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Arthropods discovered on *refugio* flowering plants in *Mangifera indica* plantation

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Abstract. *Windriyanti W, Rahmadhini N, Fernando I, Kusuma RM. 2023. Arthropods discovered on* refugio *flowering plants in* Mangifera indica *plantation. Biodiversitas 24: 4747-4754.* The diversity of *Refugio* flowering plants with different ways of flowering phenology provides alternative food sources for arthropods. Insects are the largest class of arthropods, most of which are ecological services that function as natural enemies and pollinators. Insects require an energy source over a long-term period to promote a stable and healthy population. The use of flowering plants as refugia can maximize the role of insects as ecological services in agroecosystems by providing natural enemies and pollinators with a source of food and shelter. The refugia plants used in this study were sunflowers (*Helianthus annuus*), king's salad (*Cosmos caudatus*), Peruvian zinnia (*Zinnia peruviana*), *Tridax procumbens* (Asteraceae), and *Alternanthera sessilis* (Amaranthaceae) with hedgerow and insectary bank planting patterns. Data collection and periodic direct observation (scan sampling) were conducted by transects. Observed parameters included species composition and population of arthropods in two different agro-systems, monoculture and polyculture. Identification results showed 8 orders, including 44 morphospecies, belonging to 25 families. Arthropod abundance in the on-season of the refugia was found in 1422 individuals. Ecological engineering is a habitat manipulation technology using refugia plants that has increased the composition and population of natural enemy insects and pollinators.

Keywords: Arthropods, diversity, ecological engineering, *refugio*

INTRODUCTION

Insects affect ecosystem services in several ways, both positively and negatively (Elizalde et al. 2020). Natural ecosystems have a variety of services that humans and other organisms rely on for their survival and well-being (Scudder 2017; Eggleton 2020). These services can be categorized as provision (production of food, fiber, water, and other resources), supporting (primary production, pollination, decomposition, and formation of soil necessary for resource production), and regulating (biological control and feedback mechanisms that maintain relatively consistent service delivery) (Seibold et al. 2021; Kusuma & Windriyanti 2022; Goode et al. 2022; Sogari et al. 2023). Ecosystems can also be viewed as producing "harmful" components such as pests and waste (Schowalter et al. 2018). Pollinators, predators, parasitoids, herbivores, and decomposers are the most studied functional groups, while Hymenoptera, Coleoptera, and Diptera are the most studied taxa (Jervis 2012; Rosas-Ramos et al. 2020). Experimental studies are relatively rare and only focus on biological control, pollination, and decomposition carried out in agroecosystems (Bergholz et al. 2021; Jones et al. 2021; Gagic et al. 2022). Ecological Services (ES) indicators assume or infer their provisions through indirect measurements such as species abundance, species density, species richness, diversity index, or number of functional groups (Harrison et al. 2014; Pufal et al. 2021). ES is essential to assess fully the synergies among functional ecology, community ecology, and biodiversity conservation under current global changes (Noriega et al. 2017; Díaz et al. 2019).

Coevolution between flowers and insects can be used as a rationale for strategies for utilizing and conserving natural resources of flowering plants (Angiosperms) and pollinating insects (Burkle et al. 2013; Herrera 2015). Flowers need pollination services, especially for cross-pollination, and at the same time, insects need food in the form of nectar and pollen in flowers. Most crops require the presence of pollinating insects (Katumo et al. 2022). Eighty-seven leading food crops worldwide and 35 percent of global agricultural land production depend on insect pollinators (Klein et al. 2007). Nearly 80 percent of pollinating insects play a role in the availability and stability of various agricultural production, especially food crops and horticulture (FAO 2016). Agricultural intensification has reportedly caused many problems in agroecosystems, such as loss of biodiversity, including a decrease in the provision of biocontrol services in an ecosystem (Wan et al. 2014; Raven & Wagner 2021). Ecological Engineering (EE) is the principles of ecology and engineering that are integrated into solving ecosystem problems (Bender et al. 2016; Wan et al. 2017). The EE technology component includes designing, constructing, and managing ecosystems in natural environments (Windriyanti et al. 2023). The principle of this technology can balance ecosystems, and one of its components is biodiversity, for which the concept can be used as a plant pest and disease control technique in IPM (Torres & Bueno 2018) by increasing the diversity of natural enemies to strengthen biological control by various mechanisms (Jonsson et al. 2017).

