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Diversity of hymenopteran assemblages prevailed during reproductive stage of cotton—implications for natural control

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Abstract

Hymenoptera is one of the most important insect orders contributes to pollination, biocontrol and natural control largely unexplored from Bangladesh. We, therefore, assess the diversity and relative abundance of hymenopteran insects in cotton fields from five locations of Bangladesh using five types of traps (Malaise, Yellow Pan, Fluorescent Yellow Pan, Pitfall, and Sweep Net). Analysis of samples revealed presence of diverse type of hymenopteran insects from the studied locations, with a total of 1642 insects collected, representing 28 morphospecies and 16 families of the Hymenoptera. Formicidae, Braconidae, Diapridae, Figitidae, Ichneumonidae, Scelionidae constitutes the major families comprises of 36.37, 25.09, 5.36, 5.24, 5.18 and 4.93% of the populations respectively. Among the locations, cotton fields of Rangpur showed the highest species richness, diversity indices (Simpson, and Shannon values), while cotton fields located at Jashore had the lowest. Yellow and fluorescent pan traps captured insects from nearly all families, indicating broad sampling effectiveness. Malaise traps also sampled a wide range of families except for low captures of *Formicidae*. Pitfall traps primarily targeted *Formicidae*, consistent with their ground-dwelling hymenopterans. Sweep nets captured fewer families, reflecting a more selective sampling role. Among functional guilds, parasitoids were the most abundant, followed by predators, pollinators, and herbivores of hymenopteran insects. Overall, the hymenopteran community in cotton agroecosystems from Bangladesh was dominated by parasitoid families, indicating strong potential for natural biological control. Leveraging this natural enemy dominance could significantly reduce dependence on chemical insecticides. These findings support the integration of parasitoid conservation strategies into national Integrated Pest Management (IPM) programs and landscape-level pest management policies.

Keywords Biodiversity, Hymenoptera, Bangladesh, Baseline, Conservation

1 Introduction

Hymenoptera is the third largest insect order with 27 super families and 132 families including bees, wasps, phytophagous insects, a wide range of parasitoids, and social insects [55]. It represents a highly diverse, ecologically significant and functionally



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important insect order highly abundant in tropical and sub-tropical agroecosystems offering numerous essential ecosystem services, particularly natural pest regulation and pollination ([2, 39, 50]). They also participate significantly in multitrophic interactions in natural community structure [27], considered as bioindicators [3]. These parasitoids assemblages greatly contribute to natural pest control in intensively managed crops like cotton. Thus, they provide a pathway to the chemical insecticide dependence and accelerating agroecosystem sustainability.

Hymenopteran parasitoids make up about 80% of the Hymenoptera play significant role in natural biocontrol of various insect pests. Despite the rich abundance and diversity of hymenopteran insects and their great potential as biological control agents through parasitoids in Bangladesh, only a few scattered studies have been conducted [1, 23, 24, 31]. For example, *Apanteles taragamae* is a potential parasitoid of the cucumber moth, *Diaphania indica* (Crambidae: Lepidoptera) [4], *Dinarmus basalis* (Pteromalidae: Hymenoptera) Rondani serves as a parasitoid of *Callosobruchus chinensis* (L.) in stored pulses [23], and *Trichogramma zehri* (Hymenoptera: Trichogrammatidae) is an egg parasitoid of the rice hispa, *Diuraphis armigera* (Olivier, 1808) [41]. Ichneumonidae and Braconidae are the top two major hymenopteran families prevailed from Bangladesh majority of which were recorded from rice pests [25]. Two ichneumon and thirteen braconid-flies were reported from cotton crops [5, 20], which is the second most important cash crop in Bangladesh spent on intensive insecticides and pesticides, posing significant hidden risks to health and the environment [52]. There are ten families of Hymenoptera were reported to be present from rice pests in Bangladesh [25] but there has not yet been a comprehensive report on the abundance of hymenopteran assemblages, particularly parasitoids, in Bangladesh's cotton ecosystems.

Cotton is primarily grown at diverse areas in the south-western, northern, and central areas like at Kustia, Jhenaidah, Jessore, Chuadanga, Rangpur in Bangladesh. Sampling methods varied in terms of their efficacy and composition of arthropods trapped [7, 12]. The present study is, therefore, undertaken to know about the abundance of Hymenopteran population in cotton ecosystems across different cotton-growing regions, compare the efficacy of various sampling methods, and analyze the functional group composition to better understand their potential use in biological control strategies.

2 Materials and methods

2.1 Study locations and crop production

The study was conducted at cotton research farm (government cotton research station) and one university research farm located at five districts in Bangladesh (Fig. 1; Table 1). So, a total of five research farms at five locations (Table 1) were selected. Each farm was composed multiple plots. However, Sampling was done from a fixed point of the cultivable area of a randomly selected cotton plot surrounded by other cotton plots roughly one acre size (Table 1). The primary crop under investigation was cotton (variety, CB 14), and the research fields were surrounded by different perennial trees, including jackfruit, coconut, mango, and litchi. The total and cultivable area of the farms are mentioned in Table 1. We deployed the traps either at ground or cotton crop canopy level, hence our study reflects hymenopteran communities within cotton fields rather than the inventory of the surrounding perennial vegetation landscape-level fauna. The seeds were sowed at the same time in last week of July across five locations, and the recommended agronomic

