences	3.26±0.63 ab	5.16±1.29 a	1.4±0.32 b	1.9±1.07 b
No. of X waveform occurrences	0.73±0.30 a	0.41±0.26 a	0.33±0.12 a	0.70±0.47 a
Time from 1st P to 2nd P	126.27±28.99 a	91.24±1.52 a	6.83±4.55 b	5.24±3.64 b
Duration of 1st I waveform	108.93±26.91 a	54.33±0.90 a	98.94±22.95 a	100.05±31.49 a
Time from 1st I to 2nd I	101.28±24.39 b	42.07±0.70 a	26.89±8.91 a	3.56±3.41 a

The values in this table is Mean± SE, the unit of duration variable is min. Mean followed by same letter are not significantly different at *P=0.05* among the different concentration.

If compare the time of first probe between the doses, it was revealed that in 400 mg L^{-1} (161.84±36.58 min) insect take more time to insert its stylet, although normal feeding starts after 1-2 h. Significant differences in duration from first probe to the second in 200 mg L^{-1} and 400 mg L^{-1} were also recorded. No significant differences were found in time from first probe to first ingestion, time from first ingestion to second ingestion, average and total mean duration of M and X waveforms.

Ketanserin: Among the antagonists, Ketanserin proved to be having more role in feeding behavior of *N. lugens* as it causes inhibition of feeding. It causes hesitation to insect for stylet insertion (100 mgL^{-1}= 161.84±36.58 min).

Results showed that time of np increased while time spent by insect for ingestion significantly decreased. On the other hand, there was no significant difference in percentage of P, M and X waveforms (Fig. 5)

The mean total duration of np was increased with the increase in the dose of Ketanserin (K 1.625 mg L^{-1}=162.45±22.89 min; K 25 mg L^{-1}=212.17±20.02 min; K 100 mg L^{-1}= 220.73±30.05). There are no significant differences found between the number, average duration and total duration of M and X waveforms (Table 4). Significant differences were detected in total time of feeding by the insect.

Table 4. Comparison of 6-h EPG waveforms (responses) of different doses of Ketanserin on feeding behavior of *N. lugens* adults.

ıriables	Control	Ketanserin		
		K 1.5625 mg L^{-1}	K 25 mg L^{-1}	K 100 mg L^{-1}
me to first probe	15.36±4.16 b	10.13±2.71 b	63.93±9.48 a	69.40±11.72 a
). of np occurrences	3.8±0.32 a	6.53±1.52 a	3.81±0.88 a	4.4±0.80 a
tal np duration	130.21±17.87 b	162.45±22.89 ab	212.17±20.02 a	220.73±30.05
ıration of first probe	108.55±28.72 a	78.29±22.96 a	93.28±23.01 a	84.92±27.65 a
). of probes occurrences	2.9±0.30 a	5.53±1.52 a	2.81±0.88 a	3.4±0.80 a
erage I duration	89.20±21.42 a	51.63±18.86 a	67.18±18.44 a	40.86±10.45 a
tal I Duration	192.48±17.22 a	139.31±21.63 ab	113.00±12.93 b	115.27±29.88
ngest I duration	134.19±22.94 b	85.74±18.51 ab	82.35±16.02 ab	64.23±15.37 a
). of I waveform occurrences	3.5±0.61 ab	3.86±0.59 a	2.45±0.6 a	2.53±0.72 a
me from first probe to first ingestion	6.738±4.705 a	15.04±10.47 a	5.70±4.02 a	7.29±6.83 a
). of M waveforms occurrences	3.26±0.63 ab	5.78±1.12 a	3±0.83 a	3.9±0.7 a
). of X waveform occurrences	0.73±0.30 a	3.34±0.95 a	1.45±0.86 a	1.26±0.49 a
me from 1st P to 2nd P	126.27±28.99 a	103.68±26.68 ab	86.34±27.38 ab	41.70±16.02 b
ıration of 1st I waveform	108.93±26.91 a	59.66±20.72 ab	66.47±18.64 ab	34.00±10.53 b
me from 1st I to 2nd I	101.28±24.39 a	115.24±24.96 a	59.40±24.02 ab	33.15±10.40 b

The values in this table is Mean± SE, the unit of duration variable is min. Mean followed by same letter are not significantly different at *P=0.05* among the different concentration.

