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Effect of Sowing Depth on Performance of *Quercus castaneifolia* Seedling at Different Levels of Canopy Cover

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Abstract: Due to failure of oak (*Quercus castaneifolia* C.A. Meyer) natural regeneration, investigation on the best acorn sowing depth of this species under different levels of canopy cover is an important consideration in the Caspian forests, north of Iran. For this purpose, a study site with north-facing slope, clay-loam soil and 260-280 m a.s.l. was chosen in a mixed oak forest. The experiment was conducted as a Complete Randomized Split Plot Design (CRSPD) and the measurements made in one growing season in nine fenced circular 1000 m^2 plots with three canopy densities (25, 50 and 75%) at three soil depths (5, 10 and 15 cm). The results revealed that under all canopy densities the maximum seedling emergence occurred at 5 cm depth. The highest emergence rate was appeared in June and the lowest in October. Neither canopy density nor sowing depth did prominently reduce seedling establishment, but a high quotient of mortality likely could be attributed to rodent populations, particularly *Hystrix indica*. Under all canopies, ground line diameter decreased with increasing sowing depth, the biggest being at 5 cm depth. Neither canopy density nor sowing depth influenced the seedling height. It can be concluded that the best performance of *Q. castaneifolia* seedling occurs at 5 cm sowing depth and 25% canopy cover.

Key words: Canopy density, emergence, establishment, growth, *Quercus castaneifolia*, seedling, sowing depth

INTRODUCTION

Oaks have a lot of economical and ecological importance in their dispersal regions in several continents of northern temperate zone and Polynesia (Johnson *et al*., 2001). In the absence of scientific management of wildlife as well as forest disturbance regimes, oak stands usually have poor natural regeneration and may begin to be replaced by shade tolerant species (Li and Ma, 2003). Oak natural regeneration problems, such as seed and seedling browsing, frost damage, competition from herbaceous weeds and low light levels have led forest managers to use different artificial regeneration methods, where natural regeneration is difficult to obtain (Duplissis *et al*., 2000). Caspian forests are the most valuable forests in north of Iran covering the northern slopes and foothills of Alborz mountain range in southern part of the Caspian Sea. Caucasian oak (*Quercus castaneifolia* C.A. Meyer) is a main tree in these forests with a high commercial value, particularly in forest overstorey (Jafari, 1977). On the other hand, reduction of standing volume as well as defect of natural regeneration in oak stands has greatly concerned the Iranian silviculturists (Rasaneh *et al*., 2001).

Recently, forest managers have become increasingly interested in using silvicultural systems to retain the dominant trees as shelter. Such systems often rely on regeneration development; particularly seed sowing that is the main technique to restore the forests. It is an old regeneration method (Thirgood, 1971) that has lately revived (Willoughby *et al*., 1996; Kussner and Wickel, 1998; Ammer *et al*., 2002). It makes expense as little as half of conventional planting, decreases seed predation and appropriates to gradient fields and allows seedlings extend their root naturally, contrary to root-pruned nursery seedlings (Allen *et al*., 2001). The periodical mast years may results in even lower costs since large areas may be rapidly sown when seeds can be inexpensively and perhaps locally collected. It has been also mentioned that oak direct seeding has a potential for high stock density at low costs, thereby ensuring reasonable wood quality in the future (Madsen and Lof, 2005).

Although direct seeding of oak is a fundamental measure that helps foresters for restoration of deforested areas, there is a limited knowledge regarding the appropriate canopy density and acorn sowing depth that can bring about the best emergence and performance of *Quercus castaneifolia* seedling. In this study we examined the effects of different levels of sowing depth and canopy density on emergence of *Quercus castaneifolia* acorns and determined establishment, diameter, height and mortality of seedlings in the first growing season.

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MATERIAL AND METHODS

Study area: The study was carried out in Golpoor forest, 15 km southern west of Noor town (36°28′ N, 52°03′ E, 270 m elevation), situated in watershed No. 49, north of Iran (Fig. 1). Climate is temperate and humid. Mean annual temperature averages 16.1°C (February 6.9; August 25.5°C), precipitation amounts to 803.4 mm and dry season occurs between May and August (Fig. 2). The site is an Oak-Elm stand whereas *Quercus castaneifolia, Zelkova carpinifolia* and *Carpinus betulus*, respectively with 68.5, 20.2 and 10.0% of tree basal area are laid on the north-facing mild slopes (up to 20%). Soil is forest brown with silt-clay-loam texture, more than 100 cm in depth and lime parent material.

