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Storage Fungi in Groundnut and the Associate Seed Quality Deterioration-A Review

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Abstract: Intensive crop improvement programme has resulted in the development of large number of high yielding varieties in different crops and more so in groundnut. Thus, production and distribution of quality seeds to the farmers become increasingly important. In a seed production programme, storage of seeds till the distribution during next season assumes paramount importance. Being an oil seed, groundnut losses its viability soon. Though, the initial seed quality and storage environment are important to prolong the shelf life of seeds, the invasion of fungal pathogen also play a major role in decreasing the viability of a seed lot in groundnut. So it is necessary to study the seed quality changes that occur during storage of seeds as a result of changes in biochemical constituents of seeds due to fungal infection. This review article will try to give the relationship between seed borne pathogens and seed quality deterioration and relative biochemical changes occur in seeds.

Key words: Groundnut, storage fungi, seed quality deterioration, biochemical changes

INTRODUCTION

Grain production of a country depends on good quality seeds. Quality seeds play a very important role for the production of healthy crop. Healthy and pathogen free seeds are the basic requirements for disease free crop. Seeds are stored for a considerable period of time in order to catch the correct season. It is reported that 25% of the world's crops are affected by mould or fungal growth. In India around 82% of groundnut produced is used for edible oil production, 12% as seed and 5% as feed. The seeds are found to be responsible for disease transmission because they carry a number of pathogens which get associated either in the field or in the post harvest storage condition.

Aspergillus is a common mould in tropical and sub tropical countries and causes aflatoxin contamination as a result of moulding of badly stored commodities, such as groundnut, cereal and cotton seeds. Fungi like *Aspergillus niger*, *Aspergillus flavus*, *Alternaria dianthicola*, *Curvularia lunata*, *Curvularia pellescens*, *Fusarium oxysporum*, *Fusarium equiseti*, *Macrophomina phaseolina*, *Rhizopus stolonifer*, *Penicillium digitatum* and *Penicillium chrysogenum* causes discoloration, rotting, shrinking, seed necrosis, loss in germination capacity and toxification to oil seeds (Chavan and Kakde, 2008).

Fungi growing on stored seeds, can reduce the germination rate along with loss in the quantum of carbohydrate, protein and total oil content, induces increased moisture content, free fatty acid content and enhancing other biochemical changes. The tropical climate with high temperature and high relative humidity along with unscientific storage conditions adversely affect the preservation of cereal grains, oilseeds, etc., which lead to the total loss of seed quality.

Intensive crop improvement programme has resulted in the development of large number of high yielding varieties in different crops and more so in groundnut. Thus, production and distribution of quality seeds to the farmers become increasingly important. In a seed production programme, storage of seeds till the distribution during next season assumes paramount importance. Being an oil seed, groundnut losses its viability soon. Though, the initial seed quality and storage environment are important to prolong the shelf life of seeds, the invasion of fungal pathogen also play a major role in decreasing the viability of a seed lot in groundnut. So it is necessary to study the seed quality changes that occur during storage of seeds as a result of changes in biochemical constituents of seeds due to fungal infection. This review article will try to give the relationship between seed borne pathogens and seed quality deterioration and relative biochemical changes occur in seeds.

Seed borne mycoflora: Groundnut is usually harvested and stored dry in different storage conditions. Being an

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oil seed, it losses its viability within a short period due to the irreversible phenomena of ageing. Under such conditions seeds were also susceptible to fungi, insects and other micro organisms (McDonald, 1977). A gradual decrease in field fungi followed by an increase in storage fungi was observed in groundnut by Bhattacharya and Raha (2002). Storage environment viz., temperature and relative humidity might not be conducive for the survival of field fungi (Lacey, 1975). Patra *et al.* (2000) reported that increase in storage period of groundnut seeds upto nine months, the viability decreased, while pathogen activity, moisture and sugar content in seeds increased gradually.

