Research Article

A Study on Effect of Cold Water Extracts of Biopreservatives on Germination, Plumule and Radicle Length of Mung Bean Sprouts

M.K. Ranasinghe[1, 2*] and S. Siddiqui[2]

**madukeshani@gmail.com*

[1]*Department of Animal Science, Uva Wellassa University of Sri Lanka, Passara Road, 90000, Badulla, Sri Lanka*

[2]*Centre of Food Science & Technology, Chaudhary Charan Singh Haryana Agricultural University, India*

Abstract

The present investigation was conducted with the aim to determine effective application of natural antimicrobial compounds of cinnamon, clove, ginger and garlic for mung bean sprouts and to assess their effect on germination percentage (%) and growth of plumule and radicle length of treated sprouts. Mung beans were treated at the time of germination (pre-germination mode) or its sprouts were treated for 15 minutes (post-germination mode) with 0.1% sodium benzoate as chemical preservative (Postive control) and with various bio-preservatives viz., 7.7% clove, 9.5% cinnamon, 7.9% garlic and 7.9% ginger crude extracts. The un-treated mung bean served as control (Negative control). The sprouts were packed in plastic disposable cups and stored in dark at room temperature (20±3°C) conditions and low temperature (7±1°C) conditions. In the present investigation, germination of mung beans was 99.4% in negative control and it was slightly lower in other treatments, where it was ranging from 96.0% to 98.3%. Radicle and plumule length were progressively increased during storage. In pre-germination mode of application, there were no visible plumule at low temperature storage in all the treatments except positive control. In pre-germination mode, the radicle and plumule lengths were reduced maximum by clove during storage at room temperature, while in post germination mode at low temperature conditions, no significant differences were observed in plumule length in any of the treatments. Keeping quality of mung bean sprouts remained acceptable in all the treatments till 48 hours at room temperature and for 96 hours at low temperature storage conditions.

Keywords: *Biopreservation, germination, plummule, radicle, mung bean sprouts*

1. Introduction

Mung bean (*Vigna radiata* L.) sprouts is a very popular oriental food, which has important features with respect to other legumes such as its detoxifying, anti-inflammatory, antitumourogenic, cholesterol-lowering and diuretic properties.

Recently, studies have shown that the sprouts of mung beans are having high biological activities and more secondary metabolites since relevant biosynthetic enzymes are activated during the initial stages of germination. Thus, germination is better way to improve the nutritional and medicinal qualities of mung beans (Tang *et al.*, 2014). However, during postharvest handling and storage, several types of seed sprouts have been clinically assessed to notable outbreaks of bacterial pathogens especially in mung bean sprouts. Seeds may be possibly contaminated during seed production and during sprouting contamination could be occurred due to use of non-potable water, improper handling and storage conditions (Schrader, 2002). Various researchers have revealed that the use of different physical, chemical and biological methods to reduce the microbial load and production of safer sprouts with enhanced shelf life.

Bio-preservation is an emerging concept in the world to prolong the shelf life of perishable fresh agricultural produce. Biopreservatives are a wide range of natural products that can be used to reduce or eliminate pathogen populations while increasing food quality (Ranasinghe & Siddiqui, 2018). Among this type of antimicrobials, essential oils of botanical extracts have long been applied as flavouring agents in foods, and due to their content in antimicrobial compounds, they have potential as natural agents for food preservation (Burt, 2004; Jayasinghe *et al.*, 2016). Kumar & Singhal (2009) reported that mung beans treated with three different concentrations (0.1 mg/mL, 1.0 mg/mL and 2.5 mg/mL) of methanol extract of latex of milkweed (*Calotropis procera*) and control (tap water) have produced a dose-dependent inhibitory effect on seed germination. Eighty eight percent of mung beans germinated in the control group while latex extract at higher concentration the seed germination was 48%. Gabriel (2005), observed that mung bean seeds treated with 300 ppm calcium hypochlorire for 15 hours had no significant effects on the viability of seeds. Both control (distilled water) and calcium hypochlorite soaked samples retained 100% viability. Similarly, Beuchat (1996) found that chlorine treatments did not adversely affect the viability of seed intended for sprouting. However, Penas *et al.*,(2010) showed that the combining effect of high pressure (250 MPa)/ temperature (32.5°C)/ calcium hypochlorite (18000 ppm) and high pressure (250 MPa)/ temperature (32.5°C)/ carvacrol (1500 ppm) treated mung bean seeds showed 80% and 60% of germination percentages, respectively. Ataei & Hashemloian (2006) studied the effects of different concentration of saffron water extracts on seed germination and seedling growth of mung bean in 25°C, after 6 days, it was observed that water extract from 2.5 g/L of saffron had maximal activity and increased the seed germination. The most concentrations (0.0 g/L, 0.5 g/L, 1.0 g/L, 2.5 g/L and 5.0 g/L) of the water extracts of saffron increased seed germination and seedling growth, but 5 g/L of saffron decreased length of both roots and shoots systems.