Experimental design: Acorns were collected from three mature trees of the experimental site in mid-November 2002 and stored in normal condition of forest floor in storage chamber till early January 2002. The germination rate was tested in late November. Four hundred acorns were sown in sand in a climate chamber (20°C, 90% RH; 12 h day). After 30 days, about 74% of the acorns had germinated. Weight of each acorn was 9.6 g and Water Content (WC) 43.3%. In early January acorns were sown with three levels of sowing depth under three levels of canopy cover. Examination was Complete Randomized Split Plot Design (CRSPD). Main factor was crown canopy in densities of 25, 50 and 75% in three replicates (circular main plots of 1000 m^2) and minor factor was sowing depth in levels of 5, 10 and 15 cm (Fig. 3). Main plots were established by cutting trees in 1997 and occupied by herbal vegetation following failed regeneration. Six micro plots of 1×4 m were laid out in each main plot and 40 acorns (in 20 cm spacing) were sown in each depth. In total, there were 240 acorns with three sowing depths in

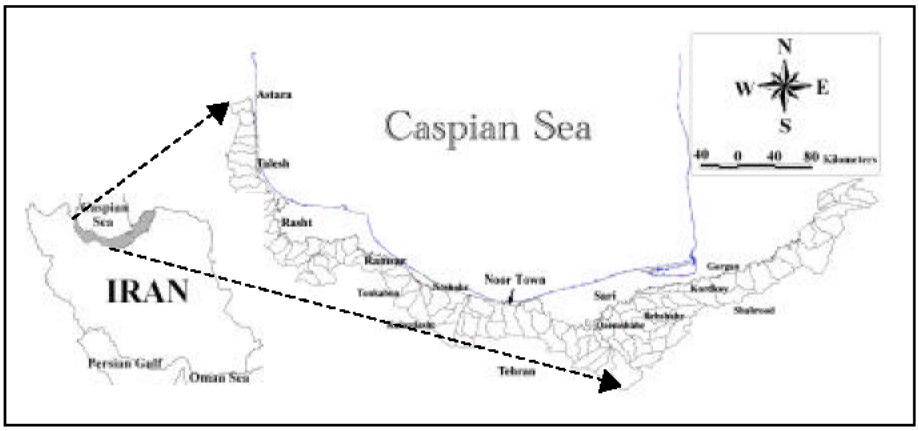

Fig. 1: Map of northern Iran watersheds covered by Hyrcanian forests

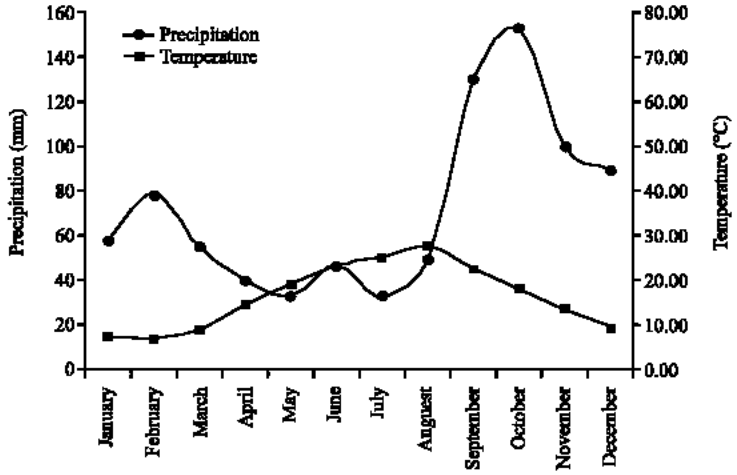

Fig. 2: Ombrothermic curve in the study area (based on 1985-1998 climatic data)

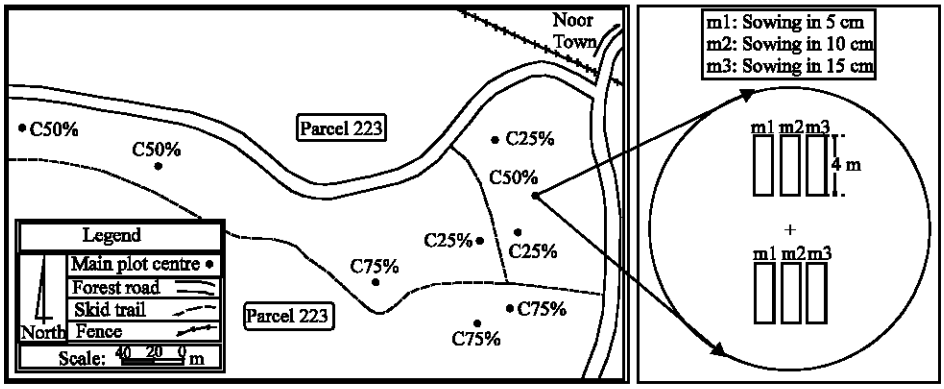

Fig. 3: Distribution of main plots in the study area (left) and the set up of micro plots in a main plot (right)-C is canopy density of main plot