About 27 species of fungi from pods and seeds of stored groundnut have been reported (Lalithakumari *et al.*, 1972). Among different species, *Aspergillus niger* and *Rhizoctonia bataticola* were serious in reducing germination of seed by about 30% after six months of storage. An increase of *Aspergillus flavus* from 40 to 78% and a decrease of *Aspergillus niger* and *Aspergillus ruber* from 55 to 40% and 45 to 0%, respectively after 10th month of storage of groundnut seeds was reported by Bhattacharya and Raha (2002). Raju and Krishnamurthy (2003) found a progressive increase in the *Aspergillus niger* in groundnut with increase in storage period. Peanuts with their subterranean growth habit were invaded by both *Aspergillus flavus* and *Aspergillus parasiticus*. According to Yu *et al.* (2004), *A. flavus* was the predominant species responsible for aflatoxin contamination of crops prior to harvest or during storage, while Rasheed *et al.* (2004) found *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium solani*, *F. oxysporum*, *Aspergillus flavus*, *A. niger* were predominant in groundnut and seed coat was greatly infected by fungi followed by cotyledon and axis. Rosetto *et al.* (2005) and Ihejirika *et al.* (2005) indicated that the fungi responsible for storage rot of groundnut were *Aspergillus flavus* and *Aspergillus niger*.

Among the species of *Aspergillus*, *A. flavus* was the most frequently isolated species in peanut and pistachio kernels, respectively, as stated by Hedayati *et al.* (2010). Aliyu and Kutama (2007) identified six fungal taxa, namely *Aspergillus*, *Rhizopus*, *Penicillium*, *Curvularia*, *Fusarium* and *Mucor* in groundnut under different storage conditions. Goncalez *et al.* (2008) stated that *Fusarium* was the most prevalent fungus in Brazilian peanut kernels from sowing to harvest. Nakail *et al.* (2008) recorded the susceptibility of peanuts to colonization of *Aspergillus flavus* especially during storage. Begum *et al.* (2008) reported that the incidence of *Colletotrichum truncatum* infection in soybean was much more in seed coat followed by cotyledon and embryonic axis without any external symptoms during incubation period.

Vikas and Mishra (2010) isolated nine species of fungi from the seeds of different varieties of groundnut during storage of one year. Chavan (2011) found that the species of *Aspergillus*, *Penicilium*, *Fusarium*, *Rhizopus* and *Alternaria* were commonly occurring post harvest moulds in storage conditions. Most of the species of *Aspergillus* were dominant and play a vital role in the seed biodeterioration. Ibiam and Egwu (2011) reported that among different species of fungal infection, *Aspergillus flavus* was the most preponderant one in groundnut.

Biochemical changes of seeds during storage: Changes in chemical constituents of cell have been related to viability of seeds. Paramasivam *et al.* (1990) reported that the germination of groundnut seed was negatively correlated with electrical conductivity of seed leachates and its soluble sugar and free amino acid concentration. Vertucci *et al.* (1994) studied the changes in lipids during storage of groundnut and other oil seeds and suggested that the changes in lipid components of seeds were associated with seed deterioration and could be measured using differential scanning colorimetry.

Braccini *et al.* (2000) observed reduction in protein, lipid and poly unsaturated fatty acids content and increased hexanal production in storage of soybean seeds. Murali *et al.* (2002) stated that germination and field emergence of the pulse seeds decreased while the electrical conductivity of seed leachate increased with increase in storage period. Peroxidation of unsaturated fatty acids led to leaching of electrolytes and other solutes in soybean (Singh and Dadlani, 2003). Verma *et al.* (2003a) reported a decrease in carbohydrates and protein content in deteriorated seeds. Narayanaswamy (2003) concluded that oil, protein and field emergence of groundnut seeds decreased but free fatty acid and EC increased with advancement of storage period. Simic *et al.* (2007) noticed a decrease in oil content of sun flower, soybean and maize seeds during storage.

