Dimethyl sulfoxide inhibits *Tilletia laevis* teliospore germination

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Abstract

Teliospore germination plays an important role in the infection and pathogenesis of smut fungi. The effect of the solvent dimethyl sulfoxide (DMSO) on teliospore germination in the wheat bunt fungus *Tilletia laevis* was investigated. DMSO concentrations greater than 2.5% significantly reduced the germination rate of teliospores on water agar. Changes in the morphology of basidia and basidiospores also were observed when germination occurred in the presence of DMSO. The current study indicates that DMSO is a potent inhibitor of teliospore germination and sporidial formation, and may be a tool for controlling *T. laevis* solely or in combination with other disease management practices.

Key words: DMSO, Teliospore germination, *Tilletia laevis.*
Subject Editor: Melanie Weckert.
Published online: 21 August 2015,

Introduction

Dimethyl sulfoxide (DMSO) is an environmentally friendly, highly polar, aprotic solvent which has been widely used for biological studies including chlorophyll extraction from plants, mosses and lichens, and as a solvent for determining the minimum inhibitory concentration of antibacterial and antifungal compounds (Santos *et al.*, 2003; Tait and Hik 2003; Voda *et al.* 2004; Randhawa 2008). DMSO binds with the plasma membrane of cells and increases membrane permeability and the leaching of low molecular weight compounds from cell tissues (Trione 1973; Hazen 2013). Although DMSO has low toxicity to many plants and fungi, the inhibitory effect of critical concentrations has been reported for a wide range of bacteria and fungi (Basch & Gadebusch 1968; Trione 1973; Randhawa 2008). DMSO was reported to have an adverse effect on teliospore germination of *Tilletia caries*, one of two species causing common bunt of wheat (Trione 1973).

Common bunt is one of the most destructive diseases of wheat world wide, and causes 25-30% yield loss in Iran (Mobasser *et al.* 2012). The germination and post-germination developmental stages may be the most susceptible to control approaches (Trione 1973). The aim of this study was to investigate the effect of DMSO on the rate of teliospores germination of *Tilletia laevis*, one of the species causing common bunt of wheat.

Materials and Methods

Samples of common bunt-infected wheat were obtained from the Agriculture Research Station in Borujerd, Lorestan Province, Iran. All collections were one-year-old and from different locations in Borujerd. Single bunt sori were placed in 0.5% sodium hypochlorite solution for two minutes, rinsed three times and ruptured in distilled water to make a teliospore suspension. Five concentrations (1, 2.5, 5, 7.5 and 10%) of DMSO (Merck, Germany) were prepared in 2% water agar (WA). Control treatments were WA with no DMSO. One half mL of teliospore suspensions were placed on each WA plus DMSO concentration or control (approximately 20 spores/mm^2) and incubated at 9 °C for 15 days. The average percent germination was determined by counting germinated and nongerminated teliospores in five random fields on each Petri dish. A total of 100 spores were examined in each microscope field (Stockwell and Trione 1986). For further study, teliospores of each isolate were harvested by gentle scraping into sterile distilled water.

A randomized complete block design was used for the experiment with three replications and two fungal isolates. Analysis of variance (ANOVA) and mean separation statistics were performed using the GLM (General Linear Model) procedure of SAS (1988).

Results and Discussion

Light microscopy revealed that DMSO inhibited teliospore germination in *T. laevis* in a dose-dependent manner. As shown in Table 1, there was significant inhibition of teliospore germination in both isolates of the fungus at 5–10% DMSO concentrations ($P < 0.05$) compared to the control treatment after 12 days of incubation. The degree of inhibition increased with the solvent concentration, and at 10% DMSO concentration, there was nearly complete inhibition of germination. At concentrations lower than 2.5%, no inhibition of germination occurred in either isolate, and there was no significant difference between these treatments and the controls.

Table 1. Inhibition levels as a function of mean teliospore germination of two *T. laevis* isolates exposed to different DMSO concentrations.

DMSO concentrations (%)	Teliospore germination (%)	Structural behavior of exposed teliospores:	
		Promycelium production	Sporidium production
Isolate TLB1012:			
0 (control)	88.31[a]	+	+
1	86.34[a]	+	+
2.5	87.97[a]	+	(+)
5	19.24[b]	(+)	r
7.5	2.94[c]	(-)	-
10	1.81c	(-)	-
Isolate TLB2012:			
0 (control)	74.14[a]	+	+
1	75.62[a]	+	+
2.5	80.70[a]	+	(+)
5	37.74[b]	(+)	r
7.5	3.74[c]	(-)	-
10	2.49[c]	(-)	-

Significant differences denoted (a-c) amongst means according to the Duncan's Multiple Range Test at $p < 0.05$. Coefficient of variation (CV) = 13.68, 20.05 for isolates TLB1012 and TLB2012 trials, respectively. (-): unusual and disorganized germination resulting in sterile and suppressed structures; r: rare production; (+): Abnormal promycelia or sporidia.

