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Smriti Singh
Research Scholar, Department of Zoology, Govt. Science P.G. College, Rewa, Madhya Pradesh, India

Vinita R Kashyap
Associate Professor of Zoology, Govt. Science P.G. College, Rewa, Madhya Pradesh, India

Corresponding Author:
Smriti Singh
Research Scholar, Department of Zoology, Govt. Science P.G. College, Rewa, Madhya Pradesh, India

Biodiversity of butterflies in endosulfan-affected areas of Rewa (M.P.)

Smriti Singh and Vinita R Kashyap

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Abstract
Butterflies are considered bioindicators of a healthy and diversified ecosystem. Endosulfan was sprayed indiscriminately on large plantations in the Rewa district of Madhya Pradesh, causing serious threat to the ecosystem. In this study, we surveyed butterflies for their abundance and diversity in three areas differentially affected by endosulfan, *i.e.*, Govindgarh-highly affected area, Jayanti Kunj-moderately affected area, Kothi Compound-unaffected area, conducted between the end of monsoon season and the beginning of the winter season, which lasts 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 ($H = 22.59$, $p<0.001$). A population of three different butterfly assemblages comprised of 2310 butterflies which represented 62 species were encountered. Our results showed that Govind Garh showed significantly lower butterfly diversity and abundance compared to the other two communities. So far, this is the first study on the effect of endosulfan on butterfly biodiversity in the affected areas of Rewa, Madhya Pradesh, India. This study may represent an indirect assessment of the persistent effects of endosulfan in affected areas, suggesting its long-term effects on the ecosystem.

Keywords: Butterflies, diversity, ecosystem, endosulfan, Rewa

Introductions

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) [1]. Endosulfan (6,7,8,9,10,10-Hexachloro 1,5,5a,6,9,9 a-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)[2]. Endosulfan kill insects by endocrine disruption (Mnif *et al.*, 2011) [3]. 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) [4]. Indiscriminately, it was sprayed aerially in mango plantations of Rewa District in Madhya Pradesh, India

Rewa district is a district of the Madhya Pradesh state in central India. Mango is the leading fruit of Madhya Pradesh and is grown in almost all the districts of the State. Its cultivation in M. P. dates back to times immemorial which is evidenced by its mention in the literature of Kalidas, culture of Shiwarinarayan, and Narasinghnath and miles and miles long very old and robust trees found growing along the Pachmarhi Hoshangabad Road and the area around Govindgarh in Rewa (Kashyap and Jyothi, 1972 and Usha Rani, 2018) [5-6].

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% diversity in the area (Mathew, 2012) [7]. As reported by the local inhabitants, forest areas having rich wildlife (predominantly birds and butterflies) as well as close to the plantation were affected.

Butterflies are considered as bio-indicators of a healthy ecosystem (Bouyer *et al.*, 2007) [8] 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 lepidic-teran diversity, in particular butterflies, will help in understanding the possible long-term effect of endosulfan on the diversity of insect populations.

Butterflies are an important group of model organisms and are widely used in biological research including pest control, navigation, mimicry, embryology, evolutionary biology, population dynamics, genetics and biodiversity conservation. They have a traceable taxonomy and can be easily researched (Syaripuddin *et al*., 2015) [9]. The study of butterflies over the years has provided unique data on a group of insects unparalleled in time and geography anywhere in the world. These data are extremely important for climate change and biodiversity research.

In this study, we used a survey method to investigate the effects of forest disturbance caused by endosulfan spraying on butterfly diversity and abundance in endosulfan-affected areas in Rewa district. Specifically, we used variation in butterfly assemblages as a proxy to measure the magnitude of disturbance associated with historical endosulfan applications and their persistence. We asked two important questions: (1) Does endosulfan continue to affect butterfly populations in affected areas? (2) If so, how does this affect differences in butterfly composition? Considering the duration since the last endosulfan spraying in 2021, our study helps to assess and understand the existing butterfly diversity in endosulfan affected areas.

