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Research Article

Aqueous plant extracts: A strategic approach to mitigate bacterial soft rot disease in onions

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ABSTRACT

The bacterial soft rot of onion caused by *Burkholderia cepacia* is a major issue in subtropical storage. At BSMRAU, Bangladesh, examined plant extracts for antibacterial impact on this disease in both lab and storage contexts. Studied 11 aqueous extracts: cheerota, jute, chatim, mander, stone apple, marigold, onion garlic, neem, lime, and turmeric, for antibacterial effects against the disease. Only the extracts from jute leaf and cheerota shown antibacterial action. Extracts from the plants cheerota and jute leaf were used to combat onion soft rot. The test involved soaking onion samples and exposing to indicator bacteria after in vitro selection. Onions were stored for 22 weeks in sterile conditions. Botanical extract application notably reduced infection, weight loss, and boosted onion's percentage disease reduction (PDR). Cheerota plant extract showed the greatest inhibitory effects for controlling soft rot bacteria of onion in-vitro and in storage conditions, respectively which may be the eco-friendly management of the onion soft rot disease.

INTRODUCTION

Onion (*Allium cepa* L.) is widely cultivated in Bangladesh as an important spice crop. In Bangladesh, it is produced widely throughout the winter months, topping all other crops in terms of both area (2.0 million hectares) and yield (2.3 million m tons) [1]. The crop suffers from various diseases caused by virus, bacteria, fungus, and nematodes [2]. Onion storage and transportation are both affected by the disease's effects, which are known as bacterial soft rot [3]. This disease reduces crop quality and quantity, which decreases crops' market value and results in significant economic losses and reduced the export potentials. Despite the lack of precise estimates, the annual global loss may range from 55×10^6 to 100×10^6 USD [4]. The USA alone suffered a 14-million-dollar loss in 1976 as a result of Erwinia soft rots [5]. Bacterial soft rot is the main reason for onion post-harvest storage loss in Bangladesh and the loss due to this disease may be 10% to 75% even up-to 100% bulbs might be damage if environmental conditions favor [6]. In Bangladesh, the disease is brought on by *B. cepacia*, *Pectobacterium carotovorum* sub sp. carotovorum (E. carotovora sub sp. carotovora) and *E. chrysanthemi* [7]. *B. cepacia* is the culprit behind the disease that affects onion bulbs that have been kept at low temperatures [8]. In fields and markets*, P. marginalis, P. syringae*, and *P. cepacia* were the culprits behind onion bulb rot [9]. *B. cepacia* and *Burkholderia cenocepacia* made up the bulk of the isolates (85% and 90%, respectively) [10].

Only wounds allow bacteria to penetrate the neck tissue and infiltrate a number of scales despite moving from one to the next. Pressing on a diseased bulb will reveal its condition; often, a foul-smelling, watery fluid will squeeze out of the bulb's neck [11]. Thus, this disease destroys a huge amount of onion in storage conditions and during transportation every year in Bangladesh. But there is no effective organic control measure available against this disease in the country. Chemical pesticides are used to control this disease, they have a serious negative impact on the environment and human health [12]. Excessive use of pesticides leads to the destruction of biodiversity which is a concern for the environment and sustainable crop production. The environment is polluted through indiscriminate use of chemical pesticides and because of their residual toxicity, pose a health risk to both humans and animals [13, 14, 15]. Considering the adverse effect of synthetic pesticides on health it is urgently necessary to find alternative means to control plant pathogenic microorganisms. Green plants can be a valuable source of natural pesticides and a reservoir of potent chemotherapeutics [16, 17, 18]. Only a small number of studies [19, 20, 21] have focused on phytopathogenic bacteria, despite the fact that numerous studies have demonstrated the effective use of extracts from plants in plant disease control. Plant extracts have been shown to effectively prevent bacterial soft rot in potatoes, according to evaluations of the effect of the extracts [22, 23, 24]. The antimicrobial properties of medicinal plants have drawn a lot of attention lately in relation to the management of plant diseases [21, 25]. Effective phytopathogenic bacterial inhibitors were assessed to be present in certain plant extracts [19, 20, 26]. Tests were conducted on the hemp flower water extract along with essential oils to combat the soft rot pathogen associated with potatoes. The protective effect of the extracts was found to be most pronounced when evaluated both in vitro upon pure cultures of bacteria and in vivo on the potatoes that were unintentionally infecting through the soft rot pathogen [22]. The significance of environmentally friendly plant protection techniques for sustainable agriculture was highlighted [27]. The creation of environmentally friendly and long-lasting controls for bacterial soft rot could reduce storage losses and enhance onion quality. However, there hasn't been any effort to look into how well plant extracts fight off bacteria that cause soft rot in onions in Bangladesh. As a result, the goal of the current study is to determine how well various aqueous plant extracts inhibit the bacteria that cause onion soft rot.