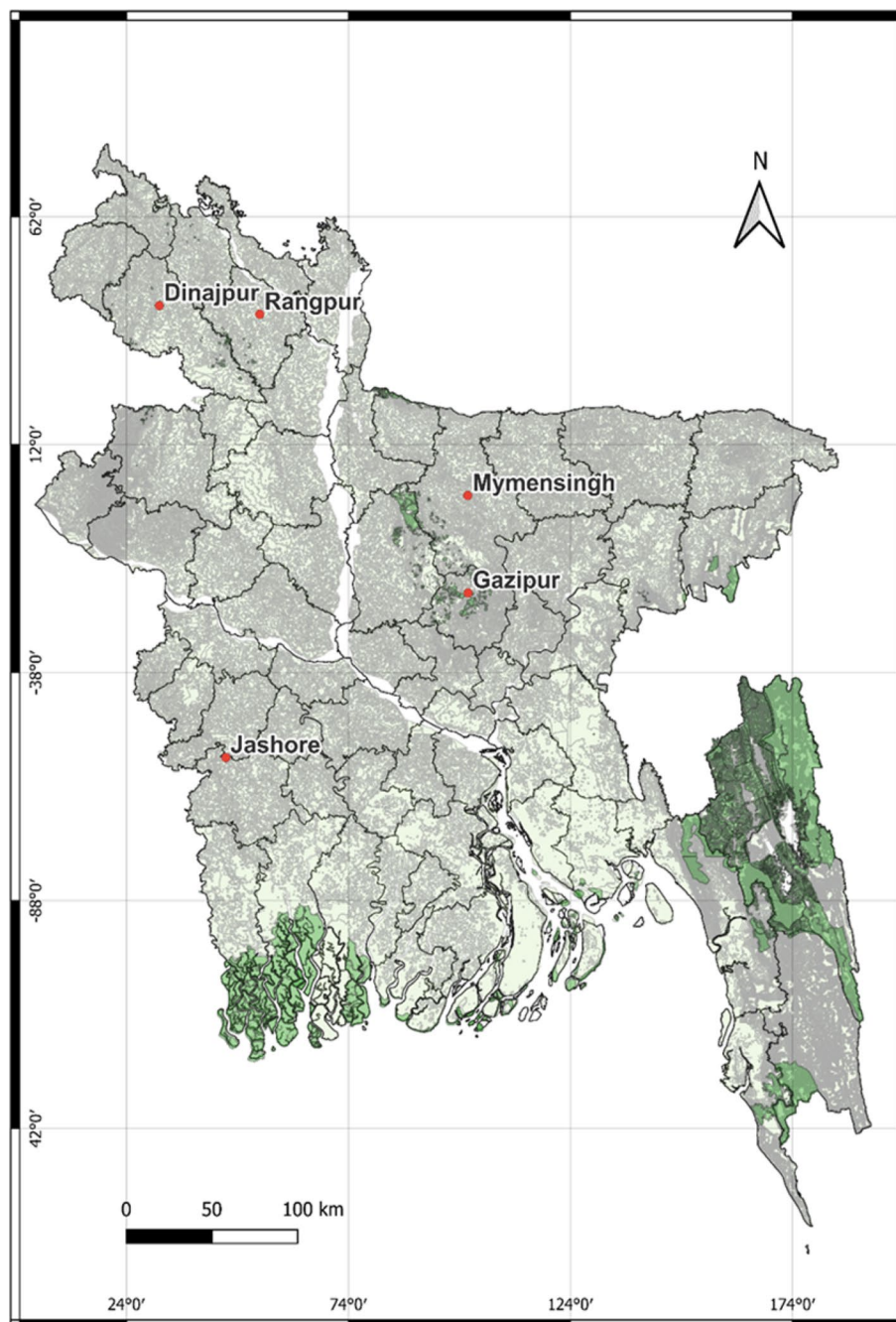


Fig. 1 Bangladesh map showing five sampling sites

practices (irrigation, weeding, fertilizations, gap-filling and others) were done according to the cotton production technology guidelines provided by the Cotton Development Board Bangladesh website (<https://cdb.gov.bd/site/page/646b1c26-e855-435e-a308-e75ab7e1be11/->).

2.2 Insect sampling

We used five kinds of traps (Fig. S1) to collect hymenopteran insects described below. From each location, three pan and pitfall traps and single malaise traps were installed to maximize collection effort of samples. The sampling was at the budding, flowering, and

Table 1 Five locations of hymenoptera sampling in Bangladesh

Sl no	Name of location	Latitude and longitude	Area (Acre)	Culti-vable area (Acre)	Sam-pling plot size (Acre)
1	Cotton research, training & seed multiplication farm (CRSMF), Sreepur, Gazipur (GA)	24°10'39.7"N, 90°25'49.9"E	150.00	112.00	0.98
2	Cotton research, training & seed multiplication farm (CRSMF), Sadarpur, Dinajpur (DI)	25° 45' 8.93" N, 88° 42' 8.34" E	128.00	84.00	1.10
3	Cotton research, training & seed multiplication farm (CRSMF), Jagadishpur, Jashore (JA)	23° 17' 30.20" N, 89° 4' 27.13" E	157.00	111.00	0.92
4	Cotton research station, Mahigonj, Rangpur (RA)	25° 42' 1 7.54"N, 89° 1 5' 48.07" E	23.50	18.00	1.02
5	Entomology research farm, bangladesh agricultural university campus, Mymensingh (MY)	24° 43' 4.71" N, 90° 25' 31.34" E	13.50	0.02*	0.80

boll development stage of the cotton plant in during November and December 2022. After collecting the insect samples from fields, they were brought to the Insect Biotechnology & Biopesticide Laboratory of the Department, Bangladesh Agricultural University, Mymensingh-2202. The detail trap constructions and installation procedures are mentioned below:

2.3 Yellow Pan traps (YPT)

Pan or bowl trapping methods was employed as different insects are attracted by color. Pan traps are the most commonly used trapping methods in monitoring and surveillance of bees, hymenopteran insects [33]. Three yellow plastic pans (diameter 28 cm and 12 cm deep) filled with 750 ml of a 2% acetic acid solution and a small amount of detergent were set up in a small hole at 20 m from each other (Supplementary Fig. S1A) in the cotton field at a distance of two meter from the edge of the field. The samples deposited in the solution were collected after 24 h of installation, sieved and preserved in 70% alcohol. Three yellow colored plastic pan traps were used for collection from each survey site.

2.4 Fluorescent yellow Pan traps (FYPT)

Three Fluorescent yellow pan traps (FYPTs) (Supplementary Fig. S1B), are made by spraying yellow fluorescent dye onto a plastic bowl (diameter 24 cm and depth of 9 cm) and letting it dry overnight. They are used to collect insects in the same way as yellow pan traps (YPTs).

2.5 Malaise trap

The malaise trap (Supplementary Fig. S1C) is a highly efficient trap for collecting hymenopteran insects [32]. Single malaise trap was set up to trap for collecting flying insects, particularly Diptera and Hymenoptera insects in the cotton field at a distance of two meter from the edge of the field. The samples were collected after 7 days from the day of installation and preserved in 70% alcohol.

2.6 Pitfall trap

A modified pitfall trap (Supplementary Fig. S1Ea and Eb) was used to collect 'ground-dwelling' insect samples as previously described in arthropod diversity studies ([11, 33, 54]. Three pitfall traps were buried at the soil level with 350 ml of 5% acetic acid solution

and lured at a distance of 15 m from each other and two meters from the edge of the field. The samples were collected after 7 days of installation, sieved and preserved in 70% ethanol.

2.7 Sweeping net (SN)

A sweeping net (Supplementary Fig. S1D) was prepared by binding a circular net of diameter 15 cm having a mesh size of diameter 0.2 cm with a stick of 90 cm in length. The sweeping net was moved to and from along one side of a row of plants and the same number of strokes back along the other side and each to and from (eight shaped) was considered as one sweep. A total of 10 sweeps [49] were taken from each site and the samples filled on the clothes were dragged at the bottom of the sweep and collected quickly into the tube containing 70% ethanol and carried in the laboratory.

2.8 Specimen identification

Specimens preserved in 70% alcohol collected by different traps were identified to family levels and different morphospecies based on different morphological characteristics such as body color, antennal shape, wing venation patterns, and other features using stereo microscope and identification keys [21, 38]. Identification of the samples were done at the morphospecies/family level. Genus-species level identifications have not yet been completed, so implications for particular species remain hypothetical or based on the previous reported species of the same family for cotton or other crops. Pictures of the specimen were taken with stereo microscope and scale bar was added using the software ImageJ [10]. Additionally, online electronic keys found on bug guide (<https://bugguide.net/>) verified this. Each voucher morphospecies were preserved and kept in 90% ethanol at Insect Biotechnology and Biopesticide Laboratory, Department of Entomology, Bangladesh Agricultural, Mymensingh. The molecular identification of the morphospecies through DNA barcoding have planned but not yet completed. Hence, molecular data were not included in this manuscript.

