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Abstract- Effect of four storage temperatures (T_1-ambient room temperature (35±5°C), T_2-Air conditioner (25±2°C), T_3- Fridge (5±1°C) and T_4-Freezer (-4±1°C) and four moisture levels M_1 (38%), M_2 (21%), M_3 (12%), M_4 (5.5%) were studied on seed longevity of seeds under controlled conditions. After setting of desired moisture content seeds were then subjected to storage treatments in 200μ thick polythene bag and kept in airtight plastic container at different temperature levels and tested upto 270 days respectively. Seeds were sown in between paper (BP) for germination study in laboratory at 30±1°C and relative humidity (90±3%).

Keywords: germination percentage, mean germination time (MGT), germination value (GV), azadirachta indica, viability.

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Storage Response of Neem (*Azadirachta Indica* A. Juss.) Seed under Different Moisture and Temperature Regime

Devendra Kumar

Abstract- Effect of four storage temperatures (T_1-ambient room temperature (35±5°C), T_2-Air conditioner (25±2°C), T_3- Fridge (5±1°C) and T_4-Freezer (-4±1°C) and four moisture levels M_1 (38%), M_2 (21%), M_3 (12%), M_4 (5.5%) were studied on seed longevity of seeds under controlled conditions. After setting of desired moisture content seeds were then subjected to storage treatments in 200μ thick polythene bag and kept in airtight plastic container at different temperature levels and tested upto 270 days respectively. Seeds were sown in between paper (BP) for germination study in laboratory at 30±1°C and relative humidity (90±3%).

Freshly collected seeds after drying at target levels showed germination from 93 to 96.5 %. Seeds having M_1, M_2 and M_3 moisture content completely lost germination well before 90 days at T_1 temperature. Seeds of M_4 moisture level showed 55.37% decline in germination upto 90 days. Seeds containing moisture M_1, M_2, M_3 and M_4 at T_2 and T_3 level showed 100%, 56.5%, 14.74%, 5.21% and 100%, 18.14%, 11.58%, 6.25% respectively decline in germination upto 90 days of storage. Seeds (M_4) stored at T_4 level showed 20.83% reduction in seed germination. After 150 days of seed storage, the rate of decline was observed higher in T_1 at all level of moistures, T_2 at M_1 (100%), M_2 (64.29%), M_3 (8.64%), M_4 (5.69%) and in T_3 at M_1 (100%), M_2 (8.61%), M_3 (16.67%), M_4 (8.89%). Rate of decline in germination percentage was lower at T_4 temperature and M_4 (3.46%) moisture level between these two periods. Seeds stored at low temperature and moisture content (T_3M_4) exhibited greater mean viability period (P_{50}, 326 days) as compared to the seeds stored at higher temperature and moisture. However, P_{50} was not increased when seeds were stored at lower temperature and moisture content (T_4M_4, 184 days). Thus present finding reveals that seeds of neem may be stored at 5±1°C (T_3) temperature with moisture 5.5% (M_4) for 326 days (P_{50}) and data also indicate the orthodox behaviour of neem seed of this region.

Keywords: germination percentage, mean germination time (MGT), germination value (GV), azadirachta indica, viability.

I. Introduction

The seed's longevity can be predicted successfully from moisture content and storage temperature specifications for most of the species. Those seeds can be dried to moisture content of 5% are generally regarded as desiccation tolerant and known as orthodox seeds. They can usually be stored for periods of many years. Longevity of orthodox seeds is increased in a specific and predictable way, over a wide range of environmental conditions by decreasing storage temperature and moisture (Roberts 1973). The seeds that require relatively high moisture content to maintain maximum viability are termed as recalcitrant. Recalcitrant seeds are sensitive to desiccation and low temperature (Chin and Roberts 1980; Hanson 1984; Pammenter and Berjak 1999). Storage life of such seeds ranges from few days to few months. These seeds are shed at high moisture contents and are intolerant of dehydration and of chilling. A further group of seeds that demonstrate behaviour intermediate between the afore-mentioned categories of desiccation tolerance and storage behaviour has been described (Ellis et al. 1990, 1991).

Neem (*Azadirachta indica*, A. Juss.) belongs to Meliaceae family, is an important multipuopose tree species for arid and semi arid regions. It is generally propagated by seeds; however, the seed have a short storage life and loose viability rapidly which is a major problem for tree planting programmes. Neem, seeds of Asian origin have been shown more or less recalcitrant (Gamene et al. 1994) while those of African provenances as orthodox (Bellefontaine and Audinet 1993). However, behaviour of neem seed has been described as short-lived (Ezumah 1986; Maithani et al. 1989). Some researchers have categorized it as having intermediate storage longevity (Sacandé et al. 1996, 1998; Hong and Ellis 1998). It has a short period of seed viability (Gamene et al. 1996) and is mainly determined by the level of desiccation tolerance of the seeds (Roberts and Ellis 1989; Kraak 1993). Berjak and Dumet (1996) also reported neem seeds having intermediate to recalcitrant storage behaviour and could be stored as intact seeds or as isolated embryonic axes while Sacandé et al. (2000) reported that storage behaviour of neem seeds has features that characterize it as orthodox. The longevity of neem seeds appears very uncertain. Thus there are many conflicting reports as to the status of seed as recalcitrant, intermediate or orthodox probably owing to the limited desiccation conditions to determine the desiccation tolerance of seed and their subsequent longevity.

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In the present study, an attempt has been made to determine the optimum temperature and moisture content during the storage of neem seed which may provide a lead for researchers to resolve of seed storage behaviour protocol of neem seed.

II. Material and Methods

Twenty middle aged neem trees were selected for seed collection around the Jodhpur city (latitude 24° 40'N and longitude 71°15'E), Rajasthan, India. Out of twenty trees, ten tree randomly taken for study. Seeds from all ten trees were collected during July, 2003 and mixed lots were prepared for experiments. The temperature was mean min 20-22°C and mean max 33-34°C and humidity between 22-59% during the entire experiment period. The green-yellow colour (Kumar and Mishra 2007) fruits were collected directly from tree branches (Kumar and Mishra 2007) of selected seed stand. Fruits were kept in gunny bags at ambient room temperature (35±5°C) for one day to soften the pulp. Seeds were extracted (Kumar et al. 2007, 2009), and Seed were dried in a dryer at 45±1°C to take various moisture level of seeds for experiment. Four initial moisture levels M_1 (38%), M_2 (21%), M_3 (12%), M_4 (5.5%) and four storage temperatures (T_1-ambient room temperature (35±5°C), T_2-air conditioner (25±2°C), T_3-referigerator (5±1°C) and T_4-fridger (-4±1°C) were taken for study (Table 1) on seed longevity under controlled conditions. Moisture content was determined as per ISTA (1993) at initial level only.