MATERIALS AND METHODS

Plant selection and preparation of extracts

A total of 11 plants extracts were used in this study listed in Table 1. The outer layer of bark of chatim as well as mandarin, the entire cheerota plant, and dried jute leaves were used to prepare extracts, which were diluted 1:10 (w/v) in a solution of distilled water. Following a 20–24-hour soak or dip of plant parts in distilled water, the water extract was gathered by running it through a double-layered cloth made of muslin at least twice. Different plant parts, such as roots, leaves, bulbs, and rhizomes, were crushed in a pestle and mortar in order to prepare extracts coming from other plants. Subsequently, distilled water and crushed materials were combined 1:1 (w/v) and blended using an electric blender. They were at least twice passed through a double-layered cloth made of muslin filter. Conical flasks were filled with the extracts, which were then used as stock solutions. For the purpose of a bacterial inhibition of growth test, the mouth of every flask was sealed with foil made of aluminum and kept cold in a freezer at 4°C [19].

Table 1. List of plants tested to prevent onion soft rot caused by bacteria.

Name of plants		Family	Plant component/s used
English name	Scientific name		
Jute	*Corchorus capsularis* L.	Tiliaceae	Dry leaves
Cheerota	*Swertia chiraita* Ham.	Gentianaceae	Whole plant
Devils tree	*Alstonia scholaris* L.	Apocynaceae	Bark
Coral tree	*Erythrina indica*	Leguminosae	Bark
Stone apple	*Aegle marmelos* L.	Rutaceae	Young fruits & leaves
Marigold	*Tagetis erecta*	Compositae	Leaves & roots
Onion	*Allium cepa*	Lilliaceae	Bulbs & leaves
Garlic	*Allium sativum* L.	Lilliaceae	Bulbs & leaves
Neem	*Azadiracta indica*	Meliaceae	Leaves
Lime	*Citrus aurantifolia*	Rutaceae	Leaves
Turmeric	*Curcuma longa* L.	Zingiberaceae	Rhizome

Isolation of onion soft rot bacteria

From soft rotted onions, 73 bacterial isolates were obtained. In various parts of Bangladesh, soft-rotted onion samples were gathered from the homes and marketplaces of farmers. The locations of the onion varieties were Santhia (Pabna), Sujanagar (Pabna), Gazipur, Rangpur, Kauranbazar (Dhaka), and Faridpur. The onion varieties were Kalashnagari, BARI-1, Taherpuri, and Faridpuri. Onion disease samples were chosen based on Rich and Shing's descriptions of the distinctive smell and outward signs of soft rot [3, 28]. Following the gathering of rotted samples, which were delivered to the Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, microbiology lab. Using the "Streak plate" method as outlined by Mortensen and Kim, soft rot bacterial isolates were extracted from diseased onion samples [29, 30]. Yeast Peptone Dextrose Agar (YPDA), a popular bacteriological medium, was employed in the isolation process.

Maintaining isolates of pathogenic bacteria

Test tubes that included sterilized distilled water and test tubes with a half-slant culture containing YPDA overlapping with disinfected liquid paraffin were used to preserve pure individual colonies for every pathogenic soft rot bacterium isolate of onion. The culture-containing tubes were kept in a cold room at 14°C.

