

**FORMULATION OF ORGANIC VASE SOLUTION FOR CUT FLOWERS
USING COMPOST AND OTHER NATURAL ADDITIVES**

by

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TABLE OF CONTENTS

LIST OF TABLES	vi
LIST OF FIGURES	viii
ABSTRACT	x
ACKNOWLEDGEMENTS	xi
CHAPTER 1.0 INTRODUCTION	1
1.1 Thesis Overview.....	1
1.2 Thesis Objectives and Organization.....	3
1.3 References.....	5
CHAPTER 2.0 REVIEW OF LITERATURE	9
2.1 Factors affecting vase life.....	9
2.1.1 Preharvest conditions affecting vase life.....	9
2.1.2 Postharvest conditions affecting vase life.....	11
2.2 Ethylene and Flower Longevity.....	13
2.2.1 Role of ethylene in senescence.....	13
2.2.2 Ethylene biosynthesis.....	14
2.3 Metabolic Changes in Cut Flowers.....	14
2.3.1 Wounding and emboli formation.....	14
2.3.2 Water balance and weight change	15
2.3.3 Senescence and abscission.....	16
2.3.4 Cellular leakage.....	16
2.3.5 Change in flower color.....	17
2.4 Role of Carbohydrates in vase solution.....	17
2.5 Compost.....	19
2.5.1 Ability of compost to replace the chemicals	19
2.5.2 Presence of carbohydrates in compost.....	20
2.5.3 Harmful substances in compost.....	21
2.5.4 Effect of compost on vase life.....	21
2.6 Compost Tea.....	23
2.6.1 Mechanism of action of compost tea.....	24

2.7 Organic Substances Used to Extend Vase Life.....	25
2.7.1 Germicides	25
2.7.2 Acidifiers.....	26
2.7.3 Coagulants.....	27
2.7.4 Hormones and Growth Regulators.....	27
2.7.5 Herbal Extracts.....	27
2.8 Conclusion.....	29
2.9 References.....	30
CHAPTER 3.0 STABILITY OF COMPOST TEA AT VARYING CONCENTRATIONS, STORAGE TEMPERATURES AND STORAGE DURATION.....	43
3.1 Abstract.....	43
3.2 Introduction.....	44
3.3 Materials and Method.....	46
3.3.1 Preparation of compost tea.....	46
3.3.2 Experimental setup.....	48
3.3.3 Municipal solid waste compost tea analysis.....	48
3.3.4 Statistical analyses.....	48
3.4 Results and Discussion	49
3.4.1 Physicochemical properties of compost tea.....	49
3.4.2 Multiple linear regression.....	57
3.4.3 Correlation among physicochemical properties.....	61
3.5 Conclusion.....	62
3.6 References.....	64
CHAPTER 4.0 EFFECT OF COMPOST TEA CONCENTRATION ON VASE LIFE OF CUT FLOWERS.....	69
4.1 Abstract.....	69
4.2 Introduction.....	69
4.3 Materials and Method.....	71
4.3.1 Preparation of compost tea concentrations.....	71
4.3.2 Experimental setup.....	71
4.3.3 Physicochemical properties	72

4.3.4 Visual analysis of cut flowers.....	72
4.3.5 Statistical analysis.....	74
4.4 Results and Discussion.....	75
4.5 Conclusion.....	82
4.6 References.....	83
CHAPTER 5.0 EFFECT OF NATURALLY AMENDED COMPOST TEA ON VASE LIFE OF CUT FLOWERS.....	86
5.1 Abstract.....	86
5.2 Introduction.....	86
5.3 Materials and Method.....	89
5.3.1 Preparation of compost tea concentration.....	89
5.3.2 Addition of putrescine.....	89
5.3.3 Addition of lemon and rosemary extract.....	89
5.3.4 Preparation of cut flowers.....	90
5.3.5 Collection of data.....	90
5.3.5.1 Physicochemical properties.....	90
5.3.5.2 Visual analysis of cut flowers.....	91
5.3.5.3 Flower longevity.....	91
5.3.5.4 Fresh weight loss.....	91
5.3.5.5 Flower head diameter.....	91
5.3.5.6 Total suspended solids (TSS).....	92
5.3.6 Data analysis.....	92
5.4 Results and Discussion.....	92
5.4.1 Addition of putrescine.....	92
5.4.2 Physicochemical properties.....	93
5.4.3 Visual analysis of cut flowers.....	97
5.4.4 Flower longevity.....	100
5.4.5 Fresh weight loss.....	101
5.4.6 Flower head diameter.....	104
5.4.7 Total suspended solids (TSS).....	106
5.5 Conclusion.....	108
5.6 References.....	109

CHAPTER 6.0 CONCLUSION AND RECOMMENDATIONS.....	114
6.1 Overview.....	114
6.2 Future Recommendations.....	115
APPENDIX 1.0 PRELIMINARY RESEARCH.....	116
REFERENCES.....	121

LIST OF TABLES

Table 3.1	Properties and features of compost used for compost tea preparation...47
Table 3.2	Properties of compost tea stock solution used for preparation of compost tea concentrations..... 47
Table 3.3	ANOVA (P-values) of the effect of compost tea concentration, storage duration and storage temperature on physicochemical properties of compost tea..... 57
Table 3.4	Descriptive statistics of variables used for multiple linear regression model development..... 58
Table 3.5	Multiple linear regression model ($k + \alpha D + \beta C + \gamma \text{ temp}$) indicating relationship of chemical properties as influenced by storage duration (D), compost tea concentration (C) and storage temperature (temp)..... 59
Table 3.6	Pearson correlation coefficient (R) for correlation plots between the measured and predicted values of electrical conductivity (EC), total dissolved solids (TDS), salinity, turbidity and pH values..... 60
Table 3.7	Pearson's correlation coefficient (R) among electrical conductivity (EC), total dissolved solids (TDS), salinity (SAL) and turbidity (TURB) and pH of compost tea..... 61
Table 4.1	Change in pH, electrical conductivity (EC), turbidity, total dissolved solids (TDS) and salinity of varying concentrations of compost tea and Chrysal..... 77
Table 4.2	Scores for visual observations of physical parameters of carnation as affected by different concentrations of compost tea and Chrysal 80
Table 5.1	Various preservative solutions from a mixture of compost tea and natural substances and the synthetic chemical, Chrysal..... 90
Table 5.2	Change in pH, electrical conductivity (EC), turbidity, total dissolved solids (TDS) and salinity of 3.5% compost tea concentration after 48 hrs of putrescine addition.....93
Table 5.3	Change in pH, electrical conductivity (EC), turbidity, total dissolved solids (TDS) and salinity of different mixed preservative solutions and the synthetical chemical, Chrysal..... 95

Table 5.4	Average scores for various physical parameters as affected by different mixture of natural preservative solutions and Chrysal.	98
Table 5.5	Assessment of vase life, flower head diameter and fresh weight loss of cut carnation in different preservative solutions.....	100

LIST OF FIGURES

Figure 3.1	Effect of storage duration and storage temperature on pH of compost tea.....	51
Figure 3.2	Effect of concentration, storage duration and storage temperature on electrical conductivity (E.C) of compost tea.....	53
Figure 3.3	Effect of concentration, storage duration and storage temperature on total dissolved solids (TDS) of compost tea.....	54
Figure 3.4	Effect of concentration and storage duration on salinity of compost tea.....	55
Figure 3.5	Effect of concentration, storage duration and storage temperature on turbidity of compost tea.....	56
Figure 3.6	Correlation plots between measured and predicted values of electrical conductivity (EC), total dissolved solids (TDS), salinity, turbidity and pH values of compost tea using multiple linear regression model.....	60
Figure 3.7	Pearson’s correlation matrix among electrical conductivity (EC), total dissolved solids (TDS), salinity (SAL) and turbidity (TURB) of compost tea.....	62
Figure 4.1	Examples of grading of cut carnations (<i>Dianthus caryophyllus</i> L. var. White Sim) scored from 0 to 5 based on visual observation of physical parameters.....	74
Figure 4.2	Initial (Day 1) and final (Day 11) values of pH, electrical conductivity (EC), turbidity, total dissolved solids (TDS) and salinity of different concentrations of compost tea preservative solution and Chrysal.	78
Figure 4.3	Initial (Day 1) and final (Day 12) scores for various physical parameters of carnation as affected by different concentrations of compost tea and Chrysal.	81
Figure 5.1	Initial (Day 1) and final (Day 11) values of pH, electrical conductivity (EC), turbidity, total dissolved solids (TDS) and salinity of different mixed preservative solutions and the synthetic chemical, Chrysal.....	96

Figure 5.2	Initial (Day 1) and final (Day 12) scores for various physical parameters as affected by different mixture of natural preservative solutions and Chrysal.....	99
Figure 5.3	Effect of different mixed preservative solutions and Chrysal on fresh weight of carnation petals during 11 days of treatment.....	103
Figure 5.4	Effect of different mixed preservative solutions and Chrysal on flower head diameter during 11 days of treatment.....	105
Figure 5.5	Effect of selected mixture of compost tea and natural substances and Chrysal on total suspended solids (TSS) of carnation cut flowers during 11 days of treatment.....	107
Figure IA.1	Effect of compost tea concentrations (a) 0% (b) 5% (c) 15% (d) 30% and (e) 100% on vase life of cut flowers during 12 days of treatment.....	118
Figure IB.1	Cut flowers placed in compost tea concentrations 1.5%, 3%, 5% (from left to right) on day 1.....	119
Figure IB.2	Cut flowers placed in compost tea concentrations 1.5%, 3%, 5% (from left to right) on day 6.....	119
Figure IB.3	Cut flowers placed in compost tea concentrations 1.5%, 3%, 5% (from left to right) on day 12.....	120

ABSTRACT

Vase life quality of cut flowers is one of the most crucial factors for customer satisfaction. This study investigated the effect of concentration, storage time and temperature on chemical properties of compost tea prepared from municipal solid waste compost, to determine the most suitable concentration of compost tea that would improve the vase life of cut flowers and to assess the effect of natural additives and compost tea mixture on vase life of cut carnation. Three-way ANOVA analysis of physicochemical properties proved significant effect of compost tea concentration, storage duration and storage temperature on physicochemical properties of compost tea. It was stated that other than chrysal, the use of a preservative solution containing 3.5% compost tea led to an increase in vase life of cut carnations. The results showed that 3.5% compost tea (C_{3.5}) amended with 1 mL lemon extract + 0.5 mL rosemary extract (R_{0.5}L₁) also increased the vase life of cut carnations and are healthy and nonhazardous alternatives to synthetic chemicals.

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CHAPTER 1.0

INTRODUCTION

1.1 Thesis Overview

Cut flowers are used to show appreciation, affection and express emotion on various special occasions. The international trade in cut flowers has expanded in recent years and is expected to increase further with the promotion and use of horticultural plants for their therapeutic benefits, as well as with the current rise in population and consumer demand. Carnation (*Dianthus caryophyllus* L.) is one of the leading ornamental crops belonging to the family *Caryophyllaceae* (Tanase et al. 2012; Khatun et al. 2018). Carnation is used mostly as cut flower, which is usually classified as standard and spray type. The standard type has only one flower that grows on a stem, and the spray type has multiple flowers that grow on a stem (Satoh et al. 2005; Boxriker et al. 2018). Flowers of most standard varieties are double with up to 120 petals (Scovel et al. 1998). Standard cultivars are harvested when flowers are half opened, or when the outer petal is perpendicular to the stem (Salunkhe et al. 2012).

Vase life quality of cut flowers is one of the most crucial factors for customer satisfaction and repeat purchase. Flowers grown for the ornamental market must be of high quality. This will increase the postharvest longevity, marketability and commercial value of cut flowers. Like the other plant parts, cut flowers are actively metabolizing organs and therefore, deteriorate with time (da Silva Vieira et al. 2014). Four major factors during both production and postharvest stages that influence vase life are water relations, carbohydrate status, ethylene and pathogens (Darras et al. 2005; Dahal 2013; Fanourakis et al. 2013). Handling techniques to achieve cut flower longevity must accomplish two seemingly

conflicting purposes: the promotion of bud growth and development of the plant part to full opening and retardation of metabolic processes leading to senescence (Jones and Hill 1993). Many researchers have shown that an appropriate chemistry of vase solution can reduce rate of respiration and senescence and extend the longevity of cut flowers (Kazemi et al. 2011). Flower preservatives are composed of carbohydrate source such as sugar, inhibitors of ethylene biosynthesis such as silver thiosulphate (STS) and aminoethoxyvinyl glycine and germicides e.g. 1-methylcyclopropene, chlorine dioxide, and 8-hydroxyquinoline sulphate to extend the vase life of cut flowers (Gorin et al. 1985; Xie et al. 2008; Macnish et al. 2008; Elhindi 2012).

Carnation is an ethylene-sensitive flower and if preservatives are not used its vase life is normally short (about 7 days) (Sato et al. 2005; Ali et al. 2012). Commercial carnation producers widely use STS to extend the vase life of cut flowers due to its outstanding effect. However, concern about potential heavy-metal contamination of the environment by waste STS solutions has increased the search for alternative methods of controlling flower senescence in carnations (Ichimura et al. 2002; Mijndonckx et al. 2013).

Compost obtained from decaying organic matter is known to have antimicrobial properties and humic substances that protect and provide nourishment to plants in many ways (de Bertoldi 2010; Steel et al. 2018). Compost stability also defined as resistance of compost organic matter to degradation is important as immature compost may lead to depletion of oxygen or nitrogen and release phytotoxic compounds (Raviv 2005).

This suggests that properly formulated mature and stable compost tea mixed with other natural additives can potentially be a substitute for synthetic chemical preservative solutions. Several natural products such as essential oils, lemon juice, apple and other fruit

extracts have been evaluated for vase life extension of cut flowers because of environmentally friendly and their antimicrobial properties (Mehraj et al. 2013; El-Moneim et al. 2018).

1.2 Thesis Objectives and Organization

The specific hypothesis tested in this study was:

- mature and chemically-stable compost tea formulation amended with natural additives at the proper dose rate can extend the vase life and postharvest quality of cut carnation flowers (*Dianthus caryophyllus* L. var. White Sim)

The specific objectives of the research project were to:

- investigate the effect of concentrations, storage time and temperature on physicochemical properties such as pH, electrical conductivity, salinity, turbidity and total dissolved solids of compost tea prepared from municipal solid waste compost.
- determine the suitable concentration of compost tea that would improve vase life of cut flowers in an optimization study.
- assess the effects of natural additives and compost tea mixture on vase life of cut flowers.

The primary goal of this research was to assess the use of compost tea and natural additives on vase life of cut carnations (*Dianthus caryophyllus* L. var. White Sim). This thesis consists of six chapters, including the present chapter. Chapter two provides the scientific background on vase life, factors affecting the vase life of cut flowers and introduces the potential of compost tea to be used for the extension of vase life of cut flowers. This chapter also introduces the history and scientific background on the uses of natural germicides, acidifiers, hormones and herbal extracts for extension of vase life of cut flowers. The effect of storage time and temperature on physicochemical properties such as pH, electrical

conductivity, salinity, turbidity and total dissolved solids of compost tea is investigated in chapter three. Chapter four and five examines the effect of compost tea and natural additives on vase life of cut flowers. Thesis is concluded in chapter six followed by appendix.

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CHAPTER 2.0

REVIEW OF LITERATURE

2.1 Factors Affecting Vase Life

Vase life refers to the time for which the cut flower retains its appearance in a vase. Vase life is determined based on attributes such as diameter and length of florets, the opening of flowers, changes in fresh weight, diameter or length of stem or pedicel, senescence pattern, the color of petals, total longevity and foliage burning (De et al. 2014). Several efforts have been directed at the evaluation of pre- and post-harvest factors affecting vase life of cut flowers.

2.1.1 Preharvest conditions affecting vase life

It has been speculated by the authors of several studies that preharvest environmental factors alter the carbohydrate status in cut flowers, which in turn affect the vase life (Halevy and Mayak 1979; Slootweg 2003; Fanourakis et al. 2013). Despite support for this hypothesis, the effect of preharvest factors on vase life of cut flowers is not well documented. Light, nutritional status of growing media, temperature and water status have been studied previously (Halevy and Mayak 1979; Rafdi et al. 2014; De et al. 2015). For instance, it is generally accepted that brighter light conditions help to produce optimal carbohydrate level in horticultural crops and flowers, although effects may vary from species to species (Han 2001; Pettersen et al. 2010). Supplementary light and elevated CO₂ levels have led to longer vase life in carnation (*Dianthus caryophyllus*), chrysanthemum (*Dendranthema grandiflora*) and rose (*Rosa* spp.) as elevated light intensity is known to affect stomatal density which in turn affects gas exchange or photosynthetic rate (Halevy and Mayak 1979; Naing et al. 2016). It was also observed that roses cut in November or

December have a shorter shelf life than those cut between July to September. Additionally, Roses cut at 8:00 in the morning have a shorter shelf life than roses cut at 4:30 after a day of sunlight. Roses cut from the lower part of the plant where leaves were shaded have shorter shelf life than those cut from the top (Boodley 1969). The same effects of low light have been found in chrysanthemums, and the effects have been totally overcome by the proper metabolic sugars in the vase water (Halevy and Mayak 1979).

In contrast to high light, elevated temperatures, can deplete carbohydrate reserves and decrease keeping the quality of flowers by increasing the respiration rates (Boodley 1969; Locke 2010). Some reports indicated that temperatures as low as 12-15°C or as high as 27°C during three weeks before harvest decreased the longevity of cut flowers (Halevy and Mayak 1979). The possible interaction of temperature with other factors complicates the understanding of temperature effects on vase life. Low temperature can also increase pigmentation such as flavonoids and anthocyanins (Rani and Singh 2014). 'Golden Wave' roses kept at low temperatures (i.e. less than 15°C) developed a greenish tint (Halevy and Mayak 1979; Rani and Singh 2014). This was because chloroplasts were incompletely converted to chromoplasts, which was thought to be caused by low carbohydrate levels.

It is usually assumed that if the fertility and structure of the growing medium are within optimal range for plant growth, there will be little or no effect on the longevity of cut flowers (Boodley 1969). High salt stress causes a change in tissue osmotic pressure. For instance, when the concentration of salts in the soil solution is high compared to that in the plant sap, it is much more difficult for the plant to uptake water. Instead, the tendency would be for exosmosis due to differences in water potential gradient (Halevy and Mayak 1979).

2.1.2 Postharvest conditions affecting vase life

Postharvest physiology of cut flowers deals with the functional processes in plant material after it has been harvested or removed from its natural growing environment until it is utilized or deteriorated (Da Silva 2003). The close association of flower respiration during storage and vase life suggests the potential use of controlled atmosphere technology (Reid and Jiang 2012). The cut flowers and foliage can be packed in a gas impermeable package to create an inside environment that can modify carbon dioxide, oxygen, nitrogen, water vapor and trace gases (Robertson 2016). To provide an optimum atmosphere for increasing the storage length and quality of produce, a modified atmosphere (MAP) is created by altering the normal composition of air (78% nitrogen, 21% oxygen, 0.03% carbon dioxide and traces of noble gases). Under controlled atmospheric conditions (CAP), the atmosphere is modified from that of the ambient atmosphere, and these conditions are maintained throughout storage. Modified atmosphere (MAP) uses the same principles as CAP; however, it is used on smaller quantities of cut flowers and the atmosphere is only initially modified (Farber et al. 2003).

The use of controlled and modified atmospheres for packaging decreases polyphenol oxidase (PPO) and ethylene production (Tian et al. 2002). Reduced amount of O₂ and elevated CO₂ reduces respiration. O₂ level below a critical level for supporting aerobic respiration may result in the glycolytic conversion of pyruvate into acetaldehyde and ethanol. This situation will lead to tissue fermentation and browning (Gran and Beaudry 1993).

Darkness or low light intensity during shipping of cut flowers and foliage can reduce flower quality due to leaf and flower abscission (Cushman et al. 1998). Moreover, dark-held plants

stored at low temperature, when moved to ambient temperatures undergo a number of changes such as rapid loss of chlorophyll, proteolysis, loss of catalase activity and increased membrane permeability (Ranwala and Miller 2005). This can cause stressful conditions to the cut flower.

