EXPERIMENTAL RESULTS

***Alternaria* Blight of Castor:**

(*Alternaria ricini* (Yoshi) Hansf.)

I) Sensitivity of *Alternaria ricini* isolates to Carbendazim.

Samples of castor leaves showing blight symptoms were collected from different five districts of **'Western Maharashtra'**, viz. Kolhapur, Pune, Sangli and Solapur. Five isolates of ***Alternaria ricini*** were obtained from these samples.

Carbendazim sensitivity of these samples was tested in both conditions i.e. *in vitro* and *in vivo.*

***In vitro* studies:**

For *in vitro* studies, sensitivity of *Alternaria ricini* to carbendazim was tested by **'Food Poisoning Technique'**, using culture medium containing castor leaf extract (150 gm of fresh castor leaves, Dextrose10gm, Agar-agar 15gm and 1000ml distilled water).

Results are as given in tables 2, 3, 4, 5 and 6 and Figs.2, 3, 4, 5 and 6.

Table 2: Sensitivity of *Alternaria ricini* isolate (AR-1) to carbendazim (*In vitro*).

Day	Growth in mm.				
	Control	**16%**	**16.5%**	**17%**	**17.5%**
1	13.75	00.00	00.00	00.00	00.00
2	22.50	8.75	00.00	00.00	00.00
3	30.00	9.00	00.00	00.00	00.00
4	45.00	11.12	9.87	9.37	00.00
5	52.50	12.37	10.25	9.87	00.00
6	67.50	12.50	11.50	10.12	7.75
7	83.758	15.00	12.25	10.37	8.50
8	100.00	16.00	12.50	10.62	8.75

Fig 2: Dose response curve of *Alternaria ricini isolate* (AR-1) to various concentrations of carbendazim on agar plates.

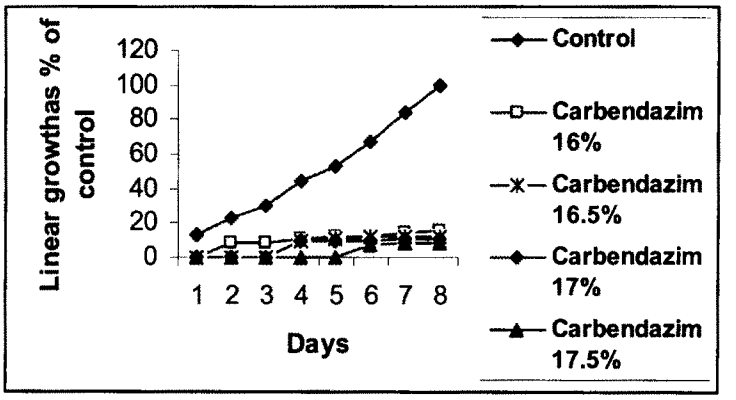


Table 3: Sensitivity of *Alternaria ricini* isolate (AR-2) to carbendazim (*In vitro*).

Day	Growth in mm.				
	Control	**10%**	**12%**	**14%**	**16%**
1	10	0	0	0	0
2	12.5	0	0	0	0
3	22.5	10	0	0	0
4	37.5	10.87	9	0	0
5	60	11.5	9.37	10	0
6	71.25	13.75	11	10.25	7.75
7	82.5	15	11.5	11.37	7.87
8	100	15.75	12.5	11.62	8.62

Fig 3: Dose response curve of *Alternaria ricini isolate* (AR-2) to various concentrations of carbendazim on agar plates.

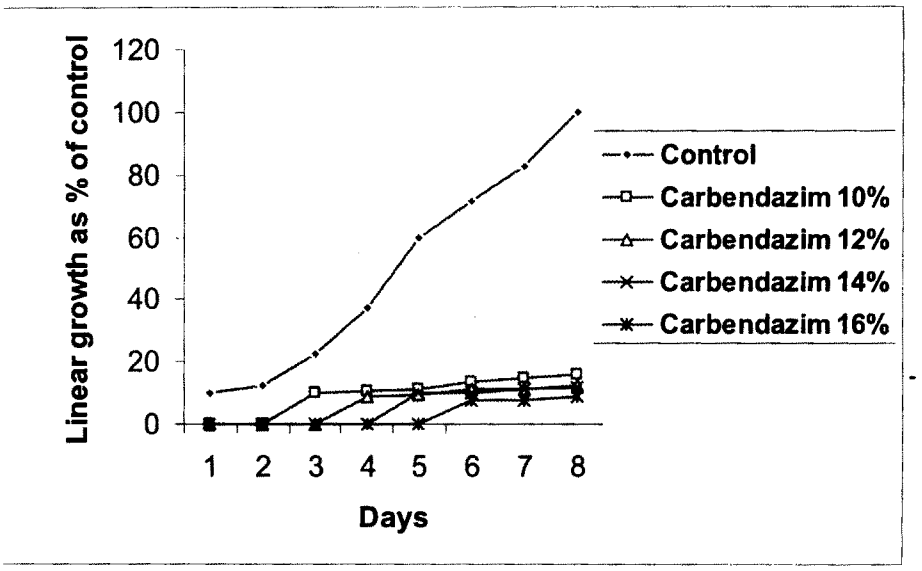


Table 4: Sensitivity of *Alternaria ricini* isolate (AR-3) to carbendazim (*In vitro*).

Day	Growth in mm.				
	Control	16%	16.5%	17%	17.5%
1	15	0	0	0	0
2	28.75	0	0	0	0
3	37.5	9.37	8.75	0	0
4	45	8.87	9	0	0
5	60	10.62	9.5	8.75	0
6	68.75	11.22	9.87	9.75	8.5
7	78.75	11.37	11.25	10.87	8.87
8	100	12.5	11.87	11.5	9

Fig 4: Dose response curve of *Alternaria ricini isolate* (AR-3) to various concentrations of carbendazim on agar plates.

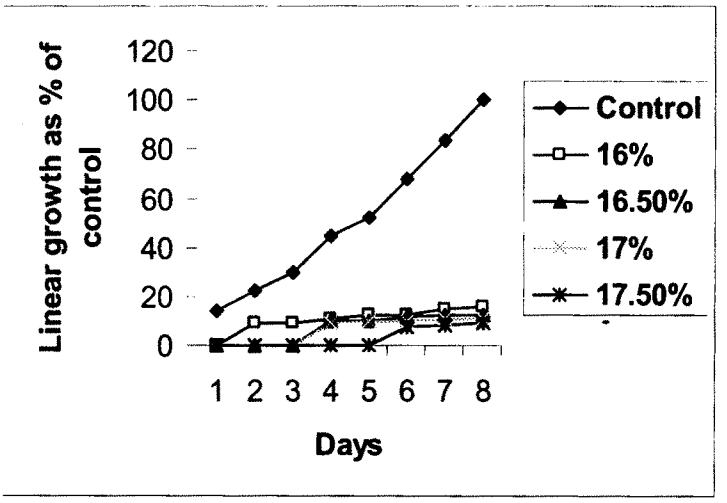


Table 5: Sensitivity of *Alternaria ricini* isolate (AR-4) to carbendazim (*In vitro*).

Day	Growth in mm.				
	Control	**5%**	**10%**	**15%**	**20%**
1	12.5	0	0	0	0
2	21.25	9.37	0	0	0
3	35	10	10	8.75	0
4	50	12.5	11.25	9.87	0
5	65	15	13.5	10.25	0
6	76.25	16.25	15	11.12	8.75
7	85	18.75	17.12	12.5	8.87
8	100	21.5	18.87	13.12	9.75

Fig 5: Dose response curve of *Alternaria ricini isolate* (AR-4) to various concentrations of carbendazim on agar plates.

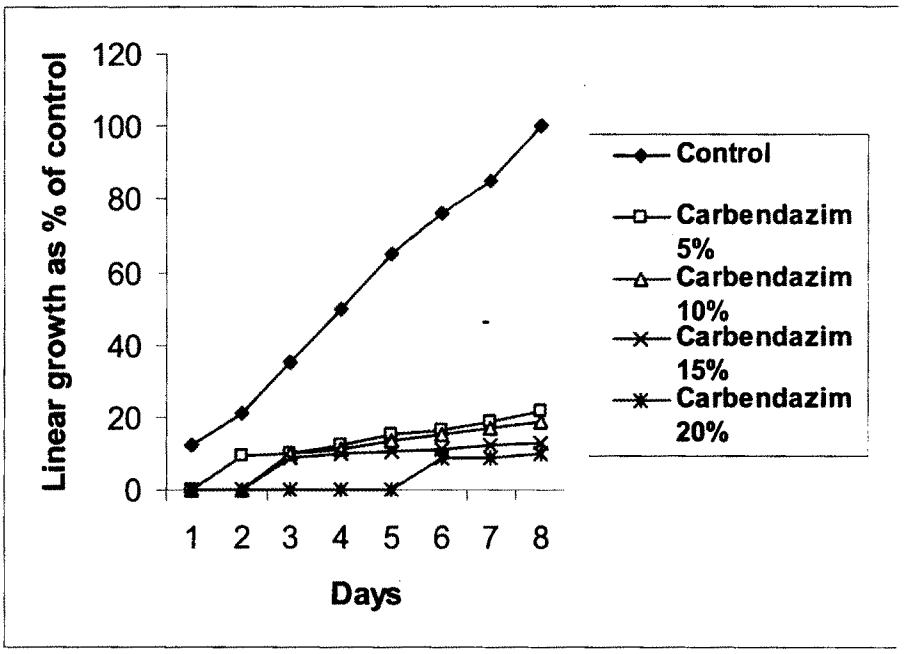


Table 6: Sensitivity of *Alternaria ricini* isolate (AR-5) to carbendazim (*In vitro*).