Pathogenicity test of soft rot bacterial isolates

Following Lelliot's standard protocol, all bacterial isolates from single colonies were examined for their capacity to cause soft rot on onion bulbs [31]. Slices of onion bulbs (about 1 cm) were aseptically cut after being sterilized with 70% ethyl alcohol and then rinsed in sterile distilled water. After CRD with three replications, the slices were placed in Petri dishes with sterilized filter paper impregnated with roughly 2 ml of sterilized distilled water. To ensure that Koch's postulates were met, the soft rot tests were conducted at least twice. The slices were pricked with a needle to inoculate them. The vaccinated slices were kept in Petri dishes that had been moistened [32] and kept at 30°C for two to three days. More virulent isolates of the bacteria were isolated and preserved for identification from the bacterial cultures that produced the typical soft rot symptoms on onion slices.

Examining and classifying the pathogenic isolates of bacteria

Three soft rot-positive isolates from onion soft rot were chosen for characterization and identification out of 73 bacterial isolates based on their higher virulence and phenotypic characteristics. In a previous study [7], a number of physiological and biochemical tests were carried out in accordance with standard procedures for the characterization and identification of particular soft rot bacterial isolates. In this work, reference strains were *B. cepacia* ATCC 25416, *E. chrysanthemi* Ura-2, and *Erwinia carotovora* sub sp. carotovora ATCC-15713, three soft-rotting bacterial strains. The most virulence isolate *B. cepacia* O-15 was selected and used for botanical control by plant extracts.

Plant extracts' bioassay for the onion-associated soft rot pathogen

The soft rot bacteria isolate with the highest virulence as test bacteria, *B. cepacia* O-15 that was isolated from onions was utilized. Plant extracts were found to have antibacterial activity following the growth inhibition technique [22, 33]. For the preparation of the inoculum, soft rot bacteria were grown on nutrient agar Yeast Peptone Dextrose Agar and cells were placed in suspension in sterile distilled water at a concentration of roughly 108 colony forming units (cfu)/ml and incubated at 28°C for a full day. The plant extracts were added to the bacterial medium YPDA at concentrations of 30, 50, 75, and 90% before being autoclaved. Twenty milliliters of the medium were added to each Petri dish. Following solidification, soft-rotted B. cepacia O-15 bacterial inocula were spot-injected into the modified medium. To ascertain the efficacy of plant extracts, the plates were placed in an incubator set at 30°C for 14 days, and the growth of the bacteria was monitored. Three replications of a complete randomized design (CRD) were used to arrange the plates in the incubator.

Effect of two plant extract on onion soft rot disease under storage condition

Dry jute leaf extracts and cheerota plant extract were chosen for further evaluation of their efficacy against soft rot bacteria of onions in storage due to the positive bioassay results. Using the previously outlined procedures, two extracts (a botanical extract and distilled water) were made at a 1:10 concentration in distilled water. We dipped seven hundred grams of onion bulbs in plant extracts. The onion bulbs were treated by dipping them in the extracts for half an hour and letting them air dry at room temperature.

Using the same steps as outlined under the in vitro test, 108 cfu/ml of the soft rot bacteria, *B. cepacia* O-15, were inoculated. Onion bulbs treated with plant extract were inoculated with *B. cepacia* O-15. Using an atomizer, the inoculum suspensions were evenly sprayed over the onion bulbs to initiate inoculation. The inoculated bulbs were allowed to air dry before being sterilised and kept at room temperature. There was an unvaccinated control group for every treatment. After 2, 6, 10, 14, 18, and 22 weeks of inoculation, visual observations were conducted. Data on the number of bulbs infected with soft rot was also recorded. The weight loss resulting from soft rot in storage was calculated and expressed as a percentage (w/w) using the formula that follows [34].

$$Infection\,\% = \frac{No.\,of\ infected\ bulbs}{Total\ no.\,of\ bulbs} \times 100$$

$$Loss\ of\ weight\,\% = \frac{Initial\ weight - weight\ after\ discarding\ the\ infected\ samples}{Initial\ weight} \times 100$$

