EFFICACY OF BIO-RESOURCES IN THE MANAGEMENT OF WHITE ROT (*Botryosphaeria dothidea*) OF APPLE

Thesis

by

KISHOR SHARMA

Submitted in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE
(AGRICULTURE)
PLANT PATHOLOGY

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Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan - 173230 (H.P.), INDIA

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Dr. H. R. Gautam
Sr. Scientist

Department of Plant Pathology
College of Horticulture
Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (HP) – 173 230, India

CERTIFICATE - I

This is to certify that the thesis entitled, **"Efficacy of bio-resources in the management of white rot (*Botryosphaeria dothidea*) of apple",** submitted in partial fulfilment of the requirements for the award of degree of **MASTER OF SCIENCE (AGRICULTURE) PLANT PATHOLOGY** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.) is a record of bonafide research work carried out by **Mr. Kishor Sharma (H-2011-58-M)** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of investigation have been fully acknowledged.

Place : Nauni, Solan
Dated

(Dr. H. R. Gautam)
Chairman
Advisory Committee

CERTIFICATE - II

This is to certify that the thesis entitled, **"Efficacy of bio-resources in the management of white rot (*Botryosphaeria dothidea*) of apple",** submitted by **Mr. Kishor Sharma (H-2011-58-M)** to Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.), in partial fulfilment of the requirements for the award of degree of **MASTER OF SCIENCE (AGRICULTURE) PLANT PATHOLOGY** has been approved by the Student's Advisory Committee after an oral examination of the same in collaboration with the internal examiner.

Dr. H. R. Gautam
Chairman
Advisory Committee

Dr. M. L. Khan
Internal Examiner

Members, Advisory Committee

Dr. I. M. Sharma
Senior Scientist
Department of Plant Pathology

Dr. J. S. Chandel
Senior Scientist
Department of Fruit Science

Dr. R.K. Gupta
Associate Professor
Department of Basic Sciences

Dr. B.C. Suman
Professor and Head
Department of Plant Pathology

Dr. H.K. Sharma
Dean's Nominee

Dean
College of Horticulture
Dr. Y.S.P.U.H.F. Nauni, Solan (H.P.)

CERTIFICATE - III

This is to certify that all the mistakes and errors pointed out by the external examiner have been incorporated in the thesis entitled, **"Efficacy of bio-resources in the management of white rot (*Botryosphaeria dothidea*) of apple",** submitted to Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.) by **Mr. Kishor Sharma (H-2011-58-M)** in partial fulfilment of the requirements for the award of degree of **MASTER OF SCIENCE (AGRICULTURE) PLANT PATHOLOGY.**

_____

(Dr. H. R. Gautam)
Chairman
Advisory Committee

_____

Dr. B. C. Suman
Professor and Head
Department of Plant Pathology
Dr Y S Parmar UHF, Nauni, Solan (H.P.), India

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I solely claim all responsibilities for the shortcomings and limitations in this work.

Place: Nauni, Solan
Dated: **(Kishor Sharma)**

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ABBREVIATIONS

%	:	Per cent
µl	:	Micro litre
µm	:	Micro meter
^{0}B	:	Degree Brix
BF1	:	Botanical Formulation 1
BF2	:	Botanical Formulation 2
Cfu	:	Colony forming unit
et al.	:	Co- worker
G	:	Gram
lbs/sq. inch	:	Pounds per square inch
ml	:	Milliliter
mm	:	Millimeter
^{o}C	:	Degree Celsius
^{o}F	:	Fahrenheit
ppm	:	Parts per million
UHF	:	University of Horticulture and Forestry
v/v	:	Volume by Volume
Viz	:	Namely
w/v	:	Weight by Volume

Chapter-1

INTRODUCTION

Apple (*Malus* x *domestica* Borkh.) belongs to family Rosaceae and it is the most important fruit crop grown extensively in temperate regions of the world. In India, apple is grown in an area of about 2, 89,000 hectare with total annual production of 28, 91,000 metric tonnes and national productivity of apple is 10.0 metric tonnes per hectare (Anonymous, 2012). It is commercially grown in the Himalayan regions including states of Jammu and Kashmir, Himachal Pradesh and Uttarakhand which together accounts for 99 per cent of the total production. The remaining 1 per cent is contributed by North-Eastern states viz. Arunachal Pradesh, Sikkim, Nagaland and Meghalaya. Himachal is having an area of 1, 03,640 hectare with total annual apple production of 4, 12,360 metric tonnes and productivity of the state is 3.79 metric tonnes per hectare (Anonymous, 2012).

Himachal Pradesh has made continuous efforts in production of high quality of apple in the last few decades which has revolutionized the socio-economic condition of the farmers. Though the area and production under apple cultivation in this state has increased during last few decades, but the productivity per unit area has not increased proportionally. It is quite low in comparison to Jammu and Kashmir and other apple growing countries of world. There are many reasons for low apple productivity and among these damage caused by diseases are one of the dominant factor. There are number of diseases which infect apple resulting in huge loss in yield. Among diseases, post-harvest diseases are also important which result in significant losses.

Post-harvest losses to varied extent have been reported from different parts of the world. Anderson (1956) reported 80 to 90 per cent losses in apple fruit due to post-harvest diseases from United States. Jijakli and Lepoivre (2004) reported 5 to 25 per cent post-harvest losses in apple. Spadaro *et al*. (2004)

recorded 50 per cent losses due to post-harvest diseases such as grey mould (*Botrytis cinerea)* and blue mould (*Penicillium expansum*) on apple.

According to a survey conducted during year 2005 to 2007, losses due to post-harvest disease ranged between 5.8 to 18 per cent resulting in estimated economic loss of Rs. 7,437 crore while in apple this economic loss consisted of Rs. 953 crore rupees (Anonymous, 2007). Development of post-harvest diseases during ripening and storage depend on a range of pre-harvest factors. The most important of these is maturity of fruits at harvest (Kvikliene, 2001; Ferguson *et al*., 1999).

Jones and Aldwinckle (1990) confirmed that among different post-harvest pathogens, fungal pathogens are most dominating. There are more than 90 fungal species have been reported to cause post-harvest apple decay. McCollum (2002) concluded that fruit rot caused by fungal pathogens can lead to considerable post-harvest losses, varying with cultivar, area of production, and season. Ilyas *et al.* (2007) reported ninety fungal species associated with post-harvest decay in apple during storage.

The major post-harvest pathogens of pome fruits are *Penicillium expansum*, *Botrytis cinerea* and *Monilinia fructigena* and other common fungal species isolated from rotten pome fruits are *Colletotrichum*, *Mucor*, *Rhizopus*, *Alternaria*, *Botryosphaeria*, *Fusarium*, *Neofabraea* (Konstantinou, 2011; Snowdon, 1990).

In Himachal Pradesh, Kaul (1979) reported 21 pathogens to cause post-harvest fungal rots and decays in apple fruits. Among different fungal post-harvest rots in apple, white rot caused by *Botryosphaeria dothidea* is one of important disease and this pathogen also cause canker on the stems.

Putterill (1919) reported fruit rot and canker of apple caused by *Botryosphaeria mali* first time from South Africa. Later, Fulkerson (1960) also observed white rot as a major pre-harvest and post-harvest problem of apple in warm and wet weather conditions during summer. Other workers have also

reported perennial cankers on the stem and rot on apple fruit in the field and during storage (Biggs and Miller, 2003).

Management of post-harvest rots is a tedious task in apple as use of chemicals results in accumulation of residues on the fruits. Residues of chemicals on the surface of fruits results in adverse effects on the health of consumers. Thus, there is need for alternative non-chemical methods like use of botanicals and other bio-pesticides for the management of post-harvest diseases in apple. In these studies, focus has been kept on the use of botanicals for the management of white rot of apple. The study has been outlined with following objectives.

i) To assess the incidence of white rot of apple in important Marketing Yards namely Narkanda, Shimla, Solan, and Parwanoo of Himachal Pradesh.

ii) To do *in-vitro* evaluation of locally available bio-resources like botanicals, cow urine in comparison to safer fungicides against the white rot pathogen of apple.

iii) To develop effective bio-formulation based on the effective bio-resources for management of white rot during storage.

Chapter-2

REVIEW OF LITERATURE

Post-harvest losses in fruits is a serious concern as after putting hard labour in production, the precious harvest is lost due to negligence in crop production and storage at various stages. Post-harvest losses in fruits are estimated at 5 to 35 per cent in developed countries and 25 to 50 per cent in developing countries. The post-harvest losses are caused by various biotic and abiotic causes. In biotic causes, several fungi have been reported to be associated with rotting of fruits during transportation; early and late stages of storage and marketing. In apple, many fungal pathogens has been reported to cause different post-harvest rots (Rosenberger, 1990; Mc Collum, 2002). Among these *Monilinia* spp., *Gleosporium* spp., *Penicillium* spp., *Botrytis* spp., *Alternaria* spp., *Botryosphaeria* spp. and *Phytopthora* spp. were the dominant ones which caused commercial fruit loss during storage (Berrie, 1993; Valuskaite, 2006). These pathogens mainly attacked injured and physically damaged fruits which resulted in severe fruit damage during storage. Among different fungal post-harvest rots, white rot caused by *Botryosphaeria dothidea* has been reported to be a serious rot of apple resulting in huge losses from United States, Japan and Korea (Hendrix *et al*., 1978; Koganezawa and Sakuma, 1984; Lee *et al*., 2001). In India, white rot (*B. dothidea*) has been reported to be a serious disease resulting in trunk death as well as fruit rot (Kaul, 1979; Sharma and Bhardwaj, 1999; Khan *et al*., 2010).

Geographical distribution

White rot (*Botryosphaeria dothidea*) was first reported by Putterill (1919) from South Africa on apple fruits as rot and also as canker on the stems. However, Birmingham (1924) reported pathogen *Dothiorella mali* associated with white rot and canker diseases of apple from Australia. Fenner (1925) established that cane current blight, soft rot and *Dothiorella* canker of apple were caused by the same fungus i.e. *Botryosphaeria ribis*. Several workers observed that *Botryosphaeria* spp. on apple were widely prevalent in apple growing regions of

the world which includes USA, Australia, South Africa and New Zealand (Heald, 1926; Anderson, 1956). White rot pathogen (*B. dothidea*) has a world-wide distribution and it is capable of infecting numerous plant species. This pathogen has been reported from 68 genera of economically important crops including pome fruits and woody plants in tropical and warm temperate regions of the world (Smith, 1934). Arx and Muller (1954) in their classification referred *Dothiorella* as conidial stage of *B. dothidea*. Hendrix *et al*. (1978) reported prevalence of *B. dothidea* causing serious fruit rot and canker on apple trees from Mid-Western and South-Eastern regions of the United States. In South Africa, Combrink *et al.* (1984) observed considerable damage of peaches and pears due to *B. dothidea* and *B. obtusa*. White rot pathogen (*B. dothidea*) has been reported to be responsible for the fruit decay and canker on different apple cultivars in Chile (Latorre and Toledo, 1984). Brown and Britton (1986) confirmed the presence of white rot and black rot of apple and peaches in Southern United States. Cho *et al*. (1986) recorded 15-16 percent severity of white rot on apple in major apple growing regions in Korea Republic and also reported that severity was most on Golden Delicious and least on Jonathan and Red Delicious cultivars.

Melzer and Berton (1988) identified *Botryosphaeria* spp. as wood attacking fungus in poorly managed apple orchards of Brazil. A study conducted by Kim and Kim (1989) revealed that Fuji, Starking Delicious and Jonard cultivars of apple were susceptible to white rot infection and Jonathan was resistant to *B. dothidea* infection in Korea Republic. In Taiwan, *Botryosphaeria* spp. has also been reported from grapevine as pathogen of grape cluster rot (Kuo *et al*., 1989). Snowdon (1990) observed prevalence of black and white rot fungi occasionally on harvested apple and pears in several areas of USA. White rot pathogen (*B. dothidea*) has been reported from different regions of Australia, Argentina and Brazil (Sutton and Arauz, 1991). Symptoms of *B. dothidea* has been reported as fruit rot and canker on Bradford pear in Louisiana (Holcomb, 1993). In Korea, Uhm (1998) reported considerable damage of apple due to white rot pathogen. Further, *B. dothidea*, *Botrytis cinerea* and *Diaporthe actinidae* were reported to be major fungal pathogens causing soft rot and decay of Kiwi fruits during storage in Korea (Lee *et al*., 2001). In Bolivia, white rot (*B. dothidea*) and

black rot (*B. obtusa*) fungi were reported from rotted fruits of Gala and Winter Banana cultivars of apple (Kaiser *et al*., 2002). Balancaluz *et al*. (2005) reported occurrence of white rot (*B. dothidea*), black rot (*B. obtusa*) and bitter rot (*Colletotrichum gloeosporiodes*) causing severe fruit loss of apple in Chile and observed that losses were more prevalent on Pink Lady, Braeburn and Fuji cultivars of apple.

In Korea, the survey of major apple growing areas during 1992 to 2000 indicated that white rot (*B. dothidea*), Valsa Canker (*Valsa ceratosperma*) and bitter rot (*Colletotrichum gloeosporiodes*) were dominant diseases of various stored fruits including apple (Lee *et al*., 2006). Moral *et al*. (2010) identified a well known isolate of *B. dothidea* causing fruit rot of olive in Spain. Tang *et al*. (2012) reported *B. dothidea* as pathogen of *Botryosphaeria* canker and apple ring rot which produces fruit rot, canker and dieback on many hosts under stress condition in Korea and China. In Spain, Roca *et al*. (2013) identified *B. dothidea* from rotten plum fruits showing soft, brown and slightly sunken necrotic lesions. In India, the disease has been reported from different states. Singh (1942) was first to report fruit rot of apple caused by *Botryosphaeria* spp. from Kumaon hills. Malik (1967) reported *B. dothidea* as apple fruit rotting pathogen from Kashmir valley and observed that this pathogen produce canker and fruit rot symptoms in commercial orchards of valley. In Himachal Pradesh, Agarwala and Gupta (1971) confirmed the presence of *Botryosphaeria ribis* synonym with *B. dothidea*. Gupta and Dutt (1972) observed *Dothiorella mali* as apple fruit rotting pathogen from Kullu valley. In Jammu and Kashmir, *B. dothidea* was reported as pathogen of white rot and stem bark canker of apple (Khan *et al*., 2010).

Losses

Post-harvest losses in fruits were estimated at 5 to 25 per cent in developed countries and 20 to 50 per cent in developing countries. Despite the modern storage facilities, post-harvest diseases caused loss of 5 to 25 per cent in fruits (Bondoux, 1992). In United States, estimated losses from post-harvest diseases ranged between 1 to 20 per cent (Janisiewicz and Korsten, 2002).

Rosenberger (1997) recorded economic loss of 4.4 million dollars per year due to post-harvest rotting of apple.

In China, Xiang (1957) reported severe loss of white rot and recorded approximately 20 per cent of fruit rot in "Yuxia" cultivar of apple before harvest and 79 per cent loss during storage. Jones and Aldwinckle (1990) observed that fruit losses due to white rot in apple can be severe under prolonged warm and wet weather condition. In South-Eastern United States, 50 per cent of fruit losses in apple are caused due to white rot or bot rot infection which was one of most destructive disease of apple in humid areas (Sutton, 1990). Kim and Uhm (2002) reported that almost 40 per cent of apple was rotted from white rot caused by *B. dothidea* in Korea. Kuijing *et al.* (2010) recorded 92 per cent of white rot incidence from Tangshan region of China.

In India, Arya (2010) recorded 10 to 40 per cent of post-harvest losses in fruits. In India, post-harvest losses to the tune of 10 to 25 per cent of total apple production have been reported (Chadha and Pareek, 1993). According to a survey conducted during year 2005 to 2007, losses due to post-harvest disease in fruits ranged between 5.8 to 18 per cent resulting in estimated economic loss of Rs. 7,437 crore while in apple this economic loss consisted of Rs. 953 crore rupees (Anonymous, 2007). Murthy *et al*. (2009) reported that India annually losses 30 per cent fruits due to post-harvest diseases.

Singh (1942) reported *Botryosphaeria* spp. to cause 10 to 15 per cent mortality of apple trees in Kumaon hills. In Kullu valley of Himachal Pradesh, *Botryosphaeria dothidea* caused 1.3 to 3 per cent of apple decay during storage (Agarwala and Gupta, 1971). Gupta and Dutt (1972) observed *Dothiorella mali* to attribute severe fruit rot on apple in Kullu valley. Kaul (1979) reported *Botryosphaeria* spp. to cause fruit rot of apple with incidence up to 1.5 per cent.

Symptomatology

Botryosphaeria dothidea has been reported to cause symptoms on fruits as fruit rot and on the stem as canker. Eid (1959) reported that fruit rot symptoms

were primarily noticed late in season after formation of the fruits but infection occurred any time after petal fall. Fulkerson (1960) observed that lesion become noticeable in 4 to 6 weeks before harvest as small circular, light green spot encircled by reddish brown halo on yellow skinned cultivars of apple while in cultivar with red skin, halo appears dark purple to black in colour. The lesion later becomes depressed and brown surrounded by alternate light green and brown margins. As lesion expand in diameter, the rotted area extended cylindrically in cross-section in the fruit and consequently entire fruit rots and become soft and mushy. White rot pathogen, *B. dothidea* is able to infect fruit through lenticels but does not move into cortex until moisture stress is available (Connor, 1968). The white rot infection appears in two phases. First, stem canker is developed on sun burnt or wounded surface of twigs and then small reddish brown lesion with purplish margin appears on fruit surface (Pathak, 1986). Sutton (1990) observed that *Botryosphaeria dothidea* infection rapidly develops in mature fruits under warm conditions and later fruit becomes tan to light brown and watery in appearance. Kaul and Sharma (1999) reported that during white rot infection flesh become soft and may take appearance of a cooked fruit with drops of liquid oozing out from the rotted areas. Further, at lower temperature white rot symptoms were indistinguishable from black rot symptoms in apple. However, Turechek (2004) also noticed that rotted fruits appear tan to light brown, soft and watery under warm conditions. This "Bleaching" action of red skinned apple cultivars during decay process has led to name "white rot".

Pathogenicity

The pathogenicity study conducted by Sutton and Boyne (1983) indicate that different isolates of pathogen *Botryosphaeria dothidea* varied in their ability to cause canker and rotting of apple fruits. They tested pathogenicity of white rot fungi (*B. dothidea*) by inoculating the mature fruits of Golden Delicious apple and characteristic fruit rot symptoms developed at 25^0 C. Rittenburg and Hendrix (1983) observed that *B. dothidea*, *B. obtusa* and *B. rhodina* cause rot of ripe fruits and confirmed that wounds are necessary for initiation of fruit rot infection of apple. Latorre and Toledo (1984) proved pathogenicity of *B. dothidea* by

inoculating different isolates of *B. dothidea* on fruits of Granny Smith and Starking Delicious cultivars of apple with conidial suspension prepared from pycnidia developed at room temperature.