After 12 days, *T. laevis* teliospores germinated and produced primary sporidia (basidiospores) on WA with no DMSO (control), 1% and 2.5% DMSO solutions, whereas in the 5–10% DMSO treatments the fungus rarely produced primary sporidia (Table 1). Teliospores exposed to 7.5% and 10% concentrations of DMSO also exhibited unusual germination behavior by production of spherical, vesicle-like, stunted, sterile or suppressed germination structures with aberrant shape or morphologically abnormally thin basidia (promycelia) (Figs. 1, 2 and 4). Teliospores exposed to 5% concentration of DMSO produced multiple branched basidia. Treatments with 2.5% and 5% DMSO concentrations also induced abnormal allantoid secondary sporidia with lobed margins on the internal side (Fig 3).

This study demonstrates that treatments with 5%, 7.5% and 10% of DMSO in water agar significantly inhibited *T. laevis* teliospore germination and sporidial formation. Based on a preliminary investigation, there were variable results for the effect of some methanolic crude plant extracts in different DMSO concentrations on teliospore germination such that the higher concentrations almost completely inhibited teliospore germination of *T. laevis* while germination rates and sporidia production were affected in a dose-dependent manner at 2.5–10% (data not shown). DMSO was shown to have inhibitory activity on organisms when tested *in vitro* with water-insoluble compounds in standard susceptibility assays (Hazen 2013).

Considerable variability has been reported among antifungal susceptibility testing methods of fungi and yeasts (Ramani & Chaturvedi 2003; Randhawa 2008). DMSO has been considered a factor that explains variability in the growth of these organisms, and was shown to inhibit the growth of fungal strains in low concentrations (Ramani & Chaturvedi 2003; Randhawa 2008). Based on our data, the use of DMSO alone in WA resulted in inhibition or prevention of teliospore germination (Table 1).

The inhibitory effect of DMSO concentrations of 1% and higher incorporated in WA on *T. caries* Race T-5 germination was reported by Trione (1973), in contrast to the lack of inhibition of DMSO concentrations of 2.5% or less in *T. laevis* in the present study. *Tilletia caries* and *T. laevis* are closely related and likely conspecific taxa that differ primarily in teliospore ornamentation (Russell & Mills 1994). Differences in germination rate due to temperature occur between isolates and races of the two common bunt fungi (Lowther 1950), and it is possible that the discrepancy between the results of

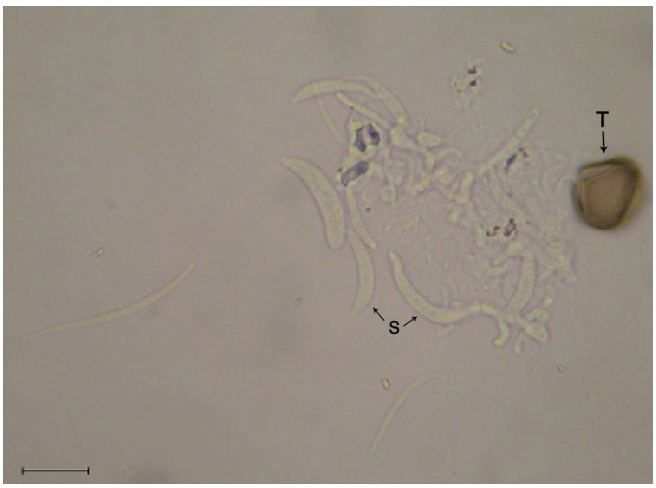

Fig 1. *T. laevis* with normal teliospore germination with basidia and sporidia (S, sporidia; T, teliospore). Bar = 20 µm.

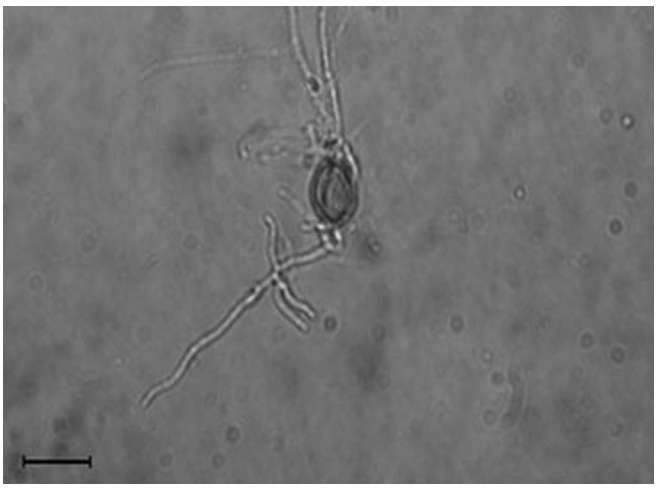

Fig 2. *T. laevis* with abnormal teliospore germination. Bar = 20 µm.

