



Assessing adverse impact of the native biological control disruptors in the colonies of the recent invasive pest *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae) in India

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ABSTRACT

New challenges appear with every biological invasion and presses need to probe their ecological interactions. In the comprehensive yet complicated food web associated with the niche of the recently invaded cassava mealybug (CMB) *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae), there was a multitrophic interaction structured vertically as well as horizontally. Altogether 45 species: thirty four species of insects from six orders (Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, and Neuroptera) and eleven species of spiders (Araneae) were grouped under four trophic levels into 11 guilds. The analysis of trophic guild structure and interaction indicated that many indigenous parasitoid species, which qualified to be placed under the fourth trophic level, actively parasitized the potential native predators of cassava mealybug (CMB) and thereby negatively impacted the natural biological control of CMB. Most of these resident hyperparasitoid species were recorded for the first time, to be associated directly or indirectly with CMB. The species diversity at fourth trophic level could be viewed as a bio-indicator and one of the most important determinant factors on the success rate of any biological control program. No indigenous primary parasitoids were documented on CMB from any of the sites sampled. In the absence of any indigenous parasitoids and high level of parasitization of the potential CMB predators, the long-term and indirect ecosystem risks will be significant until the introduction and establishment of the proven classical biological control agent, *Anagyrus lopezi* (De Santis) (Encyrtidae: Hymenoptera) from other countries.

1. Introduction

Cassava is an important cash and food crop in India. India occupies fifth place in cassava cultivation in the world whereas Thailand

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and India are the two major cassava growing countries in Asia. Cassava is ranked as the sixth most important calorific source in the human diet and fourth most important staple food in the tropics (Bellotti, 2008). In India, cassava crop is mainly grown in Tamil Nadu and Kerala, accounting for 51.9% and 31.7% of area and 57.8% and 34.9% of production, respectively (Anonymous, 2018). Barring the papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink, cassava crop in India was relatively free of pests until the arrival of cassava mealybug during the early part of 2020. The cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae), was accidentally introduced into India and was first reported from Kerala state of southern India (Joshi et al., 2020) and then in Tamil Nadu (Gupta et al., 2020; Sampathkumar et al., 2021). This species of mealybug is of Neotropical origin which got introduced into Africa from its aboriginal home in South America in the early 1970s and then in Asia (Thailand) during 2009 and became the most destructive pest on cassava worldwide (Neuenschwander, 2001; Calatayud and Le Rü, 2006; Winotai et al., 2010). Parsa et al. (2012) through CLIMEX niche model predictions suggested that *P. manihoti* imposes an important threat to cassava production in Asia with southern part of India to be at high risk of outbreaks (Ecological Index > 20) though their predictions restricted to Karnataka are questionable as the major cassava growing areas are Tamil Nadu and Kerala. Yonow et al. (2017) envisaged that even other parts of India can be at risk if cassava production is expanded. Despite warnings on bio-security threat and risk of invasion, the pest was successful to enter the country. Joshi et al. (2020) have prognosticated its chances of survival and spread on other hosts other than Euphorbiaceae for instance *Alternanthera sessilis* (Amaranthaceae), *Synedrella nodiflora* (Asteraceae) and *Blumea lacera* (Asteraceae).

Lal and Pillai (1981) mentioned about the increasing popularity of cassava as an industrial crop and as cattle feed in India and documented pests and their control measures from southern India. The cassava pest complex consists of primary pests which are likely to be co-evolved along with host as well as generalist feeders or sporadic opportunistic newly adapted pests with restricted geographic distribution (Bellotti et al., 2012). Biological control by natural enemies offers a self sustaining mechanism for the management of invasive pests. However, natural enemy abundance and performance are regulated by a variety of factors operating at field level. Natural enemies of phytophagous pests often flourish well under the least disturbed habitat and the long duration cassava ecosystem is highly amenable for conservation biological control and habitat management where the natural enemies are encouraged to proliferate more for enhanced biological control of the pests. However, the positive effects of enhanced biological control may sometimes hamper if it enhances the fitness and diversity of species at fourth trophic level (Le et al., 2018). With the accidental introduction of CMB in India, the studies on the intra-guild interactions in cassava are important especially at higher trophic levels to assess the effectiveness of native biological control agents and potential threat if any for the effectiveness of the classical biological control agent,

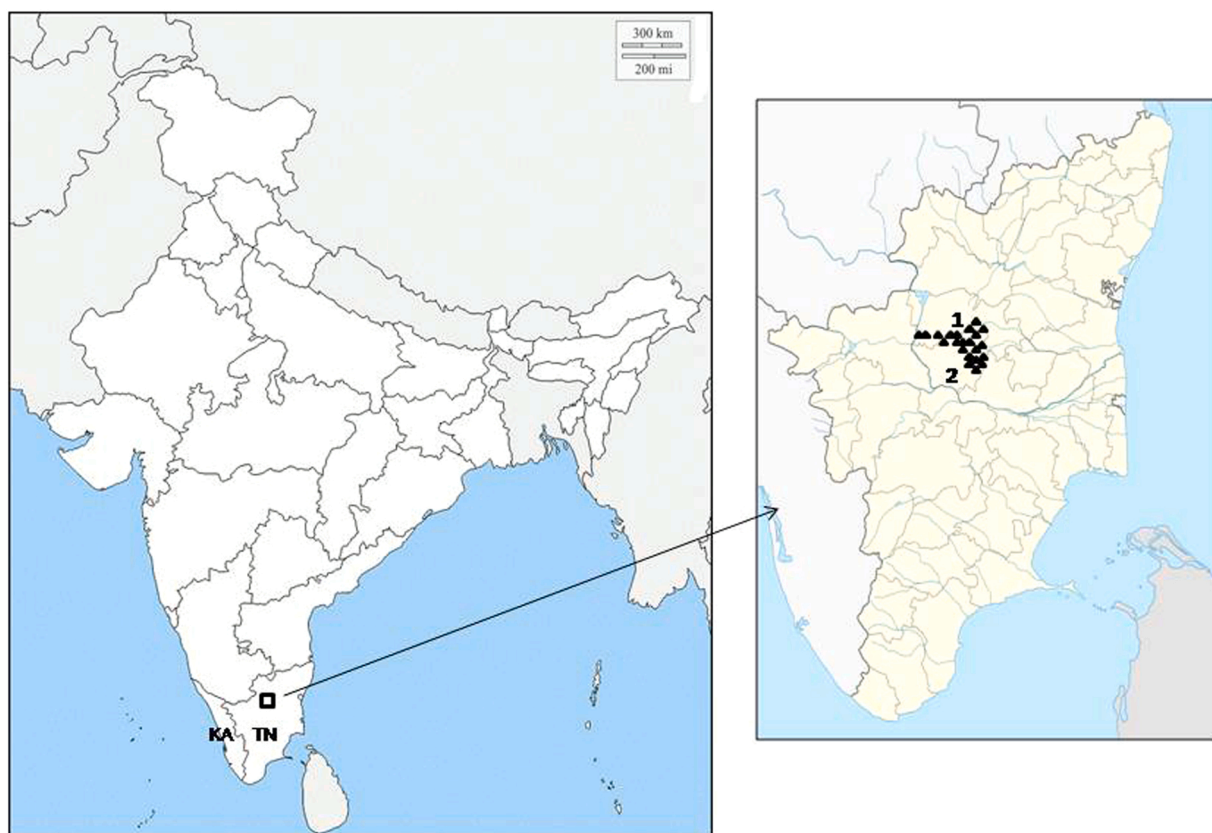


Fig. 1. A. The distribution map of CMB in Indian states (KA - Kerala, TN - Tamil Nadu) during the crop season of 2020 and delineation of the study sites (1 - Salem, 2 - Namakkal districts) in Tamil Nadu. B. The study sites at (1) Salem and (2) Namakkal districts of Tamil Nadu State in South India.

