RESEARCH PAPER:

Use of botanicals: A new prospective for enhancing fruit quality over chemicals in an era of global climate change

SANDEEP KUMAR CHAUHAN AND D.RAMESH BABU

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SUMMARY

The present study was conducted to investigate the effect of various pre-harvest treatments of neembased formulations on the storage quality of apple cv. STARKINGDELICIOUS. The pre-harvest (20-25 days harvest) treatments consisted of Nimbecidine (0.5, 1.0, 1.5%), Neemazal (1.0, 1.5, 2.0%) Neem gold (0.5, 1.0, 1.5%) with Bayistin (0.05%) as control. In another experiments the effect of post-harvest treatment with extracts of various plant leaves and with the fresh leaves as cushioning material in packages on the storage quality of apple cv. Starking delicious were studied. The various plant leaves/ flowers used as coatings were Neem leaf extracts (10, 20%), Drake leaf extracts (10, 20%), Spearmint leaf extract (10, 20%), Marigold flower extract (10, 20%), and Semperfresh (control 1.5%). Whereas, Neem, Melia, Mentha, Walnut, Banna, Basooti, and Camphor were used as cushioning material in packages. Freshly harvested fruits were treated with above treatments and were kept under refrigerated storage for analysis at a month interval upto 180 days. Nimbecidine (1.5%) was found better in reducing PLW, retaining fruit firmness, whereas, Neemazal (2.0%) was found effective in retaining maximum total soluble solids (TSS) content, starch iodine rating and pectin content at the end of 180 days storage period. Among the treatments of extracts of plant leaves/flowers, fruits treated with 20 per cent Drake leaf extract (T₄) proved to be most effective treatment in reducing weight loss, whereas, maximum retention of firmness was recorded in fruits treated with 20 per cent Neem leaf extracts (T₂). Drake (T₄) and Neem leaf extracts (T₂) were also capable to retain maximum Total soluble solid (TSS) content. Minimum decrease in starch content was recorded with 20 per cent Neem leaf extract (T₂). This treatment also retained maximum pectin content in the fruits at the end of 180 days storage. On the other hand, 20 per cent spearmint leaf extract (T_i) proved to be highly effective in reducing spoilage as no spoilage was recorded under this treatment. Whereas, among fresh leaves as cushioning material in packages it was observed after analyzing various physico- chemical parameters that fruits cushioned with camphor leaves exhibited significant superiority over the non-cushioned fruits in retaining most of quality characteristics viz., physiological weight loss (PLW), firmness, total soluble solid (TSS), starch content, pectin and spoilage at the end of 180 day of storage period.

See end of the article for authors' affiliations

Correspondence to:

SANDEEP KUMAR CHAUHAN

Botanic Garden of Indian Republic (BGIR), Ministry of Environment and Forest, NOIDA (U.P.) INDIA sandeep.chauhan @rediffmail.com

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In India, apple is the most important temperate fruit of Himalayan region. Despite spectacular progress made in the area and production, it is estimated that 20-30 per cent of the total production is lost during the post-harvest handling period due to lack of proper handling and storage facilities thereby contributing to qualitative and quantitative losses. Therefore, it is quite essential to reduce these losses. With the ever-increasing demand for good quality fruits, which are free from fungicides and pesticides residues, the growers are forced to produce quality fruit, especially after the removal of restrictions on international trade.

In the absence of highly capital intensive handling infrastructure like precooling, refrigerated transport and controlled atmospheric storage, growers will have to depend upon on alternative, simple and low cost technique till such facilities are created.

From the last few fears the use of various chemicals and waxing material at the pre and post harvest stages is becoming popular among growers in order to enhance the shelf-life of fruits. However the use of these substances have their own limitations, as some of them are believed to be ecologically unsafe and economically inviable beside leaving their

residue on the fruit surface, which may have the direct effect on human health. Additionally some of them may be associated with the changes of aroma of the fruit. In order to over come these short comings, there is an urgent need of substances which are of biological origin with growth regulating, fungicidal, insecticidal properties (Dhaliwal and Arora, 1996). Grainge et al. (1984) have documented and classified a number of plants belonging to various families having growth regulating, fungicidal properties and plant like neem, melia, mentha, lantana are under active investigation for use as a plant protection agents all over the world. Already many neem based formulations are available in the market. Owing to its various effects, azadirachtin is considered as the most active principal substance in neem which has growth regulating, fungicidal and insecticidal properties (Schmutters, 1990).

