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# Dynamics of fungicidal resistance in the agro eco-system: A review

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**ABSTRACT**

The usage of fungicides to manage plant diseases in many crops is very significant. Reduced disease control with fungicides can result from several causes including application of insufficient quantity, low effectiveness of the fungicide on the target pathogen, improper timing or method of application and excessive rainfall. Resistance is the evolutionary response of a fungus to threat its survival by the fungicides. Fungicides with single site of action are more prone to develop resistance. The quicker development of resistance and the way to control its loss solely depends both on fungicide and the target fungus. The development of fungicide resistance is complex interactions of factors involving nature of fungicide and target pathogen. Thorough knowledge about the ecology of fungicide resistance pertaining to its development and its management is crucial for ensuring sustainable disease control with fungicides.

**Key words:** Crop protection strategies, Fungicidal resistance, Management strategies, Mode of action, Risk of pathogen.

The agriculture as worldwide and particularly in India faces innumerable challenges. The highly monsoon dependent occupation has caused instability in terms of production and productivity wherein to constrain the yield at many times. With increase in growth of population, it becomes significant to enhance production. The crop production losses due to pests, diseases and weeds estimate to be around 15 to 25 %. Hence a responsible crop production and management requires adoption and implementation of practices that critically address these issues of crop loss. In this context, crop protection practices become an important aspect of agriculture. Usage of fungicides as chemical means of controlling crop diseases pertains to be the at most priority to reduce yield losses under favorable conditions. With the increase in adoption of number of fungicides in the current agricultural scenario, the pathogen population exposed to the repeated use of same fungicides develops resistance. Hence it results in the poor management of disease and to opt for alternative measures to reduce the resistance development in pathogen population. Thus monitoring the resistance development in pathogen seems important context to have effective control of the disease by efficient fungicides. In this paper, different aspects of fungicidal resistance have been addressed with special emphasis on the resistance development and strategies to control the same.

**Crop protection strategies:** Pest, disease and weeds are the frightening trio which is responsible for the considerable amount of losses in agriculture. Besides these usual suspects, rodents, nematodes, plant parasites, and snails have also been able to impose enormous losses to the crops. The damages would run to alarming numbers where the management strategies are not adopted suitably. According to the study by the Associated Chambers of Commerce and Industry of India, annual crop losses due to pests and disease amount to Rs. 50,000 crore ($ 500 billion) which is significant in a country where at least 200 million Indians sleep without food every night (Anonymous, 2018). Hence in this context, plant protection is highly significant in agriculture for developing countries like India.

Integrated Pest Management (IPM) measures includes growing resistant/tolerance varieties, cultural practices like decoy crops/trap crops, crop rotation, mixed cropping etc., Physical control includes seed treatment, soil solarization etc. chemical measures and biocontrol options. At last genetic engineering measures has been employed as crop protection measure. Chemical means of control are absolute, but possess detrimental effect on the environment and at last to humans. Thus crop protection measures have been used with all combination of available strategies in integrated manner. Availability and dissemination of appropriate technologies through several startups and extension wing in many corporate players have increased the awareness and consumption of crop protection chemicals. The pesticide products with inferior formulations constitute 40 % as spurious which are unable to kill pests effectively.

**Assessment of pesticide industry in india:** Chemical measures of control at last dealt in plant diseases and pests. Crop protection chemicals when applied in recommended doses will bring down the impact of pest and diseases to a

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considerable extent. India figures only at the end in terms of consumption of pesticides. The pesticides consumption per hectare in India found to be lowest in the world with 0.6 kg/ha against 5-7 kg/ha in UK and 13 kg/ha in China (Anonymous, 2018). The usage of crop protection chemicals will increase the productivity by 25 -50 % by mitigating crop loss due attack by pests. In order to ensure food and nutritional security, the usage of crop protection chemicals seems to be essential. India is the fourth largest global producer of agrochemicals after US, Japan and China. The annual survey of industries data showed that the industry comprises around 600 companies, of which more than 60 per cent are involved in producing insecticides, fungicides and herbicides. The total production subsidy received during the year 2014-2015 amounts to Rs. 12 crores, which is only 0.03 per cent of total output produced (Anonymous, 2018). Almost 60 % of domestic crop protection in India is dominated by the usage of insecticides. The majority application of insecticides is found in rice and cotton crops. Fungicides are the largest growing segments accounting for 18 % of total crop protection chemicals market. The usage of fungicides found to be major in fruits, vegetables and rice. The significant use of fungicides in fruits and vegetables resulted from the shift in its use from cash crops. In the view of increasing thrust in reducing crop losses and to improve crop productivity, India is a fertile market for crop protection chemicals. As on June 2017, totally 270 products (265 chemicals and 14 bio-pesticides) and 658 formulations including combinations were registered with Central Insecticides Board and the Registration Committee (CIB& RC).

