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Microarthropod Diversity, Co-occurrence and Ecosystem Impacts among Invasive and Native Plant Species

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Abstract: The study compared aerial micro-arthropod diversity in exotic and native shola species in a high-altitude shola ecosystem. The thysanoptera, hymenoptera, and ixodida were more abundant in exotic plants, while entomobryidae, paronellidae, and acariformes are more abundant in shola species and exotic *Cestrum*. The native shola dependent plant *Piper brachystachyum* has abundant entomobryidae but is less diverse. The study concluded that shola species have an indigenous population of micro-arthropods, particularly collembolans. The exotic plants act as a significant reservoir of those micro-arthropods, which could potentially damage the local ecosystem and agriculture.

Keywords: Exotic plants, Shola, Collembola, Nilgiris

The British entry to the Nilgiris Hills in the 18th century paved the way for introducing exotics or invasive plants (Nazia and Sanil 2015). The pristine primitive shola grasslands are either converted to pine forests, eucalyptus plantations, or tea estates. The vegetation of the high range (1800m above sea level) has a mosaic pattern of forest (C4 plants) locally called *"Shola"* and grasslands (C3 plants). The shola interspersed with grasslands that occur in the mountain folds is the perennial source of water and forms tributaries of rivers in the lower elevation (Thomas and Palmer 2007). The introduction of exotic/invasive plants like *Eucalyptus* and *Pinus* leads to converting forests to tea estates and residential areas, which impacted the endemic flora and fauna (Raman et al 2020a). *Cyprus* and *Acacia* were the later entrants to this fragile ecosystem, followed by the automatic invasion of orange *Cestrum*, *Lantana,* and *Parthenium* (Bhavana et al 2015). Invasive species management became a priority issue as the CBD in 1992 (Convention of Biological Diversity) identified it as a threat to the ecosystem, economics, and human health. Wind, water, and birds are the key factors that help in the dispersal of seeds of many exotic species. They sprout earlier than the native species and fall out last, ensuring a higher life span (Srivastava and Singh 2009). The exotic/invasive species can adversely affect the shola plants by competing and eliminating them, and interrupting the trophic interaction associated with them (Maron and Vila 2001).

Some exotic/invasive species have turned to weed, multiplying at an alarming pace (e.g *Parthenium*, *Lantana* and *Eupatorium*, etc.). The spread adversely affect the ecosystem by altering the geo-morphological process, hydrological cycle, bio-geochemical cycle and fire regime. The stress exerted by the invasive species devastates native species by causing changes in dominance, distribution, and shifting the entire ecosystem balance (Goyal and Brahma 2001, Jackson et al 2002). These exotics are vastly spreading in the ecologically fragile Nilgiris as they competitively eradicate the shola patches that threaten the existing shola. The leading theory behind the increase in exotics/invasive is that they escape from natural enemies that hold them back (Keane and Crawley 2002).

The exotic species suppress the growth of the native plant species as well as the behavior of native insects' guilds such as herbivore, parasitoids, and pollinators through a variety of mechanisms (Boettner et al 2000, Snyder and Evans 2006) and disturbs the agriculture sustainability and food security (Sakachep and Rai 2021). They act as the specialized antagonist and do allelopathic (chemical) interaction or release volatile compounds with native plants and variability in response and resistance (Thebaud and Simberloff 2001; Mitchell and Power 2003, Callaway and Ridenour 2004). They also act as *"evolutionary traps"* for the native aerial micro-arthropods, which readily get attracted and adapted to the food resources of exotic/invasive plants. Though the direct impacts are visible, they indirectly reduce the abundance or activity of both native plants and insects. Many aerial micro-arthropods depend on mutualism with the exotic plants depending upon the presence or absence of flowers. The relationship becomes an ecological interaction between exotic/invasive and resident micro-arthropods.

