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SOME BIOLOGICAL PARAMETERS IN *SORDARIA FIMICOLA*

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Abstract

Biological parameters associated with identification, isolation, proliferation, resistance and discharge of asci and ascosporous were investigated on *Sordaria fimicola*. As unusual habitats, the fungus was isolated from surfaces of necrotized leaf spots of *Hordeum vulgare, H. spontaneum* and *Datura inoxia* at various rates in the vegetation period of 2009 in the Şanlıurfa district, Turkey. Treatments were conducted at different temperatures and nutrition media. Together with mycelium development, the most perithecial production was observed on Potato Dextrose Agar-1 at 25ºC in shortest time. At the same temperature, the slowest mycelium growth and perithecial production was observed on Potato Dextrose Agar-2. In absence of glucose in Corn Meal Agar, perithecial production and mycelium development was also fast. Mycelia tufts of fungus were resistant to different degrees or doses of ethyl alcohol. On the mycelium appendages stored in the ethyl alcohol, the perithecium of the fungus produced. Globe, turbinate, obpyriform and clavate were respective steps in the formation of perithecium. Together with asci, ascosporous were actively thrown from bursting perithecial opening in the matured perithecium. It is considered that this fungus species may occupy the necrotized areas on living plant leaves as an alternative habitat.

Introduction

The genus *Sordaria* has important species in the saprophytic fauna. Most members of the genera grow well and fast on organic-originated materials and survive on recycling of them in nature. In the several known species, *Sordaria fimicola*, *S. macrospore* and *S. brevicollis* are genetically most studied species (Kitani, 1978; Kalogeropoulos & Kalogeropoulos 1985; Saleem *et al*., 2001). From them, *S. fimicola* and *S. macrospore* are homothallic and their distribution common and worldwide. *S. brevicollis* is heterothallic, less common but widely distributed in the hemisphere (Fields, 1970). The natural habitats of *S. fimicola* and other two species have been mainly defined in dung of herbivorous animals (Dickinson *et al*., 1981; Masunga *et al*., 2006). However, it has been isolated from different habitats. For example; it was isolated from maize stalks (Chambers & Wet, 1987). On the other hand, the fungus has decomposition effect on wood and plant wastes (Alma *et al*., 2000). In the present study, *S. fimicola* was isolated from unusual habitats of phyllosphere which were probably not reported before. It was isolated from necrotic spots of *Hordeum vulgare*, *H. spontaneum* and *Datura inoxia*. The real causes of necrotic spots were *Rhynchosporium secalis* on two barley species and an *Alternaria* sp., on *D. inoxia. H. vulgare*, is a barley culture commonly grown in the Şanlıurfa district, which is the second largest cereal district in Turkey. Also, *H. spontaneum* is a wild barley species being common in the district. The *R. secalis* is a reported-parasitic fungus causing scald symptoms on leaf and sheaths of culture and wild barley species in the Şanlıurfa district (Kavak & Katırcıoğlu, 2002; Kavak, 2003a; Kavak, 2003b; Kavak, 2004). The *D. inoxia* is a weed plant in the irrigated cultures of same district. In 2009, the necrotized spots with concentric rings were detected on leaves of many *D. inoxia* and an *Alternaria* species was also isolated from them as the real cause of the spots (Kavak, unpublished data).

Following subjects were summarized as aims of this study performed. To determination of occupation level of *S. fimicola* on the necrotic spots of some plants; effects of some nutrition and temperature on mycelium growth and perithecial production of *S. fimicola*; and investigation of resistance level of *S. fimicola* to ethyl alcohol with discharging asci and ascospores.