Material and Methods

Study area

Three places in Rewa District were selected for the study based on certain criteria viz., the area of the mango plantation, the amount of endosulfan sprayed, and the degree of biohazard it caused. The three places viz., Govindgarh, Jayanti Kunj and Kothi Compound are one of the severely affected, moderately affected, and unaffected area respectively. 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*, *Cleroterium unfortunate*, 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) [10] 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) 2022 to November (first week) 2022, for 12 surveys/transect (36 total surveys). All observations were collected between 10 AM and 12 PM while considering temperature (≥ 14 °C), wind speed (< 5 km/h), and weather (rain and > 50% cloud coverage avoided). 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) [11].

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-Wally's test. The diversity indices such as Shannon-Wiener index (H' = −S pilnpi), Simpson's reciprocal index (D = [(ni (ni − 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)[12]. 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. A total of 2310 butterflies of 62 species were encountered, Govindgarh 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 36 species and 426 specimens in Govindgarh, 42 species and 779 butterflies in Jayanti Kunj, and 38 species and 994 butterflies in Kothi Compound (refer supplementary table 1).

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′), Pileous 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	Govindgarh	Jayanti Kunj	Kothi Compound	CV
N	426	779	994	39.31
S	36	42	38	8.16
D	7.6	9.27	11.62	21.76
H'	2.72	2.74	2.85	1.96
expH'	15.52	15.58	17.14	5.65
J'	0.75	0.72	0.78	3.34
D/S	0.21	0.22	0.31	2.67

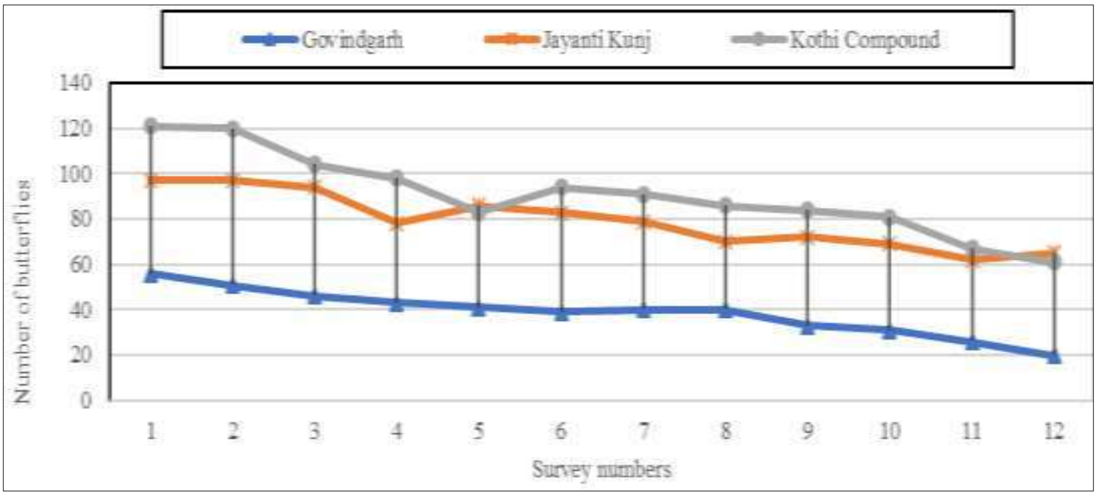

Fig 1: Twelve surveys conducted using "Pollard walk" method in all the three study areas.

