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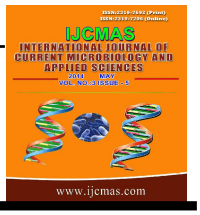

Original Research Article

Antimicrobial activity of plant extracts against post harvest spoilage of onions

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A B S T R A C T

Onion is important vegetable crop grown in India and can be used as mature and immature stages. Such an important vegetable crop is prone to many post harvest losses caused by bacterial and fungal pathogens, which in turn reduce the shelf life of onions. Onion suffers from many post harvest diseases like black mold, blue mold, neck rot, brown rot, soft rot and smudge among which, black mold ,rots and blue mold are the predominant ones which restricts the availability of onions in retail and wholesale markets. A major concern therefore is to control the growth of micro organisms in order to increase the shelf life of onions. So, a few medicinal plant extracts were screened for their antimicrobial activities against isolated spoilage organisms. The spoilage organisms were isolated from Bellary Red and Bangalore Rose varieties of onions. *Erwinia sp., Bacillus sp., Staphylococcus sp., Aspergillus sp., Pencillum sp., and Botrytis Sp.,* were dominant and frequently isolated. The antimicrobial activity of plant extracts were tested by agar cup method. Out of four plant extracts at different concentrations, 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. Turmeric leaf extract at 50% concentration inhibit *Penicillium sp.*(534sq.mm) and *Botrytis sp.*(245sq.mm), followed by *Aspergillus* sp. (308 sq. mm) .*Erwinia*(40sq.mm). Tulsi and coleus leaf extracts could not inhibit any of the cultures at all concentrations. The present investigation showed that plant extracts as potent antimicrobial activity and studied plant extracts may be the novel antimicrobial compounds to increase the shelf life of vegetables which are usually preserved under refrigerated conditions**.**

Keywords

Onion, Antimicrobial activity; Plant extracts; *In vitro*; Post harvest spoilage

Introduction

Onion (*Allium cepa* L.) is one of the major vegetable bulb crop of the world and important commercial crop grown in India. it occupies an area of 2.97 million hectares in the world with the total production of 51.9 million ton of which India ranks second in area (0.52mio hectares) and second in production (6.5 million tonnes). The major onion growing states are Maharashtra, Karnataka, Gujarat, Andhra Pradesh, Uttarpradesh and Tamil Nadu. 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. The purpose of this study was to examine *in vitro* antimicrobial activity of four plant extracts against postharvest pathogens of onions.

Materials and Methods

Isolation of spoilage organisms

The spoiled and healthy 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) to isolate bacteria, Martin's Rose Bengal Agar (MRBA) for isolation of fungi. The bacterial strains were identified based on their colony characteristics, microscopic observations and physiological characteristics. Predominant fungal isolates obtained from onion samples were maintained on Potato Dextrose Agar (PDA) slants. Slides were prepared for microscopic observation with intact fruiting bodies. The specimens were identified up to generic level based on the type of mycelium and spore as described by Benek (1957).

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 were studied against the spoilage bacteria and fungi by agar cup method. The test microorganisms were seeded into respective medium by spread plate method 10 µl (10^6 cells/ml) with the 24h cultures of bacteria growth in nutrient broth. After solidification in the centre of the medium, a cup cavity of 8 mm diameter was made with sterilized cork borer. This cup was filled with 0.1 ml of the leaf extract was incubated at 37ºC for 22-24h period. The inhibition zones were measured in diameter mm. Fungi 20 ml of PDA media was poured in sterilized petridishes and allowed to solidify same above method fallowed. The petridishes were incubated for 6 days at 30±2°C.The plant extracts

were screened at different concentrations of 10 %, 25 %, 50 %, 75 % and 100 % concentrations.

