Abstract
Stubborn caused by *Spiroplasma citri* is one of the most important diseases of citrus worldwide and in Iran. The main vector of *S. citri* in Iran is the leafhopper *Circulifer haematoceps*. In the research six isolates of *S. citri* (Fasa I, Fasa III, Darab I, Darab II, Darab III, and Darab IV), isolated from *C. haematoceps*, from Fars sesame fields and one isolate (Ghadimi isolate) from a stubborn affected sweet orange tree in Khafr region (Fars, Iran) affected to stubborn. A compeletly randomized design based on factorial experiment was performed to compare the disease severity. The main factors were season (winter and summer) and subfactors were *Spiroplasma citri* isolates including Fasa I, Fasa III, Darab I, Darab II, Darab III, Darab IV and Ghadimi. *S. citri* isolates were transmitted to young and of periwinkle plants by grafting and disease symptoms and other disease parameters such as wilting and yellowing periods (time between inoculation and initiation of wilting and yellowing) were compared, In general in winter experiment, the disease incubation period and duration of wilting and yellowing initiation were longer than those in summer season. Statistical analysis showed that duration of disease incubation, wilting and yellowing was significantly ($p<\%1$) different between summer and winter and in summer season duration of the disease parameters were significantly shorter than those with winter season. The interaction of season and isolates was significant at the ($p<\%1$). Polymerase chain reaction (PCR) assay using P89-f/r, a primer pair specific for *S. sitri*, confirmed by infection of periwinkle plants *S. citri* grafted by infected plants to *S. citri* isolates. Total DNA was separately extracted from seven periwinkle plants infected by *S. citri* and were tested for *S. citri* genetic differences by short sequence repeat (SSR) PCR using 4 SSR primers (SSR20A f/r, SSR02 f/r, SS06 f/r and SSR20B f/r). Primer pairs SSR 20A f/r, SSR06 f/r and SSR 20B f/r amplified expected fragment of chromosome from all seven isolates .The expected fragment was amplified by primer pair SSR 02 f/r with Ghadimi, Darab III and Darab isolates but not with other isolates. Therefore, on the basis of SSR PCR amplification *S. citri* isolates fell into two groups: Group one consisted of Ghadimi, Darab III and Darab I, and group two consisted of Fasa I, Fasa III, Darab II and Darab IV isolates. SSRPCR products with SSR02 f/r and SSR20A f/r were directly sequenced where fragments were used for Blast search and phylogenetic analyses. Phylogenetic analyses using sequenced fragments and the same fragment from *S. kunkelli* as outgroup indicated genetic diversity of under investigated isolates of *S. citri*. Results of the research are useful to breed resistant cultivars. Genetic diversity of *S. citri* isolates using SSR primers is reported for the first time in the Fars province.

Keywords: *Spiroplasma citri*, disease severity, SSR PCR, Genetic diversity

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Investigation of disease severity and genetic diversity of *Spiroplasma citri* isolates, causal agent of citrus stubborn disease in Fars province

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