Study area:

Ten plant gums were collected from different localities in Marathwada like viz. Gautala and Ajantha forest (Aurangabad), Bhokardan (Jalna) Mayur forest (Beed) Kinwat forest (Nanded)

A) Collection of gum from plants:

Plant gums were regularly collected for three years in all the seasons. It was done by using axe, sterilized blade. Fine cut was made at different parts of the plant, like root, stem, leaves, flower and fruits. Later on at 30, 45 and 60 days exudates gums where collected in presterlized plastic bags, kept in laboratory condition until its used.

Preparation of fine powder

The powder was prepared from collected dry gums and kept in clean glass pots. It was used for study of physical parameter like test, smell, colour, viscosity, durativity, solubility, pH, chemical property like moisture, crude protein, sugar, crude fat and fibre, phenols, alkaloids, steroids, tannins. hydrolytic enzyme (amylase, cellulase and pectinase), antifungal activity, antibacterial activity, effect of gum on seed germination and incidence of seed mycoflora and bioassay on economically important
insects like *Helicoverpa armigera*, *Plutella xylostella*, *Leucinoides orbonalis* and *Spodoptera litura* etc (Plate 5).

B) **Study of physical properties of gums**

1) **pH**

   The fresh 5 gm gum of every selected plant was taken in clean and sterilized test tube. The pH of every plant gum was checked with respect to different age with the help of pH meter and results were recorded.

2) **Colour, taste, smell and physical status (Organoleptic properties)**

   In order to study this physical parameters, fresh samples of the plant gum were used in triplicate and orally the colour, taste, smell and physical status were recorded. While, collecting the gum tribal, gum collectors also help for this type of testing the physical parameters.

3) **Durability**

   The durability of freshly collected gums was checked at normal room temperature and freeze condition.

   The fresh 1 gm gum of every selected plant was collected in 2 separate sterilized test tubes, and each test tube was properly plugged with non-absorbent cotton. After completion of plugging, one test tube of every plant was transferred to freeze and remaining one was kept at room temp. The date of transfer was recorded and at the interval of 8 days the condition of gum were recorded up to complete browning of the gum.
4) **Dry matter (DM)**

Dry matter (DM) is calculated by weighing the sample after drying to a constant weight in an oven at 95±5°C. For this purpose, 100g of sample is taken in a clean dry pre-weighed tray and is kept in oven for 48 hours or more, till constant weight. Weight of the dried sample is reported as percent dry matter (DM).

5) **Estimation of Moisture**

A noncorrodible metal container was weighed. Aluminium dish or glass with lids ensuring airtight closure, with its lid to the nearest 0.5mg was weighed into the weighed container, to the nearest 1mg, about 5gm of the sample (without grind) and spread everywhere. The container was placed without its lid in the over preheated to 103°C. To prevent the oven temperature from falling unduly introduced the container as rapidly as possible. Leaved to dry for four hours reckoned from the time when the oven temperature returns to 103°C. The lid on the container replaced and removed latter for 30-45 minutes in desiccator (a thick perforated metal or porcelain plate, containing an efficient drying agent) and weighed to the nearest 1mg.

**Calculation:**

The moisture content, as percentage of the sample as calculated by using the following formula

\[
\text{Moisture} \% = \frac{100(M1-M2)}{M1-M}
\]
Material and Methods

Where, \( M_1 \) = Mass in gm of the material before drying.

\( M_2 \) = mass in gm of the dish with the material after drying.

\( M \) = mass in gm of the empty dish.

The moisture content determination of gum of plant was carried out with the help of protocol given by ISTA (International Seed Testing Association 1966). During the determination, exposure of the sample to the atmosphere of the laboratory was reduced to the absolute minimum. The determination of moisture content was carried out by low constant temperature oven method i.e. 103°C for 17 +/- 1 hour, in following manner.

The weighing of 10 containers with their respective lids were carried out and recorded. The diameter of every container was in between 5cm to 8cm and hence 4.5gm +/- 0.5 gm of gum were collected in every container. After collection of gum the weight of every container with its lid and gum were recorded.

