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Biopreservation of Onions Using Plant Extracts and Antagonistic Organisms.

KEYWORDS: Biopreservation, Onion, plant extracts, antagonistic, shelf life, in vitro

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ABSTRACT *Maximum post harvest loss in onions is attributed to microbial spoilage. So, a few medicinal plant extracts and antagonistic organisms were screened for their antimicrobial activities against isolated spoilage organisms. It also included the study of various post harvest treatments to increase the shelf life of onions of Bellary red variety by preventing post harvest diseases by employing effective combinations of plant extracts and antagonistic organisms. Aspergillus sp. Penicillium sp. Botrytis sp. Erwinia sp. Bacillus and Staphylococcus sp.were isolated from Bangalore rose and Bellary red onion varieties. Leaf extracts like onion and turmeric leaf extract at 50 % concentration, Pseudomonas putida and Saccharomyces cerevisiae, were selected after screening based on there antimicrobial activity under in vitro conditions. Post harvest treatments to extend the shelf life of onions, turmeric leaf extract at 50 % concentration, Pseudomonas putida and onion leaf at 50 % + turmeric leaf extract at 50 % concentration were found to be more effective compared to the other treatments. There was increase in shelf life of onions by two to three weeks compared to the other treatments.*

Introduction
Onion (Allium cepa L.) is one of the major vegetable bulb crops of the world and important commercial crop grown in India. It is popularly used both immature and mature stages as a vegetables. The outstanding characteristic of onion is its pungent alliaceous odour, owing to a volatile oil known as Allyl –propyl-disulphide. This character accounts for its popularity as a food, as a spice in cooking, as a salad vegetable and certainly is the basis for its century old repute in medicines. The onion is known to possess insecticidal ,antibacterial, antifungal and antiatherosclerotic properties. These have been attributed to its sulphur containing compounds. Such an important vegetable crop is prone to several post harvest losses due to diseases like bacterial rots, smut, neck rots, etc. which in turn reduced shelf life of onions. Estimated post harvest losses are about 25-30 % due to microbial spoilage. Smut and bacterial rots are major threat to longevity of storage. Smut and rots caused by pectinolytic microorganisms are the prime causes for decay of onions in retail and whole sale markets. A major concern therefore is to control the growth of spoilage organisms in order to increase the shelf life of onions and ensure the food quality, security and safety. Many methods of preservation have been adopted, however the recent trend in the preservation of foods is biopreservation. Biopreservation technology includes the exploitation of antimicrobials of biological origin like plant extracts, microorganisms etc.

Material and methods
Biopreservation of onions was attempted using plant extracts and other antagonistic organisms. In vitro and in vivo tests were conducted with biopreservative agents against spoilage organisms associated with onions. This work was carried out in the department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bangalore.

Spoilage organisms
The spoiled samples of Bellary red and Bangalore rose onions were collected from local markets in polythene bags. They were subjected to microbiological analysis to isolate spoilage organisms by standard dilution plate count method using Nutrient Agar (NA), Martin's Rose Bengal Agar (MRBA).

Preparation of plant extract
Selected plant sample products known to contain some antimicrobial constituents were used for extraction processes by following methods like blending and squeezing of onion, coleus, tulsi and turmeric leaves.

Extraction by blending and squeezing:
Under aseptic conditions, samples were blended to get a fine paste. Using a sterilized muslin cloth, the paste was wrenched to get liquid extract. Further it was filtered through sterile filter paper to obtain a clear solution. Such extract was collected in sterilized bottle and stored under refrigerated condition for further use.

In vitro testing of antimicrobial activity of plant extracts
Antimicrobial activity of plant extracts was studied against the spoilage bacteria and fungi by agar cup method (Collins and Lyne 1970). The plant extracts were screened at different concentrations of 10 %, 25 %, 50 %, 75 % and 100 % concentrations.

Yeast suspension and Bacterial suspension preparation
Yeast suspensions were prepared using Davis Yeast Salt broth and the cultures used were Saccharomyces cerevisiae and Saccharomyces boulardii. Bacterial suspensions were prepared using Nutrient broth and the cultures used were Pseudomonas putida, and Pseudomonas fluorescens The cultures were incubated at 37°C for four days. The cultures were obtained from Department of agricultural Microbiology, GKVK, Bangalore.

In vitro testing of antimicrobial activity of antagonistic microorganisms
Inhibitory activities of antagonistic microorganisms against spoilage bacteria and fungi were studied using the agar cup method and the zones of inhibition were recorded.

