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Arthropod Biodiversity of Citrus Ecosystem with Special Reference to Citrus Leaf Miner

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Abstract

The citrus leaf miner (CLM), scientifically known as *Phyllocnistis citrella* Stainton and belonging to the Gracillaridae family, is a significant nuisance for citrus nurseries and the tender leaves of young citrus plants. Damage caused by the larvae is conspicuous especially on the young foliage during initial phase of development and the management of the pest is highly critical. The present studies were carried out at ICAR-Central Citrus Research Institute, Nagpur during 2013 to 2015 to document the species composition of bioagents (parasitoids and predators), their incidence levels and species richness/ diversity in citrus ecosystems with reference to citrus leaf miner. During the study, we documented individuals from two classes, four orders and six families of insects. Class Insecta was dominant followed by Arachnida. Among the six families, Eulophidae and Coccinellidae were the most dominant bioagent groups for parasitoid and predator, respectively. Relative abundance of the bioagents of *P. citrella* revealed that *C. phyllocnistoides* was the predominant one and constituted 30% of total bioagents collected followed by *Cirrospilus* sp. (including *C. quadristriatus*, *C. ingenuus*) (25%), *E. brevicornis* (15%) and *S. striatipes* (9%). Coccinellids constituted about 3%; whereas, chrysopid predator, *M. desjardinsi* and spider species constituted 13% and 7%, respectively of the total bioagent collection. Understanding of the bioagent complex in citrus ecosystem will help us to identify the promising ones for biological control as well as guide us the bioagent active frame to avoid pesticide sprays to sustain them naturally in the ecosystem.

Keywords: Arthropod biodiversity, Bioagents, Citrus ecosystem, Citrus leaf miner, Species composition

Introduction

Citrus leaf miner (CLM), scientifically known as *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), poses a significant pest problem in numerous regions across the globe, encompassing Asia, Australia, Africa, the Americas and Europe (Ateyyat and Mustafa, 2001; Hoy and Nguyen, 1997). This pest primarily targets plants within the Rutaceae family, with a strong affinity for the Citrus genus, particularly commercially cultivated varieties of Citrus within that genus (Argov and Rossler, 1996). It has attained importance both at national as well as international levels because of its epidemic eruptions in Florida, Australia and India. Severe infestations of *P. citrella* can cause substantial damage to young citrus trees, whether they are growing in the field or in nurseries (Argov and Rossler, 1996); while, the impact of this damage is comparatively less significant in mature citrus trees (Elnagar and Soliman, 2016; Garcia-Marí *et al.*, 2002; George *et al.*, 2018). The hatched larvae immediately promptly penetrate the leaf and create winding tunnels beneath the surface of the leaf epidermis, and these affected leaves exhibit a distinct shiny, silvery appearance (Argov and Rossler, 1996).

Effectively managing CLM presents a significant challenge due to its strong capacity for migration from external orchards and its high reproductive rate. A host of control measures including mechanical methods [manual removal of early and late growing flushes and pre-flush pruning to

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create uniform flushing, avoid pruning during active growth period, destruction of water shoots, fertilizing the trees in winter to promote flush growth in spring when the pest is absent (Ateyyat and Mustafa, 2001)], host plant resistance (resistant germplasm like Trifoliate orange), biological [use of parasitoids like *Ageniaspis citricola* Loginovskaya, *Citrostichus phyllocnistoides* (Narayanan) and *Cirrospilus quadristriatus* (Subbarao and Ramamani)], semiochemicals and chemical control have been deployed for the management of CLM (Rao *et al.*, 2017). Worldwide biological control has provided the most effective management of CLM. Across the globe, a number of parasitoids (*Aegniaspis*, *Citrostichus* and *Cirrospilus*) are utilized for the control of CLM. Globally, there have been records of over 80 hymenopteran species, primarily chalcidids, that parasitize the citrus leaf miner (Kalaitzaki *et al.*, 2011; Schauff *et al*., 1998). Consequently, these parasitoids have been integrated into several Integrated Pest Management (IPM) programs aimed at controlling the pest (Kalaitzaki *et al.*, 2011). A complex of natural enemies affecting all life stages of leaf miner would be desirable to promote population regulation below damaging levels rather than reliance on a single parasitoid species to function on its own (Browning and Pena, 1995).

