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Bio-sensitivity of some Natural Coumarins

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Bio-sensitivity of seven natural coumarins viz., 6,6'-dimethyl-dicoumarol (G_1) ; gerberinol (G_2) ; dicoumarol (G_3) ; 8,8'-dimethyl-dicoumarol (G_4) ; scopoletin (G_5) ; 3,4'-diangelayloxy—3,4'-dihydroeseselin (ST_1) and 3'-angeloyloxy, 4'-acetoxy-3', 4'-dihydroseselin (ST_2) was evaluated against five conidia and five sclerotia forming fungal plant pathogens by spore germination techniques. Only G_1 showed significant antifungal properties against *H. oryzae*, reducing the germination of conidia by ca. 60% over control at 500 μg/ml. The effects of other compounds like G_2 on *H. oryzae* and *H, sativum* ; G_5 on *A. brassicae* and *R. solani* was also of promise. While the compounds like G_3 and G_5 have meagre antifungal properties against *H. oryzae, H. sativm, R, bataticola, R. oryzae-sativae* and ST_2 on *H. oryzae* respectively.

Key words : Coumarins, bio-sensitivity, spore germination

INTRODUCTION

Antifungal compounds that are produced in plants either before or after contact with pathogens have been regarded as one of the mechanisms of plants' natural defense (Stanley and Jurd, 1971 ; Honda *et al.*, 1979). Dean (1963) has reported the widespread presence of coumarin compounds in plants of Rutaceae family,

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Scopoletin and some other compounds from sweet potato are known to posses much fungistatic effect than chlorogenic acid (Uritani and Muramatsu, 1953). The present work deals with the *in vitro* studies of bio-sensitivity of some natural coumarins against a few spore bearing fungal plant pathogens.

MATERIALS AND METHODS

Seven naturally occuring coumarins viz,, 6,6'-dimethyl-dicoumarol (G_1); gerberinol (G_2) ; dicoumerol (G_3) ; 8-8' dimethyl, dicoumerol (G_4) ; scopoletin (G_5) ; 3', 4'—diangeloyloxy -3', 4' -dihydroseselin (ST_1) and 3'-angeloyloxy -4' aceloxy -3', 4'-dihydroseselin (ST_2) were assayed for their bio-sensitivity against five conidia producing fungal pathogens like, *Helminthosporium oryzae* (Bredade Haan) causing brown spot of rice, *H. sativum* (Pammet, King and Bakke causing leaf blight of wheat, *Alternaria brassicae* (Berk) Sacc. causing leaf spot of mustard *Fusarium oxysporum* f. sp, *udum* Butler causing wilt of arhar, *Colletotrichum capsici* (Syd.) Butl. and Bisby causing ripe rot of chilli and five sclerotia forming viz., *Sclerotium rolfsii* Sacc. causing root rot of groundnut, *S. oryzae* Catt. causing stem rot of rice, *Rhizoctonia solani* Kuhn. causing sheath blight of rice, *R. bataticola* (Taub.) Butler causing seedling blight of jute and *Rhizoctonia oryzae-sativae* (Saw.) Mordue causing stem and root rot of rice.

The organisms were isolated from their respective infected host. Single spore/ hyphal tip isolation was made to get the organism in pure form. The stoek cultures were maintained on PDA slants at 5°C. For conidia producing organisms 7 d old and for sclerotia producing 15 d old culture was used for collection of sporcs and sclerotia respectively.

The bio-sensitivity of the test compounds was evaluated by 'spore germination technique' (Montogomery and Moore, 1938) with certain modifications (Pan and Sen, 1976). Different dilutions of the chemicals used were 62.5, 125.0, 250.0, and 500.0 μg/ml. Stock solutions of the assay compounds were prepared by dissolving 10 mg of each compound in 2 ml ethyl alcohol and subsequent volume was made up with distilled water in 20 ml volumetric flask. The final dilutions, with double the required strength, were prepared using distilled water. Details of the experiments has been described earlier by Pan *et. al.* (1983, 1985).