Seeds were then subjected to storage treatments in sealed 200μ thick polythene bags and kept in airtight plastic container at different temperature level and tested upto 270 days respectively. Germination test were conducted in laboratory. Seeds were sown in between paper (BP) for germination study (Kumar et al. 2007) using four replications of 100 seeds each incubated at 30±1°C and 90±5% R.H. (Relative humidity). Germination data were recorded daily up to 21 days and seed was considered germinated when radicle became 1 cm in length. Speed of germination in terms of Mean Germination Time (MGT) in days was calculated as per Rawat and Thapliyal (2003) and germination value was calculated as per the method described by Djavanshir and Pourbeik (1976). The obtained data of germination percentage, germination value (GV) and time taken to complete germination in terms of mean germination time (MGT) were analyzed through ANOVA using SPSS computer package. The P values < 0.05 were taken as significant.

Probit Analysis for Prediction of Mean viability period (P_{50})

Survival curves were derived by using probit analysis (Roberts 1973). Survival curves were drawn between expected germination and storage period (days). For all the experiment conducted in laboratory the pattern of decline in germination in each condition was subjected to probit analysis for estimating the mean viability period i.e. P_{50} (half viability period or time taken for death of 50% seeds). The germination data were transformed to probit values (Finney 1952) and then subjected to regression with storage period in days. The slope of the regression line from this probit analysis indicated the rate of deterioration (Vertucci et al. 1994). Because 0 cannot be converted into probit value, where such values were obtained experimentally. They were given a constant value of 0.5%. The P_{50} was calculated for each storage condition separately from the regression equation developed as above.

III. Results

Seeds containing different moisture levels were stored at various levels of temperature as described in materials and methods and tested up to 270 days. Freshly collected seeds after drying at target levels showed germination from 93 to 96.5 % (Table 1). Seeds having M_1, M_2 and M_3 moisture content completely lost germination well before 90 days at T_1 temperature. Seeds of M_4 moisture level seeds showed 55.37% decline in germination up to 90 days. Seeds containing moisture M_1, M_2, M_3 and M_4 at T_2 and T_3 level showed 100%, 56.5%, 14.74%, 5.21% and 100%, 18.14%, 11.58%, 6.25% respectively decline in germination up to 90 days of storage. Seeds (M_4) stored at T_4 level showed 20.83% reduction in seed germination. Seeds stored for longer period (150 days), the rate of decline was observed higher in T_1 at all level of moistures, T_2 at M_1 (100%), M_2 (64.29%), M_3 (8.64%), M_4 (5.69%) and in T_3 at M_1 (100%), M_2 (8.61%), M_3 (16.67%), M_4 (8.89%). Rate of decline in germination percentage was lower at T_4 temperature and M_4 (3.46%) between these two periods.

Germination percentage obtained after different periods of storage under each condition were transformed to probit values. These values were subjected probit analysis against days of storage for each condition separately Figure 1A-1D. With the help of regression equations, expected probit germination was calculated. The expected probit germination was transformed into expected germination and resulting survival curves were presented in Figure 2. It is evident from the survival curves that the more flat gradient, the greater variation between seeds and viability periods. At higher temperatures the gradient is steeper and exhibited rapid deterioration of seeds. The pattern of decline in germination at different level of temperature moisture is shown in Figure 2. Seeds stored at lower temperature and moisture content exhibited greater P_{50} of T_3M_4 (326 days) (Table 6) as compared to the seeds stored at higher temperature and moisture content. However, P_{50} was not increased when seeds were stored at lower temperature and moisture content (T_4M_4, 184 days). Other combinations of temperature and moisture showed P_{50} of 201 days (T_3M_2), 169 days

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(T_3M_3), 146 days (T_2M_3), 85 days (T_1M_4), 80 days (T_1M_2) and T_1M_1, T_1M_2, T_1M_3, T_2M_1 and T_3M_1 showed lowest P_{50} of 31 days for each (Table 6).

Effect of Temperature on Longevity of Seed

Seeds showed significant variation in longevity within the same temperature at varied level of moisture content. It is evident from Table 2 that seeds stored at high temperature lost viability very soon. Seeds stored at lower temperature maintained viability well. T_3 exhibited highest mean germination percentage (53.06) followed by T_2 and T_1 with 44.44 and 17.63 respectively as storage temperature reduced, the mean germination percentage increased. The pattern of decline in MGT is shown in Figure 3. Mean MGT was lowest at T_3 (11.93 days) followed by T_2 and T_1 with 12.72 and 14.59 days respectively (Table 3). The maximum mean GV exhibited by T_3 (27.28) followed by T_2 (22.20) and T_1 (10.39). The moisture decreased and GV was increased (Table 4).

Effect of Moisture on Longevity of Seed

Seeds showed significant variation in germination with moisture content reduction. Low moisture content increased and maintained viability for longer period as compared to those treatments having higher moisture content. M_4 showed higher mean germination percentage (61.47) followed by M_3, M_2 and M_1 with 41.89, 34.64 and 15.50 respectively (Table 2). The mean MGT decreased with decrease in moisture content. The moisture M_4 exhibited lowest mean MGT followed by M_3, M_2 and M_1 with 12.73, 13.30 and 14.78 respectively (Table 3). The maximum mean GV was recorded in M_4 (31.43) followed by M_3 and M_2 with 21.11, 17.68 respectively. The M_1 exhibited poorer mean GV (Table 4).

