Antifungal Activity of Trimethyltinbenzoate-4-Picoline+

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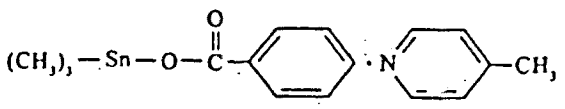
Trimethyltinbenzoate-4-picoline was screened for its antifungal activities against six fungi consisting of four moulds, *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus versicolor* and two yeasts, *Brettanomyces anomala, Saccharomyces cerevisiae* isolated from diseased grape plants (*Vitis vinifera*). *A. niger* and *A. versicolor* were inhibited by the compound at a concentration of 0.5 mg/ml, while *A. flavus* and *S. cerevisiae* required 1 mg/ml concentration. *A. fumigatus* and *B. anomala* required a higher concentration of 2.5 mg/ml for inhibition. The Minimum Inhibitory Concentration (MIC) for *A. versicolor, A. niger, A. flavus, S. cerevisiae, A. fumigatus* and *B. anomala* were found to be 0.4, 0.45, 0.8, 0.9, 2.5 and 2.5 mg/ml, respectively.

Despite the routine use of Chemical pesticides for the control of fungal diseases of crop plants, these diseases have become more severe in different parts of the world. One of the possible explanations for this is the development of tolerance by some pathogens. The suspected microbial characteristics responsible for the resistance are mutability of genes controlling the site of action of the pesticides, the rate of multiplication of the pathogens as well as the viability rate of resistant mutant[1]. These pesticides are environmental pollutants when used in excess. The production of organotin compounds is receiving more attention due to their wide range of biological properties. The biocidal activities of organotin carboxylates[2-4] and triorganotin compounds[5], the antifungal activity of n-tributyltin acetate against some common yam (*Dioscorea rotundata*, staple food in Africa) rot fungi[6], the acute toxicity of triphenyltin acetate[7], the trypanocidal activity of bis (tri-n-butyltin) oxide against *Trypanosoma* brucei[8] and the antitumor activities of organotin carboxylates[9-12] have been reported earlier.

The introduction of organotin compounds has had the effect of reducing long-term hazards, where the fact that organotin agrochemicals eventually break down physiochemically or biologically to harmless non-toxic forms of tin when released to the environment[13] is an advantage. In this study, the antifungal activity of trimethyltinbenzoate-4-picoline has been determined for its potential fungicidal activities against fungal pathogens of grapevine (*Vitis vinifera*) plants.

All chemicals were procured from BDH, England; Potato Dextrose Agar and Nurtient Broth were supplied by Oxoid Ltd., Basingstoke, Hants, England. Methanol was distilled and then dried before use.

Trimethyltinbenzoate-4-picoline complex was prepared by reacting trimethyltin chloride, sodium benzoate and 4-picoline in equimolar proportion in dry methanol at room temperature (25°) under stirring for 12 h. The precipitated sodium chloride was filtered, the filtrate was concentrated in vacuo and the product crystallized from methanol, m.p. 76-78°(I).



(I)

The IR (Nujol) spectrum of the compound (I) showed bands at 1620 (strong, -O-CO-) and 1050 cm^{-1} (weak,

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Sn-O-CO). The proton NMR spectrum (90 MHz, $CDCl_3$, TMS, δ) showed signals at 0.55 (s, 9H, $3CH_3$ attached to Sn), 2.35 (s, 3H, pyridyl CH_3), 7.2 (m, 5H, two meta pyridyl and three benzoyl protons, two meta, one para), 8.2 (m, 4H, two ortho pyridyl and two ortho benzoyl protons). C, H, N, Sn analysis: C_{16} H_{21} O_2 N Sn required were C, 50.83%; H, 5.60%; N, 3.71%; Sn, 31.40%. The values found were C, 50.76%; H, 5.72%; N, 3.63%; and Sn, 31.34%.

Six fungi consisting of four moulds, *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus versicolor* and two yeasts, *Brettanomyces anomala, Saccharomyces cerevisiae,* that were isolated from diseased grape plants (*Vitis vinifera*) collected from Ahmadu Bello University campus, Zaria during winter time (December-January). The organisms were kept in Potato Dextrose Agar (PDA) slants under refrigerated condition.

The antifungal activity of the compound was determined using agar diffusion method. The medium was prepared as follows according to standard procedure. PDA (39 g) was dissolved in boiling water (100 ml), sterilised at 121° for 15 min, the solution poured onto different petridishes (20 ml each) and then allowed to solidify asceptically. The petridishes were of 200 mm diameter size.