Day	Growth in mm.				
	Control	**1%**	**5%**	**10%**	**15%**
1	12.5	0	0	0	0
2	27.5	9	0	0	0
3	37.5	10.37	10	0	0
4	52.5	13.75	13.12	10	0
5	60	17.5	13.75	10.25	0
6	68.75	21.87	15.25	11.25	0
7	83.75	24.87	16.87	11.37	0
8	100	25	17.5	12.25	8.5

Fig 6: Dose response curve of *Alternaria ricini isolate* (AR-5) to various concentrations of carbendazim on agar plates.

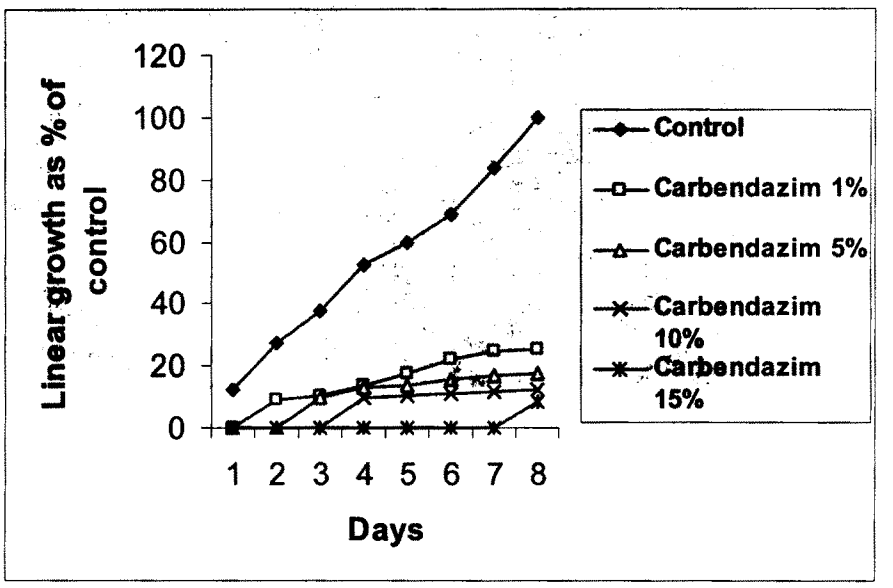
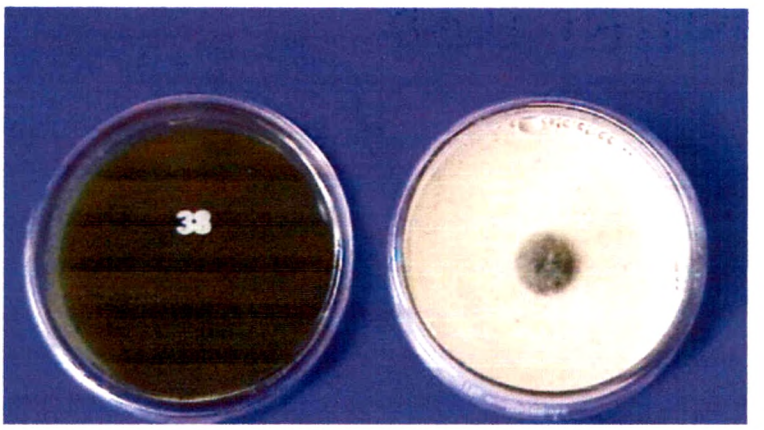
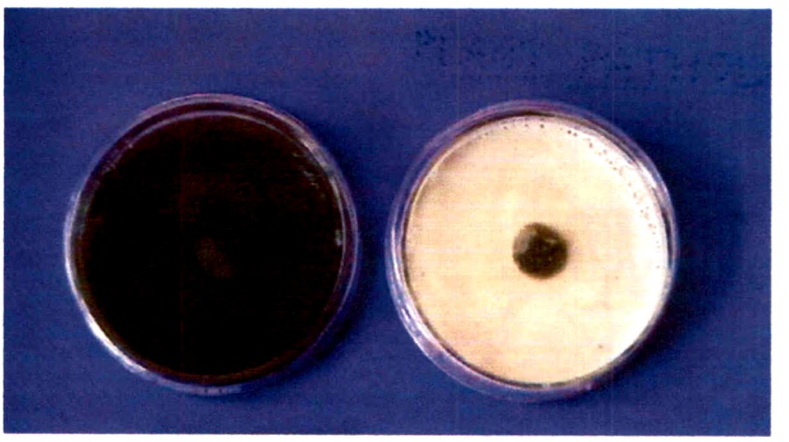


Plate 2: Sensitivity of *Alternaria ricini* isolate AR-5 to carbendazim (*In vitro*).



Control **15% Carbendazim**

Plate 3: Sensitivity of *Alternaria ricini* isolate AR-4 to carbendazim (*In vitro*).



Control **20% Carbendazim**

From the tables 2, 3, 4, 5 and 6, and Figs.2, 3, 4, 5 and 6, it was observed that, the sensitivity of *Alternaria ricini* to carbendazim ranged from 15% to 20 % on culture plates.

Among the five isolates, the sensitive isolate is AR-5 with MIC 15%. While the most resistant isolate is AR-4 with MIC 20% (Plate 2 and 3 respectively).

***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.

Results are as given in tables 7, 8, 9, 10 and 11 and Figs. 7, 8, 9, 10 and 11.

Table 7: Sensitivity of *Alternaria ricini* isolate (AR-1) to carbendazim (*In vivo*).

Day	Percentage of infection				
	Control	1.5%	2%	2.5%	3%
1	0	0	0	0	0
2	0	0	0	0	0
3	3	2	0	0	0
4	5	2	2	0	0
5	7	3	2	2	0
6	8	4	3	2	0
7	9	5	3	3	2
8	10	6	4	3	2

Fig 7: Dose response curve of *Alternaria ricini isolate* (AR-1) to various concentrations of carbendazim on castor leaves.

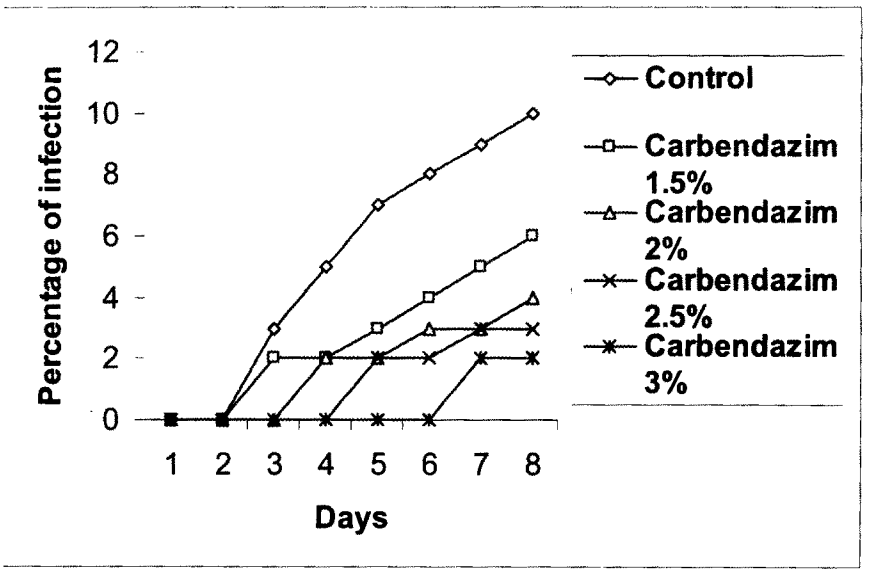


Table 8: Sensitivity of *Alternaria ricini* isolate (AR-2) to carbendazim (*In vivo*).

Day	Percentage of infection			
	Control	1%	1.2%	1.4%
1	0	0	0	0
2	0	0	0	0
3	3	2	0	0
4	5	3	2	0
5	7	3	3	0
6	8	4	3	2
7	9	5	4	2
8	10	5	4	2

Fig 8: Dose response curve of *Alternaria ricini isolate* (AR-2) to various concentrations of carbendazim on castor leaves.

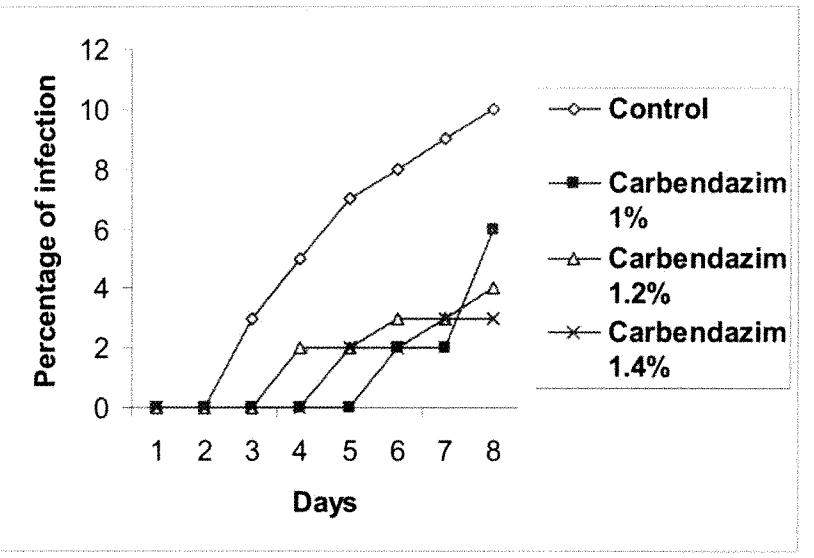


Table 9: Sensitivity of *Alternaria ricini* isolate (AR-3) to carbendazim (*In vivo*).

