academicJournals

Vol. 7(50), pp. 5742-5748, 18 December, 2013
DOI: 10.5897/AJMR2013.5763
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Effect of climatic factors on fruit rots (*Alternaria alternata* and *Aspergillus niger*) of ber (*Zizyphus mauritiana* Lamk.) and their management systems

Suresh Kumar[1*], S. L. Godara[1], S. Gangopadhyay[1], Raj Kumar[2] and Shrawan Singh[3]

[1]Department of Plant Pathology, College of Agriculture, SKRAU, Bikaner, Rajasthan, India.
[2]Krishi Vigyan Kendra, Panchmahal (Central Institute of Arid Horticulture) Godhra, Gujarat, India.
[3]Central Agricultural Research Institute, Port Blair-744101, Andaman and Nicobar Islands, India.

Accepted 8 October, 2013

The present study aimed to study influence of temperature and relative humidity on fruit rots (*Alternaria alternata* and *Aspergillus niger*) of ber (*Zizyphus mauritiana* Lamk.) and find appropriate management option during storage period. Maximum damage by *A. alternata* was observed at 25°C while *A. niger* showed maximum severity at 30°C. Positive relationship was observed between relative humidity (RH) and fruit rot by both pathogens which showed highest severity at 100% RH. The pre-and post-inoculation treatment of ber fruits with four vegetable oils, four plant extracts and four fungicides showed significant differences in effectiveness in controlling both pathogens. Neem oil and its leaf extracts significantly (p<0.05) reduced severity of fruit rots by both pathogens. Among chemicals, the carbendazim at 1000 ppm proved effective against *Alternaria* and *Aspergillus* fruit rot. Chemical fungicides reduced fungus growth significantly (p<0.05) better than botanicals; however, botanical also showed better response than control. The effectiveness of botanicals in post-harvest storage of ber can be used at commercial scale.

Key words: *Zizyphus mauritiana*, *Alternaria alternata*, *Aspergillus niger*, fruit rot, vegetable oil, botanicals.

INTRODUCTION

Ber is a tropical and subtropical fruit native to the northern hemisphere and belongs to the genus *Ziziphus* of the family Rhamnaceae and order Rhamnales. The family has 50 genera and more than 600 species (Pareek, 2001) of which the species *Z. jujube* Mill (Chinese jujube or Chinese date), *Z. mauritiana* Lamk (Indian jujube or ber) and *Z. spina-christi* (L.) wild (Christ's thorn) are the most important in terms of distribution and economic significance. Ber (*Ziziphus mauritiana* Lamk) is one of the most important fruit crops grown in arid and semi-arid regions of India (Pareek, 2001). Ber fruits are rich in dietary minerals and natural antioxidants for people in arid regions. Its fruit products are getting commercial angle in different countries, particularly for their health benefiting properties (Vithlani and Patel, 2010). Ber fruit rots have been identified as major problems next to powdery mildew in arid and semi- arid regions. Many pathogens were identified as causing agents of fruit rots of ber in storages but *Alternaria alternata* and *Aspergillus niger* were two major inciting pathogens (Sharma and Majumdar, 1993). Though, reported losses from fruit rots were 2 to 15% but this goes high during congenial climatic conditions.

Alternaria and *Aspergillus* fruit rot are the most commonly occurring fruit rots in ber. Temperature and relative humidity affect fruit rot development and establishment of pathogen infection. For this disease, the congenial temperature ranges from 20 to 30°C and high

*Corresponding author. E-mail: lorask77@gmail.com.

relative humidity (>90%). However, the temperature and relative humidity affect the severity and pathogenicity of fruit rots caused by several pathogens that is, *A*. *alternata*, *A*. *niger*, *Colletotrichum capsici*, *Drechslera australiensis*, *Fusarium moniliforme* and *F. solani* (Datar, 1995).