3 Statistical analysis

Due to the lack of independent replication within locations for each trap type and differences in effort units among sampling methods, inferential statistical analyses were not performed. The analysis was limited to descriptive statistics, with proportional CPUE used to illustrate relative patterns in insect captures among trap types.

3.1 Insect sampling and standardization

Insects were sampled from five locations using multiple collection methods, including yellow pan traps, fluorescent pan traps, pitfall traps, a malaise trap, and sweep netting. Yellow and fluorescent pan traps (three traps per location) were deployed for 24 h, pitfall traps (three traps per location) and the malaise trap (one per location) were operated for seven days, and sweep net sampling consisted of 10 standardized sweeps per location conducted within a single day.

For each trap type and location, insect counts were summed across replicate traps of the same type. To account for differences in sampling duration and effort among methods, catch per unit effort (CPUE) was calculated by dividing the total number of insects

by the corresponding sampling effort (trap-days for passive traps and number of sweeps for sweep netting).

3.2 Sampling effort and catch per unit effort (CPUE) calculation

Sampling effort for each trap type was calculated to standardize insect catch data across different trap numbers and deployment durations. For passive traps (yellow pan traps, fluorescent yellow pan traps, malaise traps, and pitfall traps), effort was calculated as the product of the number of traps deployed and the number of days they were active, expressed as trap-days. Specifically, three yellow pan traps and three fluorescent yellow pan traps were deployed for 24 h (1 day) at each location, resulting in an effort of 3 trap-days each. Three pitfall traps were deployed for 7 days (21 trap-days), and one malaise trap was deployed for 7 days (7 trap-days) per location. For sweep netting, which is an active sampling method, effort was defined as the total number of sweeps performed (10 sweeps-location) per location. Catch per unit effort (CPUE) [34] was used to determine the sampling effort.

Catch per unit effort (CPUE) for each trap type at each location was then calculated as:

$$\text{CPUE} = \frac{\text{Total number of insects caught}}{\text{Sampling effort (trap - days or sweeps)}}$$

3.3 Proportional CPUE and data visualization

To enable comparison among sampling methods with differing effort units, CPUE values were converted to proportional values within each insect family by dividing the CPUE of each trap type by the sum of CPUEs across all trap types, expressed as a percentage:

$$\text{Proportion of total CPUE (\%)} = \frac{\text{CPUE}_{\text{trap}}}{\sum \text{CPUE}_{\text{all traps}}} \times 100$$

These proportional CPUE values represent the relative contribution of each sampling method to the total standardized catch and were visualized using Sigma Plot.

3.4 Rank abundance curve and Chi-Square test for functional guilds

Based on the number of samples trapped of each morphospecies under each family for each sampling method, rank abundance curve was plotted by their relative abundance (%). In addition, the distribution of different functional guilds at different locations were tested by Chi-Square analysis.

3.5 Diversity assessment

Diversity assessment was conducted at the family level using pooled insect counts from all sampling methods. For each location, the total number of individuals per family was obtained by summing captures across all traps and sweep net samples. These pooled abundance data were used to calculate diversity indices for each location. Trap-level diversity was assessed by pooling insect abundance data across all sampling locations. For each trap type, the total number of individuals per insect family was obtained by summing captures from all locations. These pooled family-level abundance data were then used to calculate diversity indices for each trap type. Diversity was calculated by using the software Paleontological statistics software package (PAST) [22, 50].

4 Results

4.1 Morphospecies and species richness

Relative abundances were calculated by pooling individuals across all locations. Because samples were not replicated at the site level, measures of variability (e.g. confidence intervals or error bars) were not estimated. A total of 1642 hymenopteran insects were collected from five different locations over a three-month sampling period. Further grouping identified a total of 28 morphologically distinct species, representatives of 16 hymenopteran families (Table 2; Figs. 2, 3 and 4).

The most abundant morphospecies belonged to the Formicidae, representing more than one-third (36.37%) of the collected individuals. Braconidae was the second most abundant family comprising approximately 25.09% of the total collections. The other major families were Diapriidae, Figitidae, Ichneumonidae and Scelionidae comprising 5.36, 5.24, 5.18 and 4.93% of the total collections, respectively (Figs. 3 and 4). The number of specimens belonging to the family Eulophidae, Encyrtidae, Tenthredinidae, Apidae, Ceraphronidae, Mymaridae, Pteromalidae, Megaspilidae and Mymaromatidae

Table 2 Characterization and abundance of morphospecies of hymenopteran insects ($N = 1642$) across five locations (GA, Gazipur; MY, Mymensingh; RA, Rangpur; DI, Dinajpur; JA, Jashore) from cotton field in Bangladesh

Morphospecies	Family	Abundance						Percent	Functional groups
		GA	MY	RA	DI	JA	Total		
1.a	Formicidae	35	27	30	28	43	163	9.93	Predator
1.b	Formicidae	51	37	24	20	29	161	9.81	Predator
1.c	Formicidae	20	22	17	31	39	129	7.86	Predator
1.d	Formicidae	37	19	22	24	42	144	8.77	Predator
Sub-total of Formicidae		143	105	93	103	153	597	36.37	
2.a	Braconidae	70	22	43	39	17	191	11.63	Parasitoid
2.b	Braconidae	35	11	27	32	9	114	6.94	Parasitoid
2.c	Braconidae	40	18	23	15	11	107	6.52	Parasitoid
Sub-total of Braconidae		145	51	93	86	37	412	25.09	
3.a	Figitidae	26	14	17	18	11	86	5.24	Parasitoid
4.a	Eulophidae	13	4	3	5	9	34	2.07	Parasitoid
4.b	Eulophidae	9	0	5	6	6	26	1.58	Parasitoid
Sub-total of Eulophidae		22	4	8	11	15	60	3.65	
5.a	Encyrtidae	10	3	8	8	4	33	2.01	Parasitoid
6.a	Diapriidae	33	11	13	19	12	88	5.36	Parasitoid
7.a	Scelionidae	22	9	21	21	8	81	4.93	Parasitoid
8.a	Tenthredinidae	4	7	9	7	2	29	1.77	Herbivore
9.a	Ichneumonidae	4	5	11	8	7	35	2.13	Parasitoid
9.b	Ichneumonidae	2	3	5	5	4	19	1.16	Parasitoid
9.c	Ichneumonidae	4	0	4	6	0	14	0.85	Parasitoid
9.d	Ichneumonidae	0	3	7	2	5	17	1.04	Parasitoid
Sub-total of Ichneumonidae		10	11	27	21	16	85	5.18	
10.a	Apidae	10	9	13	12	4	48	2.92	Pollinator
11.a	Ceraphronidae	3	7	8	8	2	28	1.71	Parasitoid
12.a	Mymaridae	5	4	5	5	2	21	1.28	Parasitoid
13.a	Pteromalidae	4	4	5	4	3	20	1.22	Parasitoid
14.a	Chalcididae	0	0	1	0	0	1	0.06	Parasitoid
14.b	Chalcididae	0	0	1	0	0	1	0.06	Parasitoid
14.c	Chalcididae	0	0	1	0	0	1	0.06	Parasitoid
14.d	Chalcididae	0	0	1	0	0	1	0.06	Parasitoid
Sub-total of Chalcididae		0	0	4	0	0	4	0.24	
15.a	Megaspilidae	3	0	13	9	3	28	1.71	Parasitoid
16.a	Eurytomidae	4	8	5	3	2	22	1.34	Parasitoid
Grand-total		444	247	342	335	274	1642	100	

