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Research Article

The Effect of Understory Shrub Species on the Natural Regeneration of Hyrcanian Mixed Broad-Leaved Forests (Kheyrud, Iran)

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Abstract

Iran's Hyrcanian forests are part of the temperate forests and have survived since Tertiary. These forests have experienced severe damages in recent decades because of socio-economic issues such as overgrazing. This study aimed at investigating the Nurse-protégé interaction and the impact of understory shrub species on the regeneration of the Hyrcanian main tree species in Kheyrud Forests. Filed sampling was carried out using the random-systematic method. Regeneration and their features under and outside of the canopy of shrub species, as well as some environmental variables were recorded in strip plots. According to the results, the canopy cover percentage of 25-50% provides the best regeneration protection efficiency for the six nurse shrub species. Overall, regeneration density was found to be higher outside the canopy area of the shrub species than beneath their canopy area; however, regeneration under the shrub species caused higher mean height and age. Moreover, the mean cover and the depth of the litter under the shrub canopy were significantly higher than that of outside the canopy area. Although the shrub species allow more durable and complete development of seedlings by providing protection against grazing, suitable conditions for seed germination and seedling growth may not be provided because of high litter depth and insufficient sunlight due to the massive canopy of trees as well as nurse shrubs' dense foliage (especially, evergreen understory species).

Keywords*:* Nurse shrubs, Natural regeneration, Seedling, Temprate forests.

1. Introduction

Shrub species create special micro-environment under the canopy, which provides favorable conditions for the establishment and growth of some plant species. Such species also ward off wild animals and livestock that feed on forest plants. In dry environments, plants are often established in safe micro-environment beneath the canopy of nurse species which lead to reduced heat and increased soil moisture by providing shelter to the plants (Joel & Enrique, 2003). Such plant species which protect other plants regenerated beneath them are called nurse species. Shrubs are the dominant nurse life-form (Filazzola & Lortie, 2014) and these species provide more favorable micro-environment for seed germination or seedling establishment compared to their surroundings (Ren et al., 2008). Among many positive effects, nurse species are acting as a temperature buffer, cooler temperature during warm days, protection against slight frost, increasing penetration rate of water, lowering evaporation, increased amount of soil nutrients, protection from grazing and trampling, physical

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protection, and less soil compression and erosion (Flores & Jurado, 2003). Shrubs as remarkable nurse species enhanced seedling survival and growth and acorn emergence of *Quercus* spp. (Torroba-Balmori et al., 2015). O'Brien et al. (2007) indicated that Pine seedling establishment varies depending on percent canopy cover, duff and litter depth, and percent shrub cover while seedling growth is influenced by percent shrub cover. According to Da Silva Junior et al. (2021) *Psychotria* shrub densities do not decrease the natural regeneration of trees and some species of this shrub act in the understory as filters which favor ecological succession. A research conducted in Irano-Turanian semi-arid forests showed that 46% of the regenerations occurred under the protection of nurse plants or rocks (Ravanbakhsh et al., 2010). Previous studies carried out on the relationship between spiny plants and seedlings under their canopy mostly focused on spiny species of arid or semi-arid biomes (Flores & Jurado, 2003), and no such comprehensive study has yet been conducted in the Hyrcanian broad-leaved forests in Northern Iran characterized by a humed climte. Therefore, this study aimed to survey tree species regeneration traits and abundance in relation to the presence or absence of nurse shrubs, tree, shrub canopy, the litter depth, and coverage percentage. Of interest was also to see whether nurse species were involved in increasing the regeneration of tree species in the Hyrcanian forests.

