EXPERIMENTAL RESULTS

EXPERIMENTAL RESULTS:

***Alternaria* leaf spot of Gerbera:**

(*Alternaria alternata* (Fries) keisler)

Sensitivity of *Alternaria alternata* to carbendazim:

Samples of Gerbera (*Gerbera jamesonii)* infected leaves were collected from different districts of Western Maharashtra. Four isolates of *Alternaria alternata* were obtained from above samples on Gerbera leaf extract agar medium. Carbendazim sensitivity of these isolates was tested by food poisoning technique using Gerbera leaf extract agar medium.

Plates were prepared with Gerbera leaf extract medium containing different concentration of carbendazim. Each isolate was tested at different concentrations of carbendazim .The seven days old culture of pathogen was cut into 8mm disc using sterile cork borer and was placed at the center of agar plate. These treatments were maintained in triplicate.

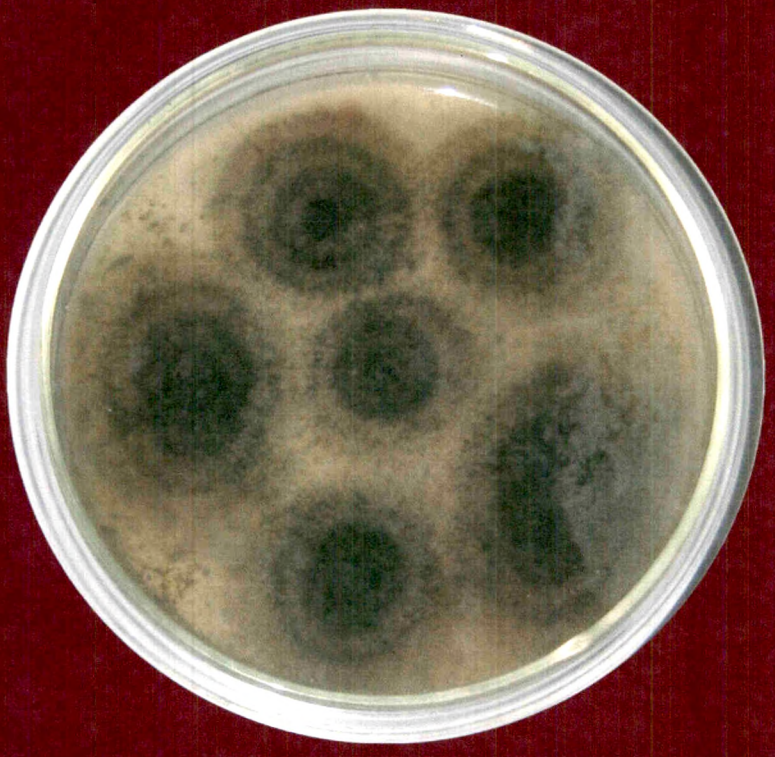
For *in vivo* experiment *Gerbera jamesonii* plants were grown in earthen pots and kept in departmental polyhouse. The plants were then inoculated by mycelial/spore suspension of *Alternaria alternata* with help of camel head brush Gerbera plants (5 replicates) were treated with different concentration of carbendazim 24 hours before inoculation. The plants after treatments were covered with sterile polythene bags. The infection was recorded after various incubation periods. The results are depicted in Tables 1 to 8. It was observed that there was variation in MIC of carbendazim against

different isolates. MIC of carbendazim on plates ranged from 10% to 15% and 5% to8% on Gerbera plants. Isolates Aa-4 from Pune appeared to having resistant factor 1.5

Dose responses cure of *Alternaria alternata* isolates against carbendazim are shown in Figs. 1 to 8. It appeared that with the increase in the concentration of carbendazim in the medium there was decrease in the radial growth of the pathogen.

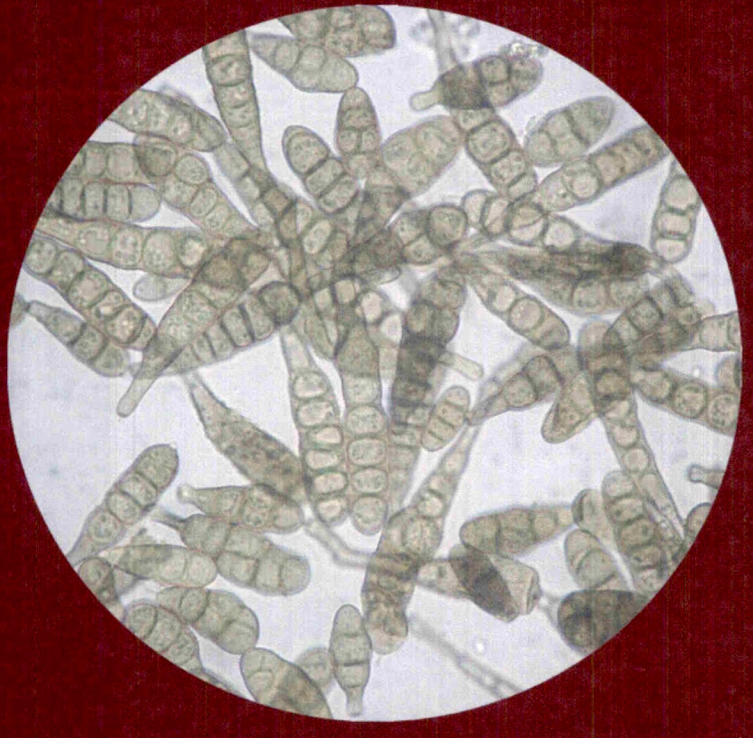
PLATE NO.1 *Habit of Gerbera jamesonii*

PLATE NO.2 *Alternaria alternata* (Fries) Keisler pure culture



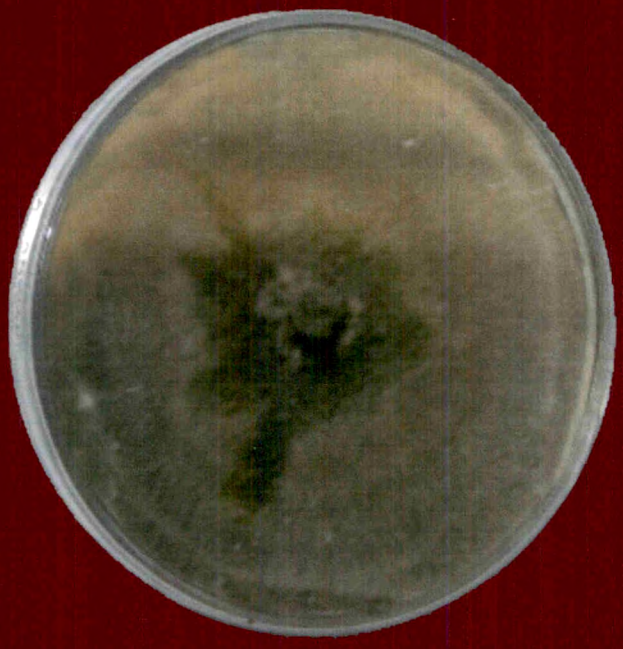
Pure Culture Plate

Pure Culture Tube



Conidia

PLATE 3 Sensitivity of *Alternaria alternata* isolate AA-1 to carbendazim



Control

10.5 % carbendazim

Table 1:Sensitivity of *Alternaria alternata* (*Aa-1*) to carbendazim (*in vitro*)

Concentration (%)	Linear growth (mm) Days							
	1	2	3	4	5	6	7	8
Control	32	48	51	56	70	80	94	100
8	0	12	15	18	20	20	21	22
8.5	0	12	15	18	20	20	21	22
9	0	12	13	13	13	16	16	16
9.5	0	0	12	13	13	16	16	16
10	0	0	0	13	13	14	14	14
10.5	0	0	0	0	0	0	0	0

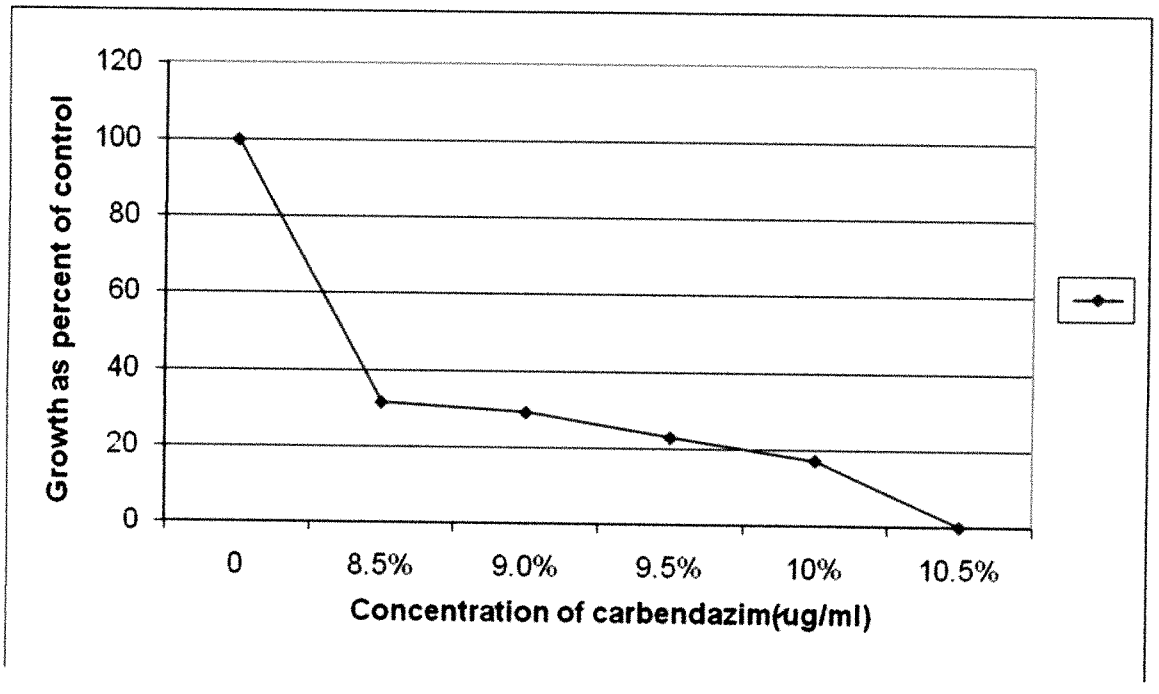


Fig:1 Dose response curve at *Alternaria alternata* (*Aa-1*) isolate causing leaf spot of *Gerbera jamesonii (in vitro)*

Table 2: Sensitivity of *Alternaria alternata* (*Aa-2*) carbendazim (*in vitro*)

Concentration (%)	Linear growth (mm) Days							
	1	2	3	4	5	6	7	8
Control	19	33	40	47	63	75	80	100
8	14	15	19	21	24	25	25	14
8.5	12	13	15	18	20	21	21	12
9	0	13	14	14	14	14	15	16
9.5	0	12	12	13	13	13	14	14
10	0	0	0	0	0	09	10	10