each main plot. The canopy density was determined by measuring the vertical canopy projection of the trees within the main plot. Ground vegetation and slash were generally removed before experiment was initiated. Since browsing damage by deer, pig, cow, sheep and goat was observed on the seedlings and saplings around the experimental site, therefore all main plots were fenced against them.

Data collection and analysis: Emergence as well as mortality was monitored during months of the first growing season. Height and ground line diameter (root collar diameter) as well as establishment rate were measured at the end of period (November). Emergence, height, ground line diameter (stem diameter on the forest floor) and establishment rate were analyzed by a Two-Way ANOVA under complete randomized split plot design. When ANOVA showed significant difference in main factor or interaction effect, Duncan multiple range test was used to ascertain between treatments difference based on the ANOVA errors mean square. In order to test ANOVA normality assumption, Shapiro-Wilk's W test was used to investigate the difference of data distribution and normal distribution in each of nine treatments. When data distributions were shown significant departure from normal distribution, logarithmic and third root transformed values were used in Analysis (Zar, 1999; Wheather and Cook, 2002). Monthly seedling emergence and mortality rates were tested in repeated measures design conducted by parametric methods using univariate and multivariate approaches (Scheiner and Gurevitch, 1993; Underwood, 1997). Because there was no unanimity among statisticians in use of these two approaches, both were used in analysis. In univariate analysis, variance-covariance matrix circularity was tested with Machly's test. Univariate analysis degree of freedom was adjusted with huynh-Feldt epsilon since the null hypothesis was rejected by the Machly's test (Scheiner and Gurevitch, 1993; Underwood, 1997). When time within the subjects effect was significant, paired samples student *t*-test were used. Regression analysis was used to analyze trend of treatment significant effects through the period of study (Scheiner and Gurevitch, 1993; Underwood, 1997).

RESULTS

Emergence: Our observation revealed that the first seedling emergence was occured in late April for shallow sown acorns (5 cm depth). It was delayed for acorns sown deep in the soil (10 and 15 cm). Two-Way ANOVA indicated that seedling emergence was significantly affected by both sowing depth and sowing depth × canopy density (Table 1). Effect of canopy density on seedling emergence varied with sowing depth such that at 5 and 10 cm pits emergence rate was highest in open canopy (Table 2). In intermediate and close canopies (50 and 75% cover) 5 cm sowing depth produced greater emergence compared to 10 and 15 cm pits. There was a significant negative correlation between sowing depth and seedling emergence under all canopy densities. Such a trend was found between time and seedling emergence in all sowing depths, too (Table 3). The repeated measures ANOVA showed a significant time × sowing depth interaction on seedling emergence (Table 4). At 5 cm sowing depth maximum seedling emergence occurred in June. Seedling emergence continued with a sharp decline in following months and reached the least value in October (Fig. 4). At 10 cm pits seedling emergence did not significantly differ between June and July but decreased till October. At 15 cm sowing depth seedling emergence was very weak; it was not different among the dates recorded. In all canopy covers the highest emergence

Table 1: The results of two-way split plot analysis of variance of characteristics measured at the end of the first growing season

Parameters	Source of variation	SS	df	MS	F	p-value
Emergence	Canopy density	0.242	2	0.121	5.033^{ns}	0.052
	Sowing depth	12.460	2	6.230	78.730**	0.000
	Canopy × Sowing depth	1.362	4	0.340	4.302*	0.022
Establishment	Canopy density	1.672	2	0.836	0.145^{ns}	0.868
	Sowing depth	8.519	2	4.260	2.153^{ns}	0.159
	Canopy × Sowing depth	3.686	4	0.922	0.466^{ns}	0.760
Height	Canopy density	65.174	2	32.587	0.818^{ns}	0.485
	Sowing depth	41.779	2	20.890	2.604^{ns}	0.115
	Canopy × Sowing depth	101.140	4	25.285	3.152^{ns}	0.055
Ground line diameter	Canopy density	1.833	2	0.917	0.755^{ns}	0.510
	Sowing depth	5.679	2	2.839	13.674**	0.001
	Canopy × Sowing depth	1.337	4	0.334	1.610^{ns}	0.235

