DISCUSSION

Blight of castor caused by ***Alternaria ricini* (Yoshii) Hansf.** is commonly occurring disease. It is very serious and destroys the crops in Maharashtra. castor is grown by intercropping along with Sugarcane. The blight of castor is managed by spraying Bordeaux mixture and other proper fungicides. The present study was therefore undertaken to examine the possibility of development of carbendazim resistance in *Alternaria ricini*. Efforts were made to employ standard techniques for studying various aspects of fungicide resistance suggested by FRAC.

In this investigation MIC of carbendazim against *Alternaria ricini* was 15%. The sensitivity of five isolates collected from various fields to carbendazim ranged from 15 to 20% on cultured plates. These results are in agreement with other workers. (Bhale, 2002; Bharade, 2002). It is essential to establish the base line sensitivity for the fungicide against a sensitive strain. (Annamalai and Lalithakumari, 1990).Therefore, on this basis the baseline sensitivity of *Alternaria ricini*, the isolates tested in this investigation. They were resistant to carbendazim. carbendazim resistance in 22 different pathogens is reported by many workers (Table: 1). All of them are of the opinion that built up of resistance in the pathogen occurs under selection pressure in the field environmental conditions.

Fungicide application programme may influence the development of resistance in the pathogen. Hence effect of successive passages on *Alternaria ricini* of carbendazim individually, alternately or in mixture of other fungicides with different mode of action was studied both *in vitro* and *in vivo*. Adaptation of *Alternaria ricini* to carbendazim during the passages might be major factor involved in the development of resistance. During the continuous passage there was increase in the carbendazim resistance in *Alternaria ricini*. While there was increase or decrease in the resistance of the pathogen during alternate passage. When carbendazim was altered with mancozeb and captan there was complete inhibition of the pathogen at passage II in mancozeb and Passage III in captan. But when carbendazim was altered with roko, the resistance of the pathogen increased. In mixed-passage there was complete inhibition of the pathogen at IInd passage. More or less similar results were obtained in *in vivo* conditions. The results are in agreement with other workers, in case of other pathogens (Reddy,1986; Horsten,1979; Griffin,1981; Gangawane and Shaikh,1988; Kareappa,1990; Waghmare,1990; Kamble,1991; Bhale,2002; Wadikar,2002; Hiwale,2003; Telmore,2004). The fungicides used alternately must have different mode of action (Griffin, 1981). In present study fungicides of different modes of action were used and therefore there must have less chance to mutate *Alternaria ricini* for the development of resistance. According to Horsten (1979) alternate use of ediphenphos in reducing carbendazim resistance in *Septoria nodorum* and *Cercosporella herpotrichoides*, Kable and Jaffery (1980) developed a mathematical model for alternate use of chemicals to reduce fungicide resistance. According to Hartill (1983) alternate use of metalaxyl with maneb controls the late blight of potato. Waghmare (1990) found that use of metalaxyl alternately with difolatan, carbendazim, thiram, captan, zineb and mancozeb reduced the metalaxyl resistance in *Phytophthora drechsleri* f. sp. *cajani* on agar plates and on pigeon pea plant as well. According to Kamble (1991) inoculation of *Phytophthora infestans* continuously on metalaxyl for four successive passages significantly increased resistance. But treatment of metalaxyl alternately with thiophanate methyl, difoltan and carbendazim completely checked the growth of the poathogen at 3rd passage only. Application of metalaxyl alternately with zineb did not exert any effect on the resistance in pathogen at 4th passage. Further he found that metalaxyl in mixture with carbendazim completely inhibited the infection of *Phytophthora infestans* on potato slices. Use of metalaxyl with mancozeb was more useful in reducing the resistance than thiophanate methyl and difoltan. According to Bhale, (2002) exposure of *Alternaria tenuissima* causing leaf spot of spinach continuously to carbendazim for eight successive passages significantly increased the resistance. But treatment of carbendazim alternatively with thiram and captafol inhibited growth of *Alternaria tenuissima.* Further he observed that exposure of *Fusarium oxysporum* f. *spinaciae* causing wilt of spinach continuously to benomyl for eight successive passages significantly increased the resistance, but treatment of benomyl alternatively with carbendazim completely inhibited growth of the pathogen at 6th passage. Use of benomyl in mixture with captan and mancozeb completely inhibited the pathogen at 5th passage. While benomyl in mixture with carbendazim completely stopped growth of the pathogen at 7th passage. Culturing of the sensitive isolate of the *Alternaria alternata* causing fruit rot of pomegnate on carbendazim continuously for seven passages significantly increased fungicide resistance (Bharade,2002). Further she noted that use of carbendazim alternately with kavach, captafol and diathane-M-45 reduced fungicide resistance in *Alternaria alternata* significantly. Carbendazim when used in mixture with kavach, captafol and diathane-M-45 inhibited the growth of the pathogen at second, third and fifth passage respectively on agar plates. But on pomegranate fruits all the three fungicides with carbendazim inhibited the growth of the pathogen at first passage only (Bharade, 2002).