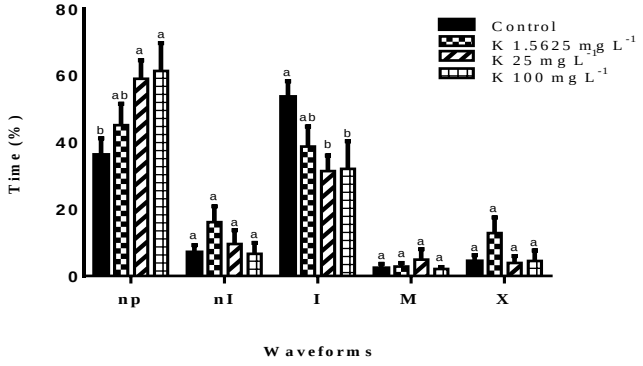


Figure 5. Percentage of time insect spent in each waveform on different doses of Ketanserin during 6-h recording.

Yohimbine hydrochloride: At 200 mg L^{-1} and 400 mg L^{-1} doses of Yohimbine, the percentage of ingestion was reduced, and np waveforms percent time was increased (Fig. 6). Significant differences in ingestion by insect were observed in 200 mg L^{-1} and 400 mg L^{-1} concentrations of Yohimbine hydrochloride.

The values in this table is Mean± SE, the unit of duration variable is min. Mean followed by same letter are not significantly different at *P=0.05* among the different concentration.

Feeding on 100 mg L^{-1} concentration of yohimbine hydrochloride showed no differences in all variables as compared to feeding on sucrose diet except in the number of occurrences of I waveform (Table 5). Time from first probe to second probe is significant in all doses of yohimbine hydrochloride.

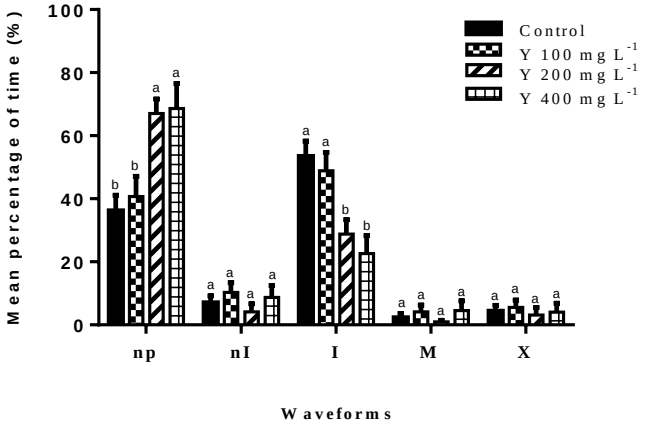


Figure 6. Percentage of time insect spent in each waveform on different doses of Yohimbine hydrochloride during 6-h recording. Np: non-probing, nI: non-ingestion (P+M+X), I: ingestion, M: Movement of stylet, X: Unknown activity of stylet.

Total mean duration of ingestion was significantly decreased in 200 and 400 mg L^{-1} but no differences was observed in mean duration of ingestion. No effect on the average ingestion was observed due to recovery of the insect although; total duration of ingestion was effected due to delay to the first probe.

Table 5. Comparison of 6-h EPG waveforms (responses) of different doses of Yohimbine hydrochloride on feeding behavior of *N. lugens* adults.

Variables	Control	Yohimbine Hydrochloride		
		Y 100 mg L^{-1}	Y 200 mg L^{-1}	Y 400 mg L^{-1}
Time to first probe	15.36±4.16 a	16.80±4.54 a	92.30±31.44 b	78.60±25.82 b
Total np duration	130.21±17.87 b	146.67±22.95 b	241.43±16.43 a	247.18±28.49 a
Duration of first probe	108.55±28.72 a	117.24±32.57 a	78.20±16.64 a	97.22±28.81 a
No. of probes occurrences	2.9±0.30 a	3.7±0.99 a	2.2±0.81 a	3.2±1.16 a
Longest I duration	134.19±22.94 a	106.54±22.12 ab	74.72±16.12 ab	62.48±17.95 b
Average I duration	89.20±21.42 a	66.18±23.82 a	48.74±11.15 a	48.09±17.74 a
Total I duration	192.48±17.22 a	175.99±20.84 a	103.60±16.53 b	81.38±20.37 b
No. of I waveform occurrences	3.5±0.61 ab	7.14±2.09 a	4.4±1.48 ab	2±0.51 b
No. of M waveforms occurrences	3.26±0.63 ab	6.64±2.19 a	3.7±1.53 a	2.8±0.74 a
No. of X waveform occurrences	0.73±0.30 a	0.64±0.24 a	0.6±0.4 a	1.2±0.69
Time from 1st P to 2nd P	126.27±28.99 a	117.07±32.56 ab	35.65±18.79 b	46.07±20.37 ab
Duration of 1st I waveform	108.93±26.91 a	84.40±23.84 a	54.13±15.34 a	58.40±18.56 a
Time from 1st I to 2nd I	101.28±24.39 a	98.39±28.99 a	60.17±17.39 a	43.80±21.67 a