Biochemical changes due to storage fungi: Ramamoorthy and Karivaratharaju (1989) noticed that there was a progressive decrease in germination percentage, oil and protein content and an increase in free fatty acids in the stored kernels than in the pods because of the invasion of storage fungi to kernels. Ushamalini *et al.* (1998) reported that protein content, total sugars and reducing sugars of cowpea seeds were reduced due to seed borne fungi in storage. Shelar (2002) recorded a higher percentage of mycoflora with the seeds that had lost its viability and had higher EC and leaching of sugars. Bhattacharya and Raha (2002) observed a decrease in carbohydrate, oil content and increase in free fatty acid content with a gradual loss followed by a small increase in protein content of maize, groundnut and soybean seeds during storage due to storage fungi.

Jain (2008) reported a rapid increase in concentration of free fatty acids in damaged seeds by fungal invasion. Embaby *et al*. (2006) observed a reduction in carbohydrate, reducing sugar and crude fat due to *Fusarium oxysporum* in legume seeds. Kakde and Chavan (2011) concluded that *Aspergillus flavus* was responsible for maximum depletion of fat content and reducing sugar in safflower, soybean, sesamum and groundnut due to *Fusarium equiseti* and *Rhizopus stolonifer* and a decrease in crude fat content by *Curvularia lunata*, *F. equiseti* and *penecillium digitatum*.

Seed quality changes during storage: Shelar (2002) reported that the germination of soybean varieties decreased during storage irrespective of varieties, threshing and processing methods and storage containers. Similar result was reported by Rajgopal and Chandran (2002) in groundnut seeds. Devi *et al*. (2003) reported that mustard seeds stored under ambient conditions recorded maximum germination%, higher field emergence and 1000 seed weight with lower electrical conductivity and sinapine leakage upto 31 months of storage. Nagaveni (2005) recorded a decline in germination, rate of germination, seedling dry weight, vigour index, field emergence and lower moisture content at the end of 10 months of storage in onion seeds. Roopa *et al*. (2006), Rao *et al*. (2006) and Haile (2006) reported a decrease in seed quality in prolonged seed storage of musk melon, onion and chickpea, respectively. Divya (2006) reported that sesamum and sunflower seeds maintained satisfactory germination upto ten months of storage, while soybean and groundnut seeds maintained viability only for seven and six months, respectively.

Balesevic-Tubic *et al*. (2007) observed a decline in seed vigour of naturally aged sunflower seeds compared to the vigour of fresh seeds. Simic *et al*. (2007) observed decrease in germination of maize, soybean and sunflower seeds after 4 years of storage. Similar results were reported by Adetumbi *et al*. (2009) in maize seeds. Khaldun and Ehsanul Haque (2009) observed a decline in moisture content, germination percentage, vigour and increase in percentage of abnormal seedling, fresh seed, dead seed, hard seed, root-shoot ratio and amount of dry matter after three months of storage in cucumber seeds. Shakuntala (2009) reported that germination of sunflower seeds declined progressively with increase in the period of storage.

The storage potential of seed is a heritable character (Justice and Bass, 1978). Therefore, information on the relative storability of the genotypes produced at different locations is important. The differential storability of genotypes of different place of origin within a species under ambient conditions has been reported in wheat by Nisha (2007). Seed quality depends on the genetic make up of seed and its interaction with the environment under which it is produced, harvested, processed and stored. It is envisaged that bulk of the carryover seeds would be stored in bags under ambient conditions because in India storage of bulk quantity of seeds under controlled conditions is neither economical nor practicable.

The storage of seeds for further sowing is warranted in sustained agriculture apart from improving the productivity. Groundnut which is one of the poor storer owing to its oil content and deteriorates faster compared to other crops. Storing seeds after harvest till the next cropping season without impairing the quality is of prime importance for successful seed production. The loss of seed viability was rapid in groundnut and about 50% viability could be lost within four to five months of storage (Nautiyal and Ravindra, 1996).

The moisture content of seed is an intrinsic seed character that is influenced by genetic and environmental fluctuations (Abdalla and Roberts, 1968). The initial moisture content of seeds plays a major role in the maintenance of seed quality during storage. This result was in conformity with previous studies carried out by Paramasivam (2005) and Gomathi (2009) in groundnut.

