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Role of Functional Groups of Dung Beetles on Secondary Seed Dispersal by Removing Sheep Dung (Case Study: Shahrekord Rangelands)

Mahdieh Ebrahimi[1]*, Iraj Rahimi Pordanjani[2] and Pejman Tahmasebi[3]

[1]*Assistant Professor, Department of Range and Watershed Management, University of Zabol, Iran*
[2] *Former M.Sc. Student of Range Management, Department of Range and Watershed Management, University of Zabol, Iran*
[3] *Assistant Professor, Faculty of Natural Resources and Earth Sciences, University of Shahrekord, Iran*

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ABSTRACT The present study was conducted to assess the effect of dung beetles on sheep dung removal and seed dispersal in semi steppe rangelands of Shahrekord province with poor condition located in Iran. Therefore, the large (1 cm^2) and small size (1 mm^2) meshes were used and filled by sheep dung in six treatments. To evaluate the role of the insects in seeds translocation, three sizes of plastic beads used as seed mimic. The results illustrated that the maximum function of the insect was observed in the possible treatment of the presence of dwellers, absent of large tuneller and large roller beetles and present of small tuneller and small roller beetles. The least dung removal was calculated in the control treatment and possible treatment of dwellers, large and small tunneler and also absent of large rollers and presence of small rollers respectively. Seed removal decreased in the order of small size (29) > medium size (5) > large size (2). In general, dung beetles play an important role in dung removal and secondary seed dispersal, but their function relates depended on habitat condition. In a degraded ecosystem, this insect will disappear. Considering the various factors affecting the function of these insects, further studies would be needed for investigating deeply different seasons and dung of different livestock feeding on the rangelands.

Key words: *Decomposers, Rangeland ecosystem, Scarabaeoidea, Seed fate*

1 INTRODUCTION

More than 57% of organisms are insects (Stork, 1997) which perform a variety of functions in ecosystems (Didham *et al*., 1996). A group of insects is dung beetles (*Coleoptera*: *Scarabaeidae*) that they are important decomposers that use faeces as a source of food and nesting material. They also provide the ecological services of secondary seed dispersal and dung removal (Davis *et al.*, 2001).

According to how the resource is used in breeding, dung beetles are divided into three functional groups: the rollers or telecoprids (those that roll balls of food on the surface of soil to some distance from the source of resource, where they bury them); tunnelers or paracoprids (those that carry food resource into the soil, making tunnels on the side or below the resource), and dwellers or endocoprids (which do not reallocate food, using it directly in the source) (Hanski and Cambefort, 1991; Halffter and Edmonds, 1982). The activities of

**Corresponding author: Assistant Professor, Department of Range and Watershed Management, University of Zabol, Iran, Tel: +98 913 146 4893, E-mail: maebrahimi2007@uoz.ac.ir*

dung beetles are linked to a wide variety of ecological processes (Nichols *et al*., 2008) such as improved soil structure (Bang *et al.*, 2005), enhancing nutrient cycling (Yamada *et al*., 2007), secondary seeds dispersal (Braga *et al*., 2013), reducing parasite populations on dung (Tyndale-Biscoe and Vogt, 1996), enhancing total nitrogen and phosphorus of plants as well as their yield (Miranda *et al*., 2001). Therefore, when evaluating the role of the other organisms, information of the ecological role of the species should be included (Nichols *et al*., 2007).