Interactive Effect of Moisture and Temperature on Longevity of Seed

The interaction of temperature and seed moisture content in maintaining viability over a period of time is quite evident in Table 2 to 4. Seed stored at higher moisture content lost germination rapidly at higher temperature as compared to seed stored at lower moisture contents. Similarly seed dried to lower moisture contents maintained viability much better even at higher temperature. Seed stored at higher temperature (T_1) with high moisture content (M_1) showed significant lower mean germination percentage (15.50) followed by M_2 and M_3 with 16.08 and 15.83 respectively. M_4 exhibited better mean germination percentage at this temperature (23.08). Significantly maximum mean germination percentage exhibited by M_4T_3 (82.50) and M_4T_2 (78.83) combinations followed by T_3M_2, T_2M_3 and T_3M_3 combinations with 62.25, 57.83 and 52.00 percent respectively (Table 2).

T_3 temperature exhibited lower mean MGT (11.93 days) followed by T_2 and T_3 with 12.72 days and 14.59 days respectively. The lower MGT was exhibited by T_3M_4 (9.91 days) followed by T_2M_4 (10.50 days), T_3M_2, T_3M_2 and T_2M_3, they exhibited significantly lower MGT with 11.21, 11.82, 11.63 days respectively and remained insignificant with each other (Table 3).. The poorer MGT exhibited by T_1M_4 (14.12 days), T_2M_2 (13.98 days) followed by T_1M_1, T_1M_2, T_1M_3, T_2M_1, T_3M_1 (Table 2) and T_1M_1, T_1M_2, T_1M_3, T_2M_1, T_3M_1 remained insignificant with each other. T_3M_4 exhibited maximum GV (43.15) followed by T_2M_4 (39.18) and remained insignificant. T_3M_2 (30.05) was followed by T_2M_3 and T_3M_3 with 27.40 and 26.32 respectively. Rest combinations of various temperatures and moistures showed poorer GV (Table 4).

When seeds were stored at very low temperature (T_4, -4°C) at low moisture (M_4), mean germination percentage (58.07) and mean germination value (28.54) were reduced. MGT was also higher (11.17 days) (Table 5) as compared to T_3M_4. However, these results were better than seeds stored at ambient room temperature (T_1M_4). Taken as a whole temperature T_3 (5°C) and moisture M_4 (5.5%) were observed the best combination of temperature and moisture.

IV. Discussion

Reduction in moisture content of seed and lowering the temperature and relative humidity in which seed is stored extends the storage life of most seeds. The moisture content of seed is directly affected by the relative humidity of the atmosphere around it. As the relative humidity increases the seed moisture percentage are also increases. Seeds are shed from the parent plant at low moisture content, having undergone maturation drying and are capable of tolerating dehydration down to 5 to 6 percent are called orthodox seed. When dry, the viability of these seeds can be prolonged by keeping them at the lowest temperatures and moisture (Roberts 1972). In contrast, recalcitrant seeds do not undergone maturation drying and are shed at relatively high moisture contents. Seeds of most tropical and subtropical trees and shrubs are recalcitrant (King and Roberts 1979; Hanson 1984) seeds are not storable under conditions suitable for orthodox seeds (Roberts et al. 1984). These seeds can only be stored in wet medium to avoid dehydration injury and at relatively warm conditions as these are mostly sensitive to chilling injury (Lyons et al. 1979; Chin and Roberts 1980; Graham and Patterson 1982). There is no successful method for long-term storage of recalcitrant seeds. Whether neem is a genuine recalcitrant or short live orthodox species, however, is still nebulous (Willan 1985). Occurrence of high moisture content in fresh seeds (Maithani et al. 1989) and manifestation of chilling damage by the seeds (Ezumah 1986; Maithani et al. 1989). But on the basis of low moisture content of seeds from a Haiti plantation it has been argued that neem is not a recalcitrant species (Chaney and Knudson 1988).

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The viability of neem seed declines rapidly with storage period increased. Most works report poor or no germination after 2-6 months of storage (Tiwari 1992). Survival of seeds with high moisture content (30-40%) is in the range of a few to 12 weeks (Chaisurisri et al. 1986). Seeds dried upto 10-12% moisture contents can be stored only a few months (Ponnuswamy et al. 1990), which led to the idea that neem seed is recalcitrant. However the storage of seeds with low moisture content of 5-8% and at 4°C resulted in 40% viability after a number of years (Roederer and Bellefontaine 1989, Dickie and Smith 1992, Bellefontaine and Audinet 1993). Nevertheless, neem seed does not behave like truly orthodox seed that can tolerate drying to very low moisture content (1-2%) and storage at low temperature.

In case of this study desiccation of seed increased the longevity brought down at moisture content upto M_4 (5.5%) in storage and these seeds were found to tolerate desiccation without significant loss in viability. Present studies are in accordance with the finding of Sacande et al. (1998), Sacande and Hoekshtra (1999) and Sacande (2000). In contrast to our studies Gamene et al. (1996) mentioned that drying of neem seed upto 6.4 and 4.8% moisture content resulted in significant loss of germination while 9-13% moisture content was best for storage. However, Pammentor and Berjak (1999) reported below 5% moisture content was sensitive to desiccation damage that occurs water, required maintaining the integrity of intracellular structure removed.

The seed stored at lower temperature (-4°C) with lower moisture content (M_4) also showed significant loss of viability due to chilling injury of seeds. In this study relatively faster loss of viability was noticed in M_1, M_2 followed by M_3 at all the temperatures. Present studies are in accordance with Berjak et al. (1995). In present studies, optimum conditions (lower moisture content (M_4 -5.5%) and temperature (T_3 5±1°C)) for storage were observed significantly better and in accordance with Hong and Ellis (1988) and Sacande et al. 1996.

Chilling sensitivity was particularly prominent in this material and also known for other intermediate and recalcitrant seed types of tropical origin (Corbineau and Come 1988, Chine et al. 1989, Tompsett 1994). The chilling sensitivity of the fruits and leaves of tropical plants has been attributed to a conformational transition in cell membranes from the liquid crystalline to gel phase (Lyons et al. 1979, Wang 1982), often followed by lateral phase separation of the components of the membrane (Platt-Alota and Thomson 1987). Diagnostic for such phenomena are leakage of cytoplasmic solutes from the cell and the dysfunction of membrane proteins (Yamawaki et al. 1983, Yoshida et al. 1986). The transition temperature (T_m) of membranes in chilling sensitive tropical plants has been estimated at approximately 10°C (Crowe et al. 1989), which means that membrane is in liquid crystalline phase under ambient conditions. In neem seeds storage below 10°C was successful than above 10°C.

