IMPACT OF HERBAL EXTRACTS ON MICROBIAL VASCULAR PLUGGING, QUALITY AND LONGEVITY OF CUT ROSES KONFETTI AND BORDEAUX

Thesis

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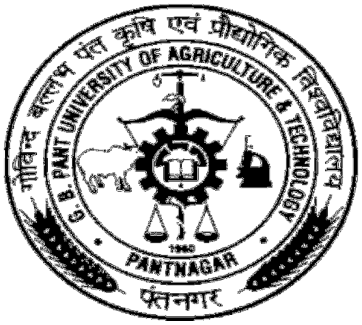

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December, 2013

(Anisha P. N.)
Authoress

CERTIFICATE

This is to certify that the thesis entitled **"IMPACT OF HERBAL EXTRACTS ON MICROBIAL VASCULAR PLUGGING, QUALITY AND LONGEVITY OF CUT ROSES KONFETTI AND BORDEAUX"** submitted in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** with major in **Horticulture (Floriculture and Landscaping)** and minor in **Vegetables Science** of the College of Post Graduate Studies, G. B. Pant University of Agriculture and Technology, Pantnagar, is a record of *bona fide* research carried out by **Ms. Anisha P.N.,** Id. No. **41126** under my supervision and no part of the thesis has been submitted for any other degree or diploma.

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We, the undersigned, members of the Advisory Committee of **Ms. Anisha P.N.,** Id. No. **41126**, a candidate for the degree of **Doctor of Philosophy** with major in **Horticulture (Floriculture and Landscaping)** and minor in **Vegetables Science** with major in **Soil Mechanics and Foundation Engineering** agree that the thesis entitled **"IMPACT OF HERBAL EXTRACTS ON MICROBIAL VASCULAR PLUGGING, QUALITY AND LONGEVITY OF CUT ROSES KONFETTI AND BORDEAUX"** may be submitted in partial fulfillment of the requirements for the degree.

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LIST OF ABBREVIATIONS

%	per cent
@	at the rate
µg	Microgram
^{0}C	degree Celcius
a.i.	Active ingredient
Ag	Silver
$AgNO_3$	Silver nitrate
AM	Ante meridian
CD	Critical difference
cfu	colony forming unit
cm	centimeter
CRD	Complete Randomized Design
cv.	cultivar
DMSO	Di methyl sulphoxide
EMB	Eosine Methylene Blue
et al	et alli (and other)
Fig.	Figure
g	gram
ha	hectare
HQC	Hydroxy quinoline citrate
hrs	hours
i.e.	id. est (that is)
kg	kilogram
Ltd.	Limited
m	meters
mg	milligram
MIC	Minimum inhibitory concentration
ml	milliliter
mM	millimolar
nm	nano meter
NS	non-significant
NA	Nutrient Agar
SPC	Standard Plate Count`
ppm	parts per million
Pvt.	Private
RH	Relative Humidity
SDA	Saboraud dextrose agar
STS	Silver thiosulphate
viz.	namely
VP	Voges-Proskauer

Introduction

Chapter 1 INTRODUCTION

Flowers are the greatest options to express the feelings rather than words. They symbolize purity, beauty, peace, love and passion. Flowers are among the loveliest objects on the earth and among these, the rose (*Rosa* spp.) has captured the hearts of all. As Hynes has said "Ever since man first beheld it and inhaled its scent", rose has captured his imagination and taken root in his thoughts (Pal, 1991). Rose has continued to play an important role in almost perfection and therefore, Sappto, the Greek poetess in her "Ode to the Rose" named it as the "Queen of flowers" in 600 B.C. (Muhammad *et al*., 1996). The position of rose is such that no garden or flower market is complete without its presence.

Rose, a symbol of affection, elegance, inspiration and source of aesthetic gratification, is most famous and popular cut flower in global floriculture trade. It belongs to family Rosaceae and genus Rosa, which contains 200 species and more than 18, 000 cultivars (Gudin, 2000). Rose enjoys superiority over all other flowers being extensively used for decorative purposes and is prized for its delicate nature, beauty, charm and aroma. Rose is recognized for their high economic value, which are used in agro-based industry especially in cosmetics and perfumes. Additionally, roses play a vital role in the manufacturing of various products of medicinal and nutritional importance. However, the main idea of rose plant cultivation is to get the cut flowers, which greatly deals with the floricultural business (Butt, 2003). It has deep relationship with human sentiments and is used on almost every event due to which it has high demand in international markets. Rose has always been the most favourite flower in the subcontinent. It has always had a special place in our culture as there is hardly any event where roses are not displayed in varied fashion.

A critical aspect of cut flower postharvest quality is longevity, which is not the length of lasting quality in itself but also consumer's satisfaction (Buys, 1978). Customer satisfaction with cut flowers is correlated with a perception of freshness (Robertson and Staby, 1977) and with adequate vase life. The longevity of cut flowers is affected by production practices as well as proper postharvest treatments.

Vase life is often used as an indicator of postharvest longevity in cut flowers, which is determined by counting number of days from harvest to flower wilting.

Flowers are extremely perishable commodities, which maintain their metabolic activities very actively even after harvesting. Short postharvest vase life is one of the most important problems in the cut flower industry. Cut roses often last a short time in vase, due to early failure of water relations, resulting at best in partial wilting of leaves and failure of bud opening and at worst in bent neck and rapid desiccation of leaves and petals. Senescence of cut flowers is induced by several factors like water stress, carbohydrate depletion, microorganisms and ethylene effects. Within these factors decays caused by bacteria and fungus, which found in high level in vase solutions used by growers, wholesalers and consumers to hydrate the cut flowers, is one of the most important factors affecting the vase life of cut flowers. Vase life of cut rose flowers is often terminated by stem wilting known as bent neck, a condition in which stem wilts just beneath flower bud. Development of such condition may be due to the presence of microorganisms which plug the vascular system particularly xylem of cut stem and inhibit water uptake by the stems (van Doorn, 1997).

Microbial development in the vase solution has a detrimental effect harming the viability and look characteristics of cut flowers (Henriette and Frank, 1989). Microorganisms which grow in vase water; including bacteria, yeasts and moulds are harmful to cut flowers through their development in, and their consequent blockage of xylem at cut ends, preventing the water absorption. Generally, cut-flowers are very sensitive to microbe gathering in the end of the stem or preservative solution and this factor results in their short postharvest life (Solgi *et al.,* 2009). Suitable germicide application might control microbial activity in the vase water (Nowak and Rundnicki, 1990).

The microorganisms on stems of cut flowers, foliage and in vase solutions are typically composed of yeasts, filamentous fungi, and bacteria (van Doorn, 1997). These microorganisms vary in their response to biocidal agents. The varied response of microorganisms to biocides may be ascribed to variations in morphological structure (e.g. vegetative cell versus mature spore) and chemical composition (e.g. different types of peptidoglycans in bacterial spores) of the individual microorganism (Maillard, 2002; Turner *et al*., 2000).

Addition of chemical preservatives to the holding solution is recommended to prolong the vase-life of cut flowers. All holding solutions must essentially contain two components viz., sugar and germicides. The sugars provide a respiratory substrate, while the germicides control harmful bacteria and prevent plugging of the conducting tissues. Therefore, developing proper techniques to prolong the vase-life of cut flowers will be a great asset to the growers and users. To be effective, an antimicrobial treatment must function in all conditions, including across varying vase solution composition (Knee, 2000), and against the prevalent microorganism, such as a specific bacterial and fungal species (Turner *et al*., 2000).

The principle antimicrobial compounds that have been used to extend the vase life of cut flowers are: hydroxyquinoline (HQ) compounds, such as 8-hydroxyquinoline citrate (HQC) (Knee, 2000) and 8-hydroxyquinoline sulphate (HQS) (Hussein, 1994) as well as silver compounds, such as silver nitrate (AgNO3) (Fujino *et al*., 1983). 8-HQ, which is broadly used, is very expensive and most harmful preservative for humans causing irritation to skin, eyes and respiratory tract. Nowadays, some of the chemical compounds, such as silver nitrate and silver thiosulfate are less applied because they cause blacking of the flower stem and are dangerous from human health and environment perspective (Damunupola and Joyce, 2008). Therefore, researchers tend to identify new, safe and less toxic materials for preservation and increment of the vase life of cut flowers (Shimamura *et al*., 1997).

The safe alternatives are the natural chemical compounds like herbal extracts and essential oils extracted from aromatic and medicinal plants. Herbal extracts and essential oils have been used for long for different purposes. The most emphasized subject is antimicrobial characteristics. Although the preservative nature of some plant extracts has been known for centuries, the antimicrobial properties of extracts of aromatic plants have received renewed attention for only a decade or so.

In recent times, the search for potent antimicrobial agents has been shifted to plants. Due to increased resistance of many microorganisms towards established antibiotics, investigation of the chemical compounds within traditional plants has become desirable. Most plants are medicinally useful in treating disease in the body and in most of cases the antimicrobial efficacy value attributed to some plants is beyond belief. Conservative estimates suggest that about 10% of all flowering plants

on earth have at one time, been used by local communities throughout the world but only 1% have gained recognition by modern scientists. There are about 120 plant-based drugs prescribed worldwide and they come from just 95 plant species. Approximately, there are 2,50,000 species of flowering plants and only 5000 have had their pharmaceutical potential assessed.

Modern science has identified several secondary metabolites of various plant species that contain antimicrobial properties. A lot of work has been done which aims at knowing the different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of microbial infections as possible alternatives to chemically synthetic drugs to which many infectious microorganisms have become resistant (Kaushik and Dhiman, 2000; Robledo *et al*., 2005). The antimicrobial activity of medicinal plants could be due to various chemical components and the presence of essential oils in adequate concentrations, which damage the microorganisms. The beneficial medicinal effects of plant materials typically result from the combinations of secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, flavonoids, resins fatty acids gums which are capable of producing definite physiological action on body (Joshi *et al*., 2009).

Plant-based antimicrobials remain a vast untapped source for medicines with enormous therapeutic potential. They are effective against many infectious microorganisms, while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. The important advantages of medicinal plants are their safety besides being economical, effective and their easy availability (Siddiqui, 1993).

Plant extracts have been well documented to inhibit microbial growth (Barkai-Golan, 2001). The effects of plant extracts on microorganisms have been studied by several researchers in different parts of the world (Reddy, 2001 and Erdoorul, 2002). Preservative solutions usually increase the vase life of cut roses but the use of plant extracts as antimicrobials in vase solutions has been rarely reported. Plant extracts can be widely used to prevent the microbial proliferation on the vase solution, which in turn will extend the flower vase life (Julia, 1992). Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it.

Keeping in view the acute need of safe antimicrobial agents to increase the vase life of cut-flower, present work was designed with the following objectives:

i. To study the cause of wilting in cut-rose by Scanning Electron Microscopy (SEM).
ii. To isolate and identify the microorganisms responsible for blockage of vascular bundles.
iii. To identify the best herbal extract inhibiting microbial vascular plugging.
iv. To determine the Minimum Inhibitory Concentration (MIC) of the best herbal extract.
v. To compare the *in-vivo* efficacy of best herbal extract with 8-HQC.
vi. To study the effect of best herbal extract on the quality and longevity of cut rose flowers kept in holding and pulsing solutions.

Review of Literature

Rose (*Rosa hybrida* L.) (Rosaceae) is the most exports of the cut flowers in the worlds (Chamani *et al*., 2004). A major from of deterioration in cut flowers is the blockage of xylem vessels by air and microorganisms that cause xylem occlusion (Elgimabi and Ahmed, 2009) as a result of which flower wilting occurs. One of most important index for senescence is significant reduction in water uptake and fresh weight of petals. Water balance is a major factor determining quality and longevity of cut flowers. It is influenced by water uptake and transpiration, being the balance between these two processes.

2.1 Plugging of vascular bundles and deterioration in cut rose

Stem plugging is one of the main factors determining longevity of roses and can be caused by physiological occlusion due to plant itself, microorganisms or air embolism (van Doorn *et al*., 1989). Proper water balance in cut stems is crucial for the flower postharvest longevity, and blockages occurring in vessels disturb it by limiting water uptake and transport to the flower. The main causes of reduced water uptake in cut stems are obstruction of xylem vessels by microbial growth, formation of tyloses, deposition of materials in the lumen of xylem vessels, and the presence of air emboli in the vascular system (Al-Humaid, 2004; Bleeksma and van Doorn, 2003; Ichimura *et al*., 2006; van Doorn, 1997; Twumasi *et al*., 2005).

Vase life of rose cut flowers is usually short. Cut flowers wilt and floral axis become bent below the flower head (bent neck). The development of such symptoms is considered to be caused by vascular occlusion which inhibits water supply to the flowers. The major form of vascular occlusion is the blockage of xylem vessels by air and microorganisms (Macnish *et al*., 2008). The rapid proliferation of microorganisms in vase water is thought to result in xylem blockage, water stress, and a subsequent reduction in cut flower longevity (van Doom and Perik, 1990). A positive correlation between the number of bacteria and water conductivity in the stem of cut flower has been reported (van Doorn *et al*., 1989).

Dineshbabu *et al*. (2002) suggested that microorganisms, especially bacteria and fungi which grow in preservative solutions have a marked adverse effect on the longevity of cut flowers. These microorganisms and their chemical products plug the stem ends and restrict the water absorption, which in turn decreases the longevity of flowers.

The study conducted by Shanan (2012) on cut rose flowers confirmed that the inhibition of water uptake occurred mainly by the occlusion of xylem vessels containing high counts of microorganisms. This inhibition of water uptake could be the reason for the unbalance in the water relations (between water uptake and water loss), which leads the flowers into early senescence and shortens its vase life.

In roses, the loss of petal turgidity and fresh weight was preceded by a decreased rate of water uptake, indicating that reduced uptake rather than excessive water loss is responsible. Since water tension in the flaccid flower is not transmitted to the base of stem a "stem blockage" was suggested within the xylem vessels (Durkin and Kuc, 1966). The reduction in stem conductivity is caused by several factors. Microbial growth paralleled the increase in stem resistance to water flow (Aarts, 1957). Therefore, microorganisms were considered to be one of the main causes of reduced water uptake by cut flower.

The development of occlusion is correlated with the growth of bacteria at the cut surface and inside the stem (van Doorn *et al*., 1989) and addition of bacteria to vase water shortens the vase life of cut rose flowers (de Witte and van Doorn, 1988; Zagory and Reid, 1986). Thus, vascular occlusion has been considered to be partially due to bacteria.

The invasion of the dead lumens of tracheary elements by living parenchyma cells (formation of tyloses) is a well known response to infection by pathogens and to wounding (Canny, 1997). It is often accompanied or followed by the transformation of gums and tannins which add to the strength and durability of the composite polymers. The nature of such material was investigated cytologically, revealing the presence of pectic elements, callose, or lignin-like molecules (Espino and Schenk, 2011). Such material is produced by the plant in response to invasion by the bacteria (Stevenson *et al*., 2004) or in response to phytotoxins produced by bacteria (Cl´erivet

et al., 2000). Microbial growth, and some metabolites produced by these bacteria, can block the xylem in the lower part of the stem. The bacteria suspected to be the biggest problems are *Pseudomonas* sp. and members of the enterobacteriaceae found in tap water (van Doorn and de Witte, 1997).

After harvest, bacteria in the vase water can have a detrimental effect on keeping quality. This effect varies according to the season, the cultivar, and the concentration of bacteria. Neck curvature in gerbera daisies increased more than 90% at bacterial concentrations of 10^6-10^9 cfu/ml (van Doorn and de Witte, 1994). This concentration range is common to vase solutions tested from floral shops. One rose in a vase might typically have a bacterial concentration of 10^6 cfu/ml after 2-3 days.

2.2 Visualisation of vascular blockage

Scanning Electron Microscope (SEM) observations are essential methods to reveal the role of micro-organisms in water stress and vessel plugging of cut flowers during their vase life (Put and Clerkx, 1988). A cause of short vase-life in cut-rose cv. Dang Gang-Galar was investigated by the SEM. In the vascular bundles of 5-day-old cut-rose stems, there were abundant hyphae of mycelium as well as bacterial cells whereas they were not found in fresh cut-rose stems (Jitareerat *et al*., 2008).

In a study conducted by Shanan (2012), samples of the stem base was taken for Scanning Electron Microscopy at the end of the vase life of cut flowers held in solution of control treatment as well as different oils of sweet basil, cinnamon and lemon grass at 25 mg/l. The results of the study showed that xylem vessels of the majority of the cut rose flowers were plugged by accumulation of microorganisms. The growth of these organisms destroyed the vessel cells, and as a result xylem occlusion took place.

Scanning Electron Microscope (SEM) observations have been made of transverse and longitudinal sections of xylem vessels of cut flowers of *Gerbera* cv. 'Fleur' and cut *Rosa* cv. 'Sonia' after a maximum of 24 h vase life. The results of the SEM observations showed that only a small fraction of the microbial cells entered into the vascular system with the normal intake of vase water; most microbial cells remained attached to the submerged cut surface while a small fraction of the initially

attached microbes were sometimes liberated into the surrounding vase water (Put and Clerkx, 1988).

Using ultrasonic acoustic emissions, Bleeksma and van Doorn (2003) found that bacteria in the stem of cut roses at 1×10^5 cfu/g fresh weight, which caused wilt and rapid deterioration due to interference in water uptake and/or possible secretion of extracellular enzymes that, could degrade the cell walls of vascular bundles.

2.3 Microorganisms responsible for vascular blockage

Microorganisms found in vase water including bacteria, yeasts and moulds are harmful to cut flowers through their development and consequent blockage of xylem at cut ends, preventing the water absorption (Nowak and Rundnicki, 1990). In past, various bacteria and fungus have been identified in the vase water of cut roses (Put, 1990; de Witte and van Doorn 1988).

van Doorn and de Witte (1997) have found that the bacteria accumulating in the vase water are primarily *Pseudomonas* sp. and, to a lesser extent, members of the enterobacteriaceae. Upon tracing the source of the contamination, it was determined that the bacteria came from the tap water, which can support their growth even though it contains only trace amounts of organic compounds. Similarly, De Witte and van Doorn (1988) found that five *Pseudomonas* spp. and one *Alcaligenes* sp. were the predominant strains in vase water of cut Sonia roses on day 6 of vase life.

van Doorn (1993) concluded that a population of bacteria, mainly *Pseudomonas,* at the cut surface and inside the xylem vessels precedes vascular occlusion. The blockage may partially be due to the copious slime produced by the bacteria. However, no evidence was found for a role of fungi and yeasts in the occlusion.

Bacterial strains found in rose stems by van Doorn *et al*., 1991were: Pseudomonads (80%), Enterobacteria (5-10%); and some other genera such as *Aeromonas, Acinetobacter,* Alcaligenes, *Citrobacter* and *Flavobacterium.*

During the first 3-6 days of vase life of rose, chrysanthemum and gerbera, *Pseudomonas cepacia, Pseudomonas fluorescens* and *Pseudomonas putida* replaced the initially dominant *Enterobacter* and *Bacillus* stem flora. However after > 6 days

of vase life, *Enterobacter* spp. again became more dominant *(E. agglomerans* and *E. cloacae).* Furthermore, *Bacillus* spp., *Erwinia* and also non-fermenting Gram-negative bacteria predominated later. Predominant mould genera were *Cladosporium, Fusarium, Pénicillium, Mucor* and *Rhizopus*. Fungal activity in vase water was low until after > 6 days of vase life of Chrysanthemum and Gerbera cultivars; *Cladosporium, Fusarium, Pénicillium* spp. and also some yeast genera (*Candida* and *Rhodotorula*) were most frequently isolated (Put,1990).

The fact that *Pseudomonas* spp. were more frequently isolated from cut-flower vase waters than *Enterobacter* spp. may be because most *Pseudomonas* spp. do not require organic growth factors and can multiply readily in diluted aqueous media (Kooy *et al*., 1982). Enterobacteriaceae often have more demanding growth requirements so their multiplication may have accelerated after the leakage of carbohydrates (glucose, fructose, and sucrose), proteins and growth factors from the disrupted plant tissues (Konings and Veldkamp, 1980; Zimmermann, 1983 and Woltering, 1987).

Zagory and Reid (1986), on their studies on the role of vase solution microorganisms on the vase life of cut flowers concluded that the microorganisms that reduced flower vase life were yeast, a fluorescent pseudomonad and a non-fluorescent pseudomonad.

Many studies have reported *Bacillus* spp. as the predominant bacterial species isolated from vase water (Jowkar, 2006; Ketsa *et al*., 1995; Put, 1990 and De Witte and van Doorn, 1988).

In the study conducted by Jowkar *et al*. (2012) to study the biocidal effect of citric acid, 'Cherry Brandy' roses, five different micro-organisms were observed in the citric acid vase solutions which were: 2 strains of *Fusarium solani* and three isolates of *Bacillus*, *Coccus* and *Streptomyces* sp. In an experiment on *Narcissus tazetta*, the only fungus found in citric acid vase solution was *Aspergillus* sp. (Jowkar, 2006).

2.4 Sources of microbial contamination

Agricultural product's microbial flora and population is determined by the product's physiological condition and mixture of bacteria, yeasts and fungi covering

the product (Zagory, 1999). It has been proved that when cut flowers are placed in vase, bacteria from flower surface transfer to vase solution. For example, van Doorn and de Witte (1997) recognized that *Bacillus* and *Staphylococcus xylosus* transfer from leaves and stems of cut 'Sonia' roses into vase solution.

Other sources of microbial contamination are vase water, contaminated vases, containers, or vessels (Hoogerwerf and van Doorn, 1992). The bacterial load encountered in practical situations may, however, be higher than in laboratory experiments. Vases may not be clean and flower stems may also have stood in solutions containing a high number of bacteria before being purchased by the consumer (van Doorn *et al*., 1995).

The microflora initially present on the stem of freshly harvested cut flowers may germinate (spores) and multiply in vase water, but exhibits a comparatively long generation time due to the nutrient-poor vase water growth medium. The predominance and shift in predominance of certain microbial genera in vase water, e.g. *Bacillus, Enterobacter, Pseudomonas* and fungi, during the course of vase life, which were mainly not correlated with the specific host plant, indicate that micro-organisms developing in the cut-flower vase fluid are more a function of the unique ecological conditions in the vase than of the micro-organisms initially present on the stems (Put, 1990).

2.5 Chemical antimicrobials and improvement of vase life in cut flowers

Several germicides, such as Aluminum Sulfate, Silver Nitrate and Hydroxyquinoline Sulphate (HQS), which are well known germicides, inhibit vascular occlusion and extend the vase life of cut roses (van Doorn, 1997).

Various studies have found that bacterial contamination is one of the most important factors in reducing postharvest life of cut flowers with the negative impact on respiration, photosynthesis and water uptake, also with increasing the evaporation, caused water imbalance and indirectly stimulates ethylene production and shortens postharvest life of cut flowers like tuberose (Balestra *et al*., 2005). Therefore, the use of antimicrobial compounds to increase postharvest life of cut flowers is recommended (Figueroa *et al*., 2005).

One of the major biocide applied in the cut flower business is hydroxy quinoline which is used as two forms of hydroxy quinoline sulphate (Liao *et al*., 2000) and hydroxy quinoline citrate (HQC) (van Doorn, 1998; Solgi *et al*., 2000). According to Marousky (1971), HQC has strong effect against fungi and yeasts, but it is highly effective in preventing bacterial activities.van Doorn and Perik (1990) studied the effects of HQC and decreasing of pH in preventing of vascular blockage by reducing the number of bacteria in Rose stems. The results showed that the use of HQC or buffer solution (citrate phosphate) with pH = 3 reduce the number of bacteria in Rose stems. Ichimura *et al*. (1999) found that treatment with HQS and HQC in combination with sucrose; both increased the vase-life of cut Rose flowers in all temperatures.

The 8-HQS is a very important germicide used in floral industry and acts as an antimicrobial agent (Ketsa *et al*., 1995; Nowak and Rudnicki 1990) which can increase water uptake (Reddy *et al*., 1996). The application of 8-HQS increases the vase life as well as the fresh weight (percentage of initial) of the cut flowers. The 8-HQS treatment also prevents growth of the microorganisms in xylem vessels of the cut flower stems and maintains water uptake.

Aluminum sulfate has been recommended for maintaining the vase life of several cut flowers (Liao *et al*., 2001) and is used as an antimicrobial compound in commercial preservative solutions (Ichimura *et al*., 2006). Aluminum Sulfate acidifies vase solution, diminishes bacterial proliferation and enhances water uptake (Hassanpour Asil *et al*., 2004; Tjeerd and Jaap, 2003).

Silver nitrate ($AgNO_3$) is one of the most common forms of silver salts used in commercial flower preservatives solutions and mostly used as ethylene binding inhibitor. Pulsing with Silver nitrate strikingly enhanced vase life and solution uptake in rose cut flowers (Singh and Tiwari, 2002). Also Darras *et al*. (2010) reported that, pulsing with 20 or 40 mg/L Silver nitrate for 24h extended vase life by 1.6 and 1.9 days, respectively, compared to the control.

Other chemical antimicrobials such as chlorine (Macnish *et al*., 2008) and silver nanoparticles (Solgi *et al*., 2009) have been shown to increase the vase life of cut flowers and reduce the bacterial population in vase solutions.

2.6 Harmful effects of chemical biocides

Inclusion of various antimicrobial compounds such as chlorine, metal salts, quaternary ammonium salts and quinoline esters in vase water can reduce the number of bacteria and thereby extend flower longevity (Macnish *et al*., 2008). But, effective concentrations of these biocides can be toxic to flowers (van Doorn *et al*., 1990 and Knee, 2000).

Synthetic germicides such as Silver thiosulphate (STS), Silver nitrate ($AgNO_3$), 8-hydroxyquinoline citrate (HQC), are expensive and not easily available in the local market. Furthermore, synthetic germicides which contained silver can pollute the environment due to its high phytotoxicity potential with harmful heavy-metal environmental contaminant (Damunupola and Joyce, 2008).

Commercial use of STS has been limited because concerns have been raised over the use of silver as it is a heavy metal salt and environmental toxin and many countries are actively working towards its elimination from commercial use (Badiyan *et al*., 2004; Bowyer and Wills, 2003; Bowyer *et al*., 2003). Disposal of waste silver solutions of STS is also a matter of concern (Macnish *et al*., 2004).

Biocides and other poisonous substances, including heavy metal compounds (e.g. Ag+), should not be disposed into the environment. Such chemicals need to be managed by an accredited/licensed waste contractor or through a chemical industry disposal program (Damunupola and Joyce, 2008).

van Doorn *et al*. (1990) noted that $AgNO_3$ cannot be used in water containing chlorine due to immediate precipitation of AgCl. Moreover, even in distilled water, $AgNO_3$ will slowly undergo photochemical oxidation leading to a black Ag_2O deposit.

Silver thiosulphate contain a heavy metal, its application as a possible environment pollutant has been banned in several countries. Therefore, scientists tend to find an effective and safe alternative for Silver thiosulphate (Serek and Reid, 1993; Cross, 1996).

Widely used 8-HQ can cause mutagenesis in *Salmonella typhimurium* and can also induce chromatid aberrations in human leukocytes (Elper *et al*., 1977).

Tribendazole is known to inhibit the assembly of mammalian and non- mammalian microtubules in vitro as well as to induce aneuploidy in hamster male embryonic lung cell lines (Antoccia *et al*., 1991). Consequently, it may be questionable as to whether HQs and TBZ should be used in commercial flower handling and vase solutions.

2.7 Herbal extracts as safe alternative biocide

Herbal medicines are traditionally used worldwide for the treatment of many infectious diseases. The use of medicinal plants as novel antibiotics have several other advantages related to safety, availability, and minimizing the risk of side effects and addiction (Lee *et al*., 2003). In India, indiscriminate and injudicious use of antibiotics in health care systems has led to the development of resistant microorganisms. Plants are rich source of active metabolites effective against variety of microorganisms. Moreover, new drugs are needed to reduce the occurrence of resistant microorganisms because existing antibacterial compounds are showing resistance against a number of microbial strains and are becoming superseded (Ekpendu *et al*., 1994). Antibacterial compounds extracted from plants are not related with side effects as the laboratory prepared compounds and can be successfully used against many infectious diseases (Iwu *et al*., 1999). The important advantages of medicinal plants are their safety besides being less expensive, efficacy and availability throughout the world (Joseph and Nair, 2013).

2.8 Medicinal plants and their antimicrobial properties

Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles, was well accepted. Since antiquity, man has used plants to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of various maladies (R´ıos and Recio, 2005). Aromatic and medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth (antimicrobial activity) (Bruneton, 1995; Chopra *et al*., 1992). Medicinal plants are considered to be very rich sources of secondary metabolites which are of immense therapeutic importance. Many plant

tissues contain a variety of compounds called "secondary" plant compounds (metabolites) grouped as glucosides, saponins, tannins, alkaloids, essential oils, organic acids and others (Fraenkel,1959). The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability (Siddiqui, 1993).

Turmeric (***Curcuma longa***)

Curcuma longa (*C. longa*), a perennial herb, is a member of the Zingiberaceae family and has a long tradition of use in the Chinese and Ayurvedic systems of medicine. Curcuminoids, a group of phenolic compounds isolated from the roots of *C. longa*, exhibits variety of beneficial effects on health and has the ability to prevent certain diseases (Joe *et al*., 2004). In East Asia, the rhizomes from *C. longa*, are considered to have natural medicinal properties, including antibacterial, anti-inflammatory, antineoplastic, and analgesic activities because they contains a number of moniterpenoids, sesquiterpenoids and curcuminoids (Fang *et al*., 2003).

Curcumin is the most important fraction and is responsible for the biological activities of turmeric. The melting point of curcumin is 184 ºC. It is soluble in ethanol and acetone, but insoluble in water. Curcumin, a potent antioxidant is believed to be the most bioactive and soothing portion of the herb turmeric and posses the properties like antioxidant, anti-inflammatory, anti-platelet, antibacterial and anti-fungal effects.

Oil extract from the turmeric oleoresin containing - Turmerone and curlone showed antibacterial activity using pour plate method against *Bacillus cereus*, *Bacillus coagulans*, *Bacillus subtilis*, *Staphylococcus aureus*, *Eshcherichia Coli* and *Pseudomonas aeruginosa* (Limtrakul *et al*., 2004; Nir *et al*., 2000). *C. longa* oil was tested against cultures of *Staphylococcus albus, Staphylococcus aureus and Bacillus typhosus*, inhibiting the growth of *S. albus and S. aureus* in concentrations up to 1 to 5,000 (Chopra *et al*., 1941).

Neem (*Azadirachta indica*)

Azadirachta indica A. Juss is well known in India and its neighbouring countries for more than 2000 years as one of the most versatile medicinal plants

having a wide spectrum of biological activity. Neem is an evergreen tree, cultivated in various parts of the Indian subcontinent. Every part of the tree is used as traditional medicine for household remedy against various human ailments, from antiquity (Thakur *et al*., 1981). Neem has been extensively used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine. The tree is still regarded as 'Village dispensary' in India (Biswas *et al*., 2002).

The chemical constituents of neem contain many bioactive compounds including alkaloids, flavonoids, triterpenoids, phenolic compounds, carotenoids, steroids and ketones. Azadirachtin is a mixture of seven isomeric compounds (Verkerk & Wright, 1993). Neem leaves have been demonstrated to possess the properties like immunomodulatory, antiinflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic (Hoque *et al*., 2007).

Extracts of neem leaf, neem oil and seed kernels are effective against certain human fungi, including *Trichophyton, Epidermophyton, Microsporum, Trichosporon, Geotricum* and *Candida* (Khan and Wassilew, 1987). Neem leaves has antibacterial properties and could be used for controlling airborne bacterial contamination in the residential premise (Saseed *et al*., 2008).

There are several reports on the antimicrobial activity of neem. Some of them have demonstrated activities of extracts from seeds and leaves against *Staphylococcus aureus*, *Escherichia coli*, as well as, negative results against *Bacillus subtillis*, *Salmonella* Paratyphi, *Shigella dysentrae* and *Candida albicans* (Almad and Beg, 2001). Irshad *et al*. (2011) demonstrated antibacterial activity of Neem against *Escherichia coli* and *Staphylococcus aureus*. Neem leaves are efficient against pathogenic fungi, such as *Trichophyton*, *Epidermophyton, Microsporum, Trichosporon* and *Geotricum* (Khan & Wassilew, 1987).The leaves also inhibited the growth of *Vibrio cholerae, Klebsiella pneumoniae*, *Mycobacterium tuberculosis* and *M. Pyogenes* in vitro (Satyavati *et al*., 1976).

Tulsi (*Ocimum sanctum*)

Among the plants known for medicinal value, the plants of genus *Ocimum* belonging to the family Labiate are very important for their therapeutic

potentials. *Ocimum sanctum* L. (Tulsi) is an important species of genus *Ocimum* that grow in different parts of the world and are known to have medicinal properties. Tulsi is a fragrant bushy perennial growing up to 1.5m in height with profusions of white blooms and slightly purple tinted leaves (Das and Vasudevan, 2006).

The leaf of *Ocimum sanctum* contains 0.7% volatile oil comprising about 71% eugenol and 20% methyl eugenol. The oil also contains carvacrol and sesquiterpine hydrocarbon caryophyllene (Shah and Qadry, 1995). Fresh leaves and stem of *Ocimum sanctum* extract yielded some phenolic compounds (antioxidants) such as cirsilineol, circimaritin, isothymusin, apigenin and rosameric acid and appreciable quantities of eugenol (Yanpallewar *et al*., 2004). The essential oils of tulsi have been equally effective against both Gram-positive and Gram-negative bacteria (Phadke and Kulkurni, 1989). Aqueous extract, alcoholic extract and seed oil of tulsi possess antimicrobial properties against enteric pathogens (Geeta *et al*., 2001).

Several studies were conducted to prove the antimicrobial activity of *O. sanctum.* Singh and co-workers (2005) suggested that higher content of linoleic acid in *O. sanctum* L. fixed oil could contribute towards its antibacterial activity. The oil showed good antibacterial activity against *Staphylococcus aureus, Bacillus pumius* and *Pseudomonas aeruginosa* with the *S. aureus* being the most susceptible organism.

Geeta *et al*., (2001) reported that the aqueous extract of *O. sanctum* L. (60 mg/kg) shows wide zones of inhibition compared to alcoholic extract against *Klebsiella, E. coli, Proteus, S. aureus* and *Candida albicans* when studied by agar diffusion method. However, alcoholic extract showed wider zone for *Vibrio cholerae*. In contrast to this, Aqil *et al*. (2005) observed that the alcoholic extract of *O. sanctum* was found to be active against multidrug resistant strains of S. aureus that are resistant to common Beta Lactam antibiotics. Similarly, *O. sanctum* was found to be active against resistant *Neisseria gonorrhea* strains (Shokeen *et al*., 2005).

Antibacterial activity of tulsi against *E. coli* and *S. aureus* at various plant concentrations of 15%, 30%, 50% and 100% showed maximum antibacterial activity against *S. aureus* followed by *E. coli* (Sagar and Thakur, 2012).

Aqueous and acetone extracts of *O. sanctum* were also found to be effective against many fungi of plant origin like *Alternaria tenuis, Helminthosporium* spp, and *Curvularia penniseli*. Antimicrobial activity of *O. sanctum* was found to be higher as compared to other commonly available species of Ocimum (i.e. *O. canum, O. Gratissimum* and *O. basilicum*).

Garlic (*Allium sativum*)

Garlic (*Allium sativum* L.) belongs to the family Liliaceae and is a common food spice used widely in many parts of the world. Garlic is one of the edible plants which have generated a lot of interest throughout human history as a medicinal panacea. A wide range of microorganisms including bacteria, fungi, protozoa and viruses have been shown to be susceptible to the crushed garlic preparations (Ankri and Mirelman, 1999).

Various garlic preparations have been shown to exhibit a wide spectrum of antibacterial activity against a variety of Gram-negative and Gram-positive bacteria including the pathogenic species of *Escherichia, Salmonella, Staphylococcus, Streptococcus, Klebsiella, Proteus, Bacillus*, and *Clostridium.* The ability of garlic to inhibit the growth of both Gram-positive and Gram-negative organisms shows that it has a broad spectrum of activity and can be used for formulation of newer broad spectrum antibacterial substances (Abubakar, 2009).

Moreover, acid-fast bacteria such as *Mycobacterium tuberculosis* are also found to be sensitive to garlic (Uchida *et al*., 1975). Garlic-rich organosulfur compounds and their precursors *viz*., allicin, diallyl sulphide, and diallyl trisulphide are thought to play a key role in these biological effects (Ankri and Mirelman, 1999).

Bacterial strains such as the mucoid strains of *Pseudomonas aeruginosa*, *Streptococcus hemolyticus* and *Enterococcus faecium* were found to be resistant to the action of allicin. The reasons for this resistance are unclear. It is assumed that hydrophilic capsular or mucoid layers prevent the penetration of the allicin into the bacteria (Ankri and Mirelman, 1999). The main antimicrobial effect of allicin is attributed to the action of biological active ingredient of allicin which exhibits its antimicrobial activity mainly by immediate and total inhibition of RNA synthesis,

although DNA and protein syntheses are also partially inhibited, suggesting that RNA is the primary target of allicin action (Deresse, 2011).

Betel leaf (Piper betle)

Piper betle L., an indigenous medicinal plant has a folk (Siddha and Ayurveda) reputation in the rural areas of southern India, a member of the Piperaceae. The plant is dioeciously, shade loving perennial root climber with glossy heart-shaped leaves. Significance of *P. betle* leaves have been explains in relation to each and every plethora of human life from the dawn of civilization (Lakshmi and Naidu, 2010).

Piper betle leaves extract contains large number of bioactive molecule like polyphenol, alkaloids, steroids, saponin and tannin (Andhikari *et al*., 1998). Betel leaves possess activities like antidiabetic, antiulcer, antiplatelet aggregation, antifertility, cardiotonic, antitumour, antimutagenic, respiratory depressant, antihelmenthetic (Andhikari *et al*., 1990), and wound healing property.

Piper betle plant leaves are rich in a wide variety of secondary metabolites such as phenolic compounds (chavicol, hydroxyl chavicol), volatile oils (safrole, eugenol, isoeugenol, eugenol methyl ester), fatty acids (stearic and palmitic) and hydroxyl fatty acids (stearic, palmitic,myristic) which in vitro illustrate the antibacterial properties and might be used as an choice,useful, cheap and safe antibacterial for the treatment of microbial infections (Bangash *et al*., 2012).

