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RESEARCH PAPER

Effect of mid storage gamma irradiation and fumigation on seed quality of soybean (*Glycine max)* and green gram (*Vigna radiata* L.)

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Abstract: An experiment was conducted at the Department of Seed and Science and Technology, College of Agriculture, UAS, Raichur to study the effect of repeated mid storage exposure to gamma irradiation and fumigation on seed longevity of soybean (*Glycine max*) and green gram (*Vigna radiata* L.). The experiment consisted of seven treatments with different dosage of gamma irradiation and fumigation including control. Among the different treatments, significantly higher seed germination (89.8 and 71.4 %), germination rate index (7706 and 4702), peak value of germination (22.7 and 12.1), shoot length (16.20 and 13.56 cm), root length (20.23 and 15.05 cm), seedling vigour index (3258 and 2051), seedling dry weight (0.409 and 0.323 g), dehydrogenase enzyme activity (2.632 and 0.319 OD value) and alpha amylase enzyme activity (27.40 and 21.78 mm) with lowest abnormal seedlings (1.1 and 11.4 %), dead seeds (5.5 and 11.9 %) and mean germination time (1.59 and 2.51) was recorded by T_7 (fumigation with aluminium phosphide @ 3 tablets / tonne of seed) compared to all other treatments and control at one and four times repeated mid storage exposure to gamma irradiation and fumigation. Whereas, seeds exposed to the gamma irradiation showed a significant reduction in seed germination, germination rate index, peak value of germination, shoot length, root length, seedling dry weight, seedling vigour index, dehydrogenase enzyme activity and alpha amylase enzyme activity with an increase in gamma irradiation dosage (200 to 1000Gy). While, the seed quality parameters such as, abnormal seedlings, dead seeds, mean germination time and electrical conductivity increased with increase in gamma irradiation dosage and also with repeated mid storage gamma irradiation and fumigation.

Key words: Fumigation, Gamma irradiation, Germination, Soybean

Introduction

In India, pulses are being cultivated over an area of 24.9 mha with an annual production of 16.3 mt and 656 kg per ha productivity (Anon., 2016). However, the per capita availability of pulses in India is 40 gram per person per day as against 140 gram per person per day as advocated by Indian Council of Medical Research (Anon., 2013). Thus, there is a challenge for agricultural scientists, extension workers, planners and farming community to enhance and sustain pulse productivity to meet national pulse requirement. Soybean [*Glycine max* (L.) Merill] is one of the most important protein rich oil seed crop used throughout the world as it possess largest component of edible oil (22 %) and protein (42-45 %). It is an important ingredient of more than 50 per cent of the world's high protein meal. It was introduced to India during 1880 and globally grown over an area of 91.40 mha with a production of 204.00 mt and with the productivity of 2233 kg per ha. In India, soybean is grown on an area of 11.60 mha with a production of 14.22 mt and productivity of 1263 kg per ha which is much below world's average productivity (Anon., 2016). Green gram (*Vigna radiata* L.) is another important pulse crop of south and south-east Asia and it is the third most widely cultivated pulse after bengal gram and pigeonpea. In India, it is grown in an area of 3.82 mha with a production of 1.59 mt and average productivity of 416 kg per ha which accounts for 65 per cent of world acreage and 54 per cent world's production (Anon., 2016).

Pulses are usually attacked in stores by different insect pests. Some of these are carried from the fields into stores (Appert, 1987). The larva feeds on the endosperm and germ of the grain and finally creates irregular holes with transparent thin outer covering (Appert, 1987). The extent of infestation by various insect pests is variable under different storage structure, conditions and practices. In each stored seeds, pest consumes approximately around 5-7 per cent resulting up to 30-50 per cent seed damage (Agrios, 1988 and Caswell, 1981).

Considering the extent of losses and damage to pulse produce, several research strategies are being adopted for management of these stored pests, The methods includes use of safe insecticides and fungicides as seed treatment chemicals and exposure of seed to fumigants during storage to protect the seeds from insects and diseases. Another method which is newly adopted to overcome the pests menace is exposing the seeds to gamma radiation.

