

Antibacterial Activity of Trimethyltinbenzoate-4-Picoline*

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The antibacterial activity of trimethyltinbenzoate-4-picoline (an organotin compound) was determined against five bacteria isolated from diseased grape plants (*Vitis vinifera*, family: Vitaceae). The zones of inhibition were observed, the Minimum Inhibitory Concentrations (MIC) against bacteria that belonged to *Pseudomonas*, *Corynebacterium*, *Bacillus*, *Klebsiella* and *Xanthomonas* species were found to be 0.5, 0.8, 2.5, 2.5 and 2.5 mg/ml respectively.

Despite the positive contribution of chemical pesticides, one important limitation of their use in the development of tolerance by some pathogens due to mutability of genes controlling the site of action of the pesticides, the rate of multiplication as well as the viability rate of resistant mutants¹. In addition, these pesticides are environmental pollutant/s, when used in excess, they usually become recalcitrant xenobiotics in the environment. The triorganotin compounds display a wide range of biological properties such as fungicides in wood preservation and miticides. The tributyl and triphenyltin compounds have been in use for thirty years in many countries and there have been very few undesirable effects reported in humans². The fungicidal activity of triphenyltin acetate and triphenyltin hydroxide in the control of fungal diseases of yams (*Dioscorea rotundata*, staple food in Africa)^{3,4} and rice⁵, the trypanocidal activity of bis (tri-n-butyltin) oxide⁶ were reported in the literature. There are five organotin compounds in commercial use as pesticides; these are fungicides, triphenyltin acetate and triphenyltin hydroxide; the miticides, tricyclohexyltin hydroxide, bis [tris (-2-methyl-2-phenylpropyl) tin] oxide and I-tricyclohexylstannyl-1,2, 4-triazole⁷. A common feature of the triorganotin agrochemicals is their ability to breakdown rapidly under the influence of sunlight and microorganisms to non-toxic inorganic tin (IV) species⁸. In this study, we report the antibacterial activity of

trimethyltinbenzoate-4-picoline an organotin compound against five bacteria isolated from diseased grape plants (*Vitis vinifera*, family: Vitaceae).

All chemicals were obtained from BDH, England. Potato dextrose agar and nutrient broth was supplied by Oxoid Ltd, Basingstoke, Hants, England. Methanol was distilled and then dried over calcium oxide before use.

Trimethyltinbenzoate-4-picoline was prepared by stirring equimolar amounts of trimethyltin chloride and potassium benzoate in dry methanol at room temperature for 10 h. Afterwards an equimolar amount of 4-picoline was added to the reaction mixture and stirring continued for another 2 h. The precipitate formed (potassium chloride) was filtered and the filtrate concentrated to give a compound which was crystallized from methanol to furnish yellow coloured crystals, mp. 76-78° (I).

The IR (Nujol) spectrum of the compound (I) showed bands at 1620 (strong, -O-CO-) and 1050 cm⁻¹ (weak, Sn-O-CO). The proton NMR spectrum (90 MHz, CDCl₃, TMS, δ) showed signals at 0.55 (s, 9H, 3CH₃ attached to Sn), 2.35 (s, 3H, pyridyl CH₃), 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 : C₁₆H₂₁O₂N Sn required : C, 50.83%; H, 5.60%; N, 3.71%; Sn, 31.40%. Found : C, 50.76%; H, 5.72%; N, 3.63%; Sn, 31.34%.

The dried diseased grape plants (*Vitis vinifera*) were collected from the Ahmadu Bello University Campus, Area-E, Zaria, Nigeria during December-January. The five

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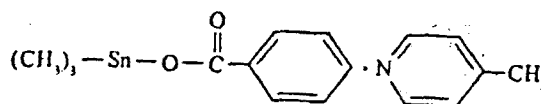
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genera of bacteria, namely, *Bacillus*, *Corynebacterium*, *Klebsiella*, *Pseudomonas* and *Xanthomonas* were isolated from the plants. After isolation the organisms were maintained on nutrient agar (NA) slants under refrigerated condition. The exact species of the bacteria were not identified.

The antibacterial activity of the organotin compound was determined using agar diffusion method. Solutions of five different concentrations of the compound ranging from 0.25 to 10 mg/ml were prepared using 10% dimethylsulphoxide (9 ml DMSO + 1 ml water) as solvent. The five bacterial isolates were streaked into five separate plates (200 mm diameter size petridish) containing freshly prepared Mueller-Hinton Agar (MHA) using sterile cotton swabs. Six wells were punched on the inoculated MHA plates using a sterilized size 8 cork borer. The wells were properly labelled according to different concentrations of the compound. Five wells in each plate were then filled with 0.5 ml of different concentrations accordingly and the sixth well contained the solvent (10 % DMSO) as a control. The plates were then incubated at ambient temperature (30°) for 24-48 h. After incubation the cultures were examined for the evidence of inhibition which appeared as clear zones of varying diameter according to concentration around the wells containing the solutions and the diameters were measured in millimeter. The control (10% DMSO) did not show any zone of inhibition. The experiment was repeated five times and the average zone of inhibition values were calculated.

The minimum inhibitory concentration (MIC) was determined using tube dilution method. Serial dilution of the test compound was prepared in test tubes containing nutrient broth as diluent. The lowest concentration that showed inhibition of growth of each organism was further diluted to different lower concentrations in order to determine the MIC. Each bacterial isolate was inoculated into different tube accordingly containing the diluted compound and then incubated at 30° for 24-48 h. The tubes were then examined for the presence of growth considering turbidity as a criteria. The highest dilution in each series that showed no growth (turbidity) of test organism was considered to be the MIC of that organism.

Pseudomonas sp. was inhibited with a zone of inhibition of 10.5, 10.7, 11.5 and 14.5 mm at 0.5, 1, 1.5 and 2 mg/ml concentrations respectively. *Corynebacterium sp.* was found to be inhibited with a zone of inhibition of 15, 15.7 and 17.7 mm at 1, 1.5 and 2 mg/ml concentrations respectively. The concentrations 2.5, 3, 5, 7 and 10



(I)

mg/ml produced the following inhibitory activity with a zone of inhibition of 10.3, 13, 13.8, 25 and 32.5 mm respectively in case of *Bacillus sp.*; 10, 13.3, 14.5, 21.8 and 28.5 mm respectively in case of *Klebsiella sp.*; 13.5, 22, 24.3, 30.5 and 34.8 mm respectively in case of *Xanthomonas sp.* The minimum inhibitory concentrations (MIC) against *Pseudomonas*, *Corynebacterium*, *Bacillus*, *Klebsiella* and *Xanthomonas* were found to be 0.5, 0.8, 2.5, 2.5 and 2.5 mg/ml respectively.

The responses of the test organisms to trimethyltinbenzoate-4-picoline suggest that the chemical has a great potential against diseases of the grape plants caused by these organisms. A very low concentration of the compound is required to inhibit any of the organisms tested and the chemical is likely to have no threat to the environment since organotin compounds break down to non-toxic forms of inorganic tin (IV) species under the influence of sunlight and microorganisms. Further work needs to be done to determine the efficacy of the compound as the activity is affected by the inoculum size, the composition of the culture medium, the incubation time and the conditions of incubation, such as temperature, pH and aeration⁹.

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