The leaf has a significant antimicrobial activity against broad spectrum of micro-organisms (Jesonbabu *et al*., 2012).The betel shows the antimicrobial activity against *Streptococcus pyrogen*, *Staphylococcus aureus*, *Proteus vulgaris*, *E.coli*, *Pseudomonas aeruginosa* etc., beside this the leaf extract also poses the bactericidal activity against the urinary tract pathogenic bacteria such as *Enterococococcus faecalis*, *C.koseri*, *C.fruendi*, *Klebsiella pneumoniae* etc. (Agarwal and Singh, 2012; Chakraborty and Shah, 2011)

Piper betle plant is effective against various bacterial strains including *Bacillus cereus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Micrococcus luteus*, *Staphylococcus aureus*, *Aeromonas hydrophila*, *Escherichia coli*, *Pseudomonas*

aeruginosa, *Salmonella enteritidis* (Suppakul *et al*., 2006), *Streptococcus mutans* (Sharma and Khan, 2010), *Streptococcus pyogenes* (Caburian and Marina, 2010), *Enterococcus faecium*, *Actinomycetes viscosus*, *Streptococcus sanguis*, *Fusobacterium nucleatum*, *Prevotella intermedia*.

2.9 Extraction method

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Organic solvents such as ethanol, acetone and methanol are often used to extract bioactive compounds (Eloff, 1998). Ethanol, however, is the most commonly used organic solvent by herbal medicine manufacturers because the finished products can be safely used internally by consumers of herbal extracts (Low Dog, 2009). Although a great amount of research has been performed to determine the antibacterial activity of medicinal plants, optimal extraction of bioactive compounds has not been well established for most plants.

For all the tested microorganisms Methanol and Ethanol showed maximum antibacterial activity in *Melia azedarach*. In Ethanol extract maximum inhibition zone diameter was obtained in *Psedomonas aeruginosa* and in *Staphylococus aureus* with diameter 22.3±0.42 mm 19.5±0.52 mm, respectively. Similarly, Methanol extract showed maximum inhibition zone with diameter of 21.5±0.86 mm in *E. coli* and 17.6±0.43 mm *B*. cereus. The Petroleum Ether (12-15 mm) and aqueous extract (8-11 mm) showed restrained and minimum activity, respectively. More specifically, aqueous extract represented higher susceptibility to all bacterial strains (Sen and Batra, 2012).

In the study conducted by Bangash *et al*. (2012), ethanol, petroleum ether and chloroform extracts of Piper betel leaf were tested against Gram positive and Gram negative bacterial strains by Agar-well Diffusion Method. The ethanolic extract turned out to be most effective for its antibacterial activity against all eleven selected bacterial strains. Percentage yield of chloroform extract was 5%, petroleum ether extract was 15% and of ethanolic extract was 39.5%. This shows that ethanolic extraction has higher percentage yield as compared to, in other two solvents.

Subashkumar *et al*. (2013) studied the antibacterial effect of crude aqueous extract of *Piper betle* L. against pathogenic bacteria and the greatest zone of inhibition among clinical strains tested was demonstrated by the extract obtained from ethanol extraction towards Gram positive and Gram negative bacteria.

The ethanol extract of Betel leaf (*Piper betle* L.), was screened for its antibacterial activity against some foodborne pathogens viz. *Vibrio cholerae* ATCC 6395, *E. coli* ATCC 25922,*E. coli* O157:H7 NCTC 12049, *Shigella dysenteriae*-1 MJ-84 and *Staphylococcus aureus* ATCC 25923. Experimental findings revealed that the ethanol extract of betel leaves potentially inhibit the growth of these food borne pathogens (Hoque *et al*., 2011).

2.10 Herbal extracts as a biocide in extension of vase life

One per cent piper extract completely inhibited microbial growth in holding solutions, delayed bent neck and ethylene production, and maintained freshness of leaves. The anthocyanin content of petals in 1% piper-treated cut-rose was not significantly different with distilled water or 250 ppm 8-hydroxyquinoline sulphate (HQS) treated cut-rose. These results implied that piper extract may be used as an alternative to HQS for inhibiting the microbial growth in the holding solution of cut-rose (Jitareerat *et al*., 2007).

The morphological, anatomical and microbial studies conducted to examine the effect of essential oils of dill, coriander and mandarin in prolonging the vase life of carnation flowers revealed that these essential oils extended the vase life of cut carnation flowers as well acted as effective antimicrobial agents in addition to the common, harmful and expensive 8-HQC (Shanan *et al*., 2010).

Hegazi and GAN (2009) studied the effect of essential oils of clove, cinnamon, ginger, marjoram and fennel on vase-life of *Gladiolus hybrid*a, l. spikes and found that using essential oils in preservative solution significantly decreased the microbial density, spike base rot and deteriorated florets and increased spikes vase-life.

Basiri *et al*. (2011) found that 25% rosemary extract increased vase life of cut carnation (*Dianthus caryophyllus* cv. 'white librity') flowers until 24.6 days in

laboratory conditions. Their results also indicated that rosemary treatments with its antimicrobial effect inhibited the growth of microorganisms in vase solution and with increasing water uptake considerably extended the vase-life of cut flowers of carnation.

In the study conducted by Solgi *et al*. (2009) various concentrations of essential oils or plant extracts such as thymol, carvacrol, thyme oil and zataria oil in combination with 6% sucrose had a positive effect on the vase life of cut gerbera (*Gerbera jamesonii* cv. Dune) flowers.

Amini *et al*. (2013) in their studies on the effect of pulse treatments and herbal medicine extracts as permanent treatments on postharvest quality of cut Gerbera flowers found that the best results in preventing fresh weight decreasing were in thyme extract with 0.2 mg/l + distilled water pulse treatment. The most lose weigh was in control treatment of Calcium chloride 4% + Sucrose 3% pulsing. However, the best result in flower diameter was found in thyme 0.1 mg/l in Calcium Chloride + Sucrose. The lowest of flower diameter was observed in control treatment of (Calcium Chloride + Sucrose pulse).

Shanon (2012) reported that natural plant essential oils found in lavender (*Lavendula angustifolia* Mill.), geranium (*Perlagonium graveolens* L.), anise (*Pimpinella anisum* L.) and cumin (*Cuminium cyminum* L.) significantly prolonged the vase life of rose cut flowers. Essential oils are safe and environmental friendly substances with anti-microbial properties.

Bazaz and Tehranifar (2011) conducted a research to study the effect of different concentrations of ethanol (4, 7, 10%) methanol (4, 7, 10%) and some essential oils (50 or 100 mg L^{-1}peppermint (*Mentha piperata* L.), thyme (*Thymus vulgaris* L.) and black cumin (*Bunium persicum* (Boiss.) B. Fedtsch)) as pulse treatments on flower longevity, solution uptake, fresh weight and SPAD value as a measure of leaf greenness of *Alstroemeria peruviana* 'santorini'. Results showed that alcohol treatments had no positive effect on increasing vase life of Alstroemeria. Applying essential oils could extend the vase-life. The greatest vase life was related to 50 mg $L^{-1}$of thyme essential oil treatment and approximately it improved inflorescence cut vase life more than 2 days longer than control treatment. The

greatest solution uptake and decrease in fresh weight were seen in 100 mg L^{-1} peppermint essential oil and 100 mg L^{-1} thyme essential oil, respectively. Essential oils could not maintain SPAD value in higher amount than control treatment.

The effect of leaf extracts of *Psidium guajava* and *Piper betle* on prolonging vase life of cut carnation flowers was studied by Rahman *et al*., (2012). The leaf extracts of *P. guajava* and *P. betle* showed highest antibacterial and antifungal activities compared to the other treatments. Both showed similar effects on flower quality as the synthetic germicide, 8-HQC.

2.11 Effect of sucrose on prolonging the vase life of cut flowers

Sugars play important role in plants as substances for respiration and cell walls as osmolytes. Since the amount of sugar contained in cut flowers is limited, the addition of sugars such as sucrose to vase water is effective in improving the vase life of some cut flowers (Halevy and Mayak, 1979).

Among the different types of sugars, sucrose has been found to be the most commonly used sugar in prolonging vase life of cut flowers and the exogenous application of sucrose supplies the flowers with much needed substrates for respiration and does not only prolongs vase life, but enables cut flowers harvested at the bud stage to open, which otherwise could not occur naturally (Pun and Ichimura, 2003).

Different concentrations of sucrose had been investigated by Butt (2005) on two cultivars of *Rosa hybirida* and results showed that sucrose at 25 g l-1 extended the vase life by 8.2 days in var. Whisk Mc and 7.5 days in var. Trika as compared to 5.3 days in control.

Carbohydrates are necessary for turgor pressure maintenance and also they are important energy sources facilitating flower opening (Sarkka, 2005). Low carbohydrate levels in stem and leaves will reduce vase life which can be partially remedied by presence of sugar in the holding and vase solutions (Sarkka, 2005; Hashemabadi and Gholampour, 2006). Sugars are essential precursors for cut flower respiration. Sucrose is the main transporting form of sugar to flower bud (Sarkka, 2005).

The postharvest life of flowers is strongly dependent on the carbohydrate status and the acceptable amount of metabolic sugars is a factor that affects the rate of senescence. Therefore, an exogenous carbohydrate supplementation would be enough to delay the senescence, considering that the main effect would be to maintain the structure and activity of the mitochondria (Kazemi and Ameri, 2012).

2.12 Combined effect of sugars and biocides

Sugars with biocides have become an important commercial preservative for several cut flowers. Treatment with sucrose in combination with 8-hydroxyquinoline sulfate (HQS) extends the vase life of cut rose flowers (Ichimura *et al*., 1999). This effect is due to supply of carbohydrates as well as inhibition of vascular occlusion by HQS.

According to the research conducted by Amini *et al*. (2013) on the effect of pulse treatments and herbal medicine extracts as permanent treatments on postharvest quality of cut Gerbera flowers, sucrose in nutritious solutions should definitely be used along with anti-bacterial factors to prevent uncontrolled bacterial growth in the end of the stem.

Flower diameter, total water uptake and vase life of rose cut flowers had increased when 8-HQS + sucrose was used in comparison to control (Sivasamy and Bhattacharjee, 1999). Also, preservative solutions containing 3% sucrose and 200 ppm 8-HQS extended the vase life and inhibited flower senescence and bent neck in rose cut flowers (Kim and Lee, 2002).

8-HQS treatment is more effective when sucrose is coupled with it (Pun and Ichimura, 2003). The concentration of glucose, fructose and sucrose in petals of cut flowers were increased with 8-HQS + 2% sucrose treatment comparing to control ones. Beura *et al*. (2001) showed that the combination treatment of 8-HQS and sucrose improved the postharvest quality of Gladiolus spikes. In Dendrobium cut flowers, holding solutions containing 8-HQS + sucrose extended the vase life and improved flower quality, water consumption, fresh weight, flower freshness, and reduced respiration rate and physiological weight loss (Dineshbabu *et al*., 2002).

Cut rose flowers were held in 200 ppm 8-HQS + 2 % sucrose. The treatment reduced both photosynthesis rate and chlorophyll content. However, it stimulated the respiration rate of leaves, as compared with the control (KiCheol *et al*. 1997).

Bhattacharjee (1994) placed cut rose flowers in distilled water or a preservative solution containing 300 ppm 8-hydroxyquinoline citrate + 10,000 ppm sucrose. The preservative solution prolonged the vase life and increased the water uptake of cut rose flowers.

The application of 200 ppm 8-HQS + 30 g/l sucrose extended the vase life of rose cut flowers and increased the fresh weight of petals. Fructose, glucose, and sucrose were the main carbohydrates in petals. The carbohydrate concentration in the control decreased sharply during first day after harvest. However, the treatment with 8-HQS + sucrose suppressed this decrease (Ichimura *et al*., 1999 a).

The maximum vase life of rose cut flowers was obtained when HQC + sucrose was used (Tiwari *et al*., 2002). The treatment of 8-HQS + sucrose prolonged the vase life of rose cut flowers (Chikkasubbanna and Yogitha, 2002).

Materials and Methods

Chapter 3 MATERIALS AND METHODS

The present investigations were carried out during 2011-12 and 2012-13 at the Model Floriculture Centre, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, District Udham Singh Nagar (Uttarakhand). The materials used and the methods adopted to record the observations during the course of this study are presented in this chapter.

3.1 Geographical Location and Climate of the Experimental Site

Pantnagar is situated at the foothills of Himalayas at 29^0 North Latitude and 79.3^0 East Longitude. The altitude of the place is 243.84 m above the mean sea level. The climate is humid sub-tropical with hot dry summers and cool winters. Some frost is common during December to February. During the whole period of experiment, the maximum temperature ranged from 26.9^0C to 39.4^0C in summer and minimum from 3.7°C to 12.9^0C in winter. The monsoon generally starts from the third week of June and recedes by the end of September. Occasional light rains are expected during winter months also. The mean relative humidity (at 7.00 a.m.) remains almost 80-90 per cent from mid June to end of February, and then it steadily decreases to 50 per cent by the mid June. The average temperature inside the post-harvest laboratory during the period of experiment was 20 ± 2^0C. The average relative humidity was $80 \pm 5\%$.

3.1.1 Soil conditions

The soil at Pantnagar comes under mollisols. The soil of experimental field was sandy-loam with adequate drainage and optimum water holding capacity. The soil samples were collected randomly from different locations of the experimental area and analyzed for the physical and chemical properties of the soil (Table) as per the methods indicated below:

Methods used for determination of different chemical properties of soil are given below:

Sr. No.	Soil property	Soil property status	Method
1.	pH	6.8	Digital pH Meter (Jackson, 1967)
2.	Electrical conductivity (dsm^{-1})	1.68 dsm^{-1}	Conductivity meter (Rhoades, 1989)
3.	Nitrogen (kg/ha)	167.34 kg/ha	Alkaline KM_nO_4 (Subbiah and Asija, 1956)
4.	Phosphorus (kg/ha)	41.25 kg/ha	Olsen's method (Olsen *et al.,* 1954)
5.	Potassium (kg/ha)	173.54 kg/ha	Flame photometer (Cooper, 1963)

3.2 Experimental material

3.2.1 Plant material

To carry out the present investigation the budded rose plants of Konfetti and Bordeaux were procured from a certified nursery at Sharanpur (Uttarakhand).

3.2.2 Chemicals/reagents/glass wares/plastic wares

All the chemicals and reagents used for the microbiological work in the study were procured from either of the firms: M/s Life-sciences, Imperial life sciences, Bangalore Genei and Merck (Bioron, Bioserve). The glassware used in the present study was procured from Borosil while the plasticware was purchased from different firms like M/s Greiner, Axygen and Tarson.

3.2.2.1 8-Hydroxy quinoline citrate (8-HQC): 8-HQC was procured from Titan Biotech limited. It is an ester of 8-Hydroxy quinoline and has bacteriostatic properties. 8-HQC has a strong inhibitory property against bacteria, yeast and fungi. Also, it lowers down the pH of vase solution, which inhibits the growth of microorganisms and thus eliminates vascular blockage and enhance water uptake in cut flowers (Burdett, 1970).

Solutions of 200 ppm of both 8-Hydroxy quinoline and citric acid were prepared separately by dissolving 200 mg, respectively, in one litre of double glass of

distilled water. Finally, both the solutions of similar concentrations were mixed to prepare 200 ppm of 8-Hydroxy quinoline citrate (8-HQC).

3.2.2.2 Sucrose : Sucrose is the main component of floral preservatives, as it is the main source of energy, necessary for maintaining all the biochemical and physiological processes after harvest.

Per cent solution of sucrose was prepared by dissolving the same number of grams of which per cent concentration is required in 100 ml of water.

3.3 Scientific equipment

The following scientific equipment were used in the present study: Scanning Electron microscope (SEM), Electronic weighing balance (Sartorius, Germany), Mechanical grinder, Portable chlorophyll meter (SPAD-502: Minolta camera Co., Osaka, Japan), Revolving electronic water bath (GFL, Merck, Germany), variable volume (adjustable) micropipettes (Nichipet, Tripette and Eppendorf), pH meter (Bangalore Genei), Hot air oven (Yorco), Autoclave (NSW), Incubator shaker (Yorco), Vortex mixer (Remi), Hand Refractometer (model RCZ and serial number SN 00850) Refrigerator (LG), and Filtration assembly (Millipore).

3.4 Experimental method

3.4.1 Preparation of experimental site

The land was brought to a fine tilth by two deep ploughing. Before leveling of experimental field all weeds and left over crop residue were removed. Beds of $1m^2$ were prepared for planting of rooted cuttings of rose plants and a spacing of 50 cm between two sub plots was provided for irrigation channel and working space.

3.4.2 Raising of crop

Rooted cuttings of rose plants were planted in the polyhouse in mid of October, 2009 at Model Floriculture Centre. Planting was done at the spacing of 25x 30 cm from plant to plant and row to row. Routine cultural operations like; weeding, hoeing, irrigation, fertilization, spraying against insect-pests and diseases were followed as per the requirement.

3.4.3 Harvesting of the flower

The flowers for experiment were harvested at early morning hours (7-8 A.M.) with the help of secateurs at a stage when calyx was fully reflexed and outer petal started to unfurl. After harvesting, cut flowers were immediately placed in a bucket containing water and taken to laboratory for further experimentation.

3.5 Experimental details

3.5.1 Experiment I: Determination of Microbial Growth in Vascular Bundles using Scanning Electron Microscope (SEM)

Stem pieces, 5 cm from the end of the stem of fresh cut-rose flowers and of 5-day-old cut-rose flowers of Konfetti and Bordeaux held in tap water were sampled. The microbial blockage in the vascular bundles was determined by Scanning Electron Microscope (SEM). Electron microscopy was done as per the standardized protocol at Electron Microscopy Facility, College of Veterinary and Animal Science, GBPUA&T, Pantnagar, Uttarakhand.

3.5.2 Experiment II: Preparation of the Herbal Extracts

3.5.2.1 Selection of herbs (medicinal plants) for this study

A number of plants have been documented for their biological and antimicrobial properties (Arora and Kaur, 2007). In an effort to expand the spectrum of antimicrobial agents from natural resources for increment of vase life of cut roses, 5 medicinal plants including *Curcuma longa*, *Azadirachta indica*, *Piper betle*, *Ocimum sanctum* and *Allium sativum* (Plate 1) were selected based on their traditional usage in India to assess their antibacterial and antifungal potential. These plants have previously been reported to have antimicrobial activity against different bacterial and fungal strains. These plants are very common and easily found in all Indian households. The list of herbs and the parts used are given below:

Sl.No.	Common name	Botanical name	Family	Plant part used
1.	Turmeric	*Curcuma longa* L.	Zigiberaceae	Rhizome
2.	Neem	*Azadirachta indica A. juss.*	Meliaceae	Leaf
3.	Betel leaf	*Piper betle* L.	Piperaceae	Leaf
4.	Tulsi	*Ocimum sanctum* L.	Lamiaceae	Leaf
5.	Garlic	*Allium sativum* L.	Amaryllidaceae	Clove

PLATE 1

Rhizomes of turmeric

Neem leaves

Leaves of betel leaf

Tulsi leaves

Garlic cloves

Plant parts used for herbal extract preparation

3.5.2.2 Collection of plant material

Rhizomes of turmeric, leaves of betel leaf and cloves of garlic were purchased from the local market and the leaves of neem and tulsi were collected from Model Floriculture Center, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar.

3.5.2.3 Preparation of Plant Material

Plant samples were washed and sanitized in sodium hypochlorite solution (50µg/ml), (Ritenour *et al*., 2002). After washing, the plant materials were left to get rid of excess water. Rhizomes of turmeric, leaves of betel leaf, neem and tulsi were thinly sliced and flatly spread in oven trays (13 in x 9 in). Further they were dried in a hot air oven at 50°C for 15 h or until dry weight did not change. Dried samples were ground to powder using a mechanical grinder and kept separately in plastic bags in dry condition until use.

The garlic cloves were peeled and grinded using ethanol in to a fine paste.

3.5.2.4 Preparation of the Extracts

Ten grams of air dried powder of rhizomes of turmeric, leaves of betel leaf, neem and tulsi as well as ten grams finely ground paste of garlic was placed in 100 ml of organic solvent (95% ethanol) in a conical flask and plugged with cotton (Plate 2). Then these conical flasks were kept on a rotary shaker at 190-220 rpm for 24 h. After 24 h, they were filtered through 8 layers of muslin cloth (Plate 2) and subsequently centrifuged at 5000 x g for 15 min. The supernatant was collected and was dried to evaporate the organic solvent at room temperature.

Yield of the extract (Chanda *et al*., 2013) was calculated as follows:

$$\text{Yield (\%)} = \frac{\text{Weight of the extract recovered}}{\text{Weight of the dry powder}} \times 100$$

The sedimented crude extracts were weighed and transferred to different glass vials and kept at 4ºC for further use. For antimicrobial testing the different extracts were dissolved in DMSO (1%), a non toxic solvent.

3.5.3 Experiment III: Determination of bacterial load in the vase solution by Standard plate Count (SPC)

Standard Plate Count (SPC) was done to determine the number of live bacteria in the vase solutions of both rose varieties *viz.* Bordeaux and Konfetti. The following procedure was used:

1. Five days old vase solutions of both varieties were mixed separately.
2. Tenfold serial dilutions of the vase solutions were made i.e., 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}.
3. Briefly, 1 ml of vase solution was transferred to a 9 ml sterile saline blank and mixed properly.
4. The 10^{-1}dilution was mixed by grasping the tube between the palms of both hands and rotating quickly.
5. Immediately after the 10^{-1} dilution has been shaken, 1 ml was aseptically transferred to a second 9ml saline blank. This second blank represented a 10^{-2} dilution of the vase solution.
6. Similarly, subsequent dilutions were made up to 10^{-6}.
7. After the dilutions were made, 1 ml of each dilution (10^{-1} to 10^{-6}) was poured onto the corresponding petri plate. Plates were poured in triplicates for each dilution to get precise results.
8. Approximately 15 ml molten nutrient agar (NA) was then poured aseptically into each plate. The agar and sample were immediately mixed gently moving the plate in a figure-eight motion or a circular motion while it rest s on the tabletop. This process was repeated for the remaining five plates. The plates were allowed to settle for 30 min.
8. Thereafter, plates were inverted and incubated at 37°C for 24 hours.
9. Next day all petriplates were observed for growth. The petriplates having bacterial colonies between 30 and 300 were selected for counting using a colony counter. Plates with more than 300 colonies were not counted and were designated too many to count (TMTC). Plates with fewer than 30 colonies were designated too few to count (TFTC).

PLATE 2

Air dried powder of rhizomes of turmeric, leaves of betel leaf, neem and tulsi and garlic paste in ethanol

Filtration of betel leaf extract through 8 layers of muslin cloth

10. The number of bacteria (CFU) per milliliter of each vase solution was determined by multiplying the number of colonies with the reciprocal of the dilution used. The following formula was used;

No. of bacteria/ ml (cfu/ml) = Number of colonies x Reciprocal of the dilution used

3.5.4 Isolation and identification of microorganisms from the vase solution

After standard plate counting (SPC), three colonies with different morphology were selected and processed further for identification. One colony was directly streaked onto the Eosin Methylene Blue plates (EMB) and incubated overnight at 37ºC for the production of metallic sheen indicative of presence of *E. coli*. Other two colonies were processed separately and differentiated according to their typical staining, cultural, morphological and biochemical characteristics. Bacterial morphological studies included: motility, cell shape, and capsule presence. The biochemical tests carried out on isolated bacterial colonies were: gram reaction, aerobic/anaerobic growth, acid production from glucose, gas production from D-glucose, pigments production, oxidase test, catalase test, growth at 5.7 pH, indol production, methyl red reaction, Voges-Proskauer Test (VP), nitrate reduction, and H2S production.

Additionally, to isolate fungal strains, a known quantity of the vase solutions was spread on the sterile Sabouraud Dextrose Agar (SDA) plates. The plates were allowed to incubate for 24-48 hrs at 30º-45ºC and individual fungus representing the most common colony morphology types were picked for further confirmation. Fungal species were identified on the basis of external morphology, growth patterns and their appearance under the microscope. Bacterial and fungal strains were purified and stored for further usage.

3.5.5 Experiment IV: Determination of antibacterial/antifungal activity of herbal extracts

Agar well diffusion method was used to determine the antimicrobial activity of the herbal extracts. This method depends on the diffusion of the various extracts from a cavity through the solidified agar layer of Petri dish to an extent such that growth of the added microorganism is prevented entirely in circular area or zone around the cavity containing the extracts.

Nutrient agar (NA) and Sabouraud Dextrose Agar (SDA) plates were swabbed using sterile cotton swabs with overnight Nutrient broth and Sabouraud Dextrose broth, respectively, inoculated with the vase solution. Sterile glass spreader was used to uniformly spread the broth culture onto the petridish. Each Petri dish was labeled with the test organism.Wells of approximate 8 mm diameter and about 2 cm apart were made in each of these plates using sterile cork borer. The wells were labeled appropriately with the respective extract. Stock solution of each plant extract was prepared at a concentration of 10% (100 mg/ml). About 100 µl of different extracts were inoculated using sterile syringe into the wells and allowed to diffuse at room temperature for 2hrs. DMSO without plant extract was used as control inoculum. The plates were incubated at 37°C for 18-24 h for bacterial pathogens and 28°C for 48 hours for fungal pathogens. The diameter of the inhibition zone (mm) was measured and the best herbal extract inhibiting the visible growth of bacteria and fungus was identified. The experiment was repeated thrice.

3.5.6 Experiment V: Determination of Minimum Inhibitory Concentration (MIC) of herbal extracts

Various concentrations (10%, 5%, 2% and 1%) were prepared to determine the Minimum Inhibitory Concentration (MIC) of the best herbal extract. Agar well diffusion method was used to determine MIC.

Briefly, Nutrient agar (NA) and Sabouraud Dextrose Agar (SDA) plates were swabbed using sterile cotton swabs with overnight cultures of respective bacteria (*Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa*) and fungi (*Mucor* sp. and *Rhizopus* sp.). Extracts of betel leaf, Neem and Garlic were used to determine MIC against above three bacteria individually. However, only betel leaf extract was used for MIC studies against fungus *Mucor* and *Rhizopus*. Sterile glass spreader was used to uniformly spread the broth culture onto the petridish. Each Petri dish was labelled with the test organism.Wells of approximate 8 mm diameter and about 2 cm a part were made in each of these plates using sterile cork borer. Stock solution 10% (100 mg/ml) of the extract was further diluted to make 5% (50mg/ml), 2% (20mg/ml) and 1% (10mg/ml) concentrations. The wells were labeled with the appropriate concentration of the extract. About 100 µl of different concentrations were inoculated using sterile syringe into the corresponding wells and allowed to diffuse at room

temperature for 2hrs. DMSO without plant extract was used as control inoculum. The plates were incubated at 37°C for 18-24 h for bacterial pathogens and 28°C for 48 hours for fungal pathogens. The diameter of the inhibition zone (mm) was measured and the best concentration of the herbal extract inhibiting the visible growth of bacteria and fungus was identified.

3.5.7 Experiment VI: Standardisation of holding solution using betel leaf extract for quality and longevity of cut roses Konfetti and Bordeaux

Factor A

Rose Cultivars (plate 3)**:** i) Konfetti

ii) Bordeaux

Factor B

Holding solutions:

i. 2 % Betel leaf extract
ii. 8- HQC 200ppm
iii. 1% sucrose and 8- HQC 200ppm
iv. 2% sucrose and 8- HQC 200ppm
v. 3% sucrose and 8- HQC 200ppm
vi. 1% sucrose and 2 % Betel leaf extract
vii. 2% sucrose and 2 % Betel leaf extract
viii. 3% sucrose and 2 % Betel leaf extract
ix. Tap water (control)

Experimental design	–	Factorial CRD
Number of treatment combinations	–	18
Number of replications	–	Three
Number of cut stems/replication	–	Three
Number of cut stems/vase	–	One

3.5.8 Experiment VII: Standardisation of pulsing solution using betel leaf extract for quality and longevity of cut roses Konfetti and Bordeaux

Factor A

Rose Cultivars (Plate 3)**:** i) Konfetti

ii) Bordeaux

Factor B

Pulsing solutions:

i. 2 % Betel leaf extract

ii. 8- HQC 200ppm

iii. 3% sucrose and 8- HQC 200ppm

iv. 6% sucrose and 8- HQC 200ppm

v. 9% sucrose and 8- HQC 200ppm

vi. 3% sucrose and 2 % Betel leaf extract

vii. 6% sucrose and 2 % Betel leaf extract

viii. 9% sucrose and 2 % Betel leaf extract

ix. Tap water (control).

Experimental design	–	Factorial CRD
Number of treatment combinations	–	18
Number of replications	–	Three
Number of cut stems/replication	–	Three
Number of cut stems/vase	–	One

3.5.9 Observations recorded

The following observations were recorded:

3.5.9.1Initial flower bud diameter (cm): The equatorial diameter of flower bud at two places before giving the treatment was recorded and average of two values was calculated.

PLATE 3

Cut rose Konfetti used for holding and pulsing studies

Cut rose Bordeaux used for holding and pulsing studies

3.5.9.2Final flower diameter (cm): The equatorial diameter of open flower at two places after full opening of bloom was recorded and average of two values was calculated.

3.5.9.3 Opening of flower bud: Flower opening was determined every second day of vase life and the maximum opening was rated using the following rating score: 1 = outer petals tightly wrapped around the bud; 2 = outer petals starting to reflex from the bud; 3 = outer petals reflexed135° to the stem; 4 = outer petals reflexed at 115° to the stem; and 5 = outer petals reflexed at 90° to the stem (modified from Kuiper *et al*., 1996).

3.5.9.4 Number of days to reach final flower diameter (days): The number of days to reach the final flower diameter from the initial flower diameter was counted.

3.5.9.5 Water uptake (g/stem): Water uptake (Wu) by the flower stem was determined by using the formula given below:

Water uptake (Wu) = [Initial weight of container (g) + Solution (g)] - [Final weight of container (g) + Solution (g)]

$$Wu = [C+S]_1-[C+S]_2$$

3.5.9.6 Water loss (g/ stem): Water loss (W_L) from the cut stem was calculated by using the formula given below:

Water loss (W_L) = [Initial weight of container (g) + Solution (g) +Flower (g)] - [Final weight of container (g) + Solution (g) +Flower (g)]

$$W_L = [C+S+F]_1-[C+S+F]_2$$

3.5.9.7 Water balance: Water balance (Wb) was calculated by subtracting the total water loss from the total water uptake.

$$Wb = Wu - W_L$$

3.5.9.8 Transpiration rate (ml/g FW): Transpiration rate was given by the amount of water loss divided by initial fresh weight of the cut flower. (Ichimura *et al*., 2002)

3.5.9.9 Fresh weight changes (%): In order to record fresh weight changes of cut flowers, flower stems were weighed by an electronic weighing balance on 2^{nd}, 5^{th} and

last day of vase life. Data were obtained to calculate fresh weight changes and consequently relative Fresh Weight (RFW) changes of the stems (Jowkar, 2006).

Relative fresh weight was calculated as: RFW (%) = (W t /W t_0) ×100; where, W t is weight of stem (g) at t = day 2, 5 and last day of vase life. W t_0 is weight of the same stem (g) at t = day 0 (He *et al*., 2006; Liu *et al*., 2009).

3.5.9.10 Vase life (days): The vase life was determined as the number of days taken from placing the cut stem in holding solution till the wilting of the outer 5 petals occurred or bent neck was observed (Bleeksma and van Doorn, 2003).

3.5.9.11 Chlorophyll content (SPAD unit): The total leaf chlorophyll (Chl.a+b) was determined with a non destructive method (Yadava, 1986) using a portable chlorophyll meter (SPAD-502: Minolta camera Co., Osaka, Japan).

3.5.9.12 Freshness of leaves and petal: Freshness of leaves and petal was evaluated using a scale of 1-5 where 5 = very fresh, 4 = moderately fresh, 3 = slightly fresh, 2 = apparent wilt and 1 = completely wilt.

3.5.9.13 Blueing of petals: Blueing of petals was evaluated using a scale of 0-3 where 0 = no blueing, 1 = slight blueing, 2 = moderate blueing and 3 = extreme blueing.

3.5.9.14 Bent neck: Cut flower in vases were observed for degree of bent neck and categorized into bent and unbent categories.

3.5.9.15 Total soluble solids (TSS) (%): Sap was obtained by squashing the petals. The TSS in the sap was measured with a hand Refractometer (model RCZ and serial number SN 00850) during the 3rdday.

3.5.9.16 pH of the vase solution: pH of all the treatment solutions was measured using a pH meter (Bangalore Genei).

3.5.9.17 Statistical analysis of data

Data recorded was subjected to statistical analysis by following Gomez and Gomez (1983). The treatments effects were tested at 1% level of significance.

Results and Discussion

The results of the investigation on the impact of herbal extracts on the microbial vascular plugging, quality and longevity of cut roses Konfetti and Bordeaux conducted during 2011-12 and 2012-13 at the Model Floriculture Centre, College of Agriculture, GBPUA&T, Pantnagar are presented in this chapter.

The observations recorded during course of investigation were analyzed statistically and are presented in the form of tables, figures and plates. The analysis of variance for parameters studied under each experiment has been presented in Appendices.

4.1 Scanning electron microscopy

The scanning electron microscopy study (Plate 4 and 5) shows the proliferation of bacteria and fungi in the xylem of untreated flowers of both Konfetti and Bordeaux, as a result of which the blockage of xylem vessels as well as water stress occurred. The xylem cells in the neighborhood of the cut were filled with bacteria as well as fungi which resulted in lesser water uptake and consequent lose of turgidity along with wilting of cut roses. The cells with the microbial occlusion did not keep their normal form. Parenchyma cells of untreated flowers had thinner cell walls and fewer starch grains at the time of senescence.

4.2 Yield of the extract

Among the five herbal extracts (Table 1 and Plate 6) viz., *Curcuma longa*, *Azadirachta indica*, *Piper betle*, *Ocimum sanctum* and *Allium sativum* prepared by ethanolic extraction the highest yield (%) was obtained for turmeric (6.06%) followed by neem (5.08%), garlic (4.25%), betel leaf (4.1%) and the least for tulsi (2.15%).

