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The resilience of Rubiaceae to anthropogenic factors: a case study from the Himalayan range of Western Bhutan

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Abstract
The study about the resilience of Rubiaceae to the influence of anthropogenic factors was conducted along the altitudinal gradient of 300–3900 m asl. in Western Bhutan. The survey covered three types of forest, categorized based on the prevalence of anthropogenic disturbances and assessed the diversity of Rubiaceae species in each forest type. The study recorded a total of 54 Rubiaceae species belonging to 41 genera from the study sites. The high diversity of Rubiaceae in the intermediately disturbed forest as revealed by Simpson and Shannon-Wiener diversity analysis and further strengthened by a between-group one-way ANOVA analysis contradicts the presumed description of Rubiaceae as ecologically sensitive. The wider adaptability range exhibited by *Ceriscoides* (Hook.f.) Tirvendadum, *Himalrandia* Yamazaki, *Uncaria* Schreber, and *Leptodermis* Wall. showing presence in all the forest categories indicates a higher survival rate of these genera. On the contrary, the species showing a higher rate of confinement to a specific habitat bears higher risk of extinction due to ever-rising anthropogenic disturbances. As such, an exhaustive research assessing the impact of different categories of anthropogenic factors on different species of Rubiaceae is required to understand the overall resilience of the family to the anthropogenic disturbances.

1. INTRODUCTION

Since not all plant species exhibit similar responses to the disturbance regime (Onaindia et al. 2004), it is imperative to understand what kind of disturbance regimes are detrimental or helpful and how various plant species respond to different types of disturbance regimes. For instance, the natural disturbances are thought as an integral part of the ecosystem (Dale et al. 2000) since they may also act as the drivers of plant species diversification (Lorimer and White 2003), while the human-driven disturbance is believed to cause exploitation of biological resources and impact species diversity to a great extent (Abadie et al. 2011). Predominantly, anthropogenic disturbances are supposed to cause greater biodiversity alteration than the one that occurs under natural conditions (Kumar and Ram 2005). On the contrary, human-driven disturbances are on the rise along with the escalating demand for forest resources (Hasenauer et al. 2012), resulting in inadvertent destruction to various plant species. Consequently, it is crucial to study the response of different plant species to different types of disturbance regimes because the consequences may be devastating for those plant species that are on the verge of extinction or endemic in nature.

The influence of anthropogenic factors on plant species diversity has been studied with reference to the Rubiaceae family as this family has been described as better fitted to be used in the ecological analysis (Delprete and Jardim, 2012). Furthermore, the Rubiaceae is described as a family exhibiting high endemism further aggravated by ecological sensitivity (Barbhuiya et al., 2014). Additionally, the species in Rubiaceae are less diverse (Davis and Bridson, 2007) and exhibit higher rate of susceptibility to extinction owing to the presence of at least 72% of the genera with lesser than 10 species further exacerbated by the presence of 211 monotypic genera of the total 620 genera (Davis et al., 2009).

Bhutan with 71% of land under forest coverage (DoFPS 2016) characterized by a high density of species richness (Bruggeman et al. 2016) that are thriving against 89.6% of the population relying on forest resources for their day livelihood (MoLHR 2011) provides ideal conditions for the study concerning the influence of anthropogenic factors on the plant species composition. Furthermore,

