Biodiversity of butterflies in endosulfan-affected areas of Kerala, India

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Abstract

Background: Butterflies are considered as bio-indicators of a healthy and diversified ecosystem. Endosulfan was sprayed indiscriminately in large plantations of Kasaragod district, Kerala which had caused serious threats to the ecosystem. In this study, we surveyed the butterflies for their abundance and diversity in three differentially endosulfan-affected areas viz., Enmakaje—highly affected area, Periye—moderately affected area, Padanakkad—unaffected area, carried out between the end of the monsoon season and the start of the winter season, lasting approximately 100 days. Seven variables viz., butterfly abundance (N), species richness (S), Simpson's reciprocal index (D), the Shannon–Wiener index (H'), the exponential of the Shannon–Wiener index ($expH'$), Pielou's evenness (J) and species evenness (D/S), related to species diversity were estimated, followed by the one-way ANOVA ($F = 25.01$, $p < 0.001$) and the Kruskal-Wallis test ($H = 22.59$, $p < 0.001$).

Results: A population of three different butterfly assemblages comprised of 2300 butterflies which represented 61 species were encountered. Our results showed that Enmakaje displayed significantly lower butterfly diversity and abundance, compared to the other two communities.

Conclusion: So far, this is the first study concerning the effect of endosulfan on the biodiversity of butterfly in the affected areas of Kasaragod, Kerala, India. This study may present an indirect assessment of the persisting effects of endosulfan in the affected areas, suggesting its long-term effects on the ecosystem.

Keywords: Butterflies, Diversity, Ecosystem, Endosulfan, Kerala

Background

The extensive use of potentially harmful pesticides is still done in many parts of the world. Endosulfan is a broad-spectrum organochlorine insecticide as well as an acaricide (Wang et al., 2012). Endosulfan (6,7,8,9,10,10-Hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine-3-oxide), comprises of two configurational isomers, alpha-endosulfan (64-67%), and beta-endosulfan (29–32%), which acts as an effective GABA-gated chloride channel antagonist and a Ca^{2+}, Mg^{2+} ATPase inhibitor (Huang et al., 2019). Endosulfan kill insects by endocrine disruption (Mnif et al., 2011). It is also a ubiquitous environmental contaminant as its semi-volatile nature makes it resistant to degradation processes in the environment.
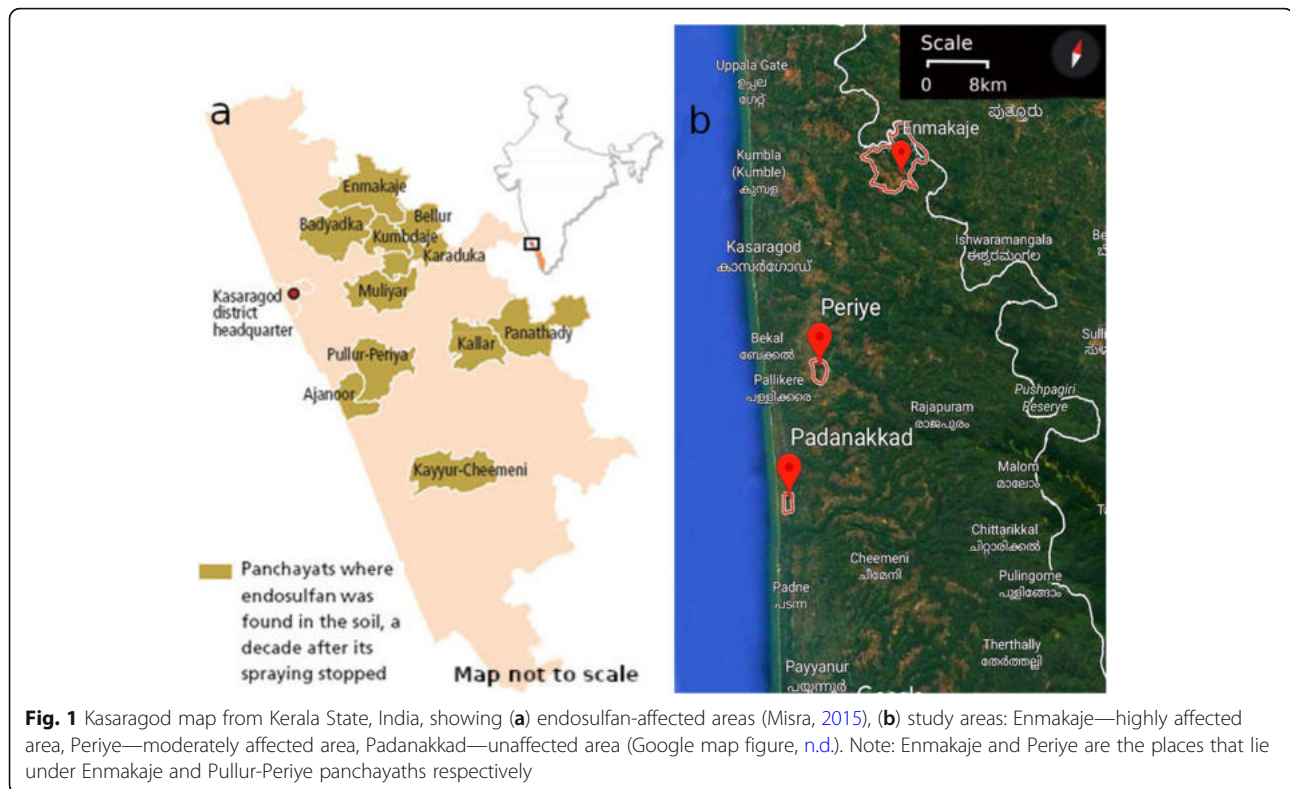
Being hydrophobic, endosulfan tends to get adsorbed into soil particles, resulting in persistence. The slow rate of degradation of endosulfan often results in the formation of endosulfan sulfate (Kullman Seth & Matsumura, 1996). Indiscriminately, it was sprayed aerially in cashew nut plantations of Kasaragod District in Kerala, India.

