APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 1976, p. 579-584
Vol. 32, No. 4

Printed in U.S.A.

Identification of Mycotoxins Produced by Species of *Fusarium* and *Stachybotrys* Obtained from Eastern Europe[1]

CS. I. SZATHMARY, C. J. MIROCHA,* M. PALYUSIK, AND S. V. PATHRE

Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota 55108; and Hungarian Academy of Science, Veterinary Medicine Research Institute, Budapest, Hungary*

Received for publication 21 June 1976

Isolates of *Fusarium* and *Stachybotrys* spp. and crude extracts from these fungi were obtained from Hungary and the U.S.S.R. and used for the evaluation of the mycotoxins they produced. The cultures were grown on millet and oats and extracted in Budapest, Hungary (Veterinary Medical Research Institute) and chemically analyzed at the University of Minnesota using thin-layer chromatography (TLC), gas-liquid chromatography (GLC), gas chromatograph-mass spectrometry (GC-MS), and the rat skin bioassay. Zearalenone was found in most of the *Fusarium* cultures. T-2 toxin, neosolaniol, T-2 tetraol, and HT-2 toxin were found in extracts of *Fusarium poae* and *F. sporotrichioides*. A special effort was made to isolate the steroid-like toxins reported in the early Russian literature as sporofusarin and poaefusarin. None of the extracts from the *Fusarium* species yielded poaefusarin or sporofusarin when analyzed by our chemical methods or by those of L. E. Olifson, S. M. Kenina, and V. L. Kartashova, 1972. We therefore accounted for the toxicity of the *Fusarium* extracts as due to the 12,13,epoxytrichothecenes. One culture of *Stachybotrys alternans* yielded a macrocyclic ester of 12,13-epoxytrichothecene which, upon hydrolysis, yielded verrucarol; a steroid-like molecule (SB-3) was also isolated. The former had skin-irritant activity but SB-3 did not; the latter exhibited cardiac activity on the heart of the cockroach.

The basic objective of this collaborative study was to investigate and clarify the identity of various mycotoxins produced by spp. of *Fusarium* and *Stachybotrys* associated with mycotoxicoses found both in Eastern Europe and the United States. The mycotoxicoses caused by the above fungi have been described as hyperestrogenism, alimentary toxic aleukia, and stachybotryotoxicosis.

The estrogenic effect of zearalenone produced by many *Fusarium* species has been reviewed by Mirocha et al. (11) and Mirocha and Christensen (10). The general effects of the toxin in swine are observed as hyperestrogenism and infertility. Mice, rats, poultry, and cattle can also be affected. Zearalenone appears to be a commonly occurring mycotoxin in the United States and in different parts of the world, including Hungary (3, 5). According to the review of Joffee (9), alimentary toxic aleukia is a lethal disease of livestock and humans in the U.S.S.R. Joffe (9) described the clinical symptoms of alimentary toxic aleukia in great detail in humans and associated the toxicosis with consumption of grain overwintered in the fields and invaded by *Fusarium poae* and *F. sporotrichioides*. Olifson (L. E. Olifson, Ph.D. thesis, Technical Institute of Food Science, Moscow, 1965) found and characterized two toxic metabolites in the lipid moiety of the grains. The first was sporofusariogenine, a steroid linked by a glycosidic linkage to two molecules of glucose and one of ribose; hydrolysis yielded the aglycone called sporofusarin. The second toxin, poaefusariogenine, has a similar structure but contains xylose in a glycosidic linkage; hydrolysis yielded poaefusarin. Olifson concluded that the causal factors of alimentary toxic aleukia were the above, as well as other toxic oxyacids and fatty acids. More recently, Pokrovskii et al. (18) reported that the aqueous solution of sporofusarin sharply inhibited the membrane-bound lysosomal enzyme, β-glucosidase, and other lysosomal enzyme preparations from rat liver; the tests were conducted in vitro.

