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Survey for the incidence of soft rot of onion: An emerging disease in Northern parts of Karnataka

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Abstract
Onion is one of the important bulbous crops that is commercially grown in India and all throughout the world. It is an indispensable part of every kitchen in Indian households and is valued for its pungency and flavors. As onion export contribute immensely to foreign exchange earnings, the production of healthy onion bulbs amenable to the export market should be a major objective. Bacterial soft rot in onion is a devastating disease as it reduces the quantity and quality of onions, thus reducing their marketability value. A roving survey in four districts of Northern Karnataka during two consecutive years revealed higher incidence of soft rot in onion in Bagalkot (31.12%) and Dharwad (36.06%) districts in 2021 and 2022 respectively. The bacteria associated with soft rot symptoms was isolated and the pathogenicity was proved.

Keywords: Onion soft rot, bacterial soft rot, roving survey, Northern Karnataka

1. Introduction
Onion (*Allium cepa* L.) is a highly valued vegetable crop used as food and medicine since antiquity. It is cultivated globally and stands second to tomato in terms of value among vegetable crops (Anon., 2021) [1]. Onion is botanically included in the family Amaryllidaceae with a chromosome number of 16 (2n). A short-duration horticultural crop, it is mostly known as 'Queen of Kitchen' owing to its exquisite, aroma, flavor and exclusive taste combined with medicinal properties
(Griffiths *et al*., 2002) [2]. India stands second in the production of onions in the world, after China and is the second largest exporter of onions. Being known for its pungency, Indian onions are widely in demand and available throughout the year. Our country has exported over 2,525,258.35 metric tonnes of fresh onion worth Rs. 4,522.79 crores during the year 2022-23. The major export destinations are Bangladesh, Malaysia, United Arab Emirates, Sri Lanka, Nepal and Indonesia (Anon., 2023a) [3]. In India, Maharashtra accounts for the highest production of onions followed by Madhya Pradesh and Karnataka. As per the advanced estimate of 2022-23, India produces 31,005,400 metric tonnes of onion with a productivity of 17.3 metric tonnes per hectare from an area of 1,791,700 hectares. In Karnataka, as per the advanced estimate of 2022-23, onion cultivation is spread over an area of 2,31,840 ha with a production of 27,79500 tonnes and productivity of 11.99 metric tonnes per hectare (Anon., 2023b) [4].
Despite the high production statistics, onion cultivation in India is restricted by countless biotic factors. The plant diseases caused by fungi, bacteria, viruses and nematodes stand out to be the most jeopardized biotic factor which limits the productivity of onion. Of these, diseases caused by phytopathogenic bacteria are gaining prevalence lately. The major bacterial diseases reported in onions are stalk rot, soft rot, bacterial streak, bulb rot, center rot, leaf blight and sour skin. Among these, bacterial soft rot is one of the destructive biotic factors causing considerable losses in onion production both under field conditions as well as in storage. Bacterial soft rot of onion initiates in the field as the bacteria enters through wounds in the neck, bulb or aged tissues. Warm, rainy weather, heavy irrigation or flooded fields are optimum conditions under which bacterial decay of onion bulbs occurs rapidly rendering the bulbs unmarketable in a short period of time. The infected bulbs become water-soaked turning pale yellow to light brown leading to the breakdown of the entire bulb. A foul-smelling liquid ooze out from the neck when squeezed and the onion core may pop out when the base of the bulb is pressed.

The above-ground symptoms initiate with water-soaked lesions on the leaves which then progress to complete wilting and yellowing of the leaves (Schwartz and Mohan, 1995) [5]. As the bacterial soft rot of onion has emerged as a devastating problem among farmers in Northern Karnataka, a roving survey was carried out in four major districts in Northern Karnataka *viz.*, Bagalkot, Belgaum, Gadag and Dharwad to assess the incidence of onion soft rot in these districts.