Environmental engineering by planting refugia is an effort to maintain environmental sustainability and a sustainable production system. With the existence of refugia plants, it is expected that an ecosystem balance can be achieved to allow natural control to work on its own (Indriyani et al. 2023). Exploration of the effect of vegetation manipulation in perennial crop systems shows that gardens with flowering shrubs have insects that provide ecological services, and their diversity is higher than gardens that are clean of plants (Ibrahim & Mugiasih 2020). Plant diversification provides spatiotemporal scale habitat heterogeneity for arthropods, changing the ability of insect herbivores and natural enemies to occupy the canopy, which is an important management strategy to improve insect biocontrol services for natural enemies (Arbi et al. 2019; Wan et al. 2019). Ecological engineering is carried out by modifying habitats using a polyculture cropping system that is easy to implement, including through inter-cropping, strip cropping, alley cropping, planting hedgerows, planting ‘flower islands’ or insectary plants in the middle of the planting area, and planting ground cover plants. These plants are flowering plants that are planted together with cultivated plants as a source of food and alternative hosts for useful insects (Horgan et al. 2016).

MATERIALS AND METHODS

The study was conducted during two distinct periods: July to September (off-season) and October to December (on-season), focusing on two different types of mango plantations—monoculture and polyculture—augmented by the presence of refugia plants. Each observation plot, covering an area of 2000 m^2, compromised a total of 20 mango trees. The research took place in Rembang Sub-District, Pasuruan Regency, East Java, Indonesia, situated at an elevation of 60 meters above sea level and geographically located at 7° 38’ 44.92” South Latitude and 112° 45’ 49.51” East Longitude. The prevailing soil type in the region is Gromosol, with average temperatures ranging from 26 to 32°C, and relative humidity fluctuating between 80% to 88%.

A combination of the hedgerows intercropping model and the planting of a ‘flower island’ or insectary bank in the center of the planting area was implemented (Musarofa et al. 2023). The insectary bank model involved planting all five types of refugia in the center of the field, each occupying a 2-meter by 2-meter square. Meanwhile, the hedgerow model consisted of planting three alternating types of refugia plants around the periphery of the experimental garden, with a planting distance of 5 cm between each plant. The three refugia plant species used were Peruvian zinnia (*Zinnia peruviana*), sunflowers (*Helianthus annuus*), and king’s salad (*Cosmos caudatus*).

A survey was conducted to observe the diversity of insects in mango plantations during the flowering period. Daily observations were conducted during the period of 7:00 AM to 3:00 PM, which corresponds to the period of peak insect activity. The study involved conducting observations to ascertain the insect species that frequented the mango plantations and the surrounding refugia plants. Observations were conducted twice a week to assess insect diversity in mango plantations during the flowering period. Sampling was performed using scan sampling, yellow traps, pitfall traps, and sweep nets. Scan sampling involved observing and collecting insect samples for 10 minutes on each tree. Observations were conducted during three periods: from 06:00 AM to 08:00 AM, then from 11:00 AM to 1:00 PM, and the third period from 3:00 PM to 5:00 PM, corresponding to the peak insect activity periods. Yellow traps were placed purposively, with 10 traps on 20 monoculture mango trees, 10 traps on 20 polyculture mango trees, and 10 traps on refugia plants (hedgerows). Pitfall traps were applied using the same method and quantity as yellow traps. Sweep netting was performed by swinging the sweep net in a zigzag pattern for 20 minutes along the observation blocks. Insect samples were collected from mango trees and five types of refugia plants. The process of collecting specimens was executed through the utilization of insect nets and color traps, as outlined in the FAO Protocol of 2011. The diversity of beneficial insects was documented through regular direct observation employing scan sampling methodology along designated transects. Multiple specimens of pollinating insects were conserved through either desiccation or immersion in a 70% ethanol solution. Additionally, photographic documentation of the insects was obtained while they were situated on refugia plants.

The preservation of insect species was carried out using conventional techniques as outlined by Upton (1991). The process of identifying insects was conducted by categorizing them into families, genera, or morphospecies, as per the guidelines provided by Borror et al. (1989), Goulet and Huber (1993), Pollination Guelph, Pollinator Identification (http://www.pollinationguelph.ca), Naumann (1991), and The Asian Honey-Bee (2007).

The metric utilized to quantify insect abundance was the mean count of individuals. Subsequently, the t-test was employed to make a comparison of the levels of insect abundance observed in monoculture and polyculture plantations. Furthermore, a diversity analysis was employed to evaluate dissimilarities in the attributes of the insect populations. The Shannon-Wiener diversity index (H′), Pielou evenness index (E), and Simpson dominance index (D) were computed. The index value that was computed underwent a Student's t-test. The statistical analysis was conducted with a significance threshold of $P < 0.05$. The statistical analyses were conducted utilizing R Statistics (R Core Team, 2020) and the vegan package was employed to examine community characteristics (Oksanen et al. 2020).

