SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 21, No. 6, pp. 1206–1212, 2002

Printed in the USA
0730-7268/02 $9.00 + .00

ANTIFUNGAL ACTIVITY OF SOME TRITYL-BASED SYNTHETIC DYES

GYULA OROS,† TIBOR CSERHATI,‡ and ESTHER FORGACS*‡
†Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary
‡Institute of Chemistry, Chemical Research Centre, Hungarian Academy of Sciences, P.O. Box 17, 1525 Budapest, Hungary

(*Received* 21 *March* 2001; *Accepted* 18 *October* 2001)

Abstract—The fungicidal activity of 10 trityl dyes and six reference compounds was determined on 36 fungal strains, and the data matrix was evaluated separately by principal component analysis (PCA) and by spectral mapping technique (SPM). The dimensionality of the maps of the principle component loadings and variables and the selectivity maps were reduced to two by varimax rotation and by nonlinear mapping. Calculations proved that both the strength and selectivity of the fungicidal activity of trityl dyes considerably depended on the chemical structure of the dye and on the type of fungi. Both PCA and SPM were suitable for evaluation of the antifungal activity of dyes; however, the strength and selectivity of the fungicidal effect can be separated only by SPM. Due to its advantageous application parameters, use of SPM in future quantitative structure-activity relationship studies is highly recommended.

Keywords—Antifungal activity Trityl-based synthetic dyes Mathematical-statistical methods

INTRODUCTION

Various synthetic dyes have been frequently employed in agrochemistry [1,2], in up-to-date food science and technology [3,4], and in numerous industrial processes [5,6].

The amount of synthetic dyes produced in the world is not exactly known. It is assumed to be more than 10,000 ton/year. Accurate data regarding the quantity of dyes released in the environment are also not available. A loss of 1 to 2% in production and of 1 to 10% in use may be a fair estimate. Because of large-scale production and use of synthetic dyes, these substances are now serious environmental pollutants [7] and health risk factors [8]. The impact and fate of dyes discharged in the environment have been widely discussed [9,10]. Because a considerable number (several thousands) of synthetic dyes are used that exert various toxicological effects, it is understandable that knowledge regarding their effects on the environment and health hazards related to their application is still incomplete. Synthetic dyes released in industrial effluents and wastewaters can contaminate soil and decrease the growth of components of soil microflora, such as nitrogen-fixing cyanobacteria [11] and the gram-negative soil bacterium *Myxococcus xanthus* [12], decreases the growth of *Bacillus subtilis* [13], etc. It has further been established that exposure to alternating current makes *Escherichia coli* susceptible to basic dyes [14].

The assessment of large data matrices containing a high number of columns and rows (as in the present study, which involves the antifungal activity of 10 synthetic dyes and six reference compounds on 36 fungi) is cumbersome or impossible with the application of linear regression analysis, as is generally used for quantitative structure-activity relationship studies. A considerable number of multivariate mathematical-statistical methods have been developed and successfully employed to facilitate elucidation of the maximal information present in such matrices. Principal component analysis (PCA), a multivariate method, makes possible assessment of the relationships between the columns and rows of any data matrix without being one of the dependent variables [15]. Because PCA is versatile and easy to carry out, it has been applied often in many fields of research [16,17]. The method clusters the elements of the matrix with simultaneous consideration of the strength and selectivity of the effect under investigation; that is, it is not suitable for data evaluation when separate calculations of the strength and selectivity of the effect are required.

The spectral mapping technique (SPM), another multivariate mathematical-statistical method, has been developed for separation of the strength and selectivity of the effect [18]. The SPM divides the information into two matrices using the logarithm of the original data. The first matrix is a vector containing the potency values related to the overall effect (in the present study, the overall antifungal effect of dyes and the overall sensitivity of fungi toward dyes). The second matrix (i.e., selectivity map) contains the information related to the spectra of activity (in the present study, the selectivity of the antifungal effect of dyes and the selectivity of the response of fungi toward dyes) [19].

The objectives of the present study were measurement of the antifungal effect of some trityl dyes and reference compounds, assessment of the data by PCA and SPM, and comparison of the results of PCA and SPM by stepwise regression analysis [20].

MATERIALS AND METHODS

Common as well as International Union of Pure and Applied Chemistry names of trityl dyes are listed in Appendix 1, and their chemical structures are shown in Figure 1. Each trityl dye was a commercial product and was used as received. The selection of this set of trityl dyes was motivated by their markedly different chemical structures (with and without quaternary amino groups, etc.) and by the fact that they are extensively used in various large-scale industrial processes. Clotrimazole (XI), fenarimol (XII), nuarimol (XIII), triarimol (XIV), blasticidin-S (XV), and trichotecin (XVI) were em-

* To whom correspondence may be addressed (forgacs@cric.chemres.hu).