At harvest, water supply to plant is interrupted, which impairs the physical development of cut flowers. It was reported that a reduced xylem water potential below the water potential of adjacent cells resulted in the withdrawal of water from these cells to cause shrinkage and wilting (Leshem and Wills 1998). Also, the quality of vase water is important for maintaining the vase life of cut flowers and foliage. Pure, clean water with limited amounts of salt and alkalinity is a requisite. Addition of Mg and Ca ions in vase solution did not affect flower vase life (Kageyama et al. 1995). The presence of salts, basic ions, and fluoride in water reduces vase life (De 2015) as salts and basic ions are responsible for changes in pH that can affect osmotic potential (Da Silva 2003). Fluorine in water causes softening of tissues, leaf browning and wilting (Eason et al. 2004). It was found that addition of small amounts of fluorine (i.e. ~1 ppm) in vase solution had injurious effects on rose cut flowers (Lohr and Pearson-Mires 1990). Some stems of cut flowers may exude sap, various cations, anions and organic acids that can be harmful to flowers themselves, so the vase water will need regular replacement (Da Silva 2003). Addition of cobalt ions to vase solution partially closes the stomata, inhibits vascular blockage in the rose stem and increases the water uptake by cut flowers (Singh and Sharma 2002).

Storage of cut flowers is economically important as it enables the producers to distribute and transport the product to its destination, and to meet the demand of buyers. But an exponential increase is seen in respiration of the cut flowers with an increase in storage

temperature (Celikel and Reid 2002). The temperature outside the physiological norm can cause damage to cut flowers by freezing injury that results in the immediate collapse of tissue or by heat injury that can heat up tissues above the thermal cell death point (Da Silva 2003). Cooling prior to packaging and transportation reduces metabolic changes such as enzymatic activity, slows down the maturation of flowers, and reduces ethylene production, metabolic processes and improves longevity (De 2015).

Several postharvest diseases such as botrytis rot, mildews and other molds affect cut flowers. The wounded stem-end placed in water quickly depletes oxygen in the vase solution due to the increased rate of respiration of cells, which provides perfect growing conditions for microbes (Reid and Jiang 2012). Microbial cells are responsible for plugging the vascular system, enzymatic damage to vascular tissue, disruption of cell membrane integrity by releasing toxic metabolites and enhance the production of ethylene (Zagory and Reid 1986; Reid 2002; van Doorn 1997).

2.2 Ethylene and Flower Longevity

2.2.1 Role of ethylene in senescence

Role of endogenous ethylene in triggering petal senescence is well known (Reid and Jiang 2012). Flowers can be classified as climacteric or non-climacteric based on ethylene sensitivity that can vary from one species to another (Redman et al. 2002). In carnation, it is observed that ethylene is first produced in the pistil and then acts on petals. This induces the expression of genes for ACC synthase, ACC oxidase and cysteine proteinase resulting in auto-catalytic ethylene production (Ten Have and Woltering 1997; Reid and Wu 1992). The physical signs of ethylene response include in-rolling of the petals and wilting of flowers (Halevy and Mayak 1979).

2.2.2 Ethylene biosynthesis

Ethylene biosynthesis is under strict metabolic pathway. The sequence of the pathway of ethylene biosynthesis is defined as methionine → S-adenosyl methionine (SAM) → 1-aminocyclopropane -1-carboxylic acid (ACC) → ethylene (Yang 1985). It was observed that ACC content was very low in freshly harvested cut carnation flowers but with the onset of senescence, it rose rapidly (Yang and Hoffman 1984).

The lifespan of cut flowers is often terminated by the abscission of petals or by petal wilting. In many species, these processes are regulated by ethylene through changes in endogenous levels (Scariot et al. 2014). Ethylene production changes during plant development and in relation to physiological status (Yang and Hoffman 1984). Ethylene production is induced during certain stages of growth like abscission of leaves and senescence of flowers along with other external factors such as mechanical wounding, environmental stress and certain chemicals like auxins (Yang and Hoffman 1984).

2.3 Metabolic Changes in Cut Flowers

2.3.1 Wounding and emboli formation

Suberisation is thought to be a general response to mechanical damage of plant tissue in which a hydrophobic polymeric material called suberin attaches to cell walls (van Meeteren and Arévalo-Galarza 2008). This material was seen to appear in intercellular spaces between xylem parenchyma cells adjacent to wounded vessels and is responsible for compartmentalization after wounding. It can, therefore, be a barrier to water uptake. On cutting off a stem, water column in plants breaks resulting drawing of air into xylem vessels which constricts water flow in xylem (Ratnayake et al. 2010). Plant tissue blockage termed as emboli is formed immediately upon cutting the stem. Emboli result in a temporary

reduction in water uptake but may become permanent if the rate of transpiration exceeds the water conductance of the wounded stem (Reid and Jiang 2012). The reversion of air emboli present in xylem vessels partly results in a positive water balance and restoration of water uptake after harvest of cut flowers and foliage (van Meeteren and van Gelder 1999). The use of techniques such as detergent dips or vase solutions, low pH, re-cutting of the stem under water and immersing the stem in deep water containing biocide may help in improving rehydration and the vase life of recalcitrant cut flowers like *Heliconia* (*Heliconia rostrate*) (Reid and Jiang 2012).

2.3.2 Water balance and weight change

The most common cause of short vase life in cut flowers was found to be water stress as steady water flow is terminated at harvest (Da Silva 2003). Undesirable appearance and short vase life of cut flowers may result from reduced water uptake by xylem blockage due to microbial growth, deposition of gum and mucilage in the lumen of xylem vessels and the presence of air emboli in vascular tissues (Da Silva 2003). This can cause competition for water among the various tissues in the cut flower shoot (Torre and Fjeld 2001). Water imbalance due to continuous loss of water through transpiration may result in drooping and pre-mature wilting of flowers making them unacceptable to consumers. Thus, the appropriate water balance and turgor level, for example, are necessary for the development of buds to bloom. Water loss also enhances premature ripening, senescence, browning, chilling injury and thus, limits the vase life of cut flowers and foliage. High humidity occurring in a greenhouse reduces vase life as it leads to early bent neck and brittle leaves in cut roses 'Souvenir' (Mortensen and Gislerod 2000). More importantly, the vase life of cut flowers depends on the rate of transpiration through stomata and the type and nature of

the solutes present in the vase water. A study showed that sugar in the vase solution helped to decrease transpiration rate (Van Doorn 1997).

2.3.3 Senescence and abscission

The cells of senescing organs undergo gradual and orderly disassembly (Rani and Singh 2014). Plant growth hormones especially, cytokinin appear to control the monocarpic senescence process. Ethylene is known to stimulate changes in membrane lipids of leaves and petals, which results in rolling of petals, drying of sepals and corollas, wilting of flower and change in color (Bartoli et al. 1996). According to Williams et al. (1995), ethylene production is centrally involved in petal senescence. van Doorn and Stead (1997) reported that protease activity increases, lipid fluidity of membranes decrease, and respiration increases in petals of cut flowers undergoing senescence. On the stem of cut flowers, there are several leaves that also suffer the degenerative process of senescence. These reports suggested that leaf senescence results in several metabolic changes including increased activity of protease, enzymes, nucleases, and chlorophyllases (Casadoro et al. 1999) and can be triggered by stress conditions such as high salinity, low light conditions, and removal of roots (Da Silva 2003).

2.3.4 Cellular leakage

Two major metabolic and biochemical changes that occur in senescing petals are increase in respiration and hydrolysis of cell components (De et al. 2015). Respiration mediates the release of for example energy, oxidative breakdown of carbon compounds, synthesis and degradation of carbohydrates, proteins, lipids, organic acids vitamins and pigments (Da Silva 2003). Most of these occurrences are undesirable to the cut flower. Cell hydrolysis includes membrane rupturing, increase in cytoplasmic debris, invagination of tonoplast,

endocytosis of cytoplasmic contents, the disappearance of microtubules, reduction in cytoplasmic volume, and change in proton flux across the plasma membrane and increase in peroxisomes (De et al. 2015). These lead to a dramatic increase in the leakage of several cellular molecules including amino acids, sugars, inorganic ions and anthocyanins, increase in the activity of petal 1-ACC synthetase, which is a precursor for ethylene production, and the disintegration of tonoplast and mitochondria (Bialeski and Reid 1992).

2.3.5 Change in flower color

Carotenoids constitute yellow, orange and red pigments whereas anthocyanins and other related compounds are responsible for the red, purple and blue coloration of plant tissues (Tyrach and Horn 1997). Anthocyanins often mask carotenoids and chlorophyll and are red at acidic pH and blue at basic pH. This gives rise to a phenomenon called ‘blueing’ that results in a shift of coloration to blue with aging due to depletion of sugars. Cellular pH is important in the regulation of metabolism, and due to the presence of large vacuoles, usually, the cellular pH is highly acidic (i.e. <5). To maintain cytoplasmic pH, metabolic processes consume or produce protons in the plasmalemma and in the tonoplast (Winkel-Shirley 2001). In senescing petals, disassembly of chloroplasts is a programmed process and thylakoid proteins are immobilized (Howard 1996). Loss of photosynthetic activity of cells begins with the orderly disassembly of thylakoids.

2.4 Role of Carbohydrates in Vase Solution

Carbohydrates have numerous roles in plants i.e. serving as photosynthetic precursors required for growth, respirable substrates, osmoregulators and sometimes as osmoprotectants. Additionally, carbohydrates can act as cellular signals by controlling gene expressions (Locke 2010). Sugar added to the vase solution is also known to extend

the vase life in several cut flowers as it helps to maintain the respiration rate by floral tissues (Da Silva 2003). Sugar content in plant organs change throughout plant development and some plant organs such as developing flowers act as a sink. With the cutting of a stem, there is a complete reduction of sucrose import to sink tissues of cut flowers. This leads to the alteration of carbohydrate metabolism. Hence, the use of sucrose in the vase solution has a significant effect on the physiological status of floral tissues and the endogenous levels of sucrose (Arrom and Munné-Bosch 2012). As such, the addition of sucrose helps in accelerating flower opening and a delay in senescence.

Sucrose addition to vase solution was followed by an increase in the length of inflorescence, development, and opening of flower heads and prolonging of the vase life of spikes of *Liatris spicata* (L.) Willd (Han 1992). Jones and Truett (1992) observed that sucrose did not extend the vase life by improving solution uptake but by delaying senescence. Despite the importance of sucrose as a substrate for respiration and as an osmolyte, other sugars and sugar alcohols (or polyols), including raffinose, trehalose, mannitol, and sorbitol, have the added function of protecting plants from stress (Loescher 1987; Bohnert et al. 1995). The polyols mannitol and sorbitol have two crucial functions. They act as both osmotic adjusters and protective compounds (Loescher 1987). Therefore, it is believed that these sugars and polyols could be beneficial when added to vase solutions or used as pulse treatments following the harvest of cut flowers.

Studies with vegetative cuttings indicated that higher levels of endogenous carbohydrates reduce sensitivity to ethylene, but do not reduce ethylene production (Rapaka et al. 2007). Therefore, in cut flowers, increased endogenous carbohydrates may not only increase vase life by increasing respirable substrate but might also decrease ethylene sensitivity.

2.5 Compost

Composting is the biological decomposition and stabilization of organic substrates. During the aerobic composting process, the elevated temperature at the thermophilic stage sterilizes the final compost product by making it free of pathogens and plant seeds (Haug 2018). The products of aerobic composting are mainly carbon dioxide, water, and heat whereas the products of anaerobic decomposition are mostly methane, carbon dioxide and low molecular weight organic acids and alcohols (Haug 2018). Application of compost is known to reduce weeds and fungal diseases in crop production systems. Brown and Tworowski (2004) reported that growth of the brown rot fungus (*Monilinia fructicola*) was significantly slower on the compost substrate. Stable compost can reduce plant pathogens and improve plant resistance to diseases. It also contains valuable nutrients like nitrogen, phosphorus and other essential micronutrients (Haug 2018). The properties of compost are known to vary according to the raw material used and the process of preparation of the compost (Raviv et al. 2005).

2.5.1 Ability of compost to replace the synthetic chemicals

Compost is known to supply nutrients as well as beneficial organic compounds to plants. Compost is rich in biomass of microorganisms and elevated levels of total extractable nutrients (16 mg N/g, 9 mg P/g, and similar levels for most other nutrients) (Ingham 2000). Several organic and inorganic substances like humic acid, sugar, and amino acids are present in compost extracts and these promote plant growth (Scheuerell and Mahaffee 2002). Recently, compost tea is being promoted to control rose (*Rosa spp.*) powdery mildew, grape (*Vitis vinifera*) mildew, leaf anthracnose, peach (*Prunus persica*) leaf curl, cherry (*Prunus cerasus*) brown rot and apple (*Malus pumila*) scab. The exact mechanism

is unknown, but it is thought that compost tea contains antibiotic compounds that suppress these plant pathogens (Lanthier 2007).

Humic acids are a mixture of naturally occurring organic materials present in composts, soils, natural waters and sediments and are generally derived from the decomposition of animal and plant residues. Humic acids promote photosynthesis (Heil 2005) and chlorophyll content (El-Ghamry et al. 2009) in plants. Humic substances serve as energy source for beneficial soil organisms which release complex polysaccharides (sugar based compounds). These compounds help in the improvement of carbohydrate contents of plants (Pettit 2004) and can thus, influence the quality and life of cut flowers because the postharvest life of cut flowers has been shown to be dependent on the carbohydrate status of the flowers (Da Silva 2003). Cordeiro et al. (2011) have also studied the effects of humic acids on antioxidative defense mechanisms, which results in stimulation of catalases (CAT). So, these facts suggest that humic acids can improve the postharvest quality of the flowers. Even the foliar application of humic acids helps to increase the chlorophyll content, rate of photosynthesis and increases flower size and fresh weight. All these factors may lead to an increase in the vase life in cut chrysanthemum flowers (Fan et al. 2015).

2.5.2 Presence of carbohydrates in compost

As mentioned in section 2.4, exogenous and endogenous carbohydrate status affects the vase life and ethylene sensitivity of cut flowers. Presence of carbohydrates in compost can be an additional advantage. Organic matter has been reported to contain organic materials such as lignin, proteins, lipids, cellulose and non-cellulosic carbohydrates (hemicellulose, starch, and mono- and oligo-saccharides) that are metabolized along different pathways (Said-Pullicino et al. 2007a). Presence of hydrolyzable carbohydrates (acidic and neutral)

has been reported in compost. Hexose carbohydrates constituted the largest proportion of neutral sugars with glucose being the most abundant (Said-Pullicino et al. 2007b). It was also reported that the proportion of acidic sugars to total carbohydrates was higher in well-cured compost. The number of hexoses and pentoses in original compost samples and humic and fulvic acids decreased during composting (Hänninen et al. 1995). D-glucitol was the main sugar alcohol in compost but its relative amount decreased during composting while the amounts of all other sugar alcohols increased (Hänninen et al. 1995).

2.5.3 Harmful pathogens in compost

It is presupposed that well degraded mature compost does not contain any harmful substances. These investigations may include some ecotoxicological tests to ensure that compost is safe to use (Kapanen and Itävaara 2001). Pathogen control and degradation of toxic organic compounds by composting are important measures of public health. It is assumed that during composting when the temperature rises between 45 to 70°C, most of the bacterial pathogens and weed seeds are killed (Chatterjee et al. 2013). The compost must be pathogen free to ensure profitable use of the product. A major concern is a potential for human pathogens to grow in compost tea. This can only be a concern if the compost is not mature. Therefore, all compost must be properly treated. If there are no detectable pathogens in the compost, and conditions during compost tea production are managed to prevent pathogen growth, then the risk involved in pathogens in compost tea are minimal (Ingham 2000).

2.5.4 Effect of compost on vase life of cut flowers

Several experiments have been conducted using compost that indicates that compost can improve the vase life of cut flowers. Compost has been used during the growth stages of

flowers to increase the vase life of cut flowers (Fan et al. 2015; Emimo and Hamilton 2003). A study was carried out using different combinations of farmyard manure, poultry manure, sand, leaf compost and coconut coir in equal proportions to check their effect on morphological parameters as well as on the vase life of tuberose (*Polianthes tuberosa*). The highest values of floral diameter, number of flowers per spike and shelf life were observed in combined sand and leaf compost medium. These components gave better quality cut flower production with maximum vase life than all other components (Ikram et al. 2012).

Postharvest vase life was also studied by applying foliar humic acid fertilizer obtained by extracting humic acids from compost to chrysanthemum flowers. Seedlings of chrysanthemum were sprayed with equal volumes of distilled water, inorganic NPK fertilizer (N: P₂O₅: K₂O = 16:6:20) and organic foliar humic acid (FHA) fertilizer every 15 days. Application of FHA resulted in increase in chlorophyll content, photosynthesis rate, soluble sugars, and soluble proteins in leaves of chrysanthemum. Also, there was an increase in vase life, flower size and fresh weight of cut chrysanthemum flowers. The vase life was 61% and 33% greater in humic acid spray than in distilled water and NPK treatment (Fan et al. 2015). Another similar experiment was conducted on tulip a cultivar viz. 'Triumph'. After application of five treatments T₀: (control), T₁: 10 g/m² NPK (17:17:17), T₂: HA 0.75 ml (8%) + 10 g/m² NPK (17:17:17), T₃: HA 1.00 ml (8%) + 10 g/m² NPK (17:17:17) and T₄: HA 1.25 ml (8%) + 10 g/m² NPK (17:17:17) in three replications under greenhouse conditions, it was observed that vegetative and reproductive attributes were significantly influenced by the addition of humic acid and NPK. Treatment T₄ was the most effective compared with the other treatments and resulted in inimitable

outcomes concerning earliest sprouting and flowering, plant height increment, leaf area expansion, stem diameter, leaf chlorophyll contents, stalk length, vase life, fresh and dry flower biomass (Ali et al. 2014). Tamrakar (2016) revealed that treatments of vermiwash, cow urine, and GA₃ have a significant effect on vegetative growth, flowering and vase life of gladiolus ‘Candyman’. Use of vermiwash and cow urine exhibited a significant improvement of post-harvest parameters such as percent opened flowers, the diameter of basal floret, shelf life and the vase life of cut spikes.

2.6 Compost Tea

Compost tea is a water extract of compost. Compost tea has been used for centuries since the Roman Empire (Ingham 2000). Compost teas are a sustainable, economic and feasible way to efficiently utilize nutrients in compost through extraction. Compost tea is an umbrella term referring to a nutrient and/or microorganism-rich solution prepared by releasing compost nutrients and its microbes into solution. To facilitate the growth of beneficial microorganisms it is prepared in the oxygenated environment as the anaerobic environment will potentially lead to the production of organic acids, which are detrimental to plants. Compost tea is gaining importance and is being used in several disciplines. There is growing interest in the use of compost tea in integrated pest management as it inhibits several diseases due to presence of high concentration of beneficial microorganisms (Ghorbani et al. 2008). Many growers are trying to reduce their use of conventional pesticides with various alternative pest management methods.

Compost tea contains many microbial components that enhance plant growth and suppress disease causing pathogens including bacteria (*Bacillus*), yeasts (*Sporobolomyces* and *Cryptococcus*) and fungi (*Trichoderma*) as well as chemical antagonists such as phenols

and amino-acids. Compost tea can be tailored for its desired use. For example, a compost tea can be specifically brewed for a use as a soil organic matter builder, a disease suppressant, or a nutrient source (Ingham 2000). A Foliar spray of compost tea made by using aerated brewing process is known to control Septoria leaf spot of tomato, which is one of the most destructive diseases of tomato (Gangaiah et al. 2004).

2.6.1 Mechanism of action of compost tea

Compost acts as a food source for the antagonists that compete with plant pathogens. The mechanism by which the compost tea works is not well known but seems to depend on host/pathogen interaction and mode of application (Ghorbani et al. 2008). These biological interactions are complex and can happen through the following non-exclusive mechanisms:

Antibiosis: Antibiosis is an antagonistic association between two organisms in which one is adversely affected. Some beneficial organisms can produce compounds that inhibit the growth of disease-causing organisms by producing antibiotics toxic to them (Ingham and Alms 2003). For example, bacteria *Pseudomonas fluorescens* strain CHAO produces hydrogen cyanide, 2,4-diacetyl phloroglucinol, and pyoluteorin, which directly interfere with the growth of various pathogens (Handelsman and Stabb 1996; Haas and Défago 2005).

Competition: When beneficial microorganisms are present in a growing medium they tend to out-compete pathogenic bacteria or fungi for food sources and infection sites leaving no food for disease-causing organisms (Hoitink et al. 1997; Ingham and Alms 2003). Even if the disease-causing organisms start to grow, they cannot penetrate the tissues of the plant.

Induced resistance: Some beneficial microbes colonizing plant roots or foliage are

documented for confer resistance to a plant by turning on plant genes that increase the plant tolerance to infection by pathogens (Haas and Défago 2005).

Parasitism: Certain beneficial microbes can feed on specific pathogens. For example, *Trichoderma* species have been shown in numerous studies, to secrete enzymes that digest the cell wall of some fungal root pathogens (Handelsman and Stabb 1996; Ingham and Alms 2003).

Nutrients: Compost tea also contains soluble nutrients that perform key functions. These nutrients feed the beneficial organisms that already exist within the compost tea, so they grow faster and are healthier and can perform disease suppressive functions. They also help in retaining the nutrients in the medium for plant uptake.