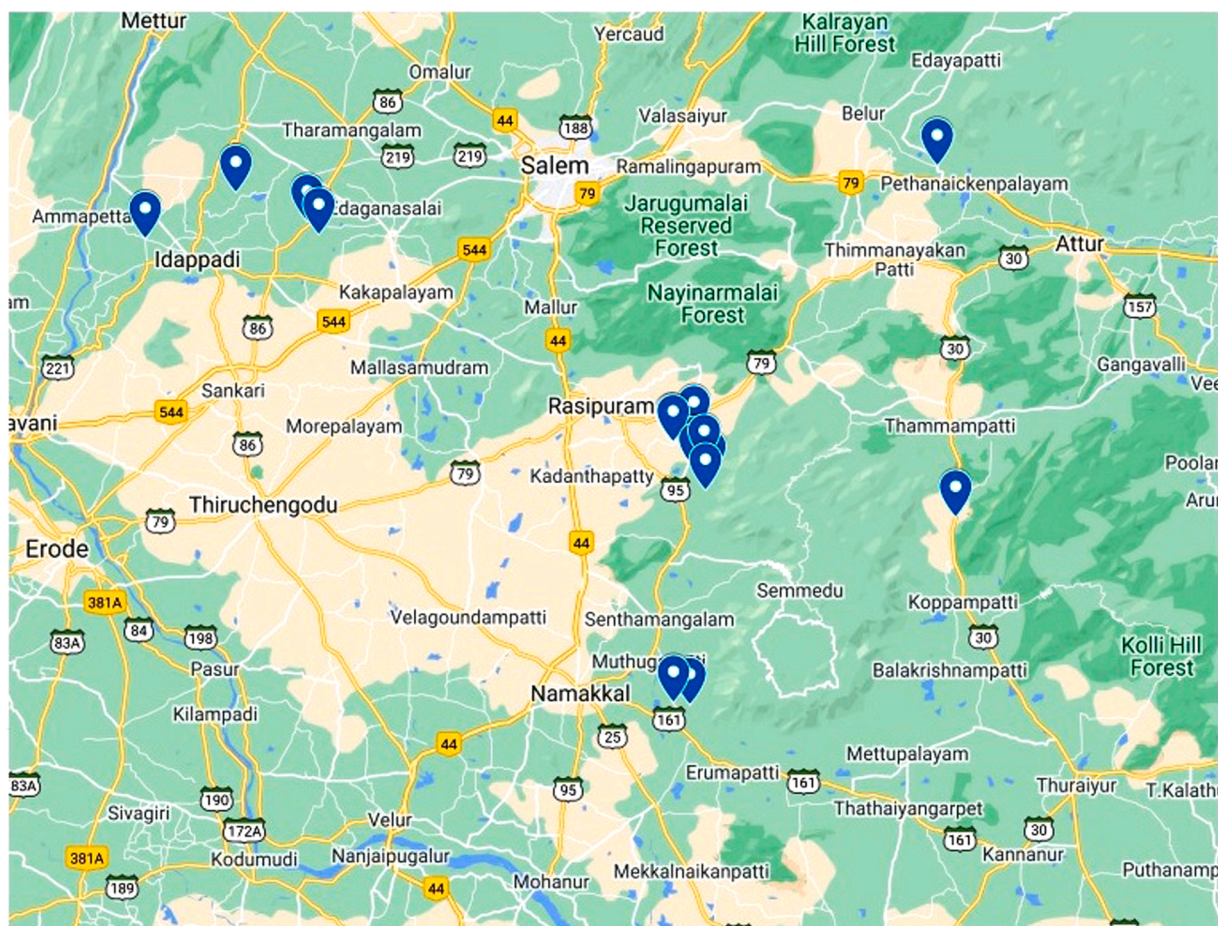


Fig. 1. (continued).

Anagrus lopezi (De Santis) (Encyrtidae: Hymenoptera) to be introduced shortly into India.

2. Material and methods

2.1. Study area

The field sites in Salem (11.6643° N, 78.1460° E) and Namakkal (11.2189° N, 78.1674° E) districts (Fig. 1A, B) of Tamil Nadu State in South India were chosen for the studies. The sites are characterized by hot and dry weather year round, staggered and intensive cultivation of indigenous (improved and local) and exotic varieties of cassava in a mono-cropping fashion. Barring the application of herbicide at initial phase and split application of fertilizers at tuber formation stage, the cassava crop was free of any other treatments including insecticidal applications. On this backdrop, the flaring up of the newly invaded cassava mealybug (CMB) population (Fig. 2) was observed during the summer season (May–July) of 2020. The maximum average temperature during the study period was above 35 °C and the minimum average temperature was above 25 °C, whereas the minimum relative humidity fluctuated between 35% and 46%. Such condition is conducive for faster multiplication of mealybugs. The occurrence and severity of CMB assemblages on different cassava varieties grown at Vellarivelli (11.6010° N, 77.7934° E), Chettimankurichi (11.6402° N, 77.8714° E), Koranampatti (11.6156° N, 77.9329° E), Kachchippalli (11.6045° N, 77.9429° E), Yethapur (11.6627° N, 78.4751° E) villages in Salem district and Thoppapatti (11.4282° N, 78.2470° E), Tho. Pachudayampalayam (11.4376° N, 78.2654° E), Kalkurichi (11.4115° N, 78.26549° E), T. Jeddarpalayam (11.4294° N, 78.2476° E), Murungapatti (11.3663° N, 78.4907° E), Bodinayakkanpatti (11.2079° N, 78.2619° E), Rootupudur (11.2101° N, 78.2482° E), Vellalapatti (11.4064° N, 78.2667° E), Valayapatti (11.4001° N, 78.2792° E), Pallipatti (11.3892° N, 78.2753° E), Eachampatti (11.4134° N, 78.2743° E) villages in Namakkal district were assessed. The distance among the collection sites varied from 2 to 46.4 km. During the survey, the cassava varieties Mulluvadi, Thailand white, H-165, Sree Vijaya and Sree Athulya were found severely infested by the CMB.



Fig. 2. A-Woolly ovisacs of CMB; B- Yellow eggs, ovoid adult females of CMB and crawlers.

2.2. Sampling procedure for CMB

Sampling started during April, 2020 and continued till November, 2020 at regular interval. In each site, 15–20 unsprayed cassava fields representing the susceptible cultivars with approximately one acre size and similar age of planting were chosen. The study also ensured sample collection on the fields with similar age of the crop. The damaged, healthy plants and plants showing bunchy top symptoms were counted by diagonal walking in each field as per our previous studies (Sampathkumar et al., 2021).

Twenty five randomly collected infested shoot tips from each field were assessed for estimating the CMB, predator and parasitoid population densities. The number of immature stages and the adult females of CMB were counted from the shoot tip both on the main stem and under surface of the leaves. The twisted shoots and shoot tip with multiple tillers were counted for the determination of bunchy top/stunted plants. The average number of CMB adults and immature stages per shoot tip was assigned into one of the following categories as per Neuenschwander et al. (1989) as 0, 1–9, 10–99, 100–999 and ≥ 1000 . The log ($X + 1$) transformed upper values of mealybugs under these categories were assumed as 0, 1, 2, 3 and 4, respectively. The same shoot tip samples were used for scoring the damage scale as described by Nwanze (1982) and PRONAM (PROGRAMME NATIONAL MANIOC) (1978) as 1: no damage, 2: slight curling of leaf margins, 3: slight bunching of the tip, 4: pronounced distortion of the tip (bunchy top), 5: severe defoliation.

2.3. Assessment of density of higher order trophic guilds

The adult and immature stages of generalist and specialist predators belonged to various insect orders viz., Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, and Neuroptera. Spiders were counted separately on CMB assemblages at the shoot tips collected from various sites. Since, the parasitism rate is not countable in the field; the documentation of the primary and secondary (hyper) parasitoids was done by separating each heavily infested cassava shoot tip in individual growth chambers at the QC-2

quarantine facility of ICAR-National Bureau of Agricultural Insect Resources. The emergence of primary parasitoids and predators was noted at regular intervals after carefully separating the parasitized hosts in individual containers. Similarly, the major predatory species from all the survey sites were sorted and later were reared individually (retaining the site codes) on CMB colonies to notice the emergence of hyperparasitoids. The percent parasitization was calculated from the number of predators reared and numbers found parasitized. The CMB nymphs and adults were also dissected under a stereomicroscope for checking the presence of immature stages of parasitoids. All the predators, parasitoids and hyperparasitoids were preserved either as dry mounting or in 70% ethanol for subsequent identification.

2.4. Identification of species

In the present study, morphological identification of the CMB samples was further confirmed with DNA barcoding. A single third instar nymph, emerged from field collected ovisacs, was subjected for DNA extraction as per OEPP/EPP0 guidelines (PM7/1291, 2016) with essential modifications according to Pacheco da Silva et al. (2014). The extracted DNA was used for polymerase chain reaction (PCR) amplification of partial mitochondrial cytochrome c oxidase 1 (CO1) gene using the forward (LCO 1490 5'-GGTCAACAAATCATAAAGATATTGG3') and reverse primer (HCO 2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3') as per the standard protocol (Hebert et al., 2003; Sampathkumar et al., 2021). Polymerase Chain Reaction (PCR) was carried out in flat capped 200 µL volume PCR tubes obtained from M/s Tarsons, Kolkata, India. 50 µL reaction volume contains: 5 µL GeNei™ Taq buffer, 1 µL GeNei™ 10 mM dNTP mix, 1 µL (20 pmol/µL) forward primer, 1 µL (20 pmol/µL) reverse primer, 1 µL GeNei™ Taq DNA polymerase (1 U/µL), 5 µL DNA (50 ng/µL), and 36 µL sterile water. Thermo cycling consisted of an initial denaturation of 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 1 min. PCR was performed using a BioRad C1000™ Thermal Cycler. The amplified products were analyzed on 1.5% agarose gel electrophoresis and amplified products were sequenced (ABI3130 platform) by M/s Eurofins Genomics, Bengaluru, India. The samples were bi-directionally sequenced and checked for homology, insertions and deletions, stop codons, and frame shifts by using NCBI-BLAST and ORF finder. The sequences showed 100% identity with GenBank accession numbers of *P. manihoti*, KY611349, KY611348, KY611347, KY611346 and MZ542529 submitted from China in National Centre for Biotechnology Information (NCBI) and the details of Genbank accession numbers and their respective locations are given in Table 1.

The primary and secondary parasitoids and predators were identified to species level based on morphological characters. The endo/ecto and solitary/gregarious nature of the parasitoids and hyperparasitoids were also noted. Microscopic images were taken with a Leica M 205 stereo zoom microscope with Leica DC 420 inbuilt camera using automontage software (version 3.8). The vouchers of the examined and DNA Barcode (Fig. 3) specimens were deposited in the National Insect Museum of ICAR- ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru.

3. Results

3.1. Density of CMB and damage on cassava

The morphological identification confirmed the sole presence of CMB in almost all the 113 field sites in the sampled villages except in one cassava field where the CMB co-existed with the papaya mealybug, *P. marginatus*; latter being heavily parasitized by *Acerophagus papayae* Noyes & Schauf (Encyrtidae). As *P. manihoti* invaded India, it started spreading rapidly in the cassava growing areas of Namakkal and Salem districts of Tamil Nadu where drought like situation occurred during the crop season of 2020. The rainfall during the study period (Jan-July 2020) was 393.1 mm as against 505.9 mm during 2019 in the study locations. The percent infested plants ranged from 5.8 to 76.9 and the plants showing bunchy top symptoms ranged from 11.6% to 56.3% (Table 2, Fig. 4). The population outbreak of CMB occurred during May 2020 and continued upto July end where the CMB density scale (scale 3–4) and shoot tip damage scale (scale 4–5) reached the maximum due to severe drought and absence of any precipitation (Fig. 5). Almost all the popular cassava cultivars such as Thailand types, Mulluvadi, Sree Atulya, Sree Vijaya and H-165 were severely infested by CMB in all the locations sampled.

