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# Effects of Biofertilizer, Cowpat Ash and Phosphorus on Seed Yield of Alfalfa

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The aim of this study was to evaluate the effects of biofertilizer (*Bacillus megatherium var phosphaticum*), cowpat ash and phosphorus fertilization on seed yield and seed weight of alfalfa. The increasing rate phosphorus fertilizer affected seed yield and seed weight of alfalfa. The highest seed yield and seed weight obtained from the application of 150 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, the lowest was in without P<sub>2</sub>O<sub>5</sub> plots. The bacterium treatment significantly increased seed yield. Seed yield and 1000 seed weight were 346 kg ha<sup>-1</sup> and 1.82 g in without bacterium plots, respectively. In bacterium treated plots seed yield was 367 kg ha<sup>-1</sup>, 1000 seed weight was 1.84 g. Application of cowpat ash increased seed yield and seed weight but the increasing is not significant.

Key Words: Alfalfa, Seed yield, Biofertilizer, Cowpat ash, Phosphorus fertilization.

# **INTRODUCTION**

Alfalfa is one of the most well known and widely grown as a forage crop due to its good quality characteristics and high adaptability<sup>1</sup>. Because of its importance among forage crops, alfalfa (*Medicago sativa* L.) is referred to as queen of forages. It is the most important high-quality forage crop for livestock because of its high protein, vitamins, energy and digestibility<sup>2,3</sup>. High yield and quality allow it to be used in feeding programs for many different types of livestock. To produce this forage, a certain degree of management is required. It is important to pay attention to details before establishment, as well as throughout the life of the stand.

However, alfalfa is cultivated mainly for forage production and seed yield is considered to be of only secondary importance<sup>1,4</sup>. Seed yield of alfalfa (*Medicago sativa* L.) is important for effective distribution of alfalfa to farmers.

Proper alfalfa variety must be seeded at proper amount and planting time must be suitable for good stand. The weed pressure should also be minimized during the season to maintain healthy and productive stands<sup>5,6</sup>.

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However, nitrogen is required by plants in larger amounts than any of the other nutrient elements, the nitrogen acquired by legumes by symbiotic fixation. In alfalfa, seedlings depend on available soil nitrogen until nodulation occurs<sup>6</sup>. The excess nitrogen at planting actually is harmful because it inhibits nodulation and stimulates competition from weeds<sup>7</sup>. Phosphorus is one of the most important nutrient elements for production and quality. Because it has numerous biochemical functions in plants<sup>8,9</sup>, high levels of available phosphorus are required for maximum nitrogen fixation by legumes and the use of phosphorus fertilizer increases seedling vigor<sup>10</sup>. It also plays various roles in seed formation<sup>11</sup>. Another important nutrient element is potassium, crucial for some metabolic activity in plants<sup>12</sup>, for alfalfa<sup>7</sup>. However potassium is important nutrient for alfalfa, need not to be applied<sup>12,13</sup>, if it is adequate in soils<sup>13</sup>.

Soils usually can supply some nutrients, but phosphorus or potassium fertilizer often needs to be applied before and during the life of the stand, after soil tests<sup>3</sup>. Phosphorus is usually the most limiting nutrient in soil<sup>13</sup> because solubility of phosphorus is low in soil. Immobility of phosphorus in soil causes concern about the effectiveness of surface applications of phosphorus. Solution of phosphorus concentration depends on soil pH, amounts of some other soluble minerals, amount of phosphorus in soil and rate mineralization of organic matter<sup>12</sup>. Phosphorus fertilization increase soil phosphorus availability, but this effect decreased year by year<sup>14</sup> because a large portion of inorganic phosphates applied to soil as fertilizer is rapidly immobilized after application.