The present study was undertaken with the objective to study the effect of pre- harvest treatment of apple CV. Starking delicious with three neem based formulations on the storage quality of apples. Apple is the most widely cultivated fruit of the temperate regions of the world and because of its importance in temperate horticulture; it is rightly called the "King of temperate fruits". Although, area and production has increased many folds but the process of development has started reversing due to one or the another reasons. In India where horticulture infrastructure is lacking, growers are liable to depend upon another sources to maintain the fruit quality, which are simple and low cost technique so that they can maintain the quality attributes of fruits. In order to develop these low cost technique, from the last few years the use of various chemicals and waxing materials at the pre and post harvest stages is gaining importance among growers in order to enhance the shelf-life of fruits as well as meeting their quality standards. But the applications of these substances are believed to be unsafe as they are prone to objectionable residue on the fruit surface, which may have the direct effect on human health. In order to over come these short comings, there is an urgent need to substitute these substances, which are ecologically and economically unsafe with substances, which are of biological origin and are also known to contain higher amounts of such principal natural substances exhibiting growth regulating, fungicidal and insecticidal properties (Dhaliwal and Arora, 1996) which can be exploited for retaining freshness and enhancing the shelf-life of horticulture crops. Among these biological substances, botanical formulations of neem, melia, mentha, and marigold as well as their applicability as a cushioning material in packages in the form of fresh leaves could be the most definate alternative to overcome the undesirable effects of chemicals and waxes. The present investigation therefore emphasized the effect of post harvest treatments of the botanicals in the form of fresh leaves and their extract on the storage quality of apple and physico- chemical characteristics of apple cv. STARKING DELICIOUS

MATERIALS AND METHODS

The experiment pertaining to the pre-harvest treatment of neem based formulations was conducted in the year 2003-04 and 2004-05 and was laid out in well maintained commercial orchard situated at an elevation of 2700 meters above msl in village Chini (Kalpa) district Kinnaur, Himachal Pradesh. Thirty well grown, uniform, 15-20 years old "Starking delicious" trees raised on seedling rootstocks, were selected for the experiment. The trees were maintained under a uniform schedule of cultural operations throughout the season and subjected to pre-harvest treatments of three commercial neem based formulations viz., Nimbecidine, Neemazal and Neemgold 20-25 days before the expected date of harvest of fruit. Each treatment was replicated thrice with each replication being applied individually to separate trees. The details of treatments under study were Nimbecidine (0.5, 1.0 and 1.5%), Neemazal (1.0, 1.5% and 2.0%), Neemgold (0.5, 1.0 and 1.5%), with Bavistin (0.05%) as control. The entire fruits from individual trees were harvested manually and only sound, medium sized fruits were selected for conducting the studies. The fruits were directly packed in corrugated fibre boxes (CFB) carton with paper moulded trays and were immediately transported to the postharvest physiology laboratory of the CA Store Rai Sonipat for observing changes in fruit quality during storage. While in second experiment, coating materials were prepared from extracts of leaves and flowers of some locally available plants which have been used traditionally for preventing spoilage in different crops. Aqueous extracts of different plant materials were prepared under laboratory conditions on per cent weight basis as per the method described by Gakhukar (1996) and Sharma et al. (1997). The method consists of collection of fresh leaves/flowers, followed by shade drying and then grinding them to powder form in an electric blender. The powdered material was stored in HDPE bottles till use. Aqueous solutions were prepared by soaking a known weight of the powdered material in an equal quantity of water and keeping it overnight. The extract was separated with the help of muslin cloth and it was considered to be of 100 per cent strength. It was diluted by adding appropriate quantity of distilled water

to make up the desired concentration. Guar gum was also added to all the coating solutions @ 2.0 per cent. Semperfresh, a waxing material generally used commercially for waxing of apples, was used to compare the effectiveness of leaf and flower extracts. The details of treatments under study were Neem leaf (*Azadirachta indica*) extract 10%, 20%, Drake (*Melia azedarach*) leaf extract 10%, 20%, Spearmint leaf (*Mentha spicata*) extract 10%, 20%, marigold flowers (*Tagetes erecta*) extract 10%, 20%, semperfresh (control, 1.5%).

