

Study of Carrots for their Storage Environment and its Effect on Biochemical Changes during Pathogenesis Cited by Post-harvest Fungi and their Control by using Low Cost Method which Prolonged Shelf-life

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Abstract: *The optimum temperature and relative humidity for the development of carrot root rot during storage were found to be 20°C and 60%, respectively, and rot development was mostly increased as these environmental factors increased in magnitude. Both, temperature and relative humidity, were responsible for rot development in diameter rotted area as a result bio-deterioration in carrot roots inoculated with R. arrhizus, S. Rolfsii and F. solani and incubated at 3 days intervals. The protein content in inoculated carrot roots at 3 days of incubation period was low significantly compared to un-inoculated carrot roots (control). However, there was significant increase in protein content in inoculated carrot roots over control at 6 and 9 days of incubation period. Carrots inoculated with three pathogens showed gradual increase in phenol contents from 3 to 9 days incubation over control. Statistically significant increase in phenol content of carrot roots inoculated with three pathogens was found from 3 to 9 days incubation period. The amounts of total sugar and non-reducing sugars in rotted carrot roots except carrot root rot with S. rolfsii, were substantially decreased over healthy carrot root whereas reducing sugars in inoculated carrot roots were significantly high than un-inoculated roots of carrot. Fluctuations in total, reducing and non-reducing sugar content was observed as the storage period was prolonged from 3-9 days. In addition, the experiment on rot management and shelf-life of carrots packed at open bunch, sealed polythene bags and perforated polythene bags was done by comparing low cost zero energy cool chamber and ambient conditions. Carrot root rot caused by three pathogens was significantly controlled at cool chamber where temperature and relative humidity were maintained at 15° C – 17° C and 85 – 98 % RH, respectively. Storage life of carrots packed was significantly increased at the cool chamber over ambient temperature. Carrots stored in perforated polythene bags showed long shelf- life*

Keywords: Carrot root rot, Temperature, RH, Biochemical changes, Post-harvest fungi and Evaporating cool chamber

1. Introduction

Carrot is a biennial herb with conical fleshy tap root and cultivated throughout India. In south and central India, it is cultivated largely in hill stations. The yield of carrot varies with the variety (20,000 to 30,000 kg/hectare). It has medicinal value and is supposed to have anti-cataract and anti-cancerous properties (Sen, 1996; Panday, 2001). Carrots are an excellent source of antioxidant compounds, and the richest vegetable source of the pro- vitamin A carotenes. Its antioxidant compounds help to protect against cardiovascular diseases, cancer, ageing process, ultraviolet and also promote good vision, especially night vision. Fruits and vegetables are best sources of food energy due to the presence of sugars amino acids, vitamins and other essential nutrients. Being perishable and vulnerable they become susceptible to invasion by post-harvest pathogens during storage, transport etc. (Llyas *et al.*, 2007). The nutrients stored in fruits and vegetables are utilized by microorganisms after converting them into simpler ones (Bagwan *et al.*, 2010). Post-harvest infection of fruits and vegetables may induce a number of alterations in their physiological and biochemical processes or in the host tissues constituents, as a result of host-pathogen interactions (Bagwan, 2006; Chourasia *et al.*, 2010). The struggle between the capacity of pathogen attack and the defense capability of the host will dictate the success or the failure of the infection. In various host

pathogen systems, fruit infection results in the decrease, increase or total disappearance of the biochemical content of the fruits (Barkai- Golan, 2005).

Numerous studies conducted during last few decades have established, beyond doubt that the infection results in both quantitative and qualitative biochemical changes (Prasad *et al.*, 1996; Odebode and Sansui 1996; Odebode and Unachulwu, 1997; Ray and Hammerschmidt, 1998; Dutta and Chatterjee, 2002; Barkai-Golan, 2005; Bagwan, 2006; Sarkar, 2009 and Rathod (2010).