Table 1: Yield (%) of various herbal extracts

Name of the extract	Weight of the extract recovered (g)	Weight of the dry powder(g)	Yield (%)
Curcuma longa L.	6.06	100	6.06
Azadirachta indica A. juss.	5.08	100	5.08
Piper betle L.	4.1	100	4.1
Ocimum sanctum L.	2.15	100	2.15
Allium sativum L.	4.25	100	4.25

4.3 Estimation of bacterial load in the vase solution by Standard Plate Count (SPC)

Standard Plate Count (SPC) was done to estimate the number of live bacteria in the vase solutions of both rose varieties *viz.* Bordeaux and Konfetti and to confirm the bacterial growth. The petriplates having bacterial colonies between 30 and 300 were selected for bacterial counting. Triplicates were maintained for each dilution (10^{-1} to 10^{-6}) and an average count was taken to get the most precise results (Table 2).The number of bacteria (CFU) per milliliter of each vase solution of Bordeaux and Konfetti was determined by multiplying the number of colonies with the reciprocal of the dilution used. Bacterial count was 2.75 x 10^5cfu/ml and 2.48 x 10^5 cfu/ml for Konfetti and Bordeaux, respectively. Following calculations were made:

Table 2: Details of bacterial counts in the vase solutions of Konfetti and Bordeaux

Plate No.	Dilution	Number of colonies/plate	
		Konfetti	Bordeaux
Plate 1	10^{-3}	281	260
Plate 2	10^{-3}	267	246
Plate 3	10^{-3}	277	238
Average no. of colonies/plate		275	248

No. of bacteria/ml (cfu/ml) for Konfetti = number of colonies x Reciprocal of the dilution

$$= 275 \times 1/10^{-3}$$

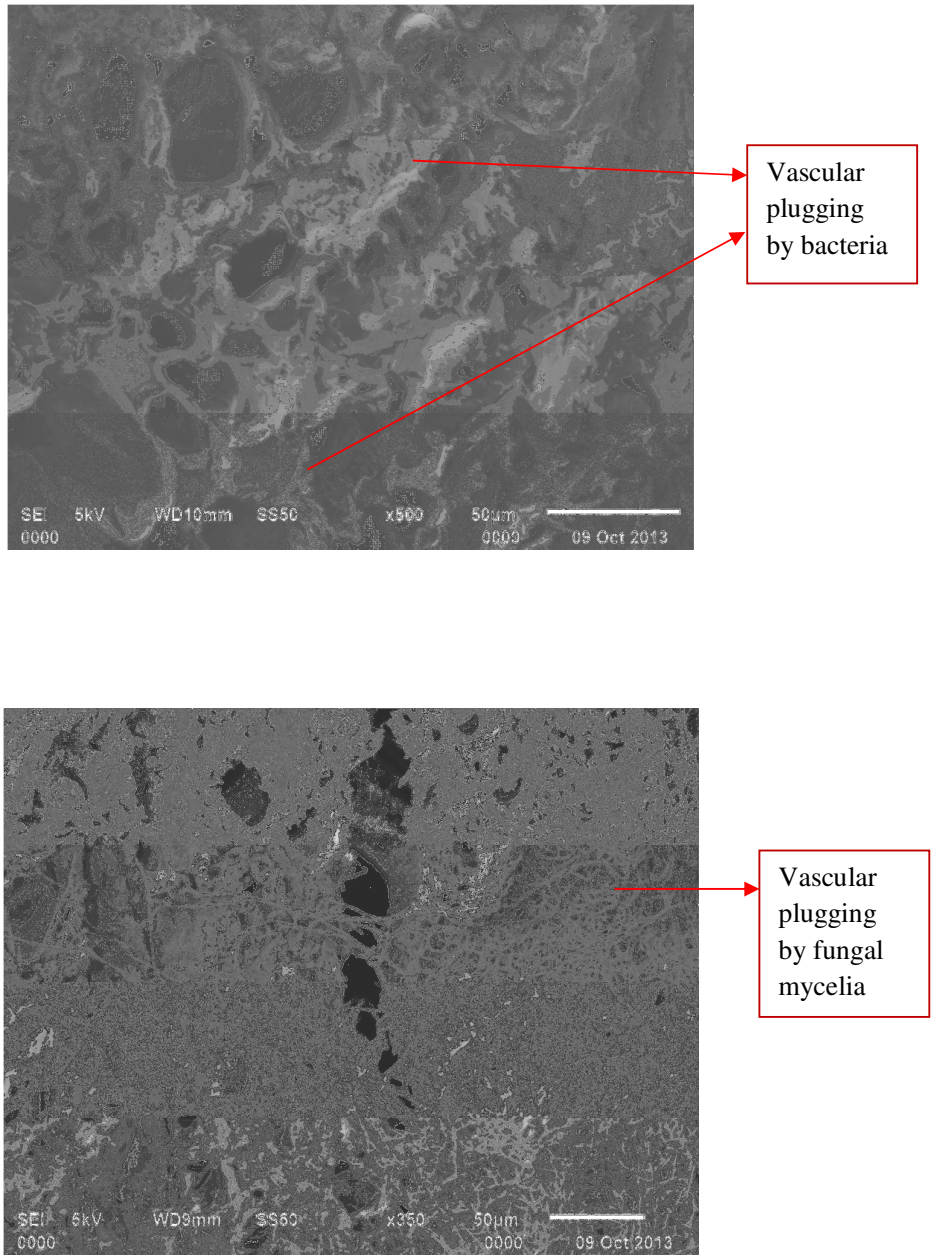
$$= 2.75 \times 10^5$$

No. of bacteria/ ml (cfu/ml) for Bordeaux = number of colonies x Reciprocal of the dilution

$$= 248 \times 1/10^{-3}$$

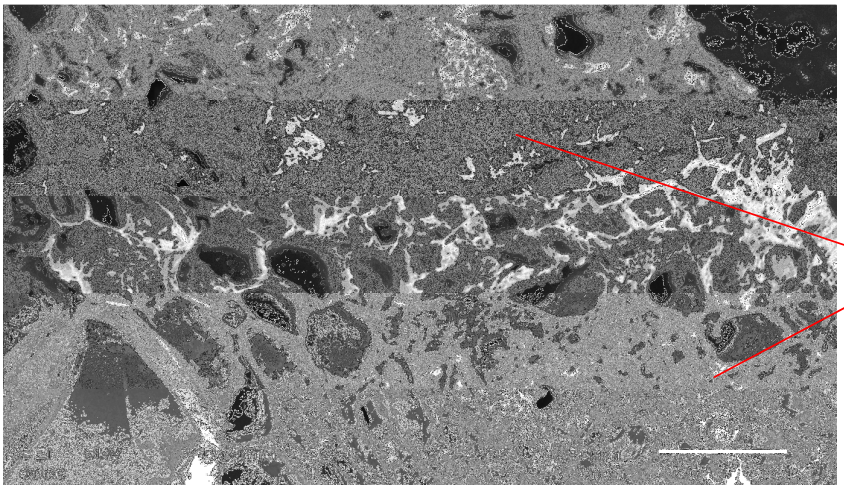
$$= 2.48 \times 10^5$$

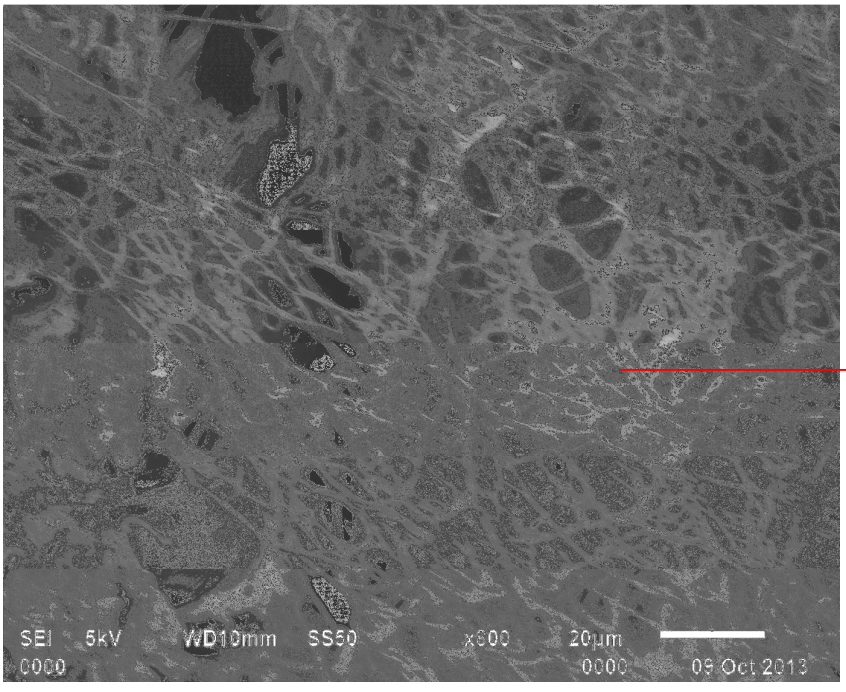
PLATE 4



SEM observations of microbial vascular plugging of 5-days-old Bordeaux cut-rose flowers held in tap water

PLATE 5





SEM observations of microbial vascular plugging of 5-days-old Konfetti cut-rose flowers held in tap water

PLATE 6

Extracts of betel leaf, garlic, neem, turmeric and tulsi after filtration

Final extracts of neem, garelic turmeric, betel leaf and tulsi

4.4 Isolation and identification of bacteria in the vase solution

Three morphologically different colonies were observed on the nutrient agar plate. These 3 colonies were picked separately and processed for further identification on the basis of gram staining, colony characteristics, motility, biochemical profiles and pigmentation.

4.4.1 Identification of *Escherichia coli*

One colony, suspected to be *E. coli* was directly streaked onto the Eosin Methylene Blue plates (EMB) and incubated overnight at 37ºC for the production of metallic sheen indicative of presence of *E. coli*. After overnight incubation clear metallic sheen was observed on the petriplate, suggesting presence of *E. coli* in the vase solutions (Plate 7). *E. coli* colonies were assessed for their morphological, physiological and biochemical characteristics. They were Gram negative, short rod, motile with polar flagella, catalase positive, oxidase negative, lactose fermenters, Methyl- Red (MR) positive, indole test positive and citrate negative. Based on the above tests the isolates were identified as *E. coli.*

4.4.2 Identification of *Bacillus subtilis*

Second colony from the nutrient agar plate was suspected to be *Bacillus* sp. on the basis of morphological characteristics. Colonies were creamy off-white, dry, rough, umbonate with irregular edges (Plate 8). Gram staining revealed gram positive large rods. Upon further processing culture was found positive to spore staining, starch hydrolysis test, Voges Proskauer (VP) test, citrate test, their ability to grow at higher salt concentration (6.5% NaCl) and no growth at 55°C. On the basis of above cultural, staining and biochemical profile, the culture was classified as *Bacillus subtilis.*

4.4.3 Identification of *Pseudomonas aeruginosa*

Third colony produced bluish coloration on the nutrient agar plates with grape like odour (Plate 9). Colonies were gram negative rods, large, mucoid with undulating edges and were found to be non-lactose fermenter. Further confirmation was made on the basis of positive oxidase test.

4.4.4 Isolation and identification of fungus from the vase solution

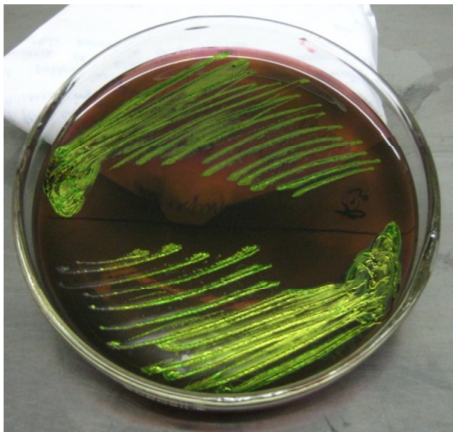
Two types of fungus were isolated from the vase solution. Colonies of *Mucor* sp. grew rapidly at 25-30°C and quickly covered the surface of the Sabouraud Dextrose Agar (SDA) (Plate 10). Growth was white in colour with fluffy appearance and resembled cotton in appearance. However, old cultures turned grey to brown in colour due to production of spores. Upon microscopic examination following observations were recorded; sporangiophores were erect, simple or branched with multispored sporangia. Moreover, apophysis, rhizoid and stolon were found absent.

Rhizopus sp. was identified on the basis of following characteristics. Colonies of Rhizopus grew rapidly, filling the petriplate completely with typical cotton like appearance. Colonies were white initially but turned grey to yellowish brown lately (Plate 11). Macrospically, sporangia were seen as black dots in the middle of white cotton like mycelia. Final confirmation was made by the presence of stolons and pigmented rhizoids.

4.5 Antibacterial/antifungal activity of the herbal extracts

Nutrient agar (NA) plates were used to detect the antibacterial activity of the herbal extracts. Zone of inhibition was measured and the best herbal extract inhibiting the visible growth of bacteria was identified (Plate 12a). Betel leaf extract (*Piper betle*) showed biggest zone of inhibition (26 mm) followed by Neem (*Azadirachta indica*) (20 mm) and Garlic extracts (*Allium sativum*) (15 mm). However, no zone of inhibition was observed for Turmeric (*Curcuma longa*) and Tulsi (*Ocimum sanctum*). For comparison, Dimethyl sulfoxide (DMSO) and 8-HQC were also added to the plates as negative and positive controls, respectively (Plate 12b).On the basis of the appearance of inhibition zones, Betel leaf (*Piper betle*), Neem (*Azadirachta indica*) and Garlic (*Allium sativum*) extracts were selected for antibacterial studies.

Similarly, antifungal property of the herbal extracts was measured against the fungus present in the vase solution. Only Betel leaf extract (*Piper betle*) showed a clear zone of inhibition measuring 28 mm and completely inhibiting any visual growth of the fungus, while other extracts did not produce any inhibition zone.



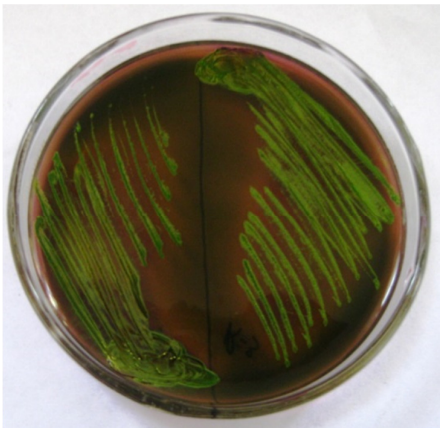


Plate 7: ***Escherichia coli*** **isolated from the vase solution of cut roses Konfetti and Bordeaux exhibiting typical Metallic sheen on Eosin Methylene Blue agar (EMB)**

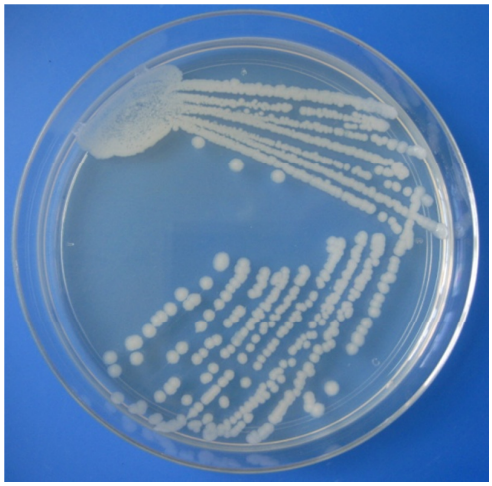


Plate 8: ***Bacillus subtilis*** **isolated from the vase solution of cut roses Konfetti and Bordeaux showing typical flat, opaque and serrated colonies on Nutrient agar (NA)**

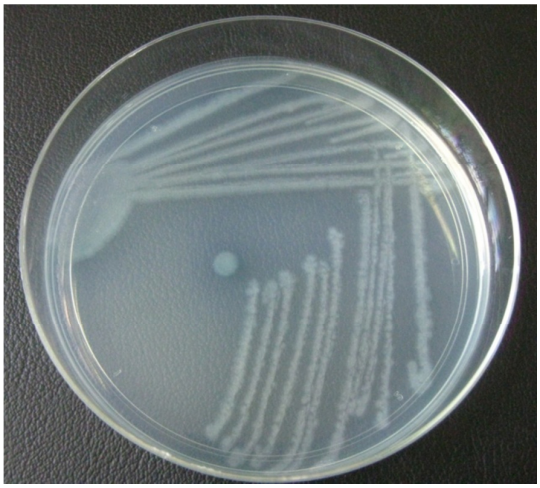


Plate 9: ***Pseudomonas aeruginosa*** **isolated from the vase solution of cut roses Konfetti and Bordeaux showing typical green discolouration on Nutrient agar (NA)**

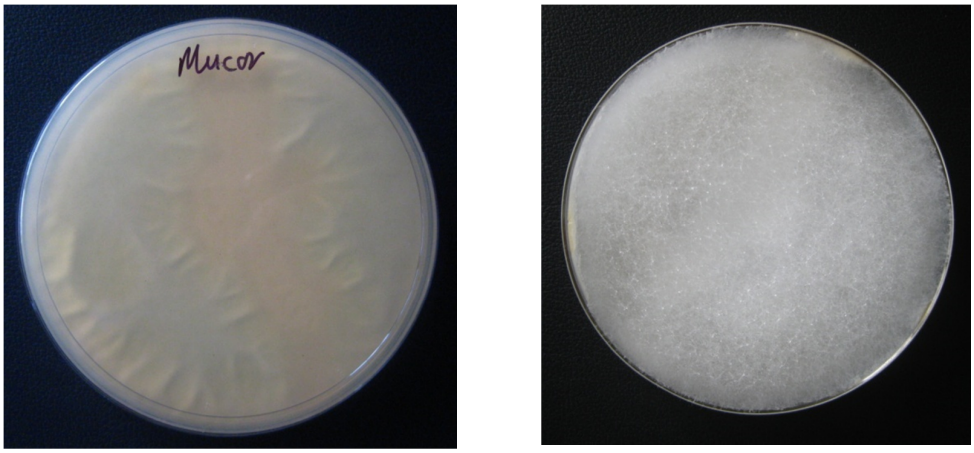


Plate 10: Growth characteristics of fungus *Mucor* spp. isolated from the vase solution

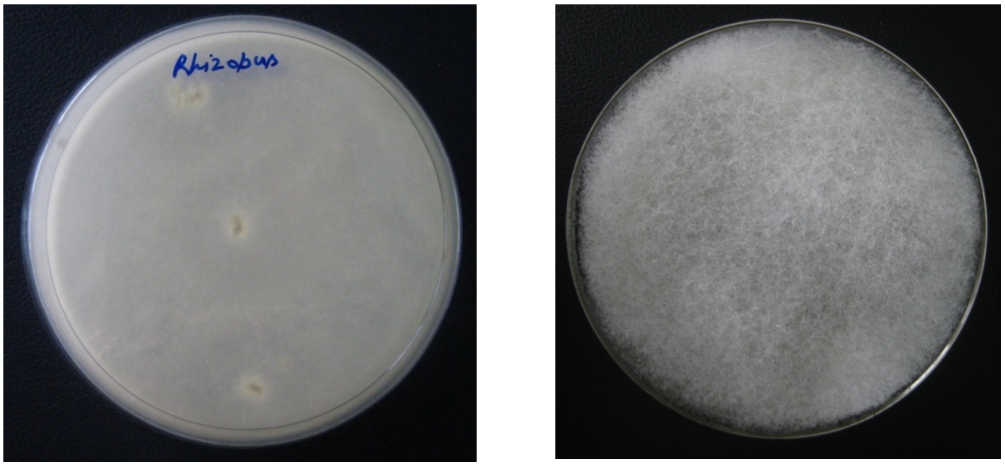


Plate 11: Growth characteristics of fungus *Rhizopus* spp. isolated from the vase solution

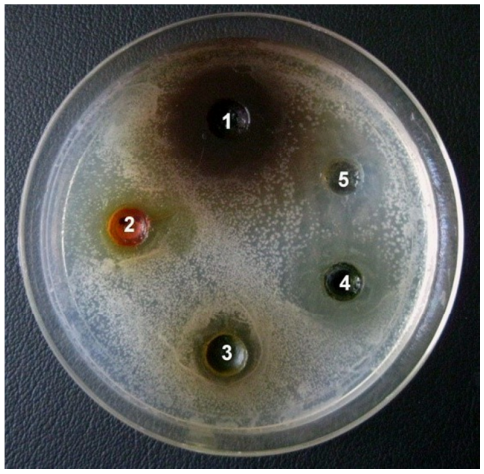
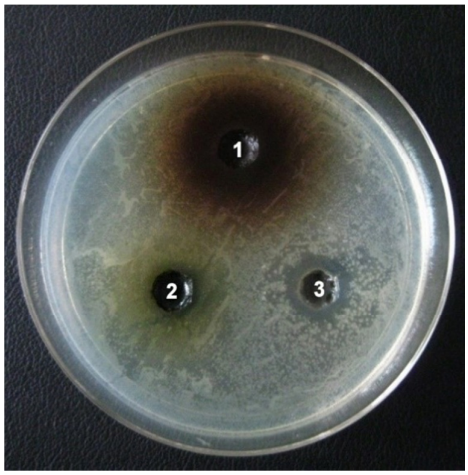


Plate 12a. Antibacterial activity of five herbal extracts against the bacteria present in the vase solution, Well 1: betel leaf extract; well 2: Turmeric extract; well 3: Tulsi extract; well 4: Neem extract and well 5: Garlic extract



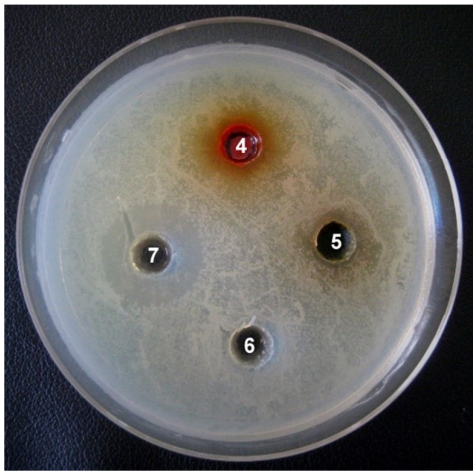


Plate 12b. Antibacterial activity of five herbal extracts against the bacteria isolated from the vase solution, Well 1: betel leaf extract; well 2: Neem extract; well 3: Garlic extract; well 4: Turmeric extract; well 5: Tulsi extract; well 6: Dimethyl sulfoxide (DMSO) and well 7: 8-HQC.

Interestingly, 8-HQC failed to prevent the fungal growth and did not produce any inhibition zone (Plate 13). Hence, only Betel leaf (*Piper betle*) extract was chosen to study the antifungal properties.

On the basis of these observations, Betel leaf (*Piper betle*) extract was concluded as the best herbal extract which could be used as an antimicrobial and antifungal agent in the vase solutions.

4.6 Minimum Inhibitory Concentration (MIC) of herbal extracts

4.6.1 Minimum Inhibitory Concentration (MIC) against bacteria

Extracts of Betel leaf (*Piper betle*), Neem (*Azadirachta indica*) and Garlic (*Allium sativum*) were selected for MIC determination against*Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* on the basis of appearance of inhibition zones (Plate 12a & b). Different concentrations (10%, 5%, 2% and 1%) of these three herbal extracts were prepared and tested individually against afore mentioned microorganisms (Table 3).

Briefly, 10% concentration of Betel leaf extract produced largest inhibition zone of 29 mm against *E. coli* followed by 5% (25 mm), 2% (18 mm) and 1% (15 mm) concentrations (Plate 14). However, 10% Neem extract showed maximum zone of inhibition of 23 mm followed by 5% (28 mm), 2% (17 mm) and 1% (15 mm) concentrations (Plate 15). Garlic extract produced zone of inhibitions measuring 17 mm and 15 mm for 10% and 5% concentrations, respectively (Plate 16). Interestingly, Garlic extract in 2% and 1% concentrations failed to prevent the growth of *E. coli* resulting in no inhibition zones (Table 3).

Similarly, against *Bacillus* sp., Betel leaf extract demonstrated inhibition zones of 24 mm, 20 mm, 17 mm and 13 mm for 10%, 5%, 2% and 1% concentrations, respectively (Plate 17).Inhibition zones of 18 mm, 17 mm, 16 mm and 11 mm were recorded for 10%, 5%, 2% and 1% for Neem extract against the *Bacillus* sp. No significance difference was observed in the sizes of inhibition zones for 10%, 5% and 2% Neem extracts against *Bacillus* sp. (Plate 18).However, none of the concentrations of the Garlic extract produced inhibition zones as they could not prevent the growth of *Bacillus* sp. (Plate 19 & table 3).

Table 3: Zones of Inhibition (ZOI) for determination of Minimum Inhibitory Concentration (MIC) of *Piper betle*, *Azadirachtaindica and Allium sativum*extracts against the microorganisms isolated from the vase solutions

Herbal extracts	Concentration of herbal extract (% w/v)	Zones of Inhibition (mm)				
		Escherichia coli	***Pseudomonas aeruginosa***	***Bacillus subtilis***	***Mucor* sp.**	***Rhizopus* sp.**
Neem (*Azadirachta indica A. Juss.)*	10	23	19	18	-	-
	5	18	17	17	-	-
	2	17	17	16	-	-
	1	15	-	11	-	-
Betel leaf (*Piper betle* L.)	10	29	22	24	30	32
	5	25	19	20	23	29
	2	18	15	17	17	30
	1	15	12	13	-	-
Garlic (*Allium sativum* L.)	10	17	-	-	-	-
	5	15	-	-	-	-
	2	-	-	-	-	-
	1	-	-	-	-	-

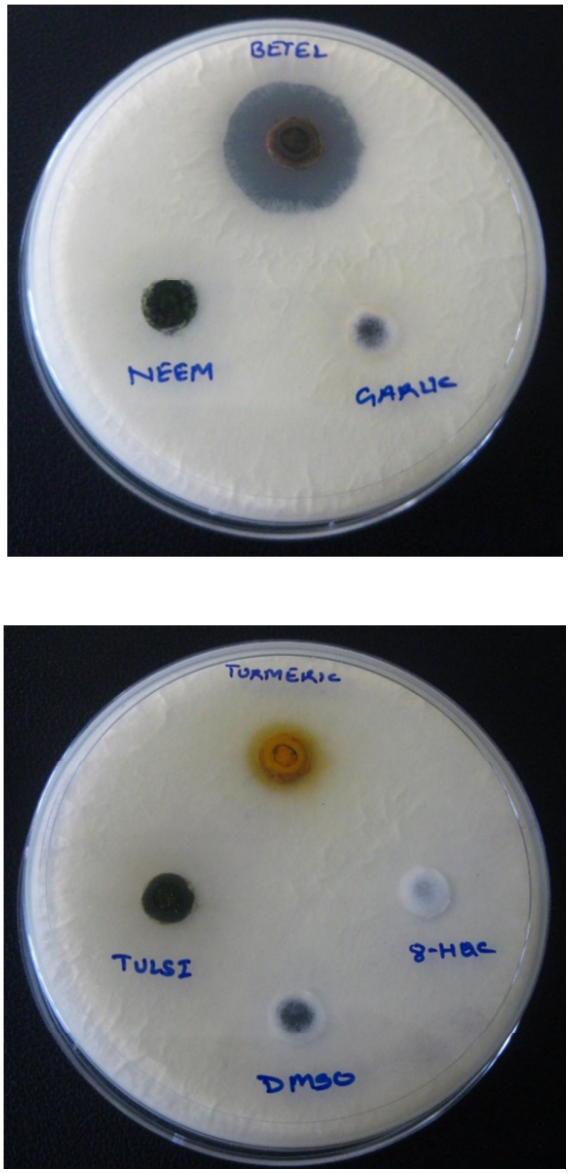


Plate 13 Antifungal activity of five herbal extracts against the fungi present in the vase solution, Well 1: betel leaf extract; well 2: Neem extract; well 3: Garlic extract; well 4: Turmeric extract; well 5: Tulsi extract; well 6: Dimethyl sulfoxide (DMSO) and well 7: 8-HQC.

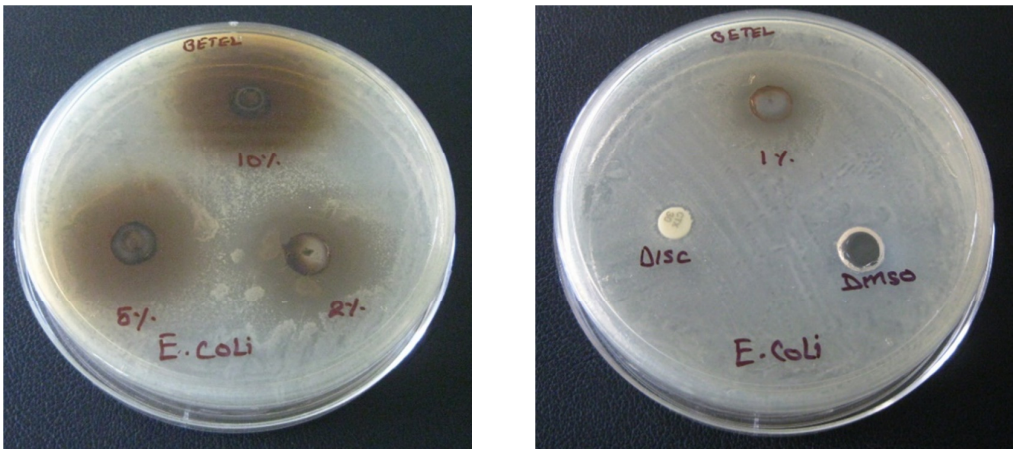


Plate 14: **Determination of Minimum Inhibitory Concentration (MIC) of the betel (*Piper betle*) leaf extract against *Escherichia coli* isolated from the vase solution, Well 1: 10% betel leaf extract; well 2: 5% betel leaf extract; well 3: 2% betel leaf extract; well 4: 1% betel leaf extract; well 5: antibiotic disc and well 6: negative control (DMSO)**

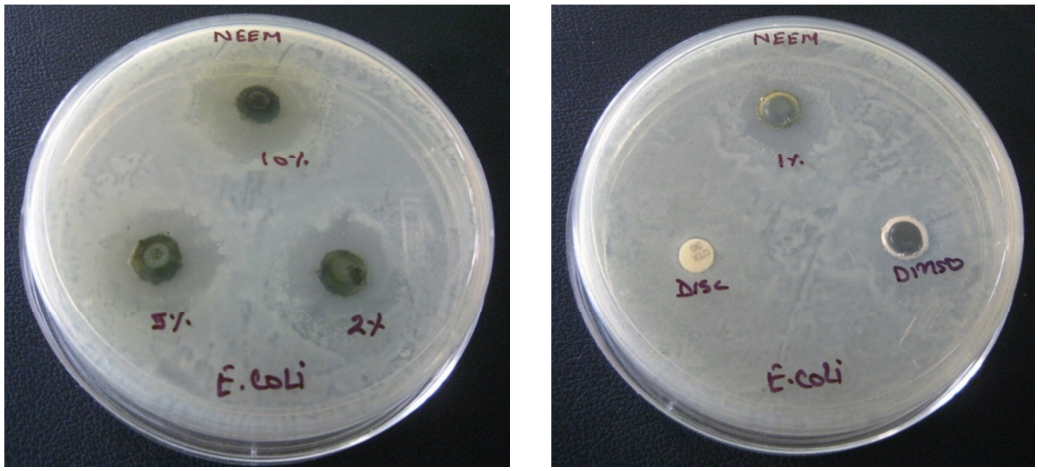


Plate 15: **Determination of Minimum Inhibitory Concentration (MIC) of the Neem extract (*Azadirachtaindica*) against *Escherichia coli* isolated from the vase solution, Well 1: 10% betel leaf extract; well 2: 5% betel leaf extract; well 3: 2% betel leaf extract; well 4: 1% betel leaf extract; well 5: antibiotic disc and well 6: negative control (DMSO)**

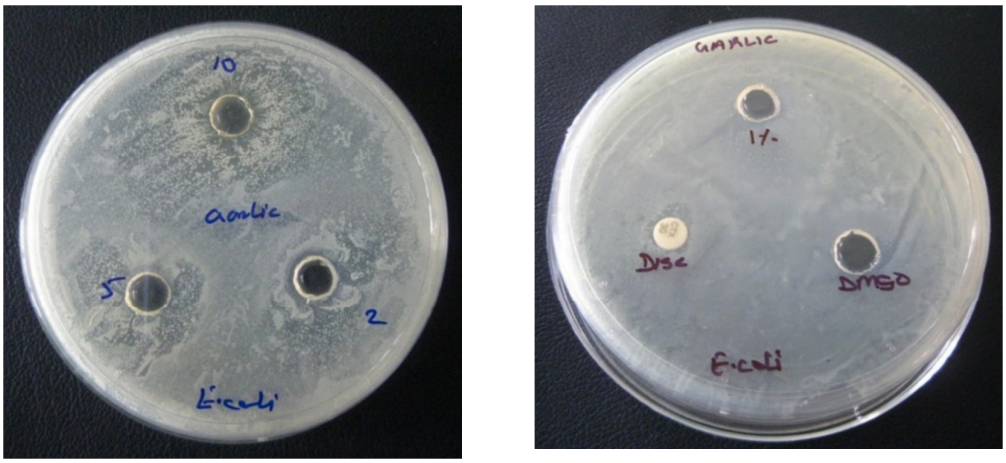


Plate 16: **Determination of Minimum Inhibitory Concentration (MIC) of the Garlic extract (*Allium sativum*) against *Escherichia coli* isolated from the vase solution, Well 1: 10% betel leaf extract; well 2: 5% betel leaf extract; well 3: 2% betel leaf extract; well 4: 1% betel leaf extract; well 5: antibiotic disc and well 6: negative control (DMSO)**

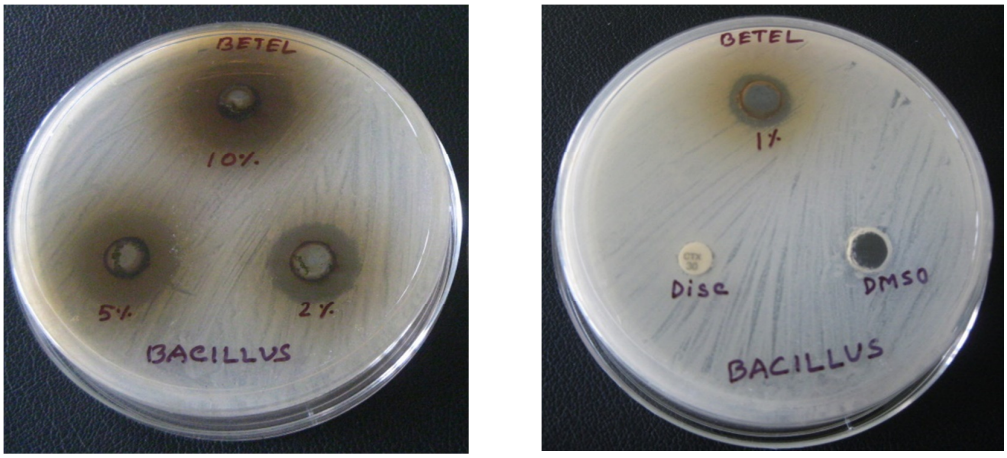


Plate 17: **Determination of Minimum Inhibitory Concentration (MIC) of the betel (*Piper betle*) leaf extract against *Bacillus subtilis* isolated from the vase solution, Well 1: 10% betel leaf extract; well 2: 5% betel leaf extract; well 3: 2% betel leaf extract; well 4: 1% betel leaf extract; well 5: antibiotic disc and well 6: negative control (DMSO)**

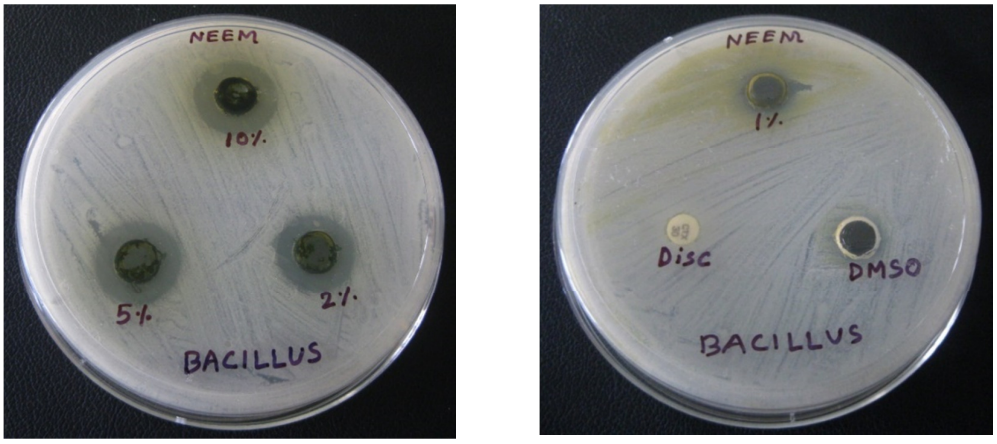


Plate 18: Determination of Minimum Inhibitory Concentration (MIC) of the Neem extract (*Azadirachta indica*) against *Bacillus subtilis* isolated from the vase solution, Well 1: 10% betel leaf extract; well 2: 5% betel leaf extract; well 3: 2% betel leaf extract; well 4: 1% betel leaf extract; well 5: antibiotic disc and well 6: negative control (DMSO)

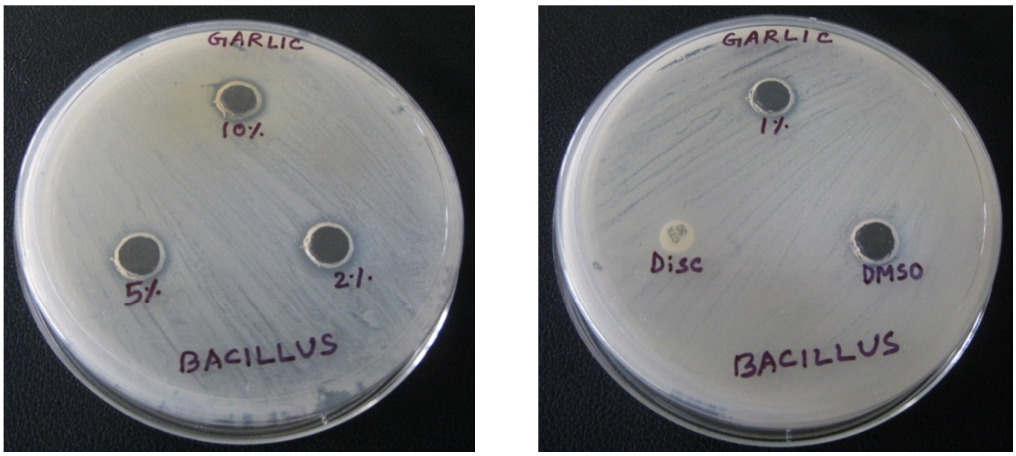


Plate 19: Determination of Minimum Inhibitory Concentration (MIC) of the Garlic extract (*Allium sativum*)against *Bacillus subtilis* isolated from the vase solution, Well 1: 10% betel leaf extract; well 2: 5% betel leaf extract; well 3: 2% betel leaf extract; well 4: 1% betel leaf extract; well 5: antibiotic disc and well 6: negative control (DMSO)

MIC studies against *Pseudomonas* sp. demonstrated maximum zone of inhibition of 22 mm for 10 % betel leaf followed by 19 mm, 15 mm and 12 mm for 5%, 2% and 1% concentrations, respectively (Plate 20). Neem extract also produced encouraging results with 10% concentration exhibiting 19 mm inhibition zone followed by 17 mm (5%) and 17 (2%). No significance difference was seen in the diameter of inhibition zones for 10%, 5% and 2% Neem extracts against *Pseudomonas* sp. (Plate 21). However, 1% Neem extract failed to prevent the bacterial growth and thus did not produce any zone. Moreover, Garlic extract could not prevent visible growth of *Pseudomonas* sp. and thus resulted in no inhibition zone (Plate 22 & Table 3).

Therefore, on the basis of above observations, 2% concentration of betel leaf extract (*Piper betle*) was found to be the best to inhibit any visible bacterial growth.

4.6.2 Minimum Inhibitory Concentration (MIC) of herbal extracts against fungus

Out of the five herbal extracts, only Betel leaf (*Piper betle*) was chosen for MIC studies against *Mucor* sp. and *Rhizopus* sp. (Plate 13). Different concentrations (10%, 5%, 2% and 1%) were prepared to determine the MIC of the Betel leaf (*Piper betle*) extract against *Mucor* sp. and *Rhizopus* sp. isolated from the vase solution.

Ten percent (10%) concentration of Betel leaf extract produced a very clear 30 mm inhibition zone against *Mucor* sp. Similarly, 5% and 2% concentrations demonstrated 23 mm and 17 mm inhibition zones, respectively. However, 1% concentration of Betel leaf extract failed to prevent the growth of fungus, thus did not produce any inhibition zone (Plate 23 & Table 3).

Similarly, against *Rhizopus* sp., 10%, 5% and 2% concentrations of the Betel leaf extract prevented the growth of *Rhizopus* sp. and produced 32 mm, 29 mm and 30 mm inhibition zones, respectively. However, similar to the activity against *Mucor* sp., 1% concentration of betel leaf extract failed to inhibit the *Rhizopus* growth on SDA (Plate 24 & Table 3).

As evident from the above results, MIC of betel leaf extract was found to be as low as 2% against both the fungus tested individually. Betel leaf extract at 2% concentration prevented any visual growth of any of the fungus on SDA.

4.7 Effect of holding solutions on the quality and longevity of cut roses

4.7.1 Initial flower-bud diameter (cm)

The data related to initial flower diameter have been presented in Table 4 and Appendix 1.

The initial flower bud diameter of cut rose stems of both the cultivars harvested for the experiment did not significantly varied in the year 2012, 2013 or in the pooled data. Among the various treatments also it was not significant. In the pooled data also maximum initial diameter (3.69 cm) was obtained for the treatment 3% sucrose+ 200 ppm 8-HQC but it was on par with all the treatments except H_1.

4.7.2 Final flower diameter (cm)

The final flower diameter (Table 5, Fig. 1 and Appendix 2) was significantly different in both the cultivars with cv. Bordeaux recording a higher value in 2012 (8.17 cm), 2013 (8.15 cm) and pooled data (8.16 cm) compared to Konfetti (7.45 cm, 7.48 cm and 7.46 cm).