Those seeds were stored at very low temperature (T_4, -4°C) at low moisture (M_4), reduced mean germination percentage (58.07) and mean germination value (28.54) was observed. MGT was also higher as compared T_3M_4. Over all temperature T_3 (5°C) and moisture M_4 (5.5%) were observed the best combination of temperature and moisture level for storage of seed in controlled conditions. Studies are in accordance with Sacande et al. 2000 as they reported that the storage behaviour of neem seeds has featured that characterize it as orthodox.

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Table 1 : Moisture and temperature levels taken for study and initial germination percentage under controlled environment. Values are means of four replications and each replication contains 100 seeds

Temperatures Level	Moistures Level	Target Moistures	Time taken to target moisture	Initial Germination
T_1 (35±5°C)	M_1	38%	12 hrs	93%
T_2 (25±2°C)	M_2	21%	17 hrs	96.5%
T_3 (5±1°C)	M_3	12%	42 hrs	96.5%
T_4 (-4±1°C)	M_4	5.5%	144 hrs	96%

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Table 2 : Effect of moisture and temperature on mean germination percentage during storage of seeds. Seed lots were stored for 270 days in a sealed polythene (200μ thick) bag in an airtight plastic container at desired incubation temperatures after drying of seeds upto indicated moisture levels. Seeds performance was taken at 0 days (fresh), 30, 60, 90, 105, 150, 180, 210 and 270 days of storage under controlled environment. Values are means of four replications and each replication contains 100 seeds. Values in parenthesis are arc sin transformed

Temperatures	Moistures				
	M_1	M_2	M_3	M_4	Mean
T_1	15.50 [f] (12.54)	16.08 [f] (13.31)	15.83 [f] (12.97)	23.08 [e] (19.95)	17.63 [c] (14.69)
T_2	15.50 [f] (12.54)	25.58 [d] (23.84)	57.83 [c] (48.29)	78.83 [a] (64.43)	44.44 [b] (37.27)
T_3	15.50 [f] (12.54)	62.25 [b] (53.45)	52.00 [c] (46.56)	82.50 [a] (66.30)	53.06 [a] (44.71)
Mean	15.50 [d] (12.54)	34.64 [c] (30.20)	41.89 [b] (35.94)	61.47 [a] (50.23)	

Table 3 : Effect of moisture and temperature on mean MGT/speed of germination (days) during storage of seeds under controlled environment. Values are means of four replications and each replication contains 100 seeds

Temperatures	Moistures				
	M_1	M_2	M_3	M_4	Mean
T_1	14.78 [a]	14.72 [a]	14.74 [a]	14.12 [b]	14.59 [a]
T_2	14.78 [a]	13.98 [b]	11.63 [c]	10.50 [d]	12.72 [b]
T_3	14.78 [a]	11.21 [c]	11.82 [c]	9.91 [e]	11.93 [c]
Mean	14.78 [a]	13.30 [b]	12.73 [c]	11.51 [d]	

Values not followed by same letter are significantly different (P>0.05)

Table 4 : Effect of moisture and temperature on mean germination value (GV) during storage of seeds under controlled environment. Values are means of four replications and each replication contains 100 seeds

Temperatures	Moistures				
	M_1	M_2	M_3	M_4	Mean
T_1	9.62 [c]	10.37 [c]	9.62 [c]	11.95 [c]	10.39 [c]
T_2	9.62 [c]	12.63 [c]	27.40 [b]	39.18 [a]	22.20 [b]
T_3	9.62 [c]	30.05 [b]	26.32 [b]	43.15 [a]	27.28 [a]
Mean	9.62 [d]	17.68 [c]	21.11 [b]	31.43 [a]	

Values not followed by same letter are significantly different (P>0.05)

Table 5 : Effect of lower moisture and lower temperature on mean germination percentage, mean germination time (MGT) /speed of germination (days) and germination value (GV) during storage of seeds. Seed lots were stored for 270 days in a sealed polythene (200μ thick) bag in an airtight plastic container at desired incubation temperatures after drying of seeds upto indicated moisture levels. Seeds performance was taken at 0 days (fresh), 30, 60, 90, 105, 150, 180, 210 and 270 days of storage under controlled environment. Values are means of four replications and each replication contains 100 seeds. Values in parenthesis are arc sin transformed

Temperature	Moisture M_4 (5.5%)		
	Germination (%)	MGT	GV
T_4 (-4±1ºC)	58.07 (50.37)	11.17	28.54

Values not followed by same letter are significantly different (P>0.05)

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Table 6 : Effect of different moistures and temperatures on the half viability period (P_{50}) on seed longevity

Temperatures	Moistures			
	M_1	M_2	M_3	M_4
T_1	31 days	31 days	31 days	85 days
T_2	31 days	80 days	146 days	278 days
T_3	31 days	201 days	169 days	326 days
T_4	-	-	-	184 days

