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Application of Eugenol for Coating Soybean Seeds as Fungal Control

AUNTIKA SAWATWANICH[1], PITIPONG THOBUNLUEPOP[2], SRISULAK DHEERANUPATTANA[1], CHAIWAT JATISATIENR[1], SUCHADA VEARASILP[3], ELKE PAWELZIK[2], ARAYA JATISATIENR[1]

[1] *Chiang Mai University, Department of Biology, Thailand*
[2] *Georg-August-University Göttingen, Department of Crop Sciences, Germany*
[3] *Chiang Mai University, Department of Agronomy, Thailand*

Abstract

Phytopathogenic molds associated with soybean seeds were determined by agar plate technique. To compare the antifungal property of eugenol with conventional substances, seeds were coated with chitosan and with chitosan plus eugenol (1%) and they were mixed with captan, respectively. Untreated seeds were used as control. The seeds from each treatment were kept in sealed plastic bags at 15ºC with 60% relative humidity for 6 months. Every month during storage, they were tested against the seed-borne fungi by the agar plate technique and the standard germination was determined by the blotter method. Seven fungal species i.e. *Aspergillus flavus*, *Aspergillus niger*, *Chaetomium* sp., *Cladosporium* sp., *Colletotrichum* sp., *Curvularia* sp. and *Macrophomina* sp., were determined in the soybean samples. After 6 months of storage, the seeds coated with chitosan plus eugenol showed similar good results as those mixed with captan. However, seeds coated with chitosan showed less efficiency. On the other hand, the efficiency of all treatments gradually decreased during storage. The germination percentage of seeds coated with chitosan was significantly at highest followed by those coated with chitosan plus eugenol and control seeds, respectively. The seeds mixed with captan lost more germination capability than the seeds from the others treatments.

Introduction

Soybean, *Glycine max* (L.) Merr., is one of the major economic crops in Thailand (DEPARTMENT OF AGRICULTURE, 2004). Soybean seeds, as all grains, begin to lose their quality after harvesting. Biological aging, microbial infection and insect attack are important factors affecting quality loss of soybean seeds during the postharvest period (LIU, 1997). Therefore, the effect of eugenol as an antifungal agent on the growth of seed-borne fungi of soybean seeds during storage was studied. Eugenol was used as seed coating agent incorporated into chitosan-lignosulphonate polymer. The germination percentage of soybean seeds and the efficiency of seed coating on storage molds were also determined.

Materials and Methods

Soybean seeds var. CM. 60 were used in this experiment. The mycoflora associated with soybean seeds samples was studied by the agar plate technique of ISTA (2006). Seeds were placed on potato dextrose agar medium supplemented with streptomycin for suppression of

bacterial growth and incubated at 25°C for 7 days. The fungi appearing on the seeds were isolated, purified and identified according to SUTTON (1980), ELLIS (1993) and BARNETT (1998).

Seed coating was prepared by adding 1 ml of eugenol in 99 ml of the seed coating substances (2% chitosan, 0.1% lignosulphonic acid, 1% acetic acid, 0.3% food color and 95.6% distilled water), mixed thoroughly with 400 g of seeds and dried at 50ºC airflow. The seed coating substances without eugenol were also prepared and the coated seeds served as control. After coating, the seeds were dried at room temperature to adjust the seed moisture content at 10±2% and kept them in sealed plastic bags at 15ºC with 60% relative humidity for 6 months. Moreover, seeds mixed with captan (2.5 grams per 1 kilogram of seed weight) and untreated seeds were also prepared to compare the antifungal property of captan with eugenol.

The presence of fungi on seeds in each group was examined and identified under a stereomicroscope by the agar plate technique (ISTA, 2006). The inhibition percentage of fungal growth was calculated by the following formula:

$$\% \text{ Inhibition} = [(f_c - f_t)/f_c] \times 100,$$

f_c is the presence of fungi on the control seed and f_t is the presence of fungi on the treated seed.

The standard germination of all treatments was determined monthly by the blotter method (ISTA, 2006). Samples of each 200 seeds per treatment were placed on 4 moist paper towels (50 seeds per towel), which were loosely rolled and maintained for 8 days at 25°C. After 3 and 8 days of incubation, seedlings with normal development were counted.

Results and Discussion

During the storage period, the following fungal species were the most abundant: *Aspergillus flavus*, *A. niger*, *Chaetomium* sp., *Cladosporium* sp., *Colletotrichum* sp., *Curvularia* sp. and *Macrophomina* sp. (Table 1).

Table 1 Occurrence of fungi on soybean seeds during storage

Fungal species	**Percentage occurrence (%)**
Aspergillus flavus	20.45
Aspergillus niger	8.28
Chaetomium sp.	5.57
Cladosporium sp.	4.14
Colletotrichum sp.	29.30
Curvularia sp.	10.12
Macrophomina sp.	26.82

After 6 months of storage, the seeds coated with chitosan plus eugenol showed similar results compared to those mixed with captan. The growth of *Curvularia* sp.; *Cladosporium* sp.; *Macrophomina* sp., *Collectotrichum* sp., and *A. flavus* could be controlled for 4, 5 and 6 months. *Chaetomium* sp. and *A. niger* were controlled in the seeds mixed with captan one month longer than those coated with chitosan plus eugenol. These results are in agreement with the studies of HITOKOTO *et al*. (1980) and KARAPINAR (1990) who found that eugenol was very effective against mycelium growth of many fungi. The seeds coated with chitosan showed less efficiency. The growth of *A. niger* could be controlled only for 2 months; *Chaetomium* sp. for 3 months; *A. flavus* and *Curvularia* sp. for 4 months; *Macrophomina* sp. for 5 months; *Colletotrichum* sp. and. *Cladosporium* sp. for 6 months. CHEAH *et al*. (1997) as well as ZHANG AND QUANTICK (1998)

reported that chitosan could be used as a natural antimicrobial coating agent against the growth of some phytopathogenic fungi. However, the efficiency of all treatments gradually decreased with the duration of storage (Figure 1).