Among the treatments 2% sucrose+2% betel leaf recorded the maximum flower diameter in 2012 (9.95cm), 2013 (9.84cm) and pooled value (9.89cm) followed by 2% sucrose + 200 ppm 8-HQC (9.09cm, 8.80 cm and 8.95 cm) with the least flower diameter for control (tap water) treatment.

Considering the interaction effect, the treatment 2% sucrose+2% betel leaf recorded the maximum flower diameter in both the cultivars in both the years 2012 (9.72cm, 10.18cm) and 2013 (9.77cm , 9.90 cm). This was followed by the treatment 2% sucrose + 200 ppm 8-HQC and the control treatment recorded the lowest value in both the years.

4.7.3 Opening of flower bud

The data regarding opening of the flower bud is represented in Table 6 and Appendix 3.

The flower opening score was not significantly different in both the cultivars in the years 2012 and 2013 (3.96, 4.26 and 4 and 4.26, respectively).

The maximum score of flower opening was observed in the treatment 2% sucrose+2% betel leaf extract with a score of 5 in 2012, 2013 and pooled but this was on par with the treatments 2% sucrose + 200 ppm 8-HQC (4.83), 3% sucrose +2%

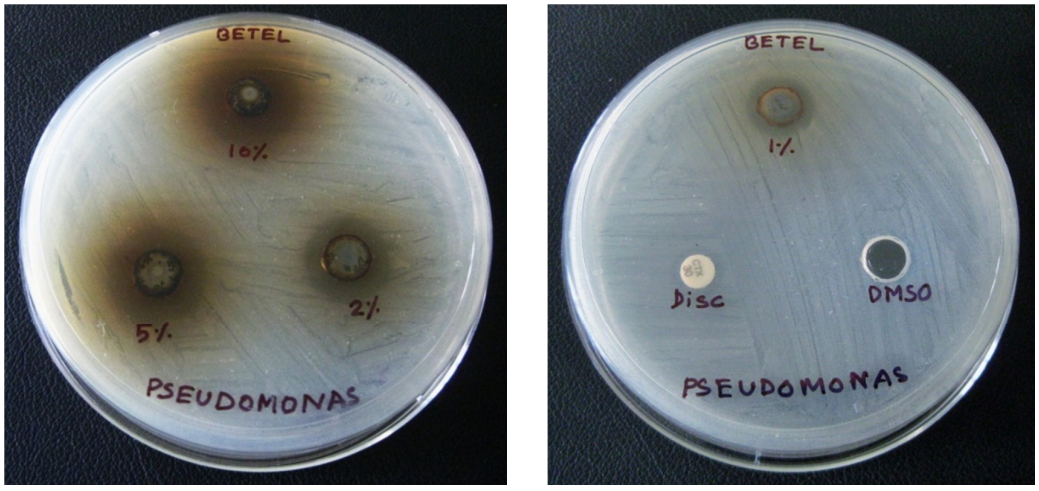


Plate 20: Determination of Minimum Inhibitory Concentration (MIC) of the betel (*Piper betle*) leaf extract against *Pseudomonas sp.* isolated from the vase solution, Well 1: 10% betel leaf extract; well 2: 5% betel leaf extract; well 3: 2% betel leaf extract; well 4: 1% betel leaf extract; well 5: antibiotic disc and well 6: negative control (DMSO)

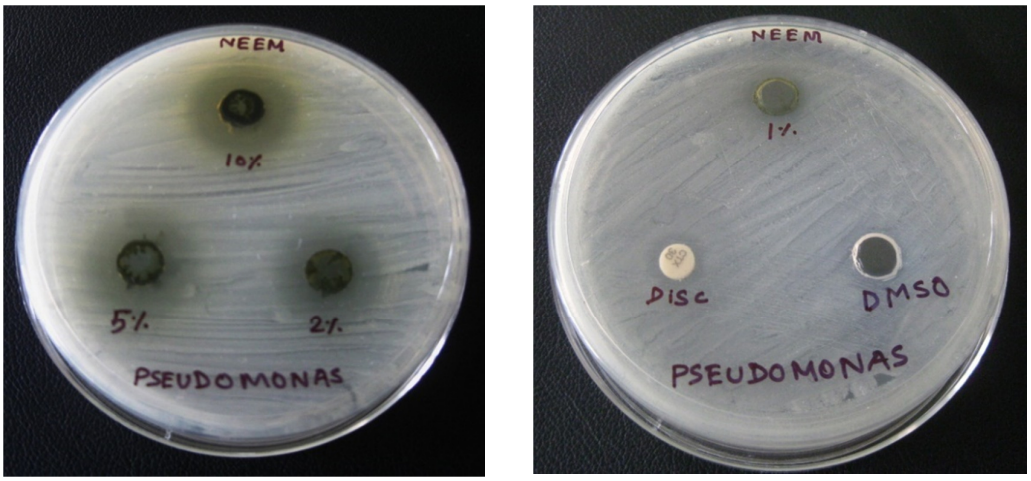


Plate 21: Determination of Minimum Inhibitory Concentration (MIC) of the Neem extract (*Azadirachta indica*) against *Pseudomonas sp.* isolated from the vase solution, Well 1: 10% betel leaf extract; well 2: 5% betel leaf extract; well 3: 2% betel leaf extract; well 4: 1% betel leaf extract; well 5: antibiotic disc and well 6: negative control (DMSO)

Plate 22: **Determination of Minimum Inhibitory Concentration (MIC) of the Garlic extract (*Allium sativum*)against *Pseudomonas sp.* isolated from the vase solution, Well 1: 10% betel leaf extract; well 2: 5% betel leaf extract; well 3: 2% betel leaf extract; well 4: 1% betel leaf extract; well 5: antibiotic disc and well 6: negative control (DMSO)**

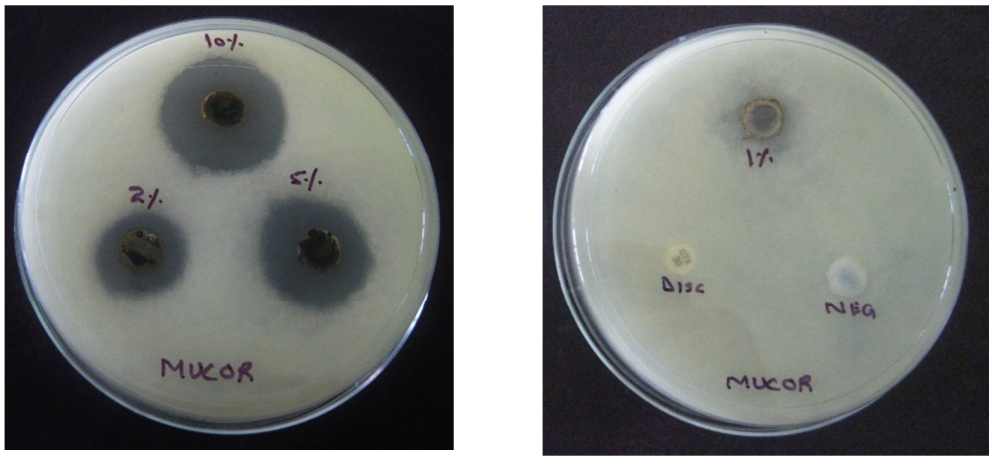


Plate 23: Determination of Minimum Inhibitory Concentration (MIC) of the betel (*Piper betle*) leaf extract against the fungus *Mucor* sp. isolated from the vase solution, Well 1: 10% betel leaf extract; well 2: 5% betel leaf extract; well 3: 2% betel leaf extract; well 4: 1% betel leaf extract; well 5: antibiotic disc and well 6: negative control (DMSO)

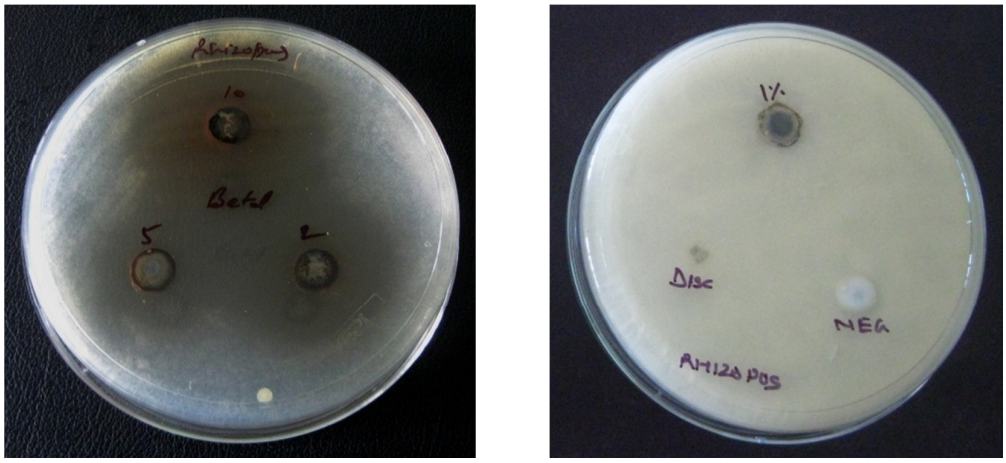


Plate 24: Determination of Minimum Inhibitory Concentration (MIC) of the betel (*Piper betle*) leaf extract against the fungus *Rhizopus* sp. isolated from the vase solution, Well 1: 10% betel leaf extract; well 2: 5% betel leaf extract; well 3: 2% betel leaf extract; well 4: 1% betel leaf extract; well 5: antibiotic disc and well 6: negative control (DMSO)

Table 4: Initial flower diameter of cut roses Konfetti and Bordeaux in the holding solutions

Treatment	Initial flower diameter (cm)								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
H_1	3.52	3.79	3.66	3.48	3.78	3.63	3.50	3.79	3.64
H_2	3.54	3.82	3.68	3.53	3.80	3.67	3.54	3.81	3.67
H_3	3.62	3.82	3.72	3.51	3.77	3.64	3.57	3.80	3.68
H_4	3.50	3.83	3.67	3.51	3.77	3.64	3.51	3.80	3.65
H_5	3.58	3.86	3.72	3.53	3.79	3.66	3.56	3.83	3.69
H_6	3.54	3.82	3.68	3.54	3.78	3.66	3.54	3.80	3.67
H_7	3.58	3.79	3.69	3.55	3.78	3.67	3.57	3.79	3.68
H_8	3.62	3.78	3.70	3.51	3.78	3.65	3.57	3.78	3.68
H_9	3.54	3.79	3.67	3.50	3.81	3.66	3.52	3.80	3.66
Mean	3.56	3.81		3.52	3.79		3.54	3.80	
	S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%	
H	0.013	0.051		0.016	0.064		0.011	0.045	
C	0.028	0.109		0.035	0.137		0.025	0.097	
H x C	0.040	0.155		0.050	0.193		0.035	0.137	

H- Holding solution , H_1-2% Betel leaf extract, H_2- 200ppm 8-HQC, H_3-1% sucrose + 200 ppm 8-HQC, H_4-2% sucrose + 200 ppm 8-HQC, H_5-2% sucrose + 200 ppm 8-HQC, H_6-1% sucrose+2% betel leaf extract, H_7-2% sucrose+2% betel leaf extract, H_8-3% sucrose+2% betel leaf extract, H_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

Table 5: Final flower diameter of cut roses Konfetti and Bordeaux in the holding solutions

Treatment	Final flower diameter (cm)								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
H_1	6.97	7.45	7.21	7.25	7.56	7.41	7.11	7.51	7.31
H_2	6.81	7.12	6.97	6.84	7.21	7.02	6.83	7.16	6.99
H_3	7.17	8.68	7.92	7.08	8.59	7.83	7.12	8.63	7.88
H_4	8.84	9.34	9.09	8.11	9.49	8.80	8.48	9.41	8.95
H_5	7.32	8.16	7.74	8.01	8.90	8.46	7.67	8.53	8.10
H_6	8.03	8.84	8.44	8.26	8.68	8.47	8.14	8.76	8.45
H_7	9.72	10.18	9.95	9.77	9.90	9.84	9.74	10.04	9.89
H_8	7.88	8.92	8.40	8.06	8.62	8.34	7.97	8.77	8.37
H_9	4.28	4.87	4.58	3.91	4.38	4.14	4.09	4.63	4.36
Mean	7.45	8.17		7.48	8.15		7.46	8.16	
	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%
H	0.069		0.267	0.104		0.401	0.063		0.244
C	0.147		0.567	0.221		0.851	0.135		0.519
H x C	0.208		0.802	0.313		1.204	0.190		0.734

H- Holding solution , H_1-2% Betel leaf extract, H_2- 200ppm 8-HQC, H_3-1% sucrose + 200 ppm 8-HQC, H_4-2% sucrose + 200 ppm 8-HQC, H_5-2% sucrose + 200 ppm 8-HQC, H_6-1% sucrose+2% betel leaf extract, H_7-2% sucrose+2% betel leaf extract, H_8-3% sucrose+2% betel leaf extract, H_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

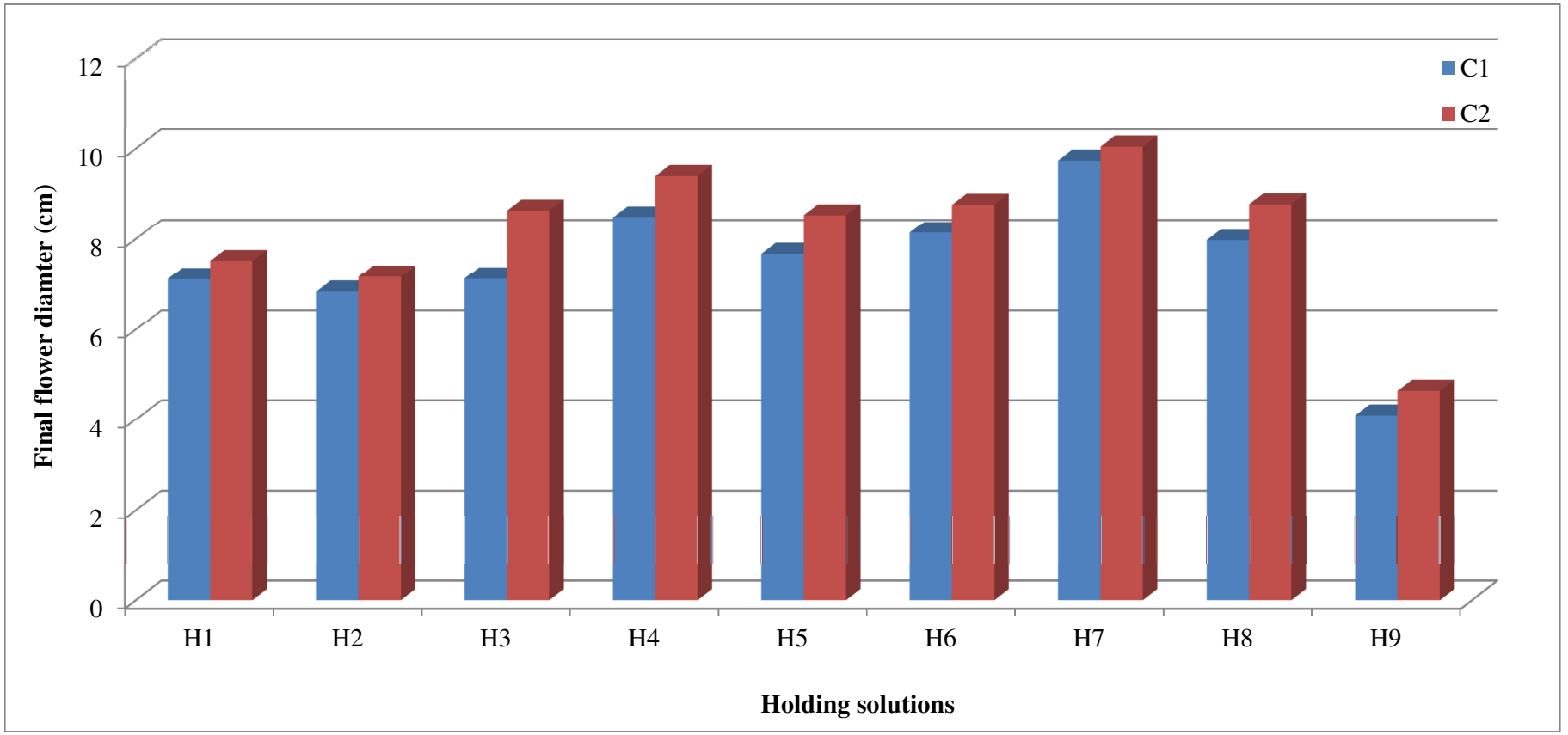


Fig. 1: Final flower diameter of cut roses Konfetti and Bordeaux in the holding solutions

Table 6: Opening of flower bud of cut roses Konfetti and Bordeaux in the holding solutions

Treatment	Opening of flower bud								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
H_1	3.33	4.00	3.67	3.33	4.00	3.67	3.33	4.00	3.67
H_2	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
H_3	4.67	4.33	4.50	4.33	4.67	4.50	4.50	4.50	4.50
H_4	4.67	5.00	4.83	4.67	5.00	4.83	4.67	5.00	4.83
H_5	4.00	4.67	4.33	4.33	4.67	4.50	4.17	4.67	4.42
H_6	4.33	5.00	4.67	4.67	5.00	4.83	4.50	5.00	4.75
H_7	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
H_8	4.67	5.00	4.83	4.67	5.00	4.83	4.67	5.00	4.83
H_9	1.00	1.33	1.17	1.00	1.00	1.00	1.00	1.17	1.08
Mean	3.96	4.26		4.00	4.26		3.98	4.26	
	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%
H	0.074		0.284	0.074		0.284	0.068		0.261
C	0.157		0.604	0.157		0.604	0.144		0.555
H x C	0.222		0.854	0.222		0.854	0.204		0.785

H- Holding solution , H_1-2% Betel leaf extract, H_2- 200ppm 8-HQC, H_3-1% sucrose + 200 ppm 8-HQC, H_4-2% sucrose + 200 ppm 8-HQC, H_5-2% sucrose + 200 ppm 8-HQC, H_6-1% sucrose+2% betel leaf extract, H_7-2% sucrose+2% betel leaf extract, H_8-3% sucrose+2% betel leaf extract, H_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

betel leaf extract (4.83) and 1% sucrose+2% betel leaf extract (4.67, 4.83 and 4.75). The lowest score was noticed in the control treatment (1.17, 1 and 1.08).

The interaction effects were significant between the treatments and the control. All the treatments having sucrose + biocide had a positive interaction and they are on par with each other with a maximum score of 5 for both the cultivars in the treatment 2% sucrose+2% betel leaf extract. The control treatment tap water) had the lowest interaction with a score of 1.33 and 1 in 2012, 1.00 and 1.00 in 2013 and 1 and1.17in the pooled data.

4.7.4 Number of days to reach maximum flower diameter

Table 7 indicates the number of days to reach maximum flower diameter.

Both the cultivars were on par with each other regarding the number of days to reach maximum flower diameter with a higher value for Bordeaux (7.52, 7.33 and 7.43 days).

2% sucrose+2% betel leaf extract was the most efficient treatment regarding the number of days to reach maximum flower diameter during both the years which were 9.67 days and 10 days respectively. In both the years this was succeeded by the treatment 2% sucrose + 200 ppm 8-HQC (8.00days and 7.83 days). Control treatment recorded the least number of days to reach maximum flower diameter which were 3.3, 3.5 and 3.42 days respectively in 2012, 2013 and pooled data (Appendix 4).

Significant interaction between the cultivars and treatments was observed during both the years. Bordeaux cut roses in combination with 2% sucrose+2% betel leaf extract showed a conspicuous increase in the number of days to reach maximum flower diameter during 2012 (10.67 days) and pooled data (9.83 days). Cultivar Konfetti (8.67 and 9.00 days) with the same treatment and Bordeaux (8.67 and 8.33 days) treated with 2 % sucrose + 200 ppm 8-HQC came next. In 2013, both Konfetti and Bordeaux treated with 2% sucrose+2% betel leaf extract were on par with each other. The lowest interaction effect was observed in the control treatment (3.33 and 3.5 in the pooled data).

Table 7: Days to maximum flower diameter of cut roses Konfetti and Bordeaux in the holding solutions

Treatment	Days to maximum flower diameter (days)								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
H_1	6.67	7.33	7.00	6.67	8.00	7.33	6.67	7.67	7.17
H_2	6.33	7.00	6.67	7.00	6.33	6.67	6.67	6.67	6.67
H_3	7.00	8.00	7.50	6.67	7.00	6.83	6.83	7.50	7.17
H_4	7.33	8.67	8.00	7.67	8.00	7.83	7.50	8.33	7.92
H_5	5.67	7.33	6.50	6.33	7.00	6.67	6.00	7.17	6.58
H_6	7.00	7.67	7.33	6.33	7.33	6.83	6.67	7.50	7.08
H_7	8.67	10.67	9.67	9.33	10.67	10.00	9.00	10.67	9.83
H_8	7.33	7.67	7.50	6.33	8.00	7.17	6.83	7.83	7.33
H_9	3.33	3.33	3.33	3.33	3.67	3.50	3.33	3.50	3.42
Mean	6.59	7.52		6.63	7.33		6.61	7.43	
	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%
H	0.167		0.644	0.143		0.551	0.103		0.399
C	0.355		1.368	0.304		1.170	0.220		0.847
H x C	0.503		1.934	0.430		1.655	0.311		1.199

H- Holding solution , H_1-2% Betel leaf extract, H_2- 200ppm 8-HQC, H_3-1% sucrose + 200 ppm 8-HQC, H_4-2% sucrose + 200 ppm 8-HQC, H_5-2% sucrose + 200 ppm 8-HQC, H_6-1% sucrose+2% betel leaf extract, H_7-2% sucrose+2% betel leaf extract, H_8-3% sucrose+2% betel leaf extract, H_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

4.7.5 Water uptake (g/stem)

Table 8 and Fig. 2 depicts the Water uptake (g/stem) of cut roses Konfetti and Bordeaux (Appendix 5).

The total water uptake significantly differed among both the cultivars in 2012 and in the pooled data where as they were on par with each other in 2013. Regarding treatments, in 2012, 2% sucrose+2% betel leaf extract (27.80 g/stem) had a significantly higher value than all the other treatments followed by 2 % sucrose + 200 ppm 8-HQC (25.97 g/stem) with least values for the control (11.67 g/stem) where as in 2013, 2% sucrose+2% betel leaf extract (26.63 g/stem) showed higher values followed by1% sucrose+2% betel leaf extract (24.83 g/stem) which was on par with by2 % sucrose + 200 ppm 8-HQC (24.63 g/stem) and 3% sucrose+2% betel leaf extract (24.10 g/stem) .In the pooled data ,the highest value was recorded for 2% sucrose+2% betel leaf (27.22 g/stem) and lowest for the control (12.07 g/stem).

Significant interaction effects were shown by both the cultivars in all the treatments compared to the control. In 2012 (30.43 g/stem) and in pooled data (27.22 g/stem) highest interaction was shown between 2% sucrose+2% betel leaf and Bordeaux. In 2013, 2% sucrose+2% betel leaf and Bordeaux (27.50 g/stem), 2% sucrose+2% betel leaf and Konfetti (25.77 g/stem) as well as 1% sucrose+2% betel leaf and Bordeaux (26.50 g/stem) were on par with each other. The lowest values were recorded for control.

4.7.6 Water loss (g/stem)

The total water loss (Table 9, Fig. 3 and Appendix 6) significantly differed among both the cultivars in 2012 (23.40, 24.10 g/stem) and in the pooled data (23.00, 21.49 g/stem) where as they were on par with each other in 2013 (23.05, 21.44 g/stem). Among the treatments, 2% sucrose+2% betel leaf extract recorded a significantly higher value in 2012 (26.43 g/stem), 2013 (25.67 g/stem) and pooled data (25.53 g/stem) where as the control treatment recorded the lowest values (13.55, 14.27 and 13.91 g/stem). Interaction effects were significant compared to control in both the years and pooled data with highest value for the interaction between Bordeaux and 2% sucrose+2% betel leaf extract (26.43, 26.47and 26.45 g/stem) and these were on par with Bordeaux and 1% sucrose+2% betel leaf extract (25.57, 25.77 and 25.67 g/stem).

Table 8: Total water uptake (g/stem) of cut roses Konfetti and Bordeaux in the holding solutions

Treatment	Total water uptake (g/stem)								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
H_1	20.37	22.63	21.50	20.03	23.27	21.65	20.20	22.95	21.58
H_2	21.73	23.53	22.63	21.90	23.77	22.83	21.82	23.65	22.73
H_3	23.23	24.00	23.62	23.03	23.93	23.48	23.13	23.97	23.55
H_4	24.10	27.83	25.97	24.57	24.70	24.63	24.33	26.27	25.30
H_5	22.23	24.63	23.43	22.53	23.27	22.90	22.38	23.95	23.17
H_6	23.70	26.67	25.18	23.17	26.50	24.83	23.43	26.58	25.01
H_7	25.17	30.43	27.80	25.77	27.50	26.63	25.47	28.97	27.22
H_8	23.60	25.20	24.40	23.77	24.43	24.10	23.68	24.82	24.25
H_9	10.90	12.43	11.67	11.70	13.23	12.47	11.30	12.83	12.07
Mean	21.67	24.15		21.83	23.40		21.75	23.78	
	S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%	
H	0.186	0.717		0.202	0.778		0.153	0.588	
C	0.395	1.521		0.429	1.651		0.324	1.249	
H x C	0.559	2.152		0.607	2.335		0.459	1.766	

H- Holding solution , H_1-2% Betel leaf extract, H_2- 200ppm 8-HQC, H_3-1% sucrose + 200 ppm 8-HQC, H_4-2% sucrose + 200 ppm 8-HQC, H_5-2% sucrose + 200 ppm 8-HQC, H_6-1% sucrose+2% betel leaf extract, H_7-2% sucrose+2% betel leaf extract, H_8-3% sucrose+2% betel leaf extract, H_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

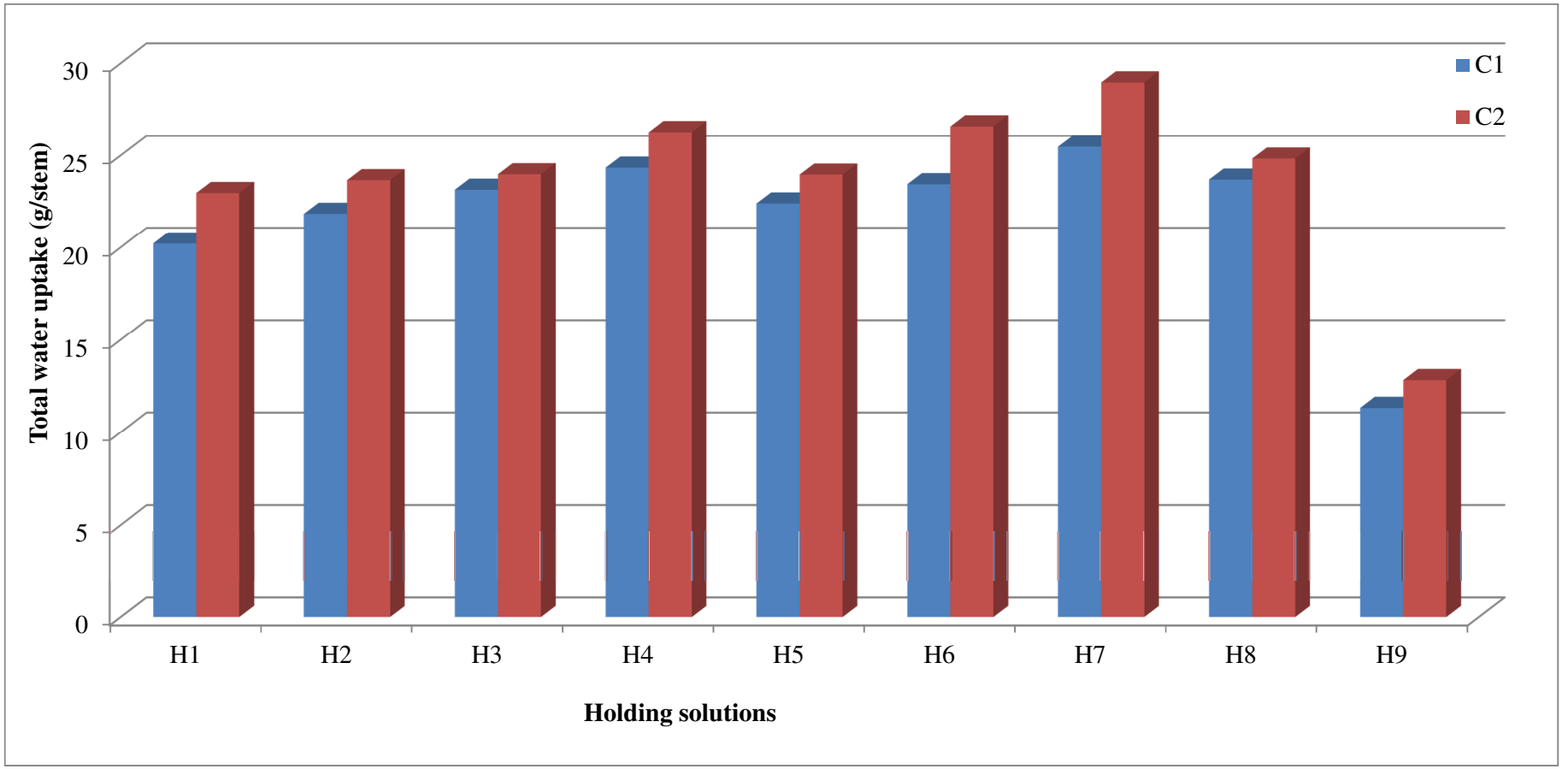


Fig. 2: Total water uptake of cut roses Konfetti and Bordeaux in the holding solutions

Table 9: Total water loss (g/ stem) of cut roses Konfetti and Bordeaux in the holding solutions

Treatment	Total water loss (g/ stem)								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
H_1	19.93	22.27	21.10	19.69	22.87	21.28	19.81	22.57	21.19
H_2	21.27	23.00	22.13	21.33	23.30	22.32	21.30	23.15	22.23
H_3	22.77	23.67	23.22	22.50	23.53	23.02	22.63	23.60	23.12
H_4	23.37	24.47	23.92	23.83	23.97	23.90	23.60	24.22	23.91
H_5	21.70	23.97	22.83	21.80	22.60	22.20	21.75	23.28	22.52
H_6	23.03	25.57	24.30	22.43	25.77	24.10	22.73	25.67	24.20
H_7	24.37	26.43	25.40	24.87	26.47	25.67	24.62	26.45	25.53
H_8	23.10	24.50	23.80	23.23	23.73	23.48	23.17	24.12	23.64
H_9	13.03	14.07	13.55	13.73	14.80	14.27	13.38	14.43	13.91
Mean	21.40	23.10		21.49	23.00		21.44	23.05	
	S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%	
H	0.161	0.620		0.208	0.803		0.137	0.527	
C	0.342	1.316		0.443	1.703		0.291	1.119	
H x C	0.483	1.861		0.626	2.409		0.411	1.583	

H- Holding solution , H_1-2% Betel leaf extract, H_2- 200ppm 8-HQC, H_3-1% sucrose + 200 ppm 8-HQC, H_4-2% sucrose + 200 ppm 8-HQC, H_5-2% sucrose + 200 ppm 8-HQC, H_6-1% sucrose+2% betel leaf extract, H_7-2% sucrose+2% betel leaf extract, H_8-3% sucrose+2% betel leaf extract, H_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

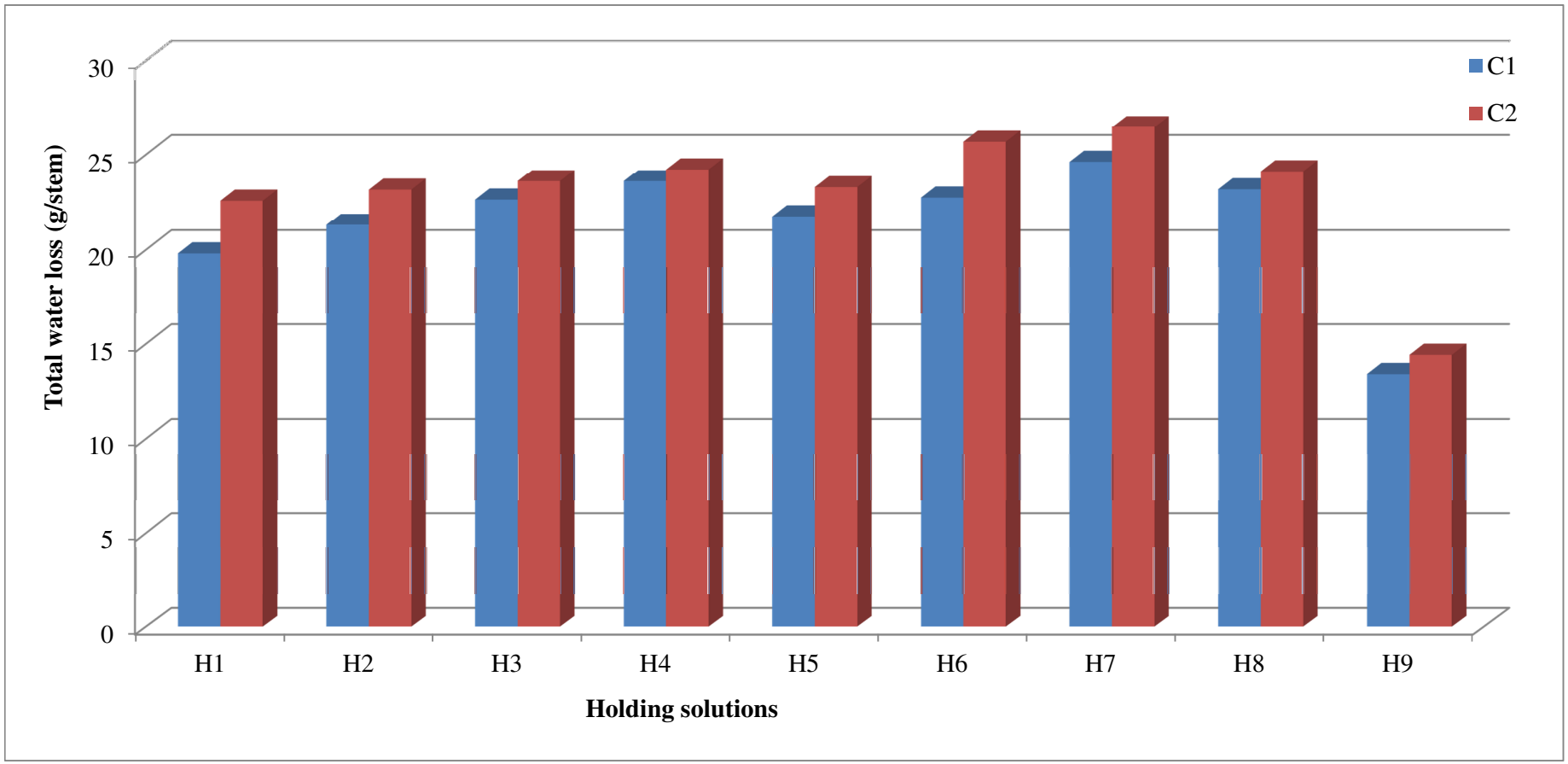


Fig. 3: Total water loss of cut roses Konfetti and Bordeaux in the holding solutions

4.7.7 Water balance

The values obtained for water balance in holding solutions are shown in Table 10 and Appendix 7.

The water balance was not significant among the cultivars. Among the treatments the highest value was recorded for 2% sucrose+2% betel leaf extract (0.90, 0.72, 0.72) which was similar to both 1% sucrose+2% betel leaf extract (0.97, 0.73, 0.73)and 2% sucrose + 200 ppm 8-HQC in 2012, 2013 and in the pooled data (0.93, 0.73, 0.73). The interaction effects were not significant among the cultivars and treatments H1 to H8 but all these were significant compared to the control in both the cultivars (-2.13, -1.63, -2.03, -1.57 and -2.08, -1.60).

4.7.8 Transpiration rate (ml/g FW)

The transpiration rate (Table 11 and Appendix 8) for both the cultivars were on par with each other in 2012, 2013 and in the pooled data (0.20, 0.19; 0.20, 0.20 and 0 .20, 0.20 ml/g FW). Among the treatments 2% sucrose+2% betel leaf extract (0.12, 0.12 and 0.12 ml/g FW) had a significantly lower value compared to all other treatments with the control having the highest values in both the years and pooled data (0.43, 0.41 and 0.42 ml/g FW).

Among the interaction effects the highest value was obtained for the control treatments of both the cultivars (0.46, 0.41; 0.42, 0.40 and0 .44 and 0 .40 ml/g FW) in 2012.2013 and pooled data and lowest was observed for the treatment 2% sucrose+2% betel leaf extract and Bordeaux.

