

Inhibition of Germination and Seedling Growth of Rice by Culture Filtrate of Aflatoxigenic *Aspergillus flavus*

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Abstract

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Aflatoxin B1 produced by *Aspergillus flavus* causes damage to crops and cereals including rice by inhibiting seed germination, elongation of the hypocotyls or roots and alters many physiological processes in developing seedlings. Rice cultivar Savitri is a popular high yielding variety in the eastern coastal part of India, where favorable growth condition for *A. flavus* exist and the harvesting practice of the farmers usually result in the infection of rice crops with this fungus. Hence, the effect of culture filtrate of *A. flavus* on cv. Savitri (CR 210-1009) was investigated. Seed germination, root shoot length and root shoot weight of Savitri seedlings were drastically reduced by dipping the seeds in the undiluted and diluted (50% and 25%) culture filtrate. In the case of undiluted culture filtrate, seed germination rate and seedlings mortality rate were 18% and 75% compared to 70% and 0.00% for the untreated control, respectively. The residual seed weight was lower (358.9mg) in the case of untreated control and higher in case of undiluted filtrate (521mg). Results indicated that the culture filtrate containing the aflatoxin, interfered with the physiological processes which prevented the use of endosperm by the developing seedlings.

Keywords: Aflatoxins, Savitri, germination, *Aspergillus flavus*, rice.

Introduction

Aflatoxins are biologically active secondary metabolites produced mainly by *A. flavus* and have been detected in cereal grains, oil seeds, fermented beverages made from grains, milk, cheese, meat, nut products, fruit juice and numerous other agricultural commodities (1). Though mycotoxins and particularly aflatoxin contamination is less commonly reported for rice than for many other cereal crops, rice represents a very good substrate for fungal growth and toxinogenesis since it is used as an ideal culture medium to test the toxigenic potential of isolated strains. The major mycotoxigenic fungi in rice are *Aspergillus* spp., *Fusarium* spp. and *Penicillium* spp. (2). Besides its toxigenic effect on animals, Aflatoxin B1 inhibits seed germination, seedling growth and alters the physiological processes of seedlings of many crops including cereals such as rice (3, 4) wheat (5) and maize (1). Aflatoxins may inhibit seed germination, elongation of the hypocotyls or roots of developing seedlings, or both, and by interference with chlorophyll synthesis in certain plants (6). The harmful effects of such fungal invasion may be glume or grain discoloration, loss in viability, quality and toxin contamination (1).

The aflatoxin producing fungi are widely distributed in nature and can grow over a wide range of environmental conditions (7). However, high relative humidity and temperature favors the growth and aflatoxin production (8, 9). In the eastern coastal part of India where such type of environmental conditions exist, farmers usually keep harvested paddy in the field for 4-5 days for proper sun drying. The occurrence of sudden heavy rain during the harvesting season resulting in flood like situation, makes

the rice crop vulnerable to infection with *Aspergillus* spp. (10) and favors the growth and spread of *Aspergillus flavus* and subsequent aflatoxin contamination of the rice crop. Among the various stages, rice at the drying stage and the stage preceding milling were shown to contain aflatoxins (11). Accordingly, the effect of dipping seeds in culture filtrate of aflatoxigenic *A. flavus* on seed germination and seedling growth of rice cultivar Savitri (CR 1009, released by Central Rice Research Institute, India) was investigated.

Materials and Methods

Aspergillus flavus isolate A129 was grown in - Cyclodextrine Potato Dextrose Broth (12) for 15 days in 150 ml conical flask at ambient temperature. The mycelial mat was separated and the broth was filtered to be used in the study. Three concentrations of this filtrate such as undiluted culture filtrate (100%) and two dilutions (50% and 25%) of culture filtrate were used. Hundred seeds of rice cv. Savitri was properly dipped in 3 ml of each solution and then plated on blotting paper in plastic petri plates. To each type of plate, 6 ml more solution was added and then kept at ambient temperature. Seed germination and seedling mortality rates, root-shoot length, root-shoot weight and residual seed weight (i.e. the weight of the seeds after removing root and shoot) were measured. The seedling mortality rate was calculated using the formula:

Seed mortality (%) = (Number of dead seeds after germination/Total number of germinated seeds) × 100

Results and Discussion

The *A. flavus* isolate (A129) used in the current study was isolated from a raw dehiscid rice sample. It was non-sclerotic type and produced 25 µg/ml of aflatoxin B1 in culture broth.

The toxic culture filtrate of A129 drastically reduced the seed germination rate, root shoot length and root shoot weight of rice cultivar Savitri and the reduction of these parameters were directly proportional to the concentration of the culture filtrate (Table 1 & Figure 1). The germination rate of seeds soaked in sterilized water (control) was 70%, with no seedlings mortality, whereas in the case of undiluted culture filtrate, germination rate was 18% and seedling mortality rate was 75%. Root growth was completely inhibited in seedlings treated with undiluted filtrate and very poorly developed in 50% and 25% filtrates. Root weight of seedlings treated with undiluted and 50% filtrate were 9 and 13 mg, whereas shoot weight was 133 and 161 mg, respectively (Table 1). These results were in accordance with those obtained previously (1). Aflatoxins in food grains interfere with protein synthesis by inhibiting the incorporation of amino acids into protein, resulting in non-germination of embryo and it also binds to DNA and thus prevents RNA synthesis (13). In some legumes, this reduction in root and shoot length was thought to be due to allantoinase (allantoina amido hydrolase) activity on germinating seeds (14). The residual seed weight was lowest (358.9 mg) in the case of untreated control and it increased with the increase in concentration of culture filtrate, i.e. highest in case of undiluted filtrate (521 mg). It indicated that the aflatoxin interfered with the physiological processes which prevented the use of endosperm by the developing seedlings.

