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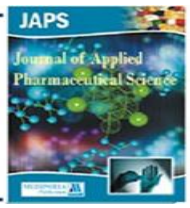

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Antifungal activity of bioactive compound of seeds of *Psoralea corylifolia* L. against seed borne *Fusarium* species of maize

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ABSTRACT

Antifungal activity of bioactive compound 2H-Furo[2,3-H]-1-benzopyran-2-one isolated from seeds of *Psoralea corylifolia* L. were tested against eight *Fusarium* species of maize seeds at 100- 1000ppm concentration. *F. moniliforme, F. oxysporum, F. semitectum and F. solani* were completely inhibited at 600ppm concentration. *F. equiseti, F. lateritium* and *F. proliferatum* were completely inhibited at 700ppm concentration and *F. graminearum* was inhibited at 800 ppm concentration. Significant inhibition was also observed from 100-500ppm concentration in all the test fungi. Compared to synthetic fungicide Thiram recorded significant antimicrobial activity than Captan tested at 2000ppm concentration. Minimum Inhibitory Concentration (MIC) was also determined for all the species of *Fusarium* tested. In dry mycelia weight analysis, the mycelia growth all the species of *Fusarium* were completely inhibited at 500-650 ppm concentration of the bioactive compound.

Key words: 2H-Furo[2,3-H]-1-benzopyran-2-one, *Psoralea corylifolia, Fusarium*, Thiram, Captan.

INTRODUCTION

Plants provide a rich source of novel biologically active compounds. Biological and chemical screenings are complementary approaches for the detection and isolation of interesting new plant constituents (Hostettmann and Wolfender, 1997). Since the beginning of human civilization, medicinal plants have been used by mankind for its therapeutic value. Plants have been a source of herbal remedies throughout the history of mankind. Various medicinal plants have been used for years in daily life to treat diseases all over the world (Koona and Budida , 2011). Plant metabolites were mainly investigated from a phytochemical and chemotaxonomic viewpoint. The interest in drugs of plant origin has been growing steadily over the last decade. Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity. It also facilitates pharmacology studies leading to synthesis of a more potent drug with reduced toxicity (Pranay and Rishabh 2011). Pure compounds are generally employed when the active principles of a medicinal plant exhibit strong, specific activity and have a small therapeutic index requiring accurate and reproducible dosage (Hamburger and Hostettmann, 1991). Several pressures have accelerated for the search of more environmentally and toxicologically safe and more selective and efficacious pesticides. Most commercial successful pesticides have been discovered by screening compounds synthesized in the laboratory for pesticides properties. The number of compounds that must be screened to discover a commercially viable pesticides has increased dramatically. The increasing incidence of pesticides

resistance is also fueling the need to search for new pesticides. Furthermore, most synthetic chemicals that have been commercialized as pesticides are with relatively long environmental half-lives and more suspect toxicological properties than the natural compounds (Duke, 1990).

Tens of thousands of secondary products of plants have been identified and there are estimates that hundreds of thousands of these compounds exist. There is growing evidence that most of these compounds are involved in the interaction of plants with other species primarily the defense of the plant from plant pests. Thus, secondary compounds represent a large reservoir of chemical structures with biological activity. This resource is largely untapped for use of pesticides (Duke, 1990). Considering the advantages of organic pesticides over synthetic pesticides, an attempt has been made to isolate the bioactive compound from *P. corylifolia* seeds and to test their efficacy for antifungal activity against different storage fungi and to determine the Minimum Inhibitory Concentration (MIC) of the bioactive compound compared to synthetic fungicides Captan and Thiram.

MATERIALS AND METHODS

Plant material

Fresh and healthy seeds of *P. 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 powered using waring blender (Waring international, new hart-ford, CT, USA).

Isolation of Bioactive compound

Bioactive active compound was isolated from seeds of *P. corylifolia* following the procedure of Harborne, 1998.

Antifungal activity assay of the bioactive compound

Test fungi: Eight species of *Fusarium* viz., *F. equiseti, F. graminearum, F. moniliforme, F. oxysporum, F. semitectum , F. solani , F. lateritium* and *F. proliferatum* isolated from maize seeds employing standard blotter technique (ISTA, 1999) served as test fungi.

Poisioned food technique: Czapek Dox Agar(CDA) 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 *Fusarium* were placed at the center of the petriplates and incubated at 25 $\pm 1^{o}$ C for 7 days. The CDA medium without bioactive compound but with the same concentration of sterile distilled water served as control. Similarly synthetic fungicides viz., Captan ($C_9H_8C_{l3}No_2S$) and Thiram ($C_6H_{12}N_2S_4$) were also tested against all the test fungi at the recommended dose of 2000 ppm concentration. For each treatment three replicates were maintained. The percent inhibition of mycelial growth if any was determined by the formula PI = 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 (Pinto et al., 1998; Bansal and Guptha, 2000). The data were subjected to statistical analysis by ANOVA and Tukey's HSD.