Table 1
Sampling locations for DNA Barcoding of invasive cassava mealybug.

Location details	Place	Name of the district in TN	Gen Bank Accession number
Location 1	Thoppapatti	Namakkal	MT895817
Location 2	T. Jeddarpalayam	Namakkal	MW039322
Location 3	Vellarivelli	Salem	OK172179
Location 4	Yethapur	Salem	OK172342
Location 5	Koranampatti	Salem	OK172561
Location 6	Thoppapatti	Namakkal	OK172562
Location 7	T. Jeddarpalayam	Namakkal	OK174324
Location 8	Kalkurichi	Namakkal	OK173048

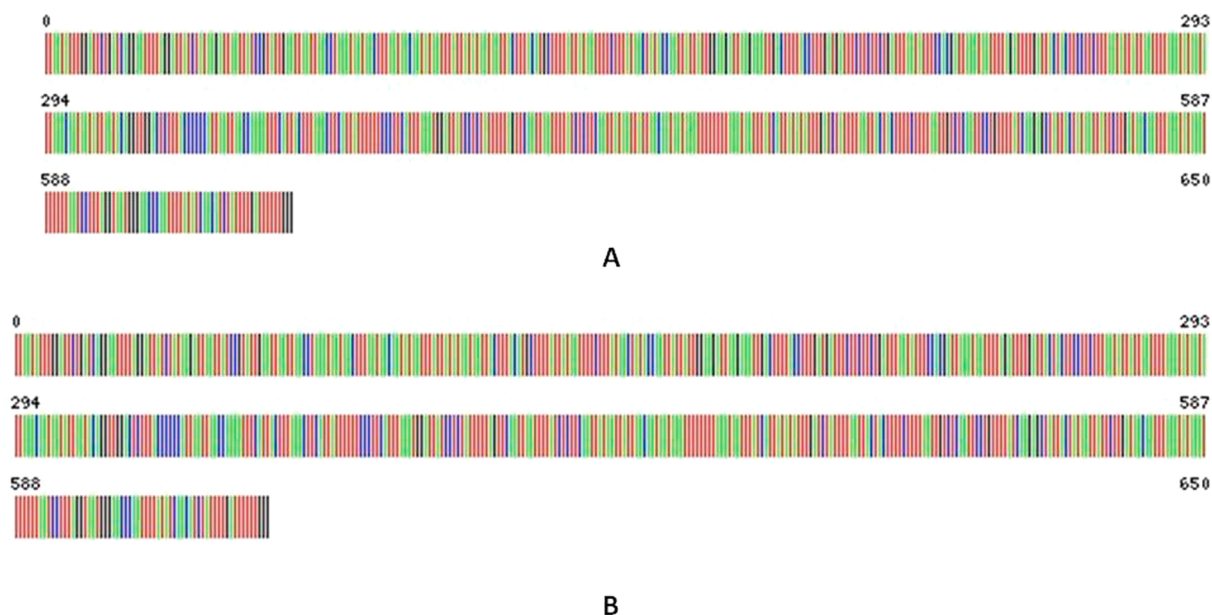


Fig. 3. DNA barcodes of *Phenacoccus manihoti* (A) NCBI Acc. No. MT895817 and (B) NCBI Acc. No. MW039322.

Table 2

Survey on the severity of cassava mealybug on cassava crop in Salem and Namakkal districts of Tamil Nadu State in India.

Month/ 2020	Cassava cultivars sampled	% infested plants	% plants with bunchy top	<i>P. manihoti</i> density scale ^a	Shoot tip damage scale ^b
April [#]	Thailand white, Mulluvadi	Moderate to severe	Moderate to severe	3–4	–
May	Sree Athulya, Mulluvadi	29.6–46.8	18.4 - 34.0	3–4	4–5
June	Thailand white, Sree Vijaya	47.6–63.7	11.6–53.6	3–4	4–5
July	Mulluvadi, Thailand white	25.7–59.2	20.6–46.3	4	4–5
August	Thailand white, Mulluvadi	33.4–76.9	26.3–56.3	4	2–4
September	Sree Athulya, mulluvadi, Thailand white	11.6–36.5	29.3–42.4	1–3	2–3
October	H-165, mulluvadi, Sree Vijaya	27.8–29.3	19.8–49.4	0–2	2–3
November	Mulluvadi, Thailand white	5.8–33.4	23.5–46.8	0–2	1–3

[#] Data not quantified.

^a 0: No mealybug, 1: 1–9 mealybugs/shoot tip, 2: 10–99 mealybugs/shoot tip, 3: 100–999 mealybugs/ shoot tip, and 4: ≥ 1000 mealybugs/shoot tip.

^b 1 = No damage, 2 = slight curling of leaf margins, 3 = slight bunching of the tip, 4 = pronounced distortion of the tip (bunchy top), 5 = severe defoliation.

3.2. Density of higher order (third and fourth) trophic guilds

The study revealed that there was an array of general predators (third trophic level) and their parasitoids (fourth trophic level) which are illustrated in Figs. 6–10 and listed in Tables 3 and 4. The predators such as *Hyperaspis maindroni* Sicard (Coleoptera: Coccinellidae), *Autoba silicula* (Swinhoe) (Lepidoptera: Erebididae), *Mallada desjardinsi* (Navas) (Neuroptera: Chrysopidae), *Spalgis epius* (Westwood) (Lepidoptera: Lycaenidae), *Cheilomenes sexmaculata* Fabricius (Coleoptera: Coccinellidae), *Pseudomallada astur* (Banks) (Neuroptera: Chrysopidae) and spiders were feeding on CMB representing interguild interactions. The average density of the young ones and adults of the *H. maindroni* per shoot tip ranged from 2.1 to 36.2 followed by *A. silicula* (0.8–21.6 larvae/shoot tip), chrysopids viz., *M. desjardinsi* and *P. astur* (0.0–13.5), *S. epius* (0.2–12.6), *Stathmopoda* sp. (Lepidoptera: Oecophoridae) (0.0–5.6), *C. sexmaculata* (0.0–4.2), and *Nola* sp. (Lepidoptera: Nolidae) (0.0–0.9). *Anatrachyntis* sp. (Lepidoptera: Cosmopterigidae), a possible scavenger on the CMB colonies also was found at the density of up to 2.6 larvae per shoot tip.

The species at fourth trophic levels were also recorded from the predator species reared on CMB colonies. Up to 29.8–63.9% parasitization of *H. maindroni* by *Homalotylus turkmenicus* Myartseva (Hymenoptera: Encyrtidae) and *Metastenus concinnus* Walker (Hymenoptera: Pteromalidae) was noticed as an independent intraguild interaction. Also 11.0–23.2% parasitization of *M. desjardinsi* by *Tetrastichus* sp. (Hymenoptera: Eulophidae) was observed as yet another independent intraguild interaction. The wasp species viz. *Apanteles* sp. (Hymenoptera: Braconidae), *Antrocephalus japonicus* (Masi) and *Brachymeria* sp. (Hymenoptera: Chalcididae), *Hockeria*

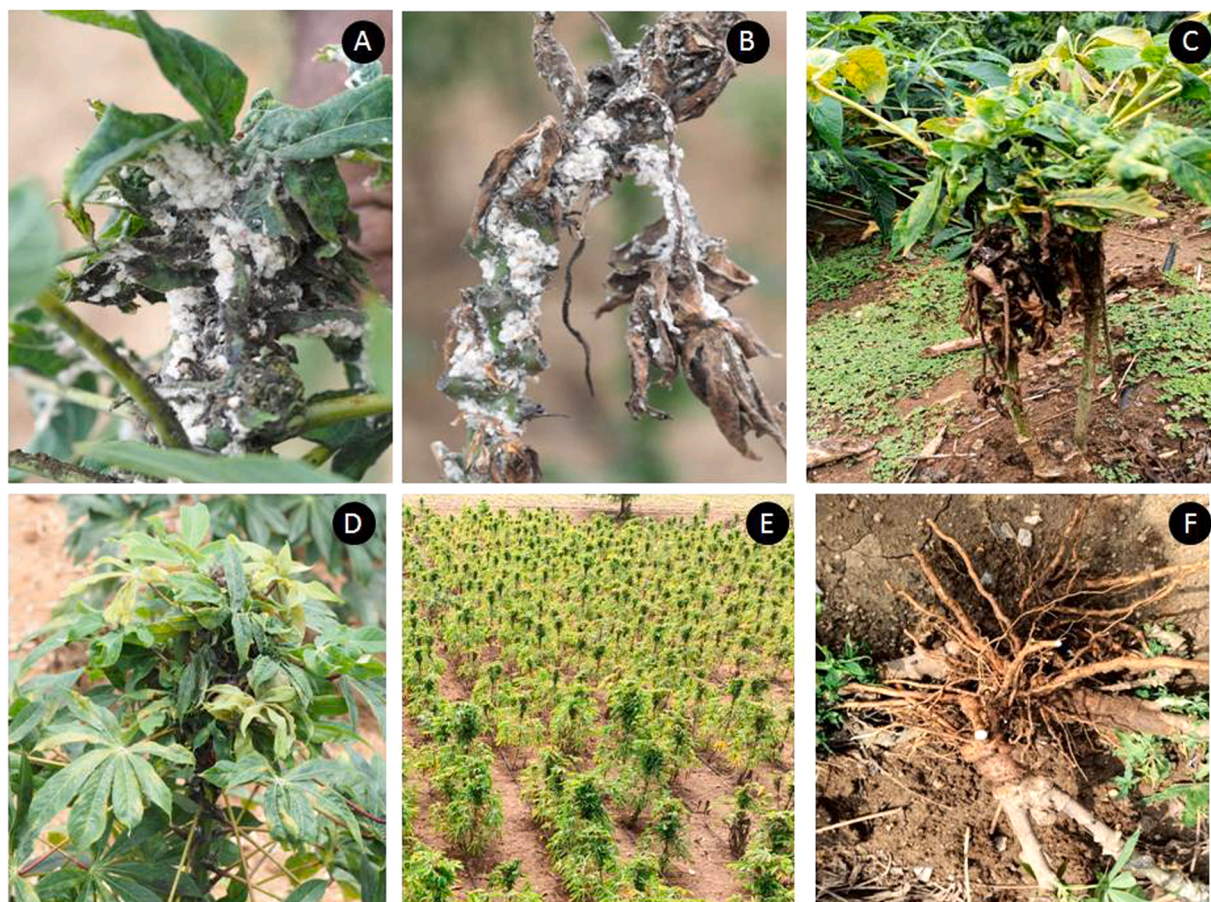


Fig. 4. Field images of damage caused by *P. manihoti* on cassava. A and B - CMB colonization on shoot tips, C and D - stunted plants with multiple shoots (bunchy tops), E - Birds eye view of severely infested cassava field with bunchy top symptom, F - plants with no or rudimentary tubers.