Radiation technology such as gamma irradiation is an effective disinfestation treatment for stored food products including seeds. Ionizing radiation has been suggested as a useful alternative to methyl bromide, as there is no development of insect resistance, absence of residue in treated food and no significant loss of nutrients in the treated commodities (Lapidot *et al*., 1991). In comparison with other physical modification methods, irradiation treatment is rapid, convenient and more extensively used because of their easy availability and power of penetration (Delia *et al.,* 2013). However, the morphological, structural and functional change in crop plants depends on the strength, duration and exposure to gamma rays. Hence, gamma radiation is a technology with immense application in agriculture and medicine. In agriculture, it is being utilised in solving various agricultural problems such as reduction of post-harvest losses through suppressing sprouting and contamination, eradication or control of insect pests, reduction of food-borne diseases, extension of shelf life and breeding of high performance well adapted and disease resistant agricultural crop varieties. But its potential exploitation in seed storage godown and the frequency of exposure for stored pest management and its subsequent effect on seed quality parameters need to be studied in detail.

Another common practice adopted to control the seed infestation during storage in most of the ware houses is fumigation. For this purpose, phosphine is successfully used as a fumigant as it is found effective against most of common storage insects. Lindgren *et al.* (1958) reported that phosphine readily penetrate flour without any apparent effect on seed germination or baking quality and produced satisfactory mortalities of storage insect. With these background in view, an experiment was carried out to study the effect of repeated mid storage exposure to gamma irradiation and fumigation on storability of soybean [*Glycine max*] and green gram [*Vigna radiata L*].

Material and methods

An experiment was conducted in the Department of Seed Science and Technology, College of Agriculture, University of Agricultural Sciences, Raichur during the year 2015-16 to study the effect of gamma irradiation and seed treatment chemicals on seed longevity of soybean (*Glycine max*) and green gram (*Vigna radiata* L.). The experiment consisted of seven treatments. *Viz.,* T_1: Control, five dosages of gamma irradiation (T_2 to T_6) T_2: 200 Gy, T_3: 400 Gy, T_4: 600 Gy, T_5: 800 Gy, T_6: 1000 Gy and T_7: Fumigation with aluminium phosiphide at 3 tablets per tonne of seed. Completely randomized design was adopted with four replications.

For imposition of gamma irradiation treatments, 3 kg seed was used for each treatment. Since, the capacity of sample chamber was 1.5 kg; the seed was divided into two parts and exposed twice. Once after filling the seeds in the container the lid was closed. Then the required gamma irradiation dosage was set as per the treatments. Later, the sample chamber (vertical drawer) moves inside cobalt-60 radiation isotope which emits gamma irradiation. The duration taken for gamma irradiation will be automatically adjusted as per the dose set.

Similarly, for imposing fumigation treatment, the two crop seeds were fumigated using aluminium phosphide tablets as per the standard dosage (3 tablets of 3gram each / tonne seed) for seven days (Rajendran, 2016). The gamma irradiation and fumigation treatments were repeated four times once in three months interval.

The germination test was conducted in four replicates of 100 seeds each following between paper method in a walk-in seed germination chamber maintained at 25 ± 2 °C temperature with 90 per cent RH for 8 days for both crops (ISTA, 2013). Similarly, the abnormal seedlings (Ab S) and dead seeds (DS) were recorded according to ISTA rules (2013). While, the mean germination time (MGT) was computed by adopting the formula suggested by Azimi *et al*. (2013). Whereas, the germination rate index (GRI) was determined as per the procedure prescribed by Mudaris (1998), peak value of germination (PVG) by Gairola *et al*. (2011), shoot length (SL), root length (RL), seedling dry weight (SDW), seedling vigour index (SVI) by Abdul-Baki and Anderson (1973), insect egg and seed damage by Tamiru *et al.* (2016), per cent weight loss by Harris and Lindblad (1978), dehydrogenase enzyme acticity (DH) by Kittock and Law (1968), alpha amylase enzyme activity by Simpson and Naylor (1962) and electrical conductivity (EC) by Milosevic *et al*. (2010) were recorded. The experimental data thus obtained were statically analysed by the procedure prescribed by Sundararaj *et al*. (1972).

