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Iprovalicarb has Potential for the Control of Downy Mildew of Pearl Millet

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The effect of Melody Duo 66.75 wp was assayed on pearl millet downy mildew (PMDM). This novel fungicide, a combined preparation of iprovalicarb and propineb, controlled all developmental stages of *Sclerospora graminicola.* The protective effect of this preparation was due to iprovalicarb and was equipotent to metalaxyl-containing Apron 35 sd and Ridomil 72 MZ wp, whereas the eradicant activity of Melody Duo exceeded that of the two latters at comparative doses. Iprovalicarb was translocated acropetally and exhibited systemically an excellent curative activity. The downy mildewed pearl millet plants recovered after foliar treatments with the preparations. The exploitation of Melody Duo 66.75 wp is recommended against pearl millet downy mildew disease.

Keywords: Pearl millet, downy mildew, Melody Duo, iprovalicarb, metalaxyl, disease management, *Sclerospora graminicola.*

Downy mildew caused by *Sclerospora graminicola* (Sacc.) Schroet., is a common disease of pearl millet [*Pennisetum glaucum* (L.) R. Br.] throughout the semiarid-tropical world. The disease was known in most of the pearl millet growing areas but it remained sporadic until the introduction of high yielding hybrids with susceptible parent line (Singh, 1995). It has been assumed that this pathogen, originally described from Europe (Schroeter, 1879), is of the same origin (Weston, 1928). Asexual spores spread the disease between plants, whilst within plants the pathogen spreads intercellularly. Sexual oospores can travel large distances and can survive several seasons (Ingram, 1981; Shetty et al., 1995). Serious grain yield losses can occur when epidemics coincide with emergence of seedlings and formation of primary tillers (Nene and Singh, 1976; Wilson, 2000). Resistance to downy mildew in some widely cultivated pearl millet cultivars was found (Talukdar et al., 1998; Thakur et al., 2001). Host-plant resistance to *S. graminicola* has been vulnerable to erosion as this pathogen shows high natural variation in aggressivity (Safeeulla, 1976a; Anon., 1977; Thakur et al., 1998; Ball and Pike, 1984). Results of biocontrol measures (Umesha et al., 1998) and enhancement of plant resistance with chemical treatment (Shailasree et al., 2001) as well as eco-friendly approaches for protection (Deepak et al., 2003; Niranjanraj et al., 2003) were reassuring, but the efficacy of these control measures failed away of the requested level, because even 10% of disease incidence can cause economic losses. Fungicides have, therefore, come to occupy a central

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role in controlling the disease. The metalaxyl has been in use since the 1990s and is still the most commonly used substance against pearl millet downy mildew (Singh and Shetty, 1990). However, constraints regarding the high cost and the residual problems and, above all, the development of resistance to acylanilides (e.g. metalaxyl) have necessitated the look for new ways of downy mildew management.

Iprovalicarb is a new fungicide that affects the growth of germ tubes of encysted zoospores and of sporangia, but also inhibits mycelial growth and sporulation of the pseudofungal oomycetes (Dutzman, 1999). Various species of oomycetes respond diversely to iprovalicarb. The sensitivity decreases with genera in the following order *Plasmopara* > *Phytophthora* > *Pseudoperonospora* > *Peronospora* > *Bremia* and the requested amount for control varies between 120 and 250 g ha^{-1} when applied as a leaf spray. In addition to direct effect on downy mildew, its systemic activity was also reported (Stubler et al., 1999; Jende et al., 1999).

The objective of our studies was the assessment of activity of iprovalicarb against *S. graminicola* and the evaluation of possibilities to imply this compound into the disease management programs.

Materials and Methods

Test compounds

Melody Duo 66.75 wp (iprovalicarb 3.5% + propineb 63.25%) was supplied by the producer (Bayer India Ltd.). Commercial preparations of Apron 35 sd (metalaxyl 35%; Novartis, Swiss) and Ridomil MZ 72 wp (metalaxyl 64% + mancozeb 8%; Novartis, Swiss) were used as reference compounds. The structures of active ingredients are shown in *Fig. 1.*

Host-parasite system

Pearl millet (HB3) highly sensitive to downy mildew and *S. graminicola* (pathotype 1) maintained on pearl millet in greenhouse were used for all the experiments. Leaf-whorls of 2-day-old seedlings grown in soil-sand culture were challenge inoculated with a suspension of zoospores (4×10^4 cell ml^{-1}) according to Singh and Gopinath (1985).

