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Antifungal potential of some natural products against *Aspergillus flavus* in soybean seeds during storage

Y.L. Krishnamurthy *, J. Shashikala, B. Shankar Naik

Department of P.G. Studies and Research in Applied Botany, Bio-Science Complex, Kuvempu University, Jnana Sahyadri, Shankaraghatta, Shimoga District, 577 451, Karnataka, India

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ABSTRACT

The inhibitory effect of cow-dung fumes, captan, leaf powder of *Withania somnifera, Hyptis suaveolens*, *Eucalyptus citriodora*, peel powder of *Citrus sinensis*, *Citrus medica* and *Punica granatum*, neem cake and pongamia cake on the growth of *Aspergillus flavus* in soybean seeds during storage was investigated. Soybean seed was treated with different natural products and the fungicide captan and was stored at ambient conditions for 6 months. Seed samples were withdrawn at monthly intervals and the incidence of seed-borne *A. flavus* and percentage germination of the seed was determined. Captan, neem cake, pongamia cake and peel powder of *C. sinensis* reduced the incidence of *A. flavus*. Leaf powder of *W. somnifera, H. suaveolens*, *E. citriodora* and peel powder of *P. granatum* also checked the frequency of *A. flavus*. All treatments maintained a high germination percentage of the soybean seeds over a storage period of 6 months. These natural products may be alternatives to chemical fungicides and provide an easy method to protect soybean and other agricultural commodities from *A. flavus* in storage.

1. Introduction

Soybean (*Glycine max* (L.) Merrill) is one of the most important food and oilseed crops cultivated and utilized in most parts of the world including India. It is widely accepted as an excellent source of nutrition to both man and poultry due to its high protein content. Several investigations have listed a large number of fungi, which could be isolated from soybean seeds during storage (Sinclair, 1982; Mukherjee and Nandi, 2001). Fungi are the most important agents in seed deterioration and hence their succession is of great significance in stored seeds. During long storage, the increase in seed moisture as well as the breakdown products or substratum offer favourable conditions for their growth (Nandi and Haggblom, 1984). It has been established that under tropical conditions seed deterioration takes place in soybean seeds during storage (Nkang and Umoh, 1996). Storage fungi cause deterioration of stored soybeans resulting in reduced seed germination and downgrading of grain because of damage and mustiness (Christensen and Dorworth, 1968). *Aspergillus flavus* Link ex. Fries is the dominant storage fungus colonizing soybean seeds under Indian conditions (Krishnamurthy and Raveesha, 1996; Syamala and Murthy, 2000). Colonization of soybean seeds with this mould is of importance because of its potential to produce aflatoxins, which are potent toxic, carcinogenic, mutagenic, immunosuppressive and teratogenic agents (Thanaboripat, 2002). *Aspergillus flavus* also cause seed rots, moulding of seeds, pre- and post-emergence damping off and reduces seed viability and seedling growth in soybean (Chandra et al., 1985).

The need for protection of food and feedstuffs against *A. flavus* is universally recognized and several approaches such as treatment of seeds with fungicides, fumigants, plant and earth products have been suggested (Bhale, 2004; Singh et al., 2004). Some common plants are known to have a high degree of resistance against fungal infection as they contain inhibitory compounds (Ghosh et al., 2000). Powders and extracts of many higher plants have been reported to offer a certain amount of protection from *A. flavus* infestation during storage and enhance seed emergence and seed germination (Nwachukwu and Umechuruba, 2001; Donli and Dauda, 2003). The present study was aimed at finding the efficacy of some naturally available materials such as cow-dung cake fumes, leaf powder of *Withania somnifera* Dunal., *Hyptis suaveolens* Poir., *Eucalyptus citriodora* Hock., peel powder of *Citrus sinensis* Osbeck., *Citrus medica* L. and *Punica granatum* L., neem (*Azadirachta indica* A. Juss.) cakes and pongamia (*Pongamia pinnata* (L.) Merr.) cakes in checking the growth of *A. flavus* on soybean seeds over a storage period of 6 months in comparison with the fungicide captan, which has been already established as a good inhibitor of *A. flavus* growth and enhancer of seedling growth.

2. Materials and methods

2.1. Source of treatment materials

Cow-dung cake used in this experiment was collected from a local household, while captan, neem cake and pongamia cake

* Corresponding author. Tel.: +91 8282 256254.
E-mail address: murthy_ylk@yahoo.co.in (Y.L. Krishnamurthy).

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were purchased from a local market in Shimoga, India. Fresh and healthy leaves of *W. somnifera* were collected from wastelands in Shimoga District and those of *H. suaveolens* and *E. citriodora* were collected in and around the Kuvempu University campus. Peels of *C. sinensis*, *C. medica* and *P. granatum* were collected from fruit markets and juice shops. All materials of plant origin were air dried at room temperature for 2–3 days and finely powdered using a Waring blender.

2.2. Soybean sample preparation

Thirty kilograms of soybean seeds (JS-335 variety) were collected from the Agricultural Research Station, Bhavikere, Karnataka and divided into 1 kg lots; each seed lot was placed in an airtight plastic container. A wooden box was divided into upper and lower compartments by means of a 2 mm size porous metallic sieve. One kilogram soybean seed was placed on the surface of the sieve in the upper compartment and the surface of the lower compartment was covered by an aluminium sheet. A dry cow-dung cake weighing either 100 g (10% w/w, cow-dung/seeds) or 250 g (25%, w/w) was placed in the lower compartment and burnt so that only fumes came out of the cakes and the soybean was exposed to the fumes through the porous metallic sieve. This process was carried out until the cow-dung cakes were burnt out completely. Then exposed soybean seeds were cooled and placed in plastic containers for storage.