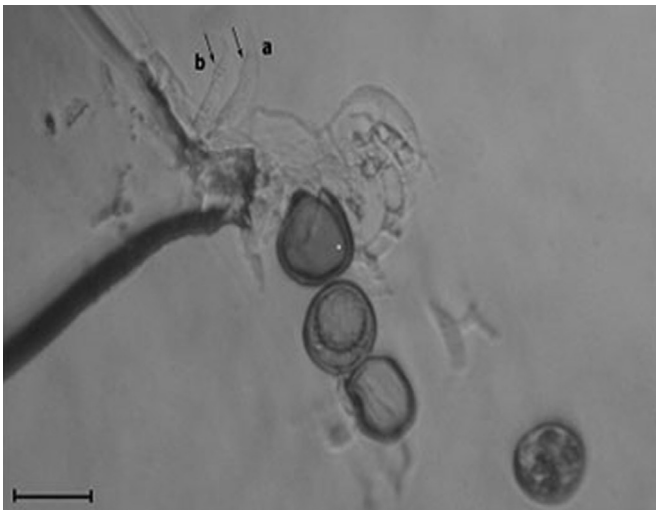

Fig 3. Abnormally-shaped sporidia with lobed margins on internal side (a, b: abnormal lobed margins). Bar = 20 µm.

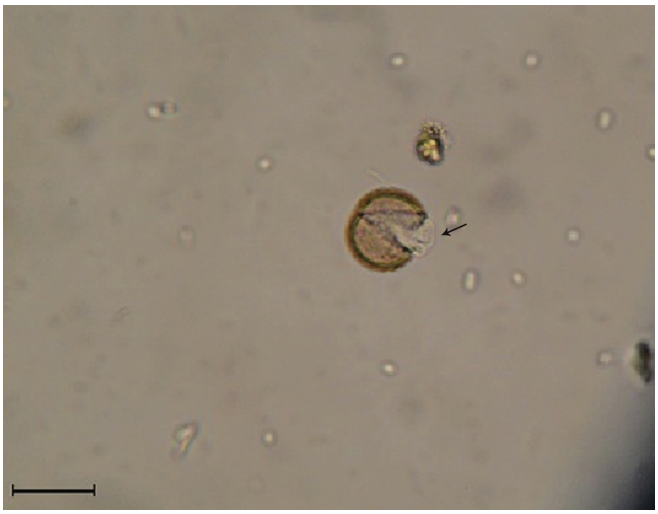

Fig 4. Nongerminated teliospore with balloon formation (arrow). Bar = 20 µm.

Trione (1973) and the present study on concentration of DMSO similarly may be strain-specific, but additional study is needed to confirm this.

Germination and post-germination developmental stages may be the most susceptible to control measures and factors affecting teliospore germination have an important role in any study of the early interaction between the pathogen and host (Trione 1973, 1977). Abnormal teliospore germination was correlated with reduction in infection in covered smut of barley (*Ustilago hordei*; Christ & Person 1988). Agricultural formulations are one of the applications of DMSO (Valencia-Quintana *et al.* 2012), and combining DMSO and surfactants may increase their effect on teliospore germination or other function in smut pathogens (Trione 1973). DMSO enhances the permeation of various chemical agents through plant, microbial and animal membranes, and increases the lipid fluidity by disrupting tightly packed lipid chains thus reducing the resistance of lipid barrier to the diffusion of drugs (Chattaraj and Walker 1995; Mishra *et al.* 2009). DMSO is also a cryoprotective substance used to allow long term storage of plant tissues (Decot *et al.* 2009). The current study indicates that DMSO could be an effective control of common bunt of wheat. In agriculture, DMSO has the potential for improving productivity in crops such as rice and wheat, increasing seed germination, and increasing the activity of certain antibiotics including fungicides (Kumar *et al.* 1976; Forwood & Owensby 1984).

Early studies indicated that DMSO had low environmental toxicity, and very low toxicity to humans and a vast range of plants and fungi (Trione 1973; Balakin *et al.* 2004; Kiefer *et al.* 2011). However, as a recent study reported that low doses of DMSO injected in rats caused cellular toxicity (Galvao *et al.* 2014), we recommend that DMSO be used as a seed treatment

rather than as a spray on wheat crops. Further work should be carried out to determine the residual quantities of this substance in the tissues. Additional investigations are required to determine the efficacy of DMSO at different concentrations alone or together with another inhibitors or seed treatments for enhancement of control measures based on low environmental and toxicological impacts.

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