Experiments related to micropropagation, callus induction, solasodine production with special reference to fungal elicitors, hairy root induction, antiaflatoxicosis studies and antimicrobial activity were carried out in Solanum melongena L.var. insanum (L.) Prain. using different field grown and callus derived samples. Results of the findings are mentioned here.

**Direct plantlet regeneration from young leaf explants**

Even though this medicinal plant belongs to the family solanaceae the young field grown explants are not much obedient in terms of multiple shoot induction. So we have selected young leaf explants for direct regeneration from seedlings.

The young leaf explants obtained from 20-25 days old young seedlings germinated in mud/plastic pots containing red soil. The excised and sterile young leaf explants inoculated on MS medium supplemented with varying concentrations of KIN and BAP individually. The inoculated explants enlarged with size within 10 days of cultures. The growth was started initially from the cut ends of the explants and covered all over the surface. Initially the growth of the explant was pale green in color and in due course of time it became dark green in color. After 15-25 days of culture, the shoot buds developed all over the surface of explants. The multiple shoots developed within 45 days of culture.

The percentage of response was varied from 60.10 - 78.32 in various concentrations of KIN and 62.92 - 80.16 in BAP. The number of shoots also varied based on the concentrations of cytokinin present in the medium. The maximum number of multiple shoots were (17.06) recorded in 5.0 mg/L of BAP followed by (11.53) KIN 5.0 mg/L. The numbers of shoots were increased with increasing concentration of KIN or BAP (Table 1, Fig. 1 & 2, Plate 1, 2 & 3).
Root induction

The well developed shoots were excised and inoculated on root induction medium containing MS half strength with B5 vitamins along with varying concentrations of IAA, NAA or IBA (0.5-2.0 mg/L). The maximum percentage of rooting was noticed in IBA 2.0 mg/L (data not shown). If MS full strength was used for root induction, there was a callus formation along with rooting. So the strength of the MS medium was reduced to half. The rooted plantlets were grown in sterile soil mixture and maintained in greenhouse conditions (Plate 4).

Callus induction and solasodine production

The young field grown young stem explant were culture on callus induction medium containing various combinations of IAA+KIN and IAA+BAP (1.0 - 3.0 mg/L + 0.50 mg/L). The stem explants responded differently in the callus induction medium. The percentage of response, the callus growth in terms of fresh and dry weight measurement and solasodine production were significantly varied based on the hormonal concentrations.

Influence of IAA with BAP

Callus was initiated within 8 days after inoculation. In all the concentrations of IAA the white greenish compact calli was observed after 20 days. Percentage of callus induction was recorded from 55.28 to 72.42. The maximum fresh weight (4.32 gm) of calli was recorded in 3.0 mg/L of IAA + 0.25 mg/L BAP. There was a significant difference among the different concentrations IAA + KIN and IAA+ BAP in respect of fresh and dry weight. Dry weight of the calli recorded from 0.05 to 0.37 (Table 2, Fig 2 and Plate 5). Solasodine content of the callus ranged from 1.75 to 2.91 mg/g dwt and maximum solasodine content (2.91 mg/g dwt) was observed from 3.0 mg/L IAA with 0.50 mg/L BAP (Table 3, Fig 2 and Plate 6).
Effect of IAA + KIN

The percentage of callus induction was recorded from 51.83-68.06. The maximum fresh and dry weight was observed in IAA with KIN (3.0 +0.50 mg/L). The fresh weight was ranged from 1.44 - 3.93 and dry weight was 0.15-0.37 gm. (Table 2, Fig 2 and Plate 5a). In the combinations of IAA + KIN, the solasodine content was ranged from 1.41-2.30 mg/dwt. Solasodine content of the callus was progressively increased due to increasing hormonal concentrations (Table 3, Fig 3 and Plate 7).