Preparation of inoculum: Leaves of downy mildewed pearl millet plants were collected in the evening hours. Previously formed zoosporangia were eliminated from the surface by washing in tap water. The excess of water was removed, then leaves were incubated in a moist chamber at 25±2 °C overnight (12–14 hours). Zoosporangia were collected by washing them off with sterile distilled water, and the resulting suspension was incubated for 15 min to release zoospores at 22±2 °C. The experimental concentration of zoospores was adjusted by adding sterile distilled water to 4×10^4 cell ml^{-1} in a haemocytometer (Safeeulla, 1976b).

Responses of the pathogen and reactions of the host plant to test compounds were studied both *in vitro* and *in vivo*. The activities estimated according to proper scale were transferred into percents of inhibiton at the given dose. The dose response lines were fitted

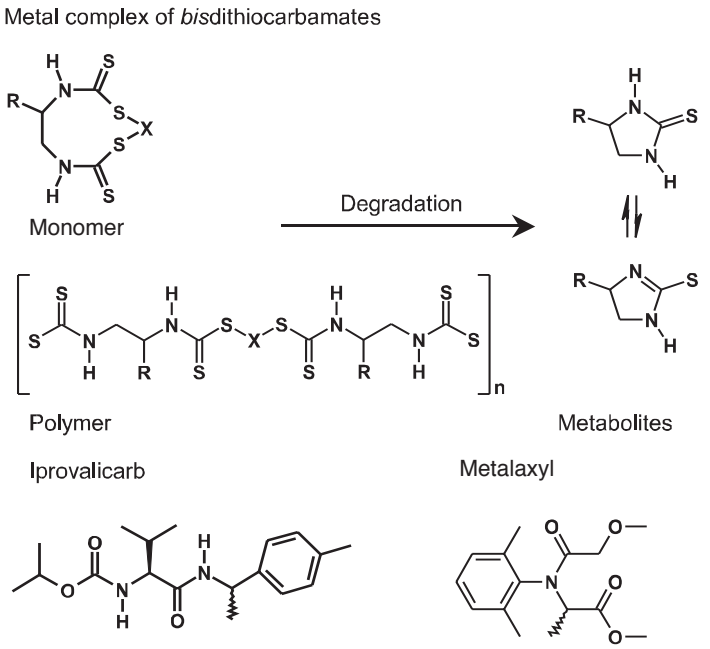

Fig. 1. Chemical structure of active substances. Metal complexes of *bis*dithiocarbamates mancozeb (R= -H, X= Mn+Zn=20:1), propineb (R= -CH_3, X= Zn) where n=20–50 in polymeric forms. The degradation products ETU (R= -H) and PTU (R= -CH_3) of mancozeb and propined respectively can be formed both *in vitro* and *in vivo*

using log/probit function and the biological activities have been expressed as EC_{50} values. The protective effect of fungicides (percents of disease inhibition) was measured both in greenhouse and field conditions.

Responses of the host plant (phytotoxicity tests)

Seeds of pearl millet were soaked in the suspensions of the fungicides at appropriate concentrations (1 g per 10 ml for 6 h). Seeds treated with sterile distilled water by the same manner served as control. After treatment, the seeds were used for further experiments.

Effects on seed germination *in vitro* were determined according to international rules for seed testing (Anon., 1993). Seeds treated with chemicals as described above were put on wet filter paper (25 seeds by Petri dish of 9 cm diameter) and incubated at 25±2 °C. The number of germinated seeds was counted after 4 days and the ratio was expressed as a percentage of control.

The length of roots and shoots was measured to nearest 1 mm and the inhibition of growth rate was calculated as a percentage of control. Four replicates of 100 seeds were used for all the treatments.

Responses of the pathogen (fungitoxicity tests)

Contact effect: 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 immerged in solution of test compound of appropriate concentration for 30 min. The surface wetness was removed and treated pieces were incubated in moist chambers (plastic trays lined with wet filter paper) at 25±2 °C in the dark for 12 h. To determine the intensity of sporulation, a 0–4 scale was used where the proportion of leaf area covered with zoosporangia was graded as follows: 0, no sporulation; 1–4, sporulation appearing on < 25, 25–75, 75–<100 and 100% of the total leaf area, respectively.