Fulkerson (1960) reported that *Botryosphaeria ribis* cause rapid rotting of apple at 65 to 70^0 F**.** Witcher and Clayton (1963) studied optimum temperature relations for growth and production of *B. dothidea* and reported 10, 28 and 32 to 35^0 C as minimum, optimum and maximum temperatures, respectively. Shandilya (1971) reported that 30^0 C was suitable temperature for mycelial growth of *Dothiorella* spp. English *et al*. (1975) worked out cardinal temperature for mycelial growth of *B. dothidea* and reported 8.5, 24-30 and 31.5^0 C as minimum, optimum and maximum temperature under *in vitro* conditions. Incidence of white rot was only 4.2 per cent when suger content of apple was 9.6^0 Brix but when suger content increased to 10.1^0 Brix disease incidence increased to 14.2 per cent (Kim *et al*., 1997).

MANAGEMENT OF FRUIT ROTS (WHITE ROT)

Effect of chemicals

Post-harvest treatment of apple fruits with tetrabenazine (TBZ) has been reported to reduce further infection of white rot disease (Beattie and Johnson, 1972). Sutton and Arauz (1981) emphasized the need for combination of cultural practices like sanitation and recommended that all colonised dead limbs affected by canker and mummified fruits which are inoculum sources of fungus should be removed from apple orchards before applying fungicide sprays. Kohn and Hendrix (1983) observed that in those areas where white rot is a serious problem, preventive spray programs should begin and continue at 10 to 14 days interval throughout the apple growing seasons. The fungicide spray programs have been reported to be initiated only when soluble solid level reaches to 10.5 per cent in apple fruits.

Different aromatic compounds such as dichloronitroanline, sodium ortho-phenylphenate and biphenyl have been reported quite effective in controlling various apple fruit rots (Kaul, 1985). Sutton *et al*. (1985) reported that Sterol-

inhibiting fungicides were more effective under *in vitro* conditions against *B. dothidea*, *B. obtusa, Glomerella cingulata* and *Gleodes pomigena*. Fungicides like ectaconazole, bitertanol and fenarimol were proved less effective against summer disease of fruits. Farr *et al*. (1989) indicated that kresoxim-methyl and trifloxystrobin were effective fungicides against canker phase of black rot and white rot of apple. Benomyl spray at 2 week intervals was reported as most effective measure for controlling summer disease caused by post-harvest pathogens (Brown and Britton, 1986).

Application of bitertanol has been found effective for management of white rot of apple (Uhm *et al*., 1995). Kim and Uhm (2002) observed that white rot of apple can be effectively controlled by a single application of ergosterol biosynthesis-inhibitors viz. bitertanol, difenoconazole and tebuconazole around mid-August in Korea Republic. Biggs (2004) reported that lesion size due to white rot of apple was slightly reduced on fruits when these were treated with calcium hydroxide or calcium silicate after wounding and inoculation with conidia of *Botryosphaeria dothidea*. Everett *et al.* (2007) found that one application of tolyfluanid, mancozeb, captan and copper at 10-14 days interval during late summer is best for the control of white rot and other summer rot diseases of apple in New Zealand. In Korea, six fungicides namely mancozeb, propineb, benomyl, folpet, azoxystrobin and iminoctadine-triacetate proved effective against white rot of apple. These six fungicides had diverse activity on disease development. Among these fungicides, folpet, iminoctadine-triacetate and azoxystrobin had after-infection activity on disease development and infection while mancozeb, propineb and benomyl showed no activity against white rot pathogen *B. dothidea* (Lee *et al*., 2007). Pitt *et al.* (2010) reported that fungicides like tebuconazole, crypoconazol, flusilazole, benzimidazole and carbendazim proved effective protectant for pruning wound infections caused by *Botryosphaeria dothidea.*

Baker (1924) and Smith (1925) advocated the use of oil wrappers in preventing the storage rots of different fruits crops. Bitter pit of apple can be effectively controlled by treatment of wraps with diphenylamine (Viney, 1963).

Jones and Burton (1973) found that brown rot of prunes, cherries, nectarines and peaches can be effectively controlled by a dip treatment of fruits in benomyl, chlorothalonil, thioflorine, dichlozine and thiophanate-methyl. Kaul and Munjal (1982) found use of botran impregnated wrappers effective against storage rot of apples. Sumbali and Mehrotra (1983) found that potassium-iodide treated wrappers provided effective control of *Geotrichum roseum* on apple which causes post-harvest disease of other temperate fruits. Koul and Kotha (1986) reported that keeping fruits in newspaper and tissue paper impregnated with carbendazim and thiabendazole checked the blue mould rot of apple in cold storage. Dip treatment of fruits in aqueous Topsin-M (0.1%) and bavistin (0.05 to 0.1%) inhibited the growth of *Aspergillus niger* in pomegranate fruits (Padule and Keskar, 1988).

Effect of plant extracts

Leaf extracts of *Ocimum sanctum, Azadirachta indica* and *Eucalyptus occidentalis* in different concentration proved effective against post-harvest fruit rot pathogens in grapes and guava (Arya, 1988). Grange and Ahmed (1988) found that plant extracts exhibited antifungal activity against wide range of fungal pathogen that caused post-harvest diseases. Amuchi (1989) observed that aqueous leaf extract of *Ocimum* spp. reduced the mycelial growth of *Rhizopus* spp. which causes avocado fruit rot. Moline and Locke (1993) reported antifungal properties of Neem (*Azadirachta indica*) seed extract against post-harvest apple pathogens. Plant extracts have been found very effective against number of plant pathogens and are more increasingly used for their control in plant diseases in number of horticulture crops (Mishra *et al*., 1993; Singh *et al*., 2001). Ryu and Holt (1993) reported efficacy of 0.1 per cent cinnamon oil against *Penicillium expansum* causing storage rot of apple. Saks and Barkai-Golan (1995) observed suppression of spore germination and growth of *Penicillium digitatum*, *P. expansum*, *Botrytis cinerea* and *Alternaria alternata* with *Aloe vera* gel at 1-10^5 µ litre concentration. Antifungal activity of *Aloe vera* gel was found against common post-harvest pathogens viz. *Penicillium digitatum*, *Penicillium expansum*, *Botrytis cinerea* and *Alternaria alternata* during storage (Adikaram *et al*., 1996). In addition, leaf

extracts of neem, eucalyptus, tulsi, datura, and bougainvillea also exhibited beneficial effects on the physical characteristics of citrus and mango fruits, as these extracts significantly reduced spoilage and moisture loss of fruits during storage (Sindhan *et al*., 1999). Sharma and Bhardwaj (2000) observed that water extract of *Artemisia vulgaris* and *Melia dubia* effectively controlled fruit rotting after 60 days of storage in apple. Cow urine and cow dung have been reported to inhibit the conidial germination and mycelial growth of fungal pathogens (Basak *et al*., 2002). Plant extract and essential oil of *Mentha arvensis*, *Zingier officinalis* were found to possess fungitoxic properties against blue mould rot (*Penicillium italicum*) of orange and lime (Tripathi *et al*., 2004). Okigbo and Ogbomaya (2006) found that *Ocimum gratissimum* leaf extract was inhibitory to post-harvest rot pathogen of yam. Patil (2007) reported antifungal activity of cow urine against various plant pathogens viz. *Fusarium oxysporum*, *Rhizopus oligosporus*, *Alternaria helianthi* and *Trichoderma viride*. Shinde *et al*. (2009) studied the efficacy of various plant extracts and oils viz. tissue paper wrapping on mango in order to increase shelf life and to minimize post-harvest losses and reported that neem oil most effective against fruit decay. Nakamura *et al*. (2010) observed antifungal activity of *Artemisia capillaries* extract against brown rot (*Monilinia fructicola*) of peach fruit in storage. Marpudi *et al.* (2011) evaluated anti-microbial activity of *Aloe* gel and reported that coating of *Aloe* gel reduce post-harvest loss through decay and extended shelf life quality of fruits. Aqueous extract of *Dedonia viscosa* has inhibitory action against *Fusarium solani* that cause dry rot disease of potato (Bhardwaj, 2012). Onyeani *et al.* (2012) observed that aqueous leaf extract of *Azadirachta indica* and *Aloe vera* were equally effective in reduction of mycelial growth and sporulation of *Aspergillus niger*. Lopez-Reyes *et al*. (2013) observed that essential oil obtained from *Ocimum basilicum* was effective against brown rot (*Monilinia laxa*) and grey mould rot (*Botrytis cinerea*) in stone fruits.

Effect of waxing

Protective skin coating with wax is one of best method to increase storage life of fresh fruits under ambient conditions. Supplementing natural waxy layer of

fruits with various synthetic edible waxes provide protection against various fruit pathogens (Dalal *et al.*, 1971; Akamine *et al.*, 1975). Skin coating with prolong (a mixture of sucrose ester of fatty acids and polysaccharides) improved storage life of apple, pear and plum and reduced growth of various decay organisms to great extent (Banks and Harper, 1981). Bancroft (1995) studied the impact of TAL-prolong coating (a sucrose ester composition) on development of some common post-harvest fungal rots and concluded that TAL- prolong had a greater effect on those rots caused by *Botrytis cinerea*, *Monilinia fructigena* and *Rhizopus nigricans*. Sumnu and Bayindirli (1996) reported that apple treated with Semperfresh (carnauba-polythene wax) and Jonfresh (based on carnauba wax) reduced spoilage of apple fruits during storage.

Coating of tomato with carnauba wax improved the fruit quality and prevented fruits from physical damage and diseases (Chiumarell and Ferreira, 2006). Abd-Allah *et al*. (2009) reported that application of carnauba wax containing *Streptomyces cervisae* or *S. cervisae* combined with peppermint oil (0.1%) effectively reduced grey mould, soft rot and black rot disease of tomato under storage. Abdel-Kader *et al.* (2011) observed that use of chemical preservatives such as chitosan and carnauba wax coating inhibited the growth of fruit rotting fungi *Penicillium digitatum* and *P. italicum* in citrus. Carnauba wax combined with acetic acid in vapour phase has also been reported effective for controlling post-harvest pathogens and maintaining fruit quality of mandarin fruits during storage (Abd-El and Rashid, 2013).

Chapter-3

MATERIALS AND METHODS

Studies on use of bio-resources in the management of white rot of apple caused by *Botryosphaeria dothidea* (anamorph *Dothiorella mali*) were conducted in the Department of Plant Pathology, Dr. Y.S. Parmar University of Horticulture and Forestry, Solan (H.P.) during 2011 to 2013. The material and methodologies adopted during the course of study are elaborated below.

Glass wares used

In different *in vitro* studies, glassware namely Petri plates (90 mm diameter), test tubes (10 and 20 ml), microscopic slides (24×7.5×1mm), Erlenmeyer flasks (100, 250 and 500 ml), beakers (500 and 1000 ml) and pipettes (1, 5 and 10 ml) were used during the course of investigations. The glassware used was of Borosil make.

Equipments, Apparatus and other material used

Weighing of different materials used in experiment was done on a single pan electrical balance (Narang Scientific Works Pvt. Ltd.). Culture of the white rot pathogen (*B. dothidea*) in different experiment was incubated in biological oxygen demand (BOD) incubator (REMI instruments Ltd.). Mixer and grinder (Whiteline) was used for grinding of different plant material for preparing botanical formulations. Sterilization of media and glassware was done in autoclave (Narang Scientific Works Pvt. Ltd.) and hot air oven (Lab Equipment industry), respectively. Studies related to isolation of the pathogen, inoculation and other studies requiring aseptic environment were done in laminar airflow (Narang Scientific Works Pvt. Ltd.). Cultures were kept in refrigerator (Godrej) at 4^0C for further use. Effigi penetrometer (FT-327) and Erma hand refractometer were used to measure fruit pressure and total soluble solid (TSS) in fruit juice, respectively. The plant materials i.e. botanicals used against pathogen were procured locally from Herbal Garden of the University and nearby forest. The fruit packaging material used *in vivo* experiments consisted of Corrugated Fibre

Board (CFB) boxes with inner dimension of 50×30×28 cm, standard apple trays of 49.5×29.5 cm and daily news paper were used to wrap individual fruit and the size of news paper sheet was 52×33 cm.

Sterilization

The sterilization of glass wares (wrapped in butter paper / brown paper) was done by autoclaving at 1.05 kg/cm^2 pressure for 20 minutes. Autoclaved glass wares were dried in hot air oven at 80^0 C for 45 to 60 minutes. Sterilization of the extracts of different plants was done at 1.05 kg/cm^2 for 5 minutes. All *in vitro* experiments were carried out under aseptic conditions using laminar airflow and spirit lamp flame was used for sterilizing inoculating needle and also inoculation studies of culture.

3. A. SURVEY AND COLLECTION OF DISEASED SAMPLES

Periodic survey of Marketing Yards of different apple growing areas in Shimla and Solan districts of Himachal Pradesh were conducted during July-September months in 2011, 2012 and 2013 for recording the incidence of white rot (*B. dothidea*) and other rots in total. Areas surveyed included important Marketing Yards namely Narkanda, Shimla (Dhalli), Solan and Parwanoo of Himachal Pradesh. Per cent disease incidence of white rot of apple in these Marketing Yards was calculated by formula given below

$$\text{Disease incidence (\%)} = \frac{\text{Number of apple fruits with white rot symptom}}{\text{Total number of apple fruits observed}} \times 100$$

During course of the survey, white rot infected fruits were collected from these areas and were kept in polythene bags and brought to the laboratory for the isolation and confirmation of associated pathogen. White rot and other pathogen related fruit rots were confirmed with the standard symptoms on the fruits and microscopic examination of the pathogen involved.

3. B ISOLATION AND IDENTIFICATION OF CAUSAL ORGANISM

(i) Isolation of the pathogen

Isolations of the pathogen involved in the rot were made from diseased portion of fruit samples of apple collected from different Marketing Yards. The

diseased fruits were surface sterilized with absolute alcohol under aseptic conditions. Small bits of 1 to 2 mm size were taken from juncture of diseased and healthy part of fruit with the help of sterilized sharp blade or scalpel. These bits were surface sterilized with mercuric chloride (0.1%) for 10 to 20 seconds and washed thrice with sterilized distilled water under aseptic conditions. The bits were then placed on the sterilized filter paper to remove the excess moisture and subsequently transferred to sterilized Petri plates containing potato dextrose agar (PDA) medium. The medium was supplemented with Streptocyclin (30 mg/lt) while pouring in Petri plates after sterilization to restrict the bacterial contaminates. The inoculated Petri plates were incubated at 25 ± 1^0 C in BOD incubator and examined daily for mycelial growth. The fungal growth developed in Petri plates was purified by hyphal tip technique and was further cultured on slants containing PDA. These slants of culture were preserved at 5^0 C in the refrigerator for further studies. Stock cultures were maintained by regularly sub culturing after 20 to 25 days.

(ii) Effect of temperature

Studies were conducted to observe the effect of temperature on the vegetative growth of *Botryosphaeria dothidea*, the pathogen involved in white rot of apple. Three Petri plates were inoculated with the test pathogen and were incubated at seven different temperature regimes at 5, 10, 15, 20, 25, 30 and 35^0 C. Each treatment was replicated thrice and observations on the radial growth of the mycelium were recorded daily till the Petri plates were completely filled with the mycelium.

(iii) Identification of pathogen

The morphological characters of the fungus were studied on host as well as in the culture grown on PDA. The causal organism was identified by studying its morphology and comparisons with standard authentic description from "Compendium of Apple and Pear Diseases" given by Jones and Aldwinckle (1990). In addition, the pure culture of this pathogen got identified through National Centre of Fungal Taxonomy New Delhi.

(iv) METHOD OF INOCULATION

Pin prick method

In different experiments, healthy fruits were inoculated by pin-prick method (Freeman *et al*., 1996; Wadia *et al*., 1983; Jadesha *et al*., 2012). In this method, four sterile insect mounting pins of (0.45 mm) diameter were taken for inoculating the fruits with the spore suspension of conidia. Spore suspension of conidia was prepared by taking 20 days old vigorously growing culture of the fungus in Petri plates. Sterilized distilled water was added to this culture to make the spore suspension (10^5/ml). Then the sterilized pins were dipped in the conidial suspension and the fruits were inoculated on the side by piercing the skin of the fruits up to 1 mm depth. Then, the inoculated fruits were further subjected to different treatments in the management studies.

3.C. Pathogenicity test

The pathogenicity test of the causal organism (*B. dothidea*) was conducted by following Koch's postulates under *in vitro* conditions by inoculating the healthy fruits (Golden Delicious) apple through pin-prick method. The inoculated portion of fruit was covered with sterilized paraffin wax and incubated at room temperature (25 ± 1^0 C). After inoculation, the fruits were immediately covered with sterilized filter paper and moist cotton to maintain the relative humidity.

3 D. Varietal behaviour

Varietal behaviour was also observed on four commercial varieties for their comparative susceptibility to white rot (*B. dothidea*). Apple fruit cultivars viz. Golden Delicious, Granny Smith, Rich-a-Red and Royal Delicious were examined for their comparative susceptibility. Ten fruits of each variety were inoculated by pin-prick method. All the inoculated fruits were kept at room temperature (25 ± 3^0 C) for comparison. Per cent rot was calculated in four different varieties after 10, 20 and 30 days of inoculation.

3 E. Fruit Qualitative Characters

Fruit qualitative characters in terms of fruit pressure, total soluble solids (TSS), titratable acidity were assayed from diseased and healthy fruits in each treatment.

(i) Fruit Pressure

The fruit pressure of apple fruits was estimated with the help of a penetrometer in all the management studies to ascertain the quality of fruits in different treatments. The skin of the fruit was removed using slicers to a 1-mm cutting depth, and flesh firmness was then measured with a penetrometer equipped with an 11-mm diameter plunger tip and the observations were noted down in lbs/sq.inch.

(ii) Total soluble solids (TSS)

The total soluble solids (TSS) content of the fruit sample were determined with the help of an 'Erma' hand refractometer. A drop of the juice squeezed from the sample was placed on the prism and viewed through the eye piece and expressed as ^{o}Brix. Five fruits were taken from each treatment for recording this observation.

(iii) Titratable acidity

Twenty five gram of fruit pulp was thoroughly homogenized with distilled water in a waring blender and the volume was made upto 250 ml. Then, the homogenized mixture was filtered through Whatman No.1 filter paper. Then 10 ml sample from the filtrate was titrated against 0.1 N NaOH solution using phenolphthalein as indicator in each treatment. The end point was noted with change in colour to pink. The total titratable acidity was calculated in term of malic acid on basis off 1 ml of 0.1 N NaOH being equivalent to 0.0067 g anhydrous malic acid. The results were expressed as per cent flesh weight of fruit pulp.

(iv) Effect on fruit weight

In the management studies, 10 representative fruits from each replication were weighed in the beginning using digital balance and then same marked fruits were weighed at an interval of 10, 20 and 30 days during the period of the experiment in each treatment. The loss in weight at each interval during storage was recorded.

3 F. DISEASE MANAGEMENT STUDIES

(i) *In vitro* evaluation of different fungicides against the white rot pathogen

Efficacy of six systemic fungicides viz., kresoxim-methyl (Ergon 44.3% w/w SC), difenoconazole (Score 25 EC), tebuconazole (Folicur 250 EC), azoxystrobin (Amistar 25 EC), trifloxystrobin (Flint 50 WG), pyraclostrobin (Insignia 20 WG) were tested under *in vitro* conditions against the white rot pathogen at 25, 50 and 100 ppm concentrations by poisoned food technique given by (Falck, 1907).