Day	Percentage of infection				
	Control	0.5%	1%	1.5%	2%
1	0	0	0	0	0
2	2	0	0	0	0
3	3	2	2	0	0
4	5	3	2	0	0
5	6	3	3	2	2
6	7	4	4	3	2
7	9	5	4	3	2
8	10	6	5	4	2

Fig 9: Dose response curve of *Alternaria ricini isolate* (AR-3) to various concentrations of carbendazim on castor leaves.

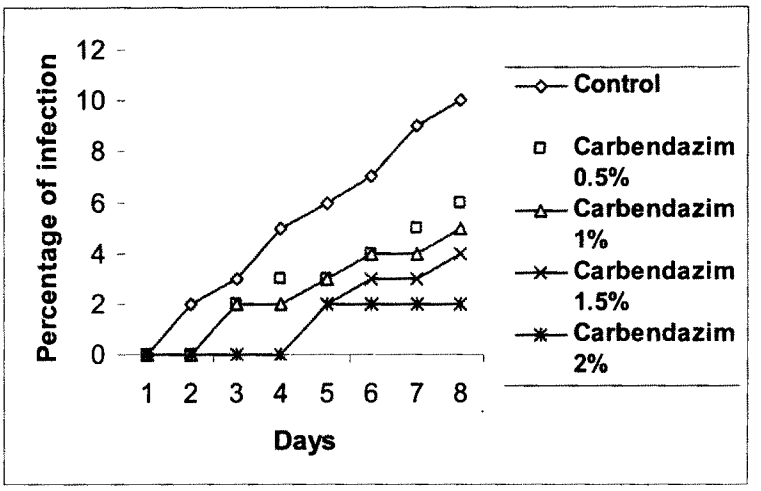


Table 10: Sensitivity of *Alternaria ricini* isolate (AR-4) to carbendazim (*In vivo*).

Day	Percentage of infection				
	Control	1%	2%	3%	4%
1	0	0	0	0	0
2	0	0	0	0	0
3	3	2	0	0	0
4	5	4	2	2	0
5	7	5	3	2	0
6	8	5	4	3	2
7	9	6	4	4	2
8	10	7	5	4	3

Fig 10: Dose response curve of *Alternaria ricini isolate* (AR-4) to various concentrations of carbendazim on castor leaves.

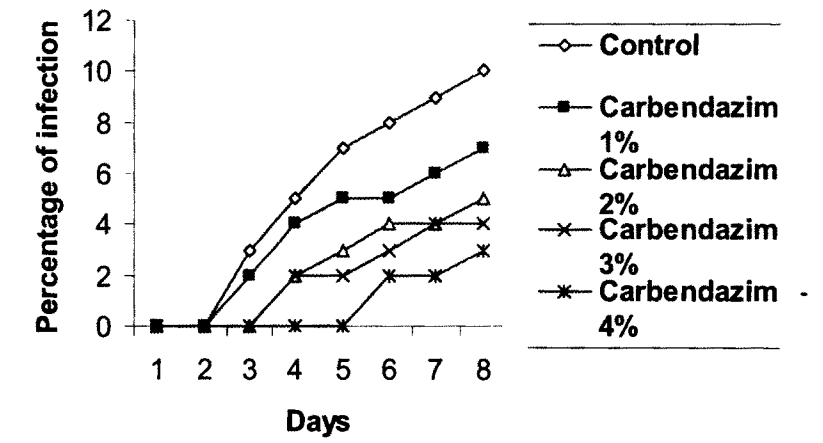


Table 11: Sensitivity of *Alternaria ricini* isolate (AR-5) to carbendazim (*In vivo*).

Day	Percentage of infection				
	Control	0.25%	0.50%	0.75%	1%
1	0	0	0	0	0
2	0	0	0	0	0
3	3	2	0	0	0
4	5	4	2	2	0
5	6	5	3	2	0
6	8	5	4	2	0
7	9	6	4	3	0
8	10	7	5	4	2

Fig 11: Dose response curve of *Alternaria ricini isolate* (AR-5) to various concentrations of carbendazim on castor leaves.

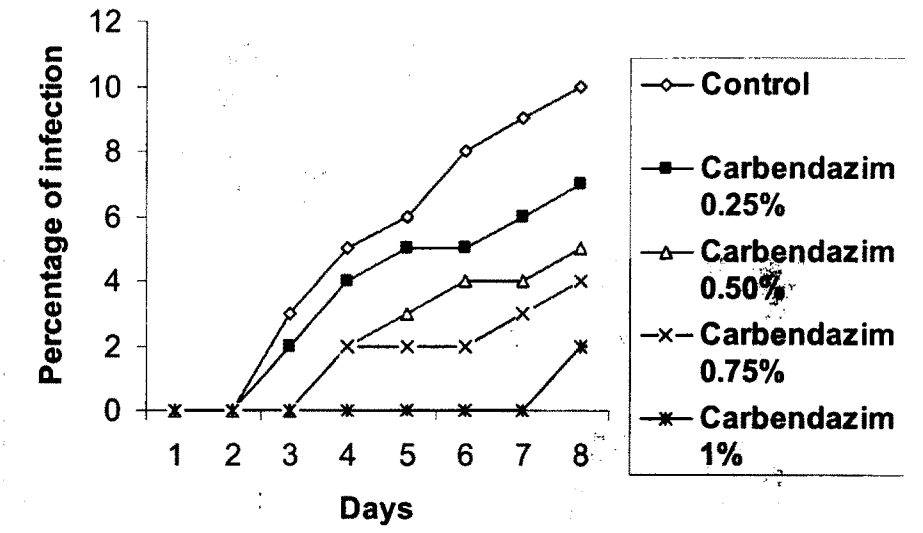


Plate 4: Sensitivity of *Alternaria ricini* isolate (AR-5) to carbendazim (*In vivo*).

Absolute Control

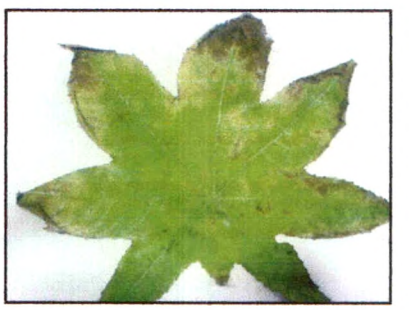
Control

1% Carbendazim

Plate 5: Sensitivity of *Alternaria ricini* isolate (AR-4) to carbendazim (*In vivo*).

Absolute control

Control



4% Carbendazim

From the tables 7, 8, 9, 10 and 11 and Figs. 7, 8, 9, 10 and 11, it was observed that, the sensitivity of *Alternaria ricini* to carbendazim on castor leaves ranged from1% to 4%.

From the above results it was observed that there was variation of MIC of carbendazim against *Alternaria ricini*, both on agar plates and castor leaves (Plate 4 and 5 respectively).

II) Effect of passage on the development of Carbendazim resistance in *Alternaria ricini:*

Effect of passage on the development of carbendazim resistance in *Alternaria ricini* was studied in both conditions i. e. *in vitro* and *in vivo.*

***In vitro* studies:**

1. **Continuous passage:**

To study the effect of continuous passage on the development of carbendazim resistance in *Alternaria ricini*, its wild sensitive isolate (AR-5) was cultured for 8 successive passages on the culture medium with carbendazim at its MIC level (15%) continuously.

2. **Alternate passage:**

To study the effect of alternate passage on the development of carbendazim resistance in *Alternaria ricini*, its wild sensitive isolate (AR-5) was cultured on the medium containing carbendazim at its MIC level (15%) altering with Captan, Mancozeb and Roko for 8 successive passages.

3. **Mixed passage:**

To study the effect of passage in mixture, on the development of carbendazim resistance in *Alternaria ricini*, its wild sensitive isolate (AR-5) was cultured on a medium containing carbendazim at its MIC level (15%) with Captan, Mancozeb and Roko, both in equal volume, for 8 successive passages.

In each type of passage, mentioned above, a 6 mm diameter agar disc from the margin of colony of the previous passage and placed at the centre of each plate in triplicate. In each passage linear mycelial growth of *Alternaria ricini* was measured after 8 days. Increase or decrease in the radial growth of *Alternaria ricini* from

passage to passage was employed as a criterion for the development of carbendazim resistance. Results are as given in table 12 and 13.

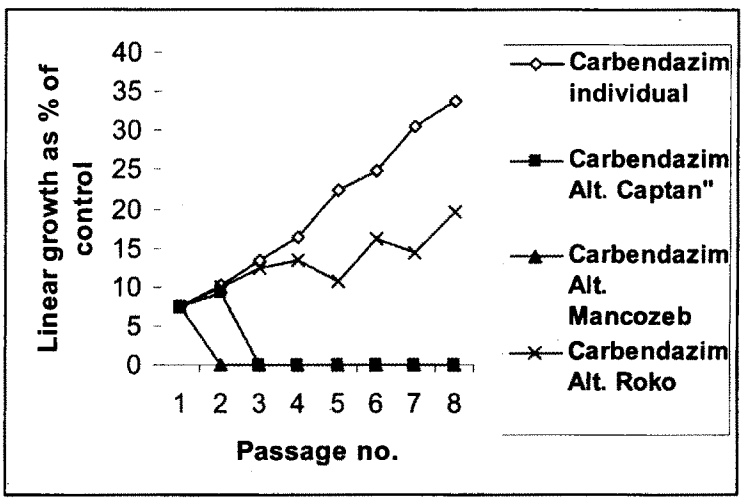
Continuous and Alternate passage:

Table 12: Effect of exposure of *Alternaria ricini* (*In vitro*) to carbendazim continuous and alternating with other fungicides on the development of resistance during eight successive passages.

