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Grain Spawn Production in *Agaricus bitorquis* (Quél.) Sacc.

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ABSTRACT

In this study the production of grain spawn prepared from *Agaricus bitorquis* (Quél.) Sacc., mycelium germinated at different temperatures was investigated. *A. bitorquis* fructifications collected from nature were grouped as A, B, C, D, and E. Malt extract agar was used as the agar media. The basidiospores of all five groups were germinated by a multispore method in malt extract agar in Petri dishes and primer mycelium was obtained. The mycelia agar discs were taken from primer mycelium and transferred to malt extract agar in Petri dishes and were developed at 30°C, 32°C, 34°C, 36°C, and 38°C separately, and secondary mycelium was obtained. For mycelium development the optimal temperature was 30°C and was therefore used as the control group. Grain spawn was prepared from this secondary mycelium. The wheat grains used for spawn production were incubated in 85-90% humidity and at 30°C. In the production of grain spawn the mycelia began to develop during the first and second shaking period, eventually covering the wheat grains completely. The covered mycelium was then incubated in the refrigerator and a grain spawn calendar was prepared.

Keywords: development of mycelium, high temperature, mushroom

INTRODUCTION

Mushroom seed is generally referred to as spawn. In nature mushrooms use spores for generative multiplication. *In vitro*, spores also need time to germinate and a pure culture of the desired mycelium is added to an appropriate substrate. The colonization of these substrates by mushroom mycelia is known as spawn running and the pure culture is called spawn (Stamets and Chilton 1983; Boztok 1987; Oei 1991; Gunay 1995; Arkan and Guler 1996; Stamets 2000). In 1932 (Stamets and Chilton 1983; Elliott 1985) and 1937 (Elliott 1985), Sinden invented grain spawn and he patented a new spawn-making process. Rye grain is the most popular substrate and corn, milo, millet, sorghum and wheat are also commonly used grains. Many large spawn producers prefer millet, a small kernel grain, because it stores well. Most small-scale gourmet mushroom growers utilize organically grown rye or wheat grain (Stamets and Chilton 1983; Elliott 1985; Erkel 1993). The distinct advantage of grain spawn is its vigour (Oei 1993). Spawn production is an important process and at spawning and during spawn running there are several factors that must be considered if yields are to be maximized. One of the factors is the temperature of the substrates (Stamets and Chilton 1983; Isık 1996).

In this study grain spawn production prepared from *Agaricus bitorquis* at different temperatures was examined.

MATERIALS AND METHODS

In this study, *A. bitorquis* fructifications as the organism and 2% malt extract agar (MEA) as the agar medium were used. The samples, which were collected from nature (*A. bitorquis* fructifications are found either above or underground singly or in groups (Guler 1999)), were grouped into five groups, labeled A, B, C, D and E. The spores, which were taken from each group (Stamets 2000), were inoculated at the agar center by the multispore method (Fritsche 1972). At the end of the 20-day incubation period, homokaryon primer mycelium was obtained which was transferred to the agar center as a single pellet, resulting in heterokaryon mycelium. Spore germination and mycelium development was carried out in the dark at 30ºC. The mycelial agar disc was taken from the primer mycelium and transferred to MEA in a petri dish, and the main culture was obtained. The mycelium of Groups A, B, C, D and E were developed at 30ºC, 32ºC, 34ºC, 36ºC and 38ºC, thus secondary mycelia were obtained. These heterokaryon mycelia were developed on wheat grain and spawn of each group was obtained separately. Regarding the use of grain spawn, the preparation was the same as for *Agaricus bisporus* (Fritsche 1978). The spawn was obtained from wheat grain because wheat is common in Turkey (Gunay 1995; Isık *et al*. 1997). In the production of grain spawn, mycelia began to develop, and during the first and second shaking periods, the mycelia covered the wheat grains completely; these were then placed into the refrigerator and the incubation period was measured and a grain spawn calendar prepared. The entire process is outlined in **Fig. 1**.

RESULTS

Spawn was developed at different temperatures (30ºC, 32ºC, 34ºC and 36ºC) for Groups A, B, C and D but at 38ºC spawn was not prepared because mycelia did not develop. In this study spawn development at 30ºC was used as the control group. 30ºC, 32ºC and 34ºC were also used on Group E but at 36ºC and 38ºC spawn was not prepared, as mycelia did not develop. During spawn development and on the first day of mycelium development, first and second shaking days and completion day of mycelium were observed.