sp., *Hockeria nikolskayae* Husain and Agarwal (Hymenoptera: Chalcididae) were recorded on the larvae of *A. silicula* to the tune of 39.4–56.8% as co-inhabitants of this intraguild. Spiders as euryphagous predators were observed in the niche of cassava mealybug colonies. Eleven species namely, *Indoxysticus* sp., *Carrhotus viduus* (C. L. Koch), *Thyene imperialis* (Rossi), *Scytodes fusca* Walckenaer, *Thomisus pugilis* Stoliczka, *Plexippus paykulli* (Audouin), *Hyllus semicupreus* (Simon), *Plexippus petersi* (Karsch), *Cheiracanthium* sp., *Cheiracanthium approximatum* O. Pickard-Cambridge and *Neoscona* sp. representing five families viz., Araneidae, Cheiracanthiidae, Salticidae, Scytodidae and Thomisidae were documented. The abundance of salticid spiders viz., *T. imperialis* and *C. viduus* were observed in the CMB infested shoot tip of the cassava plant. The mean spider density per shoot tip for *T. imperialis* and *C. viduus* was observed as 2.40 and 1.60, respectively. The other spiders observed in the niche habitat are listed in the Table 4 and Fig. 10. As spiders generally intend to capture the prey equal to its size, this specific attribute was observed where juveniles of *Indoxysticus* sp. (Thomisidae: Araneae) alone were found feeding on the nymphs of CMB.

Hyperaspis maindroni was noticed as one of the predominant predators competing with *M. desjardinsi* in the cassava mealybug niche. However, both these predators in parallel guilds were subjected to high parasitism pressure with no demarcation of niche partitioning. Three species of wasps namely *Aprostocetus* sp., *H. turkmenicus*, and *M. concinnus* parasitizing immature stages of *H. maindroni* were observed. Amongst them, *H. turkmenicus* was the predominant species followed by *Aprostocetus* sp. *Homalotylus turkmenicus* significantly reduced predatory coccinellid populations with 65.67–80.95% parasitism (Gupta et al., 2020). Also a hyperparasitoid, *Charitocerus* sp. near *walkeri* Hayat (Signiphoridae) was reared from *H. maindroni* and upto six wasps emerged from a single pupa of *H. maindroni*. *Homalotylus turkmenicus* is a gregarious endoparasitoid and upto eleven adults emerged from a single pupa of *H. maindroni*. The eulophid parasitoid *Tetrastichus* sp. was observed as the most predominant parasitoid of the dominant predator *M. desjardinsi* in the CMB colonies; about 5–30 wasps emerged from single cocoon of *M. desjardinsi*. *Brachycyrtus* sp., a solitary ichneumonid endoparasitoid, bred from the cocoons of *M. desjardinsi*, was the second species responsible to bring down the predator population. In the sites where the population of *H. turkmenicus* and *Aprostocetus* sp. was comparatively more, there *M. desjardinsi* was the dominating predator instead of *H. maindroni*.

Salticid spiders shared the niche of CMB colonies along with other insects. Spiders feeding on the CMB, grubs of *H. maindroni*, adults of *S. epius* affirmed its general predatory role. Interestingly, intra-guild predation, *T. imperialis* spider feeding on the juveniles of

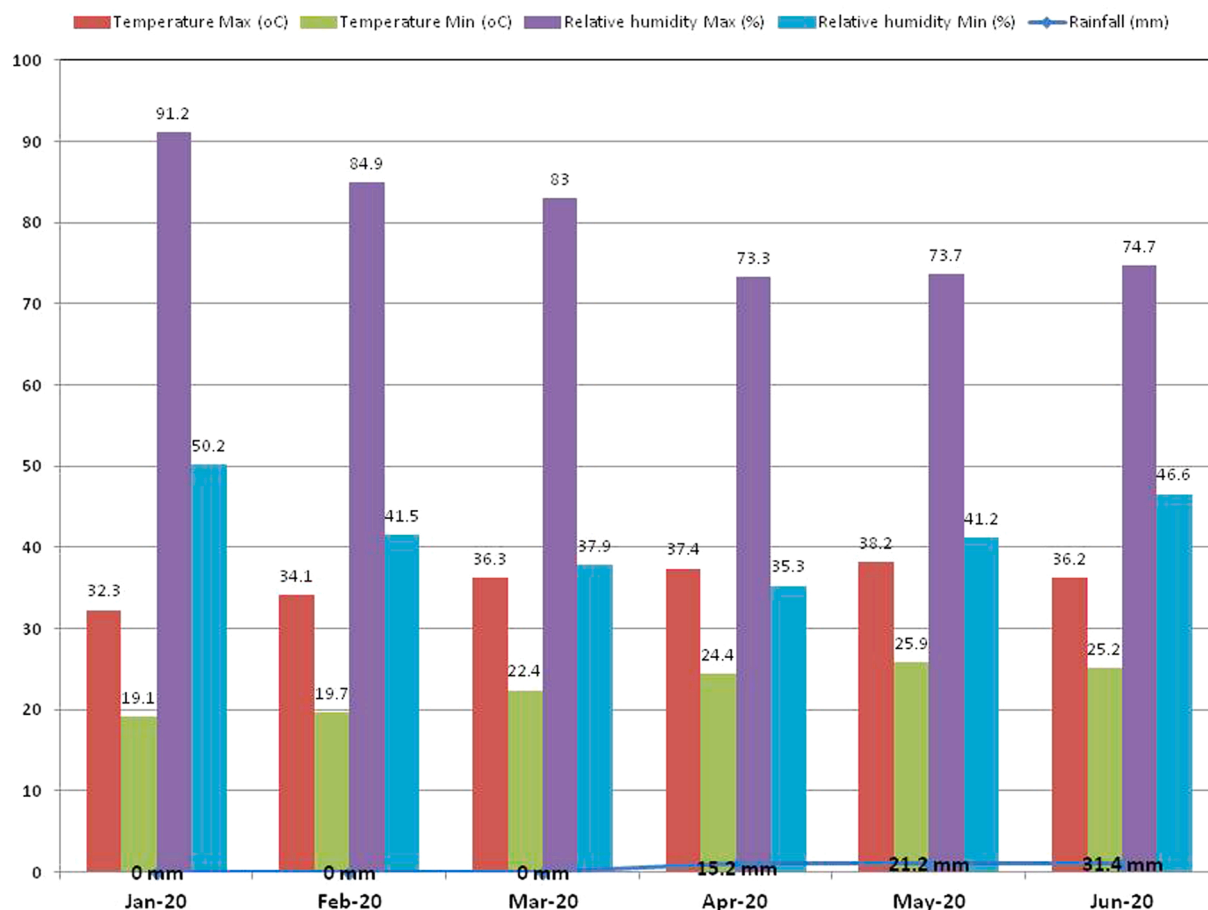


Fig. 5. Weather condition during CMB outbreak at the study area (Senthamangalam and Namagiripettai blocks of Namakkal district in Tamil Nadu).

C. viduus was observed. Salticids as opportunistic and abundance of spider juveniles at the time would have resulted in the inter/intra-guild predation. The orb-weaver spider presence in the CMB colonies was very limited except for *Neoscona* sp. This might be due to the existence of salticids, thomisids and/or probably the CMB niche is not preferred by the spiders to build its retreat because of the presence of several other arthropods as spiders exhibit type II functional response (Sinclair et al., 1998).

Autoba silicula (Erebidae) was the most abundant among all the Lepidoptera species in the CMB colonies. *Spalgis epius* (Lycaenidae) was observed actively feeding on the cassava mealybug in Tamil Nadu as well as in Kerala but was not abundantly present. The role of other species of Lepidoptera is not yet specified. In general, species of *Stathmopoda* are specialized to feed on coccids and so is the case here.

This is the first comprehensive account on the inventory of local parasitoids that have interacted with *P. manihoti* in India. Whereas *S. epius* and *Cryptolaemus montrouzieri* Mulsant are the two important predators recorded on *Paracoccus marginatus* in cassava and papaya. The introduced classical biocontrol agents such as *A. papayae*, *Pseudleptomastix mexicana* Noyes and Schauff and *Anagyrus loecki* Noyes and Menezes are well established in the study sites and keeping the *P. marginatus* population under check. Cassava whitefly and the cassava mosaic virus have not been the major issues when compared to the invasive mealybugs, *P. marginatus* and *Phenacoccus manihoti* in India. Further, the cultivars presently used such as M4, H 97, H 165, Sree Swarna, Sree Padmanabha are tolerant to cassava mosaic virus disease.

