Discussion
DISCUSSION

In the Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, while working on various aspects of seed pathology of different crops, it has been consistently observed in case of cereals (Panchal, 1984 and Khairnar, 1987), pulses (Bhikane, 1988) and oil seeds (Sandikar, 1990) that in addition to variety of fungi species of *Aspergillus* exhibited their prominent association. Therefore, in the present investigations emphasis has been given to elucidate:

1) Percent incidence of *Aspergillus* species and their relative dominance and succession and seed surface in different crops.

2) Specific association among the species of *Aspergillus* and other fungi.

3) Pathogenic nature of *Aspergilli*.

4) Ability of *Aspergilli* in the process of seed biodeterioration and role of their extracellular metabolites including hydrolytic enzymes, phyto-toxins and aflatoxins.

Totally fourteen species of *Aspergillus* from the seeds of cereals, oil seeds, pulses, vegetables and some medicinal plants were isolated. This clearly indicates
that *Aspergillus* possesses a strong ability to survive and develop association with a broad range of seeds irrespective of size, colour, age, hardness and softness of the seeds. Similar type of reports on the isolation of different species of *Aspergillus* from various crops are available in the literature. Among them prominent mention can be made of maize (Goodman and Christensen, 1952; Kang and Singh, 1976; Reddy and Reddy, 1989 and Arvinder and Rai, 1991), sorghum (Lekel and Martin, 1943; Kanaujia, 1974; Kavita Rani et al., 1978 and Bhadraiah and Ramarao, 1987), rice (Prasad et al., 1986; Singardevi and Raj, 1992), wheat (Kanaujia, 1974; Kunwar and Indira, 1989), bajra (Sharma and Basuchoudhary, 1974; Girisham Reddy, 1985), barley (Armolik et al., 1956; Kanaujia, 1974), gram (Singh and Chouhan, 1954; Singh and Dwivedi, 1992), cowpea (Zohri et al., 1992), soybean (Gowda and Sulia, 1987; Sinha et al., 1991), groundnut (Gupta and Chouhan, 1970; Cherry et al., 1975), sunflower (Solunke and Kore, 1982; Neera and Mehrotra, 1990), chilli (Deena and Basuchoudhary, 1984) medicinal plants (Dutta and Rao, 1987; Roy et al., 1991), ornamental plants (Srivastava and Gupta, 1981; Shroti et al., 1983) and from forest plants (Jammaluddin et al., 1985).

During isolation studies it has been observed that blotter method supported to grow more species of *Aspergillus*.
with minimum contaminations while, in agar plates there was always crowding species of Alternaria, Curvularia, Cladosporium, Helminthosporium, Fusarium and members of phycomycetes which ultimately made difficult to isolate Aspergilli in pure forms. Hence it can be concluded that the blotter method proved to be superior over agar plates for isolation of maximum number of Aspergillus species.

It is observed from the results that the % load of Aspergilli was found to be variable with the crop and different varieties of the same crop. This clearly indicates that the texture and chemical nature of the seed might be playing an important role for the association of Aspergilli. Similar types of variations in seed mycoflora in different varieties of bajra have been reported by Shetty et al., (1982) and Khairnar (1987) in case of bajra. Similarly, while in case of jowar, Williams and Rao (1981) found significant variation in the load of seed mycoflora among different varieties of jowar. Variations in seed mycoflora with respect to varieties have also been reported in pulses (Bhikane, 1988) and in oil seeds (Sandikar, 1990). On the contrary, Koteshwar Rao et al., (1971) claimed that there was no such variation of seed mycoflora among eight varieties of jowar.
Results about the effect of physical factors on isolation of Aspergilli are also interesting. It gave the clue that incidence of Aspergillus species was found to be possible on a broad range of pH (3.5 - 8.5) and temperature (10 to 40°C). This clearly indicates about super adaptation of Aspergilli as compared to the other members of fungi occurring on seeds.

Results regarding load of Aspergilli on different categories of seeds are very much explanatory. As the load was found to be significantly less on healthy seeds but at the same time seeds having wounds or ruptures and discolorations yielded maximum incidence of Aspergillus. This clearly indicates that initial penetration of seed coats may not be the job of Aspergilli but once they penetrate through wounds and ruptures further degradation of seed chemicals might be efficiently possible by them. Similarly, results regarding incidence of Aspergilli on different age seeds clearly showed that at early stages of seeds establishment of Aspergilli seems to be impossible. But at the same time association can be developed towards maturity of the seeds. This again indicates that Aspergilli as compared with other fungi like Fusarium, Alternaria, Curvularia may not be having abilities of infection to the developing young seed coat and at the time of seed maturity they might be establishing as successers and not as the primary invaders.
It is clear from the literature that Aspergillus are mainly storage fungi and the results given in Table 8 are in support of this fact. As load of Aspergillus was found to be increased with increase in storage period and also with the type of storage containers. The storage containers like tin box and polythene bags supported maximum multiplication of Aspergillus population as compared to the same in gunny bags. Hence, it can be concluded that by using a proper storage containers population of Aspergillus can be controled successfully without the aid of chemicals. Similarly, observations regarding use of chemicals as seed dressers for the control of Aspergillus are found to be interesting. As treatment of neem seed oil was found to be equally promising to that of thiram and vitavax in all respects. This gives a clue about the use of natural products as seed dressers in order to substitute costly and hazardous chemicals.

