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Use of Native Local Bio Resources and Cow Urine for the Effective Management of Post-harvest Diseases of Apples in Northwest Himalayan States of India

Manica Tomar[1]* and Harender Raj[2]

[1]Directorate of extension education, Dr. Y.S.P Univ. of Hort. and forestry, Nauni, Solan H.P, 173230, India
[2]Department of Plant Pathology, Dr. Y.S.P Univ. of Hort. and forestry, Nauni, Solan H.P, 173230, India

Abstract

Fungal pathogens namely *Alternaria alternata, Botrytis cinerea, Glomerella cingulata, Monilinia fructigena, Penicillium expansum* are reported to cause considerable post harvest losses in the state of Himachal Pradesh in India. Bio formulation comprising of six botanicals (seed/leaves) and cow urine were found effective for the management of post-harvest rot in apples. Fruit dip and wrappers impregnated with the bio formulations resulted in 84.7 per cent reduction in the post harvest rot after 75 days of storage at 4°C. Bio formulation treated fruits resulted in better fruit firmness and low TSS (14-16%).

Keywords: Post-harvest rots; Fungi; Cow urine; Plant extracts; Storage; Apples; Himachal Pradesh

Introduction

Apple (*Malus domestica* Borkh.) is an important fruit crop of India grown in about 1.19 lakh hectare area with a production and productivity of 25.85 lakh metric tonnes and 21.8 metric tonnes/hac, respectively (NHB, 2013-14). In India commercial cultivation of apple is confined in the states of Himachal Pradesh, Jammu and Kashmir, Arunachal Pradesh and Uttrakhand. Himachal Pradesh is situated in the heart of western Himalayas. The State is almost wholly mountainous with altitude ranging from 350 meters to 6,975 meters above the mean sea level. The State has a deeply dissected topography and rich temperate flora. About 20% of gross cropped area of Himachal Pradesh is covered with the Horticulture crops, which include the fruits, vegetables, flowers, plantations and spice crops, medicinal, & aromatic plants, roots & tuber crops cover. Apple is the major fruit crop of the state. Post-harvest losses are of major concern in the fruits in India as the total losses are to the tune of 30 percent of the total yield which are valued approximately Rs13,600 crores annually. The Indian horticulture industry is making losses estimated at more than US$32.7bn annually due to poor post-harvest practices and facilities, The Economic Times reported. In apple, post-harvest losses ranges from 10 to 25 percent in Himachal Pradesh and fungal pathogens are dominant cause of these losses. Among the different fungal pathogens, *Alternaria alternata, Botrytis cinerea, Glomerella cingulata, Monilinia fructigena, Penicillium expansum* are the dominant ones causing post-harvest losses. Fruit, due to their low pH, higher moisture content and nutrient composition are very susceptible to attack by pathogenic fungi, which in addition to causing rots may also make them unfit for consumption by producing mycotoxins [1,2]. Eckert and Ratnayake [3] estimated that out of 100,000 species of fungi, less than 10% are plant pathogens and more than 100 species of fungi are responsible for the majority of postharvest diseases. There are different strategies for the management of post-harvest losses due to biotic causes like fungal pathogens. Among these, better post-harvest handling practices and use of pre and post-harvest fungicides are the major management strategies. However, use of chemical fungicides in the management of post-harvest diseases poses a risk of residues in the harvest for the consumers. Use of chemicals in the management of post-harvest diseases is restricted due to their possible carcinogenicity, teratogenicity, high and acute toxicity, long degradation periods, environment pollution and their effects on human beings. On the other hand, bio-chemicals derived from extracts of the plants or other bio-resources have no toxic effects and their use is gaining grounds as alternatives to the prevalent chemical control measures. Plants like *Azadirachta indica, Ocimum sanctum, Eucalyptus spp, Aloe barbadensis, Vitex negundo* etc., contain an array of secondary substances like phenols, flavonoids, quinones, tannins, saponins and sterol which can be exploited for their different anti-fungal properties. The cow urine is capable of treating many curable as well as incurable diseases of human's animals and plants and has been used extensively in ayurvedic preparations since time immemorial as cited in ancient Indian holy texts like *Charaka Samhita, Sushruta Samhita, Vridhabhagabhatt, Atharva Veda, Bhavaprakash, Rajni Ghuntu, Amritasagar*, etc., [4]. Cow urine facilitated rapid and holistic recovery in disease infected combs, promoted the growth of brood, enhanced the efficiency of the worker bees in the colonies, thus revealed that the cow urine can serve as a potential eco-friendly measure for management of European foulbrood (EFB), a serious, bacterial disease of honeybee brood found throughout the world in honeybee colonies and also as an indirect control of mite diseases in colonies [5]. Achliya and coworkers [6] while working on cow urine, found many antimicrobial properties in different fractions of cow urine. Use of antifungal plant products and other bio-resources has not been studied for the management of apple post-harvest diseases. In Himachal Pradesh most of the apples are sent to different parts of the country for sale in cardboard boxes. Some of the annual produce is procured by the cold storage companies like *Adani Fresh* and *Reliance*

