SHORT REPORTS

J.Sci.Soc.Thailand, 14 (1988) 277-281

TOXIC AND PHYTOTOXIC NATURE OF SOME NEW ORGANIC COMPOUNDS AGAINST FUNGI DETERIORATING STORED MOONG SEEDS (*PHASEOLUS AUREUS* ROXB.)

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(Received 10 February 1988)

ABSTRACT

Three new organic compounds were tested for fungitoxicity against Aspergillus flavus *Link,* A. fumigatus *(Eidam) Wint.,* A. niger *V. Tiegh.,* A. parasiticus *Speare,* Cladosporium oxysporum *Bark and Curt,* Fusarium moniliforme *Sheldon and* Penicillium citrinum *Thom and for phytotoxicity. The Compound, 5-* p*-methoxyphenyl-2-* p *-ethoxyphenylthiocarbamoyl-1,3,4-oxadiazole exhibited fungicidal activity. It was non toxic to moong plants (*Phaseolus aureus *Roxb.) and also checked the appearance of fungi on the seeds.*

INTRODUCTION

Pulses are one of the major crops of India. Besides being grown over larger areas in Bengal, Bihar, Gujarat, Maharashtra, Orissa and Uttar Pradesh, it is one of the principal sources of human protein (24.6%). In the eastern U.P., largely *Cicer arietinum, Cajanas cajan* and *Phaseolus aureus* are cultivated. Storage conditions in most parts of India are very conducive to fungal growth and fungi cause appreciable deterioration in the nutritive quality of stored seeds. Both pathogenic and saprophytic fungi cause deterioration in pulses. They also reduce germination potential and secrete toxic metabolites. Such losses in seed quality can cause great economic loss. Recently synthetic heterocyclic compounds have been proved effective against bacteria, fungi and insects.[2,3,5] In the present investigation, an attempt was made to test the efficacy of newly reported organic compounds against six fungi which most frequently deteriorate pulses during storage. The

present paper reports the effect of three new organic compounds, i.e., *N,N'*-Bis [3-methyleno-5-(2,4-dichlorophenyl)-1,3,4-oxadiazole-2-thione] benzidine; 2-*n*-propyl-thio-5-*p*-hydroxyphenyl-1,3,4-oxadiazole and 5-*p*-methoxyphenyl-2-*p*-ethoxyphenyl-thiocarbamoyl-1,3,4-oxadiazole, on seven fungi in artificial medium. It also examines the effect of these compounds on the viability and growth (roots and shoots) of seeds infected with fungi.

Three new synthetic organic compounds *N,N'*-Bis[3-methyleno-5-(2,4-dichlorophenyl)-1,3,4-oxadiazole-2-thione] benzidine (compound **1**), 2-*n*-propylthio-5-*p*-hydroxyphenyl-1,3,4-oxadiazole (compound **2**), and 5-*p*-methoxyphenyl-2-*p*-ethoxyphenylthiocarbamoyl-1,3,4,-oxadiazole (compound **3**) were dissolved in acetone at one per cent concentration for analysis by the poisoned food technique.[1] Briefly, treatment sets were prepared by dissolving the requisite amount of each compound in 0.5 ml acetone and mixing it with 9.5 ml autoclaved Czapek Dox Agar medium. In controls, sterile water added to 0.5 ml acetone was mixed with 9.5 ml Czapek Dox agra medium. The plates were inoculated aseptically with the assay discs (2 mm) of each fungus viz. *Aspergillus flavus* Link, *A. fumigatus* (Eidam) Wint, *A. niger* V. Tiegh, *A. parasitious* Speare, *Cladosporium oxysporum* Bark and Curt, *Fusarium monaliforme* Sheldon and *Penicillium citrinum* Thom. The cultures were isolated from moong seeds and identified. Identifications were confirmed by the Commonwealth Mycological Institute, Kew. Plates were incubated at 24° ± 2° C. Fungitoxicity was estimated after 96 hours using the following formula:

$$\text{Per cent inhibition} = \frac{(C-T) \times 100}{C}$$

Where C = Diameter of fungus colony (mm) for control plates

T = Diameter of fungus colony for test plates after 96 hours

In vivo toxicity of all the compounds and their phytotoxic nature were investigated on moong seeds. For this experiment fresh seeds of moong *(P. aureus)* were purchased from local provision stores. Seeds were surface sterilized with 1% $HgCl_2$ solution, then washed several times in sterile distilled water. Sterilized seeds were dried in an oven at 40° C for 12 hours. In each of the following test sets 50 g of seeds were separately inoculated with a one week old culture of each test fungus. Other 50 g lots were inoculated with a mixture of all seven fungi. One test set was dressed (w/v) with a 1% concentration of each of the test compounds. One test set was not treated with any compound. One set of sterilized seeds that were undressed and uninoculated served as the control. For inoculum 5 discs (2 mm) of test fungi were used. All sets were stored for six months at room temperature (18° to 35°C). After storage, observation for the appearance and growth of test fungi were made as recommended by Neergaard and Saad.[4]

Phytotoxicity was determined by monitoring the germination of moong seeds, seedling growth, general plant morphology and general plant health.