Table 6. Comparison of 6-h EPG waveforms (responses) of different doses of Methyldopa on feeding behavior of *N. lugens* adults.

iables	Control	Methyldopa		
		MD 25 mg L^{-1}	MD 100 mg L^{-1}	MD 200 mg L
e to first probe	15.36±4.16 b	47.97±16.13 b	99.26±27.08 a	126.19±22.55
of np occurrences	3.8±0.32 a	2.9±0.43 a	2.8±0.29 a	3.45±0.45 a
ation of first probe	108.55±28.72a	139.47±29.94 a	82.16±15.75 a	78.70±9.50 a
of probes occurrences	2.9±0.30 a	1.90±0.43 a	1.8±0.29 a	2.45±0.45 a
gest I duration	134.19±22.94 a	132.47±18.23 a	98.23±13.23 a	85.88±6.70 a
rage I duration	89.20±21.42 a	80.68±19.60 a	66.55±5.16	64.25±9.52
l I duration	192.48±17.22 a	196.35±19.78 a	133.74±21.76 b	106.70±14.99
of I waveform occurrences	3.5±0.61 a	4.6±1.88 a	2.1±0.34 a	2.45±0.66 a
e from first probe to first ingestion	6.738±4.705 a	0.48±0.24 a	2.34±2.14 a	19.60±14.07
of M waveforms occurrences	3.26±0.63 ab	1.4±0.70 a	1.3±0.44 a	1.63±0.76 a
of X waveform occurrences	0.73±0.30 a	2.4±1.80 a	0.6±0.26 a	0.45±0.31 a
e from 1st P to 2nd P	126.27±28.99 a	84.17±25.01 a	80.47±29.61 a	86.51±26.73
ation of 1st I waveform	108.93±26.91 a	111.60±21.44 a	84.83±13.92 a	85.88±6.70 a
e from 1st I to 2nd I	101.28±24.39 a	122.39±27.87 a	84.25±28.80 a	84.85±26.99

The values in this table is Mean± SE, the unit of duration variable is min. Mean followed by same letter are not significantly different at *P=0.05* among the different concentration.

Methyl dopa: It is a competitive inhibitor of the enzyme DOPA decarboxylase, also known as aromatic L-amino acid decarboxylase, which converts 5-HTP into 5-HT.

Mean percentage of ingestion and non-probing waveforms of 100 mg L^{-1} and 200 mg L^{-1} doses of methyldopa showed significant differences (Fig. 7). Methyldopa showed significant differences in total duration (218.45±46.19 min) of np (Table 6), average duration (66.55±5.16 min) and total duration (133.74±21.76 min) ingestion waveforms at 100 mg L^{-1}. No significant data was found in number of occurrences of all waveforms.

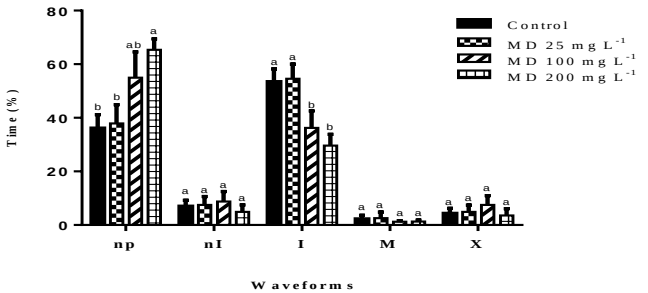


Figure 7. Percentage of time insect spent in each waveform on different doses of Methyldopa during 6-h recording. Np: non-probing, nI: non-ingestion (P+M+X), I: Ingestion, M: Movement of stylet, X: Unknown activity of stylet.

DISCUSSION

This study reported the involvement of serotonin system in feeding behavior of *N. lugens* for the first time. As we know that serotonin is ubiquitously present in both plant and animal kingdom. But in insects, little is known about its function and pharmacology. An electrical penetration graph technique was already been used to study the probing behavior of *N. lugens* (Kimmins, 1989; Seo *et al*., 2009; Ghaffar *et al*., 2011). So we use this technique to elaborate the involvement of serotonin and its receptor antagonists on feeding of *N. lugens*, as serotonin involvement in feeding behavior of aphid using EPG technique was studied by Kaufmann *et al*. (2004).