**Management of crop diseases by fungicides over decade:** To our knowledge, fungicides have been used for over 200 years in order to protect or control the plants against fungal pathogens. Ever since the Second World War, initially fungicides were used to protect seeds of cereals crops and grape vines. The number of crop disease treated, available range of chemicals, area and frequency of their use and effectiveness of treatments have been increased hugely. Extremely two very old established remedies, copper-based formulations and sulphur based formulations are widely used effectively till date. Nearly 40 years, usage of several 'middle-aged' fungicides (phthalimides, dithiocarbamates, dinitrophenols, chlorophenyls) seems to be increasing trend. By the end of 1960 and 1970 many potent fungicides with new structure and systemic nature were introduced which includes 2-amino-pyrimidines, benzimidazoles, carbo-xanilides, phosphorothiolates, morpholines, dicarboximides, phenylamides and sterol demethylation inhibitors (DMIs). Wherein the fungicides introduced in 1980s were mainly analogues of existing fungicides with improved properties, particularly DMIs. The number of novel compounds have been introduced commercially or developed at advanced stage for the past decades- these include phenylpyrroles, anilinopyrimidines, quinone outside inhibitors (QoIs, including strobilurin analogues), benzamides and carboxylic acid amides. The wide range of fungal diseases is controlled by some fungicides, wherein others have a limited spectrum of activity against one or two specific groups of plant pathogens. Although many fungicides are marketed at present, any crop disease is typically well controlled by only three or four different types of fungicides. Currently, at least forty-five different modes of action (Hollomon, 2015b) are identified for fungicides. 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).

**Definition of fungicidal resistance:** The term "fungicide resistance" considered in a different phenomenon with called as 'acquired resistance". By years of usage of commercial fungicide, populations of the target pathogen arises which are no longer sensitive to be controlled effectively. The resistant population in target organisms appears to be resulted from repeated usage of fungicides or repeated use of another fungicide with similar mechanism of antifungal action. These resistant populations are controlled and known as antibacterial drugs (e.g. sulphonamides, penicillin, streptomycin) and for agricultural insecticides (e.g. DDT) for nearly sixty years (Brent and Hollomon, 2007).

The phenomenon of development of resistant population was seemed to be called as "insensitivity" or "tolerance". The term "insensitivity" is preferred by plant pathologists to differentiate the fungicide resistance from host-plant resistance of certain fungi. Even agrochemical companies tend to use 'insensitivity', 'loss of sensitivity' or 'tolerance', because these sound less alarming than 'resistance'. At last these two terminologies suggested using the preferred term as "resistance". Earlier the term resistance has been used for many years to explain the same phenomenon in bacteriology and entomology and it is now very frequently used with reference to fungicides. The agrochemical workers recommend to use "resistance" to denote situations only failure or reduction in crop disease control emerged from change of sensitivity. Thus "Resistance" is defined as a stable, heritable trait that results in a reduction in sensitivity to a fungicide by an individual fungus. The genetic nature of fungus resulted to cause reduced sensitivity to fungicides. Reduced sensitivity seems to result from genetic mutations or natural occurrence of resistant individuals.

The resistance term should be used in correct sense without any confusion and to meet the criteria as per the need. "Field resistance" has been used to denote specifically a problem in crop disease control addressed due to resistance. It seems the use of 'field resistance' to point out simply the

presence of resistant variants in field populations was appropriate and 'practical resistance' to indicate observable loss of disease control. 'Laboratory resistance' or 'artificially induced resistance' are accurate terms which are self-explanatory. In some studies 'field resistance' known to occur when the resistance variants were detected from the field samples analyzed with exposure to fungicides. These two types of resistance are hard to categorize and differentiate (Brent and Hollomon, 2007).

**Mechanism of resistance for fungicides:** Many types of resistance mechanism are known. These include:

1. Alteration of the biochemical target site so that it is no longer sensitive;
2. Increased production of the target protein;
3. Developing an alternative metabolic pathway that bypasses the target site;
4. Metabolic breakdown of the fungicide and
5. Exclusion or expulsion of the fungicide through ATP-ase dependent transporter proteins (Brent and Hollomon, 2007).