***Corresponding author:** Manica Tomar, Directorate of extension education, Dr. Y.S.P Univ. of Hort. and forestry, Nauni, Solan H.P, 173230, India, Tel: +1792252363; E-mail: manicatomar22@gmail.com

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industries etc. Orchardists of the state face major losses due to post harvest disease during transportation and storage. Therefore, the objective of this study was to determine the effect of extracts of locally available plants and cow urine individually and in combination against major post harvest diseases of apple in India to reduce the losses by eco friendly methods.

Materials and Methods

Periodic surveys were carried out at 15 days interval in different apple growing areas in the state of Himachal Pradesh, India. Incidence of post-harvest loss due to fungal pathogens was also observed in the cold storages. Incidence of losses was recorded in major apple producing villages across the state. Four villages in district Shimla (Rohroo, kotkhai, Sarahan and Gumma), two in district Kinnaur (Nichar and Kalpa) and two in district Kullu (Bajaura and Kullu) were surveyed for recording the incidence of post harvest losses in apple. Terminal fruit markets, locally called "*Mandis*", in each district were surveyed during the season (June-oct) for recording the post-harvest losses in apple due to different post-harvest pathogens. Incidence was calculated by randomly sampling 25 apple boxes during the harvesting season (June to October). In the cold storages, incidence of post-harvest losses was recorded from November to March. Total rotting of apples was calculated by counting the rotted apples per box. The rotted apples from each location were brought to the laboratory and isolations were taken to find the associated pathogens. Each associated fungal pathogens were purified on Potato dextrose agar medium, purified cultures were then stored at 18 $\pm$ 1°C for further use.

Twelve plant species and cow urine were evaluated *in vitro* for their anti fungal activities against seven important post-harvest pathogens (*Alternaria alternata, Botrytis cinerea, Glomerella cingulata, Monilinia fructigena, Penicillium expansum, Rhizopus stolonifer* and *Tricothecium roseum*) isolated from the fruits. The fungi were purified on PDA and identified with the help of key of British Mycological society and simplified fungi identification key by Jean William Wood Ward [7]. Water extracts of twelve different locally available plant species (leaves) were prepared for evaluating their efficacy *in vitro*. Freshly harvested leaves of *Bougainvillea glabra, Dedonia viscosa, Eucalyptus globulus, Mentha piperita, Roylea elegans , Ocimum sanctum*, *Murraya koenigii, Chrysanthemum coronarium, Polyalthia longifolia, Pelargonium graveolens, Lawsonia inermis and seeds of Melia azedarach* were taken and washed twice in sterilized distilled water. Samples of each plant species were grinded in a mixer and grinder by adding equal quantity (w/v) of sterilized distilled water. Extract was filtered twice through a sterilized muslin cloth and Whatman filter paper no. 4, respectively and Refrigerated at 5°C for further use. Each extract was steam sterilized at 15 lbs pressure for five minutes in an autoclave. Plant extracts were tested against the test pathogens *in vitro* by using poisoned food technique [8] to study their inhibitory effect on the mycelial growth. Six plant extracts were selected out of twelve tested based on their efficacy to inhibit the mycelial growth of the fungi. The six selected extracts which gave more than 90 percent inhibitory effect *in vitro* were *Bougainvillea glabra, Dedonia viscosa, Eucalyptus globulus, Mentha piperita, Roylea elegans and Ocimum sanctum*.