Fungicide	Passage no.							
	1	2	3	4	5	6	7	8
Carbendazim Individual	7.5*	10.28	13.32	16.45	22.25	24.87	30.45	33.75
Cabendazim Alt. Captan	7.5**	9.12	0.0	0.0	0.0	0.0	0.0	0.0
Carbendazim Alt. Mancozeb	7.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carbendazim Alt. Roko	7.5	9.95	10.25	13.41	10.66	16.07	14.32	19.66
S. E.	0	2.46	3.46	4.35	5.30	6.17	7.25	8.23
C.D	0	6.32	8.88	11.16	13.60	15.83	18.61	21.12

*** Growth as % of control**
****Growth in mm.**

Fig 12: The effect of continuous and alternate exposure to carbendazim with other fungicides on mycelial growth of *Alternaria ricini* (*in vitro*) during 8 successive passages.



Mixed Passage:

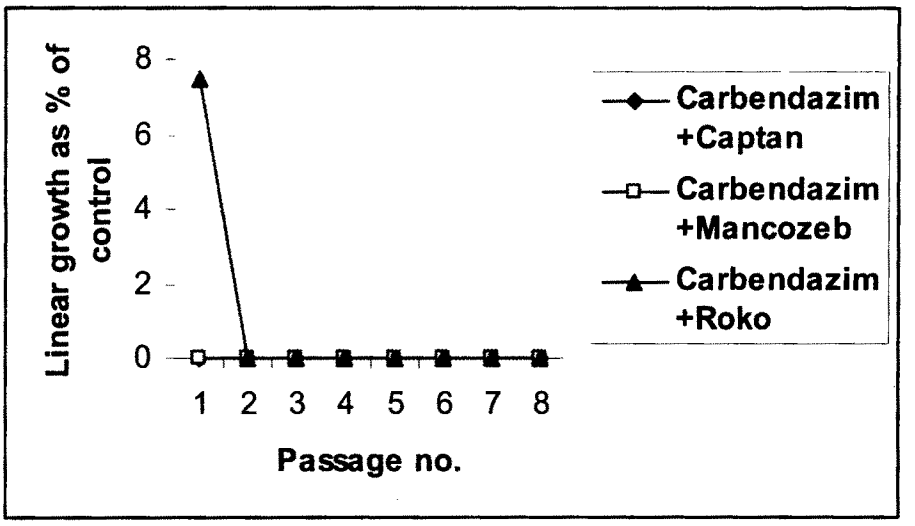
Table 13: Effect of exposure of *Alternaria ricini* (*In vitro*) to the mixture of carbendazim with other fungicide on the development of resistance during eight successive passages.

Fungicide	Passage no.							
	1	2	3	4	5	6	7	8
Carbendazim+Captan	7.5*	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Caebendazim+Mancozeb	7.5**	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carbendazim+Roko	7.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. E.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. D.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*** Growth as % of control**

**** Growth in mm.**

Fig 13: The effect of exposure of *Alternaria ricini* to carbendazim in mixture with other fungicides on mycelial growth (*in vitro*) during 8 successive passages.



From the table 12 and 13, it was observed that during the continuous passage there was significant increase in the resistance of the pathogen. 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 (Fig. 12).

During the mixed passage there was complete inhibition of the pathogen from passage II in all mixtures of fungicides (Fig.13).

***In vivo* studies:**

1. Continuous passage:

To study the effect of continuous passage, on the development of fungicide resistance, in the pathogen, *in vivo*, 10 ml mycelial suspension of one culture tube of wild sensitive isolate (AR-5) was inoculated on the healthy castor leaves, treated with 1% Carbendazim, 24hrs before, continuously for eight successive passages.

2. Alternate passage:

To study the effect of alternate passage, on the development of fungicide resistance, in the pathogen, *in vivo*, 10ml mycelial suspension of one culture tube of wild sensitive isolate (AR-5) was inoculated on healthy castor leaves, treated with carbendazim altering to Captan, Mancozeb and Roko, 24hrs before, continuously for eight successive passages.

3. Mixed Passage:

To study the effect of alternate passage, on the development of fungicide resistance, in the pathogen, in *vivo*, 10 ml mycelial suspension of one culture tube of wild sensitive isolate (AR-5) was inoculated on the healthy castor leaves, treated with solution of carbendazim along with Captan, Mancozeb and Roko, in equal volume, 24hrs before, for eight successive passages.

In each type of passage mentioned above, the castor leaves were covered with polythene bags. Percentage of the infection on castor leaves was recorded after each passage and used as a criterion for the development of resistance in the pathogen. Results are as given in table no 14 and 15.

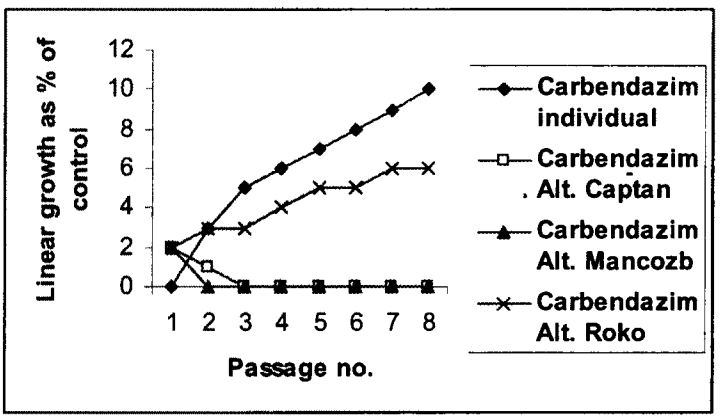
Continuous and Alternate passage:

Table 14: Effect of exposure of *Alternaria ricini* (*In vivo*) to carbendazim continuous and alternating with other fungicides on the development of resistance during eight successive passages.

Fungicide	**Passage no.**							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Carbendazim Individual	2*	3	5	6	7	8	9	10
Cabendazim Alt. Captan	2	1	0	0	0	0	0	0
Carbendazim Alt. Mancozeb	2	0	0	0	0	0	0	0
Carbendazim Alt. Roko	2	3	3	4	5	5	6	6
S. E.	0.0	0.75	1.22	1.50	1.78	1.97	2.25	2.45
C. D.	0.0	1.92	3.13	3.85	4.56	5.05	5.77	6.28

*** Percentage of infection.**

Fig 14: The effect of continuous and alternate treatments of carbendazim with other fungicides on percentage infection of *Alternaria ricini* on castor leaves for 8 successive passages.



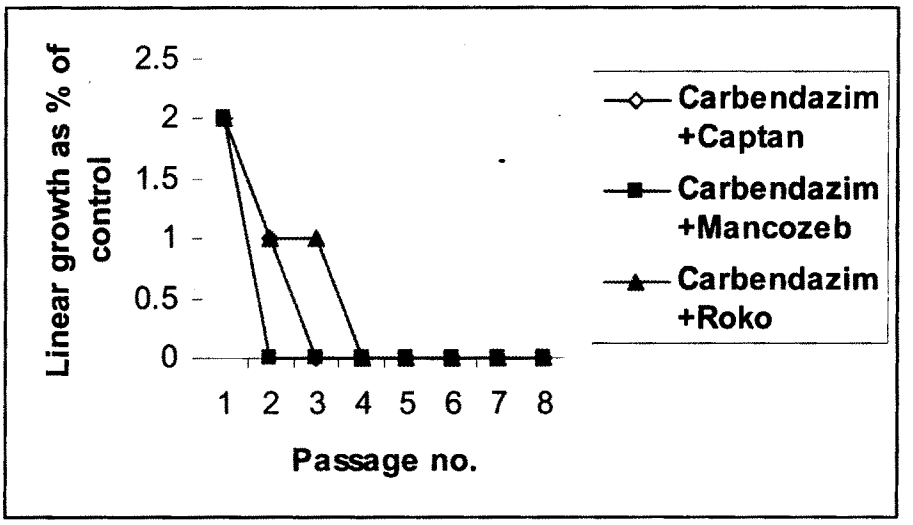
Mixed Passage:

Table No 15: Effect of exposure of Alternaria ricini (*In vivo*) to the mixture of Carbendazim with other fungicide on the development of resistance during eight successive passages.

Fungicide	Passage no.							
	1	2	3	4	5	6	7	8 -
Carbendazim+Captan	2	1	0	0	0	0	0	0
Caebendazim+Mancozeb	2	0	0	0	0	0	0	0
Carbendazim+Roko	2	1	1	0	0	0	0	0
S. E.	0.0	0.29	0.29	0.0	0.0	0.0	0.0	0.0
C. D	0.0	0.74	0.74	0.0	0.0	0.0	0.0	0.0

*** Percentage of infection.**

Fig 15: Effect of mixture treatment of Carbendazim with other fungicides on percentage infection of *Alternaria ricini* on Castor leaves for 8 successive passages.



From the table 14 and 15, it was observed that during the continuous passage there was significant increase in the resistance of the pathogen. During alternate passage there was increase or decrease in the resistance of the pathogen. 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 (Fig. 14).

During the mixed passage there was complete inhibition of the pathogen from passage II in. mancozeb, passages III in captan and at passage IV in roko (Fig. 15).

