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Highlights

Storage Response of Neem

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CONTENTS OF THE ISSUE

- i. Copyright Notice
 - ii. Editorial Board Members
 - iii. Chief Author and Dean
 - iv. Contents of the Issue
-
- 1. A New Approach to Biotechnology of Saffron (*Crocus Sativus* L.). **1-3**
 - 2. Storage Response of Neem (*Azadirachta Indica* A. Juss.) Seed under Different Moisture and Temperature Regime. **5-17**
 - 3. Effect of Different Organic Substrates and their Pasteurization Methods on Growth Performance, Yield and Nutritional values of Oyster Mushroom (*Pleurotus Ostreatus*) for Small Scale Cultivation at Arsi University, Ethiopia. **19-30**
-
- v. Fellows
 - vi. Auxiliary Memberships
 - vii. Process of Submission of Research Paper
 - viii. Preferred Author Guidelines
 - ix. Index



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A New Approach to Biotechnology of Saffron (*Crocus Sativus* L.)

By Karagyozyov T. H., Mammadova M. H., Asadova S. Sh. & Azizov I. V.

Institute of Botany of Nat. Acad. Sci. of Azerbaijan, Azerbaijan

Abstract- To develop effective approaches for biotechnological propagation of saffron (*Crocus sativus* L.) *in vitro* methods for the induction of morphogenesis and organogenesis were used. Factors influencing morphogenesis and organogenesis of saffron were considered. By using a temperature gradient under *in vitro* conditions was obtained *de novo* from 10 to 25 microcorms. Some issues related to biotechnology of saffron were elucidated. The effect of the temperature factor and gibberellin on the efficiency of callus formation and morphogenesis *in vitro* were discussed. The methodical approaches to increase the efficiency of morphogenesis and organogenesis of *Crocus sativus* L. in *in vitro* conditions were offered.

Keywords: saffron, *Crocus sativus* L., *in vitro*, morphogenesis, temperature gradient.

GJSFR-C Classification : FOR Code: 270499



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A New Approach to Biotechnology of Saffron (*Crocus Sativus* L.)

Karagyozyov T. H. ^α, Mammadova M. H. ^σ, Asadova S. Sh. ^ρ & Azizov I. V. ^ω

Abstract- To develop effective approaches for biotechnological propagation of saffron (*Crocus sativus* L.) *in vitro* methods for the induction of morphogenesis and organogenesis were used. Factors influencing morphogenesis and organogenesis of saffron were considered. By using a temperature gradient under *in vitro* conditions was obtained *de novo* from 10 to 25 microcorns. Some issues related to biotechnology of saffron were elucidated. The effect of the temperature factor and gibberellin on the efficiency of callus formation and morphogenesis *in vitro* were discussed. The methodical approaches to increase the efficiency of morphogenesis and organogenesis of *Crocus sativus* L. in *in vitro* conditions were offered.

Keywords: saffron, *Crocus sativus* L., *in vitro*, morphogenesis, temperature gradient.

I. INTRODUCTION

Saffron (*Crocus sativus* L.) as the most valuable medicinal plant known since ancient times. In recent years the area of cultivation of saffron in the world tends to increase, although in a number of European countries the production of its flower production was reduced due to the high cost and the urbanization of rural areas. Presently, demand for saffron is much higher than the norm of its reproduction. The increasing worldwide demand for floral products saffron stimulate research related to its reproduction, including with the use of biotechnological methods.

At the first international symposium on saffron biotechnology has been included in the list of priorities for the next hundred years [1]. Currently conducted biotechnology research towards the development of micro propagation techniques of different cultures, including of saffron.

Prospects for biotechnology *in vitro* considered as the basis for implementing future advances in molecular genetic improvement of saffron, as well as obtaining planting material free of pathogens.

Although research in cellular biotechnology *Crocus sativus* L. already have their own history [2-4], but so far the results achieved do not give grounds for approval of availability methodical basis, to effectively and consistently get the results that ensure the production of planting material with a sufficiently high rate of reproduction.

Analysis of the available information in the press really shows that one of the reasons for the current situation in this area is the lack of a common approach in researches on biotechnology *in vitro*. The first phase of research on the induction of callus formation, morphogenesis, organogenesis *in vitro*, which was characterized by the establishment of optimal concentrations of hormonal inductors, and combinations thereof, has been successfully implemented. Despite the large number and variety of options for phytohormones and received on that basis the effects did not contributed to the development of a single methodological approach. The next stage in the development of methodological approaches based on the use of low positive temperature (5°C) for the induction of organogenesis in the single-stage circuit producing microcorns from callus cells, which is common at this time.

However, the ability to maximize the use of the temperature factor is not fully implemented.

In this respect, the classic position on the impact of state initial explant on the progress in the implementation of morpho-physiological potency *in vitro* relative geophytes such as the crocus is the most illustrative. Numerous observations suggest that the winter months with negative temperatures lead to increased formation of floral organs of *Crocus sativus* L.

The aim of this work was to improve the method of clonal propagation applied to saffron.

II. MATERIAL AND METHODS

The starting material in our research was corns of saffron. To study the effect gibberellin on embryogenesis and organogenesis corns incubated in gibberellic acid solutions of 20-50 mg / l for 12 and 24 hours. As starting explant were used discs corns sliced in the transverse direction.

Corns sterilized in 70-80% ethanol followed by 2-3 times washing with sterile water. For further sterilization used a solution of sodium hypochlorite containing 5% of the basic substance, at various dilutions with the addition of TWEEN 20. Then were washed 3-fold with sterile water for 10 -15 minutes. Sterilized corns planted on an agar medium M-S [5].

Embodiments of the medium contained BAP (benzylaminopurine) 2.4 D (2, 4 -dichlorophenoxyacetic acid), kinetin (6-furfurylaminopurine), NAA (1-naphthylacetic acid), at various concentrations (mg /

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l), agar - 0.7-0.8%; sucrose - 30, 60, 90 g / l; pH - 5.6-5.8. Planted material was cultivated in the dark at 21° C and relative humidity of 70-80%.

III. RESULTS AND DISCUSSION

The experiments with the use of a temperature gradient revealed variants of medium, ensuring the most effective organogenesis. In conditions of incubation at a

constant temperature did not obtained organogenesis of explants *in vitro*. When had used the temperature gradient, depending on the ratios of phytohormones and initial stages of embryogenesis before temperature gradient, on medium, containing BAP (4 mg / l) and NAA (1 mg / L) had formed of 10 to 25 micro corms in a flask (Fig.1).

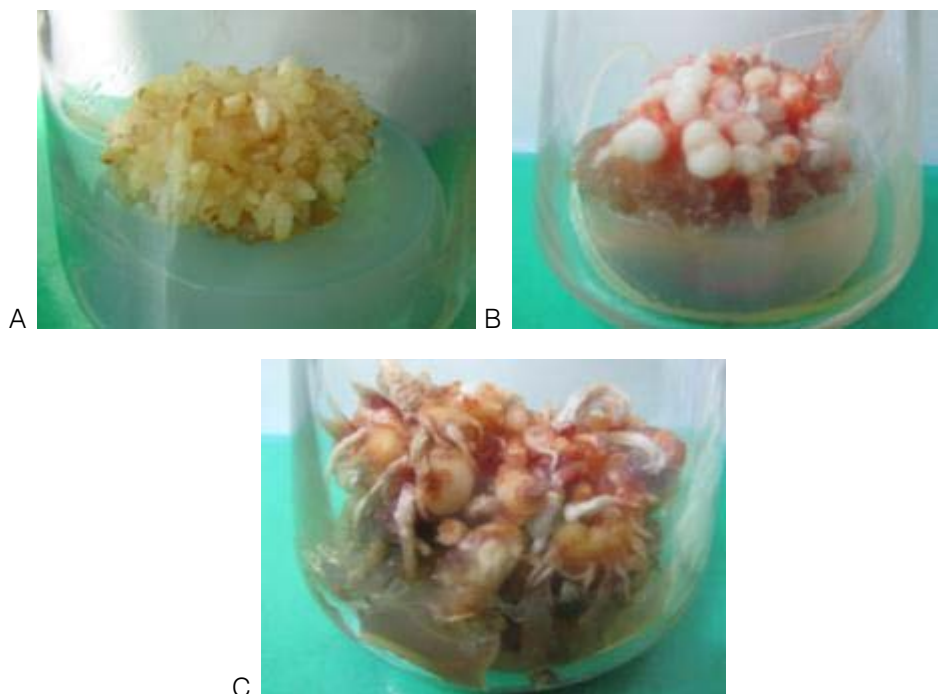


Fig.1 : Consecutive stages of embryogenesis (A), morphogenesis (B) and organogenesis (C) from *Crocus sativus* L. in culture *in vitro*

In the process of the temperature gradient, depending upon the composition of medium, was observed increase number of formed embryoids.

A similar effect has not presented in the literature. The applied temperature gradient could potentially allow reduce the time of incubation, which is currently equal to 5 weeks at a stable temperature.

Effect of temperature on themorpho - physiological processes may be associated with its impact on the level of free gibberellin when temperatures will contribute to their release from the bound state. Influence of exogenous gibberellin on activity of replacement meristems in corms saffron flowers and increasing the number of floral shoots had shown in the experiments [6].

Increasing the concentration of sucrose in a medium to 60 -90 g/l resulted in an increase of embryoids, but in this case significantly increased infection and it appeared relatively late. Apparently, it was associated with infection of internal tissues of corms.

Using gibberellin in our experiments was effective only in cases of environments in which were

present BAP, 2,4-D and kinetin. In the absence of BAP, 2,4-D and kinetin use of gibberellin has not very effective, and in some cases adversely affect the processes of organogenesis. This may reflect the complex interactions gibberellic acid with other phytohormones and perhaps may be particularly characteristic of corms *in vitro*. Regarding the callus cells from leaf explants *Crocus sativus* L. is evidence of a positive effect of exogenous gibberellin on organogenesis at various hormonal background [7,8].

IV. CONCLUSIONS

Thus, consideration of the above factors that could have some impact on the efficiency of organogenesis in conditions *in vitro*, as well as the use of a temperature gradient that was not applied early, allowed realize morphogenesis on the stage of organogenesis and *de novo* get corms of saffron *in vitro*.