Materials and Methods

Necrotized areas on leaves of *H. vulgare*, *H. spontaneum* and *D. inoxia* were materials collected randomly from plants infected with *R. secalis* and an *Alternaria* sp., during 2009 in the Şanlıurfa district, Turkey. The growth stages of *H. vulgare* and *H. spontaneum* was in the start of heading (Zadoks *et al*., 1974), and of *D. inoxia* was in the flowering when collections were made. The real causes of necrotic spots were previously confirmed by pathogen morphology and pathogenicity tests. With respect to presence of S. *fimicola* and its rate, the surfaces of necrotized leaves of related plants were investigated in the isolation treatments. The identification of fungus was made according to its anamorph and teleomorph morphology. Ten samples per plant species (*H. vulgare*, *H spontaneum* and *D. inoxia*) including necrotized spots on lower leaves were collected randomly from different fields. Exceptions of specific ones, three replicates were used in treatments. Necrotized leaf pieces with 2x4 mm^2 dimensions were sterilized in 70% ethyl alcohol for three minutes. Later, these samples were dipped in sterilized distilled water, dried with sterilized-blotting paper and placed on fresh Petri dish including PDA-1, PDA-2 and CMA. The petri dishes were incubated up to forming of teleomorphic structures. Positive results giving fungus development were statistically tested and rated. Daily colony dimension of this fungus were measured at different temperatures and nutrition media. Differences in same parameter were tested and grouped according to their significance levels. Significant levels between two parameters were determined by their comparison using t test. Mycelia tufts derived from PDA-1 were subjected to different alcohol densities at different time periods to detect resistance levels of ethyl alcohol. In addition, mycelium tufts were preserved in 70% ethyl alcohol during six month and

fungal position was observed. Perithecial- formation period and matured- perithecium number in per mm^2 were determined and grouped in different media after tested statistically. Discharge of asci and ascosporous were followed step by step and photographed. Nutrition mediums used in production of *S. fimicola* consisted of the following ingredients.

Potato Dextrose Agar-1 (PDA-1) = 200 g potato + 20 g glucose + 20 g agar + 1000 ml distilled water, and used after autoclaving at 121°C for 20 minute.

Potato Dextrose Agar-2 (PDA) = 4 g potato extract + 20g dextrose + 20 g Agar, 1000 ml distilled water, and used after autoclaving at 121°C for 20 minute.

Corn Meal Agar (CMA) =50 g corn meal + 25g agar+ 1000 ml distilled water, and used after autoclaving at 121°C for 20 minute.

Results

Anamorphic development and growth parameters in different temperatures and media: Colony of *S. fimicola* developed aerially and superficially on mediums and produced cheese like odor. They were whitish to gray in colors on PDA and transparent on CMA. Daily colony radius was highest on PDA-1 and CMA, and lowest on PDA-2. Mycelia of fungus were not affected from alcohol degrees as described in Table 2. All inoculations made from different mycelium pieces and ascosporous to PDA-1 gave fertile results, and they all were homothallic. Mycelium was hyaline, fast developed on PDA-1 and CMA in 25ºC, and lowest developed on PDA-2 in 10ºC. The solution of Potassium hydroxide (KOH) did not turn the color of the mycelia (KOH-). They developed downy and aerially, and had dichotomus branches with 40–45°, and some 90° angles. A light grey pigmentation was produced after 24 h on PDA-1 and to some extent on CMA. The color then turned into dark grey and black, respectively. Less pigmentation was observed on PDA-2.

Table 1. Growth parameters of *Sordaria fimicola* in different temperatures and media (mean ± S.E.).

▼	Temperature degrees °C				
	10	**18**	**22**	**25**	**35**
PDA-1	1.03 ± 0.06 a	9.50 ± 0.28b	19.5 ± 0.50c	26.16 ± 0.72d	13.83 ± 0.44bc
CMA	0.93 ± 0.08a	8.50 ± 0.28b	17.83 ± 0.16c	21.83 ± 0.92d	-
PDA-II	-	-	2.03 ± 0.14a	3.13 ± 0.12b	-
Comparison of PDA-I with CMA in same temperatures					-
PDA-1	1.03 ± 0.06	9.50 ± 0.28	19.5 ± 0.50	26.16 ± 0.72	-
CMA	0.93 ± 0.08	8.50 ± 0.28	17.83 ± 0.16	21.83 ± 0.92	-
t test	ns	ns	*	*	-
Comparison of PDA-II with PDA-I and CMA in same temperatures					
PDA-II	-	-	2.03 ± 0.14	3.13 ± 0.12	-
PDA-1	-	-	19.5 ± 0.50	26.16 ± 0.72	-
t test	-	-	*	*	-
PDA-II	-	-	2.03 ± 0.14	3.13 ± 0.12	-
CMA	-	-	17.83 ± 0.16	21.83 ± 0.92	-
t test	-	-	*	*	-

$p<0.05$; ▼= Daily colony radius in media (mm); *= Statistically significant; ns= Not statistically significant; - = Not treated

Means in the same line followed by the different letters are significantly different according to Tukey Test ($p<0.05$). Comparison between two media was made by t test