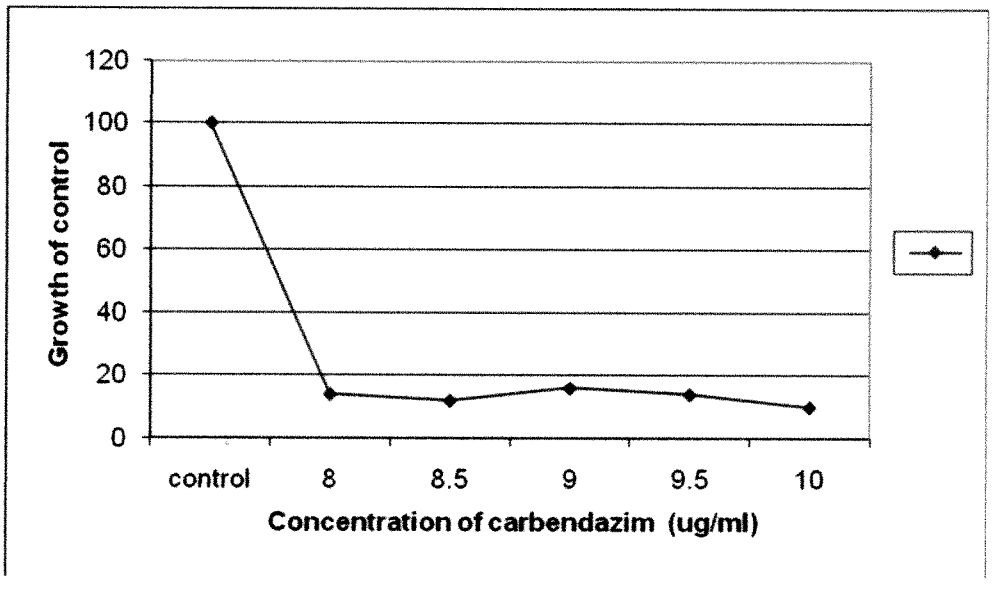


Fig:2 Dose response curve at *Alternaria alternata* (*Aa-2*) isolate causing leaf spot of *Gerbera jamesonii (in vitro)*

PLATE 4 Sensitivity of *Alternaria alternata* isolate AA-2 to carbendazim

Control

10 % carbendazim

PLATE 5 Sensitivity of *Alternaria alternata* isolate AA-3 to carbendazim

Control

10.5 % carbendazim

Table 3: Sensitivity of *Alternaria alternata* (*Aa-3*) carbendazim (*in vitro*)

Concentration (%)	Linear growth (mm) Days							
	1	2	3	4	5	6	7	8
Control	15	19	28	48	55	61	85	100
8.5	13	14	18	23	27	29	29	31
9	0	12	15	18	20	27	29	29
9.5	0	13	14	15	21	21	22	22
10	0	13	14	15	16	16	17	17
10.5	0	0	0	0	0	0	0	0

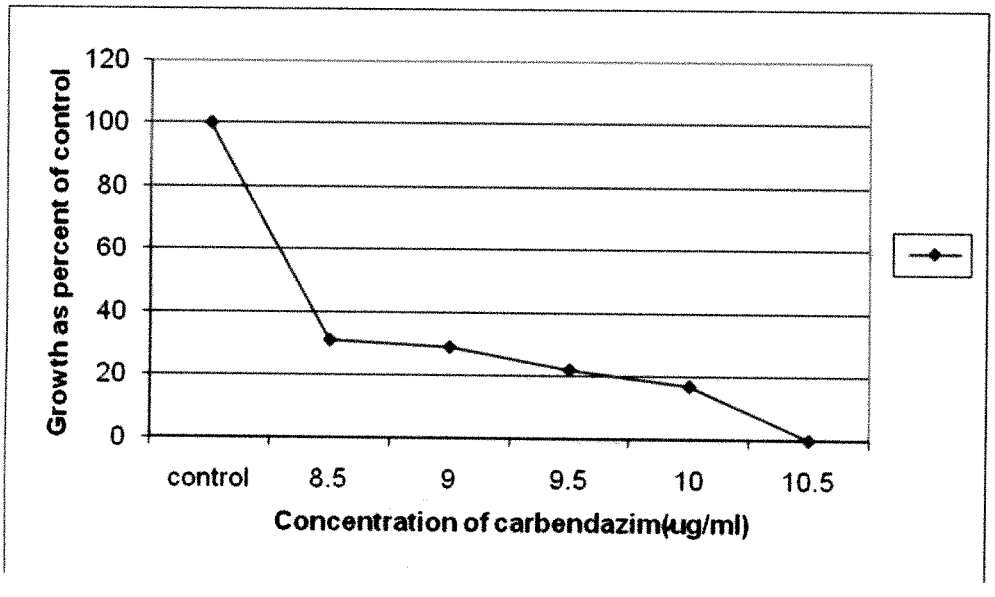


Fig: 3 Dose response curve at *Alternaria alternata* (*Aa-3*) isolate causing leaf spot of *Gerbera jamesonii (in vitro)*

Table 4: Sensitivity of *Alternaria alternata* (*Aa-4*) carbendazim (*in vitro*)

Concentration (%)	Linear growth (mm) Days							
	1	2	3	4	5	6	7	8
Control	11	29	41	52	62	72	86	100
11	12	14	17	20	21	23	28	28
12	0	13	17	19	20	22	24	26
13	0	13	17	21	23	24	25	26
14	0	13	14	16	19	19	20	20
15	0	0	0	0	0	0	0	0

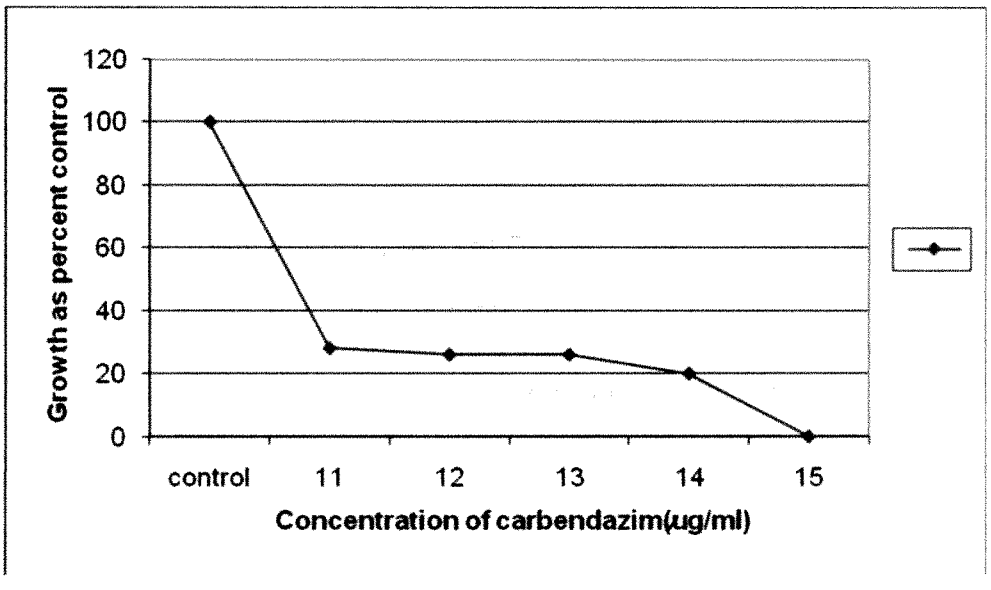
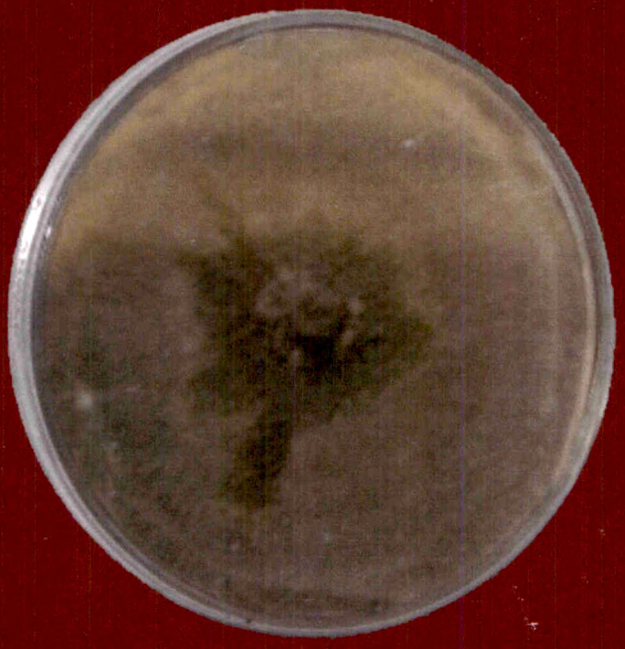


Fig:4 Dose response curve at *Alternaria alternata* (*Aa-4*) isolate causing leaf spot of *Gerbera jamesonii (in vitro)*

PLATE 6 Sensitivity of *Alternaria alternata* isolate AA-4 to carbendazim



Control

15 % carbendazim

Table 5: Sensitivity of *Alternaria alternata* (*Aa-1*) carbendazim (*in vivo*)

Sr.No.	Concentration(%)	Grade
1	Absolute control	0
2	control	4
3	1	3
4	1.5	3
5	2	2
6	2.5	2
7	3	1
8	3.5	1
9	4	1
10	5	1
11	5	1
12	5.5	0

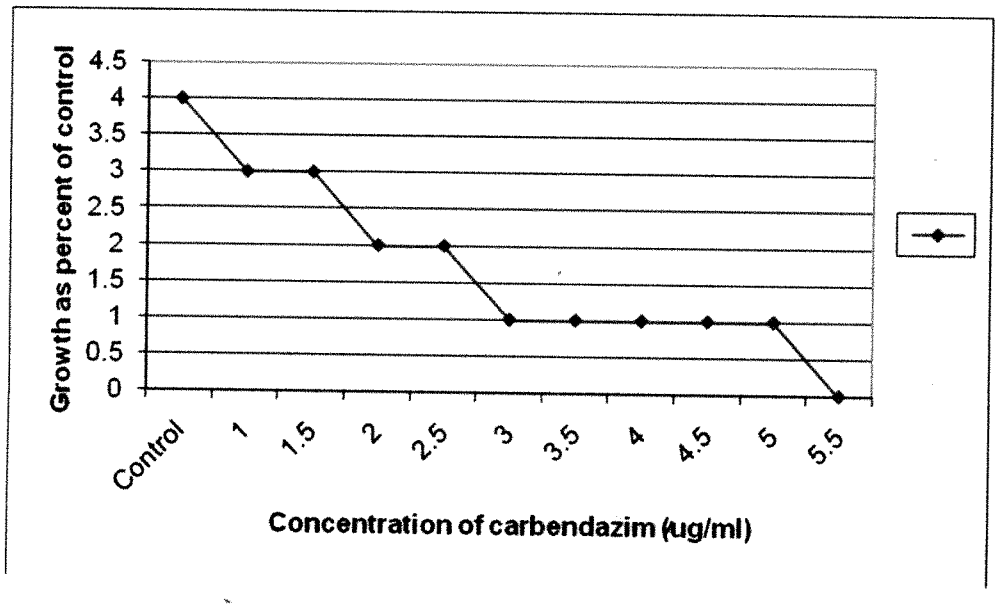


Fig:5 Dose response curve at *Alternaria alternata* (*Aa-1*) isolate causing leaf spot of *Gerbera jamesonii (in vivo)*

Table 6: Sensitivity of *Alternaria alternata* (*Aa-2*) carbendazim (*in vivo*)

Sr.No	Concentration(%)	Grade
1	Absolute control	0
2	Control	3
3	1	2
4	2	2
5	3	1
6	4	1
7	5	0