Results and discussion

Significantly higher seed germination (89.8 and 71.4 %), shoot length (16.20 and 13.56 cm) and root length (20.23 and 15.05 cm) was recorded by fumigation with aluminium phosphide with 3 tablets per tonne of seed (T_7) as compared to all other treatments and control (88.8 and 71.1 %), (16.08 and 13.34 cm) and (19.39 and 14.01 cm), respectively (Table 1). This might be due to the effect of phosphine which significantly protected the seeds from insect infestation (Gupta *et al.,* 2000). The results are in line with the earlier findings of Gupta and Kashyap (1995) who reported higher seed germination, shoot and root length of green gram seeds due to onetime fumigation compared to three times. However, the seed germination (%), shoot and root length decreased significantly with an increase in both the number of repeated gamma irradiation and fumigation and also with the dosage of gamma irradiation (T_2- T_6). Here, T_6 (1000 Gy) recorded the least seed germination (74.6 and 34.6 %), root length (14.88 and 0.74 cm) and shoot length (10.95 and 1.03) compared to control (88.8 and 71.1 %), (19.39 & 14.01 cm) and 16.08 and 13.34 cm), respectively for seed germination, root and shoot length at one and four times exposure to gamma irradiation and fumigation. This might be due to repeated fumigation might be due to higher residual effect of fumigants as well as phosphorelation activity leading to blocking of glycolysis cycle (Shadi *et al.,* 1978). The results are in line with the findings of Ramazan and Chahal (1989) who reported reduction in seed quality parameters of wheat fumigated with aluminium phopsphide (@ 1.2 g per tonne of seed) for five times. The per cent reduction in seed germination (Fig.1) due to four time exposures to gamma irradiation at higher dose (1000 Gy- T_6) was 40.0, While, it was only 18.3 per cent due to the fumigation (T_7) over one time exposure. This might be due to repeated irradiation of seeds with high dose of gamma rays disturb the synthesis of proteins (Xiuzher, 1994), hormone imbalance (Rabie *et al.,* 1996), leaf gas-exchange (Stoeva and Bineva, 2001), water exchange and enzyme activity (Stoeva

Table 1. Influence of mid storage gamma irradiation and fumigation on seed quality parameters of soybean and green gram due to one and four time exposure

Treatments	Germn (%)		Ab S (%)		DS (%)		SL (cm)		RL (cm)		SDW (g)		SVI	
Exposure time	1	4	1	4	1	4	1	4	1	4	1	4	1	4
T_1	88.8	71.1	1.5	12.0	5.8	12.3	16.08	13.34	19.39	14.01	0.398	0.291	3135	1952
T_2	85.5	53.5	3.3	19.6	6.9	24.1	15.76	7.05	19.06	7.06	0.395	0.221	2981	756
T_3	83.0	46.9	4.9	22.3	7.8	29.1	13.99	5.88	17.90	4.63	0.386	0.174	2651	488
T_4	80.9	42.9	7.9	25.6	7.9	31.3	12.95	4.14	16.86	3.81	0.371	0.146	2417	339
T_5	76.4	38.4	10.1	27.9	8.3	32.6	11.79	2.29	15.71	2.00	0.337	0.119	2106	165
T_6	74.6	34.6	14.0	31.9	9.3	33.5	10.95	1.03	14.88	0.74	0.307	0.071	1931	62
T_7	89.8	71.4	1.1	11.4	5.5	11.9	16.20	13.56	20.23	15.05	0.409	0.323	3258	2051
Mean	82.7	51.3	6.2	21.5	7.4	25.0	13.96	6.80	17.8	6.75	0.371	0.192	2639	830
S.Em.±	1.2	0.3	0.4	0.5	0.4	0.6	0.26	0.18	0.14	0.17	0.002	0.003	30	15
C.D. at 1%	3.6	1.0	1.1	1.5	1.1	1.8	0.74	0.51	0.39	0.49	0.005	0.008	86	43

T_1 - Control T_3 - 400 Gy T_5 _ 800 Gy T_7 - Fumigation (aluminium phosphide @ 3 tablets / tonne of seed)
T_2 - 200 Gy T_4 - 600 Gy T_6 - 1000 Gy