Canopy research is gaining responsiveness in present days; especially the role of aerial micro-arthropods and their ecological interactions are hazy. Micro-arthropods are omnipresent and have defined environmental roles in the soil. The antique relationship of the micro-arthropods as pollinators of moss (Rosential et al 2012), opens the curiosity on these micro-organisms. The arboreal micro-arthropod communities differ qualitatively from what is found in leaf litter at soil level, being generally dominated by a few specialist species that are uncommon in the soil (Lindow and Winchester 2006, Affeld et al 2009, Rodgers and Kitching 2011, Bolger et al 2013). Documentation of wind circulation of flightless groups such as collembolan and mites is lacking. They are hypothesisied as a pioneer community spread by a mechanism called aerial ballooning (Hawes et al 2007). Like other plant-animal relationships, the micro-arthropod community may have species-specific dominance and occupancy in different fauna. The oldest “living fossil” shola (Raman et al 2020b) may also host specific micro-arthropods species, which may have their ecological role in perpetuating the stability of ecosystem (Sharma and Singh 2021) . The present work was conducted to compare the diversity, density, and adaptive patterns of various micro-arthropod groups in shola/ native and exotic/invasive plants in the Nilgiris.

MATERIAL AND METHODS

Study area: The Nilgiri hills the Tamil Nadu state of India, is the part of the Nilgiri Biosphere reserve, a UNESCO recognized world heritage site. The Nilgiri hills is the second highest peak (~2637 asl) in the Western Ghats is a joining point of Eastern Ghats also. The region lies at a latitude of 11° 08' N to 11° 37' N and longitude of 76° 27' E to 77° 4' E, and the central location is 11°22′30″N 76°45′30″E. The area is approximately 2,479 Km^2 and the temperature reaches a maximum of 25°C in summer and up to-4°C during winter. The native vegetation is by short, stunted montane evergreen sholas and the adjoined grasslands. Plantations like tea, *Eucalyptus*, *Pinus*, *Acacia*, and exotic bushes are also common. The shola forest occurs in the higher elevations of the Western Ghats and its associated hill ranges in Southern India (Raman et al 2020a, Raman et al 2020b). Shola forests can befound at an altitude of 1800 meters above sea level. They are found only in Western Ghats regions and are always wet and contain a lot of humus, which is a suitable habitat for decomposers and wet soil dwellers. In many places, the shola regions are patchy or interspersed with exotic plantations. The invasive species like *Lantana, Parthenium, etc.,* are spreading at an alarming rate towards the shola regions.

Sample collection: The twigs with dense leaf samples were collected from five shola/native and five exotic/invasive species from various parts of the Nilgiris. *Cestrum aurantiacum, Solanum mauritianum, Polygonum divaricatum, Lantana camara,* and *Acacia dealbata* were exotic/invasive species collected. Species like *Rhodomyrtus tomentosa*, *Rhododendron nilagiricum, Photinia lasiogyna, Rubus ellipticus,* and *Piper brachystachyum* were the native/shola species. The twig samples were from twelve different sampling locations minimally separated by ~10km. The sampling regions include pristine shola regions, exotic-shola mixed regions and exotic bushy regions. Multiple samples (approximately 10-25) of each flora under consideration in each sampling locality (~2km radius) are collected mixed to maintain the homogeneity of sampling by collecting a single layer of twig with leaves in a zip lock cover of 39cm x 31cm (surface area of 1209cm^2). The total sampled area is the product of the number of samples to the surface area sampled (e.g., if N is the sample repeats in a location, the total sampled area is N x 1209 cm^2). The value is expressed in square meters using the following equation, density of aerial micro-arthropods per square meter (m^2) = No species observed / the total area sampled x10^{-4}.