Rice cv. Savitri (CR 210-1009) is a popular high yielding cultivar in the eastern coast of India, grown in shallow low-land ecosystem. It is resistant to major rice diseases and pests and of 145 days growth duration, hence largely adopted for cultivation by farmers. The current study showed that this variety was susceptible to aflatoxin

B1 produced by *A. flavus*. Hence, control measures should be adopted to prevent the contamination of this fungus to rice otherwise it may cause quantitative and qualitative changes in chemical composition (biodegradation) of the rice seeds.

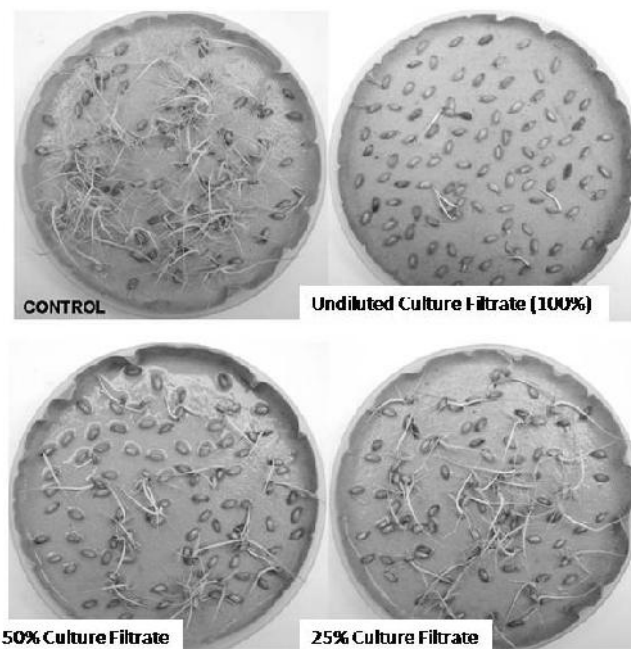


Figure 1. Plates showing germination and mortality of seedlings treated with aflatoxic culture filtrate of *A. flavus*.

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Table 1. Effect of cultural extract of toxigenic *Aspergillus flavus* isolate A129 on germination and mortality rates of rice cultivar Savitri (CR 1009).

Solution	Germination rate (%)	*Seedling mortality rate (%)	Root length (cm)	Shoot length (cm)	Root weight (mg)	Shoot weight (mg)	Residual seed weight (mg)
Control	70 ± 2.9	0.0	4.2 ± 0.19	4.3 ± 0.10	27 ± 1.2	208 ± 11.84	358.9
Cultural filtrate 100%	18 ± 1.4	75 ± 6.88	0.0	1.8 ± 0.13	9 ± 0.46	133 ± 8.24	521.0
Cultural filtrate 50%	32 ± 0.6	22 ± 2.66	0.2 ± 0.05	2.5 ± 0.12	13 ± 1.47	161 ± 1.65	495.0
Cultural filtrate 25%	35 ± 1.2	19 ± 1.45	2.3 ± 0.18	3.6 ± 0.14	22 ± 0.41	205 ± 1.45	468.0

الملخص

ناياك، اس.، ي. دهوا، س. سنكييتا، أس. سامان توس و أس. ر. دهوا. 2015. تثبيط إنبات ونمو بادرات الرز برشاحة الفطر *Aspergillus flavus* المنتج للأفلاتوكسين. مجلة وقاية النبات العربية، 33(1): 93-95.

يسبب الأفلاتوكسين B1 الذي ينتجه الفطر *Aspergillus flavus* ضرراً للمحاصيل ومحاصيل الحبوب بما في ذلك الرز عن طريق منع إنبات البذور، واستطالة السوقة الجنينية أو الجذور وتغيير العمليات الفيزيولوجية في البادرات المتطورة. يعد صنف الرز سافيتري من الأصناف الشعبية عالية الغلة في الجزء الشرقي الساحلي من الهند، حيث تتوفر ظروف ملائمة لنمو الفطر *Aspergillus flavus* وتؤدي ممارسات الحصاد التي يتبعها المزارعون عادة إلى الإصابة بالفطر. أظهرت دراسة رشاحة مزرعة الفطر *A. flavus* في الصنف سافيتري (CR 210-1009). انخفضت نسبة إنبات البذور، وطول الفروع الجذرية ووزن الفروع الجذرية للبادرات بشدة عند غمس البذور في الرشاحة الفطرية المخففة وغير المخففة (50% و 25%) وفي حالة الرشاحة غير المخففة كان معدل إنبات البذور وموت البادرات 18% و 75% مقارنة مع 70% و 0.00% لمعاملة الشاهد غير المعاملة، على التوالي. كان الوزن المتبقي للبذور أخفض (358.9 مغ) في الشاهد غير المعامل وأعلى في الرشاحة غير المخففة (521 مغ). تشير النتائج إلى أن الرشاحة المحتوية على الأفلاتوكسين تداخلت مع العمليات الفيزيولوجية والتي منعت البادرة المتطورة من استخدام السويداء

كلمات مفتاحية، أفلاتوكسين، سافيتري، إنبات، *Aspergillus flavus*، الرز.

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