It was studied and found that different species of Aspergillus responded variably to the seed extracts of cereals, pulses and oil seeds. This gives an idea about nutritional variations among species of Aspergillus. Seed extracts of oat among cereals, lentil and winged bean among pulses allowed poor and scanty growth of A. niger and A. flavus indicating that these seeds might
be having certain inhibitory compounds to prevent growth of widely occurring *Aspergillus* members.

Studies on pathogenic nature of fourteen *Aspergillus* species (Table 11) it has revealed that very few species have been found to affect seed germination adversely, but majority of them did not reduce % seed germination. This shows that *Aspergilli* may not be producing toxic substances at very early period of their growth which can enable retardation of germination and therefore seeds get germinate successfully. Next part of the experiment was found to be still interesting because there was significant root rotting in the germinating seedlings, and development of abnormal seedling due to majority of *Aspergilli*. This indicates late production of phytotoxins by majority of *Aspergillus* species which effect adversely on post germination process.

Similar type of pathogenic nature have been reported in many cases as seed rotting and root rotting due to *A. flavus* in case of jowar (Rati and Ramlingam, 1974; Panchal, 1984) due to *A. flavus* and *A. niger* in maize (Aulakh et al., 1976).

It is observed from the results that degree of seed rotting was found to be variable with the crop and varieties of the same crop. This clearly suggests
varitial resistance against *Aspergillus*. This type of varietal variation in seed rotting also have been recorded in case of jowar, groundnut and blackgram (Kanaujia, 1974; Panchal, 1984; Bhikane, 1988).

It is understood from the results (Table 17) that all the six species of *Aspergillus* employed for seed biodeterioration studies showed loss in seed weight due to their growth and association. This clearly indicates that the species are found to be capable of utilizing seed contents which might have resulted in the loss in seed weight. It was also interesting to observe that there was variation in the % seed weight loss in different crops caused by the same species. It was found to be maximum in blackgram seeds and minimum in neem seeds. This suggests preference of nutrients present in blackgram seed might be more and at the same time there must be some inhibiting factors in neem seeds due to which there was no growth of *Aspergillus* resulting it into very poor loss in seed weight. All the six species of *Aspergillus* showed variation in their rate of utilization of seed contents of the same crop. This clearly gives an idea about nutritional variation among the species. Loss in seed weight in different crops caused by different members of fungi have been observed by different workers, as Pedgaonkar (1973) in case of jowar, Sawney and Aulakh

Reports regarding the loss in seed weight specifically due to *Aspergillus* have also been made in the literature. Loss in seed weight of blackgram and green gram due to *A. flavus* (Bilgrami et al., 1966; Bhikane, 1988) due to *A. flavus* and *A. niger*, in case of jowar (Panchal, 1984), in bajra due to *A. flavus* (Girisham and Reddy, 1985), in mustard (Kumar and Prasad, 1993).

The results regarding utilization of seed protein in four different crops by six species of *Aspergillus* (Table 19) are found to be very much informative. All the species of *Aspergillus* are found to be capable of utilizing seed proteins but the rate of utilization was variable among the species. This indicates the ability of *Aspergillus* for their proteolytic nature which may also be variable for different kinds of seeds. This also gives an idea about different chemical nature of proteins in different crop seeds.

Reports about ability of *Aspergillus* species to utilize seed proteins from different crops have been made by various workers. Bilgrami et al. (1976) found
loss in protein content of blackgram and greengram due to *A. flavus*. Sinha *et al.*, (1978) observed the same in arhar seeds due to *A. flavus* and *A. niger*. Similarly, Bilgrami *et al.*, (1981) in maize seeds due to *A. parasiticus*, Neeti and Karan (1981) in sunflower and sesame due to *A. flavus* and *A. niger*, Kumar and Prasad (1993) in mustard seeds due to *A. flavus* have reported proteolytic breakdown of seed proteins.

