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RESEARCH PAPER

Free radical quenching effect of botanical treatments on storability of greengram seeds

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Abstract : Storage of seeds enhances the longevity of the seed for sowing in the following season. Seed quality is the prerequisite condition that affects the germination and yield of any crop plants. The studies were carried out to determine the effects of three botanicals on greengram seeds storage. Laboratory studies were conducted with leaf powders of three plants to show the preservative effect for maintaining the quality of greengram seeds in storage. After processing and drying, seeds were preserved with different botanicals such as *Albizia amara*, *Azadirachta indica*, *Phyllanthus emblica* at a dose of 10g/kg and halogen mixture @3g/kg stored in gada cloth bags. At the end of six and twelve months, the germination percentage and the changes in the antioxidant enzymes such as superoxide dismutase, catalase, polyphenoloxidase and peroxidase were determined in the treated seeds. The results revealed that, the botanicals and halogen mixture treatments were significantly effective in controlling and maintaining higher seed quality throughout the storage period when compared to untreated control. Among the botanicals, the Albizia *amara* was found better by recording significantly higher germination percentage and enzymatic levels were retained when compared to untreated control seeds during storage. From this study, it is revealed that uses of different botanicals are less costly, easily available to the farmers and safe to handle.

Key Words : Greengram, Botanicals, Storage, Seed quality, Biochemical analysis

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INTRODUCTION

Pulses are the major constituent of diet in India. Traditionally farmers followed various methods of seed treatment and storage. Good seed treatment helps in getting better germination and crop protection in early stage of crop growth. However, there is a growing recognition that production alone does not solve the food problem, food grains must be preserved in edible, nutritionally adequate condition until they can be distributed and con- sumed by those who need it. During storage the pulses suffer a great deal of damage due to storage moulds and insects, and the most serious results of such damage appear to be both quantitative and qualitative losses. Greengram [*Vigna radiata* Wilczek (L.)] seeds after harvest show a significant fall in germinability by the time of next planting. According to Roberts (1972) the primary cause of loss of viability may

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be developed due to its extrinsic factors and intrinsic, biochemical and molecular basis of ageing. To overcome these problems, many seed treatments have been practiced mostly in two broad groups in orthodox seed such as hydration, dehydration treatments with mild solution of inorganic salts and another by dry dressings with organic materials to slow down the ageing process at the time of storage that showed very many effective results. The beneficial effect of dry dressing helps to decrease free-radical formation due to lipid peroxidation during ageing. Very few reports were available with respect to antioxidant enzymatic levels of greengram during storage. Hence, the present study was carried out to understand the antioxidant effect of different botanicals during storage of greengram seeds.

MATERIAL AND METHODS

Studies were carried out in the laboratory of Agricultural College and Research Institute, Echangkottai, Thanjavur. Greengram seeds (Co-8) used in the study was collected from pulses research centre of Tamil Nadu Agricultural University, Coimbatore. The test botanicals were leaf powder of *Neem (Azadirachta indica)*, amla (*Phyllanthus emblica)* and arappu (*Albizia amara)* and halogen mixture. The leaves of these plants were collected from different villages of Thanjavur. The collected leaves were dried under ambient room temperature (27°C to 34°C), grinded separately by a hand grinder and passed through a 60-mesh sieve to get fine powder.

The seeds were then divided into five parts. One part was considered as control (T_1) *i.e.* without any botanical treatment. The second part was treated with halogen mixture @3g/kg. Other three parts were treated with three different botanicals of *Neem (Azadirachta indica)*, amla (*Phyllanthus emblica)* and arappu (*Albizia amara)* at the dose of 10g/kg of greengram seeds with three replications. Before storage, seeds were analyzed for germination percentage and antioxidant enzymes. Changes in the level enzymic antioxidants such as superoxide dismutase (Kakkar *et al.*,1984), catalase (Luck,1974), peroxidase (Reddy *et al.*,1995), polyphenol oxidase (Esterbauer *et al.*,1977) were analyzed. The seeds were stored in gada cloth bags for twelve months and changes germination percentage and in antioxidant enzymes such as superoxide dismutase, catalase, peroxidase, poly phenol oxidase was observed during August 2016 to July 2017.