The development of spawn prepared from mycelia in group A

In Group A the fastest spawn development was seen on mycelia cultured at 30ºC. In these cultures the first shaking was on the 15th day of incubation and on the 8th day of development. The second shaking was on the 22nd day of incubation and on the 15th day of development. The wheat grains were completed covered by the mycelium on the 29th day of incubation. In spawn cultures developed at 32ºC, the first shaking was on the 17th day of incubation and on the 8th day of development. The second shaking was on the 26th

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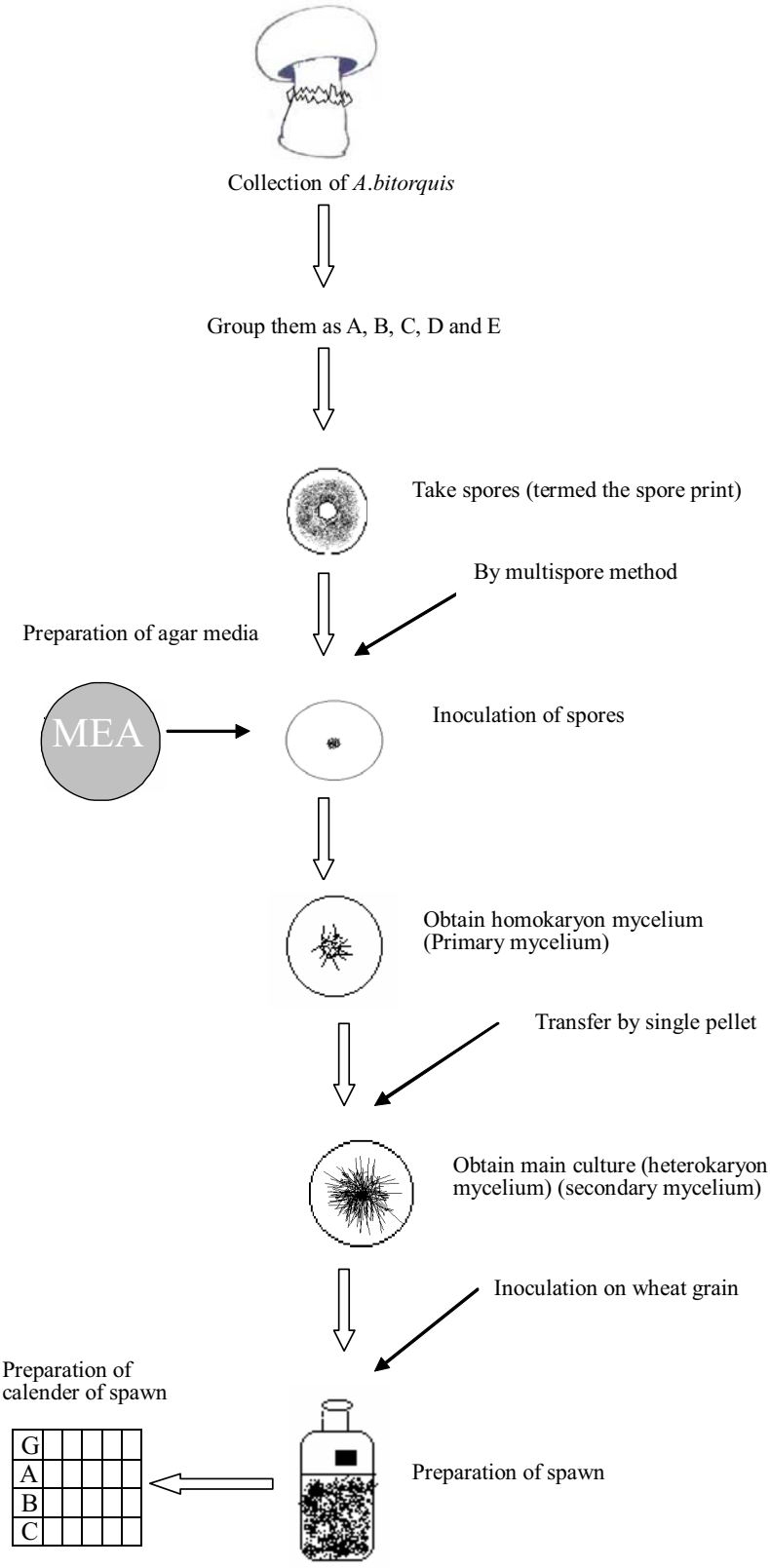

Fig. 1 Preparation of calender of spawn from the collection of *A.bitorquis* to spawn.

day of incubation and on the 17th day of development. The mycelium development was completed on the 34th day of incubation. The spawn that was cultured at 34ºC saw the slowest development in Group A. In these cultures the development was completed on the 37th day of incubation and the first shaking was on the 19th day of incubation and on the 9th day of development. The second shaking was on the 28th day of incubation and on the 18th day of development. Results from Group A are summarized in **Table 1**.