RESULTS AND DISCUSSION

Results on the effects of variable concentrations of the seven test compouds on germination of conidia have been presented in Table 1 as per cent inhibition over control. A detailed perusal of the results revealed that depending upon nature of pathogens and chemicals, the compounds had variable effects on the germination of conidia. Inhibition of over 50% germination of conidia was

Table 1. Effects of different concentrations of some natural coumarins on germination of conidia of some plant pathogenic fungi

Compounds	Concentrations (mg/ml)[a]	Percent inhibition[b] in germination of conidia over control				
		H.oryzae	*H.sativum*	*A.brassicae*	*F.udum*	*C.capsici*
6-6'dimethyl***	62.5(1.79)	25.86.(4.85)	23.01.(4.26)	4.00(3.24)	3.10(3.13)	2.91(3.11)
di-coumarol(G_1)	125.0(2.09)	36.06(4.64)	34.79 (4.6)	5.81(3.42)	5.70(3.41)	5.19(3.37)
	250.0(2.39)	45.89(4.89)	43.96(4.84)	7.20(3.53)	8.50(3.62)	7.93(3.58)
	500.0(2.69)	60.90(5.27)	58.88(5.22)	9.82(3.72)	11.20(3.78)	10.15(3.72)
Gerberinol (G_2)*	62.5(1.79)	18.67(4.11)	17.51(4.06)	3.80(3.22)	7.92(3.85)	8.25(3.60)
	125.0(2.09)	26.83(4.38)	23.90(4.29)	4.20(2.27)	8.00(3.59)	9.27(6.69)
	250.0(2.39)	37.85(4.68)	35.21(4.62)	6.75(3.50)	9.22(3.67)	11.30(3.78)
	500.0(2.69) *	53.03(5.07) *	50.97(5.03)	9.48(3.68)	14.01(3.91)	15.20(3.97)
Dicoumarol(G_3)***	62.5(1.79)	16.71(4.03)	17.82(4.08)	3.22(3.15)	6.90(3.51)	7.85(3.58)
	125.0(2.09)	21.91(4.23)	23.97(4.30)	4.38(3.29)	9.70(3.70)	10.25(3.72)
	250.0(2.39)	33.45(4.58)	35.21(4.63)	6.49(3.47)	12.25(3.83)	13.95(3.91)
	500.0(2.69)	44.64(4.87)	46.69(4.92)	10.24(3.72)	17.60(4.06)	19.27(4.12)
8-8'-dimethyl	62.5(1.79)	12.95(3.87)	11.01(3.77)	4.90(3.34)	6.60(3.49)	5.27(3.37)
dicoumarol(G_4)***	125.0(2.09)	19.71(4.15)	17.93(4.08)	8.80(3.64)	9.20(3.67)	8.01(3.59)
	250.0(2.39)	23.35(4.34)	24.82(4.32)	11.91(3.83)	13.01(3.87)	11.30(3.82)
	500.0(2.69)	35.20(4.62)	33.01(4.56)	15.82(3.99)	18.21(4.10)	16.83(4.04)
Scopoletin(G_5)*	62.5(1.79)	13.10(3.87)	11.00(3.77)	16.30(4.01)	10.39(3.73)	9.32(3.68)
	125.0(2.09)	20.25(4.16)	19.03(4.12)	26.01(4.35)	19.36(4.12)	15.36(3.96)
	250.0(2.39)	35.38(4.63)	31.60(4.32)	39.65(4.74)	28.95(4.95)	27.31(4.39)
	500.0(2.69)	48.59(4.97)	46.90(4.92)	52.20(5.05)	39.26(4.73)	38.91(4.66)
3',4'diangeloyloxy	62.5(1.79)	9.72(3.70)	7.01(3.52)	10.01(3.71)	3.85(3.22)	2.20(2.99)
3:4'-dihydrose–	125.0(2.09)	16.08(4.00)	13.90(3.91)	13.91(3.91)	6.00(3.44)	4.95(3.34)
selin (ST_1)	250.0(2.39)	25.16(4.32)	23.25(4.26)	17.31(J4.05)	9.25(3.67)	7.23(3.54)
	500.0(2.69)	39.82(4.74)	36.85(4.66)	70.50(4.18)	12.90(3.87)	10.01(3.72)
3'-angeloyloxy,4'	62.5(1.79)	28.75(4.44)	25.32(4.34)	6.67(3.49)	7.01(3.52)	4.72(3.33)
acetoxy3,4' dihy-	125.0(2.09)	33.35(4.57)	31.30(4.51)	11.67(3.78)	9.78(3.70)	6.89(3.52)
droseselin (ST_2)	250.0(2.39)	37.20(4.67)	35.01(4.61)	20.00(4.16)	11.95(3.82)	9.30(3.68)
	500.0(2.69)	41.01(4.77)	39.90(4.74)	31.21(4.62)	15.00(3.96)	12.12(3.83)

