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ORIGINAL ARTICLE

Effect of Some Agro Practices on Seed Bank, Establishment and Natural Regeneration of Annual Medics

Amini Dehaghi[1], Majid; Azizi, Khosro[2]; GHalavand, Amir[2]; Modarres Sanavy, Seyed Ali Mohammad[2]; Edalatifard[2], Leila and Etemadi, Fatemeh[2]
Department of Agronomy[1], Shahed University,
Department of Agronomy[2], Tarbiat Modares University, Iran

ABSTRACT
Recognition of critical limit of soil seed bank and influence of some agro practices of annual medics in dry lands is very essential for successful. Objective of this study was to determine the effects of different planting systems, cutting forage at different stages of growth phenology, depth of seed burial, planting seeds with pods or without pods on seed bank, emergence, seedling establishment, and natural regeneration. The experiments were conducted in 2009-2011 in Iran. Results showed that cutting forage in different phonological stages affected on seed production and seed bank of annual medics, so that cutting forage at the beginning of flowering compared to 50 percentage of flowering either produced more seed or created rich soil seed bank. The more depth of seed burial caused not only delayed seed emergence, but also seedling establishment was weaken. Planted seeds with pods in comparison with seeds without pods were caused longer existence of seeds in the soil. Method of planting with dry pressing caused early seed emergence, suitable establishment and as a result earlier flowering that produced more seed yield and created a rich seed bank. Therefore Intercropping of different species of annual medics compared with monoculture has more benefit in all different aspects and establishment of seed bank.
***Key words**: agro practices, natural regeneration, annual medics, seed bank, depth of seed burial*

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INTRODUCTION

Annual medics is particularly important as a high quality forage crops and a source of value-added products [1]. Most of annual medics grow faster than perennial medics and cultivated in more than 40 million hectares of worldwide. In some countries, annual medics are the main part of farming systems, because of nitrogen fixation and soil Fertility [2].We can use them as a fallow period, not only soil fertility occur but also can prevent of soil erosion. Development of annual medics can be lead produce significant amounts of hay for livestock's [3]. To fulfill of this assumption, abundant seed production of annual medics is essential. Seed production is not only part of the agronomic value of a forage crops but also in annual medics, it is critical in the commercial development of a new variety [4,5]. Major advances at seed production were achieved through optimization of the agronomic practices, but little progress has been achieved in breeding over the last three decades. In this regard, Soil seed bank refers to populations of viable seeds on or in the soil that act as a potential seed source for natural regeneration and restoration [6]. The soil seed bank is an important component of the forest and may impact several aspects of ecological functions; including genetic, population, community dynamics and it can play an important role in restoring former species diversity [7]. The study of seed production, soil seed bank, seed hardiness, natural establishment adaptation conditions, and forage production in annual medics are important as unavoidable issues in feasibility of ley-farming systems. Many researchers believe that [8,9], enough seed production is an essential for natural regeneration of annual medics. Also rate of success in reestablishment via natural regeneration in ley-farming system depends on potential of soil seed bank. It's one of the limiting factors in the annual medics [9]. Carter [10] showed in his experiment, Critical limit of soil seed bank for successful establishment of annual medics in ley-farming is 200kg seed/ ha at the depth of 5cm. seeds Hardiness of annual medics is necessary for stability in their seed bank too [11]. Hence, one of the most important methods for success in having a rich seed bank during legume- cereal rotation is having intercropping of different species of annual medics. Annual medics are very varied in ability for establishment of a sustainable seed bank that is applied in intercropping compared to mono cropping [12]. Baker [13] reported that the cause of having extra production in intercropping relative to mono cropping is presence of at least 25% difference at

growth period of two plants which participated at mixed cropping. Different cultivars of annual medics have diversity at type of growth semi- right to prostrate [14]. So variety of this growth diversity in annual medics increased production at intercropping relative to their monoculture. In other hand, inopportune grazing is caused delayed in flowering and reduces seed yield of annual medics [15]. Regarding to Cocks [12] report, one of the most important strategies in ley-farming system is forage production. So, determining of time and amount of grazing is important for natural regeneration. Unsuitable conditions of grazing of annual medics reduce seed production. Young [16] reported that Grazing in pod formation stage in many cultivars of annual medics declined seed yield. In addition, hardiness of seeds of annual medics is a necessary factor for sustainability in their seed bank in ley-farming systems [11]. Cocks [12] estimated that seed hardiness more than 90% is causing seed protection in the first year which wheat used in rotation system. Also depth of seed burial should be regulated, so it will be able to establishment in consecutive years. The purpose of this study was to investigate the Effect of agro practices such as: intercropping and mono cropping, natural regeneration, forage cutting at different stages, depths of burial of seed and methods of planting, on soil seed bank of annual medics in Iran.