2. Materials and Methods

2.1 Survey for assessing the incidence of soft rot of onion in Northern Karnataka

The occurrence and distribution of soft rot in onion in four districts of Northern Karnataka *viz.*, Bagalkot, Belgaum, Gadag and Dharwad were assessed in the *kharif* season during the year 2021 and 2022 through a roving survey. From each district, three taluks were chosen and from each taluk, three villages were selected from which two fields were surveyed for disease incidence. Each field selected was observed for disease incidence by walking diagonally across the field to take the count of symptomatic plants. The disease incidence (%) of each field was calculated using the formula given by Vernell and Hecloud (1975) [6].

$$\text{Disease incidence} = \frac{\text{Number of diseased plants}}{\text{Number of observed plants}} \times 100$$

During the survey, information was also collected on variety sown, area, type of sowing, irrigation method followed, soil type and stage of the crop. Along with that, a detailed account of symptoms noticed on the onion plant, both on leaves and bulb were recorded. The diseased samples were collected in polythene covers and brought to the laboratory for further studies.

2.2 Isolation of the bacteria from diseased tissues

The symptomatic onion plants were used for the isolation of the causal organism as per the protocol detailed by Masyahit *et al.* 2009 [7]. The onion bulbs showing rotting symptoms were first washed in tap water, thereafter rinsed in distilled water and air dried. The symptomatic parts of the onion bulb were sliced and disinfected with 70 percent ethanol and suspended in 5 ml of sterile distilled water taken in test tubes. Once the suspension turned turbid, a loop full of this suspension was streaked on nutrient agar plates and incubated for 48 h at room temperature.

2.3 Pathogenicity test

The purified bacterial culture was artificially inoculated on onion bulbs as per the protocol given by Gore *et al.* (2020) [8]. The bacterial culture was suspended in sterile distilled water and adjusted turbidometrically such that OD600 = 0.8-1.0 (> 10^8cfu/ml). To test the pathogenicity on bulbs, three healthy bulbs were surface sterilized with 70 percent alcohol and injected with

0.5 ml of bacterial suspension (OD600> 0.8-1.0) using a hypodermic needle and incubated in polythene covers at room temperature for 3-4 days. Uninoculated bulbs were maintained as control by injection of sterile distilled water. The inoculated bulbs were observed daily for the appearance of symptoms. The seedlings and bulbs showing the typical symptoms were subjected to re-isolation of associated bacteria on nutrient agar plates to confirm the pathogenicity.

3. Results and Discussion

3.1 Incidence of soft rot of onion in Northern Karnataka

The results of the survey conducted in *Kharif* of 2021 revealed the incidence of soft rot of onion in four districts at varied levels. Among the four districts surveyed, Bagalkot district exhibited the highest average disease incidence of 31.12 percent, followed by Dharwad (23.74%) and Gadag (21.21%) while the lowest average disease incidence was observed in Belagavi (7.10%). In Bagalkot district, among the three taluks surveyed, the maximum mean disease incidence was noticed in Mudhol (37.23%), followed by Badami (31.36%) and Bagalkot taluk (24.80%). In Mudhol taluk, having the highest disease incidence, the maximum average disease incidence was observed in Panchagav village (42.42%) which was followed by Lokapur (39.17%) and Salhalli (30.10%). In Dharwad district, Navalgund taluk exhibited the maximum mean disease incidence of 31.61 percent, which was followed by Annigeri taluk (24.60%) and Hubli (15.03%). Among the three villages surveyed in Navalgund taluk, Karlawad village recorded the highest average disease incidence of 33.59 percent which was closely followed by Belavataggi village having an average disease incidence of 32.19 percent. The least average disease incidence of 29.05 percent was observed in Navalgund, among the three villages surveyed. In Gadag district, Nargunda taluk, among all three taluks surveyed had shown the highest mean disease incidence of 23.15 percent, closely followed by Gadag (21.79%) and Rona taluk (18.70%). The highest average disease incidence of 30.15 percent was observed in Nargunda village, followed by Konnur (23.10%) and Kalakeri village (16.25%) of Nargunda taluk. Belagavi district exhibited the lowest average disease incidence, of which Saundatti taluk recorded the highest mean disease incidence of 10.77 percent, followed by Ramdurga taluk (6.17%) and Gokak (4.38%). In Saundatti taluk, Yaragatti village displayed highest average disease incidence of 12.44 percent, which was followed by Nesargi village (10.50%) and Murgod (9.35%).