Application of fungicides is a common management strategy to control the post-harvest pathogens on harvested fruits. Several fungicides have been effective in checking the storage rots of fruits and vegetables caused by different fungal pathogens (Singh and Thakur, 2005; Khokhar and Tetarwal, 2012), but, use of plant derived substances is attracting consumers and researchers for health factors. Plants rich in defence factors like seconddary metabolites reported to have strong anti-fungal, antibacterial properties (Reichling, 2010), therefore, could be used in post-harvest management of fruits and vegetables (Sable and Gangawane, 2010), but, information on their use was very meager, particularly on minor fruits like ber. Therefore, the present study was designed to determine congenial climatic factors (temperature and relative humidity) for two major pathogens that is, *A*. *alternata* and *A*. *niger* and to identify suitable botanicals (vegetable oils, leaf extracts) for management of fruit rots of ber in storage conditions.

MATERIALS AND METHODS

Inoculum preparation and inoculation

The pathogens were isolated from the infected ber fruits collected from various market and orchards of Bikaner district of Rajasthan. Pieces of transitional zone tissues of diseased fruits were scooped out and surface sterilized with 0.01% $HgCl_2$ solution for 1 to 2 min, followed by three washing in sterilized distilled water and transferred in petri-dishes containing potato dextrose agar medium (PDA). The crude cultures were incubated for growth at 26 ± 1°C and 95% relative humidity. The sporulating cultures were identified by microscopic examination for morphological characters of somatic and reproductive structures using reference isolates of *A*. *alternata* (Fr.) Keissler and *A*. *niger* van Tieghem. The isolated fungi were purified using single spore technique and then kept in a refrigerator on potato dextrose agar (PDA) slants for sporulation and further use (Chai et al., 1999). Healthy ripe fruits of ber were collected from main market of Bikaner, Rajasthan and washed with sterilized water. Excess water was removed and fruits were prepared for inoculation. Cork- borer wounding method was used for inoculation of the ber fruits. In brief, a hole of 2 × 2 mm size was made with sterilized cork borer and 2 mm disc cut of inoculum matrix of respective pathogens from inoculums petri-dishes was inserted in the hole by replacing the host pulp.

A piece of sterilized wet absorbent cotton was placed inside the surface sterilized polythene bags to create a micro-humidity chamber and mouth of the bags was loosely tied and incubated at 26 ± 1°C.

Environmental factors

The inoculated fruits were kept in polythene bags at four treatment levels of temperature that is, 20, 25, 30 and 35°C along with 95% relative humidity in four replications. Sterilized cotton swab placed inside the polythene bags to create a micro-humidity chamber. In another set, inoculated fruits were kept at six levels of relative humidity namely, 50, 60, 70, 80, 90 and 100% at 26 ± 1°C. The inoculated fruits kept in a desiccator which contained H_2SO_4 solution to produce a particular level of relative humidity, as suggested by Buxton and Mellanby (1934). The desiccators were sealed with grease and kept at 26 ± 1°C. The severity was recorded on the basis of percent infected area of fruit surface on 4^{th} and 8^{th} day of inoculation as described in Table 1.

Management options

Vegetable oil

Four vegetable oils namely, mustard oil, groundnut oil, castor oil and neem oil along with one control were used in four replications. In pre-inoculation, the fruits were first smeared with test oil and then inoculate after 12 h and vice-versa in case of post-inoculation treatment. The inoculated fruits were kept in the surface sterilized polythene bags with a piece of sterilized wet absorbent cotton to create a micro-humidity chamber and mouth of the bags was loosely tied and incubated at 26 ± 1°C. Fruit rot severity was recorded on 4 and 8 days after inoculation (DAI) as described in Table 1.

Leaf extracts

Another set of treatments with leaf extracts of *Azadirachta indica* A. Juss. (Neem), *Vinca rosea* L. (Sadabahar), *Withania somnifera* Dunal. (Ashwagandha) and *Ocimum sanctum* L. (Tulasi) and a control were tested against fruit rots. Leaf extracts were prepared by washing, air drying and homogenized for 5 min in sterile water (1:1, w/v). The mixture was filtered through muslin cloth and Whatmen No. 1 filter paper. In pre-inoculation treatments, the fruits were dipped in test extracts for 10 min and then inoculated. While post- inoculation treatments were applied after inoculation with *A. alternata* and *A. niger*; the interval between inoculation and treat-ment with plant extracts or vice-versa was 12 h. Proper controls were maintained and inoculation and observations were performed as per standard procedure (Mayee and Datar, 1986).

