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Visible/Near infrared (VIS/NIR) spectroscopy and multivariate data analysis (MVDA) for identification and quantification of olive leaf spot (OLS) disease

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Abstract: Early detection of plant disease requires usually elaborating methods techniques and especially when symptoms are not visible. Olive Leaf Spot (OLS) infecting upper surface of olive leaves has a long latent infection period. In this work, VIS/NIR spectroscopy was used to determine the latent infection and severity of the pathogens. Two different classification methods were used, Partial Least Squared-Discrimination Analysis (PLS-DA) (linear method) and Support Vector Machine (SVM) (non-linear). SVM-classification was able to classify severity levels 0, 1, 2, 3, 4, and 5 with classification rates of 94, 90, 73, 79, 83 and 100%, respectively The overall classification rate was about 86%. PLS-DA was able to classify two different severity groups (first group with severity 0, 1, 2, 3, and second group with severity 4, 5), with a classification rate greater than 95%. The results promote further researches, and the possibility of evaluation OLS in-situ using portable VIS/NIR devices.

***Keywords*:** olives, Olive Leaf Spot (OLS), disease severity, VIS/NIR spectroscopy, Multivariate Data, Analysis (MVDA) (*i.e.* chemometrics), Partial Least Squared-Discrimination Analysis (PLS-DA), Support Vector Machine (SVM)-classification

Introduction

The olive tree (*Olea eruopeas L.)* is one of the most ancient trees cultivated in the Mediterranean regions. Olive trees play an important role in the social and economical life of this region. It is one of the oldest agricultural trees cultivated over large areas in Palestine (Qutub *et al.,* 2012). The number of olive trees is exceeding 10 million (about 67.3% of horticultural trees) and occupying more than 50% of the agricultural area (Palestinian Central Bureau of Statistics, 2012). The trees comprise one of the main sources of income, reaching its contribution to the good years to about 13% of the annual agricultural production.

The olive tree is affected by many pests and diseases (Sanei and Razavi, 2011). Olive Leaf Spot (OLS) is a foliar disease widespread in all olive growing regions of the world, and has been known in the Mediterranean areas (Obanor *et al.,* 2005). The disease, also known as peacock eye disease, is caused by the fungus Spilocaea oleaginea (Cast.) Hughes (syn. *Cycloconium oleagina*) (Gonzalez-Lamothe *et al.,* 2002). Severely infected trees show defoliation, poor twig and growth. As a result of infection, yield losses may reach up to 20% (Azeri, 1993; Graniti, 1993; Rongai, 2012). Symptoms of the disease occur usually on the upper surface of the leaves, expanding and coalescing to cover a large proportion of leaf area (**Figure 1**). The symptoms form dark brown round spots (2-15 mm in diameter) which become necrotic and surrounded by concentric yellowish or pale brown haloes (Sanchez *et al.*, 1998). Leaf spot is usually more abundant on the lower parts of olive trees (Razavi and Jahany, 2009; Rongai, 2012).

Infection with S. oleaginea is normally associated with high humidity and winter conditions (cool and low light), where high temperatures restrict spore germination (Al-Khatib *et al.*, 2010; Obanor *et al.,* 2008a). OLS infection can occur at any time of the year, but usually during late autumn to early summer, if environmental conditions are favourable. In hot dry weather conditions, conidia remain viable

but inactive on infected leaves and start to germinate early in winter. Conidium production is optimal at 15^{o}C and/or temperatures ranging from 2 to 25^{o}C and high humidity (85%) (Obanor *et al.*, 2008a). Conidia of *S. oleagina* are dispersed by rain splash or wind-borne water droplets (Sanei and Ravazi 2011).

Figure 1: Symptoms of Olive Leave Spot (OLS), defoliation (top) and poor twig (bottom).

In Palestine, the disease is wide common in many areas in southern and northern parts of the country including Hebron, Bethlehem, Ramallah, Nablus, Qalqilia, Tulkarm, Salfit, Jenin and Tubas districts (Abuamsha *et al.,* 2013; Salman *et al.,* 2011). The Palestinian Ministry of Agriculture and several private institutions work on olive sector to provide guidance and information about improving olive crop production and protection.

Prevalence *S. oleaginea* in Palestine is found to be most prevalent in the period from late autumn to spring and of minor significance in the period from the beginning of July until the middle of November (Abumsha *et al.,* 2008).

The disease is chemically controlled by application of coppers fungicides directly after harvest (Sistani *et al.,* 2009). The most commonly used fungicides include copper hydroxide, copper oxide and copper oxychlorides, although some long-persisting preventative fungicides have also been used to control the disease (Sistani *et al.*, 2009). Chemical treatment appears to be rarely effective (Obanor *et al.,* 2008a). Moreover, using chemical fungicides leads to the appearance of resistant pathogen races to copper (Cu) (Vanneste *et al.,* 2003) as well as disturbance of the plant metabolism following Cu accumulation in the soil (Obanor *et al.,* 2008b).
Application of chemical fungicides to control the disease in Palestine is however not acceptable. This is due to the negative effects of these fungicide on human and environment health (Rumpf *et. al.*, 2010), as well as their effects on taste and quality of the olive oil which usually lead to low prices and low income of the yield crop.