Separation, mounting and identification: Micro-arthropods were extracted 24 hrs from the twigs-with leaves using the Berlese -Tullgren funnel (Dietick et al 1959) under a 60V light source. The upper region of the funnel kept airtight to prevent the escape of micro-arthropods. The separated micro-arthropods were collected and fixed in Gisin's fixative and preserved in 70% alcohol and made permanent mounts in the Hoyer's medium and temporary mounts in glycerol. Collected micro-arthropods using a 0.0 tip brush under Olympus Magnus MSZ-TR stereo microscope. The specimens were identified to the possible taxa (family or superorder)at high magnification using Lawrance and Mayo Model NLCD-307B digital microscope. Identified the collembolans to family level Entomobryidae, Paronellidae, hypogastruridae and neanuridae following Bellinger et al (1996-2023). The other micro-arthropods acariformes, thysanoptera, ixodida, and hymenoptera were classified following Imms et al (2012).

Diversity and multivariate analysis: Estimated the micro-arthropods diversity and evenness for each flora under consideration (α-diversity *Hα*) separately and evaluated the gamma diversity (*Hγ*) and micro-arthropod diversity and evennessin all the native/sholaorexotic flora (Hill 1973). Whittakar index was followed to estimate the beta diversity as the ratio of*Hγ*to *Hα* (*Hγ*/*Hα*) following (Whittakar 1960). Beta diversity interprets the similarity and overlap and allows us to understand the variations between distributions. Shannon equitability, Simpson's dominance (λ), Gini-Simpson index (λ-1) and Berger-parker index (BPI), Hill number-true diversity (^{q}D), and the Renyi entropy (^{q}H) in a programmed excel sheet (Goepel 2018). Principal component analysis (PCA) was performed, according to Josse et al (2014), in the R- platform (R studio version 4.0.2) using the ggplot2 package. The PCA is a type of linear transformation on a given data set. This transformation fits the micro-arthropod data set to a coordinate system and executes the most significant variance in the first coordinate and used the percentage of principal component I and principal component II variance to determine the variation of micro-arthropods in ten selected plants. To visualize the floristic dependence of the various micro-arthropods, the Non-metric multidimensional scaling (NMDS) (Oksanen et al 2005) using Bray-Curtis distance method in packages vegan and ggplot2 in R studio. NMDS is an indirect gradient analysis that produces ordination based on distance or dissimilarity matrix.

Co-occurrence of species: The species association of different micro-arthropods in two diverse vegetation were analysed using 'co-occur' package in R studio. The 'co-occur' package analyse the species co-occurrence using a probabilistic model. This method provides information such as observed co-occurrence and probability co-occurrence. This model also determines the observed frequency of co-occurrence is significantly greater (positive association, (Pgt) ≥α (α=0.05) or significantly less (negative association, (Pgt)≤α (α=0.05), or not significant (random association) (Veech 2013, Griffth et al 2016).

RESULTS AND DISCUSSION

Aerial micro-arthropods observed: A total of 8 different categories of micro-arthropods were observed from the flora under study. The observed micro-arthropods are thrips (thysanoptera), bees (hymenoptera), red mites, oribatid mites (acariformes), ixodida, and springtails (belongs to entomobryidea, paronellidae, hypogastruridae and neanuridae).The oribatid mites and the springtails such as entomobryidea and paronellidae seem to be present mainly in the shola plants. The bees, red spider mites, thrips, and the springtails of the family hypogastruridae and neanuridae are observed predominantly in the exotic plants (Table 1).

Species richness and abundance: Diversity indices (Table 2) showed a significant variance in the micro-arthropod diversity between exotic and native flora. The higher the Shannon index, the micro-arthropods may be equally distributed, while the high dominance of a particular fauna indicates a lower value. The H_{β} is a clear indicator of dominance, the higher the value higher the abundance of a particular species compared to the community (H_{γ}) (e.g. Shola/native, exotic, etc.) All the indices indicate that the shola species are more diverse (H_{γ}=1.45) than the exotic plants (H_{γ} =099). The low evenness suggests that one or two micro-arthropod communities are dominating in that particular plant. The high abundance of specific fauna was in the shola-associated native species like *Piper brachystachyum* and *Rhodomyrtus tomentosa*. In the former species was thrips, while in the latter was entomobryidea. In shola/ native plant species, micro-arthopod richness varied between 3-5, while in that of exotics it is 3-8. The flora like *Cestrum aurantiacum* (H_{β}=0.79) and *Polygonum divaricatum* (H_{β}=0.64) among the exotic species are highly diverse. The *Solanum mouritianum* is less diverse (H=0.15 λ=94.60%, H_{β}=6.60) and has high abundance of thrips. The true diversity as Hill numbers and the Renyi entropy in the order of 'q' for the shola/native and the exotic species given in Figure 1. The