III) Induction of Carbendazim resistance:

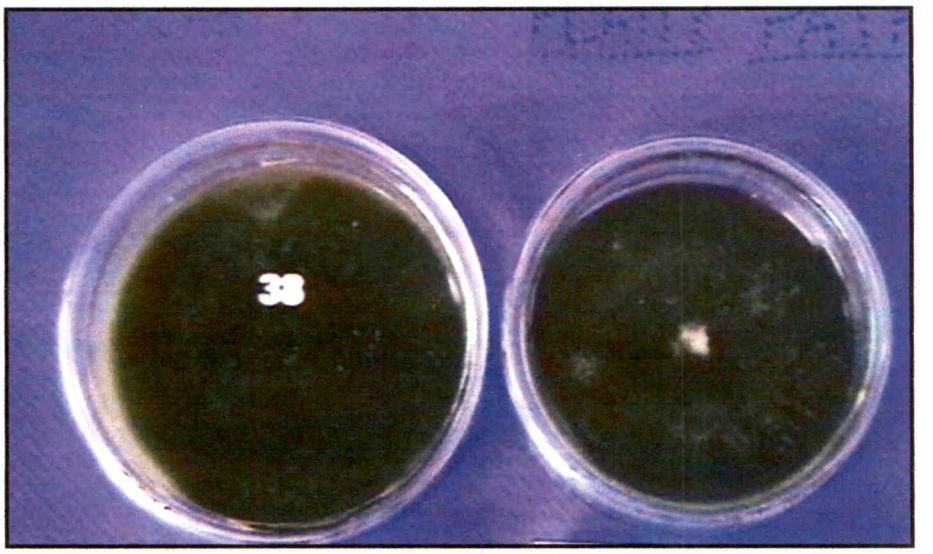
The wild sensitive isolate of ***Alternaria ricini*** **i. e.AR-5** was used for this study. The resistance was induced through **UV rays** and **Sodium azide.** Results are given in the table 16 (Plate 6).

Mycelial suspension of wild sensitive isolate of *Alternaria ricini* i. e.AR-5 was exposed to **UV rays** for 5, 10, 15 and 20 min time interval. Ten mutant colonies were obtained from it.

Mycelial suspension of wild sensitive isolate of *Alternaria ricini* i. e.AR-5 was exposed to 0.001, 0.1 and 1% solutions of **Sodium azide** for 10, 20 and 30 min. time interval. Fourteen mutant colonies were obtained from it.

It is observed that one mutant obtained from **Sodium azide** was highly resistant having **correlation factor 4.** The mutants obtained from UV rays treatment are moderately resistant. The mutants were persistant for the carbendazim resistance. Pathogenecity tests were carried out. Pathogenecity was reduced due to carbendazim resistance.

Plate 6: Induction of carbendazim resistance in wild sensitive isolate AR-5.



Wild isolate (AR-5) **Mutant**

Table 16: Development of carbendazim resistance in *Alternaria ricini* through various treatments.

Treatment	No. of mutants	Resistance factor
Sodium azide	SA-AR-1	3
Sodium azide	SA-AR-2	2
Sodium azide	SA-AR-3	2
Sodium azide	SA-AR-4	2
Sodium azide	**SA-AR-5**	**4**
Sodium azide	SA-AR-6	2
Sodium azide	SA-AR-7	2
Sodium azide	SA-AR-8	2
Sodium azide	SA-AR-9	2
Sodium azide	SA-AR-10	2
Sodium azide	SA-AR-11	2
Sodium azide	SA-AR-12	2
Sodium azide	SA-AR-13	3
Sodium azide	SA-AR-14	2
UV rays	UV-AR-15	2
UV rays	UV-AR-16	2
UV rays	UV -AR-17	3
UV rays	UV -AR-18	3
UV rays	UV -AR-19	2
UV rays	UV -AR-20	2
UV rays	UV-AR-21	2
UV rays	UV-AR-22	2
UV rays	UV -AR-23	2
UV rays	UV -AR-24	2

Synergistic effects of agrochemicals on the carbendazim resistance:

***In vitro* studies:**

Agrochemicals such as fungicides, insecticides, herbicides, antibiotics, salts, fertilizers and micronutrients were mixed with carbendazim in the culture medium. Carbendazim resistant (SA-AR-5) isolate was inoculated on the plates and its growth was observed at various incubation periods. Increased growth over carbendazim alone was considered as increase in the resistance or *vice versa.*

Fungicides:

Captan, mancozeb and zineb were used for this study. It was observed that captan at10µg/ml with carbendazim (60%) completely inhibited the growth of the pathogen. Mancozeb and zineb at 50µg/ml and 25µg/ml respectively with carbendazim (60%) inhibited the growth of the pathogen. (Table17, Fig. 16 and Plate 7).

Insecticides:

From the Table 18, it was observed that Phorate at10µg/ml with carbendazim (60%) completely inhibited the growth of the pathogen. While dimethoate and endosulphan both at100µg/ml with carbendazim (60%) inhibited the growth of the pathogen. (Table18, and Fig.17 and Plate 8).

Herbicides:

Among the herbicides, atrazine and mera-71 at 50µg/ml with carbendazim (60%) inhibited the growth of the pathogen. While 2-4-D at100µg/ml inhibited the growth of the pathogen with carbendazim (60%) (Table 19 and Fig.18).

Antibiotics:

Streptomycin, aureofungin and griseofulvin were used for this study. Results in the table 19 and Fig.19 indicates that, all the three antibiotics were not effective even at 0.4µg/ml with carbendazim (60%) (Table 20, Fig. 19 and Plate 9).

Table 17: Synergistic effects of Fungicides on the development of carbendazim resistance in *Alternaria ricini* (*In vitro*).

Fungicides with Carbendazim (60%)	Growth (mm.)
Captan (µg/ml)	
10	00.00
25	00.00
50	00.00
100	00.00
Mancozeb (µg/ml)	
10	10.00
25	08.00
50	00.00
100	00.00
Zineb (µg/ml)	
10	08.00
25	00.00
50	00.00
100	00.00
Control (60%)	15.00

Fig.16: Synergistic effect of carbendazim in combination with other fungicides on linear growth of *Alternaria ricini* resistant isolate (*in vitro*).

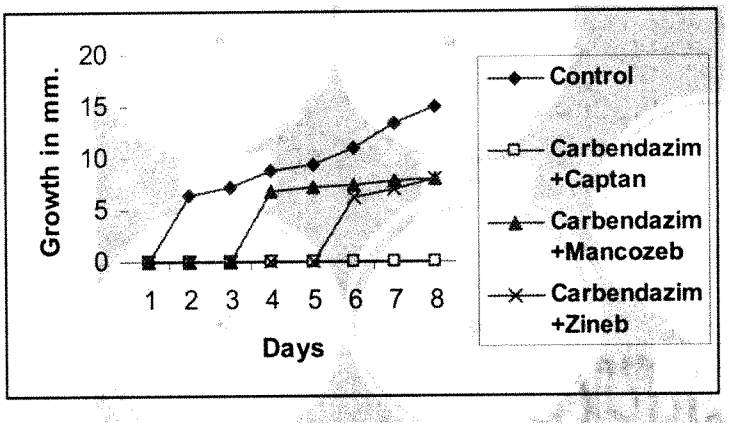


Plate 7: Synergistic effect of carbendazim in combination with other fungicide (captan) on linear growth of *Alternaria ricini* resistant isolate (*in vitro*).

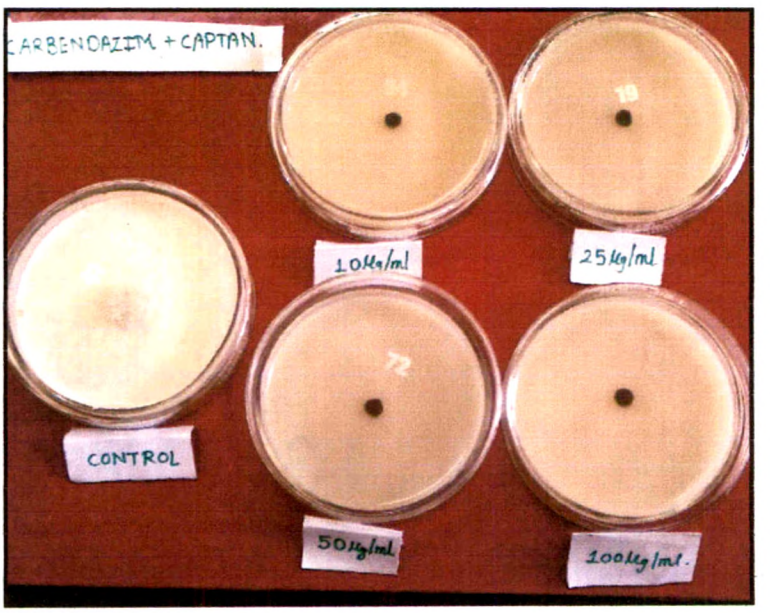


Table 18: Synergistic effects of insecticides on the development of carbendazim resistance in *Alternaria ricini* (*In vitro*).

Insecticides wit	Growth (mm.)
Carbendazim (60%)	
Dimethoate (µg/ml)	
10	22.00
25	17.00
50	10.00
100	00.00
Endosulphan (µg/ml)	
10	25.00
25	17.00
50	13.00
100	00.00
Phorate (µg/ml)	
10	00.00
25	00.00
50	00.00
100	00.00
Control (60%)	15.00

Fig.17: Synergistic effect of carbendazim in combination with other insecticides on linear growth of *Alternaria ricini* resistant isolate (*in vitro*).

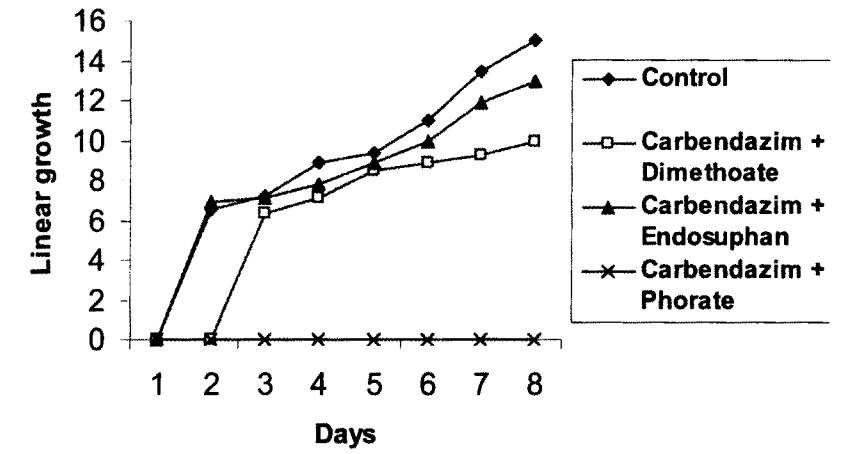


Plate 8: Synergistic effects of insecticide (endosulphan) on the development of Carbendazim resistance in *Alternaria ricini* (*In vitro*).