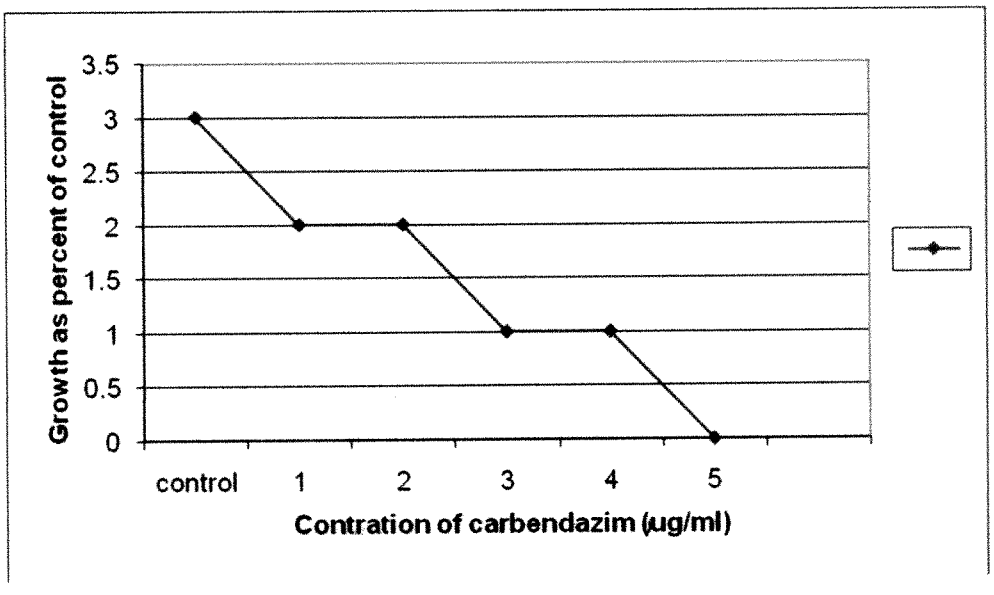
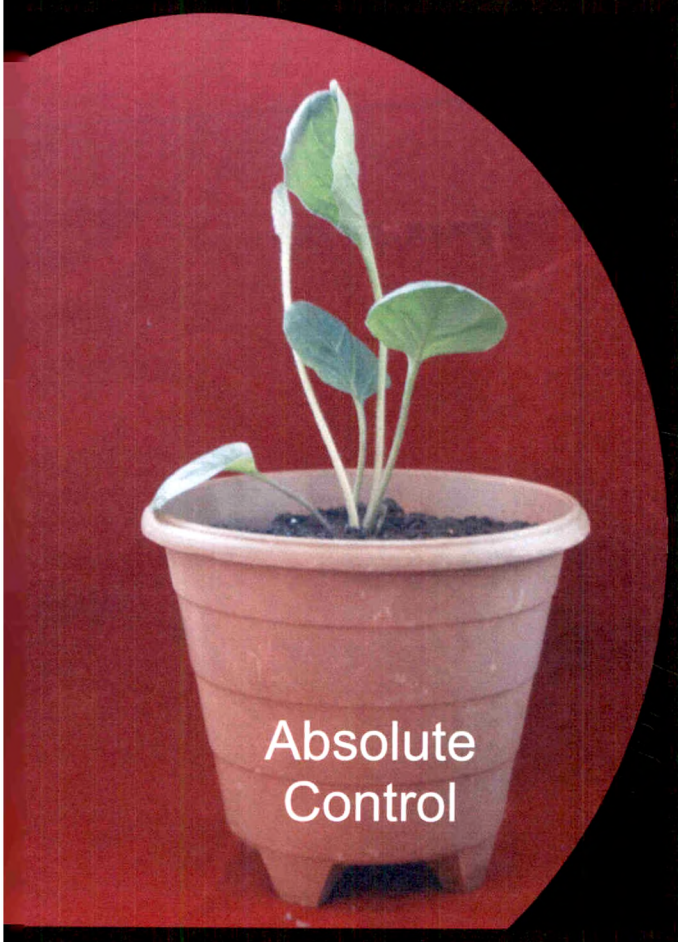
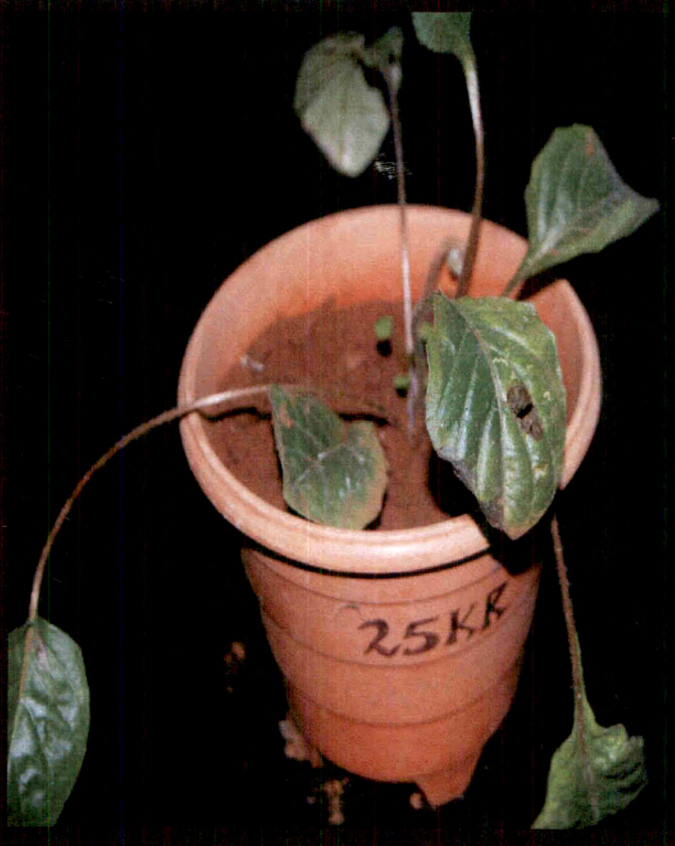


Fig:6 Dose response curve at *Alternaria alternata* (*Aa-2*) isolate causing leaf spot of *Gerbera jamesonii (in vivo)*

PLATE NO.8 Sensitivity of Alternaria alternata isolate *Aa-2* to carbendazim





5% Carbendazim

Control

Table 7: Sensitivity of *Alternaria alternata* (*Aa-3*) carbendazim (*in vivo*)

Sr.No	Concentration(%)	Grade
1	Absolute control	0
2	Control	4
3	1	2
4	2	2
5	3	2
6	4	1
7	5	0

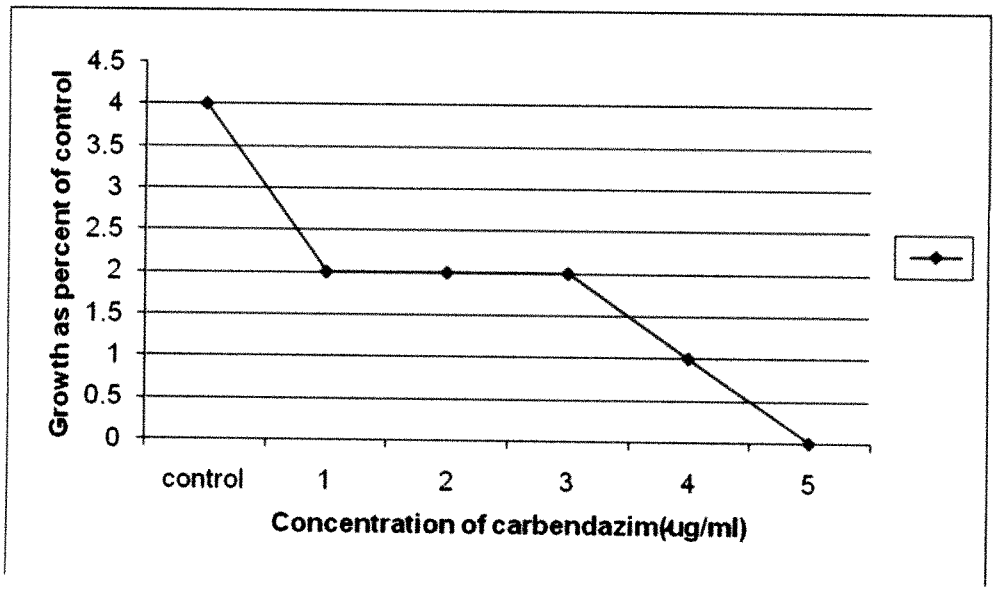


Fig:7 Dose response curve at *Alternaria alternata* (Aa-3) isolate causing leaf spot of *Gerbera jamesonii (in vivo)*

Table 8: Sensitivity of *Alternaria alternata* (*Aa-4*) carbendazim (*in vivo*)

Sr.no	Concentration(%)	Grade
1	Absolute control	0
2	Control	4
3	1	3
4	2	3
5	3	2
6	4	2
7	5	1
8	6	1
9	7	1
10	8	0

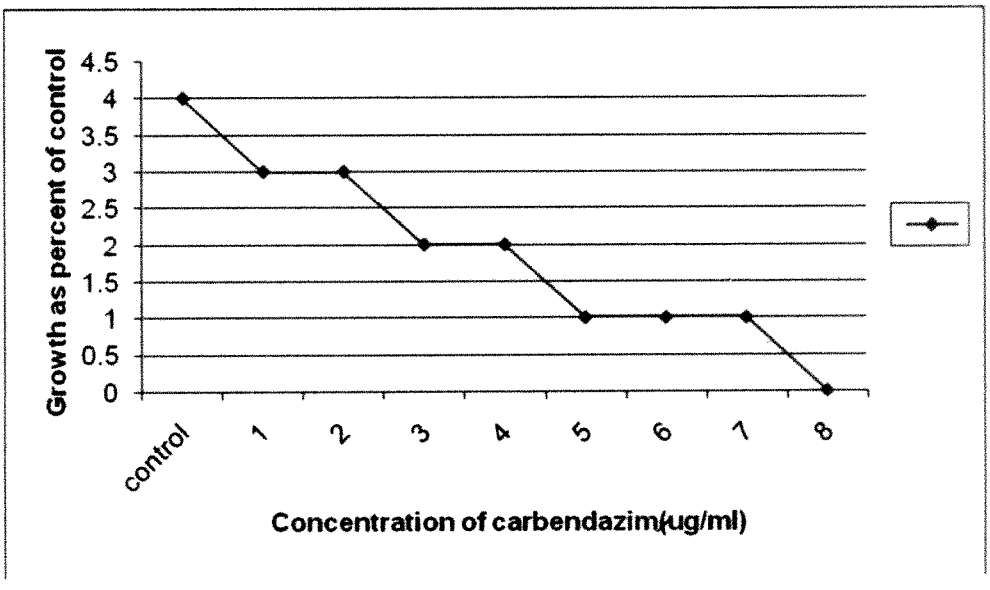


Fig: 8 Dose response curve at *Alternaria alternata* (Aa-4) isolate causing leaf spot of *Gerbera jamesonii (in vivo)*

Effect of passage on the development of carbendazim resistance in *Alternaria alternata:*

***In vitro* studies:**

The effect of continuous, alternate and mixture treatments with two fungicides having different mode of action on the development of carbendazim resistance in *Alternaria alternata* was studied. For this experiment sensitive isolates Aa-2 was cultured for eight successive passages on Gerbera leaf extract agar medium plates containing mancozeb, kocide-101 and roko alternating or mixed with carbendazim.

Continuous and alternate treatment with carbendazim:

Sensitive isolates *Aa-2* was cultured on plates containing carbendazim of MIC level (10%) for eight successive passages. 8mm diameter agar disc from the margin of actively growing colony taken from the culture of the previous passage was placed at the center of each plate in triplicate. In each passage growth of mycelium was measured after 8 days. The increase or decrease in the radial growth from passage to passage was employed as criterion for development carbendazim resistance. It was seen that culturing of the pathogen on the medium containing carbendazim for eight successive passages significantly increased the carbendazim resistance in *Alternaria alternata*. But when the pathogen was cultured alternately with mancozeb and kocide-101 there was complete inhibition of pathogen at second passage only while in case of roko, inhibition of pathogen was seen at 4^{th} passage (Table-9)

Treatment of carbendazim in mixture of different fungicide:

In this experiment sensitive isolates *Aa-2* was cultured on plates containing carbendazim at MIC level (10%) for eight successive passages. Carbendazim was mixed with mancozeb, kocide-101, and roko at same concentration. Then 8mm diameter agar disc from culture of previous passage was placed at the center of each plate in triplicate. In each passage growth of mycelium was measured after eight days.