The formula below was used to calculate the percentage of disease reduction (PDR) [35]:

$$PDR = \frac{Ack\ -\ Atr}{Ack} \times 100$$

RESULTS

Characterization of pathogenic bacterial isolates

The three pathogenic isolates of onion, O-05, O-14, and O-15, were identified as *B. cepacia* based on the results of physiological, biochemical, and carbon source utilization tests (Table 2-4). The isolates were gram negative, aerobic, and had creamy white colony morphology on YPDA, nearly identical to the reference strain of *B. cepacia* ATCC 25416. Previous reports of *B. cepacia*'s physiological, biochemical, and carbon source utilization type results [36, 37, 38] were comparable. Among the three pathogenic isolates the most virulence isolate O-15 was used for antibacterial assay by aqueous plant extracts.

The majority of the current study's characterization and identification of the onion soft rot bacteria came from conventional techniques. Because of a lack of facilities, molecular-based techniques have not been used in Bangladesh to characterize and identify the bacteria that cause soft rot in onions.

Table 2. The biochemical and physiological properties of the pathogenic soft rot bacteria that were isolated from onions.

Isolates No.	Name of Tests								
	Potato soft rot	Gram reaction	Catalase	Oxidase	OF-test OF- test	Gelatin liquefaction	Nitrate reduction	Lecithinase	Methyl red
O-05	+	-	+	-	-	+	-	v	+
O-14	+	-	+	-	-	+	-	+	+
O-15	+	-	+	-	-	+	-	v	+
Ecc ATCC 15713	+	-	+	-	+	+	+	-	+
Ech Ura-2	+	-	+	-	+	+	+	+	-
Bc ATCC 25416	+	-	+	-	-	+	-	-	+

Table 2 Contd'

Isolates No.	Name of Tests						
	Arginine utilization	Acetoin	Indole	Gas formation	Growth at 37°C	Growth in 5% NaCl	Tobacco hypersensitivity
O-05	-	+	v	-	+	-	+
O-14	-	+	v	-	+	-	w+
O-15	-	+	v	-	+	-	+
Ecc ATCC 15713	-	+	-	-	+	+	+
Ech Ura-2	-	+	+	+	+	-	+
Bc ATCC 25416	-	+	v	-	w+	-	w+

Reference/standard isolates: Ecc ATCC 15713 (Ecc=E. carotovora subsp. carotovora), Ech Ura-2 (Ech= E. chrysanthemi), Bc ATCC 25416 (Bc=B. cepacia), ATCC= American Type Culture Collection; (+) = growth positive; (-) = negative; (w+) = weakly positive; (v)= variable reaction

Table 3. Onion soft rot bacterial isolates using various sugars as a source of carbon

Isolates No.	Name of carbon sources								
	Cellubiose	Lactose	Maltose	L-Arabinose	D-Galactose	D-Xylose	Raffinose	Sucrose	Trehalose
O-05	+	-	-	+	+	+	+	+	+
O-14	+	-	-	+	+	+	+	+	+
O-15	+	-	-	+	+	+	+	+	+
Ecc ATCC 15713	+	+	-	+	+	+	+	+	+
Ech Ura-2	+	-	-	+	+	+	+	+	-
Bc ATCC 25416	+	-	-	+	+	+	+	+	+

Reference/standard isolates: Ecc ATCC 15713 (Ecc=E. carotovora subsp. carotovora), Ech Ura-2 (Ech= E. chrysanthemi), Bc ATCC 25416 (Bc=B. cepacia), ATCC= American Type Culture Collection; + = growth positive; - = negative; v= variable reaction

Table 4. Utilization of different alcohols and organic acids by soft rot bacterial isolates of onion.