Results regarding degradation of oil and fat contents of seeds from four crops due to *Aspergilli* are given in Table 20. It is understood from the results that all the species of *Aspergillus* degraded fat contents of seeds significantly. This clearly suggests their lipolytic nature. However, the fat content of neem seeds was found to be reduced poorly by all the species indicating presence of some inhibitory component in neem seeds. Reports regarding loss in fat content of seeds due to different species of *Aspergillus* has been made in many cases, Ward and Diener (1961) observed significant loss of oil content in groundnut seeds due to *A. tamari*, *A. ruber*, *A. chevalieri*, *A. restrictus*. Neera and Mehrotra (1990) observed the same in case of sunflower due to *A. flavus* and *A. fumigatus*. While, Mishra and Kumar (1993) noted in case of Sesamum due to *A. flavus*. Shaha and Singh (1993) in case of Mahua due to *A. niger*, *A. ochraceus* and *A. tamari*. 


Results summarised in Table 21 show highly amylo-
lytic nature of *Aspergillus*, because there was a signi-
ficant degradation of starch content in all the four
crops. Among six species of *Aspergillus* studied, *A. flavus*
has been found an efficient to degrade starch while,
*A. sulphureus* proved to be less efficient for the same
purpose. Reports regarding degradation of starch due
to different species of *Aspergillus* have been recorded
by different workers. Sinha *et al.* (1981) observed in
pigeon pea due to *A. niger* and *A. flavus*, Prasad and
Pathak (1987) in case of wheat seed due to *A. niger*,
*A. flavus*, *A. terreus*, *A. candidus* and *A. sydowi*.

Severity of seed biodeterioration is mainly dependent
upon the rate of degradation of seed contents like starch,
protein, lipid etc. and which is linked with the produc-
tion of hydrolytic enzymes like amylase, lipase, protease,
and pectinase by the moulds growing on the seeds. Results
regarding in this connection (Table 22) clearly suggest
that all the fourteen species have been found to be
degraded starch efficiently. Because they have pro-
duced significant amount of amylase in the culture fil-
trate. However, some species produced amylase only in
the presence of starch indicating their adaptive nature
of amylase production. Similarly, majority of the
species of *Aspergillus* have been found lipolytic and
and proteolytic in nature. Regarding lipase and protease production, majority of the species showed also adaptive nature of production. It was interesting to note that either adaptively or constitutively majority of the species did not product cellulase and pectinase. This gives an idea about their inability or non-requirement character for the survival.

Results regarding the effect of physical factors on production of hydrolytic enzymes suggest that pH 5.5 to 6.5, temperature 25 to 30 and incubation period 6 to 8 days were found to be optimum conditions for the production of hydrolytic in *Aspergillus*. All these optimum conditions have been found favourable also for maximum vegetative growth of *Aspergillus*. This clearly informs that in case of *Aspergillus* production of all hydrolytic enzymes might be taking place simultaneously or might be interdependent with maximum vegetative growth.

Reports with regards to maximum amylase production at pH 5 to 5.5 have also been made in case of *A. niger* by Ghai *et al.* (1980) in *A. awamori* by Chung *et al.* (1987) in *A. flavus* and *A. fumigatus* by Adisa (1985).

Studies regarding the effect of seed extracts, carbohydrates, nitrogen sources and amino acids which were found to be stimulatory for growth were also found
to be stimulatory for production of enzymes. Stimulation of lipase due to seed extracts of sesame and groundnut indicates the favourable chemical compositions of the seeds for growth and lipase production in both for *A. flavus* and *A. niger*. At the same time it was interesting to note that seed extracts of pulses proved slightly inferior for protease production than on the medium, supplemented with glucose. This may be due to essential requirement of glucose for protease production. Regarding the influence of carbohydrates, xylose, fructose and maltose were supporting carbohydrates for amylase production in *A. flavus* but at the same time these carbohydrates proved inferior for the production of lipase, protease and cellulase. This strongly suggests that presence of free sugars might favourable condition only for amylase production but at the same time unfavourable for other hydrolytic enzymes as observed, in the present case.

The role of nitrogen sources on production of enzymes shows that all the amino acids and nitrogen sources tested were found to be unfavourable. This indicates requirement of specific nitrogen source during the production of hydrolytic enzymes.

Studies regarding the role of different inhibitors on production of hydrolytic enzymes were carried out and
results are summarised in Table 25. It is understood from the results that respiratory inhibitors strongly affected the production of hydrolytic enzymes adversely indicating that enzyme production must be interdependent with active respiratory cycles in the fungi. Similarly, use of thiram and vitavax were found to be inhibitory for enzyme production in the fungi. Therefore, this may be helpful in order to control seed biodeterioration caused by Aspergilli. It was interesting to observe that antibiotics at 100 ppm concentration could not inhibit Aspergilli. However, this requires further investigation before drawing any conclusion.