There are more than 124 millions animal units in Iran (Azarnivand and Zare Chahouki, 2010). According to the statistics, about 52117000 of them are sheep (Sabri *et al.,* 2011). A sheep defecate usually 19-26 times per day and each dung patch covers an area of only 0.018 m^2 (Williams and Haynes, 1995). Studies showed that with an average of 10 dung events each day for sheep, 305 m^2 are fouled each year by one animal (Bornemissza, 1960). However the manure that is produced by grazing animals is a good source of organic matter (West and Nelson, 2003) and typical nutrient application rate in sheep dung patches are 130 kg N ha^{-1}, 50 kg K ha^{-1}, 35 kg P ha^{-1}, 13 kg S ha^{-1} (Chambers *et al*., 2001), sheep avoid grazing forage near their feces on rangeland (Dohi *et al*., 1991) and fouling of these areas encourages weed growth, causing a further refusal and loss of grazing area when dung is not rapidly disintegrated (Arnaudin, 2012). The dung beetles are known as decomposer organisms in natural ecosystems (Bornemissza, 1960) that dung from different herbivores species such as sheep might significantly attract different species of dung beetles (Martin-Piera and Lobo, 1995). The importance of these insects in the removal of organic matter makes them an essential element in maintaining and regulating of terrestrial ecosystems (Halffter and Matthews, 1966).

Dung beetles assume a role as secondary seed dispersers; they bury faeces in the soil with seeds inside (Andresen and Feer, 2005). Therefore, they could have also contributed to enhanced seed germination (Andresen, 2002), favoring soil seed bank development (Hondt *et al*., 2007). Thus, seeds embedded in faeces could have the advantages of decrease in seed removal by predators (Vander Wall and Longland, 2004).

Most of the studies on ecological functions of dung beetles have done in tropical and semi tropical ecosystems, whereas there is a lack of information about the insects in semi steppe ecosystems. Also, Researches indicated that dung beetles response negatively to the fragmentation and transformation of natural habitats (Chandra and Gupta, 2012) and they may be disappeared in unsuitable habitat condition. The objectives of this study were: (1) to evaluate ecological functions of dung beetles in sheep dung removal in poor condition of semi steppe rangelands of Chaharmahal va Bakhtiari Province, for the first time in Iran; and (2) to identify their effect on secondary seeds dispersal in relation to seeds dispersal related to habitat condition.

2 MATERIALS AND METHODS

2.1 The study area

The study area is located in Sharekord rangelands (50° 46′55″- 50° 55′54″E, 32° 19′ 35″-32° 26′01″N), Chaharmahal va Bakhtiari Province, Iran. The regional climate is classified as cold and arid (Köppen), with an annual average precipitation of 284.8 mm, and maximum and minimum average temperature of 20 and 2 ^{0}C. The average height of the area is 2385 meter of sea surface level. The study site is covered mainly by perennial and invasive plants with a very poor condition (Aali *et al.,* 2010).

2.2 Experimental set-up

The study was carried out during July 2013 to investigate the role of dung beetle functional groups in sheep dung removal and secondary seed dispersal. In order to avoid interference of the herbivores with the experimental set-up, an exclosure with at least a surface area of approximately 100 m^2 was erected (60 days) and surrounded by a mesh that did not let the large grazers into the exclosure. To further facilitate the measurement of ecological functions, we first cleared the soil surface of each arena of litter and vegetation (Braga *et al*., 2013).

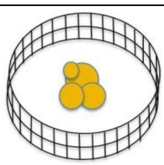
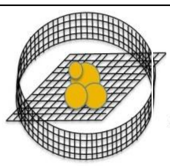
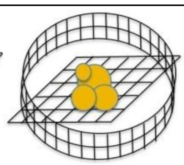
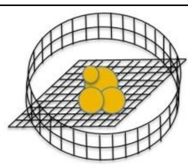
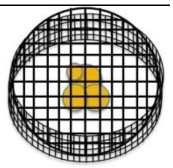
The relative impact of each dung beetle functional group was calculated in 6 possible treatment using 6 replication per treatment (Table 1). In the first treatment did not apply any mesh and in order to identify the type of dung beetles, the traps contained 100 ml of a salt solution in plastic glasses (The salt solution was used to keep the beetles from putrefying and risk of predators). Treatments of two to five were set to assess effects of various types of dung beetles on dung removal and seed dispersal.