Considering the pressing need for an effective management strategy for CLM as a major pest of nursery as well as young orchards, it becomes imperative to undertake studies on species composition of bioagents, incidence levels and the rate of parasitism of bioagents on CLM.

Materials and Methods

Surveys were conducted (October, 2013 to March, 2015) to document the species composition of bioagents (parasitoids and predators). Fixed plot sampling was done at monthly intervals, involving the collection of four flushes, each comprising 10-15 leaves, from five randomly selected trees of Nagpur mandarin, acid lime, mosambi, kinnow and pommelo for a total of 20 flushes (or 200-300 leaves) cultivar^{-1} month^{-1} at NRCC farm. In nursery rootstocks *viz.*, rough lemon and Rangpur lime, 10 leaves were selected at random from 10 seedlings for bioagent collection. The collected flushes were carefully stored into appropriately labeled paper bags (20 cm × 10 cm) in programmable environmental chamber (Remi Scientific Limited Model No. MLC 9424) in the laboratory and examined under microscope (Olympus SZX 16) for the presence of predators or parasitoids of *Phyllocnistis citrella* Stainton. Leaves containing parasitized individuals of *P. citrella* were placed in petridishes with moistened cotton beneath. Parasitoid adults on emergence were collected and preserved in 70% alcohol and got identified by Dr. Santhosh Shreevihar (Assistant Professor in Zoology, Kozhikode, Kerala). Predators including spiders (identified by Dr. Manju Siliwal, Wildlife Information Liaison Development Society, 9-A, Lal Bahadur Colony, Peelamedu, Coimbatore, Tamil Nadu, India) observed on the surveyed trees were captured using a sweep net, carefully placed in glass vials containing 70% ethyl alcohol, and then transported to the laboratory for documentation (Xiao, 2009).

The description of feeding symptoms by predators provided by Amalin *et al*. (2001) was used for differentiation. Chrysopids punctured the mine and left the dead prey larval form still visible while hunting spiders slit open the mine which resulted in necrotic marks from incomplete feeding and empty mine with the prey's crumpled skin. Ectoparasitoids also punctured the mine and typical symptom was necrotic marks from incomplete feeding and disfiguration of leaf miner larva. Alpha diversity indices were employed to evaluate and compare the diversity and distribution of arthropods within the citrus ecosystem (Senguttuvan, 2013). Species richness and diversity version II (Pisces Conservation Ltd.; www.irchouse.demon.co.uk) (Henderson, 2003) programmes were used to assess and compare the diversity of arthropods (parasitoids and predators including spiders) in citrus ecosystems (Senguttuvan, 2013).

Results and Discussion

Arthropod bioagents collection yielded a wide array of 1325 individuals from two classes, four orders and six families of insects. Among the six families, Eulophidae and Coccinellidae were the most dominant bioagent groups for parasitoid and predator, respectively (Table 1). In case of Eulophidae, 380 individuals of *Citrostichus phyllocnistoides* (Narayanan), (Hymenoptera: Eulophidae) were collected followed by 243 and 87 individuals of *Cirrospilus quadristriatus* (Subbarao and Ramamani) (Hymenoptera: Eulophidae) and *Cirrospilus ingenuus* Gahan (Hymenoptera: Eulophidae), respectively. A total of 35 unidentified individuals (parasitoids) were also collected during this period. Apart from *C. quadristriatus* and *Cirrospilus* sp., a total of 111 and 114 individuals of *Elasmus brevicornis* Gahan and *Sympiesis striatipes* Ashmead, respectively were also collected.

Among the predatory arthropods, *Mallada desjardinsi* (Navas) was the dominant predator (Neuroptera: Chrysopidae) (130 individuals) followed by 55 and 30 individuals of *Coccinella septempunctata* (Linnaeus) and *Cheilomenes sexmaculata* (Fabricius) (Coleoptera: Coccinellidae), respectively. Order Arachnida was mainly represented by Araneidae with individuals of *Neoscona* cf. *theisi* (Walckenaer), and *Neoscona* sp. (68), Clubionidae with *Clubiona* sp. (40) and Salticidae with *Thyene imperialis* (Rossi) (28) apart from some unidentified spider species.

