SUMMARY

1. A brief review of literature pertaining to the present line of investigation has been presented. The review mainly deals with the storage fungi of seeds and their harmful effects i.e. bio-deterioration of seeds by storage fungi.
2. Materials used in this investigation and the experimental procedures followed have also been described in detail.
3. Twenty six (26) fungal species were isolated from the three pulses- *Cajanus cajan, Lens culinaris* and *Vigna radiata* and these were characterised and identified. Species of *Aspergillus* was predominant in all the seeds with other genera like *Penicillium, Alternaria* and *Fusarium* showing lesser frequency.
4. Viability of seeds was shown to depend on a number of factors. i.e. storage period, temperature and humidity as well as type of seeds. Among the three pulses tested *L. culinaris* was the most resistant to deterioration as viability was lost only after 18 months of storage under ambient conditions. Whereas in case of *C. cajan* it was lost after 12 months and in case of *V. radiata* after 15 months. Storage under low temperature and humidity increased germination percentage in all seeds.
5. Effect of seed storage on two major seed components i.e. proteins and carbohydrates were determined. It was observed that after a period of storage of one year when analysis were done, significant reduction in both protein and total sugar contents were observed in all cases. This was farther confirmed by analyzing seeds artificially inoculated with *A. niger* and *A. flavus.* In this case also significant reduction was obtained. *A. falvus* was found to cause greater loss of carbohydrate in *C. cajan* in comparison to *A. niger.* However in case of protein, in *C. cajan, A. niger* caused greater reduction.

6. SDS PAGE analysis of protein pattern also showed significant reduction in the number of protein bands both during storage as well as artificial inoculation.

7. Activities of amylase as well as protease showed declining trend during storage.

8. Polyclonal antibodies (PAbs) were raised against antigen preparations from mycelia and spores of *Aspergillus niger* and *Aspergillus flavus*. These were purified by ammonium sulphate precipitation followed by DEAE cellulose chromatography. IgG obtained in each case was used for immunodiffusin and ELISA tests.

9. Agar gel double diffusion tests were performed using crude antibody as well as purified IgG prepared after four different bleedings collected for the pathogen. Strong precipitin reactions were observed from 2^{nd} bleed onwards.

10. Optimization of ELISA by using PAbs of *A. niger* and *A. flavus* and angiten preparations at variable concentration were performed. ELISA values showed both PAbs to have very high titer of which *A. flavus* gave higher absorbance values. A_{405} values decrease with antigen and IgG dilutions.

11. DAC-ELISA tests were performed separately using PAbs raised against mycelia and spore antigens of *A. niger* and *A. flavus* and antigens of seeds (Pulses) obtained from 13 different localities of West Bengal and Sikkim.

12. When seeds of all three pulses obtained from various localities were tested against PAbs of *A. niger* and *A. flavus* much higher A_{405} values were obtained with PAb of *A. niger*.

13. These results were also confirmed by Dot-Blot assays. Among the three pulses, *V. radiata* showed most reactivity to the PAbs.

Differences in ELISA values as well as intensity of colour of the dots in dot-blots also varied with the locations.

14. Mycelia and conidia of *A. niger* and *A. flavus* when treated with homologous antisera followed by FITC, bright fluorescence was noticed on young hyphae and throught the surface of conidia.

15. Fluorescence staining and immunocytochemical staining techniques were used to determine the presence of fungi within seeds. For these immunoassays sections of the stored seeds were treated with the antibodies and suitably stained. Observations under the microscope revealed that maximum reactions occur either on the seed coat surface or in the parenchymatous cells just below the seed coat.