4.7.9 Fresh weight changes (%)

The data regarding fresh weight changes are given in Tables 12, 13 & 14, Fig. 4 & 5 Appendix 9, 10 & 11**.**

The fresh weight changes among both the cultivars were not significantly different on day 2 in 2012 (111.65, 112.65%), 2013 (111.85, 113.06 %) and pooled data (111.75, 112.85%). The treatment 2% sucrose+ 2% betel leaf extract was significantly different from all the other treatments in 2012 (116.95%) and pooled data (116.72%) where as in 2013 the treatments2% sucrose+2% betel leaf extract (116.49%), 2% sucrose+200 ppm 8-HQC (115.08%), 3% sucrose+2% betel leaf extract

Table 10: Water Balance of cut roses Konfetti and Bordeaux in the holding solutions

Treatment	Water Balance								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
H_1	0.43	0.37	0.40	0.43	0.40	0.42	0.43	0.38	0.41
H_2	0.47	0.53	0.50	0.57	0.47	0.52	0.52	0.50	0.51
H_3	0.47	0.33	0.40	0.53	0.40	0.47	0.50	0.37	0.43
H_4	0.73	0.70	0.72	0.73	0.73	0.73	0.73	0.72	0.73
H_5	0.53	0.67	0.60	0.73	0.67	0.70	0.63	0.67	0.65
H_6	0.67	0.77	0.72	0.73	0.73	0.73	0.70	0.75	0.73
H_7	0.80	1.00	0.90	0.90	1.03	0.97	0.85	1.02	0.93
H_8	0.50	0.70	0.60	0.53	0.70	0.62	0.52	0.70	0.61
H_9	-2.13	-1.63	-1.88	-2.03	-1.57	-1.80	-2.08	-1.60	-1.84
Mean	0.27	0.38		0.35	0.40		0.31	0.39	

	S.Em.±	C.D. at 1%	S.Em.±	C.D. at 1%	S.Em.±	C.D. at 1%
H	0.070	0.272	0.062	0.239	0.050	0.194
C	0.150	0.577	0.132	0.508	0.107	0.411
H x C	0.212	0.816	0.186	0.718	0.151	0.582

H- Holding solution , H_1-2% Betel leaf extract, H_2- 200ppm 8-HQC, H_3-1% sucrose + 200 ppm 8-HQC, H_4-2% sucrose + 200 ppm 8-HQC, H_5-2% sucrose + 200 ppm 8-HQC, H_6-1% sucrose+2% betel leaf extract, H_7-2% sucrose+2% betel leaf extract, H_8-3% sucrose+2% betel leaf extract, H_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

Table 11: Transpiration rate (ml/g FW) of cut roses Konfetti and Bordeaux in the holding solutions

Treatment	Transpiration rate (ml/g FW)								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
H_1	0.22	0.21	0.21	0.22	0.22	0.22	0.22	0.21	0.22
H_2	0.24	0.24	0.24	0.23	0.22	0.23	0.24	0.23	0.23
H_3	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
H_4	0.14	0.14	0.14	0.14	0.15	0.15	0.14	0.14	0.14
H_5	0.16	0.16	0.16	0.16	0.18	0.17	0.16	0.17	0.17
H_6	0.15	0.15	0.15	0.17	0.16	0.16	0.16	0.15	0.16
H_7	0.12	0.11	0.12	0.12	0.12	0.12	0.12	0.12	0.12
H_8	0.14	0.16	0.15	0.14	0.16	0.15	0.14	0.16	0.15
H_9	0.46	0.41	0.43	0.42	0.40	0.41	0.44	0.40	0.42
Mean	0.20	0.19		0.20	0.20		0.20	0.20	
	S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%	
H	0.005	0.019		0.004	0.017		0.003	0.013	
C	0.010	0.041		0.009	0.038		0.007	0.029	
H x C	0.015	0.058		0.014	0.053		0.010	0.041	

H- Holding solution , H_1-2% Betel leaf extract, H_2- 200ppm 8-HQC, H_3-1% sucrose + 200 ppm 8-HQC, H_4-2% sucrose + 200 ppm 8-HQC, H_5-2% sucrose + 200 ppm 8-HQC, H_6-1% sucrose+2% betel leaf extract, H_7-2% sucrose+2% betel leaf extract, H_8-3% sucrose+2% betel leaf extract, H_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

Table 12: Relative fresh weight of day 2 of cut roses Konfetti and Bordeaux in the holding solutions

Treatment	Relative fresh weight (%)								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
H_1	109.30	108.85	109.08	109.58	111.25	110.42	109.44	110.05	109.75
H_2	111.27	110.33	110.80	112.16	112.68	112.42	111.71	111.51	111.61
H_3	111.70	113.78	112.74	111.67	113.54	112.61	111.69	113.66	112.67
H_4	114.44	116.06	115.25	114.69	115.47	115.08	114.57	115.77	115.17
H_5	112.62	113.59	113.11	112.04	112.86	112.45	112.33	113.23	112.78
H_6	113.24	115.27	114.25	113.26	115.38	114.32	113.25	115.32	114.29
H_7	115.23	118.68	116.95	115.66	117.32	116.49	115.44	118.00	116.72
H_8	113.89	114.40	114.15	114.26	115.14	114.70	114.07	114.77	114.42
H_9	103.16	102.88	103.02	103.38	103.88	103.63	103.27	103.38	103.32
Mean	111.65	112.65		111.85	113.06		111.75	112.85	
	S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%	
H	0.281	1.083		0.600	2.307		0.325	1.252	
C	0.597	2.297		1.272	4.895		0.690	2.657	
H x C	0.844	3.249		1.800	6.923		0.977	3.757	

H- Holding solution , H_1-2% Betel leaf extract, H_2- 200ppm 8-HQC, H_3-1% sucrose + 200 ppm 8-HQC, H_4-2% sucrose + 200 ppm 8-HQC, H_5-2% sucrose + 200 ppm 8-HQC, H_6-1% sucrose+2% betel leaf extract, H_7-2% sucrose+2% betel leaf extract, H_8-3% sucrose+2% betel leaf extract, H_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

Table 13: Relative fresh weight of day 5 of cut roses Konfetti and Bordeaux in the holding solutions

Treatment	Relative fresh weight (%)								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
H_1	113.22	113.08	113.15	113.90	114.15	114.03	113.56	113.62	113.59
H_2	115.08	115.04	115.06	114.94	116.38	115.66	115.01	115.71	115.36
H_3	121.36	121.68	121.52	122.27	121.42	121.85	121.82	121.55	121.68
H_4	124.38	124.06	124.22	125.05	124.95	125.00	124.71	124.50	124.61
H_5	117.56	118.25	117.91	122.16	118.62	120.39	119.86	118.43	119.15
H_6	122.33	123.02	122.67	124.48	123.49	123.99	123.40	123.26	123.33
H_7	124.12	125.19	124.66	127.43	127.26	127.34	125.77	126.22	126.00
H_8	122.68	122.68	122.68	124.78	123.17	123.98	123.73	122.93	123.33
H_9	76.19	78.66	77.43	77.06	77.97	77.52	76.63	78.32	77.47
Mean	115.21	115.74		116.90	116.38		116.06	116.06	
	S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%	
H	0.289	1.113		0.206	0.794		0.181	0.699	
C	0.614	1.362		0.438	1.685		0.385	1.483	
H x C	0.868	3.341		0.619	2.383		0.545	2.097	

H- Holding solution , H_1-2% Betel leaf extract, H_2- 200ppm 8-HQC, H_3-1% sucrose + 200 ppm 8-HQC, H_4-2% sucrose + 200 ppm 8-HQC, H_5-2% sucrose + 200 ppm 8-HQC, H_6-1% sucrose+2% betel leaf extract, H_7-2% sucrose+2% betel leaf extract, H_8-3% sucrose+2% betel leaf extract, H_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

Table 14: Relative fresh weight on the last day of vase life of cut roses Konfetti and Bordeaux in the holding solutions

Treatment	Relative fresh weight (%)								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
H_1	72.45	73.99	73.22	73.20	72.83	73.02	72.83	73.41	73.12
H_2	73.14	75.01	74.08	71.50	72.67	72.08	72.32	73.84	73.08
H_3	77.67	78.34	78.01	74.37	77.83	76.10	76.02	78.09	77.05
H_4	79.24	80.13	79.69	76.73	75.37	76.05	77.99	77.75	77.87
H_5	77.28	79.80	78.54	74.00	79.33	76.67	75.64	79.57	77.60
H_6	79.00	81.45	80.23	78.63	80.70	79.67	78.82	81.08	79.95
H_7	81.81	84.35	83.08	80.80	86.13	83.47	81.30	85.24	83.27
H_8	78.88	82.35	80.62	79.40	82.77	81.08	79.14	82.56	80.85
H_9	67.10	69.27	68.18	68.17	71.17	69.67	67.63	70.22	68.93
Mean	76.29	78.30		75.20	77.64		75.74	77.97	
	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%
H	0.183		0.707	0.505		1.945	0.281		1.081
C	0.390		1.500	1.072		4.126	0.596		2.294
H x C	0.551		2.121	1.517		5.835	0.843		3.244

H- Holding solution , H_1-2% Betel leaf extract, H_2- 200ppm 8-HQC, H_3-1% sucrose + 200 ppm 8-HQC, H_4-2% sucrose + 200 ppm 8-HQC, H_5-2% sucrose + 200 ppm 8-HQC, H_6-1% sucrose+2% betel leaf extract, H_7-2% sucrose+2% betel leaf extract, H_8-3% sucrose+2% betel leaf extract, H_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

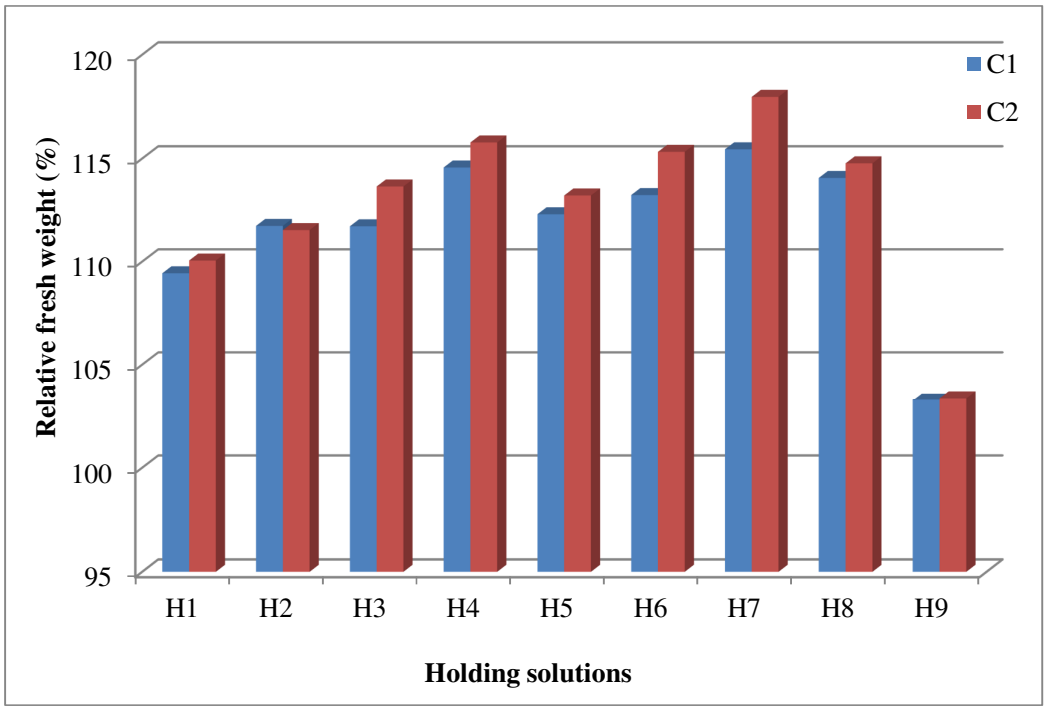


Fig. 4: Relative fresh weight of cut roses Konfetti and Bordeaux in the holding solutions on day 2

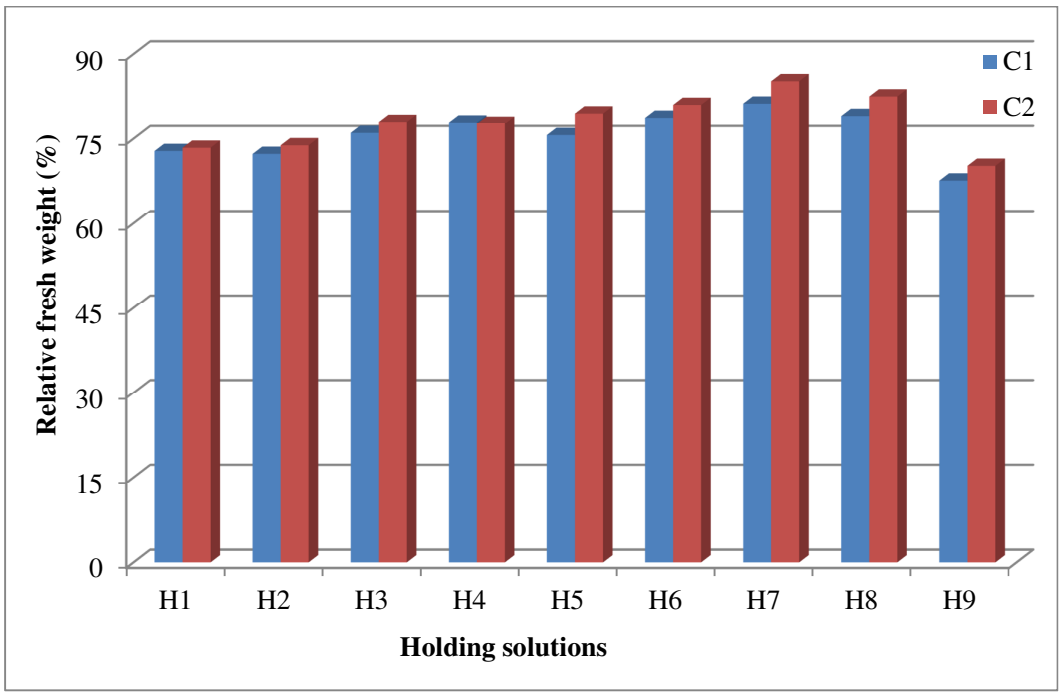


Fig. 5: Relative fresh weight of cut roses Konfetti and Bordeaux in the holding solutions on last day of vase life

(114.70%) and 1% sucrose+2% betel leaf extract (114.32%) .The interaction effects of both the cultivars and treatments was significantly different from the control with the highest value for Bordeaux +2% sucrose+ 2% betel leaf extract in 118.68%, 117.32% and 118.00% respectively in 2012, 2013 and pooled data.

On day 5, similarly, the fresh weight changes among both the cultivars were not significant in 2012 (115.21, 115.74%), 2013 (116.90, 116.38 %) and pooled data (116.06, 116.06%). 2% sucrose+2% betel leaf extract (124.66%) as well as 2% sucrose + 200 ppm 8-HQC (124.22%) had the highest fresh weight changes in 2012 where as in 2013 and pooled data the highest fresh weight changes were observed in 2% sucrose+2% betel leaf extract (127.34% and 126.00%).The lowest values were observed in the control (77.43% ,77.52% and 77.47%).The interaction effects of treatments were significant compared to control with the highest value for Bordeaux with2% sucrose+2% betel leaf extract (125.19%, 127.43% and 126.22%).

During the last day of vase life, among the cultivars, Bordeaux had a higher value (78.30%) compared to Konfetti (76.29%) in 2012 but they were not significantly different during 2013 and pooled data.2% sucrose+2% betel leaf extract exhibited greater values of 83.08%, 83.47 % and83.27% respectively and the control the lowest (68.18, 69.67 and 68.93 %) in 2012, 2013 and pooled data.

Interaction effects were significant with 2% sucrose+2% betel leaf extract (84.35, 86.13 and 83.27 %) showing higher values which was on par with 3% sucrose+2% betel leaf extract (82.35, 82.77 and 80.85 %) in 2012, 2013 and pooled data.

4.7.10 Vase life (days)

The vase life values of Konfetti and Bordeaux are indicated in Table 15, Fig. 6, Appendix 12 and Plate 25.

Concerning the vase life, among the cultivars significantly higher value was observed in Bordeaux (10.07 and 9.93 days) in 2012 and pooled data where as they were not significant in 2013.Among the treatments a significantly higher value was observed in 2% sucrose+2% betel leaf extract (11.67, 12.17and 11.92 days) followed by 2% sucrose + 200 ppm 8-HQC (10.33, 10.17 and 10.25 days) and lowest

Table 15 : Vase life of cut roses Konfetti and Bordeaux in the holding solutions

Treatment	Vase life (days)								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
H_1	9.00	10.33	9.67	9.67	10.00	9.83	9.33	10.17	9.75
H_2	8.67	9.67	9.17	8.67	9.00	8.83	8.67	9.33	9.00
H_3	8.33	10.67	9.50	8.33	9.33	8.83	8.33	10.00	9.17
H_4	9.67	11.00	10.33	10.00	10.33	10.17	9.83	10.67	10.25
H_5	7.67	9.67	8.67	8.00	9.00	8.50	7.83	9.33	8.58
H_6	9.33	10.00	9.67	9.00	9.67	9.33	9.17	9.83	9.50
H_7	11.00	12.33	11.67	11.33	13.00	12.17	11.17	12.67	11.92
H_8	9.00	10.33	9.67	7.67	10.67	9.17	8.33	10.50	9.42
H_9	6.00	6.67	6.33	6.33	7.00	6.67	6.17	6.83	6.50
Mean	8.74	10.07		8.78	9.78		8.76	9.93	
	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%
H	0.128		0.493	0.145		0.560	0.097		0.373
C	0.272		1.046	0.309		1.189	0.206		0.792
H x C	0.384		1.480	0.437		1.682	0.291		1.120

H- Holding solution , H_1-2% Betel leaf extract, H_2- 200ppm 8-HQC, H_3-1% sucrose + 200 ppm 8-HQC, H_4-2% sucrose + 200 ppm 8-HQC, H_5-2% sucrose + 200 ppm 8-HQC, H_6-1% sucrose+2% betel leaf extract, H_7-2% sucrose+2% betel leaf extract, H_8-3% sucrose+2% betel leaf extract, H_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

PLATE 25

Experimental view of Konfetti and Bordeaux held in holding solutions on 5th day of vase life

H_1.2% betel leaf extract H_2. 200ppm 8-HQC H_3. 1% sucrose and 8- HQC 200ppm

H_4.2% sucrose and 8- HQC 200ppm H_5. 3% sucrose and 8- HQC 200ppm

H_6. 1% sucrose and 2 % Betel leaf extract H_7. 2% sucrose and 2 % Betel leaf extract

H_8. 3% sucrose and 2 % Betel leaf extract H_9. Control (Tap water)

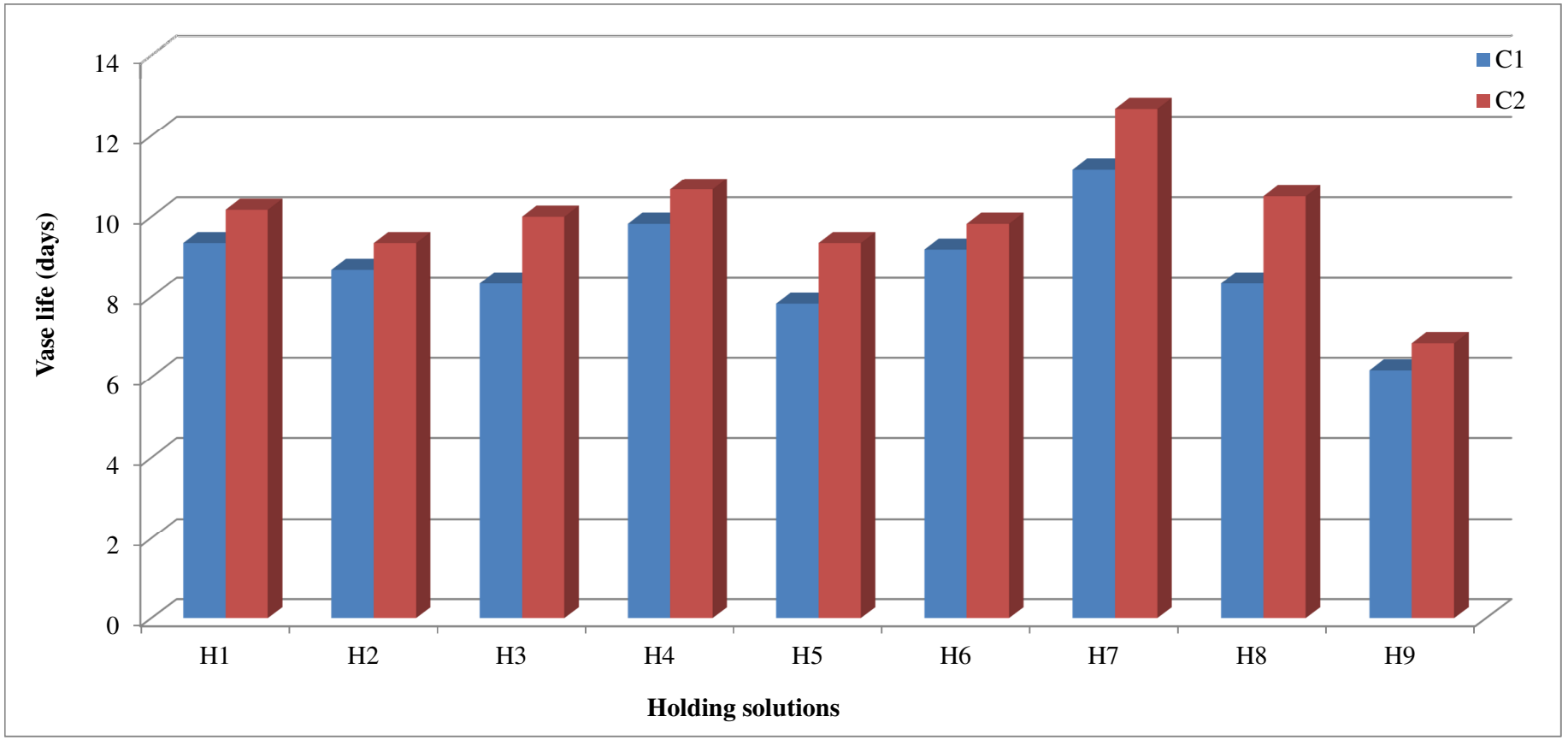


Fig. 6: Vase life of cut roses Konfetti and Bordeaux in the holding solutions

in the control treatment (6.33, 6.67 and 6.50 days) in 2012, 2013 and pooled data. The interaction effects were significant and both the cultivars treated with 2% sucrose+2% betel leaf extract were on par with each other in 2012 (11 and 12.33 days), 2013 (11.33 and 13.00 days) and pooled data (11.17 and 12.67 days).

4.7.11 Chlorophyll content

The chlorophyll content during day 2 is given in Table 16 Bordeaux (44.99 SPAD units) had higher chlorophyll content during 2012 compared to Konfetti (43.25 SPAD units) where as they were not significant in 2013 and pooled data (Appendix 13). The treatments were significantly different with a higher chlorophyll content in 2% sucrose+2% betel leaf extract in 2013 (48.50 SPAD units) and pooled data (48.45 SPAD units)where as in 2012 (47.70 SPAD units), 2% sucrose+2% betel leaf extract was similar to 2% sucrose + 200 ppm 8-HQC and the least were noticed in the control. The interaction effects were significant between the treatments and control with the highest value for interaction between Bordeaux and 2% sucrose+2% betel leaf extract in 2012 (47.70 SPAD units), 2013(49.20 SPAD units) and pooled data (48.45 SPAD units).

On day 5 (Table 17 and Appendix 14), the chlorophyll content was not significantly different between the cultivars in 2012 (37.06 and 38.58 SPAD units), 2013 (37.98 and 38.10 SPAD units) and pooled data (37.52 and 38.34 SPAD units). Among the treatments, 2% sucrose+2% betel leaf extract (43.26 SPAD units) was on par with 2% sucrose + 200 ppm 8-HQC (42.53 SPAD units) where as in 2013 and pooled data 2% sucrose+2% betel leaf extract (44.17 and 43.72 SPAD units) was the best treatment. The highest interaction effect was noticed between Bordeaux and 2% sucrose+2% betel leaf extract (44.07 and 44.17 SPAD units) in both the years and the least in the control.

On the last day of vase life (Table 18 and Appendix 15) also among the cultivars the chlorophyll content was not significant in both the years and pooled data. Between the treatments, a higher value was noticed for the treatment 2% sucrose+2% betel leaf extract in 2012 (26.47 SPAD units), 2013 (26.53 SPAD units) and pooled data (26.50 SPAD units) and the least value of 11.80, 10.57 and 11.18 SPAD units were noticed respectively in the control treatment. In the interaction

Table 16: Chlorophyll content day 2 of cut roses Konfetti and Bordeaux in the holding solutions

Treatment	Chlorophyll content (SPAD unit)								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
H_1	43.97	45.77	44.87	44.23	44.33	44.28	44.10	45.05	44.58
H_2	44.17	46.33	45.25	44.43	44.80	44.62	44.30	45.57	44.93
H_3	44.53	46.10	45.32	43.90	45.43	44.67	44.22	45.77	44.99
H_4	45.63	47.17	46.40	46.67	47.83	47.25	46.15	47.50	46.83
H_5	44.70	46.50	45.60	44.97	44.63	44.80	44.83	45.57	45.20
H_6	45.03	46.03	45.53	46.63	47.27	46.95	45.83	46.65	46.24
H_7	45.87	47.70	46.78	47.80	49.20	48.50	46.83	48.45	47.64
H_8	44.47	46.13	45.30	44.80	47.27	46.03	44.63	46.70	45.67
H_9	30.90	33.17	32.03	30.70	31.37	31.03	30.80	32.27	31.53
Mean	43.25	44.99		43.79	44.68		43.52	44.84	
	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%
H	0.183		0.707	0.255		0.981	0.143		0.550
C	0.390		1.501	0.541		2.082	0.303		1.168
H x C	0.551		2.122	0.765		2.944	0.429		1.651

H- Holding solution, H_1-2% Betel leaf extract, H_2- 200ppm 8-HQC, H_3-1% sucrose + 200 ppm 8-HQC, H_4-2% sucrose + 200 ppm 8-HQC, H_5-2% sucrose + 200 ppm 8-HQC, H_6-1% sucrose+2% betel leaf extract, H_7-2% sucrose+2% betel leaf extract, H_8-3% sucrose+2% betel leaf extract, H_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

Table 17: Chlorophyll content on day 5 of cut roses Konfetti and Bordeaux in the holding solutions

Treatment	Chlorophyll content (SPAD unit)								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
H_1	37.27	38.80	38.03	38.66	37.70	38.18	37.96	38.25	38.11
H_2	38.90	40.97	39.93	39.57	39.70	39.63	39.23	40.33	39.78
H_3	37.33	39.53	38.43	40.73	40.03	40.38	39.03	39.78	39.41
H_4	41.60	43.47	42.53	42.80	43.03	42.92	42.20	43.25	42.73
H_5	39.70	41.43	40.57	40.83	39.80	40.32	40.27	40.62	40.44
H_6	40.67	42.13	41.40	40.17	41.00	40.58	40.42	41.57	40.99
H_7	42.46	44.07	43.26	43.60	44.73	44.17	43.03	44.40	43.72
H_8	40.30	41.07	40.68	39.70	41.37	40.53	40.00	41.22	40.61
H_9	15.33	15.77	15.55	15.73	15.50	15.62	15.53	15.63	15.58
Mean	37.06	38.58		37.98	38.10		37.52	38.34	
	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%
H	0.333		1.283	0.258		0.994	0.198		0.762
C	0.707		2.722	0.548		2.109	0.420		1.618
H x C	1.000		3.849	0.775		2.982	0.595		2.288

H- Holding solution , H_1-2% Betel leaf extract, H_2- 200ppm 8-HQC, H_3-1% sucrose + 200 ppm 8-HQC, H_4-2% sucrose + 200 ppm 8-HQC, H_5-2% sucrose + 200 ppm 8-HQC, H_6-1% sucrose+2% betel leaf extract, H_7-2% sucrose+2% betel leaf extract, H_8-3% sucrose+2% betel leaf extract, H_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

Table 18: Chlorophyll content at end of vase life of cut roses Konfetti and Bordeaux in the holding solutions

Treatment	Chlorophyll content (SPAD unit)								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
H_1	18.03	19.03	18.53	16.90	16.73	16.82	17.47	17.88	17.68
H_2	19.23	20.17	19.70	17.10	18.20	17.65	18.17	19.18	18.68
H_3	20.77	21.37	21.07	19.53	20.67	20.10	20.15	21.02	20.58
H_4	24.20	25.23	24.72	25.43	25.27	25.35	24.82	25.25	25.03
H_5	21.53	22.73	22.13	22.77	23.10	22.93	22.15	22.92	22.53
H_6	21.30	23.70	22.50	22.33	22.73	22.53	21.82	23.22	22.52
H_7	26.30	26.63	26.47	27.13	25.93	26.53	26.72	26.28	26.50
H_8	22.17	23.67	22.92	23.47	23.43	23.45	22.82	23.55	23.18
H_9	11.87	11.73	11.80	10.50	10.63	10.57	11.18	11.18	11.18
Mean	20.60	21.59		20.57	20.74		20.59	21.16	
	S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%	
H	0.247	0.952		0.218	0.839		0.162	0.626	
C	0.525	2.019		0.462	1.780		0.345	1.328	
H x C	0.742	2.856		0.654	2.517		0.488	1.878	

H- Holding solution , H_1-2% Betel leaf extract, H_2- 200ppm 8-HQC, H_3-1% sucrose + 200 ppm 8-HQC, H_4-2% sucrose + 200 ppm 8-HQC, H_5-2% sucrose + 200 ppm 8-HQC, H_6-1% sucrose+2% betel leaf extract, H_7-2% sucrose+2% betel leaf extract, H_8-3% sucrose+2% betel leaf extract, H_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

effects, in 2012 and 2013 the highest value was noticed in 2% sucrose+2% betel leaf extract (26.63, 26.30 SPAD units) and 2% sucrose + 200 ppm 8-HQC (27.13,25.93 SPAD units)both in Bordeaux and Konfetti.

4.7.12 Freshness of Leaves and Petals

The freshness of leaves and petals are depicted in the Table 19 and Appendix 16. There were no significant differences between Bordeaux and Konfetti with respect to freshness of leaves and petals in 2012 (4.04 and 4.15), 2013 (4.07 and 4.19) and pooled data (4.06 and 4.17).The treatments 2% sucrose+2% betel leaf extract (5.00, 5.00), 3% sucrose+2% betel leaf extract (5.00, 5.00) and 2% sucrose + 200 ppm 8-HQC (5.00)were on par with each other in 2012 and 2013 while 2% sucrose+2% betel leaf extract (5.00), 3% sucrose+2% betel leaf extract (5.00), 1% sucrose+2% betel leaf extract (5.00) and 2% sucrose + 200 ppm 8-HQC (5.00) were similar to each other. Interaction effects were highest between Konfetti and Bordeaux and treatments 2% sucrose+2% betel leaf extract (5.00), 3% sucrose+2% betel leaf extract (5.00) and 2% sucrose + 200 ppm 8-HQC (5.00).

4.7.13 Blueing of petals

Blueing of petals (Table 20 and Appendix 17) were noticed only in Bordeaux in 2012 (0.67), 2013 (0.78) and pooled data (0.72).Very less blueing were noticed in the treatments 1% sucrose+2% betel leaf extract, 2% sucrose+2% betel leaf extract, 3% sucrose+2% betel leaf extract, 2% sucrose + 200 ppm 8-HQC and 2% sucrose + 200 ppm 8-HQC which were all on par with each other and had a score of 0.17 in 2012. In 2013 and in pooled data 2% sucrose+2% betel leaf extract (0.17) as well as 2% sucrose + 200 ppm 8-HQC (0.17) had scores similar to each other. Interactions between Bordeaux and the treatments also followed the same pattern as that of treatments with a score of 0.33.

4.7.14 Bent neck

It is evident from Table 21 that all the treatments and both the cultivars resulted in bent-neck free flowers in both the years i.e. 2012 and 2013, except the flowers which were kept in control (tap water).

Table 19: Freshness of leaves and petal of cut roses Konfetti and Bordeaux in the holding solutions

Treatment	Freshness of leaves and petal								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
H_1	3.00	3.33	3.17	3.33	3.67	3.50	3.17	3.50	3.33
H_2	3.67	3.67	3.67	3.33	3.67	3.50	3.50	3.67	3.58
H_3	4.33	4.67	4.50	4.33	4.67	4.50	4.33	4.67	4.50
H_4	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
H_5	4.67	4.67	4.67	4.67	4.67	4.67	4.67	4.67	4.67
H_6	4.67	4.67	4.67	5.00	5.00	5.00	4.83	4.83	4.83
H_7	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
H_8	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
H_9	1.00	1.33	1.17	1.00	1.00	1.00	1.00	1.17	1.08
Mean	4.04	4.15		4.07	4.19		4.06	4.17	
	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%
H	0.082		0.318	0.074		0.284	0.050		0.195
C	0.175		0.675	0.157		0.604	0.107		0.413
H x C	0.248		0.955	0.222		0.854	0.152		0.585

H- Holding solution , H_1-2% Betel leaf extract, H_2- 200ppm 8-HQC, H_3-1% sucrose + 200 ppm 8-HQC, H_4-2% sucrose + 200 ppm 8-HQC, H_5-2% sucrose + 200 ppm 8-HQC, H_6-1% sucrose+2% betel leaf extract, H_7-2% sucrose+2% betel leaf extract, H_8-3% sucrose+2% betel leaf extract, H_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

Table 20: Blueing of petals of cut roses Konfetti and Bordeaux in the holding solutions

Treatment	Blueing of petals								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
H_1	0.00	1.00	0.50	0.00	0.67	0.33	0.00	0.83	0.42
H_2	0.00	1.00	0.50	0.00	1.00	0.50	0.00	1.00	0.50
H_3	0.00	0.67	0.33	0.00	0.67	0.33	0.00	0.67	0.33
H_4	0.00	0.33	0.17	0.00	0.33	0.17	0.00	0.33	0.17
H_5	0.00	0.33	0.17	0.00	0.67	0.33	0.00	0.50	0.25
H_6	0.00	0.33	0.17	0.00	0.67	0.33	0.00	0.50	0.25
H_7	0.00	0.33	0.17	0.00	0.33	0.17	0.00	0.33	0.17
H_8	0.00	0.33	0.17	0.00	0.67	0.33	0.00	0.50	0.25
H_9	0.00	1.67	0.83	0.00	2.00	1.00	0.00	1.83	0.92
Mean	0.00	0.67		0.00	0.78		0.00	0.72	
	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%
H	0.069		0.266	0.069		0.266	0.048		0.188
C	0.146		0.565	0.146		0.565	0.103		0.399
H x C	0.207		0.799	0.207		0.799	0.146		0.565

H- Holding solution , H_1-2% Betel leaf extract, H_2- 200ppm 8-HQC, H_3-1% sucrose + 200 ppm 8-HQC, H_4-2% sucrose + 200 ppm 8-HQC, H_5-2% sucrose + 200 ppm 8-HQC, H_6-1% sucrose+2% betel leaf extract, H_7-2% sucrose+2% betel leaf extract, H_8-3% sucrose+2% betel leaf extract, H_9-Control (tap water)C- Cultivar, C_1- Konfetti, C_2- Bordeaux

Table 21: Bent neck of cut roses Konfetti and Bordeaux in the holding solutions

Treatment	Year 2012		Year 2013	
	C_1	C_2	C_1	C_2
H_1	Unbent	Unbent	Unbent	Unbent
H_2	Unbent	Unbent	Unbent	Unbent
H_3	Unbent	Unbent	Unbent	Unbent
H_4	Unbent	Unbent	Unbent	Unbent
H_5	Unbent	Unbent	Unbent	Unbent
H_6	Unbent	Unbent	Unbent	Unbent
H_7	Unbent	Unbent	Unbent	Unbent
H_8	Unbent	Unbent	Unbent	Unbent
H_9	Bent	Bent	Bent	Bent

H_1-2% Betel leaf extract, H_2- 200ppm 8-HQC, H_3-1% sucrose + 200 ppm 8-HQC, H_4-2% sucrose + 200 ppm 8-HQC, H_5-2% sucrose + 200 ppm 8-HQC, H_6-1% sucrose+2% betel leaf extract, H_7-2% sucrose+2% betel leaf extract, H_8-3% sucrose+2% betel leaf extract, H_9-Control (tap water)
C_1- Konfetti, C_2- Bordeaux

4.7.15 Total soluble solids (TSS) (%)

The cultivars did not show any significant differences as depicted in the Table 22 and Appendix 18. The treatments were significantly different with the highest value for the treatment 2% sucrose+2% betel leaf extract in 2012 (5.91%), 2013 (6.97%) and pooled data (6.44%) and least in control treatment (3.40, 3.61 and 3.53%). Among the interactions in 2012 the treatment 2% sucrose+2% betel leaf extract was equally effective for both the cultivars and recorded highest values of 6.30 and 5.52%.In 2013 and pooled data Bordeaux+2% sucrose+2% betel leaf extract had highest values of 7.52 and 6.91% respectively. The lowest values were noticed `in the control (3.30, 3.50; 3.92, 3.39 and3.61, 3.45%).

4.7.16 pH of the vase solution

The lowest pH was obtained for 2% sucrose+200ppm 8-HQC (3.68) where as highest pH was obtained for tap water (7.2) as shown in the Table 23. 2% sucrose +2% betel leaf extract had a value of 3.85.

4.8 Effect of pulsing solutions on the quality and longevity of cut roses

4.8.1 Initial flower-bud diameter (cm)

Among the cultivars there was significant difference with respect to initial flower bud diameter as shown in Table 24. Bordeaux showed a greater value compared to Konfetti in 2012 (3.79, 3.44 cm), 2013 (3.79, 3.43 cm) and pooled data (3.79, 3.44 cm). Among the treatments, in 2012, there was no significant difference where as in 2013 all treatments except P_2, P_5 and P_7 were on par with each other. Similarly in the pooled data also all treatments except P_5 and P_7 were on par with each other (Appendix 19).