The common mechanism of resistance to fungicides in plant pathogens is by altering the specific target for biochemical action for the fungicide. Due to this reason, many of older products have not encountered resistance problems. Once the older fungicide penetrated the fungal cell, it acts as general enzyme inhibitors, affecting many target sites and hence called as 'multi-site' inhibitors. These act selectively on fungi since they penetrate and accumulate more readily in fungi. Many sites in the fungus would have to change simultaneously in order to stop the action of fungicide. The chances of the many necessary genetic changes happening are insignificant and in any case an organism with so many alterations would be highly unlikely to be pathogenic or even viable. Protectant fungicides are those which tend to have a multi-site mode of action and hence encounter few problems to develop resistant strains of target fungi. Examples for such type of fungicides are copper, sulfur, chlorothalonil and mancozeb. Fungal pathogens that regularly undergo sexual reproduction are more likely to have greater variability in the population, which increases the chances of developing a strain that is less sensitive to a fungicide. When diseases have a repeating stage (polycyclic disease), the fungal pathogen may also be more likely to develop resistance to a fungicide partially due to the high number of spores that are produced within a season. Moreover the farmers have control over selection pressure, wherein variability of the pathogen is not in their hands.

On the contrary, modern fungicides act primarily at single target sites and are referred to as 'single-site' or 'site-specific' fungicides. Penetrant fungicides tend to have a single-site mode of action and allow target fungi to develop strains with resistance to the fungicide. A penetrant or systemic fungicide penetrates into plant tissue and therefore cannot have target sites that are found in plant tissue. They usually have a single site or a very few sites of action which differs in the fungus and the plant. Thus just a single gene mutation can cause the target site to alter and hence become less vulnerable to the fungicide. Over the past 10 years due to the rapid development of PCR-based diagnostic methods for detection of point mutations causing resistance has aided the identification of resistance mechanisms, especially those involving target site changes. In each case a single point mutation occurs with change in a single amino acid of the target protein so that the binding of fungicide gets less tight. The different levels of resistance occur due to changes in different amino acid of a target protein. For instance, as mentioned earlier, the G143A mutation (causing glycine to be replaced by alanine) at amino acid position 143 in the b-cytochrome of mitochondrial Complex III, causes higher levels of resistance to QoIs than the less common F129L mutation (replacing phenylalanine by leucine at position 129) (Sierotzki *et al*. 2005). Examples for single site inhibitors are Anilinopyrimidines (Amino acid and protein synthesis), Sterol biosynthesis (DMIs), Respiration (MET complex III-QoIs; MET complex II-Carboxins), Osmoregulation (Phenylpyrroles), Lipid biosynthesis (Aromatic hydro-carbons, Dicarboxymides), mRNA synthesis (Phenylamides) and Nuclear division (Benzimidazoles).

**Types of fungicidal resistance:** The type of resistance which is characterized by a sudden and marked loss of effectiveness with the presence of precise sensitive and resistant pathogen populations with widely differing responses is referred to as 'qualitative', 'single-step', 'discrete', 'disruptive' or 'discontinuous' resistance. This resistance is found to be stable and if concerned fungicide is withdrawn or used mush less, population of pathogen remains resistant for many years. Examples for this type of resistance are *Cercospora betae*, the cause of sugar-beet leaf spot to benzimidazole group of fungicides. A steady recovery of sensitive strains seems to occur in the resistance population of *Phytophthora infestans*, the potato late blight pathogen against phenylamide fungicides (Cooke *et al*. 2006).

Occasionally as in the case of the DMI fungicides and of the 2-amino-pyrimidine fungicide ethirimol, resistance has developed less suddenly. In such cases, both a decline in disease control and a decrease in sensitive strains of pathogen populations apparent gradually and are partial and variable in degree. This type of resistance is referred to as 'quantitative', 'multi-step', 'continuous', 'directional' or 'progressive'. It reverts rapidly to a more sensitive condition under circumstances where the fungicide concerned used less intensively or else alternative fungicides are applied against the target pathogen. It is prominent that rust fungi, despite their abundant sporulation and rapid spread, appear to be low-risk and rarely to induce resistance problems (Grasso *et al*. 2006).

'Cross-resistance' is a phenomenon wherein the pathogen populations which develop resistance to one group of fungicide become resistant to those other fungicides that are affected by the same gene mutation and the same resistance mechanism. Example for cross-resistance, pathogen strains that resist benomyl are almost always highly resistant to other benzimidazole fungicides such as carbendazim, thiophanate-methyl or thiabendazole. The occurrence of practical fungicide resistance in crops is furnished in Table 1.