For the application of postharvest coating treatments uniform, unblemished medium sized fruits were selected and washed in clean tap water. After air-drying, the fruits were coated with different extracts by dipping them for five minutes. The coated fruit were placed on newspaper sheet for drying in shade for half an hour at room temperature and also to remove excess coating materials. Immediately after drying the fruits they were kept with respect to their treatments under refrigerated storage. In third experiment fresh leaves of plants known to posses antimicrobial properties were collected from different parts of Himachal Pradesh during the fruit season 2003-04 and 2004-05. Approximately 25 g of fresh leaves were placed in CFB cartons (32x18x16 cm) of 3.5 kg capacities uniformly distributed from top to bottom of the trays along with the freshly harvested fruit and kept immediately after treatments under refrigerated storage. The details of treatments were: Neem (Azadirachta indica) leaves, camphor (Cinnamomum camphora) leaves, banna/China caste tree (Vitex negundu) leaves, Basooti (Adhatoda vasica) leaves and control (uncushioned). Observations regarding physico-chemical characteristics PLW, firmness, TSS, starch -iodine rating, pectin and spoilage of fruits were recorded at an interval of a month during the storage period of 180 days. PLW were weighed on a physical balance, fruit firmness were measured with an Effigi Penetrometer, TSS with the help of Erma hand refrectrometer, pectin were measured by Carra and Haynis methods as described by Ranganna (1986), starch iodine rating were measured by from starch test guide as described by Philips and Poapst (1959), whereas fruit spoilage was calculated on per cent bases..

RESULTS AND DISCUSSION

Various neem based formulations applied before harvest had significant effect on most of the parameters. In the present investigation increase in physiological loss in weight (PLW) with an increase in storage duration was observed under all treatments consisting of neem based formulations though it was relatively less than that observed in control fruits (Table 1). Among various [Asian J. Environ. Sci. (June, 2011) Vol. 6 (1)]

formulations, Nimbecidine (1.5%) was found most effective in reducing PLW, which could be due to its ability to retard moisture loss and senescence enhancing mechanism as reported by Gakhukar (1996). The reduction in PLW with neem based formulations can also be attributed to their ability to check the growth of microbes that were responsible for rotting and increasing the metabolic rate of commodities which cause loss in weight through respiration (Singh *et al.*, 2000).

During another investigation, loss in flesh firmness (Table 2) was observed to be the lowest in response to 1.5 % Nimbecidine followed by 1.0 % Nimbecidine and 2.0 % Neemazal. Retention of better firmness in Nimbecidine treated fruit can be attributed to the direct effect of azadirachtin, a principal active compound present in neem formulation on pectin molecules which are believed to regulate the calcium and pectin integrity, thereby lowering the chances of its breakdown during storage (Kleeberg, 1996) hence better firmness was recorded.

It was observed that TSS in general increased as the storage period advanced upto 120 days, registering a gradual decline thereafter (Table 3). Although, definite treatments effects were not discernible, yet the treatments 2.0 % Neemazal and 1.5 % Nimbecidine demonstrated distinct superiority over the other treatments by recording higher value for TSS at the end of 180 days storage. Higher value for total soluble solid under these treatments could be due to the maintenance of cell wall integrity for longer duration, thereby retarding ripening and senescence-related processes (Singh *et al.*, 2000).

Starch iodine rating (Table 4) indicated decline trend in the starch content of fruits with an increase in storage duration under all the treatments. The loss of starch in apple fruits during storage may be due to its hydrolysis into sugars (Wills *et al.*, 1980; Priest and Lougheed, 1981). However, 2.0 per cent Neemazal and 1.5 per cent Nimbecidine resulted in minimum loss of starch content in fruit, such an affect may be attributed to the effect of active substances especially azadirachtin present in neem formulation slowing down the changes in constituents of fruit as a result of slower ripening changes.

It was observed that pectin content (Table 5) showed a gradual decline with an advancement of storage duration under all treatments. Among various formulation 2.0 % Neemazal was best in retaining maximum pectin content and it was followed by T_5 and T_9 . The loss of pectin content may be due to breakdown of pectin during storage as claimed by Sandhu *et al.* (1990). The gradual decrease in pectin content with the advancement of storage period might be due to the action of pectin degrading enzymes