The results of the survey carried out in *Kharif* of 2022 revealed that the highest mean disease incidence in Dharwad district (36.06%) which was followed by Bagalkot (22.80%), Gadag (19.93%) and the lowest mean disease incidence was noticed in Belagavi (7.30%). In Bagalkot district, of three taluks surveyed, the highest average disease incidence was noticed in Bagalkot taluk (30.29%) which was followed by Mudhol (22.20%) and Badami (16.16%) taluks. Among the three villages considered in Bagalkot taluk, Tulsigeri recorded the highest average disease incidence of 32.13 percent, followed by Govindkopa (30.10%) and Kaladagi (28.65%). Dharwad district recorded the highest mean incidence of onion soft rot, in which Navalgund taluk displayed the highest mean disease incidence of 41.32 percent, followed by Annigeri (35.88%) and Hubli (31.00%) taluks. In Navalgund taluk, the maximum average disease incidence was observed in Navalgund village (49.17%) followed by Belavataggi (39.45%) and Karlawad (35.35%) village. In Gadag district, Nargunda taluk recorded the highest mean disease incidence of 26.20 percent, which was followed by Rona (19.79%) and Gadag (13.80%) taluks. Among the three villages surveyed in Nargunda taluk, Konnur village exhibited highest mean disease incidence of 28.62 percent, followed by Kalakeri (27.57%) and Nargunda (22.41%) village. Belagavi recorded the least mean disease incidence among the all four districts surveyed, in which, Ramdurga taluk displayed the highest

mean disease incidence of 9.50 percent, followed by Saundatti (7.47%) and Gokak (4.94%) taluks. In Ramdurga taluk, the maximum average disease incidence was observed in Kamakeri village (9.90%), followed by Ramdurga village (9.83%) and Hulkund (8.76%).

During the survey, it was observed that although the crop was cultivated in both red and black soil, higher disease incidence was associated with crops cultivated in black soil. Black soils are clayey in nature and have high moisture-holding capacity (Katti, 1978) [9] thus, encouraging the growth and multiplication of bacteria in soil (Clark, 1967) [10]. In fact, Charkowski (2018) [11] has pointed out that increased soil moisture was the most favorable factor that contributed to the prevalence of bacterial soft rot diseases in horticulture crops. Another major observation was that that disease was predominantly prevalent in plots where the method of planting was broadcasting. Since the seeds of onion are minute in size, farmers mostly go for broadcasting of seeds as compared to line sowing. But this leads to uneven spacing and clustering of plants which creates a conducive condition for the spread of the disease. It has been observed that the symptoms of the disease initiate on leaves first and then spread to the bulb. Hence, closely spaced planting further encourages the spread of the disease inoculum. The result was also supported by the observations of Nataraj (2019) [12] who also reported that onion fields that followed the method of broadcasting displayed higher onion leaf rot incidence as compared to line-sown crops in Northern Karnataka. Similarly, Balasubramanian and Hill (2002) [13] have stated that the broadcasting of seeds resulting in very high plant density can create highly humid microenvironments in fields which facilitate the rapid spread of the pathogen. Moreover, in fields showing higher disease incidence, the method of irrigation followed was flood irrigation, which further helps in the spread of the inoculum within the field.

Table 1: Disease incidence of onion soft rot in major districts of Northern Karnataka in 2021 and 2022