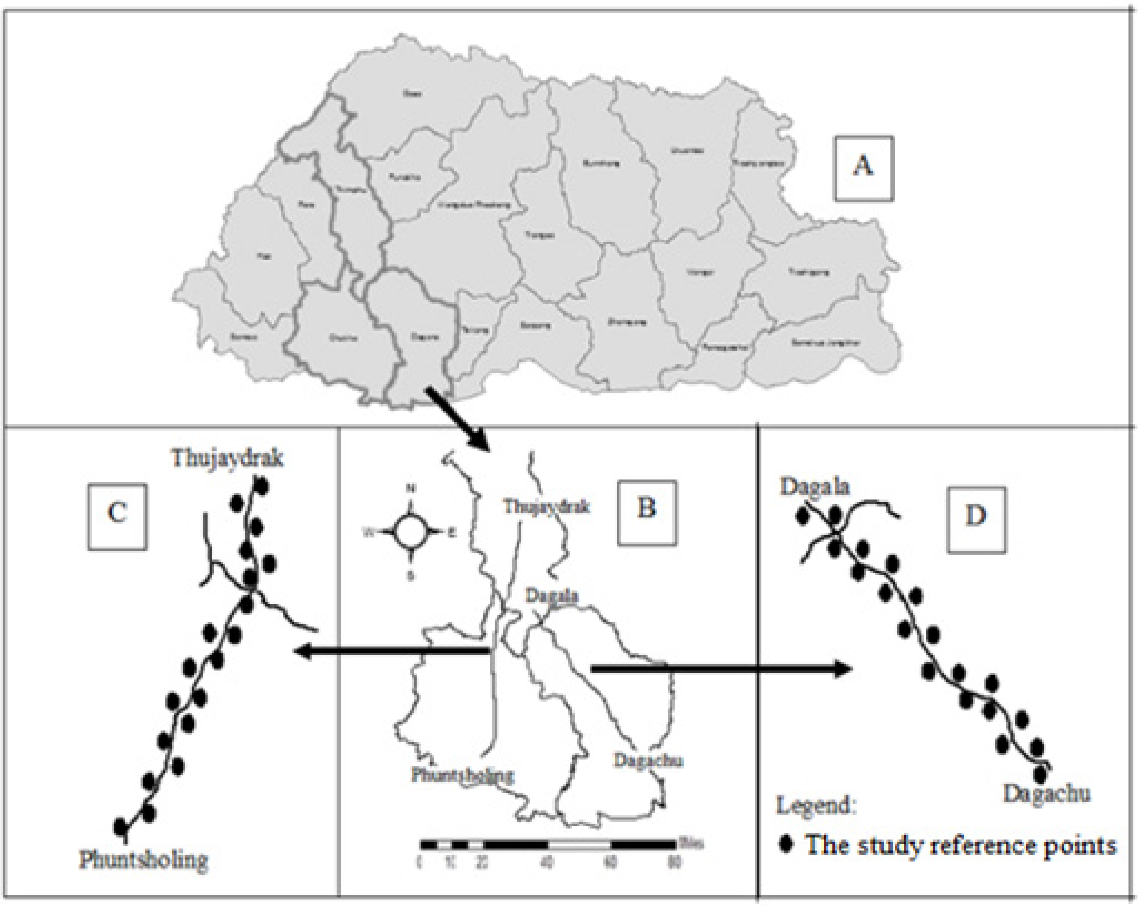


Figure 1. A map showing the study sites. (A) A map of Bhutan; (B) Map of Dagana, Chukha, and Thimphu districts; (C) Phuntsholing–Thujaydrak study area; (D) Dagachu–Dagala study area

the recognition of Bhutan as part of a global biodiversity hotspot (Myers et al. 2000) under the imminent anthropogenic threat driven by the historic human land use system and the associated disturbance regime (Siebert and Belsky 2014) provides a wide range of habitat—from disturbed to intact—for the plant species to occupy. It, therefore, creates an ideal site for the study.

Hence, this study endeavors to investigate the resilience of Rubiaceae species to anthropogenic factors. In particular, the study aims to (i) explain the variation of Rubiaceae species diversity based on the prevalence of anthropogenic factors and (ii) discuss the resilience of Rubiaceae to human disturbances.

2. MATERIALS AND METHODS

2.1 Study area

The study was conducted along Phuntsholing-Thujaydrak and Dagachu-Dagala range within the altitudinal gradient of 300–3900 m asl. (Figure 1). Both the study area encompasses the tropical, subtropical, warm-temperate, cool temperature, and subarctic zones based on the vegetation zonation of Bhutan by Ohsawa (1987). The study areas are characterized by the presence of roads, hydropower constructions, settlements, cultivation, abandoned lands, religious sites, and trekking trails, thereby, manifesting high variability of habitat—from pristine to disturbed. Furthermore, the landscape is characterized by sharp ridges, hills, valleys, thick forests, and alpine scrub and meadows.

The climate data for the past 10 years recorded at the weather stations located in the study areas, obtained from the National Center for Hydrology and Meteorology, Thimphu Bhutan, show a high variation in annual mean precipitation, relative humidity, the maximum and minimum temperature in the study sites (Figure 2). The high variability of climate, topography, elevation, and rock composition fosters the presence of high biodiversity in both the study areas.