Effect mediated by leaf tissues: Water agar (0.9% of Bacto Agar No. 1) amended with test compound was layered into clear plastic boxes. Leaves with disease symptoms were prepared as above, then segments (about 20 × 14 mm) infected at the upper half with *S. graminicola* were cut and stuck into water agar avoiding direct contact between the chlorotic part and the medium. Boxes covered were than incubated at 25±2 °C in the dark for 12 h. The intensity of sporulation was assessed as above.

Disease inhibition studies

The anti-mildew activity of test compounds was evaluated both in greenhouse and field experiments. In greenhouse the pearl millet seedlings were inoculated with *S. graminicola*. Plants with a lack of visible symptoms of disease syndrome such as sporulation, yellowing, stunted growth and malformed ear-heads, were considered healthy. The ratio of symptomless plants to those with symptoms was calculated in each case and the efficacy of treatment was calculated as a percent of decrease in disease incidence as compared to control. Field trials were conducted during rainy seasons (Monsoon) of the year 2002.

Testing protective activity: Seeds were soaked in suspension of test compounds of appropriate concentration (1 g in 2 ml for 6 h) than were sown to 4 kg clay pots containing 2:1:1 ratio of soil, sand and manure. The germination was maintained in greenhouse and 2-day-old seedlings were infected by leaf-whorl method as above. The disease incidence was assessed after 10 days.

Testing curative activity: Greenhouses-grown 21-day-old plants infected in germling stage as above were sprayed run off with 1% suspensions of the chemicals. The evolution of disease syndrome such as sporulation, chlorosis and stunting was conducted 9 days later.

The recovery of plants from downy mildew was characterized by changes in pigment constitution of leaves. Twenty-one-day-old infected and healthy plants were sprayed run-off with an aqueous suspension of Melody Duo and Ridomil MZ. Leaves were collected before and after treatments and the downy mildewed parts dissected for analysis in the laboratory. Pigment content was determined in acetonous extracts spectrophotometrically (Hitachi U-2000, Japan) as described earlier (Virányi and Oros, 1989). The following formulas were used for calculations:

Chlorophyll *a* = $9.784*OD_{662} - 0.99*OD_{644}$ mg L^{-1}

Chlorophyll *b* = $21.426*OD_{644} - 4.650*OD_{662}$ mg L^{-1}

Carotenoids = $4.695*OD_{440} - 1.348*OD_{662} - 5.416*OD_{644}$ mg L^{-1}.

Testing the effect of combined applications: Treated and untreated seeds were sowed and the germlings were infected with *S. graminicola* in greenhouse as above. Twenty-one-day-old plants were sprayed run off with 1% suspension of Melody Duo and the evolution of disease syndrome was assessed nine days later.

Persistence of the effect of iprovalicarb: In order to check the persistence of iprovalicarb activity during germination process, pearl millet seeds were treated with maximum tolerated dose of Melody Duo (60 kg t^{-1}) then were sowed and maintained in greenhouse as above. The seedlings were inoculated with zoospores of *S. graminicola* following different time intervals of 1, 2, 3, 4 and 5 days after outcropping. Disease incidence was recorded the 7th day onwards.

Translocation studies: A qualitative estimation of iprovalicarb translocation represented in the form of sporulation inhibition was conducted in 20-day-old downy mildewed plants. Dilutions of iprovalicarb viz., 10, 1, 0.1, 0.01 and 0.001% were applied with a brush to leaf below or above the nodes of the leaf to be examined for acropetal and basipetal translocations, respectively. The intensity of sporulation was evaluated one and two days later using the scale as above.

Field trials

Pearl millet seeds were treated with Melody Duo at doses of 24 and 60 kg t^{-1}, and seeds treated with Apron 35 sd (6 kg t^{-1}) were used as a reference. Seeds were sown in downy mildewed plot during Monsoon 2003 maintained by the Department of Applied Botany and Biotechnology (University of Mysore, Karnataka, India) infested with oospores for over two decades. Foliar spray was carried out 7 days after sowing, and recommended normal agronomic practices were followed during the trial. The plot size was 10 m × 5 m where plant-to-plant and row-to-row distances of 15 and 50 cm, respectively, were maintained. The experiment was designed as a Random Block Design with four replicates.