METHODS AND MATERIALS

Experimental Site: to evaluate effects of agro practices on soil seed bank, establishment and natural regeneration of annual medics a study was conducted in over two years from 2009 to 2011 in Experimental Station of Agriculture Meteorology located in north east of Khorram Abad (longitude 48^0 36', latitude 33^0 23'). Soil type was clay silt (pH 7.8).The mean monthly rainfall of the experimental site for 2009-2011 was 620 mm.

Soil Sampling and Analysis: In order to determine soil type, a chemical property of the experimental site, a composite soil sample was collected from 20 points in the entire plot and during each year of study. All soil samples were collected by bulking augured core (internal diameter 7.5 cm) from 1-30 cm soil layer. Soil depth of 30 cm is the average depth for expansion of roots (active crop root zone). The composite soil sample was analyzed in the laboratory (table1). Total N (%) was determined by the macro-Kjeldahl method, P and K were determined by spectrophotometer and flam photometer respectively.

In this research five experiments were designed and six cultivars of annual medics were used as the following:

1) *Medicago scutellata cv.* Robinson (A)
2) *Medicago scutellata cv.* Kelson (B)
3) *Medicago rigidula cv.* Rigidula (C)
4) *Medicago truncatula cv.* Caliph (D)
5) *Medicago truncatula cv.* Orion (E)
6) *Medicago truncatula cv.* Mogul (F)

Soil texture	EC	PH	Total N(%)	P (ppm)	K (ppm)	Sand%	Silt%	Clay%
Silt-clay	0.58	7.8	0.18	10	450	5	55	40

Table 1: Analysis of soil sample

All experimental treatments were planted during 14-17 March 2009 and 4-5 March 2011. The first rainfall after planting was 8 mm on 15th of March first year and at the second year was about 21mm on 8th of March. The size of each plot was 2× 6 m.

Experiment1: This experiment was planted for finding the effect of intercropping and mono cropping on seed bank and natural regeneration of annual medics. For this purpose complete block design with three replications were used. Experimental treatments were: mono cropping and intercropping systems of six cultivars. In mono cropping each cultivar was planted separately in a plot and marked with letters A, B, C, D, E, and F. In intercropping system, each cultivars of annual medics with ratio 50:50 were planted, so that the experimental treatments were : AB , AC , AD , AE , AF , BC , BD , BE , BF, CD, CE, CF, DE, DF, EF. Thus, total treatments in two systems of mono cropping and intercropping were 63 treatments.

Experiment 2: Effect of forage cutting at different stages of phenology on the regeneration of plant, seed production, total dry matter, and seed bank were studied. The experiment was conducted in a

complete block, design arrangement in split plot with three replications. Main plots were six cultivars of annual medics and sub plots were forage cutting at two stage: 1) beginning of flowering, 2) 50% of flowering. Herbage was cut from 1 m^2 area per plot then fresh and dry weight production of forage measured, so we could estimate a trend of regeneration at 1m^2 area.

Experiment 3: Evaluation of depths of burial of seed on seed bank, seedling establishment, and finally natural regeneration of annual medics were investigated. The experimental design was complete block arrangement in split factorial with three replications. Six annual medics' cultivars were randomized to the main plots and the combination of 3 planting depths (2, 4 and 6 cm) and planting with pods or without pods were located in sub plots.

Experiment 4: Methods of planting with dry pressing deeper centrifuge or hand broadcasting was studied on establishment of seed bank in the soil. The experimental was conducted in randomized complete block design arrangement in split plot with three replications. Six cultivars of annual medics were considered as main factor and two methods of planting were as sub factor. In all treatments following parameters were measured:

1) Days of flowering
2) Number of lateral branches
3) Percentage of seed emergence
4) Percentage of seedling establishment
5) Total dry weight
6) Number of seed pod
7) Number of seed in pod
8) Number of seed in soil

For estimating of Seed bank, seeds at the top of 5cm of the soil were exhumed and seedling emergence per m^2 were counted. Frequency of annual medic at different depths were estimated in each plot from 15 random placements of 1m^2 rectangular quadrate, then we dug the hole in the soil at three depth: 0-2, 2-4 and 4-6 cm. seeds were separated from soil by washing through a four-tiered sieve with 2,0.85,0.42 and 0.3mm screen opening. Size grouping allowed the seed to be more easily recognized from soil and we could count them. The experimental data were analyzed by using the SAS system (2008) and the significant differences between means were assessed by Duncan Multiple Range Test at $P \leq 0.05$.