Relative Abundance of Bioagents of P. citrella in Citrus Ecosystem

The parasitoid complex of *P. citrella* in the study was composed of *C. phyllocnistoides*, *C. quadristriatus*, *E. brevicornis*, *S. striatipes*, *etc*. *C. quadristriatus* was the predominant apart from five unidentified *Cirrospilus* sp. in the collection. Relative abundance of the bioagents of *P. citrella* revealed that *C. phyllocnistoides* constituted 30% of total bioagents collected. Diverse collection of genus *Cirrospilus* including *C. quadristriatus* and *C. ingenuus* was obtained and comprised around 25% of total collection (Figure 1). *E. brevicornis* (15%) was found as an ecto-larval parasitoid of citrus leaf miner predominant during *Hasta*

Table 1: Arthropod bioagents associated with citrus leaf miner, *Phyllocnistis citrella* during October, 2013 to March, 2015

SL. No.	Bioagent	Class	Order	Family
I	Parasitoids			
1	*Citrostichus phyllocnistoides* (Narayanan)	Insecta	Hymenoptera	Eulophidae
2	*Cirrospilus quadristriatus* (Subbarao and Ramamani)	Insecta	Hymenoptera	Eulophidae
3	*Cirrospilus ingenuus* Gahan	Insecta	Hymenoptera	Eulophidae
4	*Cirrospilus* sp. (Unidentified)	Insecta	Hymenoptera	Eulophidae
5	*Elasmus brevicornis* Gahan	Insecta	Hymenoptera	Eulophidae
6	*Sympiesis striatipes* (Ashmead)	Insecta	Hymenoptera	Eulophidae
II	Predators			
7	*Mallada desjardinsi* (Navas)	Insecta	Neuroptera	Chrysopidae
8	*Coccinella septempunctata* (Lin.)	Insecta	Coleoptera	Coccinellidae
9	*Cheilomenes sexmaculata* (Fabricius)	Insecta	Coleoptera	Coccinellidae
III	Spiders			
10	*Neoscona* cf. *theisi* (Walckenaer)	Arachnida	Araneae	Araneidae
11	*Neoscona mukerji* Tikader	Arachnida	Araneae	Araneidae
12	*Clubiona* sp.	Arachnida	Araneae	Clubionidae
13	*Thyene imperialis* (Rossi)	Arachnida	Araneae	Salticidae

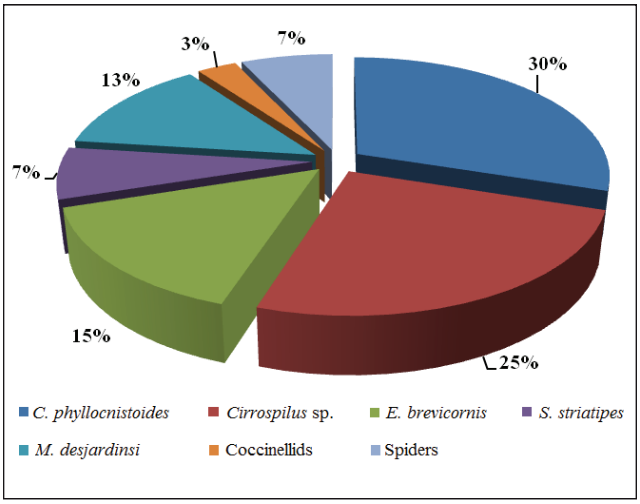

Figure 1: Relative abundance of bioagents recorded on citrus leaf miner, *Phyllocnistis citrella* Stainton from seven citrus cultivars

(spring) season. *S. striatipes* accounted for only 7% of the total bioagents collection and was found in low numbers. Coccinellids constituted about 3%, chrysopid predator, *M. desjardinsi* 13% and spider species *viz.*, *Neoscona* cf. *theisi*, *Clubiona* sp. and *T. imperialis* constituted 7% of the total bioagent collection.

Biodiversity Indices

Using the primary arthropod data as a foundation, we have computed various sets of alpha diversity indices (Figure 2-9).