District	Taluk	Village	Percent disease incidence	
			2021	2022
Bagalkot	Bagalkot	Tulsigeri	29.65	32.13
		Kaladagi	24.40	30.10
		Govindkopa	20.35	28.65
	Mean		24.80	30.29
	Mudhol	Lokapur	39.17	25.39
		Salhalli	30.10	15.62
		Panchagav	42.42	25.61
	Mean		37.23	22.20
	Badami	Badami	28.85	11.10
		Sulikeri	36.29	12.93
		Hoolageri	29.02	24.44
	Mean		31.36	16.16
	Mean disease incidence of Bagalkot district		31.12	22.80
Dharwad	Annigeri	Annigeri	21.72	41.99
		Shirguppi	26.66	35.42
		Haliyal	25.42	30.22
	Mean		24.60	35.88
	Hubli	Hebsur	13.24	21.40
		Kusugal	17.21	37.49
		Kirasur	29.28	34.11
	Mean		15.03	31.00
	Navalgund	Navalgund	29.05	49.17
		Belavataggi	32.19	39.45
		Karlawad	33.59	35.35
	Mean		31.61	41.32
	Mean disease incidence of Dharwad district		23.74	36.06
Gadag	Rona	Hirehal	15.13	24.17
		Kotabal	18.66	15.15
		Abbigeri	22.31	19.86
	Mean		18.7	19.79
	Gadag	Narayanapur	26.65	21.89
		Betageri	18.88	10.86
		Narasapur	19.85	8.66
	Mean		21.79	13.80
	Nargunda	Nargunda	30.15	22.41
		Konnur	23.1	28.62
		Kalakeri	16.25	27.57
	Mean		23.15	26.20
	Mean disease incidence of Gadag district		21.21	19.93
Belagavi	Ramdurga	Ramdurga	6.52	9.83
		Hulkund	6.37	8.76
		Kamakeri	5.6	9.90
	Mean		6.17	9.50
	Saundatti	Murgod	9.35	6.29

		Yaragatti	12.44	8.71
		Nesargi	10.50	7.41
	Mean		10.77	7.47
	Gokak	Gokak	3.97	4.89
		Arabhavi	4.43	4.65
		Mudalagi	5.02	5.26
	Mean		4.38	4.94
	Mean disease incidence of Belagavi district		7.10	7.30

3.2 Isolation of bacteria from diseased tissues and pathogenicity test

The involvement of bacteria associated with onion soft rot was confirmed by the ooze test. Further, the isolation of the bacteria from symptomatic bulbs and leaves was carried out from the collected bacterial ooze. After an incubation period of 48 h, creamy white, opaque and mucoid bacterial colonies were observed on nutrient agar. Single colonies were selected and were re-streaked on nutrient agar to obtain the pure culture of the bacterium. Similar colony morphology of bacteria isolated from soft rot symptoms was observed by various authors (Schwartz and Otto, 2007; García-González *et al.*, 2018, Nguyen *et al.*, 2021) [14, 15, 16].

To prove the pathogenicity, the isolated bacterium was challenge inoculated on onion bulbs. On bulbs, symptoms appeared three days post-inoculation where a deep depression was formed on the inoculation point and these bulbs when cut open appeared flaccid. The causal bacterium was re-isolated from infected bulbs on nutrient agar. The colonies appeared creamy white and mucoid, exhibiting similar colony characteristics to the inoculated bacterium. Thus, Koch's postulates were proved and the pathogenicity of the bacterium on onion was established.

Bishop and Davis (1990) [17] observed similar symptoms of flaccidity and discolouration on artificially inoculated onion bulbs within 72 h of incubation.

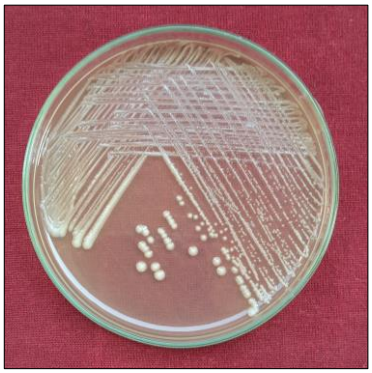

Fig 1: Bacterial colonies on nutrient agar

Fig 2: Pathogenicity test on onion

4. Conclusion

Onion soft rot is a devastating disease as it affects the economic part of the crop, that is, the bulb. The roving survey carried out focusing four districts of Northern Karnataka *viz.*, Bagalkot, Belgaum, Gadag and Dharwad during two consecutive years (2021 and 2022) revealed moderate disease incidence ranging from 7.10 percent to 36.06 percent. The bacterial pathogen associated with soft rot samples were isolated in nutrient agar and appeared as creamy mucoid colonies within 48 h of incubation in nutrient agar. The pathogenicity of the bacterium was proved on healthy onion bulbs were the symptoms appeared three days after inoculation. Thus, the study reveals the importance of an emerging disease, causing havoc among farmers. Further identification of the pathogen along with delineating necessary management practices against soft rot of onion is essential for controlling the disease.

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