According to the size of the insects (Braga *et al*., 2013; Slade *et al*., 2007); to prevent large and small rollers, large and small tuneller and dwellers (which are rather small species) to remove the dung from the site, dung was placed on 60×60 cm^2 of nylon mesh with a mesh size of 1cm^2, while the action of small and large tuneller was prevented by using a mesh size of 1 mm^2 (Table 1). Similarly, the action of small and large rollers was manipulated by surrounding sheep dung with cylinders with a mesh size of respectively 1mm^2 and 1cm^2. The combination of cylinders and squares included or excluded different functional groups, while a positive control plot estimated dung removal by all functional groups (treatment 1) and a negative control plot excluded all dung beetle groups, and admittedly all other flying and soil dung fauna (treatment 6) accounted for dung removal by other organisms or processes. Dung beetles were sampled using pitfall traps, which were plastic containers 15 cm tall and 30 cm in diameter.

2.3 Study on dung beetles function and their role in seed dispersal

In the center of each possible treatment, we placed an experimental dung pile consisting of 334.2 g of fresh sheep dung. Inside each experimental dung pile, we placed seed mimics of three sizes (assessing seed dispersal by dung beetles): 6 mm diameter (50 beads), 3 mm diameter (50 beads) and 1mm diameter (50 beads) representing large, medium and small seeds. Seeds mimic have the great advantage of not being removed by seed predators (Slade *et al*., 2007).

Table 1 All possible combinations of treatments in the dung removal experiment including and excluding different dung beetle functional groups

	1	2	3	4	5	6
Possible treatment						
Dung beetle functional group	D+ T+t+ R+r+	D+ T+t+ R-r+	D+ T-t- R-r-	D+ T-t+ R-r+	D+ T-t- R-r+	D- T-t- R-r

D=dwellers, T=large tuneller, t=small tuneller, R=large rollers, r=small rollers, + and – signs represent the contribution of the different groups (Slade *et al*., 2007).

Ecological functions were measured 60 days after the placement of the experimental dung piles. The dung remaining on the soil surface was removed and dried in a microwave oven (Memmert) at 70 ^{0}C (24h) until it reached a constant weight. The difference between secondary dry weight of dungs and primary dry weight was calculated to identify the amount of sheep dung removal by dung beetles (Slade *et al*., 2007). All seeds mimic present in the remaining dung were removed, counted and weighed. The weight of seed mimics was subtracted from the dung weight to obtain the net amount of dung remaining and then the amount of dung removed by beetles was calculated. In addition separation effect of the function group of dung beetles were estimated by subtracting the amount of dispersed seeds by the beetle (beetles) which were presented in each functional group.

2.4 Statistical analysis

All data were checked for their normality and homogeneity of variance, and where necessary, data were log-transformed before statistical analysis. All experimental results were statistically analyzed using the SPSS 18. Statistical package. Data in the text were expressed as means ±standard deviation. It is important to notice that average dry weight of secondary dungs that removed was considered to statistical analysis. In this way, weight of the seeds mimic has been deducted from the weight of initial dung. The statistical significance of the differences between groups was evaluated by analysis of variance (ANOVA). Duncan t-test between means was calculated only if the F-test was significant at the 0.05 level of probability. A probability of 0.05 or lower was considered as significant.

3 RESULTS

3.1 Impact of dung beetle assemblage in dung beetle removal

Different possible combinations in the removal of sheep dung showed significant differences among dung beetle functional groups (p=0.00) (Figure1). The maximum amount of dung removal was measured in forth treatment which investigated possible treatment of presence of dwellers, absent of large tuneller and large roller beetles and present of small tuneller and small roller beetles. Proportion of dung removal in this treatment was 41.87% of total used dung. The least dung removal was measured in control treatment (0.025%, absent of beetle) and second treatment (21.03%), respectively.

