

In Vitro evaluation of antifungal activity of Bioactive Compound 2H-FURO [2,3-H]-1-Benzopyran-2-one against seed borne fungi of maize

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Abstract

Antifungal activity of bioactive compound 2H-Furo[2,3-H]-1-benzopyran-2-one recorded a significant activity at 100-1000 ppm concentration against all the ten *Aspergillus* species tested. *A. flavus* recorded complete inhibition at 100 ppm concentration, *A. niger* at 500 ppm, *A. fumigates* at 600 ppm, *A. flavus oryzae* and *A. flavus columnaris* at 700 ppm respectively. *A. ochraceous* and *A. flavipes* recorded complete inhibition at 900 ppm concentration. Compared to synthetic fungicide Captan and Thiram at 2000ppm concentration. Minimum Inhibitory Concentration (MIC) of bioactive compound was in the range of 100-900ppm concentration against all the test fungi.

Key words:

2H-Furo[2,3-H]-1-benzopyran-2-on, *Psoralea corylifolia*, *Aspergillus*, Captan, Thiram

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INTRODUCTION: Higher plants produce hundreds to thousands of diverse chemical

compounds with different biological activities [1]. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world. The use of traditional plant extracts as well as other alternative forms of medical treatments have been getting momentum since the 1990s [2]. Medicinal plants are used by 80% of the world population as the only available medicines especially in developing countries [3]. The schematic search of higher plants for antifungal activity has shown that some plants extracts have the ability to retard fungal growth or completely inhibit the fungus [4]. Currently, medicinal plants are widely used as home remedies or as alternative treatments by both rural and urban inhabitants in developing countries [5]. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine [6,7]. Many of these synthetic fungicides are known for their non-biodegradable nature and residual toxicity. Pesticide pollution of soil and water bodies is well documented in the literature [8]. In the recent years, research on medicinal plants has attracted a lot of attentions globally. Evidence has accumulated to demonstrate the promising potential of medicinal plants used in various traditional, complementary and alternate systems of treatment of human diseases. Plants are rich in a wide variety of secondary metabolites such as tannins terpenoids, alkaloids, flavonoids, etc, which have been found *in vitro* to have antimicrobial properties [9]. Synthetic chemical fungicides form a major part of the chemical pesticides used in modern agriculture to manage diseases both in field and during storage. The ill effects associated with the use of chemical

fungicides like carcinogenicity, teratogenicity a health assaurds necessitated the search for alternative strategies for the management of pre and post harvest crop diseases. Further extensive use of chemicals leads to biohazardous effects on ecosystem, and their persistent applications lead to resistance in pathogens against these fungicides [10]. Thus alternative approaches are preferred which are ecofriendly. In the present study, The bioactive compound 2H-Furo[2,3-H]-1-benzopyran-2-one isolated from seeds of *P. corylifolia* L belongs to family Fabaceae were tested for its antifungal potentiality against ten *Aspergillus* species isolated from Maize seeds. The Minimum Inhibitory Concentration (MIC) of the bioactive compound was determined. All the results were compared to synthetic fungicide Captan and Thiram.

MATERIALS AND METHODS

Plant material: Fresh and healthy seeds of *Psoralea corylifolia* L., were washed with tap water thrice and two to three times with distilled water. The seeds were air dried at room temperature. Completely air dried seeds were powdered and used for further isolation of bioactive compound.

Isolation of the Bioactive compound: Bioactive active compound was isolated from seeds of *P. corylifolia* following the procedure of Harborne [11].

Antifungal activity assay of the bioactive compound

Test fungi: Ten species of *Aspergillus* viz., *A. flavus*, *A. niger*, *A. terreus*, *A. tamarii*, *A. flavus oryzae*, *A. fumigatus*, *A. candidus*, *A. ochraceus*, *A. flavipes* and *A. flavus columnaris* isolated from maize seeds employing Standard Blotter Technique [12] served as test fungi.