**Pathogen and fungicides properties to influence evolution and spread of resistance:** Pathogen biochemical properties include dependence on disruptible biochemical steps and availability of resistance mechanisms. Epidemiological properties consist of dispersal method e.g. wind, rain splash, soilborne; abundance of sporulation; pathogen lifecycle, short or long generation time; ability to infect all crop stages, requiring repeated treatment and isolation of pathogen populations preventing re-entry of more competitive sensitive genotypes. Genetic factors include relative abundance of genotypes with different sensitivities; fitness properties of different genotypes; sexual or asexual reproduction-influence of inheritance of resistance; Mutation rate and presence of resistance alleles (Hollomon, 2015a).

Fungicidal biochemical properties are the interaction with target metabolism and its susceptibility. Physio-chemical properties include stability, solubility, volatility, polarity, partition and transport properties. Application of fungicides such as initial dose and distribution, formulation, exclusive and repeated use of at risk mode of action, extent of treated area, integration with other disease management tools like biofungicides, resistant crop varieties, crop rotation and crop hygiene (Hollomon, 2015a).

**Detection and measurement of fungicidal resistance:** Monitoring for fungicide resistance enables to test the samples of field populations of target pathogen to their degree of sensitivity for one or more fungicides. Monitoring for resistance become crucial area since distribution, evolution and impact of resistance depends solely on it. It is done to possess early warning for the situation of resistance in future. The foremost reason to monitor is to check the working condition of management strategies. Molecular diagnostics have been successfully used to monitor the degree of success of anti-resistance strategies aimed at combating QoI resistance in powdery mildew and septoria diseases of wheat (Fraaije *et al*. 2002; 2005). Monitoring is also done at specific locations to investigate complaints from farmers with apparent loss of effectiveness of the fungicide and to provide guidance over the usage of future fungicide at their location.

The first crucial step is to develop early monitoring methods and finally to obtain base-line data on the target pathogen populations before exposing it to any widespread use of new fungicide. FRAC Monograph No. 3 Sensitivity Baselines in Fungicide Resistance Research and Management (Russell, 2003) gives a complete account of the underlying principle and methodology of baseline construction for fungicide. The second crucial activity in resistance monitoring is to have practical performance of fungicides. Systematic observations should be done routinely to evaluate disease incidence in fungicide treated commercial crops and untreated with the at-risk fungicide and in any replicated plot trials too.

**Table 1:** Occurrence of practical fungicidal resistance in crops.

| Year of observation | Fungicide/fungicide group | Years of commercial use before resistance | Crops diseases and pathogen affected |
|---|---|---|---|
| 1960 | Aromatic hydrocarbons | 20 | Citrus storage rots, *Penicillium spp.* |
| 1964 | Organo-mercurials | 40 | Cereal leaf spot and stripe *Pyrenophora spp.* |
| 1969 | Dodine | 10 | Apple scab- *Venturia inaequalis* |
| 1970 | Benzimidazoles | 2 | Many target pathogens |
| 1971 | 2-Amino-pyrimidines | 2 | Cucumber & barley powdery mildews, *Sphaerotheca fuliginea*& *Blumeria graminis* |
| 1971 | Kasugamycin | 6 | Rice blast- *Magnaporthe grisea* |
| 1976 | Phosphorothiolates | 9 | Rice blast- *Magnaporthe grisea* |
| 1977 | Triphenyltins | 13 | Sugar beet leaf spot, *Cercospora betae..* |
| 1980 | Phenylamides | 2 | Potato blight and grape downy mildew, *Phytophthora infestans* & *Plasmopara viticola* |
| 1982 | Dicarboximides | 5 | Grape grey mould, *Botrytis cinerea* |
| 1982 | Sterol Demethylation | 7 | Cucurbit and barley inhibitors (DMIs) powdery mildews, *S. fuliginea,* & *Blumeria graminis* |
| 1971 | 2-Amino-pyrimidines | 2 | Cucumber and barley, powdery mildews, *Sphaerotheca fuliginea, & Blumeria graminis* |
| 1985 | Carboxanilides | 15 | Barley loose smut- *Ustilago nuda* |
| 1998 | Quinone inhibitors (QoIs; Strobilurins) | 2 | Many target diseases and pathogens |
| 2002 | Melanin Biosynthesis inhibitors | 2 | Rice Blast- *Magnaporthe grisea* |