4. Discussion

The severity of cassava mealybug incidence was documented during the dry summer spells (April–May 2020) in almost all the surveyed locations in the Salem and Namakkal districts of Tamil Nādu. The standard week minimum average temperature recorded during March to May 2020 ranged from 22.4 to 25.9 °C, the maximum temperature ranged from 36.3 to 38.2 °C. Similarly, the minimum and maximum humidity during this period ranged from 37.9–41.2% to 73.1–83.0%, respectively. In the location Tho. Pachudayampalayam of Namakkal district during May–June 2020 period, the percent infested plants by cassava mealybug was highly correlated with the maximum temperature which prevailed during the period with *r* value being 0.81. Two continuous heavy summer

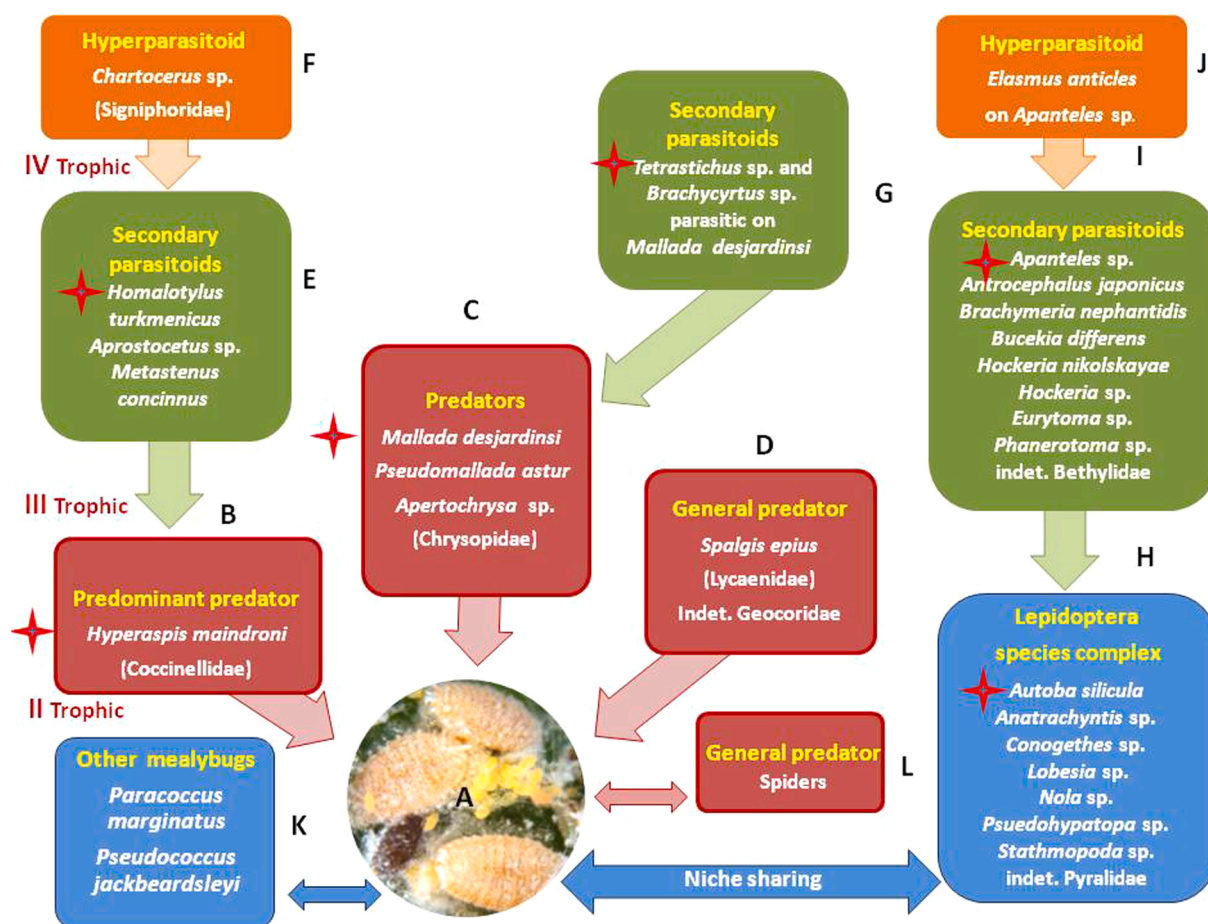


Fig. 6. Ecological interactions in the major food webs of insects associated with *Phenacoccus manihoti* showing various guilds at different trophic levels (A-L).

showers during the last week of May 2020 in Kalkurichi location, Namakkal resulted in the decline of the cassava mealybug population from first week of June. This clearly indicates the role of the abiotic factors in the regulation of cassava mealybug population density. The popular cassava cultivars such as Thailand types, Mulluvadi, Sree Atulya, Sree Vijaya and H-165 were severely infested by CMB irrespective of the survey location.

CMB is observed to share niche with the papaya mealybug *Paracoccus marginatus* (in Tamil Nadu) and *Pseudococcus jackbeardsleyi* (in Kerala) (Joshi et al., 2020) in the cassava plantation. In our surveys, we observed *P. marginatus* in the CMB colonies in one location, however, *P. marginatus* was also found on papaya plantations in addition to cassava. None of the indigenous and exotic parasitoids of *P. marginatus* such as *Acerophagous papayae* Noyes & Schauff (Encyrtidae), *Anagyrus loecki* Noyes & Menezes and *Pseudoleptomastix mexicana* Noyes & Schauff could parasitize the colonies of CMB. However, the indigenous generalist predators of other mealybugs, mainly *H. maindroni* and *M. desjardinsi*, adapted to the new invasive pest as an alternative host. Many generalist and specialist predators are reported especially on scales and mealybugs in various crop ecosystems (Pierce, 1995). The higher trophic guilds, such as third (predators) and fourth (hyperparasitoids), use variety of cues to locate their hosts (Aartsma et al., 2019). The hyperparasitoids (secondary parasitoids) live at the expense of the primary parasitoids and predators of the pest species (Sullivan, 1987).

With the shift of indigenous natural enemies to the cassava ecosystem, followed by their parasitoids and hyperparasitoids, led to the enhancement in abundance and complexity of the cassava food web. As mentioned by Neuenschwander, 1987, many insects target the damaged shoot apex caused by feeding of *P. manihoti*. The cassava mealybug niche yielded 45 species in 11 predominant guilds which are illustrated in Figs. 6–9 and listed in Tables 3 and 4. The biological information about the predominant species is mentioned in the text. Role of some species (mainly Lepidoptera species complex) is not yet understood but due to sufficient numbers collected in the rearings they are included in the study. Although ants are abundant on cassava plantation they are excluded from this study. Additionally, the guild involving the parasitoids of papaya mealybug is not included as they are not parasitic on CMB (Table 5).

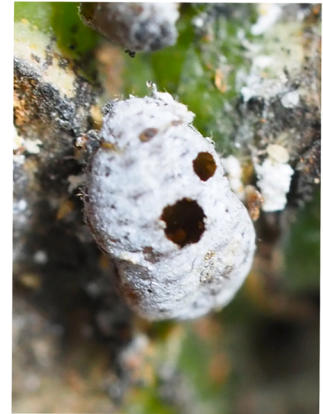
The ecological interactions in CMB niche are structured vertically as well as horizontally (Fig. 6). ‘Vertical’ represents primary interaction type (consumer–resource interactions) comprising of prey, predators and their parasitoids at different trophic levels. The different trophic levels involved in the CMB niche are: first– the autotroph (Cassava plant); second– primary pest (CMB); third–



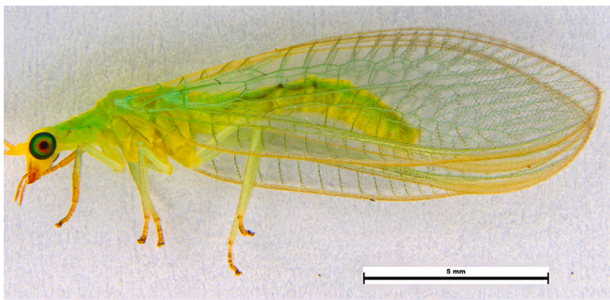
A



B



C



D



E



F



G



H



I



J



K



L

(caption on next page)

Fig. 7. Third trophic level active predators of cassava mealybug colonies - A- *Hyperaspis maindroni* Sicard adult; B- *H. maindroni* grub; C- *H. maindroni* grub with parasitoid emergence holes; D- *Mallada desjardinsi* (Navas) adult; E- *M. desjardinsi* grub; F- *Pseudomallada astur* (Banks) adult; G- *Pseudomallada* sp. grub; H- *Apertochrysa* sp. adult; I- *Spalgis epius* (Westwood) adult; J- *S. epius* grub; K- *Cheilomenes sexmaculata* Fabricius adult, L- indeterminate Geocoridae.