Chemical fungicides

The effectiveness of chemical control of fruit rots was tested using four commercially used fungicides namely carbendazim (50 WP), captan (50 WP), chlorothalonil (75 WP) and penconazole (10 EC) at 1000 ppm a.i. as in pre- and post- inoculation treatments against both pathogens separately. In pre- inoculation treatment, fruits were first dipped in the test fungicide for 5 min, air-dried and inoculated 12 h later while, 12 h after inoculation the treatments were applied in post- inoculation study. The experiment was arranged in completely randomized design. The procedures for inoculation and incubation of fruits and method of observations for fruit severity were recorded as described earlier (Mayee and Datar, 1986).

Statistical analysis

Data on fruit rots severity were recorded on the basis of percent fruit area infected. This was assessed with the help of an assessment key suggested by Mayee and Datar (1986). The analysis of variance was analysed using OPSTAT software (http//hau.ernet.in).

RESULTS AND DISCUSSION

Environmental factors

Normally, ber fruits are stored at room temperature (24°C) and 50 to 60% RH (Pareek, 2001) which found to be congenial for the fruit rots causing pathogens. The present study shows that the temperature significantly ($p<0.05$) affected growth of *A. alternata* and *A. niger* (Figure 1a

Table 1. Pathogen severity index.

Severity (%)	Character
0.0	No infection, ruptured fruits remain free from fungus after inoculation, fruit remain edible after removing inoculated area
0.1-5.0	Fungus growth remain only in inoculated area (<5%), fruit remain edible after removing inoculated area
5.1-10.0	Fungus showed slight growth to adjoining tissues (5 to 10%), fruits can be eaten after removing affected area
10.1-25.0	Fungus showed rapid growth and covered significant area (10 to 25%) of the infected fruits from inoculated area, fruit produce bad smell and not edible
>25.0	Most of the fruits surface (>25%) get affected and produce bad smell and not edible

and b).

Fruit rot severity of *A. alternata* was recorded to be highest at 25°C (16.0 and 27.5% at 4th and 8th DAI, respectively) while it was low at 20°C (9 and 16%) and 35°C (8.75 and 14.25%) (Figure 1a). In case of *A. niger*, the fruit rot severity was highest at 30°C (18.5% at 4th DAI and 28.0% at 8th DAI) followed by 25°C (13.25% at 4th and 22.75% at 8th DAI) and it was lowest at 20°C (9 at 4th DAI and 15% at 8th DAI) (Figure 1a and b). Similar observations were made in other crop species where 20 to 30°C reported to be optimum range for rapid development of fruit rot in chillies caused by *A. alternata*, *A. niger*, *C. capsici*, *D. australiensis*, *F. moniliforme* and *Fusarium solani* (Datar, 1995). The study suggests storing ber fruits at or below 20°C for longer time.

The severity of fruit rots by both fungi was increased with the increasing levels of relative humidity (Figure 2a and b). Maximum severity of *Alternaria* fruit rot was observed at 100% RH (16.25% at 4th DAI and 24.0% at 8th DAI) which was significantly higher than 3.75% at 4th DAI and 7.5% at 8th DAI at 50% level of relative humidity. Similar observations were also recorded in case of *Aspergillus* rot in ber where fruit rot severity was highest at 100% RH at both 4th DAI (21.0%) and 8th DAI (26.0%) while significantly ($p<0.05$) low at 50% RH (Figure 2a). Fruit rot severity caused by *A. alternata* and *A. niger* increased from 4th to 8th day of inoculation with increase in levels of humidity (Figure 2a) but it showed de-clining rate of increase (Figure 2b). It may be due to high level of initial severity by *A. alternata* and *A. niger* at higher levels of RH than 50% RH level. It might be due to enhanced conidial germination, infection and decreased host resistance at higher relative humidity (Oladiran and Iwa, 1993). However, significant reduction in relative humidity affects the quality of ber fruits in storage (Pareek, 2001); therefore, ber fruits should be stored at 50 to 60% for maintaining shelf life as well as reduce the chances of fruit rot development.