During the weighing, the oven was adjusted to 103°C. After completion of weighing each container was placed in oven with its lid at bottom and oven was kept at 103°C +/- 2°C for 17 +1 hour. After completion of heating the containers were closed with their respective lids and immediately transferred to desiccator for an hour. After completion of desiccation, the final weight of containers was recorded.

\[
\frac{100}{(M_2-M_3)} \frac{(M_2-M_3)}{(M_2-M_1)}
\]
Where

M₁: is the weight in grams of the container and its lid.

M₂: is the weight in grams of the container, its cover and its content before drying.

M₃: is the weight in grams of the container, cover and content after drying.

6) Solubility

The solubility of plant gum was tested in three polar and three non-polar solvents.

The freshly collected gum of different plants were measured with the help of micropipette and was poured in test tube containing 20ml solvent. This mixture was shaken for 5 minutes and solubility of gum was recorded.

C) Chemical properties of gum

1) Estimation of crude fibre

Crude fibre (CF) is determined as that fraction remaining after digestion with dilute solutions of sulphuric acid (H₂SO₄) and sodium hydroxide (NaOH) under carefully controlled conditions. The major part of it contains carbohydrates and it is valuable parameter in deciding the nutritive quality of animal feed (A.O.A.C, 1970).

2gm gum powder was taken in a 500ml spotless beaker and added 200ml 1.25% H₂SO₄ to it. Break up the lumps with the help of glass rod having a rubber policeman. Cover the beaker with a conical flask, half filled with cold water, which serves as water condenser. Boiled for 30 minutes made up any loss in volume during the boiling with hot distilled water.
Then filtered through Whatman filter paper No. 54 by washing the residue several times with hot distilled water.

Take out the residue back in the beaker with 100ml water and to it added 100ml 2.5% NaOH. Boiled for 30 minutes as earlier. Filtered through previously weighed Whatman filter paper No. 54. The residue was washed several times with hot water and lastly with 70% alcohol. Dried it over night at 100°C to a constant weight, cooled and weigh. Incinerate the residue along with filter paper in a crucible at 600±20°C for 2 hrs in a muffle furnace until all the carbonaceous matter is burnt. Cooled the crucible in a desiccator and weigh. Recorded the loss in weight as crude fibre.

2) Estimation of Crude fat:

The crude fat in the plant material was estimated by the standard Soxhlet method given in (A.O.A.C., 1970). The fat present in the gum material was extracted in the solvent consisting of chloroform (CHCl₃) and methanol (CH₃OH). This was done in Soxhlet extraction assembly and after complete evaporation of the solvent, the amount of extracted fat was measured.

2gm dry gum powder was placed into a thimble prepared with Whatman filter paper No.1. The mouth of thimble was plugged with fat free absorbent cotton. Clean, dry 250ml receiver flask from the Soxhlet assembly was taken and the solvent was added to it just to reach the level of the neck. The thimble with sample was introduced into the Soxhlet. The apparatus was
assembled and placed on heating mental with temperature controlling device. Water condenser was fitted at the top of the Soxhlet.

The fat was extracted for 8 hours at 60°C. Thimble was removed from soxhlet after extraction was over. Apparatus was again assembled and heated to recover most of the solvent from the receiver flask. When the receiver flask contains about 25ml solvent along with the extracted fat, receiver flask was disconnected. In a clean previously weighed beaker solvent was transferred with rising 2 to 3 times. Further it was dried in a hot air oven at 95°C, cooled in a dessicator and weighed. The amount of fat extracted per 2g of the sample was measured and the amount of crude fat as percent of dry matter (DM) was calculated.

3) Estimation of crude protein

This was done by estimating N content in the samples with the help of Microkjeldahl technique (A.O.A.C., 1970). The amount of N content was multiplied by 6.25 factor which gave crude protein content of the samples.

