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RESEARCH ARTICLE

Impact of leaf spot disease on beetroot production in Jaffna peninsula, Sri Lanka

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ABSTRACT

The present study focuses on the aetiology of leaf spot disease and its impact on beetroot production in the Jaffna peninsula. A survey and field study was carried out to understand present status of the disease. The fungus associated with the leaf spot lesions was isolated and identified based on morphological features and sequence analysis. The survey has shown drawbacks in the cultivation practices, such as selection of cultivation season, selection of variety and inadequate weed management practices. The initial symptoms were noted in seedling stage, and the disease incidence and severity progressed with time. The disease incidence and severity index were 85% and 2.71 at 48 days. The field experiment has revealed the negative correlation between leaf spot disease development and yield. The fungal isolates produced obclavate shape, hyaline, multiseptated conidia on dark coloured unbranched sparingly septate conidiophores. The isolates showed 99.25% sequence identity with *Cercospora beticola*.

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Introduction

The plant commonly known as beet represents four cultivated forms of *Beta vulgaris* L. (Family *Chenopodiaceae*). The most well-known beet is the purple root vegetable, also known as beetroot or table beet. The other three varieties include the leafy vegetable chards and spinach beets and the root vegetable sugar beet (Goldman and Navazio 2008). The beetroot is a biennial plant usually grown for the fleshy root and young leaves (Kikkert et al. 2010). The crop originated from Mediterranean Europe and North Africa, spread all over Europe to western India, and formed a second diversity centre in the Near East (Goldman and Navazio 2008). Even though beetroot is not one of the world's major vegetable

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crops in terms of acreage, production, or consumption, it occupies a unique niche in Europe, North America, Middle East, and Asia (Goldman and Navazio 2008).

Beetroot is especially rich in fibre and sugars but has a moderate calorie value (Kugler et al. 2007). It also consists of significant amount of Vitamin C, Vitamin B (Wang and Goldman 1996) in the root and Vitamin A in the leaves. Consuming beetroot helps cure many diseases such as anaemia, blood pressure, cancer, gastric ulcers, kidney ailments, liver toxicity or bile ailments like jaundice, hepatitis, or diarrhoea (Holmes and Assimos 2004).

In Sri Lanka, around eighty different varieties of fruits and vegetables are grown in varied agro-climatic areas. The cool-climatic conditions in the hill country are ideal for temperate crops, including beet. However, well-demarcated low country dry and wet areas are also quite suitable for beet cultivation. It is cultivated in many parts of Sri Lanka, such as Kegalle, Nuwara Eliya, Kurunegala, Moneragala, Anuradhapura, Ratnapura, Matala, Hambantota and Jaffna based on irrigation conditions. In Jaffna, the Valikamam zone is the only place where beetroot is mainly cultivated. In Sri Lanka, approximately 1700 ha are reported to have under beet cultivation, and per capita consumption is estimated to be around 0.7 kg/year.

Many fungal diseases affect cultivated beets, including mildews, damping-off, scab and leaf spot. The leaf spot disease of beet is one of the most serious problems (Weiland and Koch 2004; Koike et al. 2010), commonly caused by *Cercospora beticola*. Lesions produced by *C.beticola* involve the simultaneous collapse of cells in an area ranging upto several millimetres in diameter (Leucker et al. 2016). The disease leads to the premature death of leaves and reducing the assimilation area. Also, it causes significant loss of root yield and diminished sucrose content (Skibowska et al. 2019). Similar disease symptoms have been noted in commercial beetroot cultivations in the Jaffna Peninsula. In Sri Lanka, Even though the beetroot has been cultivated for many years, as far as our knowledge, very few studies have been done on various aspects of beetroot cultivation. The present study reveals the impact of leaf spot disease on beetroot cultivation, and the pathogen which cause the disease in the Jaffna peninsula.

Materials and methods

A survey among the farmers involved in beetroot cultivation

The survey was conducted among farmers involved in beetroot cultivation to determine the status of beetroot cultivation in the Valikamam

zone in the Jaffna peninsula. Between August to December 2019, hundred and five farmers were randomly selected from five main administrative towns: Kopay, Chankanai, Uduvil, Tellipalai and Sandilipay; this includes seventeen Grama Sevaka divisions. A questionnaire was prepared and circulated with a list of queries that fulfil the survey's specific objective. The estimated proportion of responses to each query was determined, and results were plotted on graphs.