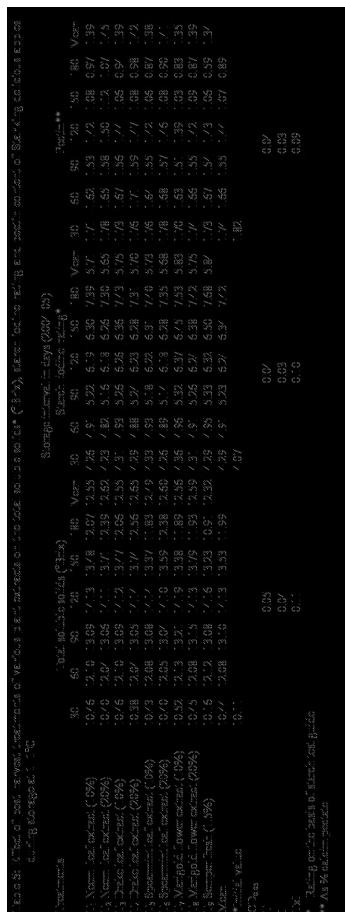
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present in fruit (Nara *et al.*, 2001). Azadirachtin a principle compound in neem formulation is reported to retard the de-estrification of pectin thereby slowing down its breakdown resulting in higher pectin content in such fruits (Gakhukar, 1996; Kleeberg, 1996; Ozdemir *et al.*, 1996 and Singh *et al.*, 2000).

Spoilage (Table 6) is one of the most important criteria in determining the effect of neem formulations in retaining the storage quality of apple and it was observed under all the treatments that spoilage was reduced significantly. Among treatments 1.5% Nimbecidine and 2.0% Neemazal decreased the spoilage considerably. Reduction in spoilage due to rotting with the use of neem formulations may be attributed to the presence of the principal compound, azadirachtin which has the ability to check the growth of microbes that are responsible for causing rotting and also to its ability to reduce the rates of respiration and transpiration in fruits (Gakhukar, 1996, Chai *et al.*, 1990).

The present study thus shows that among various pre-harvest treatments of neem based formulations, nimbecidine (1.5%) and neemazal (2.0%) retained the most of the quality characteristics of apple when compared with Bavistin.

Coating treatments with plant leaf/flower extracts caused significant reduction in PLW (Table 7)and the most effective treatments in this regard was 20 per cent Drake leaf extract . Coating of plant leaf/flower extracts might form a thin film around each fruit, which can act as a semi-permeable membrane to regulate the diffusion of $\rm O_2$ and $\rm CO_2$ into and out of the fruit, thereby reducing the rate of metabolism and also prevents water loss (Smith and Stow, 1994).

It has been observed that there was a gradual decline in fruit firmness (Table 8) under all the treatments with the progressive increase in storage durations. However, the application of 20 per cent Neem leaf extract proved to be the most effective treatment in retention of fruit firmness during storage. Retention of relatively high firmness under this treatment could be due to slower metabolic activities leading to slower ripening changes and delayed senescence (Bhardwaj and Sen, 2003). The loss of pectin substances from the middle lamella of the cell wall is perhaps the key step in the ripening process, that leads to the loss of cell wall integrity of fruits (Gross and Sams, 1984) and consequently leads to softening.

It was observed that TSS (Table 5) in general increased with the advancement of storage period up to 120 days and thereafter these constituents started declining during the remaining storage period. Among the treatments highest, mean TSS contents were recorded

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in response to coating with 20 per cent Neem leaf extract whereas, they were lowest in control fruits, which might be due to higher respiratory losses in these fruits as there was no barrier to restrict the movement of gases into and out of the fruit (Singh *et al.*, 2000). The increase in TSS and sugar contents during the earlier part of storage may be due to the hydrolysis of insoluble polysaccharides into simple sugar.

There was an increase in starch iodine rating (Table 5) indicating disappearance or loss of starch in fruits under all the treatments during storage. Minimum reduction in starch content of fruit was recorded with 20 per cent Neem leaf extract, whereas other coating materials were effective to a lesser extent in this regard. Such an effect of coating treatments may be attributed to the slower ripening changes on the metabolism of fruits and can be expected to be slower, when the fruits are treated and stored under conditions, which are not conducive to enhance ripening (Singh *et al.*, 2003).

In general, the pectin content (Table 5) in fruit exhibited a continuous decline with an increase in storage duration under all coating treatments with, 20 per cent Neem leaf extract being the most effective in retaining higher pectin content. The subsequent loss in pectin content may be due to break down of pectin during storage as claimed by Sandhu *et al.* (1990). The gradual decrease in pectin content with the advancement of storage period might be due to the result of pectin enzyme activity on natural pectin in the fruit. These findings are also in conformity with the observations of Nara *et al.* (2001) who observed similar changes in the pectin content of stored apple fruit.