Kasaragod District is located in the south Indian state of Kerala and has been a site for cashew cultivation by the plantation corporation of Kerala since 1970 (Fig. 1). Cashew cultivation has been continued in the area, however, only until 2011 endosulfan was aerially sprayed at an average height of 34 m above the tree canopy to protect the cashew trees from the tea mosquito bug (Ramesh & Vijayalakshmi, 2002; Misra & Joshi, 2018).

Besides causing endless human sufferings, endosulfan being a broad-spectrum pesticide affected a diverse group of the organism and resulted in a decline of about 40–70%

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Fig. 1 Kasaragod map from Kerala State, India, showing (**a**) endosulfan-affected areas (Misra, 2015), (**b**) study areas: Enmakaje—highly affected area, Periye—moderately affected area, Padanakkad—unaffected area (Google map figure, n.d.). Note: Enmakaje and Periye are the places that lie under Enmakaje and Pullur-Periye panchayaths respectively

diversity in the area (Mathew, 2012). As reported by the local inhabitants, forest areas having rich wildlife (predominantly birds and butterflies) as well as close to the plantation were affected. Consequently, a large butterfly population had vanished, however, the current study reports their recovery (Mathew, 2012).

Butterflies are considered as bio-indicators of a healthy ecosystem (Bouyer et al., 2007) and have an intimate relationship with plants. As endosulfan is persistent and butterflies have a close relationship with plants, they may be directly affected. An assessment of the Lepidopteran diversity, in particular butterflies, will help in understanding the possible long-term effect of endosulfan on the diversity of insect populations.

Butterflies are important groups of model organisms and are extensively used in biological research including pest control, navigation, mimicry, embryology, evolutionary biology, population dynamics, genetics, and biodiversity conservation. They have traceable taxonomy and are easily surveyed (Syaripuddin et al., 2015). The study of butterflies over the years has provided unique data on an insect group unmatched in timescale and geographical scale anywhere in the world. This data is extremely important for research on climatic change and biodiversity.