Similarly Wadikar (2002) observed that culturing of the sensitive *Macrophomina phaseolina* isolate causing charcoal rot of pigeon pea on carbendazim continuously for eight successive passages significantly increased the resistance. Further he found that use of carbendazim alternatively with captafol, mancozeb, thiram and benomyl completely reduced the resistance significantly. Carbendazim in mixture with captafol and mancozeb reduced the carbendazim resistance in the pathogen significantly. Hiwale (2003) noticed that the sensitive isolate of *Sclerotium rolfsii* causing fruit rot of *Cucumis sativus* when cultured on carbendazim containing agar medium for eight successive passages significantly increased the fungicide resistance but use of carbendazim alternately with thiram and mancozeb reduced the resistance significantly. He also found that use of carbendazim in mixture with benomyl completely inhibited the growth of the pathogen at first passage itself. When carbendazim was used in mixture with mancozeb and thiram the *Sclerotium rolfsii* failed to grow after second and third passage respectively both *in vitro* and *in vivo*.

Culturing the sensitive isolate of *Fusarium semitectum* causing leaf spot of betelvine on agar plates continuously for four passages significantly increased the benomyl resistance. (Telmore, 2004). He further observed that use of benomyl alternately with carbendazim and kavach reduced benomyl resistance in *Fusarium semitectum* significantly.

Development of resistance in *Alternaria ricini* to carbendazim through the treatment of UV and Sodium azide was demonstrated in the laboratory. Resistant factor ranged from 2 to 4. Mutants having high resistance also showed higher growth and all mutants were persistent for carbendazim resistance. A change at the target site of the carbendazim might be attributed for the induction of the resistance in the wild isolate of *Alternaria ricini*. This view can be very well supported by the findings of many workers (Fuchs *et al.*, 1977; Brown and Hall, 1979; Dekker and Gielink, 1979). Gangawne and Shaikh (1988) demonstrated the development of resistance in *Pythium aphanidermatum* causing damping off of tobacco to aluminium ethyl phosphite through various mutagens.

According to Davidse (1981) chemical mutagen affect the mutation rates by increasing the frequency of DNA replication errors and mispairs of genetic damage. Stability of the resistance in various pathogenic fungi to various fungicides has been worked out by Hollomon (1978) and Davidse (1981). They have concluded that such population is fit for its survival under field conditions.