Results are shown in Table -10 and fig 10. When carbendazim was mixed with mancozeb, kocide-101 and roko the growth of the pathogen was completely inhibited at first passage only.

Continuous and alternate passage:

Table 9: Effect of exposure of *Alternaria alternata (in vitro)* carbendazim continuos and alternating with other fungicide on the development of resistance during eight successive passages

Fungicide	Passage number							
	1	2	3	4	5	6	7	8
Carbendazim individual	11.00	14.66	21.00	25.66	33.33	39.33	43.66	50.33
Carbendazim + Mancozeb	11.00	00	00	00	00	00	00	00
Carbendazim + Kocide-101	11.00	00	00	00	00	00	00	00
Carbendazim + Roko	11.00	09.33	10.00	00	00	00	00	00

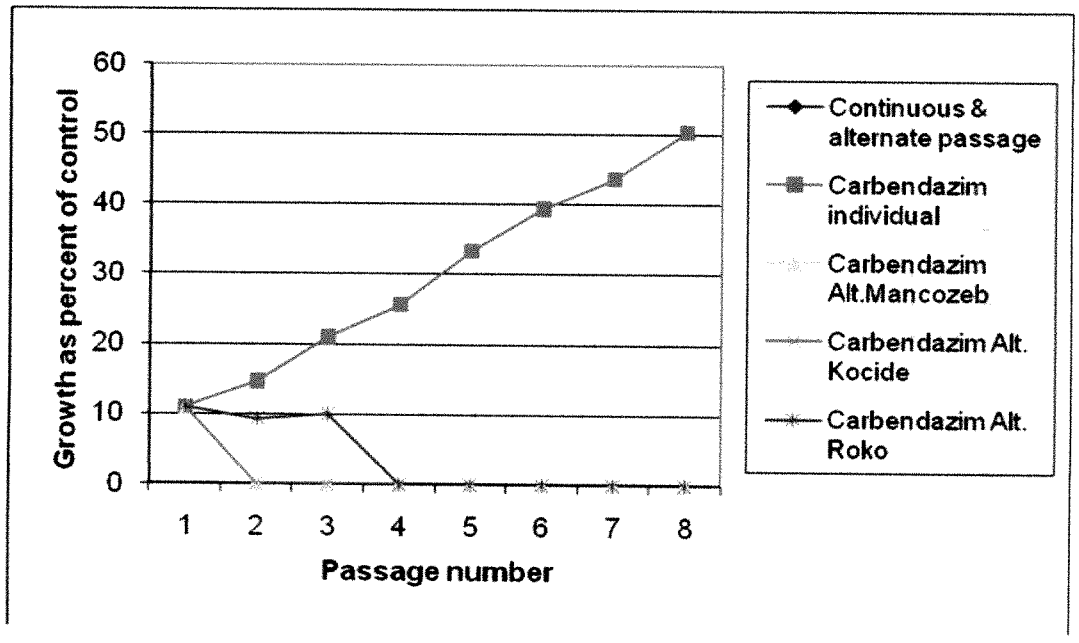


Fig 9: The effect of continuous and alternate exposure to carbendazim with other fungicide on mycelial growth of *Alternaria alternata (in vitro)* during eight successive passages.

Mixed passage:

Table 10: Effect of exposure of *Alternaria alternata (in vitro)* to the mixture of Carbendazim with other fungicide on the development of resistance during eight successive passages

Fungicide	Passage number							
	1	2	3	4	5	6	7	8
Carbendazim individual	11.00	14.66	21.00	25.66	33.33	39.66	43.66	50.33
Carbendazim + Mancozeb	00	00	00	00	00	00	00	00
Carbendazim + Kocide-101	00	00	00	00	00	00	00	00
Carbendazim + Roko	00	00	00	00	00	00	00	00

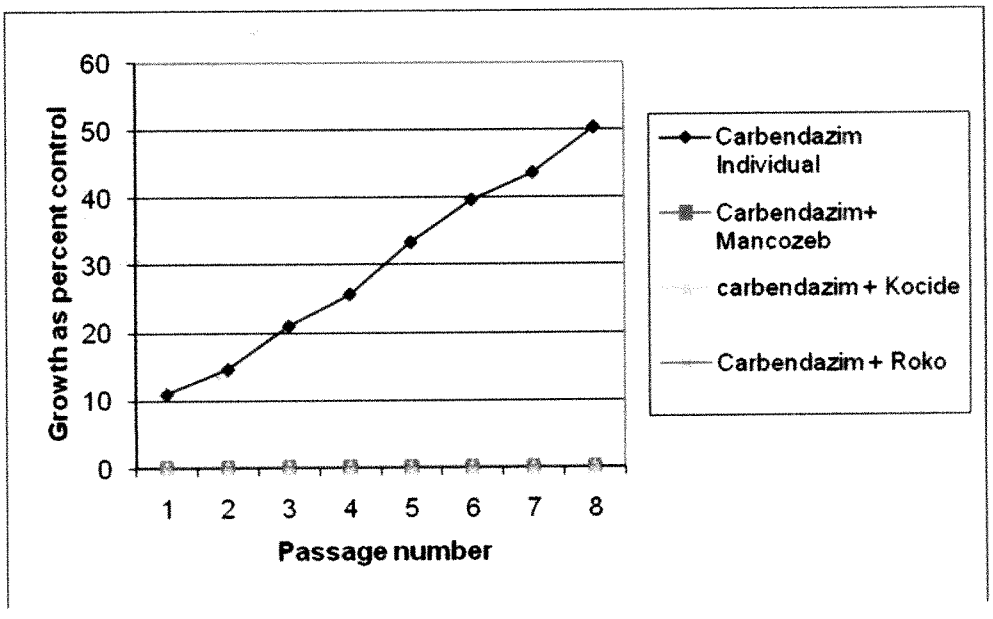


Table10: Effect of exposure of *Alternaria alternata (in vitro)* to the mixture of Carbendazim with other fungicide on the development of resistance during eight successive passages

PLATE NO.6 Effect of exposure of *Alternaria alternata* (*in vitro*) to the mixture of carbendazim with other fungicide

Control

Kocide-101

Mancozeb

Roko

***In vivo* studies:**

Continuous and alternate treatment of Carbendazim:

In vivo experiment, Gerbera plants were inoculated with mycelial suspension of *Alternaria alternata*. Before that these plants were treated with different fungicide like carbendazim, mancozeb, kocide-101, and roko. These plants were covered with polythene bag. After eight days a mycelial suspension from such infected leaves was prepared and applied to healthy Gerbera plant and treated with another fungicide at the same concentration. Same procedure followed up to eight passages.

It shows that, when carbendazim alone was used there was increase in the disease from passage to passage. When carbendazim was altered with mancozeb and kocide-101 the disease was completely controlled at second passage only. In case of carbendazim alternating with roko the disease was completely under controlled from third passage (Table 11Fig.11)

Treatment of carbendazim in mixture of different fungicide:

In this experiment, Gerbera plants were treated with the mixture of carbendazim with different fungicide. Then these plants were inoculated with mycelial suspension of *Alternaria alternata* and covered with polythene bag. After eight days mycelial suspension from such infected leaves were prepared and applied to healthy Gerbera plant and treated with another fungicide at the same concentration. Same procedure followed up to eight passages.

It shows that when carbendazim was mixed with mancozeb, kocide-101 and roko. Carbendazim mixed with kocide-101 and Roko there were complete control over the Gerbera leaf spot in first passage only (Table12 fig 12).

Continuous and alternate passage:

Table11: Effect of exposure of *Alternaria alternata(in vivo)* carbendazim continues and alternating with other fungicide on the development of resistance during eight successive passages

Fungicide	Passage number							
	1	2	3	4	5	6	7	8
Carbendazim individual	1	1	1	2	2	3	3	3
Carbendazim + Mancozeb	1	00	00	00	00	00	00	00
Carbendazim + Kocide-101	1	00	00	00	00	00	00	00
Carbendazim + Roko	1	00	00	00	00	00	00	00

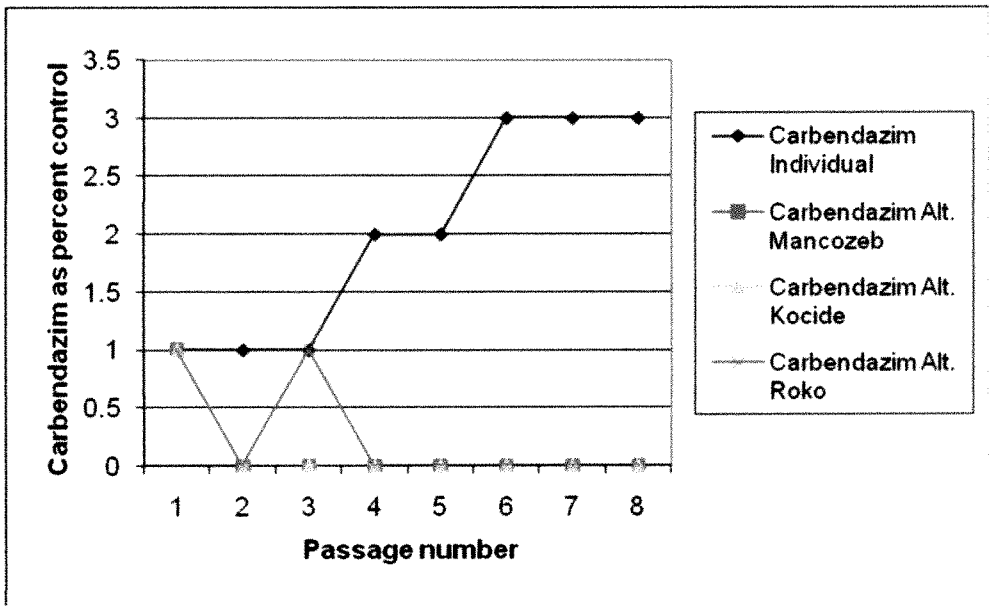


Table 11: Effect of exposure of *Alternaria alternata (in vivo)* carbendazim continues and alternating with other fungicide on the development of resistance during eight successive passages

Mixed passage

Table 12: Effect of exposure of *Alternaria alternata (in vivo)* to the mixture of carbendazim with other fungicide on the development of resistance during eight successive passages.

