Journal of Biological Control, 37(3): 181-198, 2023, DOI: 10.18311/jbc/2023/34751

Research Article

Species diversity of grasshoppers in Kamrup district of Assam and their management by aqueous extracts of *Azadirachta indica* A. Juss and *Aegle marmelos* L.

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ABSTRACT: Grasshoppers pose a significant threat to agricultural productivity worldwide, causing substantial damage to various crops such as cereals, legumes, orchards, vegetables, grasslands, and forest plantations. This research aimed to study the species diversity of grasshoppers in five selected paddy field locations of Kamrup district, Assam, and assess the efficacy of aqueous extracts from the leaves of *Azadirachta indica* A. Juss (neem) and *Aegle marmelos* L. (bael) for controlling the most common grasshopper species, *Acrida exaltata*, found in the study locations. A total of 36 species of grasshoppers were collected and identified, out of which Acrididae was the most predominant grasshopper family, constituting 77.78% of the total collected species. The experimental results demonstrated abnormal changes in behaviour, including slower movement and increased inactivity among the aqueous extracts treated insects. Both the treated groups showed morphological and behavioural change with the more prominent effects observed from the neem-treated group. Haemolymph analysis revealed the presence of severe deformities in both treated groups, such as the spindle-shaped formation of cells, nuclear membrane disintegration, cell fusion and stacking, cytoplasmic vacuole formation, and cytoplasm degeneration. These findings demonstrate the pesticidal potential of neem and bael extracts against the *Acrida exaltata* grasshopper species, following its toxic impact on haematological, physiological, and morphological behaviour, suggesting the need for further research on their use as effective and environmentally friendly control measures against grasshopper pest infestations in densely vegetated and paddy fields in the region.

KEYWORDS: *Acrida exaltata*, haemolymph, grasshopper diversity, leaf extract

(Article chronicle: Received: 15-08-2023; Revised: 27-09-2023; Accepted: 30-09-2023)

INTRODUCTION

North-East India is a great treasure of bioresources that harbours several floral and faunal species. The soil in this area is rich in nutrients, having great potential for advancing agricultural practices (Saikia *et al.*, 2017). The region produces various agricultural products such as rice, sugarcane, tea, wheat, potato, jute, Eri-silk, Muga-silk, and different vegetables (Prokop and Poreba, 2012). However, the region's extensive agricultural lands also face challenges from various types of pests. Among these pests, grasshoppers are considered as a significant imminence as they exploit a wide range of crops, including vegetables, cereals, and legumes (Chhetry and Belbahri, 2009).

Grasshoppers are classified under the superfamilies Acridoidea and Pyrgomorphoidea, which belong to the suborder Caelifera. The Acridoidea superfamily presents high diversity, consisting of eleven families, while the Pyrgomorphoidea superfamily consists of only one family called Pyrgomorphidae, which is widely distributed in the state of Assam (Song *et al.*, 2018). Grasshoppers are the major group of pests with great economic importance as they constantly threaten various crops. The impact of locusts is consistently distressing and menacing, resulting in devastating plagues. The losses incurred by locust infestations in India alone exceed one trillion dollars (USD) (Athar *et al.*, 2023). Due to their feeding habits, they are considered oligophagous, meaning they consume a limited range of food (Joshi *et al.*, 1999) and can also be classified based on their preference for grasses (graminivorous), forbs (forbivorous), or a combination of both (ambivorous or mixed feeders) (Mulkern, 1967).

In Assam, the primary crops affected by pest damage include rice, tea, leafy and green vegetables. Both the nymphs and adults of these pests can consume leaves by cutting their edges (Paul, 2008). In some situations, when the pest populations are increasingly high, they can even feed on the mid-ribs of leaves, resulting in significant defoliation of the crops as a whole (Shankar *et al.*, 2016).

Efforts have been consistently made to control and manage these pest species through the use of different chemical insecticides. However, these chemicals not only aim at controlling target pest species but also negatively affect crop production and deteriorate the quality of the soil's nutrients. Additionally, they contribute to environmental pollution and pose risks to the individuals applying them and human health in general (Jayaraj *et al.*, 2016). Therefore, to overcome this issue, different regions in India employ the use of traditional knowledge and practices involving plant materials for pest management. This serves as an efficient approach to mitigate or minimize the detrimental effects caused by chemical pesticides. Both biological control methods and indigenous practices can contribute to reduce this problem. Biological control measures maintain a delicate natural equilibrium through predator-prey relationships, while indigenous control methods that utilize plant materials enrich soil nutrients and aim to mitigate the harmful impacts on human health. (Rana *et al.*, 2022).

Research carried out in India and other countries has led to the development of effective neem formulations that are now being produced commercially. Due to the non-toxic and non-carcinogenic nature of neem, which poses no harm to birds and mammals, its demand is likely to increase. (Gupta & Dikshit, 2010). At a concentration of 500 µg/ml, the essential oils derived from the bael tree have been found to effectively fumigate stored samples of gram and wheat. The targeted insect pests included *Callosobruchus chinensis, Rhyzopertha dominica, Sitophilus oryzae, and Tribolium castaneum*. When applied at various doses, the essential oil demonstrated a significant reduction in both oviposition (egg-laying) and adult emergence of *C. chinensis* in treated cowpea seeds. The findings highlighted the effectiveness of *A. marmelos* oil as a fumigant against insect infestations in stored grains, suggesting its potential as an alternative to synthetic chemicals for preserving stored grains (Kumar *et al.*, 2008).

To manage grasshopper species, local communities and tribes employ traditional agricultural practices such as applying ash on fields, burning leaves, and using strong-smelling plants like poppy and datura, as well as bitter plants like neem and lemon leaves (Sinha, 2007).These indigenous methods have been recognized for their ability to repel or deter insect pests (Gopi *et al.*, 2016). People have long recognised and acknowledged that certain parts of trees like neem and bael, including leaves, bark, wood, and fruit, possess properties that discourage or repel insect pests (Chhetry and Belbahri, 2009). As a result of which, these plant components have been incorporated into traditional practices such as soil preparation, grain storage, and animal husbandry.

With indigenous pest control practices in mind, Neem and Bael plants were chosen for the study, as neem has been used to develop cost-effective biological pest control products, benefiting farmers and rural businesses (Gahukar, 2014). Azadirachtin in neem repels and disrupts insects, altering their behaviour, reducing crop damage, and preventing resistance due to its hormone-mimicking properties. These pesticides are water-soluble and contribute to plant growth while acting as repellents and controllers of pest reproduction (Mondal & Chakraborty, 2016). Recent research in India and other countries has resulted in the production of commercially available, effective neem formulations, with increasing demand due to neem's non-toxic and non-carcinogenic properties that pose no harm to birds and mammals (Gupta & Dikshit, 2010).

Studies have revealed that bael leaves are rich in alkaloids, such as mermesinin, rutin, phenylethyl cinnamides, anhydromarmeline, and aegelinosides, as well as sterols and essential oils. Stem barks and roots of the bael tree contain a coumarin known as aegelinol. Additionally, the roots contain psoralen, xanthotoxin, coumarins, tembamide, mermine, and skimmianine. These compounds possess significant potential as insecticides and may contribute to the control of grasshopper pests, ultimately leading to increased crop yields (Pathirana *et al.*, 2020).

The current research work intends to reveal the distribution of grasshopper species across different parts of Assam, which can be utilized to devise targeted strategies to manage their population specifically in agricultural locations. Bioinsecticides, which combine biology, agriculture, and environmental science, offer interdisciplinary solutions for pest control and sustainable agriculture, with an advantage being their minimal impact on human health, in contrast to chemical pesticides that raise concerns about toxicity to humans and non-target organisms, as bioinsecticides work by natural defences and mechanisms (Damalas and Koutroubas, 2018).