Captan (1.5% and 2%, w/w), leaf powders of *W. somnifera, H. suaveolens*, *E. citriodora*, peel powders of *C. sinensis*, *C. medica*, *P. granatum* and neem cake and pongamia cake (5% and 10%, w/w) were added individually to 1 kg soybean seed lots in plastic containers and mixed thoroughly by vigorous shaking. Untreated soybean seed was used as a control. These containers were maintained at room temperature for 6 months under ambient conditions. Treated and untreated seed samples were withdrawn at monthly intervals and the incidence of seed-borne *A. flavus* and germination percentage of the seed was determined by the Standard Blotter Method (ISTA, 1996). Four replicates of 100 seeds of each soybean sample were placed on three layers of sterilized, moistened blotter discs in sterilized plastic Petri dishes (90 mm diameter) at the rate of 20 seeds/plate. All plates were incubated for 7 days at room temperature. After incubation, samples were screened with a stereobinocular microscope for the presence of seed-borne *A. flavus.* The mould isolates were identified based on mycelial growth, colour, length and arrangement of conidia on conidiophores (Raper and Fennell, 1965; Klich, 2002). The numbers of colonies of *A. flavus* were counted and percentage occurrence was calculated using the formula

number of colonies of *A. flavus*/number of seeds plated × 100

Similarly on the 7th day of incubation, the germination percentage was calculated using the formula,

number of seeds germinated/number of seeds plated × 100

The moisture content of the initial bulk sample, each individual treated sample and the samples treated with cow-dung fumes were determined by the hot air oven method at the time of treatment and after the storage period (ISTA, 1993).

3. Results

The moisture content of the initial bulk sample was 16% and it remained the same for all treated individual samples and for the samples treated with cow-dung fumes. The moisture content did not change during the storage period and was around 16% in each sample after 6 months of storage in polythene containers.

The effect of the different treatments including cow-dung fumes and plant products on the incidence of *A. flavus* and germination of soybean seeds over a period of 6 months in ambient storage conditions is given in Tables 1 and 2, respectively. All the treatments were effective in reducing the average incidence of *A. flavus* when compared with the control after the 6-month storage period. Captan resulted in a 95% reduction at 1.5% w/w and 97% at 2% compared with the control and was most effective. Exposure of soybean seeds to cow-dung fumes reduced *A. flavus* incidence by 62% after 6 months of storage. Among the plant products, neem cake (71% reduction at 10%), pongamia cake (71% at 5% and 67% at 10%) showed maximum efficacy i n controlling *A. flavus* occurrence followed by peel powder of *C. sinensis* (59% at 5% and 61% at 10%), *P. granatum* (54% at 5% and 59% at 10%), leaf powder of *H. suaveolens* (56% at 5%) and the combination of leaf powders of *W. somnifera, H. suaveolens* and *E. citriodora* (56% at 15%). Treatments with *E. citriodora* leaf powder (54% at 5%) and *W. somnifera* leaf powder (51% at 5%) were also found to be effective. Least effective was the combination of peel powders of *C. sinensis, C. medica* and *P. granatum* (29% reduction at 7.5% and 34% at 15%). All other treatments resulted in a considerable reduction in *A. flavus* occurrence, by at least 40% compared with the control.

The average percentage germination in all treatments and the control was above the certification standard of 70% in India (Tunwar and Singh, 1988) (Table 2). The highest mean germination percentages were recorded in seeds treated with captan (94% at 1.5% and 91% at 2%). Seeds exposed to cow-dung fumes had average germination percentages of 86 and 81 at 10% and 25% concentration, respectively. Among treatments of plant origin, germination percentage was high in seeds treated with peel powder of *P. granatum* (89% at 5% and 91% at 10%), neem cake (88% at 5% and 89% at 10%), leaf powder of *W. somnifera* (87% at 5% and 90% at 10%), pongamia cake (87% at 5% and 90% at 10%) followed by leaf powder of *H. suaveolens* (89% at 5% and 86% at 10%), *C. sinensis* (87% at 5% and 85% at 10%) and the combination of leaf powders of *W. somnifera, H. suaveolens* and *E. citriodora* (86% at 5% and 10%). In all other treatments the average germination percentage was also higher than in the control except for the treatment with the combination of peel powders of *C. sinensis, C. medica* and *P. granatum* (84% at 7.5% and 82% at 15%) and cow-dung fumes (81% at 25%).

Statistical analysis by ANOVA indicated that there was a highly significant difference in the occurrence of *A. flavus* between storage months ($F_{(5,\ 120)} = 13.3$; $P<0.001$) and between different treatments ($F_{(24,\ 120)} = 15.62$; $P<0.001$). ANOVA tests also showed that percentage germination varied significantly between storage months ($F_{(5,\ 120)} = 2.93$; $P<0.001$) and between different treatments ($F_{(24,\ 120)} = 2.63$; $P<0.001$). Correlation analysis showed that there was a strong negative correlation between *A. flavus* occurrence and germination percentage during storage ($r = -0.74$, $P<0.05$).

4. Discussion

Results clearly indicated that the occurrence of *A. flavus* in soybean seeds was reduced considerably by all the treatments used. All treatments were also effective in maintaining the germination rate above 70%. The fungicide captan used in this study was found to be effective in checking the seed-borne *A. flavus* on soybean seeds during 6 months of storage and it also elevated average germination percentage when compared to the control. The same results were obtained by several other researchers with soybeans and other crops (Nasir, 2003; Chavan and Padule, 2005). The effectiveness of captan in suppressing the