Table 1. Density of micro-arthropod/ Sq.m (Mean ± standard error) observed from the native and exotic flora in the Nilgiris

Micro-arthropod fauna observed	Flora studied (Mean ± SE)									
	Exotic flora					Native/ shola flora				
	Ca	Sm	Ad	Pd	Lc	Rn	Rt	Re	Pl	Pb
Entomobryidae	9.65±1.76	0.00±0.00	0.00±0.00	98.57±3.04	0.00±0.00	50.32±4.48	35.15±3.43	0.00±0.00	16.54±2.19	232.29±3.45
Paronellidae	13.76±3.51	13.79±2.65	0.00±0.00	16.54±2.19	0.00±0.00	21.37±2.81	16.54±2.75	0.00±0.00	14.47±1.89	16.54±2.42
Hypogastruidae	6.89±3.93	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Neanuridae	1.38±1.76	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Acariformes	0.00±0.00	11.03±2.30	73.75±3.96	173.70±4.56	48.25±3.93	90.30±5.55	55.83±3.43	0.00±0.00	36.53±2.89	30.33±2.48
Thysanoptera	111.66±7.07	1204.16±11.11	29.64±3.30	194.38±6.17	148.19±6.08	4.14±2.68	15.85±2.30	137.17±3.22	0.00±0.00	0.00±0.00^{c}
Ixodida	19.99±4.92	0.00±0.00	0.00±0.00	42.74±3.61	19.99±4.35	2.76±2.68	0.00±0.00	48.94±3.30	0.00±0.00	0.00±0.00
Hymenoptera	13.10±2.51	7.58±2.30	2.07±1.89	54.45±3.22	13.10±2.02	0.00±0.00	0.00±0.00	9.65±1.76	0.00±0.00	0.00±0.00

Ca: *Cestrum aurantiacum*, Sm: *Solanum mouritianum*, Ad: *Acacia dealbata*, Pd: *Polygonum divaricatum*, Lc: *Lantana camara*, Rn:, *Rhododentron nilagiricum*, Rt: Rhodomyrtus tomentosa, Re: *Rubus ellipticus*, Pl: *Photinia lasiogyna*, Pb: *Piper brachystachyum* illustration indicates four micro-arthopods (2q=3.66) effectively using the shola/native plants, while two species (^2q=1.84) are effectively using the exotics. The micro-arthropods effectively using the shola/ native flora are the entomobryidea, paranonellidae and acariformes. The thrips (thysanurans) are the species that are commonly using the exotic species.

Floral dependency and community composition: Principal component analysis in exotic/invasive, the PCI showed 39.41% and PCII 27.01% with a total of 66.42%. In shola/ native plants, the PCI was 32.07% and PCII 26.97%, with total of 59.04% (Fig. 3). The first coordinate contain sacariformes, entomobryidae, paronellidae, and hymenoptera, the second coordinate have thysanoptera, and the fourth coordinate contains Neanuridae, Hypogastruridae, and Ixodida. In shola, the most significant variance in the first coordinate is displayed by entomobryidae and in the second coordinate containing Paronellidae and