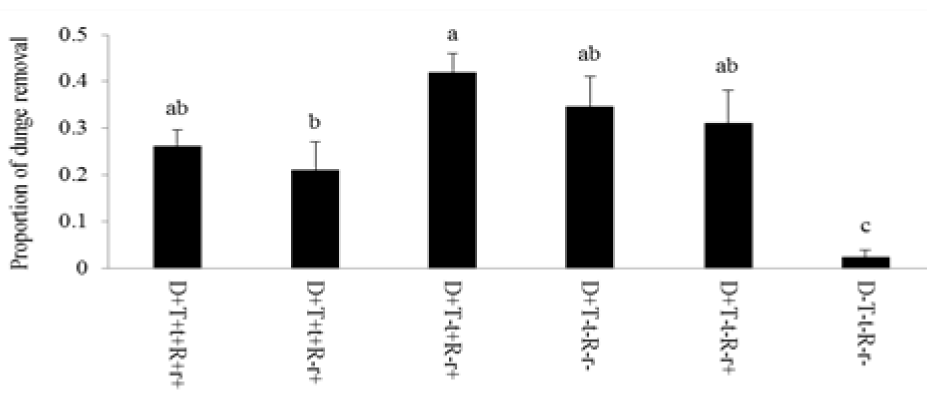


Figure 1 Proportion of dung removal by dung beetle functional groups. Vertical bars show standard deviation

*D=dwellers, T=large tuneller, t=small tuneller, R=large rollers, r=small rollers. + and – signs represent the contribution of the different groups. Values within vertical bars followed by the same letter do not differ significantly (p‹0.05, post hoc Duncan test)

3.2 Impact of dung beetle assemblage on secondary seed dispersal

The Impact of five dung beetle functional groups on the secondary dispersal of seeds indicated that there was significant differences among dung beetle functional groups in dispersal of total seed mimics (p=0.03) (Figure 2). The maximum total seed dispersal was measured in third treatment which investigated in possible treatment of presence of dwellers beetles. The least total seed dispersal was measured in second treatment, which investigated possible treatment of presence of small and large dwellers, small and large tuneller, absence of large rollers and presence of small rollers. In case of small seed mimics (Figure 3), there was significant difference amongs investigated possible treatments (p=0.03). The maximum small seeds dispersal was observed in the third treatment that 35.66% of small seeds were dispersed, and the lowest small seeds dispersal was observed (25.16) in the second treatment

Impact of dung beetle functional groups on the secondary dispersal of medium (Figure 4) and large (Figure 5) seeds size was not significant (p=0.07). The maximum of dispersed medium seeds was calculated in the third and second treatment (6% and 3%, respectively) and for the large seeds; the greatest seeds dispersal (3.50%) was calculated in forth treatment. The lowest large seeds (1.33%) dispersal was calculated in the second treatment. Impact of seed size on dung beetles efficiency in dispersal of seeds occurred in the sequence small seeds>medium seeds> large seeds (Figure 6).

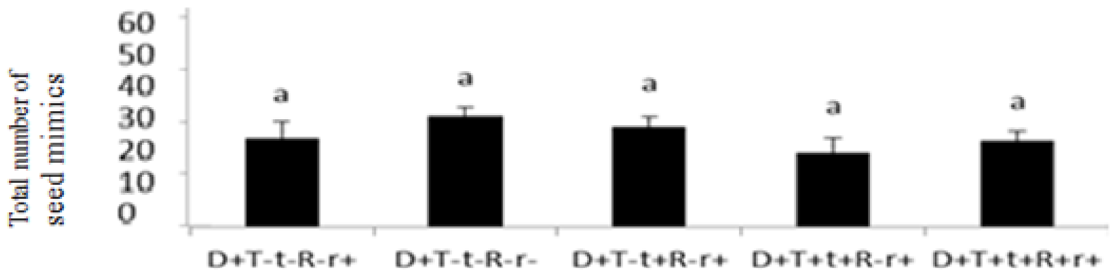


Figure 2 Number of seed mimics removal inside of sheep dung. Vertical bars show ±Standard Deviation