Carbendazim resistant mutant showed lower infection on castor leaves when compared with wild sensitive isolate in the present investigation. Most of the worker reported reduction in virulence in the resistant pathogens. Pan and Sen (1980) found that reduced virulence in *Macrophomina phaseolina* resistant to carbendazim and PCNB. Metalaxyl resistant *Phytophthora megasperma* induced through UV and MNNG treatment showed reduced virulence. Davidse (1981) advocates that reduced virulence in the resistant pathogens might be attributed to the decreased membrane permeability which often disturbs other processes. According to Kamble (1991), development of resistance in *Macrophomina Phaseolina* to the carbendazim through the treatment of MNNG and SA yielded 10 carbendazim resistant mutants. Their resistant factor ranged from 9 to 15. Here mutants having higher resistance showed higher growth and all the mutants were persistent for carbendazim resistance. Treatment of UV, NMU and SA to AT-15 isolate of *Alternaria tenuissima* causing leaf spots of spinach yielded14, 27 and 17 carbendazim resistant mutants (Bhale, 2002). He observed that resistant factor of these mutants ranged from 2 to 3. Mutants having high resistant factor showed higher growth and all the mutants were persistent for carbendazim resistance. But resistant mutant showed lower infection percentage on spinach leaves when compared with the wild sensitive AT-15. Similarly Treatment of UV, NMU and SA to *Fusarium oxysporum* f. *spinaciae* causing wilt of spinach gave 19, 17 and 21 benomyl resistant mutants. Their resistant factor ranged from 3 to 4. All mutants showed higher growth and persistence of benomyl resistance. But these mutants showed lower infection percentage on spinach roots when compared with the sensitive isolate (Bhale, 2002). According to Bharade (2002), treatment of UV, EMS, 5-bromouracil and sodium azide to sensitive isolate of *Alternaria alternata* causing fruit rot of pomegranate to carbendazim 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. Resistant mutant showed lower infection percentage on pomegranate fruits compared with the wild sensitive *Alternaria alternata* isolate. Treatment of UV, EMS and SA to *Macrophomina phaseolina* causing charcoal rot of pigeon pea sensitive to carbendazim gave 10, 24 and 17 carbendazim resistant mutants respectively (Wadikar, 2002). He observed their resistant factor between 3 to

5. Mutants having higher resistant factor showed slow growth rate and all the mutants were persistent for carbendazim resistance. Further he noted that resistant mutant showed lower infection percentage on pigeon pea roots when compared with the wild sensitive isolate. Treatment of UV and 5-bromouracil to *Sclerotium rolfsii* causing fruit rot of *Cucumis sativus* sensitive to carbendazim yielded 29 and 15 carbendazim resistant mutants respectively (Hiwale, 2003). He further observed that their resistant factor ranged from 6 to 9. Mutants having higher resistant factor showed lower growth and all the mutants were persistent for carbendazim resistance. Resistant mutant showed lower infection percentage on cucumber fruit as compared to wild sensitive isolate of *Sclerotium rolfsii.*

Fungicide mixtures are used to broaden the spectrum activity of the product or to minimize selection pressure of resistant strains. A mixture of tridemorph and carbendazim is used for controlling powdery mildew of cereals and metalaxyl and mancozeb for *Phytophthora infestans* in potato tubers. There are also theoretical models (Kable and Jeffery, 1980: Skylakakis, 1981; Levy et al.,1983) and examples (Delp,1980; Dekker,1981; Gangawane and Shaikh,1988; Kamble,1991; Gangawane et al.,1995; Bhale, 2002; Bharade ,2002; Wadikar, 2002; Hiwale, 2003; Telmore, 2004; Gangawane and Kamble,1993).