4.8.2 Final flower diameter (cm)

The data regarding the final flower diameter is given in Table 25 and Fig. 7. Among the cultivars there was significant difference with respect to final flower bud diameter. Bordeaux showed a greater value compared to Konfetti in 2012 (8.04, 7.83 cm), 2013 (7.82, 7.80 cm) and pooled data (7.93, 7.81 cm).The treatments were significant with respect to control with the highest value for 6% sucrose+2% betel leaf extract in 2012 (9.97 cm), 2013 (9.68 cm) and pooled data (9.82cm) and lowest

Table 22: TSS of cut roses Konfetti and Bordeaux in the holding solutions (day 3)

Treatment	TSS (%)								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
H_1	4.39	3.69	4.04	4.36	3.86	4.11	4.38	3.78	4.08
H_2	4.00	3.88	3.94	4.64	4.11	4.37	4.32	4.00	4.16
H_3	4.83	4.77	4.80	5.07	4.90	4.98	4.95	4.84	4.89
H_4	5.16	5.02	5.09	5.74	4.47	5.11	5.45	4.75	5.10
H_5	5.39	5.17	5.28	5.49	5.40	5.44	5.44	5.29	5.36
H_6	5.04	5.40	5.22	5.49	5.32	5.40	5.26	5.36	5.31
H_7	5.52	6.30	5.91	6.42	7.52	6.97	5.97	6.91	6.44
H_8	5.02	5.63	5.32	5.21	5.32	5.26	5.12	5.47	5.29
H_9	3.30	3.50	3.40	3.92	3.39	3.66	3.61	3.45	3.53
Mean	4.74	4.82		5.15	4.92		4.94	4.87	
	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%
H	0.074		0.284	0.077		0.297	0.050		0.195
C	0.157		0.603	0.164		0.631	0.107		0.415
H x C	0.222		0.854	0.232		0.892	0.152		0.586

H- Holding solution , H_1-2% Betel leaf extract, H_2- 200ppm 8-HQC, H_3-1% sucrose + 200 ppm 8-HQC, H_4-2% sucrose + 200 ppm 8-HQC, H_5-2% sucrose + 200 ppm 8-HQC, H_6-1% sucrose+2% betel leaf extract, H_7-2% sucrose+2% betel leaf extract, H_8-3% sucrose+2% betel leaf extract, H_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

Table 23: Initial pH of vase solution of cut roses Konfetti and Bordeaux in the holding solution

Holding solutions	pH
2% Betel leaf extract	4.00
200ppm 8-HQC	3.80
1% sucrose + 200 ppm 8-HQC	3.84
2% sucrose + 200 ppm 8-HQC	3.68
3% sucrose + 200 ppm 8-HQC	3.75
1% sucrose+2% betel leaf extract	3.94
2% sucrose+2% betel leaf extract	3.85
3% sucrose+2% betel leaf extract	3.88
Control (tap water)	7.20

Table 24: Initial flower diameter of cut roses Konfetti and Bordeaux in the pulsing solutions

Treatment	Initial flower diameter (cm)								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
P_1	3.45	3.81	3.63	3.46	3.82	3.64	3.46	3.82	3.64
P_2	3.43	3.80	3.62	3.39	3.76	3.58	3.41	3.78	3.60
P_3	3.45	3.77	3.61	3.38	3.85	3.61	3.42	3.81	3.61
P_4	3.43	3.81	3.62	3.47	3.82	3.64	3.45	3.82	3.63
P_5	3.43	3.78	3.60	3.42	3.71	3.56	3.42	3.75	3.58
P_6	3.48	3.71	3.60	3.42	3.80	3.61	3.45	3.76	3.60
P_7	3.40	3.80	3.60	3.45	3.73	3.59	3.42	3.77	3.59
P_8	3.47	3.78	3.63	3.41	3.79	3.60	3.44	3.79	3.61
P_9	3.47	3.81	3.64	3.45	3.86	3.65	3.46	3.84	3.65
Mean	3.44	3.79		3.43	3.79		3.44	3.79	
	S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%	
P	0.020	0.077		0.016	0.062		0.014	0.057	
C	0.042	0.164		0.034	0.132		0.031	0.121	
P x C	0.060	0.232		0.048	0.187		0.044	0.171	

P- Pulsing solution , P_1-2% Betel leaf extract, P_2- 200ppm 8-HQC, P_3-3% sucrose + 200 ppm 8-HQC, P_4-6% sucrose + 200 ppm 8-HQC, P_5-9% sucrose + 200 ppm 8-HQC, P_6-3% sucrose+2% betel leaf extract, P_7-6% sucrose+2% betel leaf extract, P_8-9% sucrose+2% betel leaf extract, P_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

Table 25: Final flower diameter of cut roses Konfetti and Bordeaux in the pulsing solutions

Treatment	Final flower diameter (cm)								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
P_1	7.45	7.00	7.23	7.70	7.22	7.46	7.58	7.11	7.34
P_2	6.63	6.06	6.35	6.66	6.35	6.50	6.65	6.20	6.42
P_3	8.78	7.88	8.33	8.37	8.06	8.21	8.57	7.97	8.27
P_4	8.35	8.11	8.23	8.39	8.27	8.33	8.37	8.19	8.28
P_5	9.13	8.90	9.02	8.96	8.88	8.92	9.05	8.89	8.97
P_6	8.88	8.68	8.78	8.18	8.54	8.36	8.53	8.61	8.57
P_7	9.72	10.21	9.97	9.28	10.08	9.68	9.50	10.14	9.82
P_8	9.32	8.69	9.01	8.90	8.65	8.78	9.11	8.67	8.89
P_9	4.09	4.89	4.49	3.93	4.16	4.04	4.01	4.53	4.27
Mean	8.04	7.83		7.82	7.80		7.93	7.81	
	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%
P	0.089		0.343	0.072		0.280	0.049		0.191
C	0.189		0.728	0.154		0.594	0.105		0.406
P x C	0.267		1.030	0.218		0.840	0.149		0.575

P- Pulsing solution , P_1-2% Betel leaf extract, P_2- 200ppm 8-HQC, P_3-3% sucrose + 200 ppm 8-HQC, P_4-6% sucrose + 200 ppm 8-HQC, P_5-9% sucrose + 200 ppm 8-HQC, P_6-3% sucrose+2% betel leaf extract, P_7-6% sucrose+2% betel leaf extract, P_8-9% sucrose+2% betel leaf extract, P_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

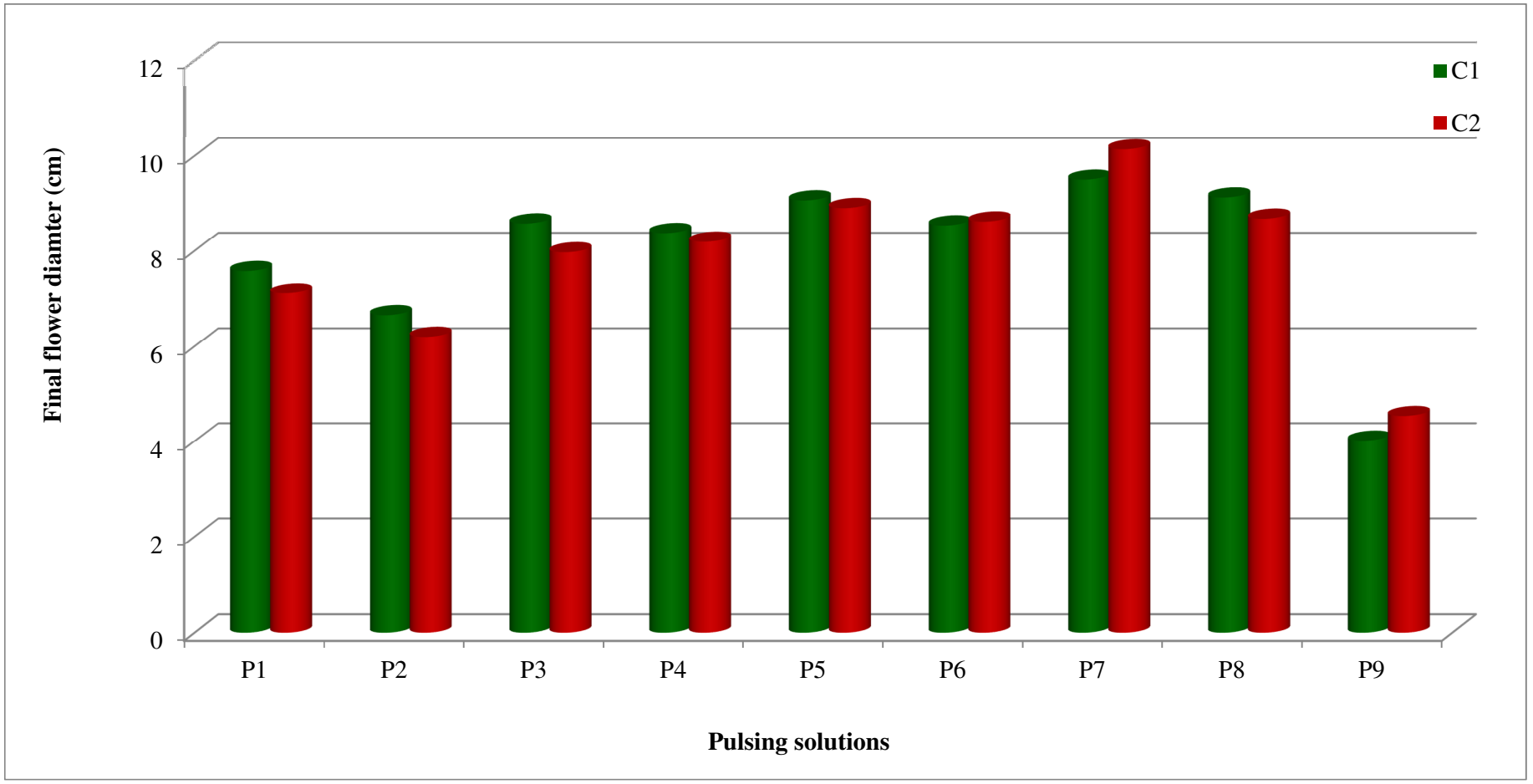


Fig. 7: Final flower diameter of cut roses Konfetti and Bordeaux in the pulsing solutions

were seen in the control (4.49, 4.04 and 4.27 cm).In 2012, the highest interactions were noticed in 6% sucrose+2% betel leaf extract + both Konfetti (9.72 cm) and Bordeaux (10.21 cm) and9% sucrose+2% betel leaf extract with Konfetti (9.32 cm) where as in 2013 the highest interactions were noticed in 6% sucrose+2% betel leaf extract + both Konfetti (9.28 cm) and Bordeaux (10.08 cm) (Appendix20).

4.8.3 Opening of flower bud

The scores obtained for the opening of flower bud is given in Table 26. The cultivars did not have any significant difference between them in both the years .Among the treatments, in 2012, P_5 (4.00), P_6 (4.00) and P_7 (4.33) were on par with each other where as in 2013, P_4 (3.83), P_5 (4.17), P_6 (3.83) and P_7 (4.17) were on par with each other. The interaction effects were significant between treatments and the control with the control having the least value for both the cultivars (Appendix 21).

4.8.4 Number of days to reach maximum flower diameter

Among the cultivars (Table 27 and Appendix 22) it was not significant. Among the treatments both P_5 and P_7 were highly effective in 2012 (12.17, 11.50 days), 2013 (11.33, 9.67 days) and pooled data (11.75, 10.58 days).

4.8.5 Water uptake (g/stem)

The total water uptake is given in Table 28, Fig. 8 and Appendix 23 which indicates that among the cultivars the water uptake was higher in Bordeaux than in Konfetti in 2012 (36.11 and 33.96 g/stem), 2013 (36.56 and 33.60 g/stem) and pooled data (36.33 and 33.78 g/stem). Among the treatments, the highest value was noticed in the treatment 6% sucrose+2% betel leaf extract in 2012 (45.34 g/stem), 2013 (45.73 g/stem) and pooled data (45.54 g/stem) while the lowest was noticed in the control treatment (9.45,9.97 and 9.71g/stem). The highest interaction effects were noticed between Bordeaux and 6% sucrose+2% betel leaf extract (47.18, 458.02 and 47.60 g/stem) and lowest in the control treatment of Konfetti (9.02, 9.75 and 9.38 g/stem).

4.8.6 Water loss (g/ stem)

The data regarding the water loss is given in Table 29 and Fig. 9. Amongst the cultivars Bordeaux recorded a higher value than Konfetti in 2012 (35.67 and

Table 26: Opening of flower bud of cut roses Konfetti and Bordeaux in the pulsing solutions

Treatment	Opening of flower bud								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
P_1	3.33	3.33	3.33	3.33	3.67	3.50	3.33	3.50	3.42
P_2	3.00	3.00	3.00	3.33	3.33	3.33	3.17	3.17	3.17
P_3	3.33	3.67	3.50	3.33	3.67	3.50	3.33	3.67	3.50
P_4	3.67	4.00	3.83	4.00	3.67	3.83	3.83	3.83	3.83
P_5	3.67	4.33	4.00	4.00	4.33	4.17	3.83	4.33	4.08
P_6	3.67	4.33	4.00	3.67	4.00	3.83	3.67	4.17	3.92
P_7	4.00	4.67	4.33	4.00	4.33	4.17	4.00	4.50	4.25
P_8	3.67	4.00	3.83	3.67	3.67	3.67	3.67	3.83	3.75
P_9	1.00	1.67	1.33	1.33	2.00	1.67	1.17	1.83	1.50
Mean	3.26	3.67		3.41	3.63		3.33	3.65	
	S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%	
P	0.090	0.349		0.094	0.363		0.068	0.261	
C	0.192	0.740		0.200	0.770		0.144	0.555	
P x C	0.272	1.046		0.283	1.089		0.204	0.785	

P- Pulsing solution , P_1-2% Betel leaf extract, P_2- 200ppm 8-HQC, P_3-3% sucrose + 200 ppm 8-HQC, P_4-6% sucrose + 200 ppm 8-HQC, P_5-9% sucrose + 200 ppm 8-HQC, P_6-3% sucrose+2% betel leaf extract, P_7-6% sucrose+2% betel leaf extract, P_8-9% sucrose+2% betel leaf extract, P_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

Table 27: Days to maximum flower diameter of cut roses Konfetti and Bordeaux in the pulsing solutions

Treatment	Days to maximum flower diameter (days)								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
P_1	7.33	8.33	7.83	8.00	8.67	8.33	7.67	8.50	8.08
P_2	7.00	7.67	7.33	7.67	8.00	7.83	7.33	7.83	7.58
P_3	8.00	9.33	8.67	9.67	9.00	9.33	8.83	9.17	9.00
P_4	9.33	10.00	9.67	9.33	8.67	9.00	9.33	9.33	9.33
P_5	11.00	12.00	11.50	9.67	9.67	9.67	10.33	10.83	10.58
P_6	9.00	10.00	9.50	9.00	9.67	9.33	9.00	9.83	9.42
P_7	11.33	13.00	12.17	10.67	12.00	11.33	11.00	12.50	11.75
P_8	9.33	11.00	10.17	10.00	10.00	10.00	9.67	10.50	10.08
P_9	2.33	2.67	2.50	3.00	2.67	2.83	2.67	2.67	2.67
Mean	8.30	9.33		8.56	8.70		8.43	9.02	
	S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%	
P	0.202	0.780		0.199	0.767		0.130	0.503	
C	0.430	1.655		0.423	1.627		0.277	1.068	
P x C	0.608	2.340		0.598	2.301		0.392	1.510	

P- Pulsing solution , P_1-2% Betel leaf extract, P_2- 200ppm 8-HQC, P_3-3% sucrose + 200 ppm 8-HQC, P_4-6% sucrose + 200 ppm 8-HQC, P_5-9% sucrose + 200 ppm 8-HQC, P_6-3% sucrose+2% betel leaf extract, P_7-6% sucrose+2% betel leaf extract, P_8-9% sucrose+2% betel leaf extract, P_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

Table 28: Total water uptake (g/stem) of cut roses Konfetti and Bordeaux in the pulsing solutions

Treatment	Total water uptake (g/stem)								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
P_1	28.65	31.13	29.89	28.20	30.96	29.58	28.42	31.05	29.73
P_2	26.45	28.01	27.23	26.10	29.51	27.81	26.27	28.76	27.52
P_3	35.27	38.79	37.03	34.83	39.35	37.09	35.05	39.07	37.06
P_4	37.09	42.82	39.95	36.63	43.65	40.14	36.86	43.24	40.05
P_5	41.50	43.87	42.68	40.72	43.83	42.27	41.11	43.85	42.48
P_6	41.77	39.80	40.78	40.56	40.14	40.35	41.17	39.97	40.57
P_7	43.50	47.18	45.34	43.45	48.02	45.73	43.48	47.60	45.54
P_8	42.41	43.54	42.98	42.20	43.35	42.78	42.31	43.45	42.88
P_9	9.02	9.87	9.45	9.75	10.18	9.97	9.38	10.03	9.71
Mean	33.96	36.11		33.60	36.56		33.78	36.33	
	S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%	
P	0.235	0.906		0.279	1.075		0.230	0.885	
C	0.499	1.922		0.592	2.280		0.488	1.878	
P x C	0.706	2.719		0.838	3.225		0.690	2.656	

P- Pulsing solution , P_1-2% Betel leaf extract, P_2- 200ppm 8-HQC, P_3-3% sucrose + 200 ppm 8-HQC, P_4-6% sucrose + 200 ppm 8-HQC, P_5-9% sucrose + 200 ppm 8-HQC, P_6-3% sucrose+2% betel leaf extract, P_7-6% sucrose+2% betel leaf extract, P_8-9% sucrose+2% betel leaf extract, P_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

Table 29: Total water loss (g/ stem) of cut roses Konfetti and Bordeaux in the pulsing solutions

Treatment	Total water loss (g)								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
P_1	28.23	30.67	29.45	27.70	30.41	29.06	27.97	30.54	29.25
P_2	26.12	27.62	26.87	25.67	36.75	31.21	25.90	32.19	29.04
P_3	34.71	38.13	36.42	34.25	38.74	36.50	34.48	38.43	36.46
P_4	36.43	42.19	39.31	35.97	42.96	39.47	36.20	42.58	39.39
P_5	40.71	42.97	41.84	39.91	42.95	41.43	40.31	42.96	41.63
P_6	41.07	39.09	40.08	39.80	39.46	39.63	40.44	39.28	39.86
P_7	42.50	46.21	44.36	42.40	46.99	44.70	42.45	46.60	44.53
P_8	41.77	42.79	42.28	41.38	42.53	41.96	41.58	42.66	42.12
P_9	10.59	11.37	10.98	11.84	11.89	11.87	11.22	11.63	11.42
Mean	33.57	35.67		33.22	36.96		33.39	36.32	
	S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%	
P	0.236	0.910		0.382	1.470		0.252	0.972	
C	0.502	1.931		0.811	3.119		0.536	2.063	
P x C	0.710	2.731		1.147	4.411		0.758	2.918	

P- Pulsing solution , P_1-2% Betel leaf extract, P_2- 200ppm 8-HQC, P_3-3% sucrose + 200 ppm 8-HQC, P_4-6% sucrose + 200 ppm 8-HQC, P_5-9% sucrose + 200 ppm 8-HQC, P_6-3% sucrose+2% betel leaf extract, P_7-6% sucrose+2% betel leaf extract, P_8-9% sucrose+2% betel leaf extract, P_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

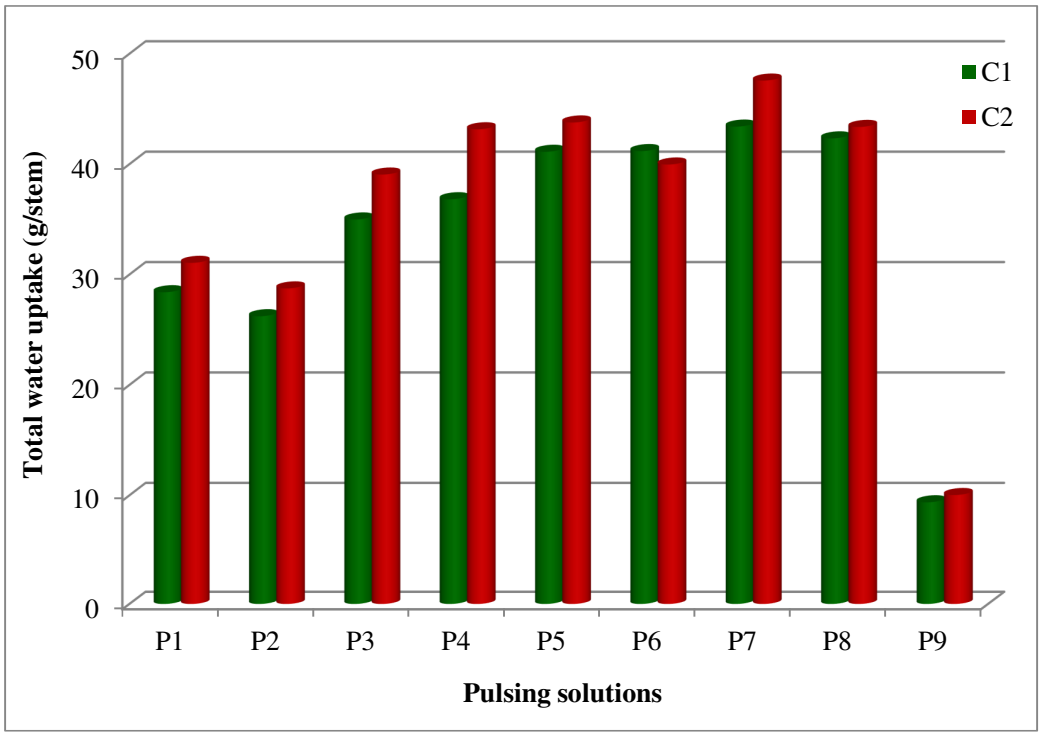


Fig. 8: Total water uptake of cut roses Konfetti and Bordeaux in the pulsing solutions

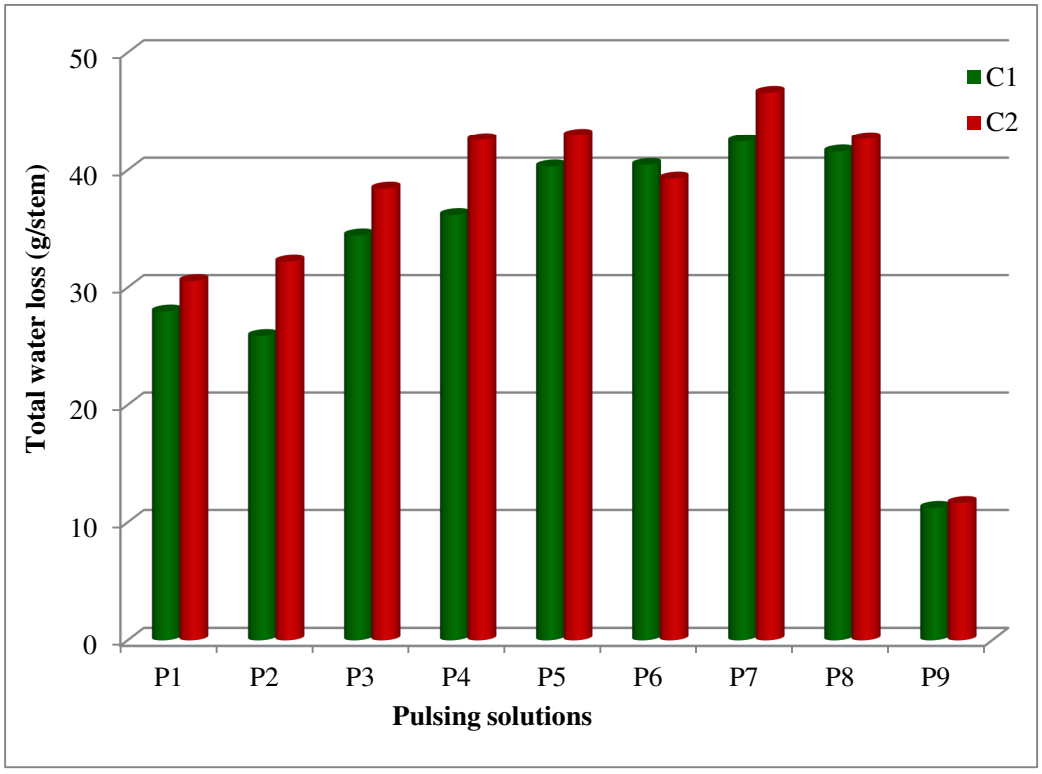


Fig. 9: Total water loss of cut roses Konfetti and Bordeaux in the pulsing solutions

33.57 g/ stem), 2013 (36.96 and 33.22 g/ stem) and pooled data (36.32 and 33.39 g/ stem). 6% sucrose+2% betel leaf extract recorded highest value in 2012 (44.36 g/ stem), 2013(44.70 g/ stem) and pooled data (44.53 g/ stem) and lowest in control (tap water).Interaction effects were highest among Bordeaux and 6% sucrose+2% betel leaf extract with a value of 46.21, 46.99 and 46.60 g/ stem respectively in 2012, 2013 and pooled data (Appendix 24).

4.8.7 Water balance

The data regarding water balance are presented in Table 30 and Appendix 25. Between the cultivars, the values were not significant but significant results were obtained for the treatments with highest values for 6% sucrose+2% betel leaf extract in 2012 (0.99), 2013 (1.04) and pooled data (1.01). The least values were obtained for the control (-1.53, -1.90 and -1.72). In the pooled data the highest interaction effects was noticed in 6% sucrose+2% betel leaf extract of both Bordeaux and Konfetti as well as 9% sucrose +200ppm 8-HQC (0.89) and lowest in the control treatments (-1.83 and -1.60).

4.8.8 Transpiration rate (ml/g FW)

The transpiration rate data are shown in Table 31 and Appendix 26. The data was not significant between the cultivars in 2012 (0.22,0.21 ml/g FW), 2013 (0.22, 0.20 ml/g FW) and pooled data (0.22, 0.20 ml/g FW).The highest values were obtained for the control and lowest for the treatment 6% sucrose+2% betel leaf extract in 2012 (0.2 and 0.14 ml/g FW), 2013 (0.41,0.14 ml/g FW) and pooled data (0.42,0.14 ml/g FW). In interaction effects the highest values were obtained in control + Konfetti (0.45, 0.44 and 0.45 ml/g FW) in both the years and pooled data.

4.8.9 Fresh weight changes (%)

Perusal of data presented in Table 32 and Fig. 10 reveals that there was a significant difference between cultivars concerning the relative fresh weight changes on day 2.A better value was obtained for Bordeaux compared to Konfetti in 2012 (114.25, 111.69%), 2013 (115.14,111.55%) and pooled data (114.70, 111.62%). As per the results the best treatment was 6% sucrose+2% betel leaf extract (119.13, 119.58 and 119.35%) and the worst was of that of the control (102.94, 104.20 and

Table 30: Water Balance of cut roses Konfetti and Bordeaux in the pulsing solutions

Treatment	Water Balance								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
P_1	0.42	0.46	0.44	0.49	0.54	0.52	0.46	0.50	0.48
P_2	0.34	0.39	0.37	0.43	0.39	0.41	0.39	0.39	0.39
P_3	0.56	0.66	0.61	0.58	0.61	0.60	0.57	0.64	0.60
P_4	0.66	0.63	0.64	0.66	0.69	0.68	0.66	0.66	0.66
P_5	0.77	0.90	0.84	0.81	0.88	0.84	0.79	0.89	0.84
P_6	0.70	0.71	0.70	0.76	0.68	0.72	0.73	0.70	0.71
P_7	1.00	0.97	0.99	1.05	1.02	1.04	1.03	1.00	1.01
P_8	0.64	0.75	0.70	0.82	0.82	0.82	0.73	0.78	0.76
P_9	-1.57	-1.50	-1.53	-2.10	-1.70	-1.90	-1.83	-1.60	-1.72
Mean	0.39	0.44		0.39	0.44		0.39	0.44	
	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%
P	0.020		0.080	0.035		0.135	0.016		0.061
C	0.044		0.171	0.074		0.287	0.034		0.130
P x C	0.062		0.241	0.105		0.406	0.048		0.185

P- Pulsing solution , P_1-2% Betel leaf extract, P_2- 200ppm 8-HQC, P_3-3% sucrose + 200 ppm 8-HQC, P_4-6% sucrose + 200 ppm 8-HQC, P_5-9% sucrose + 200 ppm 8-HQC, P_6-3% sucrose+2% betel leaf extract, P_7-6% sucrose+2% betel leaf extract, P_8-9% sucrose+2% betel leaf extract, P_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

Table 31: Transpiration rate of cut roses Konfetti and Bordeaux in the pulsing solutions

Treatment	Transpiration rate (ml/g FW)								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
P_1	0.23	0.24	0.24	0.24	0.21	0.23	0.24	0.23	0.23
P_2	0.25	0.25	0.25	0.25	0.23	0.24	0.25	0.24	0.24
P_3	0.20	0.20	0.20	0.20	0.19	0.20	0.20	0.20	0.20
P_4	0.19	0.18	0.19	0.18	0.17	0.18	0.19	0.17	0.18
P_5	0.15	0.15	0.15	0.16	0.13	0.15	0.16	0.14	0.15
P_6	0.18	0.19	0.19	0.18	0.16	0.17	0.18	0.18	0.18
P_7	0.14	0.13	0.14	0.14	0.13	0.14	0.14	0.13	0.14
P_8	0.19	0.16	0.18	0.20	0.16	0.18	0.20	0.16	0.18
P_9	0.45	0.39	0.42	0.44	0.38	0.41	0.45	0.39	0.42
Mean	0.22	0.21		0.22	0.20		0.22	0.20	
	S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%	
P	0.005	0.019		0.003	0.015		0.003	0.012	
C	0.010	0.042		0.008	0.032		0.006	0.026	
P x C	0.015	0.059		0.011	0.046		0.009	0.037	

P- Pulsing solution , P_1-2% Betel leaf extract, P_2- 200ppm 8-HQC, P_3-3% sucrose + 200 ppm 8-HQC, P_4-6% sucrose + 200 ppm 8-HQC, P_5-9% sucrose + 200 ppm 8-HQC, P_6-3% sucrose+2% betel leaf extract, P_7-6% sucrose+2% betel leaf extract, P_8-9% sucrose+2% betel leaf extract, P_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

Table 32: Relative fresh weight of cut roses Konfetti and Bordeaux in the pulsing solutions (day 2)

Treatment	Relative fresh weight (%)								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
P_1	107.71	110.37	109.04	107.47	112.30	109.88	107.59	111.34	109.46
P_2	107.38	109.92	108.65	109.00	111.70	110.35	108.19	110.81	109.50
P_3	112.50	113.10	112.80	111.73	114.70	113.22	112.12	113.90	113.01
P_4	113.35	115.89	114.62	113.30	117.77	115.53	113.33	116.83	115.08
P_5	115.50	119.13	117.31	113.93	116.43	115.18	114.72	117.78	116.25
P_6	115.10	118.02	116.56	114.50	117.40	115.95	114.80	117.71	116.26
P_7	117.60	120.65	119.13	116.83	122.33	119.58	117.22	121.49	119.35
P_8	113.52	117.80	115.66	112.93	119.53	116.23	113.23	118.67	115.95
P_9	102.52	103.37	102.94	104.27	104.13	104.20	103.39	103.75	103.57
Mean	111.69	114.25		111.55	115.14		111.62	114.70	
	S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%	
P	0.191	0.738		0.252	0.971		0.154	0.594	
C	0.407	1.565		0.535	2.060		0.327	1.260	
P x C	0.575	2.214		0.757	2.913		0.463	1.782	

P- Pulsing solution , P_1-2% Betel leaf extract, P_2- 200ppm 8-HQC, P_3-3% sucrose + 200 ppm 8-HQC, P_4-6% sucrose + 200 ppm 8-HQC, P_5-9% sucrose + 200 ppm 8-HQC, P_6-3% sucrose+2% betel leaf extract, P_7-6% sucrose+2% betel leaf extract, P_8-9% sucrose+2% betel leaf extract, P_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

Table 33: Relative fresh weight on the last day of vase life of cut roses Konfetti and Bordeaux in the pulsing solutions

Treatment	Relative fresh weight (%)								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
P_1	72.48	75.96	74.22	71.82	76.86	74.34	72.15	76.41	74.28
P_2	72.14	72.91	72.53	70.53	73.87	72.20	71.34	73.39	72.36
P_3	75.32	78.79	77.06	74.18	79.65	76.92	74.75	79.22	76.99
P_4	77.73	80.89	79.31	79.17	81.01	80.09	78.45	80.95	79.70
P_5	80.18	82.41	81.30	78.52	83.12	80.82	79.35	82.77	81.06
P_6	78.24	80.18	79.21	77.41	81.31	79.36	77.82	80.74	79.28
P_7	82.34	85.21	83.78	81.21	86.16	83.68	81.78	85.69	83.73
P_8	77.96	81.08	79.52	75.62	82.33	78.98	76.79	81.71	79.25
P_9	61.79	67.51	64.65	60.96	66.43	63.69	61.38	66.97	64.17
Mean	75.35	78.33		74.38	78.97		74.87	78.65	
	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%
P	0.201		0.774	0.292		1.123	0.223		0.859
C	0.427		1.642	0.619		2.383	0.473		1.822
P x C	0.604		2.323	0.876		3.371	0.670		2.577

P- Pulsing solution , P_1-2% Betel leaf extract, P_2- 200ppm 8-HQC, P_3-3% sucrose + 200 ppm 8-HQC, P_4-6% sucrose + 200 ppm 8-HQC, P_5-9% sucrose + 200 ppm 8-HQC, P_6-3% sucrose+2% betel leaf extract, P_7-6% sucrose+2% betel leaf extract, P_8-9% sucrose+2% betel leaf extract, P_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

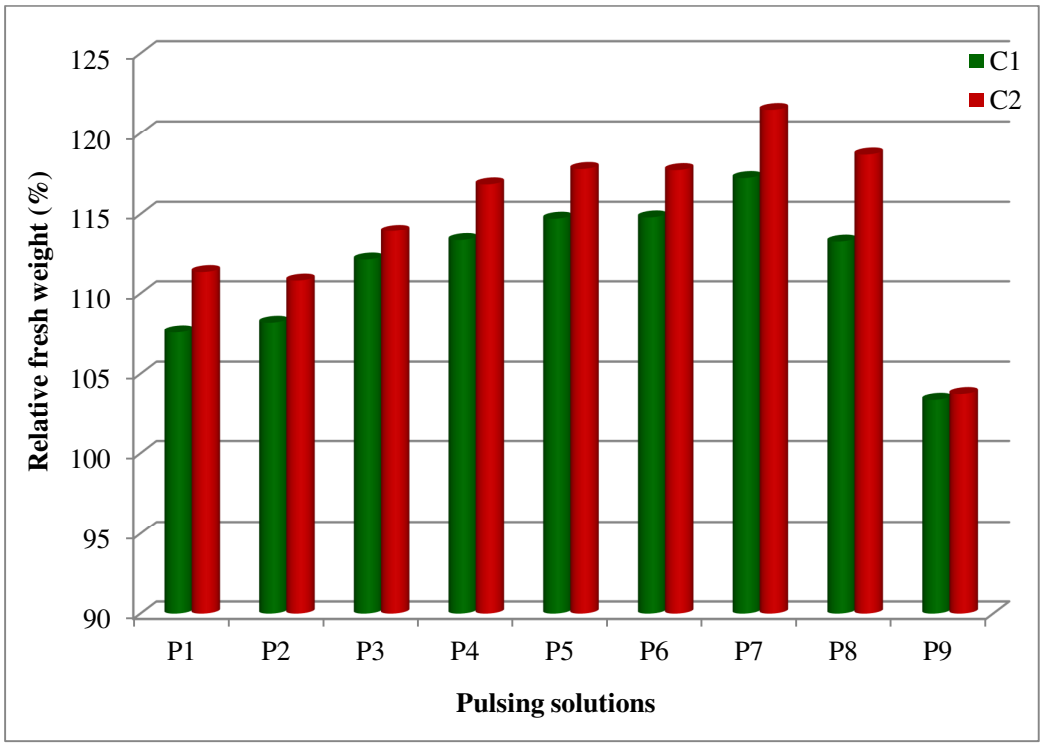


Fig. 10: Relative fresh weight of cut roses Konfetti and Bordeaux in the pulsing solutions on day 2

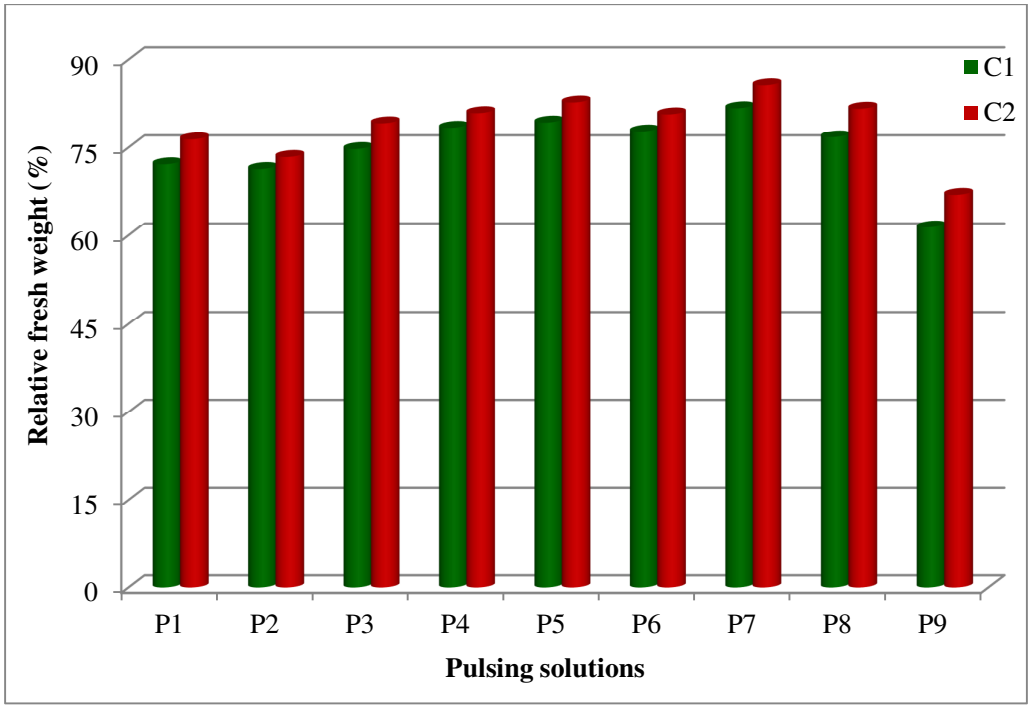


Fig. 11: Relative fresh weight of cut roses Konfetti and Bordeaux in the pulsing solutions on the last day of vase life

103.57%) in both the years and the pooled data. The interaction effects were best between Bordeaux and the treatments 6% sucrose+2% betel leaf extract (120.65%) as well as 9% sucrose +200 ppm 8-HQC (119.13%) in 2012. In 2013, 6% sucrose+2% betel leaf extract (120.65%) as well as 9% sucrose +2% betel leaf extract (119.13%) with Bordeaux showed better interaction and in the pooled data6% sucrose+2% betel leaf extract (121.49%) with Bordeaux showed better results (Appendix 27).

On the last day of vase life as seen in Table 33 and Fig. 11, it is clear that the cultivars showed significant difference with respect to fresh weight changes with Bordeaux having a higher value in 2012 (78.33%), 2013 (78.97%) and pooled data (78.65%).The treatment 6% sucrose+2% betel leaf extract depicted a higher value in 2012 (83.78%), 2013 (83.68%) and pooled data (83.73%) where as least value was observed in the control treatment (64.65, 63.69and 64.17%). The results stated that the highest interaction effects were between 6% sucrose+2% betel leaf extract and Bordeaux with a value of 85.21, 86.16 and 85.69% and lowest in Konfetti + control treatment (61.79, 60.96 and 61.38) in 2012, 2013 and pooled data (Appendix 28).