2.7 Organic Substances Used to Extend Vase Life

2.7.1 Germicides

Synthetic germicides such as silver nitrate (AgNO_3) and 8-hydroxyquinoline citrate (8-HQC) improve water uptake by preventing the blockage of xylem vessels (Rahman et al. 2014). Organic antimicrobial compounds have been reported as an alternative biocide for floriculture industry. Malic acid along with sucrose proved to be effective in controlling bacterial population in vase life preservative solution (Kazemi et al. 2011). This also limits the ACC-oxidase activity in cut carnation flowers. Antimicrobial compounds were found in methanolic and ethanolic leaf extracts of *Jatropha curcas*, *Andrographis paniculata*, and *Psidium guajava*. These were found to be effective against the microbes in vase solution of cut Mokara Red orchid flowers. The solution with leaf extract of *A. paniculata* had the lowest bacterial count compared to the other extracts. Thus, the leaf extracts can serve as a

potential solution to reduce the microbial population and can be potentially utilized to extend the vase life of flowers (Rahman et al. 2014).

2.7.2 Acidifiers

A major cause of reduction of vase life in flowers is water flow interruption due to microbial proliferation. Acidifying agents such as citric acid are used to lower the pH of vase solution. Citric acid was reported to increase vase life as well as fresh weight in cut roses. In chrysanthemum, the application of citric acid increased vase life, petal water content, initial fresh weight, and marketable value. Thus, citric acid can be used as a natural, cheap, safe and biodegradable compound that can be efficiently used as an alternative to synthetic treatments to prolong vase life of cut flowers (Vahdati Mashhadian et al. 2012). Mehraj et al. (2013) observed that sucrose treatment alone without germicides promoted bacterial proliferation and led to a shortening of vase life. However, the addition of citric acid not only an effective antimicrobial compound but is also readily available in lemons, lime and oranges and is cheap. In cut flowers of *Eustoma*, citric acid and ascorbic acid are known to extend the vase life and petal water content (Sheikh et al. 2014).

Salicylic acid has proved to increase the leaf relative water content, petal water content and initial fresh weight by 49, 73 and 23% as compared to controls (Vahdati Mashhadian et al. 2012). At a concentration of 300 ppm, salicylic acid gave the highest chrysanthemum vase life of approximately 22 days. The extension of vase life was attributed to plant regulating and anti-stress properties of salicylic acid (Vahdati Mashhadian et al. 2012). The effect of pre- and post-harvest application of salicylic acid was also reported by Soleimany-Fard et al (2013) on cut *alstroemeria* flowers. Salicylic acid (3 mM) improved the vase life, fresh

weight, water uptake, water balance, total chlorophyll content, and suppressed water loss in *Alstroemeria* (cv. 'Tampa') compared to control treatment (distilled water).

2.7.3 Coagulants

Potassium aluminum sulfate (alum) has proved to be an efficient coagulant that prolongs the vase life and increase the final weight of Sampaguita flower (*Jasminum sambac*) (Acero et al. 2016). Alum is synthetic sulphate salt of aluminium and excess use can result in some negative impacts caused by aluminum. There are several other organic coagulants available like Polyamines and Melamine. Little literature information is available regarding their use for the extension of vase life of flowers.

2.7.4 Hormones and Growth Regulators

Several growth regulators and hormones are known to extend the post-harvest life in different flowers. Kinetin is known to increase water uptake and petal growth in leafless flower shoots of cut roses. It also maintained petal turgidity for an extended period (Mayak and Halevy 1972). Gibberellins are also an important growth regulator that cause an increase in the postharvest quality in cut flowering shoots of *Alstroemeria*, *Euphorbia fulgens* and *Narcissus tazetta* var. *chinensis* (van Doorn and Woltering 1991; Ichimura and Goto 2000; Ichimura and Goto 2002). Methyl jasmonate also improved the vase life and size of fresh cut Peony flowers (Gast 1999). This is a natural plant growth regulator and is known to control *B. cinerea* in a wide range of rose cultivars (Meir et al. 1998) and cut Freesia hybrids (Darras et al. 2005).

2.7.5 Herbal extracts

Various herbal extracts like piper (*Piper magnificum*), Annona (*Annona reticulata*), curcuma (*Curcuma longa*), tobacco (*Nicotiana tabacum*) and galangal (*Alpinia galangal*)

are also known to control microbes isolated from the holding solutions of cut-roses. Piper extracts at 0 (control), 1, 3, 5, 7 or 10% (w/v) concentrations showed complete inhibition of microbial growth in holding solutions, delayed bent neck, reduced ethylene production and maintained freshness of leaves (Jitareerat et al. 2007).

Essential oils are organic, safe, environmentally friendly and contain biodegradable compounds and have been proved to be the most suitable alternative for the prolongation of vase life in several cut flowers. Trials were conducted on carnation (*Dianthus caryophyllus*) to determine the effects of essential oils from Thyme (*Thymus vulgaris* L.), Summer savory (*Satureja hortensis* L.) and Ajwain (*Carum copticum* L.) and it was observed that all the oils prolonged carnation vase life. Summer savory oil showed the highest effect by increasing the fresh weight to almost 100% in comparison to distilled water used as a control (Bayat et al. 2011). Another nine essential oils, anise (*Pimpinella anisum*), cumin (*Cuminum cyminum*), geranium (*Pelargonium graveolens*), common lavender (*Lavandula angustifolia*), sage (*Salvia officinalis*), sweet basil (*Ocimum basilicum*), cinnamon (*Cinnamomum zeylanicum*), blue gum (*Eucalyptus globulus*) and lemon grass (*Cymbopogon flexuosus*) were tested for their antimicrobial effects on the vase life of cut roses by Shanan (2012). The study indicated that lavender, geranium, and anise were not only responsible for increasing vase life, fresh weight, and water uptake but also had a superior effect on reducing the rate of water loss. They suppressed the blockage of xylem vessels by reducing the number of bacteria and fungi in the vase solution.

In cut carnations, short vase life has been reported due to the loss of carbohydrates and to stem vessel blockage. Carnation flowers, when kept in pots containing rosemary extract, water as a control along with 6% sucrose treatment, vase life was seen to extend until

approximately 25 days under laboratory conditions. It was indicated that rosemary extract has the antimicrobial effect that inhibited the growth of microorganisms in vase solution and thus, increased cut flower water uptake (Basiri et al. 2011). Rosemary essence along with cola, peppermint essence, and apple extract was investigated to verify the use of natural ingredients as flower preservatives (Babarabie et al. 2016). Different combinations of these substances caused an increase in the flower diameter, and anthocyanin and chlorophyll contents in *Alstroemeria* (Babarabie et al. 2016).

2.8 Conclusion

Though compost tea has been widely used to control diseases in several field crops the use of compost tea for vase life extension of cut flowers has not been widely evaluated. The possible utilization of compost tea and other natural substances for vase life extension is attracting several researchers. There is a good understanding of various aspects of flower senescence in common cut flowers such as rose, carnation, and chrysanthemum. However, many practical problems have not been solved yet. The properties and composition of compost tea and the effect of natural substances on vase life extension still need to be adequately defined.

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CHAPTER 3.0

STABILITY OF COMPOST TEA AT VARYING CONCENTRATIONS, STORAGE TEMPERATURES AND STORAGE DURATION

3.1 Abstract

Compost tea stability is one of the important parameters of quality. The instability can further influence the physicochemical and microbiological properties of compost tea. Usually, compost tea is prepared and utilized fresh but sometimes for research purposes preservation of sample can be necessary. Therefore, this study aimed to evaluate the effect of concentration, storage duration and temperature on physicochemical properties of compost tea and results were elaborated through different statistical analyses. In this study, different concentrations (0%, 2.5%, 5%, 10%, 15%, 20%, 30%, 50%, 100%) of compost tea were prepared by diluting stock solution and were placed at different storage temperatures (4°, 10°C, 22°C and 35°C) for 52 days. These dilutions of compost tea were arranged in a factorial randomized block design with three replications and change in pH, EC, salinity, TDS, and turbidity were monitored every 13 days. Three-way ANOVA analysis of physicochemical properties proved significant effect of compost tea concentration on all the physicochemical properties except pH, effect of storage duration was significant on all the physicochemical properties and effect of storage temperature was significant for all the physicochemical properties except salinity. Multiple linear regression models ($k + \alpha D + \beta C + \gamma \text{ temp}$) indicating relationship of response variables (physicochemical properties) to predictors (storage duration, compost tea concentration and storage temperature) were developed. The predictors whose coefficients were statistically significant at $P < 0.05$ were included in the regression model. Pearson

correlation coefficients were obtained for physicochemical properties. All the correlation coefficients were significant and positive at $P = 0.001$.

3.2 Introduction

Composting is a strategic method of organic waste management that has been adopted globally. The resultant mature and stable compost produced is rich in humic substances and nutrients and is microbiologically active. These qualities make compost a useful soil amendment for agricultural purposes (Coelho et al. 2018; Zro et al. 2018) and for soil and environmental management such as erosion control and bioremediation (Bhat et al. 2018; Kumar et al. 2018; Lee et al. 2018; Xu et al. 2018). Compost feedstocks are usually made up of various types of manure, agriculture crop residue, food wastes from kitchens and the food industry, logging and wood residues, and municipal and industrial sludges (Głąb et al. 2018; Haug 2018).

Water-based extracts from compost, commonly referred to as compost tea has recently been gaining prominence in both organic and conventional agriculture. This has led to commercial production of different formulations of compost teas. Compost tea is used as a soil drench or foliar application to enhance crop productivity and quality as well as for the control of various diseases of greenhouse and field crops (Islam et al. 2016). The efficacy of compost teas has been reported by many researchers, farmers, and landscapers. For instance, compost tea suppression of diseases on herbal plants, landscape and tomato plants (Din et al. 2018; Morales-Corts et al. 2018; San Fulgencio et al. 2018); fusarium infection in potatoes (Samet et al. 2018); and powdery mildew in rose (Seddigh and Kian 2018) have been reported. However, the type and quality of the compost feedstock, the composting process, and maturation of the compost significantly affect the properties and

efficacy of the compost tea (St. Martin and Brathwaite 2012; Marín et al. 2013; Zarei et al. 2018).

Immature compost can be phytotoxic due to the presence of toxic chemical intermediates (Butler et al. 2001) whereas, mature compost contains fewer phytotoxic organic acids and readily available nutrients for plant use (Griffin and Hutchinson 2007; Pant et al. 2012). Soluble mineral nutrients, organic acids, water-soluble plant-growth regulators and biochemical compounds are extracted into compost tea (Pant et al. 2011; 2012). Several additives such as molasses, fish hydrolysate, rock dust, soluble kelp, and humic acids can be added to compost during the fermentation process as sources of nutrients for microbes (Ingham and Alms 2003). These additives target certain groups of microorganisms and enhance their growth, which are responsible for disease suppression and improvement of growing medium fertility.

The physicochemical and microbiological stability of compost teas are important determinants of the potency of compost teas (Carballo et al. 2009; Wu and Ma 2001). However, few studies have been published that describe the impact of environmental factors on the physicochemical properties and stability of compost tea. The study by Brohon et al. (1999) demonstrated that microbial biomass of soil greatly reduced with time when storage temperature was high at 37°C, which led to a significant metabolic disorder within the microbial communities compared to storage at 4°C. Ingham and Alms (2003) observed that storage of compost at 50% moisture content at a hot temperature (~65°C) can kill the microorganisms present in the compost. These suggest that chemical and microbiological stability of compost tea can be reduced at higher temperatures when stored for a longer period.

Compost tea quality can vary considerably due to different procedures of extraction and storage conditions after preparation. It was therefore postulated that the physicochemical stability of compost tea will be reduced at higher temperatures and prolong storage. The specific objective of this study was to determine the stability of varying concentrations of municipal solid waste compost tea stored at different temperatures and times.

3.3 Materials and Methods

The study was performed at the Bio Stimulant and Compost Laboratory in the Department of Plant, Food, and Environmental Sciences between June and August 2017.

3.3.1 Preparation of compost tea

Municipal solid waste (MSW) compost was obtained from Fundy Compost Inc., Brookfield, Nova Scotia. MSW compost tea stock solution was prepared by adding 100 g of sieved compost (6.35 mm) at 35% moisture content to 2 L of distilled water in an Erlenmeyer flask. Properties and features of compost used for preparation of compost tea are reported in Table 3.1. The top of the flask was covered with parafilm and a small hole was made at the top to allow aeration. The mixture was stirred for 24 hours under room temperature and relative humidity conditions at 1100 rpm using a hot plate isothermally magnetic stirrer (Fisher Scientific, Cat No. 11-100-100SH, Toronto, ON, Canada). The mixture was allowed to stand for 24 hours to settle before filtering using a NALGENE rapid flow filter with disposable bottle top filters lined with a polyethersulfone membrane (Fisher Scientific, Toronto, ON, Canada). Compost tea concentrations of 0%, 2.5%, 5%, 10%, 15%, 20%, 30%, 50% and 100% were prepared from the stock solution using distilled water. Table 3.2 reports the characteristics of compost tea stock solution used for preparation of compost tea concentrations.

Table 3.1 Properties and features of compost used for compost tea preparation.

Compost Parameters	Unit	Value
pH		7.7
Cation Exchange Capacity	meq/100g	48.6
Organic matter	%	25.21
Total nitrogen	%	1.72
C:N ratio		9:1
E. coli	MPN/g dry	6
Fecal Coliform	MPN/g dry	6
Salmonella spp.	P-A/25g (mL)	Negative
Total Ar	mgkg ⁻¹	7.14
Total Co	mgkg ⁻¹	7.10
Total Cr	mgkg ⁻¹	17.75
Total Cu	mgkg ⁻¹	50.50
Total Hg	mgkg ⁻¹	0.11
Total Mo	mgkg ⁻¹	1.3
Total Ni	mgkg ⁻¹	14.77
Total Pb	mgkg ⁻¹	24.25
Total Zn	mgkg ⁻¹	154.75
Total P	mgkg ⁻¹	681
Total K	mgkg ⁻¹	2252
Total Mg	mgkg ⁻¹	839
Total Ca	mgkg ⁻¹	6676
Total S	mgkg ⁻¹	125
Total Zn	mgkg ⁻¹	49.5
Total Mn	mgkg ⁻¹	68
Total Fe	mgkg ⁻¹	339
Total Na	mgkg ⁻¹	592

Table 3.2 Properties of compost tea stock solution used for preparation of compost tea concentrations.

Parameters	Unit	Value
pH		7.43
Conductivity	mmhos	1.8
Nitrate	mg/L	98.4
Ca	mgkg ⁻¹	78.09
K	mgkg ⁻¹	162.78
Mg	mgkg ⁻¹	14.96
P	mgkg ⁻¹	2.00
B	mgkg ⁻¹	0.62
Cu	mgkg ⁻¹	ND
Fe	mgkg ⁻¹	0.10
Mn	mgkg ⁻¹	0.15
Zn	mgkg ⁻¹	ND

ND: Not detected

3.3.2 Experimental setup

The individual MSW compost tea concentrations were stored in closed glass bottles at four different temperatures i.e. 4°C, 10°C, 22°C and 35°C. The 4°C storage was a walk-in cooler (Barr Refrigeration, Oshkosh, USA); the 10°C and the 35°C storage were growth chamber (model LHT-2103D, Z-Sciences Crop, Quebec, Canada); and the 22°C was at room temperature. The total storage duration was 52 days from June to August 2017. This was factorial experiment arranged in randomized complete block design with three replications. Thus, the treatments consisted of nine compost tea concentrations (0%, 2.5%, 5%, 10%, 15%, 20%, 30%, 50% and 100%) replicated three times. Further treatments were four levels of storage temperatures (4°C, 10°C, 22°C and 35°C) and five levels of storage durations (0, 13, 26, 39 and 52 days).

3.3.3 MSW compost tea analysis

The MSW compost tea concentrations were analyzed for their chemical properties by collecting data on pH, electric conductivity (EC), salinity and total dissolved solids (TDS) data using PCS Testr 35 Multimeter (Oakton, model 35425-10, Vernon Hills, USA). The PCS Testr 35 was calibrated with certified accurate calibration standards before use. Turbidity was determined using a turbidity meter (Oakton, model T-100, Vernon Hills, U.S). The data were collected every 13 days for 52 days starting from June 17th.

3.3.4 Statistical analyses

Each compost tea sample was analyzed in 3 replicates. The effect of compost tea concentration, storage duration (days), and storage temperature (°C) and interaction terms on physicochemical properties were examined using three-way factorial ANOVA with replication. The analysis was done using by GLM procedure in SAS 9.4 version (SAS

Institute Inc., Cary, North Carolina, US). Graphs were plotted and polynomial quadratic equations ($y = ax^2 + bx + c$) were derived between the dependent variables (physicochemical properties and independent variables (concentration, storage duration, and storage temperature).

The data for physicochemical parameters were further explored using the descriptive statistics: maximum value, minimum value, mean value, standard error mean and standard deviation to check the validity of data for developing multiple regression models. The multiple linear regression analysis was done in SAS 9.4 version (SAS Institute Inc., Cary, North Carolina, US). Physicochemical properties were selected as response variables and storage duration, storage temperature and compost tea concentration were selected as predictors. Only the predictors whose coefficients were statistically significant at $P < 0.05$, $P < 0.001$ were included in regression model. Regression model was defined as:

$$Y = k + \alpha D + \beta C + \gamma \text{ temp};$$

where k = constant, D = storage duration; C = Compost tea concentration; temp = storage temperature; α , β and γ are the coefficients for storage duration, MSW compost concentration and storage temperature respectively.

Correlations between physicochemical properties of compost tea (EC, pH TDS, turbidity and salinity) were evaluated using Pearson correlation coefficients on all measured parameters by using MINITAB version 18.1 (Minitab, Inc., State College, Pennsylvania, U.S) (Myers and Sirois 2004).

3.4 Results and Discussion

3.4.1 Physicochemical properties of compost tea

Islam et al. (2016) reported that the storage duration significantly affects fungi and bacteria populations because of the competition for nutrients and oxygen among microorganisms and the release of metabolic toxic molecules. Compost tea temperature was considered very important for enzyme activity which could further influence the physicochemical properties of compost tea. So, the effect of these two parameters on compost tea were considered important in this study. According to the results present in Table 3.3 pH of compost tea appeared to be significantly influenced by storage duration and storage temperature. The effect of storage duration and temperature can be explained by quadratic polynomial equations: $y = 9E-05x^2 - 0.0037x + 7.5121$ and $y = 0.0005x^2 - 0.0188x + 7.6253$ respectively where pH is dependent variable and R^2 values of 0.92 and 0.74. Linear coefficients were negative in both equations indicating that pH decreases with increase in storage duration and storage temperature. No significant change in pH of the compost tea was observed with increase in concentration. Average pH of compost tea decreased from 7.51 to 7.46 in first 13 days of storage. With increase of storage duration, organic acid anions contained in compost, i.e. oxalate, citrate, and malate were released due to microbial decomposition, which balances the excess of cations due to which pH is known to decrease (Islam et al. 2016). Besides, the atmospheric CO_2 produced by microbial activities dissolves in compost tea forming carbonic acid, so determining decrease of pH over time (Islam et al. 2016). A slight decrease in pH in first 13 days of storage was followed by an increase to 7.57 on 52nd day of storage (Fig. 3.1) which is possibly due to release of ammonia from degradation of organic compounds present in compost (Majlessi et al.

2012). Similarly, effect of storage temperature was significant on pH. A slight decrease in pH was observed when the compost tea was stored at 10°C followed by a slight increase for high temperatures (Fig. 3.1). An increase in storage temperature could cause a decrease in viscosity, increase in mobility of ions in solution and an increase in ions in the same solution due to dissociation of molecules (Islam et al. 2016; Zumdahl 1993). pH change is a key factor regulating the solubility and availability of nutrients in a medium and acceptable value for microorganisms is in the range of 6.0–7.5 pH (Majlessi et al. 2012).