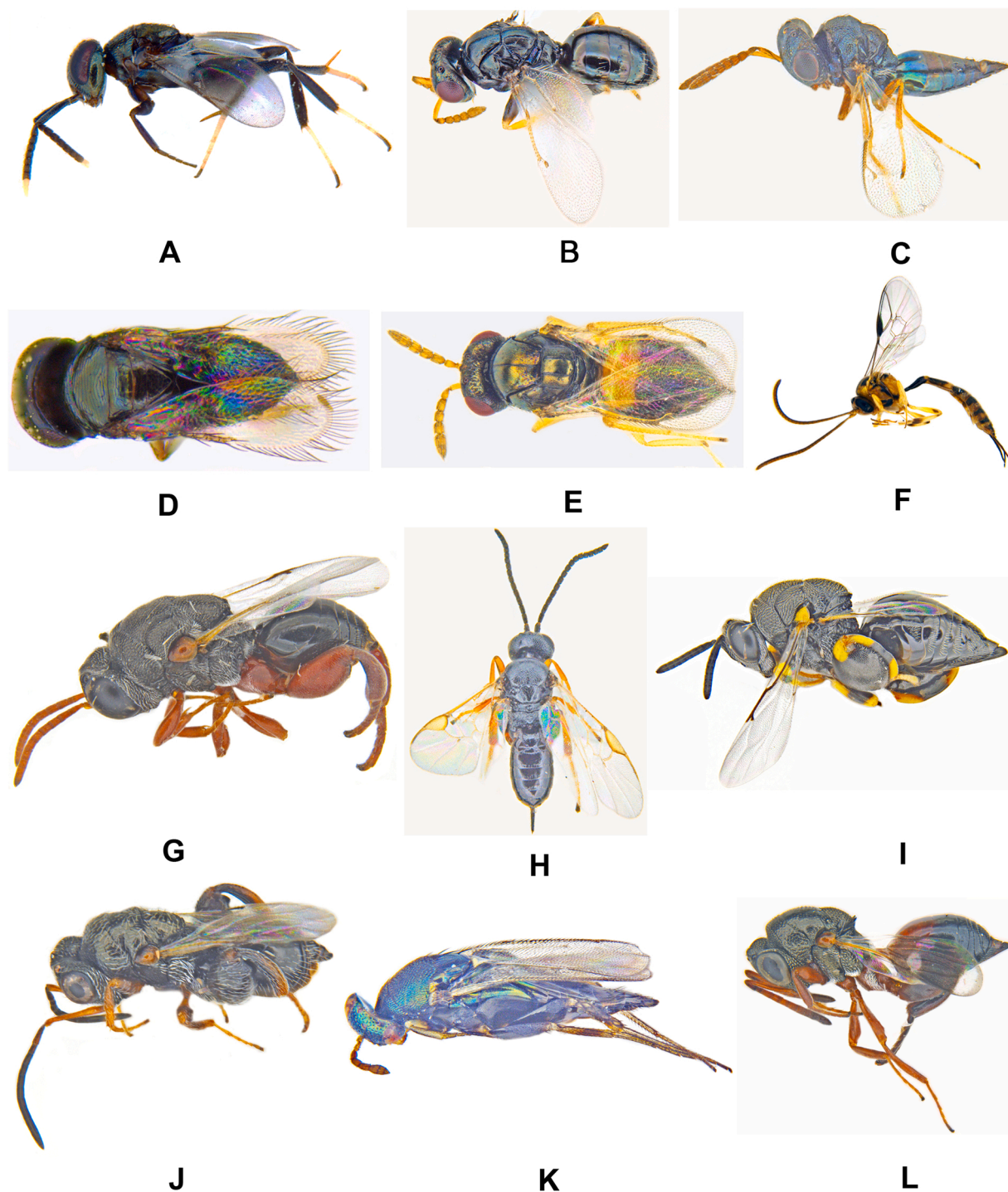


Fig. 8. Fourth trophic level hyperparasitoids (secondary parasitoids) in the CMB colonies- A- *Homalotylus turkmenicus* Myartseva; B- *Tetrastichus* sp.; C- *Metastenus concinnus* Walker; D- *Chartocerus* sp.; E- *Aprostocetus* sp.; F- *Brachycyrtus* sp.; G- *Antrocephalus japonicus* (Masi); H- *Apanteles* sp.; I- *Brachymeria* sp.; J- *Bucekia differens* (Boucek); K- *Elasmus anticles* Walker; L- *Hockeria nikolskayae* Husain and Agarwal.

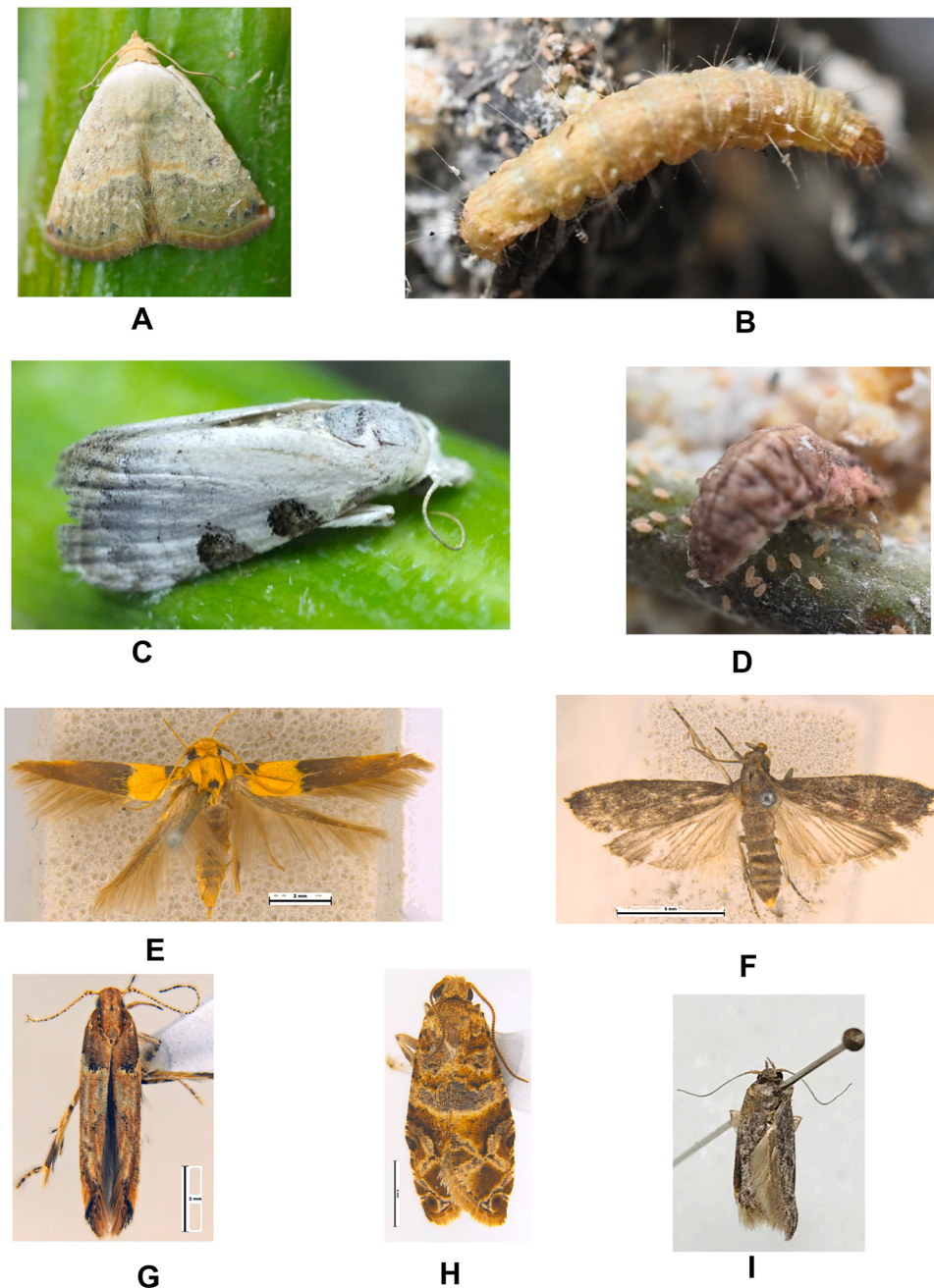


Fig. 9. Lepidoptera species complex in the CMB colonies A- *Autoba silicula* (Swinhoe) adult; B- *A. silicula* larva; C- *Nola* sp. adult; D - *Nola* sp. larva; E- *Stathmopoda* sp.; F- indeterminate Pyralidae; G- *Anatrachyntis* sp.; H- *Lobesia* sp.; I- *Psuedohypatopa* sp.

predators of CMB (B, C, D, L); fourth- predominant secondary parasitoids (hyperparasitoids) (*H. turkmenicus* and *Tetrastichus* sp.) of predominant predators (*H. maindroni* and *M. desjardinsi*), respectively and fifth- tertiary parasitoid (*Chartocerus* sp. on indet. secondary parasitoid of *H. maindroni*). There are two major networks of feeding interactions (food webs) involving two species of predominant predators actively feeding on the cassava mealybug A–B–E–F and A–C–G. ‘Horizontal’ represents herbivores and detritivores directly or indirectly associated with the cassava plant. This includes the non predatory species of Lepidoptera species complex (including scavengers) and other mealybug species sharing the niche with CMB. In the vertical trophic system, the prey (CMB) is naturally subjected to two competing predominant generalist predators (*H. maindroni* and *M. desjardinsi* observed actively feeding on CMB) co-existing with other generalist predators (*P. astur*, *Apertochrysa* sp., *S. epius* and spiders) as well as competing with other mealybug species (K) while partitioning/sharing the food resources (cassava). Six predominant species were observed in the different tri-trophic interactions involving *P. manihoti*- *H. maindroni*, *M. desjardinsi*, *H. turkmenicus*, *Tetrastichus* sp., *Autoba silicula* and *Apanteles* sp.

