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ANTIFUNGAL ACTIVITY OF SOYBEAN AND CHICKPEA ISOFLAVONES AND THEIR REDUCED DERIVATIVES

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Key Word Index—*Aspergillus ochraceus*, *Penicillium digitatum*, *Fusarium culmorum*, isoflavonoids, antifungal activity

Abstract—The fungicidal activity of the isoflavones from soybean (*Glycine max*) and chickpea (*Cicer arietinum*) has been studied on three food and forage contaminating fungi, *Aspergillus ochraceus*, *Penicillium digitatum* and *Fusarium culmorum* The reduced derivatives of the corresponding isoflavones—the isoflavanones and the isoflavans—were also included in the investigation For the first time in a comparative study it is shown that isoflavones and isoflavanones are variable in their activity whereas the isoflavans are moderately active inhibitors of fungal growth

INTRODUCTION

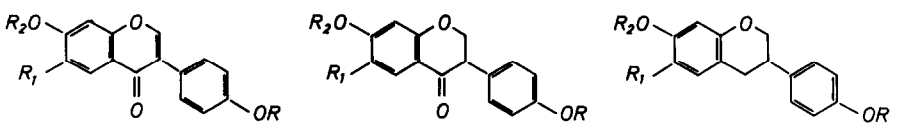
Isoflavonoids are naturally occurring substances possessing several biological properties [1] In view of phytoalexin properties their fungicidal activity has attracted the attention of some research groups [2, 3] Natural products, as potent fungal growth inhibitors, could be very useful substances in controlling plant diseases In the present investigation, some isoflavones (**1–4**) from food sources have been tested for their fungicidal activity Since most of the isoflavonoid phytoalexins possess a reduced isoflavone structure (Fig 1), we have also included the corresponding isoflavanones (**5–8**) and isoflavans (**9–12**) of the above mentioned isoflavones in our investigation The fungi of choice were *Aspergillus ochraceus*, *Penicillium digitatum* and *Fusarium culmorum* due to their food contaminating effects

RESULTS AND DISCUSSION

As expected the fungi show a differential behaviour in regard to the applied isoflavonoids (Table 1 and Fig 2) While the growth of *A ochraceus* is inhibited significantly by some isoflavonoids, *P digitatum* and *F culmorum* are inhibited as well as stimulated in their growth depending on the substances and their applied concentrations The data are tabulated below, statistically significant percentage differences in weight as compared with the control are marked with an asterisk The other values have been incorporated in the Table to indicate the trend of antifungal activity in the case of individual substances

Among the isoflavonoids, the isoflavone **1** stimulates the growth of *P digitatum* and *F culmorum* at higher concentrations and shows very low inhibitory effect on *A ochraceus* at all concentrations The isoflavanone **5** is antifungal only to *F culmorum* The isoflavan **9** actively inhibits the growth of *A ochraceus* and *F culmorum* in more than one concentration An inhibitory effect is also observed with *P digitatum*

Daidzein (**2**) inhibits *F culmorum* in all applied concentrations The isoflavanone **6** stimulates *F culmorum*, but *P digitatum* is inhibited significantly by the lowest concentration The isoflavan **10** inhibits *P digitatum* and *F culmorum* only at higher concentrations



Isoflavones
1 Gyorgy-isoflavone
2 Daidzein
3 Glycitein
4 Formononetin

Isoflavanones
5, 6, 7, 8

Isoflavans
9, 10, 11, 12

1, 5, 9 = (R R_2 = H, R_1 = OH)
2, 6, 10 = (R, R_1, R_2 = H)
3, 7, 11 = (R, R_2 = H, R_1 = OCH_3)
4, 8, 12 = (R = CH_3, R_1, R_2 = H)

Fig. 1 Chemical structures of isoflavones and their reduced derivatives

Table 1 Effect of isoflavonoids on mycelial growth of *A ochraceus*, *P digitatum* and *F culmorum* [% difference in wt as compared with the control, (+) indicates growth stimulation and (−) growth inhibition]