Double strength potato dextrose agar (PDA) medium was prepared by doubling the amount of constituents except water. PDA was sterilized for 20 minutes at 1.05 kg/cm^2 pressure in an autoclave. Simultaneously, double concentrations than what finally required was prepared of different fungicides in sterilized distilled water so as to get desired concentration of fungicide solutions in the double strength media. Double strength fungicides solution was added separately to equal quantities of double strength medium in different 250 ml conical flasks. These conical flasks were shaken well, poured separately in Petri plates and allowed to solidify. A small culture bit of 4 mm size of the test pathogen (*B. dothidea*) cut with a sterile cork borer was picked up with the help of a sterile inoculation needle and was placed in centre of each Petri plates under aseptic conditions at a laminar air flow workstation. Petri plates without fungicide in medium served as control. Each concentration of fungicide was replicated thrice and the Petri plates were incubated at 25±1^{0}C in BOD incubator.

The colony diameter of test pathogen was recorded till the control plates were fully covered with radial growth of mycelium of the test pathogen and per cent inhibition was calculated by formula given by Vincent (1947).

$$I = \frac{C-T}{C} \times 100$$

Where,

I	=	Inhibition (%)
C	=	Diametric mycelial growth in control (mm)
T	=	Diametric mycelial growth in treatment (mm)

(ii) *In vitro* efficacy of botanicals against the white rot pathogen

Water extract of leaves in case of *Karu* (*Roylea elegans* Wall.), *Artemisia* (*Artemisia roxburghiana* Wall.), *Neem* (*Azadirachta indica* L.), *Bana* (*Vitex negundo* L.), *Mehandu* (*Dedonia viscosa* (L.) Jacq.), *Bougainvillea (Bougainvillea* glabra L.), *Tulsi* (*Ocimum sanctum* L.), *Mentha* (*Mentha piperita* L.), *Safeda* (*Euclyptus globules* Labill.) *Aloe* (*Aloe vera* (L.) Burm. f); seed extract in case of *Neem* (*Azadirachta indica* L.), *Darek* (*Melia azedarach* L.) and cow urine were evauated at different concentrations (i.e. 10, 25, and 50 %) under *in vitro* condition against white rot pathogen. The extracts were tested by poisoned food technique (Falck, 1907) to observe the inhibitory effect of these extracts on the mycelial growth of *B. dothidea*.

(iii) Preparation of plant extracts

About 2 month old freshly harvested (200 g) leaves of each plant except for *Neem* and *Darek* where 200 gram mature seed were taken and then washed under tap water. Each sample was grinded in mixer and blender by adding small quantity of sterilized distilled water. After grinding, 200 ml distilled water was added and homogenized in orbital shaker at 2000 rpm for half an hour to get 100 per cent extract of each plant. The plant material was then filtered through double-layered muslin cloth. Sterilization of the extract of different plants was done in autoclave at 1.05 kg/cm^2 pressure for 5 minutes and then the extracts were kept in refrigerator for further use.

(iv) *In vitro* evaluation of botanicals against the white rot pathogen

Evaluation of botanical extracts was done by poisoned food technique. Double strength potato dextrose agar (PDA) medium was prepared by doubling the amount of all the constituents except water. PDA was sterilized for 20 minutes at 1.05 kg/cm^2 pressure in an autoclave. An equal amount of the double strength plant extract was added separately in double strength medium in the 250 ml flask. The PDA mixed with the plant extract was shaken well, poured in Petri plates and was allowed to solidify. A small culture bit of 4 mm size of the test pathogen (*B. dothidea*) cut with a sterile cork borer was picked up with the help of sterilized inoculation needle and was placed in the centre of each Petri plates under aseptic conditions in laminar air flow work station. Petri plates poured with double strength PDA inoculated as above served as control. Each concentration of extract was replicated thrice and the Petri plates were incubated at 25±1^0 C in BOD incubator. The colony diameter of test pathogen was recorded till the control plates were fully covered with radial growth of mycelium of the test pathogen. Per cent inhibition in the mycelial growth of the test pathogen was calculated as described earlier. The above experiment was carried out in Completely Randomized Design (CRD).

(v) Preparation and evaluation of Botanical Formulations (1 and 2)

Out of twelve plants evaluated for their *in vitro* efficacy in inhibiting the growth of white rot pathogen, six effective plants were selected for making two botanical formulations. Six effective plants include *Karu* (*Roylea elegans* Wall.), *Artemisia* (*Artemisia roxburghiana* Wall.), *Neem* (*Azadirachta indica* L.), *Bana* (*Vitex negundo* L.), and *Tulsi* (*Ocimum sanctum* L.). In Botanical Formulation (BF1), equal quantity (200 g) of sixty days old freshly harvested leaves of *Karu* (*Roylea elegans* Wall.), *Artemisia* (*Artemisia roxburghiana* Wall.), *Neem* (*Azadirachta indica* L.), *Bana* (*Vitex negundo* L.), *Tulsi* (*Ocimum sanctum* L.) and 200 g of mature seeds of *Darek* (*Melia azedarach* L.) were taken. These leaves and seeds were washed in the running tap water and then with distilled water. Then, the paste of all these ingredients was made in a mixer and grinder. Then, equal quantity of distilled water (200 × 6 = 1200 ml) was added to this

paste of leaves and seeds of six plants on weight and volume basis (w/v). In Botanical Formulation 2 (BF2), equal quantity (200 g) of sixty days old freshly harvested leaves of *Karu* (*Roylea elegans* Wall.), *Artemisia* (*Artemisia roxburghiana* Wall.), *Neem* (*Azadirachta indica* L.), *Bana* (*Vitex negundo* L.), *Tulsi* (*Ocimum sanctum* L.) and 200 g of mature seeds of *Darek* (*Melia azedarach* L.) were taken. These leaves and seeds were washed in the running tap water and then with distilled water. Then the paste of all these ingredients was made in mixer grinder by adding little quantity of fresh cow urine of *Jersey* cow. Then equal quantity of cow urine (200 × 6 = 1200 ml) was added to this paste of leaves and seeds of six plants on weight and volume basis (w/v). Thus, while BF1 is water based formulation, BF2 is cow urine based formulation. These formulations were sterilized at 1.05 kg/cm^2 for 5 minutes and were evaluated against the white rot pathogen in comparison with effective fungicide (Score) for their mycelium inhibiting properties of the white rot pathogen.

3 G. MANAGEMENT OF WHITE ROT

(i) Selection of fruits

Freshly harvested fruits of Golden Delicious variety were procured from the market and these fruits were spread out on the working table of the laboratory so that fruits of uniform shape, size and maturity were selected. These fruits were sorted out to discard any immature, over-ripe, bruised undersized or diseased fruit. Rest of the fruits were used for the management and other studies in the objectives. In each treatment, 45 fruits of uniform size were selected randomly.

(ii) Treatment of fruits

In these studies, Botanical formulation 1 and 2 were used either on the fruits or on the packing materials used as treatments. In comparison, fungicide was also used similarly either on the fruits or on the packing materials used. Edible wax (Carnauba wax) was also used as a treatment on the fruits for comparison.

(iii) Application of Botanical Formulations (BF1 and BF2) in different treatments

Six effective botanicals which inhibited maximum radial growth of the test pathogen under *in vitro* conditions were combined and evaluated in two combinations of Botanical Formulations 1 (BF1) and Botanical Formulations 2 (BF2) against the white rot incidence on fruits in storage. The efficacy of Botanical Formulations (BF1 and BF2) was compared with other treatments of fungicide and wax as fruit dip, dip of fruit wrappers and dip of fruit trays. Skin coating of fruits in BF1 and BF2 was done by dipping the fruits in different treatment solutions for 30 minutes. Fruits were dried after the treatment in the laboratory by spreading on working tables before packing in the trays. Fruit wrappers and trays were impregnated with botanical formulations and the test fungicide treatment for the protection of apple fruits. Daily newspaper sheets of (52 ×33 cm) dimension were used as fruit wrappers. To prepare the impregnated wrappers of BF1 and BF2 five sheets of newspaper were slowly poured with the solution (150 to 200 ml) of the different treatment separately in a way so that the sheets (wrappers) were drenched with the solution and that solution also do not spill out from set of five sheets. Uniform soaking of solution of each treatment was secured by spreading the solution smoothly over the sheets. The newspaper sheets were air dried in shade and sheets were then cut and divided in to wraps of uniform size measuring 25 cm^2 in dimension. Similarly, fruit trays were also impregnated by dipping of these trays in different treatment solutions (BF1 and BF2) for 30 minutes (Plate 4, Fig. 11). Trays were air dried after the treatment under shade in the laboratory before packing the fruits in the trays. Sterilized distilled water was taken as control for skin coating of fruits. Observations on efficacy of these treatments against white rot were recorded after 10, 20 and 30 days in storage at 25 ± 3^0 C. Observations were taken with respect to incidence of white rot on the fruits, loss in fruit weight, change in titratable acidity, change in total soluble solids (TSS), lesion size on fruits and micro-flora count on the fruits at different time interval. The total count of micro-flora present on fruit surface was enumerated by dilution plate technique (Gangopadhyay, 1984).

(iv) Application of chemical fungicide in different treatments

Most effective fungicide was mixed in distilled water in desirable concentration and final volume of chemical solution was made. Fungicide was evaluated as fruit dip. In addition, wrappers and fruit trays were also impregnated with the fungicide by dipping these in this fungicide. Skin coating of fruits in fungicide was done by dipping the fruits in fungicide solutions for 30 minutes. Fruits were then dried after the treatment by spreading them on the table before packing in the trays. To prepare the impregnated wrappers with the desired concentration of fungicide solution, five sheets of newspaper (52×33 cm) were poured with the desired fungicidal solution (150 to 200 ml) so that newspaper sheets were drenched with the solution and that solution do not spill out from set of five sheets. Uniform soaking of solution was secured by spreading the solution slowly over the sheets. The newspaper sheets were then air dried in shade and sheets were divided in to fruit wraps of uniform size measuring 25 cm^2 in dimension. Similarly, impregnation of fruit trays was done with fungicide solution by dipping them for 30 minutes. Trays were then dried after the treatment in laboratory before packing the fruits in these impregnated trays. Sterilized distilled water was taken as control for skin coating of fruits. Observation on efficacy of these treatments against incidence of white rot was recorded after 10, 20 and 30 days in storage at room temperature (25 ± 3^0 C). Observations were also taken with respect to lesion size of white rot in fruits, change in fruit weight, change in titratable acidity, change in total soluble solids (TSS) and surface micro-flora count of the fruits recorded at different time interval as described earlier.

(v) Application of Edible wax

Skin coating is known to improve the keeping quality of stored fruits in storage by decreasing water loss, retarding ripening and reducing rotting (Chiumarell and Ferreira, 2006).The edible Carnauba wax was used to study its impact on keeping quality and storage. Carnauba wax was melted at 78^0 C and the mixture was stirred continuously. Dip coating method was employed for the

surface coating of the apple fruits when mixture was completely homogenised. Skin coating of fruits with edible wax was done by dipping fruits in gently heated (40^0 C) edible wax for 3-5 minutes. Then, the treated fruits were allowed to dry under shade so that edible wax cover whole fruit skin surface. Efficacy of this treatment was observed against fruit rot and quality of fruits after 10, 20 and 30 days in storage at room temperature (25 ± 3^0 C). Observations were recorded with respect to incidence of white rot in fruits, lesion size of the rot on fruits change in fruit weight, change in titratable acidity, change in total soluble solids (TSS) and surface micro-flora count at different time interval as described earlier.

In control treatment, no treatment was given to the inoculated apples. Each treatment was replicated thrice and each treatment had 45 fruits. In this treatment, fruits were dipped in distilled water for 30 minutes. After inoculation with the test pathogen, fruits were dried and stored at room temperature (25 ± 3^0C) and consequently observations were taken at different time interval.

Micro-flora count

The total count of micro-flora present on fruit surface was enumerated by dilution plate technique (Gangopadhyay, 1984). Micro-flora count on surface of the fruits was determined by taking 1 cm diameter bits with the help of a cork borer from four sides of the fruits in different treatments and then these bits of apple skin were dipped in 10 ml of sterilized distilled water. This solution containing population of different microbes were further diluted 10^3 to 10^5 level to have a clear count of harvested fungi and bacteria. Sampling of surface micro-flora was done at 10, 20 and 30 days of storage. Each treatment contained three replication.

3 H. STATISTICAL ANALYSIS

The data recorded from various *in vitro* and laboratory experiments were subjected to statistical analysis. The differences exhibited by treatments in various experiments were tested for their significance at 5 per cent using standard procedure as described by Gomez and Gomez (1983).

Chapter-4

EXPERIMENTAL RESULTS

4A. Incidence of white rot of apple in different terminal fruit markets

The survey of different Marketing yards in Shimla and Solan districts of Himachal Pradesh were conducted during the peak harvesting season of apple during the months of July-September in 2011, 2012 and 2013 to record the incidence of white rot (*Botryosphaeria dothidea*) and other apple fruit rots in total. The survey was confined to major Marketing Yards viz. Narkanda, Shimla, Solan and Parwanoo. The study to record the incidence was based on visual symptoms of the disease which were later confirmed by the isolation of fungus from the representative samples of the diseased fruit. The number of fruits examined was selected at random from each location. Data were recorded by opening the packed fruit boxes as well as from the open heaps. Apple fruits showing white rot (*B. dothidea*) symptoms were counted and per cent disease incidence was calculated.

The data indicated that incidence of white rot varied from 2.5 to 15.5 per cent, while incidence of other fruit rots in total varied from 11.2 to 25.1 per cent during 2011, 2012 and 2013 (Table1). Maximum incidence (12.5%) of white rot was recorded at Parwanoo Marketing Yard followed by Solan (9.0%) and Shimla (4.0%) in contrast to lowest incidence (2.5%) of the white rot disease at Narkanda during 2011. During the same period, survey was conducted in 2012 and maximum incidence (15.5%) of white rot was recorded at Parwanoo Marketing Yard followed by Solan (10.5%) and Shimla (6.0 %) while lowest incidence (3.5%) of disease was observed in Narkanda. Further in 2013, white rot incidence was highest (14.5%) at fruit marketing yard Parwanoo followed by Solan (9.5%) and Shimla (5.5%) while comparatively less incidence (3.0%) of white rot was observed in fruit marketing yard Narkanda. Overall, the average incidence of white rot was higher at fruit marketing yard Parwanoo (14.1%) followed by Solan (9.7%) while fruit marketing yard at Shimla and Narkanda recorded lower

incidence of rot at 5.2 and 3.0 per cent, respectively (Plate 1, Fig. 1). The data indicated that incidence of white rot was comparatively higher ranging from 9.7 to 14.1 per cent in Marketing Yards located at lower elevations in warmer areas in comparison to fruit markets located in higher hills with comparatively cooler climate.

Table 1. Incidence of post-harvest rots in apple from 2011 to 2013

Districts	Terminal Fruit markets	Incidence (%) of post-harvest rots in apple from 2011 to 2013							
		2011		2012		2013		Mean	
		White rot	Other rot	White rot	Other rot	White rot	Other rot	White rot	Other rot
Shimla	Narkanda	2.5	11.2	3.5	13.6	3.0	15.3	3.0	13.4
	Shimla	4.0	15.3	6.0	17.2	5.5	18.3	5.2	16.9
Solan	Solan	9.0	19.8	10.5	21.3	9.5	20.4	9.7	20.5
	Parwanoo	12.5	23.9	15.5	25.1	14.5	24.6	14.1	24.5

4B. SYMPTOMATOLOGY, ISOLATION AND IDENTIFICATION OF THE PATHOGEN

(i) Symptomatology

During the course of survey, typical symptoms of the white rot disease of apple were observed in different Marketing Yards. The initial symptoms were typically comprised of formation of slightly sunken brown spots which were bordered by one or more red halo rings. As the decayed area expanded, the core became rotten in cylindrical manner (Plate 1, Fig. 2 and 3). In red skinned apple cultivars, bleaching was found during decay process and rotted portion became light brown in colour. Because of this characteristic, the disease has been referred as “white rot”. As the rot progresses, the skin colour became dark brown and flesh was soft and mushy. Syrupy beads of exudates appeared on the surface of completely rotted fruits after complete rot with this pathogen. Mature and ripened fruits were found more susceptible to white rot infection.

(ii) Isolation of the pathogen

Isolation of pathogen associated with white rot was made from diseased portion of apple fruit on potato dextrose agar (PDA) medium using standard

Fig.1. Post-harvest losses in apple in a Marketing Yard

Fig. 2. Symptoms of white rot (*Botryosphaeria dothidea*) of apple

Fig. 3. External and internal symptoms of white rot in apple

Plate 1.

technique. The white rot pathogen was cultured on PDA by taking smaller bits of host tissues from diseased fruit particularly from the infected area joining with the healthy tissues. After putting the diseased bits in the potato dextrose agar (PDA), the fungus protruded out of the infected tissues in to the medium. The isolated fungus was then purified by hyphal tip method on Petri plates containing PDA. Purity and virulence of the isolated fungus was regularly monitored and maintained by the sub culturing after 20 to 25 days.

(iii) Identification of pathogen

Disease samples, comprising of apple fruits damaged by white rot disease were examined visually followed by microscopic examination of the sections of infected tissues. The pure culture of white rot pathogen was examined for morphological and cultural characteristics. The microscopic examination of mycelium and spores indicated its resemblance with *Botryosphaeria dothidea*. The fungus produced conidia, which were hyaline, unicellular, fusoid to ellipsoidal with obtuse apex measuring 19.0 × 6.3 µm in average size (Plate 2, Fig 4). The culture of the fungus produced compact, velvety and olivaceous grey coloured fungal colonies on PDA and took 10 to 12 days to fully cover the Petri plates and then the culture finally turned to violaceous black (Plate 2, Fig 5). The morphological characters of the isolated fungus were similar to the published description of the fungus given in "Compendium of Apple and Pear Diseases" given by Jones and Aldwinckle (1990). Thus, the description indicated the presence of *Botryosphaeria dothidea*. In addition, the pure culture of this particular pathogen got identified through National Centre of Fungal Taxonomy New Delhi under Id. No. 5217.12, which identified it as *B. dothidea*.

4C. (i) Pathogenicity test

The pathogenicity test of the isolated fungus was conducted on healthy fruits of Golden Delicious apple cultivar. The fungus produced typically white rot symptoms within 25-30 days of the inoculation through pin-prick method (Freeman *et al*., 1996; Wadia *et al*., 1983; Jadesha *et al*., 2012). The pathogen was re-isolated on PDA medium from disease affected tissues of the fruits. The microscopic characters of the re-isolated fungus were same as recorded in case of

the parent culture of the test fungus. Thus, it proved the pathogenicity of fungus as stipulated in Koch's postulates.

(ii) Effect of temperature

To determine the optimum temperature required for vegetative growth of the fungus causing white rot of apple, seven different temperature regimes, ranging from 10 to 35^{0}C were evaluated in these studies. The data indicated that the fungus was capable of growing on wide range of temperature from 10 to 35^{0}C but with wide variability (Table 2).