Large quantities of chemical fertilizes, resulting in high costs and severe environmental contamination. In agriculture, it is important to make use of microorganisms in order to reduce the use of chemical fertilizers as much as possible. Increasing and extending the role of biofertilizers would reduce the need for chemical fertilizers and decrease adverse environmental effects. Several bacteria may solubilize inorganic phosphate, making soil phosphorus otherwise remaining fixed available to the plants due to excretion of organic acids and through other mechanisms<sup>15</sup>. The organic fertilizers, as animal manure are also important for environmental contamination and economics, because organic fertilizers are much more conducive to the environment<sup>16</sup> and foods than the traditional chemical fertilizers. Cowpat is used as fuel for heating in rural regions of Turkey. But using of this organic fertilizer source in crop production is more important than in heating. However, cowpat is used for heating; the leftover of burned cowpat ash is not used for anything. Since the content of nutrient elements, cowpat ash can be used in crop production, especially legumes, to increase the value of this fertilizer source.

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For seed production, alfalfa has similar fertilizer needs as for alfalfa forage<sup>17</sup> and maintain a regular irrigation schedule is critical to promote good vegetative growth, during the pre-bloom period. At peak bloom, gradually decrease irrigation because generally, high biomass production results in low seed yield. If moisture content of the seed is above 13 %, there is risk of heating, seed damage and combine losses, harvest should begin when seed moisture is 13 % or less<sup>18</sup> or two-thirds to three-quarters of the seed pods have turned dark brown or black because then the damp enough to avoid the loss of seed pods<sup>19</sup>.

The purpose of this study was to evaluate the effects of cowpat ash, *Bacillus megatherium var phospaticum* and phosphorus fertilization on seed yield of alfalfa.

## EXPERIMENTAL

This study was carried out under irrigated conditions at the Ataturk University, between 2004 and 2006. The experiment was designed in a factorial arrangement of a randomized complete design replicated three times. 'Bilensoy' lucerne was planted in early May of 2004 and 2 year (2005 and 2006) results were evaluated.

The soil in the study area is silt loam (fine-loamy, mixed) with 0.60-1.20 % organic matter. Average pH was 7.60, available P 9 kg ha<sup>-1</sup> and available K 1000 kg ha<sup>-1</sup>. Consequently, the soil was slightly alkaline, poor in organic matter, poor-middle in available phosphorus and rich in available potassium.

Plots were fertilized with 50 kg N ha<sup>-1</sup> as ammonium sulphate; 0, 50, 100, 150 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> as triple superphosphate and 0, 10, 20, 30 ton ha<sup>-1</sup> cowpat ash (C), contains 5.5 kg ton<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and 33.0 kg ton<sup>-1</sup> K<sub>2</sub>O, was applied. Bacterium (*Bacillus megatherium var. phosphaticum*) (B) was diluted with water and applied on soil surface, then the plots, with bacterium, irrigated for mixed up in soil in autumn every year. Nitrogen was given only at establishment (2004), but phosphorus (P) and cowpat ash (C) were applied in autumn every year. The alfalfa was sown at the rate of 15-20 kg ha<sup>-1</sup> with 40 cm row spacing. Plots were irrigated when 50 % of available water in the soil was utilized. Irrigation continued at flowering time, after this period the plots were not irrigated<sup>20</sup>. Individual plots were  $1.5 \times 4 \text{ m} = 6 \text{ m}^2$  in size.

The average temperatures between April and September were 14.2 and 15.7 °C for 2005 and 2006, respectively and these average temperatures are higher than the long-term mean (14 °C). The annual precipitation between April and September were 320.9, 244,6 and 252.7 mm in 2005, 2006 and long-term mean, respectively.

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In this study, 1000 seed weight and seed yield were determined. Seed yield was determined by separated from harvested and dried samples (1  $\times$  $2 \text{ m}^2$ ) from the middle of each plot. 1000 Seeds yield were determined by counted and weighed.

The results were statistically evaluated by using JMP statistical package program<sup>21</sup>. Mean separations were made on the basis of least significant differences (LSD).

## **RESULTS AND DISCUSSION**

Seed yield of alfalfa due to phosphorus and bacterium (Bacillus megatherium var. phosphaticum) applications was statistically significant (p < 0.01). Cowpat ash applications had increasing effect on seed yield but this effect did not significant. Application of 150 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> (P<sub>3</sub>) increased seed yield (411 kg ha<sup>-1</sup>) and was different from the other applications of P<sub>2</sub>O<sub>5</sub>. The lowest seed yield (287 kg ha<sup>-1</sup>) obtained from without phosphorus plots (P<sub>0</sub>). Application of bacterium increased seed yield of alfalfa. With bacterium plots seed yield was 367 kg ha<sup>-1</sup>, it was 346 kg ha<sup>-1</sup> in without bacterium (B<sub>0</sub>) plots (Table-1).