There is no generally approved nomenclature for EPG waveforms of plant hoppers because there is large variation in waveform characterization. Velusamy and Henrichs (1986) labeled waveforms as np, P, S, A, I related to activity during the feeding on plants; similar labeling used by Khan and Saxena (1988) for *N. lugens.* Kimmmins (1989) described the waveforms P1-P6. "P" referred to the plant hopper. Seo *et al*. (2009) distinguished seven waveforms appeared as a result of feeding by *N. lugens* on plants. They labelled the waveforms np, N1, N2, N3, N4-a, N4-b, N5, and the "N" prefix is related to *Nilaparvata*. Similarly, Ghaffar *et al*. (2011) characterized the waveforms as NP, N1, N2, N3, N4-a, N4-b, N5, N6 and N7. From NP to N5, waveform patterns and activities during these waveforms were similar to that of Seo *et al*. (2009). But Ghaffar (2011) combine N1-N3 into one type in order to reduce experimental work load. He also described N6 and N7 waveform. N6 same like the N5 waveform but pattern have high frequency and defined as 'derailed stylet mechanics' and N7 waveform characterized as potential drops from pathways activities. In present study, five waveforms were characterized as a result of feeding of *N. lugens* on sucrose diet were labeled as np, P, I, M, X using DC based EPG. As abbreviation indicates that np—non-probing is similar to the np as defined by Seo *et al*. (2009). In this waveform, an even base line was formed as a result of insect settle on the diet containers and voltage was remained zero. In case of movement of the insect, unstable waveforms were occurred. P is the stylet penetration in sucrose solution diet similar to the N1 waveforms appear in plants during probing (Youn and Chang, 1993, Seo *et al*., 2009; Ghaffar *et al*., 2011). The duration of P waveform was very short. When an insect inserts its stylet there is sudden increase or decrease in the voltage. I waveforms shows similar pattern like N5 in which insect feeds on xylem, regular peaks were formed with a high voltage and rapid frequency. These waveforms lasted for the longest time. It was similar to the waveform observed in whiteflies (Lei *et al*., 1999), leaf hoppers (Stafford and Walker, 2009), brown planthopper (Seo *et al*., 2009; He *et al*., 2011) and aphids (Tjallingii, 1988; Prado and Tjallingii, 1994), as in these waveform, insects ingest the sap. M waveform shows similar pattern as in N2 in which irregular peaks were formed at very low voltage (Seo *et al*., 2009; Ghaffar *et al*., 2011; He *et al*., 2011). While feeding on the plants, N2 waveform described as the salivation and stylet movement. Trebicki (2012) observed that N2 waveforms were observed when insect causes secretion on the diet side of parafilm membrane. X waveforms were occasionally occurred, pattern of these waveforms were not resemble to any other known waveform. Insect activity is still unknown. These were also referred as "Pseudo Probes" by Kindt *et al*. (2003). These were also observed by Kimmmins (1989) and Youn and Chang (1993). A detail study is needed to evaluate the activity of the insect during this waveform.

Involvement of 5-HT in regulation of feeding processes were reported in several insects; it helps in modulating different aspects of feeding like intake of food, contraction of crop (Molaei and Lange, 2003; Kaufmann *et al*., 2004; Liscia *et al*., 2012; Gasque *et al*., 2013; French *et al*., 2014). Serial concentrations of all the chemicals were made and examined for the feeding behavior of *N. lugens*. The insects which reach "I" waveforms were being used for further analysis. Comparison between the total number of insects used for probing behavior on serotonin and number of insects reaching ingestion shows significant differences. About 85% insects feed normally on sucrose diet. 5-HT (1000 mg L^{-1}) increase the first time of stylet insertion and significantly reduce the ingestion. It indicates that 5-HT is important in feeding related process of *N. lugens* but with the increase in 5-HT concentrations, it reduces the feeding. In *D. melanogaster*, appetite was increased with the decrease in levels of 5-HT in neurons and neuromodulatory actions of 5-HT were shown to depress feeding (Neckameyer, 2010). In *A. aegypti*, 5-HT helps in fluid secretion and depletion of 5-HT results in reduce feeding (Novark, 1995; Kinney *et al*., 2014). In honey bee, application of 5-HT directly into abdomen does not reduce feeding, but when applied directly into the brain, it caused significant reduction (French *et al*., 2014). 5-HT which is neuromodulator play an important role in the movement of food through the digestive tract significantly increases the pump contractions of blowfly (Liscia *et al*., 2012). 5-HT also regulates ingestion of certain nutrients in insects like; reduce the intake of carbohydrates in cockroaches, ants and flesh flies (Cohen, 2001; Dacks *et al*., 2003; Falibene *et al*., 2012). In many insects, disruption in 5-HT levels and use of serotonin receptor agonists and antagonist directly affect feeding processes (Haselton *et al*., 2009). Ketanserin, Mianserin hydrochloride and Yohimbine hydrochloride were well documented receptor antagonists used in present study. Significant increase in the first time of insertion of the stylet was found in case of serotonin receptors antagonists and methyldopa. As the chemical was not injected into the body of insect like Kaufmann *et al*. (2004), but increase the time of first probe indicates that insect facing the problem of stylet insertion. Kaufmann *et al*. (2004) observed the inhibition of stylet by 5-HT and its antagonist Ketanserin and Mianserin on