Fungicide	Passage number							
	1	2	3	4	5	6	7	8
Carbendazim individual	1	1	1	2	2	3	3	3
Carbendazim +Mancozeb	00	00	00	00	00	00	00	00
Carbendazim+Kocide-101	00	00	00	00	00	00	00	00
Carbendazim + Roko	00	00	00	00	00	00	00	00

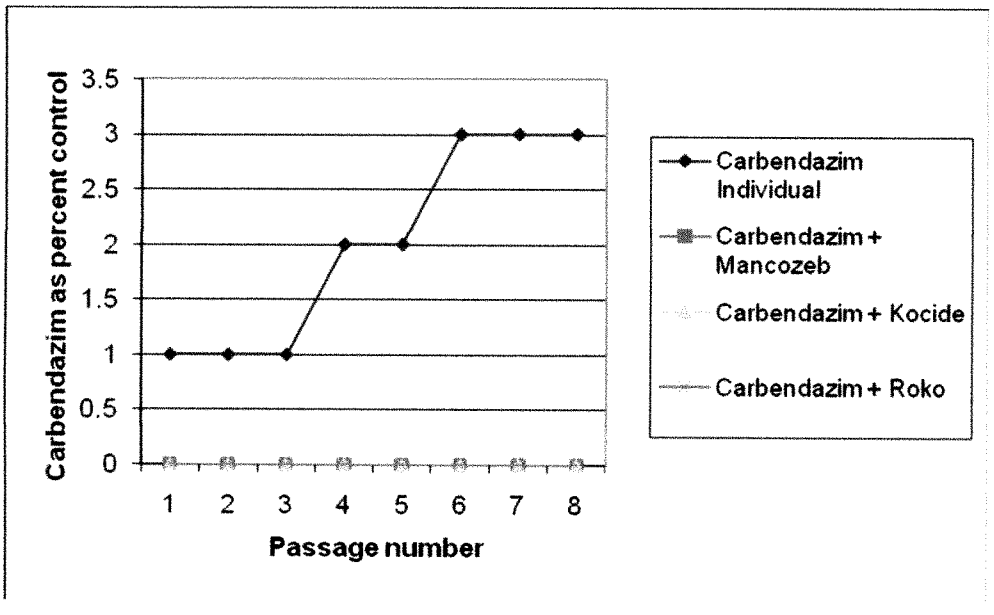


Fig 12: Effect of mixture treatment of Carbendazim with other fungicides on percentage infection of Alternaria *alternata* on Gerbera leaves for 8 successive passages

Synergistic Effect of Agrochemicals on Carbendazim Resistance

In Vitro Studies

Agrochemicals such as fungicides, insecticides, antibiotics, herbicides, salts and fertilizers were mixed with carbendazim in Gerbera leaf extract agar medium Carbendazim resistant isolate Aa- 4 was inoculated on to the plate and its growth was observed at various incubation periods. Increased growth over carbendazim control was considered as increase in the resistance and vice versa. In this experiment different fungicide, insecticides, herbicides, antibiotics, salts, fertilizers were used with Gerbera leaf extract agar medium along with carbendazim (15%) at various concentrations (25µg/ml to 100µg/ml).

Fungicide

Fungicide like kavach, mancozeb, ridomil were mixed with carbendazim (15%) at various concentrations (25µg/ml to 100µg/ml) along with Gerbera leaf extract agar media.

It was observed that carbendazim with kavach, mancozeb, ridomil 25µg/ml to 100µg/ml concentration completely inhibited growth of the pathogen. (Table13 ,fig13)

Herbicides

In these experiment different herbicides like sencor, krizin, and mera-71 were mixed with carbendazim (50%) at various concentration (25µg/ml to 100µg/ml). Sencor and krizin failed to control the growth of the pathogen while Mera-71 at 25µg/ml concentration completely inhibited the growth of pathogen. (Table14, fig14)

Insecticide

Dunet, krinet and thimate were mixed with carbendazim. Thimate and krinet with carbendazim at 100µg/ml completely inhibited the growth of pathogen, while Dunet with carbendazim did not inhibit the growth of pathogen. (Table15, fig15)

Antibiotics

Streptomycin, Taxim and C-tax were mixed with carbendazim. Out of these Taxim with carbendazim inhibited the growth of Alternaria alternata at 0.3 µg/ml and. completely. (Table16, fig16)

Salt

Potassium chloride, Sodium chloride were incorporated in Gerbera leaf extract agar medium. Potassium chloride with carbendazim at 0.4 µg/ml inhibited the growth of the pathogen. . (Table17, fig17)

Fertilizers

Urea, Potash, Phosphate were mixed with carbendazim in Gerbera leaf extract agar medium. At higher concentrations all fertilizers stimulated the growth of the pathogen. (Table18, fig18)

Table13:Synergistic effect of Fungicide on the development of Carbendazim resistance in *Alternaria alternata* (*in vitro*)

Fungicide with carbendazim (15%)	Growth in mm
Kavach(µg/ml)	
25	00
50	00
75	00
100	00
Mancozeb (µg/ml)	
25	00
50	00
75	00
100	00
Ridomil (µg/ml)	
25	00
50	00
75	00
100	00
Carbendazim	12.00

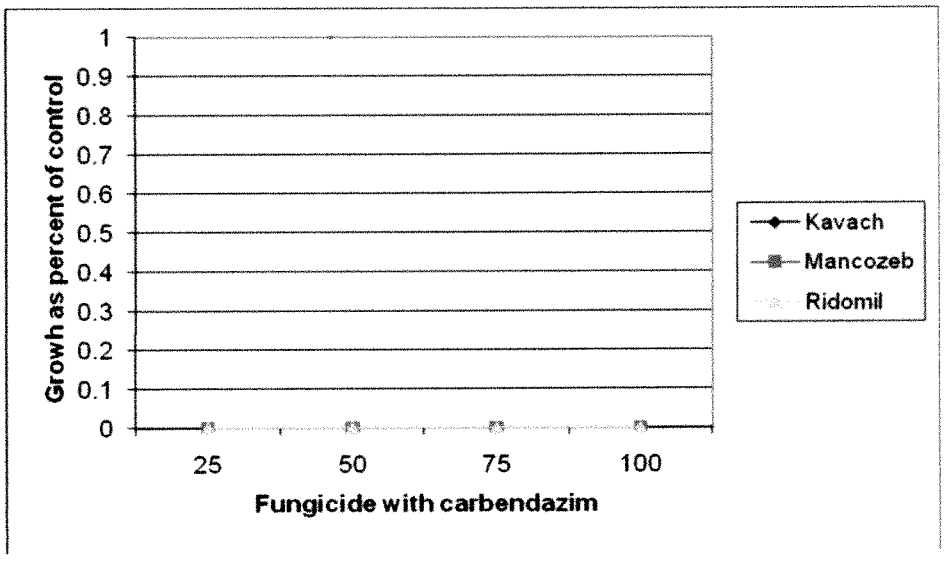


Fig13: Synergistic effect of Fungicide on the development of Carbendazim resistance in *Alternaria alternata* (*in vitro*)

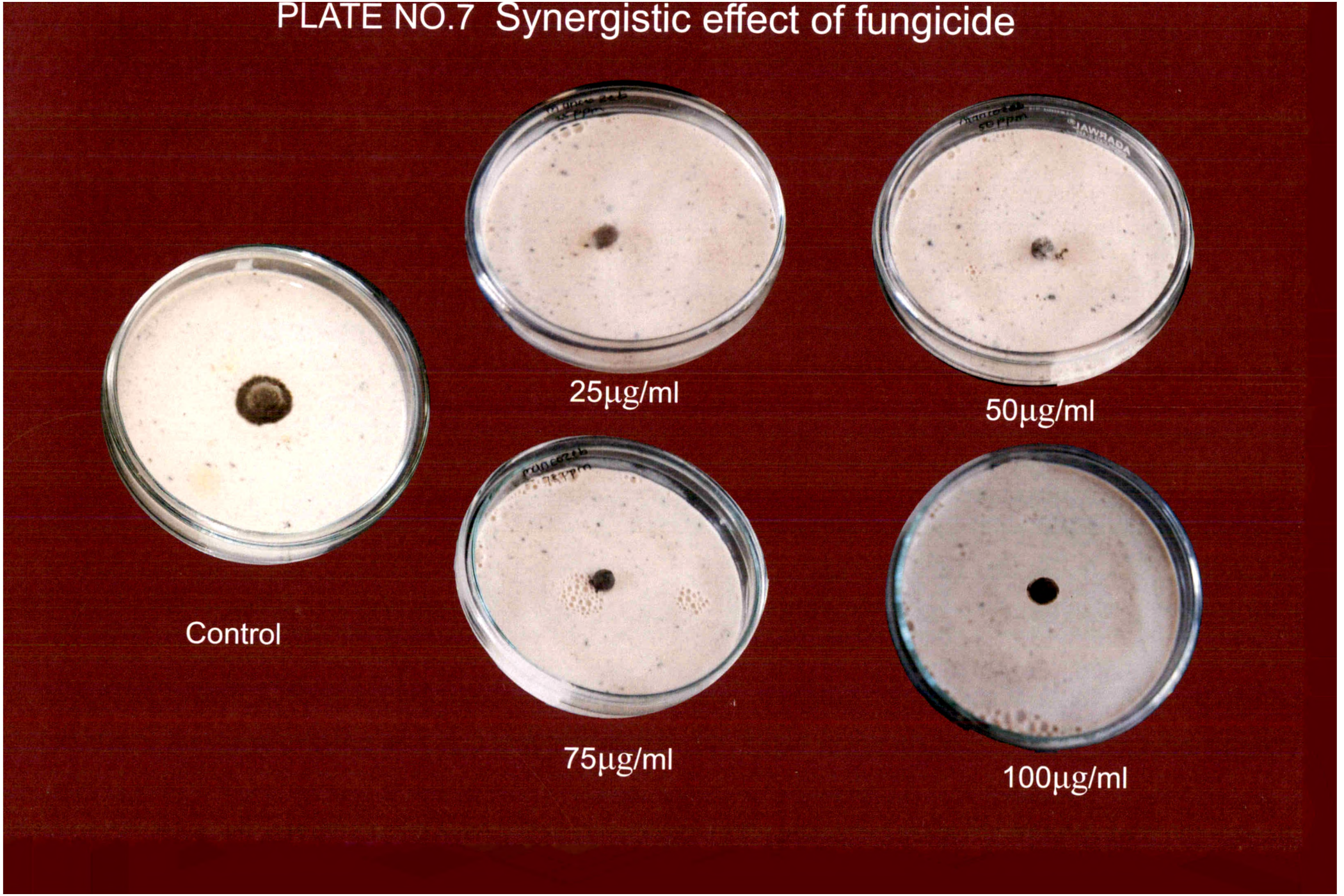


PLATE NO.7 Synergistic effect of fungicide
Control
25µg/ml
50µg/ml
75µg/ml
100µg/ml

Table 14: Synergistic effect of Herbicides on the development of Carbendazim resistance in *Alternaria alternata* (*in vitro*)

Herbicide with carbendazim (15%)	Growth in mm
Sencor(µg/ml)	
25	36.00
50	30.00
75	21.00
100	10.00
Krizin(µg/ml)	
25	30.00
50	20.00
75	15.00
100	10.00
Mera-71(µg/ml)	
25	00.00
50	00.00
75	00.00
100	00.00
Carbendazim	13.00

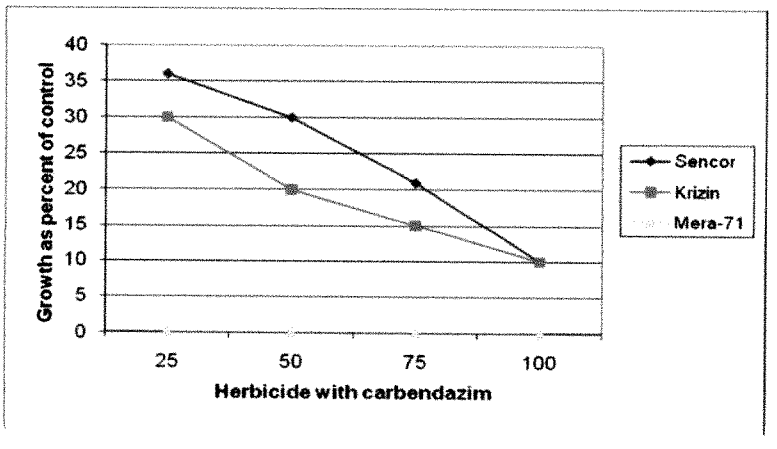


Fig.14: Synergistic effect of Herbicides on the development of Carbendazim resistance in *Alternaria alternata* (*in vitro*)

