DEVELOPMENTAL STAGE RESPONSE OF PEARL MILLET DOWNY MILDEW (SCLEROSPORA GRAMINICOLA) TO FUNGICIDES

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Abstract: Inhibitory effect of 21 commercial and 6 experimental fungicides was assessed in model experiments against pearl millet downy mildew disease. Chemicals differed strongly by their anti-mildew activity, however neither the pathogen nor the host plant showed complete tolerance to any of compounds tested. Nevertheless, the plants outgrew depressant effect of compounds observed in germling stage and this activity did not significantly influence the yield. There was a significant correlation between yield performance and disease inhibitory effects assessed either *in vitro* or *in vivo* tests. The response of pathogen to investigated compounds varied during ontogeny, where zoosporangium formation was found to be the most sensitive ontogenetic event. When comparing responses of pathogen and host with fungicides by means of principal component analysis, the presence of two independent components has been demonstrated accounting for 86% of the total variation to which responses of host and pathogen contributed differently.

The antisporulant activity of compounds evaluated on detached leaf segments and their positive effect on yield significantly correlated allowing to predict the expectable grain yield significantly ($p>0.05$). Beside acylanilides andoprim, drazoxolon and efosit offered efficacy on the level requested. Metalaxyl and tridemorph as well as andoprim and cymoxanil acted synergistically against *S. graminicola.*

Keywords: *ontogeny, fungicide screening, yield performance, synergy*

Introduction

Pear millet (*Pennisetum glaucum* (L.) R. Br.), a crop of international importance, is indigenous to areas in North Africa [2]. Today, its production is centred in semiarid-tropical zone [49] but it is a reliable double-crop after wheat for some regions of Holarctic.zone, particularly at the southern altitudes 50° in Europe and 40° in USA [16]. This plant has good drought tolerance, so it could withstand some of the late summer droughts in the marked area. The vegetation period of highly productive pearl millet varieties and hybrids is short; it is possible to harvest a mature crop 60 to 65 days after plantation. Many diseases of pearl millet have been described worldwide [65], however, catastrophic fungal diseases had not been known before Indian pandemic caused by downy mildew in 1985. The disease was known in most of the pearl millet growing areas but it remained sporadic until introduction of high yielding hybrids with susceptible parent line [49]. The causative agent *Sclerospora graminicola* (Sacc.) Schroet., originally described from Europe [44], was probably introduced into new

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areas in infected seeds various weeds contaminating grains [61]. Pearl millet downy mildew recently is distributed worldwide and one of the most important diseases causing losses up to 60% of grain yield [32].

Review of literature

S. graminicola, like other oomycetes, has a complex life cycle where developmental forms differ essentially in their anatomy and physiology, as well as having different impacts host/parasite interactions, survival and distribution in space and time. Zoosporangia produced during the night, and at over 70 percent relative humidity [60], germinate directly either by germ-tubes, or by releasing 1 to 12 zoospores, which encyst and germinate by a germ-tube. Sexual oospores are produced in colonised plant tissue and can survive to several years. Asexual spores spread the disease between plants, whilst within plants the pathogen spreads intercellularily. Sexual oospores can travel large distances and can survive several seasons [26, 47, 49]. Progenies of the same oospore could be classified into several distinct pathotype groups [55] indicating that it shows high natural variation in agressivity [7]. One could expect the similar variation in sensitivity to chemical control measures as well. Widespread and regular cultivation of susceptible F_1 hybrids led to accumulation of oospore inoculum in the soil that resulted in epidemics in 1971-72. Since than PDMD epidemics cause considerable economic losses in India [3, 43, 49]. Epidemics have also been reported from Asia and Africa [65] and this disease has been the major biotic factor affecting grain yield for the last decades [51, 53]. The pathogen can be transmitted to new areas by wind and infected seeds [53].

S. graminicola can be controlled efficiently with systemic fungicide metalaxyl [14, 31, 63]. Seed dressing with Apron 35SD (6 kg/t seeds) in pearl millet growing regions of India and foliar application of Ridomil MZ72 (2 kg/ha) for seed crop were recommended [50]. Possibilities of biocontrol measures [57] and enhancement of plant resistance with chemical treatment [46] has also been explored.

Resistance to PMDM in some pearl millet cultivars was found [54, 56] which have been cultivated widely [3, 47]. However, the vulnerability of their resistance to the disease has been a major cause of concern, as even 10 % disease incidence cause economic loss. New pathogenic races may overcome the genetic resistance. In the populations of phylogenetically related pathogens (*Bremia* [12, 62], *Peronosprora* [29], *Plasmopara* [40, 59] and *Phytophthora* [36]) resistance to metalaxyl has observed demonstrating the necessity to have alternative fungicides for PMDM control as well.

Responses of *S. graminicola* to various fungicides have been repeatedly tested, however there were difficulties to compare data obtained by different authors because of alterations in screening methods, moreover, the tests were limited in most of cases to screening only one of ontogenetic developmental stages. For this reason, anti-mildew efficacy of 21 commercial and 6 experimental compounds, with known and diverse mode of action, were compared in a highly standardized model experiment. Spectrum of activity on various developmental forms of *S. graminicola* was investigated, the responses of subsequent ontogenetic stages were compared and the effects on host plant as well as on the yield performance were evaluated.

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Materials and Methods

Host-parasites system

The downy mildew pathogen *S. graminicola* (pathotype 1) was isolated from naturally infested plants (hybrid HB3) in Bogadi village of Mysore district (Karnataka state, India) during 1970 by Shetty. The strain was maintained on greenhouse grown plants before being used as inoculum for the experiments. The pearl millet hybrid HB3 - highly susceptible to downy mildew - was used throughout the experiments. The plants for *in vitro* studies were grown on in 4 kg pots (20 plant per pot) filled with a mixture of soil, sand and manure (1:1:1) in green-house.

Preparation of inoculum. Leaves were collected from systemically infected 21 day-old plants in the evening. Previously formed zoosporangia were eliminated from the surface by washing in tap water and the exess of water was removed. Then leaves were incubated in a moist chamber at 25±2 ^{o}C overnight (12-14 hours). Zoosporangia were collected by washing them off with sterile distilled water, and the resulting suspension incubated for 15 min to release zoospores. The concentration of zoospores was adjusted by adding sterile distilled water to 4×10^4 cells/ml using haemocytometer, and this suspension was used for inoculation of the plants at first true leaf stage. The method was described in detail by Safeulla [42].

Chemicals

The compounds tested are listed in *Table 1*. Stock solutions (0.2 M) were prepared and the dilutions of the same were used for all the experiments.

Biological activity studies

Developmental stage dependent responses of the pathogen and reactions of the host plant to chemicals were studied both *in vitro* and *in vivo*. The activity of compounds determined according to appropriate scale was transformed into percent inhibiton at the given dose. The dose response lines were fitted using log probit function and the biological activities expressed as EC_{50} values. The protective effect of compounds (percent disease control) was measured both in greenhouse and field conditions at maximum dose tolerated by the host plant.

The effect of fungicides on host-dependent (HD) stages of *S. graminicola* was determined by following methods:

Sporulation (zoosporangiogenesis): Leaves with disease symptoms were collected from infected plants and washed in distilled water, exess water was then removed. The leaves were cut into ≈1 cm^2 pieces which were subsequently smeared with solution of test compound of appropriate concentration (10 μL of 0.01, 0.25, 0.5, 1.0 and 2.0 μM for a single piece). Treated pieces were incubated in moist chambers (plastic trays lined with wet filter paper) at 22 ^{o}C in the dark. To determine the intensity of sporulation, a 0-4 scale was used where the proportion of leaf area covered by zoosporangia was graded as follows: 0, no sporulation; 1-4, sporulation appearing on < 25, 25-75, 75-<100 % and total area, respectively.

Production of zoospores: Zoosporangia were collected from leaf segments in each treatment (1 mL sterile distilled water per piece). The sporangial suspension was incubated for 15 min, than centrifuged at 20-40 g for 10 min. The number of zoospores released was counted in the supernatant using a haemocytometer.