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

By Devendra Kumar

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Abstract- Effect of four storage temperatures (T_1 -ambient room temperature ($35\pm 5^\circ\text{C}$), T_2 -Air conditioner ($25\pm 2^\circ\text{C}$), T_3 - Fridge ($5\pm 1^\circ\text{C}$) and T_4 -Freezer ($-4\pm 1^\circ\text{C}$) 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 $30\pm 1^\circ\text{C}$ and relative humidity ($90\pm 3\%$).

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

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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 $5\pm 1^\circ\text{C}$ (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.

1. 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 multipurpose 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 -refrigerator (5±1°C) and T_4 -fridge (-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 200 μ 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

(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_1 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 undergo 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).

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 $5 \pm 1^\circ\text{C}$)) 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%

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 (200 μ 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				Mean
	M ₁	M ₂	M ₃	M ₄	
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				Mean
	M ₁	M ₂	M ₃	M ₄	
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				Mean
	M ₁	M ₂	M ₃	M ₄	
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 ($P>0.05$)

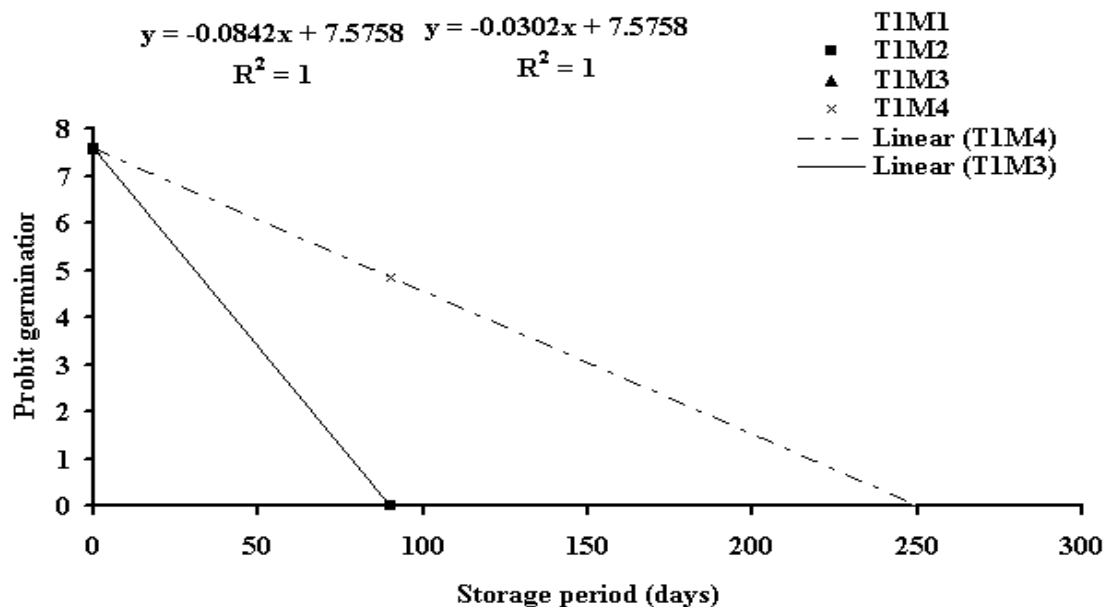
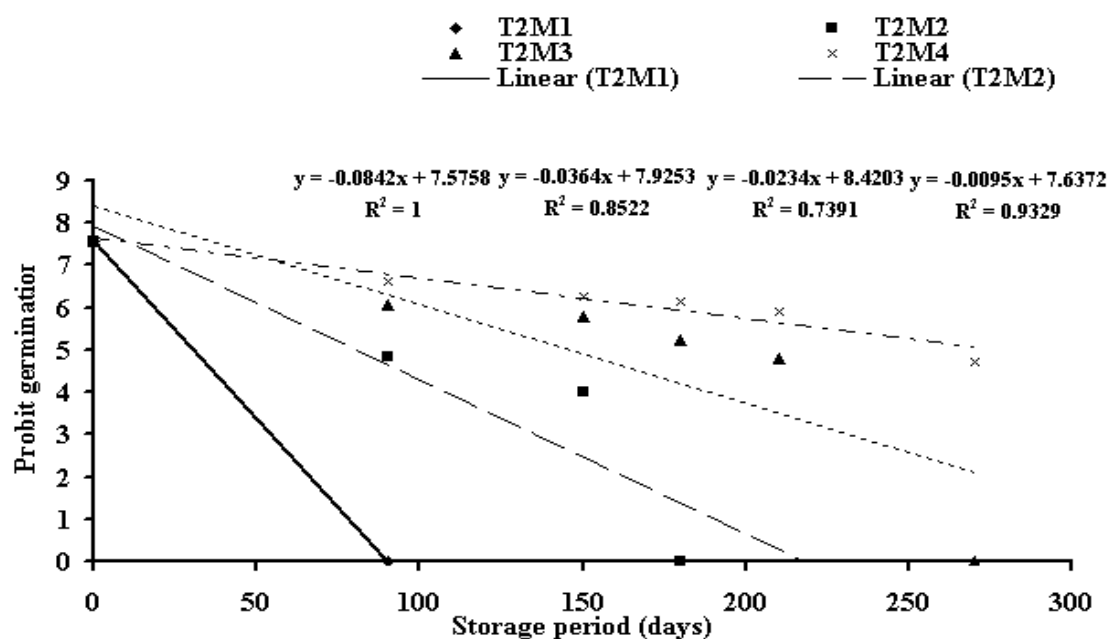
Table 5 : Effect of lower moisture and lower temperature on mean germination percentage, mean germination time (MGT) /speed of germination (days) and germination value (GV) during storage of seeds. Seed lots were stored for 270 days in a sealed polythene (200 μ thick) bag in an airtight plastic container 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 \pm 1°C)	58.07 (50.37)	11.17	28.54

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

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 , $35 \pm 5^\circ\text{C}$)Figure 1B : Regression between probit germination and storage period (days) for seeds of different moisture levels (%) stored at T_2 temperature ($25 \pm 2^\circ\text{C}$)

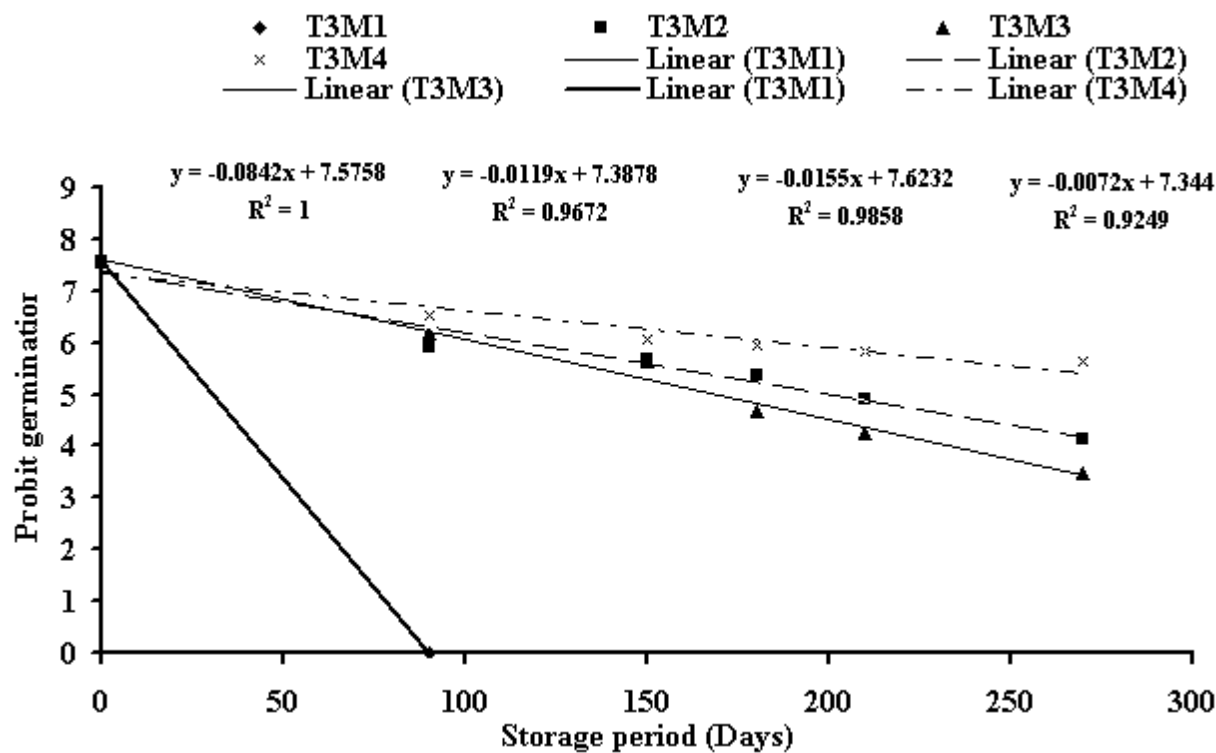


Figure 1C : Regression between probit germination and storage period (days) for seeds of different moisture levels (%) stored at T₃ temperature (5±1°C)

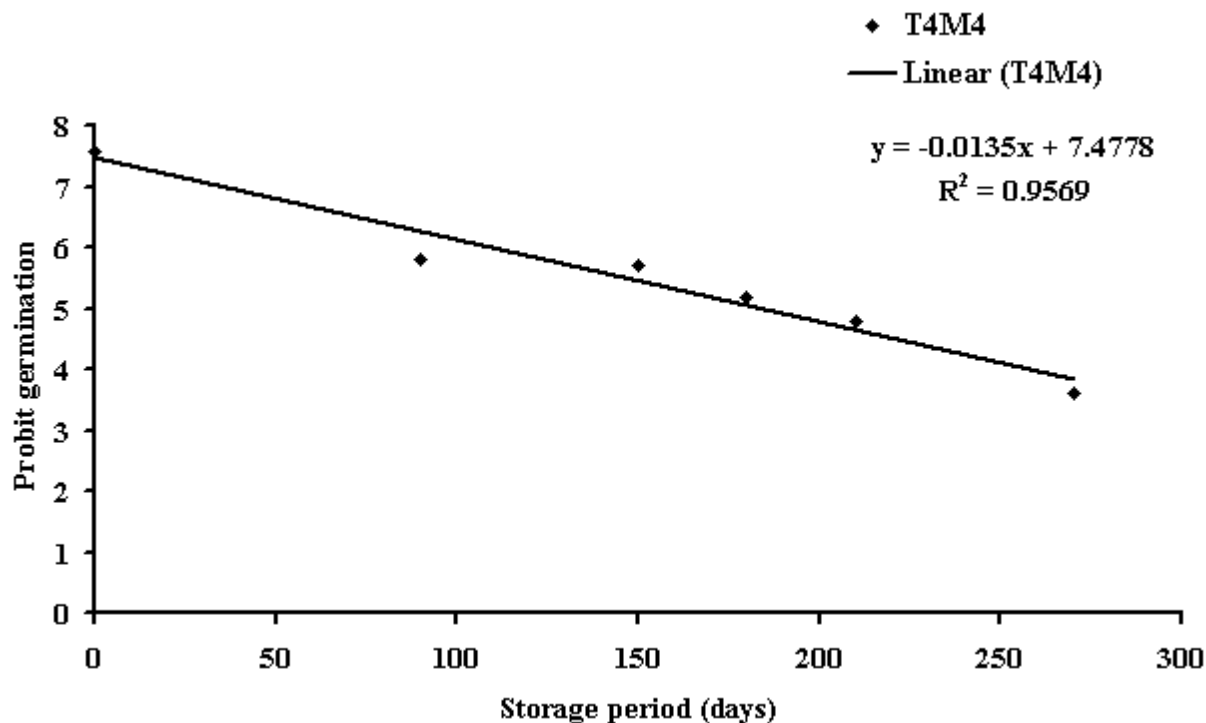


Figure 1D : Regression between probit germination and storage period (days) for seed of M4 moisture stored at T₄ temperature (-4±1°C).