Table 15: Synergistic effect of Insecticides on the development of Carbendazim resistance in *Alternaria alternata (in vitro)*

Insecticide with carbendazim (15%)	**Growth in mm**
Dunet(µg/ml)	
25	25.00
50	18.00
75	15.00
100	10.00
Krinet (µg/ml)	
25	20.00
50	15.00
75	10.00
100	00.00
Thimate (µg/ml)	
25	15.00
50	10.00
75	10.00
100	00.00
Carbendazim	11.00

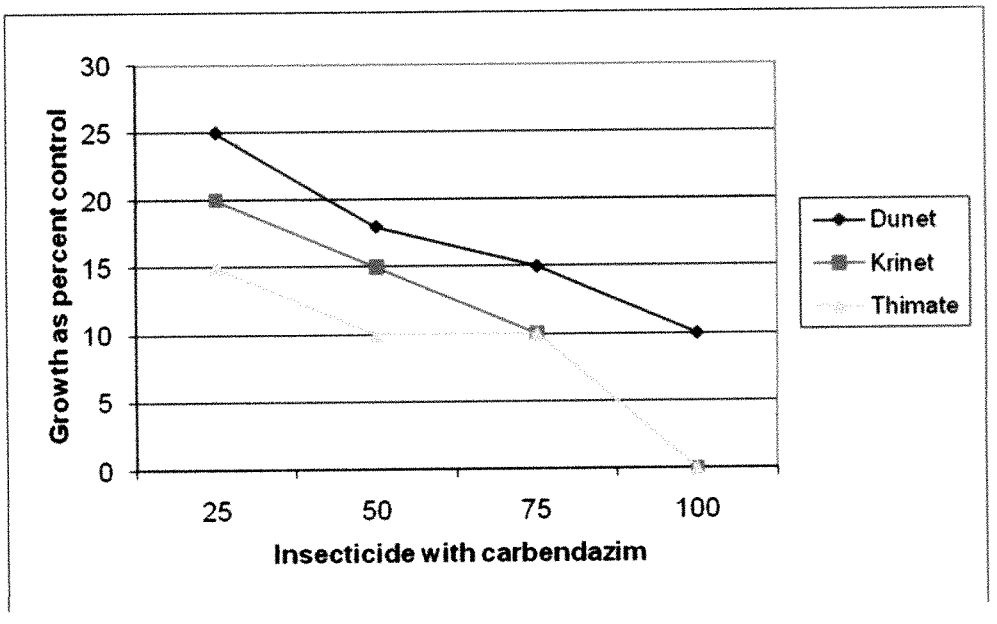


Fig15: Synergistic effect of Insecticides on the development of Carbendazim resistance in *Alternaria alternata* (*in vitro*)

Table16: Synergistic effect of Antibiotics on the development of Carbendazim resistance in *Alternaria alternata* (*in vitro*)

Antibiotics with carbendazim (15%)	**Growth in mm**
Streptomycin(µg/ml)	
0.1	22.00
0.2	18.00
0.3	13.00
0.4	10.00
Taxim (µg/ml)	
0.1	18.00
0.2	13.00
0.3	00.00
0.4	00.00
C-Tax (µg/ml)	
0.1	20.00
0.2	18.00
0.3	15.00
0.4	10.00
Carbendazim	11.00

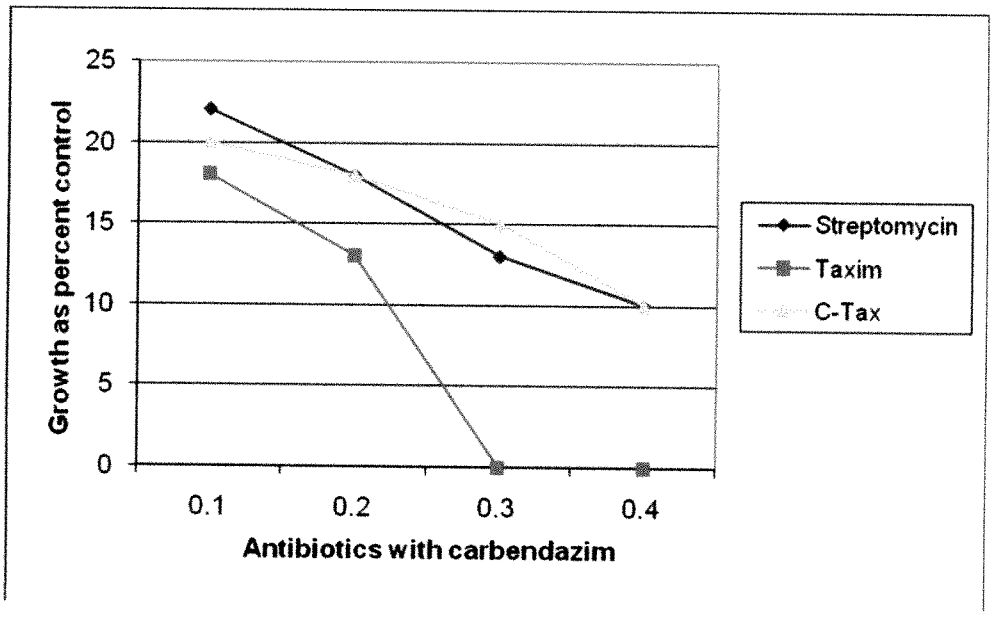


Fig. 16: Synergistic effect of Antibiotics on the development of Carbendazim resistance in *Alternaria alternata* (*in vitro*)

Table17: Synergistic effect of Salts on the development of Carbendazim resistance in *Alternaria alternata* (*in vitro*)

Salt s with carbendazim (8%)	**Grade**
Nacl (µg/ml)	
0.1	23.00
0.2	21.00
0.3	18.00
0.4	11.00
KCl(µg/ml)	
0.1	21.00
0.2	17.00
0.3	09.00
0.4	00.00
Carbendazim	12.00

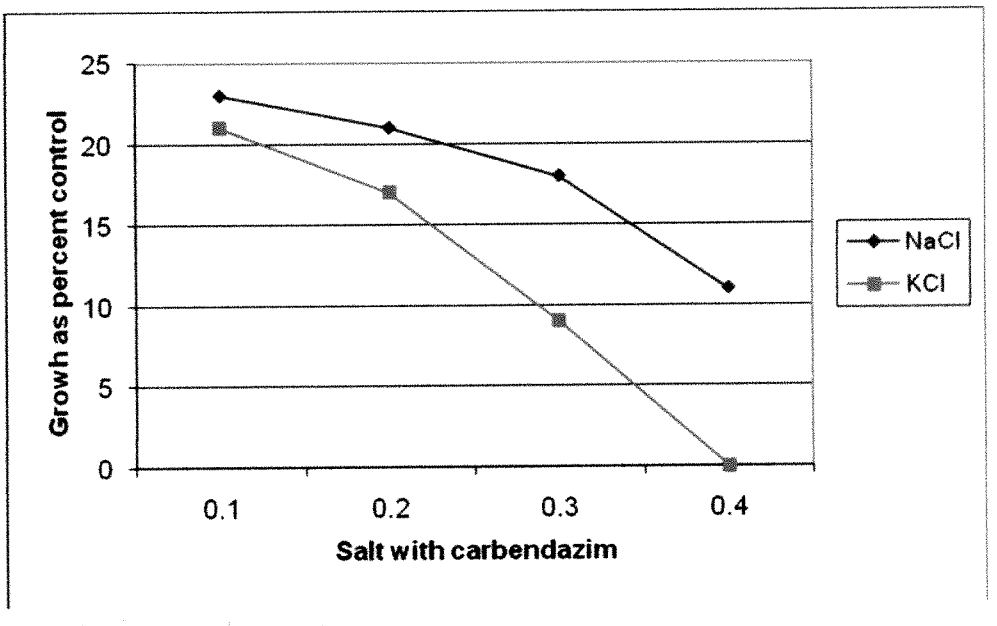


Fig. 17: Synergistic effect of Salts on the development of Carbendazim resistance in *Alternaria alternata* (*in vitro*)

Table 18: Synergistic effect of Fertilizer on the development of Carbendazim resistance in *Alternaria alternata* (*in vitro*)

Fertilizer with Carbendazim (15%)	Growth (mm)
Urea(µg/ml)	
0.1	19.00
0.2	24.00
0.3	28.00
0.4	30.00
Potash(µg/ml))	
0.1	16.00
0.2	20.00
0.3	25.00
0.4	28.00
Phosphate(µg/ml)	
0.1	20.00
0.2	25.00
0.3	27.00
0.4	30.00
Carbendazim	12.00

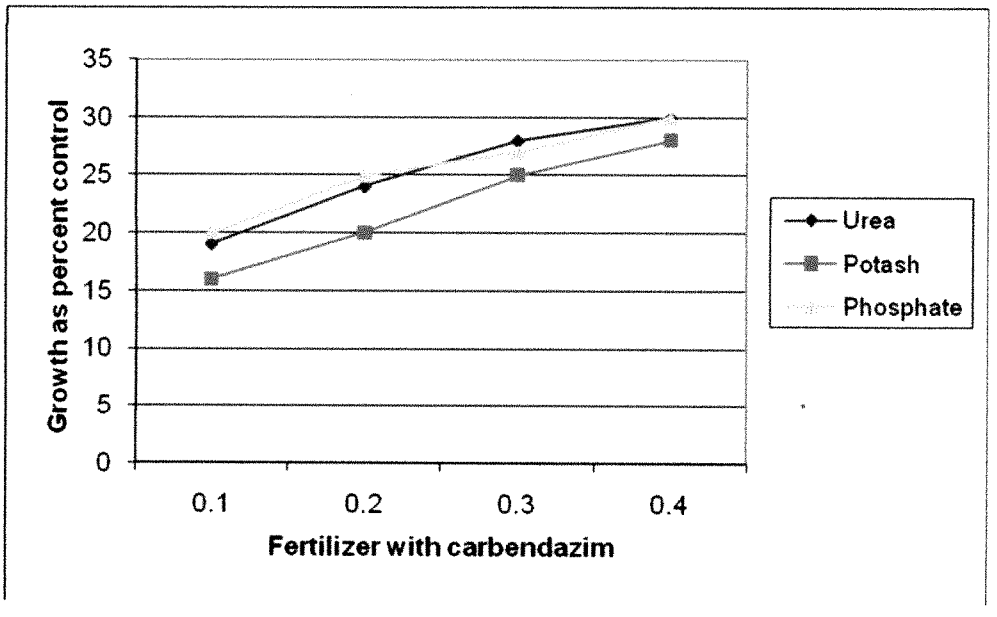


Fig18: Synergistic effect of Fertilizer on the development of Carbendazim resistance in *Alternaria alternata* (*in vitro*)

In Vivo Studies

Fungicides

Kavach, mancozeb and ridomil mixed with carbendazim (15%) at various concentrations (25µg/ml to 100µg/ml) and spreayed on Gerbera plants. After 24 hours the plants were inoculated with *Alternaria alternata* and these plants were covered with polythene bags.

It was observed that kavach, mancozeb and ridomil with carbendazim at 25 µg/ml concentration prevented the infection of *Alternaria alternata* to Gerbera plants. (Table19, fig19)

Herbicide

Sencor, krizin and mera-71 were mixed with carbendazim at various concentrations and spread on plants. After 24 hours the plants were inoculated with *Alternaria alternata* suspension

Mera-71 prevented infection of pathogen on Gerbera plants with carbendazim at 25 µg/ml concentration. It was observed that Sencor at 100 µg/ml completely stopped the infection of *Alternaria alternata*.. (Table20, fig20)

Insecticide

Dunet, krinet and thimate were mixed with carbendazim and this mixture sprayed on Gerbera plant. After 24 hours these Gerbera plants were inoculated with pathogen.