Goyal & Siddiqui (2012) studied the effect of ultraviolet irradiation, pulsed electric field, hot water dip and ethanol vapours treatments on the growth of mung bean sprouts over the storage period. In the investigation, treated sprouts which were kept at room temperature showed progressive increase in the sprout length with increasing storage period. Sprout length under various treatments ranged from 0.92 cm –3.28 cm and 0.88 cm–1.60 cm during storage period of 48 hours at room temperature and 120 hours at low temperature, respectively. There was no significant difference in the sprout length under various treatments, except in ethanol treatment, where reduced growth was observed during storage.

Ataei & Hashemloian (2006) reported the effects of different concentration of saffron water extracts on seed germination and seedling growth of mung bean in 25°C, after 6 days. Results showed that water extract from 2.5 g/L of saffron have maximum activity. The most concentrations (0.0 g/L, 0.5 g/L, 1.0 g/L, 2.5 g/L and 5.0 g/L) of the water extracts of saffron increased seedling growth, but decreased length of both roots and shoots systems in 5 g/L of saffron. Hence, the present investigation has been carried out to find out the best application of cold water extracts of biopreservatives namely, clove, cinnamon, ginger and garlic and their effect on the germination percentage (%) and radicle and plumule length of mung bean sprouts.

2. Materials and Methods

The study was carried out at the Centre of Food Science and Technology, CCS Haryana Agricultural University, Hisar, Haryana (India).

2.1 Materials

As raw materials Mung beans var. MH 421 was procured from Pulse Section, Department of Plant Breeding, CCS HAU, Hisar while available varieties of cinnamon (*Cinnamomum zeylanicum*), clove (*Syzygium aromaticum*), ginger (*Zingiber officinale*) and garlic (*Allium sativum*) were procured from local market of Hisar for extraction of antimicrobial compounds.

2.2 Method

2.2.1 Preparation of Clove and Cinnamon Extracts (Stock Solution)

Preparation of cold water extracts of clove and cinnamon were done. Clove and cinnamon were cleaned by washing in potable water. Cleaned clove and cinnamon were kept in hot air oven at 60°C for 2 days for drying and then ground into a fine powder. Each of fine powder (100 g) were weighed using electronic weighing balance and mixed with 200 mL distilled water. Solution was centrifuged at 6000 rpm for 10 minutes. Supernatant was filtered out and stored under refrigerated conditions (4°C) for further use.

2.2.2 Preparation of Ginger and Garlic Extracts (Stock Solution)

Cold water extract of both garlic and ginger was prepared using method described by Akintobi *et al.,* (2013) with slight modifications. Garlic and ginger rhizomes were peeled, washed and coarsely minced. Coarsely minced garlic and ginger were kept in hot air oven at 60°C for 2 days for drying and then ground into a fine powder. Fine powder of 100 g of each were weighed, mixed with 500 mL distilled water and stirred for 10 minutes. Solution was kept for 72 hours at room temperature (25±1°C). Solution was filtered out through a cleaned muslin cloth and stored under refrigerated conditions (4°C) for further use. As control treatments, sterilized water (Negative control) and 0.1% sodium benzoate (Positive control) were used. A preliminary study was also conducted with the effective concentration of extract and its mode of application to find out the shelf life of sprouts on the basis of overall organoleptic acceptability.

