REVIEW OF LITERATURE

Diseases of crop plants caused by pathogenic organisms are managed by following various management practices. Among various management practices, use of fungicides apart from certain drawbacks, is considered to play an important role in reducing losses due to plant diseases. According to Hewitt (1998) several incidents of devastating effects of diseases during 18^{th} and 19^{th} centuries compelled the growers to try various ordinary chemicals like Ammonium carbonate, Copper carbonate, Sulphur, Copper sulphate, Sodium chloride etc. Millardet and Gayon (1887) had discovered the use of Bordeaux mixture to control downy mildew of grapes. Since then the beginning of commercial use of fungicides on a large scale was started.

The agrochemical firms become more active and developed many types of non-systemic, protectant fungicides like Dithiocarbamates, Phthalimides, and Sulphur, copper based, Crotonates and Quintozenes. The non-systemic fungicides were unable to control pathogens already established within the plant parts. This difficulty was overcome by introducing systemic fungicides in 1960. In 1970 there were many classes of fungicides which were mostly systemic viz. Phenylamides, Benzimidazoles, Oxathiins, Morpholines, 2- aminopyrimidines, Phosphothiolates, Phenylamides (Thind, 2007).

According to Gullino *et al.* (2000) during the past decade more new compounds with different modes of action have been developed by agrochemical companies. These are Quinolines, Strobilurines, Phenylpyrrols, Anilinopyrimidines and Probenazole. These are effective at low concentrations. Presently there are more than 150 fungicides used to manage different crop diseases.

Out of total pesticides sales of $32.5 billion, the total value of fungicidal sale is $6.0 billion. European countries use highest fungicide (40%) followed by Japan (28%) and USA (21%) (Thind, 2007). In India use of pesticides is 500g/ha, in Japan 12000g/ha, in Europe 3000g/ha and 2500g/ha in USA (Singhal, 2000.) According to Krishna *et al.* (2004) the current fungicidal use in India is 19% as compared to 61% of insecticides and 17 %of weedicides.

Benzimidazoles such as carbendazim and benomyl were introduced in 1960 (Delp and Klopping, 1968). These were used to control a variety of plant diseases.

Castor (*Ricinus communis* Linn.) is one of the important oilseed crops of our country. Many varieties of castor are perennial trees and some of them are annual crops. Castor is cultivated with other crops. It is also grown on field bands and backyards. Castor oil is used for various purposes. The seedcake of castor obtained

after extraction of oil is used as manure. The castor crop suffers from rust, powdery mildew, leaf spots and blights. The **blight of castor** caused by ***Alternaria ricini* (Yoshii) Hansf.** in several parts of India including **Western Maharashtra.** This disease is managed by using carbendazim (Chatta, 2005).

Horsfall published his book entitled **'Fungicides and their action'** in 1945. He had not mentioned resistance of pathogens to fungicides in his book. But in 1956 he mentioned a topic on acquired fungicidal resistance in plant patogens. Georopoulos and Zaracovitis (1967) and Dekker (1977) had mentioned cases of fungicidal resistance. According to Horsten (1979) resistant strains of *Septoria nodorum* and *Cercosporella herpotrichoides* were developed spontaneously on the agar plates containing 2X MIC of carbendazim. According to Staron and Allard (1964) the compound Thiabendazole was first used in 1961 as an antihelminthic and later on in 1964 as an antifungal. At present carbendazim and many other chemicals are in market. The primary site of these chemicals is nucleus of pathogenic fungi. They inhibit or disrupt mitosis by forming complete subunits of microtubules and prevent normal assembly into mitotic spindle fibers (Davidse, 1973). According to Van Tuyl (1975) development of resistance in fungi is therefore mainly due to low binding activity of the tublin protein.

Resistance problem in the field has lead to breakdowns in the management of many pathogenic fungi. Many cases of carbendazim resistance have been recorded in descending order (Table 1). The countries like USA and UK have maximum cases of fungicide resistance in the world. In India attention towards fungicide resistance is paid by Gangawane (1981), Annamalai and Lalithakumari (1987, 1990), Arora *et al.* (1992), Kamble (1993), Chander and Thind (1995).