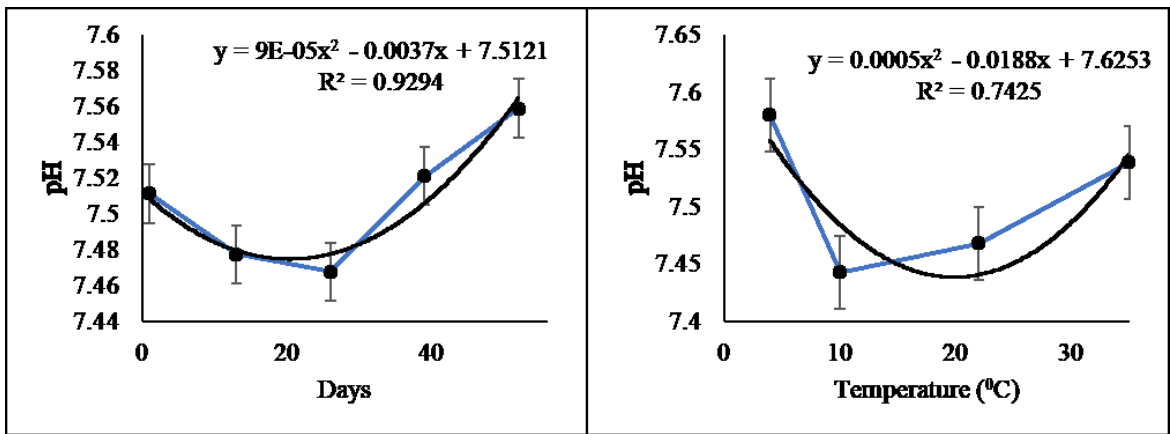


Figure 3.1 Effect of storage duration and storage temperature on pH of compost tea.

Electrical conductivity (EC) for different levels of compost tea concentration, differed significantly (Table 3.3). The effect of concentration on EC can be explained by quadratic polynomial equation: $y = -0.0032x^2 + 3.8106x + 57.195$ where EC is dependent variable and R^2 value of 0.99. Negative quadratic coefficient indicates negative correlation of EC with concentration². Electrical conductivity (EC) increased linearly from 55 $\mu\text{S}/\text{cm}$ to 406 $\mu\text{S}/\text{cm}$ as the concentration of the MSW compost tea was increased from 0% to 100% (Fig. 3.2). The increase in EC with increasing concentration can be explained by the fact that total solute concentration in compost tea increases with increase in compost quantity. Islam

et al. (2016) reported a rise in EC values with increase in compost/water ratio from 1:10 to 1:2.5 and with an increasing extraction time. The effect of storage duration and temperature on EC was observed to be significant. The effect of storage duration and temperature can be explained by quadratic polynomial equations: $y = -0.0168x^2 + 1.4227x + 130.35$ and $y = 0.0149x^2 - 0.405x + 146.81$ respectively with R^2 values of 0.99. Equations indicate negative correlation of EC with quadratic coefficient of storage duration and storage temperature. EC increased from 130 $\mu\text{S}/\text{cm}$ on day 1 to 160 $\mu\text{S}/\text{cm}$ on day 39 of storage and then became stable until the 52nd day of storage. Similar results have also been reported by Kim et al. (2015). A decline in EC value was observed when compost tea was stored at temperatures of 4°C to 10°C but significant increase in EC was recorded at a temperature between 22°C to 35°C. EC tended to be stable between 10-20°C (Fig. 3.2). EC-temperature relation is reported to be controlled by the viscosity of water (Stokes and Robinson 1966). This relation was reported to be linear in temperature range of 0–30°C in five types of waters with different salinity composition: seawater; Na-(Cl, CO₃, SO₄) lake brine; Na-(CO₃, Cl) groundwater; (Na, Mg) -(Cl, SO₄) groundwater; and (Na, Mg)-SO₄ groundwater (Hayashi, 2004). This is because the EC-temperature relation of electrolyte solutions is primarily controlled by the viscosity-temperature relation of pure water.

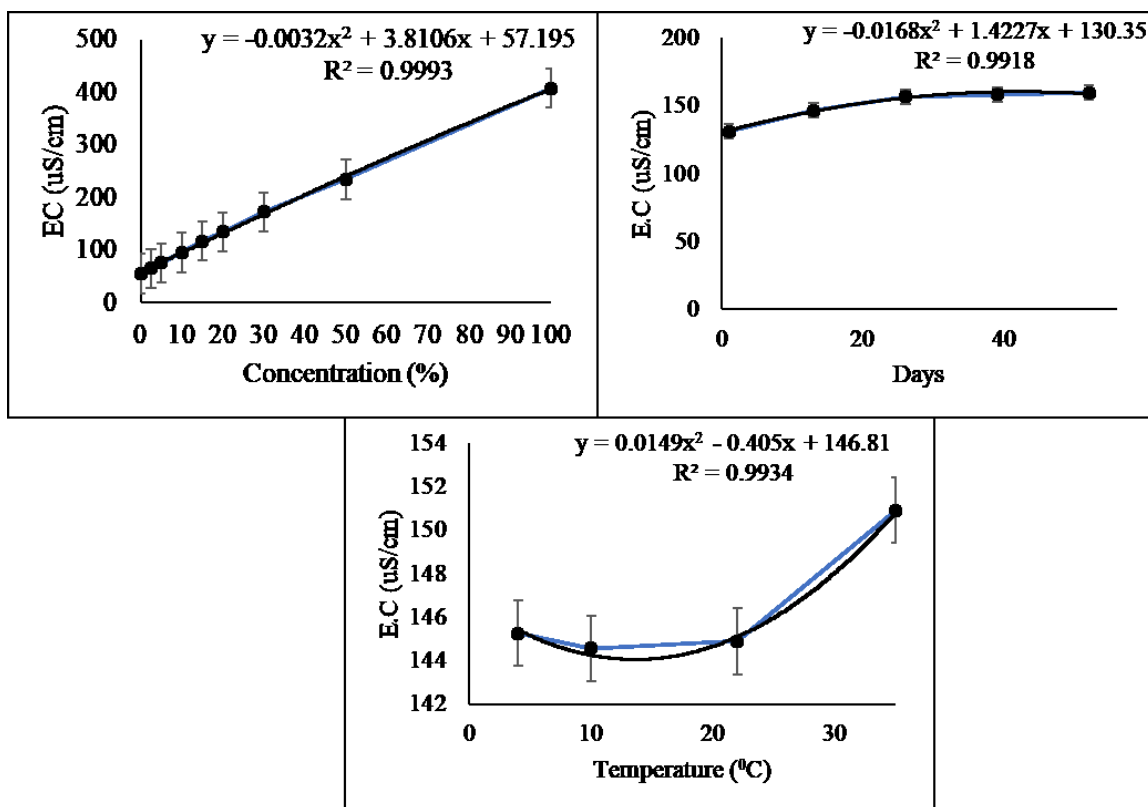


Figure 3.2 Effect of concentration, storage duration and storage temperature on electrical conductivity (E.C) of compost tea.

The effect of compost tea concentration on TDS was highly significant (Table 3.3). The increase was observed from 38 ppm in 0% (blank) to ~300 ppm in 100% compost tea concentration. An increase of about 20 ppm in TDS was observed with prolonged storage time. Although significant, the effect of number of days on TDS tended to remain constant after 13 days of storage (Fig. 3.3). A smaller decrease in TDS was observed at 10°C followed by a rise with the highest value at 35°C (Fig. 3.3). The interaction of compost tea concentration, storage duration and storage temperature were observed to effect TDS significantly. The effect of concentration, storage duration and temperature can be explained by quadratic polynomial equations: $y = -0.0026x^2 + 2.7472x + 40.287$, $y = -0.0118x^2 + 1.0033x + 92.793$ and $y = 0.0032x^2 + 0.0205x + 102.65$ respectively with R^2

values of 0.99, 0.98 and 0.97. Equations indicate negative correlation of TDS with quadratic coefficient of concentration and storage duration.

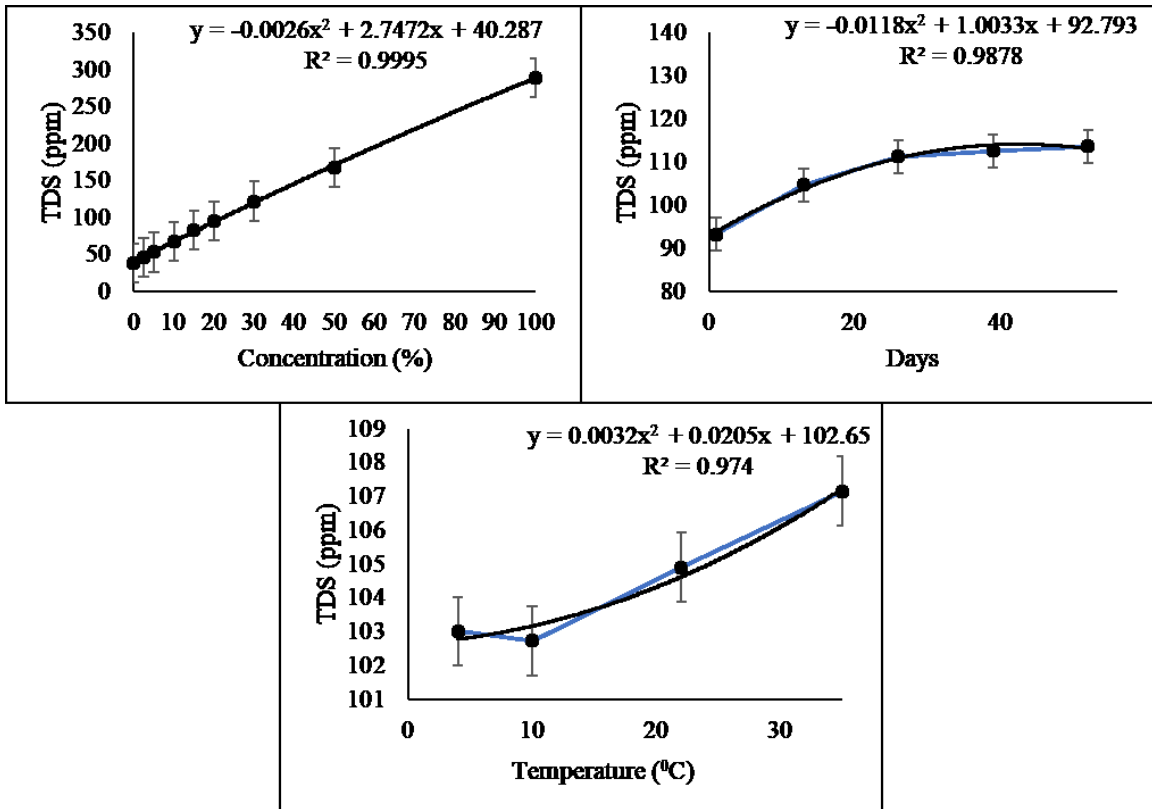


Figure 3.3 Effect of concentration, storage duration and storage temperature on total dissolved solids (TDS) of compost tea.

Trends like those observed in TDS were also recorded for salinity. The effect of compost tea concentration and storage duration was observed to be significant on salinity of compost tea whereas, storage temperature had no significant effect on salinity (Table 3.3). The effect of concentration and storage duration can be explained by quadratic polynomial equations: $y = -0.0007x^2 + 1.7094x + 31.442$ and $y = -0.007x^2 + 0.6148x + 65.48$ respectively with R^2 values of 0.99 and 0.98. Equations indicate negative correlation of salinity with quadratic coefficient of concentration and storage duration.

Salinity increased from 25 ppm in 0% (blank) to ~200 ppm in 100% compost tea concentration, as the concentration of compost tea increased. The effect of storage time on salinity, like TDS, remained almost constant after 13 days (Fig. 3.4). The minor increase from 71 to 75 ppm was observed in salinity when compost tea was stored at 5°C to 35°C which was statistically insignificant. Since TDS and salinity correlate strongly with EC, an increase in EC values explained the increase in salinity and TDS. As expected, an increase in total compost concentration in compost tea explains the increase in salinity and TDS.

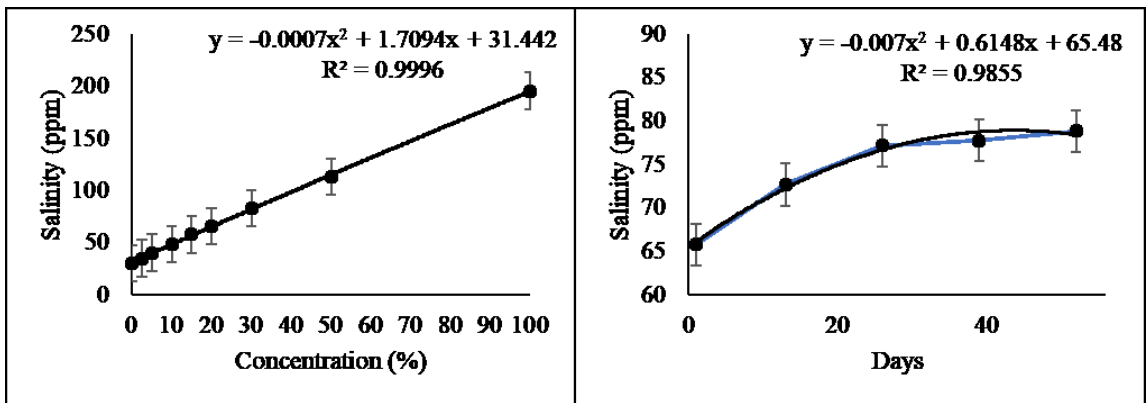


Figure 3.4 Effect of concentration and storage duration on salinity of compost tea.

Effect of compost tea concentration and storage duration was highly significant on turbidity of compost tea (Table 3.3). As expected, turbidity significantly ($P < 0.05$) increased with an increase in the concentration of MSW compost tea, which might be possible due to the presence of a larger amount of compost particles in solution (Fig. 3.5). As the concentration increased from 0 to 100%, turbidity increased from negligible to 359 NTU. Fig. 3.5 shows a rapid decrease in turbidity from 300 NTU on the first day of storage to 50 NTU on 52nd day of storage. Turbidity was highest at 4°C but was suddenly decreased at storage temperatures $\geq 10^\circ\text{C}$, followed by a slight increase between 22°C to 35°C.

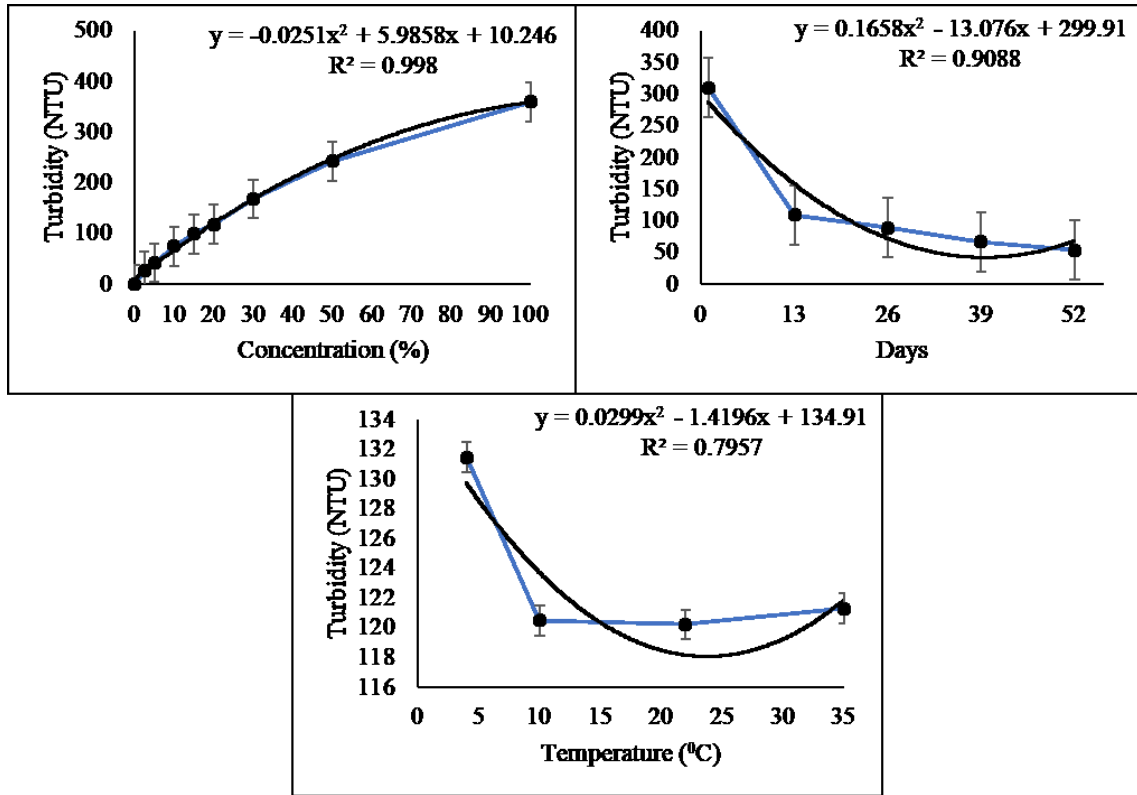


Figure 3.5 Effect of concentration, storage duration and storage temperature on turbidity of compost tea.

A rapid decrease in turbidity is due to low water solubility of organic compounds present in compost at neutral and high pH (Chiou et al. 1997). The effect of concentration, storage duration and temperature can be explained by quadratic polynomial equations: $y = -0.0251x^2 + 5.9858x + 10.246$, $y = 0.1658x^2 - 13.076x + 299.91$ and $y = 0.0299x^2 - 1.4196x + 134.91$ respectively with R^2 values of 0.99, 0.90 and 0.79. Equations indicate negative correlation of turbidity with quadratic coefficient of concentration and linear coefficients of storage duration and temperature. Individual effect of the three factors studied (i.e. concentration, storage time and storage temperature) on MSW compost tea quality parameters were further elaborated by the multiple linear regression equations.

Table 3.3 ANOVA (P-values) of the effect of compost tea concentration, storage duration and storage temperature on physicochemical properties of compost tea.

Source of variation	df	pH	EC ^a	TDS ^b	Turbidity	Salinity
Conc.^c	8	0.17	<0.0001	<0.0001	<0.0001	<0.0001
SD^d	4	0.02	0.01	0.009	<0.0001	0.008
ST^e	3	0.04	0.01	0.003	0.0004	0.31
Conc.*SD	32	0.11	0.86	0.36	0.19	0.082
Conc.*ST	24	0.13	0.89	0.16	0.87	0.1038
SD*ST	12	0.08	0.49	0.89	0.73	0.519
Conc.*SD*ST	96	0.19	0.77	0.03	0.91	0.41

Bold figures indicate significant P-values.

^a Electrical conductivity

^b Total dissolved solids

^c Compost tea concentration

^d Storage duration

^e Storage temperature

3.4.2 Multiple linear regression

The descriptive statistics of all the variables are shown in Table 3.4. The analysis includes the minimum, maximum, mean, standard error of mean and standard deviation. Standard deviation measures the amount of dispersion in data set from mean and was reported highest and lowest in turbidity and pH respectively. A wide range of variability was calculated in all the physicochemical properties except pH. Relatively slight change in pH was observed over time. A standard error of the mean indicates how far the sample mean is likely to be from true population mean. Standard error of mean for all the physicochemical properties is reported low in comparison to the mean values, lowest in pH and highest in turbidity.

Table 3.4 Descriptive statistics of variables explored for multiple linear regression model development.

Physicochemical Property	Minimum	Maximum	Mean	SEM	STD
SAL (ppm)	26.80	1098.0	76.29	2.89	67.17
TURB (NTU)	0.00	774.0	125.25	7.29	169.30
TDS (ppm)	33.50	397.0	106.60	3.29	76.34
EC ($\mu\text{S}/\text{cm}$)	49.70	562.0	150.56	4.66	108.18
pH [-log(mol/L)]	6.87	9.0	7.50	0.01	0.25

SAL= Salinity; TURB= Turbidity; TDS= Total dissolved solids; EC= Electrical conductivity; SEM= Standard error mean; STD= Standard deviation of mean.

Multiple linear regression equations were obtained for physicochemical properties of compost tea. The selected parameters are shown in Table 3.5. Evidently, all regression models explained a major part of the total variation in the data and the standard error values reported for each parameter are not significantly high. The coefficient of intercept was highly significant in regression models for all the physicochemical parameters. The effect of storage duration and concentration was highly significant whereas the effect of storage temperature was significant on electrical conductivity. Also, the effect of storage duration, temperature and compost tea concentration was positive on electrical conductivity, thus, increase in these factors result in increase of electrical conductivity. In the regression model of salinity, the effect of compost tea concentration is highly significant, effect of storage duration is significant, but storage temperature had no effect on salinity of compost tea. In the regression model of TDS, storage duration and concentration coefficient were observed

to be highly significant and storage temperature coefficient had significant effect on TDS. Storage duration had highly significant and negative effect on turbidity of compost tea. Turbidity of compost tea decreased as it was stored for longer duration. Similarly, turbidity of compost tea decreased with increase of storage temperature, but the effect of storage temperature was not significant on turbidity. Concentration effect was highly significant and positive on turbidity. In case of pH, only the effect of compost tea concentration was observed to be highly significant.

Table 3.5 Multiple linear regression model ($k + \alpha D + \beta C + \gamma \text{ temp}$) indicating relationship of chemical properties as influenced by storage duration (D), compost tea concentration (C) and storage temperature (temp).