Induction of hairy roots and solasodine production

Hairy roots were induced from multiple shoots obtained via micropropagation were infected with Agrobacterium rhizogenes ATCC 15834. The multiple shoots were infected with Agrobacterium rhizogenes with different time intervals (2, 4, 6, 8, and 10). Before infection with Agrobacterium rhizogenes the basal portions of the shoots were pricked thoroughly (0.5 cm) to facilitate the infection. After infection the infected shoots were transferred to MS basal medium for co cultivation. The co cultivation period was 24, 48, and 72 hrs. Among the various infection periods six mts duration was found suitable to induce hairy roots. The infection period beyond six mts leads to over growth to bacteria. The co cultivation period of 48 hrs facilitate hairy root induction without any over growth of bacteria. The antibiotic cefotoxime 250 mg/ L was used to control the over growth of bacteria. The same antibiotic concentration also used for washing of basal portion of the shoots, before transferred to selection media.

The fast, vigorously growing hairy roots were isolated and sub cultured on hormone free MS liquid medium for mass production (Plate 8 & 9). After 45 days the hairy roots were
harvested, dried and used for solasodine estimation by HPLC. The solasodine content in transformed hairy root were recorded as 4.52 mg/g dwt (Plate 10).

**PCR Analysis**

PCR analysis was carried out to confirm the presence of rol B gene fragment DNA of transformed hairy root. The expected 780 Pb rol B fragment was found in the positive hairy roots. Control roots did not show the fragment (Plate 11).

**Solasodine production in field grown plants**

Solasodine content was analyzed in field grown plants of *Solanum melongena* L var *insanum* (L.) Prain using HPLC. Different plant parts like leaf, stem, root, pericarp and seed were used for solasodine production. Among the various parts tested, the solasodine production in pericarp powder sample having maximum solasodine content (1.71 mg/g dwt) followed by stem powder (1.58 mg/g dwt). The results indicated that there was a lower amount of solasodine content in root powder. The solasodine content was found in increasing order as root < leaf < seed < stem < pericarp (Table 4, Fig 3 and Plate 12).

**Effect of fungal elicitors on solasodine production**

**Effect of *Aspergillus flavus* on solasodine production**

*A. flavus* broth culture (1 ml) was added with different samples like stem, stem callus and pericarp powder samples (1 gm each) increased the solasodine production when compared to control after 240 hours of incubation. The results showed that there was an increased solasodine content when the fungal culture was mixed with dried samples (Table 5, Fig 3 and Plate 13). In stem callus powder the solasodine content was increased from 2.91 to 3.40
mg/g dwt, where as it was enhanced from 1.58 to 2.89 mg/g dwt in stem powder and it was 1.71 to 2.36 mg/g dwt in pericarp powder. There was significant increase in solasodine content was observed in all the samples.

**Effect of Aspergillus nidulans on solasodine production**

Like that *Aspergillus flavus, Aspergillus nidulans* also enhanced the solasodine content in powdered samples of stem callus (4.51 mg/g dwt) and pericarp (2.52 mg/g dwt). In stem callus powder solasodine content was increased from 2.91 mg/g dwt to 4.51 mg/g dwt where as it was increased from 1.71 to 2.52 mg/g dwt in pericarp and it was increased from 1.58 mg/g dwt to 2.09 in stem. Results were noticed that solasodine content was increased significantly in all the samples than control. Solasodine was also increased in stem callus and pericarp than stem powder (Table 5, Fig 3 and Plate 14).

**Influence of Penicillium purpurogenum on solasodine production**

The broth culture of *P. purpurogenum* recorded the highest yield of solasodine production at 240 hrs of incubation period. Solasodine content of stem callus was significantly increased from 2.91 to 3.69 mg/g dwt while adding the broth culture of *P. purpurogenum*. Whereas it was increased from 1.58 to 2.79 mg/g dwt in stem and it was from 1.71 to 2.25 mg/g dwt in pericarp powder. Results recorded that higher solasodine was obtained from stem callus powder was observed (Table 5, Fig 3 and Plate 15).

**Influence of Penicillium citrinum on solasodine production**

Like that of *A. flavus, A. nudulans* and *P. purpurogenum, P. citrinum* was also enhanced the solasodine content. In stem callus solasodine was increased from 2.91 to 3.82 mg/g dwt. The
same tendency was observed in stem (1.58 to 2.61 mg/g dwt) and pericarp (1.71 to 2.66 mg/g dwt) powder. Results noticed that solasodine content in all samples like stem, stem callus and pericarp powder (Table 5, Fig 3 and Plate 16).