RESULTS AND DISCUSSION

During storage, initial seed germination percentage of 86 per cent that was declined to 72.0 per cent in control seeds at the end of twelve months of storage period. The maximum seed germination percentage (79.10%) was recorded in *Albizia amara* leaf powder treatment. (Table 1) The effects of different seed viability on superoxide dismutase activity of stored seeds was decrease in control and it was pronounced above 40 per cent in *Albizia amara* leaf treatment compared to that of 30 per cent of halogen treated seeds. Test experiments

Table 1: Different parameters of greengram seeds stored with botanicals and halogen mixture for twelve months

Treatments	Storage period (months)									
	Gernination %		Superoxide dismutase		Catalase		Polyphenol oxidase		Peroxidase	
	6	12	6	12	6	12	6	12	6	12
T_I	82.275	72.0	2.59	1.90	35.85	33.37	1.43	2.44	1.30	0.40
T_2	84.70	67.15	2.63	1.79	134.50	132.79	1.51	2.46	1.49	0.63
T_3	83.11	79.10	2.62	1.93	137.08	133.17	1.73	2.10	1.62	0.48
T_4	82.48	77.20	2.48	1.76	132.72	133.75	1.43	2.12	1.00	0.64
T_5	81.97	76.00	2.18	1.88	132.47	133.49	1.23	2.11	1.08	0.57
Mean	82.90	74.43	2.50	1.85	134.52	133.31	1.46	2.46	0.30	0.54
S.E.±	1.51	1.81	0.07	0.054	1.41	133.31	0.035	2.46	0.058	0.03
C.D. (P=0.05)	4.66	5.59	0.24	0.16	1.41	133.31	0.036	2.46	0.18	0.10

T_1= Control, T_2= Halogen mixture@3g/kg ,T_3= *Albizia amara* leaf powder @10g/kg, T_4 = *Azadirachta indica* leaf powder @10g/kg,
T_5 = *Phyllanthus emblica* leaf powder @10g/kg, powder @10g/kg
SOD = 1 Unit – Amount of enzyme that gives 50% inhibition of the extent of NBT reduction in 1 minute
CAT = 1 Unit – Amount of enzyme required to decrease the absorbance at 240nm by 0.05 units / minut
POD = 1 Unit – Amount of catechol oxidase,which transforms 1 unit of dihydrophenol to quinine/minute
PPO = 1 Unit – Change in absorbance / minute at 430nm

indicated that T_3 is the better botanical treatment for the enhancing the catalase and peroxidase activity decreases substantially with ageing in control seeds. After botanical treatments decreased enzymes levels were increased to above control seeds level.

Biochemical processes of lipid peroxidation are the major cause of seed deterioration during storage. Lipid oxidation is one of the major causes of quality deterioration in natural foods, oxidative deterioration is a large economic concern in the food industry because it affects many quality characteristics and nutritive value of foods (Chaiyasit *et al*., 2007). Lipid peroxidation and products resulting from these processes lead to DNA denaturisation, prevent translation and protein transcription and cause oxidation of the most reactive amino acids (Popovic, 2006). When these types of damages occur in seed, they may cause decrease in vigour and seed germination. Mechanism of oxidative damage is very complex and occurs as two different types of fatty acid changes. The first one is linked to the process of aging during the first week of storage and includes spontaneous oxidation of unsaturated fatty acids, with no changes occurring in saturated fatty acids. In the second type of damage the seed that has lost its ability to germinate showed oxidation of both saturated and unsaturated fatty acids. Activity of free radicals in seed may depend on water content, seed components (Laloi *et al.,* 2004).