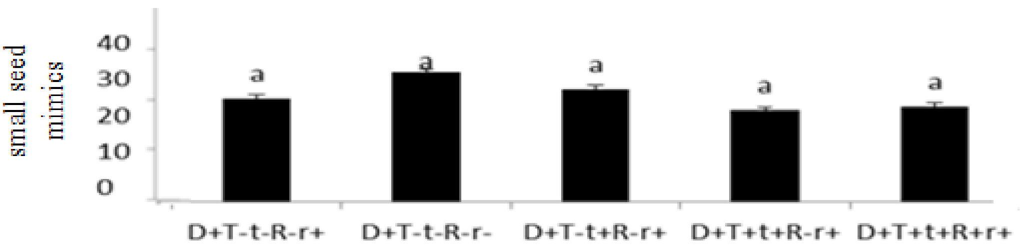


Figure 3 Number of small seed mimics removal inside of sheep dung. Vertical bars show ±Standard Deviation

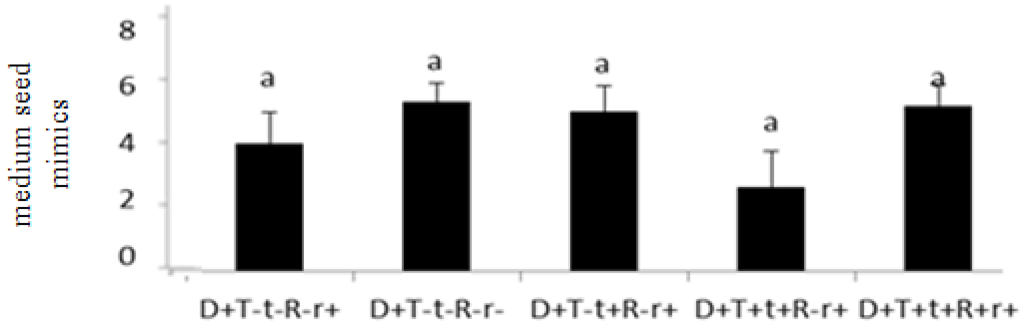


Figure 4 Number of medium seed mimics removal inside of sheep dung. Vertical bars show Standard Deviation

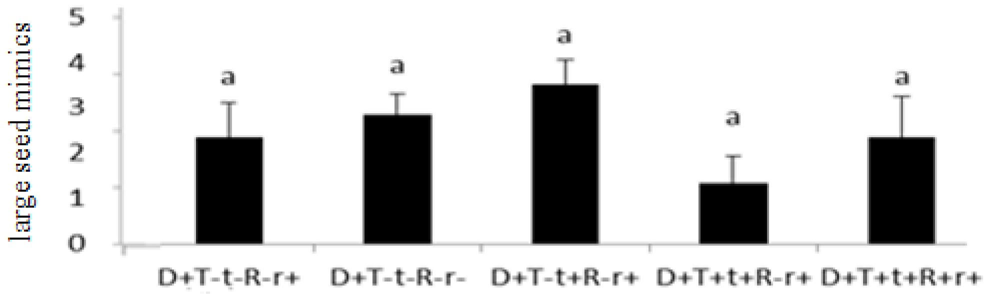


Figure 5 Number of large seed mimics removal inside of sheep dung. Vertical bars show Standard Deviation

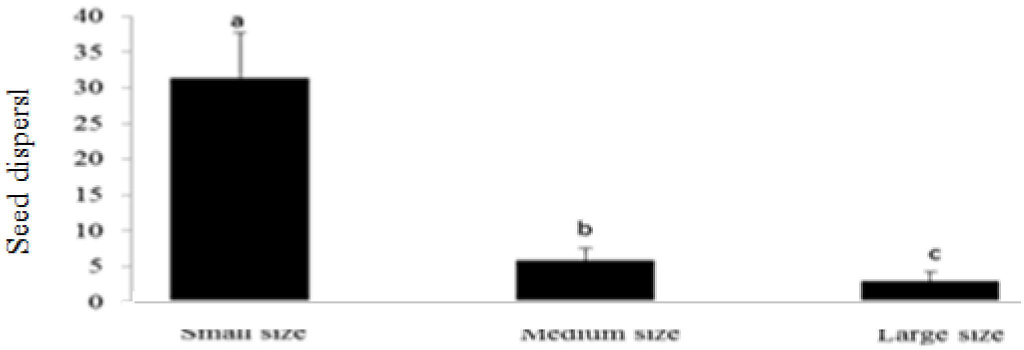


Figure 6 Impact of seed size on seed dispersal. Vertical bars show Standard Deviation