Table 2. Effect of temperature on diametric growth of *Botryosphaeria dothidea*

Temperature (^{0}C)	Average diametric growth (mm)
5	0.00
10	19.03
15	31.75
20	43.58
25	69.62
30	73.44
35	57.23
CD (0.05)	2.06

It was clear from the data that *Botryosphaeria dothidea* failed to grow at 5^{0}C. However, there was a steady and significant increase in the diametric growth after 10^{0}C onwards, touching maximum diametric growth at 30^{0}C (73.4 mm) followed by 25^{0}C (69.6 mm). Growth was abruptly reduced after 30^{0}C (57.23mm) and minimum fungal growth (19.03 mm) was observed at 10^{0}C. Thus temperature range of 25-30^{0}C is optimum for the growth of the fungus.

4 D. Susceptibility of different apple cultivars to white rot (*B. dothidea*)

Behaviour of four commercial cultivars of apple to inoculation of the white rot pathogen indicated that Granny Smith was less susceptible to white rot (*B. dothidea*), which had no rotting of fruits even after 30 days (Table 3). While, Golden Delicious cultivar was the most susceptible as it accounted for 66.45 per

cent overall rotting followed by Rich-a-Red which had 47.69 overall per cent fruit rot. While Royal Delicious cultivar of apple was less susceptible to the white rot with 7.60 per cent of overall fruit rot after 30 days of inoculation.

Table 3. Susceptibility of different apple cultivars to white rot (*B. dothidea*)

Varieties	Incidence of fruit rot after different durations (days) in storage			
	10	**20**	**30**	**Mean**
Golden Delicious	21.75	77.60	100.00	66.45
Rich-a-Red	4.12	39.95	100.00	47.69
Royal Delicious	2.0	5.10	15.71	7.60
Granny Smith	0.00	0.00	0.00	0.00
Mean	6.97	30.66	53.92	

4E. DISEASE MANAGEMENT STUDIES

(i) *In vitro* evaluation of different fungicides against the white rot pathogen (*Botryosphaeria dothidea*)

The efficacy of six fungicides viz. kresoxim-methyl (Ergon 44.3% w/w SC), difenoconazole (Score 25 EC), tebuconazole (Folicur 25 EC), azoxystrobin (Amistar 25 EC), trifloxystrobin (Flint 50 WG), pyraclostrobin (Insignia 20 WG) was tested under *in vitro* conditions against the white rot pathogen (*Botryosphaeria dothidea*) at 25, 50 and 100 ppm concentrations by poisoned food technique.

It was evident from the data that all these fungicides significantly inhibited the mycelial growth of the white rot pathogen in comparison to control (Table 4). Difenoconazole was found most effective and significantly superior among all the treatments with 75.01 per cent average inhibition in mycelial growth of the white rot pathogen followed by tebuconazole 71.68 per cent. Other fungicides effective against the white rot pathogen were trifloxystrobin and azoxystrobin which reduced the growth of the fungus by 60.12 and 60.00 per cent, respectively and both did not differ significantly with each other. Pyraclostrobin was found least effective among all the treatments with 54.32 per cent average inhibition in mycelial growth of the pathogen. It was also noticed that as the concentration of

the fungicides increased, there was corresponding increase in per cent mycelial inhibition of the pathogen.

Table 4. *In vitro* efficacy of fungicides against white rot pathogen (*Botryosphaeria dothidea*)

Fungicide	Per cent inhibition in mycelial growth at concentration (ppm)			
	25	50	100	Mean
Score 25 EC (difenoconazole)	58.71 (49.99)	77.09 (61.38)	89.25 (70.84)	75.01 (60.73)[a]
Amistar 25 EC (azoxystrobin)	48.89 (44.35)	58.15 (49.67)	72.96 (58.64)	60.00 (50.89)[c]
Folicur 250 EC (tebuconazole)	55.10 (47.91)	73.66 (59.10)	86.29 (68.24)	71.68 (58.42)[b]
Insignia 20 WG (pyraclostrobin)	35.52 (36.56)	46.6 (43.03)	56.59 (48.76)	46.23 (42.78)[e]
Flint 50 WG (trifloxystrobin)	49.26 (44.56)	54.06 (47.31)	77.04 (61.35)	60.12 (51.07)[c]
Ergon 44.3% w/w SC (kresoxim-methyl)	34.81 (36.14)	52.59 (46.47)	75.55 (60.34)	54.32 (47.65)[d]
Mean	47.04 (43.25)[c]	60.35 (51.16)[b]	76.28 (61.36)[a]	

Figures in parentheses are arc sine transformed values
Figures denoted by same letter do not differ significantly

$CD_{(0.05)}$

Fungicide	:	0.71
Concentration	:	0.50
Fungicide x Concentration	:	1.24

(ii) *In vitro* efficacy of botanicals against the white rot pathogen

Water extract of leaves in case of *Karu* (*Roylea elegans* Wall.), *Artemisia* (*Artemisia roxburghiana* Wall.), *Neem* (*Azadirachta indica* L.), *Bana* (*Vitex negundo* L.), Mehandu (*Dedonia viscosa* (L.) Jacq.), *Bougainvillea (Bougainvillea glabra* L.), *Tulsi* (*Ocimum sanctum* L.), *Pudina* (*Mentha piperita* L.), *Safeda* (*Euclyptus globules* Labill.), *Aloe* (*Aloe vera* (L.) Burm. f); seed extract in case of *Darek* (*Melia azedarach* L.) and *Neem* (*Azadirachta indica* L.) and cow urine were evaluated at under *in vitro* condition at 10, 25 and 50 per cent concentrations against white rot pathogen by poisoned food technique (Falck, 1907) to observe the inhibitory effect of these extracts on mycelial growth of *B. dothidea*.

It was evident from the data that all extracts inhibited the mycelial growth of the pathogen in comparison to control (Table 5). Leaf extract of *Ocimum*

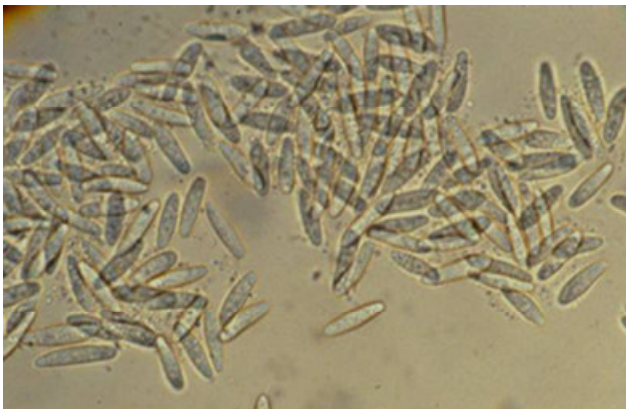


Fig. 4. Conidia of *Dothiorella mali* anamorph *Botryosphaeria dothidea*

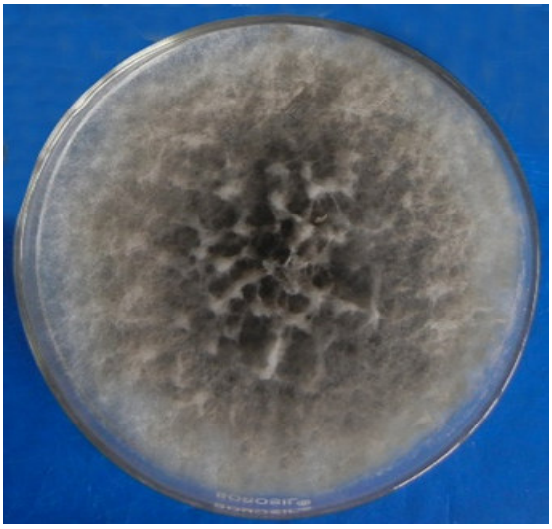
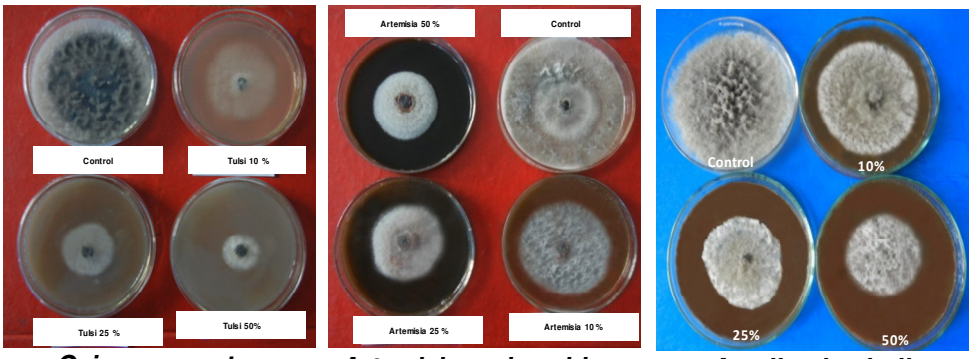


Fig. 5. Pure culture of *Botryosphaeria dothidea* on PDA



Ocimum sanctum ***Artemisia roxburghiana*** ***Azadirachta indica***

Fig. 6. *In vitro* efficacy of water extract of different plants against white rot pathogen

Plate 2.

sanctum was found most effective and significantly superior amongst all the treatments with 54.07 per cent average inhibition at different concentration in mycelial growth of the white rot pathogen followed by leaf extract of *Azadirachta indica* and *Artemisia roxburghiana* with 48.98 and 38.88 per cent mycelial growth inhibition, respectively (Plate 2, Fig.6; Plate 3, Fig. 7). Seed extracts of *Melia azedarach* followed by leaf extract of *Roylea elegans* with 31.56 and 30.46 per cent inhibition in mycelial growth of the white rot pathogen were next in efficacy. Leaf extract of *Mentha piperita* was found least effective with mycelial growth inhibition of 7.78 per cent only. The data also indicated that as the concentration increased from 10 to 50 per cent, there was corresponding increase in per cent mycelial growth inhibition of the pathogen.

Table 5. *In vitro* efficacy of botanicals and cow urine against the white rot pathogen (*Botryosphaeria dothidea*)

Botanicals/cow urine	Per cent inhibition in mycelial growth at concentration (%)			
	10 %	**25%**	**50 %**	**Mean**
Melia azedarach (S) (Darek)	11.94 (20.19)	38.60 (38.39)	44.16 (41.63)	31.56 (33.41)^{d}
Roylea elegans (L) (Karu)	21.66 (27.71)	27.49 (31.61)	42.22 (40.51)	30.46 (33.27)^{d}
Artemisia roxburghiana (L) (Artemisia)	28.33 (32.13)	38.61 (38.39)	49.72 (44.82)	38.88 (38.45)^{c}
Azadirachta indica (L) (Neem)	41.39 (40.02)	49.17 (44.50)	56.39 (48.65)	48.98 (44.39)^{b}
Vitex negundo (L) (Bana)	18.06 (25.12)	26.94 (31.25)	42.22 (40.51)	29.07 (32.29)^{e}
Dedonia viscosa (L) (Mehandu)	6.39 (14.56)	9.72 (18.13)	16.38 (23.82)	10.83 (18.84)^{k}
Bougainvillea glabra (L) (Bougainvillea)	12.22 (20.44)	16.94 (24.28)	47.50 (43.55)	25.55 (29.42)^{f}
Mentha piperita (L) (Pudina)	5.00 (12.89)	6.95 (15.24)	11.39 (19.69)	7.78 (15.95)^{l}
Ocimum sanctum (L) (Tulsi)	39.72 (39.05)	59.44 (50.43)	63.05 (52.55)	54.07 (47.34)^{a}
Euclyptus globules (L) (Safeda)	19.16 (25.94)	21.66 (27.71)	25.55 (30.34)	22.13 (27.99)^{g}
Aloe vera (L) (Ghrit Kumari)	18.89 (25.73)	20.83 (27.14)	24.72 (29.80)	21.48 (27.56)^{h}
Azadirachta indica (S) (Neem)	9.16 (17.54)	16.39 (23.85)	36.66 (37.24)	20.74 (26.21)^{i}
Cow urine	5.55 (13.59)	8.61 (17.04)	45.11 (42.17)	19.76 (24.27)^{j}
Mean	16.96 (22.49)^{c}	24.38 (27.71)^{b}	36.08 (35.38)^{a}	

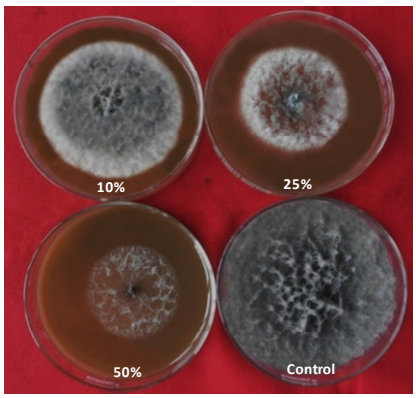
S, Seeds; L, Leaves Extract
Figures in parentheses are arc sine transformed values
Figures denoted by same letter do not differ significantly

CD $_{(0.05)}$	
Botanical	0.39
Concentration	0.85
Botanical x Concentration	1.48

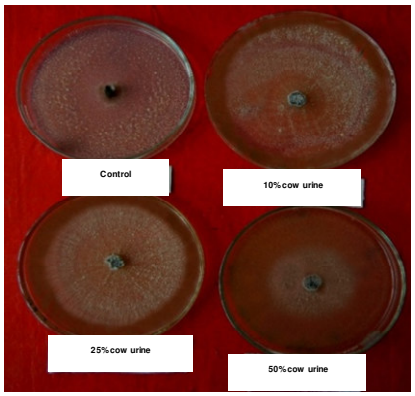
(iii) ***In vitro*** **evaluation of botanical formulations (BF1 and BF2)**

Twelve plants were evaluated for their *in vitro* efficacy in inhibiting the growth of white rot pathogen and out of these six effective plants were selected for making two botanical formulations. Six effective plants include *Karu* (*Roylea elegans* Wall.), *Artemisia* (*Artemisia roxburghiana* Wall.), *Neem* (*Azadirachta indica* L.), *Bana* (*Vitex negundo* L.), and *Tulsi* (*Ocimum sanctum* L.). In Botanical Formulation1 (BF1), equal quantity (200 g) of sixty days old freshly harvested leaves of *Roylea elegans*, *Artemisia roxburghiana*, *Azadirachta indica*, *Vitex negundo*, *Ocimum sanctum* and 200 g of mature seeds of *Melia azedarach* were taken. These leaves and seeds were washed in the running tap water and then with distilled water. Then, the paste of all these ingredients was made in a mixer and grinder. Then, equal quantity of distilled water (200 × 6 = 1200 ml) was added to this paste of leaves and seeds of six plants on weight and volume basis (w/v). In Botanical Formulation 2 (BF2), equal quantity (200 g) of sixty days old freshly harvested leaves of *Roylea elegans*, *Artemisia roxburghiana*, *Azadirachta indica*, *Vitex negundo*, *Ocimum sanctum* and 200 g of mature seeds of *Melia azedarach* were taken. These leaves and seeds were washed in the running tap water and then with distilled water. Then, the paste of all these ingredients was made in mixer grinder by adding little quantity of fresh cow urine of *Jersey* cow. Then, equal quantity of cow urine (200 × 6 = 1200 ml) was added to this paste of leaves and seeds of six plants on weight and volume basis (w/v). Thus, while BF1 is water based formulation, BF2 is cow urine based formulation. These formulations were sterilized at 1.05 kg/cm^2 for 5 minutes and were evaluated at different concentrations (10%, 25%, 50% and 100%) in comparison for their mycelium inhibiting properties of the white rot pathogen.

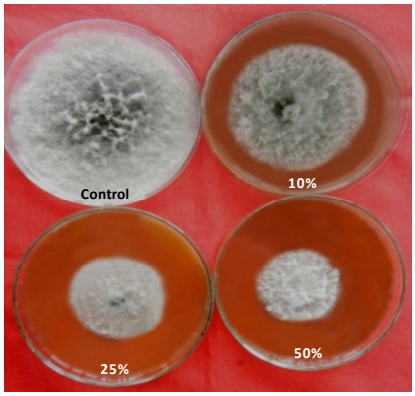
BF1, BF2 and Fungicide significantly inhibited the mycelial growth of the white rot pathogen in comparison to control (Table 6). Overall, maximum mycelial growth inhibition (64.63 %) of *Botryosphaeria dothidea* was done by Fungicide (Score) followed by Botanical formulation 2 (BF2) (48.38 %). Botanical formulation 1 (BF1) was least effective with 43.09 % overall mycelial growth inhibition (Plate 3, Fig. 8). Maximum fungal growth inhibition was achieved at 100 per cent concentration and Score, BF1 and BF2 resulted in 89.25,



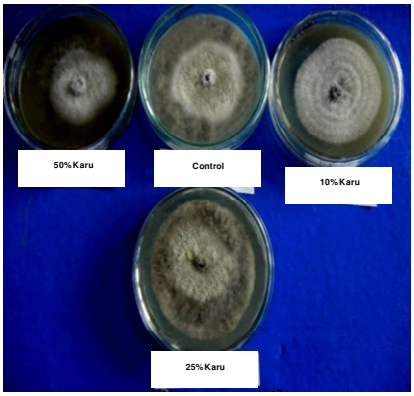
Melia azedarach



Cow urine

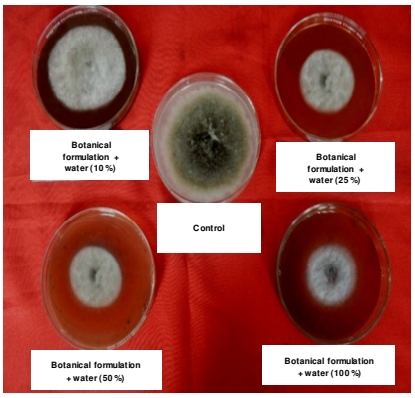


Vitex negundo



Roylea elegans

Fig. 7. *In vitro* efficacy of water extract of different plants against the white rot pathogen (*Botryosphaeria dothidea*)



BF1

Botanical formulation 1 (Water based)

Fig. 8. *In vitro* evaluation of water based (BF1) Botanical Formulation

Plate 3.

66.37 and 72.70 per cent inhibition, respectively (Plate 4, Fig. 9 and 10). The data also indicate that as the concentration increased from 10 to 100 per cent, there was corresponding increase in per cent mycelial growth inhibition of the pathogen.