Ageing is an irreversible process which decreases the seed quality as storage period increased. Decline in germination is the last physiological phenomenon in the process of ageing. The reduction in germination might be due to the depletion of food reserves and decline in synthetic activity due to ageing as reported by Heydecker (1972).

Storage fungi influence the seed quality parameters and decrease the germination potential of the seeds during storage. The reduction in germination due to storage fungi may be attributed to the production of aflatoxin in food grains interferes with protein synthesis by inhibiting the incorporation of amino acids into protein, resulting in non-germination of embryo. Aflatoxin affects the plants by inhibition of seed germination, elongation of hypocotyl or root of developing seeds (Janardhan *et al*., 2011).

Vigour is essentially a physiological phenomenon influenced by the reserved metabolites, enzyme activities and growth regulators. Vigour index value which is the totality of germination and seedling growth has been regarded as a good index to measure the vigour of seeds (Abdul-Baki and Anderson, 1973). Normally, loss of vigour precedes loss of viability. Pathogen infection also severely affects the seedling vigour during storage.

Seed quality changes due to storage fungi: Increasing the storage period of groundnut seeds upto nine months decreases the viability, while pathogen activity, moisture and sugar content in seeds increase gradually. De Frietas *et al*. (2000) reported that with increase in storage period of cotton seeds, there was a linear decrease in viability of seeds and a linear increase in incidence of storage fungi. Raj *et al*. (2002) identified that the species of *Aspergillus*, *Alternaria*, *Rhizoctonia*, *Fusarium*, *Phoma* and *Chaetomium* are affecting germination and emergence in soybean seeds.

Bhattacharya and Raha (2002) reported a gradual decrease in field fungi with simultaneous increase in storage fungi accompanied by a reduction in germinability occurred in ground nut, soy bean and maize seeds during storage. Krishnappa *et al*. (2003) reported that groundnut pods stored in gunny bag had recorded maximum infection ranged between 16 and 18% of *Aspergillus flavus*, *Aspergillus niger*, *Fusarium* spp. and *Pencillium* spp. and caused reduction in germination and vigour index. While Nargund *et al*. (2003) found the presence of *Aspergillus flavus*, *Aspergillus niger*, *Rhizoctonia* spp. *Fusarium* sp. and *Sclerotium rolfsii* in all the varieties of groundnut and they caused reduction in germination% and vigour index.

Basavaraju *et al*. (2004) revealed that *Plasmopara halstedii* was a seed borne pathogen and the seeds infected severely by this pathogen resulted in lower germination% with less vigour index in sunflower. Pre and post emergence damping off of seedlings in soybean seeds due to *Colletotrichum truncatum* were observed by Mayonjo and Kapooria (2003) and reduction in performance was also reported by Begum *et al*. (2008).

Aspergillus flavus showed 10% pre-emergence and 3% post emergence death in surface sterilized groundnut seeds (Rasheed *et al*., 2004). Malaker *et al*. (2008) observed 27.10% of *Aspergillus* spp. infection which reduced the germination to 68% at the end of tenth month of storage in wheat seeds. Kakde and Chavan (2011) observed that fungi like *Aspergillus niger*, *Aspergillus flavus*, *Alternaria dianthicola*, recorded discolouration, rotting, shrinking, seed necrosis, loss in germination capacity and toxification in oil seeds. Radha *et al*. (2011) found a reduction in seed germinability of red gram, green gram and black gram when the seeds were soaked in fungal filtrate of *Aspergillus.*

Biochemical changes due to storage fungi: Biochemical constituent of seeds is an important factor which influenced the physiological soundness of seed. Electrical conductivity of the seed leachate is a measure of membrane integrity is considered as a good index for seed viability (Matthews and Bradnock, 1968). The alteration of permeability of cell membrane for increased leakage over a period of storage could be the reason for the changes in the electrical conductivity of the seed leachate (Ching and Schoolcraft, 1968; Abdul-Baki and Anderson, 1973). Increase in electrical conductivity might have been induced by the presence of pathogen during storage which also influenced in the loss of membrane integrity during storage. Similar results were obtained by Manimurugan (2003) in black gram and Begum *et al*. (2008) in soybean. The possible reason for the increase in electrical conductivity value may be due to the development of moderate to severe fissures on the seed coat infected with disease which showed excessive electrolytes leakage and high conductivity levels as given by Loeffler *et al*. (1988).