In studies recently conducted in countries outside of Eastern Europe, investigators dealing with the same fungi have not been able to detect toxic steroid-type molecules, but only derivatives of trichothecene toxins. Bamburg and Strong (1) suspected that trichothecenes are more likely to cause alimentary toxic aleukia than other metabolic fungal products. *F. poae* and *F. sporotrichioides* strains were investi-

[1] Scientific journal series paper no. 9657, Minnesota Agricultural Experiment Station, St. Paul, MN 55108.

gated by Ueno et al. (23) and found to produce T-2 and HT-2 toxins and neosolaniol. Mirocha and Pathre (13) received unpurified poaefusarin samples from V. I. Bilai and I. P. Misiurenko. The dermatotoxic samples were analyzed by thin-layer chromatography (TLC), gas chromatography (GC), infrared (IR), ultraviolet (UV), and gas chromatograph-mass spectrometry (GC-MS). The analyses could not confirm the presence of a steroid principle though T-2, T-2 tetraol, neosolaniol, and zearalenone were found.

The different toxins responsible for the symptoms and lesions of stachybotryotoxicosis have been dealt with in comprehensive reviews (8, 19, 20). Straw and other high-cellulose-constituent foodstuffs that have great importance in feeding domestic animals in Eastern Europe, are frequently invaded by pathogenic *Stachybotrys atra*. Many field cases have been reported in Eastern Europe among horses (2, 4, 20), cattle (14), and swine (22, 24). Experimental stachybotryotoxicosis in poultry has been reported by Schumaier et al. (21) and Palyusik et al. (17).

In other studies, Eppley and Bailey (6) separated five compounds produced by *S. atra* grown on oats and toxic to brine shrimp. Three of these were identified as 12,13-epoxy-Δ^9-trichothecenes and one as Roridin E. Mirocha et al. (12) and Pathre et al. (S. V. Pathre, C. J. Mirocha, and M. Palyusik, Abstr. Proc. Second Int. Congr. Plant Pathol., Am. Phytopathol. Soc., St. Paul, Minn., Abstr. 978, 1973), reported three biologically active components from isolates of *S. atra*. One coded as SB-3 ($C_{23}H_{30}O_5$, molecular weight 386) induced cardiac activity in the guinea pig and cockroach. The others (SBF-5 $C_{23}H_{32}O_5$, molecular weight 388 and SB-4, $C_{27}H_{34}O_8$, molecular weight 486) were not tested for cardiac activity because of lack of compound, but did show skin-irritant activity in the rabbit skin test.

Our objectives were to investigate and identify the various mycotoxins produced by *Fusarium* and *Stachybotrys* species; culturing of the strains, extraction, and preparation of the crude extracts were done in Budapest. To obtain the suspected poaefusarin and sporofusarin, the crude extracts were prepared according to the original description given by Olifson (Ph.D. thesis, 1965) and Olifson et al. (15). The biologically active principles responsible for the toxicity were pursued empirically and no attempt was made to identify the nontoxic components of the extracts.

MATERIALS AND METHODS

***Fusarium* strains.** The species of *Fusarium* (see Table 1) were grown on a solid substrate of millet seeds (ca. 40% moisture content) containing 2% malt extract. The temperatures used for culturing and toxin production were: 23 to 27°C for 1 week, 5 to 8°C for 2 weeks, and room temperature (23 to 27°C) again for 1 week.

TABLE 1. *Origin of the isolates of Fusarium species used in this study*

Fungi[a]	Strains	Origin	Substrate
Fusarium culmorum	F 79	Hungary	Millet
	F 247	Hungary	Millet
F. graminearum	F 59	Hungary	Millet
	F 184a	Hungary	Millet
F. poae	5627	U.S.S.R.	Millet
	5253	U.S.S.R.	Millet
F. sporotrichioides	F 38	Hungary	Corn
	F 14	Hungary	Millet
	F 38	Hungary	Millet
	5328a	U.S.S.R.	Millet
Stachybotrys atra	Jaszapati	Hungary	Oats
	Petrivente	Hungary	Oats
	Miskolc	Hungary	Oats

[a] In the classification of *Fusarium* according to the Snyder-Hansen system, *F. culmorum* and *F. graminearum* spp. belong to *F. roseum*; whereas *F. poae* and *F. sporotrichioides* belong to *F. tricinctum*.