Ab S-Abnormal seedlings DS-Dead seedlings SL-Shoot length RL-Root length
SDW-Seedling dry weight SVI-Seedling vigour index

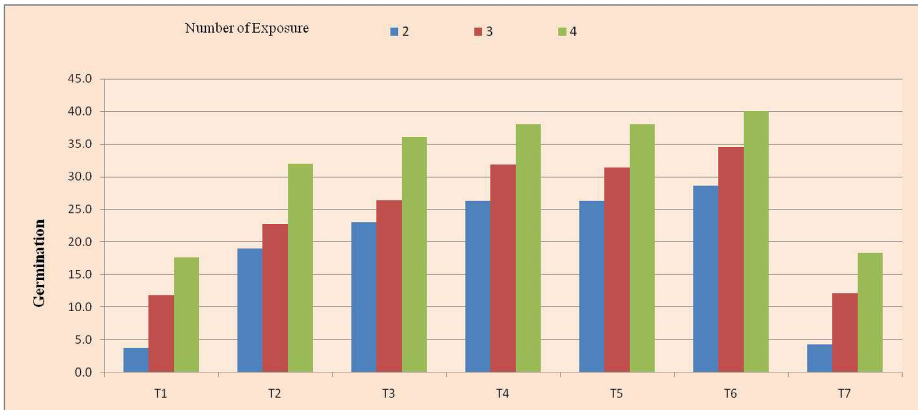

Fig. 1. Per cent decrease in seed germination due to number of mid storage exposure to gamma irradiation and fumigation over one time exposure

T_1 - Control T_3 - 400 Gy T_5 _ 800 Gy T_7 - Fumigation (aluminium phosphide with 3 tablets / tonne of seed)
T_2 - 200 Gy T_4 - 600 Gy T_6 - 1000 Gy

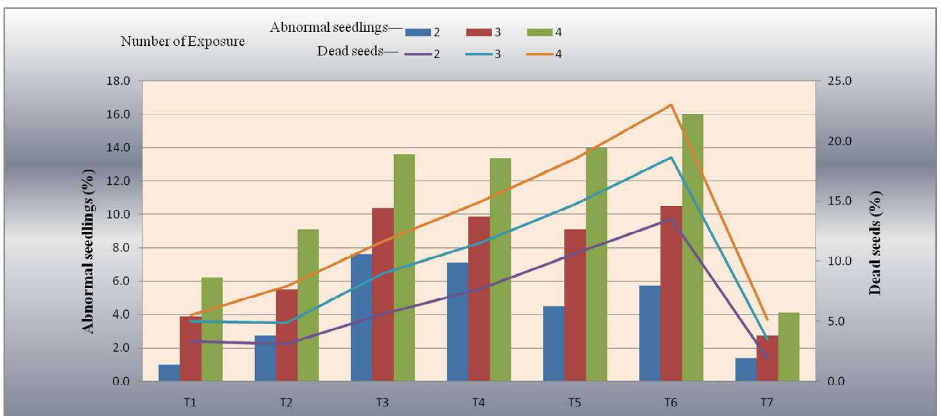

Fig. 2. Per cent increase in abnormal seedlings (%) and dead seeds (%) due to number of mid storage exposure to gamma irradiation and fumigation over one time exposure

T_1 - Control T_3 - 400 Gy T_5 _ 800 Gy T_7 - Fumigation (aluminium phosphide with 3 tablets / tonne of seed)
T_2 - 200 Gy T_4 - 600 Gy T_6 - 1000 Gy

et al., 2001). This decrease in seed quality parameters with several time exposure to gamma irradiation at a high dosage might also be due to increased plant sensitivity to gamma radiation which might have caused reduction in the amount of endogenous synthesis of growth regulators, especially cytokinins and thereby reduced the seed germination with a corresponding decline in growth of the plants (Kiong *et al*., 2008) in terms of root and shoot length.

