Discussion of *Alternaria alternata*

In the present investigation MIC of carbendazim against *Alternaria alternata* is ranging from 1.5 to 5% *in vitro* and 1 to 4% *in vivo*. According Annamalai and Lalithakumari (1990) it is essential to determine the base line sensitivity for fungicide against a sensitive strain. In this experiment the fungus *Alternaria alternata* showed variation in MIC of carbendazim both in *vitro* and in *vivo*. This type of result is also reported by many workers. MIC of carbendazim against *Alternaria tenuissima* causing leaf spot of spinach was ranging from 350 to 700 µg /ml and 110 to 321 µg /ml in *vitro* and in *vivo* respectively (Bhale, 2002). According to Wadikar (2002) MIC of carbendazim against *Macrophomina phaseolina* causing charcoal rot of pigeon pea was ranging from 80 to 140 µg /ml and 65 to 130 µg /ml in *vitro* and in *vivo* respectively. The MIC of carbendazim against *Sclerotium rolfsii* the incitant of fruit rot of *Cucumis sativus* ranged from 1300 µg /ml to 6000 µg /ml in vitro and 1500 to 4000 µg /ml in *vivo*. Telmore (2004) detected the MIC of *Fusarium semitectum* causing leaf spot of betelvine ranged from 1000 to 2000 µg /ml in *vitro* and 1000 to 1500 µg /ml in *vivo*. MIC of carbendazim against *Macrophomina phaseolina* the incitant of charcoal rot of sweet potato on agar plates ranged from 1 to 500 µg/ml while it was 0.5 to 25 µg /ml on sweet potato (Patil, 2009). More (2009) detected the MIC of *tilt* against *Phakopsora pachyrhizi* which was in the range of 3 to 36 µg /ml on soyabean leaves. Experiments were carried out on rose plant to determine whether development of carbendazim resistance in *Alternaria alternata* could be delayed or prevented by continuous alternate or in mixture of two different fungicides. In the present investigation it was found that culturing of pathogen on agar plates contain carbendazim continuously for eight successive passages significantly increased the
carbendazim resistance in the pathogen. But use of carbendazim alternately with benomyl, Ridomil MZ, Roko and Dhanuka for eight successive passages significantly reduced the carbendazim resistance both in vitro and in vivo. While in mixture with above stated fungicides carbendazim completely inhibited the pathogen. The alternatively used fungicides must have different mode of action due to which there was less chance to mutate (Griffin, 1981). Horsten (1979) observed that there was reduction in carbendazim resistance in Septoria nodorum and Cercospora herpotrichoides due to the alternate use of ediphenphos. Kabble and Jaffery (1980) developed a mathematical model to detect the development of fungicide resistance in the pathogen treated alternately with different fungicide. Hartill (1983) advised to use maneb alternately with metalaxyl to control late blight pathogen of potato. Multisite action of carbendazim with mancozeb, benomyl, captafol and thiram was responsible for the complete inhibition of Macrophomina phaseolina causing charcoal rot of potato (Kamble, 1991). According to Bhale (2002) exposure of Alternaria tenuissima continuously to carbendazim for eight successive passages significantly increased the carbendazim resistance. But treatment of carbendazim alternately with thiram, captafol, and benomyl inhibited the growth of Alternaria tenuissima causing leaf spot of spinach. Further he recorded that use of carbendazim in mixture with thiram, captafol and benomyl completely inhibited the pathogen of spinach at 7th passage. Similar results are reported by some workers in case of different fungal pathogen with different fungicides (Wadikar, 2002; Bharade, 2002; Hiwale, 2003; Telmore, 2004; Apte, 2008; Mane, 2009; Patil, 2009; More, 2009 and Patil, 2009).