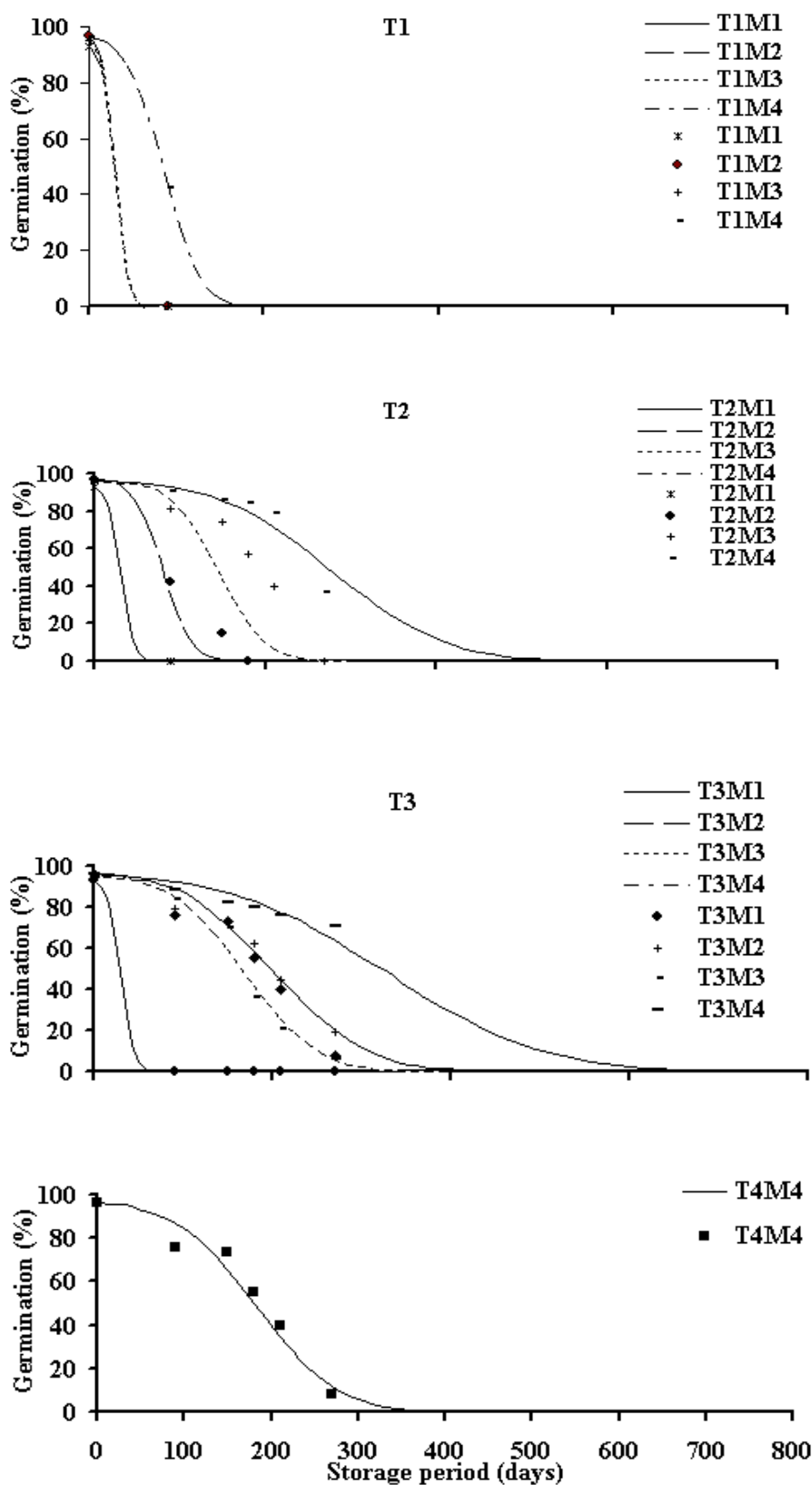
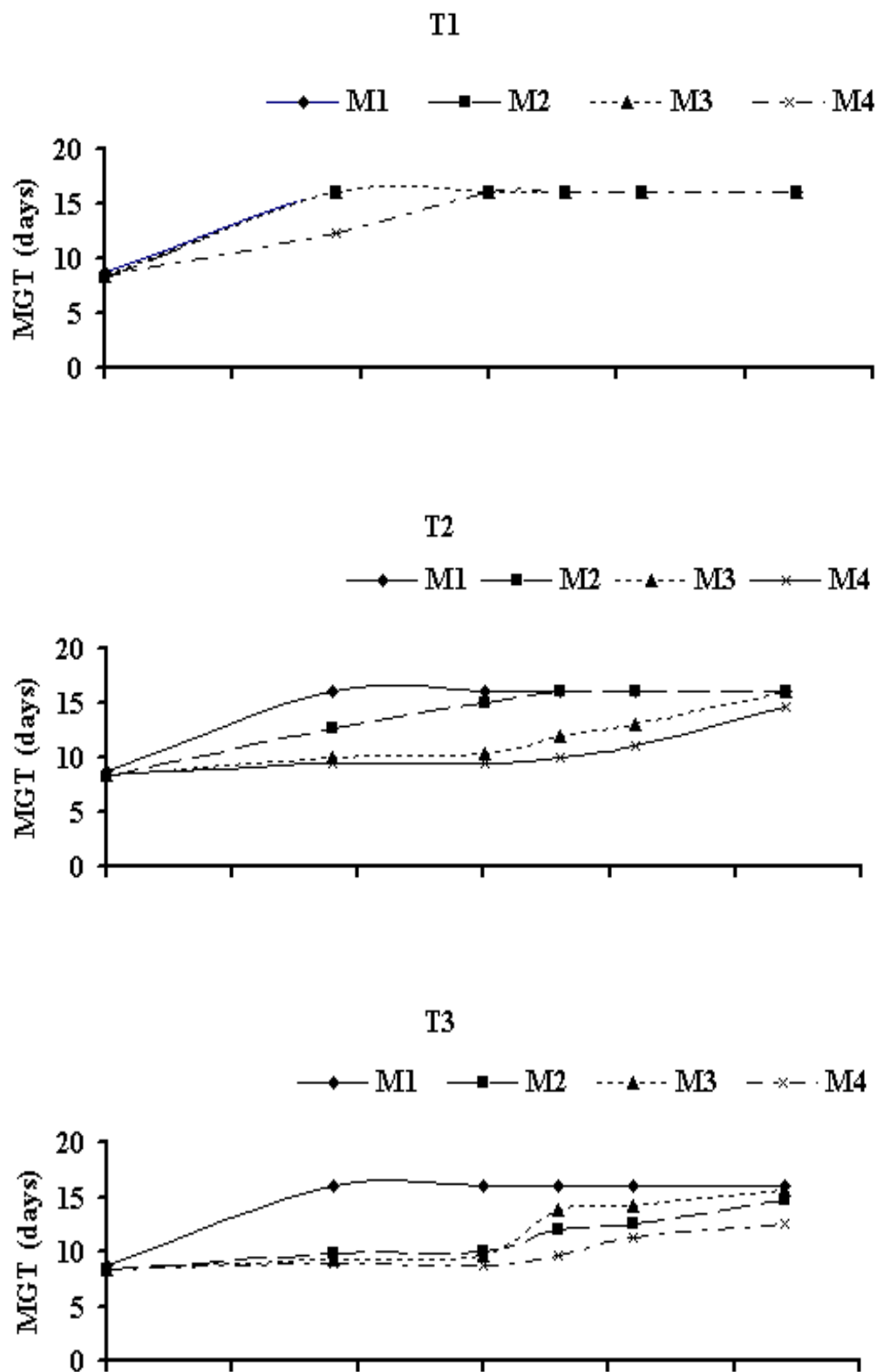


Figure 2 : Effect of different moisture and temperature on germination percentage during storage of seeds. Lines show survival curves. Points show original germination values