Isoflavonoids Concentration ($\times 10^{-4}$ mol/l)	Fungi *A ochraceus*			*P digitatum*			*F culmorum*		
	0·5	2·0	8·0	0·5	2·0	8·0	0·5	2·0	8·0
1	−5·0	−2·2	−5·5	+11·9	+31·1*	+35·4*	+1·6	+7·2*	+8·1*
5	+0·5	±0	−3·2	−2·9	+2·1	+9·8	−7·4	−9·0	−11·9*
9	−19·7*	−15·4*	−17·7*	−8·9	−5·9	−0·9	−3·2	−13·0*	−11·9*
2	+1·2	+7·5	±0	−6·5	−4·2	+4·3	−15·6*	−20·3*	−21·9*
6	+3·0	−3·2	−0·1	−13·5*	−10·9	−11·8	−1·0	+4·8	+7·0
10	+0·7	−9·3	−3·3	+3·2	+6·9	−28·2*	−5·1	+2·4	−16·5*
3	−8·8*	−4·0	−2·1	−7·4	+18·2*	+22·2*	+7·0	+19·2*	+12·9*
7	−5·4	+5·4	+5·5	−6·9	+18·0*	+12·5	−8·4	−2·6	−1·2
11	−3·3	−3·7	−28·2*	−27·4*	−36·7*	−50·8*	−4·0	+8·9	−31·9*
4	−0·3	−8·2	−4·5	+0·8	+6·8	+11·2*	+10·0	+5·8	+5·5
8	+1·2	+1·1	+2·4	+0·4	−4·3	−5·1	+4·3	+7·8*	+0·8
12	+7·0	−14·7*	−16·5*	+5·7	+2·5	−8·1	+4·8	+5·9	+8·7

Compounds **1–4**, isoflavones, **5–8**, isoflavanones, **9–12**, isoflavans

Glycitein (**3**) shows a fungal growth stimulation on *P digitatum* and *F culmorum* The isoflavanone **7** also stimulates the growth of *P digitatum* In contrast, the isoflavan **11** is the best fungicide among all the 12 substances tested here On malt extract agar plates it even produces a better inhibiting effect on *P digitatum* and *F culmorum* (58·3 and 51·6%, respectively) In view of no appreciable inhibitory activity, as found in liquid culture tests, this experiment was not performed with other substances

The results with Formononetin (**4**) are contrary to the observations of Johnson *et al* [4] and Van Etten [3]

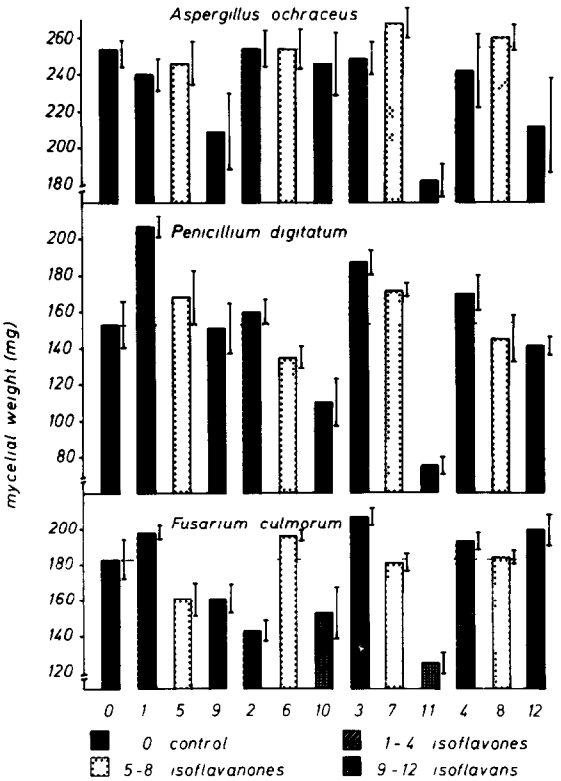


Fig 2 Mycelial dry wt (mg) of *A ochraceus*, *P digitatum* and *F culmorum* on malt extract in presence of isoflavonoids at a concentration of 8×10^{-4} mol/l

While they register a mild inhibitory activity with some fungi, in our case either an insignificant inhibition in *A ochraceus* or a low growth stimulation is encountered in *P digitatum* and *F culmorum* This discrepancy can be attributed to the variation in fungal species as well as to the differences in culture media Among the reduced derivatives **8** and **12**, the isoflavanone **8** shows virtually no inhibitory activity but the isoflavan **12** inhibits the growth of *A ochraceus*