a Figures in the parenthesis indicate log value of the corresponding dose of the test compound

b Figures in the parenthesis indicate probit value of the corresponding percent inhibition of spore germination

c Each insertation is an average of three replications

* Isolated from the plant *Cerbera lanuginosa*

** Isolated from the plant *Selinum tenuifolium*

*** Source of isolation not known

Table 2. Effects of different concentration of some natural coumarins on germination of sclerotia of some plant pathogenic fungi

Compounds	Concentrations mg/ml [a]	Percent inhibition [b] in germination of sclerotia over control				
		S.rolfsii	*S.oryzae*	*R. solani*	*R. bataticola*	*R. oryzae-sativae*
6-6' dimethyl*** dicoumarol(G_1)	62.5(1.79)	6.95(4.52)	5.90(3.43)	7.90(3.58)	6.25(3.46)	8.23(3.60)
	125.0(2.09)	8.25(3.60)	8.20(3.60)	13.12(3.87)	12.10(3.83)	14.71(3.91)
	250.0(2.39)	12.90(3.88)	12.25(3.83)	17.05(4.04)	16.55(4.02)	18.59(4.00)
	500.0(2.69)	15.10(3.96)	16.01(4.01)	21.20(4.20)	19.76(4.14)	22.70(4.25)
Gerberinol(G_2)*	62.5(1.79)	7.65(3.56)	9.25(3.67)	15.50(4.00)	14.40(3.95)	16.10(4.00)
	125.0(2.09)	9.25(3.67)	11.70(3.80)	17.50(4.01)	16.95(4.12)	18.55(4.10)
	250.0(2.39)	14.50(3.94)	16.20(4.01)	19.35((4.14)	18.25(4.10)	19.07(4.14)
	500.0(2.69)	18.10(4.08)	21.25(4.21)	23.25(4.27)	22.10(4.23)	24.02(4.30)
Dicoumarol(G_3)***	62.5(1.79)	6.50(3.48)	7.85(3.59)	11.70(3.70)	8.95(3.66)	10.78(3.76)
	125.0(2.09)	9.72(3.71)	10.29(3.73)	13.75(3.91)	12.92(3.87)	14.25(3.92)
	250.0(2.39)	13.10(3.87)	14.10(3.92)	17.01(4.04)	16.90(4.05)	17.95(4.00)
	500.0(2.69)	17.75(4.05)	17.10(4.04)	21.25(4.20)	19.70(4.15)	20.95(4.19)
8,8'-dimethyl dicoumarol(G_4)***	62.5(1.79)	8.57(3.63)	9.90(3.21)	13.29(3.88)	14.90(3.95)	12.20(3.83)
	125.0(2.09)	11.02(3.78)	13.01(3.87)	17.70(4.07)	19.01(4.12)	15.90(3.00)
	250.0(2.39)	14.91(3.95)	16.12(4.00)	21.21(4.20)	22.01(4.21)	19.97(4.15)
	500.0(2.69)	17.21(4.05)	19.25(4.13)	25.50(4.34)	27.25(4.40)	24.91(4.32)
Scopoletin(G_5)***	62.5(1.79)	9.02(3.65)	6.07(3.44)	17.05(4.05)	14.03(3.91)	17.07(4.07)
	125.0(2.09)	13.42(3.89)	11.23(3.78)	24.00(4.29)	22.87(4.25)	25.30(4.33)
	250.0(2.39)	19.83(4.15)	19.27(4.13)	33.03(4.56)	31.21(4.50)	36.02(4.64)
	500.0(2.69)	23.04(4.26)	26.15(4.33)	56.15(5.15)	44.97(4.87)	46.09(4.90)
3',4'diangeloy loxy 3', 4' dihodro-seselin (ST_1) **	62.5(1.79)	3.21(3.15)	4.90(3.34)	14.30(3.93)	10.00(3.73)	15.73(4.00)
	125.0(2.09)	6.90(3.52)	8.01(3.59)	17.73(4.01)	16.12(4.00)	19.20(4.13)
	250.0(2.39)	9.85(3.70)	11.00(3.77)	21.90(4.22)	22.21(4.21)	22.01(4.23)
	500.0(2.69)	12.90(3.87)	14.01(3.92)	25.01(4.32)	31.50(4.82)	26.02(4.36)
3'-angeloyloxy,4' aceloxy-3',4'di hydrosesin (ST_2)	62.5(1.79)	2.80(3.09)	3.01(3.12)	11.00(3.77)	15.00(3.93)	10.61(3.72)
	125.0(2.09)	4.20(3.27)	5.25(3.78)	19.10(4.12)	28.10(4.30)	20.91(4.19)
	250.0(2.39)	7.01(3.52)	8.90(3.65)	28.30(4.43)	33.01(4.56)	28.31(4.43)
	500.0(2.69)	10.10(3.72)	12.01(3.82)	35.42(4.62)	39.44(4.73)	36.39(9.65)