The grasshopper stands as a major pest species in Assam, inflicting substantial damage to crops. The overall crop damage is attributed to various types of grasshopper pest species. However, there has been a lack of comprehensive research on the different species of this pest and their control measures have not been adequately developed or implemented yet.

Based on this background, the present study was undertaken to study the diversity of grasshopper species in selected locations having dense vegetation and paddy fields of Kamrup district of Assam and evaluate the efficacy of aqueous extracts of two commonly available plants, *Azadirachta indica* and *Aegle marmelos* against laboratory-reared grasshopper pest species by spraying them on the grasses they fed upon. The study was based on their behavioural changes and changes in their body colour, body weight, food intake, mortality rate and haemolymph study.

MATERIALS AND METHODS

Selection of study locations

Different locations having dense vegetation and paddy fields in the Kamrup district of Assam were selected for the study and collection of grasshopper species (Figure 1). These locations are as follows:

Area 1: Rani paddy-field area (26°01'28.43"N, 91°36'31.00"E)
Area 2: Dimu paddy-field area (26°21'19.05" N, 91°36'43.72" E)
Area 3: Singerpara paddy-field area (26°24'07.03" N, 91°47'23.05" E)
Area 4: IIT Campus, Guwahati (26°11'43.13" N, 91°42'02.93" E)
Area 5: Boko paddy-field area (25°58'32.37" N, 91°15'21.44" E)

Sample collection and identification

The samples are collected from the rice fields of different selected locations of Kamrup districts of Assam, such as Area 1, Area 2, Area 3, Area 4 and Area 5, through the sweeping net or by hand in the morning and evening time. The collected insects are then transferred to well-ventilated chambers and carried out to the laboratory. The study was carried out during December 2022 to May 2023. Sample collection was done at fortnightly interval.

Identification of the grasshoppers to the species level was carried out, abundant species are carried to the laboratory

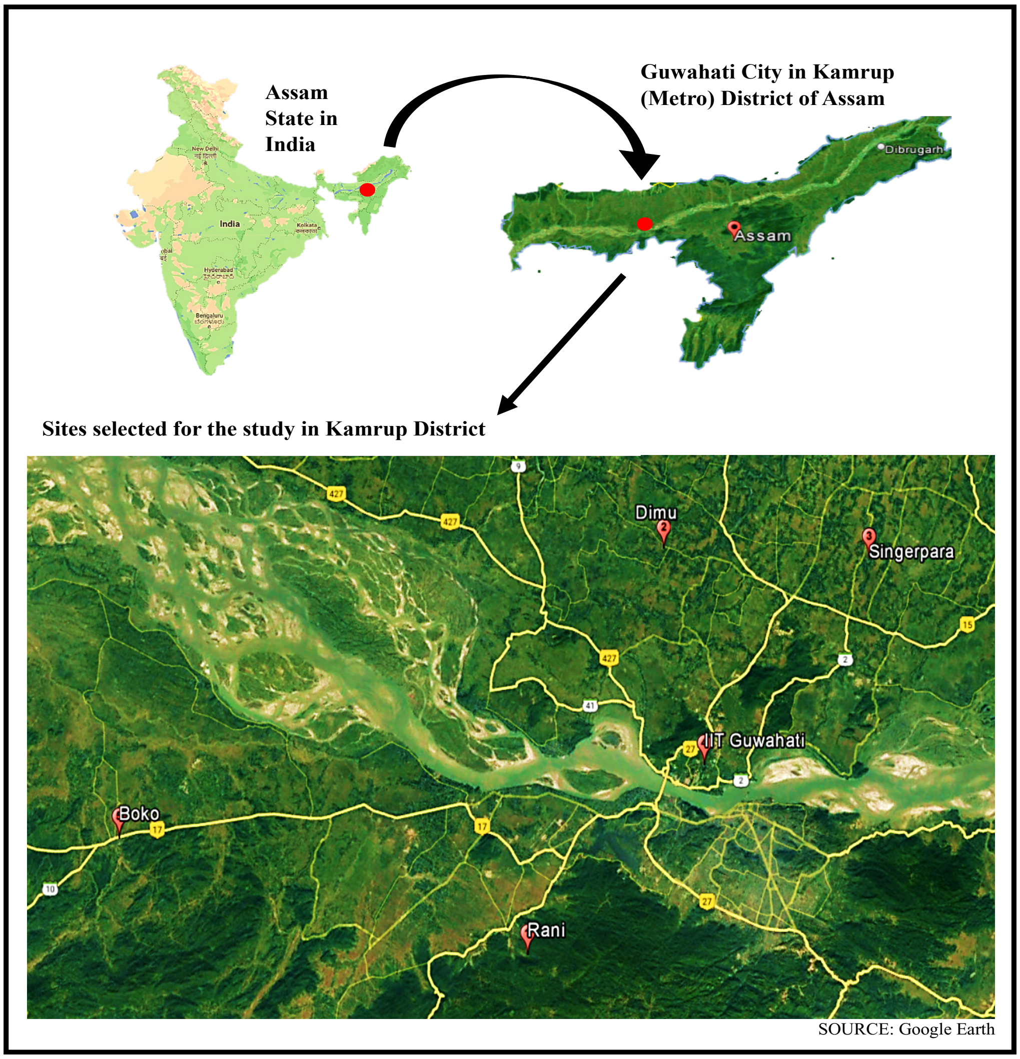

Figure 1. Map of Assam showing the sites selected for the study in Kamrup district (i) Rani, (ii) Dimu, (iii) Singerpara, (iv) IIT Campus, (v) Boko.

through a well-ventilated chamber. Identification was mostly done with the help of a Taxonomist, Prof. S. R. Hajong and several identification books and journals such as An Illustrated Key of Pyrgomorphidae (Orthoptera: Caelifera) of the Indian Subcontinent Region (Zahid *et al.*, 2020), Pictorial Handbook on Indian Short-horned Grasshopper Pests (Acridoidea: Orthoptera) (Mandal *et al.*, 2007), and A Pictorial Handbook on Grasshoppers of Western Himalayas (Srinivasan and Prabakar, 2013). Most of the collected insects after proper identification were released in the nearby flowering and park locations to prevent the unnecessary killing/torture of the species.

Experimental design

From the identified species, the most abundant species (*Acrida exaltata*) are collected for laboratory rearing under uniform husbandry experimental conditions at 25±2°C temperature, 55±2% relative humidity and 12-hour light-dark cycle. For the experimental work, the insects were kept in a transparent glass chamber of which the upper lid was covered with a white net so that it is easily accessible for food.

To observe the mortality, gain or loss of body weight and feed intake of the most commonly abundant species (*Acrida exaltata*), 3 transparent glass chambers were taken. In each chamber, 30 live insects were kept in uniform husbandry condition. One chamber was kept in controlled condition. In the second chamber, the aqueous extract of neem leaves was sprayed on the grasses. In the third chamber, the aqueous extract of bael leaves was sprayed on the leaves. The experiment was carried out for 15 days. The extracts were sprayed on the leaves every day and the grasses were changed with water ad libitum. Daily the grasses were weighed 10g and given to each chamber. The weight of the remaining grasses and the grasshoppers of each chamber was recorded. The number of dead insects and body weight of all the insects were measured from control as well as treated group. Any abnormality, morphological change or other behavioural changes are observed.

To study changes in haematological behaviour, one insect from each of the three chambers was sacrificed after 9, 12 and 15 days to observe any haematological abnormality in the haemolymph of grasshoppers of each chamber.

Ten insects were subjected to varying concentrations of the two plant leaf extracts, viz., 0.25, 0.50, 1, 3, 5, and 7mg/mL. Probit analysis was used to determine the LC_{50} (ppm) of these plant extracts against the *Acrida exaltata* grasshopper species following 24 hours of exposure.