300mg gum powder were taken in Kjeldahl flask along with 250mg K$_2$SO$_4$ and 40mg CuSO$_4$ and kept overnight. This was digested till the mixture become white. After complete digestion the flasks were allow to cool. The digest was processed for distillation with the help of markham's distillation set.

Digest was diluted to 50ml volumetric flask, 5ml aliquots were taken and introduced in distillation unit through the side tube funnel. The glass
stopper was immediately fitted. To this 10ml 40% NaOH was added into the digest. NH₃ is liberated into 10 ml 2 percent boric acid (with mixed indicator) containing 50ml conical flask. After appearance of green colour of distillation ammonium borate was titrated against 0.035 NHC1 till the end point (faint pink) was obtained (This gave 1ml 0.035 NHC1 = 0.5mg N% crude protein = % N x 6.25). Crude protein of gum was calculated as percent nitrogen liberated x 6.5.

4) Nitrogen content

Estimations of nitrogen contents were made by Microkjeldahl method (A.O.A.C., 1970). For this 300 mg dry gum samples were taken in Microkjeldahl flasks. A pinch of catalyst was added to it with the help of spatula. 7.5 ml of concentrated sulphuric acid (H₂SO₄) was added to the flask. The flasks were heated on a digestion stand until (6 to 10 hr) the mixture was clear i.e. apple green in colour or colourless. During digestion care was taken to avoid particles of indigested carbon sticking on the sides of the tube.

5 ml of the diluted material was introduced in Markham’s distillation apparatus, through the side tube funnel to which glass stopper was fitted. 50 ml conical flask containing 10 ml of 2 % boric acid solution mixed with indicator was kept at the delivery end of the condenser to collect the ammonium tetraborate [(NH₄)₃ BO₃]. It was then titrated with 0.035 N HCl till the pink colour obtained and titration values were recorded. Nitrogen
content present in mg/g dry sample was calculated by calculating the strength of NH$_3$ in the distillate using equation.

1 ml of 0.35 N HCl = 0.5 of Nitrogen.

From the above equation the amount of nitrogen for 5 ml of the sample was calculated, which will be equivalent to that of present in 300 mg of dry sample. It was recorded as percentage nitrogen of dry sample.

5) Estimation of total sugar

The sugar content in the plant gum was estimated by the procedure recommended by Oser (1979) as follows. The non reducing sugars are hydrolyzed by acid. Acid hydrolysis converts them in reducing monosaccharide. The reducing sugars were then estimated and recorded as total soluble sugars.

100-500 mg plant gum was taken into 125 ml conical flask about 15 ml distilled water was added and heat on a hot plate. Solution was allowed to boil for 1-2 minutes to gelatinize starch. One conical flask was included containing only water as the enzyme blank then cooled at room temperature. 10 ml buffer solution was pipetted out and exactly 10 ml 0.5% enzyme solution was taken into the conical flask.

The remaining amount of enzyme is sufficient to hydrolyse disaccharides and starch. The conical flask was stoppered and incubate for 44 hours at 38\(^\circ\)C. The flask was swilled occasionally to stir mixture. Solution was filtered through Whatman’s No.1 paper into a suitable volumetric flask. Conical flask and filter paper were washed several times.
with distilled water. 2 ml 10 % neutral lead acetate was added to volumetric flask, brought to volume with distilled water and mixed well. To 50 ml centrifuge tube, 30 ml of above content was decanted and centrifuged for 5 minutes. Later on decanted into a 50 ml conical flask containing 100 mg powdered potassium oxalate. It was then refrigerated for 4-5 hours or overnight. After that filtered through Whatman’s No.42 paper into a small conical flask without washing. Aliquot was directly analysed for reducing power by Shaeffer Somogyi method (3:1b) after acid hydrolysis.

6) **Estimation of reducing sugar**

The sugar content in the gum was estimated by the procedure recommended by Oser (1979) as follows.