* and ** are significant, respectively at 5% and 1% probability level ns, is non significant

Table 2: Seedlings emergence, establishment, height and ground line diameter in different sowing depths under different canopy densities (Mean±Standard Error)†

Parameters	Sowing depth (cm)	Canopy density (%) 25	50	75
Emergence (%)	5	34.5±3.8^{Aa}	16.3±3.4^{Ca}	19.1±2.7^{Ba}
	10	6.2±1.6^{Ab}	3.1±0.8^{Bb}	2.5±0.6^{Bb}
	15	0.9±0.4^{Cc}	3.3±1.6^{Ab}	2.5±1.1^{Bb}
Establishment (%)	5	76.1±5.6^{Aa}	66.3±1.9^{Aa}	70.8±3.1^{Aa}
	10	40.2±21.8^{Aa}	35.6±19.4^{Aa}	72.2±14.7^{Aa}
	15	66.7±33.3^{Aa}	46.7±29.1^{Aa}	50.0±28.9^{Aa}
Height (cm)	5	17.6±2.6^{Aa}	14.3±1.3^{Aa}	15.7±1.8^{Aa}
	10	14.4±3.8^{Aa}	14.5±2.8^{Aa}	13.6±1.6^{Aa}
	15	11.5±2.5^{Aa}	13.6±3.0^{Aa}	18.03±2.0^{Aa}
Ground line diameter (mm)	5	3.4±0.2^{Aa}	3.2±0.4^{Aa}	2.8±0.2^{Aa}
	10	2.7±0.2^{Aa}	3.1±0.8^{Aa}	2.7±0.2^{Aa}
	15	1.7±0.3^{Ab}	2.8±0.6^{Ab}	1.7±0.6^{Ab}

†Large and small Latin words respectively show significant difference among rows and columns, respectively (Based on Duncan multiple range test A>B>C and a>b>c)

Table 3: Linear correlation between seedling emergence and sowing depth under the canopy density and between seedling emergence and time in the sowing depth †

Treatments		r	a	b	F	p-value
Canopy density	25%	0.873	47.483	-3.361	51.36**	0.000
	50%	0.688	20.234	-1.295	12.57**	0.003
	75%	0.775	24.667	-1.665	24.15**	0.000
Sowing depth	5 cm	0.643	22.835	-0.098	58.40**	0.000
	10 cm	0.532	3.753	-0.016	33.97**	0.000
	15 cm	0.370	1.783	-0.007	13.18**	0.000

†r, refers to Pearson correlation coefficient and linear correlation function is y = ax+b. ** is significant at 1% probability level

Table 4: Emergence and mortality rates of seedling during the first growing season, according to ANOVA †

	Repeated measures ANOVA			MANOVA		
Source of variation	MS	F	p	Wilks' lambda	F	p-value
Emergence						
Time	2.224	57.221**	0.000	0.135	63.990**	0.000
Time × Canopy	0.036	0.920^{ns}	0.499	0.839	0.918^{ns}	0.506
Time × Sowing depth	0.404	10.395**	0.000	0.272	9.174**	0.000
Time × Sowing depth × Canopy	0.040	1.018^{ns}	0.439	0.702	941^{ns}	0.525
Mortality						
Time	1.087	2.677**	0.038	0.44	4.779*	0.011
Time × Canopy	0.645	1.588^{ns}	0.143	0.582	1.164^{ns}	0.352
Time × Sowing depth	0.294	0.725^{ns}	0.669	0.665	0.849^{ns}	0.568
Time × Sowing depth × Canopy	0.295	0.726^{ns}	0.758	0.395	1.033^{ns}	0.442

†Test degrees of freedom are adjusted with Huynh-Feldt epsilon value = 0.961 and 1.00, respectively in seedlings emergence and survival repeated measures ANOVA. * and ** are significant, respectively at 5 and 1% probability level ns, is non significant

came about in June followed a sudden fall in July (Fig. 5). Seedling emergence was not markedly different in July, August and September but it decreased in October.