Response Variable	Constant		Coefficients					
	k	SE	α	SE	β	SE	γ	SE
EC	41.19**	2.39	0.52**	0.05	3.49**	0.03	0.03*	0.08
Salinity	26.32**	4.6	0.23*	0.1	1.68**	0.06	0.01 ^{ns}	0.15
TDS	31.23**	2.04	0.33**	0.04	2.44**	0.02	0.20*	0.07
Turbidity	148.4**	11.2	-4.25**	0.24	3.52**	0.15	-0.19 ^{ns}	0.38
pH	7.42**	0.02	0.001 ^{ns}	0.0005	0.002**	0.0003	-0.0003 ^{ns}	0.00087

*, ** indicates statistical significance at $P < 0.05$, $P < 0.001$, respectively.

TDS= Total dissolved solids; EC= Electrical conductivity.

^{ns} = Not significant

The correlation graphs between the observed and predicted value using multiple linear regression model were plotted (Fig. 3.6). Pearson correlation coefficients are reported in Table 3.6.

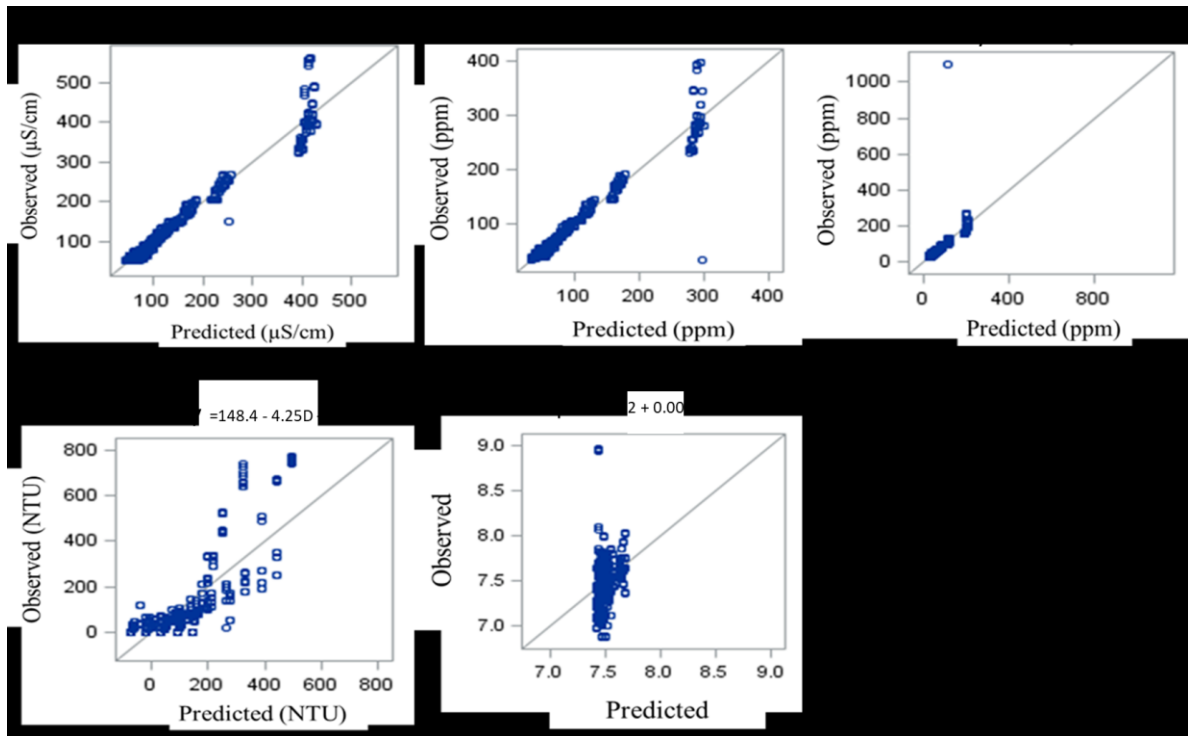


Figure 3.6 Correlation plots between measured and predicted values of electrical conductivity (EC), total dissolved solids (TDS), salinity, turbidity and pH values of compost tea using multiple linear regression model.

Table 3.6 Pearson correlation coefficient (R) for correlation plots between the measured and predicted values of electrical conductivity (EC), total dissolved solids (TDS), salinity, turbidity and pH values.

Response Variable	R- value
EC	0.97
Salinity	0.75
TDS	0.96
Turbidity	0.77
pH	0.24

3.4.3 Correlations among physicochemical properties

All Pearson's correlation coefficients (R) were significant at $P < 0.001$ although there was a wide range of correlation coefficients (Table 3.7). The strongest correlation was observed between EC and turbidity ($R = 0.98$). Similarly, a strong correlation (>0.75) was observed between salinity and EC as well as salinity and TDS. Correlations among turbidity, EC and TDS and salinity were moderate but highly significant. pH did not seem to correlate well with EC, TDS, salinity and turbidity. All the correlation coefficients were positive. Fig. 3.7 indicates Pearson's correlation matrix among electrical conductivity (EC), total dissolved solids (TDS), salinity (SAL) and turbidity (TURB) of compost tea.

Table 3.7 Pearson's correlation coefficient (R) among electrical conductivity (EC), total dissolved solids (TDS), salinity (SAL) and turbidity (TURB) and pH of compost tea.

Variable	EC	TDS	SAL	TURB
TDS	0.98			
SAL	0.77	0.76		
TURB	0.54	0.54	0.41	
pH	0.23	0.21	0.21	0.27

R is significant for all the values at $P < 0.001$.

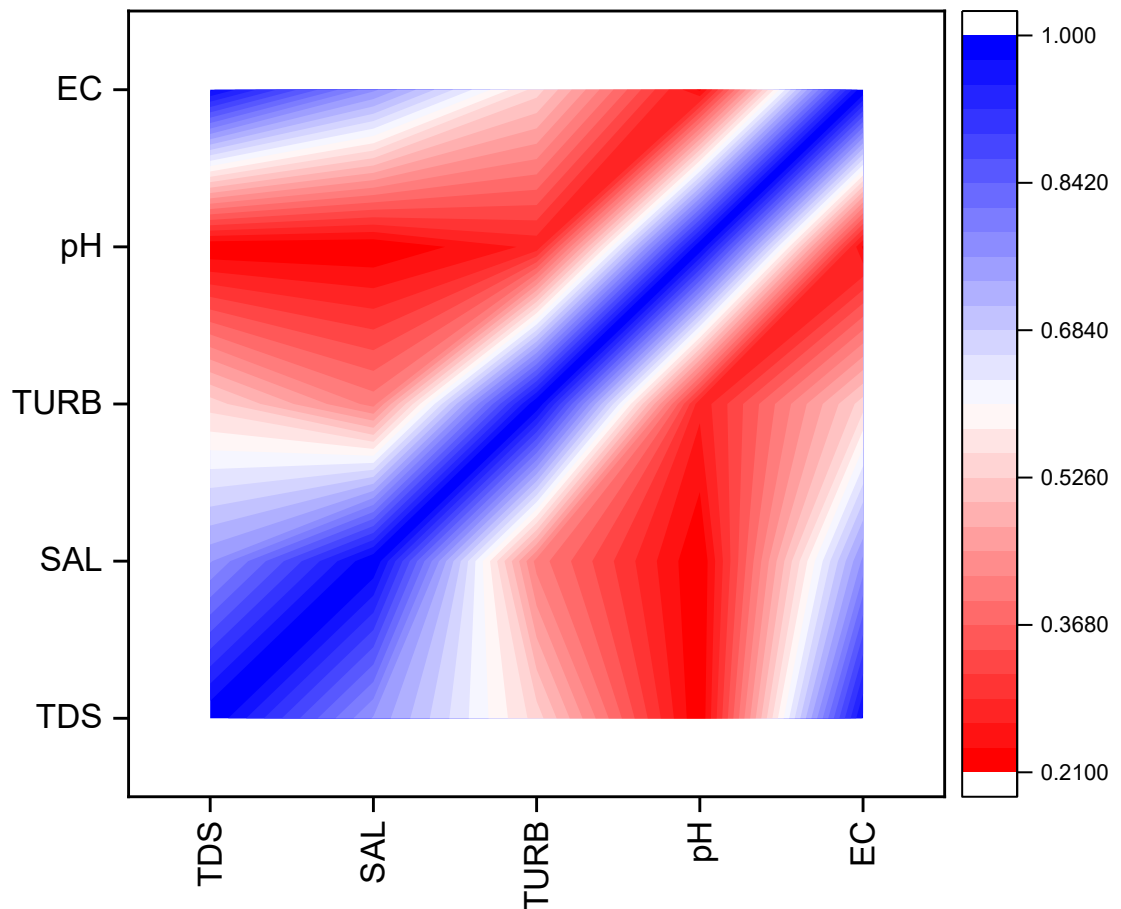


Figure 3.7 Pearson's correlation matrix among electrical conductivity (EC), total dissolved solids (TDS), salinity (SAL) and turbidity (TURB) of compost tea.

3.5 Conclusion

Many interactions and individual effect of the studied factors (compost tea concentration, storage duration and storage temperature) on compost tea physicochemical parameters were found significant and were elaborated by regression equations. The multiple regression analysis was found to be a useful method to combine all the factors.

Significance tests of predicted model and test of significance of individual factor or interaction factors at individual level revealed that many physicochemical parameters of compost tea can be predicted by compost tea concentration, storage duration and storage temperature. Since storage duration has a significant effect on the chemical properties of compost tea, it should be prepared and used as soon as possible in agriculture. Though, the effect of storage temperature on compost tea is difficult to determine, it could help to reveal information about better storage condition for compost tea after preparation.

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CHAPTER 4.0

EFFECT OF COMPOST TEA CONCENTRATION ON VASE LIFE OF CUT FLOWERS

4.1 Abstract

In this research the effect of different concentrations of compost tea were analyzed on vase life of cut carnation flowers (*Dianthus caryophyllus* L. var White Sim) in comparison to synthetic chemical vase solution (chrysal). This study investigated the use of municipal solid waste compost tea (MSW) for the extension of vase life of cut flowers. Compost tea dilutions of 0.0, 2.5, 3.5, 5.0, 6.5% were prepared from a stock solution (100g compost in 2L distilled water) and cut carnations were placed in each of the dilutions in three replicates. It was observed that the overall performance of cut carnation flowers was best in chrysal. It was also stated that other than chrysal, the performance of cut carnations was satisfactory in 3.5% compost tea concentration followed by distilled water (0%). Cut flowers treated with 5% and 6.5% compost tea concentrations had highest rate of deterioration in terms of petal blackening, petal drooping, petal wilting and petal fall. 3.5% compost tea concentration can be used as environment friendly alternative for vase life extension of cut carnation flowers. It was observed that the use of a preservative solution containing 3.5% compost tea led to an increase in vase life of cut carnations.

4.2 Introduction

The use of preservatives in vase solutions for vase life extension is a widespread practice. Commercial floral preservatives used for extension of vase life are complex mixture of sucrose, synthetic chemicals such as acidifier, anti-microbial, and anti-ethylene agents.

These preservatives help to maintain water uptake by controlling the growth of microbes. It is well documented that one of the main causes for inferior cut flower quality is the blockage of xylem vessels by microorganisms that accumulate in vase solution and in stem vessels (Basiri et al. 2011). When the vessels of stems are blocked, continuing water uptake and transpiration by leaves of cut flowers result in a net loss of water from the flower and stem tissue (Hassan 2005). It was reported that negative correlation exists between the number of bacteria and the water conductivity in the stem of a cut flowers (van Doorn et. al. 1989). The major limiting factors in postharvest management and handling of this cut flower are premature aging that reduce the vase life of flowers (van Doorn and de Witte 1994).

MSW compost is increasingly being used in agriculture as a soil conditioner but also as an organic fertilizer. Proponents of this practice consider it an important source of nutrients and recycling tool since MSW would otherwise be landfilled. The water-based extract of compost, also known as compost tea, is gaining importance as an organic pesticide and organic fertilizer for crops (Scheuerell and Mahaffee 2002). Compost tea contains plant essential nutrients as well as soluble elements required for plant growth eluted by water. Although the use of compost tea is widely gaining importance, the nutrient supplying capacity and pesticidal properties of compost tea have been sparsely discussed in the literature.

Compost tea has also been evaluated as an alternative to chemical fungicides for the control of a variety of fungus infecting plants (Al-Mughrabi 2006). Longer vase life of cut flowers is preferred in flower cultivation, marketing and use. Several preservatives are used to improve the vase life of cut flowers, but the use of natural substances is more into

consideration due to environment pollution and high cost of synthetic chemical preservatives (Babarabie et al. 2016). Use of compost tea in vase solution has not been reported yet despite the environmental friendliness, less expensive and healthy use of compost. Thus, the specific objective of this study was to determine the most suitable concentration of compost tea that will improve vase life of carnation in comparison with commercial preservative (chrysal).

4.3 Materials and Methods

4.3.1 Preparation of compost tea concentrations

Aerated MSW compost tea stock solution was prepared as previously described. Based on the preliminary research (Appendix), different concentrations (0, 2.5, 3.5, 5, 6.5%) of MSW compost tea were prepared from this stock solution. Appropriate amounts of stock solution were taken and further diluted by distilled water.

4.3.2 Experimental setup

The cut carnations (*Dianthus caryophyllus* L. var. White Sim) were purchased from a local retail superstore, Truro. The stems of the cut flowers were trimmed at the base by a slanting cut of 2 cm to provide a larger surface area for water uptake. All the leaves except the upper 2-3 leaflets were removed according to the method of Dai (1993). It was made sure that the remaining leaves on the stems were free of infection and damage of any kind. One flower stem was placed in each transparent glass beaker (1.5 L) containing 500 mL of the individual compost tea concentrations. Distilled water and chrysal were used as a control. Chrysal is a synthetic chemical preservative that contains pH regulators, water absorption promoters and flower nutrients. The experiment was conducted under room temperature

and relative conditions. The treatments were arranged in a completely randomized design with three replicates for each concentration.

4.3.3 Physicochemical properties

The different MSW compost tea concentrations were analyzed for their chemical stability by collecting data on pH, electric conductivity (EC), salinity and total dissolved solids (TDS) using PCS Testr 35 Multiparameter (Oakton, Eutech instruments, Singapore). PCS Testr 35 was calibrated with certified accurate calibration standards before measuring the pH, EC, TDS, and salinity. Turbidity was measured using Okaton turbidity meter (EUTECH INSTRUMENTS, Singapore). Data were collected every second day for 11 days as the vase life of carnation cut flowers is reported approximately 10-12 days.

4.3.4 Visual analysis of cut flowers

The flower head on each stem was evaluated every day for 12 days and graded from 0 to 5 for petal blackening, petal fall, petal freshness and petal drooping according to scales described in previous studies. The rate of damage was evaluated based on relative area of petals damaged as a percentage of total petal area.

Scales for Physical Parameter Scoring

Petal blackening (Dai 1993)

Score 0: 90-100% of flower petal area darkened

Score 1: 60-90% of flower petal area darkened

Score 2 – 30-60% of flower petal area darkened

Score 3 – 10-30% of flower petal area darkened

Score 4 – 1-10% of flower petal area darkened

Score 5 – Purchased condition

The rate of petal blackening was evaluated based upon the relative area of petals with darkened surface as percentage of total flower area.

Petal Fall (Dai 1993)

0 – Fall of 90-100% of total petals

1 – Fall of 60-90% of total petals

2 – Fall of 30-60% of total petals

3 – Fall of 10-30% of total petals

4 – Fall of 1-10% of total petals

5 – Purchased condition

Petal Drooping (Khan et al. 2015)

0 – Maximum petal shriveling

1 – Petal shriveling

2 – Noticeable in-rolling

3 – Slight petal enrolling

4 – Very slight petal enrolling

5 – Purchased condition

Petal Wilting (Khenizy et al. 2014)

0 – Extreme wilting

1 – Severe wilting

2 – Moderate wilting

3 – Slight wilting

4 – Slight discoloration

5 – Purchased condition

Time from harvest to 50% (i.e. Scale 2.5, condition between score 2 and 3) petal browning, fall, drooping and wilting was used to calculate vase life.



Figure 4.1 Example of grading of cut carnations (*Dianthus caryophyllus* L. var. White Sim) scored from 0 to 5 based on visual observation of petal blackening.

4.3.5 Statistical analysis

All experiments were arranged in a completely randomized design with three replicate vases of one stem each. Physicochemical data (pH, EC, TDS, salinity and turbidity) were subjected to analysis of variance using GLM model procedures of SAS (version 9.4) and means were separated using Fisher's least significant difference (LSD) at $\alpha = 5\%$. Ordinal data of scores for physical parameters were analyzed using rank-based non-parametric tests for repeated measures (Shah and Madden 2004). Fixed treatment effects were declared significant at $P \leq 0.05$.

4.4 Results and Discussion

There was significant difference ($P < 0.05$) in pH among all the preservative solutions (Table 4.1). All preservative solutions had an initial pH of 7.4 to 7.5 except for the chrysal for which initial pH was 2.6 (Fig. 4.2). Relatively small changes in pH (≤ 0.7 units) were recorded in solutions where stems were kept continuously in the vase solutions (Fig. 4.2). pH of floral preservative solutions became acidic in all the preservative solutions, except in chrysal in which maximum increase in pH was reported (0.7 units). However, the results showing a drop in pH in compost tea preservative solutions may be due to the accumulation of organic acids in the beaker. Organic acid anions present in the original compost i.e. oxalate, citrate and malate were probably released due to the microbial decomposition with time which balanced the excess cations (Johnson et al. 1996). These results are supported by studies conducted on storage of composts, which reported a decrease in compost pH with an increase in storage time (Rostami et al. 2010; Majlessi et al. 2012). The pH values of the compost tea preservative solutions were found in the optimum range of 6.0-7.5 for the microorganism's survival and multiplication as explained by (Boulter-Bitzer et al. 2006). Neutral to partial alkaline pH values are usually indicators of stable compost products (Majlessi et al. 2012). Initial pH of chrysal is low due to an unknown acidifier that is added to the product (Fig. 4.2). pH of vase solution is an important factor that controls water uptake, mineral uptake, reduce embolization, and slows bacterial growth.

Low to moderate vase solution EC is important for achieving the longest possible vase life of cut flowers. Many cut species perform best when solution EC is moderate (300-800 $\mu\text{S}/\text{cm}$, depending on type of flower). EC is one of the parameters evaluated as a

physical characteristic that influences the water uptake by cut flowers. When salts in the vase solution are too high, it may be detrimental to the life of many cut flower species and could cause phytotoxicity depending upon the salt tolerance of the plant species (Mengel and Kirkby 1978). pH and EC changes can be the cause of decomposition of organic acids (Majlessi et al. 2012). The EC of all preservative solutions increased during vase period and increased with increase in compost tea concentration, with a greater increase in chrysal. This was not reported due to evaporation as the beakers were covered from the top. Stems placed in chrysal had the highest initial and the final EC i.e. 693 and 1146.3 $\mu\text{S cm}^{-1}$, respectively. This can be attributed to the fact that flower nutrients are added in chrysal. Total dissolved solids (TDS) and salinity are also indicators of salt concentration in solution. TDS and salinity increased in all the preservative solutions (Fig. 4.2). Turbidity decreased from day 1 to day 11 in all preservative solutions except for the chrysal and distilled water in which the turbidity increased from 2.46 NTU to 14.02 NTU and 0.017 NTU to 0.41 NTU, respectively. Increase in EC, TDS, salinity and turbidity during vase period can be attributed to the fact that stems of flowers release various cations, anions, amino acids and organic acids (van Meeteren and Arévalo-Galarza 2008).

Table 4.1 Change in pH, electrical conductivity (EC), turbidity, total dissolved solids (TDS) and salinity of varying concentrations of compost tea and Chrysal.

