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Seasonal variation in population and biochemical contents of brown planthopper, *Nilaparvata lugens* (Stål)

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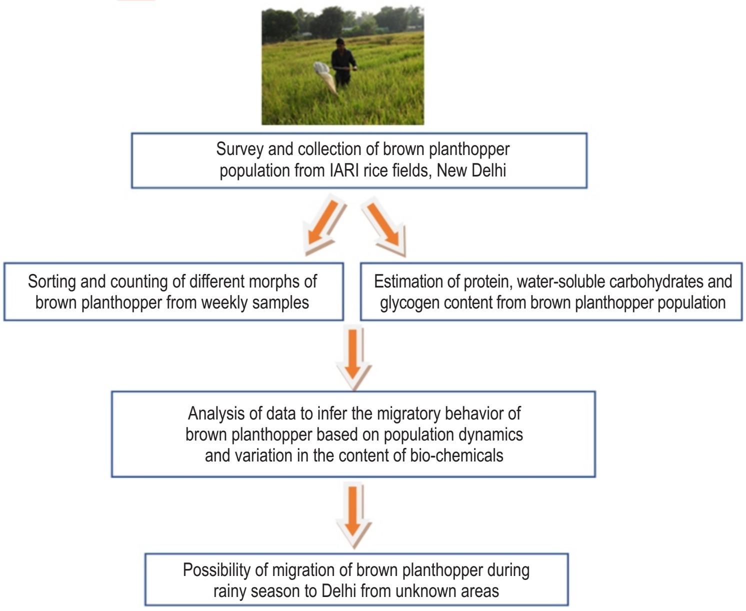
Abstract

Aim: The present study was undertaken on population dynamics and estimation of protein, water-soluble carbohydrates and glycogen contents in the brown planthopper, *Nilaparvata lugens* to explore their migratory behaviour.

Methodology: Brown planthopper populations were monitored and collected using sweep nets from 23rd standard meteorological week (SMW) to 47th SMW during 2017 and 2018 rainy seasons to understand population dynamics of the pest. The protein, water-soluble carbohydrates and glycogen contents were estimated from the pest samples collected during 36th to 44th SMW in 2017 and 2018 rainy seasons.

Results: Brown planthopper population were not observed in rice farm during 23rd SMW to 28th SMW during two years of study. However, macropterous form of the pest first appeared in the farm during 29th SMW and peaked during 43rd SMW. Thereafter, population declined and disappeared after 47th SMW during both the years. Water-soluble carbohydrates and glycogen contents varied significantly different weeks which remained low during 36th-39th SMW, however, increased gradually towards the end of the rainy season 2017 and 2018. On the other hand, protein content significantly varied among different weeks unlike the trend of water-soluble carbohydrates and glycogen.



Interpretation: The study revealed the absence of brown plant hopper during summer season preceding rainy season, and the accumulation of bio-chemical compounds towards the end of rainy season under Delhi environment is perhaps suggestive of migration of the pest from unknown areas during rainy season to Delhi and likely preparedness of the pest for emigration to safer areas from Delhi, respectively.

Key words: Brown planthopper, Glycogen, Macropterous, Migration, Protein, Water-soluble carbohydrates

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Introduction

Brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) is a serious economic pest of rice across Asia. Besides physical damage by sucking the sap, it's also a vector of rice grassy stunt and rice ragged stunt virus (Huang *et al.,* 2015). Severe outbreaks of brown planthopper have been recorded in North India in 2008 and 2013, wherein up to 70-80% crop damage was witnessed causing distress among growers (Chander and Husain, 2018). Such outbreaks of brown planthopper were also observed during 2016-2018 in different parts of India. The huge loss to rice crop from brown planthopper is attributed to prevalence of favourable weather conditions for its multiplication, absence of natural enemies, mono-cropping of rice (Hu *et al.,* 2019) and migration (Hu *et al.,* 2014). The brown planthopper does not survive during winter in the states like Uttar Pradesh, Bihar, Haryana, Punjab and union territory of Delhi, due to extremely low temperature and absence of rice crop thus, the migration of brown planthopper from eastern states *viz.,* West Bengal, Orissa and Assam in the month of June-July with the help of South-west monsoon is the likely possibility (Krishnaih and Lakshmi, 2012). Similarly, brown planthopper doesn't overwinter in temperate Asian countries like, Korea, Japan and northern China (Otuka, 2013).