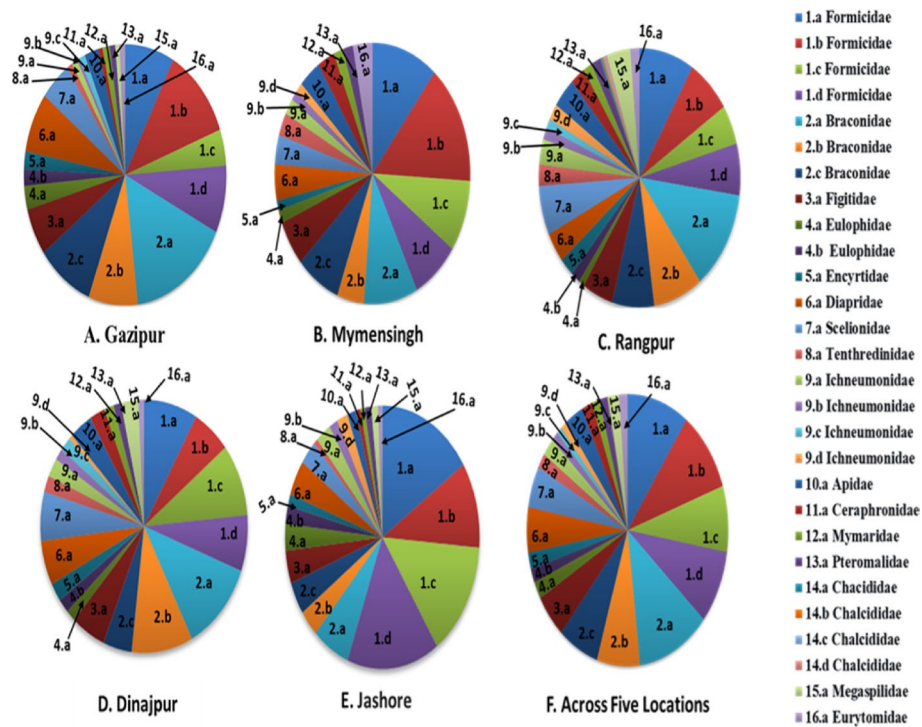


Fig. 2 Abundance of different morphospecies **A:** Gazipur; **B:** Mymensingh; **C:** Rangpur; **D:** Dinajpur; **E:** Jashore; **F:** Across five locations

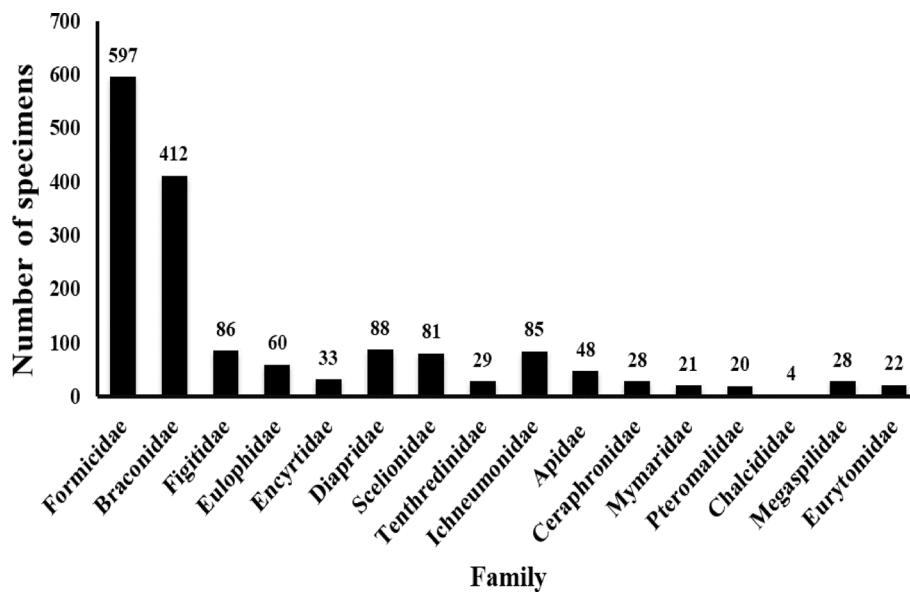


Fig. 3 Abundance of individuals (N=1642) from within each family of Hymenoptera present on five cotton fields in Bangladesh

were less than 5%. In contrast, the lowest number of morphospecies were collected from the Chalcididae family, accounting for only 0.24%. Notably, only four distinct morphospecies under Chalcididae family were recorded from Rangpur, Bangladesh (Figs. 3 and 4).

