MATERIAL AND METHODS

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Samples of *Alteraria* leaf spot of Gerbera were collected from different greenhouses in Western Maharashtra (Kolhapur, Sangli, Satara and Pune.)They were brought to the laboratory in clean, sterilized polythene bags. Infected leafs were cut into 2-3mm pieces. Then they were surface sterilized with 70 % alcohol for 2-3 min, washed three times with sterile distilled water and inoculated on Gerbera leaf extract agar medium (Gerbera leaf 300 gm juice, Dextrose 10gm, Agar 15gm). Plates were amended with 30 mg/l streptomycin sulphate to suppress the bacterial growth. The plates were incubated under 12h/12h cycles of lightness and darkness for seven days. On 8th day the plates were screened for the pathogen. From these samples four isolates of *Alternaria alternata* were obtained. Culture tubes of *Alternaria alternata* were maintained at 4°C and used for study whenever necessary.

Host plant: - *Gerbera jamesonii* H.Bolux ex J.D. Hook

Culture medium for pathogen:-

Gerbera leaf extract medium:

Gerbera leaf	- 300 gm
Dextrose	– 10 gm
Agar	– 15gm

Volume was made up to 1000 ml by using sterile distilled water.

Sensitivity of *Alternaria alternata* to carbendazim:-

***In vitro* studies:-**

Sensitivity of *Alternaria alternata* to carbendazim was determined by 'food poisoning test' Plates were prepared with Gerbera leaf extract medium along with different concentrations of carbendazim. Each fungal isolates was tested at different concentration of carbendazim. The seven day old culture of *Alternaria alternata* was cut into 8mm disc using strile cork borer & was placed at the center of agar plate containing different concentration of carbendazim. These treatments were maintained in triplicate. Plates without fungicide served as 'control'. The plates were then incubated under 12h/12h cycle of lightness and darkness and linear mycelial growth was measured at different time interval up to seven days.

***In vivo* studies:-**

In vivo sensitivity of *Alternaria alternata* to carbendazim was tested on healthy leaves of Gerbera plant. For this experiment healthy Gerbera plant (5 in numbers) were treated with different concentration of carbendazim solution after 24 hrs. These treated Gerbera plant were inoculated with 10 ml mycelial/spore suspension of *Alternaria alternata*. This spore suspension was prepared using 7 days old culture of *Alternaria alterrnata* in sterile distilled water the spore suspension was sprayed on Gerbera plant and covered with polythene bags to avoid sencondary infection. Percentage of infection was recorded after various incubation periods. Plant without fungicide treatment was treated as 'control'. Where as plants without fungicide treatment and spore suspension where treated as 'absolute control'.

Effect of Passage:-

After determination of MIC of carbendazim, the effect of continuous and alternate treatment of fungicides with two different modes of action and a mixture of both on the development of carbendazim resistance in sensitive isolates of *Alternaria alternata* (Aa-2) was studied *in vitro* and *in vivo.*

***In vitro* studies:**

Continuous Passage:

To study the effect of continuous passage on development of carbendazim resistance in *Alternaria alternata* (*in vitro*), sensitive isolate (Aa-2) in each passage was cultured on plates with carbendazim (10%) in triplicate. 8mm diameter agar disc from the previous passage of the same isolate was placed at the center of each plates of next passage. In each passage, linear mycelial growth was measured after eight days. This was repeated up to eight passages.

Alternate passage:

To study the effect of alternate passage on development of carbendazim resistance in *Alternaria alternata*, sensitive isolate (Aa-2) was cultured on plates with carbendazim (10%) in triplicate. After eight days 8mm diameter agar disc from the previous passage was transferred to the plates containing other fungicide at the same concentration (10%). The process of such alternation of carbendazim to other fungicides was continued up to eight passages.

Mixed Passage:

To study the effect of mixed passage on development of carbendazim resistance in *Alternria alternata* (*in vitro*), sensitive isolates (Aa-2) was cultured on plate containing carbendazim with another fungicide, both having equal concentration (10%) in triplicates. After eight days, 8mm diameter agar disc from the previous passage was transferred to the plate containing the same mixture of fungicides in same proportion and same concentration (10%).

The increased mycelial growth from passage was considered as criterion for the development of fungicide resistance in *Alternaria alternata.* The effect of passage on the development of fungicide resistance in the pathogen was studied up to eight passages, in each case.

***In vivo* studies:**

Continuous Passage:

To study effect of continuous passage on the development of carbendazim resistance in *Alternaria alternata.*In this, experiment mycelial/spore suspension using one culture tube of sensitive isolates (Aa-2) was prepared. Out of that suspension, 10ml mycelial/spore suspension inoculated on the Gerbera plant, treated with 5% carbendazim before 24 hours .After 8 days a mycelial suspension from such infected leaves were prepared and inoculated to healthy Gerbera plants treated with 5% carbendazim, 24 hours before inoculation. This procedure was repeated up to eight passages.

Alternate passage:

To study the effect of alternate passage, on the development of fungicide resistance in *Alternaria alternata* (*in vivo*) the Gerbera plants were treated with 5% carbendazim. After 24 hours 10 ml mycelial or spore suspension of sensitive isolate (Aa-2) was inoculated fungicide on treated Gerbera plants (5 replicates). This spore suspension was prepared using seven days old culture of *Alternaria alternata* in sterile distilled water.

After eight days a mycelial suspension from such infected leaves were prepared and applied to healthy Gerbera plants treated with another fungicide (5%).Same procedure was followed up to eight passages.

Mixed passage:

To study the effect of mixed passage, on the development of fungicide resistance *(in vivo)* in *Alternaria alternata*. Gerbera plants treated with mixture of carbendazim and another fungicide in same proportion (both 5%). After 24 hours, 10ml mycelial /spore suspension of sensitive isolate (Aa-2) was inoculated on that treated Gerbera plant (5 replicates) and covered with polythene bags.

After eight days a mycelial suspension, from such infected leaves was applied to healthy Gerbera plants treated with same mixture of fungicide in same concentration before 24 hours.