500mg of gum powder was taken in 50ml distilled water and boiled, filtered is and the filtrate was diluted upto 100ml. Three Folin-wus tube were taken and added following manner. (1) Blank tube - D. W. 2ml (2) 2ml glucose ‘C’ solution. (3) 2ml filtrate. In each tube 3ml alkaline solution of copper was added. Then tube was boiled in boiling water bath for 8 minutes. The tubes were cooled under tap water and 2ml of phosphomolybdic acid solution was added which gave blue colour. Then this solution was diluted upto 25ml distilled water and optical density was determined at 420nm and the amount of reducing sugar present in gum powder was calculated.
7) **Estimation of non-reducing sugar**

The percentage of non-reducing sugars was calculated by subtracting the value of the percentage of reducing sugars from that of total sugars.

8) **Qualitative analysis**

a) **Phenolics**

Estimation of phenols (Thimmaiah, 1999) was done by using ethanol extract (0.5 gm of samples was grinded with pestle and mortar in 10-times volume of 80% ethanol). The homogenate was centrifuge at 10,000 rpm for 20 min. The extraction was done twice. The pooled supernatant was then evaporated to dryness. The residue was dissolved in 5 ml of distilled water. Out of that 2ml was taken in different tubes. The volume was made final to 3ml with water. 0.5 ml of Folin – Ciocalteu reagent was added. After 3 min., 2ml of 20% Na$_2$CO$_3$ solution was added to each tubes. It was mixed thoroughly and then placed in boiling water for exactly one min. It was cooled and absorbance measured at 650 nm against a blank reagent. A standard curve was prepared using different concentrations (10-100 µg) of catechol.

b) **Alkaloids**

Five grams of plant gum was extracted with 50 ml of 5% ammonical ethanol for 48 hrs. The extract was concentrated by distillation and the residue was treated with 10 ml of 0.1 N H$_2$SO$_4$. The acid soluble fraction was tested with Mayer's, Wagner's, and Dragendorff's reagents.
white/coloured precipitate denoted the presence of alkaloids. The preparation of the reagents are as follows:

**Mayer's reagent:** 1.36 g of HgCl$_2$ was dissolved in 60 ml of distilled water and 5 g of KI in 10 ml of water. The two solutions were mixed and diluted to 100 ml with distilled water. A few drops of this reagent were added, as precipitates of some alkaloids are soluble when the reagent is used in excess.

**Wagner's reagent:** (Potassium Iodide) 1.27 g of I$_2$ and 2 gm of KI were dissolved in 5 ml of water and the solution diluted to 100 ml. It gave brown flocculent precipitates with most of the alkaloids.

**Dragendorff's reagent:** (Potassium bismuth iodide) 8 g of Bi(NO$_3$)$_3$. 5H$_2$O were dissolved in 20 ml of HNO$_3$ (sp. gr. 1.18) and 27.2 g of KI in 50 ml of water. The two solutions were mixed and allowed to stand when KNO$_3$ crystallised out. The supernatant was decanted off and made up to 100 ml with distilled water.

c) **Tannins**

Tannins was extracted in water and tested by treating them with protein solution when leather precipitates.

To the water extract was prepared by boiling 5 g gum in about 50 ml water, then 2% freshly prepared gelatin solution was added. The formation of a white (or milky) precipitate showed the presence of tannins in the gum.
PART - II

A) Composition of media used in isolation

For maintenance of microbial culture following standard media were used.

I) Potato Dextrose Agar (PDA)

Peeled potato – 200gm, Dextrose 20g, Agar 20 gm and distilled water 1000ml, pH 5.6. Peeled potatoes were boiled until soft and pass through muslin cloth. Then dextrose was added in it and final volume of solution was made up to 1000ml. In this solution agar was added, pH was adjusted to 5.6.

II) Czapek Dox Agar (CZA)

Sucrose – 30g, NaNO₃ – 2.0g, K₂HPO₄ – 1.0gm, MgSO₄.7H₂O – 0.5g, KCl – 0.5 gm, FeSO₄.7H₂O – 0.01gm, Agar – 15 gm and distilled water 1000 ml, pH – 5.6.