**Effect of natural plant and in vitro callus extracts on aflatoxicosis**

Aflatoxin B1 was chosen in this study. The albino rats fed with 6 ppm of aflatoxin along with feed shown significant changes in the haematological parameters such as haemoglobin, total RBC, WBC, ESR, clotting time and PCV. Treatment with the different parts of *Solanum melongena* L. var. *insanum* (L.) Prain. such as leaf, stem, root, pericarp, seed and *in vitro* raised stem callus extract to aflatoxin intoxicated rats showed a remarkable increase in the level of all tested blood parameters (Table 6 and Fig 4).

**Hemoglobin**

The haemoglobin (Hb) level was 13.86 (g %) estimated in control rats. At the end of the experimental period, the animals treated with aflatoxin for 3 weeks, the Hb level was significantly decreased to 12.42 (g %). But while, treating with the extract, the haemoglobin level was regained to 13.34 and 13.76 (g %) than the control rats respectively by 25 and 50 mg of leaf extract of *Solanum melongena* L. var *insanum* (L.) Prain. When the rats were treated with pericarp extract (25 & 50 mg), haemoglobin content was increased to 13.54 g% and 13.94 g% respectively than control. Whereas the same trends were observed in the rats treated with the extract of seed and root (13.82 and 13.75 g %). The animals treated with 50 mg of extract of stem derived calli, increased the haemoglobin content to 13.82 and 13.86 g% respectively.
Total RBC

When the white albino rats were treated with 25/mg ethanolic extract of leaf, stem, root, pericarp seed and stem derived callus of some the RBC concentration of these animals were drastically increased.

The total RBC count was decreased from 4.8 to 3.9 in aflatoxin treated rats than control. In leaf extract 50 mg of Solanum melongena L. var. insanum (L.) Prain. was increased the RBC count to 4.6. Whereas in pericarp 25 and 50 mg/kg b.wt of extract increased the RBC count to 4.4 and 4.7 ($10^6$ Cumm) respectively, as the same trend was observed in the extract of stem and seed treated animals. In root extract 25 and 50 mg administered rats shown increase in the RBC count to 4.80 and 4.84 respectively. In stem callus RBC content was increased to 4.80 and 4.66. There was a significant increase in RBC of the animal is treated with the plant extract.

Total WBC

The WBC level was 7.2 in control rats. At the end of the experimental period of aflatoxin treated animals the WBC count was recorded as 8.1. In plant extract treated animals, leaf extract 25 and 50 mg regain the WBC to 7.6, 7.3 respectively. The same trend was recorded in 25 and 50 mg of pericarp (7.5 and 7.3), stem extract (7.3 and 7.6), seed extract (7.28 and 7.14) respectively. The callus extract (IAA + BAP and IAA + Kin) also regained the WBC from 8.1 to 7.4 and 7.44 respectively. The result showed that all the extracts have the significant role in the regaining of WBC.
ESR

ESR rate was noticed as 3.4 in control rats. In aflatoxin intoxicated rat the ESR was recorded 4.1. Whereas in 25 and 50 mg of stem extract treated animals ESR was 3.7 and 3.6 respectively. Whereas 25 and 50 mg of pericarp, stem extract treated animals ESR rate was 3.8, 3.5, 3.6, and 3.5 mm/1st hr respectively.

Result showed that the rats treated with 25 and 50 mg of ethanolic extract, the ESR rate was 3.4 & 3.8. In vitro derived callus also played a significant role in ESR control. Stem callus derived from IAA + BAP and IAA + KIN treated animals ESR were recorded as 3.6 and 3.4.