Myzus persicae and *L. migratoria*. In aphid, results revealed that with the joining effect of pymetrozine with 5-HT, inhibits stylet penetration at very high concentrations. 25% insects feed on Yohimbine hydrochloride 200 and 400 mg L^{-1} doses which were the lowest among all others. In all doses of methyldopa feeding percentage was less than 40%. In Ketanserin concentrations, 60% insects reached the ingestion waveform while in mianserin hydrochloride concentrations, less than 50% insects feed during EPG (Fig. 2). We didn't find any difference in normal behavior of insects while feeding on neurotransmitters through EPG except feeding.

Our EPG data analysis of present study indicates that there is significant increase in mean total non-probing duration when treated with 5-HT and its receptor antagonists, especially with high concentrations of chemicals used in experiments, *N. lugens* adults were not active and more time spend in resting if we compared to time spent on sucrose solution diet. Significant time is decrease in mean total and average time of ingestion in high concentration of amines. Ketanserin shows high potentiating compared with other antagonists. 5-HT inhibits feeding at high doses about 6 to 10 times higher than its receptor antagonists. Mianserin and Ketanserin along with pymetrozine inhibit the feeding, disrupt the locomotion and cause death at high doses of aphids (Kaufmann *et al*., 2004). Mianserin found to be more potent antagonist and Ketanserin is reported to be a competitive antagonist of serotonin in the foregut of *S. gregaria* (Banner *et al*., 1987). Different serotonin receptor and its antagonist show different function in different insect species. As Ketanserin and Mianserin have no effect in feeding of *L. migratoria* but have significant impact on aphid feeding.

Similar results observed as 5-HT and its receptor antagonists effects causing hesitation in the stylet insertion by the insect but insect recover from it in the time span of 1-2 h. It suggested that serotonin system involve in feeding of *N. lugens*. Antagonists were more effective in inhibition of stylet activities.

Brown plant hopper is a phytophagous insect which cause significant yield loses of rice crop (Park *et al*., 2007). Difficult in management of this insect pest as it is monophagous, migratory, short life cycle, vector of viral diseases (Cheng and Zhu, 2006) and developing resistance against resistance varieties and insecticides. There is need of insecticides with novel mode of action, our study provide a glimpse of involvement of 5-HT receptor and its receptor antagonist in inhibition of feeding. No insecticides were developed targeting serotonin system, as they are involve in many important processes of insect life. Development of synthetic insecticide against armyworm, *Pseudaletia separata* target 5-HT 1A agonist (Cai *et al*., 2010), Synergism effect between pymetrozine and 5-HT against locust and aphid (Kaufmann *et al*., 2004), feeding inhibition by 5-HT antagonist methiothepin against *Drosophila* (Gasque *et al*., 2013) evidence that 5-HT, 5-HT receptors and their antagonists have potential for the discovery of new insecticides against different insect pest species.

Further detail physiological and molecular studies require for understanding the involvement of neurotransmitter in the feeding mechanism of *Nilaparvata lugens*.

***Conclusion*:** It is concluded the neurotransmitter have role in feeding of *N. lugens* and it affects feeding behavior of the insect. High accumulation of neurotransmitter inhibits the feeding of brown plant hopper. Receptor antagonist also inhibits the feeding. For the management of *N. lugens*, 5-HT, 5-HT receptors and their antagonists have possible potential for the discovery of new insecticide especially after the development of resistance against commonly used insecticides.

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