Fungicide resistance of a pathogen can be determined by many ways (Dekkar, 1982). The most common method is to grow the spore or any other propagule of the pathogen on agar medium containing different concentrations of fungicides and find out minimum inhibitory concentration (MIC). Only those cells of pathogen which have acquired fungicide resistance by mutation or in any other way will survive and form the new colony. Frequency of such mutant due to spontaneous mutation or chemical mutagens or UV etc. can be calculated. Van Tuyl (1977) obtained benomyl and thiabendazole resistant mutants of *Aspergillus nidulans*, *Cladosporium cucumerinum*, *Penicillium expansum* and *Ustillago maydis* by treating them with UV in order to study genetics of fungicide resistance. UV treatment to *Aspergillus flavus*

isolates increased the frequency of carbendazim resistant pathogen on groundnut pods. Transfer of isolate through five successive passages also increased the resistance in the pathogen (Gangawane and Reddy, 1985). According to Reddy (1986) irradiation with gamma rays at 500 rods emerged more carbendazim resistant mutant from *Aspergillus flavus* strains Af-28. Chemical mutagens such as EMS and MNNG were tested in this laboratory for carbendazim resistant isolates. EMS was more powerful in inducing carbendazim resistance in *Aspergillus flavus* than MNNG. Emergence of carbendazim resistance in *Puccinia arachidis* causing groundnut rust was higher due to spontaneous mutation (18/100 spores) than that of EMS (13/100 spores), UV and gamma rays (1/100 spores). Sodium azide did not induce any resistance in the pathogen when tested by detached leaf technique (Kareppa, 1990). *Macrophomina phaseolina* isolates causing charcoal rot of potato when treated with MNNG and SA gave 6 and 4 carbendazim resistant mutants. Resistance factor of these mutants ranged from 9 to 15. Mutants having high resistant factor also showed higher growth and all mutants were persistent for carbendazim resistance (Kamble, 1991). According to Bhale (2002) *Alternaria tenuissima* isolates causing leaf spot of spinach when treated with UV, NMU and SA gave 14, 27 and 17 carbendazim mutants. Their resistant factor ranged from 2 to 3. Mutants having high resistant factor showed higher growth and all the mutants were persistent for carbendazim resistance. Resistant mutant showed lower infection percentage on leaves when compared with the wild sensitive AT-15. *Alternaria alternata* sensitive isolate causing fruit rot of pomegranate when treated with UV, EMS, 5-bromouracil and SA gave 2, 25, 2 and 4 carbendazim resistant mutants respectively. Resistant factor ranged from 2 to 5. Mutants having higher resistant factor showed lower growth rate. All the mutants were persistent for carbendazim resistance (Bharade, 2002). According to Wadilkar (2002) *Macrophomina phaseolina* sensitive isolate causing charcoal rot of pigeon pea when treated with UV, EMS and SA gave 10, 24 and 17 carbendazim resistant mutants respectively. Resistant factor ranged from 3 to 5. Mutants having higher resistant factor showed slow growth rate. All the mutants were persistent for carbendazim resistance. *Sclerotium rolfsii* sensitive isolate causing fruit rot of *Cucumis sativus* when treated with UV and 5-bromouracil gave 29 and 15 carbendazim resistant mutants respectively. Resistant factor ranged from 6 to 9. Mutants having higher resistant factor showed slower growth rate and all the mutants were persistent for carbendazim resistance (Hiwale, 2003).

The emergence of resistant mutants in the laboratory to the fungicides does not imply that use of these fungicides in the field also lead to the failure of disease management. This will happen only after considerable proportion of the pathogen population has become resistant to the fungicides. The fungicide resistance in the pathogen depends upon many factors such as mode of action of fungicides, usage and fitness of the resistant mutants in relation to that of wild pathogen. The laboratory studies may be of great use in forecasting the resistance development in the pathogen against a particular fungicide.

Fungicide Resistance in the Field:

Until 1970, acquired resistance in the field was insignificant, but more serious problem emerged when benizimidazole fungicide **'Benomyl'** was introduced. Schroeder and Providenti (1969) were the first to ascertain high benomyl resistance in the strains of *Sphaerotheca fuliginea* causing powdery mildew of cucurbits.