Determination of the impact of leaf spot disease on beetroot cultivation

This study was done based on a field experiment. Four beetroot farms, each located about 10 km of distance, were selected for this study. The farms were in villages, namely Earlalai (nearest town Sandilipay), Inuvil (nearest town Uduvil), Maruthanarmadam (nearest town Uduvil) and Kopay. The development of leaf spot disease was monitored in the fields from seedling emergence to harvesting. In each field, plots with ten plants were chosen randomly for continuous monitoring. In 12 days of interval, disease incidence and disease severity were determined throughout the cultivation period described by Vereijssen et al. (2003). Disease severity was measured by the Agronomica whole plant diagram (DS_{AGR}). It is a whole plant assessment based on 11 classes (0–5) from healthy through to totally destroyed foliage.

The disease incidence was determined using the following equation.

$$\%\ \text{Disease incidence} = \frac{\text{Number of infected leaves}}{\text{The total number of plants observed}} \times 100$$

In addition, leaf spot symptoms in the other crop plants and weeds in the field were also monitored. Finally, during the harvesting stage, the yield was weighed from each experimental unit.

Disease incidence and severity data were subjected to ANOVA to study their variation from seed sowing to harvesting at different time points. A simple linear regression analysis was carried out to test the impact of disease incidence and the severity on yield reduction.

Isolation of the pathogen associated with beetroot leaf spot disease

Plant samples with leaf spot symptoms (with <5 mm diameter lesions) were collected from four different locations in the Jaffna peninsula (Inuvil, Earlalai, Kopay, Urumpirai) between December 2019 to March 2020. The leaf samples were observed under high power stereo dissection

microscope (AmScope SE305R-P-LED, USA) to characterize the symptoms and signs associated with the lesion. Single spores were isolated from lesions of infected leaf specimen and separately cultured on potato dextrose agar (PDA) (Oxoid, United Kingdom) medium incorporated with streptomycin (100 ppm). The plates were incubated at 25 °C with 12 h light and 12 h dark in a cool incubator. Pure cultures of the isolates were maintained on PDA.

Macroscopic and microscopic characteristics of the isolates

The morphological character of the fungal isolates was studied as described by Esh and Moghaieb (2011). Colony characteristics such as colour, texture, colony appearance and form of mycelial growth were observed. Mycelium, conidiophore and conidia of the isolates were examined under a light microscope (Olympus CX31, Japan).

Molecular identification of the isolates

For molecular identification, total DNA was extracted from mycelium of 21 isolates using a DNeasy Plant Mini kit (QIAGEN, Germany) as described in the manufacturer's guidelines. The PCR amplification was conducted using the primers ITS1-(5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4- (5′-GCATATCAATAAGCGGAGGA-3′) (White et al. 1990). PCR reactions were performed with a reaction mixture volume of 20 µL containing 10 µL of ready-to-use PCR mixture (Promega, USA), 1 µL of forward and reverse primers each (10 µM) and 1 µL DNA sample. Amplification was carried out in a thermal cycler (Techne Thermal Cycler-TC3000, UK) according to the following amplification conditions: initial denaturation at 95 °C for 3 min followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 1 min and extension at 72 °C for 1 min, and the final extension step was at 72 °C for 10 min. PCR products were electrophoresed on a 2% agarose gel, stained with ethidium bromide and examined in a gel documentation system (Enduro GDS, Labnet, USA).

The PCR products of one isolate, representing Urumpirai (Jfn03) was sequenced using forward and reverse primers by automated Sanger sequencing (Macrogen, Korea). The sequence identity search was carried out using the BLAST program available in the NCBI. The sequence of the isolate was deposited in the GenBank database.

Pathogenicity tests

Four isolates were screened to confirm their pathogenicity. The spore suspension was prepared by flooding 12 days old fungal culture with

10 ml sterile water. The suspension (10^4 spores/mL) was inoculated on the youngest leaves of one month old plants growing in pots. After the inoculation, the plants were covered with polythene bags for 24 hours to provide higher humidity. For the control experiment, leaves were inoculated with sterilized water instead of spore suspension. The development of disease symptoms was monitored, and the pathogen was reisolated and confirmed based on their morphological characteristics.