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To assess the effect of fungicides on host-independent (HI) stages in the fungal life-cycle, the effects on zoospore release and zoospore motility were recorded. Both events were observed microscopically in suspensions of spores mixed in 1:1 ratio (v/v) with fungicide solutions of appropriate concentrations to achieve a final spore number at 10-50 $\times 10^4$ cell per ml as well as 0.1, 0.5, 1.0, 2.0 and 4.0 mM concentration of test chemicals. After 15 min the proportion of empty sporangia was determined microscopically.

Zoospore motility: Released zoospores were separated from the zoosporangium suspension prepared as above by centrifugation at 20-40 g. The resulting supernant was adjusted with distilled water to 5×10^4 zoospores per ml. This suspension was treated with the test chemicals as above, and intensity of motion assessed after 15 min incubation.

Phytotoxicity limits for test compounds were determined by examining their effects on seed germination and seedling vigour. Pearl millet seeds were soaked in solutions of test compounds at appropriate concentrations (1 g per 2 ml for 6 hours). After treatment the seeds were used for further experiments. Seeds treated with sterile distilled water by the same manner served as control. Effects on seed germination *in vitro* were determined according to rules of ISTA [4] counting the number of germinated seeds after 4 days of incubation. The ratio of inhibiton was expressed as a percentage related to the watery control. The vigour index was calculated according to following formule [1];

Seedling vigour (SV) = (RL + SL) × SG where RL and SL = average length of roots and shoot of seedlings after seven days, SG = percentage ratio of germinated seeds in the given Petri dish (n=25 per set).

Preventive anti-mildew activity of test compounds was evaluated both in greenhouse and field experiments. The pearl millet seeds treated at maximum tolerated concentration of chemical were sowed into soil and the seedlings artificially infected with *S. graminicola*. All plants lacking visible symptoms (sporulation, yellowing, stunted growth and malformed ear-heads) were taken as healthy. The activity of each compound was calculated as percent decrease in disease incidence as compared to water control. Field trials were conducted in experimental station of Mysore University (N12°18', E76°39', 733 m altitude, red loam soil) during the rainy seasons Monsoon.

Greenhouse grown two-day-old seedlings were inoculated at whorl region with zoospore suspension (4×10^4 cell/ml) and maintained in daylight regime. Disease incidence was recorded after 10^{th} days. The field screening was conducted in the downy mildew nursery plot by adopting the procedures of Williams [64]. The treated seeds were sown in a randomised block design with four replicates. Normal agronomic practices were followed. The disease incidence was evaluated at 60 days after sowing. The ratio of diseased vs. healthy plants was calculated as compared to the water control in both cases.

Assessment for pearl millet grain yield. The treated seeds with untreated checks were sown in subplots (12 m^2 of each) consisting of four 4 m rows 75 cm apart. Plants were about 15 cm apart with in the rows. Four replicates were maintained for each treatment in a randomised split plot design. The grain yield was collected from the central 3.8 m of the two central rows (net plot size 5.7 m^2), measured and the result provided into kg/ha.

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Table 1. *List of chemicals tested*

No.	Common name	Chemical Name	Log P[f]	Trade name	Producer
1[b]	Metalaxyl	methyl *N*-(2-methoxyacetyl)-*N*-(2,6-xylyl)-*DL*-alaninate	1.86	Ridomil 25 wp	Ciba-Geigy, Swiss
2[c]	CGA29212	methyl *N*-(2,6-dimethylphenyl)-*N*-chloroacetyl-*DL*-alaninate	2.11	experimental	Ciba-Geigy
3[c]	RE-26745	*N*-(2,6-dimethylphenyl)-2-methoxy-*N*-(2-oxo-tetrahydro-furan-3-yl)-acetamide	1.47	experimental	Chevron, USA
4[b]	Ofurace	α-2-chloro-*N*-2,6-xylylacetamido-γ-butyrolactone	1.80	Milfuram 50 wp	Chevron
5[b]	Furalaxyl	methyl-*N*-(2,6-xylyl)-N-(2-furanylcarbonyl)-*DL*-alaninate	3.64	Fongarid 25 wp	Ciba Geigy
6[a]	LAB14202F	2-[(2,6-dimethyl-phenyl)-(oxazole-5-carbonyl)-ami-no]-propionic acid methylester	2.41	experimental	BASF AG, BRD
7[b]	Benalaxyl	methyl-*N*-phenylacetyl-*N*-2,6-xylyl-*DL*-alaninate	3.09	Galben 25 wp	Montedison, Italy
8[c]	Cyprofuram	(±)-α-[*N*-(3-chloro-phenyl) cyclopropanecarboxamido]-γ-butyrolactone	1.95	Vinicur 50 wp	Schering AG
9[a]	Oxadixyl	methoxy-*N*-(2-oxo-1,3-oxazolidin-3-yl)acet-2',6'-xylidide	1.47	Sandofan	Sandoz AG, BRD
10[a]	Dimethomorph	4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)acryloyl]morpholine	4.06	Acrobat 50 wp	ICI, UK
11[a]	Andoprim	4,6-dimethyl-pirimidin-2-yl)-(4-methoxyphenyl)-amine	2.55	experimental	Fahlberglist, BRD
12[a]	Cymoxanil	1-(2-cyano-2-methoxyiminoacetyl)-3-ethylurea	1.53	Curzate 50 wp	Du Pont, USA
13[d]	Propamocarb[e]	propyl (3-dimethylamino-propyl)-carbamic acid propyl ester	0.37	Previcur N 70 LS	Schering AG
14[a]	Prothiocarb	(3-dimethylamino-propyl)-thiocarbamic acid *S*-propyl ester	1.50	Previcur	Schering AG
15[b]	TMTD	tetramethylthiuram disulfide	2.37	Perthiram 500 SC	Bayer, BRD
16[b]	Drazoxolon	(4-(2-chlorophenylhydraxono)-3-methyl-1,2-oxazol-5(4*H*)-one	2.43	Mil-Col 300	ICI
17[d]	Efosit[e]	ethyl hydrogen phosphonate	0.41	Aliette 80 wp	Rhone Poulenc Agro, France

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No.	Common name	Chemical Name	Log P[f]	Trade name	Producer
18[a]	BKF-3[e]	propyl hydrogen phosphonate	1.49	experimental	BorsodChem, HU
19[d]	Thiomersal	ethyl-(2--mercaptobenzoato-*S*)mercury sodium salt	3.52	experimental	Sigma-Aldrich
20[c]	Ziram	zinc*bis*(dimethyldithiocarbamate)	2.04		
21[c]	$Zn(PDC)_2$	zinc*bis*(pirrolidine-1-carbodithioate)	4.58	experimental	
22[b]	Dodemorph-*cis*	4-cyclododecyl-2,6-dimethylmorpholine	6.10	Meltatox 75 ec	BASF
23[b]	Dodemorph-*trans*	4-cyclododecyl-2,6-dimethylmorpholine	6.10	Meltatox 75 ec	
24[b]	Tridemorph-*cis*	2,6-dimethyl-4-tridecylmopholine	6.54	Calixin 75 ec	BASF
25[b]	Tridemorph-*trans*	2,6-dimethyl-4-tridecylmopholine	6.54	Calixin 75 ec	
26[b]	Fenpropimorph	*cis*-4-[3-*tert*-butylphenyl)-2-methylpropyl-2,6-dimethylmorpholine	4.54	Corbel 75 ec	BASF
27[a]	Dodine	dodecylguanidinium acetate	4.42	Efuzin 500 FW	Agrokemia, HU

Stock solutions of compounds 12, 15, 16, 20 and 21 were prepared in acetone, while those of 13, 14 and 19 in water and all others in methanole.

[a] = supplied by manufacturer, [b] = extracted from marketed product, [c] = synthesised in PPI HAS by known manner, [d] = purchased, [e]= commercial preparation was used, [f]= Log P values were calculated by Interactive Analysis LogP and LogW (water solubility) predictor website [6].