These six botanicals were evaluated in two combinations as Field Formulation-I (FF-I) and Field Formulation-II (FF-II) against post-harvest fungal pathogens. Field Formulation-I was prepared by adding equal quantity of the sterilized plant extract of the six effective plants to equal quantity of sterilized distilled water (w/v). In FF-II instead of distilled water, cow urine was added (w/v). *In vitro* evaluation of FF-I, FF-II and Neemazal (neem based commercial formulation) was done at 50 percent concentration while SAAF (Mancozeb12%+Carbendazim 63%) was evaluated at 0.2 percent concentration. The efficacy of these two Field Formulations (FF-1 & FF-2) was compared with commercial neem formulation (Neemazal), edible Wax coating formulation (Carnauba wax) and fungicide (SAAF). These treatments were applied on fruits by giving them a dip treatment and the fruit wrappers were impregnated by the treatments solution.

The efficacy of FF-1, FF-II, Neemazal and SAAF was evaluated as direct skin coating of fruits and also by impregnating the fruit wrappers. Freshly harvested fruits of variety "Golden Delicious" having a TSS of 15 per cent were used for the experiment. The solution of FF-1, FF-II and Neemazal for skin coating was applied at 10 per cent concentration while SAAF at 0.2 percent concentration. Fruits were dipped in the respective solutions for half an hour. These apples were then air dried and packed in the corrugated card board boxes and stored in cold storage at 4°C. Fruit Skin coating by the edible wax was applied by instant dipping of the fruits in the warm (40°C) wax. To prepare the impregnated wrappers, five sheets of blotting paper measuring 75 × 50 cm were placed one over the other on a clean table. On each set of these five papers, 150 ml each of SAAF (500 ppm), FF-1 (10%), FF-2 (10%) and Neemazal (10%) were sprayed with hand sprayer. Uniform soaking of the solution was secured by spreading the solution smoothly over the sheets. The sheets were allowed to air dry in shade. The sheets were cut into 6 equal wraps each measuring 25 cm^2. Similarly, untreated sheets (butter paper and news paper) of the same dimension (25 cm^2) were used for comparison with the impregnated wrappers. The fruits were wrapped singly in each wrap, packed and stored in cold storage (4°C) for 75 days. Each treatment contained thirty fruits with three replications. Observations on per cent rotting of fruits were taken after 30, 45 and 75 days, respectively. Per cent rotting was calculated by the following formula:

$$\text{Rotting (\%)} = \frac{C-T}{T} \times 100$$

Where C is the control treatment where apples were not treated T is the different treatments given to the fruits.

General quality parameters analysis

After 75 days of storage, Total soluble solids (TSS) and firmness of the fruits were recorded. While TSS (%) was recorded with digital refractometre ATAGO Co Ltd (Japan), fruit firmness was recorded with help of Digital force gauge DFIS 50, USA using a piston cylinder of 4mm dia. All the experiments were conducted in completely randomized design and values of $P<0.05$ were considered significantly different.

Microbial analysis

Microbial analysis included the count of fungi, molds and yeasts on the fruit surface by dilution plate method. Potato dextrose agar medium with rose Bengal was used for enumeration of fungi while for bacteria and yeasts Nutrient agar media was used [9]. The incubation temperature for fungi, bacteria and yeast was 25 ± 1°C. Experiments were replicated thrice and results were expressed as $\log_{10}$ CFU per fruit.

Results and Discussion

Twenty one different types of post-harvest fungal pathogens were found associated with rotting of apple fruits in Himachal Pradesh (Table 1) and their average incidence varied between 0.5 to 45.6 percent.