Results

Field survey

The survey explored several critical sides of table beet cultivation in the Jaffna peninsula. Three different varieties of table beet, namely Crimson Globe (Onesh (PVT) Ltd, Sri Lanka) , Parimose and Red Ace (SPS NZ, Newzealand), were cultivated in this region. More than 67% of farmers grow the variety, Crimson Globe (Figure 1a). Red Ace is cultivated only by 6% of the farmers. *Maha* season is the primary season for beetroot cultivation in this region, about 60% of the farmers do beetroot cultivation in this season (Figure 1b). It shows that less than 10% of farmers cultivate table beet in both *Yala* (from May to the end of August) and *Maha* (from September to March) seasons. For marketing, most of the producers (82%) harvest only the plants' roots at the end of the cropping. Harvesting the whole plant, including leaf and root, for the fresh market is not popular among the farmers (Figure 1c). All the beetroot farmers are practising crop rotation. About 50% of them grow onion in the off-season, followed by chilli, carrot, brinjal, pumpkin and tomato (Figure 1d). Farmers use different kinds of pesticides to control pests and pathogens in beetroot farms. 70% of farmers use propineb (70% (W/W) WP) and profenophos (500 g/l EC) to control pests in table beet cultivation.

More than 60% farmers aware about leaf spot disease and they assume the disease causing yield reduction. Among these farmers, 84% farmers clearly described the symptoms. However, they mentioned several factors for the leaf spot disease development, such as sudden climate changes, excess of rain, hot summer and biotic factors including insect and warm. Only a 5% of farmers mentioned fungus as the disease causing agent.

Field experiment

Leaf spot lesion formation was initially observed on older leaves; later, the infection progressed to newer leaves. In the beginning, the disease symptoms developed as numerous, discrete, small circular leaf spots, as

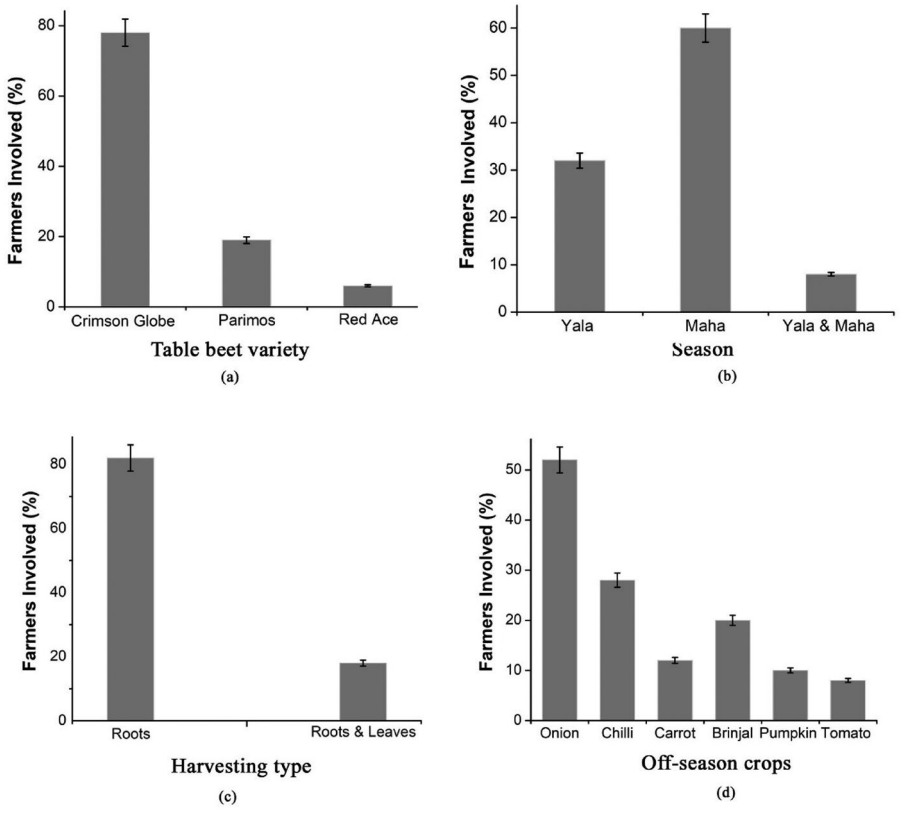

Figure 1. Involvement of farmers in the key areas of survey (a) table beet variety (b) season of cultivation (c) harvesting type (d) off-season crops. The error bars denote the standard error of the mean.