Significantly lower abnormal (1.1 and 11.4 %) and dead seedlings (5.5 and 11.9 %) were recorded by fumigation with aluminium phosphide with 3 tablets per tonne of seed (T_7) compared to all other treatments and control (1.5 and 12.0 %) and (5.8 and 12.3 %), respectively at one and four times exposure to gamma irradiation and fumigation (Table 1). Further, exposing the seeds to repeated gamma irradiation (T_2 -T_6), the per cent increase in abnormal and dead seeds seedlings increased with an increase in gamma irradiation dosage over one time exposure. The per cent increase in abnormal and dead seeds seedlings due to four time exposures to gamma irradiation (Fig. 2) at higher dose (1000Gy - T_6) was 17.9 and 24.3 per cent, while it was only 10.3 and 6.4 per cent, respectively due to fumigation (T_7)

over one time exposure. This increase in abnormal and dead seeds (%) with repeated gamma irradiation might be due to gamma irradiation induced oxidative stress with over production of reactive oxygen species (ROS) such as super oxide radical, hydroxyl radical and hydrogen peroxide which reacts rapidly with almost all structural and functional organic molecules including proteins, lipids, nucleic acids causing disturbance in cellular metabolism (Salter and Hewitt, 1992). The results are in line with the findings of Muhammad Amjad and Muhammad Akbar (2002) who noticed higher number of abnormal seedlings with an increase in irradiation dose.

Significantly higher seedling dry weight (0.409 and 0.323 g) was recorded by treatment fumigation with aluminium phosphide with 3 tablets per tonne of seed (T_7) compared to all other treatments and control (0.398 and 0.291 g) at one and four times exposure to gamma irradiation and fumigation. Similarly, the seedling vigour index was also significantly higher in T_7 (3258 and 2051) compared to all other treatments and control (3135 and 1952) at one and four times exposure to gamma irradiation and fumigation, respectively. This might be due longer shoot and root length recorded in our study by the treatment T_7 which had a direct correlation with the seedling dry weight and seedling vigour index. Similarly, Gupta *et al*. (2000) reported significantly higher vigour index in the fumigated seeds over the untreated control. However, the seedling vigour index and seedling dry weight decreased drastically with an increase in the number of exposure to gamma irradiation and fumigation. These results are in contradictory to the earlier report of Gupta *et al*. (2000) who did not observe any adverse effect due to repeated fumigation (aluminium phosphide @ 3,6 and 9 g/ cubic meter) even at three fold higher dose, on the vigour of wheat seeds indicating non sensitivity of the crop to either repeated gamma irradiation or fumigation. Similarly, repeated fumigation (@ 3 g/m^3) of wheat seed with phosphine up to four times carried out at monthly intervals for a period of seven days had no adverse effect on vigour index (Singh *et al.,* 1999).

Exposing the seeds to repeated gamma irradiation (T_2 -T_6) showed a drastic decrease in seedling dry weight and vigour index with an increase in gamma irradiation dosage (Table 1). The decrease in seedling vigour index due to four time exposures to gamma irradiation (Fig. 3) at higher dose (T_6 -1000 Gy) was 1869, while it was only 1207 due to onetime exposure to fumigation (T_7). This reduction in seedling dry weight and vigour index due to several time exposures to gamma irradiation might be due to shorter root and shoot length registered in our study which had a direct correlation with dry weight and vigour index. Several earlier studies on this aspect also indicated reduction in plant stature and reduced moisture contents in shoot due to radiation stress (Majeed *et al*., 2010), inhibition of mitosis and enzyme activities as it is more likely that the reserve food was utilized less efficiently at higher dose and repeated irradiation which might have resulted in reduction of seedling fresh and dry weight (Alduous and Stewert, 1952).

Among the gamma irradiation and fumigation treatments, significantly lower mean germination time (1.59 and 2.51) was recorded by Fumigation with aluminium phosphide with 3 tablets per tonne of seed (T_7) compared to all other treatments and control (1.68 and 2.60) at one and four time exposure (Table 2). Lesser mean germination time represents early germination. Further, several time exposure of seeds to gamma irradiation (T_2-T_6) showed an increase in the mean germination time with an increase in gamma irradiation dosage wherein, T_6 (1000Gy) recording the highest mean germination time (1.88 and 3.54) respectively at one and four times exposure compared to control 1.68 and 2.60 respectively. This increase in mean germination time with repeated exposure to gamma irradiation might be due to inhibitory effect on seed quality (Majeed *et al*., 2010).