4.8.10 Vase life

The data concerning the vase life is given in Table 34, Fig. 12 and Plate 26.

Among the cultivars, the results were significantly different in 2012 (10.44 and 11.52 days), 2013 (11.11 and 11.52 days) and pooled data (10.78 and 11.52 days). Among the treatments 6% sucrose+2% betel leaf extract showed a greater value in 2012(14.17 days), 2013 (13.67 days) and pooled data (13.92 days) where as least value were noticed in the control treatment (5.50, 6.00 and 5.58 days). Among the interaction effects, both Bordeaux and Konfetti with the treatments 6% sucrose+2% betel leaf extract and 9% sucrose +200ppm 8-HQC in 2012 (14.67, 13.67 and 13.67, 13.00 days), 2013 (14.33, 13.00 and 13.00,12.67 days) and pooled data (14.50,13.33 and 13.33 and 12.83 days) (Appendix 29).

4.8.11 Chlorophyll content

Table 35 data gives the chlorophyll content of both Konfetti and Bordeaux. The chlorophyll content of both Konfetti and Bordeaux were significantly different in

PLATE 26

Experimental view of Konfetti and Bordeaux held in holding solutions on the 5th day of vase life

P_1.2% betel leaf extract P_2. 200ppm 8-HQC P_3. 3% sucrose and 8- HQC 200ppm

P_4.6% sucrose and 8- HQC 200ppm P_5. 9% sucrose and 8- HQC 200ppm

P_6. 3% sucrose and 2 % Betel leaf extract P_7. 6% sucrose and 2 % Betel leaf extract

P_8. 9% sucrose and 2 % Betel leaf extract P_9. Control (Tap water)

Table 34: Vase life of cut roses Konfetti and Bordeaux in the pulsing solutions

Treatment	Vase life (days)								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
P_1	9.00	10.67	9.83	10.33	11.33	10.83	9.67	11.00	10.33
P_2	9.33	10.00	9.67	10.67	10.00	10.33	10.00	10.00	10.00
P_3	10.33	11.67	11.00	11.67	12.33	12.00	11.00	12.00	11.50
P_4	11.00	12.00	11.50	12.33	11.67	12.00	11.67	11.83	11.75
P_5	13.00	13.67	13.33	12.67	13.00	12.83	12.83	13.33	13.08
P_6	10.67	12.00	11.33	12.00	12.33	12.17	11.33	12.17	11.75
P_7	13.67	14.67	14.17	13.00	14.33	13.67	13.33	14.50	13.92
P_8	11.67	13.33	12.50	12.00	12.67	12.33	11.83	13.00	12.42
P_9	5.33	5.67	5.50	5.33	6.00	5.67	5.33	5.83	5.58
Mean	10.44	11.52		11.11	11.52		10.78	11.52	

	S.Em.±	C.D. at 1%	S.Em.±	C.D. at 1%	S.Em.±	C.D. at 1%
P	0.163	0.629	0.202	0.780	0.121	0.467
C	0.346	1.334	0.430	1.655	0.257	0.990
P x C	0.490	1.887	0.608	2.340	0.364	1.401

P- Pulsing solution , P_1-2% Betel leaf extract, P_2- 200ppm 8-HQC, P_3-3% sucrose + 200 ppm 8-HQC, P_4-6% sucrose + 200 ppm 8-HQC, P_5-9% sucrose + 200 ppm 8-HQC, P_6-3% sucrose+2% betel leaf extract, P_7-6% sucrose+2% betel leaf extract, P_8-9% sucrose+2% betel leaf extract, P_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

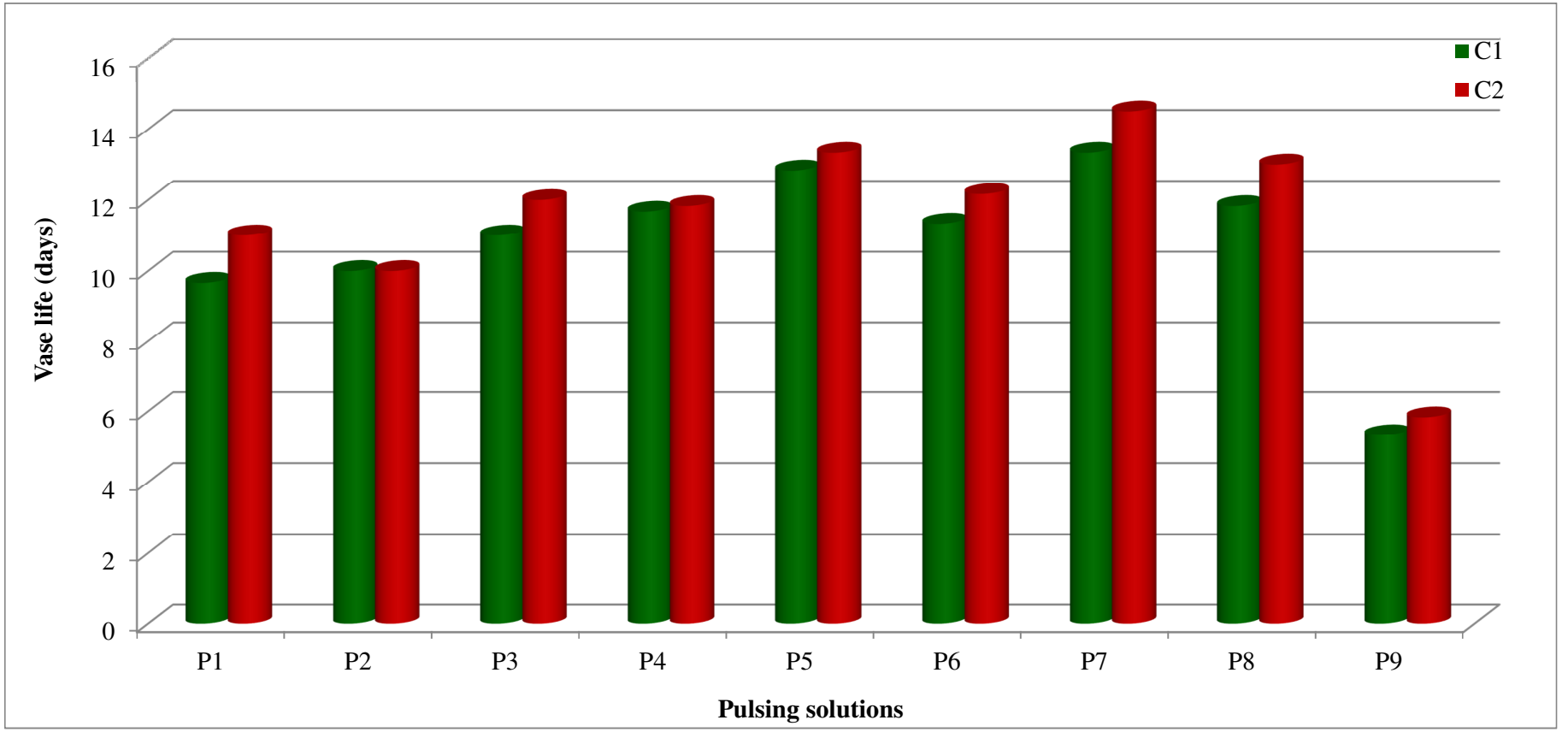


Fig. 12: Vase life of cut roses Konfetti and Bordeaux in the pulsing solutions

Table 35: Chlorophyll content of cut roses Konfetti and Bordeaux in the pulsing solutions (day 2)

Treatment	Chlorophyll content (SPAD unit)								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
P_1	45.19	45.40	45.29	44.10	45.15	44.63	44.65	45.28	44.96
P_2	44.67	44.61	44.64	43.59	44.57	44.08	44.13	44.59	44.36
P_3	44.78	45.65	45.22	43.96	45.71	44.83	44.37	45.68	45.02
P_4	45.07	46.12	45.59	43.99	47.35	45.67	44.53	46.73	45.63
P_5	45.36	47.18	46.27	43.88	46.52	45.20	44.62	46.85	45.74
P_6	45.03	46.46	45.75	44.58	45.47	45.03	44.81	45.97	45.39
P_7	46.19	48.06	47.12	45.62	49.54	47.58	45.90	48.80	47.35
P_8	44.99	47.13	46.06	43.85	46.56	45.20	44.42	46.84	45.63
P_9	30.50	37.22	33.86	31.55	36.37	33.96	31.02	36.79	33.91
Mean	43.53	45.31		42.79	45.25		43.16	45.28	
	S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%	
P	0.129	0.499		0.174	0.670		0.114	0.440	
C	0.275	1.059		0.369	1.422		0.242	0.933	
P x C	0.389	0.1497		0.523	2.011		0.343	1.320	

P- Pulsing solution , P_1-2% Betel leaf extract, P_2- 200ppm 8-HQC, P_3-3% sucrose + 200 ppm 8-HQC, P_4-6% sucrose + 200 ppm 8-HQC, P_5-9% sucrose + 200 ppm 8-HQC, P_6-3% sucrose+2% betel leaf extract, P_7-6% sucrose+2% betel leaf extract, P_8-9% sucrose+2% betel leaf extract, P_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

day 2 with a higher value for Bordeaux in 2012 (45.31), 2013 (45.25) and pooled data (45.28). The treatment 6% sucrose+2% betel leaf extract was highly effective in preserving the chlorophyll content in 2012 (47.12), 2013 (47.58) and pooled data (47.35). The interaction effects were significant with 6% sucrose+ 2% betel leaf extract recording a higher value and the control treatment the lowest value in 2012 (48.06 and 37.22), 2013 (49.54 and 36.37) and pooled data (47.35 and 33.91) (Appendix 30).

On last day of vase life (Table 36 and Appendix 31) also there was a significant difference between the cultivars with Bordeaux recording a higher value in both the years (15.89 and 16.01) and pooled data (15.95). The treatment 6% sucrose+2% betel leaf extract depicted a higher value (17.44, 18.91 and 18.18) and the control treatment the least (10.48, 9.47 and 9.98) in 2012, 2013 and pooled data respectively. In 2012 interaction effects were highest between Bordeaux and 6% sucrose+2% betel leaf extract (18.49) which was on par with the treatments 9% sucrose+2% betel leaf extract (18.18),3% sucrose+2% betel leaf extract (17.50) and 9% sucrose +200ppm 8-HQC (17.75). In 2013 and pooled data, 6% sucrose+2% betel leaf extract (20.31 and 19.40) which was on par with the treatments 9% sucrose+2% betel leaf extract (19.33 and 18.75) resulted in a higher value.

4.8.12 Freshness of leaves and petal

The Table 37 and Appendix 32 states the freshness of leaves and petal and they do not significantly differ among the cultivars in both the years. Amid the treatments the treatments 6% sucrose+2% betel leaf extract, 9% sucrose+2% betel leaf extract and 9% sucrose +200ppm 8-HQC had the highest score of 5.00 in 2012, 2013 and pooled data. The interaction effects were significant with the lowest value for the control treatment of both the cultivars (1.00).

4.8.13 Blueing of petals

The data on blueing of petals are presented in the Table 38 and Appendix 33. Among the cultivars blueing was noticed only in Bordeaux which was red coloured. Among the treatments the highest blueing was noticed in the control treatment in 2012 (0.67), 2013 (0.83) and pooled data (0.75). In the interaction effects Bordeaux + control treatment recorded the highest score in 2012 (1.33), 2013 (1.67) and pooled data (1.50).

Table 36: Chlorophyll content- last day of vase life of cut roses Konfetti and Bordeaux in the pulsing solutions

Treatment	Chlorophyll content (SPAD unit)								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
P_1	14.13	15.39	14.76	14.73	15.90	15.32	14.43	15.64	15.04
P_2	13.22	13.82	13.52	13.35	14.67	14.01	13.29	14.24	13.77
P_3	14.73	15.30	15.02	13.89	16.00	14.95	14.31	15.65	14.98
P_4	15.12	16.81	15.97	15.03	17.15	16.09	15.08	16.98	16.03
P_5	15.32	17.75	16.54	15.25	16.43	15.84	15.28	17.09	16.19
P_6	15.84	17.50	16.67	14.56	15.74	15.15	15.20	16.62	15.91
P_7	16.39	18.49	17.44	17.51	20.31	18.91	16.95	19.40	18.18
P_8	15.61	18.18	16.89	16.56	19.33	17.94	16.08	18.75	17.42
P_9	11.24	9.72	10.48	10.37	8.58	9.47	10.80	9.15	9.98
Mean	14.62	15.89		14.58	16.01		14.60	15.95	
	S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%		S.Em.±	C.D. at 1%	
P	0.144	0.557		0.097	0.375		0.094	0.364	
C	0.307	1.182		0.207	0.797		0.200	0.772	
P x C	0.434	1.671		0.293	1.127		0.284	1.092	

P- Pulsing solution , P_1-2% Betel leaf extract, P_2- 200ppm 8-HQC, P_3-3% sucrose + 200 ppm 8-HQC, P_4-6% sucrose + 200 ppm 8-HQC, P_5-9% sucrose + 200 ppm 8-HQC, P_6-3% sucrose+2% betel leaf extract, P_7-6% sucrose+2% betel leaf extract, P_8-9% sucrose+2% betel leaf extract, P_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

Table 37: Freshness of leaves and petal of cut roses Konfetti and Bordeaux in the pulsing solutions

Treatment	Freshness of leaves and petals								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
P_1	3.33	4.00	3.67	4.00	4.00	4.00	3.67	4.00	3.83
P_2	3.33	3.67	3.50	3.67	3.67	3.67	3.50	3.67	3.58
P_3	4.33	4.67	4.50	4.00	4.00	4.00	4.17	4.33	4.25
P_4	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
P_5	4.67	5.00	4.83	4.67	4.67	4.67	4.67	4.83	4.75
P_6	4.67	4.67	4.67	4.67	4.67	4.67	4.67	4.67	4.67
P_7	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
P_8	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
P_9	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mean	4.04	4.22		4.11	4.11		4.07	4.17	
	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%
P	0.074		0.284	0.078		0.302	0.062		0.241
C	0.157		0.604	0.166		0.641	0.133		0.512
P x C	0.222		0.854	0.235		0.906	0.188		0.724

P- Pulsing solution , P_1-2% Betel leaf extract, P_2- 200ppm 8-HQC, P_3-3% sucrose + 200 ppm 8-HQC, P_4-6% sucrose + 200 ppm 8-HQC, P_5-9% sucrose + 200 ppm 8-HQC, P_6-3% sucrose+2% betel leaf extract, P_7-6% sucrose+2% betel leaf extract, P_8-9% sucrose+2% betel leaf extract, P_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

Table 38: Blueing of petals of cut roses Konfetti and Bordeaux in the pulsing solutions

Treatment	Blueing of petals								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
P_1	0.00	1.00	0.50	0.00	1.00	0.50	0.00	1.00	0.50
P_2	0.00	1.00	0.50	0.00	1.33	0.67	0.00	1.17	0.58
P_3	0.00	1.00	0.50	0.00	1.00	0.50	0.00	1.00	0.50
P_4	0.00	0.67	0.33	0.00	0.67	0.33	0.00	0.67	0.33
P_5	0.00	0.67	0.33	0.00	0.67	0.33	0.00	0.67	0.33
P_6	0.00	1.00	0.50	0.00	0.67	0.33	0.00	0.83	0.42
P_7	0.00	0.67	0.33	0.00	0.67	0.33	0.00	0.67	0.33
P_8	0.00	1.00	0.50	0.00	0.67	0.33	0.00	0.83	0.42
P_9	0.00	1.33	0.67	0.00	1.67	0.83	0.00	1.50	0.75
Mean	0.00	0.93		0.00	0.93		0.00	0.93	
	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%
P	0.052		0.201	0.069		0.266	0.050		0.195
C	0.111		0.427	0.146		0.565	0.107		0.413
P x C	0.157		0.604	0.207		0.799	0.152		0.585

P- Pulsing solution , P_1-2% Betel leaf extract, P_2- 200ppm 8-HQC, P_3-3% sucrose + 200 ppm 8-HQC, P_4-6% sucrose + 200 ppm 8-HQC, P_5-9% sucrose + 200 ppm 8-HQC, P_6-3% sucrose+2% betel leaf extract, P_7-6% sucrose+2% betel leaf extract, P_8-9% sucrose+2% betel leaf extract, P_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

4.8.14 Bent neck

It is evident from Table 39 that all the treatments and both the cultivars resulted in bent-neck free flowers in both the years i.e. 2012 and 2013, except the flowers which were kept in control (tap water).

4.8.15 Total soluble solids (%)

Among the cultivars (Table 40 and Appendix 34) TSS was not significant. Among the treatments 9% sucrose+2% betel leaf extract had the highest value in 2012 (7.88%), 2013 (7.44%) and pooled data (7.66%).

Table 39: Bent neck of cut roses Konfetti and Bordeaux in the pulsing solutions

Treatment	Year 2012		Year 2013	
	C_1	C_2	C_1	C_2
P_1	Unbent	Unbent	Unbent	Unbent
P_2	Unbent	Unbent	Unbent	Unbent
P_3	Unbent	Unbent	Unbent	Unbent
P_4	Unbent	Unbent	Unbent	Unbent
P_5	Unbent	Unbent	Unbent	Unbent
P_6	Unbent	Unbent	Unbent	Unbent
P_7	Unbent	Unbent	Unbent	Unbent
P_8	Unbent	Unbent	Unbent	Unbent
P_9	Bent	Bent	Bent	Bent

P_1-2% Betel leaf extract, P_2- 200ppm 8-HQC, P_3-3% sucrose + 200 ppm 8-HQC, P_4-6% sucrose + 200 ppm 8-HQC, P_5-9% sucrose + 200 ppm 8-HQC, P_6-3% sucrose+2% betel leaf extract, P_7-6% sucrose+2% betel leaf extract, P_8-9% sucrose+2% betel leaf extract, P_9-Control (tap water)
C_1- Konfetti, C_2- Bordeaux

Table 40: TSS of cut roses Konfetti and Bordeaux in the pulsing solutions on day three

Treatment	TSS (%)								
	Year 2012			Year 2013			Pooled		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
P_1	4.73	5.23	4.98	4.86	5.76	5.31	4.79	5.50	5.14
P_2	4.44	5.20	4.82	4.63	5.90	5.27	4.54	5.55	5.04
P_3	6.78	6.25	6.52	6.37	6.75	6.56	6.58	6.50	6.54
P_4	6.88	6.24	6.56	6.70	7.04	6.87	6.79	6.64	6.72
P_5	7.58	7.21	7.40	6.86	6.46	6.66	7.22	6.84	7.03
P_6	6.56	6.35	6.46	6.53	6.65	6.59	6.54	6.50	6.52
P_7	7.17	7.48	7.32	7.34	6.89	7.11	7.25	7.18	7.22
P_8	7.97	7.79	7.88	7.77	7.10	7.44	7.87	7.45	7.66
P_9	2.94	3.16	3.05	3.21	3.37	3.29	3.08	3.27	3.17
Mean	6.12	6.10		6.03	6.21		6.07	6.16	
	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%	S.Em.±		C.D. at 1%
P	0.069		0.266	0.068		0.264	0.054		0.208
C	0.146		0.564	0.145		0.560	0.114		0.441
P x C	0.207		0.798	0.206		0.792	0.162		0.624

P- Pulsing solution, P_1-2% Betel leaf extract, P_2- 200ppm 8-HQC, P_3-3% sucrose + 200 ppm 8-HQC, P_4-6% sucrose + 200 ppm 8-HQC, P_5-9% sucrose + 200 ppm 8-HQC, P_6-3% sucrose+2% betel leaf extract, P_7-6% sucrose+2% betel leaf extract, P_8-9% sucrose+2% betel leaf extract, P_9-Control (tap water)
C- Cultivar, C_1- Konfetti, C_2- Bordeaux

5. DISCUSSION

Vase life is a desirable character for cut roses, which is often curtailed prematurely due to water deficit manifested in the form of bent neck, wilting of flowers and leaves, rapid loss of fresh weight (Halevy and Mayak, 1981). Expansion of rose cut flower industry is being retarded on account of the limitation of potential sales caused by the poor keeping quality of roses (Scholes and Boodley, 1964). Water balance is a major factor determining quality and longevity of cut flowers. It is influenced by water uptake and transpiration, being the balance between these two processes. Stem end blockage is regarded as a major cause of imbalance between water uptake and water loss from cut flowers (Da-Silva, 2003). The development of bent neck and wilting is considered to be caused by vascular plugging, which inhibits water supply to the flowers (van Doorn, 1997). This plugging is correlated with addition of microorganisms to vase water (de Witte and van Doorn, 1988) and their growth at wounded surface as well as inside the stem (van Doorn *et al*., 1989) which cause considerable reduction in vase life of cut rose flowers. These findings reveal that presence of microorganisms, bacteria and fungi, which grow in the vase solution, is a major cause of vascular plugging.

Controlling and reducing microbial proliferation is a prerequisite for extending quality and longevity of cut flowers, especially for roses. In order to prevent microbial proliferation in vase solutions of cut flowers, various compounds and chemicals have been used in cut flower vase solutions to extend vase life by improving water uptake. Application of biocides severally or moderately affect other physiological properties of cut flowers specially their photosynthetic apparatus function and membrane permeability by their toxic compounds during postharvest development and aging (Jowkar *et al*., 2012). These include silver nitrate (Torre and Fjeld, 2001), silver thiosulphate (Liao *et al*., 2000), aluminum sulphate (Ichimura and Shimizu-Yumoto, 2007), hydroxyquinoline sulphate (Liao *et al*., 2000), hydroxyquinoline citrate (Solgi *et al*., 2009) and sodium hypoclorite (Knee, 2000).

Some of these compounds such as silver nitrate and silver thiosulphate have shown environmental risks and health hazards (Damunupola and Joyce, 2006). While others such as hydroxyquinoline have shown plant phytotoxic effects. In the

cut flower market there is a great need for preserving solution biocides that control microbial contamination effectively and beside that do not show environmental risks and phytotoxicity. This need is more crucial for cut rose flowers which hold a very large portion of cut flower market and industry.

One group of naturally derived antimicrobial compounds is herbal extracts and essential oils from medicinal plants. These compounds are safe, show varying degree of antimicrobial activity, and can provide another hurdle to the growth of microorganisms, thereby improving the vase life of cut flowers. Numerous studies have reported that medicinal plants produce a large number of secondary metabolites with antimicrobial effects on pathogens (Mari *et al*., 2003; Obagwu and Korsen, 2003). Medicinal plant extracts, therefore, for the control of the growth of microorganisms are emerging as alternatives to conventional natural preservatives as they are generally safe to humans, and environment friendly (Thangavelu *et al*., 2004). Considering the fact that these extracts have no bioenvironmental harms, they have an important role in preparing organic products. Although there is no available research study on using its leaf-extracts, it is hoped that it can be used as an organic preservation solution, since it is easily accessible.

Plants are rich source of active metabolites that are effective against a variety of microbial species. The bioactive molecule thought to be responsible for anti-bacterial activity is sterol, which has been obtained in large quantities in betel leaf extracts. The mode of action may be due to surface interaction of sterol molecule present in the extracts with the bacterial cell wall and membrane leading to alteration in the primary structure of cell wall, ultimately lead to pore formation and degradation of the bacterial components. It is reported that sterol act through the disruption of the permeability barrier of microbial membrane structures (Chakraborty and Shah, 2011). The leaf has also poses the antifungal activity against many fungal infections (Ali *et al*., 2010). So betel leaf extract has a great potential to be used as an organic biocide in preservative solution. Hence this study was conducted to determine the impact of herbal extracts on the microbial vascular plugging, quality and longevity of cut roses Bordeaux and Konfetti. Results related to response of betel leaf extract as a component of preservative solution have been given in preceding chapter. In this chapter, an attempt has been made to discuss the

experimental findings to offer specific explanations for the effect of different treatments with regard to different attributes studied in the light of work done by other scientists.

5.1 Determination of Microbial Growth in Vascular Bundles using Scanning Electron Microscope (SEM)

Scanning Electron Microscope (SEM) observations are essential methods to reveal the role of micro-organisms in water stress and vessel plugging of cut flowers during their vase life (Put and Clerkx, 1988). The SEM studies confirmed the presence of microbial vascular plugging of cut roses of both Konfetti and Bordeaux kept in tap water for 5 days. The transportation of water and minerals through the xylem vessels is of vital importance for the development of cut flowers. Occlusion of these vessels by the microorganisms present in preservative solution is a common problem affecting the vase life and the quality of cut flowers. The microbial growth was clearly apparent in the untreated flowers. These results suggest that the inhibition of water uptake mainly occurred by the occlusion of xylem vessels containing high amounts of microorganisms. This inhibition of water uptake could be the reason for the unbalance in the water relations (between water uptake and water loss which leads the flowers in to early senescence and shortens its vase life.

A cause of short vase-life in cut-rose cv. Dang Gang-Galar was investigated by the SEM in the study conducted by Jitareerat *et al*., 2008. In the vascular bundles of 5-day-old cut-rose stems, there were abundant fungal myceliums as well as bacterial cells whereas they were not found in fresh cut-rose stems. Similar results were obtained by Shanan (2012) through SEM where the xylem vessels of majority of rose cut flower stems kept in the control solution or in solutions of sweet basil, lemon grass or cinnamon were conspicuously plugged by the accumulation of microorganisms (bacteria and fungi). The growth of these organisms destroyed the vessel cells and as a result, xylem occlusion took place.

5.2 Ethanolic extraction and yield (%) of extracts

It should be noted that choice of appropriate solvent is of essential importance along with application of a compatible extraction method. For selection of solvents 'like dissolves like' principle is applicable. Thus polar solvents will

extract out polar substances and non-polar material will be extracted out by non-polar solvents. Solvent extraction is the most popular method of extraction (Gupta *et al*., 2012). Ethanol is a slightly polar organic solvent that sufficiently dissolve slightly polar organic compounds. So ethanol was selected as a suitable solvent for extraction.

Maximum yield of the extract was obtained for turmeric and least for tulsi. The maximum yield of betel leaf extract was observed in case of ethanolic extract i.e. 39.5%, whereas yield for petroleum ether and chloroform extract was equivalent to 15% and 5% respectively in the study conducted by Bangash *et al*. (2005). Sasongko *et al*. (2011) reported that the absolute plant extract recovered from 20 g dry leaves of *Persicaria odorata* extracted by petroleum ether, acetone and ethanol showed a yield of 0.09%, 6.63% and 4.49%, respectively. Although acetone extraction gave the highest percentage of yield extract, ethanol was the most effective solvent because it can extracted more volatile compound compared with the other type of solvent.

5.3 Best herbal extract

Among the five herbal extracts namely, neem, garlic, betel leaf, tulsi and turmeric, betel leaf extract was most effective against the 5 isolated microorganisms, viz. *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Mucor* sp. and *Rhizopus* sp. Betel leaf extract demonstrated the highest zone of inhibition against the isolated microorganisms. Zone of inhibitions recorded for betel leaf extracts were 26 mm and 28 mm, respectively, against the collective bacterial and fungal growth in the vase solution. Similarly, in a study conducted by Jitareet *et al*. (2008) Piper extract showed the highest efficacy for control of microbial growth, followed by annona, curcuma, tobacco and galanga extracts, respectively. Piper extract completely inhibited microbial growth at 1% while curcuma, tobacco and galanga extracts inhibited completely at 3, 5, and 7%, respectively. This result is similar to To-anun (1985) who found that piper extract was able to inhibit the growth of 12 *Aspergillus* spp. isolates. Deans and Ritchie (1987) indicated that piper leaves contain phenolic compounds which function as antibacterial compounds against both gram-positive and gram-negative bacteria. Moreover, betel leaves contains fatty

acids (stearic acid and palmitic acid) and hydroxy fatty acid esters (hydroxy esters of stearic, palmitic and myristic acids). The hydroxychavicol has been reported to possess antibacterial activity. Also, Fatty acids can act as anionic surfactants and have antibacterial and antifungal properties (Subhashkumar *et al.,* 2013).

Mode of action of betel leaf extract against microorganisms

Previous studies have reported that *Piper betle* leaf extract contain a large number of bioactive molecules like polyphenols, alkaloids, steroids, saponins and tannins. Leaves of *Piper betle* are known to contain significant amount of anti-oxidants like hydroxychavicol, eugenol, ascorbic acid and b-carotene. Betel leaf extract also contains high concentration of fatty acids like palmitic acid, stearic acid and hydroxy fatty acid esters which shows potent antimicrobial activity against diverse pathogenic microorganisms (Nalina *et al*., 2007). It is also reported that *Piper betle* leaf extract exhibit biological capabilities of detoxication and antioxidation. Sterol is the bioactive molecule thought to be responsible for antibacterial activity which has been obtained in large quantities in *Piper betle* extracts. The mode of action may be due to surface interaction of sterol molecules present in the extracts with the bacterial cell wall and membrane leading to alteration in the primary structure of cell wall and membrane, ultimately leading to pore formation and degradation of the bacterial components (Chakraborty and Shah, 2011). Earlier studies have reported that sterol works through the disruption of the permeability barrier of microbial membrane structures. The terpene compounds found in leaves of betel leaf such as carvacrol, linalool and eugenol have been known to exhibit antifungal activity towards several strains of microorganisms (Friedman *et al*., 2002). In addition, there are many secondary metabolites and chemical constituents produced in *P. betle* extracts that may contribute to the observed positive antifungal effects.

5.4 Bacterial count

In this study, bacterial counts were 2.75 x 10^5 cfu/ml and 2.48 x 10^5 cfu/ml for Konfetti and Bordeaux, respectively. Microbial blockage was an important cause of short vase-life in cut-rose flowers, similarly to that reported by Bleeksma and Van Doorn (2003). They found bacteria in the stem of cut roses at 1×10^5 cfu /g fresh

weight, which caused wilt and rapid deterioration due to interference in water uptake and/or possible secretion of extracellular enzymes that could degrade the cell walls of vascular bundles. Jones and Hill (1993) reported in their study that roses are adversely affected by relatively low microbial levels ($<10^6$ cfu ml $^{-1}$) and benefit from the application of germicides. Microbial concentrations of 10^6 cfu ml $^{-1}$ were routinely recorded in the untreated tap water 3 days after flower stems were added. Based on their results lilies, gerberas, gypsophila, daffodils, freesia, and bluebell also behave in a similar manner. However, carnations appeared to be tolerant of microbial counts up to $\approx 10^7$ cfu ml $^{-1}$.

5.5 Bacterial Kind

In the vase water of cut roses, many different kinds of bacteria, yeasts and fungi have been identified (de Witte and van Doorn, 1988). While in carnation vase solution Zagory and Reid (1986) identified 25 different microorganisms, in this experiment only 5 microbial types viz., *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Mucor* sp. and *Rhizopus* sp. were seen. It seems that fewer microbe types were due to lower flower contamination and integrated management applied during flower production. The isolated microorganisms from control vase solution were 2 different kinds of bacterial isolates in the study conducted by Jowkar *et al*. (2012). The separated bacterial isolated were 2 different strains of *Bacillus*. van Doorn *et al*. (1995) found *Pseudomonas* species as the dominant microorganism in roses and carnation cut flowers. In the study conducted by Put (1990), during the first 3-6 days of vase life of rose, chrysanthemum and gerbera, *Pseudomonas cepacia, Pseudomonas fluorescens* and *Pseudomonas putida* replaced the initially dominant *Enterobacter* and *Bacillus* stem flora. However after > 6 days of vase life, *Enterobacter* spp. again became more dominant *(E. agglomerans* and *E. cloacae).* Furthermore, *Bacillus* spp., *Erwinia* and also non-fermenting Gram-negative bacteria predominated later. Predominant mould genera were *Cladosporium, Fusarium, Pénicillium, Mucor* and *Rhizopus*. Depending on experiment condition and production system, other dominant types of various microorganisms have been seen in the vase solution of cut flowers.

5.6 Minimum Inhibitory Concentration (MIC)

In the present study, MIC of betel leaf extract (*Piper betle*) was found to be as low as 2% and completely inhibiting any visual bacterial and fungal growth. The MIC for betel leaf extract was calculated individually against 3 bacterial species viz., *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and 2 fungal species namely *Mucor* sp. and *Rhizopus* sp., isolated from the vase solutions.

There is a scarcity of reports of using *Piper betle* extract as an antibacterial agent in the vase solution of cut roses. Most of the studies have investigated the antibacterial properties of *Piper betle* against food-borne or clinical pathogens. Recently, Subhashkumar *et al.* (2013) reported antibacterial activity of *Piper betle* against some of the major clinical pathogens including *Pseudomonas aeruginosa* and *E.coli* using well-diffusion method. They reported high MIC of 10% against *E.coli* with inhibition zone of 11 mm and no activity against *Pseudomonas aeruginosa.* However, in our study, we found low concentration of 2% of *Piper betle* completely inhibiting growth of bacteria and fungus including *Pseudomonas aeruginosa* and *E. coli.*

Khan and Kumar (2011) successfully used ethanolic and methanolic extracts of leaves of *Piper betle* against *E. coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Ethanolic extract showed a maximum zone of inhibition of 17 mm against E. coli and 14 mm against *P. aeruginosa*. Also, they have reported low MIC values of *Piper betle* extract ranging between 0.0021mg/ml to 8.196mg/ml in both methanolic and ethanolic extracts. Bhasabutra (1997) also reported 5 and 10% piper extracts inhibited vegetative growth and spore germination of *Colletotrichum gleosporioides*, which causes anthracnose disease of mango.

5.7 Standardisation of holding solution using betel leaf extract for quality and longevity of cut roses Konfetti and Bordeaux

5.7.1 Flower opening and flower diameter

In the present study, 2% sucrose + 2% betel leaf extract treated flowers had a larger flower diameter and flower opening compared to control. The flowers in the control treatment had very less flower diameter and flower opening. This might be due to the absence of sucrose which would provide respiratory substrate and biocide that would prevent vascular plugging. Similarly, in cut lotus (*Nelumbo nucifera*)

flowers failed to open, and petal blackening occurred due to carbohydrate depletion in the leaves and sink activity of the flowers (Jones *et al*., 1995; van Doorn, 2001). Thus, it is not practical to use tap water alone, without any food source, as a vase solution for cut rose flowers.

During this period, the diameter of the germicide-treated flowers was larger than control flowers. The larger flower diameter indicated a normal opening behavior of the flowers. The diameter opening of control flowers was not normal throughout the evaluation period. Sucrose added to the vase solution supplies cut flowers with substrates that are needed for respiration, thus enabling harvested buds to open into flowers (Pun and Ichimura, 2003). Several findings showed that flowers petal growth is associated with flower bud opening which results from cell expansion (Kenis *et al*., 1985) that requires the influx of water and osmolytes such as glucose into petal cells (Evans and Reid, 1988; van Doorn *et al*., 1991). According to Chamani *et al*. (2005) short vase life was associated with abnormal opening and inhibition of flower opening. Van Doorn *et al*. (1991) also reported that the decrease in water potential was correlated with inhibition of corolla growth and flower opening.

5.7.2 Water Relations

2% sucrose + 2% betel leaf extract markedly improved the volume of water uptake in cut rose flowers as compared to other treatments. The amount of water uptake by all flowers increased up to 5 days and then decreased with time.

The importance of improving water relations as a means of improving vase life of rose cut flowers has long been recognized and there have been substantial studies of the factors affecting water relations of different species of cut flowers (van Doorn, 1990; Hutchinson *et al*., 2003). In general, the water relations of cut flowers are determined by the difference between the amount of water lost by transpiration and the water uptake. When transpiration is high and the water uptake is low, the flowers will wilt and vice versa. When flowers are detached from the plant, water loss from these continues through transpiration. The ideal flower preservative is that which allows water absorption in flower tissues (Salunkhe *et al*., 1990). Water absorption from the preservative solution maintains a better water balance and

flower freshness and saves from early wilting resulting in enhanced vase life. Adding betel leaf extract was found to be positively and significantly correlated with water uptake of the rose cut flower. One of the greatest problems in postharvest flower physiology is the blockage of vascular system due to bacterial growth (Lutz and Hardenburg, 1968), which reduces water uptake and this blocks xylem vessels leading to water stress. Our results showed that adding betel leaf extract in vase water significantly reduced the microbial population. In addition, sucrose mainly acts as a food source or for water balance maintenance and prevents the blockage of xylem vessels (Nair *et al*., 2003).

5.7.3 Fresh weight changes

Water constitutes a large proportion of horticultural products weight. In addition to water, carbohydrates are the other major constituent of cut flowers. Flowers commonly take water and other materials from the mother plant, but when cut off, they rapidly move into senescence and death which take place due to water loss and weight reduction. This reduction is much higher in stress conditions. The decrease in relative fresh weight of cut flowers during the days after harvest could be due to the decrease in water uptake (Serek *et al*., 1995). Alaey *et al*. (2011) reported that the highest relative fresh weight of cut rose flowers was observed in vase solutions which showed the greatest water uptake. In this experiment, all treatments showed decrease in relative fresh weight, although this decrease were significantly lower in treatments of betel leaf extracts than in the control treatment. This might be due to the antimicrobial properties of betel leaf extract and consequent prevention of vascular plugging which improved water uptake. The results of experiment conducted by Basiri *et al*. (2011) in cut carnations showed that the highest fresh weight was seen 6% sucrose + 25% rosemary extract and the lowest was of that of the control treatment .

5.7.4 Vase life studies

There is a growing body of evidence that betel leaf extract can increase the vase life of both the cultivars of rose. In this experiment all the treatments showed an enhancement in vase life relative to the control. The vase life of cut rose flowers treated with 2% sucrose+ 2% betel leaf was 11.92 days while it was 6.50 days in the

control treatment. This is in agreement with the previous study conducted by Solgi *et al*. (2009) where various concentrations of essential oils or plant extracts such as thymol, carvacrol, thyme oil and zataria oil in combination with 6% sucrose had a positive effect on the vase life of cut gerbera (*Gerbera jamesonii* cv. Dune) flowers. Basiri *et al*. (2011) confirmed that 6% sucrose + 25% rosemary extract increased vase life of carnation cut flowers until 24.6 days in laboratory conditions.