Establishment and mortality: Two-way ANOVA indicated that canopy density as well as sowing depth and interaction between them did not influence on

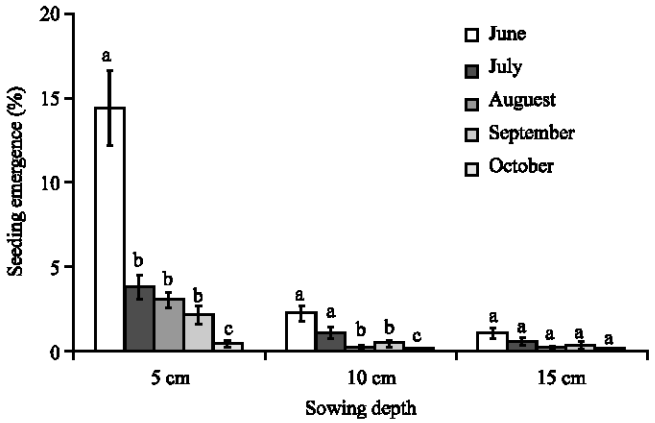

Fig. 4: Seedling emergence in different sowing depths during the first growing season (Mean Standard Error), based on Duncan multiple range test a>b>c)

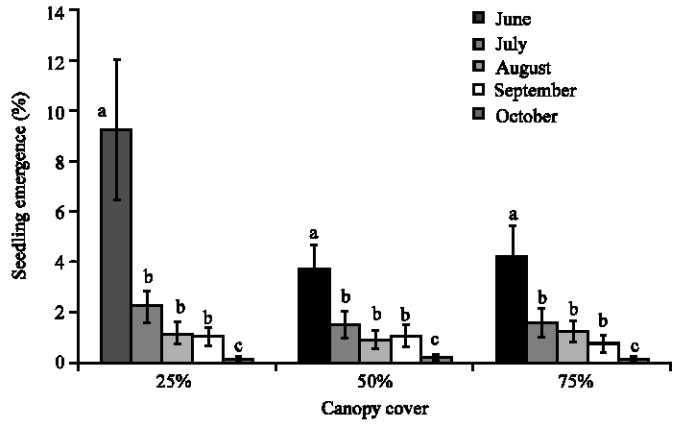

Fig. 5: Seedling emergence in different levels of canopy density during the first growing season (Mean Standard Error), based on Duncan multiple range test a>b>c)

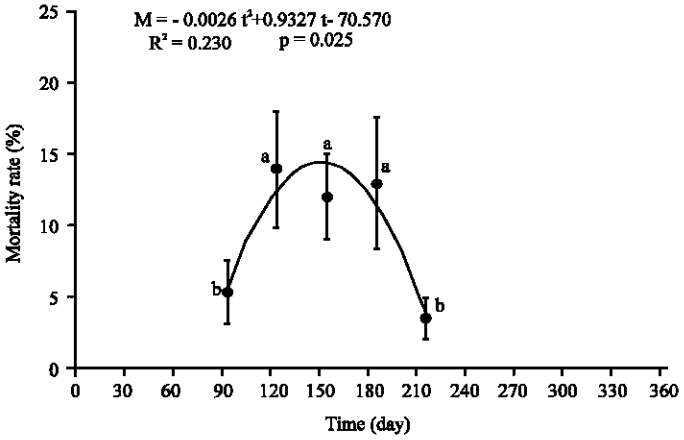

Fig. 6: Mortality rate of the experimental site (without considering sowing depth and canopy density), recorded on dates of the first growing season (Mean±SE), based on least squares regression analysis and paired samples student t-test a>b>c

seedling survival (establishment) (Table 1). Repeated measures ANOVA showed significant effect of time on seedling mortality in the study period ($p<0.05$) but no significant interaction of time × canopy or between time × sowing depth was detected on this attribute (Table 4). Contrast analysis of time, within subject factor, displayed that the maximum mortality occurred through July-September and the minimum in June and October (Fig. 6). A main part of mortality rate could be related to rodent population especially invasion of *Hystrix indica* in mid-summer.

Ground line diameter and height: According to two-way ANOVA ground line diameter was significantly different in various sowing depths but neither canopy density nor interaction between canopy density and sowing depth affected this term ($p<0.05$) (Table 1). In all canopy densities ground line diameter was lowest at 15 cm pits; other sowing depths had the same effect on this characteristic measured (Table 2). Contrary to sowing depth, canopy cover and interaction of two factors did not influence seedlings height (Table 1).