The underpinning of monitoring remains to form bioassay to identify decrease in sensitivity despite of the underlying mechanism of fungicides. Spore germination assays are done in various multi-well plate formats, permitting larger numbers of samples to be tested. Growth in a liquid medium can be measured for some fungi directly in a spectrophotometer, or by measuring respiration using reduction of a fluorophore (e.g. 4-methylumbelliferyl- N-acetyl-ß-D- glucosaminide) as an indicator (Fraaije *et al.* 2005). Bioassays can be difficult to carry out especially when applied to obligate parasites such as downy and powdery mildews. Various PCR technologies can be widely applied to detect Single Nucleotide Polymorphisms (SNPs) to understand the molecular mechanism of resistance behind point mutation (McCartney *et al.* 2003; Fraaije *et al.* 2005). The early recognition of the correlation between a single amino acid change (G143A) in the QoI target b-type cytochrome, provided the impetus for large-scale, high-throughput monitoring of QoI resistance using allele-specific real-time PCR (Collina *et al.* 2005; Kianianmomemi *et al.* 2007). By the use of real-time PCR diagnostic technologies recently to monitor for QoI resistance proved capable to detect point mutations at frequencies within field populations as low as 1 in $10^8$. The frequent reports of failure of disease control and detection of resistance to carboxylic acid amide (CAA) fungicides in *Plasmopara viticola* from three vineyards in Sangli district of Maharashtra requires intensive efforts for monitoring and mitigating the problem for successful management of downy mildew in commercial vineyards (Sawant *et al.* 2017).

**Risk of pathogen to develop resistance against fungicides:** An organization known as the Fungicide Resistance Action Committee (FRAC) was developed to address the issue of fungicide resistance. This is an international group that provides guidelines and recommendations to manage the development of fungicide resistance. This organization developed a code of numbers and letters that can be used to distinguish the different fungicide groups based on their mode of action. Different classes of pathogen also vary in their ability to become resistant to fungicides. A number of biological factors are involved in pathogen risk and can be considered to act together in an additive way. Higher pathogen risk is associated with a shorter life cycle, more abundant sporulation of the pathogen, and rapid, long-distance dispersal of spores. The different classes of pathogen risk are given in the Tables 2, 3 & 4.

**Dynamics of resistant strains over the application of fungicide:** The evolution of fungicide resistance can be divided into two phases 1. Emergence phase and 2. Selection phase. In the emergence phase, the resistant strain for fungicide occurs through mutation and consequently invades to the population of pathogen. The number of fungicide resistant lesions is very small in this phase. In spite of applying fungicides with resistant strain, it becomes extinct due to stochastic variation. The length of emergence phase

**Table 2:** Plant pathogens accepted as showing a high risk of development of resistance to fungicides (Russell, 2003).

| Plant Pathogen | Crop | Disease |
|---|---|---|
| *Botrytis cinerea* | Grapevine | Grey mould |
| *Erysiphe (=Blumeria) graminis* | Wheat/barley | Powdery mildew |
| *Mycosphaerella fijiensis* | Banana | Black sigatoka |
| *Penicillium* spp | Citrus, Various | Post harvest rot |
| *Phytophthora infestans* | Potato/Tomato | Late blight |
| *Plasmopara viticola* | Grapevine | Downy mildew |
| *Pseudoperenospora cubensisi* | Cucurbits, various | Downy mildew |
| *Pyricularia* spp | Rice, turf | Rice blast, leaf spot |
| *Sphaerotheca fuliginea* and related spp | Cucurbits, Various | Powdery mildews |
| *Venturia* spp. | Apple, Pear | Scab |

**Table 3:** Plant pathogens with medium risk to develop fungicide resistance (Russell, 2003).

| Plant Pathogen | Crop | Disease |
|---|---|---|
| *Bremia lactucae* | Lettuce | Downy mildew |
| *Cercospora* spp. | Sugar beet, Peanuts, | Various leaf spots |
| *Gibberella fujikuori* | Rice | *Bakanae* |
| *Monilinia* spp. | Various | *Monilia rot* |
| *Mycosphaerella graminicola (Septoria tritici)* | Wheat | Leaf spot |
| *Mycosphaerella musicola* | Banana | Yellow sigatoka |
| *Peronospora* spp | Various | Downy mildew |
| *Pyrenophora teres* | Barley | Net blotch |
| *Rhynchosporium secalis barley leaf blotch/scald* | Barley | Leaf blotch/Scald |
| *Sclerotinia* spp. (especially*homoeocarpa, sclerotiorum)* | Various (turf, oil seed rape) | Sclerotinia diseases, dollar spot |
| *Tapesia* spp | Wheat/barley eyespot | Eyespot |
| *Uncinula(=Erysiphe) necator* | Grapevine | Powdery mildew |