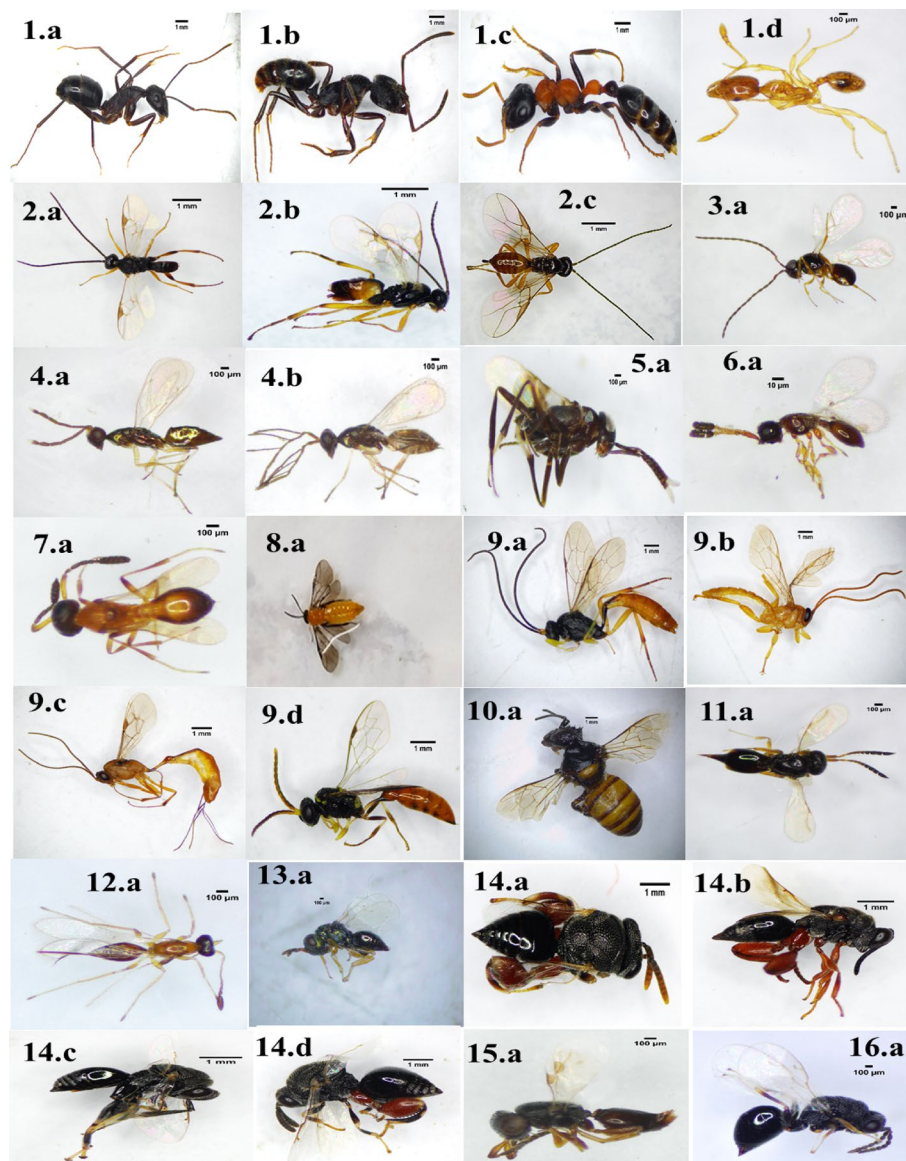


Fig. 4 Representative different morphospecies of Hymenoptera collected from cotton fields of Bangladesh. Legend: Formicidae (1.a–d); Braconidae (2.a–c); Figitidae (3.a); Eulophidae (4.a & b); Encyrtidae (5.a); Diapriidae (6.a); Scelionidae (7.a); Tenthredinidae (8.a); Ichneumonidae (9.a–d); Apidae (10.a); Ceraphronidae (11.a); Myrmecidae (12.a); Pteromalidae (13.a); Chalcididae (14.a–b); Megaspilidae (15.a); Eurytomidae (16.a)

4.2 Diversity assessment of different hymenopteran insects

The Alpha (α) diversity analyses of Hymenoptera population in the cotton field over location and trap were measured are describing in the following headings.

4.3 Alpha (α) diversity indices variation over location and trap

The results of the four alpha diversity indices (Shannon, Simpson index, Species richness, and Pielou's evenness) are presented in Tables 3 and 4. The Kruskal–Wallis tests showed significant differences among five trapping methods but not among the locations in terms of Hymenoptera diversity. Rangpur and Dinajpur exhibited the higher Simpson and Shannon index values, indicating the presence of greater hymenopteran insects' biodiversity in these locations followed by Gazipur and Mymensingh (Table 3).

Table 3 Abundance and diversity matrices for hymenopteran insects across five locations (GA, Gazipur; MY, Mymensingh; RA, Rangpur; DI, Dinajpur; JE, Jashore) from cotton field in Bangladesh

Location	GA	MY	RA	DI	JA
Dominance (D)	0.082	0.076	0.061	0.065	0.094
Simpson (1-D)	0.918	0.924	0.939	0.935	0.906
Shannon(H)	2.720	2.780	2.994	2.915	2.667
Evenness (e^H/S)	0.660	0.7672	0.713	0.7686	0.6257

Table 4 Abundance and diversity matrices for hymenopteran insects by different traps from cotton field in Bangladesh

Types of traps	Yellow pan trap	Fluorescent pan trap	Malaise trap	Pitfall trap	Sweep net	Kruskal-Wallis test
Dominance (D)	0.191	0.210	0.196	0.962	0.515	$p=0.001$
Simpson (1-D)	0.810	0.790	0.804	0.038	0.485	$p=0.001$
Shannon(H)	2.080	2.013	2.111	0.115	0.942	$p=0.00093$
Evenness (e^H/S)	0.534	0.499	0.550	0.280	0.513	$p=0.13$

On the other hand, Jessore had the lowest Shannon and Simpson indices values suggesting presence of more dominant species but less diversity, also reflected by low number of morphospecies (Table 2). A significant difference of the Alpha (α) diversity over trapping methods used for diversity measurements was found suggesting that the trapping methods differed in terms of samples trapped by them. The Malaise trap demonstrated the highest diversity among all traps, recording the highest Shannon and Simpson indexes. In contrast, the pitfall trap exhibited the lowest diversity with the lowest Shannon and Simpson diversity index. Moreover, both the yellow pan trap and the fluorescent yellow pan trap displayed almost similar diversity indices. The hymenopteran insects were more dominant in pitfall traps and evenly distributed in all the rest traps (Table 4).

Thus, YPT, FYPT and Malaise traps capture a higher number of species with relatively even distribution, as shown by their high Shannon and Simpson diversity values and moderate evenness (Table 4). They provide a more comprehensive picture of the hymenopteran community's biodiversity. On the other hand, pitfall trap captures fewer species and shows high dominance, low diversity, and low evenness, indicating dominance of a few species. Hence, the Pitfall trap may be more selective or biased toward certain ground-dwelling species, resulting in less diversity. The Sweep Net shows intermediate values, capturing some diversity but with moderate dominance and lower evenness compared to pan and Malaise traps.

4.4 Abundance of different hymenoptera functional groups

The collected insects were further grouped into four functional group, i.e., predator, parasitoid, herbivore and pollinator based on their ecological role in the ecosystem presented in Fig. 5.

Differences in hymenopteran functional guild composition among locations were analyzed using a chi-square test of independence revealed statistically significant differences present among the functional groups (χ^2 calculated value = 78.2942, p -value < 0.00001) suggested presence of significant variation among the different ecological groups of Hymenopteran insects present at the sampling sites. Higher numbers of parasitoids, with 143, 126, 227, and 213 individuals, respectively found at four locations, Gazipur, Mymensingh, Rangpur, and Dinajpur; while higher number of predator individuals (153)