Leaf extract preparation

The leaves of both *Azadirachta indica* (neem) and *Aegle marmelos* (bael) are collected and washed. Then, the leaves were left for sun drying. After drying, the leaves are then crushed and made powdered. Then, 100 mg of the powdered leaves are weighed and mixed with 100 ml of distilled water to make a concentration of 1mg/mL. Both the mixtures of neem and bael are then filtered and kept in the refrigerator at -20°C.

Phytochemical screening test

Qualitative analysis of different phytochemical constituents in the selected plant extracts was carried out on the powdered plant extracts by using standard protocols following the methods described by Harborne (1973), Trease and Evans (1989), and Sofowora (2003). The test has been performed for phytochemicals like Alkaloids, Flavonoids, Tannins, Saponins, Glycosides and Steroids found in *A. indica* and *A. marmelos.*

Feed intake and growth performance

For controlled test insects, fresh grasses were collected from the fields in the morning time. The grasses were then washed in clean water. The cleaned grasses were then weighed (10g) and a few drops of water was sprayed on the grasses. For neem-treated insects, cleaned grasses were kept in the neem-treated glass chamber. The neem extract (1ml) was sprayed on the grasses every day with a syringe. For bael-treated insects, similarly, fresh grasses were collected, cleaned and kept in the Bael-treated glass chamber. 1ml of the Bael extract was then sprayed on the grasses by a syringe. All the experiments were conducted in three replicas for accuracy.

The feeding and watering activities of all grasshopper groups were observed every day. Detailed records were maintained regarding the quantity of feed provided and the leftovers from each group. Additionally, data on the daily body weight of every individual insects was measured and recorded for a period of up to 15 days. The growth performance of the grasshoppers was closely monitored and evaluated based on parameters such as feed intake, weight loss, and mortality rate, which was determined using the following formula (Veldkamp and Bosch, 2015), where:

Feed Intake (FI) = (Feed offered – Feed leftover)

Weight Loss (WL) = (Initial body weight – Final body weight)

Haemolymph study method

A water bath was prepared and heated to a temperature ranging from 58°C to 60°C, serving as an anticoagulant. Subsequently, grasshoppers were carefully submerged in the water bath for approximately 3 mins. Upon completion of the immersion, the grasshoppers were removed from the water bath, and any adhering water on their bodies was carefully blotted using blotting paper. Next, a specific part of the grasshopper, such as the hind leg, cerci, or antennae, was incised using a sharp blade, allowing a drop of haemolymph to exude from the incision. This droplet of haemolymph was collected on a clear slide, and a haemolymph film was created with the aid of another slide. The slide was then left to dry. To enhance cell visibility, the air-dried haemolymph film was stained by applying a few drops of Methylene blue stain (prepared by mixing 3ml water with 1ml stain) and left undisturbed for approximately 3-5 minutes. After this period, the haemolymph film was rinsed with distilled water, dried once again, and subsequently observed under a microscope to study and differentiate the cells more clearly.

Differential Haemocyte Counting (DHC) method

A thin blood smear was made by placing a second slide over the first slide at 45° angle. The slides were allowed to air dry for 1 min and then fixed using drops of absolute methyl alcohol for 2 mins. Fixed cells were stained with methylene blue stain, washed several times with tap water, and dipped into distilled water. The stained smears were air dried and mounted in DPX with a cover slip. The differential counting of haemocytes was observed under a microscope. DHC was studied in both control and treated grasshoppers. The percentages of different types of haemocytes were determined using the formula provided by Ghoneim *et al.* (2017).

Statistical analysis

Data on feed intake, growth performance, and mortality were expressed as means of replicates ± SD. Significant differences between treatment groups were analysed by ANOVA using Microsoft Excel. A p-value of less than 0.05 was considered statistically significant. Probit analysis was used for the determination of LC_{50}.

RESULTS

Grasshopper species richness

Thirty-six grasshopper species, belonging to three families and eleven subfamilies were recorded from the selected study locations (Table 1). Average species richness ranged from 15 to 29 species per location among the five study locations. Low numbers of grasshopper species were found in Area-4, IIT Campus, Guwahati (15 species) and Area-3, Singerpara paddy-field area (19 species). Higher values of species richness (20-29 species) were found in Area-5, Boko paddy-field area (20), Area-1, Rani paddy-field area (23) and Area-2, Dimu paddy-field area (29). The mean species richness of all locations was 21. Among the various species found in different locations of Kamrup district, Assam, the most common and abundant were *Acrida exaltata* and *Acrida gigentea*. Among all the grasshopper species recorded, *Oxya velox, Acrida exaltata, Acrida gigantea and Phlaeoba infumata* were found in all the five selected locations of Kamrup district of Assam.

Taxonomic diversity

A total of 36 species of grasshoppers were collected and identified (Figure 2). All the grasshoppers collected are classified under three families viz., Acrididae, Pyrgomorphidae and Tettigoniidae (Table 2). Out of the three families, Acrididae was the most dominantly occurring grasshopper family with 28 species of Acridids grouped under 15 genera of seven subfamilies constituting 77.78% of the total collected species. The second largest family was Tettigoniidae with six species belonging to three subfamilies, which contributed 16.67% (six species), while Pyrgomorphidae was ranked third with 5.55% of the total two species collected with one genus of one subfamily. This is a report of its kind from Assam as no notable work has been done on this aspect.

From a taxonomic perspective, within the Acrididae, the Oxyinae and Acridinae were the most abundant and diverse subfamily (eight species each, i.e., a total of 16 species belonged to these subfamilies that represented 57.14% of the total grasshoppers' relative abundance) in our study (Figure 3), followed by the Oedipodinae (five species belonged to this subfamily, representing 17.85% of the total grasshoppers' relative abundance), Hemiacridinae (three species belonged to this subfamily, representing 10.71% of the total grasshoppers' relative abundance), Catantopinae (two species of the grasshoppers caught were catantopinae representing 7.1% of the total grasshoppers' relative abundance). Only one species in each of the Spathosterninae and Eyprepocnemidinae subfamilies was found, representing 3.5% of the total grasshoppers' relative abundance.

Detection of phytochemicals

Table 3 shows that almost all the phytochemicals, viz. Alkaloids, Flavonoids, Saponins, Tannins, Terpenoids and Cardiac glycosides are present in both the aqueous extracts of *A. indica* and *A. marmelos* at different levels of concentration, except for steroids in *A. indica.*

Behavioural change

In the control group, no behavioural change has been observed. They showed normal copulatory behaviour after seven days.

When exposed to plant extracts, grasshoppers exhibited immediate responses, including increased activity, heightened jumping behaviour, or erratic movements as they attempted to escape the treated area. In some cases, it did not cause immediate mortality in grasshoppers but showed