Shelf life study of onions
Dipping the Bellary red variety onion bulbs in a solution with effective combinations of plant extracts and antagonistic organisms with naturally occurring microflora studied the shelf life of onions. There were thirteen treatments and three replications in each treatment in the experiment. The treated onions were placed in ventilated polythene bags of 200-gauge thickness and 250 gm capacity. Bags were stored at room temperature and periodical observations were recorded upto 42nd days of storage.

Treatments employed
T_1= Control
T_2= Onion leaf extract at 50 % concentration

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T_3= Turmeric leaf extract at 50 % concentration
T_4= Pseudomonas putida
T_5= Saccharomyces cerevisiae
T_6= Onion leaf extract at 50 % concentration + Turmeric leaf extract at 50% concentration
T_7= Onion leaf extract at 50 % concentration + Pseudomonas putida
T_8= Onion leaf extract at 50 % concentration + Saccharomyces cerevisiae
T_9= Turmeric leaf extract at 50 % concentration + Pseudomonas putida
T_{10}= Turmeric leaf extract at 50 % concentration + Saccharomyces cerevisiae.

Onion bulbs were observed for physiological loss of weight and spoilage per cent during storage.

Physiological loss of weight
Samples were weighed initially and at regular intervals and loss in weight was recorded and the same was expressed in terms of per cent.

$$\% \text{ Spoilage} = \frac{\text{Number of bulbs spoiled}}{\text{Total number of bulbs}} \times 100$$

Data analysis
Data on changes in observations of different treatments during the storage period of onions were analyzed by RCBD method by following procedure outlined by Sundarraj, et al., (1972).

Results and Discussion.
In vitro testing of antimicrobial activity of plant extracts and antagonistic organisms

Onion spoilage microorganisms, which were capable of hydrolyzing pectin, were used as test organisms. They were treated with different plant extracts at different concentrations to study the antimicrobial activity of plant extracts. (Table 1).

Tulsi and coleus leaf extracts could not inhibit any of the cultures at all concentrations. Turmeric leaf extracts at 50 % and 100 % concentrations were found to be effective against spoilage organisms. At 50 % concentration the turmeric leaf extract was more inhibitory than other concentrations. Among bacteria, Erwinia sp. (40.0 sq. mm). In case of fungi, Penicillium sp. and Botrytis sp. were inhibited to the extent of 534 sq. mm, followed by Aspergillus sp. (308 sq. mm).

Onion leaf extract was found to be effective at 50, 75 and 100 % concentrations. Maximum areas of zone of inhibition were recorded in Aspergillus sp (817 sq.mm) and Botrytis sp. (817 sq.mm) followed by Penicillium sp. (377 sq.mm). Among bacteria, Erwinia sp.and Bacillus sp. were inhibited to the extent of 188.5 sq mm .Onion leaf extract showed good antimicrobial activity, which inhibited the growth of some of the bacteria and fungi, tested. The effect of onion leaf extract was due to the toxic sulfur compounds present in the extract. Indrani et al., (1992) observed the adverse effect on dough properties of wheat flour with respect to strength of gas retention capacity with the addition of onion leaf extract. In a study conducted by Benkeblia et al., red onion exhibited a better antibacterial activity than yellow one against Staphylococcus aureus and Salmonella enteritidis. The zone of inhibition increased with increasing concentration of extracts (Benkeblia, N., 2004).

Erwinia sp. and Staphylococcus sp. were inhibited to an extent of 188.5 and 74.6 sq. mm at 50 % concentration which may be due to the unfavorable conditions created for the growth of bacteria due to addition of extract. Johnson and Voughan (1969) reported that many food borne pathogens were sensitive to extracts from onions and garlic. Kishore et al., (1988) observed toxicity exhibited by turmeric leaf extract against Rhizoctonia solani and inhibited the mycelial growth completely.Huhtanen (1980) showed the inhibitory effect of turmeric leaf extract against Clostridium botulinum in culture media. The inhibition may be due to presence of aliphatic straight chain alcohols present in turmeric leaf. All the fungi and bacteria showed resistance against coleus and tulsi leaf extracts of all the concentrations might be due to the absence of antimicrobial compounds in leaf extracts. All the plant leaf extracts used in this investigation did not show inhibitory effect against all the fungi and bacteria at all the concentrations. The antimicrobal property is neither a family character nor one of genus. It varies from family to family, genus to genus and species to species (Shekawat and Prasada, 1971)

The antimicrobial activities of antagonistic organisms against spoilage organisms were tried in vitro (Table 2). Saccharomyces boulardii could not inhibit any of the cultures tested. Among bacteria, Staphylococcus sp. was inhibited to the maximum extent of 308 sq.mm by Saccharomyces cerevisiae followed by Pseudomonas putida (188sq.mm) and P. fluorescens (138.2 sq.mm).

