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Diversity of Economically Important Heteropteran Plant Bugs in Field Crops of Bangalore Region and their Molecular Identity

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ABSTRACT

Diversity of heteropteran plant bugs in field crops surrounding Bangalore region in Karnataka was assessed. The diversityof plant bugs varied in the locations surveyed and in the crops studied. Plant bugs belonging 20 genera under nine families viz., Alydidae, Coreidae, Lygaeidae, Miridae, Plataspididae Pentatomidae, Pyrrochoridae, Reduviidae and Scutellaridae were observed. Plant bugs of fami ly Pentatomidae occurred in greater abundance followed by family Coreidae. Theshield bugs (Pentatomidae) comprised of 29.85% followed by leaf footed bugs (28.35%) (Coreidae). The milk weeds bugs (Lygaeidae), predatory assassin bugs (Reduviidae) and t he Jewel bugs (Scutellaridae) comprised for 2.98% each. Among the crops, beans harbored more number of bugs than others followed by Maize. Geographic variations, habitat and food plants seemed to have influenced the diversity. Molecular characterization of the plant bugs was done and Genbankaccession numbers were obtained.

Keywords: Abundance, Diversity, Genbank accession, Molecular characterization, Plant bugs, Taxonomic composition

INTRODUCTION

The true bugs (Sub order: Heteroptera) consists of pests, predators and scavengers with a range of feeding habits *viz.*, phytophagous, zoophagous and hematophagous. The sub order has the largest group of about 45,000 described species of insects in the order Hemiptera, with 5800 genera and 140 families [1]. The sub order consists of 60% of described species which are mostly plant feeders. Heteropteran plant bugs feed on the reproductive parts of plants, flowers, ovules, ovaries, ripening and ripened seeds. In India, about 6300 species from 73 families are reported to be terrestrial in nature [2]. Many of them are important pest species of cultivated crops and some are important vectors of human diseases. The diversity, geographical variation, population dynamics of various taxa under this suborder, especially those associated with various crop ecosystems in India, is poorly documented in spite of their economic importance. They feed on almost all economically important crop plants such as pulse crops, cotton, rice, ground nut, safflower, sorghum, wheat, sugarcane and millets and causes significant crop damages. The losses have been reported to range from 14%-100% [3]. Reliable identification is the key to successful management of plant bugs. Insect identification relies on traditional taxonomy which is primarily based on external morphology [4]. However, taxonomic keys are often prepared for only certain life stages or genders, phenotypic variations in taxonomically important traits may cause significant difficulties in species identification [5]. In the present studies, the diversity of heteropteran plant bugs on different crops in areas surrounding Bangalore district was assessed with a view to understand their occurrence and their identity based on morphology and molecular taxonomy, to differentiate the morphologically cryptic species.

MATERIALS AND METHODS

Collection of plant bugs

Heteropteran plant bugs were collected from different locations surrounding Bangalore region, from vegetable and field crops. The plant bugs were collected from various locations surrounding Bangalore region, *viz.*, Attur, Bagalur, Chikkaballapur, Doddaballapur, Gowribidanur, Hebbal, Hudikeri Kanakapura, Kamannahalli and Ramnagara, The plant bugs were collected from crops *viz.*, beans, brinjal, castor, cauliflower, chilli, maize, mango, mulberry, red gram,ridge gourd, summers quash and tomato. The collection of bugs was done by sweeping with net at weekly intervals and collected insects were sorted out in respective taxa based on taxonomic characters. Bugs were collected from apical buds and flowers. Hand collection was also carried in flowers, leaf litter, bare ground and tree bases.

Morphological identification

The obtained samples were preserved in absolute ethanol. Morphological identification was done at the Division of Collection, Characterization and Documentation, ICAR-NBAIR based on the keys available in the documented literature and also on the basis of genitalia structure with the published literatures of [6-12].

Molecular characterization

Genomic DNA was extracted from ethanol preserved specimens, usually from the leg portion. . DNA was extracted using DNA kit (Quiagen) as per the manufacturer's protocol. PCR was conducted in 25 µL volume containing 2 µL of DNA, 0.2 µL Taq Polymerase (Bangalore Genei), 1.25 µL 10 µM primers, 2.5 µL 2.0 mM dNTPs and 2.5 µL 10Xbuffer. The primer pair LCO1490 (5′-ggtcaacaaatcataaagatattgg-3′) and HCO2198 (5′-taaacttcagggtgaccaaaaaatca-3′) was used [13,14]. The amplification conditions were , initial denaturation at 95°C (5 min), followed by denaturationat 95°C for 1 minute, Annealing 46°C for 30 seconds and Extension 72°C for 1 minute 35 cycles, followed by final extension at 72°C for 10 minutes (1 cycle). PCR products were identified on 1% agarose gel electrophoresis with EtBr staining under UV light, purified by PEG-NaCl method and sequenced in an automated sequencer (3730 DNA Analyser, ABI, Hitachi) using Big Dye Terminator cycle sequencing with same primers used for amplification [15].