Table 1. Number of Grasshopper species present in different selected locations

Sl. No.	Species	AREA-1	AREA-2	AREA-3	AREA-4	AREA-5
1	*Oxya velox*	+	+	+	+	+
2	*Oxya fuscovittata*	-	+	+	-	+
3	*Oxya hyla hyla*	+	+	+	-	+
4	*Oxya hyla intricata*	-	+	-	-	-
5	*Oxya japonica japonica*	+	+	-	-	-
6	*Oxya nitidula*	-	+	+	+	-
7	*Gesonula punctifrons*	+	+	+	-	-
8	*Trilophidia annulata*	-	+	+	-	-
9	*Acrida exaltata*	+	+	+	+	+
10	*Acrida gigantea*	+	+	+	+	+
11	*Truxalis indica*	+	+	-	-	+
12	*Tagasta indica*	-	-	-	+	+
13	*Chorthippus curtipennis*	+	+	-	+	+
14	*Phlaeoba infumata*	+	+	+	+	+
15	*Phlaeoba tenebrosa*	+	-	+	-	+
16	*Phlaeoba antennata*	+	-	+	-	+
17	*Aiolopus thalassinus*	+	+	+	+	-
18	*Aiolopus simulatrix*	-	+	-	-	-
19	*Aiolopus thalassinustamulus*	+	+	+	-	-
20	*Acrotylus humbertianus*	+	-	+	+	-
21	*Chortophagaviridi fasciata*	-	-	-	+	-
22	*Heiroglyphus banian*	+	+	-	-	+
23	*Hieroglyphus nigrorepletus*	-	+	+	-	+
24	*Hieroglyphus oryzivorus*	+	+	-	+	-
25	*Catanto puskarnyi*	-	+	+	+	+
26	*Catanto pusterruginuous*	+	+	-	+	+
27	*Spasthosternum prasiniferum*	+	+	-	-	-
28	*Eyprepocnemis alacris*	-	-	+	-	+
29	*Conocephalus melaenus*	+	+	+	-	+
30	*Conocephalus maculatus*	-	+	-	-	-
31	*Tettigonia viridissima*	-	+	-	-	+
32	*Euconocephalus indicus*	+	+	+	+	-
33	*Letana rubescens*	-	+	-	-	-
34	*Hexacentrus unicolor*	+	-	-	+	-
35	*Attractomorpha psittacina*	+	+	-	-	+
36	*Attractomorpha cremulata*	+	+	-	-	+
Total		23 species	29 species	19 species	15 species	20 species

+: present -: absent

Table 2. Taxonomic information of grasshopper species found in the selected locations of Kamrup district of Assam

Sl. No.	Species	Common name	Family	Sub-Family
1	*Oxya velox*	-	Acrididae	Oxyinae
2	*Oxya fuscovittata*	-	Acrididae	Oxyinae
3	*Oxya hyla hyla*	Rice grasshopper	Acrididae	Oxyinae
4	*Oxya hyla intricata*	Small rice grasshop-per	Acrididae	Oxyinae
5	*Oxya japonica japonica*	Japanese grasshopper	Acrididae	Oxyinae
6	*Oxya nitidula*	Short-horned grass-hopper	Acrididae	Oxyinae
7	*Gesonula punctifrons*	-	Acrididae	Oxyinae
8	*Trilophidia annulata*	Band-winged grass-hopper	Acrididae	Oxyinae
9	*Acrida exaltata*	-	Acrididae	Acridinae
10	*Acrida gigantea*	-	Acrididae	Acridinae
11	*Truxalis indica*	-	Acrididae	Acridinae
12	*Tagasta indica*	-	Acrididae	Acridinae
13	*Chorthippus curtipennis*	Marsh Meadow grasshopper	Acrididae	Acridinae
14	*Phlaeoba infumata*	Short-horned grass-hopper	Acrididae	Acridinae
15	*Phlaeoba tenebrosa*	-	Acrididae	Acridinae
16	*Phlaeoba antennata*	Short-horned grass-hopper	Acrididae	Acridinae
17	*Aiolopus thalassinus*	Short-horned grass-hopper	Acrididae	Oedipodinae
18	*Aiolopus simulatrix*	-	Acrididae	Oedipodinae
19	*Aiolopus thalassinustamulus*	-	Acrididae	Oedipodinae
20	*Acrotylus humbertianus*	-	Acrididae	Oedipodinae
21	*Chortophagaviridi fasciata*	-	Acrididae	Oedipodinae
22	*Heiroglyphus banian*	Rice grasshopper	Acrididae	Hemi acridinae
23	*Hieroglyphus nigrorepletus*	Rice grasshopper	Acrididae	Hemi acridinae
24	*Hieroglyphus oryzivorus*	-	Acrididae	Hemi acridinae
25	*Catantopus karnyi*	-	Acrididae	Catantopinae
26	*Catantopus terruginuous*	-	Acrididae	Catantopinae
27	*Spasthosternum prasiniferum*	Short-horned grass-hopper	Acrididae	Spathosterninae
28	*Eyprepocnemis alacris*	-	Acrididae	Eyprepocnemidinae
29	*Conocephalus melaenus*	Black-kneed cone-head	Tettigoniidae	Conocephalinae
30	*Conocephalus maculatus*	Spotted Meadow Katydid	Tettigoniidae	Conocephalinae
31	*Tettigonia viridissima*	Great green bush-cricket	Tettigoniidae	Tettigoniinae
32	*Euconocephalus indicus*	Bush cricket	Tettigoniidae	-
33	*Letana rubescens*	-	Tettigoniidae	Phaneropterinae
34	*Hexacentrus unicolor*	Balloon-winged bush cricket	Tettigoniidae	Phaneropterinae
35	*Attractomorpha psittacina*	-	Pyrgomorphidae	Pyrgomorphinae
36	*Attractomorpha cremulata*	Tobacco grasshopper	Pyrgomorphidae	Pyrgomorphinae

Figure 2. Some of the grasshopper species (Orthoptera, Acrididae) collected from the selected locations of Kamrup District of Assam (1) *Acrida exaltata*, (2) *Heiroglyphus banian,* (3) *Aiolopus thalassinus,* (4) *Oxya hyla intricate,* (5) *Oxya japonica japonica,* (6) *Aiolopus simulatrix,* (7) *Atractomorpha psittacina,* (8) *Atractomorpha crenulata,* (9) *Catantopus karnyi,* (10) *Catantopus ferruginuous,* (11) *Oxya nititula,* (12) *Trilophidia annulata,* (13) *Spathosternum prasiniferum,* (14) *Gesonula punctifrons,* (15) *Eyprepocnemis alacris*, (16) *Tettigonia viridissima,* (17) *Oxya fuscovitta,* (18) *Chortophaga viridifassiata,* (19) *Conocephalus melaenus*, (20) *Conocephalus maculatus,* (21) *Tagasta indica,* (22-23) *Truxalis indica,* (24) *Phlaeoba infumata*, (25-26) *Oxya hyla hyla*, (27) *Oxya velvox,* (28) Giant grasshopper.

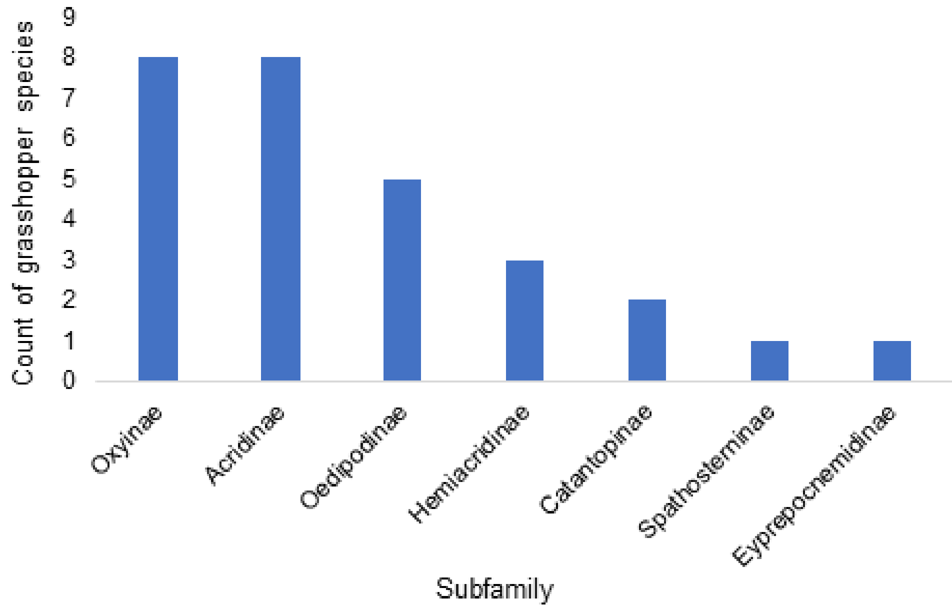

Figure 3. Acridiod family diversity in Kamrup district of Assam**.**

Table 3. Presence of different phytochemicals in the selected plant species extracts