Preservative solution	Application duration (days)	pH	EC (uS/cm)	Turbidity (NTU)	TDS (ppm)	Salinity (ppm)
0	11	7.36a	91.36a	0.23a	47.91a	42.5a
2.5	11	7.40ac	285.66b	14.31b	203.77b	134.59b
3.5	11	7.46cd	301.27b	21.30c	211.33b	130.41b
5	11	7.42ad	321.16b	31.34d	227.5b	145.36b
6.5	11	7.34a	335.55b	46.73e	235.72b	152.67b
Chrysal	11	3.03b	930.61c	8.44f	644.61c	424.00c

In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.05 level of probability

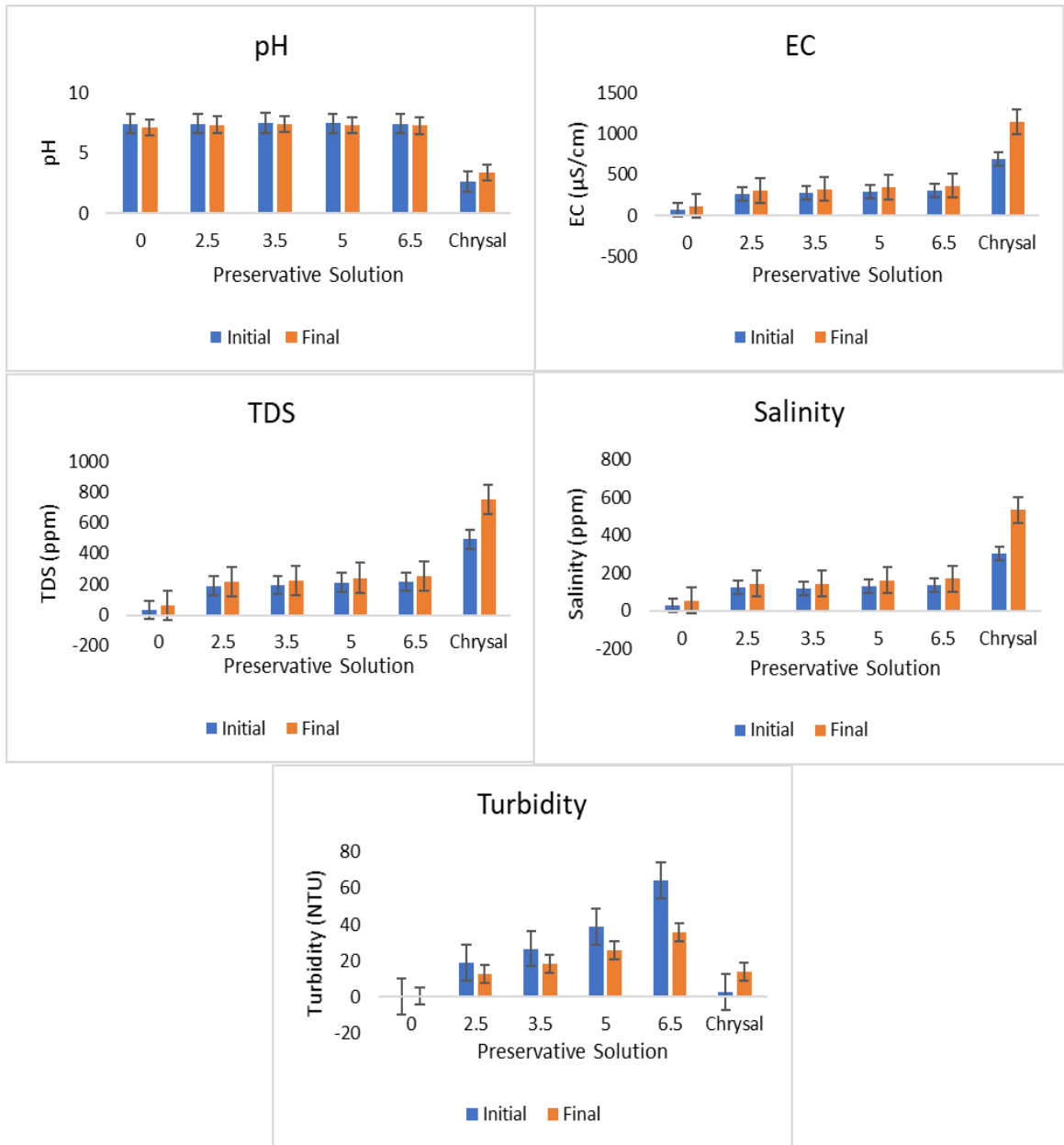


Figure 4.2 Initial (Day 1) and final (Day 11) values of pH, electrical conductivity (EC), turbidity, total dissolved solids (TDS) and salinity of different concentrations of compost tea and Chrysal.

The score for physical parameters varied significantly among different preservative solutions (Table 4.2). Drop in scores from day 1 to 12 is presented in Fig. 4.3. There was significant difference ($P < 0.05$) in petal blackening of cut carnation petals among all the preservative solutions. Minimum petal discoloration score was found in 5% and 6.5% compost tea concentrations (2.6) in which petal blackened up to 50% by day 12 followed by 0%, 2.5% and 3.5% (4.0). The maximum petal blackening score was found when cut carnations were placed in chrysal (4.3). Similarly, for petal drooping cut carnations placed in chrysal had lowest petal drooping with score of 4.3, followed by 3.5% compost tea concentration (4.0). In 5% and 6.5% compost tea concentrations highest petal drooping of cut carnation petals was observed (2.5). No petal fall was observed in cut carnation flowers placed in chrysal (Fig. 4.3). 1-10% petal fall was observed in 0, 2.5, 3.5 and 5% compost tea concentrations. Petal fall was highest in the 6.5% compost tea concentration (3.5). Minimum petal wilting was observed in cut carnations placed in chrysal (4.0), followed by 3.5% (3.6). When average of all physical parameter scores were calculated, it was observed that average score was highest for chrysal (4.4) followed by 3.5% compost tea concentration with average score of 4.0. Overall performance of cut flowers was best in chrysal as ready foods of flowers contain anti ethylene compounds and nutrients which is beneficial for maintenance of flowers as fresh as possible for a longer period (Mehraj et al. 2013).

Table 4.2 Scores for visual observations of physical parameters of carnation as affected by different concentrations of compost tea and Chrysal.

Preservative solution	Application duration (days)	Petal blackening	Petal drooping	Petal fall	Petal wilting	Average Score
0	12	4.0a	3.5ab	4.1a	2.5a	3.5
2.5	12	4.0a	3.1a	4.0a	2.5a	3.4
3.5	12	4.0a	4.0be	4.6a	3.6b	4.0
5	12	2.6b	2.5d	4.0a	2.5a	2.9
6.5	12	2.6b	2.5d	3.5a	2.3a	2.7
Chrysal	12	4.3c	4.3e	5.0c	4.0c	4.4

In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.05 level of probability

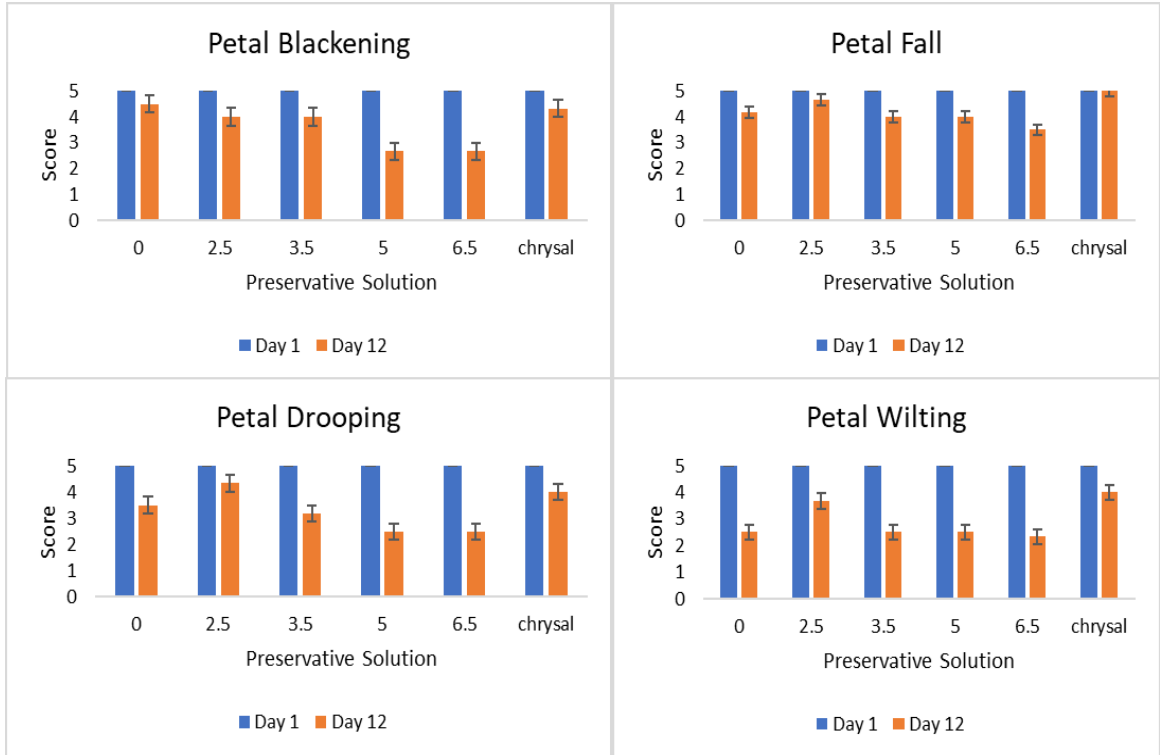


Figure 4.3 Initial (Day 1) and final (Day 12) scores for various physical parameters of carnation as affected by different concentrations of compost tea and Chrysal.

4.5 Conclusion

Carnation is one of the most popular commercial cut flowers grown on a large scale in the world. The vase life of cut flowers is an indicator of post-harvest quality and determines consumer preference of cut flowers. Apart from the external quality of the flowers, the vase life of cut flowers is one of the most crucial factors for consumers. Commercial floral preservatives have been well researched to provide an appropriate combination of sugar, acidifier, and biocide for extending vase life and, not surprisingly, they were best effective in these studies. From the above-mentioned results and discussion, it can be stated that other than chrysal, the performance of cut carnations was satisfactory in 3.5% compost tea concentration followed by distilled water (0%). Cut flowers treated with 5% and 6.5% compost tea concentrations had highest rate of deterioration in terms of petal blackening, petal drooping, petal wilting and petal fall. Other than chrysal, 3.5% compost tea concentration can be used as environment friendly alternative for vase life extension of cut carnation flowers. Any compost tea with physicochemical properties similar to 3.5% compost tea concentration prepared in this study can be used as an alternative to chrysal for vase life extension. A preservative solution containing 3.5% compost tea was selected for further amendments using natural substances (chapter 5) as it increased the vase life of cut flowers compared to other compost tea concentrations and distilled water.

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CHAPTER 5.0

EFFECT OF NATURALLY AMENDED COMPOST TEA ON VASE LIFE OF CUT FLOWERS

5.1 Abstract

This study was conducted to determine the effect of preservatives solutions containing compost tea and natural substances in comparison to a commercial floral preservative on cut carnations (*Dianthus caryophyllus* L. var. White Sim). The treatments were 3.5% compost tea (C_{3.5}), 3.5% compost tea + 1 mL lemon extract + 0.5 mL rosemary extract + 15 ppm putrescine (R_{0.5}L₁), 3.5% compost tea + 1 mL lemon extract + 1 mL rosemary extract + 15 ppm putrescine (R₁L₁), 3.5% compost tea + 2 mL lemon extract + 0.5 mL rosemary extract + 15 ppm putrescine (R_{0.5}L₂), 3.5% compost tea + 2 mL lemon extract + 1 mL rosemary extract + 15 ppm putrescine (R₁L₂). Distilled water (D₀) and chrysal were used as controls. It was observed that C_{3.5} and R_{0.5}L₁ led to increase in vase life of cut carnation flowers to 11 days in comparison to 14 days in chrysal. It was reported that C_{3.5} and R_{0.5}L₁ were equally effective in maintaining flower head diameter and fresh weight of cut carnations. The results show that C_{3.5} and R_{0.5}L₁ treatments used in this experiment are healthy, nonhazardous for the environment and appropriate to increase the vase life of cut carnations.

5.2 Introduction

Essential oils are volatile aromatic compounds obtained by hydro-distillation from plant materials such as flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots (Solgi and Ghorbanpour 2014). Several essential oils such as rosemary, geranium, mint,

eucalyptus, savory as well as acidifiers like lime, orange, citric acid, and ascorbic acid have been evaluated for their effects on extension of vase life in cut flowers (Bazaz and Tehranifar 2011). These natural substances can be used as alternative substances to synthetic chemical substances because of their antimicrobial properties against some pathogens and environmentally friendly nature (Bayat et al. 2011). Citric acid is known to extend the vase life of cut flowers by killing pathogens in the vase solution. Besides, citric acid is readily available and is cheap. It is one of the most common organic compounds used to lower the pH and control microbial population in several cut flowers such as carnation (*Dianthus caryophyllus* L.) (Kazemi and Ameri 2012), lisianthus (*Eustoma grandiflorum* Salisb.) (Kiamohammadi 2011), roses (*Rosa × hybrida* L.) (Jowkar et al., 2012), tuberose (*Polianthes tuberosa* L.) (Jowkar and Salehi, 2006), and sunflower (*Helianthus annuus* L.) (Ferrante et al. 2005). Citric acid is present in citrus fruits such as lemons, lime, and oranges. Lemon juice is known to contain 37 g/L of citric acid. Several other common products have been used in homemade floral preservatives. Some of these homemade floral preservatives include lemon/lime soda, lemon juice, household bleach and vinegar (Greer and Einert 1994), pennies, or the essential oils of several plant species (Tehranifar and Karimian 2011). However, limited information is available on effectiveness of essential oils for extending vase life of cut flowers. Commercial preservatives are well researched and effective, but not environment friendly.

Naturally occurring polyamines such as putrescine, spermidine and spermine are known to interact with a variety of biomolecules and involved in important physiological processes.

The polycationic nature of putrescine enables it to interact with negatively charged molecules. Polyamines have the kosmotropic properties. Kosmotropes cause water molecules to favorably interact, thereby stabilizing the intramolecular interactions in macromolecules such as proteins (de la Peña et al. 2000). The presence of acidic region and the hydrophobic region located between the C and N termini provide binding targets for anionic and hydrophobic molecules that could enhance the aggregation process. The potency with which polyamines facilitate aggregation correlates with their cationic charge and the number of aliphatic carbon chains between the amino groups (Antony et al. 2003). Thus, polyamine can be used as a natural way of facilitating aggregation. Particles of compost can block the xylem of cut flowers and reduce the water uptake due to it is important to place the cut flowers in clear solution.

The present study was conducted to determine the effect of floral preservatives containing compost tea and natural substances in comparison to a commercial floral preservative on carnation. The specific objectives of this study were to 1) determine the most suitable concentration of putrescine, lemon extract and rosemary extract in 3.5% compost tea concentration that will improve vase life of carnation and 2) evaluate the effects of natural additives and compost tea mixture on vase life in comparison with chrysal. It was hypothesized that the recipe would extend the vase life of carnation, similar to what was observed with commercial preservative solutions.

5.3 Materials and Methods

5.3.1 Preparation of compost tea concentration

From the stock solution, a 3.5% concentration of compost tea was prepared as stated in section 4.3.1 of chapter 4. Among all the natural additives, putrescine was selected first to check its effect on chemical properties of the 3.5% compost tea especially turbidity. It was included in the experiment to get a clear solution and reduce the turbidity of compost tea.

5.3.2 Addition of putrescine

Concentrations 10, 15, 20, 25 and 30 ppm of putrescine were prepared by addition of 0.015, 0.225, 0.3, 0.375 and 0.45 g in 1.5 L of 3.5% compost tea. Solution was prepared in three replications 1.5L each, for the control and for each concentration. Chrysal and distilled water were used as a control. The experimental design was a randomized complete block design. Electric conductivity, salinity, pH, TDS and turbidity of each solution were analyzed after 48 hrs.

5.3.3 Addition of lemon and rosemary extract

3.5% compost tea with the addition of best-selected concentration of putrescine was further amended using lemon extract and rosemary extract in 500 mL of solution. Treatments are described in Table 5.1. Two controls i.e. chrysal sachets manufactured by Oasis Company of U.S. and distilled water were used for comparison.

Table 5.1 Various preservatives solutions from a mixture of compost tea and natural substances and the synthetic chemical, Chrysal.

Preservative Solution	Preservative/ 500 mL+ 15 ppm putrescine
R _{0.5} L ₁	3.5% compost tea + 1 mL lemon extract + 0.5 mL rosemary extract
R ₁ L ₁	3.5% compost tea + 1 mL lemon extract + 1 mL rosemary extract
R _{0.5} L ₂	3.5% compost tea + 2 mL lemon extract + 0.5 mL rosemary extract
R ₁ L ₂	3.5% compost tea + 2 mL lemon extract + 1 mL rosemary extract
C _{3.5}	3.5% compost tea
D ₀	Distilled water (control)
Chrysal	Chrysal Clear Universal Flower Food (control)

5.3.4 Preparation of cut flowers

The carnation (*Dianthus caryophyllus* L. var. White Sim) was obtained from a local retail superstore, Truro. The leaves were removed from the stem of each cut flower stem, and slant cut of 2 cm was performed on each stem before placing into the transparent glass beaker of 1.5L containing different solutions.

5.3.5 Collection of data

5.3.5.1 Physicochemical properties

The compost tea dilutions were analyzed for their chemical stability by collecting data for pH, Electric Conductivity (EC), salinity and Total Dissolved Solids (TDS) using PCS Testr 35 (Multiparameter). PCS Testr 35 was calibrated with certified accurate calibration standards before measuring pH, EC, TDS, and salinity. Turbidity was measured using

Okaton turbidity meter (Model: T-100, Eutech Instruments, Singapore). Data were collected every second day for 11 days.

5.3.5.2 Visual analysis of cut flowers

The flower head on each stem was visually evaluated every day and graded from 0 to 5 for petal browning, petal fall, petal wilting and petal drooping according to the previously defined scales described in section 4.3.4 of chapter 4. The grading was done visually, and the diameters of cut flowers were recorded using an electronic caliper meter (Model: 58-6800-4, Mastercraft, Toronto, ON, Canada).

5.3.5.3 Flower longevity

Vase life was measured based on the scores defined for petal browning, petal drooping, petal fall and petal wilting. The time from purchase to 50% petal browning, fall, drooping and wilting (i.e. Scale 2.5, in between scale 2 and 3) was used to calculate flower longevity or vase life.

5.3.5.4 Fresh weight loss

The fresh weight of cut carnations was measured before imposition of the experimental treatment. The weights of carnation flowers were measured on every second day after treatment and the percentage weight loss was calculated.

5.3.5.5 Flower head diameter

The flower head diameter was measured using the index of floral wilting. The outer diameter of opened flowers was measured using an electronic caliper meter (Model: 58-6800-4, Mastercraft, Toronto, ON, Canada). The percentage decrease in flower head diameter was calculated for each flower.

5.3.5.6 Total suspended solids (TSS)

To measure total soluble solids, two petals were plucked from each cut flower and the petals were ground in distilled water to obtain an extract. A few drops of petal juice were analyzed using a multi-range digital refractometer (Model: RF15, Extech, Montreal, Canada) and the TSS value was recorded as °Brix after using standards to calibrate the instrument.

5.3.6 Data analysis

Analysis of physicochemical data (pH, EC, salinity, TDS and turbidity) and physical parameters (petal blackening, petal fall, petal drooping and petal wilting) were done as in chapter 4. Data collected on flower head diameter, fresh weight loss, and flower longevity were subjected to analysis of variance (ANOVA) procedures using GLM model procedures of SAS (version 9.4) and means were separated using Fisher's least significant difference (LSD) at $\alpha = 5\%$ (Gomez et al. 1984).

5.4 Results and Discussion

5.4.1 Addition of putrescine

There was significant difference ($P < 0.05$) in turbidity among different treatments whereas, change in other properties such as pH, salinity, EC, TDS and turbidity after 48 hours of addition of putrescine were not significant (Table 5.2).

Turbidity was lowest after 48 hrs. in 15 ppm solution. A slight reduction in turbidity was also seen in the 10 ppm concentration whereas it increased in the 20, 25, 30 ppm. Turbidity was the highest in 30 ppm after 48 hours. This can be attributed to the fact that rapid aggregation at high polyamine concentration may reflect the formation of insoluble small

amorphous aggregates and an increase in the corresponding turbidity at early time points.

Table 5.2 Change in pH, electrical conductivity (EC), turbidity, total dissolved solids (TDS) and salinity of 3.5% compost tea concentration after 48 hrs. of putrescine addition.

Putrescine (ppm)	Turbidity (NTU)	pH	Salinity (ppm)	EC (μ S/cm)	TDS (ppm)
0	34.20a	6.73a	126.63a	256.33a	202.44a
10	24.11b	6.69a	128.63a	262.72a	204.21a
15	22.68b	6.63a	130.41a	266.27a	206.48a
20	29.03a	6.61a	133.53a	264.23a	208.8a
25	30.9a	6.54a	134.8a	262.25a	206.31a
30	34.72a	6.62a	137.35a	266.88a	204.37a
Significance	0.03	NS	NS	NS	NS

In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.05 level of probability.

NS: Nonsignificant at $P < 0.05$.