In the present study, we employed a survey method to investigate the effects of forest disturbance rooted to endosulfan spray, on butterfly diversity and abundance in endosulfan-affected areas of Kasaragod District.

Specifically, we used the variation in the butterfly assemblage as a proxy to measure the magnitude of disturbance associated with historical endosulfan applications and their persistence. We did pose two important questions: (1) Is endosulfan continuing to affect butterfly populations in the affected regions? (2) If so, how does it affect the compositional differences in butterflies? Considering the duration since the last endosulfan-spray in 2011, our study helps in assessment and understanding the existing diversity of butterflies in endosulfan-affected areas.

Materials and methods
Study area
Three places in Kasaragod District were selected for the study based on certain criteria viz., the area of the cashew nut plantation, the amount of endosulfan sprayed, and the degree of biohazard it caused. The three places viz., Enmakaje ($12°62'32.05''$N$75°13'05.62''$ E), Periye ($12°39'19.51''$ N $75°08'82.36''$E), Padanakkad ($12°25'76.24''$ N $75°11'96.15''$ E) are one of the severely affected, moderately affected, and unaffected area respectively (Fig. 1) (Government of Kerala., 2003; Vijayan, 2011). To avoid the likelihood of biases in the data due to the unlikeness of vegetation among study sites including floral habitat, we conducted prior checklist surveys in different locations. The most commonly observed flowering plants in the selected study sites were *Lantana camara, Eupatorium*

odoratum, Tridax procumbens, Clerodendrum infortunatum, etc. Similar transects of around 150 m length in the evergreen forest vegetation with shrubby habitat dominated by nectar-producing plants were selected for the survey from each place.

Butterfly surveys

Butterflies were counted along 3 Pollard-style transects (Pollard, 1977) with 12 replicates for each of the 3 places by two observers. Surveys were conducted by walking 150 m long, 3 m wide transects at a constant pace ($v = 1$ km/h) while identifying butterflies within 1.5 m of each side and 3 m in front of the observer. Sampling was conducted weekly, between August (first week) 2018 to November (first week) 2018, for 12 surveys/transect (36 total surveys). All observations were collected between 10 AM and 12 PM while considering temperature (≥ 17 °C), wind speed (< 5 km/h), and weather (rain and $> 50\%$ cloud coverage avoided). While specimens were identified on spot (Bhakare M. & Ogale H, 2018), some of them which we found difficult to identify were caught for identification and released. Nevertheless, we attempted to identify and record data at the lowest taxonomic hierarchy, i.e., minimally at genus level as well as maximally at the species level.

Measure of butterfly diversity

Assemblage diversity was measured for each transect with univariate measures, with survey replicates merged into a single site observation to summarize the entire butterfly assemblage across all surveys at a site. Seven variables related to species diversity were estimated for each butterfly assemblage including butterfly abundance (N), species richness (S), Simpson's reciprocal index (D), the Shannon–Wiener index (H'), the exponential of the Shannon–Wiener index ($expH'$), Pielou's evenness (J), and species evenness (D/S). Univariate measures include butterfly abundance (number of specimens) and diversity as Hill's numbers, including species richness (Chao et al., 2014).

Statistical analysis

Analyses were performed using R version 3.5.3. To test the significance of differences in the butterfly assemblage between different study sites, one-way ANOVA was employed with p values adjusted for false discovery rate (FDR). To confirm the significance of the differences we performed the Kruskal-Wallis test. The diversity indices such as Shannon-Wiener index ($H' = -S\ p_i ln p_i$), Simpson's reciprocal index ($D = [(n_i\ (n_i - 1))/(N(N - 1))]^{-1}$) and the Pielou's evenness index ($J' = H'/(logS)$) were quantified for assemblages from each study sites using Microsoft Excel (Weibull et al., 2000). To check the evenness in diversity distribution, we plotted the

diversity profile curve and rarefaction curve using R version 3.5.3.