RESULTS AND DISCUSSION

Visitor insects in monoculture and polyculture were both subjected to identification procedures. During the mango harvesting season, the Gadung 21 plantations included sunflowers (*H. annuus*), king's salad (*C. caudatus*), common zinnia (*Z. peruviana*), *Tridax procumbens*, and *Alternanthera sessilis* from the Amaranthaceae family as refugia plants in the on-season. The results showed insects in the Arthropoda phylum, which are divided into the two classes of Insecta and Arachnida. The arthropods consisted of eight different orders: Coleoptera, Hemiptera, Diptera, Lepidoptera, Hymenoptera, Orthoptera, Neuroptera, and Araneae. The identification results showed 44 morphospecies belonging to 25 families: Curculionidae, Coccinellidae, Chrysomelidae, Carabidae, Blissidae, Aleyrodidae, Cicadellidae, Formicidae, Vepidae, Apididae, Icheumonidae, Eurytomidae, Xylocopidae, Eulophidae, Braconidae, Syrphidae, Pipunculidae, Stratiomyidae, Papilionidae, Nymphalidae, Pieridae, Lycainidae, Gryllotalpidae, Mantidae, Chrysopidae, and Qxyopidae.

The findings of this study on visitor insects in monoculture and polyculture systems contribute to the existing literature on insect diversity and composition. Similar studies have reported the importance of refugia plants in attracting and supporting a diverse range of insect species (Smith et al. 2018; Jones et al. 2020). The inclusion of sunflowers, king's salad, common zinnia, *T. procumbens*, and *A. sessilis* as refugia plants in the mango plantations aligns with the recommendations of previous research that highlight the significance of floral resources in providing food and shelter for insects (Rundlöf et al. 2015; Goulson 2019; Aminah et al. 2021).

The identification of insects belonging to the Arthropoda phylum and the presence of diverse orders such as Coleoptera, Hemiptera, Diptera, Lepidoptera, Hymenoptera, Orthoptera, Neuroptera, and Araneae. These findings underscore the importance of diverse habitats and plant communities in supporting a wide range of insect species, including pollinators and natural enemies of pests (Karp et al. 2018; Meyer et al. 2018).

The useful arthropods found in the monoculture and polyculture Gadung 21 mango plantations were Insects and Spiders. The arthropods existing in the ecosystem were generally found in the polyculture plantation with the planting of refugia plants and were mostly of groups that function as ecological services, as the groups of natural enemies and pollinating insects. The discovered natural enemy insects act as predators and parasitoids in pest control. The natural enemies found in this observation came from the orders Coleoptera, Diptera, Hymenoptera, Orthoptera, Neuroptera, and Araneae. Studies have shown that the conservation and promotion of beneficial arthropods can contribute to sustainable pest management and increase crop productivity (Bianchi et al. 2013). Furthermore, the integration of polyculture systems and the use of refugia plants have been recognized as effective strategies to enhance biodiversity and promote natural pest control (Bianchi et al. 2018).

The comparison between the polyculture field with refugia plants and the monoculture field revealed differences in the abundance and diversity of arthropod species. In the polyculture field, which provided a more diverse habitat with refugia plants, a higher number of morphospecies were observed compared to the monoculture field. In terms of overall arthropod diversity, the polyculture field with refugia plants exhibited 15 morphospecies (43%), while the monoculture field had 7 morphospecies (24%). This suggests that the presence of refugia plants in the polyculture field contributed to a more favorable environment for arthropods, leading to higher species richness. When focusing specifically on pollinator insects, the polyculture field again displayed greater diversity. The polyculture field hosted 16 morphospecies (30%) of pollinators, while the monoculture field had 9 morphospecies (22%). The pollinating insect groups identified in both fields belonged to the orders Lepidoptera, Hymenoptera, and Diptera. These findings indicate that the presence of a diverse plant community in the polyculture field attracted a greater variety of pollinators compared to the monoculture field (Tables 1 and 2). These findings align with previous studies highlighting the positive effects of polyculture and habitat diversity on arthropod abundance and diversity (Kennedy et al. 2013; Westphal et al. 2018). They support the notion that incorporating diverse plant species and creating refugia habitats can enhance ecosystem services provided by arthropods, such as pollination and pest control.