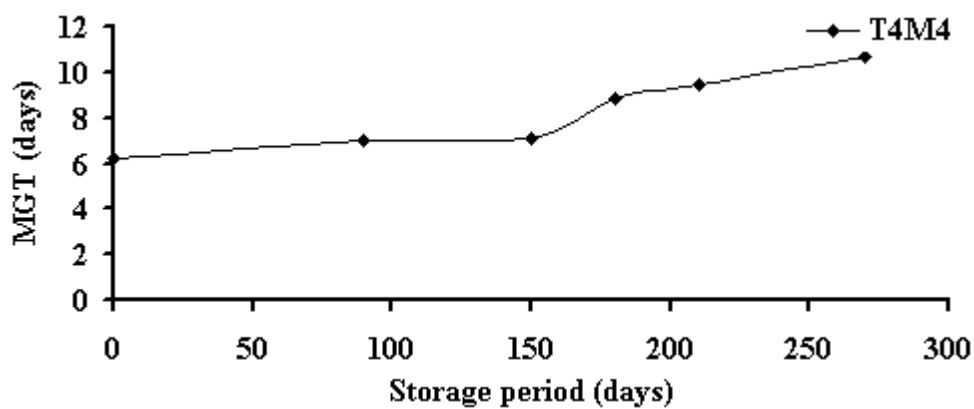
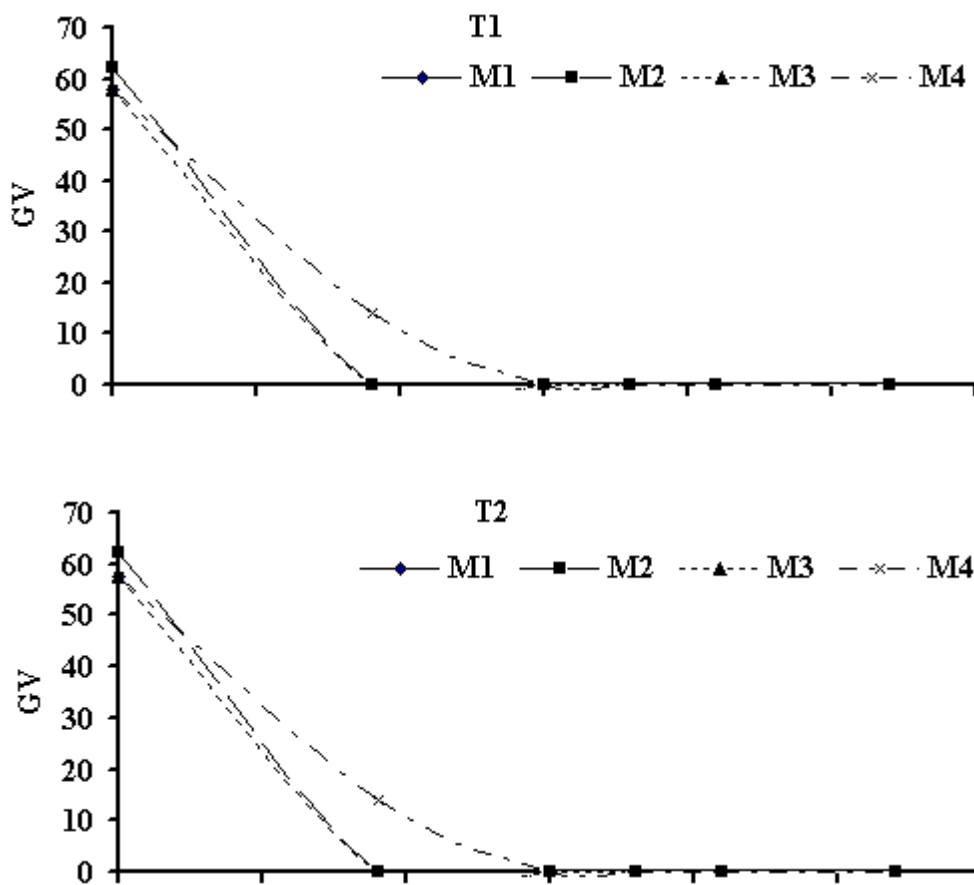


Figure 3 : Effect of different moisture and temperature on MGT (days) during storage of seeds



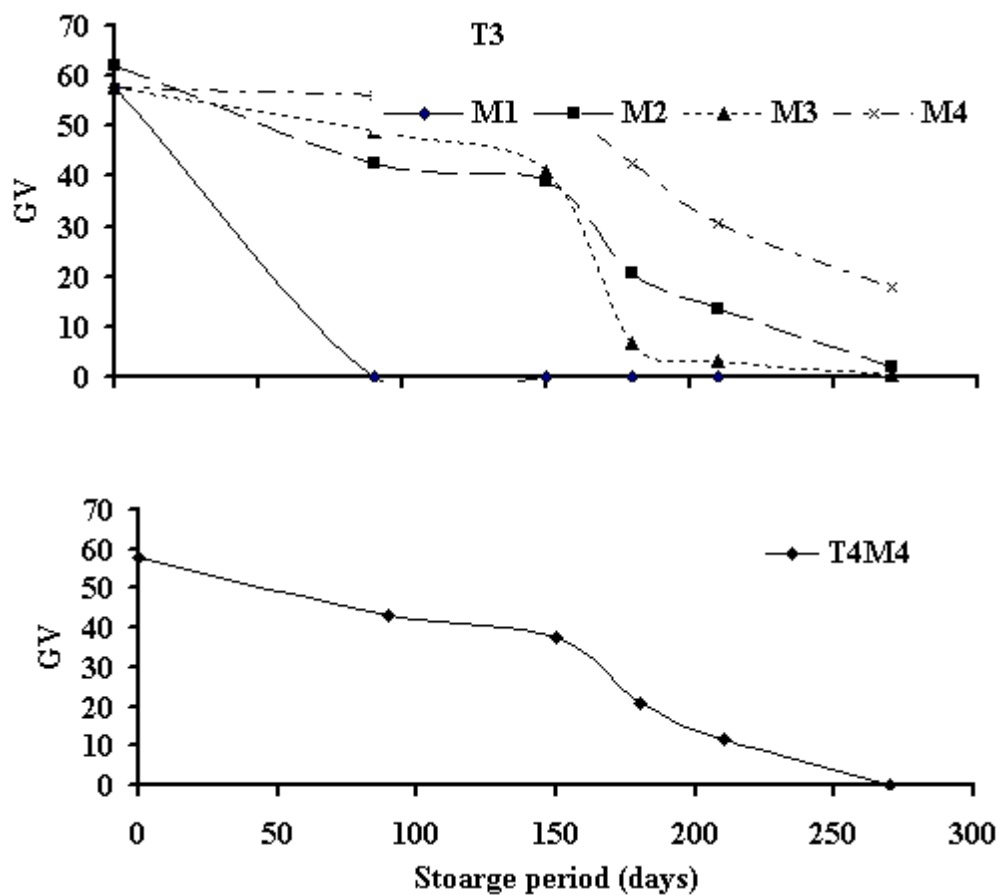


Figure 4 : Effect of different moisture and temperature and on GV during storage of seeds.

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Effect of Different Organic Substrates and their Pasteurization Methods on Growth Performance, Yield and Nutritional values of Oyster Mushroom (*Pleurotus Ostreatus*) for Small Scale Cultivation at Arsi University, Ethiopia

By Demisie Ejigu & Tadesse Kebede

Arsi University, Ethiopia

Abstract- Mushrooms are important fungi that play a great role for food self-sufficiency and to fill the gap of protein deficiency in most cereal crops, vegetables and fruits. Moreover, they are known for their flavor enhancing property, medicinal value as well as cholesterol free food. However, Mushroom production in Ethiopia is still less known due to multiple problems which include high input cost, lack of quality of spawn, lack of proper production skill, and lack of information on substrates. In view of this, the experiment was conducted at Arsi University, College of Agriculture and Environmental Science during 2013/14 Cropping years with the objectives of evaluating different organic substrates and pasteurized methods on *Pleurotus ostreatus* yield, biological efficiencies and nutritional values. Factorial combination of seven substrates (Dried faba bean stalk, Maize Stalk, Wheat straw, Teff straw, Eucalyptus Sawdust, Wheat straw + Teff straw+ Faba bean stalk and Saw dust + Faba bean stalk) and two pasteurization methods (Cold water treatment and Hot water) were used.

Keywords: oyster mushroom, substrates, yield and nutritional value.

GJSFR-C Classification : FOR Code: 060799



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Effect of Different Organic Substrates and their Pasteurization Methods on Growth Performance, Yield and Nutritional values of Oyster Mushroom (*Pleurotus Ostreatus*) for Small Scale Cultivation at Arsi University, Ethiopia

Demisie Ejigu ^α & Tadesse Kebede ^ο

Abstract- Mushrooms are important fungi that play a great role for food self-sufficiency and to fill the gap of protein deficiency in most cereal crops, vegetables and fruits. Moreover, they are known for their flavor enhancing property, medicinal value as well as cholesterol free food. However, Mushroom production in Ethiopia is still less known due to multiple problems which include high input cost, lack of quality of spawn, lack of proper production skill, and lack of information on substrates. In view of this, the experiment was conducted at Arsi University, College of Agriculture and Environmental Science during 2013/14 Cropping years with the objectives of evaluating different organic substrates and pasteurized methods on *Pleurotus ostreatus* yield, biological efficiencies and nutritional values. Factorial combination of seven substrates (Dried faba bean stalk, Maize Stalk, Wheat straw, Teff straw, Eucalyptus Sawdust, Wheat straw + Teff straw+ Faba bean stalk and Saw dust + Faba bean stalk) and two pasteurization methods (Cold water treatment and Hot water) were used. The experiment was arranged in RCBD with three replications by using the box constructed from the wood timber as blocks in cropping room. Carbon content, nitrogen content and water holding capacity of the substrates were analyzed at spawning and harvest of mushrooms and significant difference among the substrates and pasteurization methods (Hot and cold water) were observed. Phenological data including days to onset of mycelium invasion, days to appearance of pin heads and days to maturation of fruiting bodies of mushrooms and cap diameter and different nutritional analysis of mushroom for crude protein, organic carbon, organic matter and carbohydrates contents were analyzed and recorded and the significant difference among the substrates have been observed. Non-significance difference between pasteurization methods was observed on pin head formation, fruiting body formation, cup diameter, biological efficiency, Production rate, moisture content and dry matter content. To give general recommendation from this research for the producers, the highest yield of oyster mushroom (1.58kg/4 kg of substrate) was recorded from maize stalk treated with hot water and the lowest was recorded from saw dust treated with hot water (0.50 kg/4 kg of substrate). The highest protein content was recorded from faba bean stalk Pasterualized with both cold

and hot water (27.38%) and the lowest was recorded in case of wheat straw treated with hot water (17.03%).

Keywords: oyster mushroom, substrates, yield and nutritional value.

I. BACKGROUND AND JUSTIFICATION

Today as the challenge on world, the 20th century began with a world population of 1.6 billion and ended with six billion, and projected to be nearly nine billion by 2050, with most of the growth occurring in the developing country [1]. According to Population Institute of Ethiopia (2008), the Ethiopian population is 82.8 million and projected to be 149.5 million by mid of 2050. However, land in agricultural use has increased by only 11% from the early late seventeenth to the late twentieth century, meaning the land per capital declined by about 40%. Therefore, in the near future it will become increasingly difficult to produce the food required to feed the ever-growing human population [2]. Ethiopia is among few countries that failed to participate in the general thrust towards increasing average food consumption level. In 2010/2011 production season total crop production in Ethiopia was about 22.5 million tones on 13.5 million ha of land [3], making Ethiopia agriculture one of the least productive all over the world with productivity of less than 2t/ha. The food deficit of the under nourished Ethiopia people is 340 K cal/person/day and the share of cereals, root and tubers in total dietary energy supply is 79%. This problem is further aggravated by recurrent droughts [4].

