KAVAKA 47: 54 - 62 (2016)

Development of Fungicide Resistance in Plant Pathogens with Reference to Indian Scenario

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(Submitted on 05-03-2016 ; Accepted on 25-05-2016)

ABSTRACT

Fungicides are essential component of crop protection and have played significant role in managing several devastating crop diseases. However, their indiscriminate use has resulted into development of resistance in several pathogens. This has led to poor disease control in many instances. The problem is more common with site-specific fungicides and performance of many of the systemic fungicides developed in the past three decades has been adversely affected. Some of the fungicide groups such as benzimidazoles, phenylamides, dicarboximides and the recently introduced strobilurins carry high resistance risk while fungicides like sterol biosynthesis inhibitors possess moderate risk. In India, development of resistance to various site-specific fungicides is now well known in some plant pathogens under practical field situations. This calls for implementation of suitable resistance management strategies to get expected disease control levels and to prolong the active life of potential fungicides.

Keywords : Fungicides, resistance, plant pathogens, competitive fitness, pathogenic potential, site-specificity

INTRODUCTION

Fungicides serve as important tools for managing diseases in agricultural crops. Although some plant diseases may be managed through resistant varieties and alteration of cultural practices, several diseases are only managed acceptably with the application of a suitable fungicide. About 150 different chemicals belonging to different classes are used as fungicides in various countries including India.

Resistance to fungicides has become a challenging problem in the management of crop diseases and has threatened the performance of some highly potent commercial fungicides (Brent, 1995). Unlike insecticides, where resistance problems are known to occur much earlier, practical problems of fungicide resistance has emerged much later in 1970's and thereafter. Worldwide, resistance in pathogen populations to more than 100 different active ingredients has been reported. Incidence of resistance to fungicides has remained restricted mainly to systemic fungicides that operate against single bio-chemical targets also known as single site inhibitors (Dekker, 1985; Brent, 1995). These site-specific systemic fungicides were introduced in the mid 1960's onwards and include several major groups of fungicides such as benzimidazoles, pyrimidines, phenylamides, sterol biosynthesis inhibitors, dicarboximides, phenylamides, etc.. During the past decade, more novel compounds with different modes of action notably phenylpyrroles, anilinopyrimidines, strobilurins, spiroxamines, phenylpyridylanines, quinolines, etc. have been developed having bioefficacy against diverse plant diseases. Several of these modern selective fungicides have become vulnerable to the risk of resistance development in target pathogens in different countries (Brent and Hollomon, 2007)

As compared to developed countries, not much work has been done on the problem of fungicide resistance in India. This could be attributed to the lack of awareness among the Indian workers about the importance of the problem and non-availability of trained scientific manpower in this field. Most of the earlier studies done on fungicide resistance in India pertained to acquired resistance using mutagens or training (pressurization) methods under laboratory conditions, without looking into their possible implications in practical disease control. However, during the last two decades cases of resistance development in field situations have also been reported from different parts of the country (Thind, 2002; 2008)

USE OF FUNGICIDES IN INDIA

The use of fungicides in India is quite low as compared to developed countries. Overall fungicide use is less than insecticides and herbicides. The consumption of fungicides in 2009 was 8307 MT compared to 26756 MT of insecticides and 6040 MT of herbicides with market share at 19% compared to 61% of insecticides and 17 % of herbicides. The current fungicide market in India is worth Rs. 4300 millions.

Apart from conventional compounds like sulphur, dithiocarbamates, copper-based, mercurials, phthalimides, etc., several of the site-specific fungicides of the groups like benzimidazoles, oxathiins, thiophanates, organophosphorus, triazoles and related sterol inhibitors, phenylamides, strobilurins and other recently developed compounds are being used in India for controlling different diseases on a number of crops. As many as 52 fungicides belonging to different groups were registered for use in India (**Table 1**). In addition, formulations of combination products containing systemic and contact fungicides are also registered.

Crop wise consumption of fungicides in India is maximum on pome fruits (12.7%), closely followed by potatoes (12.2%), rice (12.0%), tea (9.4%), coffee (8.0%), chillies (7.6%), grapevines (6.9%), other fruits (5.9%), other vegetables (4.6%) and other crops which account for about 75% of the total fungicides used in India (Thind, 2002).

ACQUIRED RESISTANCE IN LABORATORY

In India, several cases of adaptive resistance to many fungicides including multisite-action compounds have been reported under laboratory conditions by different workers, but their possible implications in disease control have not been indicated. Various methods such as adaptation to increasing fungicide concentrations, exposure to UV radiations and chemical mutagens have been employed to study resistance development in diverse fungi to dithiocarbamates, copper-based, oxathiins, benzimidazoles,

Table 1. Site-specific fungicides registered for use against

Fungicide group	Name of the fungicide
Oxathiins	Carboxin, Oxycarboxin
Benzimidazoles	Benomyl, Carbendazim
Guanidines	Dodine
Thiophanates	Thiophanate methyl
Phosphorothiolates	Edifenphos, Iprobenfos
Dicarboximides	Iprodione
Acylalanines	Metalaxyl, Metalaxyl-M (Mefenoxam)
Cyano-acetamide oximes	Cymoxanil
Cinnamic acid derivatives	Dimethomorph
Trizoles	Propiconazole, Penconazole, Myclobutanil, Triadimefon, Bitertanol, Hexaconazole, Difenoconazole, Tebuconazole, Flusilazole
Morpholines	Tridemorph
Pyrimidines	Fenarimol
Melanin biosynthesis Inhibitors	Tricyclazole, Carpropamid
Dithiolanes	Isoprothiolane
Stobilurins	Azoxystrobin, Kresoxim methyl, Trifloxystrobin
Oxazolidinones	Famoxadone
Imidazoles	Fenamidone
Valinamides	Iprovalicarb
Anilides	Thifluzamide
Antifungal antibiotics	Aureofungin, Kasugamycin, Validamycin
Source : Central Insecticides Board (www.cibrc.nic.in)	

organophosphorus, phenylamides, alkyl phosphonates, morpholines and antifungal antibiotics in the laboratory (Thind, 1995).