Dry mycelial weight: The Czapek Dox Broth (CDB) with different concentrations of the bioactive compound of *P. corylifolia* were prepared. The concentrations chosen for each of the test fungi was based on the MIC values obtained by poisoned food technique. Thus the concentrations tested for each of the fungi varied .Five mm diameter mycelial discs of seven days old culture of species of *Fusarium* were inoculated to CD broth. The flasks containing the respective medium without the bioactive compound but with equal volume of sterile distilled water served as control. For each treatment three replicates were maintained. All the inoculated flasks were incubated at 25 $\pm 1^{o}$ C for 7 days. After the incubation period, the contents of each of the flask were filtered through a pre-weighted whattman No.1 filter paper. The filter papers with the mycelial mats were dried in an oven at 100^{o}C until constant weights were obtained. The mycelial dry weight was determined by subtracting the weight of the filter paper from the total weight (Venturini et al., 2002).

RESULT AND DISCUSSION

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^{0} C melting point.

Antifungal activity assay of the bioactive compound

Poisioned food technique: Percent inhibition of mycelial growth of species of *Fusarium* subjected to different concentrations of the bioactive compound is presented in Table 1. It was observed that all the species of *Fusarium* tested were significantly inhibited even at the lowest concentration of 100ppm. The percentage of inhibition was more than 50% at this concentration against all the species of *Fusarium*. With the increasing concentration of the bioactive compound, the percent inhibition of mycelial growth also increased.

Total inhibition of *F. moniliforme , F. oxysporum, F. semitectum* and *F. solani* was observed at 600ppm concentration. Similarly total inhibition of *F. equiseti , F.lateritum* and *F. proliferatum* was observed at 700ppm concentration and total inhibition of *F. graminearum* was observed at 800ppm concentration. Between the synthetic fungicides tested, Thiram was highly effective in inhibiting the mycelial growth of all the *Fusarium* species. The percentage of inhibition ranged between 85 to 92%. In case of Captan, the percentage of inhibition of all the *Fusarium* species was very low and it ranged between 12 and 39%. The comparative efficacy of the synthetic fungicides with that of the bioactive compound revealed that the concentration of the bioactive compound necessary for total inhibition of *Fusarium* tested is 800ppm, and which is much lower than the recommended dose of Thiram (Table 1).

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 *Fusarium* species.

Fungi	*Inhibition (%)*												
	Concentration of the bioactive compound (ppm)										MIC	Captan	Thiram
	100	200	300	400	500	600	700	800	900	1000	(ppm)	2000ppm	2000ppm
Fusarium equiseti	66.0^{a}±0.0	71.00^{b} ±0.4	71.80^{c}±0.2	74.56^{d} ±0.2	84.70^{e}±0.5	90.96^{f} ±0.2	100.0^{g}±0.2	100.0^{g}±0.2	100.0^{g} ±0.2	100.0^{g}±0.2	700	36.60^{a} ±0.1	91.62^{d}±0.0
F. graminearum	69.30^{a}±0.5	76.20^{b} ±0.5	80.80^{c}±0.5	88.10^{d} ±0.0	89.60^{e}±0.0	91.95^{f} ±0.5	91.90^{g}±0.5	100.0^{h}±0.5	100.0^{h}±0.5	100.0^{h}±0.5	800	38.50^{a}±0.0	90.82^{c}±0.0
F. lateritium	49.00^{a}±0.6	63.33^{b} ±0.5	63.70^{c}±0.6	75.40^{d} ±0.5	83.00^{e}±0.2	88.23^{f}±0.5	100.0^{g}±0.0	100.0^{g}±0.0	100.0^{g} ±0.0	100.0^{g}±0.0	700	27.10^{a}±0.0	85.12^{e}±0.0
F. moniliforme	83.83^{a}±0.9	86.80^{b} ±00.2	87.20^{c}±0.8	91.86^{d} ±0.0	93.60^{e}±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	39.60^{b}± 0.0	92.13^{d}±0.0
F. oxysporum	88.26^{a}±1.0	89.20^{b} ±0.2	89.20^{c}±0.5	92.33^{d} ±0.5	93.20^{e}±0.2	100.0^{f}±0.1	100.0^{f}±0.1	100.0^{f}±0.1	100.0^{f} ±0.1	100.0^{f}±0.1	600	12.80^{a}±0.0	90.17^{d} ±0.0
F. proliferatum	89.00^{a}±0.1	89.40^{b} ±0.0	89.40^{c}±0.5	90.13^{d} ±0.5	91.30^{e}±0.5	92.90^{f} ±0.5	100.0^{g}±0.0	100.0^{g}±0.0	100.0^{g}±0.0	100.0^{g}±0.0	700	24.80^{a}±0.0	85.15^{e} ±0.0
F. semitectum	84.50^{a}±0.0	85.70^{b} ±0.0	85.70^{c}±0.5	87.70^{d} ±0.0	91.23^{e}±0.5	100.0^{f} ±0.5	100.0^{f}±0.5	100.0^{f}±0.5	100.0^{f} ±0.5	100.0^{f} ±0.5	600	22.10^{b}±0.0	89.05^{c} ±0.0
F. solani	74.20^{a}±0.0	79.20^{b} ±0.0	82.40^{c}±0.5	83.13^{d} ±0.0	90.20^{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	600	18.40^{b}±0.0	85.13^{c} ±0.0