**Table 4:** Plant pathogens with low risk to develop fungicide resistance (Russell, 2003).

| Plant Pathogen | Crop | Disease |
|---|---|---|
| *Alternaria* spp. | Various | Leaf spots |
| *Colletotrichum* spp | Various | Anthracnose |
| *Fusarium* and related spp. | Various | Wilt |
| *Hemileia vastatrix* | Coffee | Rust |
| *Leptosphaera (=Stagonospora) nodorum* | Wheat | Leaf spot |
| *Phytophthora* spp. (soil borne), *Pythium* spp. | Various | Damping off |
| *Podosphaera leucotricha* | apple | Powdery mildew |
| *Puccinia* and related rust spp. | Wheat/barley | Various rusts |
| *Rhizoctonia* spp. | Various | Foot and root rot |
| *Sclerotium* spp. | Various | Blight |
| *Tilletia* spp., *Ustilago* spp. | Cereals | Bunts and smuts |

is defined by the time taken by the introduction for the new fungicide with mode of action till the buildup of large population of resistant strain. Then the evolution of resistance enters to the selection phase wherein the application of fungicide increases the rate of occurrence of resistance strain in the pathogen population (van den Bosch *et al.* 2011).

Due to the variations in the dynamics of the resistant strain between the emergence phase and the selection phase, the efficient resistance management strategies seems to be variable (van den Bosch *et al.* 2011). In the selection phase, the frequency of resistance in the pathogen population will generally increase faster for higher dose rates of the fungicide. On the other hand in the emergence phase, resistance evolution occurs based on effects of dose in two contrasting ways. A high dose rate of fungicide will delay the emergence of resistance by reducing the amount of sensitive pathogen population and hence increasing the number of resistant mutants. Due to the availability of less amount of pathogen population, reduction in the competition between sensitive and resistant strain to infect healthy host tissue occurs. It results in the chance of invading the pathogen population with resistant mutants. It is therefore assumed in the emergence phase, the alternative dose rate of fungicide will change the emergence time in different ways. The emergence time will decrease with increasing dose of fungicide, results in the changes in competition for healthy region of leaf.

By chance if the emergence time will increase with increasing dose of fungicide, the changes will occur in the production of mutant strains. There is a wide range of experimental studies on the development of resistance in reaction to the dose rates of a fungicide and the mixing or alternation of fungicides. Wherein many of those studies showed either introduction of resistant strains (Metcalfe *et al*. 2000; Genet *et al.* 2006; Mavroeidi and Shaw, 2006) or presence of these resistant strains at a significant frequency at the start of experiments (LaMondia, 2001; McCartney *et al*. 2007; Thygesen *et al.* 2009).

**Models used to evaluate resistant management strategies:** The available literature explains the response in development of fungicide resistance based on the dose rate, frequency of spray and coverage of fungicide (Hall *et al.* 2004; Hall *et al.* 2007; Parnell *et al.* 2005; Parnell *et al.* 2006 and Shaw, 2000). Almost the models described in all of these above studies were deterministic and hence unable to explain the stochastic nature of the dynamics of the resistant strain in the emergence phase. The literature available till now described only two models to explain the dynamics of the resistant strain during a part of the emergence phase as well as during the selection phase. In one such first model explained the dynamics of the resistant strain in the emergence phase by using a stochastic model and the dynamics of the resistant strain during the selection phase by using a deterministic model as described by Hobbelen *et al*. (2014). The other such in second model briefed about the resistant strain with introduction at the start of simulations and its dynamics in response to applications of a high-risk fungicide by using a stochastic model. There was no any apparent distinction between the emergence phase and the selection phase and the effect of the dose rate of a fungicide on the length of the emergence time in this model.

A model was developed to be used for evaluating the resistance management strategies, the effect of dose rate of a high resistance risk fungicide on the emergence time of resistance in a population of *Mycosphaerella graminicola* on winter wheat was determined for different mutation probabilities, fitness costs of resistance and sensitivity levels of the resistant strain (Hobbelen *et al.* 2014). It explains that decrease in the fitness of resistant strain to sensitive strain with increase in fitness cost of resistance or increasing the sensitivity of the resistant strain to the high-risk fungicide, increases the emergence time. This model could be applied to many foliar pathosystems on determinate crops to describe the emergence of resistance in the pathogen population. Without fungicide, the resistant strain invades by mutation in the pathogen population, but due to fitness cost of resistance makes it preventing from buildup to large number of population. Thus the temporal dynamics with alternation of short periods occurs either in the presence or absence of resistant strain without the application of fungicides.