Teleomorphic development and parameters related to isolation, resistance and perithecial formation: The *S. fimicola* was isolated from determined necrotic spots at different numbers. On PDA-1, the maturing time of perithecium was shortest and its number in per mm^2 was highest. On PDA-II the related fungal structures completed their maturing with lowest rate. Perithecium of *S. fimicola* had different shapes from beginning to maturing. On PDA-1, the first perithecia clarified as drops shapes in 3-4 days, globes form in 4-5 days, and the real perithecial form in 7-9 days, respectively. These structures were produced as dense aggregated forms on PDA-1, dense-solitaire forms on CMA and sparse solitaire form on PDA-1 in which most of them located surrounding of inoculum only. The color of perithecia during formation was light brown, dark brown and black, respectively; however, KOH solutions did not affect the color of it. After completion of globes, dark protuberances and perithecial apices that they were turbinate, obpyriform and clavate, respectively formed (Fig. 1A). Asci together with ascospores were actively hurled from bursting tips of the naturally matured perithecium to some distance (Fig. 1B). Many aggregated forms of perithecium produced on PDA-1 and formed heaps up to 2.5 mm height. Perithecium was average 03-05 x 08-1.1 mm weight/height. They had many aerial mycelium appendages giving fertile development on different mediums. On the appendages of mycelium tufts preserved in the 70% ethyl alcohol in a container, several numbers of perithecium were produced. Each perithecium had many asci and each one with eight ascosporous (Fig. 1C). Asci were long cylindrical, hyaline and had a hole on tip;

220 x 310 µm in length and 17-22 µm in width. Ascosporous were smooth ellipsoidal and their surrounds covered with a gelatinous sheath. They were green at the beginning but dark green and black in later and 20 x 22/12 x 13µm long/weight in dimensions (Fig. 1D).

Table 2. Results of *Sordaria fimicola* displaying isolation, resistance to alcohol, period of perithecium maturity and perithecial number in media (mean ± S.E.).

Species	*H. spontan*	*H. vulgare*	*D. inoxia*		
	Isolation results in PDA-I based on plant species				
	0.300 ± 0.153a	0.400 ± 0.163a	0.500 ± 0.167b		
	Test of resistance to ethyl alcohol				
	Alcohol degrees (%)	70	80	90	95
Period 1	10 minute	Resistant	resistant	resistant	resistant
Period 2	30 minute	Resistant	resistant	resistant	resistant
	Maturity period of perithecium and its type in media				
	PDA-1	CMA	PDA-II		
Day number	8.0 ± 0.57a	12.33 ± 0.66b	42.33 ± 0.88c		
Perithecial phase (type)	Aggregated	Dense solitaire	Sparse solitaire		
	Perithecium number per mm^2 in Media				
Perithecium number	96.00 ± 5.20	37.33 ± 4.33	2.67 ± 1.76		

Means in the same line followed by the different letters are significantly different according to Tukey Test (p<0.05)