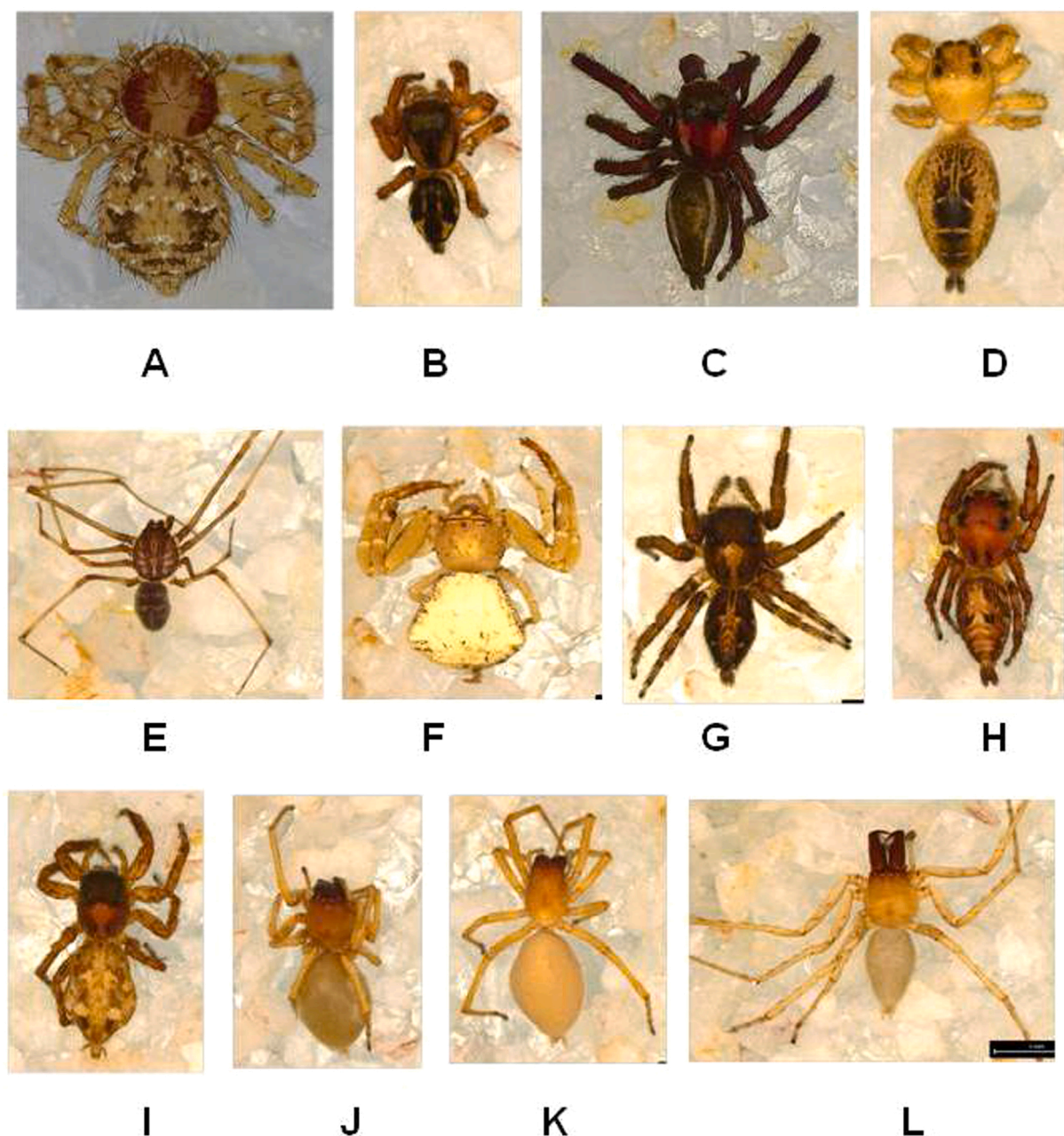


Fig. 10. Spider diversity in the complex in the CMB colonies A- *Indoxysticus* sp.; B&C-Female & male, *Carrhotus viduus* (C. L. Koch); D-*Thyene imperialis* (Rossi); E-*Scytodes fusca* Walckenaer; F-*Thomisus pugilis* Stoliczka; G-*Plexippus paykulli* (Audouin); H-*Hyllus semicupreus* (Simon); I- *Plexippus petersi* (Karsch); J-*Cheiracanthium* sp.; K & L- Female & male, *Cheiracanthium approximatum* O. Pickard-Cambridge.

The different associations (Fig. 6) found in the food webs of CMB were: The hymenopteran parasitoids - *Aprostocetus* sp. (Eulophidae), *Homalotylus turkmenicus* Myartseva (Encyrtidae), *Metastenus concinnus* Walker (Pteromalidae) and *Chartocerus* sp. (Signiphoridae) parasitizing immature stages of *Hyperaspis maindroni* Sicard (Coleoptera: Coccinellidae) while *Tetrastichus* sp. (Eulophidae) and *Brachycyrtus* sp. (Ichneumonidae) were parasitic on *Mallada desjardinsi* (Navas) (Neuroptera: Chrysopidae) actively predating on CMB.

Antrocephalus japonicus (Masi) (Chalcididae) was parasitic on pupae of *Autoba silicula* (Swinhoe) (Erebidae) while *Apanteles* sp. (Braconidae), *Brachymeria* sp. (Chalcididae), *Bucekia differens* (Boucek) (Chalcididae), *Elasmus anticles* Walker (Elasmidae), *Eurytoma* sp. (Eurytomidae), *Hockeria nikolskayae* Husain and Agarwal (Chalcididae), *Hockeria* sp., *Phanerotoma* sp. (Braconidae) and indet. Bethyridae were parasitic on immature stages of lepidopteran species complex (with *A. silicula* as the dominant species) in the CMB colonies. The following species were observed in the Lepidoptera species complex: *Autoba silicula* (Swinhoe) (Erebidae), *Anatrachyntis*

Table 3

Density of higher trophic levels on CMB infested shoot tips.

Third trophic level	Natural enemy density/ shoot tip (CMB density scale 4)	Percent hyperparasitism	Fourth trophic level	
			Hyperparasitoid species	Type of association/ parasitism (larval/pupal)
Predators of <i>P. manihoti</i>				
COLEOPTERA				
<i>Hyperaspis maindroni</i> Sicard (Coccinellidae)	2.1–36.2	29.8–63.9	<i>Homalotylus turkmenicus</i> Myartseva (Encyrtidae) <i>Metastenus concinnus</i> Walker (Pteromalidae)	Mummified grub of <i>H. maindroni</i> Mummified grub
<i>Cheilomenes sexmaculata</i> Fabricius (Coccinellidae)	0.0–4.2	Not known	–	–
NEUROPTERA				
<i>Mallada desjardinsi</i> (Navas) (Chrysopidae)	0.0–13.5	11.0–23.2	<i>Tetrastichus</i> sp. (Eulophidae) <i>Brachycyrtus</i> sp. (Ichneumonidae)	Cocoon of <i>M. desjardinsi</i>
<i>Psuedomallada astur</i> (Banks) (Chrysopidae)			Not known	–
<i>Apertochrysa</i> sp. (Chrysopidae)			Not known	–
LEPIDOPTERA				
<i>Autoba silicula</i> (Swinhoe) (Erebidae)	0.8–21.6	39.4–56.8	<i>Apanteles</i> sp. (Braconidae) <i>Antrocephalus japonicus</i> (Masi) (Chalcididae) <i>Brachymeria</i> sp. (Chalcididae) <i>Hockeria</i> sp. (Chalcididae) <i>Hockeria nikolskayae</i> Husain and Agarwal (Chalcididae)	Larval Pupal Pupal Pupal Pupal
<i>Spalgis epius</i> (Westwood) (Lycanidae)	0.2–12.6	Not known	Not known	–
<i>Stathmopoda</i> sp. (Oecophoridae)	0.0–5.6	Not known	Not known	–
<i>Anatrachyntis</i> sp. (Cosmopterigidae)	0.0–2.6 (possible scavenger)	Not known	Not known	–
<i>Nola</i> sp. (Nolidae)	0.0–0.9	Not known	Not known	–
Parasitoids of <i>P. manihoti</i>				
Nil	–	–	Nil	–

Table 4

Spider assemblages at third trophic level in the cassava mealybug niche.

S. No.	Spider species	Guild	^a Mean spider density (Density scale 4: ≥ 1000 mealybugs/ shoot tip)	Remarks
1	<i>Thyene imperialis</i> (Rossi) (Salticidae: Araneae)	Stalkers	2.40	General predator; observed all stages (male, female and juveniles) of the spider. Intra-guild predation, female spider feeding on juveniles of <i>C. viduus</i> was noticed
2	<i>Carrhotus viduus</i> (C. L. Koch Salticidae: Araneae)	Stalkers	1.60	General predator; observed all stages of spider predominantly juveniles
3	<i>Hyllus semicupreus</i> (Simon) (Salticidae: Araneae)	Stalkers	0.40	General predator; female and juvenile forms were more abundant
4	<i>Plexippus paykulli</i> (Audouin) (Salticidae: Araneae)	Stalkers	0.40	General predator; females were observed
5	<i>Plexippus petersi</i> (Karsch) (Salticidae: Araneae)	Stalkers	0.40	General predator; females were observed
6	<i>Thomisus pugilis</i> Stoliczka (Thomisidae: Araneae)	Ambushers	1.20	General predator; females only recorded feeding on the adult of <i>S. epius</i>
7	<i>Scytodes fusca</i> Walckenaer (Scytodidae: Araneae)	Ambushers	0.20	General predator; commonly called spitting spider known for its hunting. Not observed feeding any arthropods in the CMB niche
8	<i>Cheiracanthium approximatum</i> O. Pickard-Cambridge (Cheiracanthiidae: Araneae)	Foliage runners	1.00	General predator; observed male and female spiders
9	<i>Cheiracanthium</i> sp. (Cheiracanthiidae: Araneae)	Foliage runners	0.20	General predator; observed only juveniles feeding on grubs of <i>H. maindroni</i>
10	<i>Indoxysticus</i> sp. (Thomisidae: Araneae)	Ambushers	0.20	General predator; observed only juveniles feeding on cassava mealybug
11	<i>Neoscona</i> sp. (Araneidae: Araneae)	Orb-web builders	0.20	General predator; observed mummified ants from the small retreat build by the spider

^a Spider density was based on mean of five shoot tips.

Table 5
Species and their role in the cassava mealybug niche.