Food grains are the principal dietary source of calories, proteins and other nutrients but their dietary supply is not sufficient and proteins supply is not in the required amount. Besides, economic and health factors make animal protein inaccessible to the majority of the people [5]. Developing countries like Ethiopia are caught in vicious circle of poverty, shortage of food and nutritional disorder. Erratic rainfall, shortage of land, low yield of traditional crops and low status of most crops

especially in protein are among the causes of the three mentioned problems above. To tackle this problem, the Ethiopia government has been engaged in improving the agriculture sector through different initiatives. One of them being transforming the sector from subsistence to commercially oriented one by encouraging the production of high value diversified native and exotic crops.

[6] Reported productivity of fresh mushroom per kg of dry substrate was 0.8 to 1 kg. Dawit reported 7.3 kg of fresh Oyster mushroom produced from 10 kg dry cotton waste supplemented with 1% wheat bran. [7] Also grew Oyster mushroom on paddy straw, maize and sugarcane reported yield of 0.28 to 0.39 kg per kg of dry substrate.

Cultivation of oyster mushroom represents one of the major current economically profitable biotechnological process for the conversion of plant residues or a non-edible plant biomass in to nutritious / protein rich food, which will help in overcoming protein malnutrition problem in developing countries [8]. Time is, therefore ripe for developing new technologies for commodities like mushroom which increase production and productivity without using arable land. Such have great role to pay in achieving food self-sufficiency. It has wide range of substrate growth and temperature adaption. Furthermore, it is fast growing, requires no casing, less fragile than others and has market in the dry form as well as fresh form.

Mushrooms are highly perishable mostly due to moisture loss, wilting, color changes and poor preservative methods. Improving the shelf life and quality characteristics of mushroom will enhance marketability and add value in agricultural chain. Evolving preservation methods that are cheap and affordable by farmers has become imperative in order to have food sustainability [9]. The food processing industry provides a wide range of canned and processed mushroom products, including frozen, sterilized, dried, pickled, marinated, and salted mushrooms; mushroom powder; paste; and concentrates and extracts [10].

Mushrooms are a cash crop, the market for which is growing worldwide and in Ethiopia it is a new activity. In most countries, there is a well-established consumer acceptance for cultivated mushrooms. Even though, the first small research project on Oyster mushroom (*Pleurotus ostreatus*) was started in 1993 at Addis Ababa University Ethiopia, the mushroom production in Ethiopia is still less known due to many factors which include high in put cost, lack of quality spawn, lack of proper production skill and lack of information on substrate for mushroom cultivation.

Almost all producers use cotton hull as substrate, which not available for most farmers and comparatively too costly compared to other agricultural wastes. So, identification of different substrates from

different types of crops and method of pasteurization is the critical for successful mushroom cultivation.

II. OBJECTIVES

1. To evaluate different Organic residues (substrates) and their combinations for Oyster Mush room cultivation in terms of yield, biological efficiency and nutritional values.
2. To assess the possibility of using different pasteurization methods of substrates on growth performance of oyster mushroom, yield and nutritional value.

III. MATERIALS AND METHODS

a) *Experimental Site and Cropping House Construction*

The experiment was conducted at Arsi University, College of Agriculture and Environmental Science. Mushroom-growing house with a length (12 m), height (4m) and width (2.5m) has been constructed from locally available material, the wall lined with bamboo tree and wooden flat, the roof covered by iron sheet and the floor was cemented. The growing media/box with a length 70cm and width 50cm was constructed from wooden flat.

b) *Experimental materials*

The mushroom spawn produced from pure cultures of *Pleurotus ostreatus* (sajor-caju) has been collected from Ethio-mushroom and Spawn Enterprise Company.

c) *Treatments and Experimental Design*

The experimental treatments have been including Seven organic substrates (Sawdust, Faba bean stalk, Maize stalk, Wheat straw, Teff straw, Wheat straw + Teff straw and Saw dust + Faba bean stalk) and two Pasteurization methods (Cold treatment and Hot water treatments). Fourteen treatments have been laid out in a randomized complete design (RCBD), in a complete factorial arrangement with three replications.

d) *Preparation of the Substrates for Seeding*

All substrates were dried before shredding and the dried bean stalk, maize stalk, wheat straw, teff straw and saw dust were shredded or chopped into small pieces of approximately pieces of 2-4 cm size with axes. All naturally dried substrates were subjected to three days sun drying after shredded as correction factor to balance the natural difference in percentage water holding capacity.

The prepared required amount of each substrate has been subjected to different treatments as followed. For cold water treatment, the substrates used in this study were separately soaked in fresh tap water for 24 hrs using 100 liter aluminum cans and achieved sufficient imbibitions. Similarly, pasteurization of shredded substrates has been done by soaking in hot water at 70°C for one hour using aluminum cans.

The Pasteurized substrates separately kept on wire mesh tables and allowed to drain for 4 hours. The water content of the substrates was checked by squeezing in the palm and substrates were used for spawning when the water not dripped on squeezing.

e) *Seeding of Substrates and Spawn Running*

The pasteurized substrates were weighted in to small portion each weighing 4Kg dry weight basis and then filled into 50x70cm² box cemented by plastic sheet. The boxes arranged in a completely randomized block design with three replications keeping 5-10cm spacing between each box. Each box was inoculated with 50g of Spawn of mushroom strain and closed with plastic sheet to prevent possibility of contamination. The inoculated boxes were placed in spawn running room (part of the mush room house from which light excluded) until the substrate in each box fully colonized by mycelium. Mycelium invasion in the boxes visually followed in all substrates. The box has been opened and moved to the fruiting room (Mush room house fitted with fluorescent light) and allowed the development of fruiting bodies.

f) *Mushroom Crop Management and Harvesting*

The bags or substrates blocks were opened by cutting the polythene bags from sides and the plastic was removed. The exposed substrate blocks are kept

$$\text{Percent water holding capacity} = (\text{wet weight} - \text{dry weight} / \text{wet weight of substrate}) \times 100$$

Organic carbon was determined using Walkley and Black Method (Walkley and Black, 1934). In this method after adding different chemicals on ground sample it was titrated by H₂SO₄ and the organic carbon was calculated by taking the volume difference. Percent nitrogen in tissue was determined using micro Kjeldahl method [12]. In this method a ground sample was digested, distilled and titrated and nitrogen was determined by taking the volume difference during titration.

h) *Data collected*

i. *Phenological observations*

Days to the completion of invasion of mycelium (MI) on different substrates, appearance of pin heads formation (PHF), and days to maturation of fruiting bodies formation (FBF) and Cap/pileups diameter from the day of spawning in different substrates were recorded.

ii. *Yield and yield related traits*

a. *Total yield*

Weight of fresh fruiting bodies was harvested from each substrate blocks and measured using sensitive balance. Data on weight of mushrooms from each substrate blocks at first, second and third flush harvesting stages were recorded separately and their total weight was considered as total yield which was also used for calculating biological efficiency.

on their original position as before. The substrate blocks was sprayed twice a day on the bags with water in order to maintain the relative humidity in the range of 80-85% during Cropping period till pin heading and subsequent fruiting when the condition was not fulfilled. Temperature of the cropping rooms was maintained at about above 15°C and monitored by using a thermometer and also sufficient ventilation was provided during fructification to keep low CO₂ level. The fruiting bodies were harvested when the cap has opened up, the gills of the mushroom were visible and when the edge of the cap was slightly rolled inwards.

g) *Analysis of substrates*

All the substrates were analyzed for organic carbon, percentage nitrogen and water holding capacity before spawning and after harvesting of mushroom. Water holding capacity of the substrates was determined by soaking a weight amount of cold water and hot water treated substrates for 24 hrs. The moisture content was determined by weighing the pressed substrate before drying and also after drying in an oven at 70°C to constant weight. The percent moisture content of substrate was determined as given below [11].

b. *Biological efficiency*

The weight of each dry substrate and total fresh mushroom weight per box was recorded separately and then the biological efficiency (BE) of oyster mushrooms in each substrate was calculated as bellow:

$$BE = \frac{\text{Weight of fresh mushrooms}}{\text{Weight of dry substrate}} \times 100$$

c. *Production rate*

On the basis of biological efficiency on each substrate and the time taken in days from spawning to harvesting, the production rate (PR) of oyster mushrooms in each substrate was calculated as below: PR = BE / Time

i) *Nutritional Analysis of Mushroom*

i. *Crude protein*

Ground and dried samples of fruiting bodies of oyster mushroom was analyzed for crude protein (CP) using Kjeldahl method. In this method a sample was digested, distilled and titrated and nitrogen was calculated by taking the volume difference during titration. In order to determine protein, percentage nitrogen was multiplied by 6.25.

ii. *Total ash and Organic matter*

Total ash (TA) content was estimated by heating 2gm of ground mushroom sample at 550°C for 5 hrs. At this temperature all the organic matter burns off as CO₂

and the remaining matter was inorganic ash. The ash content was measured according to following formula:

$$\% \text{ Total ash} = (W3 - W1 / W2 - W1) \times 100$$

Where, W1 = Weight of crucible

W2 = Weight of crucible + Sample

W3 = Weight of crucible + Ash

Based on percentage total ash, percent organic matter will be calculated by subtracting each value from 100.

iii. Percentage moisture content and dry matter

The moisture content of the fruiting bodies was determined as loss in weight which resulted from drying fresh mushroom samples at 70°C to constant weight and this was calculated using the following formula:-

$$\text{Percentage moisture content of fruiting body} = \frac{\text{Fresh weight} - \text{Oven dry weight}}{\text{Fresh weight}} \times 100$$

Based on moisture percentage, the **dry matter percentage** was calculated by subtracting each value from 100.

j) Statistical Data Analysis

All the data collected during the study were subjected to analysis of variance (ANOVA) using SAS statistical software. LSD was used to separate the treatment means at five percent probability level and Simple correlation between the treatments was computed.

IV. RESULTS AND DISCUSSION

a) Results

The present study was conducted to evaluate the effect of different organic substrates and their pasteurization methods on yield and quality of oyster mushroom. Seven substrates (sawdust, dried faba

beanstalk, maize stalk, wheat straw, tef straw, the combination of Saw dust + faba bean stalk and wheat straw+ tef straw+ faba bean stalk) and treated with hot water and cold water as check were evaluated using RCBD design in a factorial combination with three replications.