We performed the three most commonly reported measures of species diversity, i.e., species richness, Simpson's reciprocal index, and the Shannon–Weiner index (Hill, 1973) [13]. As per the coefficients of variation (CV), Simpson's reciprocal index was observed to be the most variable (sensitive) among three assemblages (CV = 21.76). While untransformed Shannon–Wiener index appeared as the least variable among common diversity indices (CV = 1.96), 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 = 5.65) (MacDonald et al., 2017) [15]. 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 39.31, butterfly abundance was the most variable of all population variables. One-way ANOVA (F = 25.01, p<0.001) and the Kruskal-Walli's 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) [14]. Here, we investigated butterflies' response to disturbance (7-8 years of post-disturbance) from endosulfan spray in Rewa 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 Govindgarh in comparison to Jayanti Kunj and Kothi Compound. Secondly, our models reveal that type of disturbance does not have a much stronger effect on the composition of the butterfly assemblage and all three communities have similar species richness. 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) [15]. For diversity comparison, the Shannon-Weiner index (H') was calculated (Nolan & Callahan, 2006) [16]. However, the Shannon-Weiner index (H') is criticized for its potential to "compress" data (MacDonald *et al*., 2017) [15]. In order to compensate for this data compression, exponentiation of the Shannon–Wiener index (H') is criticized for its potential to "compress" data (MacDonald *et al*., 2017) [15]. 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) [17]. The butterfly communities of Jayanti Kunj and Govindgarh were found to have less exp H' values compared to Kothi Compound suggesting that they both have less diversified butterfly communities (Rolando *et al*., 2013) [18], (Table 1).

Even though the species richness was found to be high in Jayanti Kunj, Pileous evenness index was low which implies that the community is dominated by very few species (Pielou, 1966) [19]. 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) [20]. Temperature affects the growth of food plants (Hatfield & Prueger, 2015) [21], 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) [22]. Further, under favorable 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) [23-24]. We observed a negative relationship between the abundance of butterfly and measures of species evenness in Jayanti Kunj 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.

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. 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) [25]. Compared to the other two study sites, Kothi Compound has a less-steep curve, representing its balanced butterfly diversity with even distribution of most of the species. The sudden fall of the curve representing Jayanti Kunj shows a total unevenness in butterfly distribution and the community being dominated by only a few species.

To compare sample-based rarefaction curves, each species from one community were plotted against the number of individuals. 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) [26]. 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 (Senthil Kumar, 2003) [27].

Butterfly assemblage from Kothi Compound shows a decent distribution with a less-steep curve as compared to the other two study sites. While the rarefaction curves of Govindgarh and Jayanti Kunj show a gradual rise due to the presence of more singletons and doubletons species. The graph corroborates the inferences made from the diversity indices and evenness indices. Kothi Compound, 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 and indirectly. Further, the results indicate that in Jayanti Kunj, the abundance of butterflies 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. Govindgarh 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., Papillion buddha (WPA schedule 2) and Triodes Minos in Jayanti Kunj as well as and *Papilio liomedon* moore (WPA schedule 1), *Triodes Minos*, *Cirrochroa Thais*, and *Cethosia nietneri* in Govindgarh (Kunte, 2008) [28]. Furthermore, legally protected species viz., *Dophla evelina* (WPA schedule 2) and *Hypolemmal Mespilus* (WPA schedule 1) in Jayanti Kunj as well as *Tan aecia lepidic* (WPA schedule 2) in Govindgarh (Kunte, 2008) [28] were recorded. Through this study, we emphasize the importance of these species which require special care to conserve them and their habitat. Surprisingly, Kothi Compound has healthier butterfly biodiversity than Jayanti Kunj and Govindgarh, 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 in impeding the healthy growth of the butterfly population in both Govindgarh and Jayanti Kunj. Moreover, different degrees of endosulfan's effects might be the possible cause for the compositional differences between the butterfly communities. Butterfly's population of Govindgarh and Jayanti Kunj'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)" (7.6–11.62), indicating areas of high variability. Given the importance of local disturbances, we demonstrated the effects of endosulfan on butterfly populations in Rewa district. It can be inferred that endosulfan may continue to pose a threat to butterfly abundance in the communities of Govindgarh and Jayantikunj. However, the study points to sensitivity (Govindgarh and Jayanti Kunj) that can be demonstrated by their unhealthy aggregation in response to local changes in their habitat. The heterogeneity of butterfly samples showing spatial and temporal variability may further infer the possible effect of historical endosulfan sprays in Jayanti Kunj and Govindgarh. The study provides an opportunity to estimate, at best, the overall species richness of the butterfly fauna in the endosulfan-affected areas of Rewa District. We expect this study to provide the basis for a molecular approach to gain further insight into butterfly management and conservation.

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