Phytochemicals	*A. indica*	*A. marmelos*
Alkaloids	+ (**)	+ (***)
Flavonoids	+ (**)	+ (***)
Saponins	+ (***)	+ (**)
Steroids	-	+ (**)
Tannins	+ (**)	+ (*)
Terpenoids	+ (*)	+ (**)
Cardiac Glycosides	+ (*)	+ (**)

(+) - detected, (-) - not detected, (*) - Minimum intensity of reaction, (**) - Moderate intensity of reaction and (***) - Highest intensity of reaction

sub-lethal effects. The grasshoppers appeared unaffected initially for three days, but with time, it gradually affected their reproduction, development, and behaviour. Sublethal effects can contribute to the overall decline in grasshopper populations over time. Grasshoppers displayed avoidance behaviour when encountered locations treated with the extracts. They were observed to have actively moved away from such locations, trying to reduce their exposure to the chemicals. In the latter phase of the experiment, when it was observed that the test insects displayed reduced feeding activity, they were carefully relocated to a separate glass chamber containing untreated legumes and grains, such as beans, and were subsequently observed to initiate feeding. They avoided feeding on plants treated with plant extracts and sought out untreated vegetation as an alternative food source. This alteration in feeding patterns affected their nutrition and overall health throughout the study.

The behavioural changes in the grasshoppers treated with neem extract were quite prominent. Their movements became notably slower than usual, and with each passing day of treatment, they appeared increasingly inactive. Within three days of treatment, four out of 30 insects died. Only six out of the thirty insects managed to survive the full 15-day treatment period. Throughout this time, the grasshoppers exhibited reduced activity levels, sluggish movements, and a significant decline in feeding. Regarding the bael-extract treated insects, behavioural changes were also evident, but they were not as pronounced as observed in the neem-treated group. The grasshoppers appeared somewhat inactive with a slow body posture, but the effects were not as severe as those seen in the neem-treated insects.

Body colour

In the control group, the body colour remained normal and unchanged up to day-15 without any observed alterations. However, in the neem-treated group, a significant change in the abdomen colour was noticed after two days of treatment, as the green colour turned into a slight yellowish hue. One insect with a yellow abdomen succumbed to the treatment after three days. As for the bael-treated group, the change in abdomen colour to a yellowish tone was observed after three days of treatment. Furthermore, a gradual thinning of the abdomen was also recorded day by day for these insects.

Body weight

In the control group, the average body weight of 30 insects showed only a minor decrease after 15 days. However, in the neem-treated insects, a significant reduction in the average body weight of 30 insects was observed after 15 days (Figure 4). On the other hand, in the bael-treated insects, there was a reduction in the average body weight, though not as drastic as in the neem-treated group but greater than that of the control group after 15 days (Table 4).

Food intake

In the controlled test insects, food intake was normal. The average weight of food intake of 30 test insects ranged from 0.18g to 0.23g per insect in 15 days. In neem-treated insects, the food intake was greatly affected by the extract (Figure 5). The average weight of the food intake in 15 days was reduced from 0.23g to 0.005g per insect. In bael-treated insects, the food intake was also affected to much extent. The average weight of the food intake in 15 days reduced from 0.23g to 0.03g per insect (Table 5).

Mortality

In the control group, all 30 insects were observed to be alive after five days, and this pattern remained relatively stable throughout the study, with 28 insects still surviving at the end of the research period. The control group showed a mortality rate of 6.66% (Table 6).

Table 4. Average weight of Grasshoppers (g) in 15 days

Day	Average weight of grasshopper (g) [Mean ± S.D.]		
	C	T_N	T_B
1	0.230 ± 0.003	0.230 ± 0.003	0.230 ± 0.003
2	0.230 ± 0.003	0.220 ± 0.003	0.225 ± 0.003
3	0.228 ± 0.002	0.175 ± 0.002	0.200 ± 0.004
4	0.223 ± 0.003	0.164 ± 0.004	0.184 ± 0.002
5	0.221 ± 0.004	0.150 ± 0.002	0.166 ± 0.002
6	0.215 ± 0.002	0.142 ± 0.003	0.162 ± 0.003
7	0.211 ± 0.004	0.136 ± 0.002	0.155 ± 0.005
8	0.210 ± 0.003	0.125 ± 0.004	0.148 ± 0.002
9	0.207 ± 0.005	0.117 ± 0.005	0.140 ± 0.004
10	0.204 ± 0.002	0.108 ± 0.003	0.135 ± 0.003
11	0.204 ± 0.004	0.100 ± 0.002	0.132 ± 0.003
12	0.203 ± 0.003	0.092 ± 0.005	0.130 ± 0.005
13	0.201 ± 0.002	0.083 ± 0.005	0.127 ± 0.002
14	0.200 ± 0.005	0.079 ± 0.003	0.124 ± 0.002
15	0.200 ± 0.003	0.075 ± 0.002	0.120 ± 0.004

C – Control group
T_N – Neem-treated group
T_B –Bael-treated group

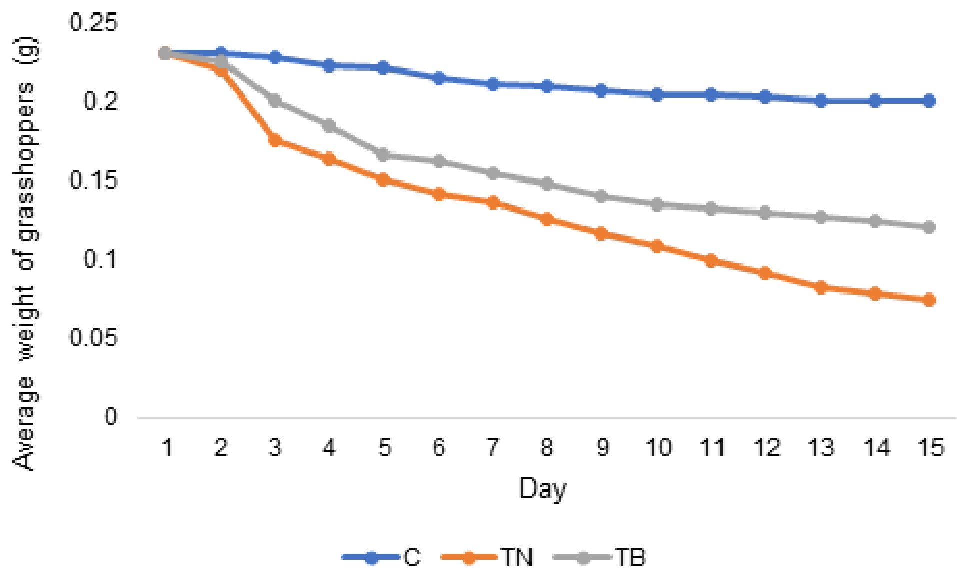

Figure 4. Trendline showing decrease in average weight of grasshoppers in control, neem-treated and bael-treated group.

