All the chemicals used during this study were procured from Hi-Media Mumbai and Sigma Aldrich, U.S.A. All the chemicals used were highest purity and of analytical grade. All glasswares used for practical work were sterilized. The work was conducted at Department of Botany, Yogeshwari Mahavidyalaya, Ambajogai Dist. Beed (Maharashtra). The required cultivars were obtained from the local market of Ambajogai. The fungal pure cultures were obtained from ARI Pune and IMTECH Chandigarh.

3.1 Preparation of culture media:

All media were prepared using distilled water. All media recipes were per liter of distilled water. pH was adjusted as per the requirement of media. Media were sterilized by autoclaving at 121° C for 15 minutes.

1. Thornton’s Agar Medium:

\[
\begin{align*}
\text{K}_2\text{HPO}_4 & \quad - \quad 1g \\
\text{MgSO}_4 \cdot 7\text{H}_2\text{O} & \quad - \quad 1g \\
\text{CaCl}_2 & \quad - \quad 0.1g \\
\text{NaCl} & \quad - \quad 0.1g \\
\text{FeCl}_2 & \quad - \quad \text{trace} \\
\text{KNO}_3 & \quad - \quad 0.5g \\
\text{Asparagine} & \quad - \quad 0.5g
\end{align*}
\]
2. Walkman’s Synthetic Agar Medium:

Dextrose - 10g
Peptone - 5.0g
KH$_2$PO$_4$; 7H$_2$O - 1.0g
MgSO$_4$; 7H$_2$O - 0.5g
Agar-Agar - 15.0g
Distilled water - 1000ml.

3. Jensen’s Agar Medium:

Dextrose - 2.0g
Caesin - 1.0g

(Dissolved in 10ml of 0.1N NaOH)

K$_2$HPO$_4$ - 0.5g
MgSO$_4$; 7H$_2$O - 0.2g
FeCl$_3$.6H$_2$O - trace
Distilled water - 1000ml.
P$H$ - 6.5 - 6.6

(Prior to sterilization)
4. Potato Carrot Agar:

- Potato (Scrubbed and diced) - 20 g
- Carrot (Peeled and grated) - 20 g
- Agar-Agar - 20 g
- Distilled water - 1000 ml

5. Potato Dextrose Agar:

- Potato (Scrubbed and diced) - 200 g
- Dextrose - 20 g
- Agar-agar - 20 g
- Distilled water - 1000 ml

3.2 Collection of seeds:

The carrot seeds of two varieties i.e. Maharani-31 [Sardar seeds] and Kohinoor -11 [Avatar Seeds] were collected from local market of Ambajogai.

3.3 Study of Rhizosphere Microflora:

The rhizosphere microflora of carrot was studied by soil dilution and plate count technique described by Timonin (1940). The healthy seeds were sown in the earthen pots. Germination of seeds occurred after 12 days after sowing. After 30 days of sowing the seeds, the plants of carrot growing in pots were carefully removed and cut at the crown to separate the roots from the shoot of the plants. The roots were transported to the laboratory in sterile petridishes. Three roots of carrot were put into 25 ml of sterile distilled water. The soil clinging to the roots was removed by shaking flaks on rotary
shaker for about 10 minutes. The plants in duplicate were inoculated with 1ml of this solution.

For the study of bacteria the samples were diluted serially with sterile distilled water so as to obtain isolated colonies. The suspension was poured on the Thornton’s agar, Walkman’s synthetic agar and Jensen’s agar media for the isolation of bacteria, fungi and actinomycetes. The plates were poured with the suspension of 1 g soil with 10 ml of distilled water. The plates were incubated for 7 days at room temperature.

3.4 Isolation of fungi associated with carrot:

Soil samples from the various sites (fields) in which carrot was cultivated were also collected. In laboratory the soil sample was used to prepare various dilutions and then different media were inoculated by using these samples. The fungi detected in different soil samples were *Rhizopus, Fusarium, Alternaria, Phoma, Mucor* and *Aspergillus* species.