Table 6. *In vitro* evaluation of fungicide and botanical formulations (BF1 and BF2)

Treatments	Per cent inhibition in mycelial growth at concentration (%)				
	10 %	**25 %**	**50 %**	**100 %**	**Mean**
BF1	14.59 (22.43)	34.04 (35.66)	57.37 (49.21)	66.37 (54.53)	43.09 (40.46)
BF2	17.23 (24.5)	39.26 (38.78)	64.33 (53.30)	72.70 (58.48)	48.38 (43.77)
Fungicide (Score)*	33.44 (35.31)	58.71 (49.99)	77.09 (61.38)	89.25 (70.84)	64.63 (54.38)
Mean	21.76 (27.41)	44.00 (41.48)	66.26 (54.63)	76.11 (61.28)	

*Score was tested at 10, 25, 50 and100 ppm
Figures in parentheses are arc sine transformed values

Effect	**$CD_{0.05}$**
Treatments	0.79
Concentration	0.91
Treatment × concentration	1.57

4F. EFFECT OF POST-HARVEST TREATMENTS ON WHITE ROT

(i) Effect of post-harvest treatments on incidence of white rot

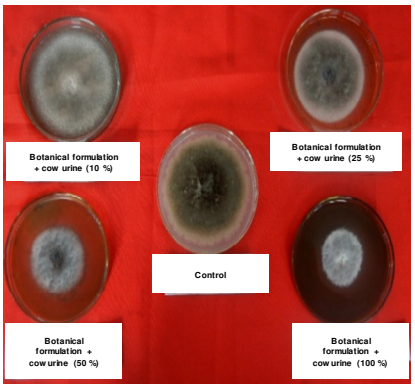
Eleven treatments were applied to the apple fruits and the fruits were stored at the room temperature (25 ± 3^0 C). The observations were recorded at 10, 20 and 30 days interval. The data of two years indicate that the no white rot incidence was recorded in treatments T3 and T5 after 10 days of storage as compared to control fruits where the rot incidence was 38.96 per cent (Table7). Thus both the treatments (T3 and T5) were statistically at par with each other. Minimum pooled white rot incidence was recorded in treatments T3 (5.61%) and T5 (5.62 %), after 30 days of storage (Plate 5, Fig.12). Fruit treated with BF1 (T1) had 16.73 per cent incidence of white rot in comparison to 100 per cent rot incidence in control fruits after 30 days of storage. Overall pooled data of two years indicate that minimum incidence (2.82%) was recorded in treatment T3 and T5 and the both treatments (T3 and T5) were statistically at par with each other (Table 7). It was followed by treatment T1 with 10.27 per cent incidence of rot. Pooled incidence of rot was maximum (67.63 %) in control fruits. The interaction between treatment, storage interval and between the years was found to be significant.

Table 7. Effect of different treatments on incidence of white rot on apple fruits at different durations in storage

Treatment	Incidence (%) of white rot at different duration in storage											Overall Mean
	2011				2012				Pooled data of 2011 and 2012 after different duration (Days)			
	Duration (Days)				Duration (Days)							
	10	20	30	Mean	10	20	30	Mean	10	20	30	
T1 (Apple treated with BF1+ Trays untreated)	0.00 (0.00)	13.89 (21.87)	16.67 (24.08)	10.18 (15.32)	0.30 (3.13)	13.99 (21.95)	16.79 (24.18)	10.36 (16.42)	0.15 (1.57)	13.94 (21.91)	16.73 (24.13)	10.27 (15.87)
T2 (Apple untreated + Trays impregnated with BF1)	16.67 (24.08)	30.56 (33.54)	38.89 (38.56)	28.70 (32.06)	16.79 (24.18)	30.68 (33.62)	39.00 (38.63)	28.82 (32.14)	16.73 (24.13)	30.62 (33.58)	38.94 (38.60)	28.76 (32.10)
T3 (Apple treated with BF2 + Trays untreated)	0.00 (0.00)	2.70 (9.45)	5.56 (13.63)	2.75 (7.69)	0.00 (0.00)	3.00 (9.96)	5.66 (13.75)	2.88 (7.90)	0.00 (0.00)	2.85 (9.71)	5.61 (13.69)	2.82 (7.80)
T4 (Apple untreated + Trays impregnated with BF2)	8.33 (16.78)	16.67 (24.08)	27.78 (31.79)	17.59 (24.21)	8.45 (16.89)	16.79 (24.18)	27.89 (31.86)	17.71 (24.31)	8.39 (16.83)	16.73 (24.13)	27.83 (31.83)	17.65 (24.26)
T5 (Apple treated with Score + Trays untreated)	0.00 (0.00)	2.70 (9.45)	5.56 (13.63)	2.75 (7.69)	0.00 (0.00)	3.00 (9.96)	5.68 (13.78)	2.89 (7.91)	0.00 (0.00)	2.85 (9.71)	5.62 (13.70)	2.82 (7.80)
T6 (Apple untreated+ Trays impregnated with Score)	5.56 (13.63)	16.67 (24.08)	25 (29.98)	15.74 (22.56)	5.68 (13.78)	16.79 (24.18)	26.02 (30.65)	16.16 (22.87)	5.62 (13.70)	16.73 (24.13)	25.51 (30.32)	15.95 (22.72)
T7 Apple treatment with edible wax	5.56 (13.63)	19.44 (26.15)	25 (29.98)	16.66 (23.25)	5.68 (13.78)	19.56 (26.23)	26.02 (30.65)	17.08 (23.55)	5.62 (13.70)	19.50 (26.19)	25.51 (30.32)	16.87 (23.40)
T8 (Wrappers impregnated with BF1)	19.44 (26.15)	38.89 (38.56)	44.44 (41.79)	34.25 (35.50)	19.56 (26.23)	39.01 (38.63)	44.56 (41.86)	34.37 (32.57)	19.50 (26.19)	38.95 (38.60)	44.50 (41.82)	34.31 (35.54)
T9 (Wrappers impregnated with BF2)	16.67 (24.08)	33.33 (35.24)	38.89 (38.56)	29.63 (32.63)	16.79 (24.18)	33.45 (35.21)	39.01 (38.63)	29.75 (32.71)	16.73 (24.13)	33.39 (35.28)	38.95 (38.60)	29.69 (32.67)
T10 (Wrappers impregnated with Score)	16.67 (24.08)	30.56 (33.54)	38.89 (38.56)	28.70 (32.06)	16.79 (24.18)	30.68 (33.62)	39.01 (38.63)	28.82 (32.14)	16.73 (24.13)	30.62 (33.58)	38.95 (38.60)	28.76 (32.10)
T11 Control	38.89 (38.56)	63.89 (53.04)	100.00 (90.00)	67.59 (60.33)	39.03 (38.64)	64.00 (53.11)	100.00 (90.00)	67.67 (60.38)	38.96 (38.60)	63.94 (53.07)	100.00 (90.00)	67.63 (60.35)
Mean	11.61 (16.89)	24.48 (28.15)	33.33 (35.64)	23.14 (26.66)	11.73 (16.70)	24.63 (28.24)	33.60 (35.48)	23.32 (26.90)	11.67 (16.63)	24.55 (28.17)	33.46 (35.54)	

Figures in parentheses are arc sine transformed values

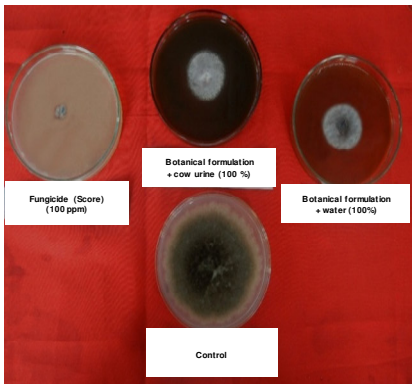
Effect	$CD_{0.05}$	Effect	$CD_{0.05}$
Year	0.02	Treatment × Storage interval	0.03
Storage interval	0.02	Treatment× Year	0.07
Treatment	0.05	Storage interval ×Year	0.09
		Year X Storage interval X Treatment	0.12

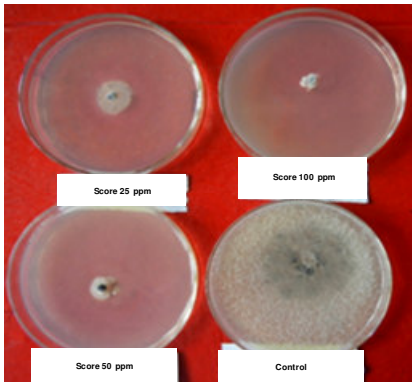


BF2

Botanical formulation 2 (Cow urine based)

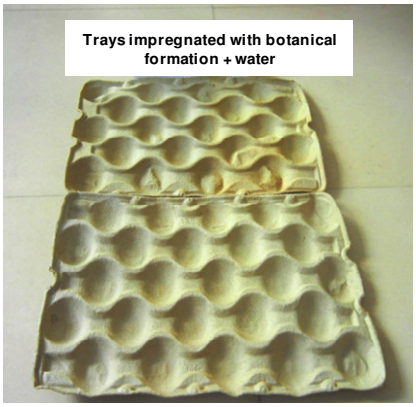
Fig. 9. *In vitro* evaluation of cow urine based (BF2) Botanical Formulation



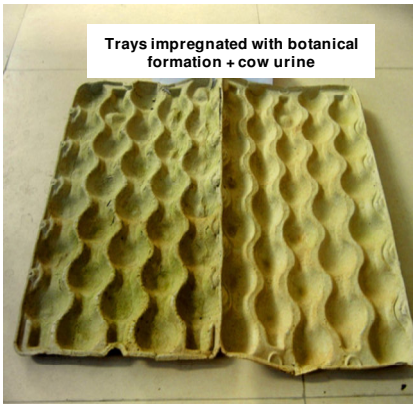


Score 25 EC

Fig. 10. Efficacy of Botanical formulations in comparison with Score



Trays impregnated with BF1



Trays impregnated with BF2

Fig. 11. Trays impregnated with BF1 and BF2

Plate 4.

(ii) Effect of post-harvest treatments on their ability to restrict infection on fruits

Different treatments i.e. direct treatment of apple fruits or impregnation of the packing material (trays or wrappers) has a different impact on disease development. Hence, the progress of the inoculated lesion was also observed in different treatments to study the efficacy in restricting the growth of the inoculated lesion on the fruits. Pooled average of the two years data indicate that maximum restriction in lesion size (100%) as compared to control was found in treatments T3 and T5 after 10, 20 and 30 days of storage (Table 8), in which fruits were treated with BF2 and fungicide (Score), respectively. Both the treatments were statistically at par with each other. Fruits treated with BF1 (T1) and edible wax (T7) resulted in 77.73 and 72.72 per cent restriction in lesion size in comparison to control after 30 days of storage. Among all the treatments, minimum per cent restriction in lesion size (59.88 %) was recorded in treatment T2 after 30 days of storage, in which fruit trays were treated with BF1. Overall pooled data of two years indicate that maximum restriction in lesion size (100%) was recorded in treatment T3 and T5 and both the treatments were found statistically at par with each other (Plate 5, Fig.13). Treatment T1 with 83.17 per cent restriction in lesion size was next in efficacy. Pooled data indicate that lesion size restriction was minimum (59.01 %) in treatment T2. The interaction between treatment, storage interval and between the years was found to be non significant.

(iii) Effect of post-harvest treatments on Total soluble solids (TSS)

Post-harvest diseases adversely affect the quality of the fruits and hence effect of different treatments was also observed on the Total soluble solids (TSS) content of the treated fruits at different time intervals in storage. Pooled average data of two years indicate that TSS content of the fruits increased with the advancement of storage period (Table 9). After 30 days of storage, treatment T5 in which fruits were treated with fungicide (Score) had minimum TSS of 11.93 ^{0}Brix. Fruits which were treated with BF2 (T3) were next in efficacy with TSS of 11.98 ^{0}Brix. Treatments T1 and T7 were proved next effective to reduce the fruit rotting with mean TSS of 12.33 ^{0}Brix and 12.35 ^{0}Brix, respectively. After 30 days of storage TSS content of rotten fruits were declined (8.82 ^{0}Brix) in control.

Table 8. Effect of different treatments on the lesion growth of inoculated apple fruits at different durations in storage

Treatment	Per cent growth reduction in lesion size in comparison to control at different durations of storage											Overall Mean
	2011				2012				Pooled data of 2011 and 2012 after different duration (Days)			
	Duration (Days)				Duration (Days)							
	10	20	30	Mean	10	20	30	Mean	10	20	30	
T1 (Apple treated with BF1 + Trays untreated)	92.30 (73.86)	80.38 (63.69)	78.18 (62.12)	83.62 (66.55)	91.40 (72.91)	79.48 (63.04)	77.28 (61.51)	82.72 (65.82)	91.85 (73.38)	79.93 (63.36)	77.73 (61.81)	83.17 (66.19)
T2 (Apple untreated + Trays impregnated with BF1)	61.52 (51.64	56.52 (48.73)	60.33 (50.95)	59.46 (50.43)	60.63 (51.11)	55.62 (48.20)	59.43 (50.42)	58.56 (49.91)	61.07 (51.37)	56.07 (48.46)	59.88 (50.68)	59.01 (50.17)
T3 (Apple treated with BF2 + Trays untreated)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T4 (Apple untreated + Trays impregnated with BF2)	73.05 (58.70)	63.01 (52.53)	63.94 (53.08)	66.66 (54.77)	72.16 (58.12)	62.11 (51.99)	63.04 (52.54)	65.76 (54.22)	72.60 (58.41)	62.56 (52.25)	63.48 (52.81)	66.21 (54.49)
T5 (Apple Treated with Score + Trays untreated)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T6 (Apple untreated + Trays impregnated with Score)	76.80 (61.18)	63.06 (52.55)	67.27 (55.08)	69.04 (56.27)	75.26 (60.15)	62.16 (52.02)	66.37 (54.53)	67.93 (55.56)	76.03 (60.66)	62.61 (52.28)	66.82 (54.80)	68.48 (55.92)
T7 Apple Treatment with edible wax	88.41 (70.06)	69.56 (56.49)	73.17 58.78)	77.04 (61.78)	87.50 (69.27)	68.66 (55.93)	72.27 (58.20)	76.14 (61.13)	87.95 (69.66)	69.11 (56.21)	72.72 (58.49)	76.59 (61.46)
T8 (Wrappers impregnated with BF1)	69.16 (56.25)	52.17 (46.22)	60.33 (50.94)	60.55 (51.13)	68.26 (55.69)	51.30 (45.72)	59.43 (50.41)	59.66 (50.61)	68.71 (55.96)	51.73 (45.97)	59.88 (50.68)	60.11 (50.87)
T9 (Wrappers impregnated with BF2)	73.05 (58.7)	56.52 (48.73)	61.81 (51.81)	63.79 (53.07)	72.15 (58.12)	56.29 (48.59)	60.58 (51.08)	63.00 (52.6)	72.59 (58.41)	56.40 (48.66)	61.19 (51.44)	63.39 (52.84)
T10 (Wrappers impregnated with Score)	73.07 (58.71)	56.52 (48.73)	63.63 (52.88)	64.42 (53.44)	72.17 (58.14)	56.62 (48.78)	62.73 (52.35)	63.84 (53.09)	72.62 (58.42)	56.57 (48.75)	63.18 (52.62)	64.12 (53.26)
T11 Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean	73.39 (60.71)	63.43 (54.22)	66.24 (55.85)	67.68 (56.93)	72.59 (59.73)	62.80 (53.37)	66.95 (55.05)	66.95 (56.05)	72.99 (60.22)	63.11 (53.79)	65.85 (55.45)	

Figures in parentheses are arc sine transformed values

Effect	CD 0.05	Effect	CD 0.05
Year	0.19	Year × Storage interval	NS
Storage interval	0.24	Year X Treatment	0.64
Treatment	0.45	Storage interval X Treatment	0.79
		Year X Storage interval X Treatment	NS

BF1 treated BF2 treated Fungicide treated

Fig. 12. Effect of different treatments on incidence of white rot after 30 days in storage

BF1 treated fruits BF2 treated fruits Fungicide treated fruits

Fig.13. Effect of different treatments on lesion size of white rot

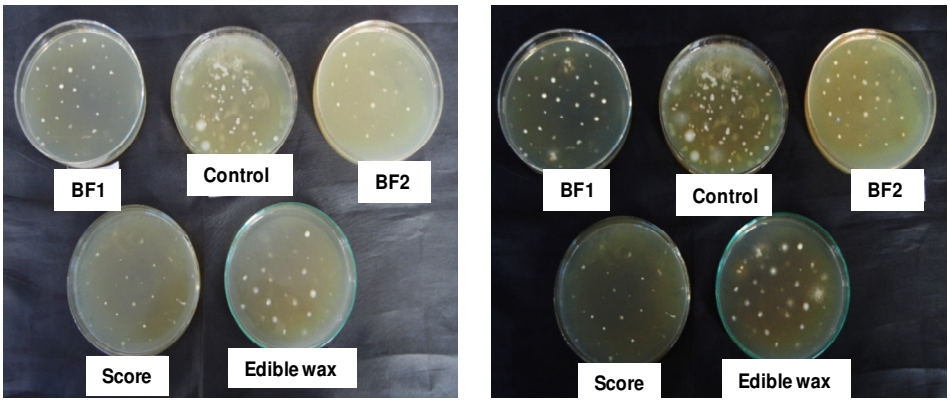


Fig.14. Effect of different treatments on micro flora count on fruit surface after 30 days in storage

Plate 5.

Table 9. Effect of different treatments on TSS (^{0}Brix) of apple fruits at different duration in storage

Treatment	TSS (^{0}Brix) of fruits at different duration of storage											Overall Mean
	2011				2012				Pooled data of 2011 and 2012 after different duration (Days)			
	Duration (Days)				Duration (Days)							
	10	20	30	Mean	10	20	30	Mean	10	20	30	
T1 (Apple treated with BF1 + Trays untreated)	10.46	11.46	12.23	11.38	10.7	11.6	12.43	11.57	10.58	11.53	12.33	11.48
T2 (Apple untreated + Trays impregnated with BF1)	10.63	11.7	12.46	11.6	10.8	11.8	12.6	11.73	10.71	11.75	12.53	11.66
T3 (Apple treated with BF2 + Trays untreated)	10.3	11.26	11.9	11.15	10.53	11.46	12.06	11.35	10.41	11.36	11.98	11.25
T4 (Apple untreated + Trays impregnated with BF2)	10.4	11.4	12.4	11.4	10.76	11.6	12.5	11.62	10.58	11.5	12.45	11.51
T5 (Apple Treated with Score + Trays untreated)	10.33	11.13	11.86	11.11	10.43	11.36	12.0	11.26	10.38	11.25	11.93	11.18
T6 (Apple untreated + Trays impregnated with Score)	10.53	11.3	12.33	11.38	10.56	11.46	12.46	11.5	10.55	11.38	12.4	11.44
T7 Apple Treatment with edible wax	10.46	11.56	12.3	11.44	10.6	11.7	12.4	11.56	10.53	11.63	12.35	11.50
T8 (Wrappers impregnated with BF1)	10.93	11.9	12.6	11.81	11.2	12.23	12.83	12.08	11.06	12.06	12.71	11.95
T9 (Wrappers impregnated with BF2)	10.7	11.83	12.53	11.68	10.9	11.93	12.56	11.8	10.8	11.88	12.55	11.74
T10 (Wrappers impregnated with Score)	10.73	11.73	12.3	11.58	10.86	11.76	12.5	11.71	10.8	11.75	12.4	11.65
T11 Control	11.93	12.94	8.27	11.05	12.2	13.4	9.36	11.65	12.06	13.17	8.82	11.35
Mean	10.67	11.65	11.92	11.42	10.87	11.84	12.15	11.62	10.77	11.75	12.04	

Effect	$CD_{0.05}$	Effect	$CD_{0.05}$
Year	0.04	Year × Storage interval	NS
Storage interval	0.05	Year × Treatment	0.14
Treatment	0.09	Storage interval × Treatment	0.17
		Year ×Storage interval ×Treatment	NS

Overall pooled data of the two years indicate that minimum rotting was recorded in treatment T5 with TSS (11.18 ^{0}Brix) which was followed by T3 with TSS 11.25 ^{0}Brix. Maximum rotting was recorded in control with TSS value 11.35 ^{0}Brix. The interaction between treatment, storage interval and year were found to be non significant.