A comparative account of physiological and biochemical characteristics of carbendazim sensitive and resistant isolates of Alternaria alternate were studied.
Growth rate of resistant isolate was higher on all sugars. Lactose inhibited growth of pathogen. The growth of resistant isolate was higher on nitrogen sources (Peptone, Potassium nitrate and ammonium nitrate), Sulphates (magnesium sulphate). There are some reports supporting this view. According to Kamble (1991) carbendazim resistant isolate of *Macrophomina Phaseolina* causing charcoal rot of potato showed higher growth than sensitive isolates on sugars (Sucrose, dextrose, fructose), nitrogen (peptone, potassium nitrate, calcium nitrate, sodium nitrate and ammonium nitrate). Phosphates (Potassium dihydrogen orthophosphate), Salts (calcium chloride, cadmium chloride and tin chloride), vitamins (vitamin B and vitamin B12). Similar findings are also observed by other workers also (Bhale, 2002; Wadikar, 2002; Bharade, 2002; Hiwale, 2003; Telmore, 2004; and Patil, 2009.) A comparison of certain biochemical characteristics of carbendazim resistant and sensitive isolate showed that total sugars, reducing sugar, DNA, RNA were reduced due to infection of *Alternaria alternata*. There are many reports indicating changes in the chemical characteristics in host due to infection by different pathogens (Johansan *et al*; 1967; Jennings *et al*; 1969; Gangawane and Datar, 1978 Salt *et al*; 1978). Total sugar, reducing sugar, non reducing sugar, nitrogen and phosphorus was reduced due to infection by early and late leaf spot pathogens of groundnut (Sindhan and Parashar, 1996). Ushamalin *et al*; (1998) found that there was decrease in total sugar, reducing sugar, non reducing sugar, and protein in cowpea seed due to seed borne fungi. The concentration of total sugar, reducing sugar, were decreased due to infection by *Colletotrichum gloeosporioides* to mungo leaves (Tofazzal Hossain, *et al*; 1999). Beniwal *et al*., (2008) observed that there was reduction in total sugar, reducing sugar in wheat plants infected by *Urocystis agropyri*. Patil (2009) reported reduction in reducing sugar,
DNA, RNA, calcium manganese and zinc in sweet potato roots due to infection by Macrophomina phaseolina causing charcoal rot of sweet potato.

Synergistic effect of carbendazim with other agrochemicals on the fungicide resistance in Alternaria alternata were observed in vitro and in vivo. This was carried out because many of these agrochemicals may be mixed with carbendazim in crop disease management. In vitro investigation it was observed that Kavach, dunet, krinet, monasan, copper, managanese, zinc, and cobalt with carbendazim inhibited the growth of the pathogen.

In vivo studies ridomyl, kavach, dunet, krinet, monasan, potassium chloride, muriate of potash, DAP, copper, manganese, cobalt, zinc, streptomycin, oflaxacine-400, cefixime, sencor, sodium chloride, calcium chloride, iron, urea, kocide, kleen, krizin, samarth, atrazin, magnesium chloride, and griseofulvin, with carbendazim prevented the infection of Alternaria alternata to rose plants. There are reports supporting to these results. Shabi and Gilpatrick (1981) reported that captan, chlorothanil and imazalil with benomyl reduced benomyl resistance in Venturia inaequalis causing apple scab. Eckert (1982) observed that there was reduction in benomyl resistance in penicillium digitatum when benomyl was used with captan, chlorothalonil and imazalil, fungicides and antibiotics used in mixture have different mode of action on target pathogen suggested by Griffin (1981). According to Gangawane and Reddy (1986) micronutrients in combination with carbendazim reduced carbendazim resistance in Aspergillus flavus the incitant of collar rot disease of Arachis hypogea. Application of fungicides (difolan, dithane, thiophonate methyl and zineb), insecticides, (paramer, endosulphan, thimate and sumicdin), antibiotics, (mycostatin, aureofungin, griseofulvin and streptomycin), weedicides (gramoxone and
atrazin), salts (barium chloride, cadmium chloride and tin chloride), fertilizers (super phosphate and muriate of potash), micronutrients (Co, Bo, Zn, and Mb) with carbendazim reduced carbendazim resistance *Macrophomina phaseolina* the incitant of charcoal rot of potato (Kamble, 1991). Bhale (2002) found that benomyl in combination with captan, carbendazim thimate, pyribon, endosulphan, 2,4-D, atrazin, stomp, ampicillin, aureofulvin, streptomycin, magnesium chloride, mercury chloride, calcium chloride, cobalt chloride, potassium chloride, and micronutrients, cobalt, chloride, manganese, molybdenum, and boron completely inhibited the growth of *Fusarium oxysporum F.spinaeae* causing wilt of spinach. Similar findings were reported by other workers (Bharade, 2002; Wadikar, 2002; Hiwale, 2003; Telmore, 2004; More, 2009; Patil, 2009) in different pathogen using different systematic fungicides with agrochemicals. Use of acibenzolar – S-methyl with fungicides and antibiotics gave good control of coffee rust (Patricio et al; 2008). There is very serious problem of development of fungicide resistance in fungal pathogen against particular fungicide used. This is because of mutation in the pathogen subjected to systematic fungicides. These resistant mutants released in the natural population. Therefore it become very important to study the survival ability of these resistant mutants, which helps the management.