The evolution of downy mildew syndrome was assessed the 10th day onwards. The disease incidence was first rated when typical symptoms (sporulation or green ear, stunting and chlorosis) appeared on control plants.

Yield performance

Ear heads from the central 3.8 m of two center rows after maturity were collected and their grain yield was weighed (Williams and Singh, 1981).

Data analysis

All tests were carried out at least in triplicates. Reliability of assessments was evaluated by analyzing variation coefficients (C. V.% = 100 × [stdev/mean]) using statistical functions of Excel 97 (Microsoft, Redmondton, USA). Averages were compared by Student's t-test and the confidence range was calculated for toxicity limits at P=5% level.

The toxicity limits were calculated from basic data expressed in percentages, using a curve-fitting method based on log/probit function. Therapeutic value of tested chemicals was calculated by the following formula:

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

where MTC_{host} = maximum tolerated concentration by germinating seeds of pearl millet, $MIC_{parasite}$ = minimum inhibitory concentration determined on sporulating of *S. graminicola.*

Bivariate regression analysis was applied for analyzing relationship between the disease inhibitory rate and yield performance of treatments, Excel 97 statistical functions (Microsoft, Redmondton, USA) and Statistica 5 program (StatSoft, Tusla, USA) were used for calculations and graphic presentation of data. Economic impact of fungicidal treatments was evaluated according to rules of cost-return budget developed by Thomas Jefferson Agricultural Institute (Anon., 2002).

Results

Reliability of assessments

The infections both in greenhouse and field conditions proved to be successful and reproducible. Downy mildew syndrome developed uniformly and 95–100% of plants were diseased in parallel series. The variation between replicates exceeded 10% only in 6 of 110 cases of disease inhibitory studies and the coefficient was less than 5% in most of cases; this verifies the reliability of the measurements (F_{exp}= 0.2–1.9 < $F_{0.1}$). All dose/response lines could be fit at P<5% level, the determination coefficients of regression were over 0.707 (r^2=0.50) verifying the steadiness of toxicological parameters; EC_{50} and slope (specific activity).

Responses of host plant

Melody Duo did not affect the seed germination at 60 kg t^{-1} when applied as seed dressing agent. Over this dose the elongation of roots contrary to coleoptiles was slightly depressed, plants however, rapidly recovered and the leaf whorls developed normally. Neither phytotoxic symptoms nor changes in pigment constitution were detected when the fungicide was applied to leaf surfaces. In contrast, Apron 35 sd was found to be toxic at doses over 6 kg t^{-1} to germinating seeds, this preparation, however, did not affect the development of seedlings at the recommended dose of 2–6 kg t^{-1}. The pigment constitution of treated leaves did not change after foliar treatment with Ridomil MZ.

Responses of the pathogen (fungitoxicity tests)

S. graminicola proved to be highly sensitive to Melody Duo *(Table 1)* upon direct contact with the chemical and Melody Duo surpassed Apron four times in this respect. Mediated by the plant's tissue, however, Apron proved to be about two times more effective. Also the efficacy of Apron was significantly more pronounced when these fungicides were applied as seed dressing agents.

Table 1

Comparative efficacy of fungicides

No.	Mode of application	Toxicity limits	
		Iprovalicarb[a]	Metalaxyl[b]
		EC_{50} mg L^{-1}	
1.	Leaf bath – contact	3.64 (3.33–3.97)	15.2 (11.1–20.8)
2.	Water Agar – mediated	198 (105–374)	103 (49–218)
		ED_{99} kg t^{-1}	
3.	Seed dressing – systemic	9.0 (8.51–9.52)	2.0 (1–6)

a: Melody Duo 67 wp, b: Apron 35 sd. The methods of application and assessment of the intensity of sporulation are detailed in Materials and Methods. Toxicity limits and confidence ranges were calculated at P=5% level using the linear regression function of log/probit dose response.