2. Materials and methods

The research was accompliahed in the Kheyrudforest research station managed by the University of Tehran. This forest with an area of about 8,000 hectares is located in the northern slopes of the Alborz Mountains, near the city of Noshahr, at longitude 51° 32' 30" to 51° 35' 00" and latitude 36° 37' 30" (Javanmiri Pour et al., 2014). The research was performed in Parcels 108, 110 and 111 in the Patom district of the aforementioned forest. The section was recognized as a suitable place to carry out the study and achieve the objectives due to the spots created by shrub species along with uncovered spots, as well as livestock movement and grazing. The elevation gradient for the study area ranged from 320 to 400 m a.s.l. and the slope ranged from 10 to 45 percent. Moreover, the annual precipitation is 1073 mm. In this study, the sampling was performed in September and October, since during this period, grazing would affect vegetation and also, it would easier to identify seedlings through their leaves. For data collecting, four strips with length of 100 m and width of two m were established in the north-south direction, placed randomly-systematic in each Parcel (with the random start point and an interval of 50 m between each two strips). In total, 12 strips of 200 square meters were completely sampled and in each one, the position (under or outside of the shrub canopy), density, height, and age of all seedlings and saplings, as well as depth and coverage of litter layer were measured and recorded. To find the age of saplings, we considered growth pattern, nodes of stem (bud scales scars), and pattern of side branches (Kimmins, 1987).

Accordingly, the normality of the data was firstly determined using the Kolmogorov - Smirnov test and then, the Student's t-Test (for normal data) and the Mann-Whitney test (for abnormal data) were used since the study variables were two independent groups. The diagrams were plotted using Excel software. Also, multivariate analysis and CCA (Canonical Correspondence Analysis) were used (Lepš & Šmilauer, 1999) to study the distribution of regeneration of various species along the gradient of the studied environmental factors. Data analysis was performed using SPSS ver. 18 and PC-Ord ver. 4 software.

3. Results

The study area includes the community of *Carpinus betulus-Parrotia persica* with the mean canopy cover of 73%. A total number of six shrub species, including *Ilex spinigera* (Loes.) Loes., *Ruscus hyrcanus* Woronow, *Crataegus pentagyna* Waldst. and Kit. ex Willd., *Mespilus germanica* L., *Rubus* sp., and *Smilax excelsa* L. were identified, acting as nurse species. Most seedlings and saplings observed in the area were related to the species *Carpinus betulus* L. followed by the *Acer* spp.

3.1. Regeneration density

Results indicated that the density of regeneration, including seedlings and saplings, was higher outside of the shrub canopy, as compared to the density under shrub canopy (Figure 1). This difference was significant for the

total regeneration and *Carpinus betulus* (Table 1).

Table 1. Comparison of the regeneration characteristics between the treatments (Outside of the shrub canopy, and under them).

Species	Density			Height			Age		
	Chi-Square	df	Sig.	U	Z	Sig.	U	Z	Sig.
***Fagus orientalis* Lipsky**	0.58	1	0.45 ^{ns}	207.50	-4.70	0.00**	284.50	-3.74	0.00**
***Carpinus betulus* L.**	3.50	1	0.05 *	165792.0	-1.37	0.16 ^{ns}	1.39	-5.66	0.00**
***Acer velutinum* Boiss.**	0.40	1	0.52 ^{ns}	9981.50	-1.02	0.30 ^{ns}	9.39	-1.69	0.09 ^{ns}
***Acer cappadocicum* Gled.**	1.08	1	0.30 ^{ns}	1274.00	-5.83	0.00**	1.91	-3.20	0.01**
***Parrotia persica* C.A.Mey.**	1.13	1	0.28 ^{ns}	68.50	-0.76	0.47 ^{ns}	79.50	-0.21	0.83 ^{ns}
***Tilia begonifolia* Steven**	0.49	1	0.48 ^{ns}	162.00	-3.29	0.00**	249.00	-1.63	0.10 ^{ns}
***Ulmus glabra* Huds**	0.37	1	0.54 ^{ns}	939.00	-3.75	0.00**	416.50	-0.13	0.89 ^{ns}
***Diospyros lotus* L.**	3.43	1	0.06 ^{ns}	1113.50	-1.68	0.09 ^{ns}	1144.00	-1.52	0.12 ^{ns}
***Quercus castaneifolia* C.A,Mey.**	1.55	1	0.22 ^{ns}	376.00	-0.85	0.39 ^{ns}	389.00	-0.79	0.42 ^{ns}
All Species	8.67	1	0.00 **	480012.50	-5.50	0.00**	4.32	-5.88	0.01*