Carbon and nitrogen content and water holding capacity of the substrates were recorded before and after harvesting of mushrooms. Phenological observations like days to complete mycelium invasion, appearance of pin head and days to maturation of fruiting bodies of mushrooms were recorded. Yield and yield related attributes such as biological efficiency and production rate and quality attributes of mushroom such as percentage moisture content, crude protein (CP) and percent total ash and organic carbon were also estimated.

Table 1 : Effect of substrate type and pasteurization methods on Water holding capacity of substrates at spawning

Pasteurization	Moisture (%) at spawning						
	Substrates						
	Saw Dust	Fababean Stalk	Maize Stalk	Wheat Straw	Teff Straw	WTF Straw	Saw Dust & Fababean
Cold water	1.50n	34.20d	22.94h	16.26i	10.62k	30.78f	7.25l
Hot water	6.20m	32.77e	54.87a	37.17c	23.85g	39.88b	15.88j
LSD(0.05)				0.32			
CV (%)				0.82			

Means with in column followed by the same letter are not significantly different at 5% probability level

Table 2 : Effect of substrate type and pasteurization methods on water holding capacity of substrates at harvest

Pasteurization	Moisture(%) at harvest						
	Substrates						
	Saw dust	Fababean Stalk	Maize Stalk	Wheat straw	Teff straw	WTF straw	Saw dust& Faba bean
Cold water	6.50n	39.13d	27.98h	21.26i	15.62k	35.78f	12.25l
Hot water	11.20m	37.57e	59.96a	42.11c	28.85g	45.00b	20.96j
LSD(0.05)				0.28			
CV(%)				0.52			

Means with in column followed by the same letter are not significantly different at 5% probability level

Table 3 : Carbon and nitrogen content of substrates at spawning and harvest

Substrates	OC(%)		N(%)		C:N	
	at Spawning	at harvest	at Spawning	at harvest	at spawning	at harvest
Saw Dust	74.97a	73.32a	0.08g	0.07f	937.18a	1024.98a
Fababean Stalk	44.85g	43.41f	1.32a	1.02a	33.93f	42.29e
Maize Stalk	50.11c	48.71c	0.65d	0.60c	77.14d	82.02d
Wheat straw	46.13d	44.64d	0.31f	0.27e	147.5b	165.54b
Teff straw	45.37f	43.98e	0.44e	0.39d	103.05c	113.89c
WTF straw	45.89e	44.44d	0.85b	0.74b	60.06e	67.23d
Saw & Faba.	59.75b	58.40b	0.80c	0.73b	74.73d	79.12d
LSD(0.05)	0.108	0.31	0.01	0.03	2.7	24.46
CV(%)	0.17	0.51	1.42	4.69	1.11	9.16

Means with in column followed by the same letter are not significantly different at 5% probability level. WTF straw =Wheat +Teff + Faba bean straw, Saw & Faba = Saw + Faba bean stalk.

Table 4 : Effect of substrate type and pasteurization methods on Growth, BE and PR of oyster mushroom

Substrates	MI(days)	PHF(days)	FBF(days)	CD(cm)	BE(%)	PR
Saw Dust	12.50cd	55.00b	68.66b	2.50b	36.10cd	0.55cd
Fababean Stalk	16.50a	56.66b	66.83bc	7.16a	51.94bcd	0.84bcd
Maize Stalk	12.00d	45.00b	54.83c	7.00a	70.87ab	1.40a
Wheat straw	16.50a	72.83a	84.83a	6.63a	64.70ab	0.79bcd
Teff straw	12.50cd	52.50b	62.50bc	6.66a	32.44d	0.51d
WTF straw	14.00b	54.33b	65.50bc	8.50a	74.59a	1.16ab
Saw & Fab.	13.00c	54.16b	64.00bc	7.16a	53.72bc	0.93bc
LSD(0.05)	0.74	11.92	13.16	3.03	20.31	0.41
Pasteurization methods						
Hot water	13.57b	53.33	64.28	7.03	55.77	0.91
Cold water	14.14a	58.23	69.19	6.00	54.05	0.85
LSD(0.05)	0.39	NS	NS	NS	NS	NS
CV(%)	4.53	18.01	16.62	39.20	31.17	39.03

Means with in column followed by the same letter are not significantly different at 5% probability level;

NS = Non- significant, Biological efficiency, Production rate, WTF = Wheat + Teff + Fababean, Mycelium invasion, Pin head formation, fruiting body formation ,cup diameter,

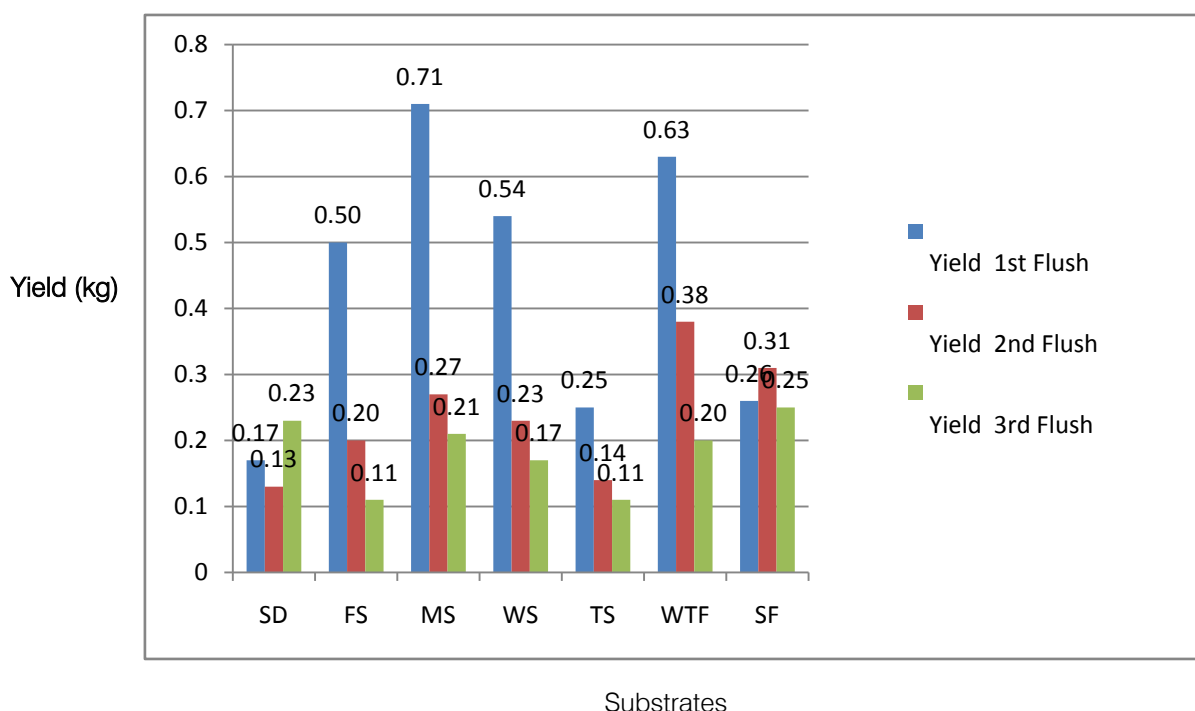


Fig.1 : Yield of Oyster mushrooms at different flushes

(SD= saw dust, FS= faba bean stalk, MS= maize stalk, WS= wheat straw, TS =tef straw, WTF= wheat + teff + faba bean, SF = saw dust + faba bean stalk; the unit of first, second and third flushes are in kg/ 4 kg dry substrate.)

Table 5 : Effect of substrate type and pasteurization methods on Total yield of oyster mushroom

Pasteurization Methods	Total Yield (Kg)						
	Substrates						
	Saw Dust	Faba bean Stalk	Maize Stalk	Wheat straw	Teff straw	WTF straw	SD+ FB
Cold water	0.58l	0.66j	0.83g	1.23c	0.35n	1.40b	0.86f
Hot water	0.50m	0.96e	1.58a	0.67i	0.62k	1.03d	0.80h
LSD(0.05)	0.01						
CV(%)	3.02						

Means with in row and column followed by the same letter are not significantly different at 5% probability level; WTF straw= Wheat + Teff + Faba bean stalk, SD+ FB+= Saw dust +Faba bean

Table 6 : Effect of Substrate type and Pasteurization methods on Protein content of oyster mushroom

Pasteurization methods	Protein Content (%)						
	Substrates						
	Saw Dust	Faba bean Stalk	Maize Stalk	Wheat straw	Teff straw	WTF straw	Saw & Fab.
Cold water	18.56h	27.38a	24.34c	18.03j	18.37i	27.37a	23.00e
Hot water	21.56f	27.38a	20.69g	17.03k	20.69g	26.47b	24.19d
LSD(0.05)	0.05						
CV(%)	0.45						

Means with in column followed by the same letter are not significantly different at 5% probability level

Table 7 : Effect of Substrate type and Pasteurization methods on some quality of oyster mushroom

Substrates	Total Ash(%)	Organic matter(%)	Moisture Cont.(%)	Dry matter cont.(%)
Saw Dust	8.40d	91.59b	85.46ab	14.53bc
Fababean Stalk	8.00d	91.99b	86.09ab	13.74bc
Maize Stalk	7.25e	92.74a	90.34a	8.99c
Wheat straw	11.08a	88.91e	88.27a	11.72bc
Teff straw	10.51b	89.48d	78.41cb	19.91bc
WTF straw	9.94c	90.06c	86.52ab	13.47bc
Saw & Fab.	8.45d	91.54b	73.73c	26.26a
LSD(0.05)	0.49	0.49	8.72	8.26
Pasteurization methods				
Hot water	9.32a	90.67b	84.86	14.61
Cold water	8.86b	91.13a	83.37	16.43
LSD(0.05)	0.26	0.26	NS	NS
CV(%)	4.58	0.45	8.74	44.87

Means with in column followed by the same letter are not significantly different at 5% probability level

V. DISCUSSION

a) Substrate Characteristics

i. Water holding capacity

Results of interaction effect of different substrates in terms of water holding capacity showed that the mean highest and the lowest water holding capacity of the substrates at spawning was for maize stalk (54.87%) and saw dust (1.5%) when treated with hot and cold water, respectively which were significantly different from other treatments (Table 1). Similarly, significantly highest and lowest water holding capacity at harvest was also observed for maize stalk treated with hot water (59.96%) and saw dust treated with cold water (6.5%). This could be due to the soft tissue of maize stalk and woody nature of saw dust, respectively (Table 2).