Table 5. Weight of food intake in 15 days

Day	Weight of food intake (g) [Mean ± S.D.]		
	C	T_N	T_B
1	1.4 ± 0.15	1.4 ± 0.15	1.4 ± 0.15
2	1.4 ± 0.15	1.2 ± 0.15	1.3 ± 0.20
3	1.4 ± 0.15	1.2 ± 0.15	1.1 ± 0.10
4	1.4 ± 0.15	1.2 ± 0.15	1.1 ± 0.10
5	1.3 ± 0.20	1.1 ± 0.10	1.0 ± 0.10
6	1.3 ± 0.20	1.1 ± 0.10	1.0 ± 0.10
7	1.3 ± 0.20	1.0 ± 0.10	0.8 ± 0.10
8	1.2 ± 0.15	1.0 ± 0.10	0.5 ± 0.10
9	1.2 ± 0.15	0.8 ± 0.10	0.4 ± 0.13
10	1.2 ± 0.15	0.5 ± 0.10	0.4 ± 0.13
11	1.2 ± 0.15	0.2 ± 0.09	0.3 ± 0.14
12	1.1 ± 0.10	0.2 ± 0.09	0.3 ± 0.14
13	1.1 ± 0.10	0.1 ± 0.10	0.3 ± 0.14
14	1.1 ± 0.10	0.08 ± 0.01	0.2 ± 0.09
15	1.1 ± 0.10	0.03 ± 0.02	0.2 ± 0.09

C – Control group
T_N – Neem-treated group
T_B – Bael-treated group

For the neem-treated group, 26 insects were alive after three days of observation. The number decreased to 11 after 10 days and finally dropped to six after 15 days. The neem-treated group showed a mortality rate of 80% (Figure 6). Similarly, for the bael-treated group, 25 insects were alive after five days, which decreased to 14 after 10 days. The number was further reduced and ultimately, only seven insects remained alive after day-15. The bael-treated group showed a mortality rate of 76.66% as shown in Table 6.

Analysis

Results of the feed intake (g), body weight loss (%), and mortality rate (%) considered for the study revealed that there was a statistically significant difference ($p<0.01$) between the control and experimentally treated groups (Table 7). Table 8 shows the results of probit analysis for the determination of LC_{50}.

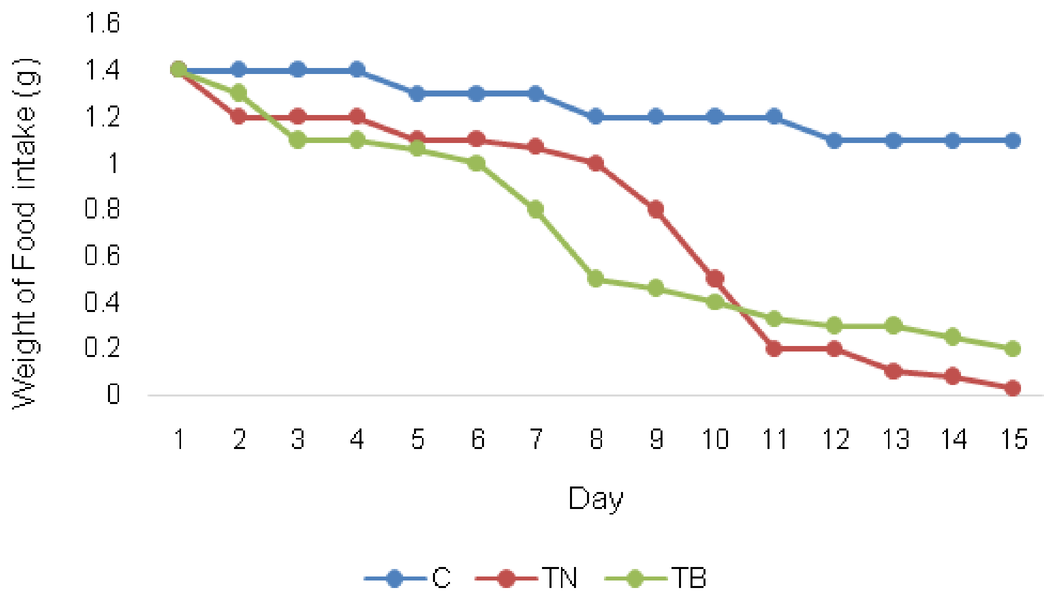

Figure 5. Trendline showing decrease in weight of food intake (g) by Grasshoppers in control, neem-treated and bael-treated group.

Table 6. Mortality rate of control and treated groups

Day	Survivability		
	C	T_N	T_B
1	30	30	30
2	30	28	29
3	30	26	29
4	30	23	27
5	30	20	25
6	29	19	22
7	29	17	21
8	29	14	18
9	29	13	17
10	29	11	14
11	29	11	12
12	29	9	11
13	28	8	10
14	28	6	8
15	28	6	7

C – Control group
T_N – Neem-treated group
T_B –Bael-treated group

Study of haemolymph

The haemolymph of grasshoppers is greenish-yellowish in colour containing a large number and varying shapes of cells or haemocytes. It constitutes about 5 to 40% of the total body weight. Five types of haemocytes were observed in the haemolymph of grasshoppers (Figure 7) viz., Prohaemocytes (spherical cells with large nuclei, granular basophilic cytoplasm, showing mitotic division), Plasmatocytes (polymorphic, ovoid, or fusiform or pear-shaped with large behaviour, nuclei, filiform cytoplasmic processes, and granular cytoplasm exhibiting phagocytic behaviour), Granulocytes (compact cells containing oval or spherical shaped small nuclei and cytoplasm, non-motile and amoeboid in function), Coagulocytes (spherical cells

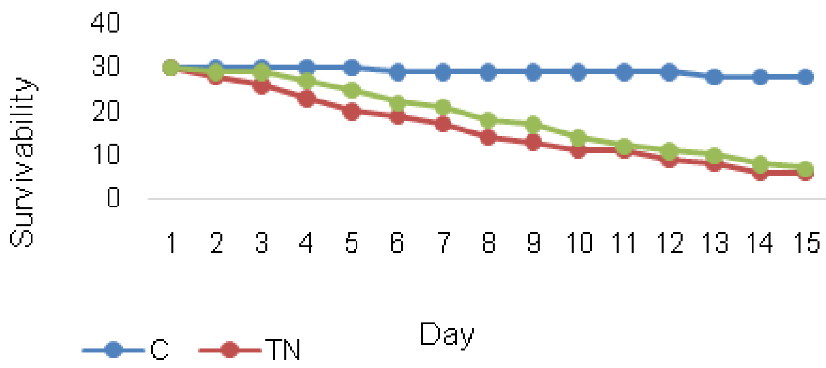

Figure 6. Trendline showing decreased survivability of grasshoppers in control, neem-treated and bael-treated group.

Table 7. Analytical result of feed intake, growth performance and mortality of control with treated grasshopper groups

Parameters	Experimental Groups			p-value
	C	T_N	T_B	
Feed intake (g)	1.24 ± 0.19	0.752 ± 0.49	0.70 ± 0.42	0.000434
Initial body weight (g)	0.230 ± 0.003	0.230 ± 0.003	0.230 ± 0.003	
Final body weight (g)	0.200 ± 0.003	0.075 ± 0.002	0.120 ± 0.004	
Weight loss (g)	0.03	0.155	0.110	
Body weight loss (%)	13.04	67.39	47.82	9.13E-07
Initial count of grasshoppers alive	30	30	30	
Final count of grasshoppers alive	28	6	7	
Count of dead grasshoppers	2	24	23	
Mortality rate (%)	6.67	80	76.67	4.98E-06

C – Control group
T_N – Neem-treated group
T_B –Bael-treated group

Table 8. Probit analysis of plant leaf extracts against *Acrida exaltata* grasshopper species following 24 hrs of exposure

Plant Extracts	LC_{50} (ppm)
Azadirachta indica	2818.38
Aegle marmelos	7413.10

with large nuclei, hyaline cytoplasm containing separately distributed granules), and Spherule cells (oval or elliptical cells with eosinophilic cytoplasm and indistinct nuclei).

In the controlled insects, all the above-mentioned types of haemocytes are found to be normal (Figure 7A). However, in the two treated groups exposed to neem extract and bael extract, the haemocytes were significantly impacted by their pesticidal nature. They exhibited enlarged and irregular shapes with non-uniform and deformed characteristics. Different grades of deformities of the haemocytes were observed throughout the period of the experiment, which gradually increased with the progress of the exposure paradigm.