Poisoned food technique: MESA (Malt Extract Salt Agar) medium with different concentrations of

the bioactive compound viz., 100,200,300,400,500,600,700,800,900 and 1000ppm were prepared and poured into sterile petriplates allowed to cool and solidify. Five mm mycelium disc of seven day old cultures of species of *Aspergillus* were placed at the center of the petriplates and incubated at 25 ±1° C and incubated at 25 ±1° C for 7 days. The MESA medium without bioactive compound but with the same concentration of sterile distilled water served as control. The colony diameter was measured in mm. Similarly, synthetic fungicides viz., Captan(C₉H₈C₁₃No₂S) and Thiram (C₆H₁₂N₂S₄) were also tested against all the test fungi at the recommended dose of 2000ppm concentration. For each treatment three replicates were maintained. The percent inhibition of mycelial growth if any was determined by the formula $PI = \frac{C-T}{CX100}$ Where C= Diameter of control colony, T=Diameter of treated colony. Minimal inhibitory concentration (MIC) for each of the test fungi was also determined [13,14]. The data were subjected to statistical analysis by ANOVA and Tukey's HSD.

RESULT:

Isolation of the Bioactive compound: The bioactive compound 2H-Furo[2,3-H]-1-benzopyran-2-one was isolated. From the observation it was recorded 0.47 *R_f* value and 138° C melting point.

Antifungal activity assay of the bioactive compound : Among the ten *Aspergillus* species tested against bioactive compound 2H-Furo[2,3-H]-1-benzopyran-2-one at 100-1000 ppm concentration, *A. flavus* recorded complete inhibition at lowest concentration of 100 ppm concentration followed by *A.niger* and recorded complete inhibition at 500 ppm concentration. *A.fumigatus* recorded 100% inhibition at 600 ppm concentration and recorded MIC at 600ppm concentration. *A.flavus oryzae* and *A. flavus columnaris* recorded complete inhibition at 700 ppm

concentration. *A. ochraceous* and *A. flavipes* recorded 100 % inhibition at 900 ppm concentration. *A. tamarii* recorded 92.90 % inhibition at 1000 ppm concentration. MIC value of all the test fungi were recorded in between the range of 100-900 ppm concentration. Compared to synthetic fungicide Captan, the inhibition percentage was recorded in between 25.30% to 80.12 % concentration and Thiram recorded 65.79 % to 80.15% inhibition at a recommended dosage of 2000 ppm concentration (Table 1).

DISCUSSION: In the present investigation for the first time the antifungal potential of this compound against eight species of *Aspergillus* known to cause many diseases in maize and other crops was tested. Further, a comparative evaluation of treatment with different concentrations of the bioactive compound has also been done to determine the minimum inhibitory concentration of the bioactive compound for each of these phytopathogenic *Aspergillus* species for the first time. A comparative evaluation of the treatment of maize seeds with the bioactive compound isolated from *P. corylifolia* and that of Captan and Thiram treatments which are generally employed in crop protection strategies has also been done. Among the minimum inhibitory concentration values determined for *Aspergillus* species, it is interesting to note that a very low concentration of 100ppm concentration of the bioactive compound is enough to bring about total inhibition of mycelial growth. In case of *A. niger*, *A. fumigatus*, *A. flavus oryzae* and *A. flavus columnaris*, the MIC values ranged between 500 to 700ppm concentration. Thus the results of the present investigation suggests that 500 to 700ppm concentration of the bioactive compound is enough to control many of the *Aspergillus* species.

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Table 1: Effect of the bioactive compound, [2h-Furo[2,3-H]-1-benzopyran-2-one] isolated from seeds of *P. corylifolia* L. on mycelial growth of *Aspergillus* species