Isolates No.	Name of alcohols and organic acids					
	Dulcitol	Inositol	Manitol	Sorbitol	Benzoate	D-Tartrate
O-05	-	+	+	-	-	-
O-14	-	+	+	-	-	-
O-15	-	+	+	-	-	-
Ecc ATCC 15713	-	+	+	-	-	-
Ech Ura-2	-	-	+	-	-	-
Bc ATCC 25416	-	+	+	-	-	-

Reference/standard isolates: Ecc ATCC 15713 (Ecc=E. carotovora subsp. carotovora), Ech Ura-2 (Ech= E. chrysanthemi), Bc ATCC 25416 (Bc=B. cepacia), ATCC= American Type Culture Collection; + = growth positive; - = negative

Antibacterial assay of aqueous plant extracts

This experiment involved testing eleven different plant extracts. Only the extracts of cheerota and dry jute leaf demonstrated antibacterial activity against the onion soft rot bacteria B. cepacia O-15 at concentrations of 75 and 90% (Table 5), as shown by the development of inhibition zones surrounding the bacteria (Fig 1-4). The extracts showed greater antibacterial activity at higher concentrations. At low concentrations 30 and 50% of extract almost no or very minute inhibition zone produced ie; soft rot bacteria grew easily, on the other hand at higher concentrations 75 and 90% of extract inhibition zones produced about 12-16 mm in diameter in both the plant extracts. In the control plate, no inhibition zone was produced. This indicated that jute leaf and cheerota plant extracts controlled the growth of onion soft rot bacteria *B. cepacia* O-15 at higher concentrations. It was also found that the cheerota plant extract was more effective than jute leaf extract against onion soft rot bacteria. Jute leaf and cheerota aqueous plant extracts were chosen for treatment of onion bulbs against soft rot disease in storage based on the results of an in vitro test. None of the other nine plant extracts exhibited any antibacterial properties (Table 6).

Effect of two aqueous plant extracts on onion bulb treatment under storage conditions

Treatment of onion bulbs with dry jute leaf extract and cheerota plant extract caused a considerable decrease in the percentage of soft rot infections and loss of bulb weight but increase the percentage of disease reduction compared to control. The mean incidence of onion soft rot was lower in case of cheerota plant extract than jute leaf extract. Under control, soft rot incidence was much higher than treated ones causing 100% damage of onion bulbs within 18 weeks of inoculation. A comparable pattern in the infection percentage was discovered when comparing the percentage of weight loss. The cheerota extract had the lowest percentage of infection, the lowest weight loss, and the highest disease reduction. In storage conditions, cheerota aqueous plant extract outperformed jute leaf extract in controlling the soft rot disease of onions (Fig. 5, 6).

Table 5. Inhibitory effect of aqueous plant extracts of dried jute leaf and cheerota against bacterial growth of *B. cepacia* O-15 isolate *in vitro* tests.

Concentration of plant extract (%)	Jute leaf extract Inhibition zones produced (mm)
30	-
50	-
75	10
90	15
Control*	--
	Cheerota plant extract Inhibition zones produced (mm)
30	-
50	-
75	10
90	20
Control*	--

-/-- = No antibacterial activity; 10 mm = Medium antibacterial activity; 15 mm/20 mm = Strong antibacterial activity; -- = Good growth of soft rot bacteria; * = Plant extract was not added in medium

Table 6. Inhibitory effect of nine aqueous plant extracts against bacterial growth of onion soft rot isolates *in vitro* tests.

Plant extract treatments (30–90% concentration range)	Antibacterial activity (B. cepacia O-15) after 14 days of incubation Inhibition zones produced (mm)
Aegle marmelos L.	-
Alstonia scholaris L.	-
Erythrina variegata	-
Tagetis erecta	-
Allium cepa	-
Allium sativum L.	-
Azadiracta indica	-
Citrus aurantifolia	-
Curcuma longa L.	-
Control*	--

-/-- = No antibacterial activity; --- = Good growth of soft rot bacteria;