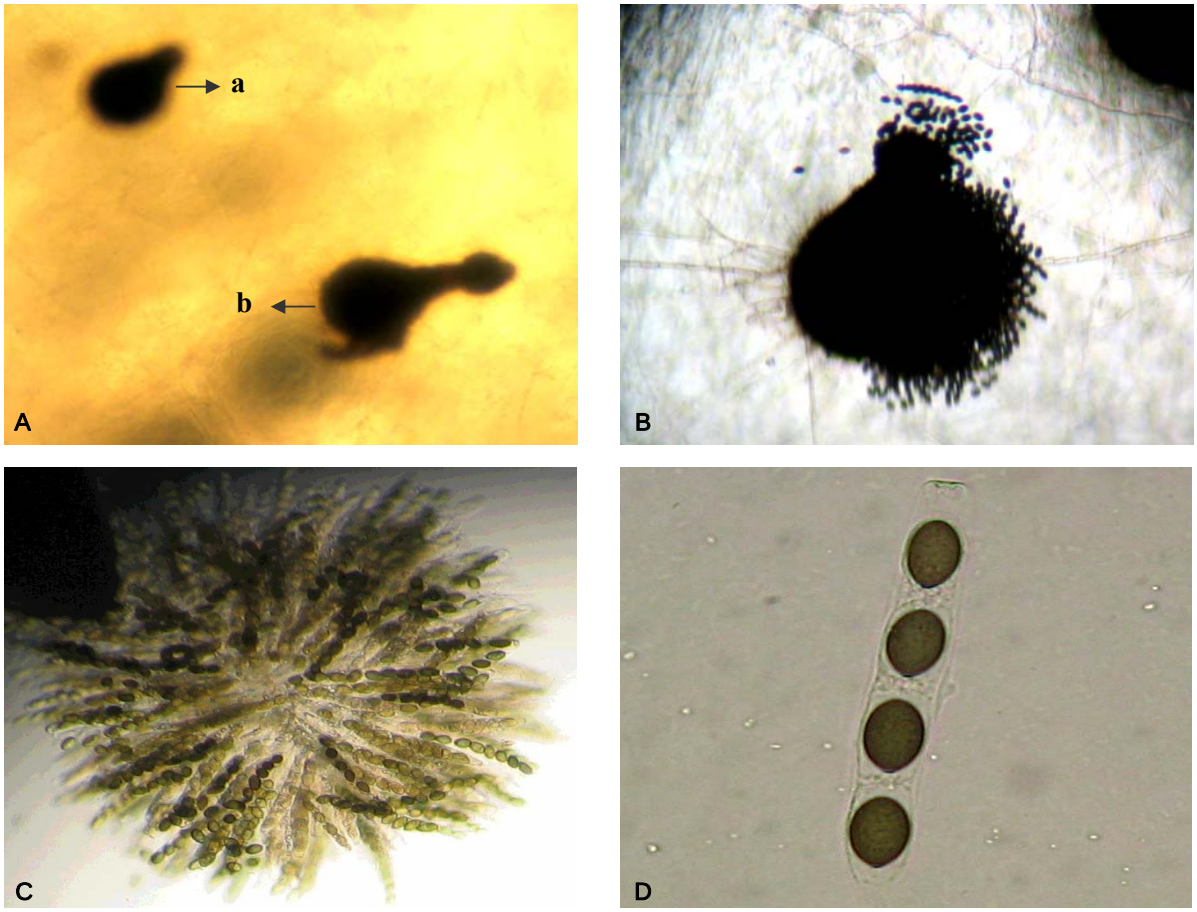

Fig. 1. Different structures in *Sordaria fimicola*
A= Obpyriform (a) and Clavate form (b) in perithecia of *S. fimicola*. b is a pre burst position of a perithecium. B= Discharging of ascus and ascospores from a matured perithecium of *S. fimicola*. C= Ascus cluster with their ascospores in *S. fimicola*. D= An asci section and its ascosporoes.

Discussion

The identification and isolation of *S. fimicola* from necrotized leaves of *Hordeum vulgare*, *H. spontaneum* and *D. inoxia* are new reports. As shown in Table 2, the highest isolation rate was made on necrotic leaves of *D. inoxia*, which probably was due to larger surfaces of infected and non-infected plant leaves. Compared to two barley species, this plant species placed in dicotyledons and constitutes plants with broad leaves. Based on teleomorph and anamorph morphology, the related fungus was identified as *S. fimicola* as described by Alexepoulus *et al.*, (2003); Ingold (1971). The colony development of the fungus on PDA-I was statistically higher than other two media at temperature of 22ºC and 25ºC (Table 1). Connected with perithecial productivity and the number of solitaire perithecium in per mm^2, total starch amount had much more importance than active glucose amount and the same observed clearly in results of three mediums as shown in Table 2. However, absence of glucose in CMA caused to decrease of perithecial productivity. In addition, in this nutrition medium, aggregated phase regressed to solitaire phase. When produced perithecia in alcohol were taken note, it is considered that this fungus could somewhat use ethyl alcohol as food and this was observed as specific properties to this fungus.

S. fimicola is a coprophilous fungus isolated frequently from herbivore and carnivore dung in the past (Iftikhar & Cain, 1972). It has generally been assumed that coprophilous fungi live in dung of herbivorous animals; specifically on dung of horses, hares and rabbits. This is due to the complex nature, possibly with the coarse nature of the cellulosic materials in the dung of these animals as suggested by Lundqvist (1972). The main habitat of *S. fimicola* is dung of herbivorous animals and it may be possibly consumed and dispersed by these animals as reported by Bell (1983). When considered isolation of *S. fimicola* from necrotized leaves, nevertheless, we suggested that this fungus may prefer an alternate life manner and can display saprophyte position on necrotic sections. Similar to this theory, the first one was suggested by Webster (1970) and supported by later findings of Chambers & Wet (1987). In addition, this ascomycete was deemed a 'cellulose-eater' by Dal-Vesco *et al*., (1967) and was able to degrade cellulose effectively as reported by Thormann *et al*., (2002). Furthermore, according to same authors it was isolated often from peat and may be more effective on the decaying of plant residues than has been supposed previously. Based on above information, it can be suggested that occupation of *S. fimicola* on necrotized areas on plant tissues may be for decomposition purposes. The mentioned fungus had properties of throwing violently their asci and ascospores. The clavate form, pre-bursting position, was evidence of it.