2.2 Study design and data collection

Based on the ocular inspection and reconnaissance survey, the study areas were pigeonholed into three as undisturbed forests (UDF), semi-disturbed forests

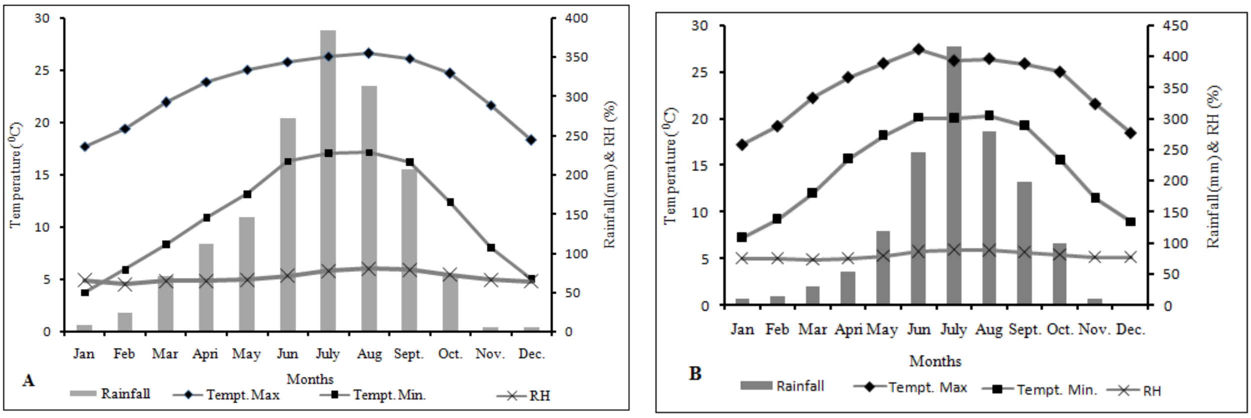


Figure 2. The summary of mean annual rainfall, relative humidity (RH), maximum, and minimum temperature of the study area during the period of 2008–2017; (A) Phuntsholing–Thujaydrak area; (B) Dagachu–Dagala area

(SDF), and highly disturbed forests (HDF). The portion of the forest where anthropogenic factors are completely absent or showing evidence of some minor disturbances lacking potential risk to deplete or alter the native state of the habitat is categorized as undisturbed forests. The forest zone experiencing timber extraction, tree felling, litter extraction, fuelwood collection, and cattle grazing is defined as semi-disturbed forests. The area characterized by settlement, road and hydro-power plant constructions, cultivated land, an abandoned area with early vegetation succession, trekking routes, and farmlands is defined as highly disturbed forests.

The species absence and presence examination were done at every 200 m elevation interval covering all the three forest types mentioned above. In this manner, a total of 38 sites, 19 sites in each study area, were set up for investigation. For every encounter of Rubiaceae species, an approximate quadrat in the manner of 10 × 10 m, 5 × 5 m, and 1 × 1 m was laid for tree, shrub, and herb species, respectively. The information on the quadrat number, species count in each quadrat, the forest type, altitude, latitude, and longitude was recorded in the field datasheet. The Rubiaceae species observed in other areas were also included in the list following the same protocol of assessment as detailed above. The data collection was done from February to December to cover the whole flowering period.

The in situ and ex situ methods of specimen identifications were adopted using the Flora of Bhutan (Grierson and Long 1999) and other available taxonomic literature. The methods of Bridson and Forman (1992) were used to prepare voucher specimens and deposited at the National Herbarium, Serbithang, Thimphu, Bhutan.