Among the different post-harvest pathogens, Blue mould rot caused by *Penicillium expansum* was found most prevalent with an incidence of 45.6 percent (Table 1). In addition, fungi namely *Monilinia fructigena*,

Common Name of the rot	Causal organism	Percent Prevalence
Alternaria rot	*Alternaria alternata* Keissl.	1.0
Black pox	*Helminthosporium populosum* Berg	0.5
Black rot	*Sphaeropsis malorum* Pk.	0.5
Black mould rot	*Aspergillus niger* van Tieghem	0.5
Blue mould rot	*Penicillium expansum* Thom.	45.6
Bitter rot	Glomerella cingulata (Ston.) Spauld. and Schren	14.0
Brown rot (Apple black)	*Monilinia fructigena* (Aderh. and Ruhl.) Honey	10.8
Cladosporium rot	*Cladosporium herbarum* Lk. ex Fr.	0.5
Core rot	*Penicillium, Alternaria, Fusarium and Trichothecium* spp	2.0
Eye rot	Cylindrocarpon mali (Allesch) Wr.	0.5
Fusarium rot	*Fusarium spp.*	0.5
Gliocladium rot	*Gliocladium viride* Matr.	0.5
Grey mould rot	*Botrytis cinerea* Pers. Ex Fr.	1.5
Pestalotia rot	*Pestalotia hartigii* Tub.	0.5
Phytophtora rot	*Phytophthora cactorum* (Leb. and Cohn) Schroe	0.5
Pink mould rot	*Trichothecium roseum* Link	12.6
Stalk end rot	*Phoma mali* Schulz. and Sacc.	1.0
Stemphylium rot	*Stemphylium congestum* Newton	0.5
Soft rot	*Mucor piriformis* Fisch.	0.5
Sour rot	*Geotrichum candidum* Link	1.0
Whisker's rot	*Rhizopus stolonifer* (Her. Ex Fr.) Lind	5.0

Table 1: Prevalence of Post-harvest rots in Himachal Pradesh.

Penicillium expansum, Glomerella cingulata, Rhizopus stolonifer and *Trichothecium roseum* were also found associated with the rotted apples.

With a mean incidence of 10.02 percent, the cumulative incidence due to various rots varied from 4.3 to12.95 percent as shown in Table 2. Highest disease incidence was recorded in Shimla district, whereas, district Kinnaur had the least incidence (4.35 %). Blanpied and Purnasiri [10] while working on apples also reported maximum incidence of *P. expansum* and *B. cinerea* in storage. Blue mould rot due to *P. expansum* was found to be the main cause of post-harvest rot both in terminal markets and in cold storage with a mean per cent incidence of 2.86 percent.

Brown rot (*Monilinia fructigena*) and bitter rot (*Glomerella cingulata)* were found to be the next important post harvest rotting diseases in the state with an incidence of 2.24 and 1.42 percent, respectively. Whisker's rot (*R. stolonifer*) and pink mould rot (*Trichothecium roseum*) were found to be more prevalent in the warmer locations of the state with an incidence of 1.03 and 0.36 percent, respectively While *M. fructigena* was of common occurrence in terminal fruit markets and cold storage units of Shimla district (>6000 a.m.s.l) indicating the affinity of this pathogen for cooler temperatures (<18°C) prevailing in these areas during September to October months. Its incidence was highest in Khashdhar (14.2%) followed by Gumma (11.50%) in Shimla district. Ivic et al. [11] while working on dynamics and intensity of apple disease development during storage found that *M. fructigena, P. expansum* and *R. stolonifer* were the major fungi causing maximum losses to apple during storage.

The efficacy of both the field formulations (FF-1and FF-2) was compared with fungicide SAAF and Neemazal in the form of skin coating and impregnated wrappers for the management of post-harvest rotting of apple due to the fungal pathogens (Table 3).

Impregnation of wrappers with these bio-formulations (FF-1 FF-2, and Neemazal) was found effective in reducing the post-harvest rotting. Perusal of data revealed that treatment of wrappers with SAAF proved to be most effective with 81.2 percent reduction in the post-harvest rot of apple after 75 days of storage at 4°C.