RESISTANCE DEVELOPMENT IN FIELD

The increase in use of fungicides, particularly of selective fungicides, on important crops caught the attention of some workers about their likely effects on pathogen populations and in the past years, cases of fungicide resistance development have also been reported under field conditions in India (Thind, 2002; 2008). Sensitivity studies through regular monitoring of conidial/sporangial populations of several pathogens have led to the detection of fungicide resistant strains with low to high resistance levels in some plant pathogens. Some reported field cases of fungicide resistance in India are mentioned in **Table 2** and are described in the following pages.

Benzimidazoles

Apple scab (*Venturia inaequalis*) : Apple scab caused by *V. Inaequalis* (Cke.) Wint. has become endemic in all the important apple growing belts covering an area of 60, 000 ha in Kashmir alone. As most of the commercial apple cultivars are susceptible to scab, orchardists mainly depend on fungicides such as mancozeb, zineb, ziram, carbendazim, benomyl, captan, triazoles, etc. for its control. At least six applications of fungicides are recommended against this disease in Kashmir and seven in Himachal Pradesh (Gupta and Gupta, 1996) at various phenological stages starting from silver tip/green tip stage till harvest. But the growers usually give 12 -15 applications of different fungicides to ensure good disease control. Based on the observations by some growers on decreased level of disease control after prolonged and exclusive usage of mancozeb and carbendazim in their orchards in Kashmir valley, sensitivity studies of conidial populations of *V. inaequalis* from 40 affected orchards were carried out. Apparently one isolate was obtained from each orchard. The results were reported to indicate mancozeb resistant strains in 12 orchards and carbendazim resistant strains in 3 orchards (Basu Chaudhary and Puttoo, 1984).

Although apparently unusual, mancozeb resistant isolates could tolerate 2.5 times higher levels of the fungicide compared to sensitive isolates and the per cent disease control ranged from 41 to 77 in these orchards. The isolates proved pathogenic on young apple foliage of cv. Red Delicious during the first and second sub-culturings only. The resistance was, however, found to be unstable as these isolates lost the character after three sub-culturings on mancozeb free medium and became non-sporulating. Since mancozeb resistance in these isolates was not found to be stable by the workers themselves and in any case was at a relatively low level, the reduction in disease control over some years could possibly be attributed to the poorly managed spray schedules of mancozeb. This fungicide stands low risk of resistance development in the pathogens due to its multi-site mode of action, and cases of practical resistance to mancozeb have not been reported elsewhere, despite its widespread use against many pathogens.

The carbendazim resistant isolates could tolerate 3-14 times higher levels of the fungicide. These are relatively low resistance factors compared with carbendazim resistance reported elsewhere. In contrast to the mancozeb resistant isolates, the carbendazim resistance was found to be stable. The isolates retained the spore producing character and were pathogenic on young apple foliage during all sub-culture

Table 2. Reported cases of fungicide resistance under field situations in India

Fungicide	Pathogen (Host)	Reference
Carbendazim	*Venturia inaequalis* (Apple)	Basuchaudhary and Putto (1984)
	Gloeosporium ampelophagum (Grapes)	Kumar and Thind (1992)
	Aspergillus flavus (Groundnut)	Gangawane and Reddy (1985)
	Cercospora beticola (Sugarbeet)	Pal and Mukhopadhyay (1983)
Edifenphos	*Dreschlera oryzae* (Rice)	Annamalai and Lalithakumari (1990)
	Pyricularia oryzae (Rice)	Lalithakumari and Kumari (1987)
Metalaxyl	*Plasmopara viticola* (Grape)	Rao and Reddy (1988)
	Phytophthora infestans (Potato)	Arora *et al.*(1992)
		Thind *et al.*(1999)
	Phytophthora parasitica (Citrus)	Thind *et al.*(2009)
	Pseudoperonospora cubensis (Cucumber)	Thind et al.(2011)
Oxadixyl	*Phytophthora infestans* (Potato)	Singh *et al.*(1993)
Triadimefon	*Uncinula necator* (Grape)	Thind *et al.*(1998)

inoculations. Strategies for the management of fungicide resistance in *V. inaequalis* involving need-based application of fungicides and the use of sanitary, physical and cultural practices to control the pathogen multiplication have been suggested by Putto and Basu Chaudhary (1986).

Grape anthracnose (*Gloeosporium ampelophagum*): Anthracnose, caused by *G. ampelophagum* (de Bary) Sacc. (syn. *Elsinoe ampelina*), poses a serious threat to grape cultivation in Punjab and other parts of India and requires regular fungicide applications for its control. A number of treatments of benzimidazole and related fungicides like carbendazim, benomyl and thiophanate methyl, as well as conventional contact fungicides (copper-based, dithiocarbamates, phthalimides, etc.) are applied repeatedly by the growers to protect the plants from this disease. Due to excessive and irrational use of benzimidazoles, development of resistance, associated with inferior disease control, has been observed in *Gloeosporium ampelophagum* and the strains with high level of resistance to carbendazim have been isolated from vineyards in the Punjab state (Kumar and Thind, 1992). Studies were conducted during 1990-97 to determine the population structure of *G. ampelophagum* with regard to fungicide sensitivity and strategies for its management.

***Screening for carbendazim resistance* :** In the preliminary screening a total of 80 isolates of *G. ampelophagum* collected from various regions in the Punjab state during 1990-97 were studied for their sensitivity to carbendazim (Bavistin 50 WP) using malt agar plates amended with 1 and 5 g/ml of carbendazim. Majority of the isolates showed sensitive or weakly resistant response and were unable to grow beyond 1 g/ml of carbendazim. However, 36 % of the isolates showed growth at 5 g/ml indicating resistant response to carbendazim (Thind and Mohan, 1998). Twenty three isolates found resistant in the preliminary screening were further grown at higher concentration up to 100 g/ml of carbendazim. Of these, all the isolates showed normal growth up to 50 g/ml while 15 were able to grow even at a higher dose of 100 g/ml of carbendazim thus exhibiting high resistance factors (**Table 3**). These isolates were obtained from vineyards receiving regular treatments of Bavistin and were mostly from areas near Ludhiana. Resistance to carbendazim was found to be persistent in nature as the resistant isolates were able to grow at 50 g/ml of carbendazim even after one year of sub-culturing on fungicide-free medium. The isolates of *G. ampelophagum*

Table 3. Structuring of *Gloeosporium ampelophagum* isolates from different vineyards for carbendazim sensitivity in Punjab (1990-1997)

Number of Isolates tested	ED50 (?g/ml)	MIC (?g/ml)	Resistance factor	Sensitivity class
36	0.02-0.04	0.05-0.1	0.0	S
21	0.04-0.16	0.20-0.5	1.8-4.5	WR
8	14-50	40-100	360-900	HR
15	69-100	> 100	> 900	HR

S = Sensitive, WR = Weekly resistant, HR = Highly resistant
Source : Thind and Mohan (1998)

were categorised into three morphological groups and majority of the resistant isolates produced reddish brown to peach red colonies (Thind *et al.*, 1994).