2.2.3 Mode of Application of Natural Antimicrobial Extracts

Through Germination Medium (Pre-germination Mode)

Raw seeds of mung bean were sorted to remove foreign material and damaged/aborted seeds and then washed under running tap water for 5 minutes. Washed seeds were divided in to 6 lots of equal amount and soaked (1:5 w/v) for overnight (10 hours) in respective antimicrobial solutions in a glass container. The Soaked seeds were then put in a sprout maker (Novelle Plast, Delhi) for 24 hours at 25±1°C and kept in dark.

By Dipping 24 Hours Sprouts for 15 Minutes in the Respective Solutions (Post-germination Mode)

Raw seeds were sorted to remove foreign material and damaged/aborted seeds and then washed under running tap water for 5 minutes. Washed seeds were soaked for overnight (10 hours) in potable water in a glass container. Soaked (10 hours) mung bean seeds were put in a sprout maker (Novelle Plast, Delhi) for 24 hours at 25±1°C and kept in dark. Sprouted mung beans were divided into 6 lots of equal amounts and dipped for 15 minutes separately in respective antimicrobial solutions.

2.2.4 Packaging and Storage

The sprouts from each treatment were packaged in plastic disposable cups (~200 mLvolume) and wrapped with 5% perforated cling films. Water soaked filter paper was placed along the inner sides of plastic cups to maintain high humidity inside. There were ~100 g sprouts/pack and the packs were stored in dark at room temperature (20±3°C) conditions and low temperature (7±1°C) conditions maintained in B.O.D. incubator.

2.2.5 Calculations and Statistical Analysis

The percentage of germination was determined only for pre application mode of antimicrobial extracts. It was determined by counting the number of germinating seeds, after 10 hours of soaked the seeds followed by 24 hours of germination period. Percentage of germination was calculated and values were expressed in %. The average radicle and plumule length of sprouts/treatment was measured in both application modes. The radicle and plumule length of 10 sprouts/treatment was measured using a measuring scale. Average length was expressed in cm.

The data obtained in the present investigation was subjected to analysis of variance (ANOVA) technique and analyzed according to two factorial completely randomized design (CRD). The critical difference value at 5% level was used for making comparison among different treatments during storage.

3. Results

3.1 Effect of Different Treatments on Physical Characteristics of Sprouted Mung Bean during Storage

Mung bean sprouts under various treatments were analyzed for following physical parameters during storage at room (20±3°C) and cold (7±1°C) temperature conditions.

3.1.1 Germination Percentage (%)

The data on germination of mung bean sprouts under various treatments is presented in Table 1. The germination of mung beans was 99.4% in control and it was slightly lower in other treatments, where it was ranging from 96.0% to 98.3%. The germination was better in chemical (Positive control) than bio-preservative treated mung beans. Amongst various biopreservatives, garlic and ginger treated mung beans were showing at par but lower germination than clove and cinnamon treatments.

Table 1: Average germination (%) of mung beans in the presence of biopreservatives in the germination medium

Treatment	Germination (%)
Negative control	99.4±0.16
Positive control	98.3±0.42
Clove	97.0±0.01
Cinnamon	97.2±0.29
Garlic	96.2±0.22
Ginger	96.0±0.36

Negative control (Distilled water); Positive control (Sodium benzoate). The values are mean± standard deviations.

3.2 Radicle and Plumule Length

3.2.1 Radicle Length

The data on radicle length of mung bean sprouts under various treatments during storage is presented in Table 2.The various preservatives when applied through germination medium (pre-germination mode), resulted in reduced radicle length as compared to untreated control mung beans. At room temperature, the radicle length under various treatments significantly increased from mean value of 0.58 cm at 0-day to 1.36 cm by 72 hours of storage. Amongst the various treatments, the radicle length was reduced maximum by clove and ginger, followed by chemical preservative (Positive control), while it was lesser affected by cinnamon and garlic. At low temperature storage conditions, the radicle length under various treatments significantly increased from mean value of 0.57 cm at 0-day to 1.27 cm by 120 hours of storage. Amongst the various treatments, the radicle length was reduced maximum by clove followed by cinnamon and ginger, while it was lesser affected by garlic and chemical preservative (Positive control). Interactions between treatment and storage were significant ($P<0.05$) both at room and low temperature conditions.