Groundnut being an oil seed, it contains lesser amount of carbohydrate than cereals but more amount of oil and protein and they are breaking down into simple sugars and amino acids is essential for germinating seed as an energy source. In the present study the carbohydrate content decreased during the course of storage and reached 13.8% at the end of storage period. Similar results were obtained by Verma *et al*. (2003b) in Brassicat. Decrease in carbohydrate content may be due to the hydrolytic breakdown of carbohydrate into simple sugars or due to exudation by loss of cell membrane integrity (Short and Lacy, 1976). In general, storability of seeds was positively influenced by protein content. However there is every possibility that protein content of seeds decreased during storage. Denaturation of protein could be considered as one of the reasons for loss of physiological vigour in the seeds. During storage, protein becomes less soluble and degraded into free amino acids (Cherry, 1983). Evidently, leaching of free amino acids affecting the viability and vigour of seeds was brought out by Bewley (1979).

Biochemical characters such as carbohydrate, protein, oil content, free amino acid, free fatty acid and electrical conductivity, activities of enzymes such as catalase, peroxidase and lipase also showed better results. The difference in storage potential of seeds produced at different locations may be due to the difference in initial seed quality under different environmental conditions and invasion of fungi in these areas (Natarajan, 1996). Hence the selection of best location for seed production is an important exercise in seed production to obtain the seeds of high quality and storability.

Ramamoorthy and Karivaratharaju (1989) noticed that there was a progressive decrease in germination percentage, oil and protein content and an increase in free fatty acids in the stored kernels than in the pods because of the invasion of storage fungi to kernels. Ushamalini *et al.* (1998) reported that protein content, total sugars and reducing sugars of cowpea seeds were reduced due to seed borne fungi in storage. Shelar (2002) recorded a higher percentage of mycoflora with the seeds that had lost its viability and had higher EC and leaching of sugars. Bhattacharya and Raha (2002) observed a decrease in carbohydrate, oil content and increase in free fatty acid content with a gradual loss followed by a small increase in protein content of maize, groundnut and soybean seeds during storage due to storage fungi.

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Seed treatments for protecting seeds from storage fungi: Use of fungicide as seed treatment is the most widely followed management practice in all crops. Fungicides form a zone of protection over the seed surface that reduces seed decay and seedling blight, resulting in healthy and vigorous seedlings (Marimuthu and Nakeeran, 2001). Fungicidal seed treatment is useful for the protection of seeds from pathogens during storage. Seed treatment become more economical and effective when it is carried out with respect to nature of pathogen and level of infection percentage (Neergaard, 1979). Carbendazim is effective against storage rot of groundnut caused by *A. flavus* (Rathod *et al.*, 2010). Since carbendazim is systemic in nature it inhibits the colony growth and sporulation of fungi and eradicates both the external and internally seed borne pathogens (Mohanna and Sharma, 1991; Habib *et al.*, 2007). Vasundhara and Gowda (1999) found that groundnut seeds treated with thiram had higher germination over control after 12 months of storage.

CONCLUSION

By understanding the need of healthy and pathogen free seeds as the basic requirement for disease free crop production, the stored commodities especially oil seeds should be taken with much care. Hence by knowing fungal pathogens that occur in storage and the related changes occur in seed quality as result of deterioration in biochemical constituent of the seeds, effective measures can be taken to protect the seeds from spoilage by means of chemical or organic seed treatment techniques and awareness should be created among farmers to store the seeds in a scientific way under optimum storage condition with suitable seed treatments.

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