Pak. J. Bot., 8 (1): 63—68, 1976.

SOME PHYTOTOXIC COMPOUNDS IN ITALIAN RYEGRASS

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Abstract

Phytotoxic compounds of Italian ryegrass were ascertained, with the help of paper chromatography. Some of these toxins were identified as caffeic aicd, ferelic acid, syringic acid and vanillic acid. The role of these phytotoxins is discussed.

Introduction

Lolium multiflorum Lamb, commonly known as Italian ryegrass, is a native of Southern and Central Europe but now naturalized in many temperate parts of the the new and old world. A very common phenomenon observed by many investigators was the capacity of *Lolium multiflorum* to furnish competition to the brush seedlings and other plants in the mixed cultures and on pasturelands (Schultz *et al.* 1957; MCkel *et al*, 1959; Naqvi, 1972). Subsequent investigations (Naqvi & Muller, 1975) confirmed the presence of strong allelopathic mechanism responsible for this observed phenomenon. Identifications of some of these phyto-toxins carried out is described in the present paper.

Material and Methods

I. *Straw extract bioassays:*

Young leafy shoots of *Lolium multiflorum* were collected from the field and dried for 72 *h* at 70°C. Ten *g* of this material was crushed and soaked in 250 *ml* of double distilled water for 2 hours and then filtered. Using a standard bioassay procedure (Muller, 1961) pieces of sponge and filter paper were soaked in filterate and germination of seeds of *Bromus rigidus* and *Lactuca sativa* examined. Double distilled water was used as control. Four replicates using 20 seeds per replicate used for the test and the control bioassays were allowed to germinate in the growth chamber for 72 *h* at 20°C. Radicle growth measurements of the two test plants were made at the end of the experiment. This experiment was repeated several times using different lots of *Lolium* straw.

II. *Chromatographic separation of compounds and their bioassays:*

After having ascertained that *Lolium multiflorum* straw has water soluble compounds some of which have toxic properties, the same extract was used for paper chromatography in order to identify some of these compounds. The extract was concentrated in a vacuum evoporator at 40°C. This concentrate was then extracted three times with 60*ml* of ether each time. After separating the two layers, the ether fraction was allowed to evaporate to dryness at room temperature. This dry residue was dissolved in 5 *ml* of 100% ethanol and used for spotting the whatman

No. 3 filter paper. With the help of one dimensional ascending chromatography using three different solvent systems (Table 3) seven compounds were detected. Most of these compounds gave phenolic indications under ultraviolet light. With the help of a bioassay technique (Mcpherson *et al*, the chromatograms were used as seed beds after cutting the spots into equal sized squares. The bioassay utilized 10 seeds of *Lactuca sativa* on each of the squares. For control, squares were cut from a non-spotted part of the same paper.

III. *Identification of toxic spots:*

A preliminary identification, using ultra violet examination and spraying reagents, suggested seven spots to be phenolic in nature. A 2x 10^{-3}M solution in ethanol was then made of each standard compound for reference purposes. They were then applied on the paper and run simultaneously with the standard *Lolium* extract in all three solvent systems. For confirmation of results, two dimensional chromatography was also used.

Results

I. The germination of *Bromus rigidus* was not affected under test conditions, while that of *Lactuca sativa* was significantly affected (Table 1). On the other hand both the species showed a significant reduction of growth under test conditions.

II. *Chromatographic separation of compounds and their bioassays:*

The results of the bioassays showed a significant reduction in radicle length and abnormality in growth pattern of the seedlings in seven spots as shown in Table 2. In subsequent experimentation therefore only the analysis of these seven spots was carried out.

TABLE 1. Results of Bioassays, *Lolium multiflorum* extract toxicity against germination and early growth of test species.

		Radicle Growth (mm)			Percentage seed germination		
Test species	Condition	Mean	±SD	% of control	Mean	±SD	% of control
Bromus rigidus	Control	39.6	2.65		95.0	0.0	
	Test	23.9	3.30	60.3*	95.0	5.8	100.0
Lactuca sativa	Control	19.8	0.75		90.0	0.0	
	Test	7.1	1.20	35.8*	20.0	7.1	22.2*

*Significant at 0.05 level of probability.
SD. Standard deviation.

TABLE 2. Relative growth of *Lactuca sativa* seedlings on various spots of the Chromatogram.