In addition that out of the various environmental factors temperature and humidity plays a significant role in the host- pathogen interaction (Patel and Pathak, 1996; Singh *et al.*, 1990; Odebode and Unachulwu, 1997; Mukherjee and Raghun, 1997; Fatima *et al.*, 2006; Cheeraninch *et al.*, 2007; Cherian *et al.*, 2007 and Yusuf and Okusanya, 2008). Therefore, refrigerated and cool storage rooms are used in order to control post- harvest decay during storage. However, cold storage methods are expensive and cannot affordable for local market vendors and farmers in developing countries like India. Hence, low cost methods are preferable which are economically feasible to handle on a small scale (Babarinsa *et al.*, 1997; Jitender-Kumar *et al.*, 1999). Roy and Krudiya (1983) and Roy (1989) worked on Post-harvest technology of vegetable crops in India by using low cost evaporative cool chamber. Pal, Roy *et al.*, (1991) reported that effect of pre- and post-

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harvest treatments on shelf-life and quality of carrot under different storage conditions by using low cost cool chamber. Also, different storage methods were studied by various workers to enhance shelf-life of carrots. Negi *et al.*, (2000) noticed that the fresh carrots (*Daucus carota* L. cv. 'Nantes') were packed in Netlon and ventilated Low-density poly ethylene bags and stored in ambient (22-37.5° C), cool chamber (17-21° C) and cool store (7.5-8.5° C) conditions. The shelf life of fresh carrots varied from 3-20 days depending on the packing and storage condition.

Singh *et al.*, (2010) reported the effect of Zero energy cool chamber and post-harvest treatments on shelf-life of fruits under semi- arid environment of Western India, part 2. Indian gooseberry fruits.

In conclusion, Little information is available on the storage diseases of *Colocasia*, onion, garlic, carrot, beet root and potato (Coursey and Booth, 1972; Ruppel, 1981; Usha Rani, 1982). The post-harvest losses are much higher (40 to 50%) in countries like India where facilities for transport and storage are inadequate or unscientific (Odebode and Unalchuvu, 1997; Sharma *et al.*, 2009; Fatima *et al.*, 2009; Sarkar, 2009 and Rathod, 2010).

In view of the magnitude of losses by post- harvest fungal pathogens, an attempt has been made in the present study to investigate the post-harvest diseases of tuber vegetable like carrot collected from Telangana. In this study the fungi responsible for the rot of carrot roots have been isolated and identified and tested its pathogenicity. The effects of temperature, relative humidity on rot development caused by fungal organisms that are responsible for nutritional changes in carrot roots during storage were studied. Also, arrest of fungal pathogens and shelf-life of carrots were studied at both condition, room temperature and zero energy cool chamber.

2. Materials and Methods

Collection and isolation of rot inducing mycoflora

Samples of rotted carrots were collected into fresh polythene bags from different local markets and storage godowns of Mahabubnagar (MBNR), Nalgonda (NLG), Nizamabad (NZN), Karimnagar (KNR) and Rangareddy (RR) districts of Telangana. Care was taken to avoid completely rotten vegetables in order to avoid secondary growth of the fungi. The disease symptoms were carefully recorded. Samples of rotted root vegetables were surface sterilized with 70% alcohol for 60 sec or 0.01 % sodium hypochlorite solution for two minutes and washed thrice with sterile distilled water and blotted dry with sterile filter paper. The infected parts were sliced into small cubes and plated onto Petri dishes having different media. Triplicates were maintained. The plates were incubated at 28±2°C for 3 days. Petri dishes were observed daily and colonies of fungi were chosen. The isolated fungi were purified using single spore technique, and then kept in a refrigerator on PDA slants in McCartney bottles (Gams *et al.*, 1998). Morphological and cultural characters of

organisms were also recorded. Pure colonies of fungal isolates were identified according to Ellis (1971, 1976), Domsch *et al.*, (1980) and Moubasher (1993), Nagamani *et al.*, (2006) and confirmed their pathogenicity as per Kochs postulates.