Sequencing

The sequencing of amplified CO1 product was carried out at M/S Eurofins Pvt. Ltd Bangalore. The sequence data was retrieved in the form of chromatograms which was then submitted to genbank for obtaining the accession numbers.

Sequence analysis and genbank submission

The ambiguous bases were removed by chromatogram editing. Sequenced data were checked for quality by BioEdit v.7.0.5 software. Homology, insertions, deletions, stop codons, and frame shifts was checked using BankIt, a WWW- based submission tool with wizards to guide the submission process was used. These edited sequences were then aligned using Basic Local Alignment Search Tool (BLAST), with the sequences of same or related species retrieved from the nucleotide database (PUBMED) of National Centre for Biological Information (NCBI). The CO1 nucleotidesequences of the hemipteran species were aligned and compared with the species obtained from PUBMED using CLUSTAL W alignment [16].

RESULTS AND DISCUSSION

The occurrence of the plant bugs varied with respect to the crop in the various locations surveyed. Eleven vegetable crops (beans, brinjal, cauliflower, cabbage, chilli, knol-khol, moringa, potato, pumpkin, ridgegourd, tomato, five field crops, one fruit crop (Mango) and one plantation crop (Arecanut) were surveyed. Plant bugs belonging to the nine families' *viz.*, *Alydidae, Coreidae, Lygaeidae, Miridae, Plataspididae Pentatomidae, Pyrrochoridae, Reduviidae and Scutellaridae* were observed. The density of insect samples belonging to the families varied based on the crop and location. Collections were made from 32 spots in Table 1.

Pentatomid bugs were recorded from nine locations. The families Coreidae and Miridae recorded 19 and 8 samples from 7 and 9 locations, respectively. Families *Plastipidae* and *Pyrrochoridae* registered the least density. Predatory bugs *Reduviidae* were also recorded in 2 locations. The collections from various crops in different locations and their

identity revealed that occurrence of plant bugs predominantly belonged to family Pentatomidae followed by Coreidae.The number of samples in the Miridae and Lygaeidae were equivalent with the number of locations. The families *Reduviidae* and *Scutellaridae* followed a similar trend (Table 1).

The species composition of plant bugs was more diversified in the family Pentatomidae followed by coriedae, where in four specimens were recorded. Two specimens of predatory bugs *Scipinia* sp and *Endochus* sp were recorded Dysdercus koenigii was the only bug observed under the family Pyrrochoridae.

Abundance of plant bugs with respect to crops and locations

The observations on occurrence of plant bugs on the various crops in the areas surrounding Bangalore region indicatedthat among the crops, beans harboured more number of plant bugs (22) than other crops. Maize and red gram recordedan incidence of 15 and 9, respectively. Crops such as moringa, potato, summer squash had the least incidence of plantbugs, similarly, the other vegetable crops *viz.*, chilli, tomato and pumpkin recorded lower incidence (Table 2). Amongthe areas from where the collections were made, Attur recorded maximum number of plant bugs (19) followed by Doddaballapura (10) and Chikkaballapura (9) and Gowribidanur (9). Locations such as Hudikeri, Kamannahallli, and Ramnagara registered lower occurrence (1-3) (Figure 1).

Taxonomic composition of plant bugs in the locations studied

Plant bugs belonging to nine families were recorded in the locations surveyed. The taxonomic composition of plant bugs varied with respect to crop and location in Table 2 and Figure 1. Among the plant bugs, the shield bugs or stink bugs (Family *Pentatomidae*) comprised of 29.85%, followed by the leaf footed bugs (Family *Coreidae*) (28.35%). The family Alydidae (broad headed bugs) and the family Miridae (Capsid bugs) accounted for 16.41 and 11.94%, respectively. The milk weed bugs (Family Lygaeidae), the predatoryassassin bugs (Family *Reduviidae*) and the Jewelbugs (Family Scutellaridae) comprised for 2.98% each , while the families Plataspidae (Kudzu bugs or globular stinkbugs) and Pyrrochoridae (red bugs/strainers) accounted for 1.49% each (Table 3 and Figure 2).

Molecular characterization

The mitochondrial DNA sequences of plant bugs belonging to different families were analyzed, their nucleotide sequence data were submitted to Genbank and the accession numbers obtained (Table 4).

