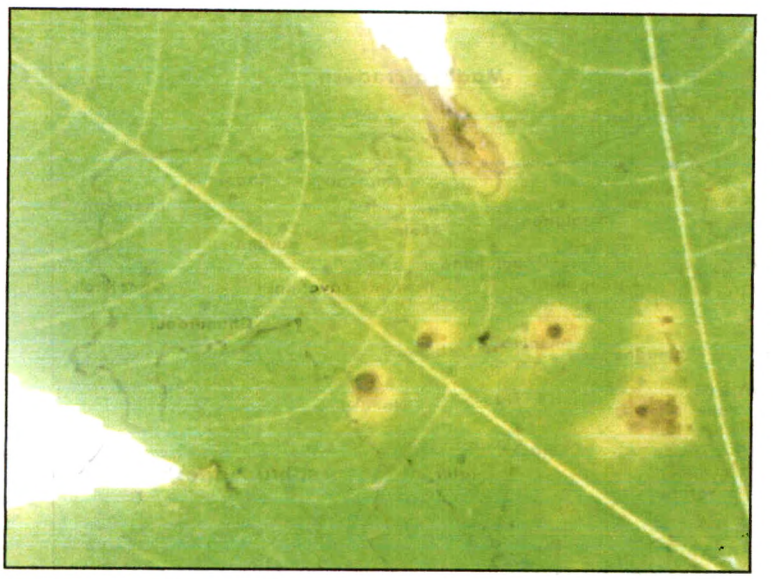
MATERIAL AND METHODS

`Fig.1: Map of Maharashtra showing region of western Maharashtra.

Plate 1: Castor leaf showing symptoms of *Alternaria* Blight.



***Alternaria* Blight of Castor:**

Diseased samples of castor leaves were collected from five districts of **'Western Maharashtra'** (Fig.1) and (Plate1) viz. Kolhapur, Pune, Sangli, Satara and Solapur. Five isolates of the pathogen were obtained from infected portion of castor leaf, on a medium containing castor leaf extract. Then the isolates were transferred on the plates using the same culture medium. Culture tubes were maintained at 4°C and used for study whenever necessary.

Host Plant:

***Ricinus communis* Linn.** was used throughout the study.

Culture Medium:

150 gm of fresh castor leaves, Dextrose10gm, Agar-agar 15gm and 1000 ml distilled water.

Sensitivity of *Alternaria ricini* to Carbendazim:

***In vitro* studies:**

Sensitivity of *Alternaria ricini* to carbendazim was determined by **'Food poisoning test'** (Dekkar and Gielink, 1979). Culture plates were prepared containing different concentrations of carbendazim. Discs (6mm) with fungal cultures, taken from the margin of an actively growing colony and these discs were placed on agar surface. The plates were then inoculated at 26±3°c.in the dark and linear mycelial growth was measured at different time intervals. Plates without carbendazim served as 'control'.

***In vivo* studies:**

In vivo studies were performed on healthy leaves of castor. For this, healthy castor leaves were treated with different concentrations of carbendazim solutions. After 24 hrs, these treated castor leaves were inoculated with 10 ml. of spore suspension of different isolates. Inoculated castor leaves were covered by polythene bags and percentage of infection was recorded after various incubation periods. Leaves treated with carbendazim without inoculations were treated as 'control'. Leaves without any treatment and inoculations were treated as 'absolute control'.

Induction of Carbendazim resistance:

Mycelial suspension of *Alternaria ricini* was treated with **UV rays** and chemical mutagen **'Sodium azide'**at different time interval and concentrations respectively. The above treated mycelium suspension was inoculated on agar plates incorporated with 2X to 6X of *in vitro* MIC of carbendazim (15%).As well as the 10 ml of same mycelial suspension was inoculated on castor leaves treated with 2X to 6X of *in vivo* MIC of carbendazim (1%), 24 hrs. before.