Table 2. *Activity of fungicides against various developmental stages of Sclerospora graminicola.*

Compounds		Developmental stages															
		Abiotrophic (HID)								Biotrophic (HD)							
No	Name	EC_{50}		m		EC_{50}		m		EC_{50}		m		EC_{50}		m	
1	Metalaxyl	236	*a*	2.841	*a*	305	*a,b*	2.747	*a*	36.2	*a,b*	1.953	*a,b*	234	*d*	4.16	*d,e*
2	CGA29212	277	*a,b*	2.783	*a*	277	*a,b*	2.783	*a*	46.4	*b*	1.815	*a,b*	202	*d*	3.85	*c,d*
3	RE26745	277	*a*	2.783	*a*	132	*a*	2.350	*a*	15.5	*a*	1.614	*a*	72.4	*b,c*	2.29	*b*
4	Ofurace	277	*a*	2.783	*a*	201	*a*	2.375	*a*	46.4	*b*	1.815	*a,b*	86.1	*c*	2.45	*b*
5	Furalaxyl	252	*a*	2.380	*a*	592	*b,c*	4.500	*b*	21.6	*a,b*	1.681	*a,b*	8.4	*a*	1.51	*a,b*
6	LAB149202F	236	*a*	2.841	*a*	277	*a,b*	2.783	*a*	46.4	*b*	1.815	*a,b*	40.3	*b*	2.03	*b*
7	Benalaxyl	236	*a*	2.841	*a*	132	*a*	2.350	*a*	46.4	*b*	1.815	*a,b*	81.4	*c*	2.38	*b*
8	Cyprofuram	236	*a*	2.841	*a*	277	*a,b*	2.783	*a*	46.4	*b*	1.815	*a,b*	46.6	*a*	2.09	*b*
9	Oxadixyl	613	*a,b*	4.424	*b*	548	*b,c*	4.402	*b*	166	*b,c*	2.936	*c,d*	100	*c*	2.57	*b,c*
10	Dimethomorph	236	*a*	2.841	*a*	277	*a,b*	2.783	*a*	36.2	*a,b*	1.953	*a,b*	65.3	*b,c*	2.33	*b*
11	Andoprim	548	*a,b*	4.402	*b*	567	*b,c*	4.523	*b*	25.8	*a*	2.182	*a,b,c*	31.6	*a,b*	2.55	*b*
12	Cymoxanil	2783	*d*	5.093	*b,c*	1496	*d*	6.860	*d*	1121	*d,e*	3.139	*d*	979	*f*	4.29	*d*
13	Propamocarb	1337	*c*	6.860	*c*	2938	*c,d*	6.644	*c,d*	2520	*f*	5.531	*e,f*	1735	*f,g*	2.16	*a,b*
14	Prothiocarb	2095	*d*	11.062	*d*	1414	*d*	7.082	*d*	585	*d*	4.670	*d,e,f*	794	*f*	7.44	*e*
15	TMTD	519	*a,b*	4.832	*b*	735	*c,d*	4.072	*b*	519	*c,d*	4.832	*e,f*	258	*d*	3.48	*c*

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Compounds		Developmental stages															
		Abiotrophic (HID)								Biotrophic (HD)							
No	Name	EC_{50}		m		EC_{50}		m		EC_{50}		m		EC_{50}		m	
16	Drazoxolon	955	*b,c*	11.062	*d*	1337	*d*	6.860	*d*	36.2	*a,b*	1.953	*a,b*	9.45	*a*	1.77	*b*
17	Efosit	644	*b*	4.870	*b,c*	669	*b,c*	4.160	*b*	134	*b,c*	2.916	*c,d*	214	*d*	3.63	*c*
18	BKF-3	562	*a,b*	4.869	*b*	1337	*d*	6.860	*d*	514	*c,d*	5.084	*e,f*	241	*d,e*	3.82	*c*
19	Ziram	519	*a,b*	4.832	*b*	1337	*d*	6.860	*d*	186	*c*	2.890	*b,c,d*	86.4	*c*	2.42	*b*
20	$Zn(PDC)_2$	1337	*c*	6.860	*c*	1414	*d*	7.082	*d*	519	*c,d*	4.832	*e,f*	243	*d*	3.39	*c*
21	Thiomersal	1496	*c*	6.860	*c*	2477	*c,d*	4.587	*b,c*	1469	*e,f*	6.644	*f*	1518	*f,g*	2.39	*a,b*
22	Dodemorph-*cis*	1337	*c*	6.860	*c*	1496	*d*	6.860	*d*	519	*c,d*	4.832	*e,f*	36.6	*b*	2.42	*b*
23	Dodemorph-*trans*	1337	*c*	6.860	*c*	2719	*d,e*	6.644	*b,c, d*	585	*d*	4.670	*d,e,f*	287	*d,e*	3.35	*b,c*
24	Tridemorph-*cis*	1337	*c*	6.860	*c*	2938	*d,e*	6.644	*c,d*	2520	*f*	5.531	*e,f*	1127	*f*	1.26	*a,b*
25	Tridemorph-*trans*	1337	*c*	6.860	*c*	1479	*d*	7.270	*d*	1360	*d,e*	6.644	*f*	351	*e*	1.16	*a*
26	Fenpropimorph-*cis*	1337	*c*	6.860	*c*	2477	*d,e*	4.587	*b,c*	2520	*f*	5.531	*e,f*	2248	*f*	1.54	*a,b*
27	Dodine	669	*b*	4.160	*b*	1414	*d*	7.082	*d*	398	*c,d*	5.546	*e,f*	198	*c,d*	3.96	*c,d*

Sensitivity to fungicides (inhibition) is expressed as EC_{50} value in micromol, m = slope of dose response line at log/probit scale.
Values labelled by the same letter are not significantly different at P=5% level.

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Data analysis

All tests were carried out at least in triplicate. Reliability of assessments was evaluated by analysing variation coefficients (C.V.%= 100×[stdev/mean]) using statistical functions of Excel 6 (Microsoft, Redmondton, USA). Disease inhibitory effects were analysed by MANOVA. Averages of grain yield were compared by Student's *t*-test.

In vitro efficacy of each chemical tested was characterised by EC_{50} value and slope of dose response line, the latter relates to specific activity of compounds. Both values were calculated from basic data expressed as a percentage, using a curve-fitting method based on log/probit function. Therapeutic value of tested chemicals was calculated by following formula (Eq.1):

$$\text{Therapeutic index (TI)} = MTC_{host}/MIC_{parasite} \quad \text{(Eq.1)}$$

where MTC_{host}=maximum tolerated concentration by germinating seeds of pearl millet and $MIC_{parasite}$=minimum inhibitory concentration determined on germinating zoosporangia of *S. graminicola* have been replaced with EC_{01} and EC_{99} values, both expressed in molar concentrations.

The experimental data were also analysed by employing bivariate linear regression, multiple correlation, multivariate linear regression and principal component analyses to disclose differences in developmental stage dependent responses. Excel97 statistical functions (Microsoft, Redmondton, USA) and Statistica5 program (StatSoft, Tusla, USA) were used for calculations and graphic presentation of data.

Results

Reliability of assessments

The coefficient of variation of the parallel measurements ranged from 0 to 6 % in most of cases. It exceeded 10 % just in 77 of 4023 cases mostly in the range of maximum tolerated concentrations when screening inhibitory effects against asexual spores, which verifies the reliability of the measurements. The correlation coefficient of dose/response lines was over 0.707 (r^2=0.50) in all cases, and it was lower than 0.775 ($r_{0.001}$=0.597) only in 10 cases. The major variation in curve fitting was found for screening activities against zoospores (*Fig 1*). The goodness of fitting of the activities of acylanilides was lower than that of other compounds. Nevertheless all lines could be fit at P<5 % level verifying the reliability of toxicological parameters; EC_{50} and slope (specific activity).

Responses of the pathogen

The responses of various developmental stages of *S. graminicola* to the tested compounds are shown in *Table 2*. Fungicides inhibited the two types (HI and HD) of ontogenetic development differently. Zoosporangium germination (ZG) was more sensitive to systemic acylanilides (**1-9**) and dimethomorph (EC_{50} < 500 μM) than to the other compounds (EC_{50}=500-2500 μM). Free moving zoospores (ZM) responded similarly. The organo-metallic compounds (**17-21**), as well as dimethyl-morpholine derivatives (**22-26**) contrary to expectations, were not most active against asexual spores

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of *S. graminicola*. These molecules exhibited high efficacy against *P. infestans* and *P. halstedii* zoospores [35], which were much less sensitive to acylanilides [37].