However, among bio-formulations, wrappers impregnated with

District/location	Altitude (ft) a.m.s.l	G. cingulata	M. fructigena	P. expansum	R. stolonifer	T. roseum	Others
Shimla							
Khasdhar*	8000	2.00	14.20	0.00	0.00	0.00	2.00
Dhambari*	6800	1.50	7.30	3.00	1.00	0.00	1.50
Sandasu*	6500	2.10	5.00	1.50	0.50	0.50	1.50
Rohru+	6000	3.50	6.20	0.50	0.00	0.00	2.50
Kotkhai+	5200	2.70	4.10	3.20	1.50	0.50	3.50
Gumma**	4750	0.00	11.50	3.60	0.00	0.00	1.00
Sarahan+	7750	4.20	2.00	1.50	0.00	0.00	3.30
Deothi*	7000	3.50	1.90	3.50	0.50	1.30	2.70
Thanedhar**	7500	0.00	3.00	1.00	0.00	0.00	0.00
Navbahar**	7000	0.00	3.00	0.50	0.00	0.00	0.00
Shimla+	7000	1.90	2.10	4.50	0.50	1.00	3.10
Mean		**1.94**	**5.48**	**2.07**	**0.36**	**0.30**	**1.92**
Kinnaur							
Nichar*	7200	0.00	3.00	0.50	0.00	0.50	2.70
Kalpa+	9000	0.00	0.00	1.00	0.00	0.00	1.00
Mean		**0.00**	**1.50**	**0.75**	**0.00**	**0.25**	**1.85**
Kullu							
Bajaura+	5165	3.90	1.70	2.60	0.50	0.50	4.00
Kullu*	5250	1.60	0.90	3.50	1.50	0.50	3.50
Mean		2.75	1.30	3.05	1.00	0.50	3.75
Overall Mean		1.42	2.24	2.86	1.03	0.36	2.11

Table 2: Incidence of post-harvest diseases of apple at various locations in Himachal Pradesh.

Wrappers impregnation treatment	Per cent rotting at different durations (days)				
	30	45	60	75	Mean
SAAF	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	5.0 (2.23)	1.25 (1.09)
Neemazal	0.00 (0.71)	6.66 (2.58)	8.33 (2.88)	11.07 (3.32)	6.51 (2.37)
Field Formulation 1	0.00 (0.71)	3.33 (1.82)	5.0 (2.23)	8.33 (2.88)	4.16 (1.45)
Field Formulation 2	0.00 (0.71)	0.00 (0.71)	3.33 (1.82)	6.66 (2.58)	2.49 (1.45)
Butter paper (without any treatment)	2.73 (1.65)	6.66 (2.58)	12.73 (3.56)	18.33 (4.28)	10.11 (3.01)
Newspaper (without any treatment)	5.0 (2.23)	8.33 (2.88)	15.0 (3.87)	21.07 (4.59)	12.35 (3.39)
Control	6.07 (2.46)	12.73 (3.56)	20.0 (4.47)	26.6 (5.16)	16.35 (3.91)

Figures in the parentheses are square root transformed values.
C.D. (0.05)
Treatment (T) 1.28
Duration (D) 1.16
Interaction (T X D) 1.48

Table 3: Effect of different impregnated wrappers on fruit rotting in storage at 4°C.

FF-2 were found most effective with 75.1 percent reduction in the fruit rotting and both the treatments FF-1 and FF-2 were found statistically at par. Untreated wrappers (butter paper and news paper) resulted in 43.9 to 54.4 per cent more rotting in comparison to apples wrapped in FF-2 impregnated wrappers. SAAF fungicide gave 92.35 percent control of rots as compared to control. Field formulation FF-2 containing cow urine was significantly better over Neemajal. Achliya and co-workers [6] while working on Antimicrobial activity of different fractions of Cow Urine reported the inhibition of several fungi and bacteria.

As percent studies cow urine has proved to be an effective pest controller and larvicide when used alone and also in combination with different plant preparations by enhancing the efficacy of different herbal preparations [12-14].

Bio-formulations were found more effective when used as skin coating as compared to their impregnation in the wrappers (Table 4).