Krinet at 50 µg/ml completely inhibited the infection of *Alternaria alternata* while thimate at 25 µg/ml concentration completely inhibited the infection of

Alternaria alternata to Gerabera plant. It was observed that Dunet at 75 µg/ml complete inhibited infection of *Alternaria alternata.* (Table21, fig21)

Antibiotics

The different antibiotics viz. Streptomycin, taxim and C-tax along with carbendazim were used in this experiment. Taxim and C-Tax with carbendazim at 0.3 µg/ml prevented the Gerbera leaf spot. Streptomycin with carbendazim at 0.4 µg/ml prevented the Gerbera leaf spot. (Table22, fig22)

Salt

In this experiment two salts (NaCl & KCl) were used. Out of these two salts, Potassium chloride at 0.2 µg/ml with carbendazim prevents the infection of Gerbera plants.Sodium chloride at 0.4 µg/ml with carbendazim prevented the infection of Gerbera plants. (Table23, fig23)

Fertilizers

In this experiment, different fungicide like Urea, Potash, and Phosphate were used along with carbendazim (8%). All these fertilizers helped to *Alternaria alternata* pathogen in infection to Gerbera plants. (Table24, fig24)

Table19: Synergistic effect of fungicides on the development of carbendazim resistance in *Alternaria alternata (in vivo)*

Fungicides with carbendazim (8%)	**Grade**
Kavach(µg/ml)	
25	00
50	00
75	00
100	00
Mancozeb (µg/ml)	
25	00
50	00
75	00
100	00
Ridomil(µg/ml)	
25	00
50	00
75	00
100	00
Carbendazim	1.00

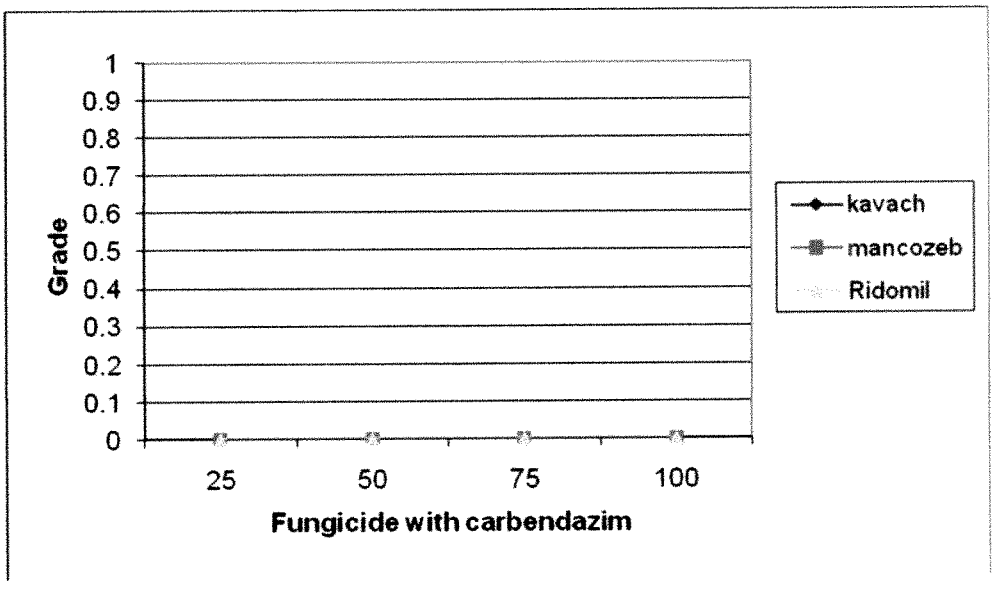


Fig19: Synergistic effect of fungicides on the development of carbendazim resistance in *Alternaria alternata (in vivo)*

Table20: Synergistic effect of Herbicides on the development of carbendazim resistance in *Alternaria alternata (in vivo)*

Herbicides with carbendazim (8%)	**Grade**
Sencor(µg/ml)	
25	2.00
50	1.00
75	1.00
100	00
Krizin(µg/ml)	
25	2.00
50	1.00
75	1.00
100	1.00
Mera-71(µg/ml)	
25	00
50	00
75	00
100	00
Carbendazim	1.00

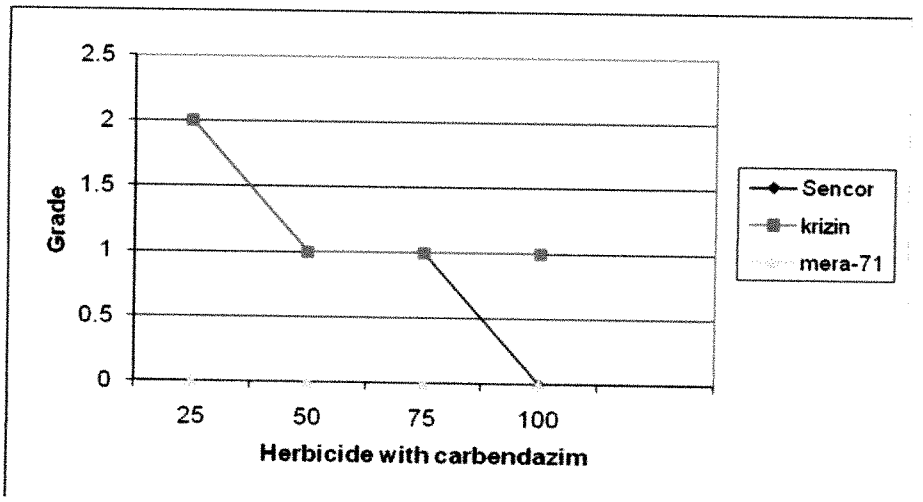


Fig 20: Synergistic effect of Herbicides on the development of carbendazim resistance in *Alternaria alternata (in vivo)*

Table21: Synergistic effect of Insecticides on the development of carbendazim resistance in *Alternaria alternata (in vivo)*

Insecticides with Carbendazim 8%	**Grade**
Dunet (µg/ml)	
25	1.00
50	1.00
75	00.00
100	00.00
Krinet(µg/ml)	
25	1.00
50	00.00
75	00.00
100	00.00
Thimate(µg/ml)	
25	00.00
50	00.00
75	00.00
100	00.00
Carbendazim	1.00

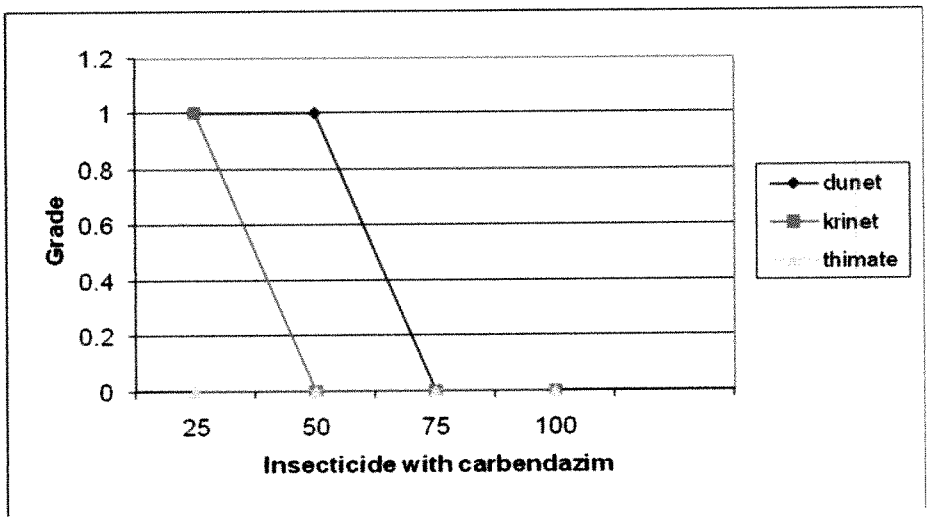


Fig 21: Synergistic effect of Insecticides on the development of carbendazim s resistance in *Alternaria alternata (in vivo)*

Table 23: Synergistic effect of Salts on the development of carbendazim resistance in *Alternaria alternata (in vivo)*

Salts with carbendazim (8%)	**Grade**
Nacl(µg/ml)	
0.1	2.00
0.2	2.00
0.3	1.00
0.4	00.00
KCl(µg/ml)	
0.1	1.00
0.2	00.00
0.3	00.00
0.4	00.00
Carbendazim	1.00

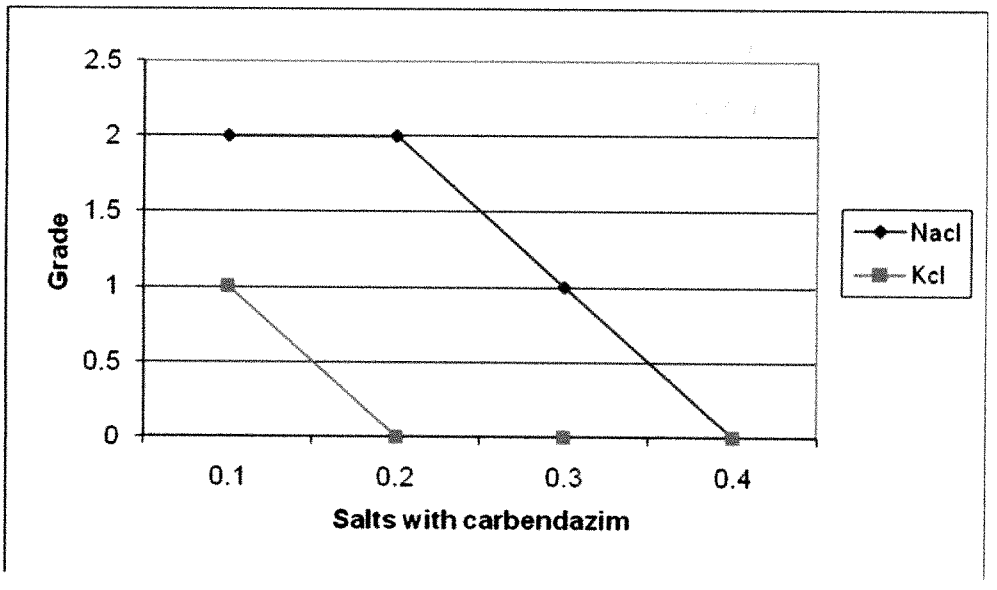


Fig23: Synergistic effect of Salts on the development of carbendazim resistance in *Alternaria alternata (in vivo)*

Table 22: Synergistic effect of Antibiotics on the development of Carbendazim resistance in *Alternaria alternata* (*in vivo*)

Antibiotics with Carbendazim (8%)	Growth in mm
Streptomycin(µg/ml)	
0.1	1.00
0.2	1.00
0.3	1.00
0.4	00.00
Taxim(µg/ml)	
0.1	1.00
0.2	1.00
0.3	00.00
0.4	00.00
C-Tax(µg/ml)	
0.1	1.00
0.2	1.00
0.3	00.00
0.4	00.00
Carbendazim	1.00