2.3 Data analysis

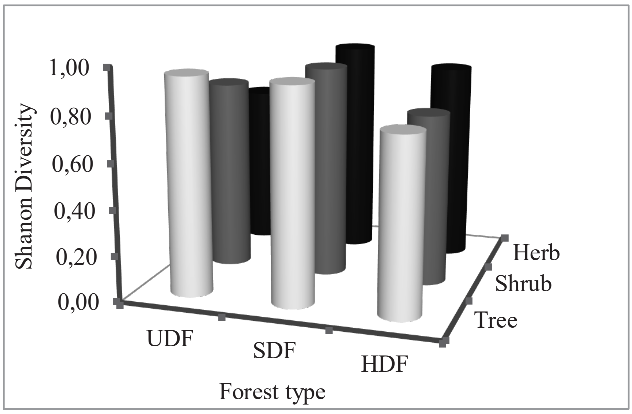
The species diversity in each forest type was calculated using Shannon-Wiener and Simpson diversity index. The climate data and species density (mean and stand deviation) were calculated through direct computation in Microsoft Excel 2007. A between-group one-way analysis of variance (ANOVA) was performed using Statistical Package for the Social Science (SPSS) version 24 to evaluate the significance of species diversity in different forest types.

3. RESULT

A total of 54 Rubiaceae species, consisting of 11 tree species, 22 shrub species, and 21 herb species, belonging to 41 genera were recorded from the study sites (Table 1). The percentage species composition was higher for semi-disturbed forests at 40.4% (51 species) against 30.4% (38 species) in undisturbed forests and 28.8% (36 species) in highly disturbed forests. In general, the Shannon-Wiener and Simpson diversity analysis revealed highest diversity for semi-disturbed forests, $H = 0.94$ (tree), 0.927 (shrub), and 0.946 (herb), $D = 0.898$ (tree), 0.937 (shrub), and 0.941 (herb), followed by undisturbed forests, $H = 0.950$ (tree), 0.831 (shrub), and 0.709 (herb), $D = 0.910$ (tree), 0.913 (shrub), and 0.876 (herb). The lowest diversity recorded was in the highly disturbed forest, $H = 0.774$ (tree), 0.751 (shrub), and 0.873 (herb), $D = 0.852$ (tree), 0.886 (shrub), and 0.925 (herb) (Figure 2).

In addition, a between-group one-way analysis of variance (ANOVA) was performed to evaluate the relationship between Rubiaceae species richness and diversity with the forest type (highly disturbed forests, semi-disturbed forests, and undisturbed forests). It is apparent that the moderate level of disturbance was associated with the highest mean level of species diversity ($M = 15.63$, $SD = 10.02$), and the highest level of disturbance was associated with the lowest mean level of species diversity ($M = 9.30$, $SD = 10.12$) as shown in Figure 3. The assumption of homogeneity of variances evaluated based on Levene's F test, $F(2, 159) = 0.239$, $p = 0.788$ was found tenable.

An independent between-group analysis of variance revealed a statistically significant difference between groups, $F(2, 159) = 5.687$, $p = 0.004$, $\eta^2 = 0.067$. However,



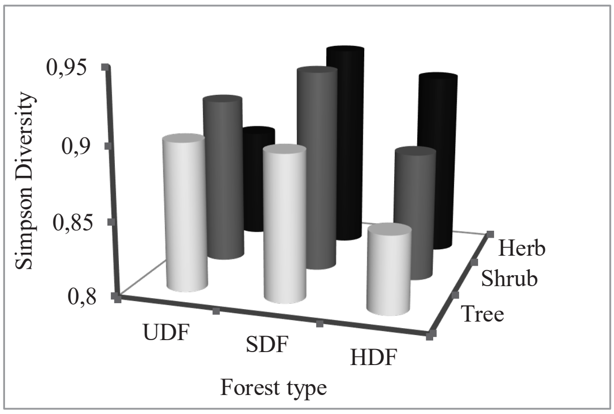


Figure 3. The diversity of trees, shrubs, and herbs in undisturbed forests (UDF), semi-disturbed forests (SDF), and highly disturbed forests (HDF) analyzed using Shannon-Wiener and Simpson diversity index

Table 1. The species density of the Rubiaceae in three different forest types based on the prevalence of anthropogenic factors (UDF: undisturbed forests, SDF: semi-disturbed forests, HDF: highly disturbed forests, SD: standard deviation)