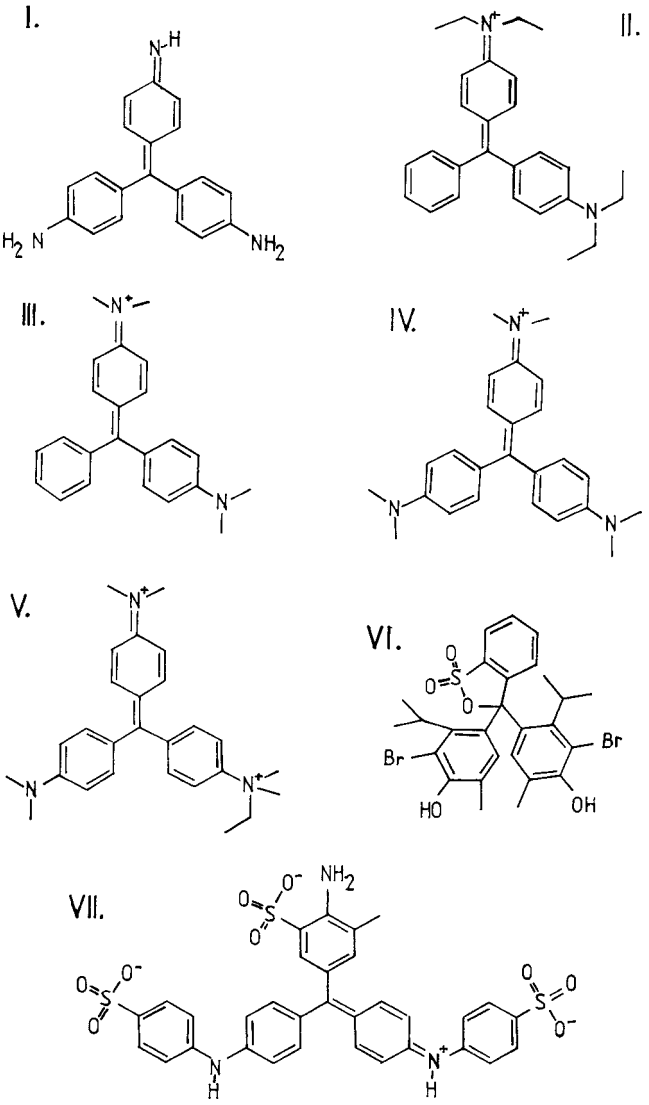

Fig. 1. Chemical structures of trityl dyes. Roman numbers refer to trityl dyes in Appendix 1.

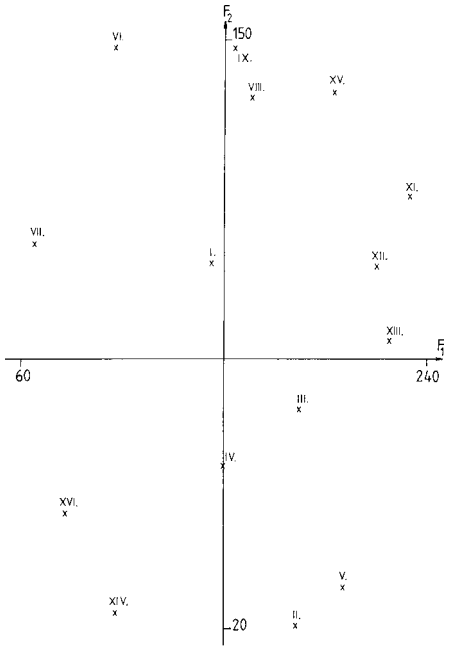

Fig. 2. Similarities and dissimilarities among the antifungal activities of trityl dyes and reference compounds as shown by a two-dimensional nonlinear map of principal component loadings (no. of iterations, 116; maximal error, 6.29×10^{-2}). Roman numbers refer to trityl dyes and reference compounds in Appendix 1 and *Materials and Methods*, respectively.

ployed as reference compounds with well-established antifungal activity. Unless otherwise noted, 0.2 mM stock solutions of test compounds were prepared in analytical-grade methanol and stored at 0 to 5°C in the dark. Analytical-grade mineral salts, glucose, amino acids (Reanal Fine Chemicals, Budapest, Hungary), vitamins (Chinoin Pharmaceutical, Budapest, Hungary), Na-β-glycerophosphate (Sigma-Aldrich, Budapest, Hungary), bacteriological agar no.1, Trypton T (L43) (Oxoid, Basingstoke, UK), yeast extract (L21; Oxoid, Basingstoke, UK), and malt extract broth (Sigma-Aldrich) were used for preparing the media.

The fungi tested and their taxonomical positions are listed according to Hawksworth et al. [21] in Appendix 2. All test organisms listed in Appendix 2 were taken from the collection of the Plant Protection Institute of the Hungarian Academy of Sciences (Budapest, Hungary). The test species represent various taxonomic groups as well as modes of habitation (saprotrophes, species parasitizing predominantly by necrotrophic, hemibiotrophic, and biotrophic modes on whole plants or aerial or underground parts of plants) [22].

