SHORT REPORT
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Efficacy of some triazoles and pyrimidine-2-one compounds against *Chaetomium*, *Cunninghamella* and *Memnoniella* found in deteriorating jute fibres

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ABSTRACT: Six organic compounds (triazoles and pyrimidine-2-ones) were tested for fungitoxicity for the first time against *Chaetomium globosum*, *C. indicum*, *Cunninghamella echinulata*, *Memnoniella echinata*, and *M. subsimplex*, which were found to be frequently responsible for the deterioration of jute fibres during storage. Two of the pyrimidine-2-one compounds exhibited a fungicidal nature and the lowest minimum inhibitory concentration against all the test fungi. These chemicals also prevented the appearance of test fungi on the surface of treated jute fibre and showed broad fungitoxic spectra. The compounds were characterized by their physico-chemical properties and spectral analysis.

KEYWORDS: fungitoxicity, physico-chemical properties

INTRODUCTION

Jute is probably used more extensively than any other fibre except cotton. Out of the total world production, a major supply is from India. India not only grows most of the jute, but it is the largest manufacturer and exporter of jute products. Indian jute fibres are abundant, but are not very strong and they have a tendency to deteriorate rapidly when exposed to moisture. Storage conditions in most parts of India are very conducive to fungal growth. If the moisture content remains high for sufficient periods, fungal growth causes appreciable deterioration and mildews, along with physical and chemical changes[1,2] which may cause the fibre to fail in service.

The maintenance of the quality of jute is vitally important and necessary in order to protect the jute materials from deterioration as well as to protect human beings who use such deteriorated jute fabric unknowingly. The fungal spores can lead to infections, predominantly of the lung and skin, and allergies[3–6].

A survey of the literature revealed that many inorganic and organic synthetic compounds have proved to be effective against biodeteriogens[7–12] but as far as we are aware no work has been done to prevent or control the fungi concerned in the deterioration of jute fibre. Hence the present study was designed to test the efficacy of newly synthesized organic compounds against the test fungi which were found to be most frequently associated with deteriorating jute fibres during storage.

The present paper reports the fungitoxic effect of six new organic compounds of triazole and pyrimidine-2-one on five test fungi in synthetic medium. The active organic compounds were characterized by their physico-chemical properties including spectral analysis (UV and IR). The fungitoxic spectrum of active compounds was tested against predominant fungi isolated from deteriorated samples of jute. The efficiency of active compounds on the appearance and growth of fungi on the surface of treated and untreated jute fabric was also examined.

MATERIALS AND METHODS

Selection of test fungi

A total of 27 fungal forms isolated from deteriorated samples of jute have been preserved in the laboratory. A continuous testing program was made in which all the isolated fungal forms were evaluated for their ability to decompose cellulose, pectin, and lignin in pure culture. In the present study, out of 27 fungal forms *Chaetomium globosum* Kunze, *C. indicum* Corda, *Cunninghamella echinulata* Thaxter, *Memnoniella echinata* (Riv.) Galloway, and *M. subsimplex* (Cooke) Deighton were selected as test fungi because they were isolated from most of deteriorated samples of jute, they exhibited predominance, and they were constant species because they appeared continuously in four or more months. They were also found either in two or more seasons. When tested in pure culture

they were shown to be cellulolytic, pectolytic and/or lignolytic.

Screening of organic compounds for fungitoxicity

The organic compounds 2-(2,4-dichlorophenyl)-1-phenyl-1,2,4-triazolo-(1,2-a) triazole (DPTT), 2-benzyl-1-phenyl-1,2,4 triazole (1,2-a) triazole (BPTT), 4-methyl-5-carboethoxy-6-phenyl-3-phenyl-acetyl pyrimidine-2-one (MCPPP), 4-methyl-5-carboethoxy-6-(4-chlorophenyl)-3-phenylacetyl pyrimidine-2-one (MCCPP), 4-methyl-5-carboethoxy-6-phenyl pyrimidine-2-one (MCPP), and 4-methyl-5-carboethoxy-6-(4-chlorophenyl) pyrimidine-2-one (MCCP) were dissolved in 1% acetone (10 000 ppm) in presterilized conical flasks. These were vigorously shaken. The stock solutions thus obtained were used to test antifungal activity against the four test fungi using the poisoned food technique of Grover and Moore[13]. For the treatment sets, 1 ml of prepared solution of each compound was mixed with 9 ml of molten Czapek Dox agar medium in presterilized Petri dishes separately and agitated in order to mix the solution homogeneously. In the control sets, a requisite amount of acetone was added in place of the solution of the compounds. Fungal discs (5 mm diameter) cut from the periphery of 7-day old cultures of each test fungus were placed on the surface of the agar medium and incubated for 6 days at 25 ± 2 °C. The colony diameter of treated as well as control sets of the fungal discs were measured on day 7. Fungitoxicity was recorded in terms of I, the inhibition of mycelia growth which is given by