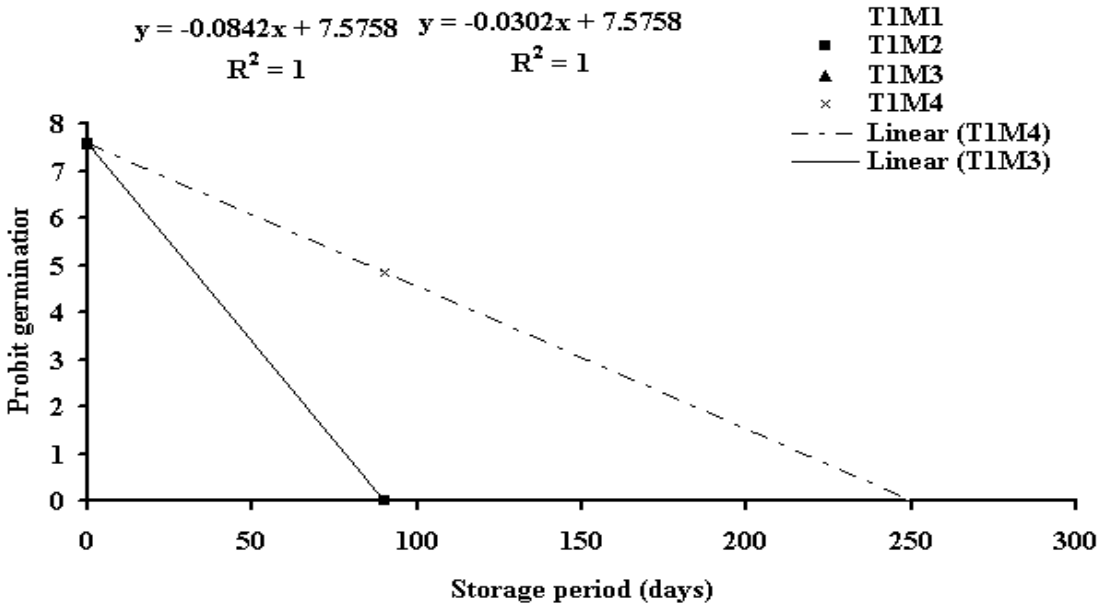

Figure 1A : Regression between probit germination and storage period (days) for seeds of different moisture levels (%) stored at ambient room temperature (T_1, 35±5°C)

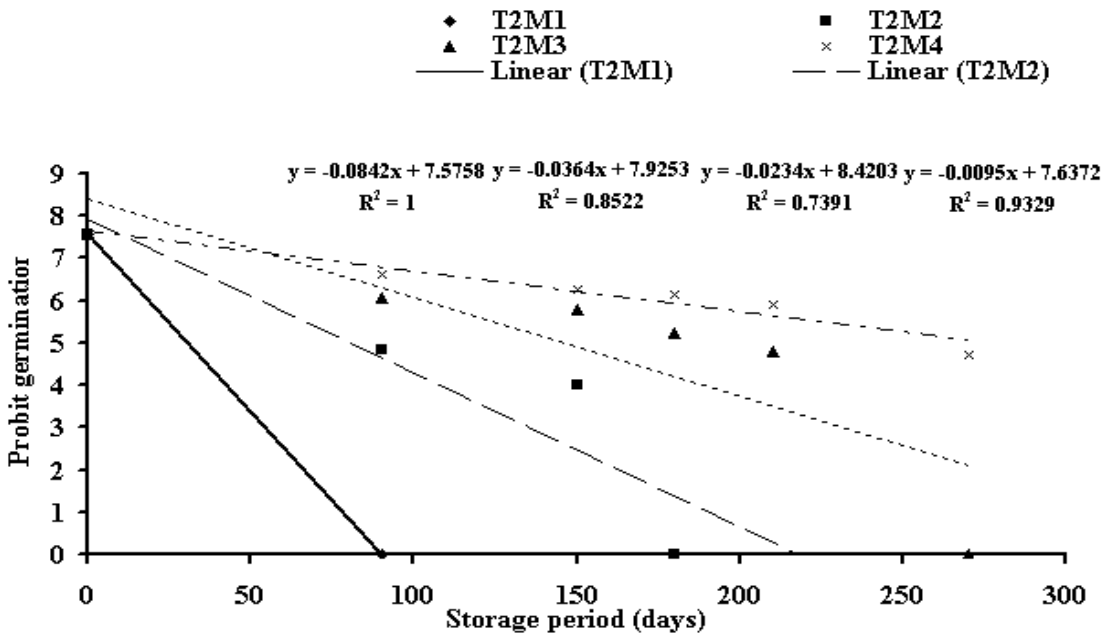

Figure 1B : Regression between probit germination and storage period (days) for seeds of different moisture levels (%) stored at T_2 temperature (25±2°C)

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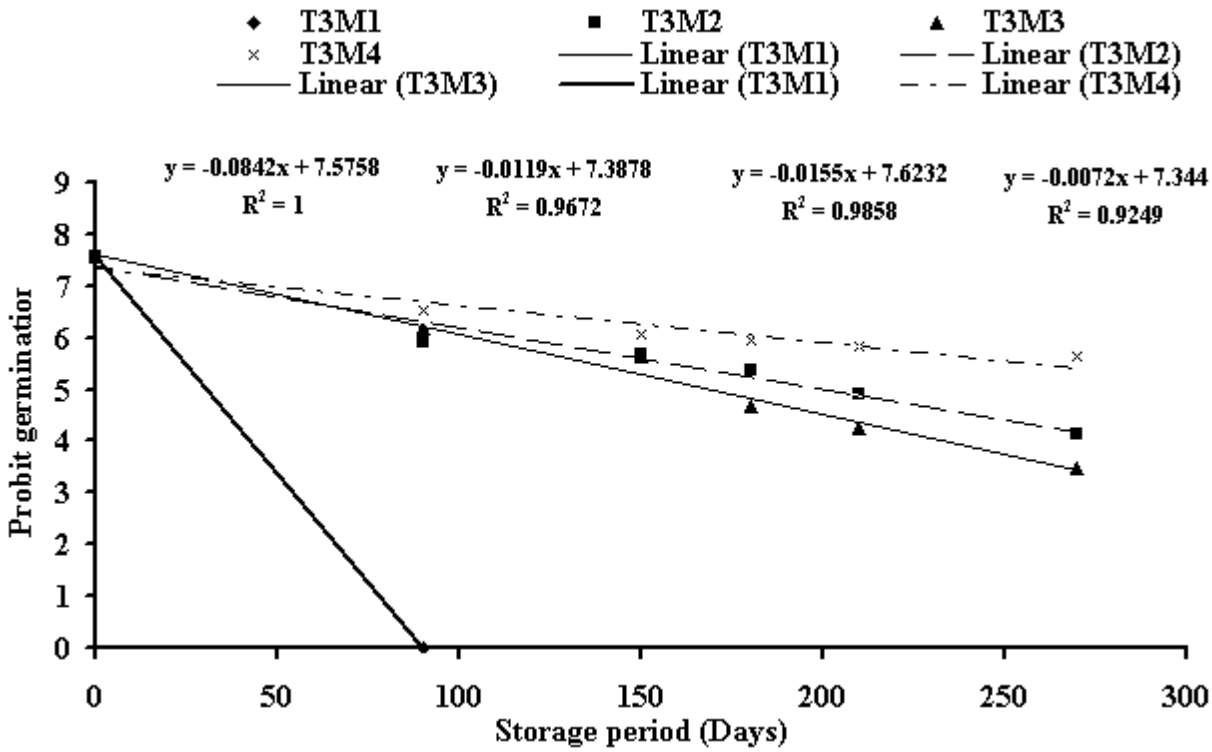