SEED YIELD OF ALFALFA (Kg ha <sup>-1</sup> )									
В	Р	$C_0$	$C_1$	$C_2$	C <sub>3</sub>	Mean			
B <sub>0</sub>	P <sub>0</sub>	240	276	310	302	282			
	$\mathbf{P}_{1}$	334	332	330	292	322			
	$P_2$	364	366	374	380	371			
	$P_3$	402	423	412	397	409			
	Mean	335	34.9	35.7	34.3	—	346 B		
	$\mathbf{P}_{0}$	253	300	308	304	291			
$\mathbf{B}_1$	$\mathbf{P}_{1}$	372	368	368	360	367			
	$P_2$	40.0	40.8	38.0	39.1	39.5			
	$P_3$	432	404	416	405	414			
	Mean	364	370	368	365	_	36.7 A		
Means	$\mathbf{P}_{0}$	247	288	309	303	287 d			
	$\mathbf{P}_{1}$	353	350	349	326	345 c			
	$P_2$	382	387	377	386	383 b			
	$P_3$	417	414	414	401	411 a			
	Mean	350	360	362	354	-	356		

TABLE-1	
SEED VIELD OF ALEALEA	(Ka ha

LSD: Bacterium (0.01) = 0.82, phosphorus (0.01) = 1.15.

Phosphorus fertilization had significant (p < 0.01) effect on 1000 seed weight of alfalfa. Seed yield was higher in plots (1.95 g) treated with 150 kg ha<sup>-1</sup>  $P_2O_5$  and was differ from the other treatments of  $P_2O_5$ . The plants in Vol. 21, No. 1 (2009) Effects of Biofertilizer, Cowpat Ash & P on Seed Yield of Alfalfa 693

without added  $P_2O_5$  fertilizer gave the lowest 1000 seed weight (1.68 g). There were no significant difference between applications of 50 and 100 kg ha<sup>-1</sup>  $P_2O_5$  with regarding to seed weight. Applications of bacterium and cowpat ash had positive effect on 1000 seed weight but there were no significant differences among the treatments (Table-2).

TABLE-2

	1000 SEED WEIGHT OF ALFALFA (g)								
		$C_0$	$C_1$	$C_2$	C <sub>3</sub>	Mean			
	$P_0$	1.60	1.70	1.60	1.70	1.65			
	$\mathbf{P}_{1}$	1.90	1.86	1.80	1.90	1.87			
$\mathbf{B}_{0}$	$P_2$	1.70	1.90	1.90	1.90	1.85			
-	P <sub>3</sub>	2.00	2.00	1.90	1.80	1.93			
	Mean	1.80	1.87	1.80	1.83	_	1.82		
B <sub>1</sub>	$P_0$	1.60	1.70	1.70	1.80	1.70			
	$\mathbf{P}_{1}$	1.90	1.80	1.80	1.90	1.85			
	$P_2$	1.80	1.70	1.90	1.90	1.83			
	P <sub>3</sub>	1.90	2.00	2.10	1.90	1.98			
	Mean	1.80	1.80	1.88	1.88	_	1.84		
Mean	$P_0$	1.60	1.70	1.65	1.75	1.68 c			
	$\mathbf{P}_{1}$	1.90	1.83	1.80	1.90	1.86 b			
	$P_2$	1.75	1.80	1.90	1.90	1.84 b			
	$P_3$	1.95	2.00	2.00	1.85	1.95 a			
	Mean	1.80	1.83	1.84	1.85	_	1.83		

LSD: phosphorus (0.01) = 0.046.