Management systems

Vegetable oils

The present study showed that pre- and post-inoculation coatings of vegetable oil on ber fruit significantly ($p<0.05$) reduced the severity of fruit rots caused by *A. alternata* and *A. niger*. Neem oil was most effective, but was at par with groundnut oil, and was significantly ($p<0.05$) better over castor and mustard oils in reducing the fruit rot severity by *Alternaria* fruit rot in pre-inoculation treatment (Table 2). The severity of *Aspergillus* fruit rot was also reduced by neem and castor oils but its efficiency was significantly ($p<0.05$) higher that groundnut and mustard oils. The post- inoculation treatments revealed that neem oil treatment was significantly better than mustard and groundnut oil in reducing the severity of fruit rot due to *A. alternata* (Table 2). Neem oil also provided effective control of *Alternaria* fruit rot as post-inocu lation agent compared to other tested oils. The oils have used commercially for storage of different fruits (Sonkar et al., 2009; Khokhar and Tetarwal, 2012) and present study indentified the potential vegetable oils for extending the storage life of pathogen free ber fruits. These oils can be used as pre- or post- inoculation treatments for effective protection against both *Alternaria* and *Aspergillus* fruit rot.

Effect of leaf extracts

The biodegradable and non-hazardous nature of botanicals increased their acceptance in post-harvest management of fruit and vegetables (Srivastava and Lal, 1997; Dargan and Saxena, 2002). The results from four botanical extracts on fruit rots severity are presented in Table 3. Pre- and post-inoculation treatments of fruits with neem leaf extract was found most effective against both *Alternaria* and *Aspergillus* fruit rots. *O. sanctum* was also found to be effective treatment against