Further studies conducted from 2000-2004 revealed that carbendazim resistant isolates of *G. ampelophagum* were persistent in natural populations and could be detected frequently in the vineyards around Ludhiana in Punjab state (Mohan *et al*., 2005).

***Pathogenic behaviour of resistant isolates*:** Pathogenic behaviour of two resistant and two sensitive isolates was studied on detached leaves of cv. Perlette treated with different concentrations of Bavistin in the laboratory. While the sensitive isolates did not produce any symptoms above 250 g/ml, both the resistant isolates Ga 28 and Ga 53 developed normal sporulating lesions at 500 g/ml and also produced mild symptoms even at 1000 g/ml of Bavistin thus confirming their resistant character.

***Cross resistance to other fungicides*:** Cross resistance to other fungicides viz. Topsin-M (thiophanate methyl) , Captaf (captan), Indofil M-45 (mancozeb), Bordeaux mixture (copper sulphate + calcium hydroxide), and Bayleton- 5 (triadimefon) was studied by growth inhibition assay as well as by detached leaf assay by taking one resistant and one sensitive isolate. Observations revealed that resistant isolate Ga 53 possessed cross resistance to Topsin-M which has a similar mode of action as Bavistin (Mohan and Thind, 1995). On the other hand both resistant and sensitive isolates exhibited sensitive response to all other fungicides tested. In another study (Thind *et al*., 1997) on cross resistance, three triazole fungicides viz. Score (difenconazol), Corail (tebuconazol) and Olymp (flusilazole) and one pyridylanine compound Dirango (fluazinam) were found to possess high inhibitory action against carbendazim resistant as well as sensitive isolates with MIC values of triazoles ranging between 1-5 g/ml for both the types. Fluazinam also exhibited good efficacy at 10 g/ml and above. By detached leaf assay difenconazole proved most effective and no symptoms developed at 25 g/ml. Fluazinam arrested diseases development completely at 500 g/ml by both the isolates and holds promise alongwith difenconazole to check resistance.

***Management of carbendazim resistance*:** Indofil M-45 and Bordeaux mixture to which carbendazim resistant isolates did not show any cross resistance were tested in a resistance affected vineyard near Ludhiana. Bavistin (0.1%) when used alone did not provide desired control of grape anthracnose. In contrast, when it was applied in alternation with Bordeaux mixture (2:2:250) or Indofil M-45 (0.3%) there was significant reduction in disease severity (Mohan and Thind, 1995). Triazole fungicides such as difenconazole, tebuconazole, flusilazole and fluazinam, a pyridylanine compound, which showed promising efficacy against resistant and sensitive isolates of *G. ampelophagum* in laboratory studies using detached leaf assays (Thind *et al.*, 1997; Thind and Mohan, 1998) are now used in field conditions as anti -resistance measures.

Application of an effective fungicide immediately after first rain shower in March/April helps in checking the primary infection and multiplication of inoculum for subsequent infections, thereby, reducing fungicide sprays and minimising the risk of resistance development to site-specific fungicides like carbendazim.

Sugarbeet leaf spot (*Cercospora beticola*) : Leaf spot of sugarbeet, caused by *C. beticola* Sacc. is a serious disease problem of sugarbeet in India. Various fungicides including carbendazim formulations are widely used to control this disease. Some natural populations of the fungus were screened for sensitivity to carbendazim (Bavistin 50 WP). It was surprising to note that a natural mutant from untreated field was able to tolerate high concentrations of carbendazim (Pal and Mukhopadhyay, 1983).

Organophosphorus Compounds

Brown spot of rice (*Drechslera oryzae*) : Organophosphorus fungicides such as edifenphos and iprobenphos are widely used in southern parts of India for the control of brown leaf spot of rice caused by *D. oryzae* (Breda de Haan) Subram. and Jain, which causes severe crop losses if not controlled in early stages. Edifenphos (Hinosan) is used quite regularly in Tamil Nadu and other rice growing states for reducing crop losses due to this disease. Risk of resistance development to edifenphos has been determined in *D. oryzae* under selection pressure of the fungicide in field during 1984 -1988 at a village farm near Chingleput, Madras (Annamalai and Lalithakumari, 1990). Field isolates of the pathogen were collected to study the base-line data on the sensitivity before commencing the application of edifenphos in 1984 and subsequently after the application of edifenphos every year up to 1988. Repeated applications of edifenphos resulted in patches of paddy crop cv. IR-50 with severe disease manifestation. The disease intensity in the treated plots was surprisingly much higher than in untreated plots. Every year 400 leaf samples were collected at random, the pathogen was isolated (one lesion/plate) and screened for edifenphos sensitivity by measuring the radial growth on PDA amended with 10, 20, 50, 200 and 300 g/ml of the fungicide. To characterise the isolates for resistance, these were grouped into four categories based on ED_{50} values i.e., sensitive, low level resistance, moderate resistance and high level resistance having ED_{50} below 50, between 50-100, between 101-150 and above 150 g/ml, respectively.

A shift in the level of sensitivity to edifenphos was noticed from year to year. In 1984, before the application of edifenphos, 96 per cent of the isolates were sensitive to edifenphos at 50 g/ml, while in 1985, 1986, 1987 and 1988 (i.e. after fungicide application), 74, 64, 50 and 48 per cent respectively of isolates showed the same level of sensitivity. The sensitivity data of the field isolates thus showed a clear shift in the level of sensitivity of *D. oryzae* due to frequent applications of edifenphos (**Table 4**). Rate of uptake of edifenphos was less in resistant strains of *D. oryzae* and the reduced membrane permeability was suggested as the mechanism of resistance.