Saprophytic and facultative parasitic fungi (species 1–34) were maintained on agarized potato broth amended with tryptophane (2 g/L), yeast extract (1 g/L), Na-β-glycerophosphate·$5H_2O$ (0.6 g/L), $MgSO_4 \cdot 7H_2O$ (0.5 g/L), KH_2PO_4 (0.25 g/L), $Na_2HPO_4 \cdot 7H_2O$ (0.25 g/L), KCl (0.15 g/L), and $FeSO_4 \cdot 5H_2O$ (0.01 g/L). The potato broth was prepared from peeled tubers (200 g) cut in pieces (~1 × 1 × 1 cm), cooked in distilled water (1 L) for 20 min, and then filtered through a Perlon meshwork (no. 500; Leverkusen, Germany). The filtrate was made up to 1 L with distilled water. The broth was solidified with 12 g/L of agar-agar (PDA) and sterilized at 120°C for 20 min. The inoculated agar slants were incubated at 21 ± 2°C. For the production of conidia, fungi (species 1–34) were grown on malt agar slants containing $NaNO_3$ (3 g/L), KCl (0.5 g/L), KH_2PO_4 (0.5 g/L), K_2HPO_4 (0.5 g/L), $MgSO_4$ (0.5 g/L), pyridoxine-HCl (1 mg/L), thiamin-HCl (10 mg/L), riboflavin (1 mg/L), nicotinamide (20 mg/L), and citric acid (0.2 g/L). Inocula were prepared by suspending conidia of vegetative cells (10^5 cell/ml) in sterile, distilled water containing glucose (2 g/L) and Tween™ 40 (0.2 g/L; ICI Americas, Bridgewater, NJ, USA).

Oomycetes (species 35 and 36) were maintained on green pea agar (GPA). The GPA was prepared by adding PDA (11 g/L), glucose (10 g/L), Trypton T (2 g/L), yeast extract (0.5 g/L), Na-β-glycerophosphate·$5H_2O$ (0.5 g/L), KCl (0.25 g/L), and $MgSO_4$ (0.15 g/L) to the broth made from green peas. The broth was prepared from sugar peas (450 g) cooked in 1 L of distilled water for 20 min, then treated in the same way as the potato broth. For both species, 4-d-old colonies, grown on cellophane film on the surface of GPA plates, were gently triturated in green pea broth, and these suspensions (50 mg mycelium/ml) were used as inoculum.

For antifungal activity tests, the PDA was inoculated with fungus (10^5 propagules per 20 ml of medium), and a layer (depth, 5 mm) was dispensed into Petri dishes (diameter, 90 mm). Filter paper discs (diameter, 5 mm) impregnated with

Table 1. Antifugnal effect of trityl dyes as the diameter of inhibitory zones (mm) caused by 10^{-6} M of dyes

	Dyes									
Fungi	I	II	III	IV	V	VI	VII	VIII	IX	PS%[b]
1	0	31	34	22	27	0		0	0	47.3
2	10	8	2	14	13	8	0	0	0	33.6
3	0	11	33	29	22	0	0	0	0	39.4
4	11	34	34	22	31	0	0	0	0	56.8
5	7	34	37	23	37	0	5	0	0	57.3
6	14	10	40	0	0	0	0	0	0	26.6
7	0	17	21	9	15	6	0	7	5	85.1
8	37	29	60	44	30	5	0	0	0	33.2
9	15	23	49	18	20	0	0	0	0	100.0
10	30	30	73	42	30	5	5	25	6	60.6
11	7	33	45	21	31	0	0	0	0	53.9
12	6	30	54	35	35	0	0	0	0	62.2
13	7	20	28	20	21	0	0	0	0	28.6
14	11	30	62	31	37	0	0	0	0	45.6
15	11	34	42	18	33	0	0	0	0	56.8
16	15	25	40	18	25	0	0	0	0	66.4
17	5	13	30	26	21	0	0	0	0	39.8
18	14	35	37	23	37	0	0	0	0	51.0
19	15	35	45	20	35	0	0	0	0	39.4
20	10	32	33	19	16	0	0	0	0	57.3
21	11	5	34	14	5	0	0	0	0	71.0
22	0	38	43	17	39	0	0	0	0	56.8
23	9	29	32	26	30	0	0	0	0	52.3
24	0	41	42	22	43	0	0	0	0	61.4
25	11	5	34	14	5	0	0	0	0	28.6
26	47	5	31	18	5	0	15	0	0	33.6
27	11	5	27	15	6	0	30	0	0	39.0
28	30	12	45	27	35	7	0	0	0	58.5
29	9	29	47	25	31	0	0	0	0	66.8
30	18	47	57	32	43	0	0	0	0	81.7
31	18	32	35	20	30	5	0	0	0	58.1
32	14	22	40	30	19	0	0	0	0	51.9
33	14	38	46	27	38	7	0	0	0	70.5
34	15	7	35	18	5	0	18	0	0	40.7
35	9	10	37	19	7	6	0	0	0	36.5
36	8	6	25	13	9	15	0	0	0	31.5
PA/%[c]	38.5	79.6	135.2	74.6	81.6	6.0	6.9	3.5	1.0	

[a] Roman and Arabic numerals refer to trityl dyes and fungi species in Appendices 1 and 2, respectively.
[b] PS% = potential sensitivity of species toward trityl dyes expressed as a percentage of the most sensitive species.
[c] PA% = potential activity of trityl dyes toward species expressed in percent of the most active trityl dyes. Least significance difference ($LSD_{5\%}$) = 1.90 ($F_{calc.}$ = 1346; $F_{5\%}$ = 1.42).

10^{-6} M of test compounds were placed centrally on the agar plate (one disk per dish), and growth-inhibition zones were measured after incubation at 22 ± 1°C for 48 h.