The development of spawn prepared from mycelia in group B

In group B, in spawn cultures improved at 30ºC, the first shaking was on the 12th day of incubation and on the 7th day of development. The second shaking was on the 19th day of incubation and on the 14th day of development. The mycelium development was completed on the 25th day of incubation. In the spawn cultures developed at 32ºC and 34ºC, the development began on the 7th day of incubation. In the spawn cultures improved at 32ºC; the first shaking was on

Table 1 Group A: growth results.

Temperature (ºC)	Condition	Day of incubation	Day of development	Observations
	1st shaking	15	8	The earliest development
30	2nd shaking	22	15	
	Put into refrigerator.	29	22	
	1st shaking	17	8	
32	2nd shaking	26	17	
	Put into refrigerator.	34	26	
	1st shaking	19	9	The latest put into refrigerator
34	2nd shaking	28	18	
	Put into refrigerator.	37	27	
36	The development of spawn was not seen.			

Table 2 Group B: growth results.

Temperature (ºC)	Condition	Day of incubation	Day of development	Observations
	1st shaking	12	7	The earliest development
30	2nd shaking	19	14	
	Put into refrigerator.	25	20	
	1st shaking	14	8	Development of beginning same as 34ºC
32	2nd shaking	21	15	
	Put into refrigerator.	28	22	
	1st shaking	15	9	Development of beginning same as
34	2nd shaking	24	18	32ºC. The latest put into refrigerator
	Put into refrigerator.	32	26	
36	The development of spawn was not seen.			

Table 3 Group C: growth results.

Temperature (ºC)	Condition	Day of incubation	Day of development	Observations
	1st shaking	17	8	The earliest development
30	2nd shaking	25	16	
	Put into refrigerator.	33	24	
	1st shaking	19	8	
32	2nd shaking	27	16	
	Put into refrigerator.	36	25	
	1st shaking	22	9	The latest put into refrigerator
34	2nd shaking	31	18	
	Put into refrigerator.	40	27	
36	The development of spawn was not seen.			

Table 4 Group D: growth results.

Temperature (ºC)	Condition	Day of incubation	Day of development	Observations
	1st shaking	9	6	The fastest development in this study
30	2nd shaking	15	12	
	Put into refrigerator.	21	18	
	1st shaking	12	7	
32	2nd shaking	19	14	
	Put into refrigerator.	25	20	
	1st shaking	11	6	
34	2nd shaking	18	13	
	Put into refrigerator.	26	21	
36	The development of spawn was not seen.			

Table 5 Group E: growth results.

Temperature (ºC)	Condition	Day of incubation	Day of development	Observations
	1st shaking	22	10	
30	2nd shaking	31	19	
	Put into refrigerator.	40	28	
	1st shaking	26	11	
32	2nd shaking	37	22	
	Put into refrigerator.	45	30	
	1st shaking	29	12	The latest development in this study
34	2nd shaking	39	22	
	Put into refrigerator.	47	30	

the 14th day of incubation and on the 8th day of development. The second shaking was on the 21st day of incubation and on the 15th day of development. The mycelium development was completed on the 28th day of incubation. The slowest development in group B was seen with spawn improved at 34ºC. In these cultures development was completed on the 32nd day of incubation and the first shaking was on the 15th day of incubation and on the 9th day of development. The second shaking was on the 24th day of incubation and on the 18th day of development. Results of group B are summarized in **Table 2**.

The development of spawn prepared from mycelium in group C

Spawn development of group C was much slower than Groups A and B. In the spawn cultures improved at 30ºC, the first shaking was on the 17th day of incubation and on the 8th day of development. The second shaking was on the 25th day of incubation and on the 16th day of development. Mycelium development was completed on the 33rd day of incubation. In the spawn cultures improved at 32ºC, development began on the 12th day of incubation. In these cultures; the first shaking was on the 19th day of incubation and on the 8th day of development. The second shaking was on the 27th day of incubation and on the 16th day of development. Mycelium development was completed on the 36th day of incubation. The slowest development in group C was seen in spawn that was improved at 34ºC. In these cultures; the development was completed on the 40th day of incubation and the first shaking was on the 22nd day of incubation and on the 9th day of development. The second shaking was on the 31st day of incubation and on the 18th day of development. Results of group C are summarized in **Table 3**.