(iv) Effect of post-harvest treatments on Titratable acidity (TA)

Titratable acidity of fruits was decreased significantly under all the treatments (Table 10). Pooled average titratable acidity was found to be maximum (0.28 %) in the treatments T5 and T3 after 30 days of storage in which fruits were treated with fungicide (Score) and BF2. Titratable acidity values of fruits treated with both these treatments (BF2 and fungicide) were statistically at par with each other. It is followed by the treatments T1and T7, in which fruits treated with BF1 (T1) and edible wax (T7) had titratable acidity of 0.27 per cent each. After 30 days of storage, minimum mean titratable acidity was recorded in control which was 0.21 per cent. Interaction among treatments, storage interval and between the two years was found to be insignificant. Over all pooled data of two years (Table10) indicate that mean titratable acidity was maximum (0.30%) in treatment T5 followed by treatments T1 and T3 with mean titratable acidity of 0.29 per cent each. Further, treatments T6 and T7 had average pooled titratable acidity 0.28 per cent each. Minimum titratable acidity was recorded in control which was 0.24 per cent. Interaction among treatments, storage interval and year was found to be non-significant.

(v) Effect of post-harvest treatments on Fruit pressure (Firmness)

Fruit firmness decrease under all treatments as the storage period progressed (Table 11). Among different treatments, maximum mean firmness (15.10 lbs/sq.inch) was recorded in T5 where the fruits treated with fungicide (Score). Treatment T3 with fruit firmness of 14.97 lbs/sq.inch was next in efficacy in which fruits were treated with BF2. Fruits treated with edible wax had 14.43 lbs/sq.inch fruit firmness. On other hand minimum mean fruit firmness was recorded in control fruits (8.52 lbs/sq.inch). The interaction between the treatments, storage interval and year were found to be non-significant.

Table 10. Effect of different treatments on titratable acidity (%) of apple fruits at different durations in storage

Treatment	Titratable acidity (%) of fruits at different durations in storage											Overall Mean
	2011				2012				Pooled data of 2011 and 2012 after different duration (Days)			
	Duration (Days)				Duration (Days)							
	10	20	30	Mean	10	20	30	Mean	10	20	30	
T1 (Apple treated with BF1 + Trays untreated)	0.29	0.27	0.26	0.28	0.31	0.29	0.28	0.29	0.30	0.28	0.27	0.29
T2 (Apple untreated + Trays impregnated with BF1)	0.27	0.26	0.24	0.26	0.29	0.28	0.26	0.28	0.28	.27	0.25	0.27
T3 (Apple treated with BF2 + Trays untreated)	0.30	0.29	0.27	0.28	0.32	0.31	0.29	0.30	0.31	0.30	0.28	0.29
T4 (Apple untreated + Trays impregnated with BF2)	0.28	0.26	0.25	0.27	0.30	0.28	0.27	0.28	0.29	0.27	0.26	0.27
T5 (Apple Treated with Score + Trays untreated)	0.30	0.29	0.28	0.29	0.32	0.31	0.29	0.31	0.31	0.30	0.28	0.30
T6 (Apple untreated + Trays impregnated with Score)	0.29	0. 27	0.26	0.27	0.30	0.29	0.27	0.28	0.29	0.28	0.26	0.28
T7 Apple Treatment with edible wax	0.28	0.27	0.26	0.27	0.30	0.29	0.28	0.29	0.29	0.28	0.27	0.28
T8 (Wrappers impregnated with BF1)	0.27	0.24	0.22	0.25	0.30	0.26	0.25	0.27	0.29	0.25	0.24	0.26
T9 (Wrappers impregnated with BF2)	0.27	0.25	0.23	0.25	0.29	0.27	0.26	0.27	0.28	0.26	0.25	0.26
T10 (Wrappers impregnated with Score)	0.28	0.26	0.24	0.26	0.30	0.27	0.26	0.28	0.29	0.26	0.25	0.27
T11 Control	0.25	0.23	0.2	0.23	0.28	0.24	0.22	0.24	0.27	0.23	0.21	0.24
Mean	0.28	0.26	0.25	0.26	0.30	0.28	0.26	0.28	0.29	0.27	0.26	

Effect	$CD_{0.05}$	Effect	$CD_{0.05}$
Year	0.01	Year X Storage interval	NS
Storage interval	0.01	Year X Treatment	NS
Treatment	0.01	Storage interval X Treatment	0.01
		Year X Storage interval X Treatment	NS

Table 11. Effect of different treatments on fruit pressure (lbs/sq.inch) of apple fruits at different durations

Treatment	Pressure (lbs/sq.inch) at different durations in storage											Overall Mean
	2011				2012				Pooled data of 2011 and 2012 after different duration (Days)			
	Duration (Days)				Duration (Days)							
	10	20	30	Mean	10	20	30	Mean	10	20	30	
T1 (Apple treated with BF1 + Trays untreated)	15.55	13.88	13.69	14.37	15.3	13.68	13.49	14.15	15.43	13.78	13.59	14.26
T2 (Apple untreated + Trays impregnated with BF1)	15.31	13.73	13.08	14.04	15.11	13.54	12.87	13.84	15.21	13.64	12.97	13.94
T3 (Apple treated with BF2 + Trays untreated)	15.67	14.94	14.59	15.07	15.47	14.75	14.39	14.87	15.57	14.84	14.49	14.97
T4 (Apple untreated + Trays impregnated with BF2)	15.42	13.97	13.42	14.27	15.19	13.79	13.19	14.06	15.30	13.88	13.31	14.16
T5 (Apple Treated with Score + Trays untreated)	15.76	15.22	14.65	15.21	15.58	15.01	14.4	14.99	15.67	15.11	14.53	15.10
T6 (Apple untreated + Trays impregnated with Score)	15.47	14.04	13.75	14.42	15.34	13.85	13.53	14.24	15.41	13.94	13.64	14.33
T7 Apple Treatment with edible wax	15.50	14.38	13.71	14.53	15.3	14.18	13.51	14.33	15.4	14.28	13.61	14.43
T8 (Wrappers impregnated with BF1)	15.04	13.64	12.9	13.86	14.84	13.45	12.70	13.66	14.94	13.54	12.80	13.76
T9 (Wrappers impregnated with BF2)	15.10	13.79	13.29	14.06	14.90	13.59	13.06	13.85	15.00	13.69	13.17	13.97
T10 (Wrappers impregnated with Score)	15.46	14.29	13.42	14.39	15.32	14.09	13.24	14.22	15.39	14.19	13.33	14.30
T11 Control	11.71	8.41	5.7	8.61	11.54	8.22	5.50	8.42	11.63	8.32	5.60	8.52
Mean	15.09	13.66	12.92	13.89	14.9	13.47	12.71	13.69	14.99	13.57	12.82	

Effect	$CD_{0.05}$	Effect	$CD_{0.05}$
Year	0.01	Year X Storage interval	0.01
Storage interval	0.01	Year X Treatment	0.02
Treatment	0.01	Storage interval X Treatment	0.02
		Year X Storage interval X Treatment	NS

(vi) Effect of post-harvest treatments on Physiological loss in weight (PLW)

Minimum mean physiological loss in weight (4.52 %) was recorded in treatment T7 where fruits were treated with edible wax (Table 12). It was followed by treatment T5 with physiological loss in weight of 4.77 per cent containing the fruits which were treated with fungicide. Fruits treated with (BF2) T3 and BF1 separately reduced physiological loss in weight to 4.87 and 5.11 per cent, respectively and both were found statistically at par. Physiological weight loss was maximum in control (11.98 %) after 30 days of storage. Over all weight of fruits decreased in storage after 10, 20 and 30 days. The interaction between treatment, storage interval and between the two years was found to be significant.

(vii) Effect of post-harvest treatments on micro flora count on fruit surface

Different treatments significantly reduced the surface micro flora (fungal and bacterial) of fruits after 30 days of storage in comparison to control decreased (Table 13 and 14). Overall, minimum mean fungal count (4.58×10^3 cfu/ml) was recorded in treatment T5, in which fruits were treated with fungicide (Score). Fruits treated with Botanical Formulation 2 (T3) and treated with Botanical Formulation 1 (T1) were next in efficacy with 6.33×10^3 cfu/ml surface fungal micro flora and 10.08×10^3 cfu/ml, respectively. Maximum overall mean fungal count (19.99×10^3 cfu/ml) in control fruits. Similarly, overall minimum mean bacterial count (22.74×10^3 cfu/ml) was recorded in treatment T3 in which fruits were treated with Botanical Formulation 2 (BF2). It was followed by treatment T5 with bacterial count of 24.91×10^3 cfu/ml where the fruits which were treated with fungicide (Score) and treatment T1 with bacterial count of 29.66×10^3 cfu/ml in the fruits treated with Botanical Formulation 1 (BF1). Maximum overall mean bacterial count (72.82×10^3 cfu/ml) was recorded in control fruits (Plate 5, Fig.14). Overall, the treatment of fruit, trays and wrappers resulted in marked reduction population of micro flora on fruit surface in comparison to control fruits. The interaction between the treatments, storage interval and year were found to be non-significant.

Table 12. Effect of different treatment on weight loss (%) of the apple fruits at different durations in storage

Treatment	Physiological weight loss (%)											Overall Mean
	2011 Duration (Days)				2012 Duration (Days)				Pooled data of 2011 and 2012 after different duration (Days)			
	10	20	30	Mean	10	20	30	Mean	10	20	30	
T1 (Apple treated with BF1 + Trays untreated)	2.15 (1.77)	4.00 (2.23)	5.05 (2.46)	5.11 (2.47)	2.41 (1.84)	4.12 (2.26)	5.17 (2.48)	3.90 (2.19)	2.28 (1.81)	4.06 (2.25)	5.11 (2.47)	3.81 (2.17)
T2 (Apple untreated + Trays impregnated with BF1)	2.38 (1.84)	4.20 (2.28)	5.80 (2.60)	5.91 (2.63)	2.63 (1.90)	4.40 (2.32)	6.03 (2.65)	4.35 (2.29)	2.51 (1.87)	4.30 (2.30)	5.90 (2.62)	4.24 (2.26)
T3 (Apple treated with BF2 + Trays untreated)	2.03 (1.74)	3.53 (2.12)	4.84 (2.41)	4.87 (2.42)	2.30 (1.81)	4.05 (2.24)	4.91 (2.43)	3.75 (2.16)	2.16 (1.77)	3.79 (2.18)	4.87 (2.42)	3.61 (2.13)
T4 (Apple untreated + Trays impregnated with BF2)	2.34 (1.82)	4.15 (2.27)	5.77 (2.60)	5.88 (2.62)	2.59 (1.89)	4.33 (2.30)	5.99 (2.64)	4.30 (2.28)	2.46 (1.86)	4.24 (2.29)	5.88 (2.62)	4.19 (2.25)
T5 (Apple Treated with Score + Trays untreated)	2.03 (1.74)	3.44 (2.10)	4.70 (2.38)	4.77 (2.40)	2.25 (1.80)	4.03 (2.24)	4.83 (2.41)	3.70 (2.15)	2.14 (1.77)	3.73 (2.17)	4.77 (2.40)	3.55 (2.11)
T6 (Apple untreated + Trays impregnated with Score)	2.26 (1.80)	4.08 (2.25)	5.74 (2.59)	5.84 (2.61)	2.56 (1.88)	4.32 (2.30)	5.95 (2.63)	4.27 (2.27)	2.41 (1.84)	4.20 (2.28)	5.84 (2.61)	4.15 (2.24)
T7 Apple Treatment with edible wax	1.86 (1.69)	3.19 (2.04)	4.42 (2.32)	4.52 (2.35)	2.21 (1.79)	3.67 (2.16)	4.63 (2.37)	3.50 (2.10)	2.04 (1.74)	3.43 (2.10)	4.52 (2.35)	3.33 (2.06)
T8 (Wrappers impregnated with BF1)	2.48 (1.86)	4.34 (2.31)	5.89 (2.62)	6.02 (2.64)	2.74 (1.93)	4.37 (2.31)	6.15 (2.67)	4.42 (2.30)	2.61 (1.90)	4.36 (2.31)	6.02 (2.64)	4.33 (2.28)
T9 (Wrappers impregnated with BF2)	2.35 (1.83)	4.18 (2.27)	5.82 (2.61)	5.94 (2.63)	2.63 (1.90)	4.36 (2.31)	6.05 (2.65)	4.35 (2.29)	2.49 (1.87)	4.27 (2.29)	5.94 (2.63)	4.23 (2.26)
T10 (Wrappers impregnated with Score)	2.32 (1.82)	4.16 (2.27)	5.77 (2.60)	5.90 (2.62)	2.60 (1.89)	4.36 (2.31)	6.03 (2.65)	4.33 (2.28)	2.46 (1.86)	4.26 (2.29)	5.90 (2.62)	4.21 (2.26)
T11 Control	4.46 (2.33)	7.53 (2.92)	11.59 (3.54)	11.98 (3.60)	5.17 (2.48)	7.92 (2.98)	12.36 (3.65)	8.48 (3.04)	4.82 (2.41)	7.73 (2.95)	11.98 (3.60)	8.17 (2.98)
Mean	2.42 (1.84)	4.25 (2.28)	5.94 (2.61)	6.07 (2.63)	2.73 (1.92)	4.54 (2.34)	6.19 (2.66)	4.49 (2.31)	2.58 (1.88)	4.40 (2.31)	6.07 (2.63)	

* Figures in parentheses are square root transformed values

Effect	$CD_{0.05}$	Effect	$CD_{0.05}$
Year	0.01	Year X Storage interval	0.01
Storage interval	0.01	Year X Treatment	0.02
Treatment	0.01	Storage interval X Treatment	0.02
		Year X Storage interval X Treatment	0.25

Table 13. Effect of different treatments on surface micro flora {fungal count (10^3 cfu/ml)} of apple fruits at different durations in storage

Treatment	Fungal count (10^3 cfu/ml)								Over all Mean
	2011			2012			Pooled data of 2011 and 2012 after different duration (hrs)		
	Duration (hrs)			Duration (hrs)					
	24	48	Mean	24	48	Mean	24	48	
T1 (Apple treated with BF1 + Trays untreated)	6.33 (8.66)	11.00 (9.27)	8.66 (8.97)	8.66 (9.07)	14.33 (9.56)	11.49 (9.31)	7.49 (8.86)	12.66 (9.41)	10.08 (9.14)
T2 (Apple untreated + Trays impregnated with BF1)	9.66 (9.13)	12.66 (9.42)	11.16 (9.27)	11.33 (9.32)	16.55 (9.70)	13.94 (9.51)	10.49 (9.22)	14.60 (9.56)	12.55 (9.39)
T3 (Apple treated with BF2 + Trays untreated)	4.44 (8.22)	6.55 (8.69)	5.49 (8.45)	6.00 (8.65)	8.33 (9.00)	7.16 (8.82)	5.22 (8.43)	7.44 (8.84)	6.33 (8.64)
T4 (Apple untreated + Trays impregnated with BF2)	9.33 (9.10)	12.66 (9.38)	10.99 (9.24)	11.66 (9.35)	14.00 (9.53)	12.83 (9.44)	10.49 (9.22)	13.33 (9.46)	11.91 (9.34)
T5 (Apple Treated with Score + Trays untreated)	2.33 (7.75)	4.33 (8.24)	3.33 (7.99)	4.66 (8.37)	7.00 (8.82)	5.83 (8.59)	3.49 (8.06)	5.66 (8.53)	4.58 (8.29)
T6 (Apple untreated + Trays impregnated with Score)	8.66 (9.02)	12.00 (9.36)	10.33 (9.19)	11.73 (9.36)	14.66 (9.58)	13.19 (9.47)	10.19 (9.19)	13.33 (9.47)	11.76 (9.33)
T7 Apple Treatment with edible wax	9.00 (9.06)	13.67 (9.50)	11.33 (9.28)	9.00 (9.08)	14.33 (9.56)	11.66 (9.32)	9.00 (9.07)	14.00 (9.53)	11.50 (9.30)
T8 (Wrappers impregnated with BF1)	12.00 (9.36)	14.66 (9.57)	13.33 (9.47)	13.00 (9.46)	17.33 (9.58)	15.16 (9.52)	12.50 (9.41)	15.99 (9.57)	14.24 (9.49)
T9 (Wrappers impregnated with BF2)	9.33 (9.10)	14.30 (9.55)	11.81 (9.32)	11.33 (9.32)	16.66 (9.46)	13.99 (9.39)	10.33 (9.21)	15.48 (9.51)	12.90 (9.36)
T10 (Wrappers impregnated with Score)	9.00 (9.04)	14.30 (9.55)	11.65 (9.29)	11.6 6 (9.35)	16.66 (9.46)	14.16 (9.40)	10.33 (9.19)	15.48 (9.51)	12.90 (9.35)
T11 Control	16.33 (10.03)	23.32 (10.06)	19.82 (10.04)	14.33 (9.56)	26.00 (10.02)	20.16 (9.79)	15.33 (9.79)	24.66 (10.04)	19.99 (9.91)
Mean	8.76 (8.95)	12.67 (9.33)	10.72 (9.14)	10.30 (9.17)	15.07 (9.48)	12.69 (9.32)	9.53 (9.06)	13.87 (9.40)	

*Figures in parentheses are natural log transformed values

Effect	$CD_{0.05}$	Effect	$CD_{0.05}$
Year	0.11	Year X Duration	NS
Duration	0.11	Year X Treatment	0.11
Treatment	0.26	Duration X Treatment	NS
		Year X Duration X Treatment	NS

Table 14. Effect of different treatments on surface micro flora {Bacterial count (10^3 cfu/ ml)} of apple fruits at different durations in storage

Treatment	Bacterial count (10^3 cfu/ml)						Pooled data of 2011 and 2012 after different duration (hrs)		Over all Mean
	2011			2012					
	Duration (hrs)			Duration (hrs)					
	24	48	Mean	24	48	Mean	24	48	
T1 (Apple treated with BF1 + Trays untreated)	19.33 (9.86)	36.33 (10.50)	27.83 (10.23)	22.66 (10.02)	40.33 (10.60)	31.49 (10.35)	20.99 (9.95)	38.33 (10.55)	29.66 (10.29)
T2 (Apple untreated + Trays impregnated with BF1)	25.00 (10.12)	40.33 (10.60)	32.66 (10.39)	29.33 (10.28)	47.00 (10.75)	38.16 (10.54)	27.16 (10.20)	43.66 (10.68)	35.41 (10.47)
T3 (Apple treated with BF2 + Trays untreated)	16.32 (9.70)	27.00 (10.20)	21.66 (9.98)	19.33 (9.86)	28.33 (10.25)	23.83 (10.07)	17.83 (9.78)	27.66 (10.22)	22.74 (10.03)
T4 (Apple untreated + Trays impregnated with BF2)	22.33 (10.01)	37.32 (10.52)	29.82 (10.29)	25.22 (10.13)	45.99 (10.73)	35.60 (10.48)	23.77 (10.07)	41.66 (10.63)	32.71 (10.39)
T5 (Apple Treated with Score + Trays untreated)	16.99 (9.74)	28.99 (10.27)	22.99 (10.04)	21.33 (9.96)	32.33 (10.38)	25.83 (10.15)	19.16 (9.86)	30.66 (10.33)	24.91 (10.12)
T6 (Apple untreated + Trays impregnated with Score)	24.33 (10.09)	40.00 (10.59)	32.16 (10.37)	27.64 (10.22)	46.33 (10.74)	36.98 (10.51)	25.98 (10.16)	43.16 (10.67)	34.57 (10.45)
T7 Apple Treatment with edible wax	23.33 (10.05)	37.00 (10.20)	30.16 (10.31)	29.32 (10.28)	46.33 (10.74)	37.82 (10.54)	26.32 (10.17)	41.66 (10.63)	33.99 (10.43)
T8 (Wrappers impregnated with BF1)	29.67 (10.29)	49.66 (10.81)	39.66 (10.58)	34.67 (10.45)	52.66 (10.87)	43.66 (10.68)	32.17 (10.37)	51.16 (10.84)	41.66 (10.63)
T9 (Wrappers impregnated with BF2)	27.00 (10.20)	42.66 (10.66)	34.83 (10.45)	31.67 (10.36)	46.99 (10.75)	39.33 (10.57)	29.33 (10.28)	44.82 (10.71)	37.08 (10.52)
T10 (Wrappers impregnated with Score)	28.31 (10.25)	43.66 (10.68)	35.98 (10.49)	33.00 (10.40)	49.66 (10.81)	41.33 (10.62)	30.65 (10.33)	46.66 (10.75)	38.65 (10.56)
T11 Control	47.70 (10.77)	93.33 (11.44)	70.51 (11.16)	50.33 (10.82)	96.66 (11.47)	73.49 (11.20)	49.05 (10.80)	94.99 (11.46)	72.82 (11.19)
Mean	25.48 (10.14)	43.29 (10.67)	34.38 (10.44)	29.50 10.29)	48.42 (10.78)	38.86 (10.56)	27.49 (10.22)	45.86 (10.73)	