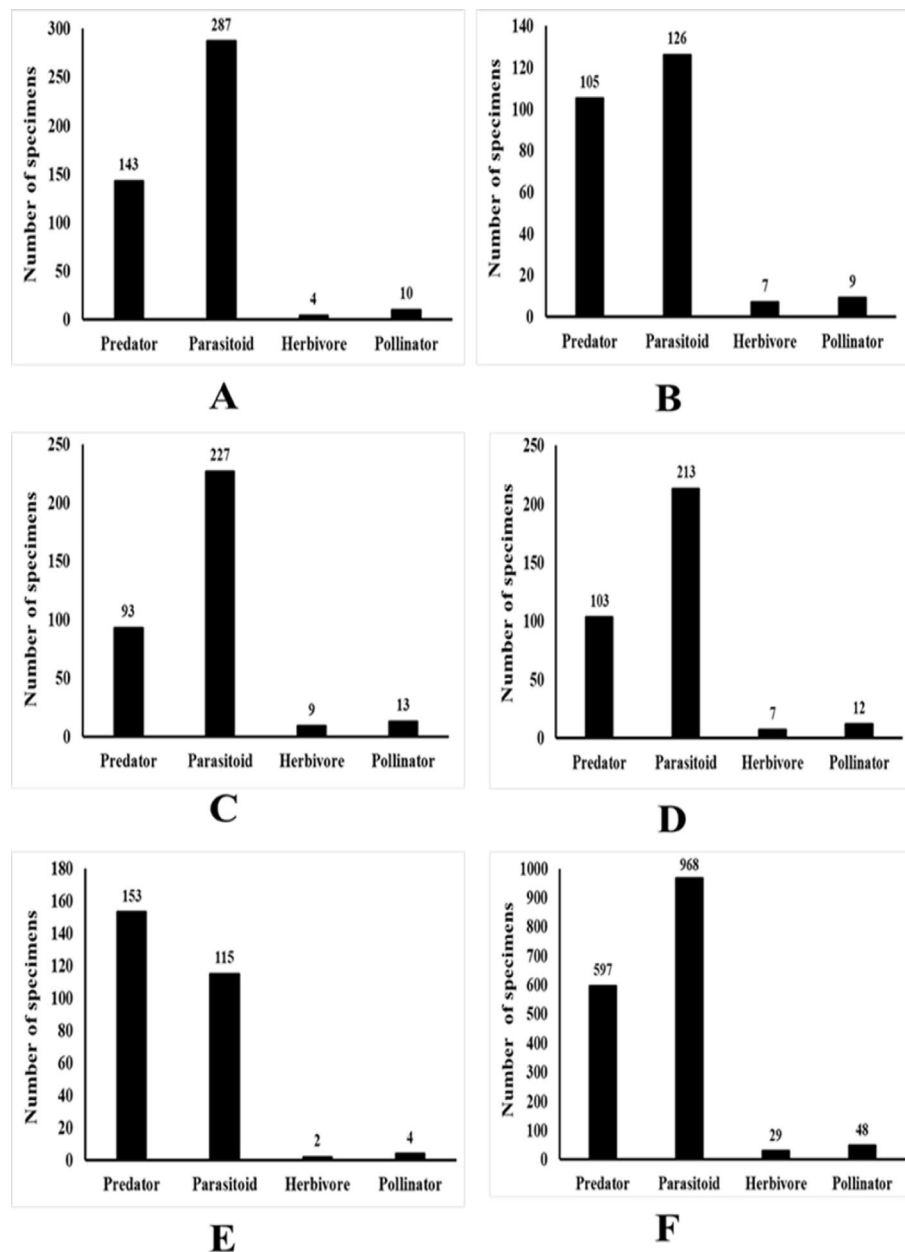


Fig. 5 Distribution pattern of Hymenopteran insects. **A:** Gazipur; **B:** Mymensingh; **C:** Rangpur; **D:** Dinajpur; **E:** Jashore; **F:** Total (χ^2 calculated value = 78.2942, p -value < 0.00001)

was recorded at the Jashore (Fig. 5). This implies that parasitoids and predators are the two main functional groups of Hymenoptera contributes to ecological functions. Herbivores and pollinators were comparatively much lower than other groups. Across all locations, prevalence of pollinators was higher than herbivores aligned the sampling of insects during reproductive phase of cotton plant, and herbivores consistently had the lowest numbers in each area (Fig. 5).

4.5 Efficacy of traps in assessing hymenopteran diversity

The bar diagram (Fig. 6) shows the proportional catch per unit effort (CPUE) of insects captured by different sampling methods, calculated using data pooled across all

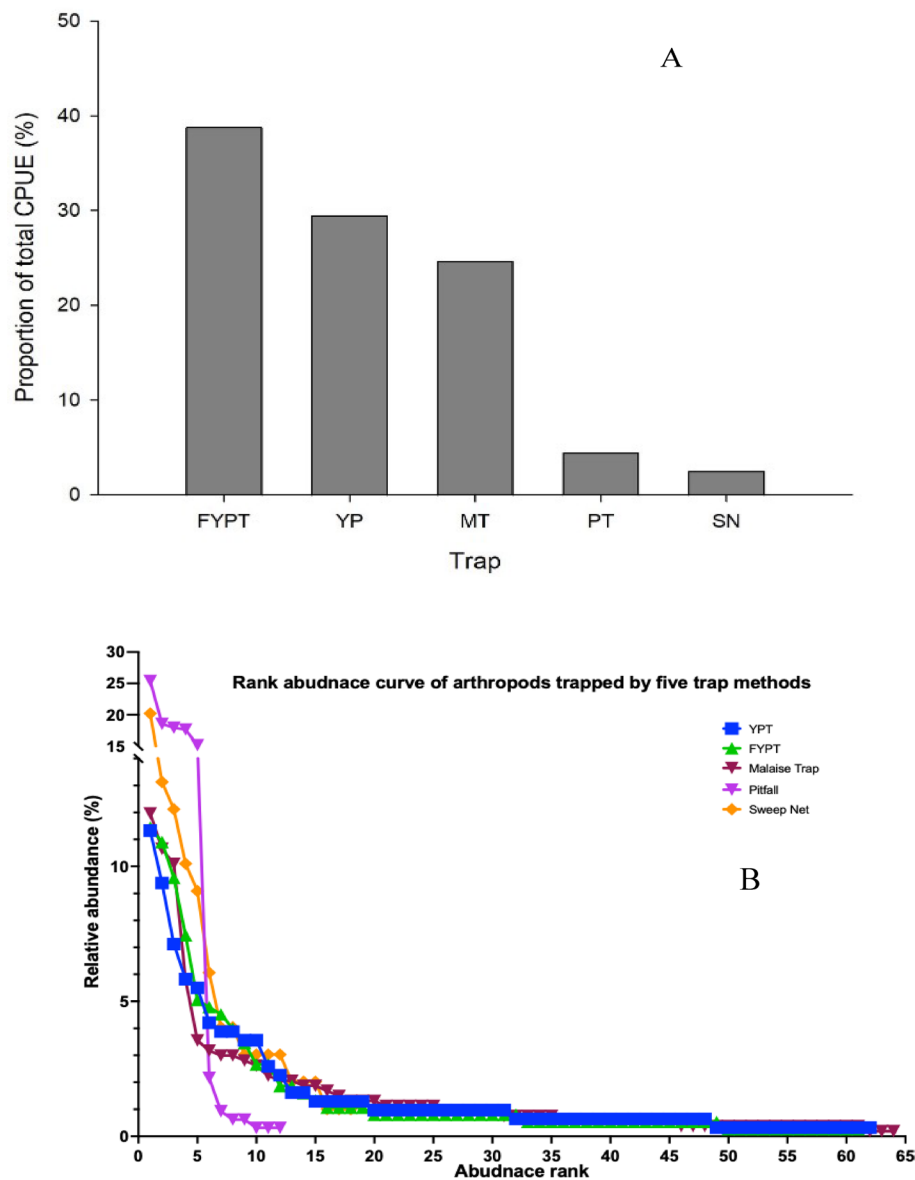


Fig. 6 Proportional catch per unit effort (CPUE) by different sampling methods (**A**) and rank abundance curve (Whittaker plot) of the shows the number of samples collected for morphospecies of insect families recorded (**B**). YPT-Yellow Pan Trap, FYPT- Fluorescent Yellow Pan Trap, Malaise Trap, PT-Pitfall Trap and SN-Sweep Net

sampling locations. Proportional CPUE represents the relative contribution of each sampling method to the total effort-standardized insect catch across the study area. Fluorescent yellow pan traps contributed the largest proportion of the total catch per unit effort (CPUE), accounting for approximately 39% of the effort-standardized insect captures. Yellow pan traps accounted for a further 29% of the total CPUE, followed by malaise trap around 24%. In contrast, pitfall traps contributed smaller proportions, representing approximately 4%, while sweep netting accounted for approximately 2.3% of the total. However, because sweep net effort was measured in sweeps rather than time-based trap-days, comparisons involving sweep net data should be interpreted with caution. Rank abundance curve (Fig. 6B). for different sampling methods aligned with the results of catch per unit effort (CPUE) (Fig. 6A).