Fungi	Inhibition(%)											MIC (ppm)	Captan 2000ppm	Thiram 2000ppm
	Concentration of the bioactive compound (ppm)													
	100	200	300	400	500	600	700	800	900	1000				
<i>Aspergillus flavus</i>	100.0 ⁱ ±0.0	100.0 ⁱ ±0.0	100.0 ⁱ ±0.0	100.0 ⁱ ±0.0	100.0 ⁱ ±0.0	100.0 ⁱ ±0.0	100.0 ⁱ ±0.0	100.0 ⁱ ±0.0	100.0 ⁱ ±0.0	100.0 ⁱ ±0.0	100.0 ⁱ ±0.0	100	57.72 ^a ±0.6	75.09 ^c ±0.3
<i>A. niger</i>	71.66 ^a ±0.5	76.23 ^b ±0.2	79.86 ^c ±0.0	85.83 ^d ±0.9	100.0 ^e ±0.3	100.0 ^e ±0.3	100.0 ^e ±0.3	100.0 ^e ±0.3	100.0 ^e ±0.3	100.0 ^e ±0.3	100.0 ^e ±0.3	500	29.57 ^a ±0.8	80.15 ^c ±0.5
<i>A. tamarii</i>	41.73 ^a ±0.0	44.06 ^b ±0.3	50.20 ^c ±0.0	62.36 ^d ±0.6	67.10 ^e ±0.5	72.26 ^f ±0.0	78.33 ^g ±0.5	82.53 ^h ±0.0	92.90 ⁱ ±0.0	92.90 ⁱ ±0.0	92.90 ⁱ ±0.0	--	72.11 ^c ±0.6	73.11 ^d ±2.2
<i>A. fumigatus</i>	39.56 ^a ±0.0	47.00 ^b ±0.2	56.50 ^c ±0.0	74.30 ^d ±0.9	83.80 ^e ±0.6	100.0 ^f ±0.0	100.0 ^f ±0.0	100.0 ^f ±0.0	100.0 ^f ±0.0	100.0 ^f ±0.0	100.0 ^f ±0.0	600	40.40 ^a ±0.8	70.65 ^b ±0.2
<i>A. ochraceous</i>	57.16 ^a ±0.2	58.10 ^b ±0.5	58.10 ^b ±0.0	61.23 ^c ±0.9	80.13 ^e ±0.0	85.53 ^e ±0.3	85.10 ^f ±0.6	89.10 ^f ±0.8	100.0 ^f ±0.2	100.0 ^f ±0.2	100.0 ^f ±0.2	900	26.16 ^a ±1.5	65.79 ^b ±1.5
<i>A. flavipes</i>	37.30 ^a ±0.1	42.70 ^b ±0.0	66.20 ^c ±0.0	74.00 ^d ±0.9	90.90 ^e ±0.2	90.90 ^e ±0.2	90.90 ^e ±0.2	90.90 ^e ±0.5	100.0 ^e ±0.5	100.0 ^e ±0.5	100.0 ^e ±0.5	900	40.20 ^b ±1.6	70.17 ^d ±1.6
<i>A. terreus</i>	49.03 ^a ±0.0	68.73 ^b ±0.0	74.50 ^c ±0.0	82.70 ^d ±0.9	87.60 ^e ±0.5	87.60 ^e ±0.1	87.60 ^e ±0.0	87.60 ^e ±0.6	87.60 ^e ±0.5	87.60 ^e ±0.5	87.60 ^e ±0.5	--	40.60 ^a ±0.5	80.16 ^d ±0.6
<i>A. flavus oryzae</i>	36.63 ^a ±0.0	40.36 ^b ±0.5	42.70 ^c ±0.0	64.53 ^d ±0.0	67.80 ^e ±0.9	67.80 ^e ±0.5	100.0 ^f ±0.0	100.0 ^f ±0.0	100.0 ^f ±0.0	100.0 ^f ±0.0	100.0 ^f ±0.0	700	80.12 ^c ±0.6	73.15 ^a ±0.8
<i>A. candidus</i>	34.23 ^a ±0.1	47.40 ^b ±0.0	63.83 ^c ±0.0	79.40 ^d ±0.0	87.60 ^e ±0.9	87.60 ^e ±1.5	87.60 ^e ±0.0	87.60 ^e ±0.2	87.60 ^e ±0.2	87.60 ^e ±0.0	87.60 ^e ±0.0	--	29.57 ^a ±0.6	80.15 ^d ±0.9
<i>A. flavus columnaris</i>	39.20 ^a ±0.0	47.66 ^b ±0.0	51.76 ^c ±0.0	71.73 ^d ±0.0	74.00 ^e ±0.8	76.76 ^f ±0.0	100.0 ^g ±0.0	100.0 ^g ±0.0	100.0 ^g ±0.0	100.0 ^g ±0.0	100.0 ^g ±0.0	700	25.30 ^a ±0.8	75.07 ^c ±0.3

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