Significantly higher peak value of germination (22.7 and 12.1) and germination rate index (7706 and 4702) was recorded by fumigation with aluminium phosphide with 3 tablets per tonne of seed (T_7) compared to all other treatments and control (22.0 and 11.7) and (7492 and 4015), respectively at one and four times exposure to gamma irradiation and fumigation, respectively (Table 2). This might be due to the fact that seeds with reduced activity of enzymes and repair system make the seed to germinate slowly and ultimately reduce vigour of seeds (Manjesh, 2016). Further, it was also noticed that, the peak value of germination and germination rate index decreased drastically with an increase in the number of exposure to gamma irradiation and fumigation. The results are in line with the findings of Polchaninova (1969) who reported slow germination rate of wheat and barley seeds where fumigated at 30 and 40 g per m^3 for two time for 72 hours exposure.

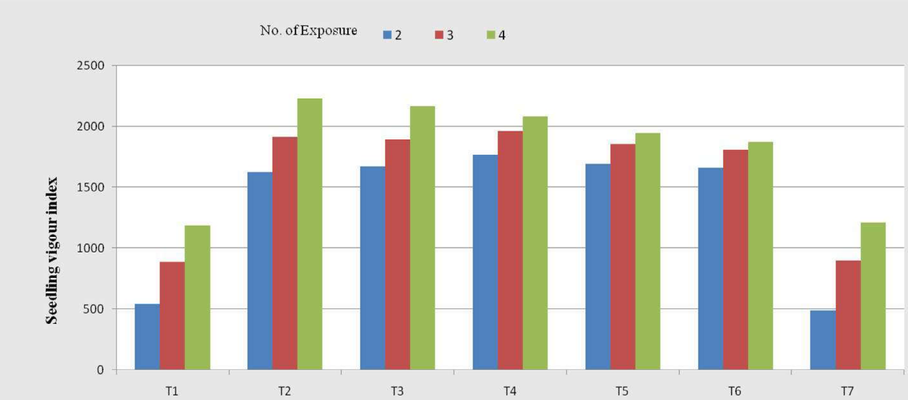

Fig. 3. Per cent decrease in seedling vigour index due to number of mid storage exposure to gamma irradiation and fumigation over one time exposure

T_1 - Control T_2 - 200 Gy T_3 - 400 Gy T_4 - 600 Gy T_5 - 800 Gy T_6 - 1000 Gy T_7 - Fumigation (aluminium phosphide with 3 tablets / tonne of seed)

Table 2. Influence of mid storage gamma irradiation and fumigation on seed vigour parameters and enzyme activities of soybean and green gram due to one and four time exposure

Treatments Exposure time	MGT		GRI		PVG		DH		α- amylse enzyme activity (mm)		EC (dSm^{-1})	
	1	4	1	4	1	4	1	4	1	4	1	4
T_1	1.68	2.60	7492	4015	22.0	11.7	2.563	0.258	26.60	18.30	0.480	0.767
T_2	1.63	2.70	7467	4308	22.0	10.3	2.499	0.143	26.50	17.10	0.497	0.907
T_3	1.75	2.82	7162	4178	21.2	9.0	2.417	0.126	26.30	15.98	0.513	0.996
T_4	1.76	3.11	6997	3930	20.2	7.7	2.236	0.112	25.08	14.28	0.523	1.020
T_5	1.78	3.21	6614	3816	18.3	5.9	2.174	0.102	24.80	11.95	0.531	1.073
T_6	1.88	3.54	6521	3603	16.5	5.3	2.054	0.089	23.65	9.53	0.533	1.141
T_7	1.59	2.51	7706	4702	22.7	12.1	2.632	0.319	27.40	21.78	0.465	0.678
Mean	1.72	20.5	7137	4078	20.5	8.9	2.796	0.164	25.76	15.56	0.506	0.940
S.Em±	0.04	0.02	99	70	0.7	0.7	0.003	0.010	0.09	0.24	0.004	0.013
C.D. at 1%	0.11	0.05	302	216	2.0	2.1	0.008	0.032	0.29	0.75	0.011	0.036