Pathogenicity test

Pathogenicity test was conducted by following the method given by Odebode and Unalchuvu (1997). Healthy vegetables were swabbed with cotton wool soaked with 70% alcohol and washed with several changes of sterile distilled water. A hole was aseptically bored into each root using a 5 mm diameter cork borer and the core was carefully removed. One 3 mm mycelial disc of the 7 day old culture was cut from the edge of the colony and inserted into the hole in the root and the core was then replaced the wound was sealed off by means of petroleum jelly. The other technique used was to dip untreated roots into a spore/mycelia suspension of the isolates. The inoculated vegetables were arranged in sets of three in clean polythene bags, containing wet absorbent cotton wool to create a micro-humidity chamber, and were incubated at 28±2° C for 7 days. Following this, cuts along the plane of inoculation were made using a sterile scalpel and rot was assessed by measuring the diameter of rot for each fungal isolate.

The pure isolated fungi were identified using cultural and morphological features such as spore size, length, conidia formation, colony colour and diameter of colonies (Barnett and Hunter, 1972 (used for genus identification); Ellis, 1971 and 1976; Domsch *et al.*, 1993; Moubasher, 1993; Pitt and Hocking, 1997; Klich, 2002; Nagamani *et al.*, 2006; Samson and Varga, 2007).

Effect of physical factors (Temperature and relative humidity) on rot enhancement

The effect of different temperatures and humidity levels on the rate of disease advancement was studied by using the methods of Odebode and Unalchuvu (1997) on commonly occurring pathogens such as, *Rhizopus arrhizus*, *Sclerotium rolfsii* and *Fusarium solani* which are causing storage rots on carrot. Carrot was artificially inoculated with above mentioned fungi individually to find the effect of different RH and temperature levels on disease advancement.

Almost of same size and same age grouped of carrots were selected from the Local markets. Apparently healthy vegetables were surfaced sterilized with 70% alcohol for 60 sec and washed in sterile distilled water and blotted dry with sterile filter paper. They were checked for internal contamination. Later they were artificially inoculated with the above pathogens, and incubated for 7 days at different temperature (10°C, 15°C, 20°C, 30°C and 35°C) and relative humidity (40%, 50%, 60%, 70%, 80% and 90%) levels. Three replicates were maintained in each case. Samples were collected at 7th day, cuts were made along the plane of inoculation using a sterile scalpel and rot was assessed by measuring the diameter of rot for each fungal isolate.

The effect of relative humidity on rot development was studied for 7 days. Different levels of relative humidity were maintained in air tight desiccators using H₂SO₄ and water at different dilutions (Buxton and Mellanby, 1934).

Biochemical changes during rot development

Biochemical changes in the host tissue due to pathogenesis were studied in relation to three post-harvest pathogens on carrot was inoculated with *Fusarium solani*, *Rhizopus arrhizus* and *Sclerotium rolfsii*. Healthy hosts were surface sterilized and artificially inoculated with the pathogens and incubated at 28[±] 2° C in moist chamber for 7 days. Five replicates were maintained for each series including the control. Ethanol extract of fresh host tissue was prepared at the beginning of the experiment, while for healthy and infected tissue; the ethanol extract was prepared at every three days interval from the incubated carrots. Ethanol extract was prepared from healthy and inoculated carrots for the estimation of biochemical changes or bio deterioration.

Preparation of homogenate

For the preparation of extracts 5g of host tissue was taken and crushed thoroughly in a ground glass homogenizer in 20 ml 70% ethanol. These homogenates were used for further estimations of proteins, phenols and sugars accordingly following the procedures

Proteins (Lowry *et al.*, 1951)

Soluble proteins were estimated by adopting the method of Lowry *et al.*, 1951 using folin-cioalteau reagent.

Estimation of Phenols

Total phenols were extracted and estimated by using the procedure of Swain and Hillis (1959).

Total sugars estimation

Total sugars were quantified by using the procedure was given by Yoshida *et al.*, (1976)

Estimation of Reducing Sugars

Reducing sugars were estimated by Nelson Method (1944). Nelson reagent was used for the estimation of reducing sugars.

Estimation of non-reducing sugars

The amount of non-reducing sugars was calculated by using the following formulae as given by Loomis and Shull (1937)

Non-reducing sugars = (Total sugars – free reducing sugars) × 0.95

The amount of non-reducing sugars was expressed as glucose equivalents in terms of mg / g fresh weight.