Results

Each transect ($n = 3$) was surveyed 12 times for a total of 36 surveys (Fig. 2). A total of 2300 butterflies of 61 species were encountered, Enmakaje was observed to display lower butterfly diversity and abundance compared to the other two communities. Table 1 summarizes estimates for seven variables relating to butterfly biodiversity: butterfly abundance (N), species richness (S), Simpson's reciprocal index (D), the Shannon–Wiener index (H'), the exponential of the Shannon–Wiener index ($exp\ H'$), Pielou's evenness (J'), and species evenness (D/S). We spotted a total of 37 species and 425 specimens in Enmakaje, 43 species and 880 butterflies in Periye, and 38 species and 995 butterflies in Padanakkad (refer supplementary table 1).

We performed three most commonly reported measures of species diversity, i.e., species richness, Simpson's reciprocal index, and the Shannon–Weiner index (Hill, 1973). As per the coefficients of variation (CV), Simpson's reciprocal index was observed to be the most variable (sensitive) among three assemblages ($CV = 21.78$). While untransformed Shannon–Wiener index appeared as the least variable among common diversity indices ($CV = 1.98$), its exponentiation expedites the discriminating power and provide an idea of the effective number of species of the biodiversity (exponential of the Shannon–Wiener index; $CV = 5.64$) (MacDonald et al., 2017). Species evenness (D/S) found to be the most variable index of the calculated indices ($CV = 23.19$) which may be attributed to the negative correlation between Simpson's reciprocal index (D) and species richness (S). With a coefficient of variation of 39.32, butterfly abundance was the most variable of all population variables. One-way ANOVA ($F = 25.01$, $p < 0.001$) and the Kruskal-Wallis test ($H = 22.59$, $p < 0.001$) showed the differences in the butterfly assemblages between different study sites were significant.

Discussion

Understanding species responses to the changes in habitat is a major focus of conservation and management of biological resources, particularly when assessing human interfered local disturbances to the landscape (Gardner et al., 2009). Here, we investigated butterflies' response to disturbance (7–8 years of post-disturbance) from endosulfan spray in Kasaragod District. We made two relevant contributions. First, we found that the endosulfan spray may have affected the butterfly assemblage by decreasing its abundance and diversity more at Enmakaje in comparison to Periye and Padanakkad (Fig. 3a). Secondly, our models reveal that type of disturbance does not have a much stronger effect on the composition of
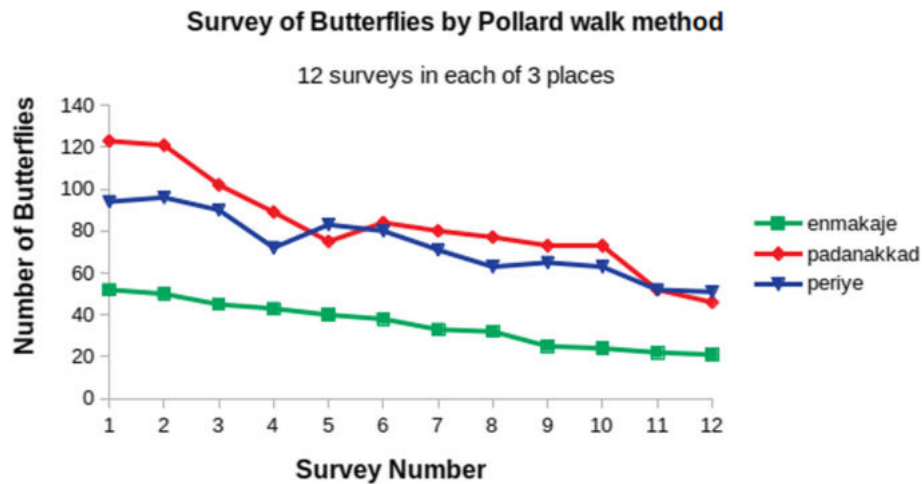
Fig. 2 Twelve surveys conducted using "Pollard walk" method in all the three study areas viz., Enmakaje, Padanakkad, and Periye. It represents a decrease in the butterfly's number from August 2018 to September 2018.

the butterfly assemblage and all three communities have similar species richness (Fig. 3b). This supports the hypothesis that the butterfly assemblages might have been shaped by the disturbances associated with endosulfan spray, responding principally to anthropogenic processes acting at a local scale.