For anti-oomycete tests, the GDA medium was inoculated with *Pythium irregulare* or *Phytophtora cactorum* mycelium suspension (1 ml/dish) and a layer of the medium (depth, 5 mm) was dispensed into Petri dishes (diameter, 90 mm). Filter paper disks (diameter, 5 mm) were impregnated with 10^6 M of test compounds and placed centrally on the agar plate (one disk per dish). The growth inhibition zones were measured after incubation at 18 ± 1°C for 72 h.

All experiments were carried out at least three times. Fisher's probe was applied for calculation of the least significant difference values of response data. Because phenolphthalein (compound X) showed no antifungal activity, it was omitted from subsequent calculations.

The data matrices used for multivariate analyses consisted of the antifungal activities (inhibitory zones in mm).

Cluster analysis (unweighted pair group average method) was employed for elucidation of the similarity of fungi in their responses toward dyes (with the 36 test species and 10 dyes being the observations and variables, respectively). Sensitivity and selectivity of the test species toward dyes were scaled by SPM, and the potential sensitivity of species toward trityl dyes and potential activity of trityl dyes toward species were calculated as percentages (with the highest sensitivity and highest activity being 100%).

The PCA was employed for assessment of the similarities and differences in 15 test compounds and 36 species simultaneously, taking into consideration simultaneously the strength and selectivity of the antifungal activity. The columns in this matrix are the activity data of test compounds, and the rows are the species listed in Appendix 2. The variance explained was set to 95%. To calculate the strength and selectivity of the antifungal effect of test compounds and the strength and selectivity of the sensitivity of test organisms, SPM was carried out on the data matrix used for PCA and on the transversed matrix. Because evaluation of the multidimensional maps of principle component (PC) loadings, PC variables, and selectivity (i.e., spectral) maps calculated by SPM is difficult, the dimensionality of the maps was reduced to two by the nonlinear map technique (NLMAP). Theoretically, NLMAP can reduce the dimensionality of any multidimensional data matrix without submitting the data to PCA. According to our previous

Table 2. Antifungal effect of reference compounds as the diameter of inhibitory zones (mm) caused by 10^{-6} M of reference compounds[a]

	Reference compounds					
Fungi	XI	XII	XIII	XIV	XV	XVI
1	12	11	8	15	5	19
2	34	19	5	23	41	30
3	22	0	10	25	44	47
4	23	21	23	24	19	38
5	23	13	16	43	13	51
6	58	38	44	23	25	8
7	5	5	5	20	34	25
8	40	41	43	14	14	26
9	42	70	82	42	31	40
10	60	81	89	5	85	9
11	16	6	19	37	11	49
12	15	13	25	71	11	50
13	17	0	7	82	11	74
14	44	30	45	14	33	21
15	18	0	5	25	5	19
16	5	0	5	5	27	17
17	8	34	43	5	8	6
18	29	42	49	5	20	0
19	83	90	90	32	41	28
20	34	38	47	34	30	18
21	5	0	7	35	5	55
22	20	25	34	90	13	85
23	30	26	35	46	15	27
24	11	23	39	0	15	17
25	15	27	17	25	13	35
26	39	31	25	39	14	30
27	6	5	5	37	5	22
28	40	65	69	18	40	10
29	13	18	14	28	43	15
30	36	28	42	5	21	0
31	8	18	16	61	12	33
32	20	19	31	13	5	22
33	0	26	43	43	27	21
34	35	15	17	20	7	11
35	0	0	0	6	0	0
36	15	7	7	0	21	16
PA%[b]	83.0	83.4	100.0	95.2	72.0	91.8

[a] Roman and Arabic numerals refer to reference compounds and fungi species in *Materials and Methods* and Appendix 2, respectively.
[b] PA% = potential activity of reference compounds toward species in percent of reference compound XIII.

experience, however, NLMAP for any original data matrix requires considerable computer time. Moreover, the resulting maps are distorted, and the data points are not well distributed on the map, irrespective of the composition of the original matrix. It must be emphasized that the conclusion drawn is based only on some practical examples and is not the result of theoretical considerations; therefore, its generalization may lead to serious misunderstanding of the results. The iteration for NLMAP was carried out to the point at which the difference between the last two iterations was lower than 10^{-8}. The PC loadings were also evaluated by varimax rotation around two axes.

The similarities and dissimilarities between the results of PCA and the two SPMs were assessed by stepwise regression analyses. For matrix A, linear correlations were calculated by the first and second co-ordinates of the two-dimensional nonlinear map of PC loadings (nlmap1 and nlmap2), the first and second rotated PC loadings around two axes, the corresponding potency values (P), and the first and second co-ordinates of the two-dimensional nonlinear selectivity map (spmap1 and spmap2, 7 × 36 matrix). The SPM was carried out on the transversed matrix. For matrix B, linear correlations were calculated by the first and second co-ordinates of the two-dimensional nonlinear map of PC variables, the corresponding potency values, and the first and second co-ordinates of the two-dimensional nonlinear selectivity map (5 × 36 matrix). The SPM was carried out on the original matrix.

Comparison of the results of PCA and SPM was motivated by the following considerations: Simultaneous or separate application of PCA and SPM for the evaluation of multidimensional data matrices is only justified when the information content of the results is different. It was assumed that the presence or absence of significant correlations is a good indicator of the difference in the results. Presumably, it can be employed as a suitable validation parameter for such types of comparison.