RESULT AND DISCUSSION

Experiment1: There was significant difference between planting systems on seed bank in the soil. The highest seed yield with 844 pods/m^2 was related to treatment of intercropping system of two cultivars *Medicago truncatula cv.* caliph and *Medicago scutellata cv.* robinson (AD) and the least seed yield with 5.33 pods/m^2 was related to treatment mono cropping *Medicago scutellata cv.* Keelson(B) (table 2).Similar results were reported by other researchers [15]. Baker [13] suggested that intercropping relative to mono cropping had superiority in production. This study also showed that in AD treatment with two cultivars of A and D were different at period of growth as that each plant completed its growth at 93 and 123 days, respectively. Fukai and Trenbath [17] also suggested that the relative yield of crops at mixed cropping can be greatly affected by small changes in the growth duration. Seed yield of two cultivars *Medicago truncatula cv.* caliph and *Medicago scutellata cv.* robinson in mono cropping system were 654.67 and 485.33 pods / m^2, respectively while seed yield of these two cultivars at intercropping were more than their mono cropping.

The results showed that seed bank enriched of ley farming system for natural regeneration were more appropriate in intercropping than mono cropping system [12]. Crawford [18] suggested that for success in ley farming system, using a planting system to produce abundant seed is necessary. Two cultivars E and B were late maturing relative to other cultivars thus they could produce higher dry matter due to their longer period of growth but could not complete their phenology growth at spring planting and could not produce suitable seed yield (144.67 pod/m^2), so that seed yield of two cultivars B and E in mono cropping system were 5.33 and 278 pod/m^2 respectively. Results showed that although intercropping was more suitable than mono cropping, because of seed bank in order to natural regeneration in ley- farming system, it depends on growth and maturity duration.

Experiment2: Reaction of different cultivars to cutting at beginning at flowering (T1) and at 50% flowering (T2) was significant (Table 3) and caliph produced the richest seed bank about 611 and 313 pods / m2 at two stages, because this cultivar produced more number of branches during the growing season. Superiority of this cultivar was reported previously by Loi [19]. Results showed that yield of dry

matter during regeneration at T1 were more than T2. Similar results were reported by other researchers. Altinok et al. [15] reported that yield of dry matter increased but rate of regeneration after forage cutting highly reduced. This study showed that seed yield after regeneration at stage T1was more than T2, because of growth period of T2 that had interaction with moisture deficiency in the farm. Forage cutting at the beginning of flowering produced more seed yield than forage cutting at pod formation stage. The highest seed bank due to regeneration was 226.00 pods /m2 that was related to T1 (table 4). Among six cultivars of annual medics in this study, *Medicago truncatula* cv. caliph had the highest seed bank in soil with 494 pods / m2 that had superiority to other cultivars(table 4). This result has been reported by other researchers. Loi [19] reported that among several cultivars of annual medics in spring planting, cultivar *Medicago truncatula* cv. Caliph had the highest seed yield. Caterton [20], Dear [22] and Walsh [22] also reported about high vigor of seed production of this cultivar. Comparisons showed that cultivars E and F and cultivars B and C were in different groups. These cultivars were late maturing, and despite regeneration after cutting, could not produce reasonable flower thus could not produce acceptable seed. Results also showed that late maturity treatment at 50% flowering had the weakest seed bank in the soil, for example *Medicago rigidula* cv. Rigidula produced 39.30 pod/m2 while *Medicago scutellata* cv. Robinson produced 234.80 pod/m2 at 50% flowering Similar results was reported by Sahbani [23].

Table 2: comparison of total dry matter, dry forage, and seed yield in cropping system experiment

Cropping systems	cultivars		Seedyield pod/m²
Mixed cropping	AB	327.00^{efg}	
	AC	458.33^{cde}	
	AD	844.00^{a}	
	AE	445.33^{def}	
	AF		474.00^{cde}
	BC	558.67^{cd}	
	BD	462.00^{cdef}	
	BE	144.67^{ghi}	
	BF	209.33^{gh}	
	CD		589.00^{cd}
	CE		224.00^{gh}
	CF	334.67^{efg}	
	DE	641.33^{bc}	
	DF	774.00^{ab}	
	EF	326.00^{efg}	
Mono cropping	**A**	485.33^{cde}	
	B	5.33^{l}	
	C	88.00^{hi}	
	D	654.67^{bc}	
	E	278.00^{fg}	
	F	167.33^{ghi}	

Means of each column having similar letters are not significantly different (Duncan 1%)