The Simpson's index (Figure 2), calculated at the ordinal level, displayed its highest value during the first week of September, 2014 (3.889) and minimum during first week of November, 2013 (1.123). Familial based values ranged from

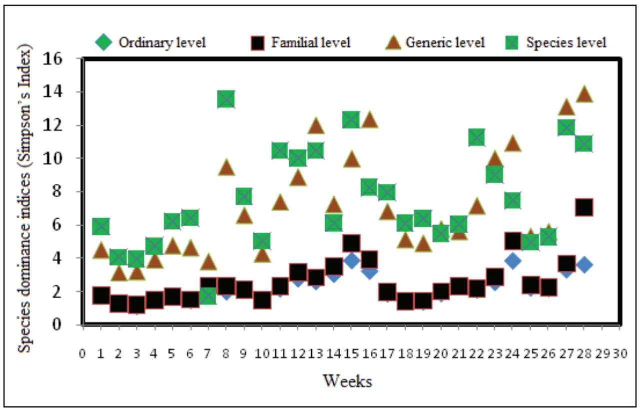

Figure 2: Simpson's index

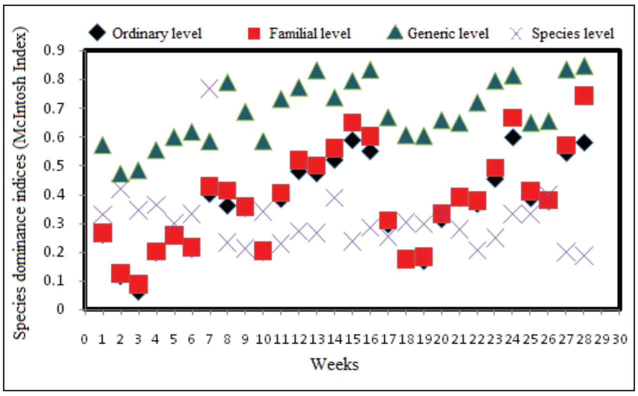

Figure 3: McIntosh index

1.174 during first week of November, 2013 and 7.059 in the month of March, 2015. When analyzing values at the generic and species levels, the highest values recorded were 13.909 and 13.6, respectively during second week of March, 2015 and third week of January, 2014. McIntosh index showed

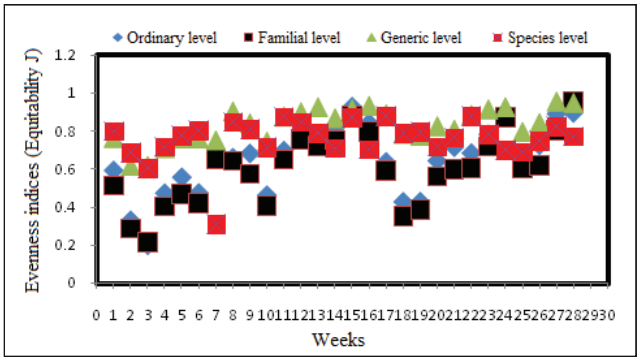

Figure 4: Equitability J

no clear variation among the values (Figure 3). However, evenness indices (Equitability J) showed clear variation in the values of generic level (Figure 4). Similar experiment was conducted by (Senguttuvan, 2013).