All substrates were significantly different from one another in terms of water holding capacity both at spawning and harvest. The water holding capacity of all substrates increased after growing mushroom which could be due to the frequent watering of substrates and loosening effect on tissue (Table 1 and Table 2).

The main effects of treatment methods on water holding capacity of substrates indicated that substrates treated with hot water showed significantly more moisture at spawning and harvesting as compared to cold water except in the case of fababean stalk, it was the reverse. This could be due to the ability of hot water to make the tissues of substrates more soft in order to observe more water and due to the decay of substrate by unwanted fungi on substrates treated with cold water (Table 1 and Table 2).

b) Chemical composition of the substrates

i. Carbon content of the substrates

The results of carbon content of the substrates measured before spawning and after harvest are presented in Table 3. Maximum carbon was recorded for saw dust at spawning (74.97%) and after harvesting (73.32%) which was significantly higher than all other treatments. Similar results for saw dust were reported [13]. The lowest carbon content at spawning (44.85%) and after harvesting (43.41%) was observed for faba bean stalk. In all organic substrates a slight reduction of organic carbon was observed from spawning to harvest which may be due to the utilization of carbon by the growing mushroom. Cold water treatment and hot water treatment did not significantly influence the carbon content of the substrates.

ii. Nitrogen content of the substrates

Results of measurement of nitrogen in different substrates showed maximum nitrogen content was recorded for the faba bean stalk at spawning (1.32%) and harvest (1.02) which was significantly higher than nitrogen content of all other substrates (Table 3). This result is in line with [14] who reported the nitrogen content of leguminous substrates was higher than that of non-leguminous substrates. In contrast to the nitrogen content of dried faba bean stalk, the significantly lower nitrogen content was recorded for saw dust both at spawning (0.08%) and harvest (0.07%). All substrates are significantly different from each other in terms of their nitrogen contents at spawning. Significant difference was not observed between the combination of wheat + teff straw + fababean stalk and saw + faba bean stalk in terms of nitrogen content at harvesting. In all organic substrates a reduction in

nitrogen content was recorded from spawning to harvesting of mushrooms which may be attributed to the utilization of nitrogen by the growing mushroom. Because of high carbon content and low nitrogen, C:N ratio for saw dust was found to be widest and that of dried faba bean stalk was narrowest both at spawning and harvest (Table 3). These results were in line with Richard who reported a C:N ratio of 600:1 and 16:1 for sawdust and dried bean pod husk, respectively. Significant difference was not observed between maize stalk and saw dust + faba bean in terms of C:N ratio at spawning and between wheat +teff + faba bean and Saw dust + faba bean at harvest statically. In all organic substrates a significant increase in C:N ratio was recorded from spawning to harvesting.

c) *Effect of Substrates and Pasteurization Methods on Growth and Yield*

i. *Mycelium invasion*

The fastest days to mycelium invasion (MI) of different organic substrates was recorded for maize stalk (12.00 days) however it was statistically at par with saw dust and bean straw (Table 4). Faba bean stalk and wheat straw were found to be invaded by inoculated mushroom spawn at the slowest rate (16.50 days).

Hot water treated substrates had significantly earlier invasion by mycelium than cold water. This could be due to the release of easily available nutrients from substrates and the reduction of unwanted green mould by effective hot water pasteurization. The pasteurization methods recorded significant differences with regard to days to mycelium invasion (Table 4). Interaction of substrates with pasteurization methods was non-significant for days to mycelium invasion.

ii. *Pin head formation*

With respect to the effect of different substrates on pin head formation, the slowest pin head formation (PHF) after spawning was observed in wheat straw (72.83 days). Significant difference was not observed among other substrates statically but Maize stalk recorded early pin head formation compared to others.

Significant difference was not observed between cold water and Hot water methods with regard to pin head formation statically (Table 4). But Hot water treated substrates had significantly earlier pin-head formation than cold water. This could be due to effect of hot water on chemical composition of substrates which may have increased availability of essential nutrients to the growing mushroom. There is no interaction effect between different substrates and pasteurization methods on pin head formation of the mushroom.

iii. *Fruiting body formation*

There was also no interaction effect between the substrates and pasteurization methods on fruiting body formation of the mushroom. The fastest fruiting body formation (FBF) after spawning was observed on

maize stalk (54.83 days) followed by teff straw however; teff straw was statistically at par with the other substrates except wheat straw. The slowest fruiting body formation was recorded on wheat straw (84.83 days). No significance difference was observed between Hot water and cold water treated substrates statically but numerically when it was observed Hot water treated substrates had significantly earlier ($p < 0.05$) fruiting body formation than cold water (Table 4). This could be due to the effective pasteurization and increase in availability of nutrients due to hot water treatment of substrates.

iv. *Yield of oyster mushroom*

Harvesting of the mushrooms growing in different substrates was completed in three consecutive harvesting operations till all the mushrooms were harvested from each substrate. The yield of mushrooms in each flush and the total yield for each substrate are presented in Fig.1. The highest yield of mushrooms in first flush was obtained in maize stalk (0.71kg), in second flush was obtained in, Wheat + Teff + Fababean stalk (0.38 kg) and in third flush was obtained in saw dust + faba bean stalk (0.25kg). The lowest yield during the first and second flush was obtained for mushroom grown on saw dust 0.17 and 0.13 kg respectively, whereas the lowest yield in third flush was obtained for mushroom grown on faba bean stalk (0.11kg). In all cases yield showed a declining trend from the first flush to third flush. This could be due to the decreasing nutrient content of the substrate consumed by mushroom during growth from one flush stage to the next. But in the case of saw dust and saw dust + fababean stalk the highest yield was obtained in third and second flush respectively. This could be due to the hardness and less consumable of saw dust in the first flush.

v. *Total yield, biological efficiency and production rate*

Significant ($p < 0.05$) variation was observed in total yield of oyster mushrooms as affected by the interaction of different substrates and Pasterualization methods used in this study (Table 5). The maize stalk Pasterualized with hot water gave significantly the highest yield (1.58 kg/ 4kg of dry substrate) while teff straw treated with cold water resulted in the lowest yield (0.35 kg/ 4kg of dry substrate). The highest yield of mushroom grown on maize stalk treated with the hot water and the lowest yield grown on teff straw treated with cold water may be due to the narrow C:N ratio of the maize stalk which provided enough nutrients and could be due to the effective pasteurization of the substrate with hot water in order to protecting unnecessary fungal pathogens that may occurred during the growing season of mushrooms.

Biological efficiency of various treatment combinations was calculated on the basis of percentage fresh mushroom production per unit dry weight of

substrate. Significantly ($p < 0.05$) higher biological efficiency (74.59%) was recorded for mushrooms grown on the combination of wheat + teff + faba bean stalk (Table 4). This value is close to that mentioned by Kumar [15] who observed a biological efficiency in the range of 80 to 150% for oyster mushrooms grown in leguminous substrates. Similarly a Biological efficiencies range of 73 to 100 was reported by Tripathi. Significantly lower biological efficiency (32.44%) was recorded for the mushrooms grown on teff straw (Table 4). High value of biological efficiency was observed for mushrooms grown on narrow C:N ratio substrates such as faba bean stalk and Maize stalk and low value of BE was observed for mushrooms grown on wide C:N ratio substrates such as saw dust (937:1). This implies that nitrogen is also important for improving the yield (Table 4).

Results from main effect of pasteurization methods revealed that no-significant difference among pasteurization methods was observed in terms of BE statically. But hot water (55.77%) proved to be superior to cold water (54.05%) treatments in terms of BE (Table 4). This may be due to the effective pasteurization and increasing the availability of nutrients due to hot water treatment which contributed towards high biological efficiency.

Significantly ($p < 0.05$) the higher production rate (1.40) was recorded for maize stalk substrate followed by the mixture of wheat + tef straws + faba bean substrates (Table 4). The lowest production rate (0.51) was recorded for teff straw. However, significant difference was not observed between the wheat straw, faba bean stalk and the mixture of saw dust + faba bean stalk in terms of production rate statically. Teff straw, Saw dust and wheat straw showed lower PR as compared to the others. There was no significance difference ($p < 0.05$) was observed between pasteurization methods statically but hot water treatment gave significantly ($p < 0.05$) higher production rate compared to cold water (Table 4). This could be due to the effective pasteurization which reduced competition for the growing mushrooms and also the release of available nutrients due to hot water treatment.

d) *Effect of Substrates and Pasteurization Methods on Quality Parameters of Oyster Mushrooms*

In the present study, crude protein, total ash, organic matter, moisture content and dry matter percentage of oyster mushrooms at harvest were measured to assess quality of mushrooms grown on different substrates treated /Pasteurized with hot water and cold water.

i. *Crude protein*

The interaction effect (Table 6) of substrates and pasterualization methods on crude protein content of oyster mushroom in this study showed that the protein content (27.38%) of mushrooms grown on dried

faba bean stalk treated by cold water and cold water were the highest and significantly ($p < 0.05$) different from other combinations except in the case of the mixture of wheat, teff and faba bean stalk treated with cold water statically. The nitrogen contents of substrates directly correlated with the protein content of the oyster mushroom (refer Table 3 and 6). Similar values for crude protein of oyster mushrooms have been reported [16]. The positive correlation of high protein content with high nitrogen content of substrates implies that adequate nitrogen in the substrate is essential for synthesis of protein in mushroom fruiting bodies. The lowest value of crude protein was observed in the cold water treated saw dust (18.56%). Significant differences were also not observed between hot water treated maize stalk and teff straw.

ii. *Ash content*

The highest ash content (11.08%) was recorded for mushrooms grown on wheat straw followed by ash content of mushroom grown on tef and the mixture of wheat, teff and faba bean straw, respectively. This value was close to the value mentioned [17] who reported 11.5% ash for mushrooms grown on wheat straw. Contrary to the ash value of mushroom grown on wheat straw, lower ash content (7.25%) was recorded for mushroom grown on maize stalk (Table 7). There was no significance difference observed among ash content of mushroom grown on saw dust, faba bean and the mixture of fababean and saw dust. The ash content of oyster mushroom recorded in this study ranged from 7.25% to 11.08%. In contrary, [18] reported 6.20% and 5.90% ash for oyster mushrooms grown on paddy straw and corn stalk, respectively. This difference could be due to straw type and method of analysis. On the other hand the result of this study is in agreement with [19] who reported ash content of 8.80% for oyster mushroom and 7.20% for *P. sajor-caju* respectively. Significant difference was observed among substrates.