Squares	Control	1	2	3	4	5	6	7	8	9	10	11
Mean growth	20.2	18.6	18.5	17.6	16.3	14.2	10.8	10.4	9.4	8.6	8.0	5.2
L. S. D.		0.26	0.25	1.82	1.30	1.20	0.53	0.42	1.39	0.20	0.86	0.28
% of control		91.5	91	85.6	81.6	69.9*	53.50*	50.70*	45.9*	41.7**	40.6**	24.5**

* Significant at 0.05 level of probability.

** Significant at 0.01 level of probability.

TABLE 3. Rf values of the 4 identified phenolic acids in 3 different solvent systems.

		Solvent 1	Solvent 2	Solvent 3
Caffeic acid	Standard	29.0	79.0	8.0
	Test 1	29.0	79.0	8.0
		30.0	80.0	7.0
Ferulic acid	Standard	33.0	85.0	68.0
	Test 2	32.5	85.0	68.0
Syraingic acid	Standard	48.0	86.0	73.0
	Test 3	48.0	87.0	72.0
Vanillic acid	Standard	56.0	90.0	62.0
	Test 4	56.0	90.0	62.0

a = Standard reference compound.

b = Test material i.e. ryegrass extract.

Solvent systems

1. 2% acetic acid.
2. Butanol: acetic acid: water (68 : 10 : 27)
3. Chloroform: methanol: water: formic acid (1000 : 100 : 96 : 4)

III. *Identification of toxic spots:*

Table 3 shows a comparison of the Rf values of the standard reference compounds with those of the ryegrass extract in 3 solvent systems. Table 4 shows the confirmation of these results on the basis of colour reactions under UV light and with various reagents. These confirm the identification of caffeic acid, ferulic acid, syringic acid and vanillic acid, to be present in ryegrass straw. Two other spots were tentatively identified as chlorogenic acid and phydroxy benzoic acid. The seventh spot remained un-indentified.

All these compounds are very well known growth and germination inhibitors; however, an experiment was set up in which a series of concentrations of these 4 Phenolic acids ranging from 400 ppm to 12.5 ppm. were used in a standard bioassay of the *Lactuca* seeds. All these compounds were found to be toxic to growth in relatively low quantities and that there was an increase in the inhibition with increase in the amount of toxin present in the media.

Conclusion

The presence of toxic phenolic acids in *Lolium multiflorum* straw and the fact that they are capable of being extracted in an aqueous medium suggests that the allelopathic effects of this grass species are presumably due to these compounds.

TABLE 4. Comparative identifications of 4 phenolic acids in regrass straw with the standard reference compounds.

Phenol	Condition	Ultraviolet radiation		1Ferric chloride-Ferricynaide	2Diazotized P-nitranaline
		shortwave	longwave		
Caffeic acid	Reference	B. blue flor	B. blue flor	Deep blue	Light grayish
	Extract	B. blue flor	B. blue flor	Deep blue	Light grayish
Ferulic acid	Reference	L. blue flor	L. blue flor	Light blue	Light blue
	Extract	L. blue flor	L. blue flor	Light blue	Light blue
Syringic acid	Reference	L. blue flor	L. blue flor	Sky blue	Sky blue
	Extract	L. blue flor	L. blue flor	Sky blue	Sky blue
Vanillic acid	Reference	Absorbent	———	Sky blue	Violet
	Extract	Absorbent	———	Sky blue	Violet

1. Ferric chloride-Ferricyanide Reagent. (Smith, 1960)
   FeC1, 3 percent in water
   K Fe3 (CN)6, 3 percent in water
   equal volume of each diluted 10 times and sprayed.

2. P-Nitranitine Reagent (Chuang and Wang, 1966)
   P-nitraniline saturated-1 part
   1%NANO2-1 part
   5% urea-1 part
   water—7 parts
   Sprayed the papers with this mixture and later
   Sprayed with 10% $NaCO_3$.

They are leached out of the straw with rain or dew. Once they reach the soil they can suppress the growth and germination of the susceptible species in the vicinity, if accumulated in biologically significant quantities.

The idea of a biochemical antagonism among grasses existed long before the term "allelopathy" was coined. The presence of anillic acid and ferulic acid from the straw and litter of wheat, berley and rye (Massart, 1957), the identification of caffic acid along with some other phenolic acids from rice straw (Koves, 1958), and the isolation of syringic acid from sugar cane litter (Wang *et al*, 1967), are known. Most of these researchers gave strong evidences of the germination and growth inhibiting properties of these phenolic acids in extremely low quantities. It is concluded, therefore, to consider phenolic phyto-toxins as important habitat variable in grassland ecology and community dynamics.

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