All the bio-formulations were found effective in the management of post-harvest rotting of apple in storage. Skin coating with FF-2 was found effective with 84.7 percent reduction in the post-harvest rotting in the storage after 75 days at 4°C. However, the fruits dipped in fungicidal solution Saaf gave maximum protection against post-harvest rots. Skin coating with FF-2 resulted in more reduction in the post-harvest rotting in comparison to impregnation of wrappers with FF-2. Skin coating with Neemazal was also found effective with 71.1 percent reduction in the post-harvest losses in the storage. The fruits coated with edible carnauba wax gave 75.02 percent reduction in disease as compared to control. Bio formulations were found to be equally effective against the rots.

The mechanism of disease suppression by plant products and biocontrol agents have suggested that the active principles present in them may either act on pathogen directly or induce systemic resistance in host plants resulting in reduction of disease development [15]. The antifungal compounds present in this leaf extract may have prominent effect in inhibiting the mycelial growth of the pathogen [16].

Plants have been shown to produce pectinase and proteinase inhibitors which restrict the microbial development [17-20]. Albersheim and Anderson [17] showed that proteins from plant cell walls inhibited polygalacturonases (PG) secreted by plant pathogens. Fielding [18] found similar inhibitors in extracts of peach and plum fruit. The level of the PG-inhibitor activity was correlated negatively with the rate of fungal rot development in apple fruits.

To develop an eco-friendly strategy for the management of post-harvest diseases of apple, most effective treatments were combined to test their efficacy against post harvest rots in storage and also to check the quality parameters like shelf life, Fruit Firmness and Total Soluble Substances (TSS). Perusal of data in Table 5 reveals that fungicidal solution of SAAF was most effective in controlling storage rots as compared to other treatments. However, Field Formulation-2 when given as fruit dip was able to reduce drastically with 80 percent disease control after 75 days of fruit storage at 4°C (Table 5). FF-2 containing cow urine was significantly helpful in managing storage rots when applied on fruits as well as on wrappers. Edible fruits wax was found to be significantly less effective in managing storage rots as compared to other treatments.

Quality parameters of the fruits like TSS and fruit firmness were found to be better in the treated fruits. Wax coating of fruits with carnauba wax showed best Fruit firmness (15.0 lbs per square inch) after 75 days of storage. Fruit firmness of 14.0 lbs per square inch after 75 days of storage was recorded in fruits which were dipped in with FF-2 as compared to 11.0 lbs per square inch in control.

Similarly, fruit wrapped in FF-2 impregnated wrappers were also effective with fruit firmness of 14.5 lbs per square inch. Fruits dipped in these bio-formulations were having a good shining and less wrinkles on the fruit skin in comparison to the other treatments.

Total soluble solids were the lowest (13.5) in wax coated fruits. In the fruits dipped in Field Formulation 2, level of TSS increased from 13.0 to 14.5 after 75 days of storage, indicating a slower ripening of the fruits. Fruits dipped in fungicidal solution SAAF indicated maximum TSS of 16.5 percent. These results indicated that a coating of field formulation on fruits not only prevented the losses from storage rots but also helped in maintaining the firmness and quality of fruits.

Firmness is an important quality parameter for fresh fruit, which decreases during storage as a result of cell wall degradation and loss of turgor. Rojas-Graü et al. [19-21] found that alginate edible coatings enriched with vanillin (up to 6%) and oregano (1%) applied to fresh-cut apples were effective in improving firmness. However, lemongrass containing coatings and oregano at 5% induced severe texture softening. They attributed it to the lower pH of those edible coating

Treatment	Storage rot(%) due to post-harvest diseases		
	30 days	45 days	75 days
Fruits coated with wax	2.66 (1.82)*	5.33 (2.49)	6.66 (2.74)
Fruits dipped in Neemazal (0.1%)	1.33 (1.41)	5.33 (2.49)	8.0 (3.0)
Fruits dipped in *Saaf* (0.2%)	0.00 (1.0)	1.33 (1.41)	2.66 (1.82)
Fruits dipped in Field Formulation-1 (10.0%)	1.33 (1.41)	4.0 (2.23)	6.66 (2.74)
Fruits dipped in Field Formulation-2 (10.0%)	1.33 (1.41)	2.66 (1.82)	4.0 (2.07)
Control	9.33 (3.20)	17.33 (4.27)	26.67 (5.23)

Figures in the parentheses are square root transformed values.
C.D. (0.05)
Treatment (T) 0.48
Duration (D) 0.23
Interaction (T X D) 0.58

Table 4: Effect of different dip treatments on fruit rot in storage.