Ten different concentrations in the range of 0.25 to 10 mg/ml were prepared by dissolving the compound in 10% dimethyl sulphoxide (DMSO in water). Each fungal isolate was streaked onto separate plate of freshly prepared sterile PDA using sterile cotton swabs. Six wells were punched on each inoculated PDA plate using sterilised No. 8 cork borer and properly labelled according to different concentrations of the compound. The wells were filled with 0.5 ml of different concentrations of the test compound with a control (10% DMSO) in each plate and then incubated at ambient temperature (29±1°) for 48-72 h. After incubation period the cultures were examined for inhibition growth which appeared as clear zones around the wells. The diameter of zones of inhibition were measured in mm (Table 1).

The Minimum Inhibitory Concentration (MIC) was determined using serial dilution method in test tubes containing nutrient broth. The medium was prepared according to specification. Nutrient broth (13 g) was dissolved in distilled water (1 litre), sterilised at 121° for 15 min and then distributed into the final container. The lowest concentration which showed inhibition in agar diffusion assay for each organism was serially diluted, each fungal isolate was inoculated into different tubes containing the compound at different concentrations and then incubated at ambient temperature (29±1°) for 48-72 h. After incubation period the tubes were examined for the growth of organisms. The highest dilution in the series for each organism showing no growth was considered to be the MIC of the test organism (Table 2).

All the organisms tested were inhibited by trimethyltinbenzoate-4-picoline and variations were observed as regards to the lowest concentration of the compound. For instance, *A. niger* and *A. versicolor* were inhibited when tested against a concentration of 0.5 mg/ml, whereas, *A. flavus* and *S. cerevisiae* were inhibited

TABLE 1: RESPONSES OF FUNGAL ISOLATES TO TRIMETHYLTINBENZOATE-4-PICOLINE

Name of fungi	Concentration in mg/ml								
	0.5	1	1.5	2	2.5	3	5	7	10
	Average diameter of zone of inhibition in mm								
A. versicolor	15	32.5	35	37	NT	NT	NT	NT	NT
A. niger	10.5	35.8	40.3	42.8	NT	NT	NT	NT	NT
A. flavus	-	35.3	40	44.8	NT	NT	NT	NT	NT
S. cerevisiae	-	13.3	14.3	23.3	NT	NT	NT	NT	NT
A. fumigatus	-	-	-	-	17.8	21	23.8	25	28.5
B. anomala	-	-	-	-	10.8	14.3	16	16.8	19.3

10% DMSO (control) and 0.25 mg/ml concentration did not show any inhibition in all cases. - sign indicates no inhibition, while, NT indicates not tested.

TABLE 2: ANTIFUNGAL ACTIVITY OF TRIMETHYLTINBENZOATE-4-PICOLINE

Name of fungi	Concentration in mg/ml		
	0.4	0.45	0.5
A. versicolor	+*	+	+
A. niger	-	+*	+
	0.8	0.9	1
A. flavus	+*	+	+
S. cerevisiae	-	+*	+
	2.3	2.4	2.5
A. fumigatus	-	-	+*
B. anomala	-	-	+*

Minimum inhibitory concentrations of trimethyltinbenzoate-4-picoline in mg/ml (*) against various fungal isolates tested '+' sign indicates inhibition and '-' sign indicates no inhibition.

at a concentration of 1 mg/ml. In the case of *A. fumigatus* and *B. anomala*, a higher concentration of 2.5 mg/ml was required for inhibition. Increasing size in the diameter of zones of inhibition with increase of concentration was observed for all the isolates. The maximum concentration of 10 mg/ml was used in the case of *A. fumigatus* and *B. anomala* (Table 1).

In all cases, the MIC was either the same or lower than the lowest concentration that showed inhibition when the compound was tested using agar diffusion method. The MIC values for *A. versicolor, A. niger, A. flavus* and *S. cerevisiae, A. fumigatus* and *B. anomala* were found to be 0.4, 0.45, 0.8, 0.9, 2.5 and 2.5 mg/ml respectively.

The responses of the fungal isolates to trimethyltinbenzoate-4-picoline suggest that the compound has great potential as a chemical protectant against diseases likely to be caused by these organisms as only a low concentration of the chemical is required to inhibit any of the organisms tested. Organotin compounds break down to harmless non-toxic forms of tin after bio-degradation or physico-chemical reaction and therefore this compound is expected to have no threat to the environment when used as a crop protectant chemical. Further work needs to be carried out to determine the factors that affect the efficacy of the compound. This is because the efficacy of any potential crop protection chemical is known to vary with factors such as pH, temperature, aeration, the nature of disease agent and the size of inoculum[14]. The results obtained in the study clearly show that the organotin compound tested has a great potential as a fungicide against fungal diseases of grapevine.

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