The results of statistical analysis showed that the total numbers of visiting insects on the polyculture and monoculture fields were not significantly different in value ($P=0.001$, $P<0.05$). However, when viewed from the number of individuals, the total number of visiting insects in the polyculture field was higher in comparison to the monoculture field (Figure 1). The total number of observed insects included herbivorous insects, predators, and pollinators. Figure 1 shows that the number of herbivorous insects was higher in monoculture, while the number of predatory insects and pollinators was higher in polyculture. Herbivorous insects are plant-eating insects that mostly act as plant pests. The high number of herbivorous insects on the monoculture field proves the dominance of a group of insects on the monoculture field (Ghazali et al. 2016; Ikhsan et al. 2020). In polyculture, the number of individual herbivorous insects was not significantly different when compared to monoculture. However, the polyculture field had higher numbers of predators (8.04 ± 1.33) and pollinators (8.37 ± 0.85) than predators (3.57 ± 0.49) and pollinators (5.35 ± 0.79) in the monoculture field. This proves that the polyculture system helps to suppress the dominance of a group of insects in the agroecosystem (Ghazali et al. 2016; Ikhsan et al. 2020).

Table 1. Average abundance of individual arthropods visiting mango plantations (monoculture and polyculture)

Genera	Role	Monoculture	Polyculture
Araneae			
Argiope	Natural enemy	2.4±1.35	0±0.00
Oxyopes	Natural enemy	0±0.00	3.9±1.91
Phintelloides	Natural enemy	3.2±1.75	0±0.00
Coleoptera			
Carabid	Natural enemy	0±0.00	8.3±4.45
Cheilomenes	Natural enemy	2.7±0.67	13.3±5.93
Chrysomelid	Herbivore	4.9±2.08	5.8±2.04
Coleophora	Natural enemy	2.5±0.97	5.1±2.18
Hypomeces	Herbivore	0±0.00	11.6±6.55
Diptera			
Bactrocera	Herbivore	15.6±7.97	0±0.00
Eristalis	Pollinator	6.6±4.55	3.9±1.85
Hermetia	Pollinator	3.8±2.78	10±3.94
Pipunculus	Natural enemy	0±0.00	2±1.41
Hemiptera			
Bemisia	Herbivore	0±0.00	29.6±5.23
Blissus	Herbivore	0±0.00	3.1±1.37
Bothrogonia	Herbivore	3.6±2.32	0±0.00
Idiocerus	Herbivore	55.8±22.65	8.7±2.58
Ricanula	Herbivore	2.8±0.79	0±0.00
Hymenoptera			
Amegilla	Pollinator	0±0.00	2.1±0.74
Apis	Pollinator	3.2±2.35	9±3.89
Brachymeria	Natural enemy	0±0.00	3.9±1.79
Ceratina	Pollinator	0±0.00	7.4±3.06
Charops	Natural enemy	0±0.00	1.8±0.63
Crematogaster	Natural enemy	18.2±9.04	22.2±8.31
Dolichoderus	Natural enemy	0±0.00	8.1±3.31
Eurytoma	Natural enemy	0±0.00	1.6±0.84
Myrmicaria	Natural enemy	8.5±1.96	7.2±3.79
Oecophylla	Natural enemy	0±0.00	10.5±3.57
Polistes	Natural enemy	0±0.00	3.9±1.66
Tetrastichus	Natural enemy	0±0.00	2.5±1.27
Trigona	Pollinator	12.6±7.55	13.7±3.71
Vespa	Pollinator	2.1±1.60	2.8±1.14
Xylocopa	Pollinator	1.9±0.88	1.7±0.67
Lepidoptera			
Borbo	Herbivore	4±1.83	0±0.00
Catopsilia	Pollinator	0±0.00	1.4±1.26
Deramas	Pollinator	0±0.00	3.6±1.65
Elymnias	Pollinator	0±0.00	2±1.05
Eurema	Pollinator	0±0.00	2.6±1.84
Graphium	Pollinator	0±0.00	2.4±1.17
Hypolininas	Pollinator	4.4±2.07	3.1±0.99
Leptosia	Pollinator	0.9±0.74	0±0.00
Melanitis	Pollinator	2.6±1.43	1±0.82
Neptis	Pollinator	0±0.00	0.9±0.88
Mantodea			
Hierodula	Natural enemy	0±0.00	1.7±0.82
Neuroptera			
Chrysoperla	Natural enemy	1.4±0.84	0±0.00