Effect of brick wall evaporative cool chamber

In order to find out reduction in rot development and enhance shelf-life of carrots, experiment was carried out by using brick wall evaporative cool chamber which was constructed with modified method of Babarinsa *et al.*, (1997) with locally available materials in rural areas in various agroclimatic conditions where humidity is low and temperatures are high, materials like generated from a mixture of formaldehyde and KMnO₄ crystals. Inoculated and un-inoculated carrot samples were examined daily for decay and enhancement of shelf-life, respectively.

3. Results and Discussion

Fungi were isolated from rotten carrot roots and tested for its pathogenicity

Three different fungi were isolated from rotten carrot roots collected from local markets of Telangana, India. The isolated fungi were *Rhizopus arrhizus*, *Sclerotium rolfsii* and *Fusarium solani*. Three fungi were pathogenic with rot diameter of 182.66 mm, 147.66 mm and 141.33 mm respectively, at room temperature (Table-1).

Temperature

At 10^oC, 15^oC and 40^oC there was no rot development (Table-1). The optimum temperature for rot development was between bricks and bamboo frame, khus-khus and gunny bags were used. This chamber can provide inside temperature and relative humidity in between 12° C - 15° C and 85- 90%, respectively. Carrots were washed 20 C -35 C, while maximum rot appeared at 35^oC. *Rhizopus arrhizus* and *S. rolfsii* showed maximum rot at 35^oC whereas *F.solani* exhibited at 30^oC. The maximum and minimum rot was observed by *S. rolfsii* at thoroughly under tap water, any vegetables with skin blemishes were removed. 35^oC and 20^oC, respectively. In the present study, the effect of temperature on the development of rot showed that no fungal.

In order to find rot development and shelf-life at both conditions, Inoculated carrots for rot and un-inoculated carrots for shelf-life were placed in ambient conditions (open laboratory) and brick wall cool chamber. Each treatment was replicated three times, and each replicate contained 20 bags of three carrots. Each treatment replicate was scored in one of two systems: ambient, 17 - 34° C and 16 -40 % relative humidity (RH); in brick cooler, at 15° C – 17° C and 85 – 98 % RH.

Before loading, the storage chamber of the brick cooler was sterilized with fumes pathogen caused carrot root rot at 10 C, 20 C and 40^oC. This lower and higher temperature is known to cause a reduction in respiration activity and other enzyme reactions which can help to fungal growth.

Relative Humidity (RH)

The relative humidity had a great impact on the subsequent rot development within 7 days. The maximum

rot development occurred between 70% - 90% RH (Table-2). *Rhizopus arrhizus* and *F. solani* showed maximum rot development at 90% whereas *S.rolfsii* had significant rot at 70% RH. Minimum rot development was observed with *F. solani* at 40% RH. For the development of rot, *R. arrhizus* needs 35^oC and 90% RH, *S. rolfsii* requires 35^oC and 70% of RH and *F. solani* needs 30^oC & 90%. For the development of rot, *R. arrhizus*, *S. rolfsii* and *F. solani* require 35^oC and 90% RH, 35 C and 70% of RH and 30^oC & 90%, respectively. The results showed that there is a positive and significant relationship among RH, temperature and rot development.

Proteins

Results of biochemical changes in carrot inoculated with *Fusarium solani*, *Rhizopus arrhizus* and *Sclerotium rolfsii* and incubated for 3, 6 and 9 days are presented in the Table 3. In general, carrots collected from market showed maximum protein content at 3 days incubation gradually reducing with increase in incubation period. The protein content in carrot inoculated with *F. solani*, *R. arrhizus* and *S. rolfsii* at 3 days of incubation period was low significantly compared to healthy un- inoculated carrot. However, there was significant increase in protein content in the pathogen inoculated carrot over control at 6 and 9 days of incubation period over un- inoculated control.

Phenols

It is clear from the data presented in the Table 8 that there was significant increase in phenol content with increase in period of incubation recording maximum at the 9 days incubation comparatively in all the un-inoculated controls of carrot. Carrots inoculated with three pathogens showed gradual increase in phenol contents from 3 to 9 days incubation over un-inoculated control.