Treatments on established thallus of *S. graminicola* inhibited the intensity of sporulation (SP) within large limits (EC_{50}=15-2500 μM). In addition to acylanilides (**1-9**) andoprim, dimethomorph and drazoxolon proved to be highly active (EC_{50}=15-50 μM). The remaining compounds were two or three order of magnitude less effective in this respect (EC_{50}=100-2500 μM). The number of zoospores (PZ) arising from zoosporangia originated from the exposed leaf areas was also decreased by various degrees. Furalaxyl and drazoxolon separate from others with particularly high (EC_{50} < 10 μM) while propamocarb, thiomersal and fenpropimorph with low activity (EC_{50} > 1500 μM) on zoospore production.

When evaluating similarity of responses of various developmental stages to fungicides on the base of EC_{50} values (*Table 5*) the correlation did not deviated significantly from the linear one (P=0.1-5 %). The similarity in spectrum of sensitivity was significant ($r_{0.001}$=0.597 < $r_{SP,PZ}$= 0.816 < $r_{ZG,ZM}$=0.871 < $R_{[ZG+ZM],[SP+PZ]}$= 0.901) between HD and HID stages. Differences in the slope of dose response lines indicate qualitative dissimilarities in reaction of the parasite to test compounds in dependence of its ontogenetic stage. In general, the differences in steepness of dose response lines for activities against asexual spores (HID) were smaller for acylanilides than for other compounds (*Table 2*). The spectrum of sensitivity in HD stages as evaluated by specific activities differed greatly ($r_{SP,PZ}$= 0.051 < $r_{0.001}$=0.597 < $r_{ZG,ZM}$=0.729).

Responses of the host plant

Phytotoxity of tests compounds varied within large limits (EC_{50}=0.5-280 mM), and the systemic anti-oomycete fungicides were less toxic than the other ones (*Table* 3). Pearl millet did not tolerated well (EC50 < 10 mM) the thiocarbamate derivatives (**15**, **19**, **20**), thiomersal, tridemorph isomers (**24**, **25**) and fenpropimorph. Cymoxanil and drazoxolon proved to be also surprisingly toxic. Variations in the structure of acylanilide derivatives greatly influenced their phytotoxicity; replacement of methoxyacetyl group to chloroacetyl one (**2**, **4**) increased, while coupling methylester moiety into butyrolactone ring (**3**, **4**) decreased this activity. The cationic tenside type dodemorph isomers (**22**, **23**) and dodine as well as the phosphonic acid salts (**17**, **18**) were also well tolerated by pearl millet germlings. With few exceptions (**1**, **13, 15, 17** and **20**) the retardant effect of fungicides (EC_{50} mM) applied as seed dressing was slightly increased during first steps of seedling growth (EC_{50} for SG=1-115 mM > EC_{50} for SV=0.5-42 mM, $t_{SG,SV}$=2.90 > $t_{0.01}$=2.80).

The slope of dose response lines showed great variations (SG=1.29-5.45 and SV=1.28-6.88 probits in both cases), and except compounds **1**, **5**, **7, 13** and **24** either decreased or increased (14 and 8 cases, respectively) by development of seedlings. Nevertheless pear millet plants outgrew the depressant effect of fungicides. None of compounds exhibited phytotoxic effects when applied on leaf surface (2 mM).

Activity against downy mildew disease

Only acylanilide derivatives exhibited adequate protective effect (<95%) against downy mildew of pearl millet (*Table 4*). Although the activity of oxadixyl was slightly lower than other members of this group of fungicides, there were not significant differences in their efficacy. Among the other test compounds andoprim, drazoxolon

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and salts of phosphonic acid (**17**, **18**) exhibited some disease control (>30%) while all others proved to be ineffective. The field activity of fungicides with few exceptions (**10**, **11**, **17**, **18**) was lower than the greenhouse one but this difference was not significant ($\Delta_{GH,F}$=(1-10)% < $LSD_{0.05}$=12%). Otherwise the field activity of compounds was closely related to their greenhouse effect (F_{regr}=927.6, p<0.001).

The therapeutic index of compounds (*Table 3*) varied between 0.02-29. Differences in the structure of acylanilides characteristically influenced this parameter; the presence of chloroacetyl group (**2**, **4**) decreased their therapeutic value. Changes in structure increasing the hydrophobicity of molecules (**2**, **4**, **7**, **18** and **21**) lead to increase in phytotocity and a reduction of the therapeutic value.

Seed treatments influenced radically the grain yield of pearl millet (*Table 4*). With exception of cationic surfactant type molecules **24-26** all compounds increased the yield. The acylanilide derivatives (**1-9**) were the most effective increasing yield by more than 60%. Significant correlation was revealed between antimildew efficacy of compounds and their effect on the yield with special regard to disease inhibition as evaluated either in greenhouse or in provocation field ($F_{Y,GH}$=927,7; $F_{Y,\ F}$=118.5 > $F_{0.001}$=13.8). Contrarily, reverse correlation was revealed between the yield of plants treated on the level of maximum tolerated concentrations of conpounds and their depressant on host plant effect ($r_{Y,\ [SG+SV]}$= -0.747; F_{regr}=15.12 > $F_{0.05}$=3.40).

The effect of treatments correlated significantly ($r_{lg(TI),Y}$=0.728 > $r_{0.001}$=0.59) with therapeutic value of fungicides: Y= [4.369±0.108] + [0.873±0.159]*X (F_{regr}=30.27 > $F_{0.001}$=13.61, t_a=38.83, t_m=5.502 > $t_{0.001}$=3.73); where Y= yield increase in percent (probits) related to the untreated control, X = logarithm of therapeutic index). The compound with higher therapeutic index tends to influence more beneficially onto the grain yield of downy mildewed pearl millet than those of lower index (p <0.001).

The synergetic action of combined preparations containing systemic fungicides metalaxyl+tridemorph as well as andoprim+cymoxanyl has been demonstrated against oomycetes [18, 58]. In our model experiments the joint action of these fungicides against *S. graminicola* proved to be also synergetic (*Table 6*).

The compounds for present investigations were selected on the basis of studies with other oomycetes [34, 59, 62] that made possible to compare sensitivity spectra of various genera. There were significant differences between responses: asexual spores of *S. graminicola* were more sensitive to acylanilides than the analogous cells of *P. halstedii* or *P. infestans* while the cationic surfactant type molecules (**23-26**) exhibited much less activity to *S. graminicola*. The sensitivity of the biotrophic *S. graminicola* to acylanilides was non selective contrary to *P. halstedii* [62] and *Phytophthora* species [59]. Cymoxanil exhibited good activity against SDM [62] and poorly inhibited the PMDM while drazoxolon acted oppositely. The sunflower tolerated all compounds better than pearl millet. The rank order of therapeutic values for PMDM shows few similarities with that established for sunflower downy mildew ($\rho_{PMDM,SDM}$=0.517, F_{regr}=4.36, P=5-10%). Nevertheless, some similarities occurred; the replacement of methoxylacetyl group for chloroacetyl one decreased the therapeutic value for SDM as well [62].