Hot water treated substrates produced mushroom with significantly ($p < 0.05$) higher ash content compared to cold treatment ones. It may be due to increased availability of mineral elements because of hot water treatment (Table 7).

iii. *Percentage organic matter*

Organic matter content of oyster mushrooms was significantly ($p < 0.01$) affected by different substrates and their pasteurization methods. The highest organic matter (92.74%) was recorded for mushrooms grown on maize stalk substrate whereas the lowest organic matter (88.26%) was recorded for mushroom grown on wheat straw (88.91%). This may be due to highest and the lowest ash content of mushroom grown on maize stalk and wheat straw respectively (Table 7). No-significant difference was observed among the substrates such as saw dust, faba bean stalk

and the mixture of saw dust + faba bean stalk in relation to organic matter (Table 7).

Cold water treatment resulted in the highest organic matter (91.13%) while hot water treatment was the lowest (90.67%). This may be due to highest and the lowest ash content of mushroom treated by hot water and cold water, respectively (Table 7).

iv. Percentage moisture content

Percentage moisture content of oyster mushrooms grown in different substrates ranged from 73.73-90.34%. The highest moisture content of fresh mushrooms (90.34%) at harvest was recorded for mushroom grown on maize stalk which was significantly higher than other substrates which was at par with wheat straw (Table 7). The moisture content obtained in this study is close to the value mentioned [20], who reported 89% moisture for mushrooms grown on paddy straw. The lowest moisture content (73.73%) was recorded for mushroom grown on the substrate mixture of saw dust and faba bean stalk. These results are in line with that of [21] who reported a range of 70-90.9% moisture.

Non-significant difference was observed for moisture content of mushroom between the pasteurization methods statically but the mean value for moisture content of mushrooms under pasteurization methods of substrates resulted in higher moisture (84.86%) was recorder for mushrooms grown on hot water treatments (Table 7).

v. Dry matter percentage

The highest dry matter percentage of oyster mushroom (26.26%) was recorded for mushroom grown on substrates mixture of saw dust and faba bean stalk (Table 7). This may be due to the lowest moisture content of fresh mushroom (73.73%) grown on this substrate. The lowest percentage dry matter (8.99%) was found for mushroom grown on maize stalk in apart with other substrates. The dry matter percentage of oyster mushroom found in this study ranged from 8.99 - 26.26 g. These results are in agreement with [22] who reported a range of 5.3-14.8g dry matter percentage.

The mean value for dry matter percentage of mushrooms under two pasteurization methods of substrates showed that non-significant statically but cold treatment resulted in the highest percentage dry matter (16.43%) while hot water treatment was the lowest (14.61%).

VI. SUMMARY AND CONCLUSIONS

Growth, yield and quality of mushrooms are substantially influenced by the substrate type on which it is grown. Studies on the effect of locally available substrates along with pasteurization methods on yield and quality of mushroom through systematic research are vital for optimizing productivity and choice of best quality mushrooms. In view of this, the present research

work was conducted at the Arsi University, College of Agriculture and Environmental Science Research and Training Center during the year 2013-2014. A factorial experiment involving two treatment methods (cold water, and hot water) and seven substrates (sawdust, dried faba bean stalk, shredded maize stalk, wheat, tef straw, wheat + teff + faba bean stalk, and Saw dust + faba beanstalk + Saw dust) in a randomized complete block design with three replications was used.

All substrates were dried before shredding and the dried bean stalk, maize stalk, wheat straw, teff straw and saw dust were shredded or chopped into small pieces of approximately pieces of 2-4 cm size with axes. The required amount of each substrate has been subjected to different treatments such as cold water treatment, the substrates used in this study were separately soaked in fresh tap water for 24 hrs using 100 liter aluminum cans and achieved sufficient imbibitions. Similarly, pasteurization of shredded substrates has been done by soaking in hot water at 70°C for one hour using aluminum cans.

After proper draining, the pasteurized substrates were weighted in to small portion each weighing 4Kg dry weight basis and inoculated with oyster mushroom spawn made on wheat grains and then filled into 50x70cm² box cemented by plastic sheet. The temperature of the cropping room was maintained around 15-20°C and the relative humidity (80-85%) was adjusted by frequently spraying water over the wall of cropping room and on the boxes.

Substrates were analyzed for water holding capacity before and after growing the mushroom, Organic carbon was determined by Walkley and Black Method (Walkley and Black, 1934) and percent nitrogen in tissue was determined using micro Kjeldahl method (Black, 1965) both before and after growing mushroom. Days to complete mycelium invasion, appearance of pin heads, and onset of fruiting bodies in different substrates were recorded. Yield parameters such as biological efficiency (BE) and production rate (PR) of oyster mushrooms in each substrate was calculated after weighing fresh fruiting bodies using weight balance and dividing by dry weight of substrates. Quality parameters such as crude protein (CP), and total ash (TA) content were analyzed using Kjeldahl method and by heating known weight of grinded mushroom sample at 550°C for 5 hrs, respectively. Data were subjected to analysis of variance (ANOVA) using SAS statistical software. LSD was used to separate the treatment means at five percent probability level and Simple correlation between the treatments was computed.

The highest and the lowest water holding capacity of the substrates were recorded in maize treated with hot water (54.87 % and 59.96%) and saw dust treated with cold water (1.5% and 6.5%) both at spawning and harvesting respectively. Maximum carbon content was recorded for saw dust both at spawning

(74.97%) and harvest (73.32%) while lowest carbon at spawning (44.85%) and at harvest (43.41%) were observed for dried faba bean stalk while lowest nitrogen (0.08%) at spawning and harvest (0.07%) were observed for saw dust. Therefore widest C:N ratio was recorded for saw dust at spawning (937.18:1) and harvest (1024.98:1) while the narrowest C:N ratio was for faba bean stalk at spawning (33.93:1) and harvest (42.29:1).

The fastest mycelium invention (12 days), pin head formation (45 days) and fruiting body formation (54.83 days) was recorded for maize stalk straw while the lowest mycelium invention (16.50 days), pin head formation (72.83 days) and fruiting body formation (84.83 days) was recorded in wheat straw. The highest cup diameter (8.50cm) and the lowest (2.50cm) was observed in the substrate mixture of wheat+ teff+ faba bean stalk and saw dust respectively. Hot water treated maize stalk followed by cold water treated the mixture substrates of wheat +teff+ faba bean stalk gave significantly higher total yield of (1.5 kg/ 4 kg of dry substrate) and (1.4 kg/4 kg of dry substrate), respectively, while the lowest yield was obtained on cold water treated sawdust (0.35 kg / 4kg of dry substrate). The mixture of substrates of wheat +teff +faba bean stalk was significantly better (74.59%) compared to all other substrates in terms of biological efficiency and the lowest biological efficiency (32.44%) was recorded in the case of teff straw. Significantly the highest production rate (1.40) was recorded in maize stalk, while the lowest production rate was observed in teff straw (0.51).

The protein content of mushrooms grown on bean pod husk treated with cold and hot water (27.38%) was the highest in par with the substrate mixture of wheat + teff + faba bean stalk treated with cold water statically and significantly different from other treatment combinations. The lowest value of crude protein was observed in the hot water treated wheat straw (17.03%).

Ash content of mushrooms grown on wheat straw was the highest (11.08%) and significantly different from other substrates. Whereas, the lowest ash content (7.25%) was observed in mushroom grown on the maize stalk and the hot water treated substrates gave the higher ash content (9.32%) as compared to the cold water treated substances (8.86).

Organic matter content of mushrooms grown on maize was highest (92.74%) and significantly different from other substrates. While the least value (88.91%), was observed in mushroom grown on wheat straw. The mushroom grown on cold water treated substrates gave the higher organic matter content as compared to hot water treated substrates. Moisture content of fresh mushrooms grown on maize stalk was the highest (90.34%) and significantly different from other substrates. The lowest significantly different moisture content (73.73%) of fresh mushrooms was observed in substrate mixture of saw dust + faba bean stalk and inversely the highest (26.26%) percentage dry matter of

mushrooms grown this substrate was recorded and significantly ($p < 0.05$) different from other substrates and substrate combination. The lowest significantly ($p < 0.05$) different percentage dry matter (8.99%) was observed in mushroom grown on maize stalk.

In general, results of the present study showed that hot water pasteurization of the substrates followed was effective to reduce competent micro organisms. Organic residues having wide C:N ratio and excessive WHC reduced the yield and quality of mushrooms, however, the level of contamination in substrates with wide C:N ratio was relatively less. On the other hand, crop residues with narrow C:N ratio such as dried faba bean pod stalk and maize stalk had a positive correlation with yield and quality of mushrooms but with some risk of relatively higher contamination due presence of readily available nutrients. Thus, a sort of compromise should be reached for yield and selecting different substrates for growing mushrooms, so as to obtain economical yield without much effect on the quality. The present study was conducted with a limited number of substrates. However there is a lot of scope for conducting similar studies on different substrates which are easily available locally to the farmers interested to grow mushrooms under different agro climatic conditions across the country.

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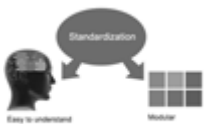
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- Submitting a manuscript with pages out of sequence

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- Use standard writing style including articles ("a", "the," etc.)
- Keep on paying attention on the research topic of the paper
- Use paragraphs to split each significant point (excluding for the abstract)
- Align the primary line of each section
- Present your points in sound order
- Use present tense to report well accepted
- Use past tense to describe specific results
- Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives
- Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.



Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript-- must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
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- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
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- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

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Approach:

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This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

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- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
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- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

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- Report the method (not particulars of each process that engaged the same methodology)
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- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
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What to keep away from

- Resources and methods are not a set of information.
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The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently. You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.



Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
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- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

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- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

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- Put figures and tables, appropriately numbered, in order at the end of the report
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- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
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- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.



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Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
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References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring



INDEX

A

Azadirachta · 7, 8, 13, 14, 15, 16

I

Invasion · 25, 28, 30, 31, 34, 37

M

Meliaceae · 8

Mycelium · 25, 28, 30, 34, 37, 39

O

Ostreatus · 25

P

Pleurotus · 25, 27, 40, XLI

R

Recalcitrant · 8, 10, 12, 13, 14, 15, 16

S

Shed · 8, 10

Symposium · 1



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