Table 2. Abundance of individual arthropods (insects, herbivores, natural enemies, and pollinators) that visit mango plantations (monoculture and polyculture)

	Monoculture	Polyculture	t value (df = 18)	P value
Total insects	163.7±62.68	222.4±39.07	2.51	0.021
Total herbivores	86.7±31.74	58.8±12.29	2.59	0.018
Total natural enemies	38.9±14.05	96±21.58	7.01	< 0.001
Total pollinators	38.1±19.93	67.6±9.28	4.24	< 0.001

The results of statistical analysis showed that the diversity of insect natural enemies and pollinators in monoculture and polyculture fields had a non-significant value of P=0.001 (P<0.05), which means that there was a difference between the diversity of visiting insects to the polyculture and monoculture fields. There was no significant difference between monoculture and polyculture visiting insect diversity in the Gadung 21 mango plantations, which was caused by the factor of herbivore visiting insect diversity being quite low.

A community is said to have high species diversity if the community consists of many species with the same or almost the same species abundance. Conversely, if a community consists of very few species and only a few species are dominant, then species diversity is low (Soegianto 1994) (Table 3). Insects are the most diverse multicellular group of organisms on planet Earth. Insects have a key role in the regulation and multi-dynamics of ecosystem services (ES). Pollination, biological control, food supply, and recycling of organic matter are the most studied parts of ES. Based on prior information, Hymenoptera, Coleoptera, and Diptera are the taxa that possess the most roles in ecosystem services (ES) (Noriega et al. 2017). These ecosystem services can be categorized as provisional (production of food, fiber, water, and other resources), cultural (non-material benefits, such as recreation, spiritual, and other aesthetic values), support (primary production, pollination, decomposition, and formation of necessary soils for resource production) and regulatory (biological control and other feedback mechanisms that maintain relatively consistent service delivery) (Schowalter et al. 2018). Yumura et al. (2016) revealed that the number of pollinators and the macro-scale landscape structure are indicators of ES quantity.

Table 3. Abundance of visiting arthropods (insects, herbivores, natural enemies, and pollinators) based on indices of diversity (H'), dominance (D), and evenness (E) in monoculture and polyculture Gadung 21 mango plantations

Sub-community	Index	Monoculture	Polyculture	t value (df = 18)	P value
Total insects	H'	2.40±0.09	3.14±0.07	20.14	< 0.001
	1/D	6.55±1.01	17.06±1.92	15.27	< 0.001
	E	0.78±0.03	0.88±0.01	9.03	< 0.001
Herbivores	H'	1.13±0.12	1.29±0.07	3.41	0.003
	1/D	2.26±0.33	2.94±0.36	4.37	< 0.001
	E	0.63±0.06	0.80±0.04	6.44	< 0.001
Natural enemies	H'	1.53±0.09	2.33±0.09	18.84	< 0.001
	1/D	3.57±0.49	8.04±1.33	9.95	< 0.001
	E	0.79±0.04	0.86±0.03	4.24	< 0.001
Pollinators	H'	1.86±0.11	2.36±0.07	11.66	< 0.001
	1/D	5.35±0.79	8.37±0.85	8.13	< 0.001
	E	0.89±0.04	0.87±0.02	0.70	0.488

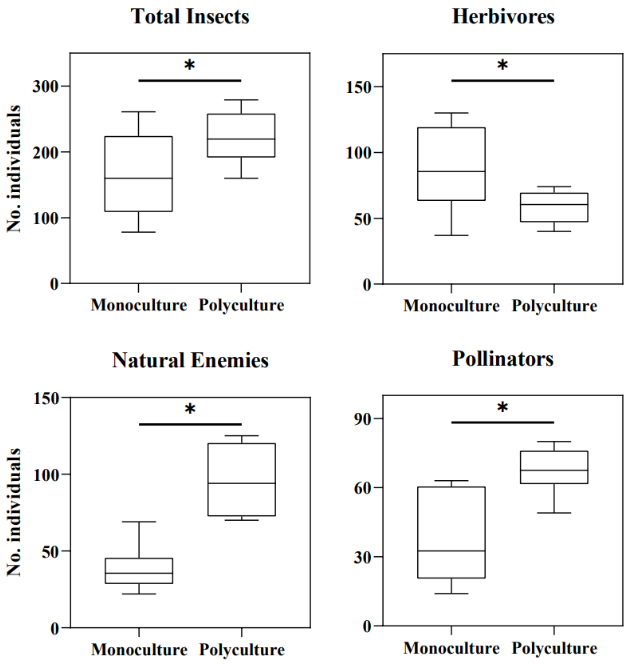

Figure 1. Comparison of visiting arthropods (insects, herbivores, natural enemies, and pollinators) based on indices of diversity (H'), dominance (D), and evenness (E) in monoculture and polyculture Gadung 21 mango plantations