*Figures in parentheses are natural log transformed values

Effect	CD 0.05	Effect	CD 0.05
Year	0.61	Year × Duration	0.84
Duration	0.60	Year × Treatment	NS
Treatment	1.42	Duration × Treatment	1.98
		Year × Duration × Treatment	NS

Chapter-5

DISCUSSION

Apple (*Malus* x *domestica* Borkh.) is the most important fruit crop grown extensively in temperate regions of the world. In India, apple is grown in an area of about 2, 89,000 hectare with total annual production of 28, 91,000 metric tonnes (Anonymous, 2012). In Himachal Pradesh, the area and production under apple cultivation state has increased during last few decades, but the productivity per unit area has not increased proportionally. Among major factors responsible for low productivity, post-harvest diseases were also important which result in significant losses (Kaul and Munjal, 1982). Among different post-harvest pathogens, more than 90 fungal species have been reported to cause different types of post-harvest apple fruit rots, which lead to considerable losses in apple during storage (Jones and Aldwinckle, 1990; McCollum, 2002; Ilyas *et al.*, 2007). Among different fungal post-harvest rots in apple, white rot caused by *Botryosphaeria dothidea* is one of the important diseases and this pathogen is reported to cause fruit rot and canker on the stems (Sharma and Bhardwaj, 1999; Travis *et al.,* 1995). Putterill (1919) for the first time reported the canker phase of apple caused by *Botryosphaeria mali* from South Africa. Whereas, fruit rot caused by *B. ribis* was first reported by Fenner (1925) from the United States.

Management of post-harvest rots is a tedious task in apple as use of chemicals as pre-harvest sprays or post-harvest treatment results in accumulation of residues on the fruits. Residues of chemicals on the surface of fruits results in adverse effects on the health of consumers (Tripathi *et al*., 2008). Thus, there is need for alternative non-chemical methods like use of botanicals and other bio-pesticides for the management of post-harvest diseases in apple. In these studies, focus has been kept on the use of botanicals for the management of white rot of apple.

In the present study, survey of the important Marketing Yards in Shimla and Solan districts of Himachal Pradesh was conducted during the peak

harvesting season of apple to record the incidence of white rot (*Botryosphaeria dothidea*) and other rots. Incidence of white rot was recorded in all the Marketing Yards, which were surveyed. Incidence of white rot was more severe in Marketing Yards located at lower elevations in warmer areas at Parwanoo (14.1%) and at Solan (9.7%), in comparison to Marketing Yards at Narkanda (3.0%) and in Shimla (5.2 %). In last few years, white rot of apple has been reported to occur in serious proportion from Jammu and Kashmir (Khan *et al*., 2010). Malik (1967) also reported 15-18 per cent incidence of white rot in Kashmir. Kaul (1979) reported *B. dothidea* causing fruit rot of apple with incidence up to 1.5 per cent from Kullu valley of Himachal Pradesh. Sutton (1990) reported 50 per cent apple fruit loss due to white rot infection in warm and humid areas of South-Eastern United States.

In the present study, the symptoms of the white rot of apple were studied in detail. The initial symptoms were typically comprised of formation of slightly sunken brown spots which were bordered by one or more red halo rings and similar type of symptoms on fruit have been reported in literature (Parker and Sutton, 1993; Kim *et al.,* 2001). As the decayed area expanded, the core became rotten in cylindrical manner. In red skinned apple cultivars, bleaching was found during decay process and rotted portion became light brown in colour. Because of this characteristic, the disease has been referred as "white rot". As the rot progresses, the skin colour becomes dark brown with soft and mushy flesh. Similar description of symptoms of this disease has been provided by different workers (Fulkerson, 1960; Malik, 1967; Brown and Hendrix., 1981).

The pathogen (fungus) associated with the infected fruits was isolated in pure culture and examined under the compound microscope. Numerous conidia were observed under the microscopic field which were hyaline, unicellular, fusoid and ellipsoidal with obtuse apex, measuring 19.0 × 6.3 µm in average size. The morphological characters of the isolated fungus were similar to the published description of the fungus given in "Compendium of Apple and Pear Diseases" given by Jones and Aldwinckle (1990). English *et al*. (1975) also reported that

conidia of *B. dothidea* were ellipsoidal to fusoid, hyaline non-septate with average dimension of 21.5 (12.5- 33.7) µm × 5.9 (3.8-7.7) µm in size.

Temperature is a critical factor for the optimum growth of any fungus and *B. dothidea* could grow on wide range of temperature ranging from 10^0 C to 35^0 C. However, 30^0 C was found as optimum temperature for vegetative growth. The growth was significantly reduced at temperature below or above the optimum temperature. English *et al.* (1975) reported 8.5, 24-30 and 31.5^0 C as minimum, optimum and maximum temperatures, respectively for the mycelial growth of *B. dothidea* which further corroborates these findings. In other similar studies, temperature of 30^0 C has been reported as optimum for the maximum vegetative growth on PDA medium (Kohn and Hendrix, 1982; Michailides and Morgan, 1992; Brooks and Ferrin, 1994).

In the present study, Golden Delicious was found the most susceptible cultivar followed by Rich-a-Red. In other studies, Golden Delicious has been reported as most susceptible cultivar of apple to white rot pathogen (Mc Vay *et al*., 1993; Parker and Sutton, 1993; Biggs and Miller, 2003).

Among six fungicides evaluated under *in vitro* conditions against the white rot pathogen (*B. dothidea*), difenoconazole was found most effective and significantly superior among all the treatments with 75.01 per cent average inhibition in mycelial growth followed by 71.68 per cent inhibition in tebuconazole. Other fungicides effective against the white rot pathogen were trifloxystrobin and azoxystrobin which reduced the growth of the fungus by 60.12 and 60.00 per cent, respectively. Kim and Uhm (2002) also reported that white rot of apple can be effectively controlled by application of difenoconazole. Savocchia *et al*. (2005) reported the efficacy of tebuconazole under *in vitro* conditions against mycelial growth of black rot pathogen (*B. obtusa*). Difenoconazole has been reported to effectively check the conidial germination and radial mycelial growth of *Diplodia* spp. and *B. obtusa* under *in vitro* conditions (Auger *et al*., 2004; Diaz *et al*., 2011; Morales *et al*., 2012). Liu *et al.* (2013) also reported effectiveness of difenoconazole against *B. dothidea* under *in vitro* condition followed by flusilazole.

In the present study, six effective botanicals namely *Karu* (*Roylea elegans*), *Artemisia* (*Artemisia roxburghiana*), *Neem* (*Azadirachta indica*), *Bana* (*Vitex negundo*), *Tulsi* (*Ocimum sanctum*) and Darek (*Melia azedarach*) were used for preparation of water based and cow urine based botanical formulations. Among different treatments, *O. sanctum* (50%) leaf extract was found most effective with 63.05 per cent average inhibition of pathogen followed by leaf extract of *Azadirachta indica, Artemisia roxburghiana, Roylea elegans, Vitex negundo* and seed extract of *Melia azedarach* with 56.39, 49.72, 42.22, 42.22 and 44.16 per cent inhibition, respectively. Leaf extract of *Mentha piperita* was found least effective against the test pathogen. Arya (1988) reported effectiveness of leaf extracts of *O. sanctum*, *A. indica* against post-harvest fruit rot pathogens in grapes and guava. Faria *et al.* (2006) reported antifungal activity of *Ocimum spp*. against *Botryosphaeria rhodina*. Ethanol extract of ripe fruits of *Melia azedarach* is reported to exhibit fungistatic activities against fungi like *Aspergillus niger*, *Fusariuum moniliformae*, *Microsporium canies* and *Candida albicans* (Carpinella *et al*., 1999). Lopez-Reyes *et al.* (2013) observed that essential oil obtained from *Ocimum basilicum* was effective against brown rot (*Monilinia laxa*) and grey mould rot (*Botrytis cinerea*) pathogens in stone fruits. Onyeani *et al.* (2012) observed that aqueous leaf extract of *Azadirachta indica* and *Aloe vera* were equally effective in reduction of mycelial growth of *Aspergillus niger*.

In the present study, both the botanical formulations i.e. made with water (BF1) or cow urine (BF2) were found effective in inhibiting the mycelial growth of white rot pathogen (*B. dothidea*). BF2 was found more effective than BF1. Botanical Formulation 2 (BF2) inhibited the overall mycelial growth of the test pathogen by 48.38 per cent in comparison to Botanical Formulation 1 (BF1) with 45.34 per cent overall mycelial growth inhibition. Further, BF2 inhibited mycelial growth by 72.70 in comparison to 66.37 per cent mycelial inhibition in BF1 at 100 % concentration. Gautam (2011) reported that bio-formulation which has been made from leaves or seeds of plant species like *Bougainvillea glabra*, *Eucalyptus globules*, *Mentha piperita*, *Melia azedarach*, *Roylea elegans*, *Dedonia viscosa* and cow urine was effective against five major post-harvest rots in apple viz., blue mould rot (*Penicillium expansum*), bitter rot (*Glomerella cingulata*),

brown rot (*Monilinia fructigena*), pink mould rot (*Tichothecium roseum*) and whisker's rot (*Rhizopus stolonifer*). Raj and Tomar (2013) also reported the efficacy of aqueous and cow urine based plant extracts and between these cow urine based bio-formulation was reported to be more effective in inhibiting mycelial growth of different post-harvest pathogens of apple. Similarly, cow urine based botanical formulation has been reported more effective against grey rot disease of strawberry (Raj and Sharma, 2013).

Cow urine and cow dung have been reported to inhibit the conidial germination and mycelial growth of fungal pathogens like *F. oxysporum* f.sp. *cucumerinum, F. solani* f. sp. *cucurbitae* and *S. sclerotiorum* (Basak *et al*., 2002). Okigbo and Ogbomaya (2006) found that leaf extract of *Ocimum gratissimum* was inhibitory to post-harvest rot pathogens (*Aspergillus niger, A. flavus, Fusarium oxysporum, Rhizopus stolonifer, Botryodiplodia theobromae* and *Penicillium chrysogenum*) of yam. Antifungal activity of cow urine has been reported against various plant pathogens viz., *Fusarium oxysporum*, *Rhizopus oligosporus*, *Alternaria helianthi* and *Trichoderma viride* (Patil, 2007). Sathasivam *et al*. (2010) and Sharma *et al*. (2010) also reported the anti-microbial properties of cow urine and composted cow dung on clinical pathogens and phyto-pathogenic fungi, respectively.

In the present studies, different treatments of BF1 and BF2 along with treatment of edible wax and chemical fungicide (Score) were either applied as fruit dip or these were used for impregnation of trays/ wrappers used for the packing of the fruits. All the treatments reduced the incidence of the white rot in the storage. However, fruit dip in BF2 and Score was found most effective with 94.3 per cent reduction in the incidence of white rot after 30 days of storage. Impregnation of trays with BF2 also reduced the incidence of white rot by 72.2 per cent in comparison to control. Though, BF1 was found less effective than BF2 but still treatment of fruits in BF1 and packing of fruits in trays impregnated with BF1 resulted in 83.3 and 61.1 per cent reduction in the incidence of white rot in comparison to control after 30 days of storage. Raj and Tomar (2013) also reported effectiveness of fruit dip in botanical formulation with cow urine in

reducing disease incidence of post-harvest rots of apples in storage. Raj and Sharma (2013) also reported effectiveness of cow urine based bio-formulation consisting of leaf extract of *Bougainvillea glabra*, *Ocimum sanctum*, *Artemisia roxburghiana*, *Roylea elegans*, *Cryptolepsis buchanani* and seed extract of *Melia azedarach* against grey mould incidence in strawberry. Use of chitosan and grape fruit extract in apple reduced incidence of grey rot (*B. cinerea*) when used as post-harvest treatments under controlled condition (Montealegre *et al*., 2010).

In the present study, treatment of apple fruits with Score and BF2 completely restricted the lesion size (100%) during storage. Trays impregnated with BF2 also restricted the lesion size by 63.5 per cent. Botanical Formulation 1 (BF1) was found less effective than BF2. Fruit dip of fruits and packing of fruits in trays impregnated with BF1 resulted in 77.7 and 59.9 per cent restriction of lesion size in comparison to control, respectively after 30 days. Ikeura *et al*. (2011) also reported that application of plant extracts restricted the lesion growth of *Penicillium expansum* on apple fruits. Treatment of fruits with neem seed kernel extract significantly reduced lesion diameter on plum fruits inoculated with *Monilinia fructicola* (Wang *et al*., 2010). Cosoveanu *et al*. (2013) also reported the efficacy of *Artemisia* spp. extract against *Penicillium expansum* and growth of lesion size was restricted by 88.5 per cent after 17 days of inoculation.

Infection of white rot on fruits drastically reduced the firmness of fruits from 16.54 lbs/ sq. inch at the time of the storage to 5.60 lbs/ sq. inch after 30 days of storage in control. However, different treatments appreciably improved the fruit firmness. Minimum loss in fruit firmness 12.39 per cent (from 16.54 lbs/ sq. inch in the beginning to 14.53 lbs/sq. inch) was recorded in the fruits treated with Score followed by 12.40 per cent loss in firmness of fruits treated with BF2. In fruits treated with edible wax, loss in fruit firmness was 17.71 per cent. Fruits treated with BF1 had 17.84 per cent loss in firmness as compared to control after 30 days of storage. Impregnation of trays with BF2 reduced the firmness by 19.5 per cent. Although BF1 was less effective than BF2, but packing of fruits in impregnated trays with BF1 result in 21.6 % reduction in fruit firmness in comparison to control after 30 days. In earlier studies, Raj and Tomar (2013)

reported that fruit dip in botanical formulation with cow urine was equally effective in retaining firmness of fruits during storage. Combination of chitosan and aqueous extracts of leaves of custard apple, leaves and seeds of papaya has inhibitory effect on the development of anthracnose (*Colletotrichum gloeosporioides*) in papaya and it also resulted in retaining the fruit firmness as rotting progresses (Bautista-Banos *et al*., 2003).

Total soluble solids (TSS) content of the fruits have been reported to increase during storage (Rivera, 2005). All the treatments were effective in delaying the ripening of the fruits with TSS ranging from 11.93 to 12.71^0 Brix after 30 days of storage in comparison to control with highest TSS of 13.17 ^{0}Brix after 20 days. In the present study, fruits treated with Score and BF2 had TSS of 11.93 and 11.98^0 Brix, respectively, which were 9.4 and 9.0 per cent less in comparison to control after 20 days of storage. While, treatment of edible wax was also proved effective in retaining fruit TSS (12.35^0 Brix) in comparison to control (8.82^0 Brix), after 30 days of storage. Thus, these treatments were effective in delaying the ripening process. However, as the fruits in control completely rotted after 20 days, TSS declined to 8.82 after a peak of 13.17^0 Brix. The increase in TSS during rotting by pathogens could be attributed to the breakdown of starch into sugars or the hydrolysis of cell wall polysaccharides (Ben and Gaweda, 1985; Beaudry *et al*., 1989). In earlier studies, Kim *et al.* (1997) also reported that at lower incidence of white rot (4.2 %), TSS content of the apple fruits was 9.6^0 Brix but as the incidence of rot increased to 14.2 per cent, the TSS of the fruits also increased to 10.1^0 Brix. Tzortzakis (2007) reported that treatment of strawberry fruit with cinnamon (*Cinnamomum zeylanicum*) and eucalyptus (*Eucalyptus globulus*) vapours increased the fruit TSS during storage. Fruit coating of apple with neem oil has been reported to provide better retention of physico-chemical characteristics of fruits including firmness, total soluble solids, and titratable acidity of fruit (Chauhan *et al*., 2008; Wijewardane and Guleria, 2009).

Titratable acidity of the fruits have been reported to gradually decline with the advancement of rotting of fruits in storage (Soliva and Martin, 2003). Different treatments appreciably improved the titratable acidity of fruits.

Minimum reduction in titratable acidity 15.2 per cent (from 0.33 % at beginning to 0.28 %) was recorded in the fruits treated with Score and BF2. Impregnation of trays in BF2 was also found effective with reduction of 21.2 per cent in titratable acidity in comparison to the fruits at time of storage. Although, BF1 was found less effective than BF2 but packing of fruits in impregnated trays with BF1 resulted in 24.2 per cent reduction in titratable acidity after 30 days of storage. Maximum reduction in titratable acidity 36.4 per cent was recorded in control after 30 days of storage. Similarly, Wijewardane and Guleria (2009) also reported maximum titratable acidity (0.30 %) in apple fruits treated with neem oil. Shinde *et al.* (2009) also reported fruit dip treatment with neem oil (10%) was highly effective in retaining maximum titratable acidity of mango fruits in storage. Ergun and Satici (2012) reported that higher concentration of *Aloe vera* gel delayed titratable acidity in Granny Smith variety of apple.

Higher rates of respiration during post-harvest rotting of fruits has been reported to result in physiological loss in weight which is accelerated by the high temperature (Ghafir *et al*., 2009). Infection of white rot also resulted in loss in weight of fruits after 30 days of storage in control. In the present study, all the treatments reduced the loss in weight of the fruit in the storage. However, minimum reduction in weight of fruits (4.52 %) was recorded in the fruits treated with edible wax followed by 4.77, 4.87 per cent loss in fruits treated with Score and BF2, respectively in comparison to control after 30 days of storage. Post-harvest treatment of fruits with antifungal compounds like eugenol, thymol and menthol vapors is reported to reduce weight loss percentage in cherries and grapes (Serrano *et al*., 2005). Similarly, less reduction in physiological weight loss has been reported in strawberry fruits treated with cinnamon (*Cinnamomum zeylanicum*) and eucalyptus (*Eucalyptus globulus*) oil (Tzortzakis, 2007; Tian *et al*., 2011). Chauhan and Babu (2011) also reported that apple treated with leaf extract of *Melia azedarach* (20%) proved to be most effective treatment in reducing physiological weight loss in apple fruits. Treatment of apple with neem based formulations proved effective in reducing the physiological weight loss of fruits and also reduced the growth of pathogens responsible for rotting (Singh *et al*., 2000).