Total sugars (mg/g)

It is evident that from the data presented in Table 4 that the amounts of total sugars in rotted carrot roots, except carrot root rot with *S. rolfsii*, were substantially decreased over un-inoculated carrot roots. Total sugar content was gradually decreased from 3 to 9 days of incubation period in healthy and carrot roots inoculated with *F. solani*. However, carrots inoculated with *R. arrhizus* showed gradual decrease in total sugars from 3 to 6 days and later subsequent increased amount was observed at 9 th day of incubation period.

In *S. rolfsii* inoculated carrots there was a reduction in total sugars from 3 and 9 days incubation with a subsequent increase at 6 days incubation period, compared to un- inoculated control.

Reducing sugars

Table 4 revealed that all the three pathogen inoculated carrots had higher amount of reducing sugars over un-inoculated control at all the three incubation periods. In *R. arrhizus* and *F. solani* inoculated carrots, there was significant increase in reducing sugar content with increase in incubation period. *S. rolfsii* inoculated samples showed considerable reduction from 3 to 6 days incubation subsequently increased at 9th day of incubation.

Non-Reducing sugars (mg/g)

All inoculated carrots except *S. rolfsii* inoculated carrots, showed significant reduction in non-reducing sugars over un- inoculated controls (Table 4). Non-reducing sugar content was gradually decreased from 3 to 9 days of incubation period in healthy as well as inoculated carrot roots except carrots inoculated with *S. rolfsii*. However, carrot roots inoculated with *S. rolfsii* showed rapid increase in the amount of non-reducing sugars from 3 to 6 day incubation with subsequent reduction in 9 days incubation.

Table 1: Mean diameter (mm) of rotted area developed on carrot inoculated with the three fungi incubated at different temperatures.

Fungi	Mean diameter (mm) of rotted area at different Temperatures					
	10°C	15°C	20°C	30°C	35°C	40°C
<i>Rhizopus arrhizus</i>	0.00 ^a	0.00 ^a	132.66 ^b	160.00 ^c	166.00 ^c	0.00 ^a
<i>Sclerotium rolfsii</i>	0.00 ^a	0.00 ^a	24.66 ^b	131.32 ^c	184.66 ^d	0.00 ^a
<i>Fusarium solani</i>	0.00 ^a	0.00 ^a	29.32 ^b	93.33^c	60.00 ^d	0.00 ^a

The same superscript letters between different temperatures did not differ significantly at 5% level by DMRT.

Table 2: Mean diameter (mm) of rotted area developed on carrot inoculated with the three fungi incubated at different relative humidities.

Fungi	Mean diameter (mm) of rotted area at different relative humidity levels					
	40%	50%	60%	70%	80 ³⁶ %	90%
<i>Rhizopus arrhizus</i>	112.66 ^c	128.00 ^c	140.66 ^b	150.00 ^{ab}	151.32 ^{ab}	159.32 ^a
<i>Sclerotium rolfsii</i>	156.00 ^c	173.32 ^b	173.32 ^b	193.32^a	186.66 ^b	186.33 ^b
<i>Fusarium solani</i>	13.32 ^d	21.32 ^d	32.66 ^c	37.40 ^{bc}	46.66 ^{ab}	54.00 ^a

The same superscript letters between relative humidity levels did not differ significantly at 5% level by DMRT.

Table 3: Food nutrients of un-inoculated and inoculated carrots roots with three fungi and inoculated for nine days (values are means of 3 replicates calculated as mg/g and µg/g for proteins and phenols, respectively).

Fungi	Food nutrient content at 3, 6 and 9 days of incubation					
	Proteins (mg/g)*			Phenols (µg/g)		
	3	6	9	3	6	9
Control	2.47	1.62	0.83	15.6 ^c	208.4 ^b	366.3 ^a
<i>Rhizopus arrhizus</i>	2.40 ^a	2.27	2.05	39.9 ^c	379.2 ^b	462.9 ^a
<i>Sclerotium rolfsii</i>	2.44	2.10	1.88	114.3 ^b	360.0 ^a	372.9 ^a
<i>Fusarium solani</i>	2.31	1.78	1.59	32.8 ^c	360.0 ^b	430.9 ^a

* - Differences in protein contents between incubation days is insignificant; The same superscript letters between incubation days did not differ significantly at 5% level by DMRT.