In present investigation it is observed that on the untreated rose plant the resistant population of *Alternaria alternatea* in the presence of sensitive population was reduced from passage to passage. But on the carbendazim treated rose plants the population of resistant population in mixture with sensitive population of *Alternaria alternata* increased from passage to passage. Similar observations are recorded by many workers in different pathogens (Davas, et.al; 1976; Holloman, 1978; Horsten,
spinosa, Alternaria tenuis, Curvularia lunata and Helminthosporium sps (Kolte and Salunkhe, 1973). According to Khanna and Chandra (1977) homeopathic drugs (Arsemicum albura, Thuja accidentalis and Biota orientalis) completely inhibited the spore germination in Alternaria alternata the incitant of leaf blight of wheat. Out of 10 medicinal plants used for antifungal activity against Curvularia lunata only two plants i.e. Cinnamomum comphora and Catharanthus roseous inhibited growth and sporulation in test fungus (Bhowmick and Vardhan, 1981). According Bhowmick and Choudhary (1982) there was complete inhibition of Alternaria alternata by the leaf extract of Acalypha indica. Shrivastava and Keoliyal (1984) observed that litter extracts of Adiantum edgewerthii, Chelianthes alabomarginata, polypodium steartii and Pteris cretica were inhibitory to the pathogenic fungi Alternaria alternata and Biopolaris oryzae. They further observed that leaf extracts of these plants were less effective against the fungi. According to Tiwari and Dath (1984) out of 23 plants only Ocimum sanctum, Lowsonia inermis, Nyctanthus arbortrits and Piper betle showed inhibitory effect against Pyricularia oryzae the incitant of blast of rice.

Out of 15 Mediterranean algae only Falkenbergia rufolumosa showed antifungal activity against 4 dermatophytes and 05 pathogenic yeast (El-Shami et al; 1986). According to Prasad and Ojha (1986) leaf extracts of Azadirchta indica, Catharanthus roseous, Cinnamomum camphora, Ocimum sanctum, plumbago zeylanica. Strychnos nuxvomica, Lantana camara and Vitex negundo were very effective against post harvest decay pathogens (Fusarium equisetii, F. semitectum and Curvularia lunata) of cucurbits. Sinha and Saxena (1990) observed that leaf extract of Chenopodium album was inhibitory to Aspergillus niger the incitant of fruit rot of tomato in presence of Drosophila buscleci. Out of 20 plants only Seseli indicum
showed inhibitory action against *Rhizoctonia solani*. (Khisore et al; 1989). Khan and Kumar,(1990) reported antifungal activity of *Azadirachta indica* against seed mycoflora of wheat. Latex of *Euporbia hirta* was very effective against *Aspergillus niger* causing fruit rot of tomato. Dubey and Dwivedi (1991) observed growth inhabitation of *Macrophomina phaseolina* by leaf extracts of *Acacia Arabica, Allium cepa and Allim sativum*. Ethanol leaf extract of *Croton sparsiflorus* was effective against *Pyriculari oryzae,Drechslera oryzae,Rhizoctonia solani,sarocladium oryzae,Alternaria tenuis, Colletotricthum capsici. C. coffeeanum and fusarium javanicum.* According to Manian and Udanian (1991)out of 50 angiospermic plants only *Allium sativum* and *Eugenia caryaphyllata* showed inhibitory action against pathogenic fungi (*Botryodiplodia theobromac, Corynespora cassicola, Drechslera oryzae, Pythium aphanidermatum, Sclerotium rolfsii and Thanetophorus cucumeris.*)

Ghewande et al., (1989) reported that leaf extract of neem reduced early leaf spot (ELS) of ground nut by 13.64% and late leaf spot (LLS) of the same crop by 18.73% at the same time Karani leaf extract reduced the disease by 18.9 and 15.73% respectively. According to Dubey (1991) fresh leaf extract of *Ocimum canum* and *Pinus rouxborghii* and fruit extract of *Medica Citrus* were very effective against *Macrophomina phaseolina*. Chandrasekhar and Balasubramanian (1991) noticed inhibitory activity of crude leaf extract of *Vinca rosea, Ocimum sanctum, Allium sativum, Partenium hysterophorus, Datura strumarium, Azadirachta indica and Thuja sinensis* against Downey mildew of Pearl millet.