Lesser inhibition was observed in case of fungi. Aspergillus sp. was inhibited by Saccharomyces cerevisiae (94.2 sq mm) followed by Pseudomonas putida (56.5 sq mm). Only Aspergillus sp was inhibited by P. fluorescens (25.1sq.mm) Test antagonistic organisms of yeasts were employed against bacteria and fungi. Saccharomyces cerevisiae was found to be effective against all bacteria and fungi tested. Maximum area of zone inhibition was (308 sq. mm) recorded against Erwinia sp. followed by Bacillus sp. Debaromyces hansenii exhibited the highest antagonistic activity against Penicillium sp. (Chalutz and Wilson, 1990). Sporobolomyces roseus inhibited blue mold Penicillium expansum from 100 to 0 % and gray mold Botrytis cinerea from 78 to 0 %.

Shelf life study of onions
Physiological loss of weight (PLW)
The PLW of onions were recorded for 42 days from the day of inoculation are presented in Table 3.There was significant difference in mean physiological loss of weight in the onions subjected to all the 12 treatments and control upto 42nd days of treatment. On 42nd day after treatment, the mean physiological weight loss recorded ranged between 5.12 %to 9.07%. The onions treated with Pseudomonas putida showed minimum weight loss of 4.99 % followed by onions treated with onion leaf extract at 50 % concentration with mean weight loss of 5.12 %. Maximum mean weight loss of 9.07 % was recorded in control, which differed significantly higher over all other treatments. . Hittalmani (1986) reported that the decline in quality of stored vegetables and fruits as due to build up of spoilage microflora and found that physiological loss of weight increased with storage duration. Maina (2000) working with tomato and beans found that PLW increased as storage days progressed and decline in quality of vegetable due to microbial population was observed.

Spoilage per cent
Spoilage per cent of onions under natural conditions as influenced by different treatments at different storage period are presented in Table 4. There was significant difference among treatment upto 42nd days treatments. The onions treated with onion leaf extract at 50 %, turmeric leaf extract at 50 %, Pseudomonas putida, onion leaf extract at 50 % + turmeric leaf extracts at 50 % and turmeric leaf extract at 50 % + Pseudomonas putida remained unspoiled even after 14 days of treatment. On 42nd day, the onions that were treated with onion leaf extract at 50 % with mean spoilage of 20.8 % differed significantly with other treatments and control (100%). Maximum spoilage of 100 % was recorded in control, and minimum of 20.8 % in onions treated with onion leaf extract at 50 %. where as onions treated with turmeric leaf extract at 50 % concentration + Saccahromyces cerevisiae recorded

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spoilage of 25 %. The increase in shelf life of onions may be attributed to the inhibitory effect of onion and turmeric leaf extracts on the naturally occurring microflora. Fan and Chen (1999) found inhibitory activity of welsh onion leaf extract against aflatoxin production by Aspergillus flavus and A.parasiticus.

From the present investigation, it can be concluded that plant extracts such as onion and turmeric extract at 50 per cent concentration, antagonistic organisms Saccharomyces cerevisiae and Pseudomonas putida generally are capable of inhibiting the growth of spoilage organisms of varying degrees of efficiency. Effective combinations of bioagents can be used for prolonging the shelf life of onions.

Table: 1 In vitro testing of antimicrobial activity of bacterial and yeast cultures against onion spoilage organisms (Area of zone of inhibition in sq.mm)

Sl.No.	Test Organisms	Pseudomonas fluorescens	Pseudomonas putida	Saccharomyces cerevisiae	Saccharomyces boulardii
1.	Spoilage Bacteria				
	Erwinia Sp.	138.2	11.7	188.5	0.0
	Staphylococcus Sp.	138.2	188.5	308.0	0.0
	Bacillus Sp.	0.0	56.5	25.1	0.0
2.	Spoilage Fungi				
	Aspergilus Sp.	0.0	56.5	94.2	0.0
	Botrytis Sp.	0.0	25.1	11.7	0.0
	Pencillium Sp.	0.0	11.7	25.1	0.0

*** Values represent mean of four replication**

Table: 2 In vitro testing of antimicrobial activity of plant extracts against onion spoilage organisms (Area of zone inhibition(sq.mm))