To measure diversity, the three well-known approaches, viz., evenness, diversity and richness, and indices (including their transformations to Hill numbers) are essential. Individual assessment of the three approaches aids to highlight different information on distinct, however related properties of butterfly assemblages (MacDonald et al., 2017). For diversity comparison, the Shannon-Weiner index (*H'*) was calculated (Nolan & Callahan, 2006). However, the Shannon-Weiner index (*H'*) is criticized for its potential to "compress" data (MacDonald et al., 2017). In order to compensate for this data compression, exponentiation of the Shannon–Wiener index

was done which resulted in true diversity (effective number of species) for a butterfly assemblage (Jost, 2006). The butterfly communities of Periye and Enmakaje were found to have lesser *exp H'* values compared to Padanakkad suggesting that they both have less diversified butterfly communities (Rolando et al., 2013), (Table 1).

Even though the species richness was found to be high in Periye, Pielou's evenness index was low which implies that the community is dominated by very few species (Pielou, 1966). This may be due to the extensive availability of host plants for these butterflies and their ability to survive in harsh conditions. Furthermore, the number of butterflies encountered in the survey decreased from August to November which may infer the response of butterflies towards the seasonal increase in the temperature at all three places (Forrest, 2016) (Fig. 2). Temperature affects the growth of food plants (Hatfield & Prueger, 2015), and therefore should be negatively correlated with the numbers of individuals and species.

Depression of species-evenness is expected to occur due to the disproportional increase in the abundance of common species in relation to other species of the assemblage and the relative magnitudes of the population (Gosselin, 2006). Further, under favourable environmental conditions, butterfly assemblages may become "less even". Abundance distribution may not result primarily due to niche overlap and interspecific competition, given the fact that butterfly species differ in both host and nectar plant species (Hawkins & Porter, 2003; Kitahara et al., 2008). We observed a negative relationship between the abundance of butterfly and measures of species evenness in Periye which may infer that the differences in reproductive potential across species coupled with interspecific variation in environmental preference and may be correlated to the effect of endosulfan.

Table 1 Butterfly abundance (*N*), species richness (*S*), Simpson's reciprocal index (*D*), the Shannon–Wiener index (*H'*), the exponential of the Shannon–Wiener index (*exp H'*), Pielou's evenness (*J'*), and species evenness (*D/S*) calculated for 3 months of butterfly surveys completed within the three areas of study. Coefficient of variation (*CV*) is given for each metric

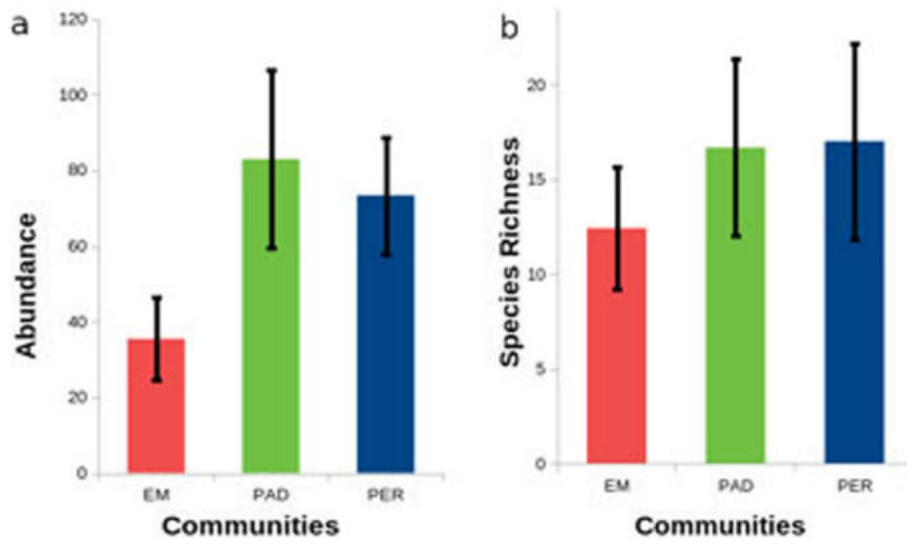| Communities	Enmakaje	Periye	Padanakkad	CV
N	425	880	995	39.32
S	37	43	38	8.17
D	7.5	9.28	11.61	21.78
H'	2.74	2.75	2.84	1.98
expH'	15.53	15.59	17.13	5.64
J'	0.76	0.73	0.78	3.32
D/S	0.2	0.22	0.3	23.19