Spoilage (Table 6) due to rotting did not occur under any of the treatments during the first four storage intervals. Thereafter, some spoilage was recorded under most of the treatments and a significant increase was observed as the storage period increased to 180 days. Among treatments, 20 per cent spearmint leaf extract proved to be highly effective in reducing fruit spoilage as no spoilage was recorded under this treatment. It was followed by the treatment consisting of coating with 20 per cent Neem leaf extract. On the other hand, maximum mean fruit spoilage was recorded with coating 1.5 per cent Semperfresh, which was the control treatment for the experiment.

Among fresh leaves, it was observed that cushioning with camphor and spearmint leaves resulted in lower physiological loss (Table 7) weight. Reduction in weight loss due to these treatments might be due to their effect on slowing down of physiological processes responsible for weight loss (Sharples and Johnson, 1977), beside this,

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inclusions of fresh leaves in packages might have resulted in the maintenance of high relative humidity with in packages, thereby reducing respirational and transpirational losses (Singh *et al.*, 2000).

Among fresh leaves decrease in fruit firmness (Table 7) during storage was observed to be lower in fruit that were cushioned with camphor and Vasaka leaves. The active principal compound present in these plants are believed to maintain calcium integrity in fruit cellular compartments thereby retaining cellular integrity of fruits, thereby resulting in retention of better flesh firmness (Sandhu *et al.*, 1990).

It was observed that with the use of fresh leaves as cushioning material in packages, the total soluble solid (Table 8) increased during the initial storage period up to 120 days where as in case of non cushioned fruits this increase was observed only after 90 days. After these periods, a decline was observed in the constituents during the remaining storage period. Camphor leaves (T_5) in general retained higher amount of these constituents, which might be due the ability of the volatiles generated by the cushioning material to slow down the rate of metabolism of fruit hence utilizes the respirable substrate at a slower rate (Singh *et al.*, 2000 and Bhardwaj and Sen, 2003).

The initial increase might be due to the fact that during storage, starch gets hydrolyzed into mono and disaccharides, which lead to an increase in TSS and sugars content (Aly et al., 1981) and after complete hydrolysis of starch, no further increase in these constituents occur, but subsequently a decline due to utilization as primary substrate for respiration (Wills et al., 1980).

Among all the fresh leaves treatments, decrease in starch iodine rating (Table 8) was observed with an increase in storage duration. Drake leaves retained maximum starch content in apple fruits during 180 days of storage, such an effect may be attributed to the ability of principal compound to act as an antisenscent agent (Grainge *et al.*, 1984). The loss of starch in apple fruit during storage may also be attributed due to its hydrolysis to sugar (Wills *et al.*, 1980), which act as intermediatry substrate for catabolic and anabolic pathway.

Pectin content (Table 9) in general showed a decline with an increase in storage duration under all treatments of fresh leaves as a cushioning material in packages. However, fruit treated with spearmint leaves retained higher pectin contents throughout storage. The decrease in pectin levels during storage may be due to its breakdown during storage as claimed by Sandhu *et al.* (1990). The shower and gradual decline in pectin content of fruits

cushioned with spearmint leaves during storage may be attributed to lower respiration rates, proteolysis and tissue breakdown in such fruits as menthol is believed to deactivate the removal of galacturonic acid residue from pectin molecule, thereby retaining higher pectin content in fruits (Sonkar and Ladaniya, 1999).

Among fresh leaves (Table 9) during first 30 days there was no spoilage of fruits under any treatment. However, thereafter a slight increase in spoilage was recorded under some treatments and after 120 days spoilage was observed under all the treatments. Among cushioning treatments with fresh leaves, camphor leaves (T₅) delayed the appearance of spoilage symptoms till 120 days and exhibited the lowest mean fruit spoilage and therefore was the most effective treatment in controlling spoilage during the entire storage duration of 180 days. A possible reason for low spoilage could be the active principal compound present in botanicals which imparts resistance against infection and generally acts as antifeedent and antirepellent agents thereby lowering down pathogen infestation on fruit surface (Gakhukar, 1996).

Authors' affiliations:

RAMESH BABU, Fresh and Healthy Enterprises Limited, Container Corporation of India, Ministry of Railways, CA Store RAI, SONIPAT (HARYANA) INDIA

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