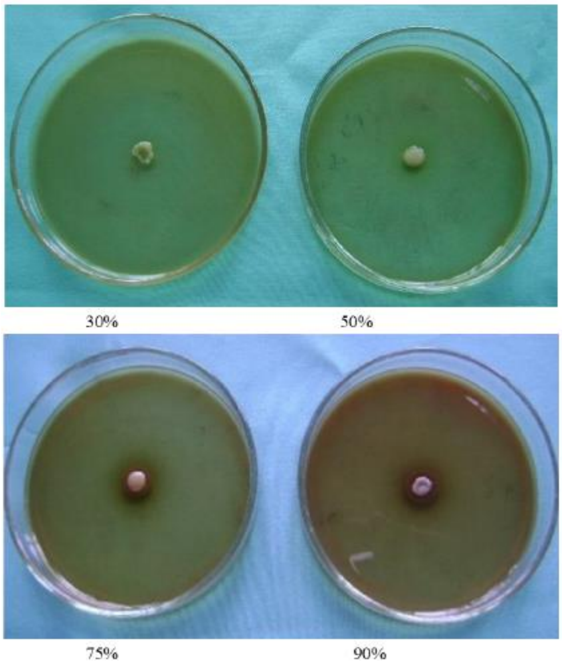

Fig. 1. Jute leaf extract's antibacterial activity against the *B. cepacia* O-15 bacteria that causes onion soft rot at different concentrations showing growth inhibition.

Fig. 2. Control plates (without botanical extract) showing thriving onion soft rot bacterium

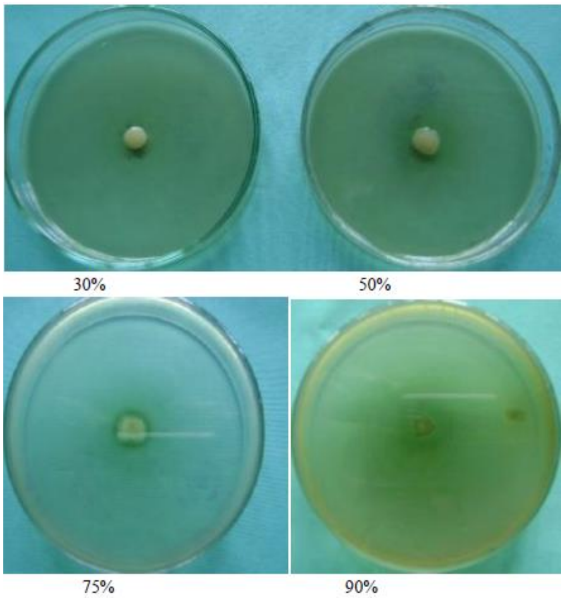

Fig. 3. The cheerota plant extract exhibits antibacterial activity against the B. cepacia O-15 bacteria that cause onion soft rot at different concentrations showing growth inhibition

Fig. 4. Control plates (without botanical extract) showing thriving onion soft rot bacterium

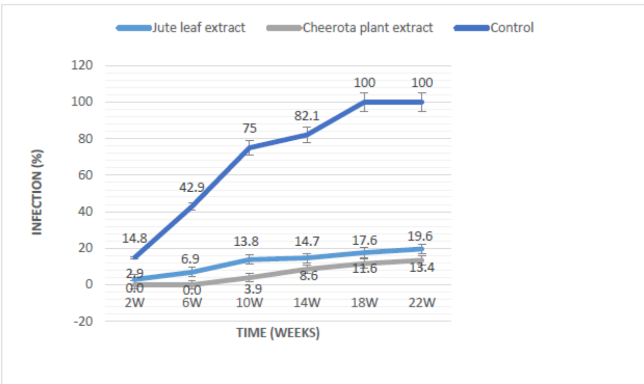

Fig. 5. Impact of treating onion bulbs for soft rot disease during storage with a 1:10 (w/v) concentration of distilled water and jute leaf aqueous extract and cheerota aqueous extract

DISCUSSION

Bacterial soft rot of onion is a major and typical post-harvest disease in Bangladesh. It is noted that every year huge amount of onion is damaged in the country. But no effective control measures are being practiced in Bangladesh. Botanical extracts are a safe and environmentally friendly substitute for chemical pesticides when controlling plant diseases. We discovered the botanicals in the current investigation as cheerota aqueous plant extracts and jute leaves for management the post-harvest disease of onion soft rot successfully under storage condition. In a similar study, the authors [24] investigated the efficiency of some botanicals against soft rot bacteria of potatoes and found satisfactory results. But investigation against onion soft rot disease by using botanicals had not been conducted in Bangladesh earlier since the study was performed and we found satisfactory results as ecofriendly management strategies.