Hobbelen *et al.* (2014) presented for the first time a model structure to describe the emergence of resistance to high-risk fungicides in a pathogen population. The model consists of a deterministic sub-model to describe the dynamics of the host and the sensitive pathogen population. The effect of the dose rate of a high-risk fungicide was determined on the number of years concluded that high-risk fungicide can provide efficient disease control in the selection phase for the same host-pathogen system (Hobbelen *et al.* 2011; Hobbelen *et al.* 2013). A mathematical model was developed to simulate an epidemic and the resistance evolution of *Zymoseptoria tritici* on winter wheat, which was used to compare different combinations of seed and foliar treatments by calculating the fungicide effective life, i.e. the number of years before effective disease control is lost to resistance (Kitchen *et al.* 2016).

**Scenario of fungicidal resistance in india:** In India, development of resistance to various site-specific fungicides is well known to occur in some plant pathogens under field conditions. Several cases of adaptive resistance to many fungicides including multi site-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 by increase in fungicide concentrations, exposure to UV radiations and chemical mutagens have been employed to study resistance development in diverse group of fungi to dithiocarbamates, copper-based, oxathiins, benzimidazoles, organophosphorus, phenylamides, alkyl phosphonates, morpholines and antifungal antibiotics in the laboratory (Thind, 2002). Reported cases of fungicide resistance under field situations in India are as follows; Fungicide Carbendazim against pathogens *Venturia inaequalis* (Apple), *Gloeosporium ampelophagum* (Grapes), *Aspergillus flavus* (Groundnut) and *Cercospora beticola* (Sugarbeet). Fungicide Edifenphos against pathogens *Bipolaris oryzae* and *Pyricularia oryzae* (Rice). Metalaxyl against *Plasmopara viticola* (Grapes), *Phytophthora infestans* and *Phytophthora parasitica* (Citrus), *Pseudoperonospora cubensis* (Cucumber). Fungicide Oxadixyl against *Phytophthora infestans* (Potato) and Triadimefon against *Uncinula necator* (Grapes) (Thind *et al.* 2008, 2009, 2011 & 2016).

Studies conducted during the year 2000 to 2004 revealed that carbendazim resistant isolates were persistent in natural populations and could be detected frequently in the Grape vineyards around Ludhiana in Punjab state (Mohan *et al.* 2005). During the year from 2005 to 2008 crop seasons in potato, *Phytophthora infestans* populations collected from different potato growing areas were tested for metalaxyl sensitivity, among these 10 populations showed resistant response causing infection at 200 ìg/ml of metalaxyl (Thind *et al.* 2001; Kaur *et al.* 2010). Phenylamide fungicides are frequently used by farmers in various states of India to manage downy mildew infection on cucurbits such as cucumber and melons. During the year 2007 and 2008, sensitivity changes were found to occur in metalaxyl against *Pseudoperonospora cubensis* populations collected from cucumber and muskmelon fields (Thind *et al.* 2011). Metalaxyl based fungicides seems to commonly used for managing foot rot of citrus, *Phytophthora parasitica* in different states of India. A significant reduction in fungicide efficacy has been observed in many orchards over the years. Cymoxanil is providing effective control of foot rot in the orchards where metalaxyl resistance found to be 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).

**IMPACT OF FUNGICIDAL RESISTANCE**

**On the farmer:** Several cases of serious resistance have been occurred in practice. The fungicide resistance has caused impact of farmer thereby to lose his profit due to the attack by the resistant strains on the crop. Example for such cases is resistance to pyrimidines, then to benzimidazoles and to acylalanine metalaxyl group. Pathogen such as apple scab (*Venturia inaequalis*) developed resistance to benzimidazole group, late blight pathogen *(Phytophthora infestans*) developed resistance to metalaxyl group of fungicides. Before discussing about the economic impact of resistance on farmers, precise loss of crop should be understand thoroughly.

**On the consumer:** The consumer may not be immediately affected by the mechanism of fungicide resistance. The consumer will realize that resistance has become problem in practice when due to severe losses caused by resistant pathogens, affects either cost of food or commodities like flowers or fruits, locally and temporarily unavailable. In the developed countries, with their broad international base of food supply, the consumer may not be affected. Wherein in developing countries, if the resistance occurred in staple crop or high value export crop, it might have immediate impact on the nation populations and its economies.