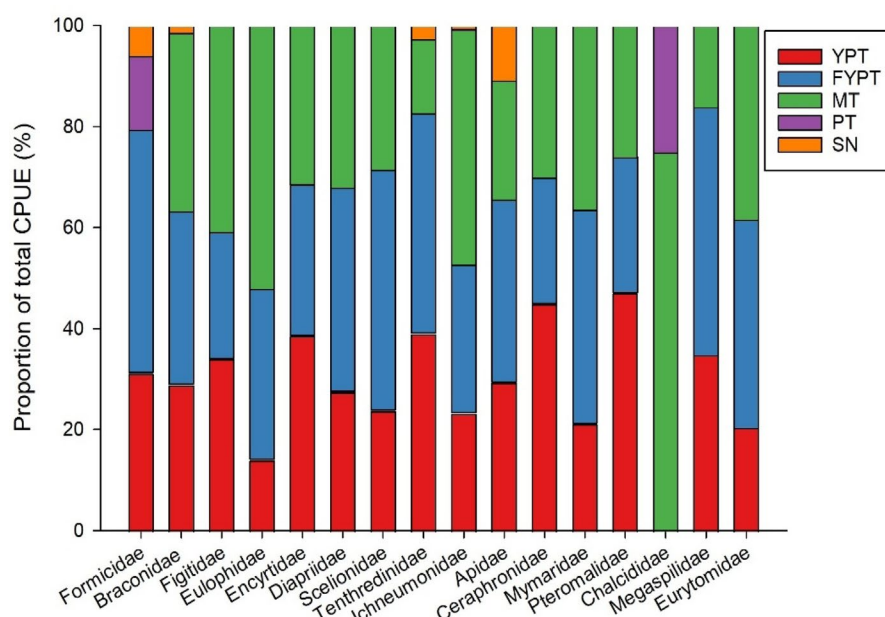


Fig. 7 Proportional catch per unit effort (CPUE) of insect families collected using different sampling methods. For each family, insect counts were standardized by sampling effort and expressed as proportions of the total CPUE across all sampling methods. Stacked bars illustrate the relative contribution of each method. Sweep net data are included for comparative purposes but should be interpreted cautiously due to differences in effort units. YPT- Yellow Pan Trap, FYPT- Fluorescent Yellow Pan Trap, MT- Malaise Trap, PT- Pitfall Trap and SN- Sweep Net

We further compared the effectiveness of different traps for capturing insects based on various identified families using proportional CPUE (Fig. 7). Yellow and fluorescent pan traps contributed to insect captures across nearly all families sampled, demonstrating broad coverage and high relative efficiency. Malaise traps also showed wide sampling breadth, with the exception of notably low proportional CPUE for *Formicidae*. Pitfall traps predominantly contributed to captures of *Formicidae*, reflecting their effectiveness for ground-dwelling insects. Sweep nets contributed proportionally to only a few families, indicating a more limited but targeted sampling scope.

5 Discussion

5.1 Role of hymenopteran insects in biodiversity

Biodiversity refers to the range of all kinds of life forms found in nature, shaped by evolutionary processes. It is critical for the proper ecosystem functioning, stability of ecosystem functions over time [8] and proper functioning of agricultural ecosystem [8, 30, 53]. A rich hymenopteran biodiversity particularly parasitoid and predator groups, play a critical role in maintaining the stability of cotton agroecosystems by naturally regulating pest populations [29]. In our study, among the functional guilds, parasitoids group was found as the most dominant group (about 60% of total) comprising of 13 families among the 16 families recorded in our study suggests a substantial potential of using them for the biological control of insects through the utilization of parasitoid wasps found in each location. We also found significant numbers of Apidae from all the studied places, which plays a crucial rule in pollination of crops [18]. Presence of low pollinators may due to use of pesticides and difference in cultural practices [37].

5.2 Role of hymenoptera in sustainable pest management

Parasitoids act as biological control agents for insect pests [15], predators like ants often play a key role in regulating pest populations [14], pollinators are crucial for increasing crop productivity [44], and herbivores are phytophagous pests of agricultural, ornamental, and forest crops [51]. These results indicate that the type of group significantly affects the measured variable, suggesting ecological or functional differences among the groups that may have implications for biological control and maintaining of ecosystem in the cotton field. Further investigation is warranted to explore these differences in more detail.

The members of Braconidae, Encyrtidae, Figitidae, Ichneumonidae, Megaspilidae, Pteromalidae, Ceraphronidae, Scelionidae, Eurytomidae and Formicidae have reported contributing in various biological control program of different pests. We found Formicidae, or ants, as the most abundant family of ground-dwelling insects. Ants play a diverse role in ecosystem such as fire ants “nurture” aphids and either attack or eliminate any potential predators [17]; indirectly lower herbivore populations, serving important biological control functions in agroecosystems.

Our results based on morphospecies showed presence of highest percent of Braconid parasitoids. Braconidae is one of the important family for biocontrol program around the world, specifically, against lepidopteran and coleopteran larvae [48]. We have identified 3 morphospecies of braconid wasps from cotton field, which emphasizes the greater potential for biological control of insect pests, since a number of braconid wasps have been reported as parasitoids of cotton insect pests, such as *Bracon vulgaris* Ashmead (Hymenoptera: Braconidae), a gregarious larval ectoparasitoid that attacks the boll weevil larvae, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae) [13], and *Microplitis mediator* Haliday (Hymenoptera: Braconidae), which parasitizes *Helicoverpa armigera* [58]. Similarly, our study records four morphospecies of Ichneumonidae, suggesting that there is a huge scope for biological control of insect pests in cotton such as *Campoletis chlorideae* (Ichneumonidae) had reported to be parasitized the cotton bollworm *Helicoverpa armigera* (Noctuidae) [26] and *Campoletis perdistinctus* is biological control agent of cotton bollworm and tobacco worm [9].

Similarly, Encyrtid parasitic wasp *Aenasius arizonensis* (Girault) can be used as a natural enemy for controlling the cotton Mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) [36]; members of the Megaspilidae family, particularly *Dendrocerus laticeps*, have significant potential for the biological control of cotton aphids, which are major agricultural pests, by parasitizing the primary parasitoid *Aphidius colemani* (Mitsunaga, [35]). Scelioninae holds considerable economic importance since they have been effectively utilized as classical biological control agents [45] such as *Telenomus ullyett* parasitized cotton bollworm [40]).