In the neem-treated group, the haemocytes were greatly affected as compared to the bael-treated group. Extensive protoplasmic extensions were visible in plasmatocytes in earlier days of exposure, i.e., the 3rd, 6th, and 9th days (Figure 7B). Obliteration of the nucleus with degeneration of cytoplasm was also noted in some prohaemocytes after 12 days. The proportion of spherule cells with scanty cytoplasm was comparatively reduced in number as compared to the control group after 15 days of treatment (Table 8). Extensive degeneration of the nucleus along with the membrane deformation in some haemocytes was also recorded side by side. In some haemocytes, the presence of

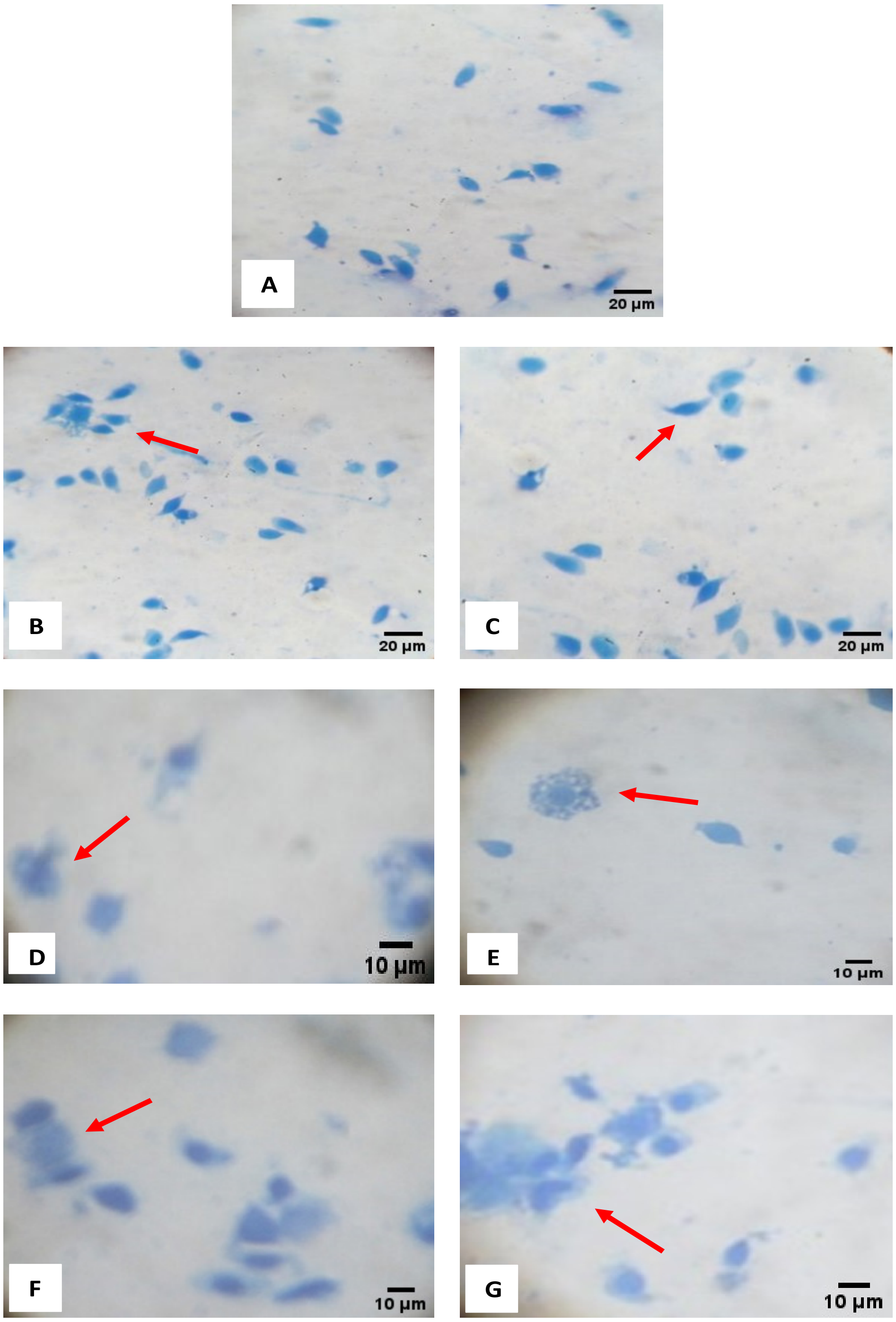

Figure 7. (A) Normally distributed cells in Control group. 10X. (B) Spindle-shape formation and aggregation of cells in Neem-treated group after 9 days. 10X. (C) Spindle-shape formation of cells in Bael-treated group after 9 days. 10X. (D) Nuclear membrane disintegration and increased number of spindle-shaped cells in Neem-treated group after 12 days. 40X. (E) Cytoplasmic vacuole and pseudopodial structure formation of cells in Bael-treated group after 12 days. 40X. (F) Enlargement of the plasmatocytes with membrane deformation, cell fusion and cluster formation of deformed cells in Neem-treated group after 15 days. 40X. (G) Nuclear membrane and cytoplasm degeneration, fusion of cells and increased number of spindle-shaped cells in Bael-treated group after 15 days. 40X.

mitotic figures was recorded. It is encircled with network fibre-like structures, which indicates the floating nature of haemocyte. Enlargement of the plasmatocytes with membrane deformation was recorded (Figure 7F). In the bael-treated group, the haemocytes were not normal, rather, larger than the cells of the control group. Networking-like structures encircling a cell and more pseudopodial structures were recorded in some haemocyte cells (Figure 7C). Nuclear and cytoplasmic degeneration were so extensive that makes a naked appearance in some haemocyte cells after 12 days of treatment. The presence of fibre-like cells that gives a podocyte-like appearance along with different shapes of coagulocyte cells and spherulocyte cells with deformed membrane was observed after 12 days of treatment. The coagulocyte cells having much larger nuclei occupying almost the entire cytoplasm leaving very little space for the granules of the hyaline cytoplasm were noticed after 15 days of exposure paradigm.

In both the treated groups, the alteration in the cells of the haemolymph progressively increased throughout the period of study (Figures 8 and 9).

DISCUSSION

Grasshoppers are widely distributed across the globe and represent one of the largest and most diverse groups of insects. In our current study or survey, we have identified a total of 36 grasshopper species in selected regions, underscoring their abundance in these locations. The study revealed a diverse array of grasshopper species thriving in the dense vegetation and paddy fields of certain selected locations in Kamrup district.