Table 2: Effect of bio-preservatives on mean length of radicle (cm) of mung bean sprouts during storage

Treatment	Storage Duration (h)											
	Room temperature					Cold temperature						
	0	24	48	72	Mean	0	24	48	72	96	120	Mean
Pre-germination												
Negative control	0.95	1.22	1.30	1.45	1.23	0.94	1.20	1.28	1.32	1.40	1.44	1.26
Positive control	0.50	0.70	1.18	1.30	0.92	0.50	1.15	1.20	1.20	1.34	1.39	1.13
Clove	0.50	0.60	1.20	1.25	0.89	0.40	0.65	0.65	0.75	0.82	0.85	0.69
Cinnamon	0.60	0.80	1.25	1.47	1.03	0.60	0.60	1.00	1.12	1.18	1.32	0.97
Garlic	0.45	1.25	1.35	1.42	1.12	0.50	1.10	1.22	1.31	1.38	1.41	1.15
Ginger	0.50	0.83	1.01	1.28	0.91	0.50	1.00	1.00	1.12	1.18	1.21	1.00
Mean	0.58	0.90	1.22	1.36		0.57	0.95	1.06	1.14	1.22	1.27	
CD at 5%	T =0.06 S =0.05 TxS=0.12					T =0.07 S =0.07 TxS=0.17						
Post-germination												
Negative control	1.05	1.25	1.32	1.45	1.27	1.05	1.25	1.32	1.40	1.45	1.48	1.33
Positive control	0.96	1.30	1.35	1.40	1.25	1.00	1.20	1.30	1.38	1.40	1.43	1.29
Clove	0.95	1.05	1.22	1.40	1.16	0.95	1.01	1.11	1.24	1.28	1.33	1.15
Cinnamon	0.95	1.10	1.50	1.50	1.26	0.95	1.10	1.21	1.33	1.38	1.46	1.24
Garlic	0.95	1.05	1.20	1.30	1.13	1.00	1.23	1.28	1.32	1.36	1.45	1.27
Ginger	1.00	1.00	1.10	1.30	1.10	1.00	1.20	1.28	1.39	1.44	1.45	1.29
Mean	0.98	1.13	1.28	1.39		0.99	1.17	1.25	1.34	1.39	1.43	
CD at 5%	T =0.06 S 0.05 TxS=0.11					T =0.06 S =0.06 TxS=NS						

Negative control (Distilled water); Positive control (Sodium benzoate); T=Treatment; S=Storage; NS=Non-significant

The various preservatives when applied by soaking treatments (post-germination mode) resulted in lesser reduction in radicle length than pre-germination mode. At room temperature, the radicle length under various treatments significantly increased from mean value of 0.98 cm at 0-day to 1.39 cm by 72 hours of storage. Amongst the various treatments, the radicle length was reduced maximum by ginger, garlic and clove, while it was lesser affected by cinnamon and chemical preservative (Positive control). At low temperature storage conditions, the radicle length under various treatments significantly increased from mean value of 0.99 cm at 0-day to 1.43 cm by 120 hours of storage. Amongst the various treatments, the radicle length was reduced maximum in clove, while it was not significantly affected by other various bio-preservatives. Interactions between treatment and storage were significant at room temperature, while it was non-significant at low temperature conditions.

3.2.2 Plumule Length

The data on plumule length of mung bean sprouts under various treatments during storage is presented in Table 3.