The results of our investigation allow the conclusion that isoflavones and isoflavanones do not possess any remarkable fungicidal activity, whereas the isoflavans are comparatively good inhibitors of mycelial growth This partially corroborates the hypothesis of Perrin and Cruickshank [5] that the isoflavans, due to a planar conformation, fit into the probable receptor sites in the cells of sensitive fungi [3] The fungicidal activity of Daidzein (**2**) for *F culmorum* or of the isoflavanones **5** and **6** for *P digitatum* and *F culmorum* respectively shows a deviation indicating that other factors may be important for the antifungal property of the isoflavonoids Regarding the substitution pattern within the tested isoflavans, we observe that the methoxy group at the 6-position in **11** has a positive effect on fungal inhibition In the case of the methoxy group at the 4'-position in **12** no inhibition is observed with *P digitatum* and *F culmorum* The compound **10** without a methoxy group at the 4'-position shows a rather better activity against these fungi It seems that the fungicidal property of the isoflavonoids is specific to individual fungi, substances and their concentrations and no absolute generalization is possible in this context Ward *et al* [6] came to the same conclusion in their investigation with differently substituted sesquiterpenes

EXPERIMENTAL

Isoflavonoids Compounds from a food source like soybean and chickpea were selected for the present study 7,4'-Dihydroxy- and 7,4'-dihydroxy-6-methoxyisoflavones (**2** and **3**, respectively) occur in *G max* [7] Gyorgy *et al* [8] isolated 6,7,4'-trihydroxy-isoflavone (**1**) from soybean fermented with *Rhizopus oligosporus* 7-Hydroxy-4'-methoxy-isoflavone (**4**) is found in *C arietinum* [9] As the quantities obtained from natural sources were meagre, the isoflavones were synthesized in our laboratory In order to study the influence of gradual reduction on the antifungal activity, the isoflavones were catalytically (Pd–C, H_2) reduced to isoflavanones and isoflavans The purity and authenticity of these products were verified by physical methods (NMR and MS)

Fungi and culture The soil borne fungus *A ochraceus* was isolated in Syria from fruits of Lady Fingers (*Hibiscus esculentus*) and kept in the fungus collection of the Institut fur Pflanzenkrankheiten at Bonn *P digitatum* has been recently isolated from lemon peel (*Citrus limon*) bought at the local market in Bonn *F culmorum* was found in panicles of winter barley (*Hordeum vulgare*) grown in Germany Single spore cultures were prepared and preserved in a refrigerator at 8° The effect of isoflavonoids on the mycelial growth of these fungi was investigated in liquid culture The medium contained 30 g malt extract and 3 g peptone per l distilled H_2O Me_2CO served as solvent for the isoflavonoids The solvent concn in the medium was maintained at a 1 1% level Medium (20 ml) including the particular isoflavonoid in proper concn was transferred to 100 ml Erlenmeyer flasks and inoculated with one small piece of mycelium (4 mm in diameter) The flasks were incubated at 23–25° on a reciprocal shaker for 7 days Malt extract agar plates (30 g malt extract, 3 g peptone and 15 g agar per l distilled H_2O) was also used to test the glycitein-isoflavan (**11**) with *P digitatum* and *F culmorum* In this expt the isoflavan soln in a concn of 5 $\times 10^{-4}$ mol/l was added to the medium after the sterilization process at a temperature of $<60°$ After inoculation the radial mycelial growth was measured for 7 days

Concentrations of isoflavonoids From phytoalexin experiments [3, 10] it is known that concns in the range of 10^{-5} to 10^{-3} mol/l are effective in showing fungal inhibition With 5,7-dihydroxy-isoflavone as a reference substance the concns of 0 5, 2 0 and 8 0 $\times 10^{-4}$ mol/l were determined to be suitable for our expts

Evaluation of results Round filter papers (Schleicher & Schull No 595) were weighed after drying for 24 hr at 105° After filtration of the culture the residue was dried and weighed as described above The difference of the weighings gave the dry wt of the fungi The mean value of eight repetitions for each concn and fungus was used for calculation The data were evaluated by analysis of variance Probability of single differences was calculated at the 5% level

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