The migration of brown planthopper from Philippines to Vietnam and South China through southwest monsoon (Hu *et al.,* 2017) vis-a-vis from Taiwan to Japan and Korea during May month has been well documented (Pender, 1994). The morphological attributes accounting for the migratory behaviour of brown planthopper reported earlier include wing polymorphism, characterized as long-winged (macropterous) and short-winged (brachypterous) forms (Tufail *et al.,* 2010). The macropterous are migratory in nature and are responsible for infesting new habitats, whereas brachypterous form is adapted to breed under a suitable habitat (Lin *et al.,* 2016). To study the migratory behaviour of brown planthopper, aerial trapping (Reynolds *et al.,* 1999), meteorological analyses (Hu *et al.,* 2014), radar observations (Qi *et al.,* 2014), and mark-recapture experiments (Nanjing college *et al.,* 1981) have been commonly used. However, recently, molecular markers were also used to study the rice planthoppers migration (Matsumoto *et al.,* 2013; Yang *et al.,* 2020).

Proteins, carbohydrates and lipids play an important role in growth and development of insects including energy for flight. The migratory locust (*Locusta migratoria*) utilized carbohydrates during initial phase flight and lipids during sustained flight (Pener *et al.,* 1997), while proline is utilized by *Aedes aegypti* (Scaraffia and Wells, 2003) and combination of proline and carbohydrates is used in some hymenopterans as fuel during flight (Teulier, 2016). Likewise, in brown planthopper, the flight fuel is the combination of carbohydrate and lipid (Padgham, 1983). In India, no reports are available regarding migratory behaviour of brown planthopper, therefore, in view of the knowledge, present investigation was undertaken on population dynamics of different forms of brown planthopper and estimation of biochemicals *viz.,* protein, water-soluble carbohydrates and glycogen as an indicator of migratory behaviour of the pest.

Materials and Methods

Experiments were conducted in the field and laboratory of Entomology Division, ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India during rainy season of 2017 and 2018.

Collection of brown planthopper: Brown planthoppers were collected from rice fields of ICAR-IARI at weekly interval using light traps and sweep net from 23^{rd} standard meteorological week (SMW) to 47^{th} SMW during both the rainy seasons. The sample size consisted of 20 sweeps per week and weekly insect collections were pooled, which formed the sample of the week. The collected samples were frozen at -80°C for few minutes in order to kill insects. Later, the specimens were sorted and their forms (brachypterous and macropterous) were segregated, counted and further stored at -20ºC for biochemical analysis.

Estimation of biochemical constituents in brown planthopper: The biochemical constituents were estimated from the brown planthopper samples collected during 36^{th} to 44^{th} SMW of rainy seasons 2017 and 2018. The macropterous females were used for biochemical analysis during 36^{th} to 39^{th} SMW and during 43^{rd} to 44^{th} SMW while, in other SMWs analysis was carried out using brachypterous female. In males, only macropterous adults were used for biochemical analysis throughout the study. Ten females and males were used in each replicate and biochemical analysis values for each SMW were average of 3 replicates.

Sample homogenisation: Ten males and females each were taken separately in a 1.5 ml microcentrifuge tube and 180µl of aqueous lysis buffer (100 mM KH_2PO_4, 1mM DTT and 1mM EDTA at P^H 7.4) was added and insects were homogenised for 2 min with a handheld motorized homogenizer (Cordless pestle) followed by centrifugation of each homogenate at 3000 rpm at 4°C for 2 min.

Protein quantification: A small quantity of supernatant was used for the estimation of protein content using Bradford reagent (Bradford, 1976) with bovine serum albumin (BSA) (1mg ml^{-1}) as standard.

Water-soluble carbohydrates and glycogen estimation: A 200µl of 2% Na_2SO_4 was added to the supernatant followed by 1500 µl of chloroform: methanol (1:2 v/v) solution to solubilize the total lipids as well as water soluble carbohydrates (Van Handel, 1965; Van Handel and Day, 1988). Further vigorous vertexing and centrifugation at 3000 rpm at 4°C for 10 min was done to remove the glycogen from supernatant. Thereafter, the pellet containing glycogen was transferred into a new eppendorf tube for subsequent analysis. A 500 µl of supernatant from each sample was taken in a separate glass tube (20ml) and incubated at 90°C to 100°C till complete solvent was evaporated to 100µl. Further it was cooled on ice followed by addition of 5ml of anthrone reagent (fresh anthrone reagent was used for analysis during each week with a concentration of 1.42 g/l with 70% sulphuric acid) and incubated again at 90°C to 100°C for 15 min. It was further cooled on ice/cold water to stop the reaction and absorbance was measured at 620nm using spectrophotometer (Bio Tek, 800TS, microplate reader) against the blank and glucose (1mg ml^{-1}) as a standard (Foray *et al.,* 2012).