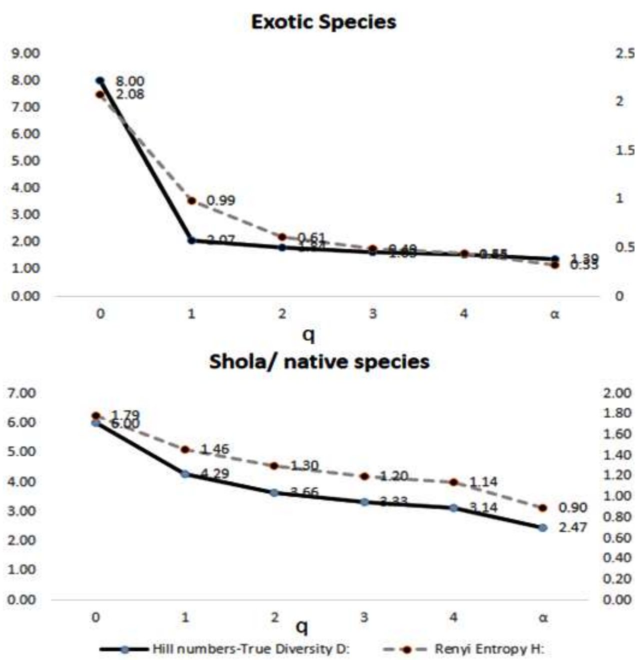

Fig. 1. Hill numbers of true diversity (^qD) and the Renyi entropy (^qH) in shola/native and exotic plants

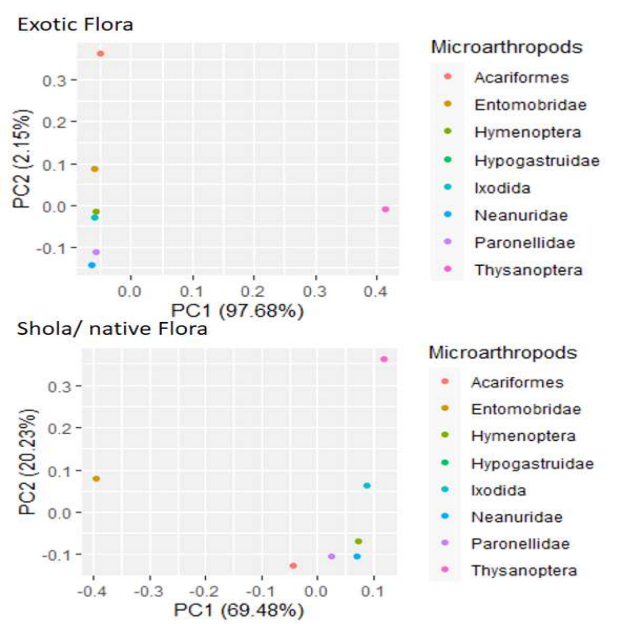

Fig. 2. Principal component analysis of microarthropods in native/shola and exotic flora

Table 2. Diversity analysis of micro-arthropods in native and exotic flora

Flora	Faunal richness	Diversity index	Faunal evenness	Shannon equitability (%)	Simpson dominance (%)	Gini-Simpson index (%)	Berger-Parker index (%)	β - diversity
Exotics (Hγ: R=8; H= 0.99; λ= 54.30% ;λ-1 = 45.70%; BPI = 72.15%)								
Cestrum aurantiacum	7	1.26	0.50	64	42.10	57.90	62.40	0.79
Solanum mouratianum	4	0.15	0.29	11.	94.60	5.40	97.30%	6.60
Polygonum divaricatum	6	1.55	0.78	86.50	24.40	75.60	33.30	0.64
Lantana camera	4	0.97	0.65	70.00	47.80	52.20	64.90	1.02
Acacia delbata	3	0.66	0.64	59.60	58.00%	42.00	70.90	1.50
Native/ Shola (Hγ: R = 6; H= 1.45; λ= 27.40% ; λ-1 = 72.60%; BPI = 40.50%)								
Rubus ellipticus	3	0.75	0.70	67.90	55.50	44.50	70.00	1.94
Rhodomyrtus tomentosa	4	1.24	0.87	89.60	32.30	67.70	45.10	1.17
Rhododendron nilgiricum	5	1.16	0.64	71.80	37.50	62.50	51.70	1.25
Photinia lasiogyna	3	1.01	0.91	91.80	39.80	60.20	54.00	1.44
Piper brachystachyum	3	0.55	0.58	49.80	71.80	28.40	83.70	2.65