Influence by the mode of application

As it was revealed in model experiment, the mode of application significantly influenced the performance of antimildew activity of Melody Duo *(Fig. 2)*. Foliar application proved to be essentially more effective than seed dressing (F_{exp}=157.5, p<0.01). There was, however, no difference between performances in greenhouse and in field (F_{exp}=0.7). Leaf treatments with Melody Duo were equipotent to seed dressings with Apron. The correspondence of greenhouse and field data shows that the mode of application can be optimised in greenhouse (p <0.01).

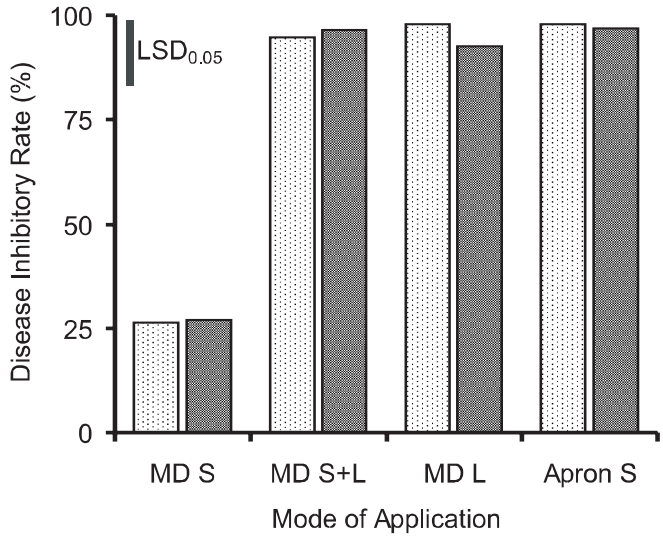

Fig. 2. Influence of the mode of application on performance of Melody Duo.
Melody Duo 67 wp (MD) was applied either as seed dressing (S) at 4 kg t^{-1} or leaves of 21-day-old plants were sprayed (L) with 1% solution. Apron 35 sd was applied as seed dressing (6 kg t^{-1}).
The effect of treatments was evaluated at the 30th day after sowing

Melody Duo, in comparative dose, protected pearl millet germlings from *S. graminicola* infection at the level of Apron *(Table 2).* The protective effect of both preparations started declining after five days of outcropping.

Melody Duo proved to be as efficient as Ridomil MZ when applied as foliar spray, regardless the time of application. Applied either to new or well-established infections, Melody Duo inhibited the zoosporogenesis by equal power 98–100%.

Table 2

Persistence of protective effect of fungicides applied as seed dressing agents

Time of inoculation next to outcropping (days)	Fungicides	
	Melody Duo 67 wp	Apron 35 sd
3	100	100
4	92 (86–98)	100 (98–100)
5	90 (86–95)	100 (97–100)

Confidence limits are given at P=5%. Both fungicides were applied at doses maximum tolerated by germinating pearl millet.

Translocation studies

Iprovalicarb translocated very rapidly within the plant and the acropetal translocation dominated over the basipetal one *(Table 3).* On the first day, the systemic activity of iprovalicarb was similar to that of metalaxyl, which also moved rapidly to other areas. Later however, the anti-mildew activity of metalaxyl as compared to that of iprovalicarb increased more intensively and its transit to basipetal direction was also more pronounced.

Table 3

Intensity of translocation of systemic fungicides in downy mildewed pearl millet plants

Side of translocation	Time of assessment (days)	Anti-mildew activity (EC_{50} mM)	
		Iprovalicarb	Metalaxyl
Acropetal	1	1.116b	0.926b
	2	0.479a	0.435a
Basipetal	1	354.3e	27.2c
	2	113.8d	17.6c

Values labelled by the same letter do not differ significantly at P=5%. Determination coefficients of dose response lines in each case were over 0.92 ($p<0.001$).

On plants sprayed with 0.25% solution of Melody Duo, the pathogen was efficiently inhibited at acropetal parts (at about 90 and 99% within the next two days), whereas the systemic activity remained poor at basipetal side (< 5%), which is negligible from points of view of PMDM control. The acropetal systemic activity of Ridomil MZ applied at comparative dose did not significantly surpass the Melody Duo but basipetally it was more active (12–20%), although this effect is also insignificant for controlling *S. graminicola.*

Recovery effect of systemic fungicides

When diseased plants were sprayed with Melody Duo *(Fig. 1),* they recovered from downy mildew, and in addition to complete inhibition of sporulation the whole outlook of downy mildewed areas was changed. Notably, the disease dramatically decreased the pigment content of leaf tissues *(Table 4).* The reduction of pigment content was expressed more profoundly in the level of chlorophylls than carotenoids and the ratio of chlorophyll species was also altered significantly. The disease process seemingly more heavily affected the photosystems II and I than the light harvesting protein complex (LHPC).