** Significant at P<0.01 level * Significant at P<0.05 level ^{ns} no significant (P>0.05)

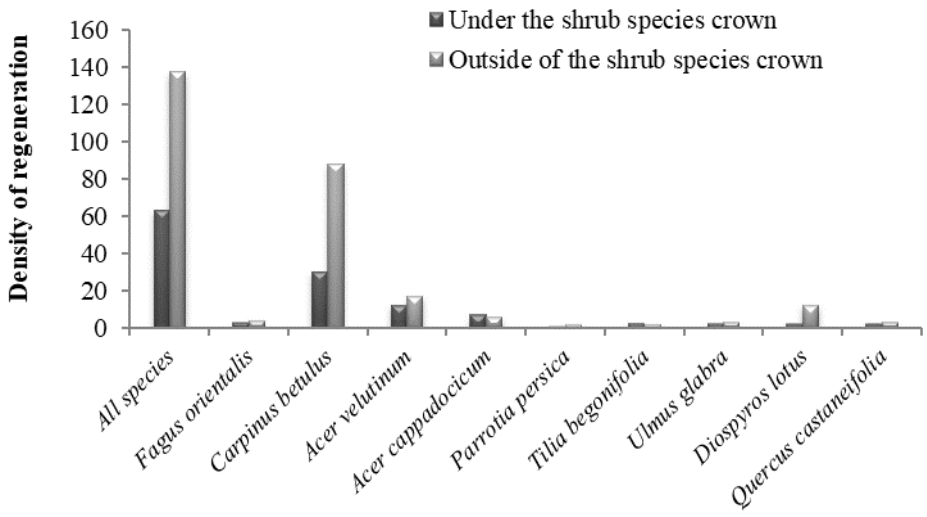

Figure 1. The density of regeneration (number per 100 square m) under and outside of nurse canopy.

3.2. Quantity features of regeneration

In total, all the regeneration protected by the shrub species had a height more than or equal to those that were outside of the canopy of shrub species (Figure 2). The difference was statistically significant for *Fagus orientalis*, *Acer cappadocicum*, *Tilia begonifolia* and *Ulmus glabra*, as well as for the total species (p<0.05) (Table 1).

Moreover, the mean age of all the regeneration protected by the shrub species was significantly higher than that of the unprotected ones (Figure 3). Similiarly, the difference was significant for *Fagus orientalis*, *Carpinus betulus* and *Acer cappadocicum* (p<0.05) (Table 1).

3.3. Shrub canopy

The canopy of the shrub species varied from 20 to 80%, and the mean canopy was calculated as 43%. Moreover, the shrub spots with 25-50%

canopy protected the highest rate of seedlings and saplings (Figure 4) and the difference was significant at the level of P<0.01 (Table 2). This comparison was carried out within the standardized areas for the canopy classes.