Figure 1C : Regression between probit germination and storage period (days) for seeds of different moisture levels (%) stored at T_3 temperature (5±1°C)

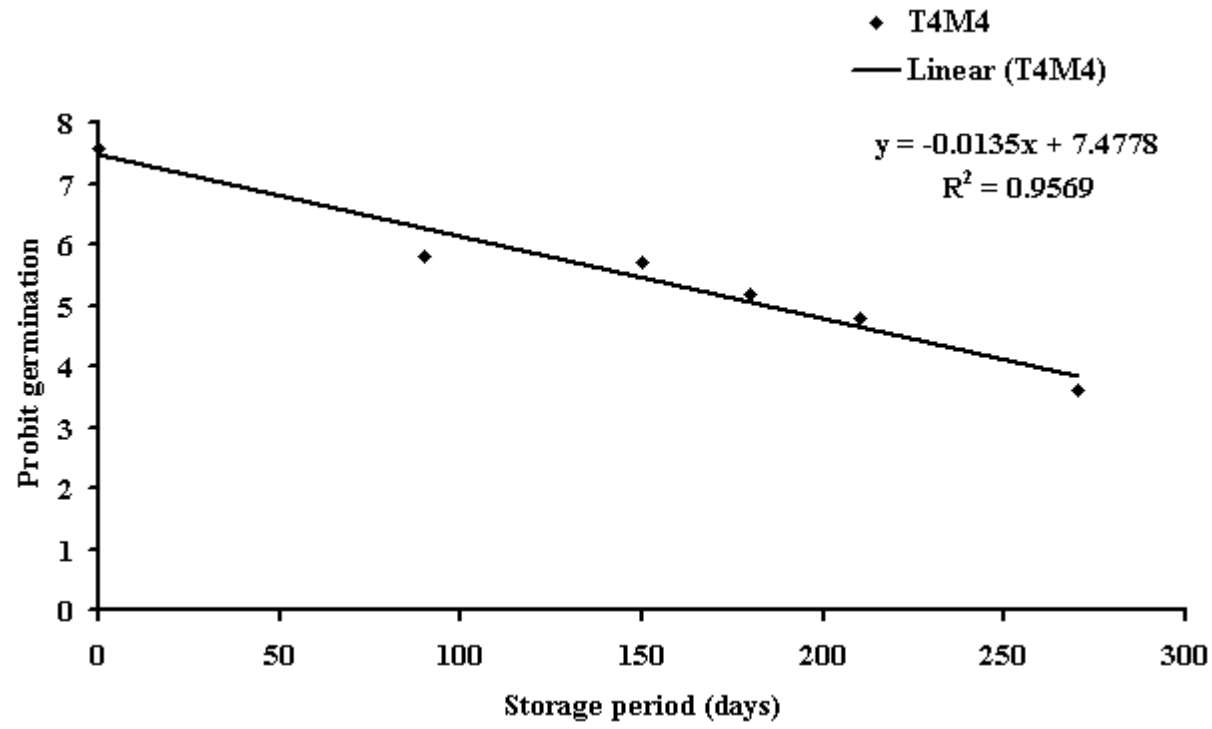

Figure 1D : Regression between probit germination and storage period (days) for seed of M4 moisture stored at T_4 temperature (-4±1°C).

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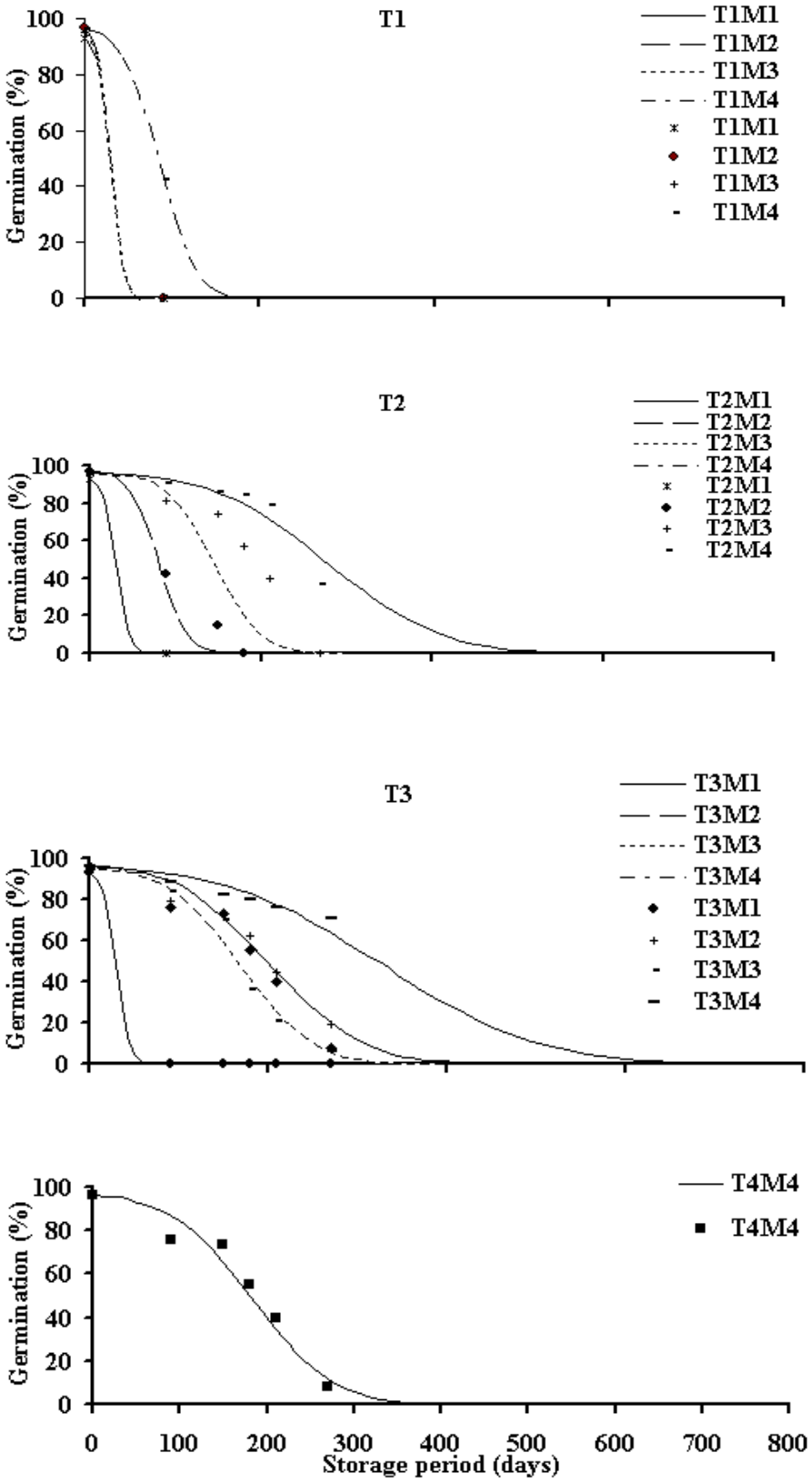