Table 4

Influence of downy mildew on pigment constitution of pearl millet leaves

Pigments	Pigment content in leaf areas (mg cm^{-2})	
	Healthy	Diseased
Chlorophyll *a*	0.968±0.014	0.166±0.018
Chlorophyll *b*	0.151±0.024	0.063±0.035
Chlorophyll *a/b*	6.41	2.63
Carotenoids	0.386±0.009	0.105±0.017
Chlorophylls/Carotenoids	2.90	2.18

As opposed to diseased leaves, the recovering ones started with a massive *de novo* synthesis of photosynthetic pigments in formerly chlorotic areas *(Fig. 3).* The level of chlorophyll *a* increased more rapidly than the other two pigment groups indicating the reconstruction of the photosystems. There were no differences in the curative effect of iprovalicarb and metalaxyl in this respect; the differences among variants were not significant at P=5%.

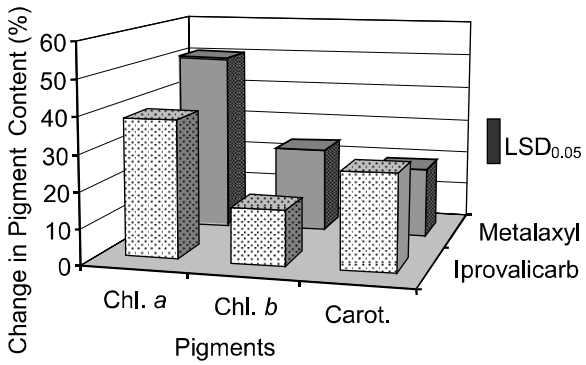

Fig. 3. Changes in pigment content of downy mildewed pearl millet leaf tissues following sprays with systemic fungicides.The changes in contents of chlorophyll *a* (Chl. *a),* chlorophyll *b* (Chl. *b)* and carotenoids (Carot.) were related to untreated chlorotic areas of downy mildewed plants

Field activity studies

Treatments with Melody Duo significantly inhibited the downy mildew incidence in provocation field *(Table 5).* No case of phytotoxicity was observed. The efficacy of single seed treatment with 24 kg t^{-1} failed behind the single leaf spray (4 kg ha^{-1}) carried out before the formation of primary tillers. The combined use of seed treatment and foliar spray proved to be more effective than the seed treatment only with Apron. Melody Duo did not exhibit any toxicity to pearl millet even at highest doses tested verifying the high therapeutic index found in the greenhouse.

Table 5

Field performance of fungicide treatments

Treatments		Effect		Net return (Total return) USD[b]
Fungicides	Dose[a]	Disease Inhibition (%)	Yield (t/ha)	
Melody Duo 66.75 wp				
Seed treatment	24 kg t^{-1}	89 (87–91)	1.67 (1.65–1.69)	+35 (220)
Seed treatment	60 kg t^{-1}	97 (94–99)	1.85 (1.83–1.87)	+54 (244)
Leaf spray	4 kg ha^{-1}	92 (91–94)	1.65 (1.62–1.67)	+34 (218)
Seed treatment+Leaf spray	24 kg t^{-1} + 4 kg ha^{-1}	99 (99–100)	1.89 (1.87–1.92)	+63 (249)
Seed treatment+Leaf spray	60 kg t^{-1} + 4 kg ha^{-1}	99 (99–100)	1.87 (1.86–1.89)	+56 (247)
Apron 35 sd				
Seed treatment	6 kg t^{-1}	97 (95–99)	1.84 (1.82–1.85)	+59 (243)
Control		0	1.13 (1.04–1.20)	–33 (150)

Confidence limits were calculated at P=5% level.
a: Quantity of Melody Duo 66.75 wp was related to Apron 35 sd on the base of the content of systemic partner in the combination.
b: Net return was calculated according to Cost-Return Budget proposed by Thomas Jefferson Institute.