	Species name	Density (Mean + SD)		
		UDF	SDF	HDF
Trees	*Ceriscoides campanulata* Roxb.	1.3 + 0.5	2.3 + 1.9	2.3 + 1.9
	Hymenodictyon flaccidum Wall.	2.0 + 0.8	2.5 + 0.3	0
	Ixora undulata Roxb.	0	2.3 + 1.0	1.3 + 0.5
	Tarenoidea wallichi Hook.f.	1.3 + 0.5	0	0
	Wendlandia coriacea DC.	3.0 + 0.8	3.3 + 1.3	2.3 + 0.9
	Wendlandia grandis Cowan.	2.8 + 0.9	2.3 + 1.5	0
	Wendlandia heynei Stantapau & Marchant.	4.0 + 0.8	4.5 + 1.3	2.8 + 1.7
	Wendlandia puberula DC.	3.0 + 0.8	4.3 + 2.2	2.3 + 1.5
	Wendlandia speciosa Cowan.	1.8 + 0.9	2.0 + 0.8	0.5 + 0.6
	Wendlandia tinchoria DC.	2.5 + 0.6	3.0 + 0.8	1.5 + 0.6
	Wendlandia wallichi Wight & Arnott.	2.3 + 0.9	1.3 + 0.9	0
Shrubs	*Catunaregam longispina* Link.	2.0 + 0.8	1.5 + 0.6	0
	Chassalia curvifolia Wall.	9.3 + 1.0	8.3 + 1.2	0
	Himalrandia tetrasperma Roxb.	3.5 + 1.0	4.0 + 1.8	2.8 + 0.9
	Leptodermis amoena Springate.	1.8 + 0.9	3.0 + 1.4	5.8 + 0.9
	Leptodermis kumaonensis Parker.	2.3 + 0.5	3.8 + 1.7	1.8 + 1.0
	Leptodermis stapfiana Winkler.	1.3 + 0.5	2.8 + 1.7	0
	Luculia grandifolia Ghose.	0.8 + 0.5	1.3 + 0.5	0
	Luculia gratissima (Wall.) Sweet.	3.8 + 1.3	1.8 + 0.9	1.8 + 0.9
	Morinda angustifolia L.	0	5.8 + 1.3	2.0 + 0.8
	Mussaenda frondosa L.	0	2.3 + 0.5	0.5 + 0.6
	Mussaenda glabra Vahl.	2.5 + 1.3	3.5 + 1.3	2.3 + 1.3
	Mussaenda macrophylla Wall.	0	1.8 + 1.0	3.8 + 1.0
	Mussaenda roxburghii Hook.f.	0	4.8 + 1.7	5.3 + 2.8

Continued **Table 1.** The species density of the Rubiaceae in three different forest types based on the prevalence of anthropogenic factors (UDF: undisturbed forests, SDF: semi-disturbed forests, HDF: highly disturbed forests, SD: standard deviation)