The microtube containing glycogen pellet was washed with 800 µl of 80% methanol by two steps. First step involved vigorous vertexing and second step was centrifuging at 13000 rpm, 4°C for 10 minutes. Further, the glycogen pellet was carefully transferred to glass tubes (20 ml) and 5ml of anthrone reagent was added followed by incubation at 90 °C to 100° C for 15 min. It was later cooled on ice to stop the reaction and finally absorbance was measured using spectrophotometer Bio Tek, 800TS, microplate reader) at 620nm against blank with glucose (1mg/ml) as a standard (Foray *et al.,* 2012).

Statistical analyses: Weekly changes in protein, water-soluble carbohydrates and glycogen content of brown planthopper population were analysed using one way analysis of variance (ANOVA) followed by Tukey's honest significant difference at 5% level of significance to compare the treatment means using statistical software SAS 9.3. (http://support.sas.com/software/ 93/)

Results and Discussion

Both light trap and sweep net were used for collecting brown planthopper during 23rd to 30th SMW during two years of study. However, light traps didn't prove suitable for only brown planthopper collection, because it attracted other phototropic insects in large quantity and sorting of 4-6 mm size brown planthopper from large sample size became difficult and cumbersome. Hence, in the present study population dynamics of different morphs of brown planthopper was studied using sweep net collection. Observations on brown planthopper incidence were recorded from 23rd SMW onwards. Initially during first six

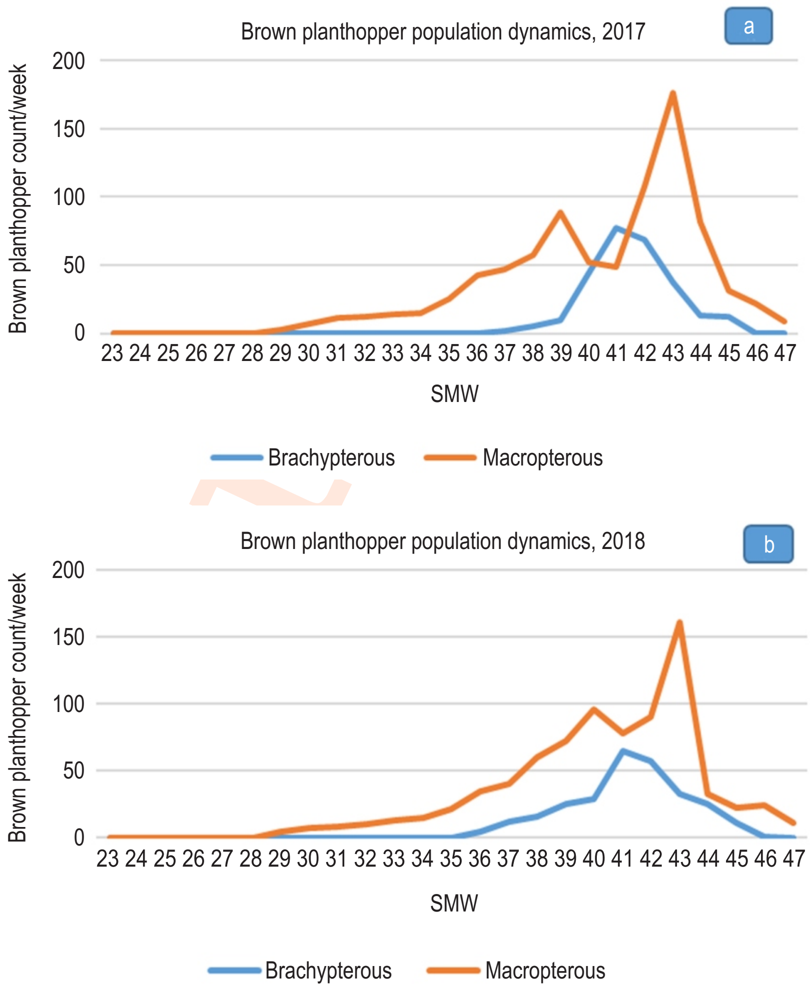

Fig.1: Population dynamics of brown planthopper during rainy season (a) 2017 and (b) 2018; SMW: Standard Meteorological week.