Biological control emerges as one of the most promising methods for managing citrus insect pests (Tahir *et al.*, 2011). Therefore, the primary aim of the current study was to document the various arthropod bioagents including parasitoids, predatory insects and spiders might be promising, safe and feasible tool to manage citrus pests. In various regions around the world, citrus pests have been effectively managed through the utilization of natural predators such as spiders and beetles (Tahir *et al.*, 2011). Additionally, a diverse array of hymenopterous parasitoids has been reported to attack *P. citrella*, as documented by Ohbayashi and Mafi (2010), Pena *et al.* (1996) and Schauff *et al.* (1998). These parasitoids primarily belong to the eulophid family, with some representatives from encyrtids, elasmids, eurytomids, eupelmids, and pteromalids, as reported by Ohbayashi and Mafi (2010) and Ujiye *et al.* (1996). Among eulophids, *Citrostichus phyllocnistoides* (Narayanan) was the predominant one (30%) apart from *Cirrospilus quadristriatus* (Subbarao and Ramamani)*, Cirrospilus ingenuus* Gahan and other unidentified *Cirrospilus* spp. (25%) in the collection (Figure 1). *Elasmus brevicornis* Gahan and *Sympiesis striatipes* Ashmead constituted 15 and 7%, respectively of the total collection. Similar results of over 80 hymenopteran species, mostly chalcidids: eulophids have been documented on citrus leaf miner throughout the world, and as a result, these parasitoids have been incorporated into several Integrated Pest Management (IPM) programs (Argov and Rossler, 1996; Hoy *et al.*, 1995; Kalaitzaki *et al.*, 2011; Neale *et al.*, 1995; Noyes, 2008; Smith and Beattie, 1996). *Citrostichus phyllocnistoides* is an ectoparasitic wasp that lays its eggs during the prepupa stage of the citrus leaf miner, and the adult wasp subsequently emerges from the pupa of the host citrus leaf miner (Argov and Rossler, 1996). Parasitism by *C. phyllocnistoides* commenced towards the end of July, with a notable increase occurring in August, resulting in a parasitism rate ranging from 40% to 50% (Elekcioglu and Uygun, 2013; Subba Rao and Ramamani, 1965). Throughout all seasons, the most abundant parasitoid was consistently *C. phyllocnistoides* (Elekcioglu and Uygun, 2013). Following *C. phyllocnistoides*, the second most prevalent species was *C. quadristriatus*, followed by *C. ingenuus*, which was the third most frequently encountered species in this study. Wang *et al*. (1999) also reported that *C. phyllocnistoides* as the dominant species from southeast China; 99.4% of the parasitoids collected in Spain (Elekcioglu and Uygun, 2013; Karamaouna *et al*., 2010); 80% of the collection in Western Crete followed by *S. petiolatus* (Elekcioglu and Uygun, 2013; Kalaitzaki *et al*., 2011). In central India, *C. quadristriatus* and *C. phyllocnistoides* were found prolific on leaf miner causing parasitism to the extent of 6.2% to 13.1% (Rao and Shivankar, 2002).

Browning *et al.* (1996) recorded *Cirrospilus* sp., *Pnigalio minio* (Walker), *Closterocerus cinctpennis* Ashmead, *Horismenus* sp., *Elasmus tischeriae* (Howard), *Sympiesis* sp., and *Zagrammosoma multilineatum* (Ashmead) on CLM. *E. brevicornis* Gahan and *S. striatipes* Ashmead wasps were also collected from citrus leaf miner infested leaves during the above period. *S. striatipes*, an ectoparasitoid belonging to the polyphagous eulophid group, is known to parasitize various gracillariid leaf miners, including *Acrocercops* spp. and *Phyllonorycter* sp., in addition to its activity against the citrus leaf miner (Ohbayashi and Mafi, 2010), while Genus *Elasmus* are mostly primary gregarious larval ectoparasitoids of lepidopteran larvae of families: Coleophoridae, Gracillariidae, Cosmopterygidae, Gelechiidae, Heliozelidae, Figurellidae, Psychidae, Pyralidae and Tortricidae (Herting, 1975; Herting, 1977; Thompson, 1954; Trjapitzin, 1978).

Among the predatory arthropods, *Mallada desjardinsi* (Navas) was the dominant predator (Neuroptera: Chrysopidae) followed by coccinellid beetles consisting of *Coccinella septempunctata* (Linnaeus) and *Cheilomenes sexmaculata* (Fabricius) (Coleoptera: Coccinellidae). Class Arachnida was mainly represented by Araneidae with individuals of *Neoscona* sp., Clubionidae., *Clubiona* sp. and Salticid: *Thyene imperialis*. Numerous predatory arthropods have been documented by studies as consumers of P. citrella, including lacewing larvae, ants and hunting spiders (Amalin *et al.*, 2001; Argov and Rossler, 1996; Hoy *et al.*, 2007; Xiao and Fadamiro, 2010; Xiao *et al.*, 2007). Past research has consistently identified predation as the most significant natural factor contributing to the mortality of *P. citrella* across various regions around the world (Amalin *et al.*, 2001; Chen *et al.*, 1989; Hoy *et al.*, 2007; Xiao and Fadamiro, 2010; Xiao *et al.*, 2007).