Figure 2 : Effect of different moisture and temperature on germination percentage during storage of seeds. Lines show survival curves. Points show original germination values

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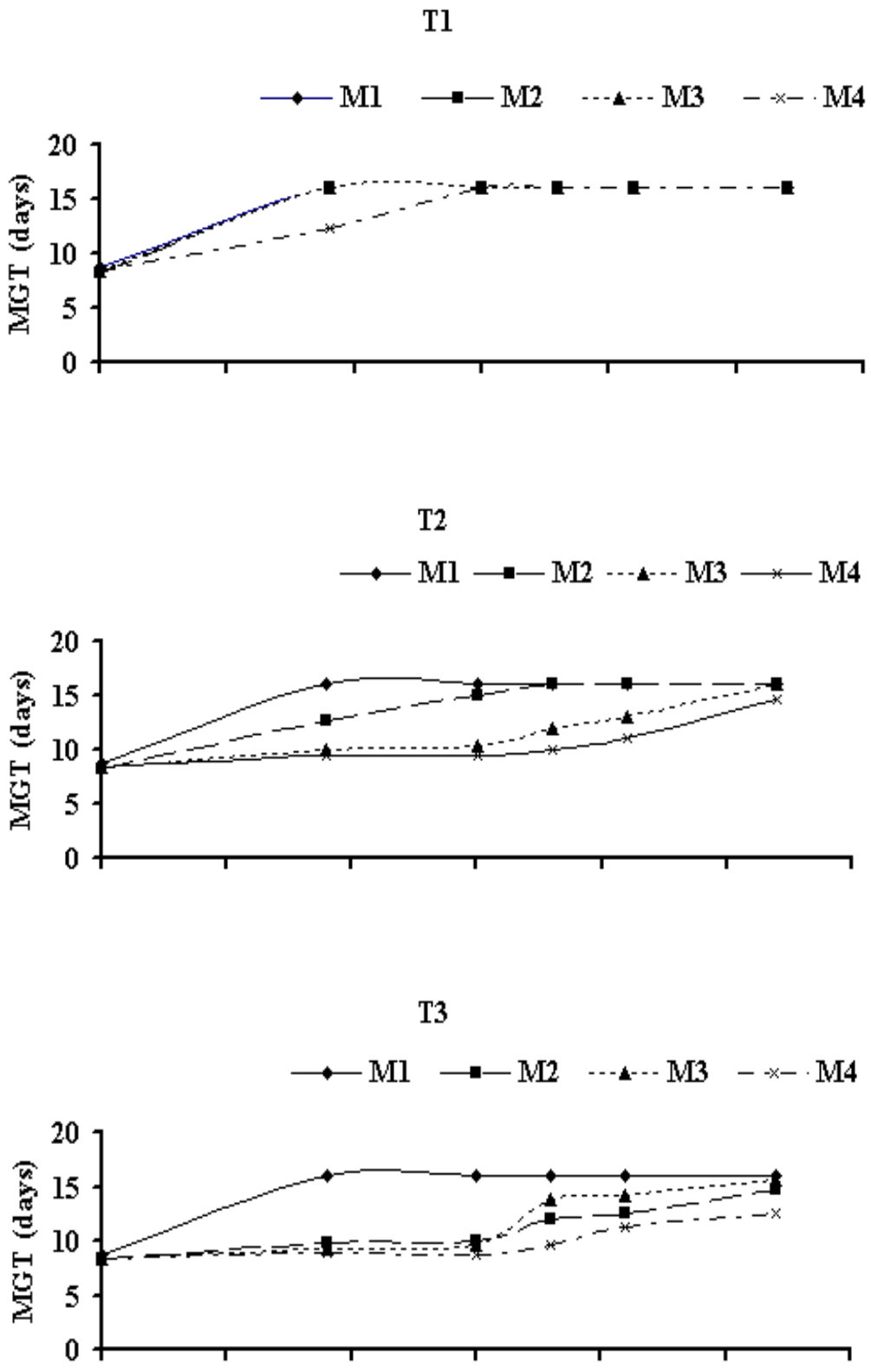