Clotting time

In control rats, clotting time was recorded as 110 (Sec), whereas, it was 121(Sec) in aflatoxin treated animals. In leaf extract treated animals the clotting time recorded as 115 & 112 in the concentration of 25 and 50 mg/kg b.w.t respectively. The same trend was observed in pericarp (113 and 111), stem (104 and 109), seed (114 and 108) and root extract (112 and 112) respectively in the concentration of 25 & 50 mg/Kg b.w.t. When the rats treated with 50 mg of callus extract (IAA + BAP and IAA+KIN), the clotting time recorded as 114 and 110 respectively.

PCV

Packed cell volume was recorded as 40.20% in control rats. In aflatoxin intoxicated rats, PCV was 47.20%. In plant extract of S.melongena L. var. insanum (L.) Prain. leaf extract treated animals, PCV was recorded as 45 and 44 in the concentration of 25 and 50 mg/ Kg b.w.t. The same trends were observed on the rats which were treated with pericarp (43 & 42 %), stem
(43 & 45 %), seed (42&40 %) and root (42 & 42%) respectively in the concentration of 25 and 50 mg/kg b.wt. In 50 mg/kg b.wt of extract (IAA+ BAP & IAA+KIN) treated animals, PCV was controlled to 40.20 & 42.24% respectively.

**Antimicrobial activity**

The antimicrobial activity of ethanolic extracts of leaf, stem, root, pericarp, seed and stem callus were assayed under *in vitro* conditions by agar well diffusion method against bacterial and fungal pathogens. The data pertaining to the antibacterial and antifungal potential of the callus culture and adults plants extracts of *Solanum melongena* L. var. *insanum* (L.) Prain. are presented in Table.

**Antibacterial activity of leaf extract**

Antibacterial activity of leaf extract of *Solanum melongena* L. var. *insanum* (L.) Prain. against gram positive and gram negative bacteria was given in the (Table 7 and Fig 5). The maximum inhibition zone was recorded in 100 µg/ml of leaf extract against *K. pneumoniae* (3.26 mm) followed by 75 µg/ml leaf extract against *S.dysentriae* (3.23 mm). The minimum inhibition zone was noticed that leaf extract 25 µg/ml against *S.epidermidis*.

**Antibacterial activity of stem extract**

Among the pathogens tested *S.typhi* was highly sensitive for 100 µl of stem extract (4.43 mm) followed by *E.coli* (4.16 mm); while 25 µg/ml of stem extract did not show any activity against *V.cholarae, S.epidermidis, Pseudomonas sp, S.dysentriae* and 50 µg/ml in *S.epidermidis*. The lowest level of inhibition was observed in 25 µg/ml of stem extract against *S.typhi* (0.23 mm) (Table 8 and Fig 6).
Antibacterial activity of seed extract

Ethanolic extract of Solanum melongena L. var. insanum (L.) Prain. seed exhibited varying degree of antibacterial activities against test organisms. The 100 µg/ml of seed extract had higher antibacterial activity with mean zone of inhibitions 5.96 mm in K.pneumoniae followed by 5.03 mm S.typhi. Besides that the 25 µg/ml ethanolic stem extract had a low antibacterial activity against V.cholarae (0.96 mm) and 50 µg/ml in S.aureus (Table 9 and Fig 7).

Antibacterial activity of root extract

Among the pathogens tested most susceptible bacterium was V.cholarae (4.06 mm) in 100 µl of seed extract followed by S.aureus (3.53 mm) at 100 µg/ml concentration. The low amount of inhibition was found 25 µl of seed extract in S.epidermidis (0.03 mm) (Table 10 and Fig 8).

Antibacterial activity of pericarp extract

The pericarp ethanol extract of Solanum melongena L. var. insanum (L.) Prain. was found to be as effective as 100 µg/ml against S.dysentriae (6.00 mm). But the ethanolic extract of pericarp showed minimum inhibition 0.46 mm. The 25 µg/ml of root extract were inactive against the following three organisms viz E.coli, S.aureus, Streptococcus sp (Table 11 and Fig 9).