RESULTS AND DISCUSSION

The antifungal effects of trityl dyes and reference compounds are compiled in Tables 1 and 2, respectively. The coefficient of variation of the parallel measurements ranged from 0 to 7.2%, depending considerably on the compound and the species. It exceeded 4% in only 45 of the 360 cases. The highest value was achieved in the measurement of the effect of methyl green against *Trichoderma harzianum*. No significant differences were observed among the parallel determinations ($F_{calc.} = 0.34$, $F_{10\%} = 2.71$), which verifies the reliability of the measurements. The sensitivity of species toward dyes showed significant differences ($F_{calc.} = 91.5$, $F_{5\%} = 1.54$), indicating that the various dyes exert different impacts on the fungi in the environment. None of the species showed complete tolerance, and *Alternari solani*, *Colletotrichum graminicola*, and *Mycophaerella tulasnei* displayed the lowest sensitivity. In general, saprotrophic and dominantly necrotrophic species were less sensitive than hemibiotrophic and biotrophic ones, and species parasitizing mainly the underground parts of plants were less sensitive than those parasitizing on the aerial parts.

The antifungal activity of dyes also showed marked differences ($F_{calc.} = 3{,}828$, $F_{5\%} = 2.46$), indicating that various dyes may exert different impacts on the fungi in the environment. Cationic dyes were more active than the sulfone derivatives or those without alkyl substituents. The activity of malachite green even surpassed that of the reference compound with the most potential activity.

The results of cluster analysis showed that neither the taxonomical position of a species nor its mode of habitation related to its sensitivity toward trityl dyes (cluster not shown). The individual clusters contained species belonging to different higher taxa and species with different modes of habitation.

The results of PCA indicated that seven hypothetical (i.e., background) variables explained the overwhelming majority of total variance with only 9.22% loss of information, suggesting that the 15 original variables can be reduced to seven theoretical variables. Both dyes and reference compounds have high loadings in more than one PC component, indicating that they show different effects when the strength and selectivity of their antifungal activity are taken into consideration simultaneously.

The two-dimensional nonlinear map of PC loadings is presented in Figure 2. Neither trityl dyes nor the reference compounds form clear-cut clusters on this map, which suggests that the antifungal activity of dyes and reference compounds deviate considerably. The effect of triarimol and trichotechin was similar, whereas the points of the other reference compounds are located at the opposite end of the map. The two-

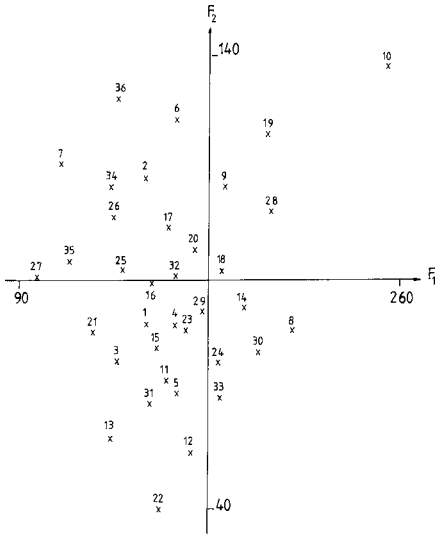

Fig. 3. Similarities and dissimilarities among the sensitivities of fungi toward trityl dyes and reference compounds as shown by a two-dimensional nonlinear map of principal component variables (no. of iterations, 101; maximal error, 5.32×10^{-2}). Numbers refer to fungi species in Appendix 2.

dimensional nonlinear map of PC variables is shown in Figure 3. Fungi did not form well-defined clusters according to their taxonomical position. This finding supports our previous conclusion, according to which the strength and selectivity of the sensitivity of fungi toward trityl dyes and reference compounds do not depend on their taxonomical position. However, it cannot be excluded that the modes of action by trityl dyes and reference compounds are different, and that the distribution of the fungi on the map reflects not only the different sensitivities of fungi but also the different modes of action of the compounds.

The potency values of individual synthetic dyes and fungi calculated by SPM are compiled in Table 3. The data in Table

Table 3. Strength of antifungal activity of trityl dyes and reference compounds and sensitivity of fungi[a]

Dyes and reference compounds							
No.	Potency	No.	Potency	No.	Potency	No.	Potency
I	68.17	V	144.33	IX	1.83	XIV	168.33
II	140.83	VI	10.67	XI	146.83	XV	127.33
III	239.17	VII	12.17	XII	147.50	XVI	162.33
IV	131.83	VIII	6.17	XIII	176.83		
Fungi							
No.	Potency	No.	Potency	No.	Potency	No.	Potency
1	47.51	10	147.17	19	132.71	28	104.05
2	60.16	11	71.00	20	80.30	29	70.23
3	62.74	12	89.08	21	45.44	30	84.95
4	73.59	13	74.10	22	104.31	31	74.36
5	76.69	14	92.44	23	78.75	32	60.68
6	67.13	15	54.22	24	65.32	33	85.21
7	44.93	16	46.99	25	51.90	34	52.41
8	98.89	17	51.38	26	66.87	35	24.27
9	112.83	18	75.14	27	44.93	36	36.66

[a] Potency values (arbitrary units) are calculated by the spectral mapping technique. Roman and arabic numbers refer to trityl dyes in Table 1 and to reference compounds in *Materials and Methods* and fungi species in Appendix 2, respectively.