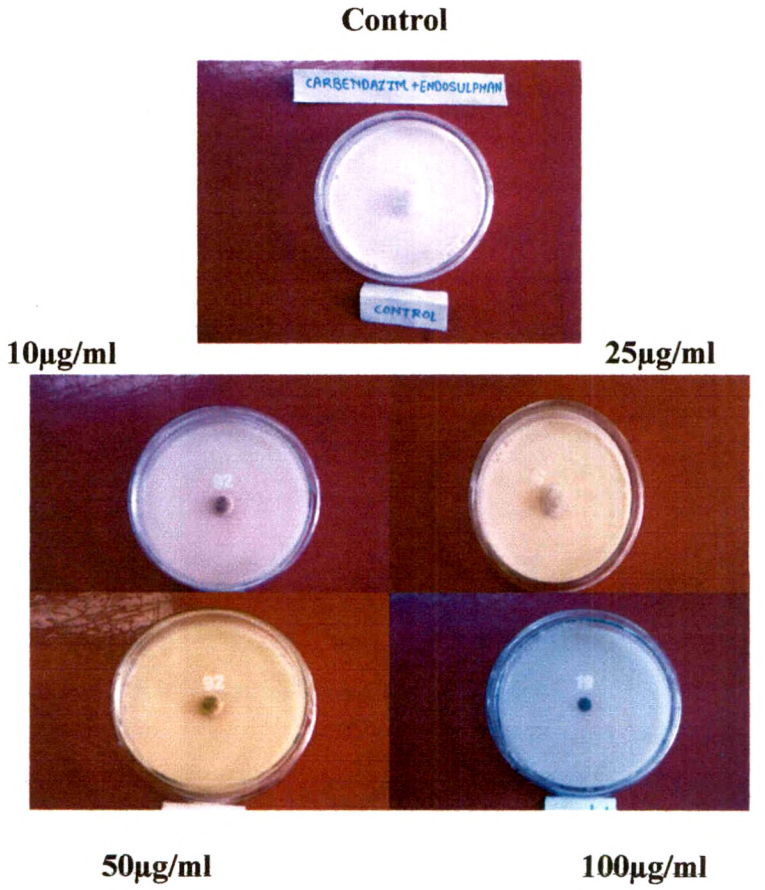


Table 19: Synergistic effects of herbicides on the development of carbendazim resistance in *Alternaria ricini* (*In vitro*).

Herbicides with Carbendazim (60%)	Growth (mm.)
Atrazine (µg/ml)	
10	10.00
25	08.00
50	00.00
100	00.00
2-4-D (µg/ml)	
10	30.00
25	25.00
50	10.00
100	00.00
Mera-71 (µg/ml)	
10	11.00
25	10.00
50	00.00
100	00.00
Control (60%)	15.00

Fig.18: Synergistic effect of carbendazim in combination with other herbicides on linear growth of *Alternaria ricini* resistant isolate (*in vitro*).

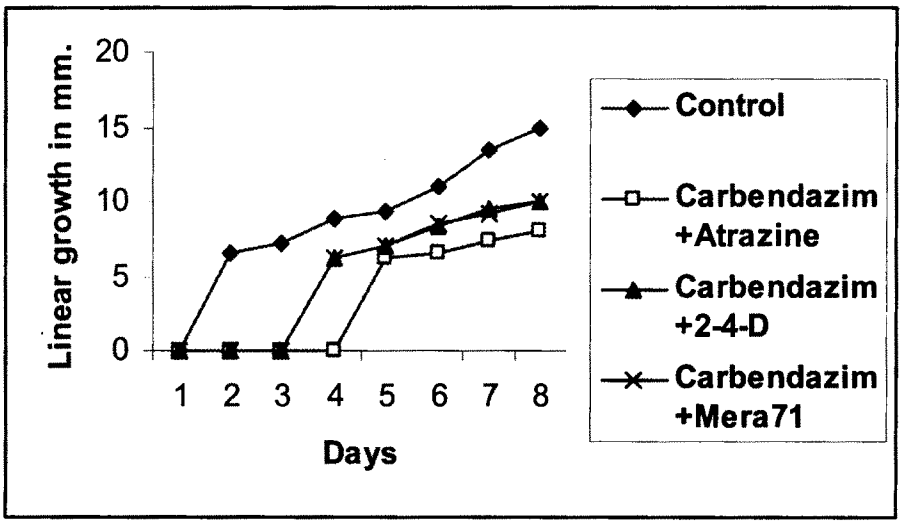


Table 20: Synergistic effects of antibiotics on the development of carbendazim resistance in *Alternaria ricini* (*In vitro*).

Antibiotics with Carbendazim (60%)	Growth (mm.)
Streptomycin (µg/ml)	
0.1	28.00
0.2	22.00
0.3	11.00
0.4	9.00
Aureofungin (µg/ml)	
0.1	10.00
0.2	9.00
0.3	9.00
0.4	7.00
Griseofulvin (µg/ml)	
0.1	12.00
0.2	11.00
0.3	9.00
0.4	8.00
Control (60%)	15.00

Fig.19: Synergistic effect of carbendazim in combination with other antibiotics on linear growth of *Alternaria ricini* resistant isolate (*in vitro*).

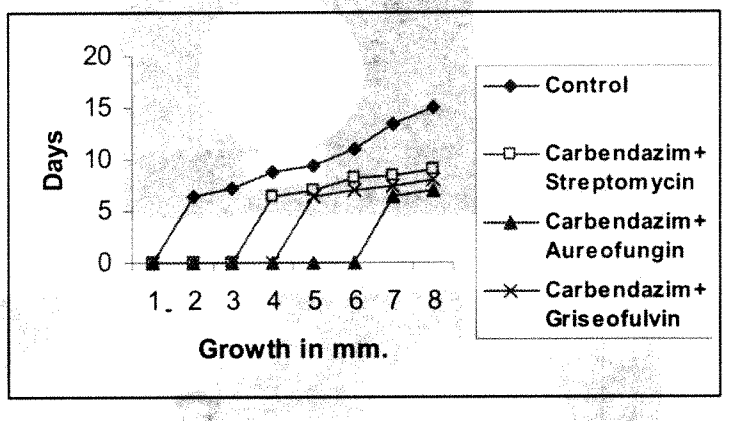
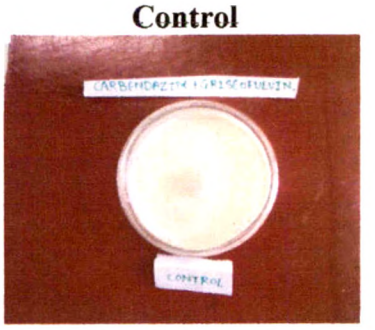


Plate 9: Synergistic effects of antibiotic (griseofulvin) on the development of carbendazim resistance in *Alternaria ricini* (*In vitro*).

Control



0.1µg/ml **0.2µg/ml**

0.3µg/ml **0.4µg/ml**

Table 21: Synergistic effects of salts on the development of carbendazim resistance in *Alternaria ricini* (*In vitro*).

Salts with Carbendazim (60%)	Growth (mm.)
KCl (µg/ml)	
0.1	20.00
0.2	15.00
0.3	11.00
0.4	9.00
NaCl (µg/ml)	
0.1	00.00
1.2	00.00
0.3	00.00
0.4	00.00
Control (60%)	15.00

Fig.20: Synergistic effect of carbendazim in combination with other salts on linear growth of *Alternaria ricini* resistant isolate (*in vitro*).

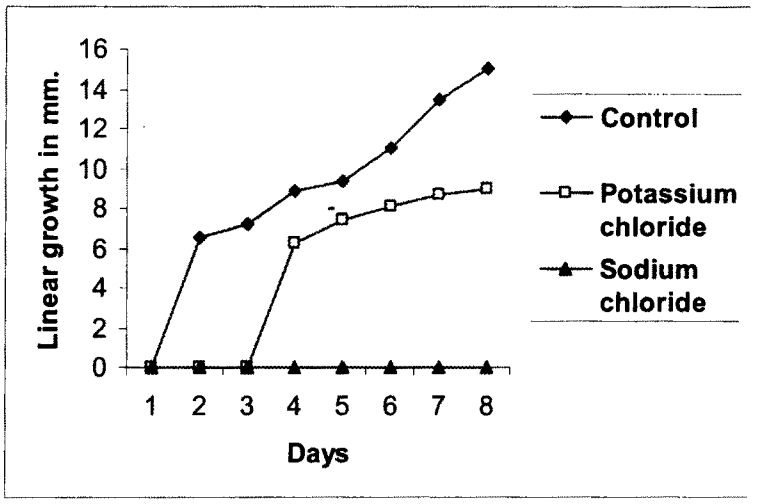


Table 22: Synergistic effects of fertilizers on the development of carbendazim resistance in *Alternaria ricini* (*In vitro*).

Fertilizers with	Growth (mm.)
Carbendazim (60%)	
Muriate of Potash (%)	
0.1	10.00
0.2	13.00
0.3	16.00
0.4	18.00
18:18:10 (%)	
0.1	10.00
0.2	12.00
0.3	20.00
0.4	25.00
Urea (%)	
0.1	9.00
1.2	10.00
0.3	13.00
0.4	14.00
Control (60%)	15.00

Fig. 21: Synergistic effect of carbendazim in combination with other fertilizers on linear growth of *Alternaria ricini* resistant isolate (*in vitro*).