Table 3: interaction between cultivars and forage cutting stage in different growth stages of annual medices cultivars

cultivars	Forage cutting stage	Seed bank Pod/m²
A	T_1	404.5b
	T_2	234.8d
B	T_1	23.31j
	T_2	8.15j
C	T_1	106.65fg
	T_2	39.3ij
D	T_1	611.3a
	T_2	313.15c
E	T_1	135.3f
	T_2	53.5hi
F	T_1	191.6e
	T_2	81.5gh
cultivars*forage cutting		**
different of year		n.s

Table 4: Description of seed bank at different forage cutting

Treatments		No. of branches	Seed bank Pod/m²
cultivars	A	20.00[b]	347.67[b]
	B	13.66[c]	12.67[d]
	C	12.83[c]	10.33[d]
	D	28.16[a]	494.00[a]
	E	9.00[d]	104.67[c]
	F	12.00[c]	154.00[c]
Cutting stage	T_1	20.00[a]	226.00[a]
	T_2	11.88[b]	148.33[b]
cultivars		**	**
forage cutting		**	**

Experiment3: Results showed that there were significantly difference between seed planting with and without pod of six cultivars for seed bank, emergence, and seedling establishment. Cultivar *Medicago truncatula cv.* caliph and *Medicago scutella cv.* robinson had the highest emergence with 50.34% and 44.79% respectively. Robinson compare with other cultivars had larger seeds with less hardness so percentage of emergence was more. The least number of emergences about 29.57% was related to cultivar *Medicago rigidula cv.* Rigidula (table 5). This cultivar produced very small seeds with high hardness as that the water couldn't penetrate to it [23]. Cocks [24] suggested that at deep tillage (25 cm) and buried seeds in an unsuitable depth, seedling established were reduced. He also showed that planting seeds of annual medics with pod due to seed hardiness had more stability in soil. Percentage of emergence and established seedling in seeds planting without pod was more than planting with pod. So, seedling which emerged and established earlier because of optimum use of growth factors during growth period, had more seed yield and as a result had a positive effect on seed bank in soil.

Results also showed there were significant differences between depth of burial and percentage of emergence and the depth of 2 cm have superiority to the other depth (table 6). Cultivar *Medicago scutella cv.* Robinson and *Medicago truncatula cv.* Caliph had the highest percentage of emergence at depth of 2cm with 69.80% and 66.11% respectively and the least emergence about 6.84% was related to *Medicago rigidula cv.* Rigidula at depth of 6cm. Cultivar *Medicago truncatula cv.* Caliph also produced most seed bank in the soil with 798 seed/pod at the depth of 2cm and the least seed bank at this depth produced by *Medicago scutellata cv.* Kelson with 172 seed/pod. *Medicago rigidula cv.* Rigidula at depth of 6cm produced the least seed bank with 48.14 seed/pod (table 6).

Interactions of burial depth of seeds in soil and seed planting with and without pod showed that the highest percentage of emergence was about 67% belongs to seed planting without pod in depth of 2 cm of *Medicago scutella cv.* Robinson (Table 7). Although This cultivar produced seed bank about 422 pods /m², *Medicago truncatula cv.* Caliph had the highest seed bank with 529 pods/m². This cultivar was the best cultivar for successful rotational in cereal–legumes system, and the weakest seed bank in the soil was related to *Medicago Scutellata cv.* Kelson with 113.61 pod/m². Amezine [26] reported that same results. The least percentage of seed emergence and seedling establishment belongs to *Medicago rigidula cv.* Rigidula (about 19%) with planting in depth of 6 cm and with pod.

Although Seed planting with pod kept viability for successful natural regeneration in ley–farming system [25] seed planting without pods helped them for having easily emergence and establishment. This experiment showed that seed planting without pod at depth of 2cm had the highest seed emergence and seeds planted at more depth not only delayed seed emergence, but also seedling establishment was weaken and planting at more than 2cm has a negative effect on seed bank. Late season is one of the most reasons for producing weak seed bank in this cultivar, so it couldn't complete its growth and produced enough seeds.

Table 5: Interaction of depth seed burial, seeds planting with and without pod of annual medices seeds germination and established seedlings.

cultivars	Seeds germination and Established seedlings (%)	seed bank pod/m^2
A	**50.34^{a}**	353.85^{ab}
B	41.18^{b}	**113.61^{bc}**
C	29.57^{c}	126.68^{bc}
D	**44.79^{ab}**	**425.42^{a}**
E	41.78^{b}	216.77^{b}
F	44.58^{ab}	220.97^{b}

Table 6: Means of seed bank, seed germination and established seedlings at depth of burial and planting with and without pod of annual medics cultivars