***Stachybotrys* strains.** The cultures were propagated for 1 month on sterile oat grains and were thereafter transferred to oat grains again. Culturing was made at room temperature for 2.5 weeks and at 5 to 8°C for 1.5 weeks. The culture medium contained 40 to 45% water (fresh weight basis).

Extraction of cultures. (i) ***Fusarium* spp.** *Fusarium* spp. extraction was done according to the original description of Olifson (Ph.D. thesis, 1965). Each culture (300 × *g*) was extracted with diethyl ether for 6 h in a Soxhlet extractor, the ether was evaporated and the free-lipid (FL) fraction was obtained. The residue was refluxed in 96% ethanol and filtered; the ethanol was evaporated to separate the fraction designated as the alcoholic intermediate (IM). The residue retained on the filter paper was extracted again with diethyl ether; this yielded the bound-lipid (BL) fraction.

(ii) ***Stachybotrys.*** *Stachybotrys* extraction was done by diethyl ether in a Soxhlet apparatus according to the description of Fialkov and Serebriani (7) as modified by Palyusik (16).

Clean-up procedure. The FL, IM, and BL fractions were prepared for chemical and biological analyses as follows: defatted by partitioning against petroleum ether (boiling point, 60 to 70°C) and methanol-water (4:1, vol/vol), the aqueous methanolic fraction was concentrated under a vacuum and dissolved in pure methanol. The methanol-nonsoluble fraction was separated by filtration; the filtrate was concentrated and adjusted to 100 μg of solids/μl. One-hundred microliters of this solution was applied to a TLC plate.

TLC. Separation of various components present in the defatted extracts was carried out by TLC using silica gel G (Uniplate, Analtech) and Kieselgel 60 F-254 (Merck); the thickness was measured as 250 μm. Developing systems were as follows: (i) petroleum ether-diethyl ether-acetic acid (70:30:2,

vol/vol/vol); (ii) benzene-acetone 12:7, vol/vol); (iii) chloroform-isopropanol (96:4, vol/vol). Developing system (i) was used for the identification of sporofusarin and poaefusarin. For qualitative and quantitative chemical evaluations as well as for biological screening, system (ii) was used as follows: 10 mg of defatted extract of each sample was applied to a preparative silica gel plate; an appropriate amount of the extract was cospotted with a standard mixture (for R_f values see Table 2). Three bands were scraped off (R_f: 0,7-0,5, 0,4-0,2, and 0,1-0,06, respectively) and checked for biological activity. A portion was saved for quantification by GC and GC-MS. System (iii) was used for the separation of *Stachybotrys* samples. The components of the mixture were made visible by spraying with 50% sulfuric acid in methanol and charring. Zearalenone fluoresces when irradiated by UV; the trichothecenes fluoresce and can be seen in UV after charring.

The *p*-anisaldehyde reagent (0.5 ml of sulfuric acid, 9 ml of ethanol, 5 drops of glacial acetic acid, 0,5 ml of *p*-anisaldehyde) was also used for the detection of trichothecenes; the plates were heated after spraying.

The *Stachybotrys* component (designated SB-3) has a quenching effect under short (254 nm) UV when separated on silica gel having a fluorescent background.

GLC. A Varian 1800 series chromatograph equipped with FID detector was used for gas-liquid chromatography (GLC). The carrier gas used was nitrogen with a flow rate adjusted to 20 ml/min. The column (90 by 8 mm) consisted of 3% OV-17 adsorbed on Gas-Chrom Q 100 to 120 mesh. The temperature of the injector block was 285°C, with a temperature program of 180 to 280°C; increments of 4°C/min were used and the detector was set at 285°C. The toxins were analyzed as their trimethylsilyl ether derivatives by using Tri-Sil-BT or Tri-Sil-TBT as silylating reagents (Pierce Chemical Co.). Table 2 shows the retention times of the standards used. Quantification of unknown components was done by comparison with the peak height of standards at different concentrations.