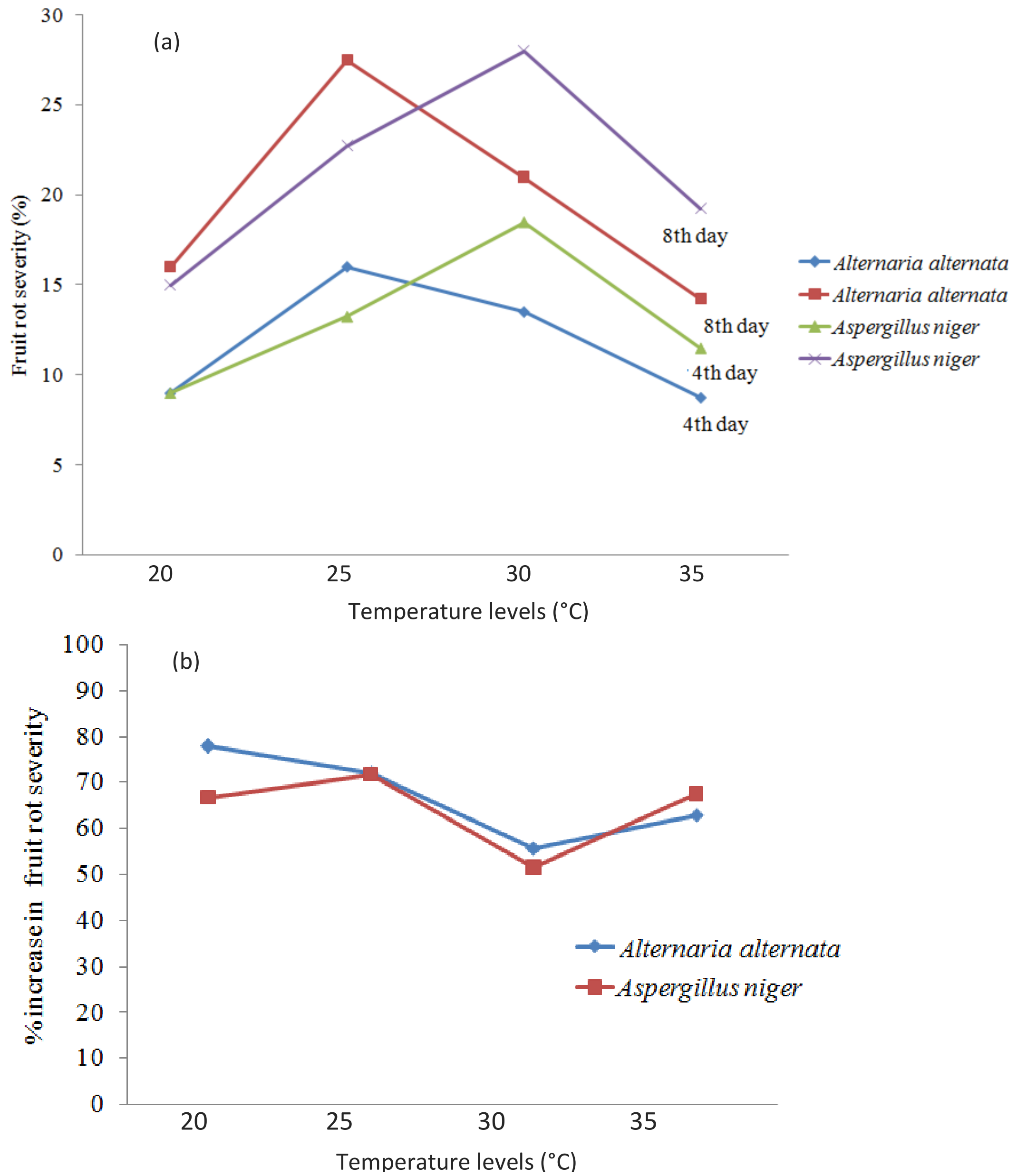

Figure 1. Effect of temperature on fruit rots by *Alternaria alternata* and *Aspergillus niger* (a) and their progress in ber (b).

these two post- harvest rots. While the performance of *W. somnifera* extract as pre- and post- inoculation treatment was relatively low against both rots. Similar reports were made by Meena et al. (2009) and Kumar et al. (2010). The variation in response of plant extracts may be due to differences in phytochemical profiles of tested plants and presence of anti-fungal compounds in leaf extracts (Cowan, 1999).

Effect of fungicides

Several kinds of synthetic fungicides had been successfully used to control the post-harvest decay of fruits and vegetables (Adaskaveg et al., 2004; Kanetis et al., 2007). But, chemical fungicides are acceptable in field level crop protection practices but their use at storage level in fruit and vegetables is discouraged for consumer and environmental benefits. However, present study tested four different fungicides against *Alternaria* and *Aspergillus* fruit rots in ber and found comparatively better than botanicals (Table 4 and Figure 3). Among organic protectants, vegetable oils found effective than plant extracts against *A. alternata* while *A. niger* but differences were non-significant. All fungicides were significantly ($p<0.05$) superior in reducing fruit rots severity as compared to control in both pre- and post-inoculation treat-

