SUMMARY
Pulses play an important role in Indian diet and are the major source of protein. In India about 12.65 million tones of different pulses are produced per year. Pulses are rich in proteins, vitamins and minerals and they form the protein source for the vegetarian population in India. Qualitative and quantitative losses of pulses occur to an extent of 8.5 percent due to poor post storage handling and attack by the beetles (Bruchidae – Coleoptera). In India, pulses are an important but cost prohibitive source of proteins in human diet.

The Pulse beetle *Callosobruchus maculatus* is one of the most destructive pests of stored pulses. This pest multiplies rapidly under warm humid conditions and cause serious damage within a short time. Several species of bruchids in the genus *Callosobruchus* are known to damage grains of legumes up to 93.3 percent during storage. Among the five species of *Callosobruchus* three species viz., *Callosobruchus maculatus*, *Callosobruchus chinensis* and *Callosbruchus analis* are commonly found in India. Controlling bruchids through use of synthetic insecticides leads to several problems like resistance and residual hazards rendering the product unfit for human consumption.

*Sitophilus oryzae* is also considered as a major pest of stored grains. Control of this insect relies heavily on the use of synthetic insecticides and fumigants. But their widespread use has led to some serious problems. Different types of plant preparations such as powders, solvent extracts, and whole plants are being investigated for their insecticidal activity including their action as fumigants, repellants, antifeedants, anti-ovipositors, and insect growth regulators. Botanicals and entomopathogenic microflora are used in recent years to control the pests.
The main objective of the present study is to screen the entomopathogenic potential of selected plants viz., *Azadirachta indica*, *Calotropis gigantea*, *Catharanthus roseus*, *Cynodon dactylon*, *Morinda pubescens*, *Ocimum tenuiflorum*, *Phyllanthus amarus*, *Sesbania grandiflora*, *Tephrosia purpurea* and *Vitex negundo* to control the stored grain pests *Callosobruchus maculatus* and rice weevil *Sitophilus oryzae*.

To find out the possibility of utilizing the entomopathogenic microbes (bacteria *Serratia marcescens* and fungus *Beauveria bassiana* to control the *Callosobruchus maculatus* and *Sitophilus oryzae*.

Biochemical characterization of the stored products that got damaged due to pest attack.

To identify suitable biopesticide that can alternate chemical method of controlling stored grain pest.

1. Identification and collection of ten different plants from various places.
2. Characterization of the secondary metabolites of selected plants.
   2.1. Phytochemical analysis -TLC
   2.2. Phytochemical analysis - HPLC
3. Efficacy of selected plants powder and plant extracts against the pulse beetle, *C. maculatus*
   3.1. Preparation of plants powder and plants extract
   3.2. Effect of selected plants powder and plants extract on oviposition
   3.3. Effect of selected plant powder and extract on adult emergence
   3.4. Effect of selected plants powder and extract on adult mortality.
4. Evaluation of entomopathogenic bacteria and fungus on bruchid beetle, *C. maculatus*

4.1. Oviposition activity

4.2. Adult mortality

5. Biochemical analysis of *C. maculatus* treated with botanicals and microbes

5.1. Protein analysis and protein profile using SDS PAGE

5.2. Carbohydrate analysis

5.3. Lipid analysis

6. Effect of selected botanicals and microbes on rice weevil, *Sitophilus oryzae*.

**Characterization of the secondary metabolites of selected plants:**

Aerial parts of 10 selected plants were collected from Parvathiapuram, Kaduvetti, Konarkulam, Kattabomman nagar, Tirunelveli district of Tamilnadu, India. They were washed thrice with distilled water and once with tap water and were shade dried for two weeks. 20 g each of the aerial parts powder samples of *Azadirachta indica, Calotropis gigantea, Catharanthus roseus, Cynodon dactylon, Morinda pubescens, Ocimum tenuiflorum, Phyllanthus amarus, Sesbania grandiflora, Tephrosia purpurea* and *Vitex negundo* were taken separately and successively extracted with methanol and water in a soxhlet apparatus. The extracts were tested for alkaloids, phenol compounds and flavonoids. The various phytochemical tests were performed to find out the secondary metabolites. The presence of bioactive phytocompounds i.e the secondary metabolites from the leaves of selected plants were qualitatively analysed by Thin layer chromatography and High Performance Liquid Chromatography.
Collection and preparation of plants powder and plants extract:

The healthy leaves of selected plants were collected from selected areas in early morning and were washed in tap water and shade-dried for 10 days. The shade dried plant material was powdered using kitchen blender and that powder was subjected to Soxhlet extraction with petroleum ether (60°C) and methanol (60°C) for 24 h. Each solvent extract was distilled and condensed at 40°C. The condensed extract was stored at room temperature in air tight bottles and was used.

Five pairs of one or two days old adults of *C. maculatus* were released in each jar (6.5 x 8cm) containing 25g of sterilized black gram grains. The jars were sealed for a maximum of seven days and tested at different doses viz., 1%, 3% and 5% per 25 g of seeds. A control was also maintained by mixing the seeds with distilled water. The treatments were replicated thrice. Oviposition deterrence, adult emergence and adult mortality of *C. maculatus* was studied.

Collection and rearing of test insects:

A total quantity of 25g of sterilized black gram (*Vigna mungo*) grains were mixed with different concentrations like 30%, 60%, 90% of respective plant powders and five pairs of freshly emerged pulse beetle, *Callosobruchus maculatus* were introduced into each plastic container under ambient conditions of 28± 2°C and 65±5% RH for about 3 months. The experimental jars (6.5x8cm) were covered with muslin cloth. A control set was also maintained without any treatment powder. The insects emerged after four weeks were used in the entire investigation. Three replications were maintained for each treatment.
Oviposition deterrence activity:

Laboratory tests for oviposition inhibition effects were conducted. Five pairs of *C. maculatus* were released in each plastic container which was covered for the next 7 days allowing them to lay eggs. One week after oviposition the number of eggs laid were counted using handlens. Experiment was replicated thrice. The number of eggs laid on treated seeds (ET) and control seeds (Ec) were recorded and the percentage of oviposition deterrence (POD) was calculated.

Effect of plans powder and extract on oviposition of *C.maculatus*:

The reduction in the egg laying capacity of *C.maculatus* due to the treatment of the powders of *P.amarus, C.dactylon, C.roseus, T.purpurea, M.pubescens, A. indica* and *C.gigantea* is a new observation.

Maximum reduction in oviposition was noticed in the treatment with plant extracts of *C. dactylon, O. tenuiflorum, P. amarus, C. gigantea, C.roseus, V.negundo, S.grandiflora, T.purpurea, A. indica* and *M.pubescens* at higher concentration.

Adult emergence activity:

Starting from 20th day after oviposition, the number of adult emerged at alternative days were counted. Total number of adult emerged in each treatment was counted after 25 days of release. The number of adult emerged from the control seeds and the treated seeds were recorded. The reduction in the percentage of adult emerged was calculated.
Effect of plants powder and extract on adult emergence of *C. maculatus*:

In the treatment with higher concentration (90%) of plant powders, the adult emergence reduction percentage was higher in *S. grandiflora* followed by *V. negundo*, *C. gigantea*, *O. tenuiflorum*, *C. roseus*, *C. dactylon*, and *A. indica*.

Reduction of adult emergence was higher in the grains treated with 5% of *C. gigantea* extracts followed by *S. grandiflora*, *V. negundo*, *C. dactylon*, *O. tenuiflorum*, *P. amarus*, *C. roseus*, *T. purpurea* and minimum was recorded in case of *A. indica* and *M. pubescens*.

Adult mortality:

The adult mortality was recorded at alternate days. The adult mortality of *C. maculatus* was observed after 1, 3, 5, 7 and 9 days treatment.