Shenoi et.al (1993) noted that, leaf extracts of *Azadirachta indica* and *Lawsonia inermis* were very effective against *Pythium aphanidermatum, Phytophthora parasitica var. nicotioni, Colletotrichum tobacum* and *Alternaria alternata* all
pathogens infecting tobacco seedlings in nursery. Leaf extracts (in cold water, hot water and ethanol) of *Prosopis julifera*, *Adhatoda vasica*, *Vitex negundo*, *azadirachata indica* and *Polyalthia longifolia* showed inhibition of spore germination, mycelial growth and biomass production of *Pyricularia oryzae* causing blast of rice (Rajeshwari and Marriappa, 1993). Maharshi (1993) observed that neem formulations such as fresh neem leaf aqueous extracts, margocide Ck 80 EC and margocide CK 20EC were inhibitory against chilli diseases (dieback, fruit rot and bacterial blight). Thongvela et al. (1995) found that out of four neem formulations (neemgold, margocide, 20 EC, Hinosen and Repilin R.D. was significantly superior in inhibiting spore germination in *pyricularia oryzae*. According to Gohil and Valu (1996) only non sterilized phytoextracts of *Allium sativum* and *sapindus trifolia* showed antifungal activity against *Fusarium moniliformae* the incitant of wilt of Sugarcane. Qussem (1997) noticed that leaf extracts of *Anagalis foemina*, *Cerastium dichotomum*, *Falcaria vulgaris*, *Ranunculus asiaticus*, *Scorpius muricans* and *Solanum niger* were toxic to *Fusarium oxysporum F.sp.lycopersici* causing wilt of tomato.

Out of five plant extract only *Allium sativum* extract reduced the growth of *Pythium aphanodermatum* causing damping off chilli (kuruchere and Padmavati, 1998). Gehlot and Bohra (1997) observed that out of 11 plant extracts obtained from various organs of these plants only stem extract of *Hyponylon recurvum* inhibited the growth of *Alternaria solani*.

Tiwari (1997) found that volatile oil isolated from bark of *Cinnamomum zeylanicum* was antifungal to *Aspergillus flavus* and *Aspergillus niger* causing infection to paddy and maize in storage condition. Shivpuri et al., (1997) stated that ethanol extract of 10 flowering plants (*Allium cepa*, *Allium sativum*, *Calotropus*...
procera, Tagetes erecta, Vinca rosea, Datura strumarium, Azadirachta indica, Ocimum sanctum, Polyalthia longifolia and Withania sominifera) were inhibitory to Alternaria Brassicola, Colletotrichum capsulifer, fusarium oxysporum, Rhizoctonia solani and Sclerotinia Sclerotium. Leaf extracts of Moringa oelifera was antifungal against Thanetoporus cucumeris the incitant of bandol leaf blight of rice (Dubey, 1998). Sindhan et al. (1999) reported that onion bulb extract and Datura leaf extract were useful in controlling powdery mildew of pea. Out of 10 different leaf extracts only leaf extract of Hyptis suaveolens and Adencolyma allices gave maximum inhibition of mushroom bed weed fungi (Sing, 1999). Sharma and Nanda (2000) reported that garlic bulb extract completely inhibited the spore germination in Neossia indica the incitant of kernel bunt of wheat. Among the ten leaf extracts only Lantana camera, Eucalaptus citrodora, Eucalaptus adenophorum and Agave americana leaf extracts prevented the infection of rice blast pathogen to rice crop (Ashok kumar, 2006). Polyalthia longifolia, Allium sativum, Partenium hysterophorus were very effective against Macrophomina phaseolina the incitant of Charcoal rot of Sorghum sps. (Datar, 1999). Among the 20 leaf extracts, leaf extract of Oxyspora paniculata was most effective against pythium aphanidermatum (Bhat and Shukka, 2001). Withania sominifera leaf extract was found to be very effective against growth and spore germination in Aspergillus niger the incitant of tomato fruit rot (Dorgan and saxena, 2002). According to more (2009) alcoholic leaf extracts of Azadirachta indica, Polyalthia londifolia, Hyptis suaveolens, Mentha spicata and Ocimum sanctum significantly reduced the percent infection of Phakopsora pachyrhizi infecting soybean plants.