Results and Discussion

Results on the experiments conducted, to isolate the post harvest spoilage organisms from onions of two varieties namely Bangalore rose and Bellary red, the *in vitro* antimicrobial activity of plant extracts were tested. Based on microscopic observations some physiological and biochemical tests, the isolated bacteria were found to be *Bacillus* sp., *Erwinia* sp. and *Staphylococcus* sp.(Table 1) Fungi isolates were characterized based on their morphological structures viz., fruiting body and mycelial characteristics. The dominant ones belonged to the genera *Aspergillus, Penicillium and Botrytis*.Their morphological characteristics are described in Table 2. Microorganisms are associated with vegetables throughout the process of their development and also during their post harvest handling. Increase in the microbial load favours earlier spoilage of vegetables. A significant portion of the losses of vegetables during post-harvest period is attributed to spoilage caused by fungi and other bacteria. (Madan *et al.,* 1993) Hiremath and Govindu (1975) reported it from Karnataka. *Penicillium* generally causes a soft watery rot on the surface of, which are borne broom like conidiophores covered by masses of blue green conidia. These symptoms were caused by *Penicillium cyclopium* in stored onions (Wijeratnam and Lowings, 1978). The organism was repeatedly isolated and identified as *Pencillium digitatum* Sacc. (Raju and Naik, 2006)The bacterial cultures were characterized as *Erwinia* sp. *Bacillus* sp. *Staphylococcus* sp. Liao and Wells (1987) isolated *Pseudomonas* sp. *Bacillus* sp. *Erwinia carotovora*, *Xanthomonas campestris* which were pectinolytic and were known to cause soft rots in fresh vegetables at produce markets. *Bacillus, Pseudomonas* and *Erwinia* were responsible for causing severe post harvest spoilage of vegetables.

Surface microflora of onions

Bacterial and fungal populations were enumerated in both the varieties. Total bacterial counts varied from 9.5×10^3 CFU/g to 81.9×10^3 CFU/g. Highest population was recorded in Bellary red onions (81.9×10^3 CFU/g). Lowest bacterial counts were in Bangalore rose (9.5×10^3 CFU/g). Fungal population ranged from 5.3×10^2 CFU/g to 16.09×10^2 CFU/g. Maximum fungal population was recorded in Bellary red onions (16.09×10^2 CFU/g) and minimum in Bangalore Rose (5.3×10^2 CFU/g). (Table 3) High bacterial and fungal population were recorded in Bellary Red onions, since they are underground bulb vegetables in contact with soil and are more susceptible for microbial spoilage. *Pseudomonas allicola* and *P.cepacia* have been isolated from soil in which onions were grown (Kawamoto and Lorbeer, 1967). Maude (1976) demonstrated experimentally that wintered bulb onions could act as an infection bridge for *Botrytis alli* between one spring sown crop and the next.

In vitro testing of antimicrobial activity of plant extracts

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 4).

Table.1 Characteristics of isolated bacterial cultures

Sl. No	Organism code	Source	Colony character	Gram reaction	H_2S formation	Gelatin Hydrolysis	Catalase activity	CHO utilization		Type of Isolates
								Production of acid	Production of gas	
1	B_1	Bellary red and Bangalore Rose bulbs	Small round, Dull white, water droplets like	Gram –ve, straight rods, occurring in pairs/ singly	+	+	+	+	-	*Erwinia* sp.
2	B_2	Bellary Red bulbs	Dull white, medium sized, colonies with irregular margin	Gram +ve, rods, endospore in centre in centre position	+	-	+	+	+	*Bacillus* sp.
3	B_3	Bellary Red bulbs	Lemon yellow pigmented, medium sized	Gram +ve cocci in bunches	+	+	+	+	+	*Staphylococcus* sp.

Table.2 Characteristics of isolated fungal cultures

Sl. No	Organism code	Source	Colony character	Mycelium	Fruiting body	Type of isolates
1	F1	Onion bulbs and soil	Black velvety to cottony surface, profused growth	Septate	Conidophore with vesicle like tip, surface with flask shaped sterigmata and chains of conida	*Aspergillus* sp.,
2	F2	Onion bulbs	Shades of green white at surface velvety to powdery	Septate	Brush like conidophore chains of conida cut from flask shaped sterigmata	*Penicillium* sp.,
3	F3	Bulbs and leaves	Grayish to black colour	Septate	Stout,dark branched conidophore bearing clusters of conidia on dentricles arising form apical ampullae	*Botrytis* sp.,

Table.3 Surface microflora of Bellary red and Bangalore rose onions

Sl.no	Source	Bacteria CFU 10^3/g		Fungi CFU 10^2/g	
		Bangalore rose	Bellary red	Bangalore rose	Bellary red
1	Bulbs(spoiled)	64.8	71.4	14.06	16.09
2	Bulbs(healthy)	23.1	45.8	5.3	7.30
3	Soil	55.25	81.90	9.7	12.49
4	Leaves	9.5	13.90	9.70	6.43

Table.4 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

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. Maximum inhibition (817 sq.mm) was recorded against *Aspergillus* sp. at 50 % concentration, where as *Penicillium* sp. were inhibited to an extent of 377 sq. mm. 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. Turmeric leaf extract was tested against fungi, it was found to be effective and inhibition was observed against all tested fungi. Maximum inhibition (534 sq.mm) was seen against *Penicillium* sp. at 50% concentration. *Botrytis* sp. was found to be quite resistant against the turmeric leaf extracts. 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)

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