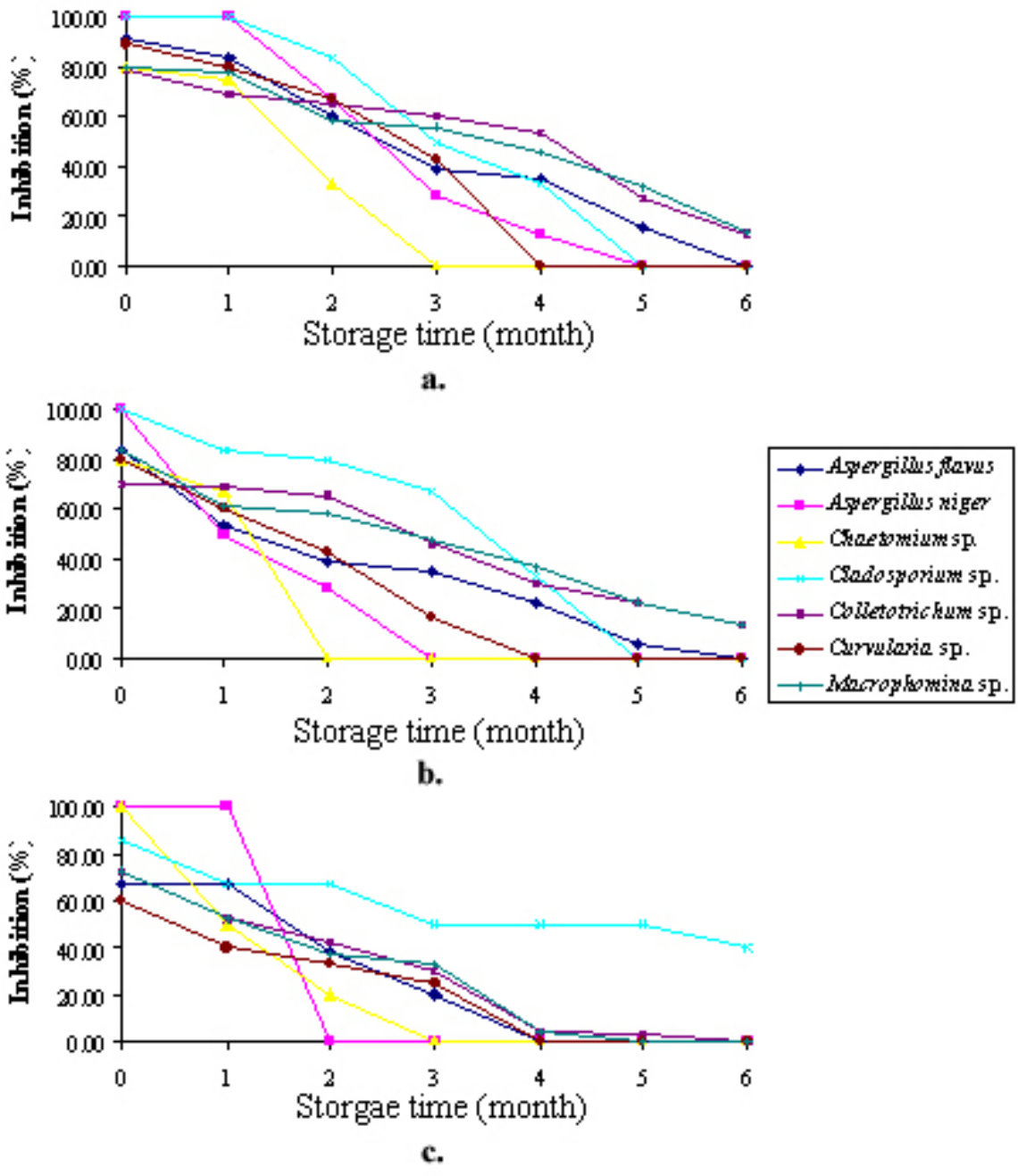

Figure 1 Inhibition percentage of seed-borne fungi during storage
a: seeds mixed with captan
b: seeds coated with chitosan plus eugenol
c: seeds coated with chitosan

Standard germination test showed that the percentage of germination of all treatments was 90 to 92 % at the beginning. After 6 months of storage, the germination of seeds coated with chitosan (74%) was significantly higher than in other treatments followed by those coated with chitosan plus eugenol (72%) and control seeds (70%), respectively. However, the seeds mixed with captan gave only 68% germination. This result corresponded with the work of AGARWAL AND SINCLAIR (1997) and GOULART *et al* (2002). Therefore, eugenol could be used as a seed coating substance because it is able to protect soybean seeds for some months from fungal infection and does not affect germination. However, more investigations should be carried out for more conclusive information.

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