Antibacterial activity of stem callus extract

From this results it is evident that the ethanolic extract of stem callus (table no) was highly active against Pseudomonas sp, V.cholarae (4.00 mm) followed by S.aureus (2.46 mm) at 100 µl concentration. While 25 µg/ml of stem callus extract could inhibit lowest amount
represented in *E.coli* (0.03 mm). But 25 µg/ml of stem callus extract exhibited no antibacterial activity against the following organisms *S.typhi*, *S.epidermidis*, *Pseudomonas sp* and *Streptococcus sp* (Table 12 and Fig 10).

**Antifungal activity of leaf extract**

The susceptibility of the fungi towards various concentration of the leaf extract of *Solanum melongena* L. var. *insanum* (L.) Prain. is summarized in (Table 13 and Fig 11). Results of the well diffusion assay showed that ethanolic extract of *Solanum mellongena* var. *insanum* L Prain. has antifungal activity against all six investigated fungi and the greatest inhibition was observed with 100 µl of the extract. The diameter inhibition zones ranged from 0.60±0.15 to 0.3.73±0.17 with the hogh zone value observed against *T.tonsurans* (0.3.73 mm). Dose of 25 µg/ml extract showed smaller zones of inhibition in the range of 0.33 mm to 0.53 mm. The lowest concentration of the 25 µg/ml of the extract showed no inhibitory effect against *T.mentagrophyte, T.tonsurans, M.mycetoma* and *A.flavus*.

**Antifungal activity of stem**

The ethanolic extract of stem was screened for the activity against six fungi by agar well diffusion method. Among the fungi tested, *E. flocossum* (3.86 mm) registered most susceptible at a concentration of 100 µg/ml followed by *T.tonsurans* (3.66 mm). There was no activity found against *M.mycetoma* and *A.flavus*. The minimum activity was exhibited by the ethanolic extract of the stem at concentration of 25 µg/ml extract against *T.rubrum*. The 25 µg/ml of extract did not exhibit any activity against *M.mycetoma, A.flavus* (Table 14 and Fig 12).
Antifungal activity of root

Antifungal activity recorded in terms of average zones of inhibition in millimeter was noted in Table 15 and Fig 13. The ethanolic extract of *Solanum melongena* L. var. *insanum* (L.) Prain. exhibited very strong activity against with three fungi with maximum activity ranged from 3.76 mm, 3.80 mm, 3.80 mm and 4.43 mm in *T. tonsurans*, *A. flavus*, *M. mycetoma* and *T. mentagrophyte* respectively. The extract is found to be lowest inhibitory activity in *T. rubrum* (0.63 mm).

Antifungal activity of seed extract

Ethanolic extract of seed of *Solanum melongena* L. var. *insanum* (L.) Prain. showed high antifungal activity against *A. flavus* (4.90 mm) at concentration of 100 µg/ml. At a concentration of 25 µg/ml of seed extract, there was no inhibition zone was observed against *T. tonsurans* and *T. rubrum*. The ethanolic extract showed lower activity against *M. mycetoma* (0.26 mm). Ethanolic extract of seed did not prevent the growth against *T. tonsurans* (25, 50 µg/ml), *T. rubrum* (25 µg/ml) (Table 16 and Fig 14).

Antifungal activity of pericarp extract

The higher concentration of pericarp extract (100 µg/ml) showed maximum activity against *T. rubrum* (5.50 mm). The least activity (0.50 mm) was recorded by the ethanolic extract against *E. fllocossum* at 50 µg/ml (Table 17 and Fig 15). At 25 µg/ml pericarp extract was not showed the inhibitory zone against *E. fllocossum*. 
Antifungal activity of stem callus extract

The ethanolic extract at 100 µg/ml, 3.36 mm was recorded as diameter zone of inhibition against *M. mycetoma* and this was followed by 2.00 mm zone of inhibition found against *A. flavus* (Table 18 and Fig 16). Among the organisms tested for their susceptibility to the stem callus extract *M. mycetoma* was showed most susceptible at 25, 75, 50 and 100 µg/ml in the zone of inhibition ranged from 0.63, 1.20, 1.96 and 3.36 mm respectively. At higher concentration (100 µg/ml) maximum inhibitory activity was noticed against *A. flavus*. 