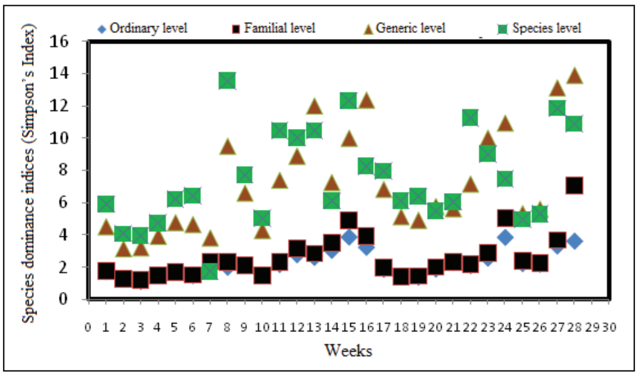

Figure 5: Simpson index (Species dominance)

Predatory spiders have the capacity to detect and engage with the larvae by piercing through the leaf epidermis (Amalin *et al.*, 2001). These spiders have poor vision and rely more on visual cues in prey capture sequences, such as detecting vibrations in the substrate where their prey is concealed. The continuous movement of *P. citrella* larvae as they feed on tissues beneath the leaf epidermis likely generates the vibrations in the leaf substrate (Amalin *et al.*, 2001). Even though the proportions of predators like beetles, spiders and chrysopids were less n during the study compared to parasitoids on citrus leaf miner, their impact being lessened due to the generalist nature of feeding of predators. These predators have a wide array of host preference in the citrus ecosystem rather than high specificity or narrow host range as observed in the case of parasitoids of citrus leaf miner. It cannot be denied that predation is one of the most important natural mortality factors acting on *P. citrella* in the citrus ecosystem (Xiao, 2009) and could be complementary in managing the citrus leaf miner. Higher levels of parasitisation of eulophiod parasitoids under field conditions will definitely have practical application for CLM management in future.

Arthropods are commonly employed as ecological indicators due to their high representation, accounting for over 80% of global species richness (Senguttuvan, 2013). In this study, we have conducted an alpha diversity analysis on the collected arthropod data. Alpha diversity was assessed using a variety of metrics, including species number, Fisher's alpha index, Margalef's D index, Q statistic, Brillouin index and the Shannon-Weiner index. Dominance, on the other hand, was determined using metrics such as Simpson's index, McIntosh index and Berger-Parker index. Among the bioagents recorded, parasitoids were the dominant group. Diverse collection of Genus *Cirrospilus* including *C. quadristriatus* and *C. ingenuus* was obtained and comprised around 25% of total collection. Coccinellids constituted about 3% and spider species *viz.*, *Neoscona* cf. *theisi, Clubiona* sp. and *T. imperialis* constituted 7% of the total bioagent collection. Species number in the present study ranged from 4 during January, 2014 and to 13 during October, 2014. This again confirmed the finding that bioagent mainly parasitoid activity was more during *Hasta* season (October-December). Species Richness, represented by Margalef's D index, is indeed a measure that accounts for the number of species present while also considering some adjustment for the abundance or number of individuals within those species. Margalef' D index values in our study was almost static in variation which indicates that there was minimum variation in the group of bioagents especially ordinal and familial level variation over the period with only the species number increasing or decreasing.

Simpson index values were calculated and in our studies, at species level higher values indicated greater diversity of bioagents (Figure 5). For example during the third week of January, 2014 an index value of 13.60 was observed followed by 12.35 during September, 2014. Index value was very low during second week of January, 2014.

Shannon-Wiener Index incorporates both species richness and equitability components. The Shannon index is a measure of evenness and calculated as the ratio between the observed diversity and the maximum diversity (Pieloue, 1981). Shannon-Weiner index (Figure 6) based on ordinal (0.276-1.171), generic (1.423-2.203), familial (0.390-1.717) and species levels (0.794-2.261) indicated minimum variation. This evenness measure at species level, *i.e.*, the ratio was found between zero and one with the highest value during first week of January, 2015 (0.99) and the lowest during second week of January, 2014 (0.35). Equitability J values were also less than one. Evenness index were more close to 1 indicating that the each species/ category consisted of the same number of individuals.

