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Original Contribution

EFFICIENCY OF *SEPTORIA TRITICI* SPORULATION ON DIFFERENT CULTURE MEDIA

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ABSTRACT
The solid and liquid media were investigated for their efficiency in quantitative sporulation of Septoria tritici. The inoculated culture media were incubated at 18 to 20 ˚C with a photoperiod of 12 h light and 12 h darkness and investigated after 4 to 6 days. Among solid media, YMDA (yeast extract + malt extract + dextrose + agar) and liquid media YMB (yeast extract + malt extract + glucose in 1 lit distilled water) were the best with conidia production rates of 1.7×10^9 spore/ ml and 2.3×10^9 spore/ ml, respectively. The pathogenicity tests using conidia produced on PDA, YMDA and YMB showed that the inoculum produced on YMDA and YMB are more successful in establishing disease in wheat than inoculum produced on PDA. These results indicate that YMDA and YMB are the most efficient culture media inoculum production and the most reliable inoculum production method for screening of wheat cultivars for resistance to Septoria leaf blotch.

Key words: Mycosphaerella graminicola, inoculum, culture media, needle–like conidia, secondary conidia

INTRODUCTION

Septoria leaf blotch causing by Septoria tritici (teleomorph Mycosphaerella graminicola) is one of the most important wheat disease throughout the world and causes severe yield loss. The researchers always consider different aspects of this disease such as genetic variation, biology, resistant cultivars and effect of fungicides on disease control. For all mentioned cases we need to produce a large amount of spores in laboratory. Up to now several culture media for spore production were introduced (1, 2).

Eyal (1987) introduced some solid media such as PDA (potato dextrose agar amended with 4.5 percent yeast extract (YE), YMDA (YE + malt extract (ME) + dextrose + agar), and also liquid media like YS (YE + sucrose + distilled water), Modified Fries liquid medium, Potato dextrose yeast liquid medium (1). Guo and Verreet (2008) used YMDA, PDA and CA (carrot extract + agar), WLA (wheat leaf extract + agar) and Czapek Dox Agar for spore production in Septoria tritici (3). This study was conducted to investigate quantitative efficiency of Septoria tritici sporulation on several culture media. Since the quality and quantity of inoculum is very important for obtaining reliable results in cultivar screening experiments, this research was conducted to examine the best inoculum production culture media.

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MATERIALS AND METHODS

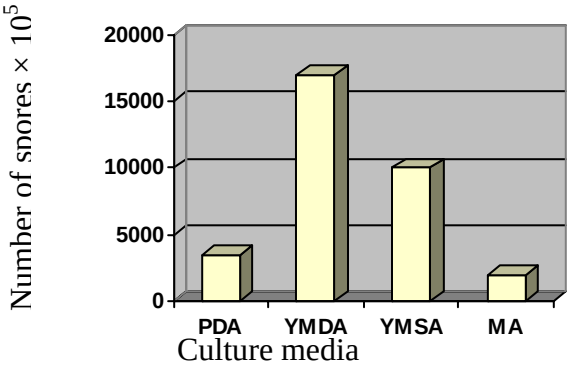
Some solid and liquid media included PDA + 4.5 % YE, YMDA (4 g YE + 4g ME +10g dextrose + 15g agar in 1 L distilled water), YMSA(4 g YE + 4g ME + 4g sucrose + 15g agar in 1 lit distilled water), MA (30g ME +3g peptone, 15g agar in 1 L distilled water) , YSB (10g sucrose + 10g YE in 1 lit distilled

water), YMB(4g YE + 4g ME + 10 g glucose in 1 L distilled water) and PDYB(extract of 200g potato + 20g dextrose + 20g YE in 1 L distilled water) were used. To prevent bacterial contamination, 200 mg mixture of streptomycin sulfate with penicillin (1000000 unit of penicillin in 3g streptomycin sulfate) and 10 mg/l gentamycin were added to warm medium after autoclaving (approximately at 45-50˚C). The gentamycin can be added with the other ingredient prior to autoclaving. The Petri dishes were filled by media. After cooling, each Petri dish was streaked by spores of a high virulent isolate of S. tritici collected from Izeh in Khuzestan province. After inoculation plates were incubated at 18-20 ˚C with a photoperiod of 12 h light and 12 h darkness. To prepare spore suspension, 1-2 ml distilled water were added to PDA plates colonized with the fungus and shook horizontally on the bench. The resulting spore suspension distributed on medium uniformly. Then Petri dishes were incubated in mentioned conditions for 4- 6 days. The pH of all culture media was adjusted to 5.8 to 6. The antibiotics were added to liquid media the same as solid media and then were inoculated by suspension of secondary conidia (3) that obtained previously on WA (agar + distilled water). The liquid media were kept in a shaker-incubator at 18-20˚C and 120 rpm with a photoperiod of 12 h light and 12 h darkness. After 4 to 6 days spore production of different media were examined by counting spores on haemacytometer slide.