$$I = \frac{\Delta d_0 - \Delta d}{\Delta d_0},$$

where Δd_0 and Δd are the average increases in diameter of the fungal colonies in the control and treatments sets, respectively.

Physico-chemical properties

The compounds which exhibited absolute toxicity against all the test fungi were tested further. Their solubility was determined by the methods of Langeneu[14]. To determine the solubility, 1 mg of each compound was taken separately in a separating funnel and dissolved in different organic solvents one by one in increasing order of polarity. The funnel was shaken properly after each such addition. The organic solvent in which the compound dissolved completely to give a transparent solution was recorded. Their melting point was determined by using a melting point apparatus (Tempo Instrument); the requisite amount of organic compound was placed in a capillary tube and then heated until the chemical melted and this melting temperature was recorded. Spectral analyses (UV and IR) were also carried out on the compounds.

Fungitoxic properties

The minimum inhibitory concentration (MIC) of the compounds was determined by using the poisoned food technique[13].

To determine the nature of toxicity of the compounds, test experiments were designed according to the method described by Garbour and Houston[15]. The treatment sets were prepared by supplementing 1 ml of the 100 ppm solutions of the compounds with 9 ml of molten Czapek Dox agar medium in presterilized Petri dishes separately and agitating in order to mix the solution homogeneously. In the control set, acetone was used in place of the active compound solution. The assay Petri dishes were inoculated with fungal discs 5 mm in diameter cut from the periphery of the 7-day old culture and incubated for 6 days at 25 ± 2 °C. On day 7, the fungal disc of the treatment sets exhibiting complete inhibition of mycelial growth were separately taken out from the Petri dishes, washed thoroughly with sterilized water, and reinoculated in separate sets of Petri dishes containing fresh Czapek Dox Agar medium only. Reinoculated dishes were again incubated for 6 days at 25 ± 2 °C. Any revival of mycelial growth on day 7 was recorded. Experiments were repeated twice and each set contained three replicates.

The fungitoxic spectrum of active organic compounds was determined by the poisoned food technique[13] at their MIC against some fungi isolated from deteriorated samples of jute.

Efficacy of active organic compounds

Active organic compounds were further tested for their efficacy on the appearance of test fungi on samples of jute. For this, 5 cm^2 pieces of jute samples were surface sterilized by dipping them in 95% ethyl alcohol for 5 min. Sterilized pieces were left for half an hour in aseptic conditions and then dressed or treated with active organic compounds at their MIC for 5 min. Control sets in which the jute samples were not treated with solution of the active compounds were also maintained. Test fungi were inoculated aseptically in treated and untreated samples and incubated at a temperature of 25 ± 2 °C for 6 days. Appearance of fungi was observed by using the standard blotter method[16]. After day 3 of the incubation period, the Petri dishes were observed daily for the growth of the test fungi. Data relating the presence of the fungi on the pieces of control and treatment jute fabrics was

Table 1 Screening of compounds for their toxicity against the test fungi *Chaetomium globosum* (C.g.), *C. indicum* (C.i.), *Cunninghamella echinulata* (C.e.), *Memnoniella echinata* (M.e.), *M. subsimplex* (M.s.).

Compound	*I* (%)				
	C.g.	C.i.	C.e.	M.e.	M.s.
DPTT	30	37	45	0	0
BPTT	22	20	25	0	0
MCPPP	98	90	100	100	100
MCCPP	100	92	100	100	100
MCPP	100	100	100	100	100
MCCP	100	100	100	100	100

Table 2 Spectral analysis of MCPP and MCCP.