When tested for cross resistance to other fungicides, mancozeb showed significant inhibitory effect on the growth of edifenphos resistant isolates compared with the sensitive

Table 4. Sensitivity range of *Drechslera oryzae* field isolates against edifenphos

ED_{50} Range (μg/ml)	Per cent of isolates				
	1984	1985	1986	1987	1988
20 - 50	96	74	64	50	48
51 - 100	4	26	16	18	16
101 - 150	0	0	20	18	10
151 - 180	0	0	0	14	26

Source : Annamalai and Lalithakumari (1990)

strain and exhibited ED_{50} value of 45-50 M. Cross resistance was observed to iprobenphos, an organophosphorus fungicide with same mode of action. Other fungicides tested such as copper oxychloride, benomyl, bitertanol, carbendazim and pyroquilon were less effective against resistant isolates and inhibited the growth of the fungus at higher concentrations (Annamalai and Lalithakumari, 1992). Cross resistance studies and field treatments indicated that edifenphos resistance in *D. oryzae* can be counteracted by spraying mancozeb as an alternative (replacement) fungicide. However, edifenphos is still used against *D. oryzae* and is working well in most of the areas.

Blast of rice *(Pyricularia oryzae)* : Blast disease of rice caused by *Pyricularia oryzae* Cav. (syn. *P. grisea* Cav. and *Magnaporthe oryzae*) is a serious disease in rice - growing areas of South Indian states. Edifenphos (Hinosan) is widely used in foliar applications for the effective control of this disease. A preliminary study has been done to estimate the sensitivity of natural populations of *P. oryzae* isolates to edifenphos (Lalithakumari and Kumari, 1987). Diseased leaves of rice cv. IR 50 were collected from fields sprayed regularly with edifenphos and fifty monosporic isolates of the pathogen were obtained. Sensitivity of these isolates to edifenphos was tested at ten concentrations ranging from 10 to 100 m on oat meal agar by mycelial growth inhibition technique. The ED_{50} values were compared with a sensitive isolate, unexposed to edifenphos.

The sensitive isolate had ED_{50} value of 36.3 M. Seventeen isolates from treated fields showed a shift in their ED_{50} values above 50 M and out of these 17 isolates, 9 isolates had ED_{50} values above 60 M (up to 75.8 M) thus indicating resistant response to edifenphos. Not much variation was observed in the growth pattern, conidial morphology and pathogenicity among the fifty isolates tested. The resistant isolates were equally pathogenic when inoculated on one month old seedlings of IR 50 rice cultivar. In cross resistance studies to other fungicides the resistant isolates showed positive cross resistance to iprobenphos, a related fungicide. Ziram effectively inhibited the growth of all the resistant isolates and its use was suggested as a companion fungicide in mixture with edifenphos or as alternate spray fungicide (Lalithakumari and Mathivanan, 1990).

Phenylamides

Grape downy mildew (*Plasmopara viticola*) : Grape downy mildew caused by *P. viticola* (Burk. & Curt.) Verl. & de Toni causes severe losses in southern states of India such as

Maharashtra, Andhra pradesh and Karnataka, especially on two commercial grape varieties Anab-e-Shahi and Thompson Seedless affecting the production and quality of grapes. When metalaxyl became available in late 1970s, it caught the attention of Indian farmers who found it miraculous in controlling grape downy mildew which was earlier difficult to be controlled by the traditional contact fungicides.

The grape growers around Hyderabad started using metalaxyl (Ridomil 25WP) in 1981 to control downy mildew. Based on reports by some grape growers in 1986 regarding the loss of effectiveness of metalaxyl in controlling grapevine downy mildew in areas around Hyderabad, monitoring and sensitivity studies were undertaken for ascertaining the cause for reduced efficacy of metalaxyl (Rao and Reddy, 1988). Infected leaves were collected from three affected orchards situated at three villages near Hyderabad which had received metalaxyl applications for 3, 4 and 3 years, respectively. Sporangial populations from these samples were assayed for sensitivity to metalaxyl (Ridomil 25 WP) at 25, 50, 100 and 250 g a.i./ml following detached leaf method of Pappas (1980). The preliminary sensitivity assays with *P. viticola* populations indicated that the loss of efficacy of metalaxyl in these vineyards was attributed to the development of resistance to metalaxyl which had been used frequently by the growers (Rao and Reddy, 1988). Considerable difference in minimal inhibitory concentration was seen among the three populations.

Metalaxyl was almost completely inactive against pathogen populations collected from two of the three villages which confirmed the reports of grape growers regarding the loss of efficacy of metalaxyl in controlling grape downy mildew (**Table 5**). The continuous and exclusive use of metalaxyl (Ridomil 25 WP) by grape growers at these villages for 3-4 years had led to the development of resistant populations of *P. viticola*. No further work has been done on this problem after this report. Several rounds of metalaxyl based combination products with mancozeb viz. Ridomil-MZ (now Ridomil Gold) are used for the control of grape downy mildew in India. Although these combination products are known to minimise the risk of resistance development, regular monitoring for determining the changes in sensitivity levels of pathogen populations is necessary where these fungicides are used frequently. A simple laboratory technique based on sporulation on leaf discs has been developed for laboratory testing of fungicides (Thind *et al*., 1988) which requires less space and can be easily employed for determining fungicide resistance in a large number of *P. viticola* populations.

Table 5. Sensitivity of *Plasmopara viticola* populations to metalaxyl by detached leaf assay

ED_{50} Range (μg/ml)	Per cent of isolates				
	1984	1985	1986	1987	1988
20 - 50	96	74	64	50	48
51 - 100	4	26	16	18	16
101 - 150	0	0	20	18	10
151 - 180	0	0	0	14	26
Source : Annamalai and Lalithakumari (1990)					

Potato late blight (*Phytophthora infestans*) : For the management of late blight of potato caused by *P. infestans* (Mont.) de Bary, traditional fungicides such as mancozeb, zineb, copper oxychloride, chlorothalonil, etc. have been in use since many years in India. However, these fungicides provided poor disease control under heavy disease pressure. The introduction of phenylamide fungicides provided much needed relief to the Indian farmers as these provided excellent control of late blight even under severe disease conditions. Metalaxyl in combination with mancozeb (Ridomil MZ 72WP) was commercially introduced in India during autumn 1988 and since then is being widely used for the control of late blight in different potato growing areas of the country. Now Ridomil Gold having metalaxyl-M (also called mefenoxam) has been introduced recently in India for control of late blight. Although the mixture fungicides are expected to delay the onset of resistance build up, their use does not guarantee prevention of resistance development (Gisi and Staehle-Csech, 1989).