Fig. 3 Univariate measures of butterfly diversity and abundance across all the study sites (EM; Enmakaje, PAD; Padanakkad, and PER; Periye). From left, butterfly abundance (**a**) and species richness (**b**) (average ± standard deviation) for cumulative assemblages from all transects from each study sites

Also, lack of correlation of species richness with Simpson's reciprocal index, the Shannon–Weiner index, or the exponential of the Shannon–Wiener index may be attributed to the unevenness of the butterfly assemblages. Further, based on the coefficients of variation calculated for all the diversity indices, Simpson's reciprocal index showed more variability than others. The lowest coefficient of variation owing to the Shannon–Weiner index tends to conceal the variability in species diversity. The diversity profile curve was plotted using Hill

numbers of order 0, 1, and 2 for all the butterfly assemblages (Fig. 4a). The steepness of the curve clearly demonstrates more balanced diversity, and a line parallel to the *x*-axis is an ideal condition (Rousseau et al., 1999). Compared to the other two study sites, Padanakkad has a less-steep curve, representing its balanced butterfly diversity with even distribution of most of the species as indicated in Fig. 4a. Sudden fall of the curve representing Periye shows a total unevenness in butterfly distribution and the community being dominated by only a few species.
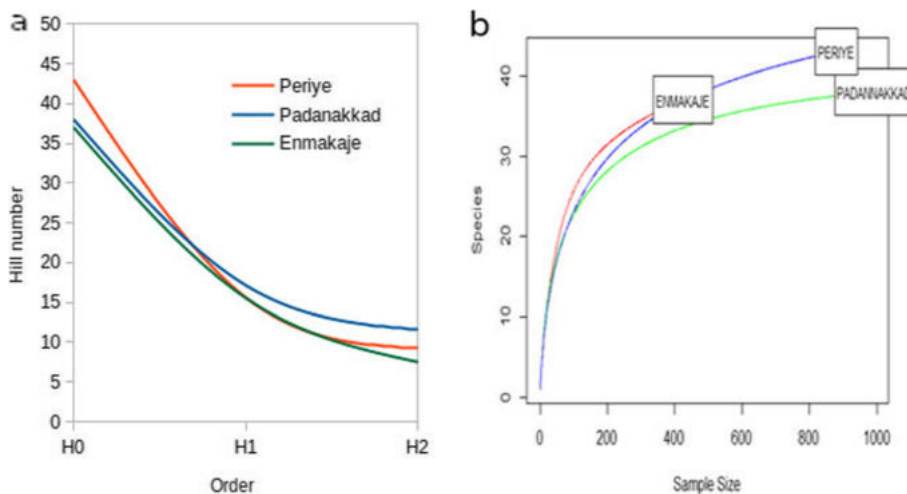

Fig. 4 a Diversity profile curve; diversity curves of the cumulative assemblage of each transect type (Hill's number of order 0 is species richness, 1 is the exponential of Shannon's entropy, and 2 is the inverse of Simpson's concentration). **b** Rarefaction curve of each butterfly assemblages for the given number of samples as a function

To compare sample-based rarefaction curves, each species from one community were plotted against the number of individuals (Fig. 4b). This depiction plot provides a measure of species diversity, which is strongly built on the sample size effects, permitting comparison between communities. Steeper curves denote more diverse communities. Richness estimators were highly influenced by rare species (Crist Thomas & Veech, 2006). In our study, out of 61 species, 12 species were singletons and 4 species were doubletons (represented by two individuals). The difference between observed and the true species richness of the butterfly assemblage would be greater with more singletons within a sample, for a given number of doubles (Senthilkumar, 2003).