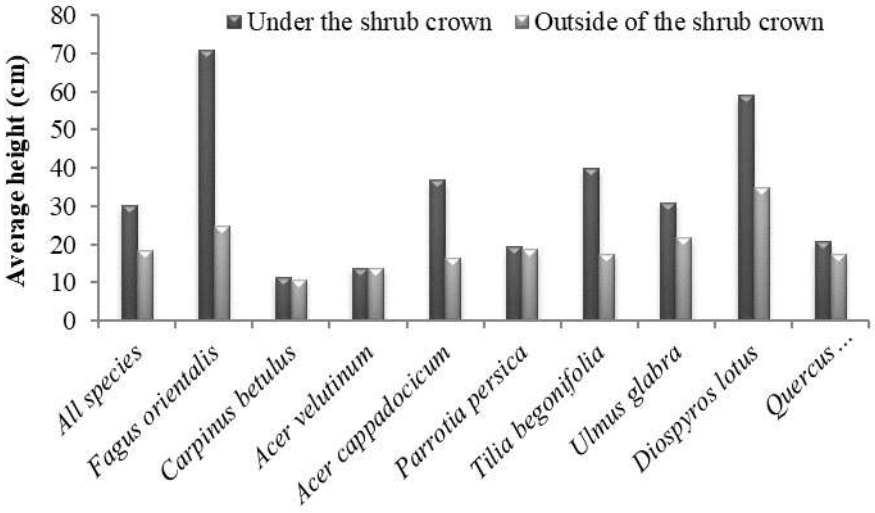

Figure 2. The regeneration mean height between under and outside of the canopy of nurse species.

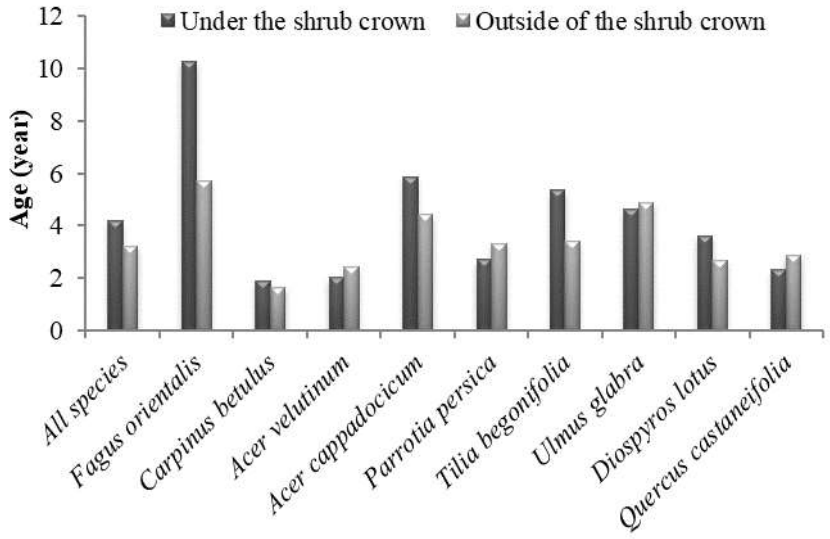

Figure 3. The regeneration mean age between under and outside of the canopy of nurse shrub species.

Table 2. Statistical comparison of the regeneration density in different canopy classes of the nurse species (Kruskal-Wallis test).

Chi-Square	df	Sig.
16.72	3	0.001 **

** Significant at P<0.01 level

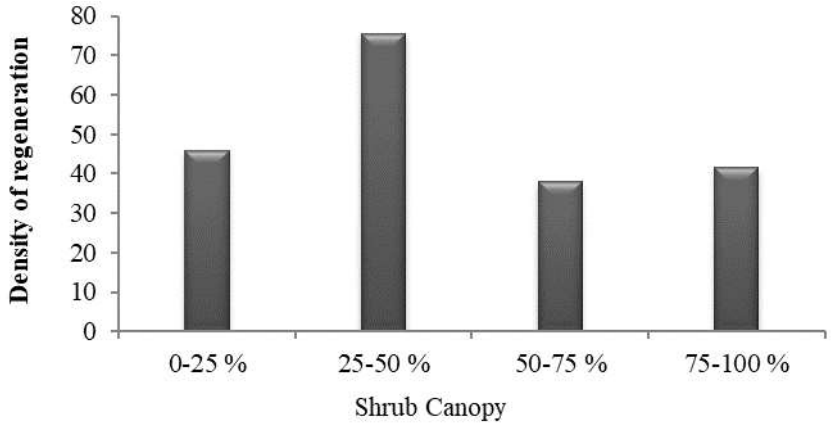

Figure 4. Density of tree regeneration in different canopy classes of the nurse species, per 1 ar.