The increased vase life might be attributed to the presence of two components: sugar and germicides. The sugar provides a respiratory substrate, while the germicides control harmful bacteria and prevent plugging of the water-conducting tissues. It is well known that sugar supply increases the longevity of many cut flowers, since sucrose act as a source of nutrition for tissues approaching carbohydrate starvation; it may also act as an osmotically active molecule there by having a role in flower opening and subsequent water relations (Kuiper *et al*., 1995). The dissolved sugars in the cells of the petals are osmotically active substances that draw into the corolla-cells, making the cell turgid and hydrolyzing the sucrose for respiration. Similar findings were obtained by Nichols (1973) and Ichimura and Korenaga (1998).

Cloudiness of the vase solution due to the activities of microbes could contribute to early senescence of the flowers. The reasons for the short vase life of the control flowers were associated with the presence of microbes in the vase solution. Metabolites produced by certain bacteria also reduced longevity and water conductivity (Mayak and Accati-Garibaldi, 1979). A similar observation was found in carnation whereby the flowers only lasted for 5-6 days in tap water (Salunkhe *et al*., 1990).

5.7.5 Chlorophyll content

Previously it has been shown that leaf chlorophyll content decreases during senescence (Tang *et al*., 2005). In this experiment also the chlorophyll content decreased in both the cultivars of cut rose with increase in vase life. Various treatments with betel leaf extract lead to a considerable delay in degradation of chlorophyll compared to control. Abadi *et al*. (2009) showed that the chlorophyll content in cut carnation flowers treated by antimicrobial compounds was

considerably improved. A positive effect of antimicrobial compounds on the chlorophyll content of cut carnation cv. 'White liberty' was reported by Basiri and Zarei (2011). Results of the experimentation by Basiri *et al*. (2011) indicated that the highest content of chlorophyll a and b was seen in treatment with 6% sucrose + 25% rosemary extract and the lowest was control treatment in carnation cut flowers.

5.7.6 Freshness of leaves and petal

The effects of sugars on the maintenance of flower freshness have been reported by Liao *et al*. (2000) and Pun and Ichimura (2003). Liao *et al*. (2000) suggested that the beneficial effects of sugars in prolonging flower vase life in rose have been attributed to the suppression of ethylene biosynthesis or sensitivity to ethylene. Hammer *et al*. (1990) also reported that provision of sucrose in the vase solution raised the steady state respiration level of Grevillea 'Sylvia'. In carnation cut flower cv. Yellow Librity, Basiri *et al*. (2011) observed that the highest quality of leaves was for 6% sucrose+ 20% rosemary extract treatment and the highest quality of flower was of that of 6% sucrose +25% rosemary extract treatment. The lowest quality of flowers and leaves were obtained with control treatment. Comparable results were obtained in this study also where 2% sucrose +2% betel leaf extract treated flowers of both the cultivars of cut roses had the highest freshness of leaves and petal and lowest were obtained with control treatment.

5.7.7 Blueing of petals

Petal blueing was observed in all the treatments but the intensity was higher in cut roses kept in tap water (control) as it did not have any external sucrose or biocide supply. The high degree of postharvest blueing in rose petals can be linked to the increase in pH level (Asen *et al*., 1971). Aging petals of cut roses rapidly increase in ammonia, due to the proteolysis, resulting in an increase of cell sap pH, which is one of the physiological processes accompanying senescence (Kuiper *et al*., 1996). Similarly, in cut 'Mercedes' and 'Sweet Promise' rose, cell sap pH increased with ageing of petals during vase life (Barthe and Vaillant, 1993; Oren-Shamir *et al*., 2001) and blueing occurred. Jitareerat *et al*., 2008 confirmed that though somewhat more blue, the anthocyanin content of petals in 1% piper-treated cut-rose was not significantly

different with distilled water or 250 ppm 8-hydroxyquinoline sulfate (HQS) treated cut-rose.

5.7.8 Bent neck

Only the control treatment demonstrated bent neck in both the cultivars Konfetti and Bordeaux. Bent neck of cut rose flower is caused by inadequate water transport through the neck tissue and tends to be varietal characteristics. Hence, the water is an important component of cut flowers and loss of water without replenishment causes the flower to wilt and drop. So one cannot exclude the possibility that the antisenescence factor is water and the degradative changes in cut flower are results of water imbalance (Thwala *et al*., 2013). Inadequate water supply in the control treatment might be due to the absence of a suitable biocide that might prevent the microbial growth and subsequent vascular blockage. Jitareerat *et al*., 2008 found that the percentage beck neck of cut-rose cv. Dang Gang-Galar flowers was lowest when used 1% piper extract as holding solution. Use of 3% annona extract in holding solutions increased bent neck to 50% within 3 days and to 100% within 4 days.

5.7.9 TSS

It was observed that 2% sucrose + 2% betel leaf extract exhibited the highest TSS value while control (tap water) the lowest in both the rose cultivars. One of the important factors that affect longevity of cut flowers during vase life is diminishing of respiratory substrates (Rogers, 1973), whose speed of change depend, at least in part, on the amount of reserves that are present in the flower when they are cut and on the exogenous sugar application to the vase solution (Pun and Ichimura, 2003). Carbohydrates are important reserve compounds, being sucrose the most abundant soluble carbohydrate, sometimes the only one in the phloem sap. In the trial performed by Basiri *et al*. (2011) in carnation cut flowers, the highest total soluble solids (TSS) content were in the 6% sucrose + 25% rosemary extract treatment whereas the lowest TSS was obtained with control treatment.

5.7.10 pH of the vase solution

Measurement of pH of vase solutions, in initial of assay showed that the betel leaf extract preservative solutions had lower pH levels in comparison with the control. It is determined that the pH of holding solution had significant effect on longevity of cut flowers because enzymes involved in polymerization processes leading to deposition of lignin and suberin are inhibited at low pH (Vámos, 1981). Therefore, it could be speculated that one reason for high effectiveness of betel leaf extract in the improvement quality of cut roses such as fresh weight, solution uptake and vase life may be related to their property in induction of low pH in vase solution. According to cut flower preserving solution recommendations, decreasing (acidifying) vase solution pH, microbial proliferation is controlled (Nowak and Rudnicki, 1990). Van Doorn (1998) has showed that low pH prevents vascular blockage in cut rose stems by reducing the bacterial population in the vase solution. Jowkar *et al*. (2012) also found that with decrease in pH, by increasing citric acid concentration, microbial population decreases with a significant correlation in cut rose flowers.

5.8 Standardisation of pulsing solutionusing betel leaf extract for quality and longevity of cut roses Konfetti and Bordeaux

5.8.1 Flower opening and flower diameter

It is well known that sugar supply increases the longevity of many cut flowers, since sucrose act as a source of nutrition for tissues approaching carbohydrate starvation; it may also act as an osmotically active molecule thereby having a role in flower opening (Kuiper *et al*., 1995). The dissolved sugars in the cells of the petals are osmotically active substances that draw into the corolla-cells making the cell turgid and hydrolyzing the sucrose for respiration. Similar findings were obtained by Nichols (1973) and Ichimura and Korenaga (1998). It seems that high water uptake, supply of sucrose and lack of contamination in the treatment 6% sucrose + 2% betel leaf allowed the maximum flower opening. Similar results were obtained Hajizadeh *et al*. (2012) in rose hybrid cv. Black magic using ethanol and aluminium sulphate. This is in agreement with the previous study conducted by Rahman *et al*. (2012) in cut carnation flowers. The flowers treated with leaf extract

of *P. betle* had significantly larger flower diameter compared to flowers treated with 8-HQC by day 9. Diameter of flowers treated with 8-HQC, copper coin and leaf extract of *P. guajava* were not significantly different while the control flowers had the smallest diameter. The cloudiness of the control vase solutions (tap water) indicated that there was an abundance of microbes in the vase solutions. These microbes clogged the xylem vessels of the flower stem. Similarly, all flowers were partially opened on day 3and there were rapid increases in diameter of flowers treated with 8-HQC, copper coin and leaf extracts of *P. guajava* and *P. betle* from 3-5 days. On day 5, control flowers had already attained the maximum diameter opening. Flowers treated with 8-HQC, copper coin, leaf extracts of *P. guajava* and *P. betle* obtained maximum diameter opening on day 5 and the diameter opening level off until day 9 by which time the flowers began to senesce.

5.8.2 Water relations

A water deficit may develop only when the rate of water uptake is lower than the rate of transpiration, and a high rate of transpiration disrupts the water balance, which may then shorten the vase life of cut roses. Hence, the onset of water stress can be delayed by reducing water loss (van Doorn, 1997). In the present experiment, 6% sucrose +2% betel leaf treatments decreased water loss and maintained optimal water balance and therefore extended the vase life of cut rose flowers. Germicides control the microbial growth and partially decrease the resistance to water uptake (Jones and Hill, 1993). The control solution was full of bacteria cells since no germicide was used to control the microbial growth. This might have caused the vascular plugging and subsequent lower water uptake. Rahman *et al*. (2012) in their trial to prolong the vase life of cut carnation flowers affirmed that flowers treated with leaf extracts of *P. guajava* and *P. betle* had the highest water uptake, followed by 8-HQC and copper coin by day 11. Flowers in tap water had the lowest water uptake.

5.8.3 Fresh weight changes

Fresh weight (%) was highest for the treatment 6% sucrose + 2% betel leaf when compared to control. Fresh weight decreased gradually towards the end of vase life in all the treatments with the lowest value corresponding to the control

treatment (tap water) in both the cultivars of rose. Fresh weight is attributed to the higher uptake of water and better development of corolla (Hutchinsen *et al*., 2005). Flower fresh weight is lost gradually due to loss in available carbohydrates through the process of respiration. So the recorded improvement of fresh weight in these findings can be attributed to the continuous supply of sucrose. Furthermore, betel leaf can be prized as a very good biocide which prevents microbial plugging of vascular bundles and consequent increase in water uptake. Likewise, Da Silva, (2003) reported that sucrose delays the onset of hydrolysis of structural components of cell, decrease ethylene production and sensitivity which means that flower structural integrity is maintained and fresh weight is increased. Sucrose may have, also, a beneficial effect on maintaining higher fresh weights in cut flowering stems by inducing stomata closure in the leaves and thus, reducing water loss (Marousky, 1972). Furthermore, pulsing cut flowers in sucrose resulted in higher longevity period, which might indicate that sucrose played a critical role in promoting water absorption and metabolic processes within flower (Kim and Lee, 2002).Similar to our observations, Pirpour *et al*. (2013) in their study on *Lilium santander* found that on the sixth day, the use of thyme with a concentration of 900 mg/L, and the peppermint with concentrations of 300 and 900 mixed with sucrose showed a considerable increase in the flowers' wet weight than on the 3rd day. They conclude that when sucrose was used with peppermint and thyme, the resultant substances prevented the growth of bacteria, while sucrose improved water absorption through osmotic pressure that it exerts, resulting in an increased wet weight.

5.8.4 Vase life

The vase life of cut rose flowers was significantly extended by the different concentrations of sucrose and 2% betel leaf used. The vase life was longer in 6% sucrose + 2% betel leaf, which gave 13.92 days in comparison to 5.58 days for the control treatment. The previous results show that adding sucrose extended the vase-life and improved the quality of cut roses. Adding a carbohydrate source such sucrose to the holding solution resulted in an extension of vase-life if the growth of microorganisms was controlled by a suitable biocide. The increased flower longevity in the betel leaf extract which acted as a biocide was due to the inhibition of vascular blockage and increased water absorption (Marousky, 1971). The pulsed rose cut

flowers for 24h in 6% sucrose +2% betel leaf resulted in a longer vase life compared to ther pulsing treatments. This is in harmony to the recommendations of Halevy and Kofranek (1984) where treated Lisianthus flower by 6% sucrose for 24h, considerably increased the flower opening and longevity. This is in agreement with the previous study conducted in carnation by Rahman *et al*., 2012 where longevity of the Carola and Pallas Orange carnation flowers doubled when treated with leaf extracts of *Psidium guajava* and *Piper betle* compared to the flowers placed in the control solution. These results indicated that leaf extracts of *P. guajava* and *P. betle* were able to prolong the vase life of carnations flowers.

5.8.5 Chlorophyll content

The maintenance of green colour in the leaves is an important quality in the economically significant ornamental plants. It has been shown that leaf chlorophyll content decreases during senescence (Tang *et al*., 2005; Ferrante *et al*., 2009; Guiboileau *et al*., 2010). In the current trial also the chlorophyll content gradually decreased in all the treatments with sucrose and biocide but it was more rapid in control treatment. Senescence delay and chlorophyll preservation has been achieved by various compounds which mostly have growth regulatory behavior such as: GA (Ferrante *et al*., 2009; Tiwari *et al*., 2010), benzyladenine (Petridou *et al*., 2001) and tidiazuron (Ferrante *et al*., 2009; Tiwari *et al*., 2010). Few reports have studied the effect of preservative biocides on chlorophyll content changes.

5.8.6 Freshness of leaves and petals

Petals and leaves of a cut flower are the main ornamental parts and turgidity of these parts is important for a good looking product. Petal turgidity depends largely on water uptake and maintenance in treatments used. Results of this experiment show significantly higher water uptake and water maintenance in cut flowers, which subsequently increase the cut flower fresh weight. The increases in water uptake and subsequently cut flower fresh weight area was apparently due to the acidifying and stress alleviating properties of betel leaf. According to our results, we can generally discuss that the major part of the absorbed water is gathered in the

petals and leaves which in fact helps to have a better visual quality in betel leaf treated cut flower samples. Similar results were obtained by Mashhadian *et al*. (2012) in their study with salicylic and citric acid treatments to improve the vase life of cut chrysanthemum flowers.

5.8.7 Blueing of petals

Petal blueing is regarded as a characteristic of deterioration in cut red roses (Halevy and Mayak, 1981). In this study also all the treatments of red cultivar showed blueing of petals but the intensity was higher in the control treatment. Weinstein (1957) suggested that petal blueing was due to pH increase resulting from increased free cellular ammonia accompanying proteolysis. The well-known bluing phenomenon in senescing red roses is tightly linked to a shift into the blue range of some anthocyanins when pH is increased. Asen *et al*. (1971) reported that cyanidin-3,5-di-O-glucoside was colorless at lower pH levels and formed complexes with quercetin derivates when pH was increased. These complexes can modify the color of the plant tissue to bluish hues.

5.8.8 Bent neck

The vase life of cut rose flowers often terminates by bending the floral axis just below the flower head, which is called bent-neck. In the present study bent neck was observed only in the control treatment. The development of such symptoms is considered to be caused by vascular occlusion, which inhibits water supply to the flowers (Mayak *et al*., 1974; De Stigter, 1980; Van Doorn, 1997). Bent neck occurs not only by the reduction of water uptake due to the blockage, but also by water stress caused by elevated transpiration rate and competition for water between leaves and petals. Bending rate increased in the 3 first days in the preservative solutions containing benzyl adenine, nano silver, 8-hydroxyquinolin sulfate, and sucrose in Dianthus cv. Cream Viana but this increase was remarkably lower in all preservative concentrations compared to control in which rise of bending rate happened from the 0^{th} day. Lack of bent neck in the treatments containing Betel leaf extract or 8-HQC might be due to their antimicrobial properties.

5.8.9 TSS

It was observed that 6% sucrose + 2% betel leaf extract exhibited the highest TSS value while control (tap water) the lowest in both the rose cultivars. This might be due to the availability of external sucrose for respiration. Moradi *et al*. (2012) found that the amount of soluble solids remarkably increased for all concentrations of benzyl adenine in solutions containing 2 and 4 ppm nano silver, 300 ppm 8-Hydroxyquinoline sulfate and 3% sucrose in comparison with control flowers.

Considering the findings of this study, one can argue that the use of such natural compounds such as extracts can help decrease the microorganisms existing in preservative solutions. Microorganisms can play a negative role in decreasing the degree of flowers' solution absorption and wet weight, while causing vascular blockage, all which can degrade the longevity and external quality of flowers (Azizi *et al*., 2007).

Summary and Conclusion

Chapter 5 SUMMARY AND CONCLUSION

The present investigation entitled impact of herbal extracts on the microbial vascular plugging, quality and longevity of cut roses Konfetti and Bordeaux was carried out during 2011-12 and 2012-13 at the Model Floriculture Centre, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, District Udham Singh Nagar (Uttarakhand).

The cause of short vase-life in cut roses Konfetti and Bordeaux was investigated by the SEM. In the vascular bundles of 5-days old cut-rose stems, there were abundant hyphae of mycelium as well as bacterial cells .Thus, microbial blockage was an important cause of short vase-life in cut-rose flowers since it inhibited water uptake.

In order to replace chemicals with natural compounds as antimicrobial preservatives used in solutions for cut flowers due to their safety five herbs namely *Curcuma longa*, *Azadirachta indica*, *Piper betle*, *Ocimum sanctum* and *Allium sativum* were selected based on their traditional usage in India to access their antibacterial and antifungal potential. Ethanolic extracts of all the herbs were prepared and the highest yield (%) was obtained for turmeric (6.06%) followed by neem (5.08%), garlic(4.25%), betel leaf(4.1%) and the least for tulsi(2.15%).

The microbial count of the vase solution (tap water) in which cut rose flowers were kept for 5 days was analysed using serial dilution technique and bacterial count was 2.75 x 10^5cfu/ml and 2.48 x 10^5 cfu/ml for Konfetti and Bordeaux, respectively. Thereafter, the microbial kind was studied based on their morphological, physiological and biochemical characteristics. in this experiment only 5 microbial types viz, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Mucor* sp. and *Rhizopus* sp. were obtained.

Nutrient agar (NA) plates were used to detect the antibacterial activity of the herbal extracts. Zone of inhibition was measured and the best herbal extract inhibiting the visible growth of bacteria was identified. Betel leaf extract (*Piper betle*) showed biggest zone of inhibition (26 mm) followed by Neem (*Azadirachtaindica*) (20 mm)

and Garlic extracts (*Allium sativum*) (15 mm). However, no zone of inhibition was observed for Turmeric (*Curcuma longa*) and Tulsi (*Ocimum sanctum*).

Similarly, antifungal property of the herbal extracts was measured against the fungus present in the vase solution. Only Betel leaf extract (*Piper betle*) showed a clear zone of inhibition measuring 28 mm and completely inhibiting any visual growth of the fungus, while other extracts did not produce any inhibition zone. Interestingly, 8-HQC failed to prevent the fungal growth and did not produce any inhibition zone. Hence, only Betel leaf (*Piper betle*) extract was chosen to study the antifungal properties.

10% concentration of Betel leaf extract produced largest inhibition zone of 29 mm against *E.coli* followed by 5% (25 mm), 2% (18 mm) and 1% (15 mm) concentrations. However, 10% Neem extract showed maximum zone of inhibition of 23 mm followed by 5% (28 mm), 2% (17 mm) and 1% (15 mm) concentrations. Garlic extract produced zone of inhibitions measuring 17 mm and 15 mm for 10% and 5% concentrations, respectively. Interestingly, Garlic extract in 2% and 1% concentrations failed to prevent the growth of *E. coli* resulting in no inhibition zones.

Similarly, against *Bacillus* sp., Betel leaf extract demonstrated inhibition zones of 24 mm, 20 mm, 17 mm and 13 mm for 10%, 5%, 2% and 1% concentrations, respectively. Inhibition zones of 18 mm, 17 mm, 16 mm and 11 mm were recorded for 10%, 5%, 2% and 1% for Neem extract against the *Bacillus* sp. No significance difference was observed in the sizes of inhibition zones for 10%, 5% and 2% Neem extracts against *Bacillus* sp..However, none of the concentrations of the Garlic extract produced inhibition zones as they could not prevent the growth of *Bacillus* sp..

MIC studies against *Pseudomonas* sp. demonstrated maximum zone of inhibition of 22 mm for 10 % betel leaf followed by 19 mm, 15 mm and 12 mm for 5%, 2% and 1% concentrations, respectively. Neem extract also produced encouraging results with 10% concentration exhibiting 19 mm inhibition zone followed by 17 mm (5%) and 17 (2%). No significance difference was seen in the diameter of inhibition zones for 10%, 5% and 2% Neem extracts against *Pseudomonas* sp. However, 1% Neem extract failed to prevent the bacterial growth and thus did not produce any zone. Moreover, Garlic extract could not prevent visible growth of *Pseudomonas* sp. and thus resulted in no inhibition zone.

Therefore, on the basis of above observations, 2% concentration of betel leaf extract (*Piper betle*) was found to be the best to inhibit any visible bacterial growth.

Ten percent (10%) concentration of Betel leaf extract produced a very clear 30 mm inhibition zone against Mucor sp. Similarly, 5% and 2% concentrations demonstrated 23 mm and 17 mm inhibition zones, respectively. However, 1% concentration of Betel leaf extract failed to prevent the growth of fungus, thus did not produce any inhibition zone.

Similarly, against Rhizopus sp., 10%, 5% and 2% concentrations of the Betel leaf extract prevented the growth of *Rhizopus* sp. and produced 32 mm, 29 mm and 30 mm inhibition zones, respectively. However, similar to the activity against *Mucor* sp., 1% concentration of betel leaf extract failed to inhibit the *Rhizopus* growth on SDA. Betel leaf extract at 2% concentration prevented any visual growth of any of the fungus on SDA.

Among the holding solutions, 2% sucrose+2% betel leaf extract recorded the highest value followed by 2 % sucrose + 200 ppm 8-HQC and the least effectiveness was noticed in the control solution. 2% sucrose+2% betel leaf extract showed better results regarding vaselife, water relations, fresh weight changes, chlorophyll content, flower diameter and flower quality.

Among the pulsing solutions, 6% sucrose+2% betel leaf extract recorded the highest value followed by 9 % sucrose + 200 ppm 8-HQC and the least effectiveness was noticed in the control solution. 6% sucrose+2% betel leaf extract increased the vase life, water relations, fresh weight changes, chlorophyll content, flower diameter and flower quality.

On the basis of the results summarized above it can be concluded that holding, pulsing treatments were effective in increasing the vase life and presentability of the rose cut flowers Konfetti and Bordeaux. 2% sucrose+2% betel leaf extract was the best treatment among holding solutions and6% sucrose+2% betel leaf extract among pulsing solutions. Considering the findings of this study, one can argue that the use of such natural compounds such as betel leaf extract can help decrease the microorganisms existing in preservative solutions and increase the postharvest quality and longevity of cut roses.

Future lines of work:

Based on the results obtained from the present study, it would be worthwhile to investigate the possibility of the following aspects in the future:

1. Screening of betel leaf extract as a biocide for the cut flowers other than cut roses
2. Identification of other herbal extracts which can be potentially used as antimicrobial agents in the vase solution and their comparison with betel leaf.
3. Betel extract can be tested against other bacteria/fungi commonly found in vase solution. Moreover, MIC can be determined against these microorganisms.
4. Transfer of technology to the floriculture industry, so that they can use herbal extracts as vase solution biocides.

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Appendices

Appendix 1: Analysis of variance for initial flower diameter

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	0.841**	0.967**	0.903**
Holding solution	8	0.003	0.001	0.001
Rose culture × Holding solution	8	0.004	0.001	0.001
Error	36	0.004	0.007	0.003
Total	53			

Appendix 2: Analysis of variance for final flower diameter

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	7.149**	6.093**	6.610**
Holding solution	8	13.888**	15.342**	14.454**
Rose culture × Holding solution	8	0.206	0.350	0.217
Error	36	0.130	0.294	0.109
Total	53			

Appendix 3: Analysis of variance for opening of flower bud

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	1.185**	0.907*	1.041**
Holding solution	8	8.416**	9.407**	8.896**
Rose culture × Holding solution	8	0.185	0.074	0.093
Error	36	0.148	0.148	0.125
Total	53			

Appendix 4: Analysis of variance for days to maximum flower diameter

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	11.573**	6.685**	8.962**
Holding solution	8	16.874**	16.768**	16.622**
Rose culture × Holding solution	8	0.615	0.768	0.379
Error	36	0.759	0.555	0.291
Total	53			

Appendix 5: Analysis of variance for total water uptake

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	83.127**	33.297**	55.408**
Holding solution	8	127.420**	99.112**	112.598**
Rose culture × Holding solution	8	2.755*	1.877	1.251
Error	36	0.939	1.106	0.633
Total	53			

Appendix 6: Analysis of variance for total water loss

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	39.348**	30.842**	34.974**
Holding solution	8	73.204**	63.455**	68.098**
Rose culture × Holding solution	8	0.579	1.904	0.997
Error	36	0.702	1.177	0.508
Total	53			

Appendix 7: Analysis of variance for water balance

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	0.155	0.031	0.081
Holding solution	8	4.280**	4.148**	4.212**
Rose culture × Holding solution	8	0.052	0.051	0.049
Error	36	0.135	0.104	0.068
Total	53			

Appendix 8: Analysis of variance for transpiration rate

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	0.0006	0.0000007	0.0001
Holding solution	8	0.0553**	0.0445**	0.0497**
Rose culture × Holding solution	8	0.0004	0.0002	0.0003
Error	36	0.0007	0.0005	0.0003
Total	53			

Appendix 9: Analysis of variance for relative fresh weight on day 2

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	13.416*	19.615	16.395*
Holding solution	8	102.760**	85.348**	93.127**
Rose culture × Holding solution	8	3.072	0.563	1309
Error	36	2.141	9.721	2.864
Total	53			

Appendix 10: Analysis of variance for relative fresh weight on day 5

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	3.643	3.891	0.101
Holding solution	8	1320.089**	1403.857**	1360.729**
Rose culture × Holding solution	8	1.127	3.221*	1.221
Error	36	2.263	1.152	0.892
Total	53			

Appendix 11: Analysis of variance for relative fresh weight on last day

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	54.755**	80.519**	67.172**
Holding solution	8	128.147**	118.660**	119.584**
Rose culture × Holding solution	8	1.160	8.099	3.108
Error	36	0.913	6.906	2.134
Total	53			

Appendix 12: Analysis of variance for vase life

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	24.000**	13.500**	18.375**
Holding solution	8	12.171**	12.958**	12.337
Rose culture × Holding solution	8	0.458	1.124	0.458
Error	36	0.444	0.574	0.254
Total	53			

Appendix 13: Analysis of variance for chlorophyll content on day 2

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	40.728**	10.655*	23.303**
Holding solution	8	125.420**	159.617**	140.854**
Rose culture × Holding solution	8	0.205	1.078	0.268
Error	36	0.913	1.758	0.553
Total	53			

Appendix 14: Analysis of variance for chlorophyll content on day 5

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	31.139**	0.176	9.070**
Holding solution	8	435.932**	442.531**	438.257**
Rose culture × Holding solution	8	0.506	1.358	0.322
Error	36	3.005	1.804	1.062
Total	53			

Appendix 15: Analysis of variance for chlorophyll content on last day

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	13.104**	0.386	4.503*
Holding solution	8	107.436**	149.229**	126.599**
Rose culture × Holding solution	8	0.776	0.754	0.453
Error	36	1.654	1.285	0.715
Total	53			

Appendix 16: Analysis of variance for freshness of leaves and petals

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	0.166	0.166	0.166
Holding solution	8	9.671**	10.532**	10.052**
Rose culture × Holding solution	8	0.041	0.041	0.031
Error	36	0.185	0.148	0.069
Total	53			

Appendix 17: Analysis of variance for blueing of getals

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	6.000**	8.166**	7.041**
Holding solution	8	0.333**	0.375*	0.333**
Rose culture × Holding solution	8	0.333*	0.375*	0.333**
Error	36	0.129	0.129	0.064
Total	53			

Appendix 18: Analysis of variance for TSS

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	0.084	0.698*	0.074
Holding solution	8	3.949**	5.485**	4.550**
Rose culture × Holding solution	8	0.315	0.607**	0.375**
Error	36	0.147	0.161	0.069
Total	53			

Appendix 19: Analysis of variance for initial flower diameter

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	1.581**	1.800**	1.689**
Holding solution	8	0.001	0.005	0.002
Rose culture × Holding solution	8	0.003	0.004	0.001
Error	36	0.010	0.007	0.005
Total	53			

Appendix 20: Analysis of variance for final flower diameter

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	0.628	0.003	0.183
Holding solution	8	16.736**	16.800**	16.67**
Rose culture × Holding solution	8	0.440	0.248	0.298**
Error	36	0.215	0.143	0.067
Total	53			

Appendix 21: Analysis of variance for opening of flower bud

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	2.240**	0.666	1.337**
Holding solution	8	4.782**	3.393**	4.025**
Rose culture × Holding solution	8	0.115	0.124	0.087
Error	36	0.222	0.240	0.125
Total	53			

Appendix 22: Analysis of variance for days to maximum flower diameter

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	14.518**	0.296	4.740**
Holding solution	8	48.393**	34.282**	40.343**
Rose culture × Holding solution	8	0.310	0.671	0.334
Error	36	1.111	1.074	0.462
Total	53			

Appendix 23: Analysis of variance for total water uptake

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	62.431**	117.544**	87.807**
Holding solution	8	773.373**	746.899**	759.895**
Rose culture × Holding solution	8	6.972**	8.032**	7.304**
Error	36	1.499	2.109	1.431
Total	53			

Appendix 24: Analysis of variance for total water loss

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	59.57**	189.800**	115.491**
Holding solution	8	679**	609.127**	640.975**
Rose culture × Holding solution	8	7.078**	19.541**	9.816**
Error	36	1.512	3.947	1.727
Total	53			

Appendix 25: Analysis of variance for water balance

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	0.033	0.031	0.032*
Holding solution	8	3.414**	4.724**	4.039
Rose culture × Holding solution	8	0.005	0.028	0.010
Error	36	0.011	0.033	0.006
Total	53			

Appendix 26: Analysis of variance for transpiration rate

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	0.0016	0.0088**	0.0045**
Holding solution	8	0.0432**	0.0410**	0.0420**
Rose culture × Holding solution	8	0.0007	0.0003	0.0004
Error	36	0.0007	0.0004	0.0002
Total	53			

Appendix 27: Analysis of variance for relative fresh weight on day 2

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	88.567**	174.192**	127.812**
Holding solution	8	160.700**	125.096**	140.976**
Rose culture × Holding solution	8	2.106	5.960**	3.179**
Error	36	0.994	1.721	0.644
Total	53			

Appendix 28: Analysis of variance for relative fresh weight on last day

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	119.246**	284.628**	193.170**
Holding solution	8	195.720**	213.431**	204.201**
Rose culture × Holding solution	8	2.729*	3.002	2.015
Error	36	1.094	2.304	1.347
Total	53			

Appendix 29: Analysis of variance for vase life

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	15.574**	2.240	7.407**
Holding solution	8	38.601**	32.726**	35.112**
Rose culture × Holding solution	8	0.324	0.699	0.334
Error	36	0.722	1.111	0.398
Total	53			

Appendix 30: Analysis of variance for chlorophyll content on day 2

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	42.940**	81.522**	60.727**
Holding solution	8	97.157**	91.073**	93.732**
Rose culture × Holding solution	8	5.965**	2.955**	3.855**
Error	36	0.454	0.820	0.353
Total	53			

Appendix 31: Analysis of variance for chlorophyll content on last day

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	21.570**	27.509**	24.449**
Holding solution	8	28.284**	42.545**	33.984**
Rose culture × Holding solution	8	2.396**	2.846**	2.376**
Error	36	0.566	0.257	0.242
Total	53			

Appendix 32: Analysis of variance for freshness of leaves and petal

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	0.462	0.00002	0.115
Holding solution	8	10.199**	9.666**	9.855**
Rose culture × Holding solution	8	0.087	0.000002	0.021
Error	36	0.148	0.166	0.106
Total	53			

Appendix 33: Analysis of variance for blueing of petals

Source of variance	d.f.	Mean sum of square		
		2012	2013	Pooled
Rose culture	1	11.574**	11.573**	11.572**
Holding solution	8	0.074	0.199	0.116
Rose culture × Holding solution	8	0.073	0.197	0.115
Error	36	0.074	0.129	0.069
Total	53			

Appendix 34: Analysis of variance for TSS

Source of variance	d.f.	Mean sum of square		
		2012	**2013**	**Pooled**
Rose culture	1	0.003	0.463	0.099
Holding solution	8	14.279**	10.023**	11.915**
Rose culture × Holding solution	8	0.351*	0.605**	0.348**
Error	36	0.129	0.127	0.079
Total	53			

VITA

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ABSTRACT

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Advisor	:	Dr. Santosh Kumar			

The present investigation was carried out at the Model Floriculture Centre, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, during 2011-12 and 2012-13 to study the impact of herbal extracts on the microbial vascular plugging, quality and longevity of cut roses Konfetti and Bordeaux. The major cause of vase life reduction in cut flowers is water relation interruption which is mostly due to vase solution microbial proliferation and consequently vascular occlusion resulting in solution uptake reduction. The microbial vascular plugging of cut roses Konfetti and Bordeaux was confirmed through the findings from SEM. A broad range of chemical biocides has been suggested to prevent the proliferation of microorganisms in vase solutions. In order to replace chemicals with natural compounds as antimicrobial preservatives used in solutions for cut flowers five herbal extracts namely, *Curcuma longa*, *Azadirachta indica*, *Piper betle*, *Ocimum sanctum* and *Allium sativum* were assessed for their antimicrobial properties against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Mucor* sp. and *Rhizopus* sp. isolated from vase solution of cut roses. Among these betel leaf had the highest zone of inhibition against all these microorganisms and its MIC was found to be 2%. Further, in order to compare the in-vivo efficacy of betel leaf extract with 8-HQC as well as to assess the effect of betel leaf extract on quality and longevity of both the cultivars of rose in holding and pulsing solutions, experiments were laid out in Factorial CRD. 2% sucrose + 2% betel leaf extract and 6% sucrose + 2% betel leaf extract were respectively the best among the holding and pulsing solutions. They improved the vase life, water relations, fresh weight, flower diameter and chlorophyll content compared to 8-HQC. Among the cultivars Bordeaux showed better response in improvement of quality and longevity. Thus it is evident from this study that betel leaf extract can be used as an organic biocide in the vase solutions for the improvent of quality and longevity of cut roses.

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सारांश

नाम : अनीषा पी०एन० **परिचयांक** : ४११२६

सत्र एवं वर्ष : प्रथम, २०१०–११ **उपाधि** : पी०एच०डी०

प्रमुख विषय : पुष्प विज्ञान एवं भूदृश्य **विभाग** : उद्यान विज्ञान

गौण विषय : वनस्पति विज्ञान

शोध शीर्षक : ''कट गुलाब कोनफेटी एवं बोर्डो के सूक्ष्म जैवीय संवहन अवरोध, गुणवत्ता एवं लंबी उम्र पर हर्बल अर्कों का प्रभाव''

सलाहाकार : डा० संतोष कुमार

वर्तमान जांच गोविन्द बल्लभ पंत कृषि एवं प्रौद्योगिक विश्वविद्यालय पंतनगर के आदर्श पुष्प विज्ञान केन्द्र पर वर्ष 2011–12 एवं 2012–13 के दौरान कट गुलाबों कोनफेटी एवं बोर्डो के सूक्ष्मजैविक संवहन अवरोध, गुणवत्ता और लंबी उम्र पर हर्बल अर्कों के प्रभाव का अध्ययन करने के लिए की गई थी। कट फूलों के फूलदान जीवन में कमी का मुख्य कारण पानी संबंधी रूकावट होता है, जो मुख्यतः फूलदान घोल में सूक्ष्म जीवाणुओं के प्रसार और उसके फलस्वरूप होने वाली संवहन बाधा की वजह से होता है। जिसके परिणामस्वरूप फूलदान घोल के संवहन में कमी होती है।

कट गुलाबों कोनफेटी और बोर्डो के सूक्ष्मजैविक संवहन अवरोध की पुष्टि स्कैनिग इलेक्ट्रान सूक्ष्मदर्शी (एस.ई.एम.) के निष्कर्षों के आधार पर की गई। रासायनिक जीवाणुनाशकों की एक विस्तृत रेंज फूलदान घोल में सूक्ष्म जीवाणुओ के प्रसार को रोकने के लिए प्रस्तावित की गई है। कट फूलों के फूलदान घोल में रासायनों के स्थान पर प्राकृतिक जीवाणुनाशक यौगिकों का उपयोग करने के लिए पांच हर्बल अर्कों अर्थात *कुरकुमा लोंगा अजाडिरैक्टा इंडिका, पाइपर बीटल, ओसिमस सैंक्टम* और *एलियम सैटाइवम* का आंकलन किया गया। इन पाँचों हर्बल द्रव्यों की जीवाणुनाशक गुणवत्ता की जांच फूलदान घोल से अलग किए गये ई० कोलाई, स्यूडोमोनास एरोजिनोसा बेसिलस सबटीलिस, म्यूकर स्पी० और राइजोपस स्पी. के विरूद्ध की गई। इनमें पान के पत्ते के अर्क ने 2 प्रतिशत न्यूनतम निरोधात्मक मात्रा के साथ सभी पांच सूक्ष्मजीवियों के विरूद्ध उच्चतम निषेध क्षेत्र का प्रदर्शन किया। इसके अतिरिक्त 8 एच.क्यू.सी. के साथ पान के पत्ते के अर्क की इनविवो प्रभावकारिता की तुलना करने के लिए और साथ ही होल्ड़िग और पलसिंग घोलों में दोनो कट गुलाबों की गुणवत्ता और दीर्घायु पर पान के पत्ते के अर्क के प्रभाव का आंकलन करने के लिए क्रमगुणित सी.आर.डी. का प्रयोग किया गया। सभी होल्ड़िग और पलसिंग घोलों में 2 प्रतिशत सुक्रोज + 2 प्रतिशत पान के पत्ते का अर्क और 6 प्रतिशत सुक्रोज + 2 प्रतिशत पान के पत्ते का अर्क क्रमशः सर्वोत्तम पाये गये। 8 एच.क्यू.सी. की तुलना में उनके उपयोग से फूलदान जीवन, जल संवहन, ताजा वजन, फूल व्यास और क्लोरोफिल मात्रा में महत्वपूर्ण सुधार हुआ। हालांकि, बोर्डो प्रजाति ने गुणवत्ता और दीर्घायु के सुधार में बेहतर प्रतिक्रिया दिखाई।

इस प्रकार, इस अध्ययन से सिद्ध होता है कि पान के पत्ते के अर्क का उपयोग फूलदान घोल में कार्बनिक जीवाणुनाशक की तरह कट फूलों की गुणवत्ता और दीर्घायु बढ़ाने में सहायक हो सकता है।

(संतोष कुमार)
सलाहाकार

(अनीषा पी०एन०)
लेखिका