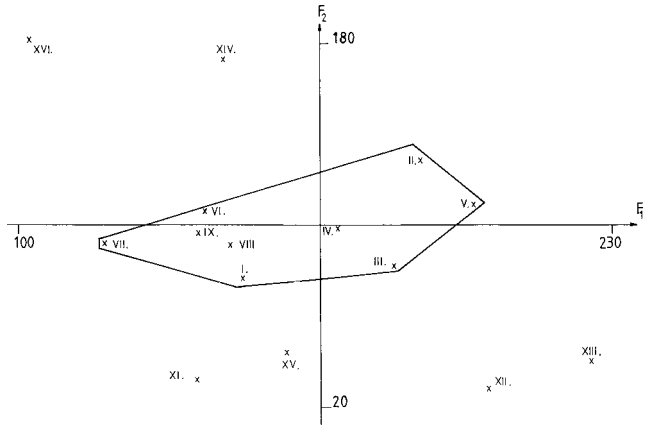

Fig. 4. Similarities and dissimilarities among the selectivities of the antifungal activities of trityl dyes and reference compounds as shown by a two-dimensional nonlinear selectivity map (no. of iterations, 195; maximal error, 3.02×10^{-2}). Roman numbers refer to trityl dyes and reference compounds in Appendix 1 and *Materials and Methods*, respectively.

3 support our previous qualitative conclusions. The strength of the overall antifungal activity of dyes shows high variation among the dyes, proving again that their biological activity strongly depends on their chemical structure. Interestingly, the potency values of dyes are commensurable with those of reference compounds, indicating that trityl dyes accumulated in the environment may cause serious pollution. The response of fungi toward dyes also exhibits considerable diversity, indicating that some fungi can tolerate this class of environmental pollutants, with the sensitivity depending on the character of the fungi.

The dyes form a concise cluster on the two-dimensional nonlinear selectivity map (Fig. 4), indicating that their selectivity is more similar than their overall biological activity.

The two-dimensional nonlinear selectivity map of species is given in Figure 5. Fungi do not form clear-cut clusters according to their taxonomical position on the selectivity map, which proves again that the taxonomical position does not

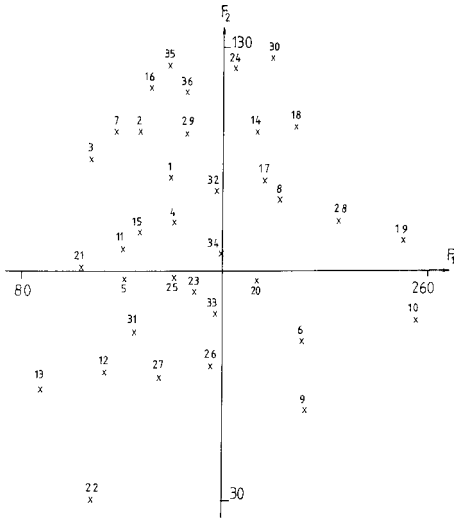

Fig. 5. Similarities and dissimilarities among the selectivities of sensitivities of fungi toward trityl dyes and reference compounds as shown by a two-dimensional nonlinear selectivity map (no. of iterations, 164; maximal error, 5.12×10^{-2}). Numbers refer to fungi species in Appendix 2.

Table 4. Coefficients of correlations of linear relationships between the first and second co-ordinates of the two-dimensional nonlinear map of principal component (PC) loadings and variables (nlmap1, nlmap2), first and second rotated PC loadings (var1, var2), the potency values (P), and first and second co-ordinates of the two-dimensional nonlinear selectivity map (spmap1, spmap2). Italicized data show significant correlations

Trityl dyes and reference compounds (n = 15, $r_{99.9\%}$ = 0.7603)						
	nlmap1	nlmap2	var1	var2	P	spmap1
nlmap2	0.0438					
var1	0.6759	0.6559				
var2	0.5162	*−0.7930*	−0.1226			
P	0.4229	−0.6880	−0.1544	*0.7885*		
spmap1	0.7373	−0.2951	0.4033	0.6495	0.4802	
spmap2	−0.7110	−0.5242	*−0.8469*	0.0876	−0.0368	−0.4097

Fungi (n = 36, $r_{99.9\%}$ = 0.5189)				
	nlmap1	nlmap2	P	spmap1
nlmap2	0.1392			
P	*0.8288*	0.0480		
spamp1	*0.7495*	*0.6244*	*0.5873*	
spmap2	0.0423	0.2122	−0.4035	0.1074

define the selectivity of the antifungal effect of trityl dyes and reference compounds.

The correlation coefficients of linear relationships between the first and second co-ordinates of the two-dimensional nonlinear map of PC loadings and variables, the first and second rotated PC loadings, the potency values, and the first and second co-ordinates of the two-dimensional nonlinear selectivity map are compiled in Table 4. No significant correlation was found between the first and second co-ordinates of rotated PC loadings, with nonlinear and spectral maps proving the real orthogonality of each calculation method. Significant relationships were, however, found between the co-ordinates, which verifies the similarity of the methods.