Interaction of phosphorus and bacterium levels showed significant influence on seed yield.  $P_3B_0$  application produced highest seed yield (411 kg ha<sup>-1</sup>), whereas lower rate of phosphorus fertilizer produced lower seed yield, the lowest seed yield (282 kg ha<sup>-1</sup>) obtained from without fertilizer plots ( $P_0B_0$ ), did not differ from  $P_0B_1$  treatment plots. There was no significant difference among the  $P_3B_0$  and  $P_3B_1$  treatment plots. Between  $P_2B_1$  and  $P_3B_0$ ,  $P_1B_1$  and  $P_2B_0$  there were no significant differences (Fig. 1). Also interaction of phosphorus and cowpat levels had significant influence (p < 0.01) on seed yield. In addition to cowpat, increasing rate of phosphorus applications increased seed yield of alfalfa. Mean seed yield varied from 247 to 417 kg ha<sup>-1</sup> due to different levels of phosphorus and cowpat (Fig. 2).

Mean seed weight varied from 1.60 to 2.00 g, the lowest recorded for without phosphorus and cowpat application plots, application of phosphorus and cowpat tended to increase 1000 seed weight upto  $150 \text{ kg ha}^{-1} P_2 O_5 + 20$  ton ha<sup>-1</sup> cowpat application (P<sub>3</sub>C<sub>2</sub>) and it was differ from P<sub>3</sub>C<sub>3</sub> application (Fig. 3).

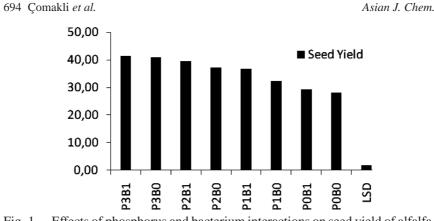


Fig. 1. Effects of phosphorus and bacterium interactions on seed yield of alfalfa. LSD bar is given at the end of treatments

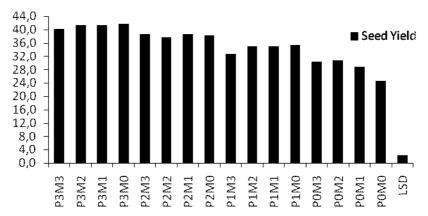


Fig. 2. Effects of phosphorus and cowpat interactions on seed yield of alfalfa. LSD bar is given at the end of treatments

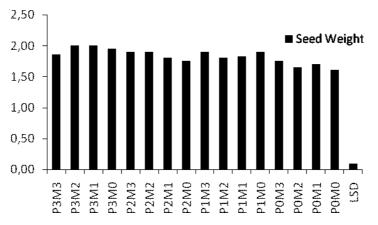


Fig. 3. Effects of phosphorus and cowpat interactions on 1000 seed weight of alfalfa. LSD bar is given at the end of treatments

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The results, obtained from present study showed that applications of phosphorus and bacterium increased seed yield. Seed weight was increased by only phosphorus fertilization. Cowpat ash increased seed yield and seed weight of alfalfa but these effects did not statistically significant. These results can most probably result from the increasing effects of phosphorus and potassium on seed production. Since alfalfa requires application of phosphorus and potassium for seed yield<sup>19,22,23</sup>. Phosphorus plays various roles in seed formation<sup>11</sup>, potassium effects seed production by effecting plant density. Since under potassium-deficient conditions, the legume plant density was reduced, thus potentially reducing seed production<sup>23</sup>. Bacterium (Bacillus megaterium var phosphaticum) have effects on solubility of phosphorus<sup>15</sup>, thus increased available phosphorus can be affected positively on seed yield of alfalfa. The interaction of cowpat ash and phosphorus also affected seed yield and seed weight, with related to combine effects of cowpat ash and phosphorus on seed yield, because cowpat ash contains phosphorus and potassium. Likewise, some research showed that phosphorus fertilization increased seed yield of some legume species<sup>11,24</sup>. These values were also similar with results of the study of Mermer and Serin<sup>25</sup> that applied the same rate phosphorus on alfalfa.

### Conclusion

The application of phosphorus fertilizer is important for seed yield of alfalfa. Biofertilizer (*Bacillus megatherium var phosphaticum*) and organic fertilizers as cowpat ash have increasing effects on seed yield of alfalfa. Biofertilizer (*Bacillus megatherium var phosphaticum*) increases availability of phosphorus in soil, by the effects on solubility of phosphorus. The use of biofertilizer and organic fertilizer is important both high yield and the need for reducing of chemical fertilizer.