Effect of plants powder and extract on adult mortality:

The effect of plants powder on adult mortality of pulse beetle shows a significant reduction among the treatment on their respective concentrations. There was a progressive increase in mortality of *C. maculatus* from 1 to 9 days treatment. The highest mortality was observed in *C. maculatus* treated with *P. amarus*, *M. pubescens*, *T. purpurea*, *A. indica* and *V. negundo* and these treatments were significantly different from each other in all concentrations.

*A. indica*, *T. purpurea* and *M. pubescens* at 1%, 3% and 5% (v/w concentrations) were very effective to cause 100% mortality of *C. maculatus* on the 5th day. In the control group the mortality is very less and there was zero mortality on the 5th day.
Evaluation of entomopathogenic bacteria and fungus on bruchid beetle, *C. maculatus*

To study the bio-safety of microbes against *C. maculatus* an experiment was conducted in the laboratory with 2 treatments. Tested microbes were *S. marcescens* and *B. bassiana*. A total of 10 newly emerged females of *C. maculatus* was introduced in an experimental (6.5x8 cm) jars covered with a net and supplied with 25g treated *V. mungo* grains. All experiments were performed at 28±2 °C and 65±5% R.H.

**Microbes on oviposition of *C. maculatus***

The efficacy of the biocontrol agents *S. marcescens* and *B. bassiana* were tested in different test doses and compared with a control group. The oviposition efficiency in *C. maculatus* treated with *S. marcescens* got reduced in a bacterial dose dependent manner. In the test dose of $2.5 \times 10^4$ CFU/ml the mean oviposition was 18.66±1.81. This was 78.82% reduction when compared with the oviposition potential of *C. maculatus*. Treatment with different conidial concentrations of *B. bassiana* indicated that reduction in oviposition was maximum in a dilution of $1 \times 10^4$. In this treatment the mean oviposition of *C. maculatus* was 35.56±2.93 (60.58% reduction in oviposition) when compared with the control.

**Mortality response of *C. maculatus* treated with microbes***

Percent mortality of *C. maculatus* showed a cumulative increase in the mortality with increase in exposure time in both the cases. Mortality of *C. maculatus* was monitored at 24 h interval up to 120 h (seven days). Treatment with *S. marcescens* indicated that the mortality was 100% at 72 h in (Treatment 1) where higher numbers of bacterial colonies were formed. In the treatment (T2) 100% mortality was obtained at 92 h and in T5, 100% mortality was noted at 120 h. Treatment with *B. bassiana* indicated that it can be used as a biocontrol agent for stored
pests. In the dilution $1 \times 10^{-6}$, 100% mortality was observed at 92 h. In the treatment T4, $1 \times 10^{-7}$ the cumulative mortality was 10 (100% mortality at 120 h).

**Biochemical analysis of *C. maculatus* treated with botanicals and microbes**

The test insects (adults of *C. maculatus*) were treated with respective botanical powder and extract at 5% concentration level and microbes at different concentrations. After five days treatment standardized biochemical protocol was followed for estimating total carbohydrate, protein and lipid. The whole body protein profile of the insect was analysed by SDS-PAGE method.

**Chemical composition of *C. maculatus* treated with plants powder and extract**

**Protein content of *C. maculatus***

The protein content was reduced in *C. maculatus* treated with plants powder and extracts. The change in the protein content varied according to the type of plant powder used. The maximum reduction was observed in *C. maculatus* exposed to *C.gigantea, P.amarus, A.indica, S.grandiflora, O.tenuiflorum* and *T.purpurea*. The decrease in the protein content due to the treatment of plant extract was in the following order *V.negundo, T.purpurea, A.indica, C.gigantea, M.pubescens, S.grandiflora, O.tenuiflorum, C.roseus, C.dactylon* and *P.amarus*. *C.gigantea* was found to be effective in reducing the protein content of *C. maculatus*.