GC-MS. An LKB 9000 instrument was used for GC-MS. A glass column (90 by 8 mm) consisting of 3% OV-17 packing material adsorbed on Gas-Chrom Q, 100 to 120 mesh, was used. One-microliter samples of the silylated components (previously resolved by TLC) were injected into the GC-MS system for qualitative analysis. Mass spectra were recorded in 4 to 6 s on the apex of the GC peaks. The mass spectral data were normalized and plotted by using a computer-driven plotter.

In the studies involving hydrolysis of the TLC bands of *S. atra* toxin in the rat skin bioassay, the following procedure was followed: a portion of the resolved bands was transferred into a small vial; 60 μl of 0.6 N K_2CO_3 in methanolic water (4:1) was added and held overnight at 50°C. The solvent was evaporated by a stream of nitrogen and 20 μl of Tri-Sil-BT (Pierce Co.) reagent was added, and portions were injected into the GC-MS.

Bioassay (rat skin test). An area (2 by 3 cm) was shaved on the back of a rat (50-g Holtzmann, white, female); the various bands separated by TLC were applied in acetone with a micropipette. The rates of assay were examined daily for 5 days following administration. The rat uterotropic assay was used for the screening of *F. culmorum* and *F. graminearum* strains for estrogenic components. Twenty-day-old white female weanling rats were used in this bioassay.

TABLE 2. *Resolution of various mycotoxins by TLC and GLC*

Mycotoxins	R_f, using TLC in solvent system:[a]			t_R (min) using GLC
	(i)	(ii)	(iii)	
Zearalenone	0.20	0.66	0.57	21.9
T-2 toxin	0.00	0.53	0.60	20.7
Diacetoxyscirpenol	0.00	0.51		13.8
Monoacetoxyscirpenol	0.00	0.36	0.32	11.6
Neosolaniol	0.00	0.33		15.5
HT-2 toxin	0.00	0.28	0.33	19.0
Deoxynivalenol	0.00	0.25		8.3
Scirpenetriol	0.00	0.08		8.6
T-2 tetraol	0.00	0.06		9.9
Poaefusarin (presumably)	0.22			
Sporofusarin (presumably)	0.06			
SB 3			0.48	28.0
Roridin A			0.52	
Verrucarin A			0.65	
Verrucarin B			0.73	
Verrucarol				6.0
Stachybotriotoxin found in Jaszapati strain			0.27	

[a] (i) Petroleum ether, diethyl ether, glacial acetic acid (70:30:2). (ii) Benzene, acetone (12:7). (iii) Chloroform, isopropanol (96:4).

RESULTS

The crude extracts were separated into fractions designated as FL, BL, and IM and separated into their individual components by TLC. Solvent system (ii) was used for resolution of the trichothecenes and zearalenone as shown in Table 2. After separation on TLC plates, three distinct bands having R_f values of 0.50 to 0.70 (zearalenone, T-2 toxin, and diacetoxyscirpenol), 0.20 to 0.4 (monoacetoxyscirpenol, neosolaniol, HT-2 toxin, and deoxynivalenol), and 0.06 to 0.1 (scirpenetriol and T-2 tetraol) were eluted with acetone off the silica gel and tested for biological activity in the rat skin test. The constituents of the bands showing biological activity were analyzed further by TLC, GLC, and GC-MS.

The mycotoxins found in the various cultures and fractions obtained from the *Fursarium* spp. are recorded in Tables 3 and 4. In all cases, the results of the bioassay (uterotropic or dermati-

tic response) supported the findings in chemical analyses. One strain of *F. poae* (no. 5627), in which no toxins were found chemically, was also negative in the biological tests. Strains of *F. culmorum* and *F. graminearum* (Table 3), when fed to rats, caused an enlargement of the rat uterus, but they did not produce a dermatitic response; correspondingly, they were positive for zearalenone but negative for trichothecenes when analyzed chemically.