Table 1: Protein content (µg mg^{-1}) of brown planthopper, *Nilaparvata lugens* during rainy season 2017 and 2018 under Delhi environment

SMW	Protein (µg mg^{-1})			
	Rainy season 2017		Rainy season 2018	
	Male	Female	Male	Female
36	96.4±1.2^{cde}	129.1±2.0^{c}	135.4±1.6^{cd}	160.9±0.2^{d}
37	104.6±10.2^{c}	157.2±6.7^{bc}	140.5±1.3^{bc}	170.6±2.2^{d}
38	97.11±1.5^{cde}	165.9±9.7^{bc}	144.5±0.8^{b}	154.7±3.0^{d}
39	98.39±2.7^{cd}	109.6±5.7^{c}	142.1±2.1^{bc}	161.0±1.3^{d}
40	82.17±0.9^{de}	155.8±8.4^{bc}	126.7±0.9^{d}	423.8±5.6^{b}
41	134.8±0.1^{b}	138.0±1.7^{c}	235.5±1.4^{a}	429.8±15.3^{b}
42	75.96±0.7^{ef}	199.2±4.5^{bc}	144.0±2.4^{bc}	271.0±11.2^{c}
43	187.69±6.5^{a}	250.2±16.7^{ab}	102.7±3.2^{e}	250.8±7.7^{c}
44	55.06±2.4^{f}	317.1±56.8^{a}	80.4±1.3^{f}	594.9±16.2^{a}
S.Em±	4.3	20.47	1.8	8.99
F value	79.1	10.3	540.6	305.8
P value	<0.001	<0.001	<0.001	<0.001
Tukey's HSD at 5%	12.8	61.29	5.44	26.94

Different lowercase letters in same column differ significantly at P<0.05, SMW: Standard meteorological week

weeks of study period (23rd to 28th SMW) brown planthopper population was not observed in the farm in both the years. Macropterous adults that most probably immigrants were observed during 29th SMW during both the years of study (Fig. 1). Thereafter, macropterous form of the pest was observed throughout the remaining rainy season. The macropterous forms peaked during 43rd SMW (176 and 161 brown planthoppers respectively) in both the years, suggesting the likely preparation of brown planthopper for emigration to safer places due to impending weather. On the other hand, brachypterous form of brown planthopper (consisting of only females) were observed from 36th SMW and 37th SMW onwards in 2017 and 2018 respectively (Fig. 1) which peaked during 41st SMW (77 and 65 brown planthoppers) in two years. After 47th SMW, the brown planthopper population declined and disappeared in both the years.

This could be attributed to either death or emigration of population to unknown area possibility because in North India rice is grown only during kharif season and brown planthopper is being a monophagous pest of rice (Sogava, 1982) is not known to have an alternate host to survive. Besides, temperature reaches freezing point in winters and it can't survive with temperature <10°C in tropical condition (Krishnaih *et al.,* 2005). The lower temperature thresholds for egg and nymphal development in brown planthopper are reported to be 8.4°C and 8.24°C respectively (Krishnaih, *et al.,* 2005). Therefore, the increase in the proportion of macropterous forms during end of rainy season or onset of winter suggests that the pest might be preparing for emigration to certain safer areas. Earlier, collection of brown planthopper in November month in West Bengal using aerial net placed 150m above ground level confirmed extensive nocturnal migration with associated predators indicating migratory behaviour (Riley *et al.,* 1995). The brown planthopper appearance during 29-30th SMW and its disappearance after 47th SMW in Varanasi region of Uttar Pradesh (Sharma *et al.,* 2018) has been reported. Likewise, it has been observed that brown planthopper cannot overwinter in temperate part of China and Japan and, the infestation in rice starts with windborne summer or spring migrants from southern parts (Wang *et al.,* 2011) and by the end of October it migrates back to safe overwintering sites in Indo-China peninsula (Hu *et al.,* 2011).