It can be concluded from the data that trityl dyes show marked antifungal activity; therefore, they may be considered as environmental pollutants when accumulated in groundwaters and soil. Various multivariate methods, such as PCA and SPM, combined with nonlinear mapping and varimax rotation can be successfully employed for the study of their antifungal effect. Because of its capacity to separate the strength and selectivity of the antifungal effect, SPM is highly recommended.

Acknowledgement—This study was supported by a grant from the Ministry of Environmental Protection (HP-206).

REFERENCES

1. Cook SMF, Linden DR. 1997. Use of Rhodamine WT to facilitate dilution and analysis of atrazine samples in short-term transport studies. *J Environ Qual* 7:1438–1441.
2. Kross BC, Nicholson HF, Ogilvie LK. 1996. Methods development study for measuring pesticide exposure to golf course workers using video imaging techniques. *Applied Occupational and Environmental Hygiene* 11:1346–1351.
3. Turnipseed SB, Roybal JE, Plakas SM, Pfenning AP, Hurlbut JA, Long AR. 1997. Determination of methylene blue in channel catfish (*Ictalurus punctatus*) tissue by liquid chromatography with visible detection. *JAOAC Int* 80:31–35.
4. Plakas SM, El-Said KR, Stehly GR, Gingerich WH, Allen JL. 1996. Uptake, tissue distribution, and metabolism of malachite green in the channel catfish (*Ictalurus punctatus*). *Can J Fish Aquat Sci* 53:1427–1434.
5. Knittel D, Schollmeyer E, 1996. Prevention of water pollution in dyeing processes of synthetic textiles. *European Water Pollution Control* 6:6–10.
6. Petek J, Glavic P. 1996. An integral approach to waste minimization in process industries. *Resource Conservation Recycling* 17:169–189.
7. Sahu RK, Acharya NN, Mishra RN. 1987. Cytomorphological effects of some dyes released with paper mill effluents of *Allium cepa* root meristems. *Indian Bot Rep* 6:73–79.
8. Gatto C, Milanick MA. 1993. Inhibition of the red cell calcium pump by eosin and other fluorescein analogs. *Am J Physiol* 246: C1577–C1586.
9. Calin C, Miron M. 1995. Where to the dyers? The textile finishing of the 2000 year. *Ind Text (Bucharest)* 46:140–147(in Romanian).
10. Hunger K. 1995. The organic pigments and their effects on toxicology and environment. *Pitture e Vernici Europe* 71:30–32(in English and Italian).
11. Pandey KD, Kashyap AK. 1992. Induction of mutation in the cyanobacterium *Anabaena doliolum*. *Folia Microbiol (Prague)* 37:377–380.
12. Arnold JW, Shimkets LJ. 1988. Inhibition of cell-cell interactions in *Myxococcus xanthus* by Congo red. *J Bacteriol* 170:5765–5770.
13. Ogawa T, Shibata M, Yatome C, Idaka E. 1988. Growth inhibition of *Bacillus subtilis* by basic dyes. *Bull Environ Contam Toxicol* 40:545–552.
14. Shimada K, Shimara K. 1987. Effect of alternating current exposure on the resistivity of resting *Escherichia coli* B cells to crystal violet and other basic dyes. *J Appl Bacteriol* 62:261–269.
15. Mardia KV, Kent JT, Bibby JM. 1979. *Multivariate Analysis*. Academic, London, UK.
16. Héberger K, Lopata A. 1998. Assessment of nucleophilicity and electrophilicity of radicals and of polar and enthalpy effects on radical addition reactions. *J Org Chem* 63:8646–8653.
17. Héberger K, Görgényi M. 1999. Principal component analysis of Kováts indices for carbonyl compounds in capillary gas chromatography. *J Chromatogr A* 812:21–31.
18. Lewi PJ. 1976. Spectral mapping, a technique for classifying biological activity profiles of chemical compounds. *Arzneim-Forsch* 26:1295–1300.
19. Lewi PJ. 1989. Spectral map analysis. Factorial analysis of contrast, especially from log ratios. *Chemom Intell Lab Syst* 5:105–116.
20. Mager H. 1982. *Moderne Regressionsanalyse*. Salle, Sauerländer, Frankfurt am Main, Germany, pp 135–157.
21. Hawksworth DL, Kirk PM, Sutton BC, Pegler DN. 1995. *Ainsworth and Bisby's Dictionary of the Fungi*, 8th ed. CAB International, Wallingford, UK, p 616.
22. Parberry DG. 1996. Trophism and ecology of fungi associated with plants. *Biol Rev* 71:473–527.