3.3 Separation effect of functional group of dung beetles in seeds dispersal

For estimating separation effect of functional group of dung beetles, for example by subtracting the first column (DTtRr) and second (DTtr), the role of large rollers (R) was founded (in this case, DTtRr-DTtr=R). Comparison among treatments for separating the effect of functional group of dung beetles in seeds dispersal (Table 2) showed that in small and medium seeds size and total translocated seeds, functional group of large and small tunllers and small rollers had the greatest role (-10.5%, -3%, -15% respectively) in seeds dispersal. In case of large seed mimics, large tunellers (2.1%) showed the most important role. The lowest role in seed dispersal for all seed size and total translocated seeds related to small rollers (-7.16%), large rollers (2.83%), small tunellers (1.16%) and small rollers (-9.16%).

Table 2 Separation effects of functional group of dung beetles in seeds dispersal

		DTtRr	DTtr	D	Dtr	Dr
DTtRr	S					
	M					
	L					
	T					
	F					
DTtr	S	1				
	M	2.83				
	L	1				
	T	4.83				
	F	R				
D	S	-9.50	-10.50			
	M	-0.16	-3			
	L	-0.50	-1.50			
	T	-10.16	-15			
	F	TtRr	Ttr			
Dtr	S	-4.83	-5.83	-4.63		
	M	0.16	-2.66	-0.33		
	L	-1.16	-2.16	0.66		
	T	-5.83	-10.66	-4.33		
	F	TR	T	tr		
Dr	S	-2.33	-3.33	-7.16	2.50	
	M	1.33	-1.50	-1.50	1.16	
	L	0	-1	-0.50	1.16	
	T	-1	-5.83	-9.16	4.83	
	F	TtR	Tt	r	t	

* In each row and column of the table, only the probability of the presence of different functional groups of dung beetles was considered to compare the functional groups that likely play a role in the considered treatment.

4 DISCUSSION

The results indicated that the maximum amount of dung removal was measured in the fourth treatment which investigated possible treatment of the presence of dwellers, absent of large tuneller and large roller beetles, and present of small tuneller and small roller beetles.

In the study, rangeland vegetation cover was poor in condition and in some areas of the rangeland, degradation was observed. Several studies indicated that dung beetles response negatively to the fragmentation and transformation of natural habitats (Chandra and Gupta, 2012) and the large dung beetles may be disappeared in unsuitable habitat condition. Therefore, due to the possible presence of small dung beetles, dung removal was not high. Because, small beetles remove more smaller piece of dung than large piece (Ong *et al.*, 2013).

There are significant physical and chemical differences in dung quality between dung types (Louzada and Silva, 2009). However dung beetles have used dung of different animals such as cattle, hours, goat and sheep, knowledge on the dung beetles assemblage that utilized sheep dung as a resource is scarce through the world; this lack of information is even more pronounced for the neo-tropical region (Correa *et al*., 2013).

Some studies showed that larger beetles require and use large amounts of dung and bury larger number of large seeds, but smaller amounts of dung which often contain large quantities of small seeds seem to attract beetles of small size (Andresen, 2001, 2002, 2003).

Faeces from sheep were considered only in a few studies. One quite obvious feature of sheep dung is that sheep produce two different types of droppings: 1. compact lumps, which vary widely in size, or 2. small pellets, which are deposited in groups or isolated as single pellets. In contrast to cow dung, which develops a compact crust on its surface, sheep droppings are able to rehydrate by dew or during rainfall (Sowig and Wassmer, 1994).