DISCUSSION

In the present experiment we found a significant negative correlation between seedling emergence and sowing depth with *Q. castaneifolia* acorn. Under all canopy covers the maximum emergence rate was found at 5 cm sowing depth. This is in agreement with Fattahi *et al.* (1996)'s findings, who noted the best sowing depth for *Q. persica* and *Q. infectoria* acorns was 5-7.5 cm. Similarly, Nilsson *et al.* (1996) showed that the highest emergence of *Q. robur* occurred at 5 cm depth. On the other hands, Lalhal *et al.* (1996) and Guo *et al.* (1999) proved that the highest emergence appeared in 1.5-2 cm holes, respectively for *Q. semecarpifolia* and *Q. nigra*. This is while that Wood (1998) found this at 10 cm depth for *Q. phellos* acorns. In this study the highest seedling emergence of *Q. castaneiflia* took place at 5 and 10 cm depths under open canopy (25% cover). This is partly in line with Ashton and Larson (1996) and Li and Ma (2003), who found that seedling emergence of *Q. coccinea*, *Q. rubra*, *Q. velutina* and *Q. liaotungensis* was greatest in open stands. In contrast, Nilsson *et al.* (1996) asserted that emergence did not differ between acorns sown inside the shelterwood and those on the clearcut.

It should be underlined that deeper seeding usually prevents some predation, but will be delayed the emergence of seedlings (Johnson, 1981; Nilsson *et al.*, 1996; Tomlinson *et al.*, 1997). Our study can be confirmed by this reality, so we observed the commencement of seedling emergence in early June for acorns sown deep in the soil but in late April for acorns sown shallow in the soil. The main part of emergence rate happened in June; however, it continued decreasingly till October. This means that *Q. castaneifolia* acorn can survive beneath the soil surface during one growing season, although it is supposed that, generally, oak seedling emergence may appear in following year (Nilsson *et al.*, 1996).

The same as finding of Lalhal *et al.* (1996) on *Q. semicarpifolia*, a part of emergence failure during the period could be as a consequence of unfavorable climate conditions (low temperature) come about in spring, but the main cause was related to rodent population. The signs of rodent activity look liked intimately connected with the poorest direct sowing results. Some of the more unspecific evidence could be small holes in the seed-bed where acorns had been sown and sometimes with remnants of the seed coat spread out on the soil surface near seed points. Our findings are in accordance with working of Johnson (1981), Bullard *et al.* (1992), Nilsson *et al.* (1996) and Madsen and Lof (2005) on direct seeding in forested areas, where too much rodent damage could be accepted as a main reason of early losses. In reality it can be stated that the success for rodents in finding seeds usually decreases as the depth of burial increases (Nilsson *et al.*, 1996; Kussner and Wickel, 1998: Vander Wall, 1998). It may be also supported by Watt (1919) and Shaw (1968) who report that covering with soil protects the acorns from being eaten by rodents and that predation may be substantially lowered if the acorns are sown at 5 cm depth in the soil.

Under all canopy levels, the greatest ground line diameter was produced in 5 cm holes; it was decreased with increase of sowing depth. The same as Duplissis *et al.* (2000, on *Quercus rubra*) and Cardillo and Bernal (2005, on *Quercus suber*) findings, in our investigation no significant effect of shading was detected on ground line diameter; similar result was observed on seedlings height. This means that *Q. castaneifolia* does not need much light intensity to grow in initial years. In other words, it can be emphasized that like many shade tolerant oaks (Joyce, 1998; Johnson *et al.*, 2002) *Q. castaneifolia* can also bear shade in the seedling stage.

In this study, seedling mortality in *Q. castaneifolia* did not appreciably differ in various canopy covers such that Negreros and Hall (1996); Fuchs (2000) and Li and Ma (2003) found with *Q. garryana* and *Q. liaotungnsis* seedlings. The existence of a distinctive nonlinear correlation between time (months of recording) and seedling mortality implies that the highest mortality, caused by rodents and in particularly *Hystrix indica*, appeared in July-September.

The resultant of the present study indicates that sowing of *Q. castaneifolia* acorn has the potential to become an effective regeneration method, where the canopy opening is greater and the seeds and seedlings are protected from rodents. It can be also mentioned that increased amounts of sown acorns may provide the alternative food for rodents and as a result to some extent guarantee the success of regeneration establishment, though it would raise the overall costs. Generally, it can be affirmed that in the site study the most suitable performance of *Q. castaneifolia* was where the acorns were planted at 5 cm holes beneath open canopy (25% cover). In canopy covers of 50 and 75% employment of natural regeneration along with soil scarification can be more proper than direct seeding.

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