The protein content of male brown planthopper significantly varied among different weeks (55.06±2.4 to 187.69±6.5 µg mg^{-1}; F=79.1; p<0.001) during 2017 and 2018 (80.4±1.3 to 235.5±1.4 µg mg^{-1}; F=540.6; p<0.001). On the other hand, in female brown planthopper it was lowest during 36th-39th SMW, gradually increased later and was highest during 44th SMW (317.1±56.8 µg mg^{-1}) during 2017 as well as 2018 (594.9±16.2 µg mg^{-1}) (Table1). The water-soluble carbohydrates content in male brown planthopper also significantly varied among different weeks (10.4±0.7 to 164.5±3.2 µg mg^{-1}; F=194.06; p<0.001) during 2017 and 2018 (0.4±0.3 to 124.0 µg mg^{-1}; F=208.1; p<0.001) and it was found to be lower than protein and glycogen during both years of study. Similarly, in female also it varied among different weeks (25.6±3.9 to 296.8±4.7 µg mg^{-1}; F=103.6; p<0.001) during 2017and 2018 (7.3±0.3 to 374.0±6.0 µg mg^{-1}; F=1543.2; p<0.001) and was recorded lowest during 36th to 39th SMW and highest during 43rd SMW in both male (164.5±3.2 µg/mg and 124.0±0.7 µg mg^{-1}) and female (296.8±4.7 µg mg^{-1} and 374.0±6.0 µg mg^{-1}) during 2017 and 2018 respectively (Table 2). Similarly, the glycogen content was also found to be lower during 36th to 39th SMW, that gradually increased and found peaked during 43rd SMW in both male (635.9±25.0 µg mg^{-1} and 549.2±7.3 µg mg^{-1}) and female (823.7±39.8 and 1007.0±56.8 µg mg^{-1}) during 2017 and 2018 respectively (Table 3). The

Table 2: Water-soluble carbohydrates content (μg mg^{-1}) of brown planthopper, *Nilaparvata lugens* during rainy season 2017 and 2018 under Delhi environment

SMW	Water-soluble carbohydrates (μg mg^{-1})			
	Rainy season 2017		Rainy season 2018	
	Male	Female	Male	Female
36	10.4±0.7^{f}	25.6±3.9^{d}	0.4±0.3^{e}	7.3±0.3^{f}
37	13.7±1.8^{f}	41.8±4.6^{d}	4.5±0.6^{e}	32.9±0.7^{e}
38	21.2±1.1^{f}	28.4±2.2^{d}	53.1±2.6^{c}	68.1±3.7^{d}
39	38.7±5.9^{de}	54.0±1.5^{d}	24.8±1.5^{d}	43.1±0.6^{e}
40	23.7±1.0^{ef}	40.6±1.2^{d}	29.8±0.3^{d}	43.1±0.7^{e}
41	56.8±7.1^{bc}	97.0±23.9^{c}	94.3±7.1^{b}	138.4±2.9^{bc}
42	43.7±0.7^{cd}	147.0±4.3^{b}	23.4±3.5^{d}	151.8±3.5^{b}
43	164.5±3.2^{a}	296.8±4.7^{a}	124.0±0.7^{a}	374.0±6.0^{a}
44	64.0±1.2^{b}	116.5±0..6^{bc}	37.0±0.7^{d}	85.4±1.0^{c}
S.Em±	3.39	8.58	2.91	2.84
F value	194.06	103.6	208.1	1543.2
P value	<0.001	<0.001	<0.001	<0.001
Tukey's HSD at 5%	10.15	25.70	8.72	8.51

Different lowercase letters in same column differ significantly at P<0.05, SMW: Standard meteorological week

Table 3: Glycogen content (μg mg^{-1}) of brown planthopper, *Nilaparvata lugens* during rainy season 2017 and 2018 under Delhi environment

SMW	Glycogen (μg mg^{-1})			
	Rainy season 2017		Rainy season 2018	
	Male	Female	Male	Female
36	193.7±8.0^{c}	225.4±12.8^{d}	97.6±9.0^{e}	274.0±4.9^{d}
37	94.0±3.9^{d}	244.0±18.3^{d}	95.4±1.1^{e}	235.9±4.8^{d}
38	137.9±3.9^{d}	218.7±14.6^{d}	96.5±1.2^{e}	231.8±7.2^{d}
39	260.4±7.8^{b}	233.4±9.4^{d}	139.5±3.2^{d}	449.0±24.2^{bc}
40	210.4±3.2^{bc}	631.2±18.8^{b}	211.8±3.6^{c}	455.6±3.5^{bc}
41	216.8±9.6^{bc}	476.5±17.6^{c}	290.4±11.5^{b}	542.3±15.0^{b}
42	196.5±6.0^{c}	664.3±5.0^{b}	188.7±1.2^{c}	461.2±3.6^{bc}
43	635.9±25.0^{a}	823.7±39.8^{a}	549.2±7.3^{a}	1007.0±56.8^{a}
44	261.5±10.6^{b}	292.6±3.8^{d}	135.1±5.1^{d}	386.2±5.1^{c}
S.Em±	13.02	18.51	5.96	21.56
F value	211.09	157.09	598.8	120.2
P value	<0.001	<0.001	<0.001	<0.001
Tukey's HSD at 5%	39.01	55.43	17.85	64.56

water-soluble carbohydrates and glycogen content showed a gradual increasing trend from the 36th SMW onwards reaching their peak in 43rd SMW and thereafter content of these biochemicals declined in 44th SMW that corresponded to the increasing trend of appearance of macropterous forms.