A special effort was made to identify other toxic metabolites, besides those well characterized in the extracts of *F. poae* and *F. sporotrichioides*. Two fractions were obtained by the clean-up procedure described; one was soluble in petroleum ether (boiling point, 60 to 70°C) and the other was soluble in aqueous methanol. No toxicity was found in any of the ether fractions (lipids) of the *Fusarium* species tested. The aqueous methanolic fraction was developed in petroleum ether-diethylether-acetic acid (70:30:2, vol/vol/vol) as described by Olifson (15) for the resolution of poaefusarin. Individual components readily visible on the TLC plate were separated into their respective bands, as well as the remaining portion of the plate, and eluted from the silica gel with acetone or methanol and tested for biological activity. Although the amount spotted on the TLC plate was five times the amount used for the detection of the trichothecenes, no biological activity was detected except for the band at the origin of the plate. In the developing system described, all the known trichothecenes remain at the origin. In a few cases, bands having low R_f values (0.06 to 0.08) caused inflammation of the skin of the rat but after subsequent analysis by GLC, only T-2 toxin was found. The T-2 toxin, being less polar than most of the trichothecenes, migrates slightly in the developing system, particularly when higher concentrations cause overloading. Olifson (15) described toxicity associated with a fluorescent band at R_f 0.22 when developed in this system. The fluorescent band was found but no toxicity was associated with it.

TABLE 3. *Chemical analysis of the fungi F. culmorum and F. graminearum*

Strains	*Fusarium* sp.	Fractions	Mycotoxin detected (zearalenone, μg/ml)
F 79	*F. culmorum*	BL	540
		IM	3,400
F 247	*F. culmorum*	FL	3,000
		BL	340
		IM	200
F 59	*F. graminearum*	FL	200
		BL	730
		IM	
184a	*F. graminearum*	FL	350
		BL	1,900

TABLE 4. *Chemical analysis of F. poae and F. sporotrichioides*

Strains	Fractions	Mycotoxins detected (μg/g)				
		Zearalenone	T-2	HT-2	Neosolaniol	T-2 tetraol
5253[a]	FL		520	580	540	50
	BL		300	1,600	400	
	IM		200	200	600	
No. 38[b] (corn)	FL	200	400	900	300	300
F 14[b]	FL		1,460	400	600	50
	BL		1,070	270	1,480	50
	IM		1,510	380	780	
F 26[a]	FL		260	50	50	
	BL		1,560	500	810	
	IM		1,500	500	420	
F 38[b] (millet)	FL	1,200	2,300	1,000	100	
	BL	990	1,550	460	420	
	IM	1,500	1,300	800	500	
5328a[b]	FL	340	960	420	430	
	BL	1,300	3,000	650	1,750	

[a] *F. poae*.
[b] *F. sporotrichioides*.

After separation by TLC, the dermal toxicity test demonstrated that the Jaszapati strain of *Stachybotrys* contained a biologically active component. The R_f value of this toxic principle was different from Verrucarin A and B and Roridin A, but similar to Satratoxin G reported by Eppley and Bailey (6) and Rodricks and Eppley (19). The component was isolated, hydrolyzed, silylated, and analyzed by GC and GC-MS. When analyzed by GC, after addition of the silylating reagent, a single peak was noted. This observation, as well as the fact that no other impurity was located when resolved by TLC, verified purity of the component and implicated it as the toxic component. Hydrolysis in alkali, which resulted in the formation of verrucarol, was verified by GC-MS analyses of the TMS ether derivative of the hydrolysate. The data indicated that the toxic component was a macrocyclic ester similar to Verrucarin and Roridine.

The other *Stachybotrys* strain called "Miskolc" yielded a biologically active component with a steroid-like structure previously reported as SB-3 by Mirocha et al. (12) and Pathre et al. (Abstr. Second Int. Congr. Plant Pathol., Am. Phytopathol. Soc., St. Paul, Minn., Abstr. 978, 1973). The molecular weight of this component was 386 and an empirical formula of $C_{23}H_{30}O_5$ was determined by high-resolution mass spectroscopy. The latter component had no dermal activity but showed cardiac activity in a guinea pig and cockroach.