III) Martin’s Rose Bengal Agar (RBA)

Glucose – 10gm, Peptone – 5.0gm, K₂HPO₄ – 1.0gm, MgSO₄.7H₂O – 0.5g, Rose Bengal – 0.0001 gm, Agar – 20 gm and distilled water 1000 ml, pH – 5.6.

IV) Glucose nitrate agar (GNA)

Glucose – 10gm, KNO₃ – 2.5gm, KH₂PO₄ – 1.0gm, MgSO₄.7H₂O – 0.5g, Agar – 20 gm and distilled water 1000 ml, pH – 5.6.
B) **Bioactivity of plant gum against fungi**

The bioactivity of plant gum was studied against economically important fungi like *Alternaria alternata, Aspergillus niger, Curvularia lunata, Curvularia pellescence, Fusarium equiseti, Fusarium oxysporum, Macrophomina phaseolina, Penicillium digitatum, Penicillium chrysogenum, Rhizopus stolonifer*. The bioactivity of selected plant gum was studied at different concentrations of gum like 1%, 5% and 10%.

Fungitoxicity of plant gum was studied by the poisoned food technique described by Nene and Thapliyal (1993). Glucose nitrate medium was prepared in flasks and sterilized. To this medium, the requisite quantity of the plant gum was added. Plant gum was prepared by collecting fresh plant parts, washed thoroughly in distilled water and grinned in distilled water. The plant gum was thoroughly mixed by stirring. The medium was then autoclaved at 15 lb pressure for 20 minutes. After cooling the medium, fungi were inoculated in aseptic condition and incubated for 6 day at room temperature. Suitable checks were kept where the fungi were grown under the same conditions on glucose nitrate without plant gum. Mycelial growth and sporulation of the test fungi was measured after harvesting. The mycelial growth of the fungi compared with check, was taken as a measure of the fungitoxicity.

C) **Antibacterial activity of plant Gum**

For evaluation of antibacterial activity of gum, agar well diffusion assay (Navarro *et. al.*, 1996) method was used using Nutrient Agar Medium
for antibacterial bioassay, the pH of medium was between 7.2 to 7.4 after equilibration at room temperature. The freshly prepared and cooled medium was poured into clean autoclaved plates as a level horizontal surface, so as to give a uniform depth of approximately 4mm. After that the medium was allowed to cool at room temperature. After completion of cooling, the petriplates were stored in refrigerator between 2 to 8°C until use. Just before the use, the plates were placed in an incubator (37°C) with lids ajar until excess surface moisture on the surface of the medium or on the petriplates cover was lost by evaporation.

Inoculation of test plates were done by using intended bacterial cultures (Procured from eminent microbiological institute). The entire surface of the plate was inoculated.

The antibacterial efficacy of gum was checked. The well was made in the centre with the help of cork borer and defined concentration of gum were added in each well. After 48 hours, the antibacterial activity of gum was checked by means of measuring the zone of inhibition and results were recorded.

D) Study of hydrolytic enzymes

I) Production of amylase

Production of amylase(s) was studied by growing the fungi in liquid medium containing starch 1%, KNO$_3$, 0.25%, KH$_2$PO$_4$, 0.1% and MgSO$_4$.7H$_2$O, pH of the medium was adjusted at 5.5. 25 ml of the medium was poured in 100ml conical flasks autoclaved and inoculated separately
with 01 ml spore suspension of the fungi which were grown for 7 days on PDA slants. Unless otherwise stated, the flasks were incubated for 6 days at 25 ± 1°C with diurnal periodicity of light. On 7th day, the flasks were harvested by filtering the contents through Whatman filter No.1. The filtrates were collected in presterilized bottles and termed as crude enzyme preparation.

Composition of media used for Amylase production

i) Starch nitrate medium

Soluble starch 10gm, KNO₃ 2.5gm, KH₂PO₄ 1gm, MgSO₄.7H₂O 0.5gm, dissolved in 1000ml distilled water.

ii) Glucose nitrate medium:

Glucose 10g, KNO₃ 2.5gm, KH₂Po₄ 1.0gm, and MgSO₄.7H₂O 0.5gm, dissolved in 1000ml distilled water.