Values are the mean of three replicates, ± standard error. The means followed by the same letter (S) are not significantly different at P<0.05 when subjected to Tukey's HSD. Pattern of percent Inhibition increase is not uniform for all the microorganisms.

Dry mycelial weight: The concentration of the bioactive compound needed for total inhibition of mycelial growth of *F.moniliforme* was 450ppm and 650ppm for *F.equiseti* and *F.proliferatum. F.graminearum, F.semitectum* and *F.solani* were completely inhibited at 600ppm concentration. *F.lateritium and F.oxysporum* were completely inhibited at 500ppm concentration (Table 2).

The earlier workers have isolated this compound employing a lengthy procedure and the yield of the compound isolated is also less. Where as in the present investigation a modification of the procedure has been employed which involves usage of less volume. Thus in present investigation a modified procedure for isolation of 2H-Furo[2,3-H]-1-benzopyran-2-one compound has been developed and standardized. Evaluating the efficacy of the bioactive compound 2H-Furo[2,3-H]-1-benzopyran-2-one for antimicrobial property has been done by (Takizawa et al., 2002; Pescitelli et al., 2002) . All these workers have evaluated the compound for antibacterial and antifungal activity only against a few human pathogenic bacteria species and *Canida albicans.*

None of the earlier reports have evaluated the antifungal potency of this compound against a wide range of pre harvest and post harvest phytopathogenic fungi. In the present investigation for the first time the antifungal potential of this compound against eight species of *Fusarium* known to cause many diseases in maize and other crops. 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 *Fusarium* 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. Experiments conducted in the present investigation to determine the MIC values for the eight *Fusarium* species tested revealed that 600 to 800ppm concentration is enough to bring about total mycelial growth inhibition of these test *Fusarium* species. A comparative evaluation of treatment of different concentrations of the bioactive compound with that of the recommended dose of the synthetic pesticides by poisoned food technique reveal that a very low concentration (half of the synthetic fungicide) of the bioactive compound is enough to bring about total inhibition of mycelial growth. Experiments have also been conducted in the present investigation to know the antifungal efficacy of this bioactive compound by dry mycelial weight technique employing the same pathogenic fungi. The data revealed that, the MIC dosage arrived at based on poisoned food technique is appropriate as evidenced by total inhibition of growth in dry mycelial technique.

Table 2: Concentrations of the bioactive compound, [2h-Furo[2,3-H]-1-benzopyran-2-one] isolated from seeds of *P. corylifolia* for total inhibition of *Fusarium* species in dry mycelial weight technique

Microorganisms	Concentration (ppm)	Dry Mycelial weight(gms)		Inhibition (%)
		Control	Treated	
Fusarium equiseti	650	0.280^{a}± 0.0	0.00^{b}± 0.0	100
F. graminearum	600	0.071^{a}± 0.0	0.00^{b}± 0.0	100
F. lateritium	500	0.604^{a}± 0.0	0.00^{b}± 0.0	100
F. moniliforme	450	0.544^{a}± 0.0	0.00^{b}± 0.0	100
F. oxysporum	500	0.502^{a}± 0.0	0.00^{b}± 0.0	100
F. proliferatum	650	0.287^{a}± 0.0	0.00^{b}± 0.0	100
F. semitectum	600	0.235^{a}± 0.0	0.00^{b}± 0.0	100
F. solani	600	0.299^{a}± 0.0	0.00^{b}± 0.0	100

Values are the mean of three replicates, ± standard error. The means followed by the same letter (S) are not significantly different at P<0.05 when subjected to Tukey's HSD. Pattern of percent Inhibition increase is not uniform for all the microorganisms.

CONCLUSION

Form the observation it can be concluded that the isolated bioactive compound form seeds of *P. corylifolia* showed a significant antimicrobial activity against seed borne fungi of maize. Further work is necessary to test the potential of this bioactive compound against different seed borne fungi and to test its potency in many biological assays.

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