The macropterous forms of brown planthopper peaked in 43rd SMW and started declining thereafter towards the end of the rainy season. Insect migration is a complex physiological process characterized by well-developed flight muscles, female biased sex ratio (Guo *et al.,* 2019) and high energy consumption (Kent and Rankin, 2010). Many studies suggest that protein, carbohydrates (glycogen and trehalose) and lipids are the fuel for flight in the insects. Out of which, carbohydrates, especially glycogen supports during initial phase of flight and lipids during sustained phase of flight in locust, *Locusta migratoria* (Pener *et al.,* 1997) and in cutworm, *Spodoptera litura* (Murata and Tojo, 2004) while in bees only carbohydrates fuel their flight due to hovering habit (Suarez *et al.,* 2005). The present study revealed that biochemicals such as water-soluble carbohydrates (Table 2) and glycogen (Table 3), except protein (Table 1) in brown planthopper showed an increasing trend from 36th to 43rd SMW. However, these were found in less quantity in the pest during 36th to 39th SMW (Table 2, 3) that probably could be related to consumption of these compounds for pest's flight from original habitat to new habitat *i.e.,* Delhi study area. It has been observed earlier, the migration of insects such as brown planthopper (Zheng *et al.*, 2014), rice leaf folder, *Cnaphalocrocis medinalis* (Zhang *et al.,* 1979) and Silver Y moth, *Autographa gamma* (L.) often occurs during pre-reproductive stage and their fecundity in the new invaded area always less due to diversion of stored energy for flight and reproduction (oogenesis-flight syndrome) (Lorenz, 2007). Further, build-up of these carbohydrates that eventually peaked during 43rd SMW (Table 2, 3) corresponded with peak of macropterous form of brown planthopper (Fig. 1), indicating likely preparation of brown planthopper for migration to safer areas to tide over lower temperature during winters under Delhi environment.

The female rice leaf folder, *C. medinalis* which being migratory in nature had more glycogen and lipid reserves than male counterparts, suggesting that female migrates earlier and can fly farther (Guo *et al.,* 2019). Monarch butterfly, *Danaus plexippus* also during their fall migration to Mexico from Eastern America accumulated more lipid reserves to use them as energy during 5 months over wintering period (Brower *et al.,* 2006). Similarly, the protein (Table 1), water-soluble carbohydrates (Table 2) and glycogen contents (Table 3) found to be higher in female brown planthopper than male. Glycogen is an important storage polysaccharide that is converted quickly to dextrose or trehalose as and when needed in various tissues including flight muscles as a flight fuel (Storey, 1985).

In the present study also, glycogen was found to be higher in brown planthopper towards the end of rainy season suggesting its accumulation for pest's future consumption during emigration flight to safer areas or it's conversion to lipids when it is in excess. Conversion of excess carbohydrates to lipids is a common metabolic phenomenon in living organisms including insects (Briegel, 1990). The study revealed the absence of brown planthopper during summer season preceding rainy season and the accumulation of bio-chemical compounds towards end of rainy season under Delhi environment is perhaps suggestive of migration of the pest from unknown areas during rainy season to Delhi and likely preparedness of the pest for emigration to safer areas from Delhi, respectively.

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Add-on Information

Authors' contribution: S. Narayana: Designing of experiment, research work undertaken, preparation of manuscript and statistical analysis; **S. Chander:** Designing of experiment, guidance to conduct research, statistical analysis and correction of manuscript; **S. Doddachowdappa:** Guidance to research work, Statistical analysis; **S. Sabtharishi:** Statistical analysis, Data interpretation, and correction of manuscript and **P. Divekar:** Sample collection, writing manuscript.

Research content: The research content of manuscript is original and has not been submitted or published elsewhere.

Ethical approval: Not applicable

Conflict of interest: Authors declare that there is no conflict of interest.

Data from other sources: Not applicable

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