Treatments	Storage rot (%) due to post-harvest diseases			Fruit firmness (lbs) at zero day	Fruit firmness (lbs) after 75 days of storage	TSS (%) at zero day	TSS (%) after 75 days of storage
	30days	45days	75days				
Fruits dipped in Field Formulation-2 (10.0%)	0.00 (1.0)*	2.85 (1.58)	5.71 (2.16)	16.0	14.0	13.0	14.5
Wrappers impregnated with Field Formulation-2 (10.0%)	2.85 (1.58)	5.71 (2.16)	8.56 (2.74)	16.0	14.5	13.0	15.0
Fruits coated with wax	2.85 (1.58)	5.70 (2.16)	11.42 (3.32)	16.0	15.0	13.0	13.5
Fruits dipped in Saaf (0.2%)	0.00 (1.00)	2.85 (1.58)	4.54 (1.39)	16.0	13.0	13.0	16.5
Control	8.56 (2.74)	20.0 (4.52)	28.57 (5.36)	16.0	11.0	13.0	20.0

*Figures in the parentheses are square root transformation values.
CD (0.05)
Treatment 0.93
Days 0.72
Interaction (Treatment × Days) 1.14

Table 5: Effect of best post- harvest fruit treatments on fruit rot due to diseases and shelf life in storage.

Treatments	No. of fungal and bacterial microorganisms (Log $_{10}$ CFUg^{-1}) on the fruit surface per cm^2 after different durations (days) in storage					
	0		45		75	
	Fungal	Bacteria	Fungal	Bacteria	Fungal	Bacteria
Fruits dipped in Field Formulation-2 (10.0%)	6.8 ± 0.0	7.9 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.3	0.7 ± 0.3
Wrappers impregnated with Field Formulation-2 (10.0%)	6.8 ± 0.0	7.9 ± 0.0	7.4 ± 0.0	8.0 ± 0.0	7.5 ± 0.0	8.1 ± 0.0
Fruits coated with wax	6.8 ± 0.0	7.9 ± 0.0	7.9 ± 0.3	8.9 ± 0.4	8.3 ± 0.0	9.2 ± 0.0
Fruits dipped in Saaf (0.2%)	6.8 ± 0.0	7.9 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.0	0.8 ± 0.4
Control	6.8 ± 0.0	7.9 ± 0.0	7.9 ± 0.1	9.0 ± 0.1	9.1 ± 0.0	11.0 ± 0.0

*Figures in the parentheses are logarithmic transformation values.
C.D. (0.05)
Treatment 0.290
Days 0.225
Interaction (T × D) 0.355

Table 6: Effect of different post- harvest fruit treatments on fungal and bacterial surface micro-flora.

solutions. Also, lemongrass has as main compound citral, confirming our results. Guerreiro et al. [22] also reported a better effect of Eugenol than Cit on *Arbutus unedo* fresh fruit storage when using alginate based edible coatings.

According to Duan et al. [23] for blueberries, Soluble Solid Content (%) was not significantly affected by cold storage or coating (sodium alginate and chitosan) treatments. These results differ from those reported by Gol et al. [24] and Velickova et al. [25] who showed a decrease in the total soluble solids content in strawberries, at the end of storage, and attributed it to respiration, when using other edible coatings.

In our case the fruits before storage had a TSS of 13.0 percent which increased significantly in untreated control as compared to those dipped in cow urine based plant extract formulation. Cow's urine is not a toxic effluent as 95% of its content being water, 2.5% urea and the remaining 2.5%, a mixture of minerals, salts, hormones and enzymes [26]. The biochemical estimation of cow urine has shown that it contains sodium, nitrogen, sulphur, Vitamin A, B, C, D, E, minerals like manganese, iron, silicon, chlorine, magnesium, calcium salts, phosphate, lactose, carbolic acid, enzymes, creatinine and hormones [27]. Fractions of cow urine obtained by solvent extraction possess antimicrobial activity due to presence of aforesaid components those are solely responsible for the action [28].