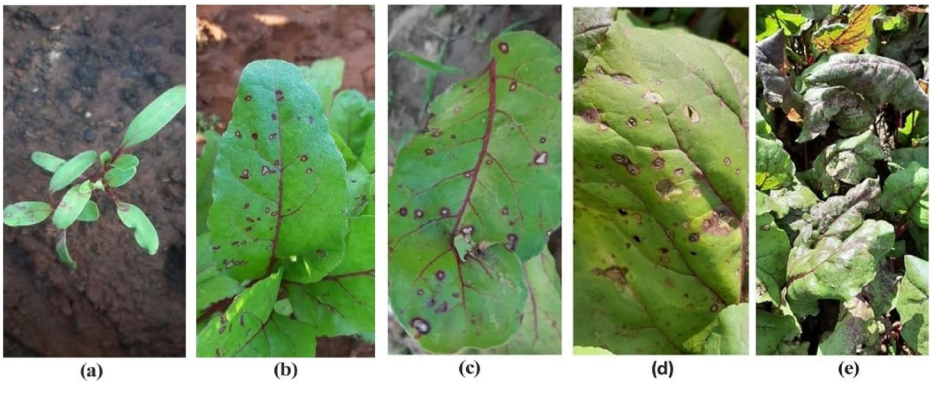

Figure 2. Leaf spot disease on table beet leaves. The gradual expansion of lesions in leaves showed in 10 days of intervals (a–e).

shown in Figure 2. Each spot has a circular centre with a grey to brown dead lesion surrounded by an undefined red to purple margin. Lesions expand in size, merge, and turn grey with time. This lesion expansion resulted in extensive foliage loss.

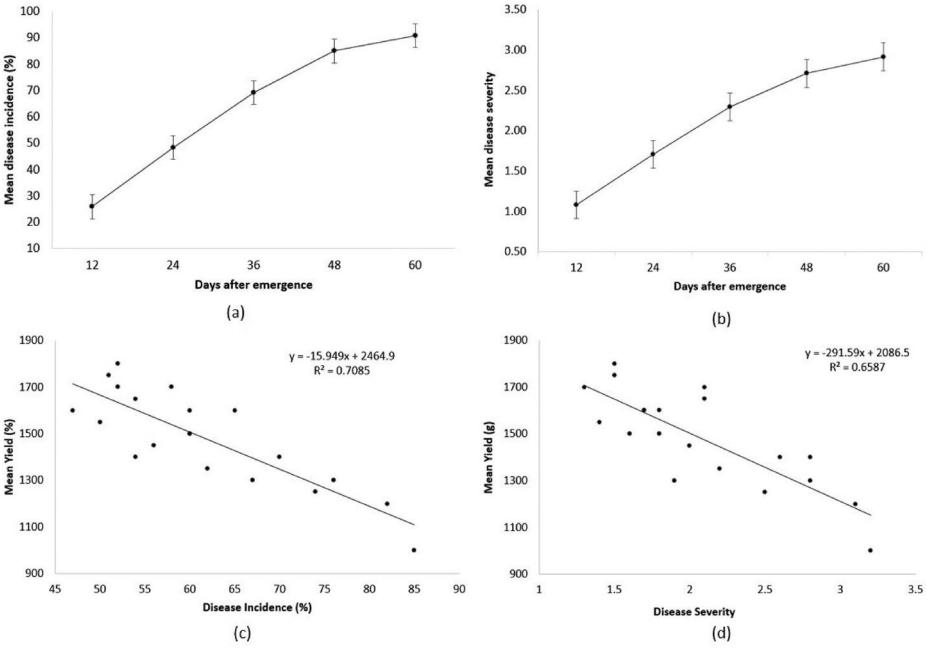

Figure 3. The results obtained in the field study. (a) mean disease incidence at 12 days intervals from 12 days after seedling emergence (ASE) to harvesting at 60 days ASE. (b) disease severity at 12 days intervals from 12 days after seedling emergence (ASE) to harvesting at 60 days ASE. The error bars denote the standard error of the mean. (c) the correlation between disease incidence and mean yield at the time of harvesting. (d) the correlation between disease severity and mean yield at the time of harvesting.