According to Brent (1978) fungicide resistance in *Sphaerotheca fulginea* was reported from several countries. Vargas (1973) reported benomyl resistance in *Erysiyphe graminis* in U.S.A. Dekker (1977) found acquired resistance in *Botrytis cinerea, Penicillium corymbiferum, Cercospora spp.* and *Venturia inaqualis* in glass house ornamentals. Dovas *et. al.* (1976) found that *Cercospora beticola* causing sugar beet disease was resistant to benomyl and benomyl resistance was persistent even after the use of benomyl was discontinued. Jones and Ehert (1981) detected carbendazim and thiophanate and benomyl resistance in strains of *Coccomyces liemalis* causing leaf spot of cherry. Strains of *Penecillium digitatum* were tolerant to benzimidazole fungicide than the wild sensitive (Gutter *et al.*, 1981). Leroux and Besselat (1984) reported the benzimidazole resistance in *Botrytis cinerea* causing diseases of grapes. Gullino and Garibaldi (1986) devised a method for detection benzimidazole resistance in *Botrytis cinerea. Penicillium* digitatum causing citrus disease was also resistant to benzimidazoles (Kiely, 1971; Eckert, 1982; Wild, 1984; Loyan, 1978; Gullino and Garibaldi, 1986). Pan and Sen (1980) reported carbendazim resistance in *Macrophomina phaseolina.* Gangawane and Saler (1981) reported fungicide resistance in *Aspergillus flavus.* Chaudhary and Putto (1984) reported fungicide resistance in *Venturia inaequalis.* Arora *et. al.* (1992) reported fungicide resistance in *Phytophthora infestans* in Nilgiri Hills of Southern India.

Characteristics of Resistant mutants:

Fungal mutants resistant to a particular fungicide may vary in pathogenecity and fitness. According to Van Tuyl (1977) there was wide variation in pathogenecity of strains of *Penecillium expansum* resistant to Benomyl. Horseten (1979) observed that carbendazim resistant strains of *Septoria nodorum* were also pathogenic to wheat plant at the same level of sensitive strains. He also found that carbendazim resistant strain of *Cercosporella herpotrichoides* produced their spores at 1000µg/ml carbendazim *in vitro.* They also produced more spores / more mycelium when cultured in the presence of carbendazim than its absence. It seems the characteristics of carbendazim resistant strains were very similar to carbendazim sensitive strain in the absence of fungicide.

Venturia inaequolis resistant to carbendazim was pathogenic but differed in nature and virulence. Benomyl resistant isolates of *Cylindrocarpon sp.* and *Fusarium oxysporum* were highly pathogenic to *Lillium speciosum* (Bollen, 1983). Wild and Eckert (1982) found that 96% of benzimidazole resistant isolates of *Penicillium digitatum* were pathogenic as benzimidazole sensitive ones on orange, but 4% isolates of *Penicillium digitatum* were less pathogenic than benzimidazole sensitive ones. *Penicillium digitatum* strains resistant to carbendazim appeared to be more virulent than sensitive isolates (Kuramoto, 1976). According to Schriber and Gregory (1980) there was no difference in the pathogenecity of benzimidazole resistant and sensitive strains of *Certocystis ulmi.* Carbendazim resistant strains of *Aspergillus flavus* were more infective while some of them have lost their infectivity. (Gangawane and Reddy, 1986).

Management of Carbendazim resistance in plant pathogens:

To avoid fungicide resistance in pathogens, one should consider type of fungicides, type of pathogen and alteration in method of application (Dekker, 1981). Genetic changes in a pathogen leading to fungicide resistance do occur more rapidly with a specific site inhibitor.

Accessibility of the pathogen to fungicide multiplication, infection threshold, fitness and life cycle are important criteria to be considered to avoid fungicide resistance. In addition to above mentioned things amount of frequency of fungicide treatment, efficacy of treatments and altered / combined application of fungicides has been found to be more useful in managing resistance in carbendazim. Combination of

benomyl with captan reduced the rate of infection of plants by *Venturia inaqualis* (Shabi and Gilpatrick, 1981).

According to Horseten (1979) *Cercosporella spp.* were cultured on medium containing **carbendazim** and **naurimol** alternatively were unable to grow after first being cultured on plates containing **naurimol**.

Wild and Eckert (1982) suggested different plans for the management of benzimidazole resistant strains of *Penicillium* causing rot of citrus fruit. These included sanitation of packing house with formaldehyde and quaternary ammonium compounds. These compounds reduced the build up of resistance in pathogen. Hot solutions of boron and sodium carbonate also reduced the citrus fruit infection. A mixture of captan and benomyl was also able to prevent penetration of benzimidazole resistant strains of *Penicillium* into treated citrus fruits. Mixture of imazalil and benomyl also control the resistant strains of *Penicillium*. According to Eckert (1978) *Penicillium* infection of citrus fruit can be controlled by treating the fruit with soap and secondary butyl amine before and after storage and also with soap and benomyl. benzimidazole resistant strains of *Penicillium italicum* and *Penicillium digitatum* can be controlled by treating them with unrelated chemicals like eta conazole flusilazole. Hartill (1979) found that use of procymidose with vinclozoline can control benzimidazole resistant strains of *Botrytis cinerea*. Benomyl and captafol in combination reduced the infection of *Cylindrocarpon spp* on Lily (Bollen and Van Zaayen, 1975).