S. No.	Taxa/species	Host association	Remarks
I	HYMENOPTERA		
1	<i>Antrocephalus japonicus</i> (Masi) (Chalcididae)	Reared from pupae of <i>Autoba silicula</i> (Swinhoe) (Erebidae)	Solitary parasitoid
2	<i>Apanteles</i> sp. (Braconidae)	Reared from immature stage of ? <i>A. silicula</i>	Gregarious parasitoid
3	<i>Aprostocetus</i> sp. (Eulophidae)	Immature stage of <i>Hyperaspis maindroni</i> Sicard (Coleoptera: Coccinellidae)	Gregarious parasitoid
4	<i>Brachymeria</i> sp. (Chalcididae)	Reared from pupae of <i>A. silicula</i>	Solitary parasitoid
5	<i>Brachycyrtus</i> sp. (Ichneumonidae)	Reared from cocoon of <i>Mallada desjardinsi</i> (Navas)	Solitary parasitoid of <i>M. desjardinsi</i>
6	<i>Bucekia differens</i> (Boucek) (Chalcididae)	Reared from immature stage of indet. Lepidoptera	Solitary parasitoid
7	<i>Chartocerus</i> sp. (Signiphoridae)	Pupae of <i>Hyperaspis maindroni</i> Sicard (Coleoptera: Coccinellidae)	Upto six individuals emerged per pupae of <i>H. maindroni</i> when reared separately
8	<i>Elasmus anticles</i> Walker (Elasmidae)	Reared from <i>Apanteles</i> sp. cocoons on larvae of indet. Lepidoptera	Secondary parasitoid of <i>Apanteles</i> sp.
9	<i>Eurytoma</i> sp. (Eurytomidae)	Reared from immature stage of indet. Lepidoptera	Hyperparasitoid
10	<i>Hockeria nikolskayae</i> Husain and Agarwal (Chalcididae)	Reared from immature stage of ? <i>A. silicula</i>	Solitary parasitoid
11	<i>Hockeria</i> sp. (Chalcididae)	Reared from immature stage of ? <i>A. silicula</i>	Solitary parasitoid
12	<i>Homalotylus turkmenicus</i> Myartseva (Encyrtidae)	Mummified grubs of <i>H. maindroni</i>	Gregarious parasitoid
13	<i>Metastenus concinnus</i> Walker (Pteromalidae)	Pupae of <i>H. maindroni</i>	Solitary parasitoid of <i>H. maindroni</i>
14	<i>Tetrastichus</i> sp. (Eulophidae)	Cocoons of <i>M. desjardinsi</i>	Predominant gregarious parasitoid of <i>M. desjardinsi</i>
15	<i>Phanerotoma</i> sp. (Braconidae)	Reared from immature stage of indet. Lepidoptera	Less in number
16	indet. Bethyidae	Reared from larvae of indet. Lepidoptera	–
II	LEPIDOPTERA		
17	<i>Autoba silicula</i> (Swinhoe) (Erebidae)	Predominant species amongst the entire Lepidoptera species complex	Maximum specimens reared
18	<i>Conogethes</i> sp. (Crambidae)	Unspecified role; could be a chance visitor	One specimen reared
19	<i>Anatrachyntis</i> sp. (Cosmopterigidae)	Unspecified role; could be a scavenger	Multiple specimens reared
20	<i>Lobesia</i> sp. (Tortricidae)	Unspecified role	One specimen reared
21	<i>Nola</i> sp. (Nolidae)	Unspecified role	Multiple specimens collected
22	<i>Spalgis epius</i> (Westwood) (Lycaenidae)	Observed actively feeding on CMB	General predator; less in numbers when compared with <i>H. maindroni</i> and <i>M. desjardinsi</i>
23	<i>Stathmopoda</i> sp. (Oecophoridae)	Unspecified role	Multiple specimens reared
24	indet. Pyralidae	Unspecified role	Multiple specimens reared
III	NEUROPTERA		
25	<i>Mallada desjardinsi</i> (Navas)	Predominant chrysopid predator of CMB	Even after parasitism by <i>Tetrastichus</i> sp. and <i>Brachycyrtus</i> sp. many adults were reared from multiple colonies
26	<i>Pseudomallada astur</i> (Banks)	Predator of CMB	Less in number
27	<i>Apertochrysa</i> sp.	Predator of CMB	Less in number
IV	COLEOPTERA		
28	<i>Hyperaspis maindroni</i> Sicard (Coccinellidae)	Predominant predator of CMB	Maximum parasitism of <i>H. maindroni</i> by <i>H. turkmenicus</i> was observed
29	<i>Cheilomenes sexmaculata</i> Fabricius (Coccinellidae)	General predator	Less in number
30	<i>Carpophilus mutilates</i> Erichson	Unspecified role	Few specimens recovered from colonies
V	DIPTERA		
31	indet. Diptera	Can be saprophagous	One specimen reared
VI	HEMIPTERA		
32	<i>Paracoccus marginatus</i> Williams & Granara de Willink	Sharing niche with CMB	In Tamil Nadu
33	<i>Pseudococcus jackbeardsleyi</i> Gimpel & Miller	Sharing niche with CMB	In Thrissur, Kerala
34	indet. Geocoridae	General predator	Less in number

sp. (Cosmopterigidae), *Conogethes* sp. (Crambidae), *Lobesia* sp. (Tortricidae), *Nola* sp. (Nolidae), *Psuedohypatopa* sp. (Blastobasidae), *Spalgis epius* (Westwood) (Lycaenidae), *Stathmopoda* sp. (Oecophoridae) and indet. Pyralidae. Among all the moth species, *S. epius* was found most actively predating on CMB. The neuropteran predators associated with CMB were: *Mallada desjardinsi* (Navas), *Pseudomallada astur* (Banks) and *Apertochrysa* sp. and among them *M. desjardinsi* was observed as the most predominant predator of CMB. The other miscellaneous species associated were: *Cheilomenes sexmaculata* Fabricius (Coccinellidae), *Carpophilus mutilates* Erichson (Nitidulidae) and two indeterminate species of Diptera and Hemiptera (Geocoridae), respectively.

As aptly explained by Olff et al. (2009), the interaction webs are often highly variable in space and time. In some sites (Tamil Nadu),

where the CMB was competing with the other species of mealybug *P. marginatus*, the latter was heavily parasitized by *A. papayae*. However in (Kerala), CMB was observed sharing niche with *P. jackbearsleyi* (Joshi et al., 2020) with no signs of parasitism of the latter. Both the species of mealybugs- *P. marginatus* and *P. jackbearsleyi* were never observed coexisting and competing with CMB in the same location.

In general where heavy infestation of CMB is observed leading to distortion/curling/crinkling of the leaves into bunchy top, the dominance of the predators observed in the descending order of their abundance was: *H. maindroni* ~ *M. desjardinsi* > *A. silicula* > *S. epius*. Full grown spiders were omnipresent in the CMB infested fields playing role in intraguild predation however only spiderlings were noticed inside the rosette.

5. Conclusions

Based on our study we conclude that drought like situation favor CMB infestation in India. The percent infested plants and the plants showing bunchy top symptoms ranged from 5.8 to 76.9% and 11.6 to 56.3%, respectively. The population outbreak of CMB occurred during May 2020 and continued upto July end where the CMB density scale (scale 3–4) and shoot tip damage scale (scale 4–5) reached the maximum. Most of the popular cassava cultivars - Thailand types, Mulluvadi, Sree Atulya, Sree Vijaya and H-165 were severely infested by CMB.

Altogether 45 species: thirty four species of insects from six orders (Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, and Neuroptera) and eleven species of spiders (Araneae) were grouped under four trophic levels into 11 guilds. Overall, both the efficient primary predators (*H. maindroni* and *M. desjardinsi*) in the CMB niche were observed to be under tremendous pressure due to predator-prey competition, competition with other coexisting predators and high parasitism pressure by predominant secondary parasitoids (hyperparasitoids) - *H. turkmenicus* and *Tetrastichus* sp., respectively. The secondary parasitoids, *H. turkmenicus* and *M. concinnus* significantly reduced the population of primary predator *H. maindroni* with 29.8–63.9% parasitism and upto 11.0–23.2% parasitism of *M. desjardinsi* by *Tetrastichus* sp. was observed.

The conclusion drawn based on our observations indicate that the prey (CMB) is getting benefited owing to absence of the CMB primary parasitoid *Anagyrus lopezi* (De Santis) (Hymenoptera: Encyrtidae) and due to comparatively less efficient primary intraguild predators (*H. maindroni* and *M. desjardinsi*) which are subjected to high parasitism pressure and intra-specific competition from coexisting predators (*A. silicula*, *S. epius*, spiders, etc.). The results from our surveys and analysis show that there was no indigenous species of primary parasitoid attacking *P. manihoti*, while there were four dominant species of Hymenoptera in the CMB niche. First one- *Acerophagus papayae* as primary parasitoid of *P. marginatus*; two secondary parasitoids - *H. turkmenicus* and *Aprostocetus* sp. parasitizing *H. maindroni* and the fourth species - *Tetrastichus* sp. parasitic on *M. desjardinsi*, respectively. The conclusions which emerge in the form of findings of these four major parasitoids - *Acerophagus papayae*, *H. turkmenicus*, *Aprostocetus* sp. and *Tetrastichus* sp., reveal that none of them could parasitize CMB.

Unlike the other recent invasive pests in India, for instance *Spodoptera frugiperda* (J. E. Smith) (which can fly hundreds of kilometers) or *Tuta absoluta* (Meyrick) (infested fruits and the packing containers can act as carrier), cassava mealybug requires an aided introduction (especially through the infested plant parts). Hence, the likely mode of entry for CMB in India should be through infested plant parts however it is interesting to note that the parasitoids did not accompany the CMB possibly because the humidity plays a major role in emergence of parasitoids and this could have been a major constraint.

With additional pest load and with no indigenous parasitoid currently targeting the invasive pest, we assessed that the long-term and indirect ecosystem risks due to increased pesticide applications in cassava plantation would be significant. Hence, *Anagyrus lopezi* (De Santis) (Encyrtidae), a proven successful primary parasitoid of CMB, is attempted for introduction in India.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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