**On the adviser:** The main duty of an extension officer is to advise the farmer which fungicides to use at what time with what spraying schedule, to obtain an optimal yield. The adviser collects or generates his knowledge from technical information from the manufacturer and from his own experimental work. Further, he relies on the findings of workers active in fungicide research. Nobody can blame advisers for not having foreseen the risk of resistance to the first systemic fungicides appearing on the market, the benzimidazoles and pyrimidines, which were widely used by farmers because of their attractive qualities as fungicides.

**On the agrochemical industry:** The agrochemical industry has been exposed to resistance to insecticides for several decades. During the time development of resistance may have meant a short commercial life for a new product in a certain

market segment. However, it never led to the complete disappearance of an insecticide from the market. Indeed, some observers believe that resistance was one of the driving forces for the development of new insecticides. In addition to this, increasing research efforts have been made over the past years to understand the various aspects of resistance as a biological phenomenon and to develop strategies. Thus industry could adopt a simple 'wait and see' attitude on fungicide resistance. Chemical companies need the collaboration of agricultural advisers to encourage and enforce promising strategies in farming practices and to educate the farmer in usage of new concepts.

**Fungicide resistance management strategies:** The best approach for resistance management to fungicide is to devise spray plan consisting of both combinations and alternating of fungicides with different modes of action. An at-risk fungicide should be used only in the season when absolutely necessary. In order to use at-risk fungicide, another product with different mode of action should be combined. The combination helps to slow the selection of the rate of resistance and control of resistant strains already present. The following are the points to be remembered for planning spray program includes 1. The number of applications with high and medium-risk fungicides to be limited, 2. Usage of fungicides with different modes of action, 3. Usage of the right dose to control the disease, 4. Alternation or rotation of fungicide with different classes and 5. Fungicides from different classes are mixed that are effective against the same disease. Integrated Disease Management (IDM) offers an important contribution to delay and possibly to avoid fungicide resistance. Cultivation of resistant cultivars, agronomic and crop hygienic measures and biocontrol agents aids to reduce level of disease and represent that fungicides are used less frequently to decrease selection pressure over the pathogen. Evaluation and introduction of anti-resistance management strategies is the responsibility of manufacturers. In order to succeed, strategies should be applied over large areas and then to monitor their performance of fungicide. The biggest challenge remains with farmers to integrate resistance management within effective and profitable disease control strategies (Hollomon, 2015b).

## CONCLUSION

Fungicide resistance is one of several possible causes for reduction in disease control. Fungicide resistance not only threatens the usefulness of individual fungicides, but also the farm economy because of impending yield losses due to reduced control of disease. For the newly developed fungicides, assessment of risk seems to be significant. Mode of action group and resistance management strategies are now clearly included on the registration labels of most site-specific fungicides. As fundamental research in genetics, biochemistry and epidemiology will increase understanding of factors which influence risk in usage of fungicides. In future research should focus to search new modes of action of fungicides involving inhibition of metabolic processes to enhance lower risk of resistance development for fungicides. Still more studies to be focused on the field nature of resistant biotypes, biochemical and genetic basis of resistance, with their mechanism of interaction with other strategies. Several site specific fungicides are used in Indian agriculture to control different crop diseases. Since the report of fungicide resistance for commonly used fungicides under field conditions shown that the new generation fungicides can be given utmost priority in managing plant diseases. Before the introduction of such new generation fungicides for farmers usage, risk assessment seems to be crucial. New technologies are required to monitor the performance of resistance management strategies and to predict problems before they occur. Utmost priority should be given to know about the nature of genetic causes for resistance and that is based on the studies of laboratory mutants and also field isolates. There is no doubt at all that method of chemical control will always be required to maintain reliable crop yields of good quality. Major improvement in research effort, better interaction between industry, quality research in public-sector, registration authorities and valid publication of information seems to be beneficial in near future. Through the integration of usage of fungicide along with other approach of disease management which includes agronomic practices and use of resistant cultivars, farmers have a key role to play in delaying selection for resistance by reducing the application of fungicides. Moreover the farmers should have the awareness to manage the field level occurrence of fungicide resistance by adopting the integrated disease management strategies effectively. Thus by adopting effective fungicide resistance management strategies, the residual hazards to the environment due to repeated use of fungicide can be minimized wherein the agro-ecosystem can be protected in near end and with aim to double the farmer's income by the year 2020.

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