In study on role of dung beetles in French Mediterranean region Lumaret and Kirk (1987) defined two principal models of faeces, one represented by cow pads and the other by sheep droppings. They showed that sheep dung does not remain usable over a long period, while cow pads are used in all seasons by burying species of large.

Approximately 80% of the nitrogen content in sheep dung is denatured by bacteria and volatilization when left on the rangeland surface (Gillard, 1967). Several studies have shown that grass growth was benefited when the nutrients present in dung was quickly recycled within the rangeland ecosystem by dung beetles (Bornemissza, 1960; Holter, 1977). Yamada *et al*. (2207) report a significant positive relationship between the magnitude of released inorganic N and available P and K in sheep dung and dung beetle abundance. Martín-Piera and Lobo (1996) in the study of diversity and ecological role of dung beetles in Iberian grassland reported that these insects have significant impact on yield of rangeland ecosystems through live stocks dung decomposition. The researchers showed that a diverse population of these insects could remove 80 percent of live stocks dung on the rangeland and thus, increase nutrient cycling and energy flow in the other levels of the food chain.

In the present study, small beads were buried more often than large beads that it depends on beetle size or bait size. Feer *et al*. (2013) in their study on effect of dung beetle activities on the soil seed bank structure by monkey dung using plastic beads (1.3-5.8 mm) reported that smaller seeds are buried in greater amount and at greater depth than larger seeds.

The results revealed that the lowest seed mimics dispersal was observed in the first treatment (presence of dwellers, small and large tuneller and small and large rollers).

Andresen (2003) in a study of dung beetles in a central Amazonian rainforest and their ecological role as secondary seed dispersers using monkey dung with three different seed species: *Helicostylis scabra* (seed size: 5±0.2), *Pourouma guianensis* Aubl (seed size: 11±0.8 mm), and *Pouteria durlandii* (seed size: 27±0.7) reported that dung beetles could significantly increase seed removal and burial and probability of seedling successful establishment. Larger size of beetles is capable than the small size of beetles in terms of seeds burial. Additionally, they are likely to be more important in secondary seed dispersal. Data showed that the control treatment had the lowest proportion of dung removal. This treatment was set to control the effect of the other dung beetle functional group. Therefore, in the treatment, dungs may be moved by secondary dispersal agent (environmental factors).

The results indicated that in the absence of large species, the other functional group of beetles shows more competition for food sources. In other words, larger-bodied dung beetles limit activity and competition among the smaller species (<10 mm). To better understand of function of dung beetles, more studies would be needed for investigating different seasons on rangelands. Because, the activity of these insects depends on many factors such as seasonal changes (Hanski and Cambefort, 1991), vegetation cover (Escobar *et al*., 2007), light intensity (Ratcliffe and Paulsen, 2008), temperature (Atkinson, 1994) and habitat structure (Nichols *et al*., 2008; Davis *et al*., 2001).

In general, according to a variety of researches about dung beetles, these insects play an important role in live stocks dung removal, seeds dispersal (Andresen, 2002) and regeneration of plant species (Vega *et al*., 2011). Relationship between the amount of dung and the probability of a seed being buried is ecologically important because it links secondary seed dispersal (movement of seeds by dung beetles) to primary seed dispersal (defecation of seeds by arboreal mammals) (Andresen, 1999). Secondary dispersal of seeds occurs in many dispersal systems, and although less studied than primary dispersal, it is becoming evident that this phase can add great complexity to the seed dispersal ecology of plants (Andresen, 2001).

Seed dispersal is advantageous for plants because the seeds maybe deposited in sites in which conditions are appropriate for seed survival (Howe and Smallwood, 1982) and seedling establishment (Vander Wall and Longland, 2004). Studies have shown that seed burial may even help maintain viability of dormant seeds (Borchert and Davis, 1989), decrease the negative effects of seed clumping and seedling competition (Howe, 1989), seeds survive (Estrada and Coates-Estrada, 1991), and rangelands cleaning (Jin *et al*., 2004).