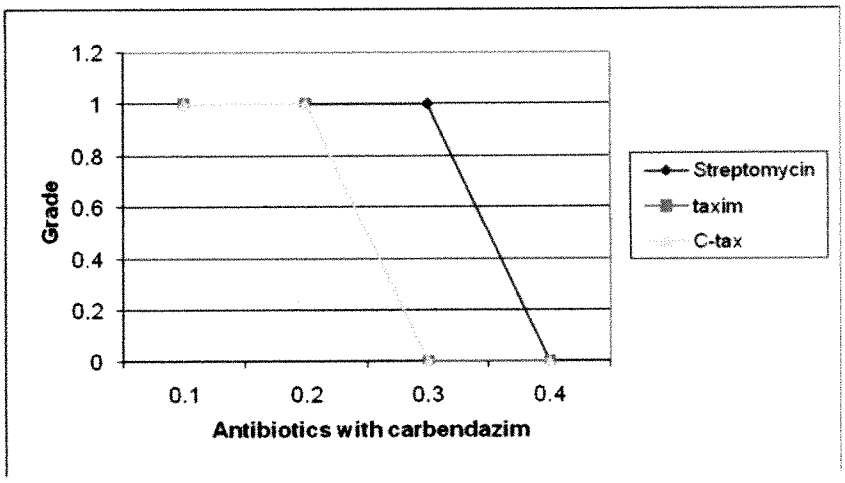


Fig 22: Synergistic effect of Antibiotics on the development of Carbendazim resistance in *Alternaria alternata* (*in vivo*)

Table 24: Synergistic effect of Fertilizer on the development of Carbendazim resistance in *Alternaria alternata* (*in vivo*)

Fertilizer with Carbendazim (15%)	Growth (mm)
Urea(µg/ml)	
0.1	1.00
0.2	1.00
0.3	2.00
0.4	2.00
Potash(µg/ml)	
0.1	00.00
0.2	1.00
0.3	1.00
0.4	2.00
Phosphate (µg/ml)	
0.1	1.00
0.2	1.00
0.3	2.00
0.4	2.00

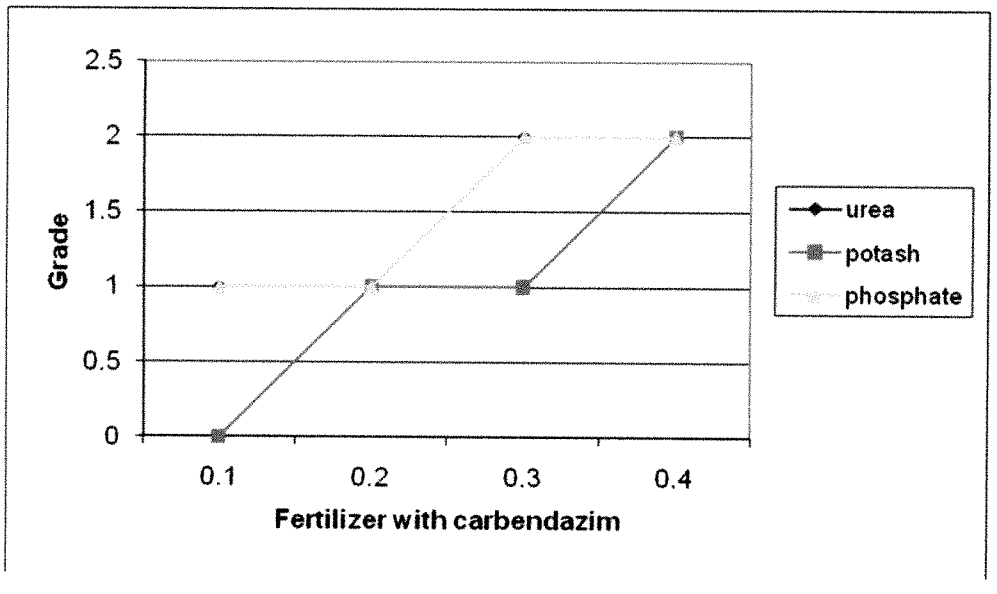


Fig24: Synergistic effect of Fertilizer on the development of Carbendazim resistance in *Alternaria alternata* (*in vivo*)

Percentage Control Efficacy

The Percentage Control Efficacy of each agrochemical in mixture with carbendazim was calculated as follows:

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

Where,

X = the diameter of colony lesion amended/ treated with agrochemical.

Y = the diameter of colony lesion on the medium / Gerbera plant.

The results in tables(25-36) indicates that disease caused by resistant isolates of *Alternaria alternata* controlled by 100% due to application of kavach, mancozeb, ridomil at 25 µg/ml, mera-71 at 25 µg/ml, krinet and thimate at 100 µg/ml, potassium chloride at 0.4 µg/ml, taxim at 0.3 µg/ml(*in vitro*).

100% due to application of kavach, mancozeb and ridomil at 25 µg/ml; sencor and mera-71 at 100 µg/ml; NaCl & KCl at 0.4 µg/ml, taxim at 0.3 µg/ml (*in vivo*).

Table 25: Percent control efficacy (PCE) of Fungicides mixture in controlling the carbendazim resistant isolate of *Alternaria alternata* on agar plates (*in vitro*)

Fungicides with Carbendazim (15%)	**PCE**
Kavach(µg/ml)	
25	100
50	100
75	100
100	100
Mancozeb(µg/ml)	
25	100
50	100
75	100
100	100
Ridomil(µg/ml)	
25	100
50	100
75	100
100	100
Carbendazim	85.00

Table 26: Percent control efficacy (PCE) of Herbicides mixture in controlling the carbendazim resistant isolate of *Alternaria alternata* on agar plates (*in vitro*)

Herbicides with Carbendazim (15%)	**PCE**
Sencor(µg/ml)	
25	55.00
50	62.50
75	73.75
100	87.50
Krizin(µg/ml)	
25	62.50
50	75.00
75	81.50
100	87.50
Mera-71(µg/ml)	
25	100.00
50	100.00
75	100.00
100	100.00
Carbendazim	83.75

Table 27 : Percent control efficacy (PCE) of Insecticides mixture in controlling the carbendazim resistant isolate of *Alternaria alternata* on agar plates (*in vitro*)

Insecticides with Carbendazim (15%)	**PCE**
Dunet(µg/ml)	
25	68.75
50	77.50
75	81.25
100	87.50
Krinet(µg/ml)	
25	75.00
50	81.25
75	87.50
100	100.00
Thimate(µg/ml)	
25	81.25
50	87.50
75	87.50
100	100.00
Carbendazim	86.25

Table28: Percent control efficacy (PCE) of Antibiotics mixture in controlling the carbendazim resistant isolate of *Alternaria alternata* on agar plates (*in vitro*)

Antibiotics with Carbendazim (15%)	**PCE**
Streptomycin (µg/ml)	
0.1	72.45
0.2	77.50
0.3	83.75
0.4	87.50
Taxim (µg/ml)	
0.1	77.50
0.2	83.75
0.3	100
0.4	100
C-Tax (µg/ml)	
0.1	75.00
0.2	77.50
0.3	81.25
0.4	87.50
Carbendazim	86.25

Table 29: Percent control efficacy (PCE) of Salts mixture in controlling the carbendazim resistant isolate of *Alternaria alternata* on agar plates (*in vitro*)

Salts with Carbendazim (15%)	**PCE**
Nacl (µg/ml)	
0.1	71.25
0.2	73.75
0.3	77.50
0.4	86.25
KCl (µg/ml)	
0.1	73.75
0.2	78.75
0.3	88.75
0.4	100.00
Carbendazim	85.00

Table30: Percent control efficacy (PCE) of Fertilizers mixture in controlling the carbendazim resistant isolate of *Alternaria alternata* on agar plates (*in vitro*)

Fertilizers with Carbendazim (15%)	**PCE**
Urea(µg/ml)	
0.1	76.25
0.2	70.00
0.3	65.00
0.4	100.00
Potash(µg/ml)	
0.1	80.00
0.2	75.00
0.3	68.75
0.4	65.00
Phosphate(µg/ml)	
0.1	75.00
0.2	68.75
0.3	66.25
0.4	62.50
Carbendazim	85.00

Table 31: Percent control efficacy (PCE) of Fungicides mixture in controlling the carbendazim resistant isolate of *Alternaria alternata* on agar plates (*in vivo*)

Fungicides with Carbendazim (15%)	**PCE**
Kavach(µg/ml)	
25	100
50	100
75	100
100	100
Mancozeb(µg/ml)	
25	100
50	100
75	100
100	100
Ridomil(µg/ml)	
25	100
50	100
75	100
100	100
Carbendazim	80.00

Table 32: Percent control efficacy (PCE) of Herbicides mixture in controlling the carbendazim resistant isolate of *Alternaria alternata* on agar plates (*in vivo*)

Herbicides with Carbendazim (8%)	**PCE**
Sencor(µg/ml)	
25	60.00
50	80.00
75	80.00
100	100.00
Krizin(µg/ml)	
25	60.00
50	80.00
75	80.00
100	80.00
Mera-71(µg/ml)	
25	100.00
50	100.00
75	100.00
100	100.00
Carbendazim	80.00

Table 33: Percent control efficacy (PCE) of Insecticides mixture in controlling the carbendazim resistant isolate of *Alternaria alternata* on agar plates (*in vivo*)

Insecticides with Carbendazim (15%)	**PCE**
Dunet(µg/ml)	
25	80.00
50	80.00
75	100.00
100	100.00
Krinet(µg/ml)	
25	80.00
50	100.00
75	100.00
100	100.00
Thimate(µg/ml)	
25	100.00
50	100.00
75	100.00
100	100.00
Carbendazim	80.00

Table 34: Percent control efficacy (PCE) of Antibiotics mixture in controlling the carbendazim resistant isolate of *Alternaria alternata* on agar plates (*in vivo*)

Antibiotics with Carbendazim(8%)	**PCE**
Streptomycin (µg/ml)	
0.1	80.00
0.2	80.00
0.3	80.00
0.4	100.00
Taxim (µg/ml)	
0.1	80.00
0.2	80.00
0.3	100.00
0.4	100.00
C-Tax (µg/ml)	
0.1	80.00
0.2	80.00
0.3	100.00
0.4	100.00
Carbendazim	80.00

Table 35:Percent control efficacy (PCE) of Salts mixture in controlling the carbendazim resistant isolate of *Alternaria alternata* on agar plates (*in vivo*)

Salts with Carbendazim (15%)	**PCE**
Nacl(µg/ml)	
0.1	60.00
0.2	60.00
0.3	80.00
0.4	100.00
KCl (µg/ml)	
0.1	80.00
0.2	100.00
0.3	100.00
0.4	100.00
Carbendazim	80.00

Table 36: Percent control efficacy (PCE) of Fertilizers mixture in controlling the carbendazim resistant isolate of *Alternaria alternata* on agar plates (*in vivo*)

Fertilizers with Carbendazim (8%)	**PCE**
Urea(µg/ml)	
0.1	80.00
0.2	80.00
0.3	60.00
0.4	60.00
Potash(µg/ml)	
0.1	100.00
0.2	80.00
0.3	80.00
0.4	60.00
Phosphate(µg/ml)	
0.1	80.00
0.2	80.00
0.3	60.00
0.4	60.00
Carbendazim	80.00

Synergistic effect of Agrochemicals on the development of carbendazim resistance in *Alternaria alternata*

***In vitro* studies:**

To study the synergistic effect of different agrochemicals on the development of carbendazim resistance in *Alternaria alternata* .This experiment was studied by mixing different agrochemicals such as fungicides, insecticides, herbicides, antibiotics, salts and fertilizers with carbendazim. For this study resistant isolate (Aa-4) was used. It was grown on medium containing equal volume of agrochemicals at four different concentrations (25, 50, 75, 100µg/ml) with carbendazim (15%).

***In vivo* studies:**

To study synergistic effect of agrochemicals on the development of resistance in *Alternaria alternata* against carbendazim. Gerbera plants treated with resistant concentration of carbendazim (8%) and other agrochemicals with above concentration, in equal volume. After 24 hours 10 ml mycelial/spore suspension of resistant isolate (Aa-4) was inoculated on treated plants. Each Gerbera plant was covered with polythene bag. Percentage of infection on Gerbera leaves was measured after eight days.