The development of spawn prepared from mycelia in group D

The fastest spawn development in this study was obtained in group D. Mycelium development began on the 4th day of development at 30ºC and on the 6th day of development at 32ºC and 34ºC. At 30ºC the first shaking was on the 9th day of incubation and on the 6th day of development; the second shaking was on the 15th day of incubation and 12th day of incubation. In this group mycelium development was com-

Table 6 The spawn calendar of all groups.

		Incubation Period (day)																													
Groups	**Temperature (ºC)**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
	30	-	-	-	-	-	-	-	+	+	+	+	+	+	+	1S	+	+	+	+	+	+	2S	+	+	+	+	+	+	R	
	32	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	1S	+	+	+	+	+	+	+	+	2S	+	+	+	+
	34	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	1S	+	+	+	+	+	+	+	+	2S	+	+
	36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A		**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**													
	30																														
	32	+	+	+	R																										
	34	+	+	+	+	+	+	R																							
	36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-													
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
	30	-	-	-	-	-	+	+	+	+	+	+	1S	+	+	+	+	+	+	2S	+	+	+	+	+	R					
	32	-	-	-	-	-	-	+	+	+	+	+	+	+	1S	+	+	+	+	+	+	2S	+	+	+	+	+	+	R		
	34	-	-	-	-	-	-	+	+	+	+	+	+	+	+	1S	+	+	+	+	+	+	+	+	2S	+	+	+	+	+	+
	36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B		**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**													
	30																														
	32																														
	34	+	R																												
	36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-													
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
	30	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	1S	+	+	+	+	+	+	+	2S	+	+	+	+	+
	32	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	1S	+	+	+	+	+	+	+	2S	+	+	+
	34	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	1S	+	+	+	+	+	+	+	+
	36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C		**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**													
	30	+	+	R																											
	32	+	+	+	+	+	R																								
	34	2S	+	+	+	+	+	+	+	+	R																				
	36	-	-	-	-	-	-	-	-	-	-	-	-	-	--	-	-	-													
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
	30	-	-	-	+	+	+	+	+	1S	+	+	+	+	+	2S	+	+	+	+	+	R									
	32	-	-	-	-	-	+	+	+	+	+	+	1S	+	+	+	+	+	+	2S	+	+	+	+	+	R					
	34	-	-	-	-	-	+	+	+	+	+	1S	+	+	+	+	+	+	2S	+	+	+	+	+	+	+	R				
	36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	--	-	-	-	-	-	-
D		**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**													
	30																														
	32																														
	34																														
	36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-													
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
	30	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	1S	+	+	+	+	+	+	+	+
	32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	1S	+	+	+	+
	34	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	1S	+
E		**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**													
	30	2S	+	+	+	+	+	+	+	+	R																				
	32	+	+	+	+	+	+	2S	+	+	+	+	+	+	+	R															
	34	+	+	+	+	+	+	+	+	2S	+	+	+	+	+	+	+	R													

(+) = development; (-) = no development; 1S = first shaking; 2S = second shaking; R = put into refrigerator

pleted on the 21st day of incubation. The earliest mycelium development occurred at 30ºC. In the spawn cultures improved at 32ºC, the first shaking was on the 12th day of incubation and on the 7th day of development. The second shaking was on the 19th day of incubation and on the 14th day of development. Mycelium development was completed on the 25th day of incubation. The slowest development in group D was seen in spawn improved at 34ºC. In these cultures development was completed on the 26th day of incubation and the first shaking was on the 11th day of incubation and on the 6th day of development. The second shaking was on the 18th day of incubation and on the 13th day of development. Results of group D are summarized in **Table 4**.

The development of spawn prepared from mycelia in group E

The slowest spawn development in this study was determined in group E, except at 30ºC. In spawn cultures improved at 30ºC, the first shaking was on the 22nd day of incubation and on the 10th day of development. The second shaking was on the 31st day of incubation and on the 19th day of development. Mycelium development was completed on the 40th day of incubation. In spawn cultures improved at 32ºC, development began on the 16th day of incubation. In these cultures the first shaking was on the 26th day of incubation and on the 11th day of development. The second shaking was on the 37th day of incubation and on the 30thday of development. Mycelium development was completed on the 45th day of incubation. The slowest development in group E and in this study was seen in spawn improved at 34ºC. In these cultures development was completed on the 47th day of incubation and the first shaking was on the 29th day of incubation and on the 12th day of development. The second shaking was on the 39th day of incubation and on the 22nd day of development. In group E spawn was not prepared at 36ºC because mycelia did not develop at this temperature. Results of group E are summarized in **Table 5**.

The spawn calendar for all groups in this study is summarized in **Table 6**.