Assay method for amylase enzymes (Cup-plate method)

Determination of amylase activity was done with the help of cup-plate method which was adopted by Singh and Saxena (1982), where 20ml of starch agar assay medium (soluble starch – 10gm, Na₂HPO₄ – 2.84gm, NaCl – 0.35gm, Agar agar 20gm, distilled water 1000ml and pH 6.9) was poured in each petriplate. On solidification of the medium, a cavity (08 mm diameter) was made in the centre with the help of a cork borer (No.4) and was filled with 1ml culture filtrates (crude enzyme preparation) of the test fungi. The plates were incubated at 28°C for 24 hours, then they were flooded with Lugol’s iodine solution as an indicator. A clear, non blue,
circular zone obtained surrounding the central cavity, diameter of the zone was measured (mm) as the amylase activity zone. Similar procedure was followed for the control except pouring of culture filtrates in the central cavity instead of the activity enzyme.

II) Production of cellulase

Production of cellulase was made by growing the fungi on liquid medium containing CMC – 10gm, KN03 – 0.25%, KH2PO4 – 0.1% and MgSO4.7H2O – 0.05%, pH – 5.0. Out of which 25ml of medium was poured in 100 ml Erlenmeyer conical flasks and autoclaved at 151bs pressure for 15 minutes. The flasks on cooling were inoculated separately with 1ml spore suspension of test fungi prepared from the 7 days old cultures grown on PDA slants. The flasks were inoculated for 6 days at 25 ± 1°C with diurnal periodicity of light. On the 7th day the flasks were harvested by filtering the contents through Whatman’s filter paper No.1. The filtrates were collected in the pre-sterilized bottles and termed as crude enzyme preparation.

Assay for cellulase (cup-plate method)

The cup-plate method followed by Dingle et. al., (1953) and Szecsi (1969) was used. The assay medium contains 1% CMC and 2% difco agar, was poured in petri dish (20ml/plate) and allowed to solidify. In the centre, a 6 mm diameter cup/cavity was made with pre-sterilized cork borer (No.4). The cup was filled with 0.1m culture filtrate and incubated at room temperature for 48 hours. The activity zone was developed flooding the plates with 3% lead acetate solution (10-15ml/plate). Milky white coloured
activity zones were clearly seen on removing lead acetate solution with distilled water after a period of 30 minutes. The diameter of zone was measured in mm.

**III) Production of pectinase**

Production of pectinase was made by growing the fungi on liquid medium containing pectin – 10gm, KNO₃ – 0.25%, KH₂PO₄ – 0.1%, MgSO₄.7H₂O – 0.05%, pH – 5.0. Out of which 25 ml of medium poured in 100 ml Erlenmeyer conical flasks and autoclaved at 15 lbs pressure for 20 minutes. The flasks on cooling were inoculated separately with 01 ml standard spores / mycelial suspension of test fungi prepared from 7 days old cultures grown on PDA slants. The flasks were incubated for 6 days at 25±1°C with diurnal periodicity of light. On 7th day, the flasks were harvested by filtering the contents through Whatman’s filter paper no.1. The filtrates were collected in the presterilized bottles and termed as crude enzyme preparation.

**Assay for pectinase (Viscometer method)**

The Oswald’s viscometer was thoroughly cleaned with distilled water and dried before use; 6 ml of 1% pectin in 2 ml of 0.2 m Acetate buffer pH 5.2 and 4ml of enzyme source was taken in viscometer and were thoroughly mixed and incubated at 25°C temperature. The efflux time of the mixture at 0, 5, 10, 20, 30, 40 minutes was recorded with the help of stop watch.
The percent loss of viscosity was calculated by using the formula.

\[
\text{Percent loss of viscosity} = \frac{\text{To} - \text{T}_x}{\text{To} - \text{Tw}} \times 100
\]

Where,
- \( \text{To} \) - Flow time in seconds at zero time
- \( \text{T}_x \) - Flow time of the reaction mixture at time 'T'
- \( \text{Tw} \) - Flow time of distilled water.