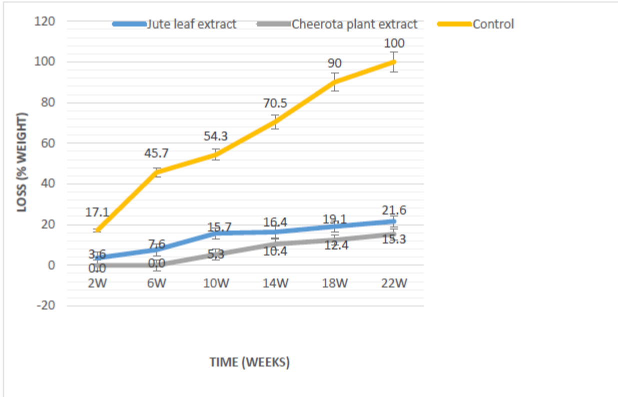

Fig. 6. The impact of treating bulbs with aqueous extracts of jute leaves and cheerota plants at 1:10 (w/v) (leaf extract and distilled water) concentration on the percentage weight loss of onion

Numerous researchers examined the effectiveness of plant extracts in preventing bacterial soft rot in potatoes and found satisfactory control of the disease earlier [7, 22, 23]. The antimicrobial properties of plant materials for the management of plant diseases have attracted a lot of attention in recent decades. Numerous researchers have reported that certain plant extracts are efficient at inhibiting the growth of phytopathogenic bacteria [19, 20, 22, 39] also looked into the effectiveness of a hemp flower water extract and essential oils against the potato soft rot-causing *E. carotovora*. Potatoes were used to test the extracts both in vitro and in vivo. Hemp flower extract was found to have a protective effect. The soft rot pathogens have been largely controlled by very few botanical extracts in the world but some other works have been done against other bacterial pathogens [21, 40, 41].

Plant extracts' inhibitory activity was probably caused by antimicrobial substances found in the extracts. A phytochemical analysis was conducted [42] on the leaves of *C. capsularis* (white jute), revealing the presence of two functional compounds: monogalactosyl diacyl glycerol (1,2-di-O- ±-linolenoyl-3-²-D-galactopyranosyl-glycerol) and phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol) and phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol). However, it is discovered that flavone compounds from *C. olitorius* have also been shown to inhibit microbes by preventing the bacterial enzymes from performing vital metabolic processes [43]. The use of plant extracts against onion soft rot bacteria in vitro has produced encouraging results in the current study, suggesting that they may also be useful in managing the disease while it is being stored. It was still unknown what chemical components and mechanism the botanical extracts used to prevent soft rot contained. Therefore, there is a need to explore the potential usage of these antimicrobial compounds to control bacterial soft rot disease of the onion because a huge amount of onions is damaged by this disease in the world. If onion growers use these botanicals to control bacterial soft rot disease of onion it will be eco-friendly management as because these botanicals are used as a very good herbal extract for human health as well as environment friendly.

CONCLUSION

Eleven aqueous plant extracts were tested for the environmentally friendly management of onion soft rot disease under storage conditions. Out of eleven aqueous plant extracts, cheerota and jute leaf extracts demonstrated antibacterial activity against *B. cepacia* O-15, the soft rot bacteria, in vitro and successfully decreased the bacterial soft rot disease of onions under storage conditions. These encouraging results show the potential of these extracts in the management of onion soft rot disease. In the current study, the cheerota aqueous plant extract's effectiveness in managing the soft rot disease of onions was particularly noteworthy. The approach is very cheapest and novel management strategy for controlling onion soft rot disease under storage conditions.

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