Following the reports of resistance development to metalaxyl in other countries (Davidse *et al*., 1981; Davidse, 1987), monitoring for metalaxyl resistant strains of *P. infestans* was carried out in Nilgiri hills from 1989-91 (Arora *et al.*, 1992). Sporangial populations of *P. infestans* collected from potato fields sprayed with Ridomil MZ were analysed for their response to metalaxyl by detached leaf method. Metalaxyl resistant isolates of the pathogen were absent from early to mid summer potato crop seasons. These, however appeared towards the end of summer season starting from last week of July and a maximum frequency of 13% in the autumn. Variations in tolerance to metalaxyl from 50 to 700 g/ml were observed among different isolates resistant to metalaxyl.

The highly tolerant isolates (300 to 900 g/ml) were observed only during the autumn season and comprised up to 6% of the total samples examined. The resistant isolates could be obtained in plots with a combined spray of metalaxyl and mancozeb and also in plots with individual sprays of mancozeb or chlorothalonil and the control plots later during the season. The resistant isolates were found to be more aggressive in traits like short incubation period, quick germination of sporangia to zoospores, and ability to cause larger lesions, as compared to the sensitive isolates (Arora, 1994). In another study of resistant monitoring in Nilgiri Hills following leaf disc assay, Gangawane *et al.* (1995) have reported that out of isolates of *P. infestans* tested, 82% were sensitive, 5% were moderately resistant (RF 15-40) and 4% were highly resistant (RF 60-70). Use of metalaxyl in mixture with chlorothalonil was highly effective against both sensitive and metalaxyl resistant isolates. Resistance to metalaxyl has also been reported in *P. infestans* from Shimla hills after 5 years of use of Ridomil MZ by Singh *et al*. (1993) but the resistance level reported is quite low. They have also reported isolates of this pathogen developing moderate resistance to oxadixyl under experimental conditions.

In the Punjab state of India, metalaxyl in mixture with mancozeb (Ridomil MZ, Matco 8-64) is being widely used for the control of late blight of potato since 1989. Quite often, farmers also use self-prepared mixtures (tank-mixed) of metalaxyl (35% SD) and mancozeb in various proportions. Sensitivity levels of 68 *P. infestans* populations collected during 1996-1999 crop seasons from various fields treated with fungicides in Punjab were monitored for their sensitivity to metalaxyl following detached leaf method (Thind *et al.*,1989). Thirty one populations, mostly from Hoshiarpur district, showed mild to severe infection at 10 g/ml, while 12 populations, collected mostly during 1998-99 showed varying levels of infection at 50 g/ml in the initial screening. When tested at higher concentraions of metalaxyl, three populations were able to produce symptoms at 100 and 200 g/ml thus showing higher resistant response to metalaxyl (Thind *et al.,* 2001). The resistance factors of populations with varying levels of decreased sensitivity to metalaxyl ranged between 2.8 to 28.5. A marked decrease in the efficacy of Ridomil MZ was also observed in the field from where highly resistant populations were collected.

During 2005-2008 crop seasons, 48 sporangial populations of *P. infestans* collected from different potato growing areas in Punjab were tested for metalaxyl sensitivity among these 10 populations showed resistant response causing infection at 200 µg/ml of metalaxyl with resistance factor up to 60 (Kaur *et al.,* 2010). The resistant population exihibited competitive fitness in a mixture with sensitive population (**Table 6**). RAPD analysis of metalaxyl resistant populations of *P.infestans* was done with 10 oligonucleotide primers. Of 50 primers initially used for amplification, 23 showed

Table 6. Competitive fitness of metalaxyl resistant and sensitive populations of *Phytophthora infestans*

Metalaxyl concs. (µg/ml)	PDI with different combinations of R and S populations				
	R	S	R (50): S (50)	R (25): S (75)	R (75): S (25)
0	86.0	85.0	74.8	74.1	75.6
10	65.3	13.4	38.5	29.3	71.1
50	49.2	0.0	31.2	23.3	46.6
100	44.2	0.0	22.1	7.2	33.5

R = Resistant strain, PI-24; S = Sensitive strain, PI-31; PDI=Per cent disease index
Source : Kaur *et al.* (2010)

polymorphism and 10 were able to distinguish resistant and susceptible populations producing 2-3 unique bands. Information on banding pattern for all the primers was used to determine genetic distance between resistant and sensitive isolates and to construct a dendrogram. RAPD data distinguished the test isolates into two groups thus separating the resistant and susceptible isolates. Using primer P 9 a unique band of 100bp was found in susceptible (S) isolates indicating that these isolates are different from resistant ones in this 100bp region (**Fig.1**).

The resistant populations were found to be highly pathogenic when inoculated on potato leaves of cv. Kufri Chandramukhi. Their disease severities (78 - 90 per cent) were comparable with those of sensitive populations (82 - 100 per cent). Incubation period varied from 4 to 5 days in both resistant and sensitive populations. Sporulation was also comparable in both types of populations. No cross resistance was observed to dimethomorp, mandipropamid, cymoxanil, benalaxyl, previcur, fluopicolide, azoxystrobin and multisite contact fungicides chlorothalonil, fluazinam and mancozeb. Dimethomorph has been reported to be effective in controlling late blight of potato and tomato caused either by

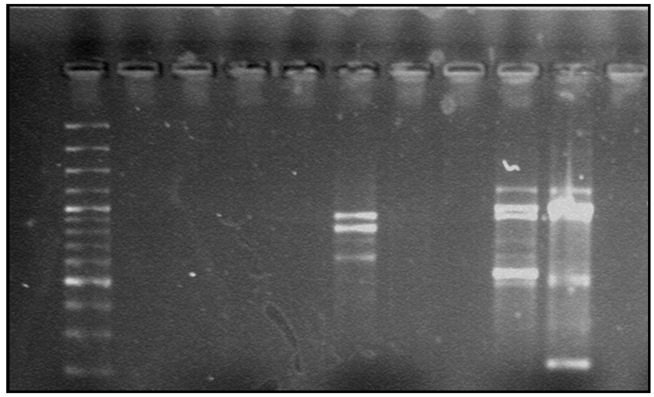

Fig. 1 Metalaxyl resistant and sensitive strains of *Phytophthora infestans* showing amplification with P9

Source: Kaur *et al*.(2010)

metalaxyl sensitive or tolerant strains of *P. infestans* (Cohen *et al.,* 1995). Metalaxyl resistance was effectively managed under field conditions through application of novel action fungicides such as Infinito 68.75 SC (fluopicolide+ propamocarrb chloride), Amistar 25 SC (azoxystrobin), Acrobat 50 WP (dimethomorph), Mandipropamid 250 SC and Curzate M-8 72 WP (cymoxanil + mancozeb). Combination of fungicides with different modes of action retards development of resistance and ensure sustainable management of late blight. The potential of several of these new fungicides has been documented in a recent review (Stevenson, 2009).