Gangawane and Reddy (1986) observed that application of chemicals other than fungicides in combination with carbendazim gives promising results in management of carbendazim resistant *Aspergillus flavus*. Gangawane and Kamble (2001) reported that carbendazim in combination with Cu, Mo, Al and Co decreased the resistance in *Macrophomina phaseolina* causing charcoal rot of potato. 'Al' with carbendazim was more powerful in reducing carbendazim resistant *Aspergillus flavus* (Gangawane and Reddy 1988).

According to Elad et al. (1992) benzimidazole resistant strains of *Botrytis cinerea* can be controlled by using a mixture of benzimidazole with dietofencarb. Gangawane and Kamble (1993) found that use of carbendazim with difolatan, dithane M-45 completely inhibited the carbendazim resistant isolates of *Macrophomina phaseolina* causing charcoal rot of potato. Use of carbendazim alternately with difolatan, zineb and mancozeb significantly reduced the carbendazim resistance at 8th

passages. Carbendazim in combination with mancozeb and zineb completely inhibited the growth of *Macrophomina phaseolina* isolates causing charcoal rot of potato resistant to carbendazim (Kamble and Gangawane, 1999). Latha et al. (2000) reported that a mixture of carbendazim and carbofuran prevented the infection of *Macrophomina phaseolina* to *Vigna mungo*.

Application of carbendazim with insecticides, antibiotics, weedicides, salts, fertilizers and micronutrients inhibited the growth of *Macrophomina phaseolina* causing charcoal rot of potato both *in vitro* and *in vivo* (Gangawane and Kamble, 2001).

According to Bhale (2002) synergistic effects of Agrochemicals inhibited the growth of carbendazim resistant isolates of *Alternaria tenuissima* causing leaf spot of of spinach.

Table 1: Acquired Resistance of pathogens to Carbendazim Fungicides in different countries.

Sr.No	Carbendazim	Authors and countries
1	*Aspergillus flavus*	Gangawane and Saler (1981); Gangawane and Reddy (1985), India.
2	*Aspergillus nidulans*	Davidse (1976), Netherlands; Georgopoulos (1982), Greece.
3	*Botrytis cinerea*	Geeson (1978), U.K.; Tripathi and Schloesser (1982), W. Germany; Delen and Yildiz (1981), Turkey; Leroux and Clerjeau (1985), France.
4	*Ceratocystis ulmi*	Gibbs and Brasier (1980), U.K.
5	*Cercosporidium personatum*	Katan and Shabi (1981), Israel.
6	*Cladosporium spp*	Delen and Yildiz (1981), Turkey.
7	*Coccomyces hicmalis*	Jones and Ehret (1981), U.S.A.
8	*Colletotrichum coffeanum*	Okigga (1976), Kenya.
9	*Fusarium oxysporum* f. sp. *dianthi*	Laeski (1977), Poland.
10	*Fusarium oxysporum f. sp.curcumerinum*	Delen and Yildiz (1981), Turkey.
11	*Macrophomina phaseolina*	Pan and Sen (1980); Kamble

		(1991); Gangawane and Kamble (1993), India.
12	*Pseudocercosporella herpotrichoides*	Horsten and Fehrmann (1980), Netherlands.
13	*Puccinia arachidis*	Gangawane *et al.* (1988), India.
14	*Rhizoctonia solani*	Delen and Yieldiz (1981), Turkey.
15	*Sclerotinia sclerotiorum*	Delen and Yieldiz (1981), Turkey.
16	*Septoria leucanthemi*	Paulas *et al.* (1976), U.S.A.
17	*S. nodurum*	Horston and Fehrmann. (1980), Netherlands.
18	*S. tritici*	Fisher and Griffin. (1984), U.K.
19	*Sporobolomyces roseus*	Nachmias and Barash (1976), Israel.
20	*Venturia inaequalis*	Kiebacher and Haffmann. (1976), W. Germany; Chaudhary and Putto (1984), India.
21	*V. pirina*	Shabi and Ben Yephet (1976); Shabi and Katan (1980), Israel.
22	*Verticillium fungicula*	Bollen and Van Zaayen (1975), Netherlands.