Fungicide treatments significantly increased the amount of grain yield. The combined application of Melody Duo as a seed dressing and foliar treatment in early stage of vegetation was optimal. The yield performance of treatments positively depended on their disease inhibitory rate *(Fig. 4).* By means of this experiment, the economic limit requests about 52–60% level of disease control. The minimum disease inhibitory rate was between 25 and 30% for perceptible changes in grain yield. All variants of treatments adequately controlled the pathogen. Determination coefficient for saturation type of regression between yield increase versus Disease Inhibition Rate (DIR) was 0.8275 ($P<2\%$), that indicates the dominant role of disease in yield loss or reversibly, the dominant role of chemical treatment in yield increase.

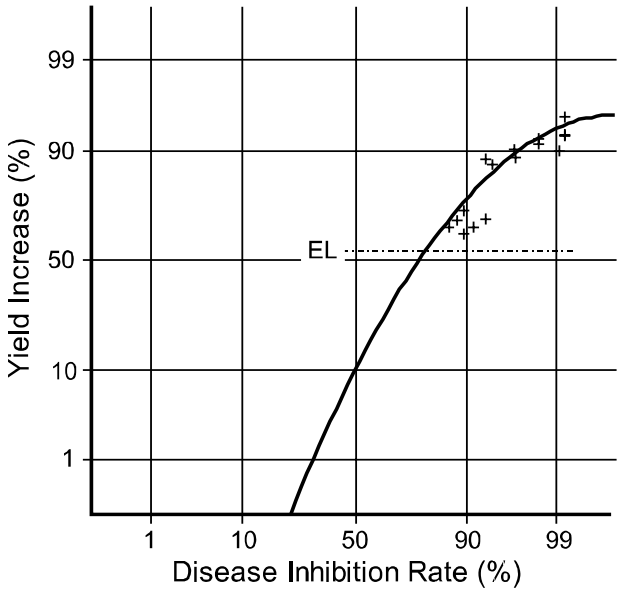

Fig. 4. Relationship between the anti-mildew activity of fungicides and their effect on the yield of pearl millet *in vivo.*Yield increase in percent (probits) related to the untreated control. Baule–Mitscherlich function was applied to calculate the regression between variates (Sváb, 1981). EL= economic limit for yield increase. This is the amount of harvested grain yield, which returns all expanses for producer in financial conditions of USA

Discussion

Melody Duo 67 wp exerted different impacts on PMDM depending on the mode of application. As a seed dressing agent, it exhibited lower protective effect than Apron 35 sd, when both were tested in fields heavily contaminated with oospores of metalaxyl sensitive *S. graminicola.* One can assume that small sized pearl millet seeds adsorbed less amount of iprovalicarb than metalaxyl because the water solubility of the two substances significantly differs (0.02 and 7.1 g L^{-1}, respectively). Otherwise, protective effect of Melody Duo when applied as foliar spray was at the level of Apron and Ridomil MZ. In all cases in which propineb or mancozeb were in contact with the thallus of *Sclerospora,* they acted simultaneously with organic fungicides. However, the effect of *bisdithiocarbamates* might be negligible when only systemic activity could perform (or some barrier counteracted). Thus the higher efficacy of Melody Duo to eradicate *S. graminicola* as compared to Apron and Ridomil MZ can be related to the anti-mildew activity of iprovalicarb.

Protective and curative ability of iprovalicarb was reported to be at 5 ppm against *Phytophthora infestans* colonizing epidermis cells and palisade parenchyma; it prevented further pathogen ingress in tomato plants. The protective ability *in vivo* was due to inhibition of early developmental stages of oomycetes, like *P. infestans* (Jende et al., 1999). In model experiments the systemic activity of iprovalicarb at acropetal side of pearl millet was equipotent to metalaxyl while at basipetal side lagged behind. This difference can be related either to a rapid translocation of iprovalicarb into leaf margins or to more intensive metabolism as compared to metalaxyl. Stubler et al. (1999) have shown that after spot application in the axil of a tendril to grape vines, the ^{14}C labeled iprovalicarb was transferred in the direction of the transpiration stream (tendril and shoot above) while basipetal translocation against the direction of the flow in the xylem was virtually or entirely absent. This fungicide undergoes progressive translocation in treated shoot, which starts from basally inserted leaves and results in uniform distribution throughout the shoot and leaves followed by translocation in the veins into spaces between them and finally accumulates in the leaf margin region (Stubler et al., 1999). The improvement of curative effect in leaves above the level of handling can be related to dominant acropetal translocation of iprovalicarb in pearl millet. The effect of iprovalicarb on the recovery of downy mildewed pearl millet plants was commensurable to that of metalaxyl. Analysis of the ratio of chlorophylls *a* and *b* showed that LHPC was less affected by pathogenesis than the photosystems I and II. The iprovalicarb treatment resulted more rapid reconstitution of photosystems than LHPC. The systemic spread of iprovalicarb can induce recovery of tissues far from the treated areas as well.