Table 1: Plant bugs recorded in different locations

Sl.No,	Family	No. of locations	No. of insect samples	Species of plant bugs recorded
1	*Alydidae*	2	11	*Riptortus pedestris*
2	*Coreidae*	7	19	*Cletus signatus* *Cletus bipunctatus* *Clavigralla gibbosa Spinola* *Gralliclava horrens horrens* *(Anoplocnemis phasianus*
3	*Lygaeidae*	2	2	*Graptostethus servus*
4	*Miridae*	8	8	*Mircarvalhoia arecae* *Nesidiocoris tenuis*
5	*Pentatomidae*	9	20	*Halyomorpha picus* *Megacopta cribrarium* *Nezara vitridula* *Bagrada hilaris* *Halys serrigera Westwood* *Tolumia baslis*
6	*Plastaspidae*	1	2	*Coptosoma. sp*
7	*Pyrrochoridae*	1	1	*Dysdercus koenigii(Fabricius)*
6	*Plastaspidae*	1	2	*Coptosoma. sp*
7	*Pyrrochoridae*	1	1	*Dysdercus koenigii(Fabricius)*
8	*Reduviidae*	2	4	*Scipinia sp.,* *Endochus sp.*
9	*Scutellaridae*	2	4	*Hotea curculionoides* *Chrysocoris stockerus*

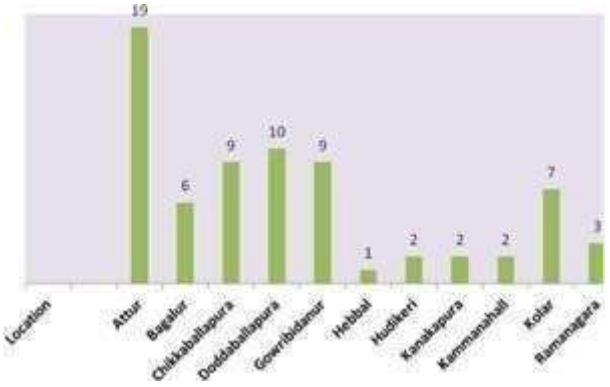

Figure 1: Abundance of plant bugs in different locations under Bangalore region

Table 2: Abundance of plant bugs in relation to crops

Sl.No.	Crop	No. of plant bugs collected
1	Arecanut	1
2	Beans	22
3	Castor	4
4	Chilli	2
5	Maize	15
6	Mango	3
8	Mulberry	3
9	Potato	1
10	Pumpkin	2
11	Rice	1
12	Red gram	9
13	Tomato	2
14	Safflower	2
15	Summer squash	1

Table 3: Taxonomic composition of plant bugs

Sl.No.	Family	Common name	Composition (%)
1	*Alydidae*	Broad headed bugs	16.41
2	*Coreidae*	Leaf footed bugs	28.35
3	*Lygaeidae*	Milk weed bugs	2.98
4	*Miridae*	Capsid bugs/grass bugs	11.94
5	*Pentatomidae*	Shield bugs/Stink bugs	29.85
6	*Plataspidae*	Kudzu bugs/globular stink bugs	1.49
7	*Pyrrochoridae*	Red bugs/Stainers	1.49
8	*Reduviidae*	Assasin bugs/ Ambush bugs	2.98
9	*Scutellaridae*	Jewel bugs/ Metallic sheild bugs	2.98

Figure 2: Taxonomic composition of Plant bugs (%)

Table 4: Molecular identityof plant bugs recorded in different crops, their identity and Genbank accession