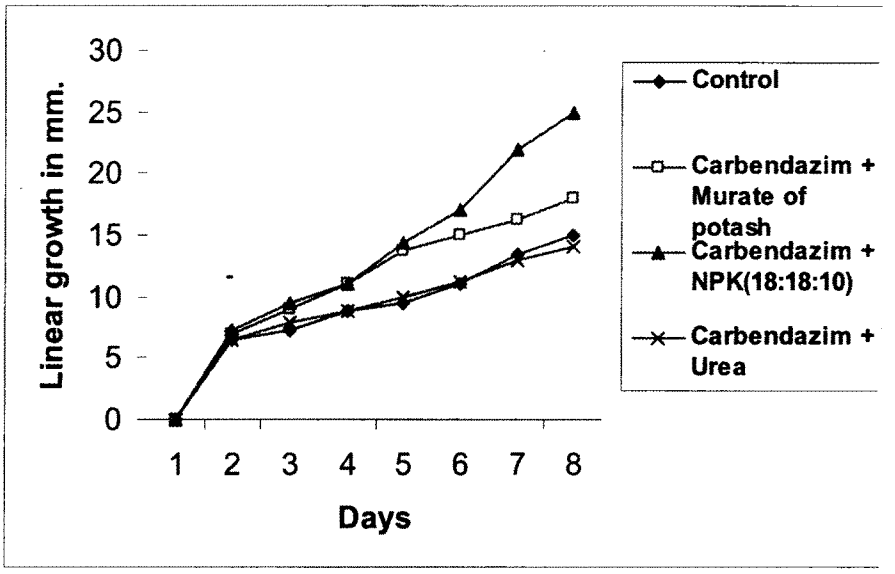
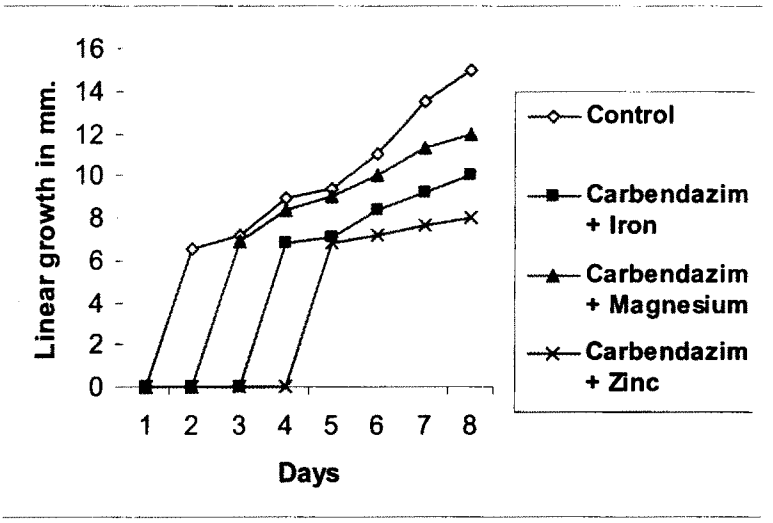


Table 23: Synergistic effects of micronutrients on the development of carbendazim resistance in *Alternaria ricini* (*In vitro*).

Micronutrients with Carbendazim (60%)	Growth (mm.)
Fe (µg/ml)	
0.1	18.00
0.2	17.00
0.3	13.00
0.4	10.00
Mg (µg/ml)	
0.1	20.00
0.2	15.00
0.3	15.00
0.4	12.00
Zn (µg/ml)	
0.1	12.00
1.2	10.00
0.3	9.00
0.4	8.00
Control (60%)	15.00

Fig.22: Synergistic effect of carbendazim in combination with other micronutrients on linear growth of *Alternaria ricini* resistant isolate (*in vitro*).



Salts:

Among the salts, Sodium chloride at 0.1µg/ml with carbendazim (60%) completely inhibited the growth of the pathogen while potassium chloride was not effective (Table 21 and Fig.20).

Fertilizers:

Muriate of potash, 18:18:10 and urea were used for this study. It was observed that all the three fertilizers i. e. muriate of potash and 18:18:10 and urea stimulated the growth of the pathogen with increase in their percentage (Table 22 and Fig.21).

Micronutrients:

Iron, magnesium and zinc were used for this study. All the micronutrients were not effective to control the pathogen (Table 23 and Fig.22).

***In vivo* studies:**

To study synergistic effect of agrochemicals on the development of resistance in *Alternaria ricini* against carbendazim, 10 ml mycelial suspension of one culture tube of resistant mutant isolate (AR-SA-5) was inoculated on healthy castor leaves treated with resistant dose of carbendazim (5%) and other agrochemicals at different concentrations, 24 hrs before. Each castor leaf was covered with polythene bag. Percentage of infection on castor leaves over carbendazim alone was considered as increase or decrease in the resistance respectively.

Fungicides:

Among the fungicides, captan at10µg/ml with carbendazim 5% completely checked the infection of the pathogen to caster. Mancozeb at100µg/ml, with carbendazim 5% protected the plant from infection of the pathogen. Zineb was not effective to control the pathogen. (Table24).

Insecticides:

Among insecticides, only Phorate was only effective to check the growth of the pathogen completely at 100µg/ml with carbendazim (5%) while dimethoate and endosulphan were not effective (Table 25).

Herbicides:

From the results of table 26, it was observed that among the herbicides atrazine was effective which checked the infection of the pathogen at 25µg/ml, with carbendazim (5%).As well as a new herbicide mera-71 was also able to protect the castor from pathogen at 100µg/ml, with carbendazim (5%). But 2-4-D was not effective.

Antibiotics:

All the three antibiotics viz. streptomycin, aureofungin and griseofulvin were unable to stop the infection of castor plant (Table 27).

Salts:

It was observed that, among the sodium chloride and potassium chloride both were not effective to check the infection of the pathogen (Table 28).

Fertilizers:

It was observed that all the three fertilizers stimulated the infection of the pathogen with increase in their percentage (Table 29).

Micronutrients:

Iron, magnesium and zinc were used for this study. All the micronutrients were not effective to control the pathogen (Table 30).

Table 24: Synergistic effects of fungicides on the development of carbendazim resistance in *Alternaria ricini* (*In vivo*).

Fungicides with	Percentage of infection.
Carbendazim (5%)	
Captan (µg/ml)	
10	0
25	0
50	0
100	0
Mancozeb (µg/ml)	
10	3
25	2
50	2
100	0
Zineb (µg/ml)	
10	4
25	3
50	3
100	2
Control (5%)	5

Table 25: Synergistic effects of insecticides on the development of carbendazim resistance in *Alternaria ricini* (*In vivo*).

Insecticides with Carbendazim (5%)	Percentage of infection
Dimethoate (µg/ml)	
10	5
25	4
50	4
100	3
Endosulphan (µg/ml)	
10	4
25	3
50	2
100	2
Phorate (µg/ml)	
10	3
25	2
50	2
100	0
Control (5%)	5

Table 26: Synergistic effects of Herbicides on the development of carbendazim resistance in *Alternaria ricini* (*In vivo*).

Herbicides with	Percentage of infection.
Carbendazim (5%)	
Atrazine (µg/ml)	
10	2
25	0
50	0
100	0
2-4-D (µg/ml)	
10	3
25	3
50	2
100	2
Mera-71 (µg/ml)	
10	3
25	2
50	2
100	0
Control (5%)	5

Table 27: Synergistic effects of antibiotics on the development of carbendazim resistance in *Alternaria ricini* (*In vivo*).

Antibiotics with Carbendazim (5%)	Percentage of infection.
Streptomycin (µg/ml)	
0.1	4
0.2	4
0.3	3
0.4	3
Aureofungin (µg/ml)	
0.1	4
0.2	3
0.3	3
0.4	2
Griseofulvin (µg/ml)	
0.1	4
0.2	3
0.3	3
0.4	2
Control (5%)	5

Table 28: Synergistic effects of salts on the development of carbendazim resistance in *Alternaria ricini* (*In vivo*).

Salts with Carbendazim (5%)	Percentage of infection.
KCl (µg/ml)	
0.1	3
0.2	3
0.3	2
0.4	1
NaCl (µg/ml)	
0.	2
0.2	2
0.3	2
0.4	1
Control (5%)	5

Table 29: Synergistic effects of fertilizers on the development of carbendazim resistance in *Alternaria ricini* (*In vivo*).

Fertilizers with Carbendazim (5%)	Percentage of infection.
Murate of Potash (%)	
0.1	2
0.2	2
0.3	3
0.4	4
18:18:10 (%)	
0.1	2
0.2	3
0.3	3
0.4	4
Urea (%)	
0.1	2
1.2	3
0.3	4
0.4	4
Control (5%)	5

Table 30: Synergistic effects of micronutrients on the development of carbendazim resistance in *Alternaria ricini* (*In vivo*).

Micronutrients with Carbendazim (5%)	Percentage of infection.
Fe (µg/ml)	
0.1	4
0.2	3
0.3	3
0.4	2
Mg (µg/ml)	
0.1	3
0.2	2
0.3	2
0.4	2
Zn (µg/ml)	
0.1	3
1.2	2
0.3	2
0.4	2
Control (5%)	5

PCE of Agrochemicals:

The percentage control efficacy of each agrochemical was calculated as follows:

$$PCE = 100(1-X/Y)$$

Where:

X=is the diameter of the colony on medium amended with fungicides.

Y= is the diameter of the colony on the medium alone.

The results are as given bellow:

***In vitro* studies:**

The results in the table 31 indicate that, captan showed 100% PCE at10µg/ml while mancozeb and zineb showed 100% PCE at 50µg/ml and 25µg/ml respectively.