3.4. Litter

The results showed that the litter beneath the shrub canopy was significantly (P<0.01) higher than the litter outside of the shrub clumps (Table 3). Also, the mean depth of the litter beneath the shrub canopy (3.5 cm) was significantly (P <0.01) higher than the outside of the shrub clumps (1.8 cm) (Table 3).

Table 3. Comparison of the percentage of litter cover and depth between the two treatments.

Variable	Treatment	Mean (±St. Dev.)	t	df	Sig.
Litter Coverage%	Under the canopy of shrubs	94.1±8.3	3.45	21.63	0.002 **
	Outside of the shrub crown	81.6±9.4			
Depth of Litter (cm)	Under the canopy of shrubs	3.5±0.8	21.57	5.16	0.000**
	Outside of the shrub crown	1.8±0.7			

** Significant at P<0.01 level * Significant at P<0.05 level ns no significant (P>0.05)

3.5. Comparison of species regeneration

The ordination results demonstrated that greater regeneration of *Acer cappadocicum*, *Tilia begonifolia* and *Quercus castaneifolia* had been established under the shrub canopy with higher litter density, whereas *Carpinus betulus* and *Diospyros lotus* created more regeneration in lands without shrubs canopy or with a low shrub canopy and less litter density (Figure 5).

4. Discussion

Understory shrubs can have a multi-functional effect on the survival and growth of regeneration and the outcomes were not the same for different species. The positive effect of the nurse shrub species on regeneration has been addressed in numerous studies (Go'mez-Aparicio, 2009; Filazzola & Lortie, 2014; Torroba-Balmori et al., 2015; Da Silva Junior et al., 2021). However, We detected that) the regeneration density under the canopy of the nurse shrub species was lower than that outside of the canopy area when all species were included (First column in Fig. 1). This is related to the type and structure of the ecosystem influenced by the prevailing climate. In sparse and degraded forests as well as in semi-arid woodlands, nurse species play an important role for the survival of seedlings and saplings (Flores & Jurado, 2003; Ravanbakhsh et al., 2010; Yirdaw et al., 2017) and increase seed germination and seedling survival compared with open spaces (Torroba-Balmori et al., 2015). This kinds of ecosystems are exposed to high-stress and harsh conditions, such as direct sunlight, extreme evapotranspiration, erosion and soil instability**,** and shrub species protect seedlings against these damages by creating a protective shelter**.** Filazzola & Lortie (2014) showed that

nurse plants demonstarted positive plant interactions mostly occurred in highly stressed areas. Caldeira et al. (2014) stated that facilitating effects of nurse species are greater as habitat conditions become more unfavorable. Further, Anthelme et al. (2014) expressed that the establishment of nurse plants is considered a restoration method for the degraded forests, especially in cases of absence of nurse trees and shrubs. Therefore, research showed that nurse-protégé interaction is mainly established in arid and semi-arid or disturbed environments.