Legend

T_1 - Control T_3 - 400 Gy T_5 _ 800 Gy T_7 - Fumigation (aluminium phosphide @ 3 tablets / tonne of seed)

T_2 - 200 Gy T_4 - 600 Gy T_6 - 1000 Gy

MGT-Mean germination time   GRI-Growth rate index   PVG-Peak value of germination   DH-Dehydrogenase enzyme activity

EC- Electricity conductivity

Exposing the seeds further to repeated gamma irradiation (T_2-T_6) showed a significant decrease in peak value of germination and germination rate index with an increase in gamma irradiation dosage wherein, T_6 (1000 Gy) recorded the lower peak value of germination (16.5 and 5.3) and (6521 and 3603) respectively at one and four times exposure to gamma irradiation and fumigation compared to control (22.0 and 11.7) and (7492 and 4015), respectively. Similar reports of low peak value of germination and germination rate index with an increase in gamma dose were earlier reported by Din *et al*. 2003 in wheat.

The results of the present study also revealed higher dehydrogenase activity (2.632 & 0.319 OD value) and alpha amylase enzyme activity (27.40 and 21.78 mm) by fumigation with aluminium phosphide with 3 tablets per tonne of seed (T_7) compared to all other treatments and control (2.563 and 0.258 OD value) (26.60 and 18.30 mm), respectively at one and four times exposure to gamma irradiation and fumigation. The results are in line with the findings of Kamble *et al*. (2013) who reported that groundnut seeds fumigated with ethylene dibromide at 30 and 90 days after harvest retained higher value for dehydrogenase enzyme activity even up to six months of storage.

Exposing the seeds to gamma irradiation several times showed a drastic reduction in dehydrogenase and alpha amylase enzyme activity with an increase in gamma irradiation dosage (Table 2). This decrease in dehydrogenase and alpha amylase enzyme activity with repeated gamma irradiation may be due to decline in the activity of amylases in seed which reduces the rate of starch hydrolysis and thus would be expected to slow down the germination (Koksel *et al.,* 1998). The results are in agreement with the findings of Ivan *et al*. (2012) who reported that irradiation of malt caused a significant reduction in alpha and beta amylase activity. Significantly lower electrical conductivity (0.465 and 0.678 dSm^{-1}) was recorded by fumigation with aluminium phosphide with 3 tablets per tonne of seed (T_7) compared to all other treatments and control (0.480 and 0.767 dSm^{-1}) at one and four times exposure to gamma irradiation and fumigation, respectively. However, the electrical conductivity of seed leachates increased due to repeated exposure to gamma irradiation and fumigation. This might be due to the cumulative injury to the seeds and some residue of fumigants retained after first fumigation which again gets accumulated in each subsequent fumigations that led to drastic reduction in seed quality parameters like germination, vigour and viability of seeds due to repeated fumigation (Kamble *et al*., 2013).

Exposing the seeds several times to gamma irradiation (T_2-T_6) showed an increase in electrical conductivity with an increasing gamma irradiation dosage wherein, T_6 (1000 Gy) recorded the higher electrical conductivity (0.533 & 1.141 dSm^{-1}), respectively at one and four times exposure to gamma irradiation and fumigation compared to control (0.480 and 0.767 dSm^{-1}), respectively. This increase in electrical conductivity with repeated exposure to gamma irradiation is attributed to increased membrane permeability which enhanced leakage of leachates (Krishnaswamy and Seshu, 1989).

Conclusion

Fumigating soybean and green gram seeds before storage with aluminium phosphide at 3 tablets of 3 g each per tonne of seed for seven days under ambient conditions maintained better longevity of the two crops. Further, exposing soybean and green gram seeds to repeated mid storage gamma irradiation had significantly reduced the seed quality parameters. Hence, it was concluded that repeated mid storage gamma irradiation technology is not suitable for the management of stored grain pests in soybean and green gram.

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