RESULT AND DISCUSSION

The culture media were investigated after 4 to 6 days. The results showed that the greatest amount of spores were produced on YMDA (1.7×10^9 spore/ ml) and YMB (2.3×10^9 spore/ ml) **(Figure 1a and b)**.

(a)



(b)

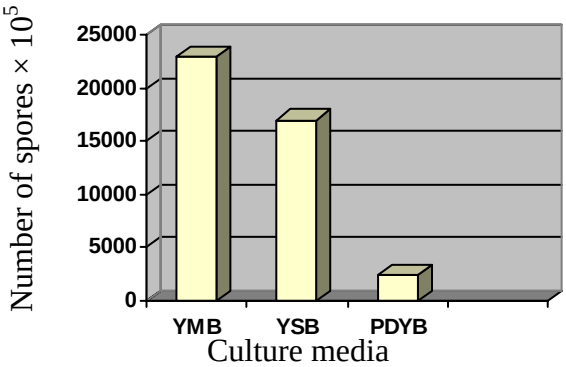


Figure 1. Number of *Septoria tritici* spores on different solid (a) and liquid (b) culture media

The fungus grew yeast like on YMDA and YMSA and produced a large amount needle–like spores and secondary conidia **(Figure 2 to 5)**. Cultures with yeast-like growth produced hardly any pycnidia or pseudopycnidia, while cultures with intermediate or mycelial growth types produced them frequently. Incubation in continuous darkness induced intermediate to mycelial growth types rather than yeast-like growth type in some cultures (4) .The fungus on PDA formed a little dark mycelia and then needle-like spores were produced. Meanwhile

on MA it formed dark condensed mycelial mat after 2 days and needle-like spores were produced after 7 to 10 days but their amount were inconsiderable **(Figure 6)**. This observation is supported by the data of Kema and Annone, 1991 (4).

Secondary conidia were observed on YSB and YMB too. The liquid media were clear at first but took cloudy appearance after four days and light pinkish flour like mass on the bottom of flask was aggregated after turning off the shaker **(Figure 7)**. These results were similar to Guo and Verreet, 2008; Kema and Annone, 1991 and showed that the ingredients of culture media can be important factor in spore production in *S. tritici* (3, 4). In addition to ingredients of culture media other agents such as light and pH may be affected production of conidia (3). Light affects colony color and conidial number significantly. The visible light may be essential for the formation of secondary conidia (3, 5). The pH can be impacting factor on spore formation. Guo and Verreet , 2008 showed that the suitable pH for formation of secondary conidia was between 5 and 9 and at pH 2, 10 and 11 conidia hardly budded, so we adjusted pH to 5.6 to 6 (3). The pathogenicity tests using conidia produced on PDA, YMDA and YMB showed that the inoculum produced on YMDA and YMB are more successful in establishing disease in wheat than inoculum produced on PDA (data not shown). These results indicate that YMDA and YMB are the most efficient culture media inoculum production and the most reliable inoculum production method for screening of wheat cultivars for resistance to Septoria leaf blotch.