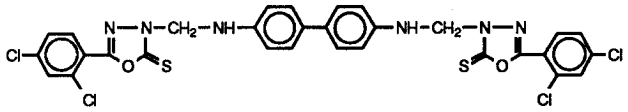
All the experiments were repeated twice and each test consisted of three replicates.

The effect of temperature on the toxicity of compounds was studied by keeping them for one hour at temperatures ranging between 15° to 40° C. The compounds were stored for 365 days to study the effect of storage on their toxicity.

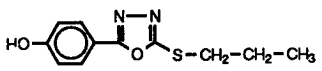
TABLE 1 Percent inhibition of fungal growth by 1,3,4-oxadiazoles **1** to **3**

Fungi	Inhibition (%)		
	Compounds		
	1	**2**	**3**
A. flavus	2	2	50
A. fumigatus	100	100	100
A. niger	100	100	100
A. parasiticus	80	2	100
C. oxysporum	100	100	100
F. moniliforme	100	2	50
P. citrinum	100	100	100

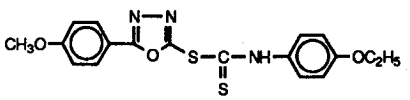
Compounds: 1. *N,N'*-Bis [3-methyleno-5-(2,4-dichlorophenyl)-1,3,4-oxadiazole-2-thione] benzidine.



2. 2-*n*-Propylthio-5-*p*-hydroxyphenyl-1,3,4-oxadiazole



3. 5-*p*-Methoxyphenyl-2-*p*-ethoxyphenylthiocarbamoyl-1,3,4-oxadiazole.



Substitution in 1,3,4-oxadiazole compounds alters the fungitoxic spectrum. Results in Table 1 reveal that the growth of *A. fumigatus, A. niger, C. oxysporum* and *P. citrinum* was completely inhibited by all three compounds, while growth of *A. parasiticus* and *F. moniliforme* was completely inhibited by compounds **3** and **1** respectively. The growth of *A. flavus* was found to be least affected by any compound. Minimum inhibitory concentrations of the compounds were found to be 1%. Compounds **2** and **3** were fungicidal for *A. fumigatus, A. niger, C. oxysporum* and *P. citrinum* but fungistatic for *A. flavus.* The fungitoxicity was not affected either by temperature or by storage.

Seeds inoculated and treated with the three compounds and stored for six months showed the appearance of fungi only in the case of compound **1.** All other treatments inhibited fungal growth completely. All untreated seeds showed fungal growth. Compound **1,** showed only partial inhibition of growth of *A. parasiticus* (6%), *C. oxysporum* (15%), *F. moniliforme* (10%) and *P. citrinum* (50%). Thus, compounds **2** and **3** were most effective in preserving the seeds.

TABLE 2 Effect of 1,3,4-oxadiazoles on the shoot length (mm) of *P. aurens* Roxb.

Fungi	Compound 2	Compound 3	Control
A. flavus	12.8 ± .058 *	1.8 ± .097	2.0 ± .146
A. fumigatus	13.4 ± .068	21.8 ± .068	3.0 ± .073
A. niger	11.4 ± .115	20.2 ± .056	2.3 ± .163
A. parasiticus	13.6 ± .068	21.3 ± .115	0.8 ± .093
C. oxysporum	14.0 ± .058	22.3 ± .150	1.2 ± .103
F. moniliforme	12.2 ± .052	17.2 ± .115	1.4 ± .077
P. citrinum	10.2 ± .106	20.6 ± .063	1.9 ± .093
Mixture	9.6 ± .106	18.4 ± .086	1.4 ± .077
Sterilized seeds	13.0 ± .058	20.0 ± .153	2.8 ± .068

* Standard error of mean

$P < .001$ for compounds **2** and **3**

Results from phytotoxicity tests showed that compound **1** was phytotoxic since it inhibited germination of seeds by 10% when used alone. In combination with fungi inhibition was 100%. By contrast, compounds **2** and **3** allowed 100% seed germination, as did sterilization. Seeds inoculated with fungi in the absence of compounds **2** and **3** all showed various degrees of germination inhibition ranging from 95% for the fungal mixture to 71% for *A. parasiticus.* Thus, compounds **2** and **3** show promise as seed preservatives.

Results from tests on the effect of compounds **2** and **3** on plant growth after germination are show in Table 2 for shoot growth. It can be seen that both compounds allow good shoot growth, observed to be even better than in the untreated, sterilized control seeds. A similar pattern was found with root growth tests using these two compounds. Because of their lack of phytotoxicity and effectiveness in preventing mold growth, compounds **2** and **3** may be tested as seed preservatives.

ACKNOWLEDGEMENT

The authors are thankful to Dr. S.C. Bahel, Reader in Chemistry, Gorakhpur University, Gorakhpur for providing the compounds.

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บทคัดย่อ

บทความนี้รายงานคุณสมบัติในการฆ่าเชื้อราและความเป็นพิษต่อพืชของสารอินทรีย์เคมีที่สังเคราะห์ขึ้นมาใหม่ 3 ตัว