Butterfly assemblage from Padanakkad shows a decent distribution with a less-steep curve as compared to the other two study sites (Fig. 4b). While the rarefaction curves of Enmakaje and Periye show a gradual rise due to the presence of more singletons and doubletons species (Fig. 4b). The graph corroborates the inferences made from the diversity indices and evenness indices. Padanakkad, comparatively with the higher butterfly abundance, can be considered as a diversified community with evenly distributed species. This brings about a balance to their community and other organisms interacting with them both directly or indirectly. Further, the results indicate that in Periye, the abundance of the butterfly is observed to be remarkable. However, species distribution is found to be uneven and the population is predominated by a few species. Also, it constitutes a comparatively higher number of singleton and doubleton species. Enmakaje has the lowest abundance and diversity among the three butterfly communities of study.

For conservation priorities, along with endemic butterflies, those which are protected under WPA (wildlife protection act), 1972 has been given additional importance. In our study, we recorded a total of five endemic butterfly species viz., *Papilio budha* (WPA schedule 2) and *Troides minos* in Periye as well as and *Papilio liomedon moore* (WPA schedule 1), *Troides minos*, *Cirrochroa thais*, and *Cethosia nietneri* in Enmakaje (Kunte, 2008). Furthermore, legally protected species viz., *Dophla evelina* (WPA schedule 2) and *Hypolimnas misippus* (WPA schedule 1) in Periye as well as *Tanaecia lepidea* (WPA schedule 2) in Enmakaje (Kunte, 2008) were recorded. Through this study, we emphasize the importance of these species which require special care to conserve them and their habitat. Surprisingly, Padanakkad has healthier butterfly biodiversity than Periye and Enmakaje, however, such that no endemic species or species that are legally protected under WPA, 1972 was recorded.

Based on the results, it can be hypothesized that the effects of endosulfan might be playing a role to impede the healthy growth of the butterfly population in both Enmakaje and Periye. Moreover, different degrees of endosulfan's effects might be the possible cause for the compositional differences between the butterfly communities. Butterfly's population of Enmakaje and Periye's might have migrated from nearby healthy ecosystems or might have gradually developed resistance against endosulfan, during the course of 8 years after the banning of endosulfan spray. Through this study, we anticipate the conservation of butterflies by preserving their habitat through awareness of the current diversity patterns.

Conclusion

Our study shows a high value of 'Simpson's reciprocal index (*D*)' (7.5–11.61), which points towards the areas with high variation. Given the importance of local disturbances characteristics over the landscape factors (known to affect butterfly assemblages), we demonstrated the effects of endosulfan on butterfly populations at Kasaragod District. It may be inferred that endosulfan might be continuing to pose a threat to the butterfly's abundance in Enmakaje and Periye communities. Nevertheless, the study points towards sensitiveness (Enmakaje and Periye) which can be evinced with their unhealthy assemblage in response to the local changes in their habitat. Heterogeneity of the butterfly samples showing spatial and temporal variability may further infer that the possible effect of historical endosulfan sprays at Periye and Enmakaje. The study provides an opportunity to estimate at best, the overall species richness of butterfly fauna in endosulfan-affected areas of Kasaragod District. We anticipate that this study would provide a base towards a molecular approach to gain further insight into butterfly management and its conservation.

Supplementary information

Additional file 1. Supplementary Table 1.

Abbreviations

N: Butterfly abundance; *S*: Species richness; *D*: Simpson's reciprocal index; *H*': Shannon–Wiener index; *expH*': Exponential of the Shannon–Wiener index; *J*: Pielou's evenness; *D/S*: Species evenness; ANOVA: Analysis of variance; GABA: Gamma-Aminobutyric Acid; FDR: False Discovery Rate; *CV*: Coefficients of variation; *WPA*: Wildlife Protection Act

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