Table 4: Food nutrients of un-inoculated and inoculated carrots roots with three fungi and inoculated for nine days (values are means of 3 replicates calculated as mg/g for total sugars and reducing sugars and non-reducing sugars).

Fungi	Food nutrient content at 3, 6 and 9 days of incubation								
	Total sugars (mg/g)*			Reducing sugars (mg/g)			Non-reducing sugars (mg/g)*		
	3	6	9	3	6	9	3	6	9
Control	6.84	6.76	6.32	0.11 ^b	0.15 ^{ab}	0.19 ^a	6.39	6.18	5.82
<i>Rhizopus arrhizus</i>	6.49	5.76	6.08	0.17 ^c	0.38 ^b	1.35 ^a	5.99	5.11	4.49
<i>Sclerotium rolfsii</i>	6.49	6.94	6.26	0.89 ^a	0.17 ^b	0.24 ^b	5.31	6.43	5.71
<i>Fusarium solani</i>	6.61	5.99	5.78	0.22 ^c	0.64 ^b	0.80 ^a	6.07	5.08	4.73

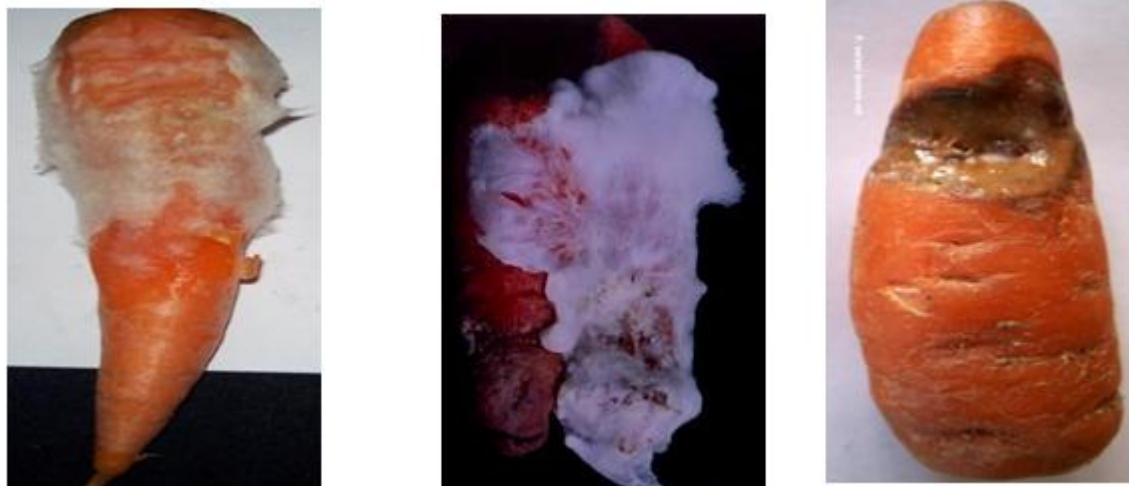
* - Differences in the nutrient contents between incubation days is insignificant; The same superscript letters between incubation days did not differ significantly at 5% level by DMRT

Table 5: Diameter of root rot on carrots inoculated with three pathogens were packed in open bunch, sealed polythene and perforated polythene bags and stored at ambient condition and zero energy cool chamber

Fungi	Ambient condition (Room temperature) (17-34C and 16-40%)			Zero energy cool chamber (15° C – 17° C & 85 – 98 % RH)		
	Unpacked (open bunch)	Sealed polythene bags	Perforated polythene bags	Unpacked (open bunch)	Sealed polythene bags	Perforated polythene bags
	<i>Rhizopus arrhizus</i>	0.00	164.66	0.73	0.00	0.00
<i>Sclerotium rolfsii</i>	0.00	118.33	0.66	0.00	0.00	0.00
<i>Fusarium solani</i>	0.00	161.0	0.5	0.00	0.00	0.00

Table 6: Shelf-life of carrots packed in open bunch, sealed polythene and perforated polythene bags and stored at ambient temperature and zero energy cool chamber.