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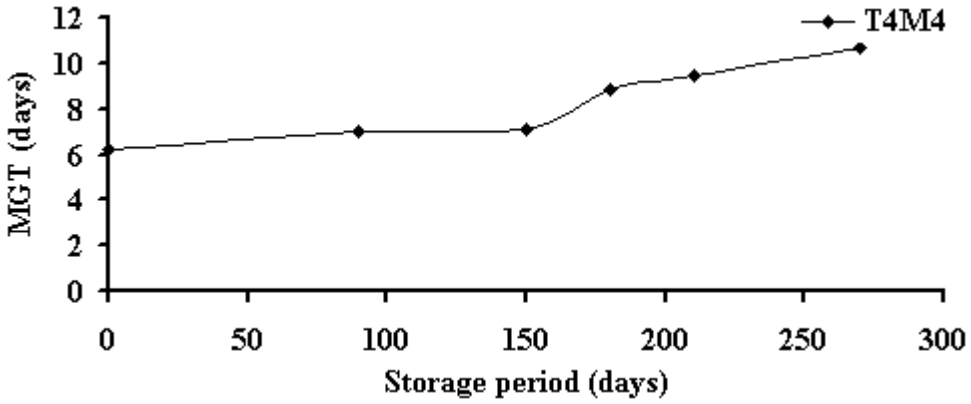
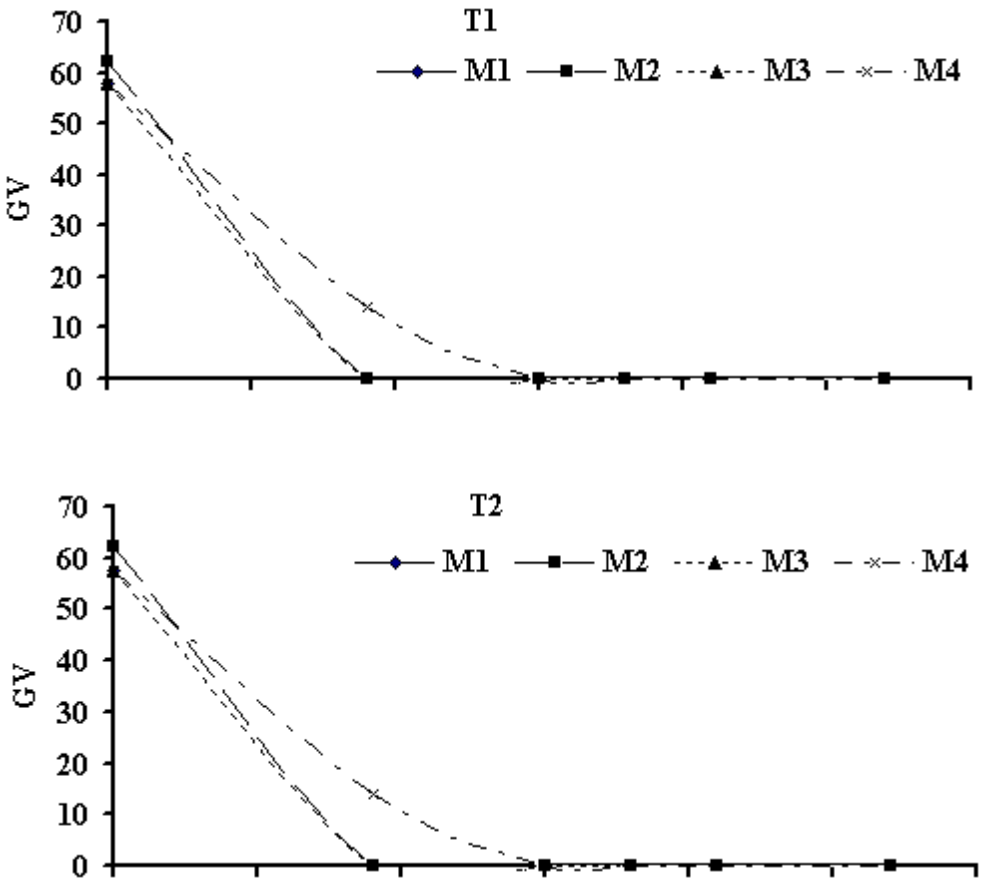


Figure 3 : Effect of different moisture and temperature on MGT (days) during storage of seeds



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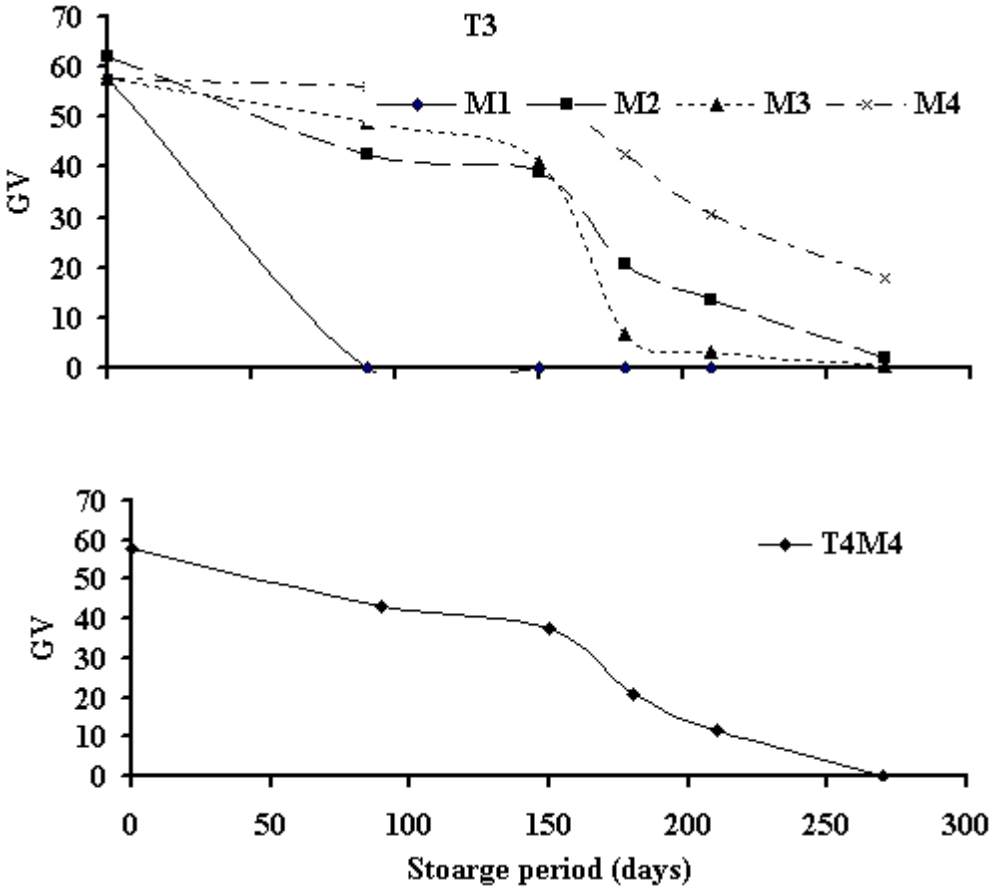

Figure 4 : Effect of different moisture and temperature and on GV during storage of seeds.

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