The initial visible symptoms were noted about seven days after the emergence of seedlings (AES). At ten days AES, about 26% of the seedlings had at least a single point lesion (Figure 3a), and the severity index was about 1.08 (Figure 3b). Later, the disease incidence and severity significantly ($p < 0.05$) increased up to 48 days AES. At that point, the disease incidence and severity reached about 85% and 2.71, respectively. After that, until harvesting at 60 days AES, the disease incidence and severity increased slightly up to 91% and 2.92, respectively.

A simple linear regression was conducted to test if disease incidence significantly predicted yield reduction (Figure 3c). The regression results indicated that the model explained 70.9% of the variation and that the model was significant, $F(1,18)=43.76$, $p < 0.001$. It was found that disease incidence significantly predicted yield reduction ($\beta 1 = -15.9$, $p < 0.001$). The final predictive model was: proportion of yield reduction = 2464.9 + (-15.9*disease incidence). Similarly, a simple linear regression was carried out to test if disease severity significantly predicted yield reduction (Figure 3d). The results of the regression indicated that the model explained 65.9% of the variation and that the model was significant, $F(1,18)=34.74$, $p < 0.001$. It was found that disease severity significantly

predicted yield reduction (β1 = −291.6, p<.001). The final predictive model was: proportion of yield reduction = 2086.5 + (-291.6*disease severity). The weed Punarnava (*Boerhaavia diffusa*), *Tridax*, parthenium and some grasses and some crops, including tomato, spinach, corn, bean and chilli, growing adjacent to the table beet field, showed leaf spot symptoms similar to the one observed in beet (Figure 4).

Macroscopic and microscopic features the isolates

Morphological characteristics of 21 isolates were studied. The fungal isolates produced olivaceous-grey on PDA with irregular patches of white or smoke-grey colonies (Figure 5d-g). The colonies had smooth, erumpent and regular, even margins and sparse to moderate aerial mycelium. The microscopic observation of the isolates revealed branched, septate, slender intercellular and brown coloured mycelium. Dark coloured unbranched sparingly septate conidiophores were observed. Conidia are obclavate shape, hyaline, acicular, multiseptated (4 to 12 septate present).

Identification and characterization of pathogen based on molecular methods

The PCR amplification of the ITS region of the 21 isolates yielded about 600 bp products (Figure 6). The result is similar to the length reported to the species *Cercospora*. The isolate jfn03 showed 99.25% in BLAST search with *Cercospora beticola* isolate Cer 75-18 (GenBank accession number: MN209928), isolated from leaf spot disease of *Beta vulgaris*. Finally, the sequence of the isolate jfn03 has been submitted in the GenBank database (MZ540796).

Pathogenicity test

Four isolates were tested to confirm the pathogenic potential. The isolates produced characteristic symptoms of leafspot disease. The leaf spots were small circular lesions at the beginning. The dead central region was surrounded by undefined red to purple margin. The re-isolation and morphological studies of the isolates from inoculated plants confirmed the pathogenicity of the isolates.

Discussion

Beetroot cultivation has been practised for many years in Jaffna Peninsula in small farms by conventional methods. In recent days, leaf spot disease

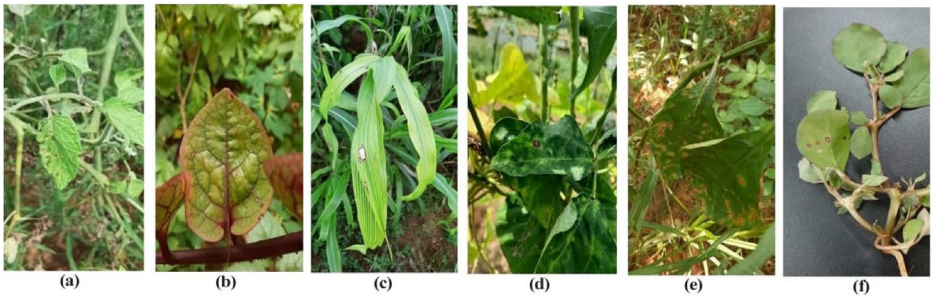

Figure 4. Leaf spot lesions were observed in weed and other crops growing adjacent to table beet (a-Tomato, b- Spinach, c- Corn, d- Bean, e- Chilli, f- Punarnava).