MCPP		MCCP	
IR Data		IR Data	
Significant bands (cm^{-1})		Significant bands (cm^{-1})	
Band (cm^{-1})	Assignment	Band (cm^{-1})	Assignment
3247.8	N−H	3243.9	N−H
3119.8	C−H	3118.5	C−H
2978.7	C−H	2956.2	C−H
1723.9	C=O	1704.9	C=O
1645.9	C=O−N	1648.6	C=O−N
1488.5	Aromatic ring	1688.5	Aromatic ring
1461.8	Aromatic ring	1461.8	Aromatic ring
1425.2	Aromatic ring	1424.2	Aromatic ring
1222.1	C−O	1222.1	C−O
UV Data		UV Data	
λ-max (nm)	ABS Assignment	λ-max (nm)	ABS Assignment
205	1.041 π- π* C=C	207	1.041 π- π* C=C
279	1.097 π- π* C=O	267	1.097 π- π* C=O
359	1.241 η- π* C=O	364	1.241 η- π* C=O

recorded. All the experiments were repeated twice and each set contained three replicates.

RESULTS

Out of six organic compounds which were screened, MCPP ($C_{14}H_{17}N_2O_3$, mol. wt. 261) and MCCP ($C_{14}H_{16}N_2O_3Cl$, mol. wt. 294) showed absolute toxicity against all four test fungi (Table 1). Both compounds were shown to be fungidical with an MIC of 100 ppm. They have an aromatic odour, a ketone functional group, and were soluble in acetone, benzene, methanol, phenol, and xylol. Both had similar melting points and colour (200 °C, mustard for MCPP; 196 °C, yellow for MCCP). The results of the spectral analysis are given in Table 2.

Both compounds also completely inhibited growth of the fungi *Alternaria alternata*, *A. fumigatus*, *A. nidulans*, *A. ochraceus*, *A. sydowi*, *A. terreus*, *Penicillium notatum* and *Stachybotrys theobromae* isolated from deteriorated samples of jute.

In the tests on jute samples, MCPP inhibited the growth of 4 out of 5 of the test fungi (Table 3). On the other hand, MCCP was found to be less effective in the sense that it inhibited the growth of *Chaetomium globosum*, *Memnoniella echinata*, *M. subsimplex* and did not prevent the growth of *Chaetomium indicum* and *Cunninghamella echinulata* on treated samples of jute. However, the growth of these test fungi was much less in treated sets than in untreated sets (Table 3).

Table 3 Efficacy of active organic compounds at their MIC on jute fabric.

Fungi	Compounds		
	Untreated	Treated	
		MCPP	MCCP
Chaetomium globosum	+++	-	-
C. indicum	+++	-	++
Cunninghamella echinulata	+++	+	+
Memnoniella echinata	+++	-	-
M. subsimplex	+++	-	-

+++: maximum growth
++: moderate growth
+: minimal growth
-: no growth

DISCUSSION

In the present study, all the test fungi were selected on the basis of specific criteria which revealed that these species play a vital role in the deterioration of jute fibre. Usually there are no definite criteria for the selection of the test organism[17].

Fungitoxic properties of organic compounds have been found to vary from group to group. In the present study six organic compounds were screened against all the test fungi. Two of them are derivatives of triazole and the remaining four are derivatives of pyrimidine-2-one. The absolute toxicity against all the test fungi was found in two compounds of pyrimidine-2-one derivatives having methyl, carboethoxy, phenyl, and chlorophenyl as additional groups.

Evaluation of antifungal activity has been done by earlier workers on the basis of either inhibition of spore germination or mycelial growth. In the present study, because the spores of most of the test fungi are very small, inhibition of mycelial growth was taken for antifungal testing. Park et al[18] reported the antifungal efficacy of some novel indazole-linked triazoles against a variety of fungal cultures (*Candida* spp. and *Aspergillus* spp.) which supports the result of present investigation.

For the characterization of organic compounds,

physico-chemical properties are the best criteria. The study of the chemicals for antifungal activity without providing their physico-chemical characteristics may lead to variable fungitoxic results, because the quality of different chemical compounds may vary for various reasons. It was for these reasons that the physico-chemical properties and spectral data were recorded.