Cucumber downy mildew (*Pseudoperonospora cubensis*) : Phenylamide fungicides are regularly used by farmers in various states of India including Punjab to manage downy mildew infection on cucurbits such as cucumber and melons. Sensitivity changes to metalaxyl in *Pseudoperonospora cubensis* populations collected from cucumber and muskmelon fields were monitored during 2007 and 2008. Maximum number of sporangial populations (12 out of 25) exhibited resistant response were collected in district Amritsar with ED_{90} values of metalaxyl ranging between 30-150 g/ml and resistance factor (RF) between 6-30. Most of the populations from Jalandhar and Kapurthala showed normal sensitive response. Resistant populations possessed normal pathogenic potential and exhibited strong competitive fitness when inoculated in mixture with sensitive populations (**Table 7**). Resistant populations did not show cross resistance to fungicides with different modes of action such as azoxystrobin, cymoxanil, dimethomorph, fluopicolide, propamocarb, chlorothalonil and mancozeb. These fungicides were also found effective against metalaxyl resistant populations under field condition and can form a part of the strategy to manage metalaxyl resistance in practice (Thind *et al.*,2011)

Citrus foot rot (*Phytophthora parasitica*): Metalaxyl based fungicides are commonly used to manage foot rot of citrus (*P. parasitica*) in different states of India. A significant reduction in fungicide efficacy has been observed in many orchards over the years. In Punjab state, investigations were carried out

Table 7. Pathogenic potential of metalaxyl resistant (R) and sensitive (S) population of *Pseudoperonospora cubensis*

***P.cubensis* population**	**MIC (µg/ml)**	**Incubation period (Days)**	**Disease severity (%)**	**Sporulation (spores/cm²)**
DM-12 (R)	100	5	80	75.5×10^3
DM-13 (R)	150	4	85	73.0×10^3
DM-15 (R)	10	4	90	75.0×10^3
DM-16 (R)	10	4	80	72.0×10^3
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R=Resistant population, S= Sensitive population
Source : Thind *et al.* (2011)

to determine changes in sensitivity levels of *P. parasitica* isolates from different citrus orchards where reduced efficacy have been reported after metalaxyl applications. Of the 56 isolates of the fungus tested, 9 isolates showed resistant response with ED_{50} values of metalaxyl ranging between 38-200 g/ml. Pathogenic potential, colony growth and sporulation of the resistant isolates were comparable with sensitive isolates (Thind *et al*., 2009). The resistant isolates did not show cross resistance to azoxystrobin, cymoxanil, fluopicolide and previcur. Cymoxanil is providing effective control of foot rot in the orchards where metalaxyl resistance has been a problem. A leaf disc assay involving fungicide treated leaf discs of rough lemon rootstock placed on soil slurry has been developed for early detection of metalaxyl resistance in citrus orchards (Thind *et al.*, 2015).

Triazoles

Grape powdery mildew (*Uncinula necator*) : Powdery mildew, incited by *U. necator* (Schw.) Burr., is another serious disease of grapevine in India causing more damage to the developing berries. For the past 20 years, various DMI fungicides, in addition to the traditional sulphur and dinocap, are being used for controlling this disease. Apart from triadimefon, which is widely used against this disease in India, other DMI fungicides like penconazol, flusilazole and fenarimol are also applied. In the recent years, azoxystrobin has also been introduced for the control of powdery mildew and also downy mildew in grapevine. Conidial populations of this fungus collected from various regions during 1995-97 were studied for detection of resistant strains. The first case of development of resistance in *U. necator* to triadimefon was reported by Thind *et al*. (1998) in India.

Fifteen populations of *U. necator*, each obtained from five infected leaves bearing profuse sporulation, were collected from treated vineyards in Punjab, Maharashtra and Karnataka. These were studied for determining their sensitivity levels to triadimefon (Bayleton 25) following criteria of conidial germtube length and sporulation on leaf discs treated with different concentrations ranging from 0.01 to 10 g/ml of this fungicide. Criterion of germtube length of more than 250 at 0.3 g/ml or above was taken for determining resistance to triadimefon (Steva and Clerjeau, 1990 ; Thind and Mohan, 1995). Majority of the populations showed typical sensitive reaction as their germtubes measured less than 250 at 0.3 g/ml which was taken as the discriminatory concentration to distinguish resistant strains in the conidial populations. Germtubes of such conidia were distorted and deformed at the tips and comparable in their response with the reference sensitive strain Ane-17. However, three populations showed 4 to 6 per cent conidia with normal germtubes measuring more than 250 at a higher concentration of 3 g/ml of triadimefon. Two of these populations, 1a from Bangalore and 7a from Pune, showed one per cent conidia producing normal germtubes at a still higher concentration of 10 g/ml thus demonstrating low to moderate levels of resistance development in these populations (Thind *et al*., 1998). These three populations also developed sporulating colonies on the leaf discs at 3 and 10 g/ml thus confirming resistance to triadimefon. However, no apparent decline in the field performance of this fungicide was observed except in the vineyard at Bangalore where a reduced disease control was recorded.

***Cross resistance to other SBI fungicides* :** One isolate each from populations 1a and 7a, showing moderate resistance to triadimefon, was further studied for sensitivity to two other sterol inhibiting fungicides, triadimenol (Baytan 5) and fenarimol (Rubigan 4) by sporulation test on treated leaf discs. Compared to the sensitive strain Ane-17, which showed negligible sporulation at 0.3 g/ml of triadimenol, isolate 1a and 7a produced some sporulation even at 1 g/ml thus confirming cross resistance to triadimefon. However, on fenarimol treated discs, the two isolates were found to be equally sensitive as the reference strain, thereby, showing no cross resistance to fenarimol (Thind *et al*., 1998). Fenarimol is now being used where reduced sensitivity has been observed to triazole fungicides.