The McIntosh index and Berger-Parker index which are measures of dominance indicated the values closer to zero except for second week of January, 2014 at species level analysis conducted under McIntosh index indicated the dominance of parasitoid species (*C. phyllocnistoides* and *Cirrospilus* sp.) among the bioagents. Minimum variation was observed in case of Brillouin diversity index based on ordinal (0.235-1.082), familial (0.329-1.333), generic (1.276-1.906), and species level (1.345-2.082) (Figure 7). Analysis based on Q-statistic showed variation based on ordinal level ranged from 0.401 to 4.933 and on the generic level, the value was maximum in the first week of January, 2015 (7.399) and zero during first week of January, 2014 (Figure 8). The species richness was not clear in variation from the Fisher's alpha index values, followed by (Senguttuvan, 2013; Figure 9), based on ordinal level and familial level analysis. Thus species richness indices (species number, Fisher's alpha index, Margelef's D index, Q statistic, Brillouin index and Shannon-Weiner index) were low to moderate indicating

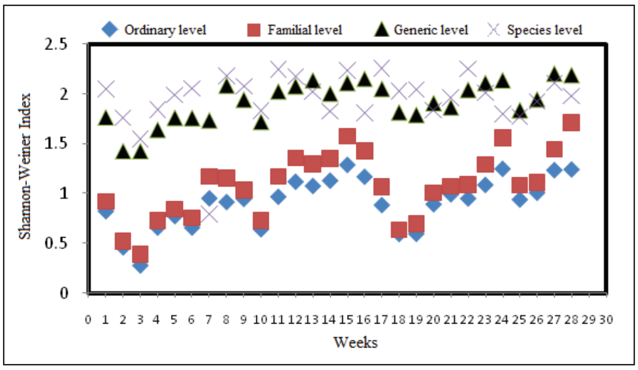

Figure 6: Shannon-Weiner index

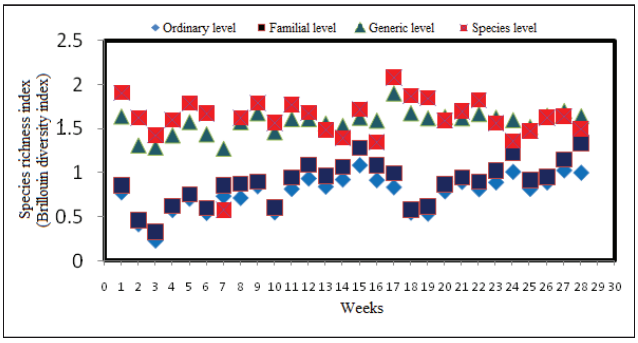

Figure 7: Brillouin diversity index

low diversity levels which while dominance based indices (Simpson's index, McIntosh index and Berger Parker index) remarkably showed the dominance of a particular group (eulophid parasitoids) in the study.

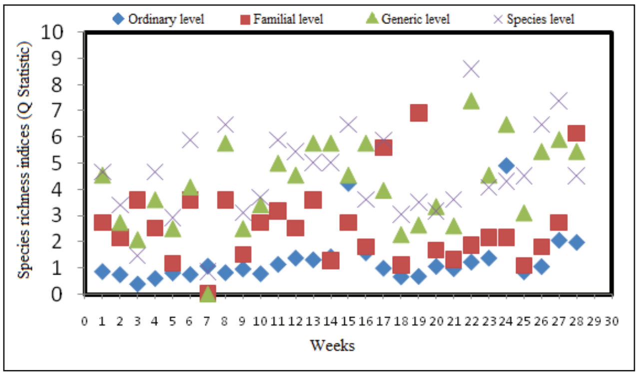

Figure 8: Q Statistic

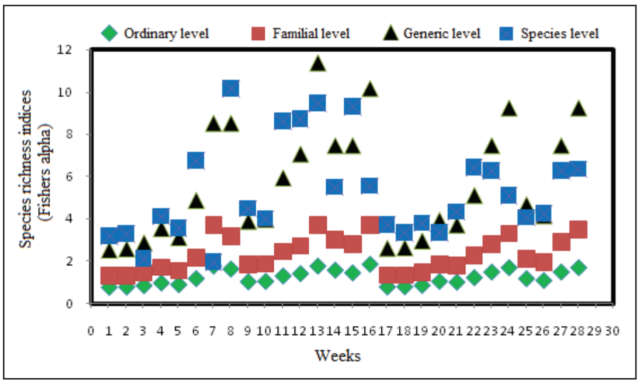

Figure 9: Fishers alpha

Conclusion

The conclusions of the above study gave an insight of the relative abundance of different native bioagents of citrus leaf miner present in the citrus ecosystem which could possibly provide an option to proceed further for implementation of a mass scale management strategy for this pest. Awareness about the bioagent active time frame helps us to boost the natural progeny by providing shelters or any food niches so that natural control could be sustained.

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