Hγ is the gamma diversity, R is the micro-arthropod richness, H is micro-arthropod diversity index, λ is the Simpsons dominance, λ-1 is the Gini-Simpson index and BPI is the Berger-Parker index)

Thysanoptera, and third coordinates with Acariformes and Ixodida. Non- metric multidimensional scaling (NMDS) ordination produced a stress value of 0.108979. The stress versus dimension plot indicates that two dimensions were the best suited for presenting our data. The NMDS plot shows an apparent clustering of the shola/native plants and the exotics and indicates its dissimilarity in micro-arthropod diversity. Except for the *Rubus elipticus*, the other shola species share the same type of micro-arthropod faunal composition. The exotic species do not have a unique kind of micro-arthropod composition, and it varies from plant species to species.

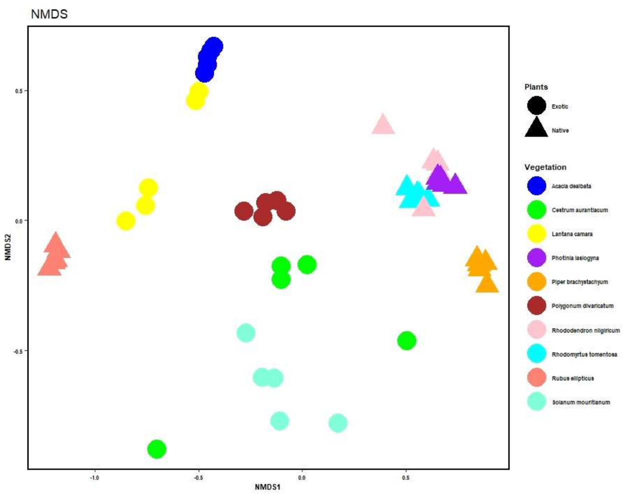

Fig. 3. NMDS of various microarthropods in relation the shola/ native and exotic plants

Co-occurrence of species: The co-occur results suggest that 16 pairs of species association were observed from shola and exotic vegetation (Table 3). All the associations are random except that of the entomobryidae and paronellidae. They are predominantly present in the shola species and a few exotics.

Micro-arthropods are omnipresent, and the literature on aerial micro-arthropods is scanty owing to the lack of proper taxonomical descriptions. The ecological role of these animals in the aerial environment is yet unknown. The curiosity about the ecology of these groups increased as Rosenstiel et al (2012) showed oribatids and collembola have role in moss pollination described the relationship as an antique one, and relationships exist much before the evolution of flowering plants. In the soil, micro-arthropods act as decomposers and maintain nutrient cycles, but such type of role in aerial habitat is unclear. The collembolans are considered as fungal feeders by Jorgensen et al (2005). It may be assumed that they check the fungus growth in the old plant twigs and protect them. Acariformes like Oribatids are predators and feed on the collembola and other micro-arthropods in an aerial ecosystem. The epiphytes, mosses, and lichens are common in the shola fauna (Bunyan et al 2012). Hence the existence of this species has a significant role in maintaining epiphytic, moss, and lichen growth. If collembola and mites are absent, the mosses will not proceed to the sporophyte generation (Rosenstiel et al 2012).