DISCUSSION

Our results, obtained from both chemical and biological assays, could not confirm the presence of poaefusarin or sporofusarin in toxic cultures of *Fusarium* species as reported by Olifson (Ph.D. thesis, 1965) and Olifson et al. (15); only the well known mycotoxins (zearalenone and trichothecenes) were found. The species of *Fusarium* used in this study included authentic toxic isolates (*F. poae* 5627 and *F. sporotrichioides* 5328a) obtained from the U.S.S.R.

Olifson et al. (15) reported that the R_f values of sporofusarin and poaefusarin were 0.06 and 0.22, respectively, when resolved by TLC in a petroleum ether-diethyl ether-glacial acetic acid (70:30:2, vol/vol/vol) developing system. We found that T-2 toxin could migrate on the TLC plate up to 0.08 (R_f) depending on the concentration of T-2, quality of the thin layer, saturation in the TLC tank, and purity of solvents. The other members of the trichothecene group tested (HT-2, T-2 tetraol, nesosolaniol, deoxynivalenol) did not migrate from the origin. The other component reported by Olifson (Ph.D. thesis, 1965) at R_f 0.22 had a strong fluorescence under UV irradiation and proved to be identical to zearalenone when analyzed by GC-MS. No dermatitis was noted with substances from this band in the rat skin test. We conclude that the T-2 toxin is probably responsible for the biological activity attributed to the steroids by Olifson et al. (15).

Ueno et al. (23) analyzed similar isolates of *F. poae* and *F. sporotrichioides* and found trichothecene toxins.

This study also suggests that stachyobotryotoxicosis can be caused, at least partially, by the macrocyclic esters of 12,13-epoxytrichothecene (Roridin E) as implied by Eppley and Bailey (6). The strains of *Stachybotrys* used in our study were authentic isolates obtained from Eastern Europe and associated with toxicoses in farm animals. It appears that the macrocyclic ester type of trichothecene may be only partially responsible for the toxicoses reported in Hungary as this fungus produces other toxins as well.

ACKNOWLEDGMENTS

This research was supported by National Science Foundation research grant GF-42346 and the financial aid of the Cultural Institute for International Relations in Budapest. Permission number of the Hungarian Academy of Science is 5193/73-6230/73.

We are indebted to Professor Sarkisov, All-Union Research Institute of Veterinary Medicine, Moscow, for providing the strains of *Fusarium* spp. from which we prepared the crude extracts in Budapest.

LITERATURE CITED

1. **Bamburg, J. R., and F. M. Strong.** 1971. 12-13-Epoxytrichothecenes, p. 207-292. *In* S. Kadis, A. Ciegler, and S. Ajl (ed.), Microbial toxins, vol 7. Academic Press Inc., New York.
2. **Bernard, A.** 1947. Fatal cases of Stachybotriomycosis equi, a disease caused by the spores of a *Stachybotrys* sp. in horses. Mag. Allatorv. Lapja 2:39-40.
3. **Dankó, Gy., and P. Áldási.** 1969. Mycotoxin induced vulva swelling in pigs. Mag. Allatorv. Lapja 24:517-519.
4. **Dankó, Gy., and J. Tanyi.** 1968. On Stachybotryotoxicosis of horses. Mag. Allatorv. Lapja 23:225-229.
5. **Debreczeni, I., and Gy. Rejtö.** 1969. Toxicosis of pigs caused by feeding mouldy corn. Mag. Allatorv. Lapja 24:520-523.
6. **Eppley, R. M., and W. J. Bailey.** 1973. 12-13-Epoxy-Δ^9-trichothecenes as the probable mycotoxins responsible for Stachybotryotoxicosis. Science 181:758-760.
7. **Fialkov, J. A., and S. B. Serebriani.** 1949. Toxic substances from *Stachybotrys alternans*. *In* V. G. Drobotko (ed.), Diseases of horses and humans caused by fungi. Stachybotryotoxicosis. Kiev, Russia.
8. **Forgács, J.** 1972. Stachybotryotoxicosis, p. 95-126. *In* S. Kadis, A. Ciegler, and S. J. Ajl (ed.), Microbial toxins, vol. 7. Academic Press Inc., New York.
9. **Joffe, A. Z.** 1974. Toxicity of *Fusarium poae* and *F. sporotrichioides* and its relation to alimentary toxic aleukia, p. 229-262. *In* I. F. H. Purchase (ed.), Mycotoxins. North-Holland Publishing Company, Amsterdam.
10. **Mirocha, C. J., and C. M. Christensen.** 1974. Oestrogenic mycotoxins synthesized by *Fusarium*, p. 129-148. *In* I. F. H. Purchase (ed.), Mycotoxins. North-