Among many factors responsible for affecting quality of fruits, presence of micro-flora on fruit surface is also one of the important factor (Brackett, 1992). In the present study, fungal and bacterial micro-flora count was recorded from the fruit surface to ascertain the quality of the fruits. Fruits treated with Score had minimum fungal count (4.58×10^3 cfu/ml) followed by BF2 (6.33×10^3 cfu/ml).

Similarly, fruits treated with BF2 had minimum count of bacterial population (22.74×10^3 cfu/ml) followed by Score (24.91×10^3 cfu/ml). Fruit dip in Score was found most effective with reduction of 76.13 per cent in fungal micro-flora and 65.69 per cent reduction in bacterial micro-flora. Botanical formulation 2 was next in efficacy with 67.0 per cent reduction in fungal micro-flora and 68.8 per cent reduction in bacterial micro-flora. Thus, fruits treated with the BF2 were better due to lower surface micro-flora than untreated apple fruits in control. In other study Gautam (2011) also reported that treatment of apple fruits and impregnation of trays /wrappers reduced the micro-flora population on fruit surface. Similarly, Raj and Tomar (2013) reported that apple fruits treated with cow urine based botanical formulation comprised of six effective botanicals has less micro-flora population and better quality of fruits. Atress *et al*. (2010) reported that coating of strawberry fruit with essential oil of thyme resulted less microbial count on fruit surface as compared to untreated fruit surface. Marpudi *et al*. (2011) reported that edible coating of papaya fruits with *Aloe vera* gel and papaya leaf extract based formulation resulted less microbial population on fruit surface.

Chapter-6

SUMMARY AND CONCLUSION

Apple is the most important fruit crop grown extensively in temperate regions of the world. In India, the productivity is quite low in comparison to other apple growing countries of world. There are many reasons for low apple productivity and among these diseases are one of the dominant factors. There are number of diseases which infect apple resulting in huge loss in yield. Among diseases, post-harvest diseases are also important which also result in significant losses. Among different fungal post-harvest rots in apple, white rot caused by *Botryosphaeria dothidea* is the most serious. Therefore, the present investigations were undertaken with the objectives to isolate and identify the pathogen causing white rot of apple, record its incidence in different Marketing Yards and to devise an eco-friendly management strategy during storage based on effective bio-resources.

Survey was conducted in important Marketing Yards of the Himachal Pradesh to record the incidence of white rot of apple and maximum incidence (14.1%) of the white rot was recorded at Parwanoo Marketing Yard followed by Solan (9.7 %), while Narkanda (3.0 %) Marketing Yard had the minimum disease incidence during the harvesting season from 2011 to 2013.

The fungus associated with the white rot was isolated in pure culture and was identified as *Botryosphaeria dothidea* on the basis of morphological characters i.e. the culture of the pathogen, size and shape of the conidia of the fungus. The symptoms of the disease were characterized by formation of slightly sunken brown spots which were bordered by one or more red halo rings. As the decayed area expanded, the core became rotten in cylindrical manner. In red skinned apple cultivars, bleaching was found during decay process and rotted portion became light brown in colour. Because of this characteristic, the disease has been referred as “white rot”. Mature and ripened fruits were found more susceptible to white rot infection.

Under *in vitro* condition, temperature range of 25-30^0 C was found most suitable for the growth of the fungus (*Botryosphaeria dothidea*). Minimum growth of the fungus was recorded at 10^0 C.

Golden Delicious was most susceptible to white rot pathogen followed by Rich-a-Red cultivar of apple during 30 days of storage.

Among six fungicides evaluated under *in vitro* conditions against the white rot pathogen, difenoconazole (Score) was found most effective and significantly superior among all the treatments with 75.01 per cent average inhibition in mycelial growth while pyraclostrobin was found least effective among all the treatments with 54.32 per cent average inhibition in mycelial growth of the pathogen.

Out of twelve botanicals evaluated under *in vitro* conditions against the white rot pathogen, leaf extract of *Ocimum sanctum* was found most effective and significantly superior amongst all the treatments with 54.07 per cent average inhibition at different concentration in mycelial growth of the white rot pathogen followed by leaf extract of *Azadirachta indica* and *Artemisia roxburghiana* with 48.98 and 38.88 per cent mycelial growth inhibition, respectively. Leaf extract of *Mentha piperita* was found least effective with mycelial growth inhibition of 7.78 per cent only.

Twelve plants were evaluated for their *in vitro* efficacy in inhibiting the growth of white rot pathogen and out of these six effective plants (*Karu*, *Artemisia*, *Neem*, *Bana*, *Tulsi* and *Darek*) were selected for making two botanical formulations (BF1 and BF2). While, BF1 was water based formulation, BF2 was cow urine based formulation. BF1, BF2 and Score significantly inhibited the mycelial growth of the white rot pathogen in comparison to control. Score resulted in 89.25 per cent mycelial growth reduction of white rot pathogen followed by 72.70 per cent in BF2 and 66.37 per cent in BF1 at 100 per cent concentration.

Eleven treatments were applied to the apple fruits and the fruits were stored at the room temperature (25 ± 3^0 C). Among these, overall minimum white rot incidence (2.82%) was recorded in treatment where fruits were treated with BF2 and Score. Incidence of white rot was maximum (67.63 %) in control fruits. Thus, treatment of BF2 was equally effective as Score in controlling fruit rot incidence after different duration during storage.

Different treatments i.e. fruit dip or impregnation of the packing material (trays or wrappers) were effective in restricting the growth of fungus on the fruits. Among eleven treatments maximum restriction (100%) in lesion size as compared to control was found in fruits treated with BF2 and Score. Restriction in lesion size was 59.01 per cent in fruit trays treated with BF1. Treatment with BF2 was found equally effective as Score in restricting lesion development after 30 days during storage.

Post-harvest diseases adversely affect the quality of the fruits. Total soluble solid (TSS) content of the fruits increased with the advancement of storage period. After 30 days of storage, fruits treated with Score had minimum TSS (11.93 ^{0}Brix) and the fruits treated with BF2 were next in efficacy with TSS of 11.98 ^{0}Brix. TSS content of fruits declined to 8.82 ^{0}Brix after complete rotting in control after reaching to 13.7 ^{0}Brix in 20 days of storage. Treatment with BF2 was found equally effective as Score in retaining fruit TSS after different durations during storage.

Titratable acidity of the fruits declined with the advancement of fruit rotting during storage. Titratable acidity was found to be maximum (0.28 %) in fruits treated with Score and BF2 after 30 days of storage. Minimum titratable acidity of rotten fruits (0.21 %) in control was observed after 30 days of storage. Treatment with BF2 was found equally effective as Score in retaining fruit titratable acidity after different durations during storage.

Fruit firmness decreased under all treatments as fruit rotting progressed during storage. Among different treatments, maximum fruit firmness (15.10 lbs/sq.inch) was recorded in fruits treated with Score. Treatment T3 with fruit

firmness of 14.97 lbs/sq.inch was next in efficacy in which fruits were treated with BF2. On other hand, minimum fruit firmness was recorded in control fruits (8.52 lbs/sq.inch). Thus, treatment with BF2 proved equally effective as Score in retaining fruit firmness after different duration during storage.

Different treatments i.e. fruit dip or impregnation of the packing material (trays or wrappers) were effective in reducing the physiological weight loss. While, minimum physiological loss in weight (3.33 %) was recorded where fruits were treated with edible wax. Which was followed by treatment where the fruits were treated with Score and Botanical Formulation 2 with loss in weight of 3.55 and 3.61 per cent, respectively. Physiological weight loss was maximum in control (8.17 %). Treatment with BF2 and Score was effective as edible wax coating with less reduction in weight loss after different duration during storage.

Minimum fungal count was observed on fruit surface in which fruits were treated with Score (4.58×10^3 cfu/ml) followed by BF2 (6.33×10^3 cfu/ml). Minimum bacterial count was recorded from fruit surface which treated with BF2 (22.74×10^3 cfu/ml) followed by Score (24.91×10^3 cfu/ml).

Chapter-7

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Dr. Y.S. Parmar University of Horticulture and Forestry
Nauni, Solan 173230 (H.P.) India
Department of Plant Pathology

Title of Thesis	**:**	**Efficacy of bio-resources in the management of white rot (*Botryosphaeria dothidea*) of apple**
Name of the student	**:**	Kishor Sharma
Admission Number	**:**	H- 2011- 58- M
Major Advisor	**:**	Dr. H. R. Gautam
Major Field	**:**	Plant Pathology
Minor Field	**:**	Fruit Science
Degree awarded	**:**	Master of Science (Agriculture) Plant Pathology
Year of Award of Degree	**:**	2013
No. of pages in thesis	**:**	71 +V
No. of words in Abstract	**:**	335

ABSTRACT

The present investigation entitled "Efficacy of bio-resources in the management of white rot (*Botryosphaeria dothidea*) of apple" was conducted in the laboratories of the Department of Plant Pathology. During the survey of the disease, 3.0-14.1 per cent incidence of white rot was recorded in important Marketing Yards. Among different fungicides tested *in vitro*, Score was found most effective with 75.01 per cent average inhibition in mycelial growth of the white rot pathogen. Out of twelve botanicals evaluated under *in vitro* conditions against the white rot pathogen, leaf extract of *Ocimum sanctum* was found significantly most effective among all the treatments with 54.07 per cent average inhibition at different concentration in mycelial growth. Out of twelve plants evaluated for their efficacy, six effective plants *Karu* (*Roylea elegans*), *Artemisia* (*Artemisia roxburghiana*), *Neem* (*Azadirachta indica*), *Bana* (*Vitex negundo*), *Tulsi* (*Ocimum sanctum*) and Darek (*Melia azedarach*) were selected for making two botanical formulations (BF1 and BF2).While, BF1 was water based formulation and BF2 was cow urine based formulation. BF2 inhibited mycelial growth of white rot pathogen by 72.70 per cent and BF1 66.37 per cent at 100 % concentration. Eleven treatments evaluated for the management of white rot in storage, minimum incidence (2.82%) was recorded in treatment where fruits were treated with BF2. Different treatments i.e. fruit dip or impregnation of the packing material (trays or wrappers) were effective in restricting the growth of fungus on the fruits. Maximum restriction (100%) in lesion size in comparison to control was found in fruits treated with BF2. Fruits treated with BF2 were also found equally effective in retaining minimum fruit TSS (11.98 ^{0}Brix), maximum titratable acidity (0.28 %) and fruit firmness (14.97 lbs/sq.inch) after 30 days of storage. Treatment with BF2 was found effective as edible wax coating in reducing the physiological weight loss by 3.61 and 3.33 per cent respectively, during storage. Treatment of fruits with BF2 also reduced surface micro flora count (fungal and bacterial) in comparison to untreated apples in control, thus improving the quality of the fruits.

Signature of Major Advisor **Signature of Student**

Countersigned

Professor and Head
Department of Plant Pathology
Dr. Y.S. Parmar University of Horticulture and Forestry
Nauni, Solan-173230 (H.P.)

APPENDIX –I

Culture media used for growing micro-organisms

1) Potato Dextrose agar medium

Pealed potato	:	250 g
Dextrose	:	20 g
Agar	:	20 g
Distilled water	:	1000 ml

2) Nutrient agar medium

Beef extract	:	3 g
Peptone	:	5 g
Agar	:	15 g
Distilled water	:	1000 ml

APPENDIX- II

ANOVA TABLES

Anova 1: Analysis of variance for effect of temperature on mycelial growth of pathogen (Table 2)

Sources of variance	DF	SS	MSS	F	P
Treatment	6	13147.0	2191.1	1610.7	0.00
Growth	14	19.0	1.4		
Total	20	13166.1			

Anova 2: Analysis of variance for *in vitro* efficacy of fungicides against white rot pathogen (Table 4)

Sources of variance	DF	SS	MSS	F	P
Treatment	2	2968.1	1484.1	2657.0	0.00
Concentration	5	2010.7	402.2	720.0	0.00
Treatment x Concentration	10	216.1	21.6	38.7	0.00
Treatment x Concentration x Replication	36	20.1	0.6		
Total	53	5215.1			

Anova 3. Analysis of variance for *in vitro* efficacy of botanicals against white rot pathogen (Table 5)

Sources of variance	DF	SS	MSS	F	P
Treatment	2	4703.2	2351.6	2119.7	0.00
Concentration	13	22274.7	1713.4	1544.4	0.00
Treatment x Concentration	26	2409.5	92.6	83.5	0.00
Treatment x Concentration x Replication	126	139.7	1.1		
Total	167	29527.2			

Anova 4: Analysis of variance for *in vitro* efficacy of BF1 and BF2 against white rot pathogen (Table 6)

Sources of variance	DF	SS	MSS	F	P
Treatment	3	6065.3	2021.8	2347.9	0.00
Concentration	2	1269.4	634.7	737.1	0.00
Treatment x Concentration	6	22.5	3.8	4.4	0.00
Treatment x Concentration x Replication	24	20.7	0.9		
Total	35	7377.8			

Anova 5: Analysis of variance for effect of per cent rot incidence of white rot apple fruit at different duration in storage (Table 7)

Sources of variance	DF	SS	MSS	F	P
Year	1	3.0	3.0	130.6	0.00
Duration	2	11395.8	5697.9	240409.1	0.00
Year X Duration	2	0.5	0.2	12.4	0.00
Treatment	10	38016.3	3801.6	160399.7	0.00
Year X Treatment	10	3.9	0.3	16.4	0.00
Duration X Treatment	20	4563.0	228.1	9626.3	0.00
Year X Duration X Treatment	20	5.1	0.2	10.8	0.00
Year X Duration X Treatment X Replication	130	3.0	0.1		
Total	197	53991.3			

Anova 6: Analysis of variance for effect of different treatments on the lesion growth of inoculated apple fruits at different duration in storage (Table 8)

Sources of variance	DF	SS	MSS	F	P
Year	1	38.1	38.1	78.8	0.00
Duration	2	1471.4	735.7	1519.9	0.00
Year X Duration	2	0.2	0.1	0.2	0.30
Treatment	10	97408.9	9740.8	20124.4	0.00
Year X Treatment	10	92.9	9.2	19.1	0.00
Duration X Treatment	20	902.2	45.1	93.2	0.00
Year X Duration X Treatment	20	3.0	0.1	0.3	0.6
Year X Duration X Treatment X Replication	132	63.8	0.4		
Total	197	99980.9			

Anova 7: Analysis of variance for effect of different treatments on TSS of apple fruits at different duration in storage (Table 9)

Sources of variance	DF	SS	MSS	F	P
Year	1	2.0	2.0	91.7	0.00
Duration	2	58.4	29.2	1295.4	0.00
Year X Duration	2	0.0	0.0	0.4	0.70
Treatment	10	8.7	0.8	38.9	0.00
Year X Treatment	10	0.9	0.1	4.1	0.00
Duration X Treatment	20	92.9	4.6	205.8	0.00
Year X Duration X Treatment	20	0.6	0.0	1.5	0.1
Year X Duration X Treatment X Replication	132	2.9	0.0		
Total	197	166.8			

Anova 8: Analysis of variance for effect of different treatments on titratable acidity of apple fruits at different duration in storage (Table 10)

Sources of variance	DF	SS	MSS	F	P
Year	1	0.01	0.02	199.09	0.00
Duration	2	0.04	0.02	253.61	0.00
Year X Duration	2	0.00	0.01	0.83	0.43
Treatment	10	0.05	0.01	68.79	0.00
Year X Treatment	10	0.01	0.01	0.34	0.96
Duration X Treatment	20	0.01	0.01	2.50	0.01
Year X Duration X Treatment	20	0.02	0.00	0.43	0.91
Year X Duration X Treatment X Replication	132	0.01	0.00		
Total	197	0.12			

Anova 9: Analysis of variance for effect of different treatments on fruit pressure of apple fruits at different durations in storage (Table 11)

Sources of variance	DF	SS	MSS	F	P
Year	1	1.9	1.9	8227.1	0.00
Duration	2	160.9	80.4	339962.0	0.00
Year X Duration	2	0.0	0.0	28.2	0.00
Treatment	10	581.7	58.1	245702.2	0.00
Year X Treatment	10	0.0	0.0	8.2	0.00
Duration X Treatment	20	54.7	2.7	11557.8	0.00
Year X Duration X Treatment	20	0.0	0.0	1.5	0.09
Year X Duration X Treatment X Replication	132	0.0	0.0		
Total	197	799.50			

Anova 10: Analysis of variance for effect of different treatment on weight loss of the apple fruits at different durations in storage (Table 12)

Sources of variance	DF	SS	MSS	F	P
Year	1	0.19	0.19	794.21	0.00
Duration	2	18.92	9.46	39029.28	0.00
Year X Duration	2	0.01	0.01	23.14	0.00
Treatment	10	10.98	1.09	4532.45	0.00
Year X Treatment	10	0.02	0.00	7.55	0.00
Duration X Treatment	20	0.81	0.04	167.56	0.00
Year X Duration X Treatment	20	0.02	0.00	5.05	0.00
Year X Duration X Treatment X Replication	132	0.03	0.00		
Total	197	31.00			

Anova 11: Analysis of variance for effect of surface micro flora (fungal count) of apple fruits at different durations in storage (Table 13)

Sources of variance	DF	SS	MSS	F	P
Year	1	1.1	1.1	11.4	0.00
Duration	1	3.8	3.8	38.8	0.00
Year X Duration	1	0.0	0.1	0.2	0.61
Treatment	10	22.2	2.2	22.2	0.00
Year X Treatment	10	1.4	0.1	1.4	0.16
Duration X Treatment	10	0.3	0.1	0.3	0.94
Year X Duration X Treatment	10	0.3	0.1	0.3	0.96
Year X Duration X Treatment X Replication	88	8.8	0.10		
Total	131	38.3			

Anova 12: Analysis of variance for effect of different treatments on surface micro flora (bacterial count) of apple fruits at different durations in storage (Table 14)

Sources of variance	DF	SS	MSS	F	P
Year	1	0.46	0.46	113.12	0.00
Duration	1	5.91	5.91	1434.66	0.00
Year X Duration	1	0.06	0.06	15.01	0.00
Treatment	10	8.89	0.88	215.64	0.00
Year X Treatment	10	0.04	0.00	1.13	0.34
Duration X Treatment	10	0.20	0.02	4.88	0.00
Year X Duration X Treatment	10	0.03	0.00	0.79	0.63
Year X Duration X Treatment X Replication	88	0.36	0.00		
Total	131	15.98			

CURRICULUM VITAE

Name	**:**	Kishor Sharma
Father's Name	**:**	Sh. Khem Raj Sharma
Date of Birth	**:**	02 September, 1989
Sex	**:**	Male
Marital Status	**:**	Unmarried
Nationality	**:**	Indian

Educational Qualifications **:**

Certificate/ degree	Class/ Grade	Board/ University	Year
Matric	First	HPBSE	2005
10+2	First	CBSE Board	2007
B.Sc. Horticulture	First	Dr. Y.S. Parmar UHF, Nauni, Solan	2011

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(Kishor Sharma)