Table 3: Effect of biopreservatives on mean lengh of plumule (cm) of mung bean sprouts during storage

Treatment	Storage Duration (h)											
	Room temperature					Cold temperature						
	0	24	48	72	Mean	0	24	48	72	96	120	Mean
						Pre-germination						
Negative control	0.00 (0.00)	0.80 (5.13)	0.85 (5.29)	0.90 (5.44)	0.64 (3.96)	0.00 (0.00)	0.44 (3.80)	0.50 (4.04)	0.56 (4.29)	0.76 (4.99)	0.84 (5.26)	0.62 (3.73)
Positive control	0.00 (0.00)	0.82 (5.19)	0.70 (4.78)	0.82 (5.19)	0.59 (3.79)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Clove	0.00 (0.00)	0.00 (0.00)	0.60 (4.44)	0.65 (4.85)	0.31 (2.32)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Cinnamon	0.00 (0.00)	0.00 (0.00)	0.73 (4.89)	0.85 (5.29)	0.39 (2.55)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Garlic	0.00 (0.00)	0.00 (0.00)	1.00 (5.74)	1.05 (5.74)	0.51 (2.89)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Ginger	0.00 (0.00)	0.00 (0.00)	0.75 (4.97)	0.81 (5.16)	0.39 (2.53)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean	0.00 (0.00)	0.27 (5.19)	0.77 (5.02)	0.85 (5.28)		(0.00)	(0.63)	(0.67)	(0.71)	(0.83)	(0.88)	
CD at 5%	T =(0.09) S =(0.08) TxS=(0.20)					T =(0.06) S =(0.06) TxS=(0.15)						

	Post-germination											
Negative control	0.00	0.80	0.82	0.95	0.64	0.00	0.50	0.55	0.64	0.82	0.90	0.68
	(0.00)	(5.13)	(5.19)	(5.59)	(3.98)	(0.00)	(4.05)	(4.12)	(4.59)	(5.19)	(5.44)	(3.90)
Positive control	0.00	0.65	0.68	0.70	0.51	0.00	0.52	0.56	0.58	0.60	0.66	0.58
	(0.00)	(4.62)	(4.72)	(4.78)	(3.54)	(0.00)	(4.13)	(4.29)	(4.36)	(4.44)	(4.66)	(3.65)
Clove	0.00	0.00	0.61	0.65	0.32	0.00	0.50	0.55	0.60	0.60	0.65	0.58
	(0.00)	(0.00)	(4.48)	(4.62)	(2.27)	(0.00)	(4.05)	(4.12)	(4.44)	(4.44)	(4.62)	(3.61)
Cinnamon	0.00	0.62	0.64	0.65	0.48	0.00	0.50	0.60	0.62	0.64	0.65	0.60
	(0.00)	(4.51)	(4.59)	(4.62)	(3.43)	(0.00)	(4.05)	(4.44)	(4.51)	(4.59)	(4.62)	(3.70)
Garlic	0.00	0.75	0.81	0.86	0.61	0.00	0.55	0.55	0.64	0.70	0.83	0.65
	(0.00)	(4.96)	(5.16)	(5.32)	(3.86)	(0.00)	(4.25)	(4.12)	(4.59)	(4.78)	(5.22)	(3.87)
Ginger	0.00	0.80	0.80	0.95	0.64	0.00	0.50	0.60	0.62	0.73	0.85	0.66
	(0.00)	(5.13)	(5.13)	(5.59)	(3.96)	(0.00)	(4.05)	(4.44)	(4.51)	(4.89)	(5.29)	(3.86)
Mean	0.00	0.60	0.73	0.79		0.00	0.51	0.57	0.62	0.68	0.76	
	(0.00)	(4.06)	(4.89)	(5.09)		(0.00)	(4.10)	(4.25)	(4.49)	(4.76)	(4.97)	
CD at 5%	T =(0.10) S =(0.09) TxS=(0.21)					T =(0.11) S =(0.11) TxS=(0.28)						

Negative control (Distilled water); Positive control (Sodium benzoate); T=Treatment; S=Storage; NS=Non-significant; *The values present in the brackets are the actual lenghs of plumule in cm, which are of 10 replicates.

The various preservatives when applied through germination medium (pre-germination mode), resulted in reduced plumule length as compared to untreated control mung beans. At room temperature, the plumule length under various treatments significantly increased from mean value of 0.27 cm at 24 hours to 0.85 cm by 72 hours of storage. Amongst the various treatments, the plumule length was reduced maximum by clove followed by, cinnamon and ginger, while it was lesser affected by garlic and chemical preservative (Positive control). At low temperature storage conditions, there were no visible plumules under various treatments except in Negative control, where it appeared by 24 hours and progressively increased with increasing storage period. Interactions between treatment and storage were significant at room temperature conditions.