References

Alexepoulus, C.J., C.W. Mims and M. Blackwell. 2003. Genus *Sordaria* In: *Introductory Mycology*. John Wiley & Sons Inc. 869 p.

Alma, M.H., M. Dığrak and I. Bektaş. 2000. Deterioration of wood wastes- based molding materials by using several fungi. *J. Mat. Sci. Let*., 19: 1267-1269.

Bell, A. 1983. *Dung fungi*. Victoria University Press, Wellington, New Zealand.

Chambers, K.R. and D.C. De Wet. 1987. Isolation of *Sordaria fimicola* from Maize Stalks. *J. Phytopathol*., 120: 369-371.

Dal Vesco, G., B. Peyronel, M.T. Barge and N. Volpiana. 1967. Sulla micoflora dello sterco di coniglio ("Oryctolagus cuniculus"). *Allionia*, 13: 107-127.

Dickinson, C.H., V.S.H. Underhay and V. Ross. 1981. Effect of season, soil fauna and water content on the decomposition of cattle dung pats. *New Phytol*., 88: 129-141.

Fields, W.G. 1970. An introduction to the genus *Sordaria*. *Neurospora Newsletter*, 16: 14-17.

Iftikhar, A. and R.F. Cain. 1972. Revision of the genera *Sporormia* and *Sporormiella*. *Can. J. Bot*., 50: 417-477.

Ingold, C.T. 1971. *Fungal spores: their liberation and dispersal*. 302 p. Clarendon press, Oxford.

Kalogeropoulos, A. and P. Kalogeropoulos. 1985. Gene conversion at the gray locus of *Sordaria fimicola*: fit of the experimental data to a hybrid DNA model of recombination. *Genetics*, 109: 599-610.

Kavak, H. and Z. Katırcıoğlu. 2002. Determination of response of some barley cultivars to *Rhynchosporium secalis* depending upon different inoculation sources and methods. *J. Turkish Phytopathol*., 31: 117-124.

Kavak, H. 2003a. First record of leaf scald caused by *Rhynchosporium secalis* in natural population of *Hordeum vulgare* subsp. *spontaneum* in Turkey. *Plant Pathol*., 52: 805.

Kavak, H. 2003b. First record of scald disease caused by *Rhynchosporium secalis* in a population of *Hordeum murinum* in Turkey, Australas. *Plant Pathol*., 32: 567.

Kavak, H. 2004. Effects of different sowing times on leaf scald and yield components of spring barley under dry-land conditions. *Aust. J. Agr. Res*. 55: 147-153.

Kitani, Y. 1978. Absence of interference in association with gene conversion in *Sordaria fimicola*, and presence of interference in association with ordinary recombination. Ge*netics*, 89: 467-97.

Lundqvist, N. 1972. Nordic Sordariaceae *s. lat.* Symb Bot Ups 20.

Masunga, G.S., Ø. Andresen, J.E. Taylor and S.S. Dhillion. 2006. Elephant dung decomposition and coprophilous fungi in two habitats of semi-arid Botswana. *Mycol. Res*., 110: 1214-1226.

Saleem, M., B.C., Lamb and E. Nevo. 2001. Inherited differences in crossing over and gene conversion frequencies between wild strains of *Sordaria fimicola* from Evolution Canyon. *Genetics*, 159: 1573-93.

Thormann, M.N., R.S. Currah and S.E. Bayley. 2002. The relative ability of fungi from *Sphagnum fuscum* to decompose selected carbon substrates. *Can. J. Microbiol*., 48: 204-211.

Webster, J. 1970. Coprophilous fungi. *Trans. Br. Mycol. Soc*., 54: 161-180.

Zadoks, J.C., T.T. Chang and C.F. Konzak. 1974. A decimal code for the growth stages of cereals. *Weed Res*., 14: 415-421.

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