Slno	Test organisms	Area of zone inhibition (sq.mm)																			
		Plant extracts (percent)																			
		Onion leaf extract					Turmeric leaf extract					Tulsi leaf extract					Coleus leaf extract				
		10	25	50	75	100	10	25	50	75	100	10	25	50	75	100	10	25	50	75	100
1	Spoilage bacteria																				
	Erwinia sp.	00	00	188.5	56.5	94.2	00	00	40.0	25.1	40	00	00	00	00	00	00	00	00	00	00
	Bacillus Sp.	00	00	25.1	56.5	00	00	00	11.7	25.1	40	00	00	00	00	00	00	00	00	00	00
	Staphylococcus Sp.	00	00	00	00	188.5	00	00	00	25.1	25.1	00	00	00	00	00	00	00	00	00	00
2	Spoilage fungi																				
	Aspergilus Sp.	00	94.5	817	245	94.2	94.2	188.5	308	188.5	188.5	00	00	00	00	00	00	00	00	00	00
	Pencillium Sp.	00	245	377	138	94.2	308	188.5	534	245	138	00	00	00	00	00	00	00	00	00	00
	Botrytis Sp.	188.5	245	452	534	817	25.1	25.1	245	245	534	00	00	00	00	00	00	00	00	00	00

*** Values represent mean of four replication**

Table 3: Physiological loss of weight (PLW) in onions with naturally associated microflora at weekly intervals (%)

Treatments	7days	14 days	21 days	28 days	35 days	42 days
T_1	1.45(6.91)	3.00(9.97)	3.55(10.8)	4.48(12.2)	5.98(14.1)	9.07(17.5)
T_2	0.79(5.08)	1.40(6.79)	1.90(7.97)	2.23(8.58)	3.55(10.8)	5.12(13.0)
T_3	1.60(7.26)	1.62(7.34)	2.03(8.19)	2.38(8.88)	4.30(11.9)	6.27(14.6)
T_4	0.37(2.81)	1.48(6.98)	1.75(7.58)	2.80(9.63)	3.60(10.9)	4.99(12.9)
T_5	1.45(6.91)	1.77(7.63)	2.11 (8.32)	2.75(9.55)	3.68(11.0)	5.17(13.1)
T_6	1.70(7.48)	1.85(7.81)	2.30(8.71)	2.72(9.48)	4.85(12.7)	6.09(14.4)
T_7	1.50(7.02)	1.55(7.14)	1.75(7.57)	3.30(10.4)	4.30(11.9)	6.03(14.2)
T_8	2.30(8.63)	2.46(9.07)	2.85(9.64)	3.80(11.2)	5.25(13.2)	6.79(15.0)
T_9	1.30(6.54)	1.36(6.70)	1.47(6.97)	2.63(9.33)	5.66(13.7)	7.76(16.1)
T_{10}	1.45(6.91)	1.49(7.01)	2.85(9.71)	3.80(11.2)	6.46(14.7)	7.93(16.5)
SEM	0.641	0.362	0.339	0.330	0.190	0.199
CD	1.87	1.06	0.989	0.965	0.579	0.583

***Weight loss is expressed as % of weight loss**
***Figures in parenthesis indicate values after Arc sine transformations**
***Values represent mean of four replications.**

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Table 4: Spoilage of onions with naturally associated microflora at weekly intervals (%)

Treatments	7day	14 day	21 day	28 day	35 day	42 day
T_1	16.6(23.8)	45.8(45.5)	50.0(45.0)	62.5(52.2)	87.5(77.0)	100(89.4)
T_2	0.00(0.00)	0.00(0.00)	0.00(0.00)	8.33(13.8)	12.5(20.7)	20.8(26.9)
T_3	0.00(0.00)	0.00(0.00)	0.00(0.00)	8.33(13.8)	12.5(20.7)	25.0(30.0)
T_4	0.00(0.00)	0.00(0.00)	12.5(20.7)	12.5(20.7)	16.6(23.8)	25.0(30.0)
T_5	0.00(0.00)	4.16(6.90)	12.5(20.7)	25.0(30.0)	25.0(30.0)	25.0(30.0)
T_6	0.00(0.00)	0.00(0.00)	8.33(13.8)	12.5(20.7)	25.0(30.0)	41.6(40.0)
T_7	16.6(23.8)	25.0(30.0)	33.3(35.0)	33.3(35.0)	54.1(40.1)	91.6(79.6)
T_8	12.5(20.7)	16.6(23.8)	33.3(35.0)	50.0(45.0)	58.0(47.1)	75.0(59.9)
T_9	0.00(0.00)	0.00(0.00)	8.33(13.8)	12.5(20.7)	33.3(35.0)	66.6(59.8)
T_{10}	0.00(0.00)	8.33(13.8)	12.5(20.7)	25.0(30.0)	29.1(32.5)	75.0(59.9)
SEM	2.31	3.67	3.97	3.23	3.87	5.78
CD	6.74	10.71	11.60	9.45	11.30	16.78

***Values represent mean of four replications**
*** Spoilage is expressed as % onions spoiled**
***Figures in parenthesis indicate values after Arc sine transformations**

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