5.4.2 Physicochemical properties

The values of all the physicochemical parameters of each preservative solution are shown in Table 5.3. All compost tea preservative solutions had an initial pH ranging from 6.78 to 7.5 except for chrysal for which the initial pH was 2.56 (Fig. 5.1). Lemon extract was added to reach the target pH of 6.2 to 6.5 (Cross 2001). Initial pH for distilled water was 7.05. The change in pH was recorded for solutions when the flower stems were kept continuously

in preservative solutions. pH of amended compost tea preservative solutions (R_{0.5}L₁, R₁L₁, R_{0.5}L₂, R₁L₂, C_{3.5}) decreased from day 1 to day 11 thus, the solutions became more acidic. This decrease in pH has been reported in citric acid or lemon juice amended preservative solutions when cut flower stems were placed in the solutions until termination of cut flowers as citric acid or lemon juice are acidic in nature (Ahmad and Dole 2014). Increase in pH was recorded in chrysal and distilled water from 2.56 to 3.17 and from 7.05 to 7.22, respectively (Fig. 5.1). This change in physicochemical properties from day 1 to day 11 is shown in Figure 5.1. TDS, EC, and salinity increased in all the preservative solutions. The highest change was observed in chrysal among all the preservative solutions and smallest in the C_{3.5} treatment (Table 5.3). Carnation cut stems placed in chrysal had the highest initial and final EC (686 and 1277 μ S/cm respectively). Turbidity decreased from day 1 to day 11 in all preservative solutions except in chrysal and distilled water in which turbidity increased from 2.32 NTU to 13.3 NTU and from 0.6 NTU to 1.41 NTU, respectively. The effect of the preservative solution was observed to be significant for pH, salinity, EC, TDS and turbidity.

Table 5.3 Change in pH, electrical conductivity (EC), turbidity, total dissolved solids (TDS) and salinity of different mixed preservative solutions and the synthetic chemical, Chrysal.

Preservative solution	Application duration (days)	pH	EC (uS/cm)	Turbidity (NTU)	TDS (ppm)	Salinity (ppm)
R _{0.5} L ₁	11	5.34de	175.49c	30.58c	123.12cd	79.45c
R ₁ L ₁	11	5.72e	163.57c	33.90cd	117.11c	73.83c
R _{0.5} L ₂	11	5.07d	175.25c	38.05d	122.87cd	76.67c
R ₁ L ₂	11	4.97d	188.52c	35.16cd	135.13d	83.56c
C _{3.5}	11	7.45a	288.23a	21.31a	212.08a	130.43a
D ₀	11	7.13a	61.09b	1.05b	43.40b	30.24b
Chrysal	11	2.94c	914.00d	8.78d	628.21d	389.99d

In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.05 level of probability.

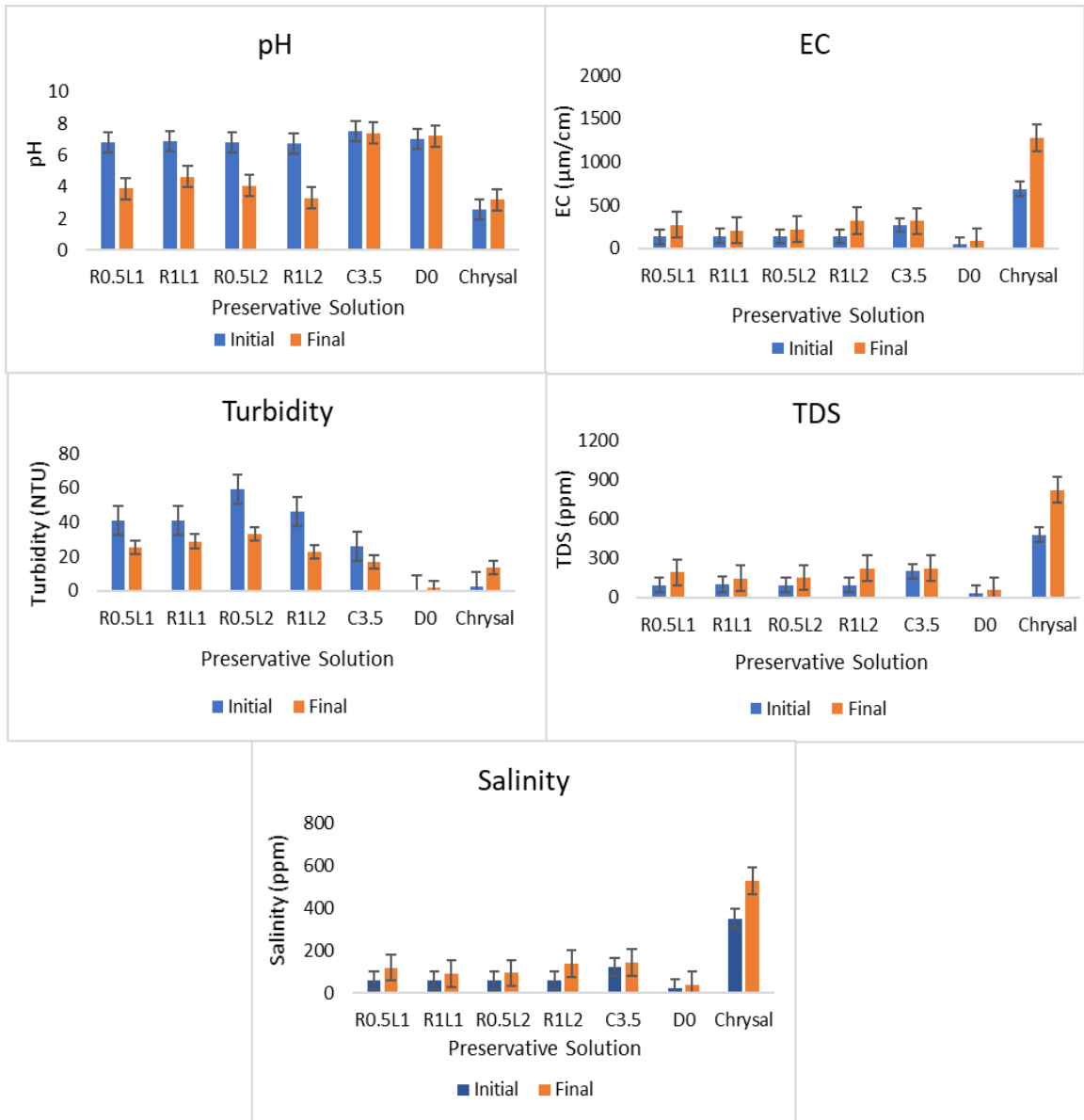


Figure 5.1 Initial (Day 1) and final (Day 11) values of pH, electrical conductivity (EC), turbidity, total dissolved solids (TDS) and salinity of different mixed preservative solutions and the synthetic chemical, Chrysal.

5.4.3 Visual analysis of cut flowers

Petal blackening: Significant differences ($P < 0.05$) were observed for petal blackening in different preservative solutions (12 days of treatment) (Table 5.4). Petal blackening or discoloration was 50% in D_0 , R_1L_1 , $R_{0.5}L_2$, R_1L_2 preservative solutions (Fig 5.2). Early petal discoloration denoted the lowest quality of cut carnation in flower vase whereas the delayed petal discoloration denoted better quality of cut carnation in flower vase. Petal discoloration increased with increased duration. Minimum petal blackening was found in chrysal and $C_{3.5}$ with scores of 4.3 and 4 respectively.

Petal drooping: Significant differences ($P < 0.05$) were observed for petal drooping in different preservative solutions. Petal drooping was least in chrysal followed by $C_{3.5}$ with 10-30% petal damage respectively. $C_{3.5}$ preservative solutions provided delayed petal drooping of cut carnations denoting improved vase life, comparable to chrysal. Petal drooping was observed to be 50% in D_0 , R_1L_1 , $R_{0.5}L_2$, R_1L_2 preservative solutions (Fig. 5.2). Therefore, it could be stated that chrysal and $C_{3.5}$ are the best among all the treatments used in this experiment followed by other preservative solutions. Maximum petal drooping was observed in D_0 and R_1L_1 (Table 5.4).

Petal fall: Petal fall or shatter can be attributed to lack of food in the solution thus, forcing the flower to use all the reserves (Thwala et al. 2013). Petal fall did not illustrate significance in different preservative solutions after 12 days of treatment. There was no petal fall (score 5) in any of the flowers placed in different preservative solutions (Fig. 5.2).

Petal wilting: There was significant ($P < 0.05$) difference in petal wilting of carnation cut flowers among different preservative solutions after 12 days of treatment (Table 5.4). Petal wilting increased in cut carnations with increased duration. After 12 days of treatment,

maximum wilting was observed in cut flowers treated with R₁L₁, R₁L₂, R_{0.5}L₂, and D₀ with statistically no significant difference (P<0.05) (Fig. 5.2). The lowest wilting was scored in chrysal and C_{3.5} with a score of 4. Petal wilting is due to depleted plant food and the inability of the plant to draw up water which leads to subsequent color change and flaccidity of cell and hence the manifestation of flower death. Flaccid cells give the flower a wilting appearance which may be improved by adding effective bactericide in the preservative solution to eliminate the accumulation of bacteria (Thwala et al. 2013).

Table 5.4 Average scores for various physical parameters as affected by different mixture of natural preservative solutions and Chrysal.

Preservative solution	Application duration (days)	Petal	Petal	Petal	Petal	Average Score
		blackening ^a	drooping ^a	fall ^a	wilting ^a	
R _{0.5} L ₁	12	3.3c	3.3c	5.0a	3.6c	3.8
R ₁ L ₁	12	2.3a	2.3a	5.0a	2.5b	3.0
R _{0.5} L ₂	12	2.6a	2.6ac	5.0a	2.6b	3.2
R ₁ L ₂	12	2.0a	2.3a	5.0a	2.5b	2.9
C _{3.5}	12	4.0b	4.0b	5.0a	4.0a	4.2
D ₀	12	2.6a	2.3a	5.0a	2.6b	3.1
Chrysal	12	4.3b	4.3b	5.0a	4.0a	4.4

In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.05 level of probability.

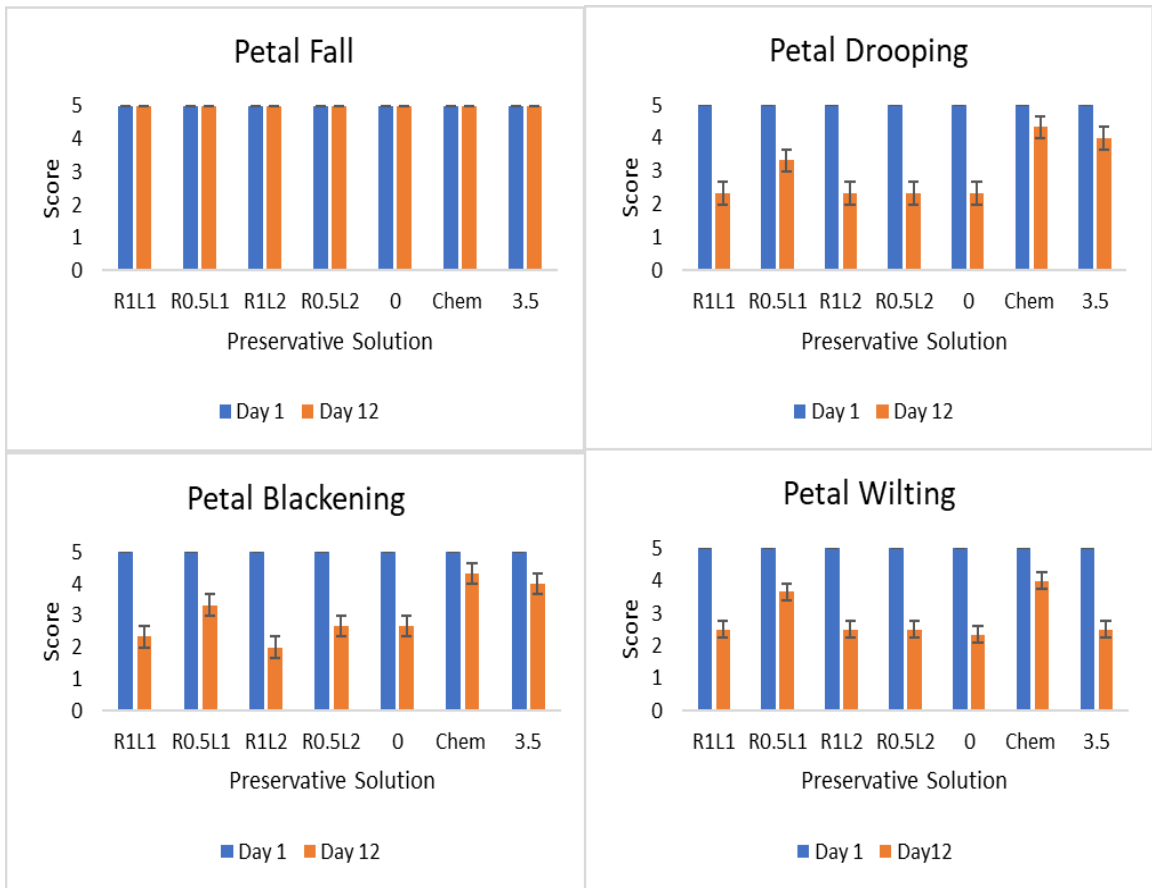


Figure 5.2 Initial (Day 1) and final (Day 12) scores for various physical parameters as affected by different mixture of natural preservative solutions and Chrysal.

5.4.4 Flower longevity

Significant differences ($P < 0.05$) were observed for flower longevity in different preservative solutions (Table 5.5). The longest vase life (i.e. 14 days) was recorded for chrysal treatment followed by C_{3.5} and R_{0.5}L₁ (~11.0 days with no significant difference between two treatments) whereas minimum (7 days) was counted for the R₁L₂ treatment and distilled water (8 days). This indicates that the addition of an appropriate amount of compost tea, rosemary and lemon extract, as the preservative solution was superior to water. It also indicates that 3.5% compost tea solution without any amendment performed comparably to (R_{0.5}L₁). Bayat et al. (2011) reported an increase in vase life of carnations using essential oils such as Thyme (*Thymus vulgaris* L.), Summer savory (*Satureja hortensis* L.) and Ajwain (*Carum copticum* L.).

Table 5.5 Assessment of vase life, flower head diameter and fresh weight loss of cut carnation in different preservative solutions.

Preservative solution	Application duration (days)	Flower longevity ^a	% Decrease in flower diameter	Fresh weight loss (%)
R _{0.5} L ₁	11	10.66a	4.05a	8.29c
R ₁ L ₁	11	9.0b	5.32c	10.14a
R _{0.5} L ₂	11	9.33b	4.28a	8.9a
R ₁ L ₂	11	7.66c	5.68c	10.96a
C _{3.5}	11	11.0a	2.57b	5.9bc
D ₀	11	8.33c	4.21a	12.5a
Chrysal	11	14.33d	2.54b	2.7b

In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.05 level of probability

5.4.5 Fresh weight loss

Percentage of fresh weight loss varied significantly among the treatments during 11 days of treatment using different preservative solutions (Table 5.5). Flowers reduced their quality rapidly due to the fresh weight loss. Minimum weight loss of cut carnations (~3%) was found in chrysal followed by C_{3.5} and R_{0.5L₁} with fresh weight loss of 6% and ~8% with statistically no difference. All other preservative solutions had weight loss ranging from 9-12.5%. The maximum loss in fresh weight of cut carnations was obtained in distilled water (12.5%) (Fig. 5.3). Similar results showing the maximum weight loss of cut flowers in tap or distilled water have been reported as compared to other preservative solutions (Mehraj et al. 2013; Penniston et. al. 2008).

Postharvest water relations of cut flower are considered the major factors determining flower vase life (Rogers 1973; Coorts 1975; Halevy and Mayak 1979, 1981). The water balance is affected by two phenomena, water uptake and transpiration (Da Silva 2003). A water deficit and wilting in a cut stem in a vase solution will develop when the rate of transpiration exceeds the rate of water uptake (van Doorn 1997). Positive effects of lemon extract on vase life of cut flowers could be attributed to the antimicrobial effects of citric acid present in lemon extract that act as a biocide in preservative solution and increase water uptake. Redman (1994) showed that carnation flowers are sensitive to high populations of bacteria in vase solution. When cut flowers are placed in a vase containing water, the cell sap oozes out and microbes start multiplying in the vase water which results in xylem vessel clogging (Thanusha 2017). The presence of the bacteria causes vascular blockage, prevents water uptake and consequently results in the reduction of fresh weight. Solgi et al. (2009) reported that flower preservatives allow water absorption through flower

tissues. Water absorption from the preservative solution maintains a better water balance and flower freshness which saves the cut flowers from early wilting and improves the vase life. Antibacterial agents keep the water free from bacteria and other harmful microorganisms by keeping check on their growth which can otherwise form occlusion inside the stem obstructing the flow of water to the flower (Redman 1994). Halevy and Mayak (1981) demonstrated that the vase-life of rose, gypsophila, gerbera, carnation, and chrysanthemum was improved significantly with germicide solution whereas increasing microbial contamination in the vase water resulted in poor vase life of many cut flowers. A reduction in membrane integrity, destruction of enzymatic systems involved in energy production and cellular structure components are the main mechanisms of these compounds in mitigating microbial infection (Sikkema et al. 1994). Basiri et al. (2011) and Rahman et al. (2012) indicated that rosemary extracts inhibited the growth of microorganisms in vase solution and increased water uptake of carnation (*Dianthus caryophyllus*) cut flowers. The anti-bacterial activity of essential oils is associated with the presence of high monoterpene concentrations. The solutions were very effective as antimicrobial agents in inhibiting the growth of microorganisms and consequently, preventing the occlusion of xylem vessels. The number of bacteria in distilled water (control) was increased more than the natural and synthetic chemical solutions (El-Moneim et al. 2018). Application of compost tea and in combination with different concentrations of lemon extract and rosemary extract in preserving solution prolonged the vase-life of carnation cut flowers and reduced fresh weight of flowers as compared to control. Based on the present results, appropriate combination of all three natural substances: putrescine, lemon extract and rosemary extract can be an appropriate alternative compound in

improving of vase-life of carnation cut flowers. These materials can be both safe and environmentally friendly.

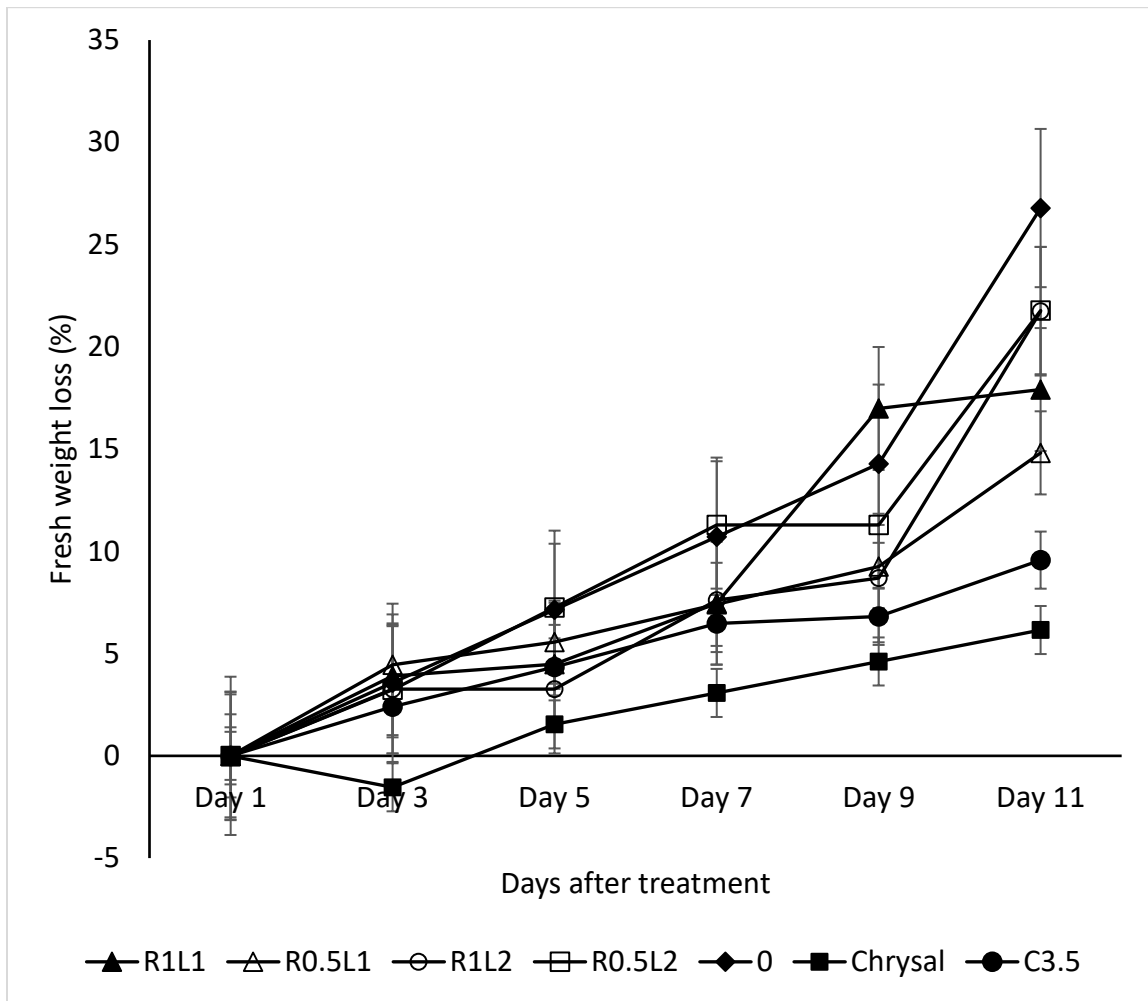


Figure 5.3 Effect of different mixed preservative solutions and Chrysal on fresh weight of carnation petals during 11 days of treatment.