Vegetables	Ambient condition (Room temperature) (17-34C and 16-40%)			Zero energy cool chamber (15° C – 17° C & 85 – 98 % RH)		
	Unpacked (open bunch)	Sealed polythene bags	Perforated polythene bags	Unpacked (open bunch)	Sealed polythene bags	Perforated polythene bags
	Carrot	5 days	10 days	15 days	22 days	20 days



Wooly soft rot of carrot by *R. arrhizus* Watery soft rot of carrot by *S. rolfsii* Root rot of Carrot by *F. solani*
Figure 1: Carrot root rots caused by post-harvest fungi like *Rhizopus arrhizus*, *S. rolfsii* and *F. solani*

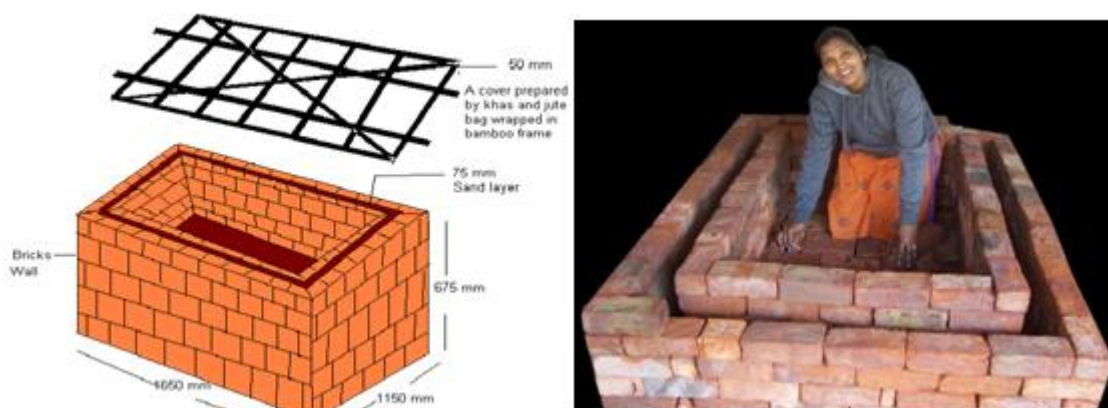


Figure 2: Zero energy brick wall cool chamber (low cost method) used for controlling of three post- harvest fungi and extending shelf life of carrot roots
 Zero energy cool chamber model constructing double wall for leaving cavity



Storage area for vegetables in cool chamber Top of the cool chamber & water supply

Storage Methods

Rot development at ambient and cool chamber

Table -5 provides data on rot development of carrots at both, room temperature and brick wall cool chamber. Carrots inoculated with three pathogens were placed in open (unpacked), sealed polythene bags, perforated polythene bags and stored at both Room temperature and brick wall cool chamber in order to find out rot development and to know the efficacy of cool chamber in the controlling of carrot root rots caused by three

pathogens during storage. Maximum rot was observed in carrots placed in sealed polythene bags due to high moisture while minimum rot was found in carrots packed in perforated polythene bags stored at ambient temperature.

No rot was found in carrots placed in open, polythene and perforated bags stored at brick wall cool chamber as well as carrots stored in open condition at ambient temperature. This could be lack of appropriate temperature for rot development at cool chamber and due to desiccation at ambient temperature. Table 1 and 2 revealed that the

optimum temperature and Rh for *R. arrhizus*, *S. rolfsii* and *F. solani* to develop of rot in carrots were 35°C and 90% RH, 35°C and 70% of RH and 30°C & 90%, respectively. Though cool chamber provides high RH (85-90%), pathogens could not show any rot at cool chamber. This could be due to low temperature 10°C-17°C in cool chamber.

Shelf-life of carrot

Table -6 presents data on shelf-life of carrots packed in open bunch, sealed polythene bags and perforated polythene bags stored at both, room temperature and cool chamber. Shelf- life of carrots was significantly more at cool chamber than room temperature. Among three packing systems, perforated polythene bag system showed maximum shelf-life. However, brick wall cool chamber is the best storage method for controlling root rot and enhancement of shelf-life of carrot. It is the best storage method and also affordable to the farmers and local market venders.

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