The enzymes play an important role in the progress of seed deterioration and changes in their activity can be an indication of quality loss (Copeland and McDonald, 1995). There is relationship between seed deterioration and the enzymes involved in lipid peroxidation (Shen and Odén, 1999). All enzyme activity is positively correlated with germination of seed, as ageing progressed, germination decreased and enzyme also activity also decreased which showed significant deterioration in natural aged seeds.Many hypotheses have been proposed regarding causes of seed ageing such as lipid peroxidation mediated by free radicals, inactivation of enzymes or decrease in proteins, disintegration of cell membranes and genetic damage (McDonald, 1999 and Murthy *et al.,* 2003). Degradation and inactivation of enzymes due to changes in their macromolecular structures is one of the most important hypotheses proposed regarding causes of ageing in seeds (Bailly, 2004; Goel *et al.*, 2003; Lehner *et al*., 2008 and McDonald, 2004).

Most of these studies suggested that decreases occur in the activity of enzymes such as superoxide dismutase, catalase, peroxidase and glutathione reductase in aged seeds. Results of this study shows that storage en- hances the role of the lipolytic activity of grains, which results in a decrease of superoxide dismutase, catalase, polyphenoloxidase and peroxidase values. The investigation clearly indicates the effect of storage on the deactiva- tion of seed lipases in biodeterioration of lipids during storage. Pulse grains such as greengram are important sources of proteins and lipids and also natural isoflavinoid antioxidants such as daidzein, genistein and their glucosides daidzin and genistin, which can protect them against oxidation (Lapcik *et al*., 1999) for human consumption as well as animal feed.

The general decrease in enzyme activity in the seed lowers the respiratory capacity, which in turn lowers both the energy (ATP) and assimilates supply of the germinating seed. Therefore, several changes in the enzyme macromolecular structure may contribute to their lowered germination efficiency. As evidence mounts, the leading candidate causing seed deterioration increasingly appears to be free radical production. Free radical production, primarily initiated by oxygen, has been related to the peroxidation of lipids and other essential compounds found in cells. This causes a host of undesirable events to include decreased lipid content. Lipid peroxidation begins with the generation of a free radical either by autoxidation or enzymatically by oxidative enzymes such as lipoxygenase present in many seeds. Peroxidative changes in fatty acid composition of membrane lipids exert influence on viscosity, permeability and membrane cell function. Also, decrease in mitochondrial respiration during storage could be associated with peroxidative changes in lipid mitochondria that lead to loss of seed vigour (Ferguson *et al.*,1990).

The beneficial effect of dry dressing in maintaining vigour and viability of greengram seed due to anti-oxidant property of plant material as well as inhibition in aldehyde production during ageing process (De *et al.*,1999). Peroxidase enzyme activity was studied to ascertain the mechanism of action of treatment effect on free radical quenching. Result showed that peroxidase activity was significantly higher in seeds treated with *Albizia amara* over control (Table1). This increase in peroxidase activity indicates that the treatments induce the resistance against the production of free radicals and protect the seeds from faster ageing process.

All seeds undergo aging process during long-term storage which leads to deterioration in seed quality, especially in the humid tropical regions. However, the rate of seed deterioration can vary among various plant species (Merritt *et al.*, 2003). Aged seeds show decreased vigour and produce weak seedlings that are unable to survive once reintroduced into a habitat (Atici *et al.*, 2007). Some protective mechanisms involving free radical and peroxide scavenging enzymes, such as catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) have been evaluated within the mechanism of seed aging (Hsu *et al.*, 2003; Goel *et al.*, 2003 and Pukacka and Ratajczak, 2007). Scialabba *et al.* (2002) reported that peroxidase activity decreased in aged seeds as compared to fresh seeds in radish. Pallavi *et al.* (2003) studied that sharp decline in peroxidase emzyme during ageing.

The effects of different botanicals on seed viability might be due to activity of enzymes, superoxide dismutase, poly phenol oxidase and the antioxidant enzymes levels was pronounced above 40 per cent in *Albizia amara* leaf treatment compared to that of control seeds. Test experiment indicated that *Albizia amara* leaf treatment was superior botanical treatment for the enhancing the antioxidant enzymes activity compared to control seeds. Based on the present findings of twelve months of storage period in greengram seeds, it was concluded that there is a great scope of using botanicals that have equal effectiveness to that of chemicals during storage of green gram seeds.

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