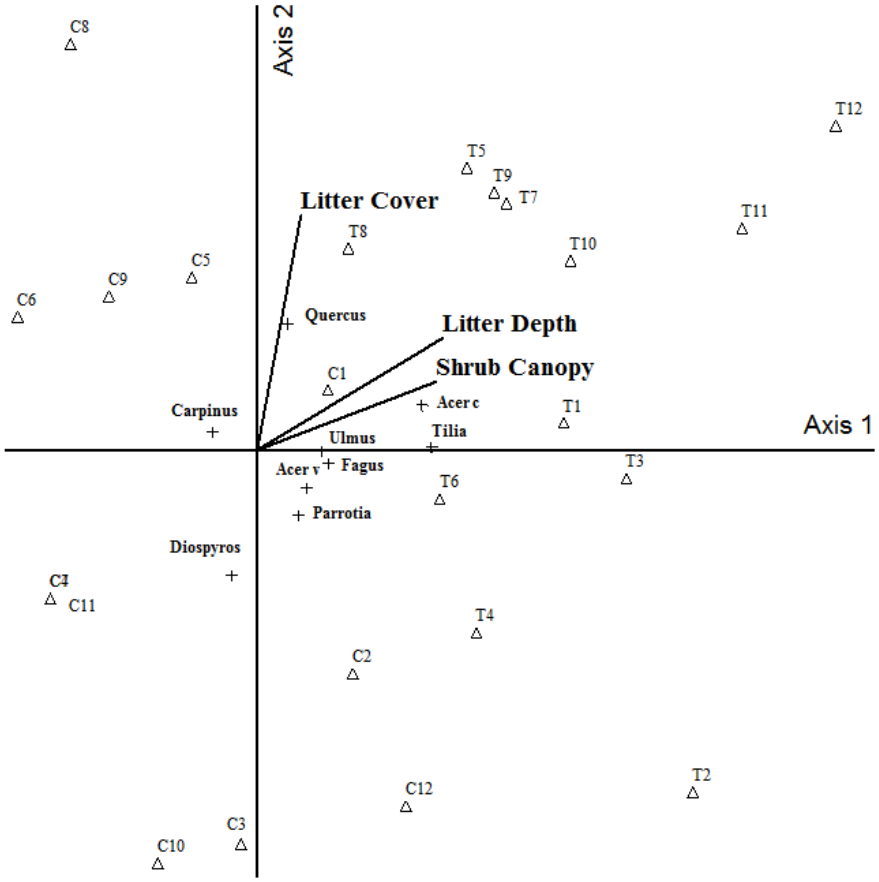

Figure 5. The ordination (CCA) graph and distribution of regeneration of tree species along the environmental variables gradient (T: samples located under the shrub canopy; C: samples located outside of shrub canopy).

On the other hand, in healthy, dense forests with humid climates, nurse species play a different role. In such forests, dense upper and mid-story vegetation prevents the light to reach the forest floor (Kimmins, 1987). Therefore, understory shrub plant canopy causes extremely low light to reach the floor. Moreover, litter trapped in a dark and cool space beneath shrubs decomposes slowly, resulting in a thick layer of litter. Insufficient sunlight and high litter depth make the conditions difficult for tree seed germination and seedling growth under the shrub canopy in dense forests.. In such conditions, regeneration density under shrubs is lower than that of the surrounding open space. The results also showed significant differences between the coverage and depth of the litter under the shrubs and in open space, which has been also confirmed by Stark et al. (2015). Litter may have a positive effect on seedling surviving in dry grasslands or under water-limited conditions, or in the presence of low to medium litter amounts. However, high litter amounts will inhibit seedling survival (Loydi et al., 2013). Furthermore, excessive accumulation of litter is also harmful to nurse species (Kuiters, 1990). Therefore, Litter accumulation is one

limiting factor for regeneration in dense and dark forests.

Our study showed *Quercus castaneifolia*, *Tilia begonifolia* and *Acer cappadocicum* have greater regeneration beneath the shrub canopy and the *Diospyros lotus* and *Carpinus betulus* have greater regeneration in the surrounding open space. The ability of seed emergence beneath deep litter was apparently related to seed size, so that large-seeded species have greater emergence under litter rather than small-seeded species (Wotton & McAlpine, 2013). Small seeds of *Carpinus betulus* were unable to grow in the litter thick bed and access to mineral soil, while *Quercus castaneifolia* and *Tilia begonifolia* with larger seeds was able to grow. Wotton & McAlpine (2013) showed that seed size is related to light requirements for germination: small-seeded species failed to germinate in the dark, while large-seeded species did. *Tilia* is a thermophilous tree (Heinrichs et al., 2021). Therefore, under the canopy of shrubs with thicker litter layer and microbial activity and as a result warmer condition, seedings growth becomes more possible. As well, *Q. castaneifolia*, and *A. cappadocicum* are more demanding than *C. betulus* and have more regeneration rate in the advanced stages of succession and closer canopy (Jafarzade et al., 2022).