S.No	Classification	Taxanomic identity	Location	Crop	Genbank Acc. No.
1	*Alydidae*	*Riptortus pendestris (Fabr.)*	Attur,	Beans	KA051737
2	*Pentatomidae*	*Menida formosa (Westw)*	Attur	Beans	-
3	*Pentatomidae*	*Menida formosa (Westw)*	Chikkaballapura	Maize	-
4	*Coreidae*	*Cletus sp.*	Doddaballapura	Maize	GU247497
5	*Coreidae*	*Anoplocnemis phasianus (Fabr)*	Doddaballapura	Maize	MG838335
6	*Pentatomidae*	-	Attur	Mango	-
7	*Pentatomidae*	*Nezara vitridula var. torquata (Fabr.)*	Gowribidanur	Beans	MT179300
8	*Coreidae*	*Clavigralla gibbosa Spinola*	Gowribidanur	Beans	KY274846
9	*Pentatomidae*	*Nezara vitridula var. smaragdulla (Fabr.)*	Doddaballapura	Beans	KR028339
10	*Alydidae*	*Riptortus pedestris (Fabr.)*	Doddaballapura	Beans	KA051738
11	*Lygaeidae*	*Graptostethus servus (Fabr.)*	Doddaballapura	Beans	-
12	*Plataspididae*	*Coptosoma sp.*	Ramnagara	Mulberry	-
13	*Coreidae*	*Cletus rebidiventris*	Kanakapura	Maize	GU247497
14	*Pentatomidae*	*Tolumia baslis (Dallas)*	-	Beans	-
15	*Pentatomidae*	*Nezara vitridula var. smaragdulla*	Attur	Beans	KR028338
16	*Pentatomidae*	*Halyomorpha picus (Fabr.)*	Hebbal	Castor	-
17	*Pentatomidae*	*Nezara viridula*	Kolar	Redgram	OK357905
18	*Pentatomidae*	*Nezera viridula*	Kolar	Beans	OK 357906
19	*Pentatomidae*	*Halyomorpha picus*	Chikkabalapura	Chilli	OK284413
20	*Reduviidae*	*Endochus sp.*	Attur	-	OK 493374
21	*Pyrrhocoridae*	*Dysdercus cingulatus*	Kolar	-	OK483371
22	*Coreidae*	*Coreidae(Cletus sp)*	Doddaballapura	-	OK135978
23	*Pentatomidae*	*Nezara viridula vouhes*	Komamanahalli	-	OK210086
24	*Pentatomidae*	*Nezara vitridula var. torquata(Fabr)*	Gowribidnur	-	OK271466
25	*Pentatomidae*	*Nezara viridula*	Kanagapura	-	OK273825
26	*Pentatomidae*	*Tolumia baslis(Dallas)*	Attur	-	OK284409
27	*Pentatomidae*	*(Nezara vitridula var. smaragdulla(Fabr)*	Attur	-	OK284410
28	*Miridae*	*Nesidiocoris tenuis*	Bagalur	-	OK448180
29	*Pentatomidae*	*(Halyomorpha picus)*	Chikkabalapur	-	OK284413
30	*Pentatomidae*	*Nezara viridula*	Kolar	-	OK357905
31	*Pentatomidae*	*Nezara viridula*	Kolar	-	OK357906
32	*Reduviidae*	*Endochus sp.*	Attur	-	OK493374

Molecular characterization

The collected plant bugs were also characterized using molecular tools and Gen Bank accession numbers were obtained(Table 2). To confirm that the desired portion of COI gene has been amplified, gel electrophoresis was conducted. Thermo Fisher GeneRular 100 bp was used as ladder. The gel documentation image obtained by BioDoc Analyzer shows that all the samples selected for gel electrophoresis gave bands between 600 and 700bp of DNA ladder (Figure 1). It reveals that desired COI gene of mtDNA were properly polymerased. The visualized PCR product contained no double bands on agarose gel, thus indicating that sequences obtained were targeted mitochondrial DNA. Basic local alignment search tool (BLAST) was used to check homology between the retrieved sequences and Genbank library or database of sequences, which helped to identify sequence similarity across genomes. Plant bugs are known to damage a wide range of plants by sucking nutrients from them [17]. The heteropteran bugs include pests, predatorsand scavengers. The diversity of plant bugs varied in the locations surveyed and in the crops studied. The present studies indicated 20 genera, representing nine families in the areas surrounding Bangalore region. The bug fauna was dominated by species belonging to family Pentatomidae followed by family Coreidae and the least was represented by families, Pyrrochoridae and Plastaspidae [1,4,6]. Similar observations were made by in their studies on faunal diversity of heteroptera in the Punjab region. Our observations also corroborate with the reports of in their studiesin Madhya Pradesh and in Daman and Diu [18-21]. Species richness is determined by floristic composition and vegetation structure reported that the composition could be related to plant community in which the insects live [22-24]. Similarly, the type of landscape and surrounding natural habitat has been reported to have a positive effect on theprobability of occurrence of the heteropteran bugs [25]. Study on the diversity plant bugs in the Tibetan plateau was influenced by both contemporary environmental and historical factors (Habitat heterogeneity, climatic stability and energy availability) and species richness is affected by micro-ecological variations such as altitude, mean temperaturerange and population density [26].

CONCLUSION

The observations made in the present studies corroborate with the views of the earlier workers. The diversity of economically important heteropteran plant bugs in field crops of areas surrounding Bangalore region varied. Bugsof family Pentatomidae were more predominant in occurrence than others. Geographic variations, landscape, natural habitat and availability of food plants appear to have influenced the abundance and diversity. The plant bugs were morphologically identified and characterized using molecular tools.

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