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Table 3. *Phytotoxic effect of various fungicides on pearl millet*

Compounds		Inhibition of								Therapeutic
		Seed germination				Seedling vigour				Index
No.	Name	EC_{50}		m		EC_{50}		m		SG/SP
1	Metalaxyl	69.7	*h*	1.697	*a,b,c*	26.3	*f*	1.391	*a*	5.23
2	CGA29212	17.5	*e*	1.768	*b*	12.8	*e*	3.346	*c,d*	0.945
3	RE26745	64.1	*g,h*	3.305	*c,d,e*	87.9	*i*	1.276	*a*	29.4
4	Ofurace	38.2	*f*	1.974	*b,c*	20.6	*e,f*	3.870	*c,d,e*	2.83
5	Furalaxyl	80.7	*h,i*	1.641	*a,b*	32.4	*g,h*	1.445	*a*	5.84
6	LAB149202F	113.4	*i*	1.286	*a*	33.3	*g,h*	1.778	*a,b,c*	1.97
7	Benalaxyl	41.5	*f,g*	1.857	*a,b,c*	28.9	*g,h*	1.636	*a,b*	2.59
8	Cyprofuram	56.4	*f,g*	1.519	*a,b*	20.3	*e,f*	2.776	*b,c,d*	1.85
9	Oxadixyl	93.9	*i*	1.334	*a*	28.8	*f,g*	1.648	*a,b*	1.63
10	Dimethomorph	65.2	*g,h*	1.394	*a,b*	27.6	*f,g*	1.574	*a*	2.46
11	Andoprim	69.7	*h*	1.697	*a,b,c*	26.9	*f,g*	5.282	*e,f*	9.79
12	Cymoxanil	14.2	*d,e*	1.994	*b,c*	7.22	*d*	2.144	*b*	0.156
13	Propamocarb	69.3	*h*	1.378	*a*	41.9	*h,i*	1.396	*a*	0.212
14	Prothiocarb	22.4	*e,f*	5.448	*e*	17.8	*e*	2.454	*b,c*	4.53
15	TMTD	2.56	*b*	5.081	*e*	1.68	*b*	3.895	*d*	0.566
16	Drazoxolon	14.2	*d,e*	1.994	*b,c*	13.9	*d,e*	3.270	*c,d*	1.71
17	Efosit	29.1	*e,f*	2.133	*b,c*	19.2	*e,f*	6.876	*f*	2.79
18	BKF-3	44.8	*f,g*	1.585	*a,b*	21.5	*e,f*	5.614	*e,f*	1.03
19	Ziram	12.2	*d*	2.227	*c*	9.24	*d,e*	2.916	*c*	0.923
20	$Zn(PDC)_2$	4.01	*c*	3.988	*e*	4.99	*c*	4.916	*e*	0.663
21	Thiomersal	2.53	*b*	4.947	*e*	1.83	*b*	3.626	*d*	0.260
22	Dodemorph-*cis*	30.2	*e,f*	2.395	*c,d*	11.1	*e*	1.807	*b,c*	2.04
23	Dodemorph-*trans*	26.1	*f*	4.701	*e*	18.9	*e,f*	1.605	*a,b*	4.52
24	Tridemorph-*cis*	1.09	*a*	2.993	*d*	0.874	*a*	2.962	*c*	0.0273
25	Tridemorph-*trans*	1.08	*a*	4.971	*e*	1.09	*a,b*	3.829	*d,e*	0.120
26	Fenpropimorph	1.31	*a*	4.527	*e*	0.574	*a*	3.102	*c,d*	0.0603
27	Dodine	15.2	*d,e*	1.750	*b*	14.7	*d,e*	2.310	*b,c*	0.675

Sensitivity to fungicides (inhibition) is expressed as ED_{50} value in millimol, m = slope of dose response line at log/probit scale. Values labelled by the same letter are not significantly different at P=5% level. Therapeutic index = the ratio (EC_{01} for seed germination)/(EC_{99} for intensity of sporulation).

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Table 4. *Antimildew activity of compounds and their effect on the grain yield of pearl millet*

No.	Compound	Conc.[a] (mM)	Activity (%)[b] in the Glasshouse	Field	Yield[c] kg/ha	Increase[d] kg/ha
1	Metalaxyl	10	98.0	97.9	1864±88	728*f*
2	CGA29212	5	98.4	98.7	1864±96	728*f*
3	RE26745	10	99.3	98.8	1875±94	739*f*
4	Ofurace	10	97.3	97.5	1856±96	720*f*
5	Furalaxyl	10	98.6	98.7	1906±94	770*f*
6	LAB149202F	10	97.0	98.0	1838±90	702e*f*
7	Benalaxyl	10	97.9	97.9	1864±88	728*f*
8	Cyprofuram	10	99.1	98.7	1872±96	736*f*
9	Oxadixyl	10	95.8	94.4	1842±102	706*f*
10	Dimethomorph	10	12.8	26.0	1427±64	291*c*
11	Andoprim	10	35.9	46.7	1474±69	338*d*
12	Cymoxanyl	10	14.7	3.9	1272±64	136*bc*
13	Propamocarb	10	12.2	9.5	1346±70	210*bc*
14	Prothiocarb	10	20.1	13.4	1400±64	264*c*
15	TMTD	1	27.3	26.4	1456±72	320*c*
16	Drazoxazolon	10	32.9	28.5	1600±80	464*d*
17	Efosit	10	32.4	40.0	1496±72	360*cd*
18	BKF-3	10	44.4	50.4	1384±64	248*bc*
19	Ziram	1	13.1	14.7	1288±64	152*bc*
20	Zn-PDC	1	16.5	22.4	1288±67	152*bc*
21	Thiomersal	1	13.7	18.1	1256±58	120*ab*
22	Dodemorph-cis	10	13.0	14.8	1360±56	224*c*
23	Dodemorph-trans	10	8.3	8.8	1288±64	152b*c*
24	Tridemorph-cis	0.2	3.1	4.3	1139±53	3*a*
25	Tridemorph-trans	0.5	6.6	12.4	1192±64	56*a*
26	Fenpropimorph	0.5	9.6	3.5	1208±64	72*ab*
27	Dodine	10	9.0	8.7	1256±64	120*ab*

[a]= Maximum tolerated doses by pearl millet. Preventive seed treatments were made in each case (see Materials and Methods).
[b]= For efficacy of compounds in different locations $LSD_{5\%}$= 11.9 (F_{exp}= 43.9 < $F_{5\%}$=1.26).
[c]= Yield of the untreated control was 1136±56 kh/ha. [d]= as compared to untreated control plots. The values marked by the same letter are not significantly different at P=5% level.

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Table 5. *Correlation in sensitivity to fungicides between different developmental of Sclerospora graminicola and pearl millet basen on their responses to fungicides*

	Matrix A (FG=27) Inhibition of					
Matrix B (FG=27)	**Zoospore Release**	**Zoospore Motility**	**Sporulation**	**Zoospore production**	**Seed germination**	**Seedling vigour**
Inhibition of	**1**	**2**	**3**	**4**	**5**	**6**
1. Zoospore release	1.0	0.872	0.793	0.630	-0.600	-0.579
2. Zoospore motility	0.7296	1.0	0.872	0.653	-0.597	-0.607
3. Sporulation	0.5670	0.6734	1.0	0.854	-0.644	-0.649
4. Zoospore production	0.2167	0.1725	0.0513	1.0	-0.571	-0.523
5. Seed germination	0.5520	0.3069	0.6257	0.2027	1.0	0.933
6. Seedling vigour	0.1547	0.1517	0.1987	0.0962	0.1496	1.0

Matrix A = Pearson's correlation coefficients calculated on the base of log EC_{50} values.
Matrix B = Pearson's correlation coefficients calculated on the base of slope values of dose respose lines. ($r_{0.001}$=0.597)
The underlined coefficient indicates significant relationship between the activities of compounds evaluated by the given parameter ($100 \times R^2 > 50$).

Table 6*. Joint action of systemic fungicides against Sclerospora graminicola*

	Treatments	**Efficacy (mgl^{-1})**	
No.	**Compounds**	**EC_{99}**	**Co.T.I.[a]**
1	Metalaxyl (M)	72.9	
2	Tridemorph (T)	640.7	
	1M+4T[b]	62.3	3.36
3	Andoprim (A)	117.8	
4	Cymoxanil (C)	1788.9	
	3A+7C[b]	105.4	3.43

[a]=Co.T.I. = Comparative toxicity index according to Sun [52]. The inhibition of sporulation was determined on detached leaf segments (see Materials and methods).
[b]= Weight parts.

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***Table 7**. Principal components of the Spearman's correlation matrix between responses of downy mildew fungus and pearl millet to fungicides*

No.	Evaluated events[a] (variable)	Principal component[b] 1	2
1	Zoosporangium germination (ZG)	0.822	0.314
2	Zoospore motion (ZM)	0.892	0.299
3	Intensity of sporulation (SP)	0.859	0.382
4	Production of zoospores (PZ)	0.695	0.354
5	Seed germination (SG)	-0.368	-0.911
6	Seedling vigour (SV)	-0.351	-0.916
7	Disease inhibition in vitro (GH)	0.856	0.301
8	Disease inhibition in vivo (F)	0.892	0.335
9	Yield increase (Y)	0.859	0.414
	Eigenvalue	5.215	2.504
	Percentage of variation	57.9	27.8
	Cumulative percentage	57.9	85.7

[a]= Events correspond to those given in Tables 2, 3 and 4.
[b]= The Varimax rotated principal components are shown in order of the amount of variation they represent; eigenvalues having percentage variation less than 5 are omitted.