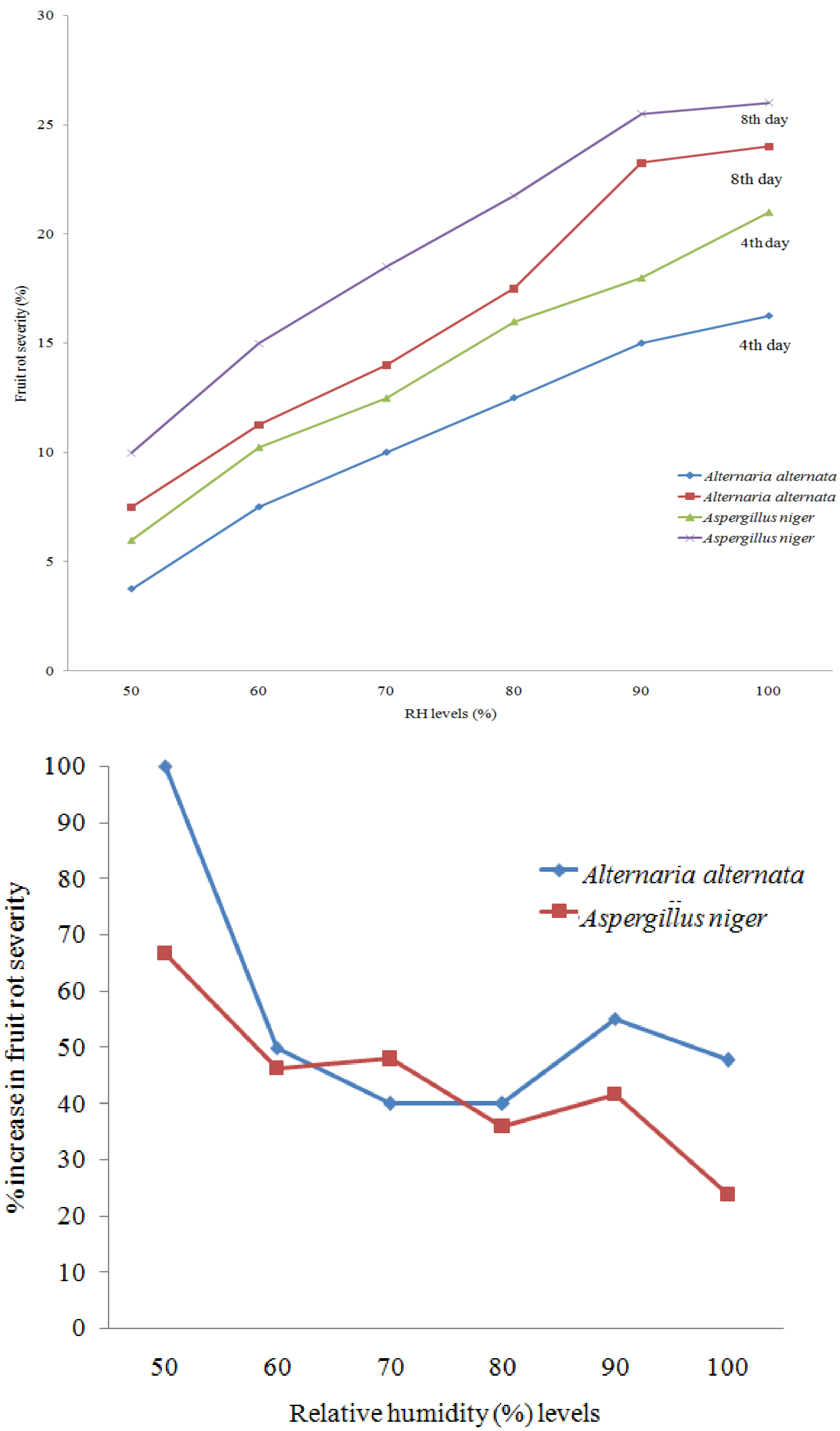

Figure 2. Effect of relative humidity (RH; %) on fruit rots by *Alternaria alternata* and *Aspergillus niger* (a) and their progress in ber (b).

ments at 4th and 8th days of inoculation. Carbendazim and chlorothalonil were found most effective against *Alternaria* fruit rot while carbendazim and penconazole for *Aspergillus* fruit rots. Similarly, different fungicides

Table 2. Effect of vegetable oils on fruit rot severity (%) by *Alternaria alternata* and *Aspergillus niger* in ber.

Vegetable oil	Pre-inoculation treatment				Post-inoculation treatment			
	A. alternata		*A. niger*		*A. alternata*		*A. niger*	
	4th DAI	8th DAI	4th DAI	8th DAI	4th DAI	8th DAI	4th DAI	8th DAI
Mustard oil	5	8	6.5	9	3.7	7.5	10	15
Groundnut oil	3	5.5	5	7.3	6.5	8	9.5	12.5
Neem oil	2.7	4	3.3	4.5	4.5	6.3	5.5	7.5
Castor oil	3.3	5.5	3.7	5.2	8	12	7.5	9.5
Control	15.5	25	17	26.5	17.5	24.5	16	25
CD (p = 0.05)*	1.9	1.9	1.5	1.9	1.1	0.8	0.9	0.8

*CD, Critical difference.

Table 3. Effect of plant extracts on fruit rot severity (%) by *Alternaria alternata* and *Aspergillus niger* in ber.