For any chemical, it is very important to know the exact concentration at which it just kills or stops the growth of the fungus and the nature of toxicity. This helps in describing its efficacy as well as in prescribing its appropriate dose. A high dose will decrease its rational value, increases its wastage, and may cause harm to the material which is being protected.

In the present study, the two active organic compounds exhibiting the lowest MIC were taken for further studies. It is always to the benefit of the masses that an antifungal compound should be efficacious against several organisms. During this study, the active compounds exhibited a broad range of antifungal activity, completely inhibiting the mycelial growth of 8 fungal species and inhibiting a further 3 species by more than 50%. These compounds also inhibited the growth of test fungi in situ on samples of jute.

From the literature available, it is evident that there is no record on antifungal activity of these organic compounds against the test fungi examined in the present study. Therefore, the organic chemical compounds discussed above are being reported as new compounds as far as their fungitoxic properties and efficacy are concerned. The present investigation suggests that the two most active organic chemical compounds, on account of their best fungitoxic property, may be recommended for use, after testing on a large-scale basis, as disinfectants at the stage of manufacturing of jute fabric or jute products from jute fibre.

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REFERENCES

1. Basu SN, Ghosh SN (1952) Fungal decomposition of jute fibre and cellulose. *J Textile Inst* **43**, 355–61.
2. Bhattacharya JP, Bose RG (1954) Fungi growing on rot proofed jute cloth. *Sci Cult* **20**, 189–91.
3. Bunse T, Merk H (1992) Mycological aspects of inhalative mould allergies. *Mycoses* **35**, 61–6.
4. Gravesen S (1979) Fungi as cause of allergic disease. *Allergy* **34**, 135–64.
5. Issac S (1996) To what extent do airborne fungal spores contribute to respiratory disease and allergic reaction in humans? *Mycologist* **10**, 31–2.
6. Lacey J (1991) Aerobiology and health: The role of airborne fungal spores in respiratory disease. In: Hawsworth DL (ed) *Frontiers in Mycology*, CAB International.
7. Gupta RC, Nath R, Shanker K, Bhargava KP, Kishore K (1978) Biologically active thiazolidinone. *J Indian Chem Soc* **55**, 832–4.
8. Khan N, Misra A, Singh N (2007) Fungitoxic properties of some organic chemical compounds against *Epidermophyton floccosum*. In: Sharma N, Singh HB (eds) *Biotechnology: Plant Health Management*, Int Book Dist Co, Lucknow, pp 563–71.
9. Male O (1991) The significance of mycology in medicine. In: Hawsworth DL (ed) *Frontiers in Mycology*, CAB International.
10. Misra N, Batra S (1987) Efficacy of some oxadiazoles against *Aspergilli* and *Penicillia* deteriorating stored spices. *Indian J Mycol Plant Pathol* **16**, 217–9.
11. Okeke CN, Gugnani HC (1990) In vitro activity of seven azole compounds against some clinical isolates of non-dermatophytic filamentous fungi and some dermatophytes. *Mycopathologia* **110**, 157–61.
12. Rathore A, Misra N (1988) Toxic and phytotoxic nature of some new organic compounds against fungi deteriorating stored moong seeds (*Phaseolus aureus* Roxb.). *J Sci Soc Thailand* **14**, 277–81.
13. Grover RK, Moore JD (1962) Toxicometric studies of fungicides against brown rot organisms *Sclerotinia fructicola* and *S. laxa*. *Phytopathology* **52**, 876–80.
14. Langenau EE (1948) The examination and analysis of essential oils, synthetic and isolates. In: Guenther E (ed) *The Essential Oils*, Vol 1, Robert E Krienger, New York, pp 227–348.
15. Garber RH, Houstan BR (1959) An inhibitor of *Verticillium alboratum* in cotton seeds. *Phytopathology* **49**, 449–50.
16. Neergaard P, Saad A (1962) Seed health testing of rice. A contribution to development of laboratory routine testing methods. *Indian Phytopathol* **15**, 85–111.
17. Mahadevan A (1982) *Biochemical Aspects of Plant Disease Resistance. Part I. Performed Inhibitory Substances – Prohibitins*, Today and Tomorrow, New Delhi.
18. Park JS, Yu KA, Kang TH, Kim S, Suh YG (2007) Discovery of novel indazole-linked triazoles as antifungal agents. *Bioorg Med Chem Lett* **17**, 3486–90.