A multitude of vegetation will increase the complexity value of a landscape, which means many types of resources that can be utilized by insects (Godfray 1994). The abundance and diversity of insects are closely related to the existence and types of vegetation in fields because the diversity of vegetation can provide food for imago, alternative hosts, and shelter for insects (Riyanto et al. 2011). The ecological services carried by biodiversity in an agricultural agroecosystem are described by Landis (2017); these include pollination services, decomposition services, and biological control services (predators, parasitoids, and pathogens) to control pests, which are very important for sustainable agriculture (Altieri et al. 2005; Mayer et al. 2011). In the last decade, there have been reports of declining diversity and population of useful insects (pollinating insects and natural enemies of insects) globally (Steffan-Dewenter et al. 2005; Biesmeijer et al. 2006; Klein et al. 2007). Therefore, it is quite necessary to save insects that are useful as ecological services in order to prevent the continued decline in diversity and population (Snyder 2019). The diversity and population of useful insects in nature are closely related to the diversity and population of flower-producing plants as a food source in the form of pollen and nectar. This includes the use of refugia plants, as continuous efforts to control insect pests will eliminate other beneficial pest populations such as predators, insect pollinators, and decomposers of organic matter.

Various flowering plants will be able to become food sources at any time and throughout the year because of the different ways of flowering phenology among plants. One of the technologies that have been applied by organic farmers is called Ecological Engineering (EE), in which habitat manipulation can be performed by planting flowering plants (insectary plants) that function as food sources, alternative hosts or prey, and refugia for natural enemies and pollinating insects. Most of the insects prefer small flowers that tend to open and possess a long flowering time, which is usually the case for flowers from the Asteraceae and Apiaceae families (Altieri et al. 2005). Flowering plants or what are called refugia plants, are a form of ecological service for the restoration of plant agroecosystems. Flowering plants or weeds that play an important role in the conservation of these natural enemies include the sunflower (*H. annuus*) and its family of plants, as well as king's salad (*C. caudatus*) and Peruvian zinnia (*Z. peruviana*).

To conclude, the applied planting model of refugia plants represents a combination of hedgerows and insectary plants. The diversity of visiting insects on refugia plants in the Gadung 21 mango plantations that were observed consists of 7 orders belonging to 24 families, as Curculionidae, Coccinellidae, Chrysomelidae, Carabidae, Blissidae, Aleyrodidae, Cicadellidae, Formicidae, Vepidae, Apididae, Icheumonidae, Eurytomidae, Xylocopidae, Eulophidae, Braconidae, Syrphidae, Pipunculidae, Stratiomyidae, Papilionidae, Nymphalidae, Pieridae, Lycainidae Gryllotalpidae, Mantidae, and Qxyopidae. These are divided into 38 morphospecies, with 1422 individuals in total. These insects play a role in ecological services as beneficial insects (predators, parasitoids, and pollinators). The study on visitor insects conducted in monoculture and polyculture environments, particularly within gadung 21 mango plantations, has revealed intricate insights into the dynamics of arthropod communities and their multifaceted interactions. The incorporation of refuge plants such as sunflowers, king's salad, common zinnia, *T. procumbens*, and *A. sessilis* in the polyculture system has played a pivotal role in attracting a diverse array of arthropod species spanning various orders, including Coleoptera, Hemiptera, Diptera, Lepidoptera, Hymenoptera, Orthoptera, Neuroptera, and Araneae. This diversified habitat has contributed significantly to the richness of morphospecies from distinct families, thereby enriching our understanding of insect diversity and composition. Notably, the heightened diversity of insect species within the polyculture field underscores the crucial significance of habitat diversity and the inclusion of refuge plants in supporting a wider spectrum of beneficial arthropods, encompassing both natural predators and pollinators. These findings underscore the value of embracing polyculture systems and strategically integrating refuge plants to enhance biodiversity and bolster the provisioning of vital ecosystem services for sustainable agriculture. Nevertheless, it remains imperative to diligently conserve and promote these advantageous insect populations, counteracting the ongoing decline in their diversity and abundance, thus safeguarding the equilibrium and resilience of agroecosystems.

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