Table 3. Association of micro-arthropods. Fauna A and B are the comparing micro-arthropods in a locality

Fauna A	Fauna B	Obs	P(obs)	Exp	P (lt)	P (gt)	Faunal association
Entomobridae	Paronellidae	6	0.42	4	1.0000	0.0333	Positive
Entomobridae	Acariformes	5	0.48	5	0.8667	0.6667	Random
Entomobridae	Thysanoptera	5	0.54	5	0.6000	1.0000	Random
Entomobridae	Hymenoptera	2	0.36	4	0.0714	1.0000	Random
Entomobridae	Ixodida	5	0.42	4	0.9667	0.3333	Random
Paronellidae	Acariformes	6	0.56	6	0.9333	0.5333	Random
Paronellidae	Thysanoptera	6	0.63	6	0.7000	1.0000	Random
Paronellidae	Hymenoptera	3	0.42	4	0.1667	1.0000	Random
Paronellidae	Ixodida	5	0.49	5	0.8167	0.7083	Random
Acariformes	Thysanoptera	7	0.72	7	0.8000	1.0000	Random
Acariformes	Hymenoptera	4	0.48	5	0.3333	1.0000	Random
Acariformes	Ixodida	5	0.56	6	0.4667	1.0000	Random
Thysanoptera	Hymenoptera	6	0.54	5	1.0000	0.4000	Random
Thysanoptera	Ixodida	6	0.63	6	0.7000	1.0000	Random
Hymenoptera	Ixodida	4	0.42	4	0.6667	0.8333	Random

Observed: The observed number of sites having both A and B. P(obs); Probability that both A and B occur at a site. Exp: Expected co-occurrence of A and B. P(lt): The probability that A and B would co-occur in a site at a frequency lesser than P(obs), if distributed independently.P(gt): The probability that A and B would of co-occur at a frequency greater than the observed frequency. If P(lt) ≤ α two species are negatively associated (α = 0.05). (Refer Griffith et al. 2016 for more details).

The present study demonstrates the pattern of micro-arthropod occupancy varies from species to species and collembolans and oribatid mites are present in much higher density and diversity in shola plants than the exotics/invasive. Collembolans, in particular, seems to be absent in almost all of exotic/invasive species, except the *Cestrum aurantiacum* and *Polygonum divaricatum*. The exotics selected for the present study are bushes, while shola species are stunted woody. As the epiphytic growth is absent in the bushy exotics the collembola and mites may not colonized. Zeppelin et al (2009) opined that in thorny plants, springtails are absent owing to their soft bodied nature. The *Rubus ellipticus* and *Lantana camara* are spiny plants where springtails are absent. *Cestrum aurantiacum* and *Polygonum divaricatum* are exotics having nectar rich flowers (Bhavana et al 2015; Wanner and Dorn 2006). The presence of Entomobryidea springtails in these exotics can be attributed to their nectar content.

The changing pattern of exotic invasion and the depletion of the shola plants thereby increase harmful insects. Thysanoptera is one of the most significant agricultural pests globally, altering the micro-arthropod community by competitive replacement (Reitz, 2009).The native mountain shola plants are primitive, short, stunted semi-evergreen vegetation (Jose 2012), and the species-specific relation can also be old. Shola forests maintain a unique humid atmosphere, which is highly favorable for soft-bodied springtails prefer relatively high humidity and show an inverse relationship with the temperature (Hayward et al 2001, 2003). The native species *Piper brachystachyum* have dense populations of springtails (e.g. Entomobryidae) and are more diverse than the other shola species. The pepper plant contains many specific alkaloids, as collembola and oribatids are attracted chemically (Ratnayake 2014, Rai and Singh 2020).

CONCLUSION

The springtails and the oribatids mainly depend on the shola plants and absent in the exotic/invasive species. An in-depth taxonomic analysis is needed to anlyse the specificity of species present. The presence of Thysanoptera and Ixodida seems to be more in exotic/invasive plants. In exotic/invasive plants having nectar, the presence of collembolan is there, and in thorny plants is absent. The presence of thrips in the exotic plants raises doubts that they act as hosts for the thysanopetra, which is a threat to agriculture and indigenous biodiversity.

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