***Table 8**. Variations in the efficacy of seed dressings against pearl millet downy mildew in various regions of India*

Location[a]		Geographic coordinates		Disease Incidence (%)[b]		Disease Inhibition
No.	Name	N	E	Untreated Control	Apron 35SD 3 kg/t	Rate (%)[c]
1	Jaipur	26°55'0"	75°49'0"	24.3	4.3	82
2	Gwalior	25°55'60"	78°28'0"	81.8	62.4	24
3	Jamnogar	22°28'0"	70°4'0"	85.5	82.2	4
4	Anand	22°13'0"	71°10'0"	82.2	91.2	1
5	Aurangabad	19°52'60"	75°19'60"	85.3	9.8	89
6	Patancheru	17°31'60"	78°16'0"	9.0	5.0	44
7	Mysore	12°18'0"	76°39'0"	27.0	7.7	71
8	Coimbatore	11°0'0"	76°58'0"	82.0	14.0	83

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In accordance to official reports [5] and multitudinous observations cited the standard metalaxyl treatment (2 kg/t) showed in earlier times excellent protective effect in all plots inhibiting the PMDM at rate >95%.

[a]The trials were conducted in ICAR-AICPMIP research stations [5].

[b]Disease incidence was recorded at tillering, $LSD_{0.05}$= 10.2 ($F=231.7$, $p<0.001$).

[c]The disease inhibition rate was calculated following formula DIR=100*(C-T)/C, where C and T are disease incidences in control and treated variants, respectively.

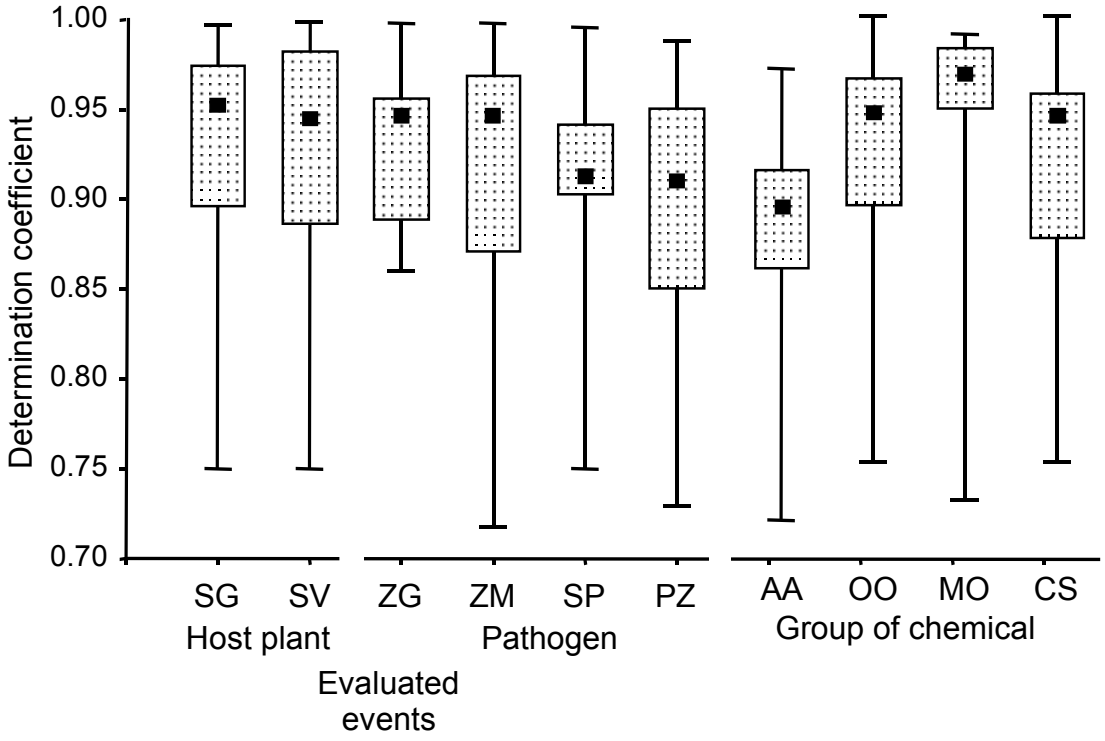

Figure 1. *Evaluation of the goodness of curve fitting of dose response lines for characterising activity of test compounds screened by different methods.*
The graph was built up based on median (central point), second and third quartiles (box) and extremum values (whiskers) of determination coefficients of log/probit function used for calculation of dose response lines (Tables 2, 3 and 4). Evaluated events are SG = seed germination, SV = seedling vigour, ZG = zoosporangium germination, ZM = zoospore motility, SP = intensity of sporulation, PZ = zoospore production. Responses of pathogen to the chemicals tested are grouped as follows; AA = acylanilides, OO = other antioomycete compounds, MO = metallo-organics, CS = cationic type surfactants.

Multivariate analysis

The responses of parasite and host plant to fungicides evaluated by different manners were compared and subjected as variates to PCA (*Table 7*). The first PC accounted for 58 % of the total variation comprising all events where the pathogen was presented with positive weights and plant responses with negative ones. The second PC accounted for less of the variation (28 %) and related mainly to host plant which was less sensitive to test compounds than the parasite. The two organisms have been presented in both cases

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with opposite signs that relates to the reverse relationship between groups of variates; observations for pathogens and for host plant.

The multivariate regression analysis revealed positive correlation of different power between disease inhibitory effect on the field (F) and the capacity of compounds to inhibit host independent and host dependent developmental form of *S. graminicola* as well as their phytotoxicity; $R_{F,[ZG+ZM]}=0.884 > R_{F,[SP+PZ]}=0.815 > r_{0.001}=0.652 > R_{F,[SG+SV]}=0.614 > r_{0.05}=0.427$. All these are in accordance to the result of bivariate regression and PC analyses (see *Tables 5 and 7*). When omitting from calculation observations for disease inhibitory actions determined in greenhouse (G) and field (F) as well as yield increase (Y) the resulted PC-s are derived only from *in vitro* activity data. The first PC in this case accounted 74.5 % of variation of the correlation matrix of EC_{50} values (reduced *Table 5*). Plotting effects of treatments on the yield of pearl millet versus variables of this first PC (*Fig 2*) revealed significant linear correlation ($p < 0.001$).

The comparative analysis of multivariate determination coefficients showed that the number of parameters in screening might be decreased, and involvement of two, correctly selected variables was satisfactory for prediction of the effect on the yield of pearl millet ($\rho_{Y,[SP+SG]}=0.84$, $p<0.01$). All the above indicates the possibility of limiting the number of parameters for selection of compounds in primary screening against PMDM.