E) Studies on Phytotoxins

1) Production of phytotoxins

The test fungi isolated from different seeds were grown on G.N. medium. 25 ml of the medium was added in 100 ml conical flasks and autoclaved at 15 lbs pressure for 15 min. On cooling, flasks were inoculated separately with 1 ml of spore suspension of test fungi prepared from 7 days old cultures grown on PDA slants. The flasks were incubated at 25°C for six days and were harvested by filtering their contents through Whatman No.1 filter paper. The filtrates were collected in presterilized bottles and termed as crude toxin preparations. These preparations were tested for their toxicity.

2) Composition of media used for phytotoxin production

**Glucose nitrate medium**

Glucose - 10 g, \( \text{KNO}_3 \) - 2.5 g, \( \text{KH}_2\text{PO}_4 \) – 1.0 g, \( \text{MgSO}_4 \cdot 7\text{H}_2\text{O} \) - 0.5 g, dissolved in 1000 ml distilled water.

3) Assay method

The toxicity of culture filtrate was determined by using following methods.
a) **Seed germination method**

Surface sterilized hundred seeds of each variety were soaked in crude toxin preparation for 24 hours. They were then placed on moist blotter in petriplates. Seed soaked similarly in freshly prepared uninoculated liquid medium served as control. Percent germination or percent inhibition of germination, root and shoot length of seedlings were measured after 7 days of incubation at room temperature.

F) **Effect of different concentrations of plant gums on seed germinability and incidence of seed mycoflora.**

a) **Seed Treatment:**

The freshly collected gums were used for preparing 1% concentrations of gum at different time periods. The 1% concentrations of gum were prepared by adding required amount of distilled water. After preparation of different concentrations of gum, it was poured in petri dishes under aseptic condition. After completion of seed counting, the seeds were transferred in the petri dishes containing gum. The seeds were allowed to soak for an hour in gum. After completion of seed soaking the seeds were removed and dried under aseptic condition for 2 hours.

b) **Seed plating for mycoflora and germinability**

The cutting of blotting paper was carried out in accordance with the size of petriplates. The resultant blotting paper was dipped in sterilized D. water until saturation and it was transferred to each plate. The plating of 10
dried seeds of each crop were carried out per plate, as per the protocol of ISTA (International Seed Testing Association 1966) i.e. 9 seeds at periphery and one seed in the centre at equidistance. After putting seeds the petriplates were kept in incubator i.e. 20°C for 7 days. After completion of 7 days the plates were removed from incubator and results were taken for the parameters like % germination, shoot length, root length and % incidence of fungi.

H) Bioassay of plant gum against the economically important insects

The bioassay of plant gum against the economically important insects like *Helicoverpa armigera, Leucinoides orbonalis, Plutella xylostella* and *Spodoptera litura* were carried out by using the pure gum.

Prior to initiation of experiments, the availability of second instar larvae of *Helicoverpa armigera, Leucinoides orbonalis, Plutella xylostella* and *Spodoptera litura* were checked. The fresh gum of selected plants were collected by using the standard method followed for experiments. The different dilutions of gum were prepared by using sterilized distilled water and the sterilized distilled water was used as a control.

The fresh leaves were collected and were used for bioassay of plant gum. After preparing the pieces of leaves, the 10 pieces were dipped in each selected concentration of gum and 10 pieces (control) were dipped in sterilized distilled water for an hour. After completion of an hour treatment
of gum concentration, the pieces were taken out and dried on the polythene sheet for an hour.

After complete drying of gum each piece was transferred to separate box and the box was closed with lid. After filling up of pieces in boxes the single larvae per box was allowed to feed on the piece for 48 hour and after the completion of 48 hour the results were recorded.