Effect of passage:

After determination of MIC of carbendazim, the effect of continuous and alternate treatments of fungicides with two different modes of action and a mixture of both on the development of resistance in wild sensitive isolate of *Alternaria ricini* (AR-5) was studied *in vitro* and *in vivo.*

***In Vitro* studies:**

Continuous passage:

To study the effect of continuous passage, *in vitro*, wild sensitive isolate (AR-5) in each passage was cultured on plates with carbendazim (15%) in triplicate. Six mm diameter agar disc from the previous passage of the same isolate was placed at the centre of each plate in triplicate. In each passage, linear mycelial growth was measured after 8 days.

Alternate passage:

To study the effect of alternate passage *in vitro*, wild sensitive isolate (AR-5) was cultured on plates with carbendazim (15%), in triplicates. After 8 days, 6 mm diameter agar disc from the previous passage was transferred to the plates containing another fungicide at the same concentration (15%). The process of such alteration of carbendazim to another fungicide was continued up to 8^{th} passage.

Mixed passage:

To study the effect of mixed passage *in vitro*, wild sensitive isolate (AR-5) was cultured on plates with carbendazim with another fungicide, both having equal concentration (15%), in triplicates. After 8 days, 6 mm diameter agar disc from the previous passage was transferred to the plates containing the same mixture of fungicides, in same proportion and same concentration (15%).

In each type of passage, mentioned above, the increased mycelial growth from passage to passage was considered as criterion for the development of fungicide resistance. The effect of passage on the development of fungicide resistance in the pathogen was studied up to 8th passage, in each case.

***In vivo* studies:**

Continuous passage:

To study the effect of continuous passage, on the development of fungicide resistance, in the pathogen, *in vivo*, mycelial suspension using 1 culture tube of wild sensitive isolate (AR-5) was prepared. 10 ml mycelial suspension was inoculated on the castor leaves, treated with 1% carbendazim, 24hrs before it. After 8 days a mycelial suspension from such infected leaves was prepared and applied to healthy castor leaves treated with 1% carbendazim, 24hrs before inoculation. Same procedure was followed up to 8th passage.

Alternate passage:

To study the effect of alternate passage, on the development of fungicide resistance, in the pathogen, *in vivo*, 10ml mycelial suspension using 1 culture tube of wild sensitive isolate (AR-5) was inoculated on the healthy castor leaves, treated with 1% carbendazim 24hrs before it. After 8 days a mycelial suspension from such infected leaves was prepared and applied to healthy castor leaves treated with another fungicide (1%). Same procedure was followed up to 8th passages.

Mixed passage:

To study the effect of mixed passage, on the development of fungicide resistance, in the pathogen, *in vivo*, mycelial suspension using 1 culture tube of wild sensitive isolate (AR-5) was inoculated on the healthy castor leaves, treated with mixture of carbendazim and another fungicide in same proportion (both 1%), 24hrs

before it. After 8 days a mycelial suspension from such infected leaves was prepared and applied to healthy castor leaves treated with same mixture of fungicides, in same concentration before 24 hrs.

In all above types, the castor leaves were covered with polythene bags.

Synergistic effects of Agrochemicals on the development of Carbendazim resistance in *Alternaria ricini:*

***In vitro* studies:**

Effects of various agrochemicals on the development of resistance in *Alternaria ricini* against carbendazim was studied by mixing different agrochemicals such as fungicides, insecticides, herbicides, antibiotics, salts, fertilizers and micronutrients with carbendazim. Resistant mutant isolate 'SA-AR-5' was used for this study. It was grown on the medium containing equal volume of chemical (10, 25, 50 and 75µg/ml) and a resistant dose of carbendazim (60%). Increase in the growth of the organism over control was considered to be the increase in the resistance. While decrease in the growth of the organism over control was considered to be the decrease in the resistance. Plates containing 60% carbendazim were served as 'control'.

***In vivo* studies:**

To study synergistic effect of agrochemicals on the development of resistance in *Alternaria ricini* against carbendazim, 10 ml mycelial suspension of resistant mutant isolate (SA-AR-5) was inoculated on healthy castor leaves treated with resistant dose of carbendazim (5%) and other agrochemicals with above concentrations, in equal volume 24 hrs, before it. Each castor leaf was covered with polythene bag. Percentage of infection (Datar, 1982) on castor leaves was measured after 8 days.