	Species name	Density (Mean + SD)		
		UDF	SDF	HDF
Shrubs	*Mycetia longifolia* (Wall.) Kuntze.	7.0 + 1.8	0	0
	Neohymenopogon parasiticus Wall.	5.5 + 1.3	4.0 + 1.8	0
	Oxyceros fasciculata Roxb.	3.0 + 2.2	1.3 + 0.5	0
	Pavetta subcapitata Hook.f.	3.8 + 1.8	5.8 + 1.3	0
	Psilanthus bengalensis (Sc.) Leroy.	0	6.8 + 1.7	2.8 + 1.7
	Rubiaceae sp. (Pentas)	5.8 + 1.1	4.0 + 1.8	0
	Spemadictyon suaveolons Roxb.	0	9.5 + 1.3	9.3 + 1.7
	Uncaria scandens (Sm.) Hutchinson.	1.3 + 0.5	1.8 + 1.0	1.0 + 0.8
	Uncaria sessilifructus Roxb.	1.8 + 0.9	1.3 + 0.5	1.5 + 0.6
Herbs	*Argosteamma sarmentosum* Wall.	8.3 + 2.6	4.0 + 2.6	0
	Argosteamma verticillatum Wall.	9.8 + 2.2	5.8 + 2.8	0
	Galium aparine L.	5.8 + 1.0	4.8 + 0.9	5.3 + 2.6
	Galium asperuloides Edgeworth.	0	7.3 + 2.6	2.8 + 1.3
	Galium craticulatum Mill.	3.0 + 0.8	3.5 + 1.7	3.8 + 0.9
	Galium sp.	0	7.5 + 1.3	3.0 + 1.8
	Hedyotis auricularia L.	0	3.3 + 1.5	4.3 + 0.9
	Hedyotis scandens Roxb.	0	5.3 + 1.7	4.0 + 0.8
	Hedyotis verticillata (L.) Lam.	0	5.3 + 2.6	4.0 + 1.4
	Neonatis ingrata Lewis.	0	9.0 + 2.2	9.5 + 2.1
	Oldenlandia corymbosa L.	0	1.5 + 0.7	3.8 + 0.9
	Ophiorrhiza fasciculata Roxb.	10 + 1.8	9.8 + 0.9	0
	Ophiorrhiza mungos L.	10 + 0.8	9.8 + 1.7	0
	Ophiorrhiza rugosa Wall.	5.3 + 1.5	4.8 + 1.7	0
	Paederia foetida L.	3.8 + 1.0	5.8 + 1.7	4.3 + 1.5
	Richardia brasillensis Gomes.	0	0	9.5 + 1.9
	Rubia cordifolia auct.	2.3 + 1.3	2.8 + 1.0	2.0 + 0.8
	Rubia manjith Roxb.	0	2.5 + 1.3	2.8 + 1.7
	Rubia sikkimensis Kurz.	1.8 + 0.9	1.8 + 0.9	1.5 + 0.6
	Spemacocea latifolia Aublet.	0	5.8 + 2.1	6.0 + 2.6
	Spermacoce mauritiana Gideon.	0	5.5 + 3.5	6.3 + 1.3

based on the conventions for interpreting effect size (Cohen 1988), the magnitude of the difference between a mean score and the effect size was very small. The post-hoc analysis with Tukey's HSD, to evaluate the differences among three group means, revealed a statistically significant differences in Rubiaceae species diversity between undisturbed forest ($M = 10.22$, $SD= 11.43$) versus semi-disturbed forest ($M = 15.63$, $SD = 10.02$), $p = 0.023$, and semi-disturbed forests ($M = 15.63$, $SD = 10.02$) versus highly disturbed forests ($M = 9.30$, $SD = 10.12$), $p = 0.006$. On the contrary, the undisturbed forests versus highly disturbed forests did not show statistically significant differences, $p = 0.892$.

4. DISCUSSION

In total, these results suggest that the prevalence of a moderate form of disturbance enhances species diversity of Rubiaceae. Similar findings, though not for Rubiaceae species, were reported by other researchers (Connell 1978; Shrestha et al. 2013; Rahman et al. 2009) claiming higher plant diversity in the forest characterized by an intermediate level of disturbance. Nonetheless, a higher disturbance regime in the form of complete habitat alteration or destruction is compatible neither for species conservation nor for diversification. The higher species diversity recorded in the forest experiencing a moderate

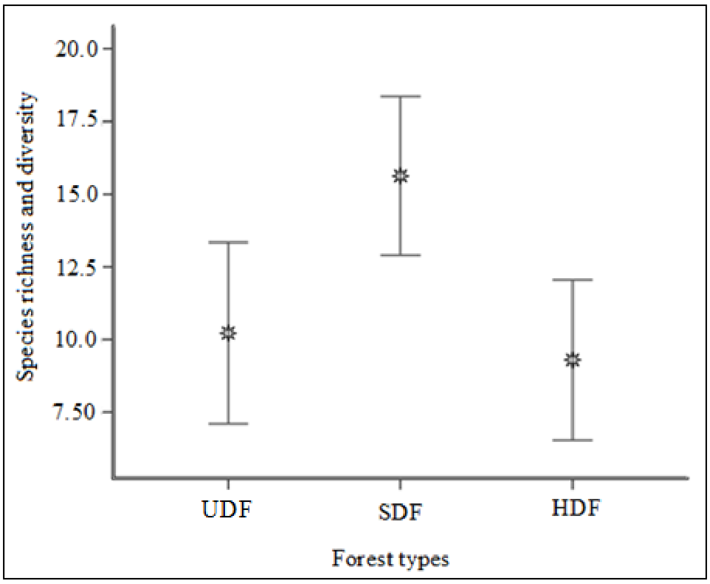


Figure 4. The species richness and diversity mean and 95% confidence interval across forest types