Pearl millet 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. This crop 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. For this reason the remediative effect of plant is not enough to decompose pesticides; residual amounts can thus be translocated into seeds if these were applied after development of secondary tillers. Detailed analysis showed that the infection of germlings and basal tillers of pearl millet is detrimental, while infection of secondary tillers has negligible influence on the yield (Singh and Shetty, 1990). Consequently, the pest management should concentrate on the possible control of seedlings and primary tillers.

Pearl millet downy mildew recently is distributed worldwide and one of the most important diseases causing losses up to 60% of grain yield (Nene and Singh, 1976). Field trial with Melody Duo showed significant positive effect on the yield of pearl millet grown under high infection pressure of *S. graminicola.* The economic impact of optimized applications were equal to those carried out with Apron and Ridomil MZ. Pearl millet however, tolerated Melody Duo at higher extent in our experiments.

The present control technologies of downy mildews disrupt infection cycles either by killing asexual spores or by inhibition of the parasitizing thallus. In the case of soil-borne endobiotrophes only the latter method is conducive to economically estimable results. Traditional fungicides (Bordeaux mixture, various metal complexes of *bis*dithiocarbamates)

exhibiting high activity against the majority of plant pathogenic fungi, do not control efficiently endobiotrophic pathogens that, like *S. graminicola,* invade their host plant systemically. When the systemically active substance is applied as a seed-dressing agent, it inhibits both seed- and soil-borne infections. Furthermore, the substance translocating into the shoots will be present in maximally susceptible phenophase of pearl millet thereby preventing air-borne infections. In our experiments both incidence and severity of PMDM were significantly decreased by a single seed treatment with either iprovalicarb or metalaxyl.

Resistance to systemic fungicides occurs worldwide and is increasing as the use of these chemicals grows. Observation made in recent vegetation on the decline of metalaxyl sensitivity of *S. graminicola* has been alarming in north-west region of India (Anon., 2001). The potential for metalaxyl resistance to develop on the crops we do grow is real. Although the details of the biochemical mechanisms need to be determined, it has been stated that iprovalicarb does not interfere with respiration or with the nucleic acid or lipid metabolism. This clearly reveals that iprovalicarb has a novel specific mode of action against oomycetes (Jende et al., 1999). Therefore, it is a potential candidate for replacement of acylanilides (e.g. metalaxyl) in control programs of PMDM.

Seed treatment in general is the technology easiest to transfer to the farmers to promote sustained millet production (Nyemba, 1997), and the costs of treatment are low, nonetheless, the contamination with residual amounts of fungicide is negligible (Reddy et al., 1996; Andersen et al., 2002). However, the application of highly active fungicides, like iprovalicarb in early stage of vegetation as opposed to seed treatment, leads to the same result in yield performance. The differences in persistence of the anti-mildew effects of iprovalicarb and metalaxyl seemingly did not influence the development of healthy inflorescence and, as a result, the yield performance of Melody Duo was similar to that of Apron 35 sd or Ridomil 72 MZ, independently of the mode of application. All this is in accordance with earlier observations on the dominant role of infections in early developmental stages of pearl millet (Singh and Shetty, 1990).

Although Melody Duo 66.5 wp seems not be suitable for controlling *S. graminicola* as a seed dressing agent, it has significance in controlling pearl millet downy mildew as a foliar spray alone. Taking the levels of activity of iprovalicarb against *S. graminicola* into consideration, the exploitation of this systemic fungicide is recommended against pearl millet downy mildew disease.

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