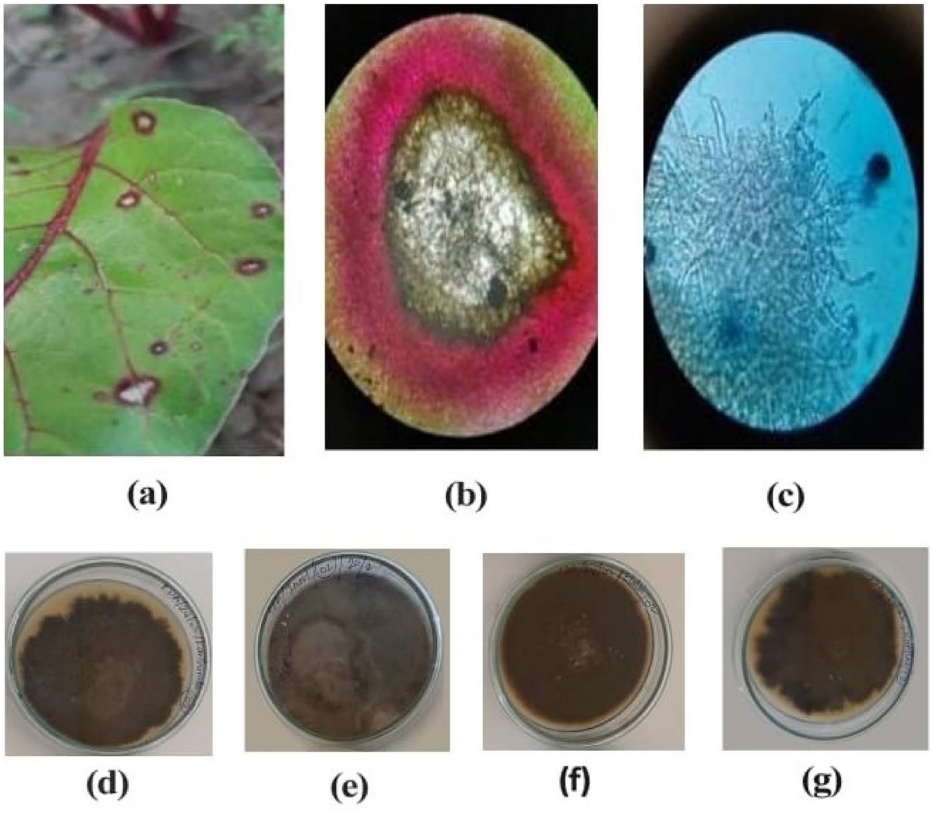

Figure 5. a: Leafspot lesions on table beet leaves b. Dissection microscopic view of leaf spot lesion c: Light microscopic view of stromata and conidiophores d,e,f,g: Culture Morphology of the selected *C. beticola* isolates grown on PDA plates.

is an emerging threat in beetroot cultivation in this region. This study has experimentally proved that the development of the disease significantly reduces the root yield in this region. The severe stage of the disease reduces the photosynthetic leaf area and reduces the amount of

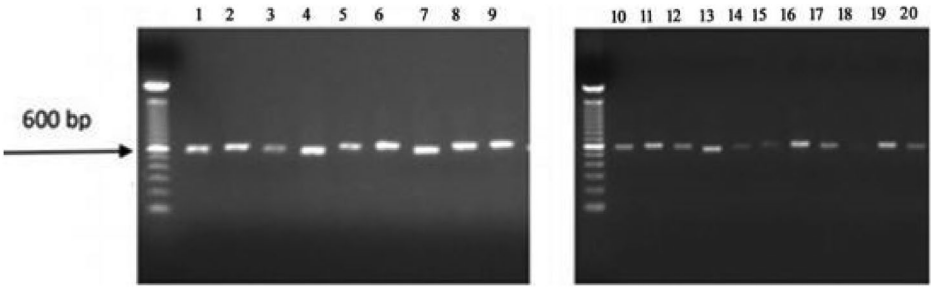

Figure 6. The gel- electrophoresis image obtained with twenty isolates represents four different regions in the Jaffna peninsula. Lane 1- DNA Marker, lane 2 to 21- PCR products obtained by amplifying ITS region of the twenty isolates. The PCR product size is 600 bp. 1-5 obtained from Jfn 01, 6-10 obtained from Jfn 02, 11 - 15 obtained from Jfn 03 and 16 to 20 obtained from Jfn 04.