APPENDIX 1

Common and International Union of Pure and Applied Chemistry (IUPAC) names of trityl dyes

No. of dyes	Common name	IUPAC name
I	Pararosaniline	4-((4-Aminophenyl)(4-imino-2,5-cyclohexadien-1-ylidene)methyl)benzenamine monohydrochloride
II	Brilliant green	*N*-[4-[[4-(diethylamino)phenyl]phenylmethylene]-2,5-cyclohexadiene-1-ylidene]-*N*-ethyl-ethanaminium sulfate
III	Malachite green	*N*-[4-[[4-(dimethylamino)phenyl)phenylmethylene]-2,5-cyclohexadiene-1-ylidene]-*N*-methyl-methanaminium chloride
IV	Crystal violet	*N*-[4-[bis[4-(dimethylamino)phenyl]methylene]-2,5-cyclohexadien-1-ylidene]-*N*-methyl-methaminium chloride
V	Methyl green	4-[[4-(Dimethylamino)phenyl][4-(dimethylimino)-2,5-cyclohexadiene-1-ylidene]methyl]-*N*-ethyl-N,N-dimethylbenbenzaminium bromide chloride
VI	Bromthymol blue	3′,3-Dibromothymolsulfonphthalein
VII	Cotton blue	Aminomethyl[[4-((sulfophenyl)imino]-2,5-cyclohexadien-1-ylidene)methyl]-benzenesulfonic acid disodium salt
VIII	Brilliant Blue R-250	*N*-[4-[[4-[(4-ethoxyphenyl)-amino]-phenyl][4-[ethyl[(3-sulfophenyl)methyl]-amino]-phenyl] methylene]-2,5-cyclohexadien-1-ylidene]-*N*-ethyl-3-sulfo-benzenemethanaminium inner salt (monosodium salt)
IX	Brilliant Blue G-250	*N*-[4-[[4-((4-ethoxyphenyl)-amino]phenyl][4-[ethyl[(3-sulfophenyl)methyl]-amino]-2-methylphenyl]methylene]-3-methyl-2,5-cyclohexa-di-en-1-ylidene]-*N*-ethyl-3-sulfobenzenemethanaminium inner salt (monosodium salt)
X	Phenolphthalein	2-[Bis(4-hydroxyphenyl)methyl]benzoic acid

APPENDIX 2

List of species, their origin, and taxonomical position according to Hawksworth et al. [21][a]

No.	Species	Source
	Mucorales (Oomycetes)	
1	*Mucor racemosus* Fresenius	Air
2	*Rhisopus stolonifer* (Ehrenberg:Fries) Lind	Sunflower
	Saccharomycetales (Saccharomycetes)	
3	*Saccharomycetes cerevisiae* (Hansen)	Baker's yeast
	Eurotiales (Eurotiomycetes)	
4	*Aspergillus niger* van Tieghem	Onion
5	*Penicillium oxalicum* Currie&Thom	Cucumber
	Pleosporales (Dothideomycetes)	
6	*Alternaria solani* (Ellis&Martin) Sorauer	Potato
	Dothideales (Dithideomycetes)	
7	*Didymella applanata* (Niessl.) Sacc.	Raspberry
8	*Botrydosphaeria rhodina* (Berk.&Curt.) von Arx	Coconut
9	*Venturia inaequalis* (Cooke) Winter emend.	Aderhold apple
10	*Ascochyta pisi* Lib.	Pea
11	*Septoria lycopersici* Speg.	Tomato
12	*Fulvia fulva* (Cook) Ciferri	Tomato
13	*Mycophaerella tulasnei* (Janz.) Land	Air
14	*Cladosporium cucumericum* Eliis&Arth.	Cucumber
	Hypocreales (Sordariomycetes)	
15	*Nectria haematococca* Berk.&Br.	Potato
16	*Fusarium graminearum* Schwabe	Maize
17	*Fusorium oxysproum* Schlecht.:fr.f.sp. *lycospersici* (Sacc.) Snyder&Hansen	Maize
18	*Trichoderma harzianum* Rifai	Soil
19	*Verticillium albo-atrum* Reinke&Berthold	Tomato
20	*Magnaporthe grisea* (Hebert) Barr	Rice
21	*Trichotecium roseum* (Pers.:fr.) Link (mitosporic)	Apple
	Phyllacorales (Sordariomycetes)	
22	*Colletorichum coccodes* (Wallr.) Hughes	Tomato
23	*Colletotrichum lindemuthianum* (Saccardo&Magnus) Briosi&Cavara	Bean
24	*Colletotrichum dematium* (Pers.:fr.) Grove	Soya
25	*Colletotrichum graminicola* (Cesati) Wilson	Wheat
26	*Colletotrichum musae* (Berk.&Curt.) v. Arx	Banana
27	*Myrothecium roridum* Tode:fr. (mitosporic)	*Primula* sp.
28	*Thielaviopsis basicola* (Berkeley&Broome) Ferraris	Tobacco
	Diapothales (Sordariomycetes)	
29	*Cryphonectria parasitica* (Murrill) M.Barr	Chestnut
	Heliothiales (Leotiomycetes)	
30	*Botrytis aclada* Fresenius	Onion
31	*Botryotinia fuckeliana* (de Barry) Whetzel	Grape
	Ustilaginales (Ustilaginomycetes)	
32	*Schroeteria decaisneana* (Boudier) De Toni	*Viola tricolor*
33	*Ustilago maydis* (de Candolle) Corda	Maize
34	*Sorosporium cenchri* P. Hennings	*Cenchrus* sp.
	Pythiales (Oomycetes)	
35	*Pythium irregulare* Buisman	Pea
36	*Phytophtora cactorum* (Lebert&Cohn) Schroeter	Apple

[a] Species 1 and 2, 3−31, 32 and 33, and 35 and 36 belong to divisions (phyla) Zygo-, Asco, Basidio- and Oomycota, respectively.