Studies were carried out in order to understand the role of toxic compounds produced by Aspergilli on seed germinability and seedling vigour in different crops. Results summarised in Table 27 show that among the three crops tested against 14 species of Aspergillus, jowar was found to be affected by more number of species than the other two crops. This indicates that every crop might be having a different degree of tolerance for the toxins produced by seed borne fungi. Similarly, it has also been observed that a particular group of Aspergilli including A. flavus, A. niger, A. fumigatus, A. versicolor and A. carbonarius was found to be equally toxic for reduction in seed germination of all the crops. This
indicates non specific nature of toxins to different hosts, which has been considered to be a saprophytic behaviour of the fungi.

Results summarised in Table 28 regarding the effect of toxins on seed germination in different varieties of the same crop are found to be still more clear and enlight that the toxins of *A. flavus* and *A. niger* showed variations in the degree of damage of seeds and seedlings in different varieties of both groundnut and blackgram. This clearly indicates varietal resistance to the toxins of *Aspergillus* at minute levels.

Reports regarding lethal effects on seed and seed embryos due to culture filtrates of *Aspergillus* in case of different crops have been made by various researchers. This was reported by Tripathi (1974) due to *A. flavus* in case of jowar, by Mathur and Sinha (1977) due to *A. chevalieri*, *A. flavus* and *A. terreus* for bajra, by Bose and Nandi (1985) due to *A. flavus* and *A. fumigatus* for Sesamum and due to *A. flavus* and *A. sydowi* and *A. candidus* for safflower.

In order to understand effect of culture filtrates on leaf surface tissues and vascular system experiments were undertaken and results are summarised in Table 29
and 30. Culture filtrates from most of the species of *Aspergillus* caused at least watersoaked lesions as minimum damage while at further level reaching to chlorosis and necrosis in all the four crops tested. This gives an idea that although the species of *Aspergillus* are known as non-pathogens to green plants but treatment of their toxic compounds to healthy tissues proved to be pathogenic. In a similar way the culture filtrates of *Aspergillus* damaged internal tissue of treated shoot cuttings. Majority of the species showed water soaked lesions, leaf drying and tip drying in the test plant. While, very few species showed stem necrosis and wilting. This shows that toxins produced by different species of *Aspergillus* may not be very efficient for the damage of internal tissue as that of *Fusarium* and other wilt causing pathogens.

It is clear from the literature that all the species of *Aspergillus* are not found to be potential to produce aflatoxins. This has also been observed in the present investigation (Table 31). Because when all the 14 species were screened for aflatoxin production only six of them were found to be aflatoxigenic in nature. It is also observed that *A. flavus* and *A. versicolor* produced aflatoxin in seeds of groundnut, black gram and
jowar while *A. fumigatus, A. terreus, A. ruber* and *A. nidulans* produced aflatoxin only in groundnut. This clearly suggests that aflatoxin production in these later two species must be substrate specific while in *A. flavus* and *A. versicolor* it might be non specific. Variations in aflatoxin production on different crop seeds has been reported in case of *A. flavus* by Hesseltine et al. (1966).

Results regarding ability of aflatoxin production in two selected isolates of *A. flavus* on different crop seeds are found to be interesting. Isolate No.80 which was selected as virulent produced aflatoxin significantly in majority of crops but at the same time it failed to produce on the seeds of mustard and neem. This might be due to presence of some inhibitory compounds in neem and mustard seeds which might be playing very important role for the control of aflatoxin production. However, this needs further confirmation prior to draw any conclusion. Similarly, isolate No.110 which was selected as an avirulent could produce only on three crop seeds among the totally 14 crops tested. This suggests that isolates which are known for their poor efficiency to produce aflatoxin also get stimulated with supply of favourable nutrients or seed chemicals.
Studies were undertaken to understand the influence of interactions of other fungal populations with *A. flavus* on aflatoxin production. Results are found to be very much surprising because *A. flavus* when grown in the presence of *A. niger*, *A. ruber* and *A. versicolor* showed synergistic effect while, in the presence of species of *Alternaria*, *Fusarium*, *Helminthosporium*, *Cladosporium*, *Curvularia* and a bacterium it failed to produce aflatoxin. This clearly suggests that in nature this factor might be playing very important role to control aflatoxin production by species, the species of *Aspergillus*. This type of studies if undertaken in future shall definitely enlight about biocontrol of aflatoxin production as well as helpful for biodegradation of aflatoxins by making use of various microorganisms rather than the use of poisonous and costly chemicals.