In case of insecticides, it was observed that, phorate at 10µg/ml showed 100% PCE. While dimethoate and endosulphan showed 100% PCE at 100µg/ml (Table 32).

From the table 33, it was observed that, among the herbicides, atrazine and mera-71 showed 100% PCE at 50µg/ml.While 2-4-D at 100µg/ml showed 100% PCE.

Among the antibiotics, viz. streptomycin, aureofungin and griseofulvin showed 88.75, 91.25 and 90% PCE at 0.4% respectively (Table 34).

Among the salts, Sodium chloride showed 100% PCE at 0.1µg/ml.While Potassium chloride at 0.4µg/ml showed 88.75% PCE (Table 35).

From the table 36, it is observed that, among the fertilizers, muriate of potash, urea and 18:18:10 showed 77.50%, 68.75% and 82.50% PCE at 0.4%.

From the table 37, it is observed that, all micronutrients did not show 100% PCE.

Table 31: Percentage control efficacy (PCE) of fungicide mixtures in controlling the carbendazim resistant isolates of *Alternaria ricini* (*in vitro*).

Mixture dose with Carbendazim	PCE
Captan (µg/ml)	
10	100
25	100
50	100
100	100
Mancozeb (µg/ml)	
10	87.5
25	90
50	100
100	100
Zineb (µg/m)	
10	90
25	100
50	100
100	100
Control (60%)	81.25

Table 32: Percentage control efficacy (PCE) of insecticide mixtures in controlling the carbendazim resistant isolates of *Alternaria ricini* (*in vitro*).

Mixture dose with Carbendazim	PCE
Dimethoate (µg/ml)	
10	72.50
25	78.75
50	87.50
100	100
Endosulphan (µg/ml)	
10	68.75
25	78.75
50	83.75
100	100
Phorate (µg/ml)	
10	100
25	100
50	100
100	100
Control (60%)	81.25

Table 33: Percentage control efficacy (PCE) of herbicide mixtures in controlling the carbendazim resistant isolates of *Alternaria ricini* (*in vitro*).

Mixture dose with Carbendazim	PCE
Atrazine (µg/ml)	
10	87.50
25	90.00
50	100
100	100
2-4-D (µg/ml)	
10	62.5
25	68.75
50	87.50
100	100
Mera-70 (µg/ml)	
10	86.25
25	87.50
50	100
100	100
Control (60%)	81.25

Table 34: Percentage control efficacy (PCE) of antibiotics mixtures in controlling the carbendazim resistant isolates of *Alternaria ricini* (*in vitro*).

Mixture dose with Carbendazim	PCE
Streptomycin (µg/ml)	
0.1	65.00
0.2	72.50
0.3	86.25
0.4	88.75
Aureofungin (µg/ml)	
0.1	87.50
0.2	88.75
0.3	88.75
0.4	91.25
Griseofulvin (µg/ml)	
0.1	85.00
0.2	86.25
0.3	88.75
0.4	90.00
Control (60%)	81.25

Table 35: Percentage control efficacy (PCE) of salts mixtures in controlling the carbendazim resistant isolates of *Alternaria ricini* (*in vitro*).

Mixture dose with Carbendazim	PCE
KCl (µg/ml)	
0.1	75.00
0.2	81.25
0.3	86.25
0.4	88.75
NaCl (µg/ml)	
0.1	100
0.2	100
0.3	100
0.4	100
Control (60%)	81.25

Table 36: Percentage control efficacy (PCE) of fertilizers mixtures in controlling the carbendazim resistant isolates of *Alternaria ricini* (*in vitro*).

Mixture dose with Carbendazim	PCE
Muriate of Potash (%)	
0.1	87.50
0.2	83.75
0.3	80.00
0.4	77.50
18:18:10 (%)	
0.1	87.50
0.2	85.00
0.3	75.00
0.4	68.75
Urea (%)	
0.1	88.75
1.2	87.50
0.3	83.75
0.4	82.50
Control (60%)	81.25

Table 37: Percentage control efficacy (PCE) of micronutrients mixtures in controlling the carbendazim resistant isolates of *Alternaria ricini* (*in vitro*).

Mixture dose with Carbendazim	PCE
Fe (µg/ml)	
0.1	77.50
0.2	78.75
0.3	83.75
0.4	87.50
Mg (µg/ml)	
0.1	75.00
0.2	81.25
0.3	81.25
0.4	85.00
Zn (µg/ml)	
0.1	85.00
1.2	87.50
0.3	88.75
0.4µ	90.00
Control (60%)	81.25

***In vivo* studies**

The results in the table 38 indicate that, captan showed 100% PCE at 10µg/ml while mancozeb showed 100% PCE at 100µg/ml. But zineb at100µg/ml showed 83.33 % PCE.

From the table 39 it is observed that, among the insecticides, phorate at 100µg/ml showed 100% PCE. While dimethoate and endosulphan showed 75 and 83.33% PCE at 100µg/ml respectively.

From the table 40 it is observed that, among the herbicides, atrazine showed 100% PCE at 25µg/ml.While mera-71 at 100µg/ml showed 100% PCE. But 2-4-D showed 83.33% PCE at 100µg/ml.

From the table 41 it is observed that, among the antibiotics, streptomycin, aureofungin and griseofulvin showed 75, 83.33 and 83.33% PCE at 0.4µg/ml respectively.

From the table 42 it is observed that, among the salts, potassium chloride and sodium chloride showed 91.66% PCE at 0.2, 0.4 and 0.3µg/ml respectively.

From the table 43 it is observed that, among the fertilizers, muriate of potash, 18:18:10 and urea showed 66.67% PCE at 0.4% respectively.

From the table 44 it is observed that, among the micronutrients, iron, magnesium and zinc at 0.4µg/ml showed 83.33% PCE.

Table 38: Percentage control efficacy (PCE) of fungicide mixtures in controlling the carbendazim resistant isolates of *Alternaria ricini* (*in vivo*).

Mixture dose with Carbendazim	PCE
Captan (µg/ml)	
10	100
25	100
50	100
100	100
Mancozeb (µg/ml)	
10	75.00
25	83.33
50	83.33
100	100
Zineb (µg/ml)	
10	66.67
25	75.00
50	75.00
100	83.33
Control (5%)	58.33

Table 39: Percentage control efficacy (PCE) of insecticides mixtures in controlling the carbendazim resistant isolates of *Alternaria ricini* (*in vivo*).

Mixture dose with Carbendazim	PCE
Dimethoate (µg/ml)	
10µg/ml	58.33
25µg/ml	66.67
50µg/ml	66.67
100µg/ml	75.00
Endosulphan (µg/ml)	
10µg/ml	66.67
25µg/ml	75.00
50µg/ml	83.33
100µg/ml	83.33
Phorate (µg/ml)	
10µg/ml	75.00
25µg/ml	83.33
50µg/ml	83.33
100µg/ml	100
Control (5%)	58.33

Table 40: Percentage control efficacy (PCE) of hHerbicide mixtures in controlling the cCarbendazim resistant isolates of *Alternaria ricini* (*in vitro*).

Mixture dose with Carbendazim	PCE
Atrazine (µg/ml)	
10µg/ml	83.33
25µg/ml	100
50µg/ml	100
100µg/ml	100
2-4-D (µg/ml)	
10µg/ml	75.00
25µg/ml	75.00
50µg/ml	83.33
100µg/ml	83.33
Mera-70 (µg/ml)	
10µg/ml	75.00
25µg/ml	83.33
50µg/ml	83.33
100µg/ml	100
Control (5%)	58.33

Table 41: Percentage control efficacy (PCE) of antibiotics mixtures in controlling the carbendazim resistant isolates of *Alternaria ricini* (*in vivo*).

Mixture dose with Carbendazim	PCE
Streptomycin (µg/ml)	
0.1	66.67
0.2	66.67
0.3	75.00
0.4	75.00
Aureofungin (µg/ml)	
0.1	66.67
0.2	75.00
0.3	75.00
0.4	83.33
Griseofulvin (µg/ml)	
0.1	66.67
0.2	75.00
0.3	75.00
0.4	83.33
Control (60%)	58.33

Table 42: Percentage control efficacy (PCE) of salts mixtures in controlling the carbendazim resistant isolates of *Alternaria ricini* (*in vivo*).

Mixture dose with Carbendazim	PCE
KCl (µg/ml)	
0.1	75.00
0.2	75.00
0.3	83.33
0.4	91.66
NaCl (µg/ml)	
0.1	83.33
0.2	83.33
0.3	83.33
0.4	91.66
Control (5%)	58.33

Table 43: Percentage control efficacy (PCE) of fertilizers mixtures in controlling the carbendazim resistant isolates of *Alternaria ricini* (*in vivo*).

Mixture dose with Carbendazim	PCE
Murate of Potash (%)	
0.1	83.33
0.2	83.33
0.3	75.00
0.4	66.67
18:18:10 (%)	
0.1	83.33
0.2	75.00
0.3	75.00
0.4	66.67
Urea (%)	
0.1	83.33
1.2	75.00
0.3	66.67
0.4	66.67
Control (5%)	58.33

Table 44: Percentage control efficacy (PCE) of micronutrients mixtures in controlling the carbendazim resistant isolates of *Alternaria ricini* (*in vivo*).

Mixture dose with Carbendazim	PCE
Fe (µg/ml)	
0.1	66.67
0.2	75.00
0.3	75.00
0.4	83.33
Mg (µg/ml)	
0.1	75.00
0.2	83.33
0.3	83.33
0.4	83.33
Zn (µg/ml)	
0.1	75.00
1.2	83.33
0.3	83.33
0.4	83.33
Control (5%)	58.33