As mentioned above, increase or decrease of the canopy coverage can disrupt the protective function of nurse species. According to the results, the canopy coverage rate of 25-50% of nurse species have the best performance in studied temperate broadleaf forests; however, higher or lower canopy coverage rate has less efficiency due to avoiding the passage of sufficient light and reducing protective effect.

Although the density of regeneration under the canopy of nurse shrub species was lower than that of outside the canopy area, regeneration had higher mean height and age. This suggests that seeds which succeeded to germinate beneath the shrub canopy and passed the preliminary growth stages were not grazed because of the support of nurse species and managed to reach the higher age and height, as compared to the unprotected seedlings. Accordingly, the successful seedlings in terms of light competition benefited the supportive role of the shrubs against grazing and thus, further grew. Anthelme et al., (2014) stated that compressed, massive groups of nurse plants in the Andean cloud forests reduced the detection ability in herbivores and thusreduced the effects of grazing by providing physical protections and establishing a shelter for seedlings. They showed that nurse plants significantly increased seedling growth of *Ceroxylon echinulatum*.

5. Conclusion

Nurse shrub species have a bilateral effect on regeneration, either as facilitating or inhibiting, depending on the type of ecosystem and canopy. In arid and semi-arid forest ecosystems and woodlands, highly stressed areas, extreme habitats and degraded forests with open canopy, shrub bases as nurse species can play a facilitating role in seed germination and seedling growth. However, in humid forest ecosystems, climax and mass forests can have an inhibiting role. In the Hyrcanian forests, nurse evergreen shrubs with very dense and rough leaves make the competition more difficult for light, space, and growing seedlings. Also, high litter thickness negatively affects the seedlings germination and growth. However, even in these conditions, shrub understory could yet be beneficial in terms of protecting the regeneration against grazing. In these ecosystems, the shrub canopy of 25-50% has the best efficiency.

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چکیده

جنگل‌های هیرکانی بخشی از جنگل‌های معتدله کره‌زمین هستند که قدمت آن‌ها به ترشیاری می‌رسد اما در دهه‌های گذشته به‌دلیل مسائل اقتصادی- اجتماعی از جمله دامداری، آسیب جدی دیده‌اند. این پژوهش به بررسی اثر محافظت گونه‌های پرستار و تاثیر گونه‌های درختچه‌ای زیراشکوب بر تجدیدحیات گونه‌های درختی اصلی هیرکانی در جنگل‌های خیرودکنار ایران می‌پردازد. آماربرداری با روش منظم تصادفی انجام شد و نوع و مشخصات زادآوری در زیر تاج و بیرون تاج گونه‌های درختچه‌ای زیراشکوب به همراه برخی از متغیرهای محیطی در قطعات‌نمونه نواری مطالعه و ثبت شدند. بر اساس نتایج به‌دست‌آمده، شش گونه درختچه‌ای به‌عنوان گونه پرستار شناسایی شدند که تاج‌پوشش درختچه‌ای مناسب جهت عملکرد حفاظتی مطلوب آن‌ها، ۲۵ تا ۵۰ درصد بود. در مجموع، تراکم زادآوری در بیرون از سطح تاج درختچه‌ها بیشتر از زیر تاج بود اما ارتفاع متوسط و سن زادآوری در زیر تاج بیشتر بود. همچنین، متوسط پوشش و عمق لاشبرگ در زیر تاج درختچه‌ها به‌طور معنی‌داری بیشتر از خارج تاج بود. اگرچه گونه‌های درختچه‌ای باعث بقا و دوام بیشتر زادآوری به دلیل حفاظت آن‌ها در برابر چرای دام می‌شوند، اما به دلیل عمق لاشبرگ و نور ناکافی، که خود نتیجه لایه‌های پوشش توام درختی و درختچه‌ای است، شرایط مساعدی را برای جوانه‌زنی و رشد نونهال‌ها فراهم نمی‌کنند (به ویژه در گونه‌های درختچه‌ای همیشه سبز).

واژه‌های کلیدی: زادآوری طبیعی، گونه پرستار، جنگل‌های معتدله، نونهال.

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