The fungal and bacterial count increased with time in some of the treated fruits in storage (Table 6). In control this increase was constant up to 75 d storage. However, when FF-2 was applied on fruits, no fungal and bacterial development on fruits was recorded after 45 d of storage but 0.8 ± 0.3 and 0.7 ± 0.3.

Discussion

Chemical treatment of plant diseases especially in edible commodities has been drawing concerns due to their residual properties and human health issues. This has led to the introduction of eco friendly holistic approaches for the management of these diseases. The mechanism of disease suppression by plant products and bio control agents have suggested that the active principles present in them may either act on pathogen directly or induce systemic resistance in host plants resulting in reduction of disease development [15]. Plant extracts have been considered as an alternate and efficient way of plant disease management. In the present studies, twelve plant extracts were studied *in vitro* against the major post harvest pathogens of apple out of which only best six were further used. The present study also incorporated the use of cow urine for plant disease management. In India Cow is considered as pious animal and cow urine is known for its therapeutic properties. The cow urine is not only used against human ailments as therapeutic agent but also has several other uses as in agriculture and sericulture sectors [5]. The biochemical estimation of cow urine has shown that it contains sodium, nitrogen, sulphur, Vitamin A, B, C, D, E, minerals, manganese, iron, silicon, chlorine, magnesium, citric, succinic, calcium salts, phosphate, lactose, carbolic acid, enzymes, creatinine and hormones [27]. The present studies showed that the formulations with cow urine inhibited the fungal growth more efficiently as compared to the formulations containing

water. Wrappers impregnated with FF-2 containing cow urine were found most effective with 75.1 percent reduction in the fruit rotting. Though the chemically treated fruits were better than cow urine based field formulation but statistically they were at par with each other. However, the results of water-based formulation (FF-1) were statistically different from both the chemically treated fruits as well as cow urine based formulation treated ones, there by clearly indicating that cow urine based formulation had more inhibitory effect on the fungi which was quite similar to the inhibition rendered through chemicals. Fruit dip in Saaf, Neemajal, edible wax, FF-1 and FF-2 gave maximum control of post harvest diseases through Saaf followed by cow urine based FF-2. Achliya and co-workers [6] while working on Antimicrobial activity of different fractions of Cow Urine reported the inhibition of several fungi and bacteria. As per recent studies cow urine has proved to be an effective pest controller and larvicide when used alone and also in combination with different plant preparations by enhancing the efficacy of different herbal preparations [12-14].

Shelf life of apples is an important attribute in storage. The apples in India are stored in cold storage chains and then supplied in the markets at an appropriate time for earning profits. During marketing of storage apples, TSS and fruit firmness decide the fruit price. In the present study it was observed that though the chemically treated fruits had comparatively lesser storage rots but its firmness had decreased over a period of 75 days in storage and the TSS content had also risen from 13.0 to 16.5 percent, indicating the ripening process of the fruits. However, The TSS and fruit firmness was 14.5 percent and 14.0 lbs respectively, in FF-2 treated apples which were comparatively better than other treatments used. In agrarian country like India, where majority of rural population have cows as their additional source of income, Cow urine based formulations would definitely prove to be a potential medicine, which in turn would reduce the pressure on the existing use of chemicals.

Conclusion

The present study tried to integrate the holistic approaches for the important storage rots of apples in the North West Himalayan states of India. Use of cow urine for management of plant diseases has been explored in this research. There is need for further research in this regard, as cow urine can be easily available, cheap, unharmful potential substitute for chemicals especially in the edible products. Protection of stored apples from post harvest diseases by chemicals lead to residual toxicity in human body. Therefore, plant extracts and cow urine can be utilised as potential source of inexpensive and efficient source of crop protection from post-harvest diseases.

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