Discussion

The tested fungicides exerted different impacts on *S. graminicola* at different stages in its life cycle, where the zoosporangiogenesis was more sensitive than other events. Inhibition of asexual spores of *S. graminicola* is important, because these provide the greatest opportunity for rapid built up in the number of infective propagules and subsequently, the high potential for infection of new plants. Therefore, any chemical that significantly suppresses the zoosporangium formation reduces the ability of disease expansion as well. Indeed, there was a significant correlation between the inhibition of this event and the effect of a single seed treatment on the grain yield ($r_{SP,Y}=0.83 > r_{P=0.1\%}=0.54$). The sensitive response of asexual spores to acylanilides is particularly interesting property of *S. graminicola*. These chemicals are thought to inhibit a single enzyme system, the α-amanitine RNA polymerase [15]. The inhibition of this receptor site leading to stop of protein synthesis and consequently to discoupling of the life cycle dominates over other metabolic effects. There was demonstrated that transcription in *Peronospora tabacina* started before the differentiation of sporangia [25] and that mRNA continued to be synthesised during HD stages. Similarly, this pause occurred also with *Bremia lactucae* sporangia [13]. Although the zoosporangium formation of *S. graminicola* was found to be more sensitive to the tested set of chemicals than other ontogenetic events, the developmental stage dependent variations in its response were less pronounced than in the case of *Plasmopara* or *Phytophthora*. Contrary to the latter species the ontogenetic forms of *S. graminicola* had many commons in their sensitivity to fungicides. The high activity of acylanilides against PMDM is likely to involve active transcription and translation (protein synthesis) in parasiting thallus, while the sensitivity of asexual spores indicates that both ratio and intensity of the above steps in protein synthesis would occur at the previous level during host independent stages. However, the selective inhibition of mitochondrial respiration by phenylamide

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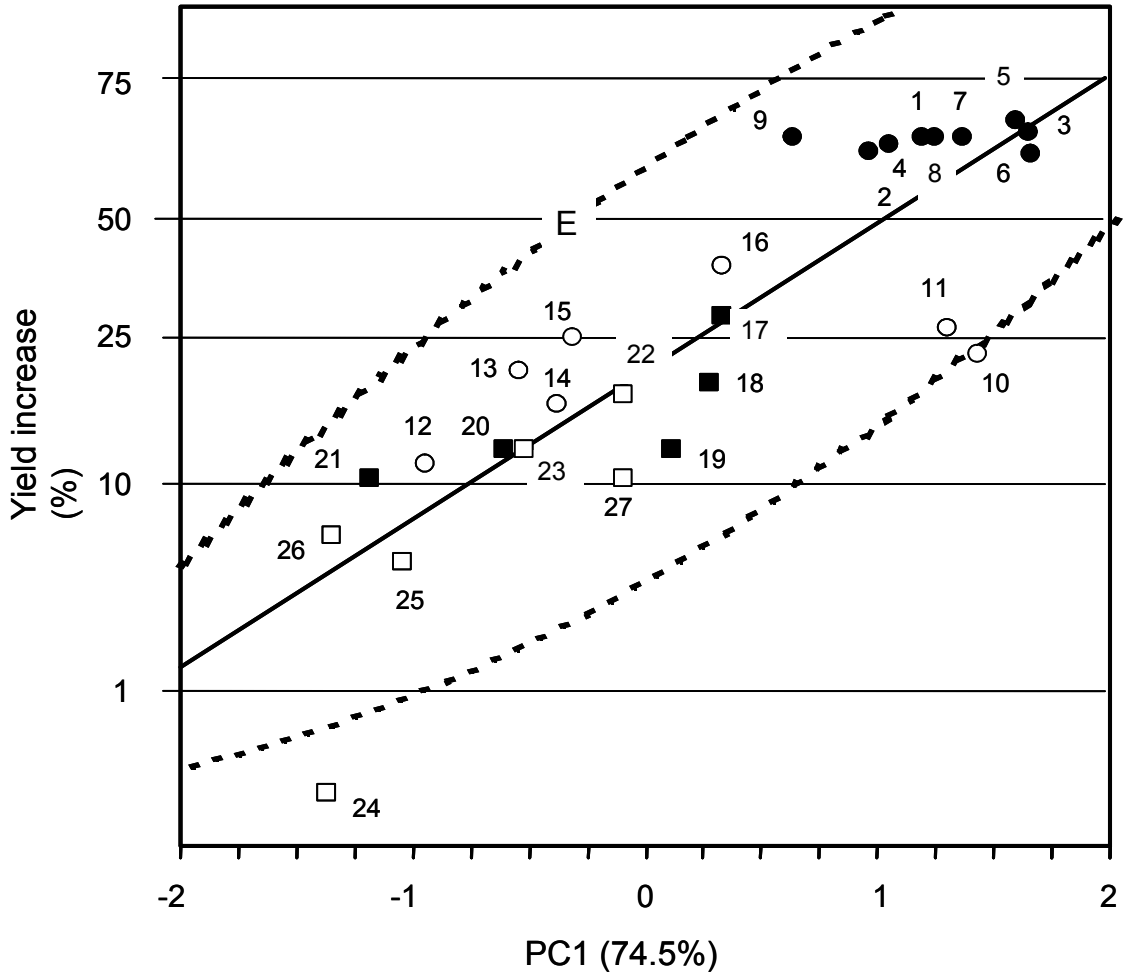

Figure 2. *Relationship between the effect of fungicides on the yield of pearl millet and their antimildew activity measured in vitro.*
Arabic numerals represent compounds and correspond to those given in Table 1. Chemical groups of acylanilides and other antioomycete fungicides are marked with filled and opened circles while metallo-organics and cationic tensides with filled and opened squares, respectively. The trend line was drawn according to the equation y=b+mx as follows: Yield increase (%) = [4.4289±0.0806]+[0.7039±0.0821]×PC1; (F_{regr}=73.51 > $F_{0.1}$= 13.74, R^2_{adj}= 0.7361, t_a=54.98, t_b=8.57)
where Y= Yield increase in percent (probits) related to the untreated control (Derived from data of Table 4), X = Principal component (PC1) of Spearman's correlation matrix calculated from in vitro response data (Tables 2-3). E = 99.5 % normal bivariate confidence ellipse.

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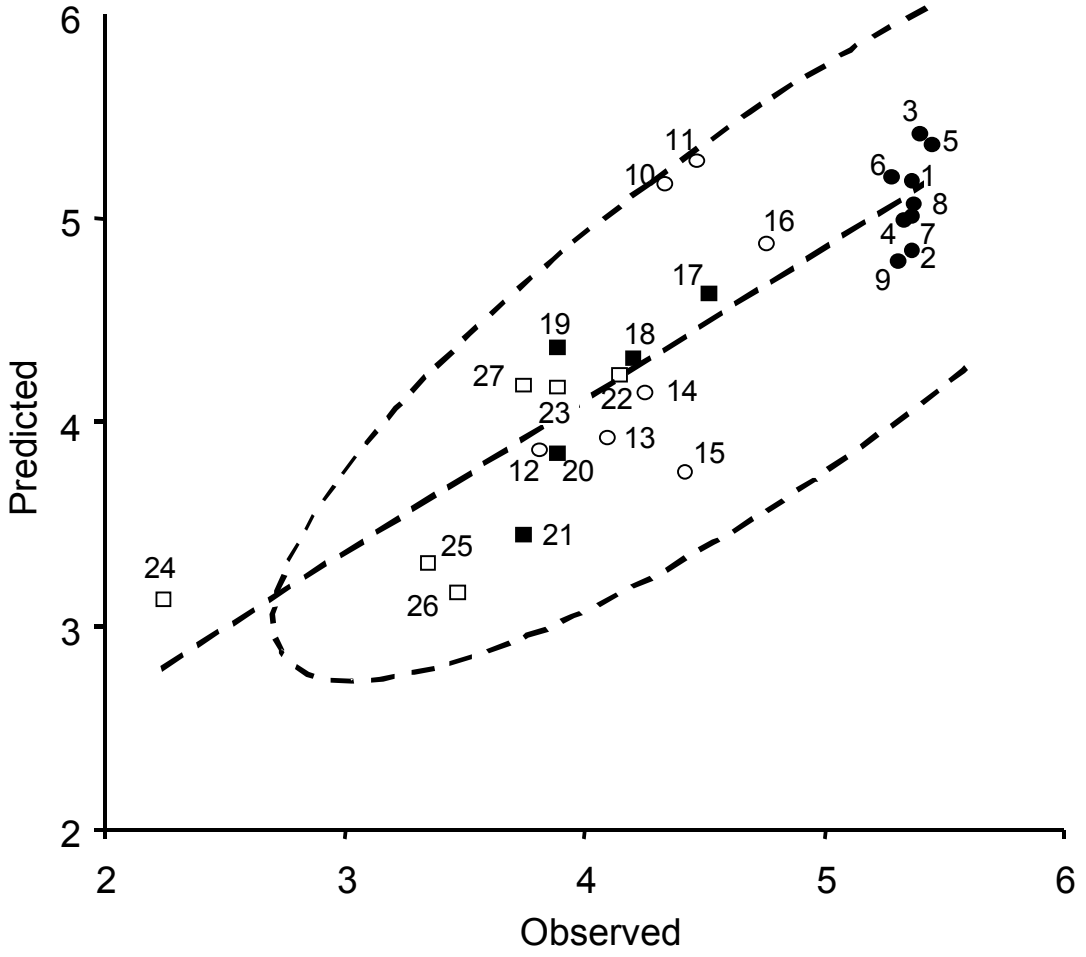