Plant extract	Pre-inoculation treatment				Post-inoculation treatment			
	A. alternata		*A. niger*		*A. alternata*		*A. niger*	
	4th DAI	8th DAI	4th DAI	8th DAI	4th DAI	8th DAI	4th DAI	8th DAI
Vinca rosea	3.5	5.7	7	11	8	11.5	10	14
Withania somnifera	6	8.5	4.3	6.3	9.5	14	8.2	12
Azadiracta indica	3	4.7	3.5	5	5	7	5.3	8.5
Ocimum sanctum	3.3	5.8	4	6	5.7	8.2	6.5	9
Control	14.5	24	18.5	27	17	26.3	16.5	28
CD (p = 0.05)*	2.1	1.4	2	1.6	1	0.9	1	0.9

*CD, Critical difference.

Table 4. Effect of chemical fungicides on fruit rot severity (%) by *Alternaria alternata* and *Aspergillus niger* in ber.

Fungicide	*A. alternata*		*A. niger*		*A. alternata*		*A. niger*	
	4th DAI	8th DAI	4th DAI	8th DAI	4th DAI	8th DAI	4th DAI	8th DAI
Carbendazim	1.3	2.5	1.0	2.3	2.3	4.5	1.5	3.0
Captan	4.0	7.5	4.8	6.0	3.0	5.3	5.5	8.5
Chlorothalonil	2.0	3.3	3.5	5.5	3.5	5.5	4.5	7.0
Penconazole	3.3	5.0	2.0	3.0	4.3	7.0	2.0	3.3
Control	16.0	26.0	16.5	25.0	16.5	27.3	17.0	27.5
CD (p = 0.05)*	1.9	1.5	1.9	1.6	1.8	1.6	2.4	2.9

*CD, Critical difference.

namely; Bavistin, Benlate, Thiobendazole, Dithane M-45, Blitox and Aureofungin have been indentified against storage rots of apple and ber (Singh and Thakur, 2005; Khokhar and Tetarwal, 2012).

A comparative analysis of three sets of anti-fungal agents indicates superiority of chemical fungicides, but their use is discouraged for consumer and environment reasons and also in organic fruits and vegetables. Therefore, the present study suggest use of vegetable oils of neem and ground nut and leaf extract of *A. indica* or *O. sanctum* as pre-infection treatment for keeping ber fruits for longer period. The same may be used for post-infection control of *A. alternata* and *A. niger* in ber and similar fruits.

ACKNOWLEDGEMENTS

The authors thanks the Dean, College of Agriculture, Rajasthan Agricultural university, Bikaner, Rajasthan, India for financial and laboratory facilities and also acknowledge the support of farmers and fruit sellers in

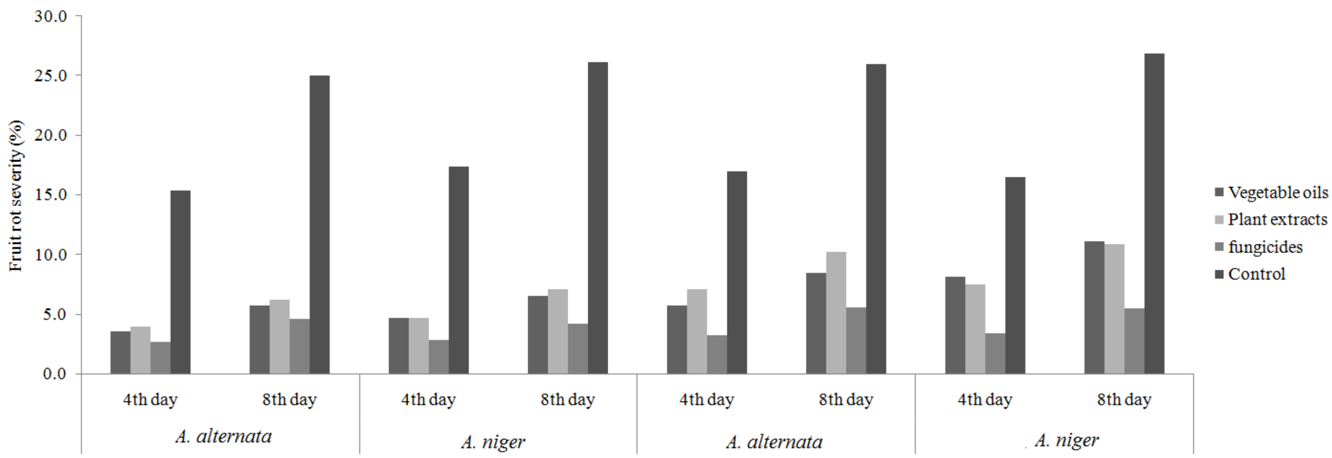

Figure 3. Relative efficacy of different treatments on fruit rot severity (mean values) of ber.