Triazoles and other sterol inhibiting fungicides such as bitertanol (Baycor), hexaconazole (Contaf), myclobutanil (Systhane), penconazole (Topas) and fenarimol (Rubigan) are also being used at present for controlling apple scab in India. Reduced sensitivity to these fungicides in this pathogen has not yet been reported from Indian orchards Since *Venturia inaequalis* is reported to encounter resistance development to DMI fungicides in other countries (Thind *et al*., 1986), it is necessary to initiate monitoring programmes for determining changes in the sensitivity levels of *V. inaequalis* populations to these fungicides. A simple and quick method based on spore germination, germ tube length and morphology has been developed which can be effectively used for determining resistance to DMI fungicides (Thind *et al.*, 1987).

CONCLUSIONS AND FUTURE OUTLOOK

Several site specific fungicides are used in Indian agriculture for managing different crop diseases. Reports of resistance build up to some commonly used fungicides in field populations of certain pathogens indicates the likely risk these new generation fungicides may pose in managing plant diseases more effectively. Risk assessment is crucial for the newly developed fungicides before these are introduced for the commercial use by the farmers. New research initiatives need to be developed to predict the actual risk of resistance.

New technologies are required for monitoring the performance of resistance management strategies and to help predict problems before they occur. New techniques based on molecular biology may prove handy for rapid detection of resistance in pathogen population and may provide necessary information about performance of an anti-resistance strategy. Apart from using at-risk fungicides in mixture or alternation with compounds from different modes of action, their integration with other control methods may help greatly in resistance management by keeping disease pressure at low level.

REFERENCES

Annamalai, P., and Lalithakumari, D. 1990. Decreased sensitivity of *Drechslera oryzae* field isolates to edifenphos. *Indian Phytopath*ology **43**(4) : 553-558.

Annamalai, P. and Lalithakumari, D. 1992. Development of resistance in *Drechslera oryzae* to edifenphos - monotoring and control strategies. *Tropical Pest Management* **38**(4) : 349-353.

Arora, R.K. 1994. Aggressiveness of metalaxyl resistant and sensitive isolates of *Phytophthora infestans*. In : *Potato*: *Present and Future* (Eds.: Shekhawat, G.S. , Paul Khurana, S.M., Pandey, S.K. and Chandra, V.K.). Indian Potato Association, Shimla, pp. 179-183.

Arora, R.K., Kamble, S.S and Gangawane, L.V. 1992. Resistance to metalaxyl in *Phytophthora infestans* in Nilgiri Hills of southern India. *Phytophthora Newsletter* **18** : 8-9.

Basu Chaudhary, K.C. and Puttoo, B.L. 1984. Fungicide resistant strains of *Venturia inaequalis* in Kashmir - a prediction. *Brit. Crop Prot. Conf. - Pests and Diseases*, pp. 509-514.

Brent, K.J. 1995. *Fungicide Resistance in Crop Pathogens : How can it be Managed*? FRAC Monograph No. 1, GCPF, Brussels, pp. 48.

Brent, K.J. and Hollomon, D.W. 2007. *Fungicide Resistance in Crop Pathogens: How can it be Managed* ? FRAC Monograph No.1 (2nd edition), Crop Life International, Brussels, Belgium. pp.55.

Cohen, Y., Baider, A. and Cohen, B.H. 1995. Dimethomorph activity against Oomycete fungal pathogens. *Phytopathology* **85** : 155-1506.

Davidse, L.C. 1987. Resistance to acylalanines in *Phytophthora infestans* in Netherlands. *EPPO Bulletin* **15** : 129.

Davidse, L.C., Looijen, D., Turkensteen, I.J. and Van der Wal, D. 1981. Occurrence of metalaxyl-resistance strains of *Phytophthora infestans* in Dutch potato fields. *Netherland Journal of Plant Patholog* **87**: 65-68.

Dekker, J. 1985. The development of resistance to fungicides. *Progress in Pesticide Biochememistry and Toxicology* **4**: 165-218.

Gangawane, L.V. and Reddy, B.R.C. 1985. Resistance of *Aspergillus flavus* to certain fungicides. *ISPP Chemical Control Newsletter* **6** : 23.

Gangawane, L.V., Kamble, S.S. and Arora, R.K. 1995. Synergistic effect of other fungicides on metalaxyl resistant isolates of *Phytophthora infestans* from Nilgiri Hills. *Indian Journal of Plant Protection* **23** : 159-162.

Gisi, U. and Staehle-Csech, U. 1989. Resistance risk evaluation of new candidates for disease control. In : *Fungicide resistance in North America* (Ed.: Delp, C.J.). APS Press, pp. 101-106.

Gupta, V.K. and Gupta, A.K. 1996. Use of forecasting system in rational use of fungicides for apple scab control. In: *Rational use of Fungicides in Plant Disease Control* (Eds.: Sokhi, S.S. and Thind, T.S.). National Agricultural Technology, Information Centre, Ludhiana, pp. 121-131.

Kaur, R., Thind, T.S. and Goswami, S. 2010. Profiling of *Phytophthora infestans* populations for metalaxyl resistance and its management with novel action fungicides. *Journal of Mycology and Plant Pathology* **40** (1) : 14-21.

Kumar, S., and Thind, T.S. 1992. Reduced sensitivity in *Gloeosporium ampelophagum* to carbendazim in Punjab. *Plant Disease Research* **7** : 103-105.

Lalithakumari, D., and Kumari, D.S. 1987. Screening for field resistance in *Pyricularia oryzae* to edifenphos. *Indian Phytopath*ology **40** (3) : 342-347.

Lalithakumari, D. and Mathivanan, K. 1990. Strategies to avoid or delay or break resistance in *Pyricularia oryzae* to edifenphos. *Journal of Ecobiology* **2** (2) : 155-160.

Mohan, C., and Thind, T.S. 1995. Development of carbendazim resistance in *Gloeosporium ampelophagum* and strategies for its management. *Indian Journal of Mycology and Plant Pathology* **25** : 25-33.