The various preservatives when applied by soaking treatments (post-germination mode) resulted in similar trend in plumule length as pre-germination. At room temperature, the plumule length under various treatments significantly increased from mean value of 0.60 cm at 24 hours to 0.79 cm by 72 hours of storage. Amongst the various treatments, the plumule length was reduced maximum by clove and cinnamon followed by chemical preservative (Positive control), while it was lesser affected by ginger and garlic. At low

temperature storage conditions, the plumule length under various treatments significantly increased from mean value of 0.51 cm at 24 hours to 0.76 cm by 120 hours of storage. Amongst the various treatments, there were no significant differences in plumule length. Interactions between treatment and storage were found significant at room temperature, while it was non-significant at low temperature conditions.

4. Discussion

4.1 Germination Percentage (%)

The germination percentage (%) of mung beans was 99.4% in control and it was slightly lower in other treatments, where it was ranging from 96.0 to 98.3% (Table 1). Amongst various biopreservatives, garlic and ginger treated mung beans were showing at par but lower germination than clove and cinnamon treatments. These reduced germination in treated mungbeans could be due to the effect of phytochemicals of the respective biopreservatives. These results are in conformity of the earlier findings of Van der Wolf *et al.,* (2008), where thyme, oregano, cinnamon and clove extract treated cabbage (*Brassica oleracea*) seeds were showing lower germination percentage. Similarly, Seth *et al.,* (2014) reported that the effect of different plant and plant part extracts (garlic, datura, neem, onion, papaya, ginger, parthenium, and turmeric) had significant effect on germination of wheat seeds. Kumar & Singhal (2009) reported that there was a dose-dependent inhibitory effect of methanol extract of latex of milkweed (*Calotropis procera*) on mung bean seed germination.

4.2 Radicle and Plumule Length

In both the modes of application, the progressively increased radicle and plumule length was observed during storage while reduced lengths were observed amongst treatments compared to untreated control mung beans (Tables 2 & 3). However, in post-germination mode of application, lesser reduction in radicle and plumule length was observed. In pre-germination mode, the radicle and plumule lengths were reduced maximum by clove during storage at room temperature. However, no visible plumule was observed throughout the storage in any of the treatment except control in pre-germination mode and low temperature storage. However, in post germination mode at low temperature conditions, no significant differences were observed in plumule length in any of the treatment. In the present investigation, the inhibitory effect of clove treatment on sprout growth could be due to phytotoxicity of eugenol, the major chemical component in clove extract. Similarly, longer exposure in different bio-preservatives during soaking and low temperature storage in pre-germination mode probably reduced the growth of plumule adversely and hence no visible plumules were observed during storage. Similar decrease in sprout

lengths by various biopreservatives has been reported by other workers also. Ataei & Hashemloian (2006) reported that water extract from saffron at 5 g/L decreased length of both roots and shoots systems in mung bean sprouts. Lower concentrations, however, were showing promotery or non-significant effect on sprout growth. Vokou *et al.,* (1993) reported the significant reduced sprout length in potato by essential oils of lavender, mint, spearmint, Turkish oregano, Greek oregano, rosemary, and sage.

5. Conclusion

The germination of mung beans was 99.4% in control and it was slightly lower in other treatments, where it was ranging from 96.0 to 98.3%. Garlic and ginger treated mung beans were showing at par but lower germination than clove and cinnamon treatments.The various preservatives resulted in reduced radicle and plumule lengths length as compared to untreated control mung beans, the reduction being more in pre-germination mode of application. In pre-germination mode, the radicle and plumule lengths were reduced maximum by clove during storage at room temperature. However, no visible plumule was observed throughout the storage in any of the treatment except control in pre-germination mode and low temperature storage. However in post-germination mode at low temperature conditions, no significant differences were observed in plumule length in any of the treatments. Mung bean sprouts remained acceptable in all the treatments till 48 hours at room temperature and for 96 hours at low temperature storage conditions.

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