Bikaner market for sharing ber fruits for the study.

REFERENCES

Adaskaveg JE, Kanetis I, Soto-Estrada A, Förster H (2004). A new era of post-harvest decay control in citrus with the simultaneous introduction of three new "reduced-risk" fungicides. Proc. Int. Soc. Citricult. 999-1004.

Buxton PA, Mellanby K (1934). Measurement and control of humidity. Bull. Entom. Res. 25:171-175.

Choi YW, Hyde, KD, Ho WH (1999). Single spore isolation of fungi. Fungal Divers. 3:29-38.

Cowan MM (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12(4):564-82.

Dargan P, Saxena SK (2002). Effect of extract of *Withania somnifera* on fruit rot of tomato caused by *Aspergillus niger* in presence of *Drosophila busckii*. Indian Phytopathol. 55(1):112-113.

Datar VV (1995). Pathogenicity and effect of temperature on six fungi causing fruit rot of chilli. Indian J. Mycol. Plant Pathol. 25(3):195-197.

Kanetis L, Förster H, Adaskaveg JE (2007). Comparative efficacy of the new post-harvest fungicides azoxystrobin, fludioxonil, and pyrimethanil for managing citrus green mold. Plant Dis. 91:1502-1511.

Khokhar MK, Tetarwal JP (2012). Management of post- harvest black mould fruit rot of pomegranate (*Punica granatum* L.) caused by *Aspergillus niger* (Tieghem). Agric. Res. Rev. 1(5):162 -165.

Kumar A, Shukla R, Singh P, Dubey NK (2010). Chemical composition, antifungal and antiaflatoxigenic activities of *Ocimum sanctum* L. essential oil and its safety assessment as plant based antimicrobial. Food Chem. Toxicol. 48:539-543.

Mayee CD, Datar VV (1986). Phytopathometry Tech. Bulletin. 1 (Special Bulletin-3). Maradhawada Agriculture University, Prabhni, Maharashtra, India.

Meena OP, Godara SL, Rathore GS, Vijay P (2009). Eco-friendly management of post- harvest fruit rot of guava caused by *Pestalotiopsis palmarum.* J. Mycol. Plant Pathol. 39(3):445-448.

Oladiran AO, Iwu LN (1993). Studies on the fungi associated with tomato fruit rots and effect of environment on storage. Mycopathologia. 121(3):157-161.

Pareek OP (2001). Ber: International Centre for Underutilized crops, Southampton, U.K. pp. 248 - 266.

Reichling J (2010). Plant-microbe interactions and secondary metabolites with antibacterial, antifungal and antiviral properties. In Annual Plant Reviews (39): Functions and biotechnology of plant secondary metabolites, Second edition (ed M. Wink), Wiley-Blackwell, Oxford, UK. doi: 10.1002/9781444318876.ch4.

Sable P, Gangawane LV (2010). Studies on integrated management of ber [*Zizyphus*] diseases using plant extract combination with carbendazim. Asian J. Exp. Biol. Sci. Spl:35-39.

Sharma M, Majumdar VL (1993). Some new post- harvest diseases of ber fruits in India. Indian Phytopath. 46(4):415.

Singh D, Thakur AK (2005). Effect of fungicides on spoilage caused by mycoflora in kinnow (*Citrus reticulata* Blanco.) fruits during storage. J. Mycol. Pl. Pathol. 35:125-127.

Sonkar RK, Sarnaik DA, Dikshit SN, Saxena RR (2009). Individually stretch cling film wrapped kinnow mandarin under ambient storage. Indian J. Hort. 66:0972-8538.

Srivastava AK, Lal B (1997). Studies on bio fungicidal properties of leaf extracts of some plants. Indian Phytopath. 50(3):408-411.

Vithlani VA, Patel HV (2010). Production of functional vinegar from Indian jujube (*Zizyphus mauritiana*) and its antioxidant properties. J. Food Tech. 8(3):143-149.