Synergistic effects of carbendazim with other agrochemicals on the fungicide resistance in *Alternaria ricini* were seen *in vitro* and *in vivo*. Because it is possible that many of them may be mixed in carbendazim in the various field operations during the management of crop diseases. Thus in this investigation, *In vitro*, it was noted that application of carbendazim with fungicides (Captan, Mancozeb and Zineb), insecticides (Phorate, Endosulphan and Dimethoate), herbicides (Atrazine Mera-71 and 2-4-D), salts (Sodium chloride) inhibited the growth of the resistant isolate which indicates reduction in the carbendazim resistance. These compounds when mixed with carbendazim also inhibited the infection of resistant *Alternaria-ricini* on castor leaves. There are reports that benomyl with captan, chlorothalonil and imazalil reduced benomyl resistance in *Venturia inaequalis* (Shabi and Gilpatrick, 1981) and in *Sclerotinia homeocarpa* and *Penicillium digitatum* (Eckert, 1982).Certain micronutrients when used in combination with carbendazim reduced resistance in *Aspergillus flavus* causing aflaroot disease of groundnut (Gangawane and Reddy, 1988). According to Griffin (1981), fungicides and antibiotics used in a mixture have different mode of action and therefore this combination controls the resistant pathogens. Samoucha and Cohen (1989) noted that mixtures of mancozeb and cymoxanil and mixtures of mancozeb and cymoxanil and oxadixyl were highly effective and synergistic in controlling the phenylamide resistant isolate of *Phytophthora infestans* causing late blight of potatoes in the fields. They also suggested that higher concentrations of fungicides are essential to 100% control of resistant isolates than sensitive isolates. Gissi et al (1985), Grabaski and Gisi (1987); Samoucha *et al.* (1987) reported combined interaction in the mixtures of mancozeb plus oxadixyl and mancozeb in late blight of potato. Suggesting that there is a significant delay of resistance build up in the pathogen when mixtures of fungicides with different mode of action were used. Kamble (1991) noted that application of carbendazim with fungicides (Difoltan, Diathane M-45, Thiophanate and Zineb), Insecticides (Paramar, Endosulphan, Thimate and Sumicidin), Antibiotics (Aureofungin, Streptomycin, Griseofulvin and Mycostatin), Weedicides (Gramoxone and Atrazine), Salts (Barium chloride, Murcuric chloride, Cadmium chloride and Tin chloride), Fertilizers (Superphosphate and Muriate of Potash) and Micronutrients (Co, Bo, Zn and Mb) inhibited the growth of resistant isolate of *Macrophomina phaseolina* causing charcoal rot of potato. Further he observed that these compounds when mixed with Carbendazim also inhibited the infection of resistant *Macrophomina phaseolina* on potato tubers.

Use of metalaxyl with mancozeb, emisan, aureofungin, Co, Cd, Mg and Cu reduced metalaxyl resistance in *Phytophthora drechsleri* f sp *cajani* on pigeon pea. (Waghmare,1990).Build up of fungicide resistance among the pathogen will be less if a combination of agrochemicals used is of systemic nature and a multisite inhibitor (Dekker, 1981). Bhale (2002) reported that a carbendazim with captafol, thiram, mancozeb, benomyl, endosulphan, pyribon, thimate, stomp, ampicilline, aureofungin, streptomycin, cobalt chloride, calcium chloride, magnesium chloride, potassium chloride, barium chloride, Cu, Co, Mn, Bo, muriate of potash superphosphate, NPK, and urea inhibited the growth of *Alternaria tenussima* causing leaf spot of spinach completely/partially. He was further observed that when carbendazim was used with 2-4-D and atrazine then there was increase in the growth of the pathogen. In *in vivo* studies all above mentioned combinations protected spinach leaves from the infection of the pathogen. When benomyl is used with captan, carbendazim, mancozeb, thimate, pyribon, endosulphan, 2-4-D, atrazine, stomp, ampicillin, griseofulvin, aureofungin, streptomycin, magnesium chloride, cobalt chloride, calcium chloride, barium chloride, Cu, Co, Mn, Mo and Bo inhibited the growth of *Fusarium oxysporum* f. *spinaciae* causing wilt of spinach (Bhale, 2002). *In vivo* studies gave similar effects. According to Bharade(2002) when carbendazim was used with captan, ziram, diathane-Z-78, blue copper, topsin-M, captafol, fytolan, mnocrotophos, atrazine, endosulphan, gramoxone , streptomycin, aureofungin, griseofulvin, manganese chloride, ferric chloride, superphosphate, ammonium phosphate, muriate of potash, , Cu, Co, Mn, Mo, Bo and Zn inhibited the growth of *Alternaria alternata* causing fruit rot of pomegranate both *in vitro* and *in vivo*. Hiwale (2003) found that use of carbendazim with captan, nuvon, endosulphan, phorate, streptomycin, aureofungin, ampicillin, algrip, goal, barium chloride, Bo, superphosphate completely inhibited the growth of *Sclerotium rolfsii* causing fruit rot of *Cuccumis sativus* both *in vivo* and *in vitro*.