Although, we have not identified the identified morphospecies to genus or species level, but the examples reported above are the natural biocontrol agents of cotton insect pests infesting during the reproductive phase. Given that the sampling was conducted during the flowering to ball formation stages, we hypothesize that the morphospecies reported here are likely belongs to similar taxonomic groups associated with cotton agroecosystems at comparable phenological stage. An in-depth taxonomic and biological studies are required before these morphospecies can be deployed in pest management programs.

5.3 Distribution of hymenopteran insects

The hymenopteran insects present in different locations are significantly differed. Various environmental parameters such as temperature, moisture, light greatly governed the distribution and abundance of insects due to their influence on physiological functions and life-cycle [57, 59]. We, therefore, analyzed the Karl Pearson's correlation coefficient whether there is a relationship between various weather parameters, the number of Hymenoptera insects caught by various traps, and diversity indices. We found that most correlations were not significant except Pitfall vs. minimum relative humidity ($r=0.93$, $P<0.05$) and minimum temperature ($r=0.90$, $P<0.05$) were found (Supplementary Fig. S2) suggesting that these parameters have a great impact on the number of trapping soil-dwelling insects.

Although correlations were observed with average relative humidity, maximum relative humidity, and minimum relative humidity between number of insects caught by other traps, but these were not statistically significant. The total number of insects (TNI) exhibited negative correlation with average relative humidity, maximum relative humidity, and minimum relative humidity (correlation coefficients of -0.69 , -0.71 , and -0.23 , respectively). Conversely, it showed a positive but non-significant correlation with average temperature, maximum temperature, and minimum temperature (correlation coefficients of 0.50 , 0.45 , and 0.11 , respectively). The Shannon index (HI) and Simpson index (SI) had non-significant negative correlations with average relative humidity and minimum relative humidity but non-significant positive correlations with average temperature, maximum temperature, minimum temperature, and maximum relative humidity (Supplementary Fig. 1). The absence of precipitation during this time frame could have contributed to the decreased variety within the hymenopteran family [42]. Since, correlation analyses involving multiple weather variables without correction for multiple testing may produce false-positive results and majority of the correlations in our study were found not-significant, these results must be interpreted cautiously. To understand the distribution of hymenopteran insects in relation to weather parameters, an in depth and multiyear study is therefore recommended.

5.4 Hymenoptera in Bangladesh

Bangladesh has vast diversity of fauna and flora. Our findings indicate a rich and diverse presence of hymenopteran taxa within the cotton ecosystems of Bangladesh. In addition, members of the families Ampulicidae, Bembicidae, Colletidae, Crabonidae, Halictidae, Megachilidae, Pemphredonidae, Philanthidae, Bethylidae, Dyrinidae, Chrysididae, Pompilidae, Scoliidae, Tiphidae Vespidae [32], Aphelinidae [6]; Mymaridae [24] has been reported from Bangladesh [1, 24, 43, 56]. Although, we have recorded a total of 16 hymenopteran families from cotton field, 173 genera across 23 families [30] were reported from a survey at Rajshahi University and Chittagong University, Bangladesh clearly indicated presence of vast diversity of hymenopteran insects present in Bangladesh. The disparity in diversity between the cotton fields and university campuses may be attributed to the monoculture practices and pesticide usage in the fields. In contrast, the university campuses boast a greater variety of undisturbed vegetation. It is well-established that undisturbed habitats foster heightened biodiversity and abundance [28]. Additionally, the application of insecticides has been shown to lead to a decrease in both wasp abundance and richness [46]. These findings emphasize the value of incorporating

semi natural habitats such as field margins, hedgerows, and flowering strips around cotton fields to provide essential resources and refuges for beneficial insects, thereby promoting greater Hymenoptera diversity and enhancing their role in biological pest control.

5.5 Variation of traps

To effectively study and monitor different types of insects for biodiversity research, it's crucial to use appropriate sampling methods. The choice of sampling method depends on the specific environment being studied, as different environments require different strategies. In our study, we utilized five types of traps to collect hymenopteran insects. Notably, malaise traps, yellow pan traps, and fluorescent pan traps demonstrated higher catch per unit effort (CPUE) and greater sampling efficiency compared to other methods. It is worth mentioning that the malaise trap, the fluorescent trap, and the yellow pan trap demonstrated the highest and similar taxa richness. Our results align with the findings of a study that compared the sweep net, yellow pan trap, and malaise trap for sampling parasitic Hymenoptera in a backward habitat in Kerala, [49]. The fluorescent and yellow pan traps demonstrated comparable taxa richness and nearly similar Shannon and diversity indices, although the fluorescent trap captured higher number of individuals which is statistically not significant suggesting suitability of using any one of the colour trap based on availability in arthropod diversity study as reported previously [19]. Furthermore, pitfall trap is highly effective in trapping ground-dwelling insects, especially in collecting Formicidae is a contrasting result compared to a study conducted on urban green rooftops, where numerous families were recorded in pitfall traps [16]. This disparity in results could potentially be attributed to the differences in the irrigation practices and vegetation present in the survey areas employed in the crop field [47]. However, given the limitations of our study design, including the lack of sufficient replication and varying trap numbers, future research should incorporate increased replication and standardized trap deployment to more accurately assess the relative effectiveness of these sampling methods. Expanding trap numbers and consistent effort across locations will improve the reliability of comparisons and help optimize sampling protocols for monitoring insect communities in cotton agroecosystems.

6 Conclusions

Our results indicate Bangladesh cotton ecosystems harbors a rich diversity of Hymenopteran assemblages during reproductive stage majority of which belongs to the parasitoids, suggesting significant potential for biological control of insect pests. Additionally, our data demonstrate that yellow pan traps, fluorescent yellow pan traps, and malaise traps are highly effective for capturing a greater number of parasitoids and other insects under different Hymenopteran families, while pitfall traps are particularly effective for capturing ground-dwelling insects. This data could provide a solid foundation for future studies on the diversity of Hymenopteran insects in cotton ecosystems in Bangladesh and support the development of sustainable biocontrol management strategies. However, since there is no comprehensive identification key for species-level classification in Bangladesh, comprehensive taxonomical and molecular analysis experiments through DNA barcoding are planned as future research topics.

Supplementary Information

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Supplementary Material 1

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Author contributions

M.M.R.: Methodology, Investigation, Formal analysis, Visualization, Writing—Original Draft T.H.: Formal analysis, Writing—Review & Editing, M.T.H.H.: Conceptualization, Formal analysis, Resources, Writing—Review & Editing, Funding acquisition, Project administration.

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Data availability

All available data generated by experiments mentioned in this article are included either in the manuscript or in the supplementary material. Raw datasets used and/or analyzed during the current study are available from the corresponding authors upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent to publish

All authors have read and approved the final version of the manuscript and consent to its publication in *Discover Sustainability*.

Competing interests

The authors declare no competing interests.

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