#### REFERENCES

- 1. A.N. Iannucci and P.M. Di Fonzo, Field Crops Res., 78, 65 (2002).
- D.K. Barnes and C.C. Sheaffer, in eds.: R.F. Barnes, D.A. Miller, C.J. Nelson, Forages: An Introduction to Grassland Agriculture, Iowa State University Press, Ames, IW, USA, Vol. 1, pp. 205-216 (1995).
- 3. Anonymous, Oklohoma Cooperative Extension Services. Available: http://alfalfa.okstate.edu/pub/alfalfa-production/queen\_nav.htm (2001)
- 4. E.D. Bolanos-Aguilar, C. Huyghe, C. Ecalle, J. Hacquet and B. Julier, *Crop Sci.*, **42**, 45 (2002).
- 5. G. Bates, Agricultural Extension Service the University of Tennessee, SP434-C (1998).
- D.H. Smith, G.K. Beck, F.B. Peairs and W.M. Brown, Colorado State University Cooperative Extension. 10/99. No: 0703. www.colostate.edu/Depts/CoopExt (1999).
- 7. D. Cash and Z. Jingzhong, United Nations Food and Agriculture Organization and the Peoples Republic of China, pp. 4-11 (2003).
- D.C. Whitehead, Nutrient Elements in Grassland, Soils-Plant-Animal Relationships, CABI Publ., New York, USA (2000).

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- 9. K.W. Berg, S.M. Brouder, B.C. Joern, K.D. Johnson and J.J. Volenec, *Better Crops*, **87**, 20 (2003).
- 10. Anonymous, Food and Rural Initiatives. Manitoba Forage Council, Available: www.mbforagecouncil.mb.ca/Repository (2006a).
- 11. M.A. Hossain, A. Hamid and S. Nasreen, J. Agric. Res., 45, 119 (2007).
- 12. R.F. Follett and S.R. Wilkinson, in eds.: R.F. Barnes, D.A. Miller and C.J. Nelson, Forages, Iowa State University Press, Ames, Vol. 2, edn. 5, pp. 55-82 (1995).
- J.G. Davis, D.H. Smith and R.L. Croissant, Fertilizing Alfalfa and Grasses, Quick Facts, Colorado State University Extension, No. 0537 (1996).
- 14. A.M. María and A. Berardo, Better Crops, 89, 22 (2005).
- 15. R. Çakmakçi, Selçuk Üniversitesi Ziraat Fakültesi Dergisi, 19, 93 (2005).
- 16. A. Hasanzadeh-Gorttapeh, H. Doulati, A. Ebadi and H. Ranji, Available: http://www.fertilizer.org/ifa/publicat/PDF (2007).
- 17. D. Cash, United Nations Food and Agriculture Organization and the Peoples Republic of China, pp. 3-11 (2003).
- S.H. Husman, The University of Arizona Cooperative Extension, Available: ag.arizona.edu/pubs/crops/az1129.pdf (1999)
- 19. Anonymous, Manitoba Agriculture, Food and Rural Initiatives office, http://www.gov.mb.ca/agriculture/crops/forages/bjb00s02.htmL (2006b).
- 20. Y. Serin and M. Tan, Ataturk University, Publication No.190. Erzurum, Turkey, Ataturk University Press, p. 177 (2001).
- 21. SAS Institute, Cary, NC, USA: SAS Institute, Inc., p. 707 (2002).
- D.A. Miller and J.F. Stritzke, in eds.: R.F. Barnes, D.A. Miller, C.J. Nelson, Forages: An Introduction to Grassland Agriculture, Iowa State University Press, Ames, IW, USA, Vol. 1, pp. 89-105 (1995).
- 23. S.S. Malhi, K.M. Panchuk and M. Tremblay, Agriculture and Agri-Food Canada, Melfort, Saskatchewan. http://www.agriculture.gov.sk.ca. (2004).
- 24. B. Çomakli, Atatürk Üniversitesi Ziraat Fakültesi Dergisi, 21, 43 (1990).
- 25. A. Mermer and Y. Serin, Türkiye VII, Tarla Bitkileri Kongresi, 25-27 Haziran, Erzurum, 1, 277-280 (2007).

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