Mohan, C., Singh, J. and Thind, T.S. 2005. Prevalance of grape anthracnose and carbendazim resistance in *Gloeosporium ampelophagum* in Punjab. *Plant Disease Research* **20** (2): 194-195.

Pal, V. and Mukhopadhyay, A.N. 1983. Occurrence of strains of *Cercospora beticola* resistant to carbendazim(MBC) in India. *Indian Journal of Mycology and Plant Pathology* **13** (3) : 333-334.

Pappas,A .C. 1980. Effectiveness of metalaxyl and phosethyl-Al against *Pseudoperonospora cubensis* (Berk. and Curt.) Rostow isolates from cucumbers. *Proc. 5th Cong. Medi. Phytopath. Union*, Patras, Greece, 21-27 Sept., Pp. 146-148.

Putto, B.L. and Basu, Chaudhary, K.C. 1986. Management of Apple scab pathogen resistant to fungicides. In: *Vistas in Plant Pathology* (Eds.: Verma, A. and Verma, J.P.) Malhotra Publishing House, New Delhi, pp. 361-366.

Rao, C.S., and Reddy, M.S. 1988. Resistance to metalaxyl in isolates of grapevine downy mildew fungus (*Plasmopara viticola*) around Hyderabad. *Indian Journal of Plant Protection* **16** : 297-299.

Singh, B.P., Roy, S. and Bhattacharrya, S.K. 1993. Scheduling of systemic fungicides and development of fungicide resistant strains in *Phytophthora infestans*. *Indian Journal of Potato Association* **20** : 48.

Steva, H. and Clerjeau, M. 1990. Cross resistance to sterol biosynthesis inhibitor fungicides in strains of *Uncinula necator* isolated in France and Portugal. *Med. Fac. Landbouww. Rijksuniv. Gent*. **55** : 983-988.

Stevenson WR. 2009. Late blight control strategies in the United States. *Acta Hort. (ISHS)*. **834** : 83-86.

Thind,T.S. 2002. *Fungicide Resistance in India*. Technical Bulletin, Crop Life India, Mumbai, pp. 50.

Thind, T.S. 1995. Fungicide resistance in plant disease control with reference to Indian situation. *Indian Journal of Mycology and Plant Pathology,* **25** : 121-122.

Thind, T.S. 2008. Fungicide resistance : A perpetual challenge in disease control. *Journal of Mycology and Plant Pathology* **38** (3): 407-417.

Thind, T.S., Attri, Monica and Singh Amarjeet 2015. A simple method for early detection of metalaxyl resistance in *Phytophthora parasitica* Dast. causing foot rot of citrus. *Journal of Mycology and Plant Pathology* **45** (2) : 215 217.

Thind, T.S., Clerjeau, M. and Olivier, J.M. 1986. First observations on resistance in *Venturia inaequalis* and *Guignardia bidwelli* to ergosterol biosynthesis inhibitors in France. *Proceedings of British Crop Protection Conference - Pests and Diseases*, 4C, **1**: 491-498.

Thind, T.S., Clerjeau, M. and Olivier, J.M. 1987. A rapid method for detecting resistance to ergosterol biosynthesis inhibitors in *Venturia inaequalis*. *ISPP Chemical Control Newsletter* **9** : 28-29.

Thind, T.S., Clerjeau, M., Sokhi, S.S, Mohan, C. and Jailloux, F. 1998. Observations on reduced sensitivity in *Uncinula necator* to triadimefon in India. *Indian Phytopath*ology **51**(1) : 97-99.

Thind, T.S., Goswami, S., Thind, S.K. and Mohan, C. 2009. Resistance in *Phytophthora parasitica* against metalaxyl in citrus orchards. *Indian Phytopathology* **62** (4) : 536-538.

Thind, T.S., Goswami, S., Kaur, R., Raheja, S. and Mohan, S. 2011. Development of metalaxyl resistance in *Pseudoperonospora cubensis* and its management with novel action fungicides. *Indian Phytopatholgy* **63** (4) : 387-391.

Thind, T.S. and Mohan, C. 1995. A laboratory method for evaluating fungicides against *Uncinula necator*. *Indian Phytopath*ology **48** (2) : 207-209.

Thind, T.S. and Mohan, C. 1998. Management of carbendazim resistance in *Gloeosporium ampelophagum* in India. 9th *International Congress of Pesticide Chemistry - The Food Environment Challenge*, London, U.K., Aug. 2-7, 1998, p. 4D-022.

Thind, T.S., Mohan, C., Kumar, S. and Azmi, O.R. 1994. Observations on field isolates of *Gloeosporium ampelophagum* with reduced sensitivity to carbendazim in Punjab. *Indian Journal of Mycology and Plant Pathology* **24**(1): 46-50.

Thind, T.S., Mohan, C., Clerjeau, M., Sokhi, S.S and Jailloux, F. 1997. Management of carbendazim resistance in *Gloeosporium ampelophagum* with new fungicides. Inter. Conf. Integ. Pl. Dis. Manag. Sust. Agric., 10-15 Nov., 1997, New Delhi. Abstract p. 324.

Thind, T.S., Mohan, C., Clerjeau, M., Sokhi, S.S. and Jailloux, F. 1998. Resistance to triazole fungicides in *Uncinula necator* in India. *7th International Congress of Plant Pathology*, Edinburgh, Scotland, Aug. 9-16, 1998, p. 5.5.24.

Thind, T.S., Mohan, C., Sokhi, S.S. and Bedi, J.S. 1989. A detached leaf technique for maintenance and multiplication of *Phytophthora infestans* and evaluation of fungicides. *Current Sci*ence **58** : 388-389.

Thind, T.S., Munshi, G.D. and Sokhi, S.S. 1988. A method for maintenance of *Plasmopara viticola* and laboratory evaluation of fungicides. *Current Science* **57** (13) : 734-735.

Thind, T.S., Singh, Lakhvir and Mohan, C. 1999. Behaviour of *Phytophthora infestans* populations to metalaxyl and management of resistance in Punjab. XIV International Plant Protection Congress, Jerusalem, 25-30 July, 1999, Abstract p. 152.

Thind, T.S., Singh, Lakhvir, Mohan, C. and Pal, Joginder. 2001. Monitoring for metalaxyl resistance in populations of *Phytophthora infestans* from 1996 to 1999 in Punjab and their characteristics. *Indian Phytopathology* **54** (1): 68- 74.