**Carbohydrate content of *C. maculatus***

The carbohydrate content was reduced in *C. maculatus* treated with plants powder. The decrease in the carbohydrate content was in the following order, *A.indica* followed by *M.pubescens, V.negundo, C.gigantea, T.purpurea, S.grandiflora, C.dactylon* and *C.roseus*.
The carbohydrate content was also reduced due to the treatment of plants extract. Order of decrement was *A.indica* followed by *M.pubescens, V.negundo, C.roseus, C.gigantea, T.purpurea, P.amarus, C.dactylon, O.tenuiflorum* and *S.grandiflora*. *A.indica* played a significant role in reducing the carbohydrate content of *C. maculatus*.

**Lipid content of *C. maculatus***

In the present study the highest reduction percentage of lipid content in *C. maculatus* was noticed due to the treatment of *A.indica*. It was followed by *V.negundo, M.pubescens, O.tenuiflorum, C.dactylon, P.amarus, C.gigantea* and *T.purpurea*.

Among the plants extract tested *V.negundo* and *A.indica* extract was effective in reducing the lipid content of the treated insects. Among the other plants the order of decrement was *C.roseus, P.amarus, C.gigantea, M.pubescens, T.purpurea, S.grandiflora, C.dactylon* and *O.tenuiflorum* extract. Lipid content was also greatly reduced by *A.indica*.

**Chemical composition of *C. maculatus* treated with microbes**

**Total body protein of *C. maculatus*** and Protein profile using SDS PAGE

Proteins are the major biological factor that plays an important role in insect growth, development and various physiological processes. The present study result suggested that the selected plants altered the normal physiological activity. In the present study the protein content of *C.maculatus* was reduced due to the treatment of *S.marcescens* and *B.bassiana*.

**Lipid Content of *C. maculatus***

In the present study the microbes *S.marcescens* and *B.bassiana* were found to be effective in reducing the lipid content of *C. maculatus*. 
Carbohydrate content of *C. maculatus*

The amount of carbohydrate was drastically reduced in the treated pest, percent reduction was observed in *C. maculatus* treated with *S. marcescens* and *B. bassiana*. The present study indicates that both microbes have caused significant alterations in carbohydrate, protein, and lipid content of *C. maculatus*.

Effect of selected botanicals and microbes on rice weevil, *Sitophilus oryzae*

*S. oryzae* was mass cultured and the insect culture was further multiplied on paddy grains in plastic containers in the laboratory. *S. oryzae* were maintained at ambient laboratory temperature (28 ± 2°C) and relative humidity (65 ± 5%) conditions. Plant parts of different species *Azadirachta indica*, *Calotropis gigantea*, *Tephrosia purpurea* and *Vitex negundo*, were collected, shade dried and powdered using pulverizer. Four plant powders (2.5 and 5 per cent) were tested for their insecticidal action in comparison with untreated control. Twenty g of paddy grains were taken in petridishes. The plant powders @ 2.5 per cent and 5 percent (w/w) were added to paddy grains and shaken thoroughly. Ten newly emerged adults were released into each petridish and kept in the laboratory. Three replications were maintained for each treatment. Adult mortality observations were recorded on 1, 3, 5 and 7, days after the treatment.

In another experiment 20g of paddy grains were taken in glass bottles and the microbial cultures of (*S. marcescens* and *B. bassiana*) @ T1 to T3 were added to paddy grains and shaken thoroughly. Then the glass bottles were covered firmly using muslin cloth. Five pairs of newly emerged adults of *S. oryzae* were released to each glass bottle, covered firmly and kept in laboratory conditions. Mortality was recorded every 24 hrs, for a total period of nine days. Three replications were maintained for each treatment.
Mortality response of *S. oryzae* treated with botanicals and microbes

The present study indicated that the mortality of *Sitophilus oryzae* was higher when treated with powders of *V. negundo* and *A. indica*.

The mortality was recorded at 24 h, 48 h, 72 h, and 96 h. The adult mortality of *S. oryzae* was observed on 72 h after treatment with *S. marcescens*. 100% mortality was observed at higher concentration. But in *B. bassiana*, cent percent mortality was observed in 48h at higher concentration. The overall results of bioassay revealed that both the microbes were found to be highly pathogenic to *S. oryzae*. 