***Figure 3**. Relationship between observed and predicted effects of fungicides on the yield of downy mildewed pearl millet.*
Arabic numerals represent compounds and correspond to those given in Table 1. Chemical groups of acylanilides and other antioomycete fungicides are marked with filled and opened circles while metallo-organics and cationic tensides with filled and opened squares, respectively. E = 99.5 % normal bivariate confidence ellipse. The trend line was drawn according to the equation y=b+mx as follows:
$Y= [1.0961\pm0.3384] + [0.7525\pm0.0863]*X$; ($F_{regr}=76.02 > F_{0.001}=13.61$, $R^2_{adjusted}=0.7426$, $t_b=2.82$, $t_m=8.72 > t_{0.01}=2.80$) where X= Yield increase in percent (probits) related to the untreated control (derived from data of Table 4), Y = the predicted yield increase calculated from activities against SP and SG stages.

fungicides [41] might also be the factor of sensitivity of asexual spores of *S. graminicola*. The extent of lipophilic properties of different phenylamide fungicides varies; this is probably the reason why the most lipophilic member of this group (benalaxyl) is active also against zoospores of *Phytophthora* and *Plasmopara* [11, 24, 62]. Although the importance of zoospores in sustaining of disease cycle of PMDM is reduced because of dual character of zoosporangia, the high tolerance of *S. graminicola* spores to cationic type surfactants is very interesting, with special regard of its cell wall

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less zoospores, that might be related to alterations in membrane structure as compared to other peronosporas.

None of the compounds tested was tolerated completely by HB3 hybrid, however the depression observed on germinating seeds was soon overcome; the negative effect on the grain yield was seemingly unimportant. Nevertheless, an antimildew compound to be used in the control of pearl millet should provide better therapeutic properties than majority of acylanilides (*Table 3*). The influence of the host plant on the performance of biological activity of any compound can be determinative in the case of biotrophic endoparasites. By this reason it is very important to study the effects on the host plant. The risk of application of compounds shown to prove satisfactory control of other Peronosporas can be high when applied against PMDM because of different therapeutic indices. The ratio of non-effective dose on host and lethal dose on parasite is an important measure (T.I.). If it is high, than the pesticide is relatively safe. This means that there is a big difference between these parameters of the compound affecting two partners at the same time. The anilopyrimidine derivative (andoprim) and the dimethomorph being highly active in other relations [21, 28, 45, 56] exerted, in our experiments, good activity against *S. graminicola in vitro*. Nevertheless, they can't be recommended for controlling PMDM because of their low T.I. Similar studies on metalaxyl have been reported wherein, the higher concentrations, which offered higher protection but had effect on seed quality parameters [48]. The other compounds exhibited good sporocidal and antisporulant activity against taxonomically related pathogens [11, 20, 22, 30, 63], however, for controlling *S. graminicola* these compounds were not efficacious at the requested level.

Among systemically active antimildew compounds only the acylanilides were efficient at economically acceptable levels (*Table 4*). Regrettably, apart of their high efficacy these compounds (**1-9**) possess some unfavourable properties when using them against PMDM. Metalaxyl has low therapeutic value because of the sensitivity of pearl millet [53]. Although modifications in molecule of metalaxyl advantageously affected the therapeutic value (*Table 3*), the cross-tolerance has been complete for these analogues [19, 59]. Consequently new compounds with different mode of action should be developed for resolution of this problem. In populations of phylogenetically related to *S. graminicola* pathogens this type of tolerance was reported with probability between 10^{-6}–10^{-7} in model experiments [59, 62]. Although in the case of PMDM has not been reported yet, one can expect the appearance of strains of *S. graminicola* acquired tolerance to acylanilides in near future. Significant shift of PMDM incidence was in the recent vegetation recorded [5] in some plantations treated with metalaxyl (*Table 8*). There is, therefore, a need to find new systemic fungicides with activity against *S. graminicola*. The high variation in genetic background of *S. graminicola* [8, 27, 47, 55] also impress on searching new possibilities.

The screening activity against an obligate endoparasite is complicated. The comprehensive analysis of the effect against *S. graminicola* involve, in our opinion, evaluation effects both on host and parasite (zoosporangiogenesis, zoosporogenesis), disease syndrome and grain yield. Measurements on intact plants are time and work consuming. Moreover, the costs are also higher than studying responses of spores and germinating seeds or assessments of detached leaf segments. Similarities in the response of various developmental forms of *S. graminicola* to diverse chemicals as well as the close relationship between predicted activities and experimental values indicate the possibility of reduction of parameters for characterising the effect of compounds for use

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against PMDM. The results of calculations showed that characterisation of biological activity by two parameters (SP and SG) gave possibility ($p < 0.01$) to discriminate between compounds with acceptable or negligible antimildew effect (*Fig 3*).

The yield loss due to PMDM disease is attributed to loss of diseased plants during early developmental stages, poor tillering, and ear-head malformation [63]. Singh (1983) has made observation on grain yield increase upon seed treatment with metalaxyl [28]. It was shown that greatest yield increase was occurred in PMDM susceptible varieties. The present study also demonstrated the dominant effect of disease inhibition by fungicides, as it was indicated by a positive relation between efficacy of downy mildew control and yield enhancement (*Table 4*). The compounds with efficacy over 30% against PMDM in vitro may be useful for protecting pearl millet as seed applicants, while the compounds with disease inhibitory activity less than 25 % at maximum tolerated dose by pearl millet are considered useless as protectants. Therefore, the compounds with ED_{50} values more than 50 μM (about 10-20 mgL^{-1}) in test for sporulation are ineffective to prevent economic loss caused by *S. graminicola*.

Basing on our results the use of some acylanilides (furalaxyl, RE26745) is proposed for controlling PMDM. The chemicals with different mode of action that offered protection to considerable extent were andoprim (45%), efosit (39%), BKF-3 (49%) and drazoxolon (27%). The possible exploitation of them in the form of combinations with other fungicides would be promising for preventing the risk of resistance development in pathogen populations to fungicides. However, there are no such preparations available in the control of pearl millet downy mildew pathogen, by our results (*Table 6*) the effect of synergetic mixtures was reassuring. The value of these combinations for PMDM control should be rectified.

Conclusions

The present control technologies of downy mildews disrupt infection cycles either by killing asexual spores or by inhibition of growth of the parasite within its host. The infection of germlings and basal tillers of pearl millet is detrimental, while infection of secondary tillers does not contribute greatly to yield [63]. In our experiments both incidence and severity of PMDM were dramatically decreased by a single seed treatment, protecting germlings from seed and early soil borne infections. The phenological phase related susceptibility of pearl millet [50] was revealed with maximum in germling stage, which finding supports the importance of seed treatment as well. Consequently, the primary screening in the case of PMDM should concentrate on prevention of soil- and seed-borne infections as well as early air-borne infections of seedlings, because the control of diseases of primary tillers is fundamental in the production of pearl millet. On our opinion the effects on zoosporangiogenesis, zoosporogenesis, disease syndrome, seed germination and grain yield should be studied for screening compounds to be included into PMDM management. However, the expected yield performance can be roughly estimated ($P<1\%$) evaluating effects on seed germination and sporulation on detached leaf segments that can short-circuit the process for decision from 70 days to 4 days in pesticide development.

Seed treatment is the only feasible method to this disease [63]. This technology easiest to transfer to the farmers to promote sustained millet production [33], because it requests minimum participation and collaboration of farmers as well as the performance of the effect and impact of results do not depend essentially on participants in

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production. Moreover, the contamination with residual amounts of fungicide is also negligible [39] and the exposition of beneficial soil microflora associated to pearl millet [29] remains at low level. In arid climate and poor nutritional conditions the beneficial microflora associated to plants is extremely important [23, 38] so the use of compounds with broad spectrum of activity against soil borne pathogens should be excluded. Although the downy mildew tolerance of pearl millet can be enhanced by diverse methods [17, 66] the use of selective antimildew compounds with systemic activity can not be evaded.

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