Holland Publishing Company, Amsterdam.
11. **Mirocha, C. J., C. M. Christensen, and G. H. Nelson.** 1971. F-2 (zearalenone) estrogenic mycotoxin from *Fusarium*, p. 107-138. *In* S. Kadis, A. Ciegler, and S. J. Ajl (ed.), Microbial toxins, vol. 7. Academic Press Inc., New York.
12. **Mirocha, C. J., M. Palyusik, S. Pathre, and B. Schauerhamer.** 1972. Mycotoxins from *Stachybotrys alternans* grown on oats. Phytopathology 62:778-779.
13. **Mirocha, C. J., and S. Pathre.** 1973. Identification of the toxic principle in a sample of poaefusarin. Appl. Microbiol. 26:718-724.
14. **Mitroiu, P., G. V. Stoicea, P. Jivoin, and I. Cerni.** 1973. Stachyobotriotoxicosis in cattle. Revta Zootech. Med. Vet. Bucuresti 23:66-74.
15. **Olifson, L. E., S. M. Kenina, and V. L. Kartashova.** 1972. Chromatographic method to identify toxicity of grain of grasses (wheat, rye, millet and others) affected by a toxigenic strain of *Fusarium sporotrichiella*, p. 3-8. Orenburg Regional Administration of D.I. Mendelieev, All-Union Chemical Association.
16. **Palyusik, M.** 1970. Biological test for the toxic substance of *Stachybotrys alternans*. Acta Vet. Acad. Sci. Hung. 20:57-67.
17. **Palyusik, M., K. Bamberger, and Z. Hagy.** 1971. Experimental stachybotryotoxicosis of day-old and growing chickens with special regards to its diagnostic differentiation from fowl pox. Mag. Allatorv. Lapja 26:304-306.
18. **Pokrovskii, A. A., V. A. Tutelyan, L. D. Olifson, and L. V. Kravchenko.** 1974. Action of the mycotoxin of *Fusarium sporotrichiella* (V) *sporotrichioides* on lysosomal membranes. Byull. Eksp. Biol. Med. 78:38-41.
19. **Rodricks, J. V., and R. M. Eppley.** 1974. *Stachybotrys* and Stachybotryotoxicosis, p. 181-197. *In* I. F. H. Purchase (ed.), Mycotoxins. North-Holland Publishing Company, Amsterdam.
20. **Sarkisov, A. Kh.** 1954. Stachybotryotoxicosis of horses. *In* A. Kh. Sarkisov (ed.), Mycotoxicosis. State Publishing House for Agricultural Literature, Moscow.
21. **Schumaier, G., H. M. DeVolt, N. C. Laffer, and R. D. Creck.** 1963. Stachybotryotoxicosis of chicks. Poult. Sci. 42:70-74.
22. **Szabó, I., F. Ráta, P., Aldásy, P., Szabó, and L. Gaál.** 1970. Scaly and scabious skin affection and rhinits (stachybotryotoxicosis) in pig stocks. I. Clinical observations and aetiological investigations. Mag. Allatorv. Lapja 25:633-637.
23. **Ueno, Y. N., Sato, K. Sakai, and M. Enomoto.** 1972. Toxicological approaches to the metabolites of Fusaria. V. Neosolaniol, T-2 toxin and butenolide, toxic metabolites of Fusarium sporotrichioides NRRL 3510 and Fusarium poae 3287. Jpn. J. Exp. Med. 42:461-472.
24. **Vachev, V. L., X. X. Dyakov, P. Peichev, and B. Tabakov.** 1970. Abortion caused by *Stachybotrys* toxin in swine. Vet. Sbir. 67:7-10.