food that transfer to the root. Ultimately, due to this stress condition plant failed to produce the expected yield. Therefore, one of the primary challenges in enhancing the profitability of table beet production is the effective management of plant diseases (Abawi et al. 1986; Shah and Stivers-Young 2004). For this disease management, a clear understanding of the pathogen's origin, biology, pathogenicity, and ecology is essential (Crous et al. 2015). The field isolation of leaf spot causing agent revealed that leaf spot was associated with morphologically similar types of fungi in all four zones. The isolates produced branched, septate brown coloured mycelium, and pigmented conidiophores bearing obclavate shape, hyaline, acicular and multiseptated conidia. The PCR based amplification of the ITS region also confirmed the similarity by yielding about 600 bp products. Finally, the pathogen has been confirmed as *C.beticola* according to sequence homology search using BLAST. The leaf spot of beetroot caused by *C.beticola* has been reported in several previous works in many other countries (Weiland and Koch 2004; Koike et al. 2010; Pethybridge et al. 2017). However, the present study is the first report from Sri Lanka, based on the results of molecular sequencing. A recent study has shown that in addition to *C. beticola*, *Cercospora apii* and *C.* cf. *flagellaris* were pathogenic to beetroot (Vaghefi et al. 2018).

The conidiospores produced by *C. beticola* dispersed short distances by wind, rain, or both (Franc et al. 2010). Studies have shown that the *C.beticola* may overwinter on plant debris and soil for several months, and it may initiate epidemics by splash dispersal of inoculum to leaves (Solel 1970; Khan et al. 2008; Franc et al. 2010). Since beetroot cultivation is not conducted throughout the year in Jaffna, the pathogen must survive in the field in alternative hosts. During the field studies, it has been noted that weeds, namely *Tridax*, parthenium and some grasses, showed symptoms characteristic to the one observed in beetroot. The

fungus isolated from the lesions is also identical to the isolates obtained from diseased beetroot. Due to the overwhelming cost of weed management, farmers nowadays reduced the weeding frequencies even during the cultivation seasons. Farmers also carry out crop rotation. The study has shown that some cultivated crops such as tomato, spinach, corn, bean, and chilli also have similar symptoms and carry morphologically identical pathogens. Therefore, for efficient disease management, farmers need to consider weed management not only in cultivation season also during off-seasons. It is also important to consider the crop rotations with nonhost of the *C. beticola*. For this aspect, further studies are needed.

The survey denotes the need for changes in cultivation and harvesting practices to reduce the disease incidence. The variety "Red Ace" has been recently introduced as a beetroot variety with features such as all-year-round production and intermediate resistance to *Cercospor*a leaf spot disease. However, farmers are not aware of this variety. Similarly, the climatic conditions in the Jaffna region favour beetroot cultivation in both *Yala* and the *Maha* seasons. But the farmers mainly focusing the *Maha* season since the cultivation of beetroot in other parts of Sri Lanka is less in the *Maha* season than the *Yala* season. However, the rainy, cool climate prevailing in the *Maha* season favours fungal diseases, including leaf spot disease.

A two-stages of harvesting and marketability is possible for beetroot since it has several marketable units (foliage, roots or both). However, in Jaffna, most of the farmers harvest only mature roots for selling. Depends on the season, the root is harvested between 60 to 80 days at complete maturation. In this harvesting manner, the plants being exposed to microbial infection for an extended period. Therefore, harvest dates have a significant impact on disease incidence. However, early harvesting may lead to economic detriments to the growers (Kikkert et al. 2010).

The high frequency of resistance to multiple fungicide modes of action is the key factor that responsible for the uncontrol nature of the disease. Hence, consideration of effective disease management practices is needed in order to increase production. Rotation to nonhosts between table beet crops, optimal weed management, and suitable resistant variety are crucial factors in conventional farms to overcome the leaf spot disease.

Conclusion

The table beet cultivation experienced severe threat by the leaf spot disease in Jaffna Peninsula. Leaf spot disease in table beet is caused by *Cercospora beticola* in the Jaffna peninsula. The present survey and field study confirmed that disease incidence and severity impact the yield of table beet cultivation. The losses can be prevented by selecting

recommended varieties, cultivation in both *Maha* and *Yala* seasons, implementing effective weed management practices and altering harvesting time.

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Authors' contribution

CJE conceived and designed the experiments, received the grant and supervised the project. NS conducted survey, and experiments. ECJ performed the sequence analysis. ECJ and NS prepared the manuscript. Both authors read and approved the final manuscript.

Disclosure statement

All authors declare that they have no conflict of interest.

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