DISCUSSION

A model for *Agaricus bisporus* cultivation was proposed by Zadrazil *et al*. (1973), Vedder (1978), Fritsche (1981), Stamets and Chilton (1983), Boztok (1987), Erkel (1993), Gunay (1995) and Stamets (2000), and was modified for mycelium development and yield applications for *Agaricus bitorquis*. 30ºC was considered to be the optimum temperature for mycelium development of *A. bitorquis* (Zadrazil *et al*. 1973; Vedder 1975; Raper 1978; Vedder 1978) and was therefore taken as the control temperature in this study. In this paper the culture of mycelium was labeled as Groups A, B, C, D and E was developed at different temperatures (30ºC, 32ºC, 34ºC, 36ºC and 38ºC) and the spawn of groups from these mycelia were prepared at different temperatures. The spawn of Groups A, B, C and D were developed at 30ºC, 32ºC and 34ºC and 36ºC but in these groups spawn was not prepared at 38ºC. Similarly, Group E spawn was developed at 30ºC, 32ºC and 34ºC but not at 36ºC or 38ºC. The thermal lethal point for mycelium of Groups A, B, C and D was determined to be 38ºC. Likewise, the thermal lethal point for the mycelium of Group E was determined as 36ºC.

The spawn of all groups was prepared. As shown in **Table 6**, the fastest mycelium development was observed in Group D and the slowest mycelium development was observed in Group E. Guler and Arkan (2000) examined spawn development at four different groups of temperatures and they found both the best and the slowest growth of the spawn to be that of *A. bitorquis*.

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REFERENCES

Arkan O, Guler P (1996) *Agaricus bitorquis* (Quel.) Saccardo yetiştiriciliği, *Türkiye 5. Yemeklik Mantar Kongresi*, Yalova, pp 166-172

Boztok K (1987) Mantar Üretim Tekniği*, E.Ü. Ziraat Fakültesi Yayınları,* No: 489, Bornova-İzmir

Elliott TJ (1985) *Spawn Making and Spawns, The Biology and Technology of the Cultivated Mushroom,* Chapter 8, pp 131-140

Erkel İ (1993) Kültür Mantarı Yetiştiriciliği. Kocaoluk Yayınevi, Yalova, 85 pp

Fritsche G (1972) Experiments on the maintenance of strains of cultivated mushroom III. Propagation by multispore culture. *Mushroom News* **20**, 4-19

Fritsche G (1978) Breeding work. In: Chang ST, Hayes WA (Eds) *The Biology and Cultivation of Edible Mushrooms*, Academic Press, New York, pp 239-248

Fritsche G (1981) Some remarks on the breeding, maintenance of strains and spawn of *Agaricus bisporus* and *Agaricus bitorquis*. *Mushroom Science* **XI**, 367-385

Guler P, Arkan O (2000) The spawn development in *Agaricus bitorquis* (Quel.) Sacc. mycelium germinated at different temperatures. *Hacettepe Bulletin of Natural Sciences and Engineering* **29**, 35-40

Gunay A (1995) Mantar Yetiştiriciliği, *İlke Kitabevi Yayınları*, No: 22, Ankara

Isık SE (1996) Kültür mantarının ekolojik istekleri, *Türkiye 5. Yemeklik Mantar Kongresi*, Yalova, pp 14-24

Isık SE, Aksu S, Damgacı E, Ergun C, Erkal S (1997) Mantar Yetiştiriciliği, *Tarım ve Köyişleri Bakanlığı Atatürk Bahçe Kültürleri Merkez Araştırma Enstitüsü,* Yalova, 77 pp

Oei P (1991) *Mushroom Cultivation*, Publisher, Amsterdam, Chapter 4, pp 33-38

Raper AC (1978) Biological nature. In: Chang ST, Hayes WA (Eds) *The Biology and Cultivation of Edible Mushrooms*, Academic Press, New York, pp 365-369

Stamets P, Chilton JS (1983) *The Mushroom Cultivator*, Agarikon Press, Olympia, Washington, 415 pp

Stamets P (2000) *Growing Gourmet and Medicinal Mushrooms*, Ten Speed Press, Berkeley, CA

Vedder PJC (1978) Cultivation. In: Chang ST, Hayes WA (Eds) *The Biology and Cultivation of Edible Mushrooms*, Academic Press, New York, pp 377-392

Vedder PJC (1975) Practical experiences with *Agaricus bitorquis*. *The Mushroom Journal* **32**, 262-269

Zadrazil F, Schneidereit M, Pump G, Kusters H (1973) Ein Beitrag zur Domestikation von Wildpilzen. *Champignon* **138**, 17-19, 22-34