5.4.6 Flower head diameter

Flower head diameter varied significantly among the treatments during 11 days of treatment (Table 5.5). Results reveal that average percent decrease in flower diameter was least in chrysal and C_{3.5} (2.54% and 2.57%, respectively) followed by R_{0.5}L₁, D₀ and R_{0.5}L₂ (4.05%, 4.21% and 4.28%, respectively) (Fig. 5.4). Average percent decrease in flower head diameter of the cut flowers placed in R₁L₁ and R₁L₂ was high among all the preservative solutions (5.32% and 5.68%) (Table. 5.5).

In-rolling and wilting of petals, discoloration and necrosis of the petal margins, which gradually spread to the remaining petal portions are the typical symptoms of senescence induced by ethylene in carnation flowers (Sato et al. 2005). Reduction flower head diameter in flower vase denoted the quality reduction of cut carnation. Increasing flower diameter denotes that opening of flower occurred. In case of cut carnation, as it was assumed that the cut flowers were picked at full blooming stage, so quality is maintained by the diameter of flower head.

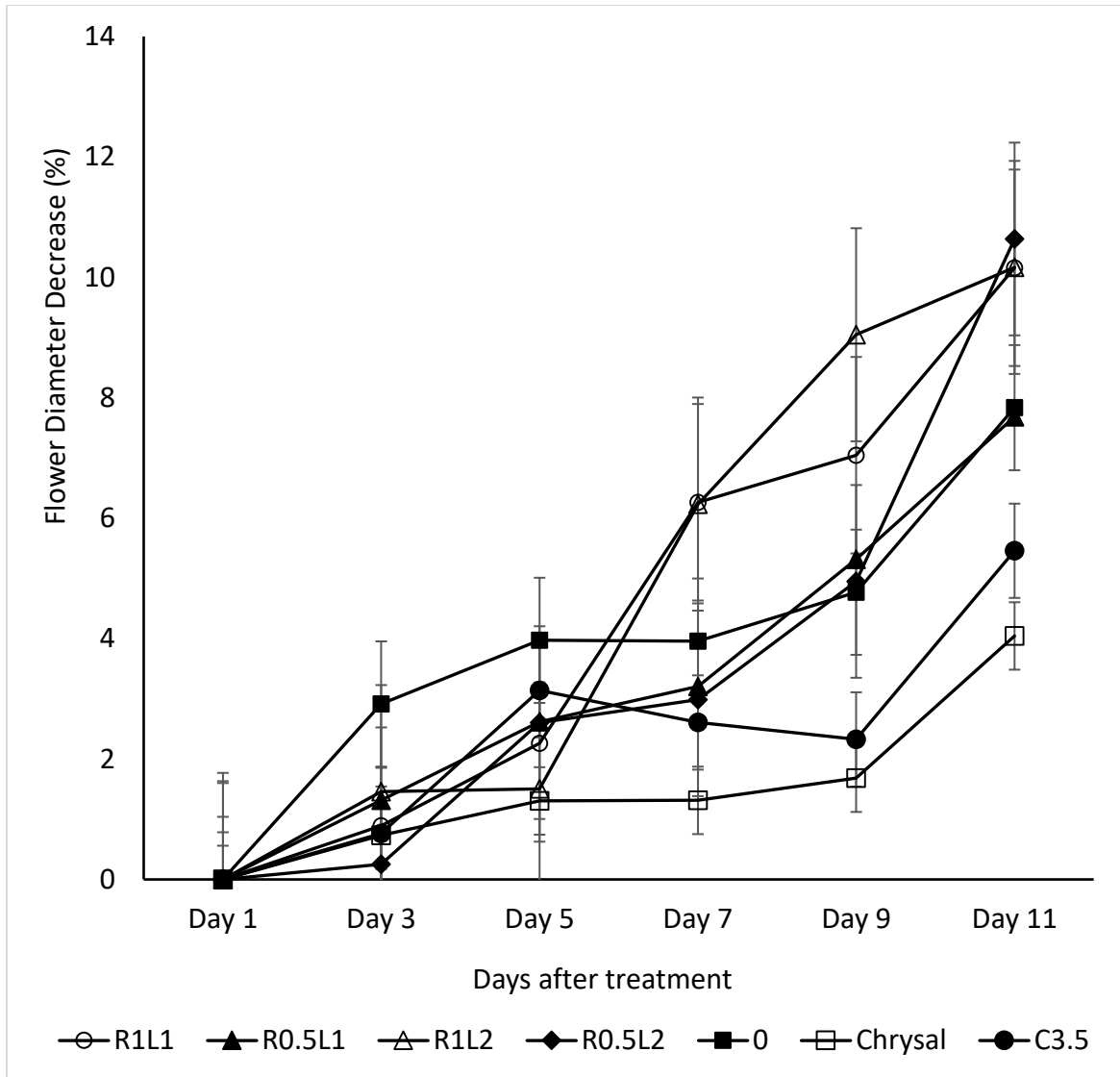


Figure 5.4 Effect of different mixed preservative solutions and Chrysal on flower head diameter during 11 days of treatment.

5.4.7 Total suspended solids (TSS)

In this study, the TSS content of the flowers decreased from day 1 to day 11. Maximum decrease in brix % was observed in distilled water (3%) followed by R_{0.5}L₁, C_{3.5} and chrysal (Fig. 5.5). Minimum reduction in sugar content of flower petals was obtained in chrysal solution (0.7%). Changes in soluble carbohydrate concentrations in petals was observed. In the petals of control flowers, the concentration of TSS i.e. glucose, fructose, and sucrose gradually decreased with time and this decrease has been associated with a climacteric-like increase in ethylene production in previous studies (Pun et al. 2016). Thus, the decrease in the sugar concentrations may trigger increase in ethylene production, leading to petal senescence. Similarly, a climacteric-like increase in ethylene production was associated with decrease in soluble carbohydrate levels in cut sweet pea (Ichimura and Suto 1999) and Delphinium flowers (Ichimura et al. 2000). In contrast, treatment with 5% sucrose has been reported to increase TSS content in petals and delayed climacteric-like increase in ethylene production (Pun et al. 2016). Sugars have been proposed to be signal molecules received by some sensor proteins, such as hexokinase and sucrose transporter (Smeekens 2000; Hanson and Smeekens 2009) and sucrose treatment may reproduce the internal conditions of intact flowers.

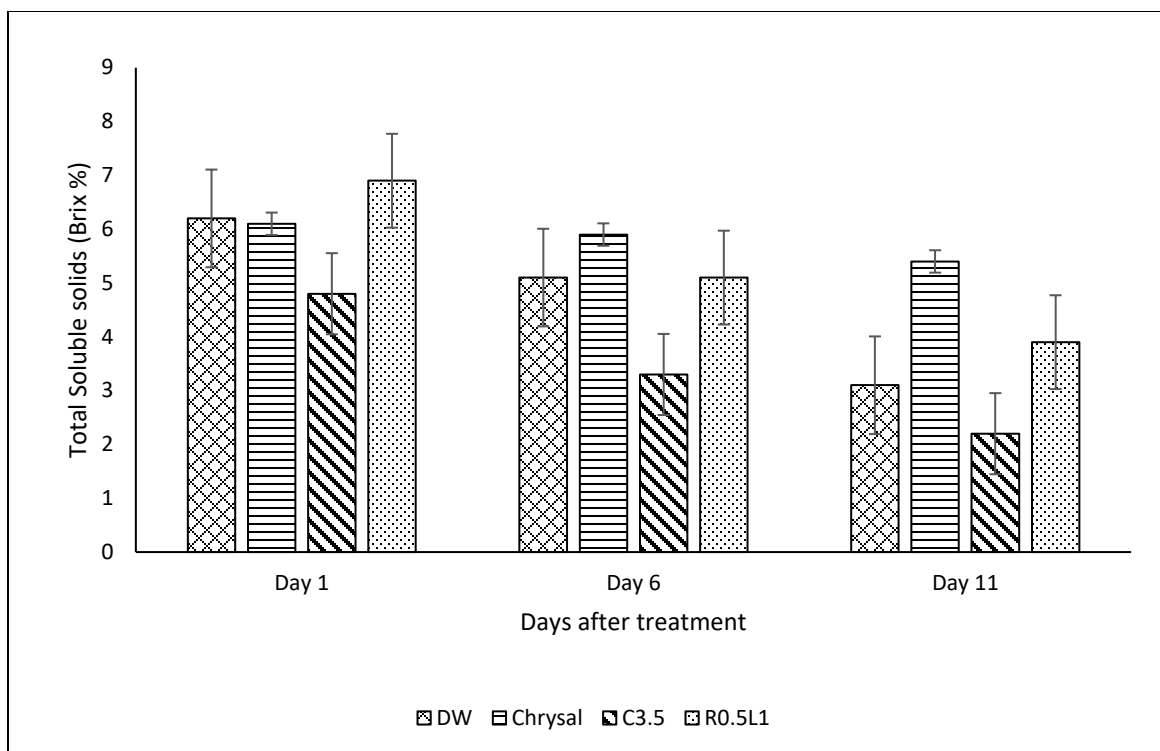


Figure 5.5 Effect of selected mixture of compost tea and natural substances and Chrysal on total suspended solids (TSS) of carnation cut flowers during 11 days of treatment.

5.5 Conclusion

It was observed that the preservative solution containing 3.5% compost tea and the solution containing 3.5% compost tea amended with 0.5 mL rosemary extract and 1 mL lemon extract led to increase in the vase life of cut flowers. Compost tea concentrations prepared from different composts and natural additives with similar physicochemical properties can be used for vase life extension of cut carnations. The commercial floral preservative (chrysal) with sugar, acidifier, and an appropriate biocide produced the longest vase life of cut carnations. The treatment combinations with compost tea contained acidifier (lemon extract), biocide (rosemary extract and antimicrobial properties of compost tea) but lacked in sugar to some extent which resulted in a lower performance of cut flowers, but improved performance compared to distilled water. 15 ppm of putrescine successfully reduced the turbidity of compost tea concentrations to desirable extent. Producers of organically grown flowers have long searched for an organically certified floral preservative. However, some studies have shown that acidifiers alone are not effective flower preservatives due to microbial contamination. Dense microbial colonies have been observed (visual observation) in vase solutions without biocides when used until termination, which may have reduced solution uptake. Preservative solutions containing compost tea, lemon extract as an acidifier, and rosemary extract as a biocide, were more effective than distilled water. However, cost effectiveness of essential oils such as rosemary may be an issue, as the product is generally more expensive than commercial flower food. Maintenance of low pH in preservative solutions containing lemon extract might have maintained continued metabolic activities and water uptake, resulting in maintaining higher dry matter in the cut stems until termination.

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CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Overview

Carnations are becoming increasingly popular cut flowers worldwide due to diversity in color pattern, petal shape and size. At present, only chemical preservatives are widely used, popular, and established preservatives known to extend the vase life of cut flowers. In these studies, attempts were made to develop readily available, low-cost, environment friendly floral preservative solutions to maximize postharvest life and quality of cut flowers. The vase life of cut flowers is an important parameter for the evaluation of cut flower quality for domestic and international markets. The current study focused on the effect of postharvest treatments on the vase life of cut flowers that were assumed to be cultivated under the same environmental conditions. Even though the conditions were the same in our experiments, the variability in pre-harvest conditions can have influence on the present results. Many factors during production may influence vase life, water uptake, and other measured parameters. The vase life of carnations has been reported to be affected by vascular blockage not only due to microbial contamination but also, several physical factors e.g., vascular occlusion due to air embolisms and genetically controlled factors e.g., loss of membrane permeability. If solution uptake is not blocked by vascular blockage, genetically controlled loss of membrane integrity may hinder uptake and ultimately, lead to senescence. The present study demonstrated the potential impact of natural preservatives on the postharvest longevity of cut carnation stems. The continuous use of preservative solution containing 3.5% compost tea or the solution containing 3.5% compost tea mixed with 0.5 mL rosemary extract and 1 mL lemon extract increased vase life of cut flowers.

Although the overall performance of cut flowers was best in the commercial preservative (chrysal), but still the performance of cut flowers was comparable and better in the above mentioned natural preservative solutions compared to distilled water.

6.2 Future Recommendations

Based on the findings of the present investigation the following future lines of research work are proposed:

- The preservative solutions should be tested for microbial contamination. Presence of microbial colonies in vase solutions have been reported to block the vascular system, causing reduction in water uptake and rapid water loss and ultimately reducing fresh weight and longevity.
- Sucrose acts as a food source or respiratory substrate and improves the water balance of cut flowers. Inclusion of sugar may be tested for extension of vase life of cut carnations.
- Study on ethylene production and respiration can be related to sensitivity and vase life of cut carnations.

APPENDIX 1.0

PRELIMINARY RESEARCH

Materials and Methods

Preparation of stock compost tea

Municipal solid waste (MSW) compost was obtained from Fundy Compost Inc., Pleasant Valley Rd, Brookfield, Nova Scotia. MSW compost tea stock solution was prepared by adding 100 g of sieved compost at 20% moisture content to 2 L of distilled water in Erlenmeyer flask. The top of the flask was covered with parafilm and a small hole was made at the top to allow aeration. The mixture was stirred for 24 hrs. under room temperature and relative humidity conditions at 1100 rpm using Isotemp magnetic stirrer (Cat No. 11-100-100SH; Fisher Scientific, Toronto, ON, Canada). The mixture was allowed to stand for 24 hrs. to settle before filtering using a NALGENE rapid flow filter with disposable bottle top filters lined with polyethersulfone membrane (Fisher Scientific, Toronto, ON, Canada). Desired concentrations were prepared from stock compost tea.

Preparation of plant material

Flowers of a carnation (*Dianthus caryophyllus* L.) that belong to the standard type were used. Flowers were harvested from a field of commercial grower at normal harvest maturity. The flowers were placed in distilled water immediately after harvest in a bucket and then transported in a personal vehicle to the lab (10 km distance) for the experiment. The flowers were then stored in a refrigerator at 4-5°C overnight and were used in the morning. Before conducting the experiment, the flower stems were trimmed 2 cm at the base and all the leaves except the upper 3-5 leaflets were removed. It was made sure that the remaining leaves on the stems were free of infection and damage of any kind.

Part-IA

Compost tea concentrations of 0%, 5%, 15%, 30%, and 100% were prepared from the stock solution using distilled water.

Determination of the vase life of carnation flowers

For the determination of the vase life observed by senescence profiles, two cut flowers with single flower at their fully open stage (day 0) were placed in 300 mL of compost tea concentrations (0%, 5%, 15%, 30%, and 100%). Distilled water was used as a control. The experiment was conducted in the laboratory at room temperature conditions with relative humidity and photoperiod conditions according to prevalent weather conditions. Each flower was observed daily to record senescence symptoms, *i.e.*, in-rolling and subsequent wilting of petals, desiccation, discoloration of the petal margins and any kind of pathogen infection. The cut flowers were left under the conditions described above for 12 days. The experiment was conducted in two replicates for qualitative observation.

Observations

After 12 days, the flowers in all five compost tea concentrations (0%, 5%, 15%, 30%, and 100%) were wilted but no petal fall was observed. The flowers were scored from **0** to 5 according to the scale defined for petal wilting in Chapter 4. Maximum wilting was observed in 100% compost tea concentration with a score of 2, followed by 15% and 30% with flowers scored as 2.5, followed by 0% with flowers scored as 3. The least wilting was observed in flowers placed in the 5% compost tea concentration with a score of 3.5.

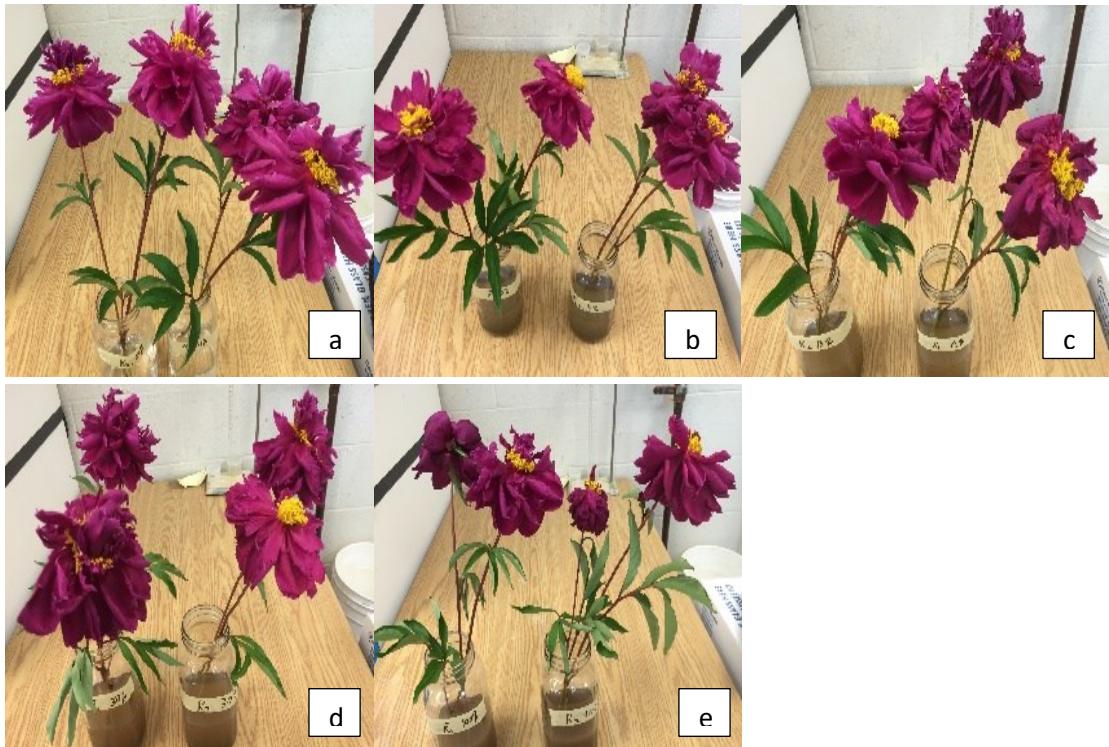


Figure IA.1 Effect of compost tea concentrations (a) 0% (b) 5% (c) 15% (d) 30% and (e) 100% on vase life of cut flowers during 12 days of treatment

Part-IB

Compost tea concentrations of 1.5%, 3% and 5% were prepared from the stock solution using distilled water.

Determination of the vase life of carnation flowers

As the performance of cut flowers was best in 5% concentration of compost tea among the five concentrations prepared in part 1A (0%, 5%, 15%, 30%, and 100%), 1.5, 3, 5% concentrations were prepared, and two flowers of variety B were placed in each of the dilutions. The flowers were again observed for 12 days for petal wilting, petal fall and any kind of pathogen infection. Based on the following observations, it was assumed that a range of 0-5 compost tea concentration should be used in subsequent studies.

Observations

No petal fall was observed in cut flowers except for 5% compost tea concentration by 12th day of experiment. Some aphids appeared in the 1.5% concentration on day 6. The quality of the flowers was in a slight decline, stems and leaves had no obvious change till day 6. The stem became dry and yellow, receptacle became dry and some flowers were dead in 1.5% compost tea concentration by day 12. No major change was seen in flowers in the 3% compost tea concentration on day 12. Though, the stem became dry and yellow, but performance of the cut flowers was better than in the 1.5% compost tea. Performance of cut flowers in the 5% compost tea concentration was similar to the 3%.



Figure IB.1 Cut flowers placed in compost tea concentrations 1.5%, 3%, 5% (from left to right) on day 1.



Figure IB.2 Cut flowers placed in compost tea concentrations 1.5%, 3%, 5% (from left to right) on day 6.



Figure IB.3 Cut flowers placed in compost tea concentrations 1.5%, 3%, 5% (from left to right) on day 12.

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