5 CONCLUSION

As seen in this study, dung beetles in rangelands have great potential to affect sheep dung removal and thus plant regeneration, through their role as secondary seed dispersers. However, the correlation between dung removal and seed dispersal is not simple, and many factors add complexity to this interaction. The exact and complex mechanisms leading to this flexibility remain to be studied. In addition to, the results of the present study were to investigate a season (summer) of the year in the rangeland with poor conditions. Monitoring during different seasons can provide more certainty about the function of different groups of dung beetles and more accurate assessment about their activities. Overall, The earth would be one global ball of dung if it weren't for the activities of dung beetles and other insects.

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نقش گروه‌های عملکردی سوسک‌های سرگین‌خوار در پراکنش ثانویه بذرها به‌وسیله برداشت سرگین گوسفند (مطالعه موردی: مرتع شهرکرد)

مهدیه ابراهیمی[1]*، ایرج رحیمی پردنجانی[2] و پژمان طهماسبی[3]

۱-استادیار گروه مرتع و آبخیزداری، دانشگاه زابل، ایران

۲-دانش آموخته کارشناسی ارشد مرتع‌داری، گروه مرتع و آبخیزداری، دانشگاه زابل، ایران

۳-استادیار، دانشکده منابع طبیعی و علوم زمین، دانشگاه شهرکرد، ایران

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چکیده مطالعه حاضر به‌منظور بررسی تأثیر عملکرد سوسک‌های سرگین‌خوار در برداشت سرگین گوسفند و پراکنش بذور در مراتع نیمه استپی شهرکرد با شرایط فقیر، ایران انجام شد. بدین منظور توری‌های با اندازه سوراخ‌های بزرگ (یک سانتی‌متری) و کوچک (یک میلی‌متری) استفاده شد که به‌وسیله کود گوسفندی در شش حالت مختلف به‌عنوان تیمار پر شدند. برای ارزیابی عملکرد اکولوژیکی این حشرات، مهره‌های پلاستیکی در سه اندازه به‌عنوان بذرهای شبیه‌سازی شده استفاده شد. نتایج نشان داد که حداکثر عملکرد سوسک‌های سرگین‌خوار مربوط به تیمار احتمال حضور سوسک‌های اقامت‌گر، عدم حضور تونل‌گرهای بزرگ و غلتان‌گرهای بزرگ و حضور تونل‌گرهای کوچک و غلتان‌گرهای کوچک بود. حداقل مقدار سرگین برداشت شده مربوط به حالت شاهد (عدم حضور سوسک) و تیمار احتمال حضور سوسک‌های اقامت‌گر، تونل‌گرهای بزرگ و کوچک و هم‌چنین عدم حضور غلتان‌گرهای بزرگ و حضور غلتان‌گرهای کوچک بود. بذرهای جابه‌جا شده به‌ترتیب به‌صورت بذر کوچک (۲۹ عدد)> بذرهای متوسط (۵ عدد)> بذرهای بزرگ (۲ عدد) بود. به‌طورکلی این حشرات در برداشت سرگین دام و پراکنش ثانویه بذرها نقش مهمی ایفا می‌کنند، اما عملکرد آن‌ها وابسته به شرایط زیستگاه است. یک اکوسیستم تخریب‌یافته منجر به ناپدید شدن این حشره می‌شود. با توجه به تاثیر عوامل متعدد در عملکرد این حشرات، انجام مطالعات بیش‌تر طی فصول مختلف سال و هم‌چنین برای بررسی عملکرد این حشرات در سرگین دام‌های مختلف تغذیه کننده از پوشش گیاهی مراتع تأکید می‌شود.

کلمات کلیدی: *Scarabaeoidea*، اکوسیستم مرتعی، تجزیه‌کنندگان، سرنوشت بذر