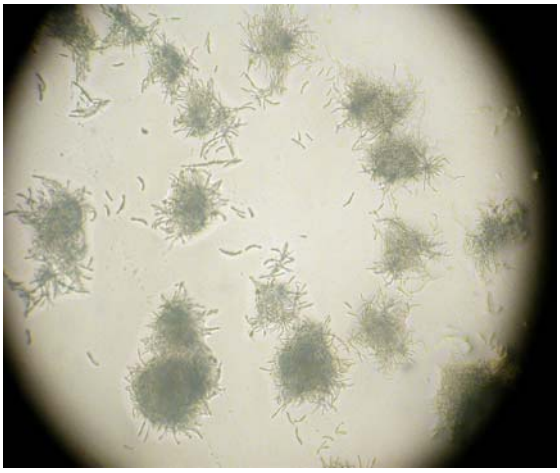


Figure 2. Needle-like spores of *Septoria tritici* on YMSA

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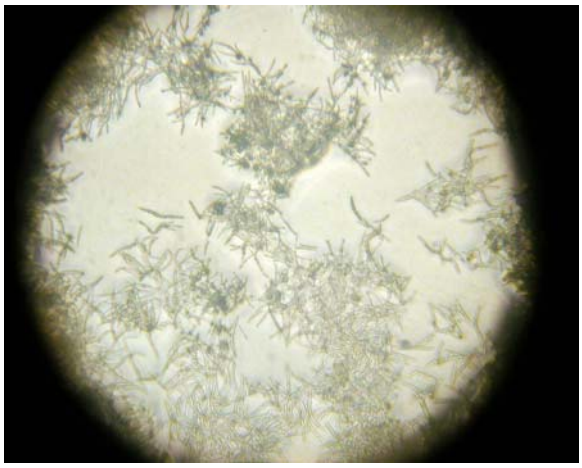

Figure 3. Needle-like spores of *Septoria tritici* on YMDA

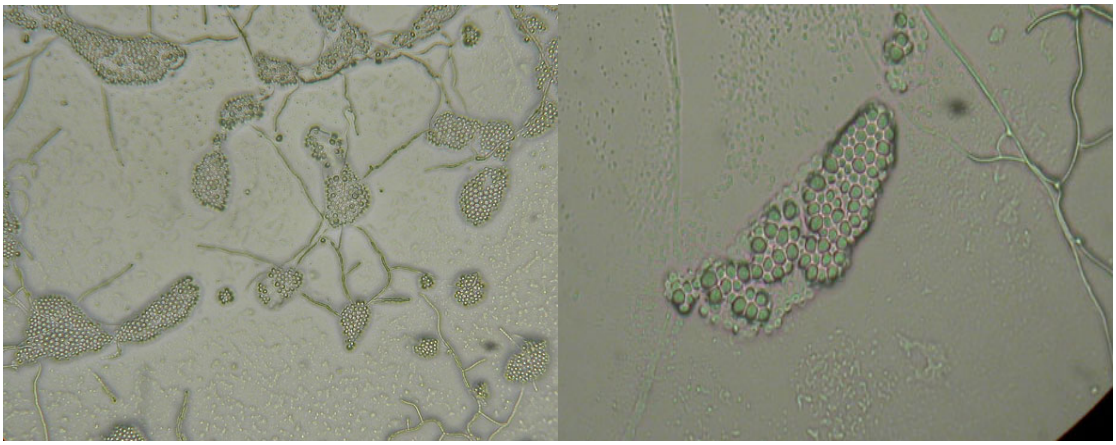

Figure 4. Secondary conidia of *Septoria tritici* on media culture

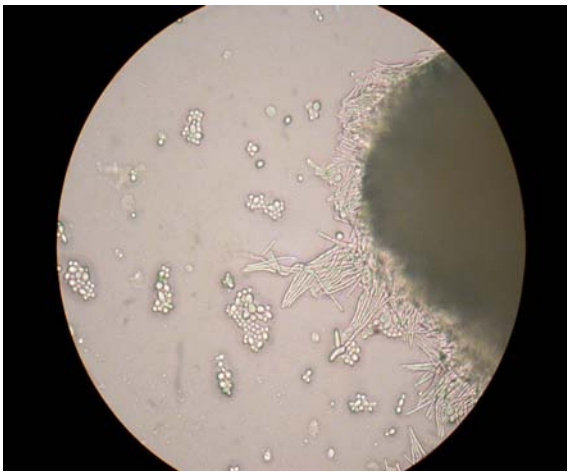

Figure 5. Simultaneously formation of secondary conidia and needle-like spores of *Septoria tritici* on YMDA.

Figure 6. Dark condensed mycelial mat of *Septoria tritici* on MA

Figure 7. Formation of light pinkish cloudy and flour like mass of spore of *Septoria tritici* on the bottom of flask in YMB.

CONCLUSION

Production of inoculums on different culture media influences on successful establishment of disease by *Septoria tritici*. Therefore, this must be considered in screening of wheat lines and cultivars against this pathogen. Our results showed that YMDA and YMB are the most efficient culture media inoculum production. Meanwhile the inoculums produced on these media can efficiently establish disease.

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