In this study, all the collected grasshoppers were classified into three families: Acrididae, Pyrgomorphidae, and Tettigoniidae. The most dominant family was Acrididae,

Table 8. Percentage of deformed haemocytes in controlled, Neem-Treated (T_N) and Bael-Treated (T_B) Grasshoppers (%)

Groups Prohaemocytes		Cells					
		Plasmatocytes	Granulocytes	Coagulocytes	Spherule cells	Deformed cells	
Controlled		26	40	10	10	5	9
9 days	T_N	15	31	8	9	3	34
	T_B	20	39	10	7	4	20
12 days	T_N	15	28	5	8	3	41
	T_B	18	30	9	7	3	33
15 days	T_N	12	20	6	4	2	56
	T_B	17	22	7	7	3	44

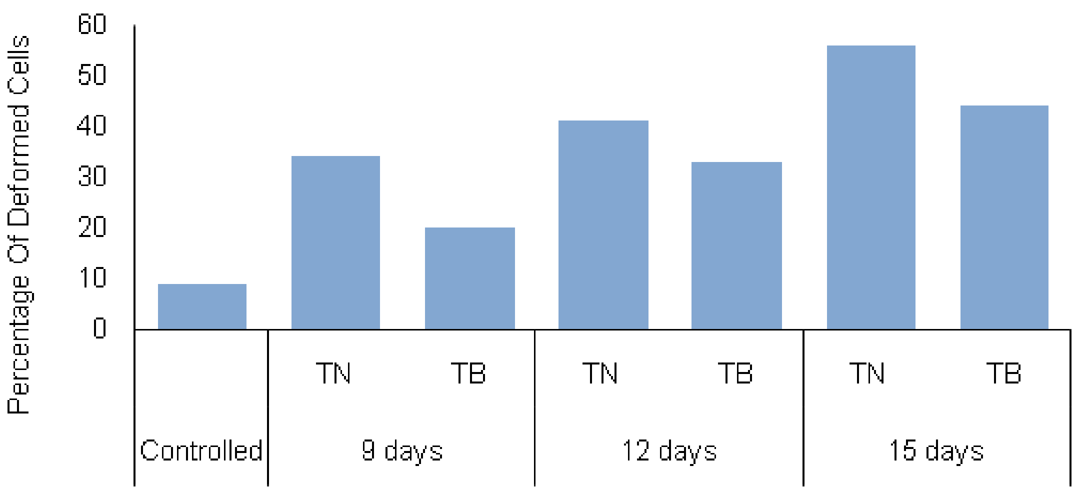

Figure 8. Percentage of deformed cells in the control group and neem- and bael-treated group after 9, 12, and 15 days.

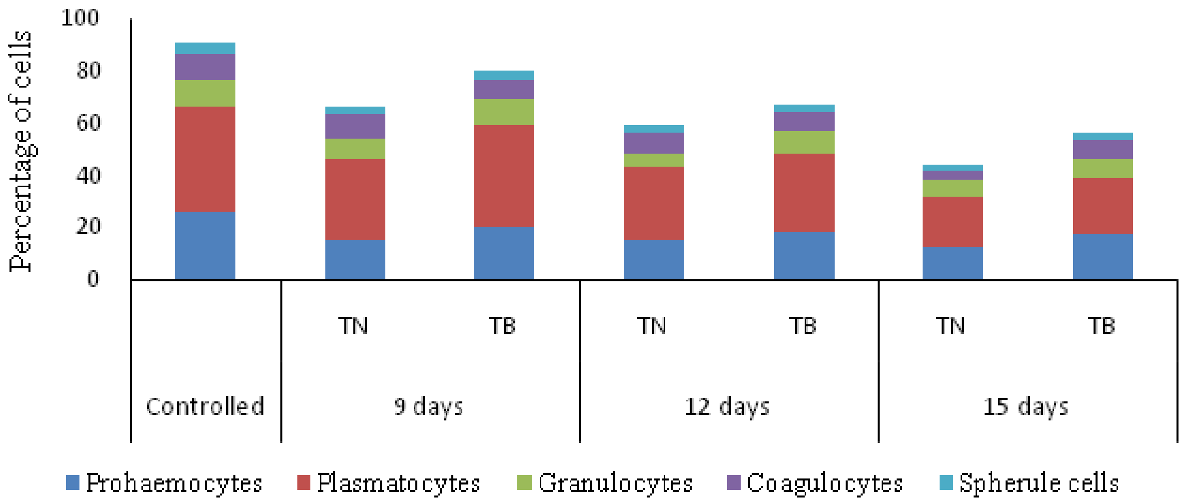

Figure 9. Distribution of different types of cells observed in the control group and neem- and bael-treated group after 9, 12, and 15 days.

comprising 28 species, with the majority found in the Dimu area. These species were further categorized into 15 genera and seven subfamilies, which aligns with findings from previous studies (Capinera *et al.*, 1997; Kandibane *et al.*, 2004). The second largest family, Tettigoniidae, consisted of six species across five genera and three sub-families, while Pyrgomorphidae ranked third with a total of three species belonging to one genus and one subfamily. Similar observations were reported in Kakadu National Park, Australia, by Andersen *et al.* (2000). Grasshoppers can significantly disrupt agriculture and husbandry, as they tend to breed and multiply rapidly in suitable environments. Consequently, it becomes essential to study the potential habitats for grasshoppers, analyze their spatial-temporal characteristics, and investigate how key environmental factors affect them in both meadow and typical steppe landscapes (Guo *et al.*, 2023). Various reports by Kandibane *et al.* (2004), Thakur *et al.* (2004), Mayya *et al.* (2005), and Paulraj *et al.* (2009) have highlighted the diversity of grasshopper species in different crops and agricultural fields, supporting the findings of our present study.

In our study, we observed a statistically significant mortality rate ($p<0.05$) of grasshoppers exposed to neem and bael plant extracts, with percentages of 80% and 76.66%, respectively. This demonstrates the significant role of neem and bael extracts in controlling grasshopper populations impacting their overall species diversity rate. Moreover, we found that food intake and body weight were decreased in the exposed grasshoppers, suggesting that these plant extracts may interfere with their metabolic activities. It is possible that the repelling odour of these extracts impairs the grasshoppers' appetite. In laboratory-reared grasshoppers exposed to neem extract, copulatory behaviour decreased compared to the control group and the group exposed to bael extract, indicating that neem extract might be more effective in this regard. This aligns with the effects of Azadirachtin, the principal active product of neem, which repels insects from feeding and disrupts their growth and reproduction. The neem-based formulation generally does not directly kill insects but significantly alters their behaviour, particularly reducing their reproductive potential (Isman *et al.*, 1990), as observed in our study. The components of neem extract can mimic natural hormones, affecting the physiology of insects (Mordue, 2004). When laboratory-reared grasshoppers, specifically *Acrida exaltata*, were exposed to bael extract, decreased levels of reproduction, food intake, and body weight was observed, suggesting the potential of bael extract as a pesticide.

Exposure to both neem and bael plant extracts led to variations in the cytomorphological architecture of different haemocytes in grasshoppers. The neem extract-treated grasshoppers showed more significant variations in the number and architecture of haemolymph compared to those exposed to the bael extract and the control group. This indicates that both extracts impact the cell membrane integrity of grasshopper haemolymph, leading to impaired cell membrane permeability of haemocytes, which may, in turn, affect their reproductive physiology, copulatory behaviour, and metabolic metabolism. The presence of various deformities in the haemocyte membrane in the treated groups also suggests the toxic effects of these plant extracts. Further long-term studies are necessary to understand the mechanisms of these plant extracts on the physiology and haemocytology of grasshopper species, enabling effective species control without compromising their biodiversity.

CONCLUSION

In conclusion, grasshoppers, while being essential components of the ecological network, can turn into a tough challenge when their populations are left unchecked. This unchecked growth poses a significant threat to agriculture, with potentially catastrophic consequences and adverse socio-economic impacts. Our study has highlighted the rich diversity of grasshopper species in Kamrup District, underscoring the region's biodiversity. It emphasized the potential for substantial crop losses and socio-economic adversity resulting from uncontrolled grasshopper abundance. The investigation into the effects of Neem and Bael extracts on grasshoppers suggests their promise as organic pesticides. In light of these findings, it is imperative to deepen our understanding of grasshopper habitat, species types, and distribution. This knowledge will enable the implementation of ecologically-based pest management strategies, crucial for safeguarding crop health and ensuring the well-being of agricultural communities in the region. Effective control measures and further detailed studies are paramount in mitigating the looming threat posed by grasshoppers in Kamrup District and similar agricultural landscapes worldwide.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Zoology, Cotton University, for providing administrative and laboratory support to carry out the research work. The authors are grateful to S.R. Hajong, Professor, Department of Zoology, North-Eastern Hill University, for his valuable help in identification of grasshopper species.

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