3.5 Collection of Pure Fungal Cultures:

Pure cultures of fungi were obtained from the different institutes. The cultures of *Alternaria, Rhizoctonia* and *Fusarium* were obtained from the Maharashtra Association for the Cultivation of Science’s Agharkar Research Institute, Pune. The pure sample of *Ceratocystis fimbriata* was obtained from the Microbial Type Culture collection and Gene Bank, Institute of Microbial Technology, Chandigarh.
3.6 Collection of Farm Yard Manure and Vermicompost:

The vermicompost was obtained from the Krishi Vigyan Kendra, Ambajogai. The Farm yard manure was obtained from the farmers.

3.7 Collection of Pesticide and Biopesticide:

The pesticide, Bavistin and biopesticide, Mahaneem was obtained from the local market of Ambajogai.

3.8 Study on phytoalexin production:

Seeds of two varieties i.e. Kohinoor-11 and Maharani-31 were collected from the local market of Ambajogai. Earthen pots were filled with the sterilized garden soil. The seeds were surface sterilized with HgCl₂ and washed with distilled water. The seeds of each variety were soaked in the spore suspension of different fungi for an hour and thereafter sown in the earthen pots. The seeds were germinated after 12 days. The pots were irrigated every day regularly. The growth was recorded in terms of height of the plant, number of leaves and biomass of the plant after 60 days. The phytoalexins synthesized were analyzed using gas chromatography and mass spectrometry (GCMS).

3.9 Effect of Vermicompost on Phytoalexin production:

Seeds of two varieties i.e. Kohinoor-11 and Maharani-31 were collected from the local market of Ambajogai. Earthen pots were filled with the garden soil and vermicompost (3:1 proportion). The seeds were surface sterilized with HgCl₂ and washed with distilled water. The seeds of each variety were sown in the earthen pots. The seeds were germinated after 12 days. The pots were irrigated every day regularly. The
growth was recorded in terms of height of the plant, number of leaves, biomass of the plant after 60 days.

3.10 Effect of biofertilizer (Azotobacter) on Phytoalexin production:

Seeds of two varieties i.e., Kohinoor-11 and Maharani-31 were collected from the local market of Ambajogai. Earthen pots were filled with the garden soil and biofertilizer (Azotobacter). The seeds were surface sterilized with HgCl₂ and washed with distilled water. The seeds of each variety were sown in the earthen pots. The seeds were germinated after 12 days. The pots were irrigated every day regularly. The growth was recorded in terms of height of the plant, number of leaves, and biomass of the plant after 60 days.

3.11 Effect of Farm Yard Manure (FYM) on Phytoalexin production:

Seeds of two varieties i.e., Kohinoor-11 and Maharani-31 were collected from the local market of Ambajogai. Earthen pots were filled with the garden soil and Farm yard manure (FYM). The seeds were surface sterilized with HgCl₂ and washed with distilled water. The seeds of each variety were sown in the earthen pots. The seeds were germinated after 12 days. The pots were irrigated every day regularly. The growth was recorded in terms of height of the plant, number of leaves, and biomass of the plant after 60 days.

3.12 Effect of biopesticide on Phytoalexin production:

Seeds of two varieties i.e., Kohinoor-11 and Maharani-31 were collected from the local market of Ambajogai. Earthen pots were filled with the garden soil. The seeds were
surface sterilized with HgCl₂ and washed with distilled water. The seeds of each variety were sown in the earthen pots after soaking in the solution of biopesticide, Mahaneem for one hour. The seeds were germinated after 12 days. The pots were irrigated regularly. The growth was recorded in terms of height of the plant, number of leaves, and biomass of the plant after 60 days.

3.13 Analysis of the phytoalexins:

For the identification of different phytoalexins the GCMS analysis was performed. The extracts of carrot roots treated with various fungi along with the control were subjected for GCMS profiling.