a Concentration figures in the parenthesis indicate log value of the corresponding dose of the test compound

b Figures in the parenthesis indicate probit value of the correspondings percent inhibition of spore germination

c Each insertation is an average of three replications

* Isolated from the plant *Cerbera lanuginosa*

** Isolated from the plant *Selinum tenuifolium*

*** Source of isolation not known

recorded for *H. oryzae* and *H. sativum* with G_1 and G_2 respectively at 500μg/ml. Scopoletin (G_5) at 500 μg/ml also had almost similar effect on *A. brassicae* (52.2%) followed by *H. oryzae* (48.59%) and *H. satlvum* (46.9%). However, all the assay compounds, in general, showed very poor to marginal effecte an *F. o. udum* and *C. capsici*.

On the contrary, most of the compounds, except G_5 at 500 μg/ml on *R. solani* (56.1%), *R. oryzae-sativae* (46 0%) and *R. bataticola* (44.9%) failed to inhibit the germination of sclerotia irrespective of their dosage (Table 2).

The present examination revealed a moderate (for some of the compounds) to poor (for majority of compounds) antifungal properties against the selected fungal plant pathogens.

Although the natural coumarins including scopoletin, furocoumrins and umbelliferone are known to play significant roles in plants natural defense mechanisms (Wain, 1969), non toxic effects of furanocoumarins towards 16 strains of *G. pulicaris* has also been reported (Desjardins *et al.*, 1989). Knypl (1963) observed that coumarins can inhibit germination of spores only at higher concentrations.

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REFERENCES

Dean, F. M. (1963). "Naturally occuring Oxygen Ring Compounds", Butterworth, London, pp. 176-250.

Desjardins, A. E., Spencer, G. F., Platiner, R. D. and Beremand, M. N. (1989). Furanocoumarin phytoalexins, trichothecene toxins and infection of *Paslinaca sativa* by *Fusarium sporotrichioides*. *Phytopathology*, **79** : 170-175.

Honda, G., Tabata, M. and Tasuda, M. (1979). The antimicrobial specificity of Tryptanthrin, *Plants Medica*. **37** : 172-174.

Knypl, J. S. (1963). A fungistatic action of coumarin. *Nature* (London), **200** ; 800-802.

Montogomery, H. B. S. and Moore, M. H. (1938). A laboratory method for testing the toxicity of protective fungicides. *P. Pomol.* **15** : 253-266.

Pan, S. and Sen, C. (1976). Fungitoxic properties of four new fungicides. *Z. Pfian, Krank. Pflschutz.* **83** : 577-583.

Pan, S., Bhattacharya, A., Ghosh, P. and Thakur, S. (1983). Studies on the antifungal activity of naturally occurring coumarins. *Z. pflenzen, Krank.* **90** : 265-268.

Pan, S., Mukherjee, B., Ganguly, A,, Mitra, S. R. and Bhattacharya, A. (1985). Antifungal activity of naturally occurring flavonoids. *Z. Pflenzen. Krank.* **92** : 392-395.

Stanley, W. L. and Jurd, L. (1971). Citrus coumarins. *J. Agr. Food Chem,* **19** : 1166-1110.

Uritani, I. T. and Muramatsu, K. (1953). Pathological Chemistry of black rotted sweer potato. Isolation and identification of polyphenols from injured sweet potato. *J. Agr. Chem. Soc.* Japan, **27** : 27.

Wain, R. L. (1969). Proc. symposium on potentials in crop protection, New York. Agri. Exp. Station, Cornell Univ. Geneva, pp. 26.

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