level of disturbance regime could be ascribed to creation of ample gap for increased light penetration to the forest floor and reduced competition (Pedreira et al. 2000). In contrast, lower species diversity in the undisturbed forest and highly disturbed forest could be construed as a restriction triggered by increased competition in the former and amplified degrees of stress to the species diversification in the latter condition. While the phenomenal diversity of Rubiaceae in the semi-disturbed forest is unusual for the family that has been described as ecologically sensitive and rare in the secondary forest (Davis et al. 2009), the creation of suitable and variable micro-habitat (Brosofske et al. 2001) from disturbed to intact in the semi-disturbed forest may have enhanced diversity.

The detection of higher woody Rubiaceae diversity in the undisturbed forest indicates a higher rate of tolerances of the woody Rubiaceae to the competition as well as the ability to compete at par with other woody species. Along these lines, the dominance of herbaceous Rubiaceae in the highly disturbed forests could signal higher resource availability for the herbs and lesser competition from superior species like trees and shrubs. Correspondingly, the higher diversity of shrubby Rubiaceae in the semi-disturbed forest describes the ability of the shrub to take advantage of the overstory openings and grow into the layer where light is available. Such a pattern of species diversity variation based on the life form is observed due to the influence of higher vegetation layers like overstory trees on the distribution and abundance of subordinate species like shrubs and herbs (McKenzie et al. 2000).

The findings also suggest that the impact of anthropogenic factors on Rubiaceae species diversity is inconsistent. The species like *Argostemma* Wall., *Mycetia* Reinwardt., and *Chassalia* Poiret. are confined mostly to the nondisturbed areas while the distribution of *Ceriscoides* (Hook.f.) Tirvengadum, *Himalrandia* Yamazaki, and *Uncaria* Schreber, *Leptodermis* Wall. ranges from highly disturbed forests through the semi-disturbed forest into the undisturbed forests. And it is interesting to note that the *Rubia* L., *Mussaenda* L., *Spemacocea* L., and *Oldenlandia* L. are diverse in highly disturbed forests. Furthermore, the dominance of *Rubia manjith* Roxb. in swidden and secondary forests and their absence in undisturbed forests testify the importance of a disturbance regime for the existence and diversification of some species. In general, species showing a higher rate of adaptability to different environmental and ecological conditions may exhibit a higher probability of survival in the world where disturbance regimes are on the rise. On the other hand, species showing higher confinement to natural habitat may experience a higher risk of extinction.

The effect of anthropogenic factors on the diversity of endemic and endangered Rubiaceae species requires further investigation. The presumed sensitivity of Rubiaceae to the ecological disturbance and the vulnerability of endemic and monotypic Rubiaceous species, calculated at 34.5% of genera (211 monotypic genera) to extinction (Davis et al. 2009) is a cause of concern requiring thorough investigation and documentation. However, the current research findings contradict this proposition revealing higher Rubiaceae diversity in the semi-disturbed forest. Such contradictory findings could be associated with the absence of endemic species in the current survey or may have been obscured by the research design.

Since plants exhibit species-specific tolerances and adapt based on the suitability of conditions (Kikvidze et al. 2005), not all the species would respond equally to the disturbance regimes. For that matter, the effect relies upon the type and intensity of disturbances that may augment or reduce diversity. Thus, there is a need for a careful examination of the impact of different anthropogenic factors on the resilience of different species of Rubiaceae.

5. CONCLUSION

The diversity of Rubiaceae species, as revealed by Shannon-Wiener and Simpson diversity index, further validated by in between-group one-way ANOVA analysis is higher in the forest experiencing an intermediate form of disturbances. The finding suggests that the tolerance of species to the disturbance regime is specific, and it, therefore, defines habitat preferences of different species. Ultimately, the survival rate and extinction risk of the species may be defined by the adaptive potential of the species to varying conditions created by anthropogenic influences.
Subsequently, striking a good balance between the forests managed through the influence of human activities and keeping the virgin forest intact is found imperative to ensure the continuance of the species diversity of Rubiaceae.

DECLARATION OF INTERESTS

The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results. The paper is original and has so far not been published, in part or in entirety.

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