Treatments		Seeds germination and established seedlings (%)	seed bank pod/m^2
cultivars	Depth of burial		
	2cm	**69.80^{a}**	555.08^{ab}
A	4cm	49.01^{b}	380.50^{c}
	6cm	21.11^{f}	106.76^{h}
	2cm	59.45^{bc}	172.65^{fgh}
B	4cm	40.49^{cd}	115.91^{hi}
	6cm	23.88^{f}	52.25^{i}
	2cm	50.94^{bc}	194.58^{fg}
C	4cm	30.65^{ef}	138.33^{gh}
	6cm	**6.84^{g}**	**48.14^{i}**
	2cm	**66.11a**	**798.75^{a}**
D	4cm	41.40^{cd}	435.67^{c}
	6cm	26.92^{f}	63.75^{i}
	2cm	53.45^{bc}	385.66^{cd}
E	4cm	64.77^{cd}	208^{fg}
	6cm	25.48^{f}	56.16^{i}
	2cm	58.20^{bc}	376^{cd}
F	4cm	50.34^{d}	213.^{6fg6}
	6cm	24.63^{ef}	73.25^{i}

Table 7: Interaction of depth seed burial, seeds planting with and without pod of annual medices on seed bank

	Planting seeds with pod		Planting seeds without pod	
Cultivars	Seeds germination and established Seedlings (%)	seed bank pod/m^2	Seeds germination and established Seedlings (%)	seed bank pod/m^2
A	33.60^{ef}	285.38^{bc}	67.09^{a}	422.33^{ab}
B	24.80^{fg}	85.90^{ef}	57.55^{ab}	141.27^{ef}
C	19.02^{g}	101.94^{ef}	40.15^{cde}	151.44^{de}
D	25.64^{fg}	21.79^{b}	63.96^{ab}	529.17^{a}
E	31.28^{ef}	113.86^{ef}	52.46^{bc}	319.61^{b}
F	42.36^{c}	170.88^{de}	46.57^{bc}	271.06^{c}

Experiment4: Methods of planting showed that there was a significant difference between dry pressing deeper (S_1) and planting with centrifuge (S_2). In planting method S1 maximum of seed bank and seed yield were related to *Medicago truncatula cv.* Caliph with 188 g/m^2 and 990pod/m^2.These Results were similar with other researchers [19,22]. *Medicago scutellata cv.* Kelson in both planting methods(S1,S2) had the least seed bank with 46 and 12 pod/m^2 respectively, thus it couldn't make a rich seed bank in order to natural regeneration in ley –farming system (table 8). Similar results have been reported by other researchers [21].

Influence of method S1 was more than method S2 on enrichment of seed bank, because of seed planting at the center of the furrow and compacting soil on seeds, which made a good situation for seed

emergence at field. Seed growth period was simultaneous with favorable rainfall conditions, thus seed bank obtained, increased (Table 8). Young et al. [16] suggested that delayed emergence and seedling establishment reduced seed yield. Methods S2 in comparing with methods S1 due to delaying at seeds emergence produced low seed and dry matter. At planting method S1, growth period of cultivars due to earlier emergence and suitable establishment of seedling was longer than (about 22 days). Crawford *et al.* [18] suggested that emergence and production of vigorous seedlings and earlier suitable establishment made better condition for using of production factors during growth period and as a result seed yield increased. They also reported that the more growth period, the more dry matter produces.

Table 8: Description of total dry matter, dry forage, seed bank and seed yield in planting methods experiment

treatment	seed bank pod/m^2	seed yield G/m^2
APS_1	458.17^{b}	139.93^{b}
APS_2	285.17^{d}	76.18^{cd}
BPS_1	46.67^{gh}	34.41^{fg}
BPS_2	12^{h}	11.84^{g}
CPS_1	101.67^{fg}	70.66^{cd}
CPS_2	60.33^{gh}	26.73^{fg}
DPS_1	960.33^{a}	188.23^{a}
DPS_2	370.67^{c}	84.03^{c}
EPS_1	178.83^{e}	111.15^{c}
EPS_2	121.17^{efg}	73.55^{cd}
FPS_1	158^{ef}	103.15^{c}
FPS_2	88^{fg}	56.99^{de}

S1=Deeper pressing method
S2=Centrifuge method

CONCLUSION

It is recommended that fallow–wheat system can be replaced by wheat–annual medics system. Also, it's better to use intercropping for seed bank enrichment of annual medices. In addition, forage yield of *Medicago truncatula cv.* caliph at the beginning of flowering was reasonable related to 50% flowering, because of better regeneration after cutting and can produce enough seed for seed bank in order to natural regenerate. Agronomical activates such as planting depth in 2cm and planting methods with deeper dry land pressing should be considered in planting annual medics.

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