Visible/Near infrared (VIS/NIR) spectroscopy is a mature sensor technology. It has a big succeed for non-invasive analysis detection of may agricultural commodities' parameters, e.g. quality, safety, stress and disease (Alander *et al.* 2013; Garrigues and de la Guardia, 2013; Levasseur-Garcia 2012; Mahlein *et al.* 2012; Rumpf *et al.* 2010; Sankaran *et al.* 2010). This sensor technology was used for some applications related to olives (De Luca *et al.*, 2011, 2012; Salguero-Chaparro *et al.*, 2013). Portables VIS/NIR spectroscopes are marketable and used for different agricultural and food industries (dos Santos *et al.* 2013; Tiwari *et al.* 2013).Interpretation of VIS/NIR spectroscopy signals is carried out using multivariate data analysis (MVDA) (*i.e.* chemometrics). The latter used different modelling and classification methods for linear and non-linear models (Abu-Khalaf *et al.*, 2013; Natsheh *et al.*, 2013).

A feasibility study for using VIS/NIR spectroscopy to detect plant diseases was carried out in our biotechnology laboratory (Abu-Khalaf and Salman, 2013). In this contribution, further steps for investigating OLS disease were carried out, taking into consideration healthy leaves and the whole range of OLS disease severity. Two classification methods namely Partial Least Squared-Discrimination Analysis (PLS-DA) (linear method) and Support Vector Machine (SVM) (non-linear) were carried out. These classification methods were used in many different applications (Abu-Khalaf *et al.* 2004; Kalinowski *et al.* 2013; Luna *et al.* 2013; Peerapattana, 2013; Szymanska, 2012; Talens *et al.* 2013; Yang *et al.* 2013). The aim of this work was to

develop an early detection method of the latent infection with OLS disease using VIS/NIR spectroscopy.

Material and methods

Olive leaves

Leaves from different olive trees cultivar Nabali were collected randomly from orchards in Tulkarm district (32°18′25.80″N 35°07′01.00″E). The leaves were brought directly to the laboratory. Some leaves showed clear symptoms, while others were expected to be infected. Leaves were ready, without any pre-treatment, for spectra acquisition using VIS/NIR spectroscopy.

Spectroscopy

A VIS/NIR spectroscopy, a USB2000+ miniature fiber optic spectrometer (OceanOptics, USA) with Vivo light source, was used for spectra acquisition. Leaves were placed on the top of Vivo light source, and spectra were acquired in triplicates for each leaf, covering whole leave area.

The spectroscopy has a 550-1100 nm wave length, and a resolution of 0.35 nm full width at half maximum (FWHM). Spectroscopy has 2-MHz analog-to-digital (A/D) converter, a 2048-element CCD-array detector, and a high-speed USB 2.0 port. The USB2000+ can be controlled by SpectraSuite software. Vivo system contains four tungsten halogen bulbs that can be turned on or off individually. The risk of overheating the sample is mitigated through active cooling. This protects the sample and ensures accuracy every time. The four halogen tungsten light sources make the Vivo a high-powered VIS/NIR source, which allows a shorter integration time than conventional methods (OceanOptics, USA). The integration time used in this investigation was 1 ms.

Evaluation of OLS severity

After spectra acquisition of each leave, severity of OLS was determined using NaOH solution. Each leaf was immersed in 5% NaOH at 50°C for 2 min. Disease severity was calculated by counting the number of lesions per leaf. Severity was then graded; 1 (1 lesion), 2 (2 lesions), 3 (3–5 lesions), 4 (6-10 lesions) or 5 (greater than 11 lesions) (Abuamsha *et al.*, 2013; Salman *et al.*, 2011).

Multivariate Data Analysis (MVDA)

Classification efficiency of leaves according to their severity using VIS/NIR spectra was carried out using MDVA. Two supervised classification methods were used, namely Partial Least Squares-Discrimination Analysis (PLS-DA) (linear method) and Support Vector Machine (SVM) (non-linear method). Unscrambler software was used for analysis (version 10.3, CAMO Software AS, Oslo, Norway).

PLS-DA consists of a classical PLS regression, where VIS/NIR spectra represented X-data matrix, and the dependent variable (Y) is categorical and represented samples class membership (*i.e.* Y with values of -1 and 1, where 1 represents each sample belonging to the targeted class and -1 represents each sample belonging to the other classes). Random cross validation, with segmentations was used.

SVM method was firstly proposed by Vapnik (1995). SVM-classification algorithms try to find patterns in empirical data (*i.e.* training data) with regard to label classes. SVMs have many advantages; the main advantages are mainly referred to their generalization ability, which is achieved by using the maximum margin hyperplane for separation and the application of non-linear discriminant functions. In addition to that, SVM can handle convexity discrimination problems. This classification method used cross validation (Rumpf *et al.* 2010; Suphamitmongkol *et al.*, 2013).
In SVM-classification, VIS/NIR spectra represented X-data matrix, and leaves severity levels were used as the response factor (Y-data matrix, label classes). Unscrambler uses one column for a classifier factor. For SVM-classification, a 15-fold cross validation was performed for the training data. Polynomial degree three was used for classification. VIS/NIR spectra (X-data matrix) were normalized and auto-scaled before analysis.

Results and discussion

Severity levels were evaluated as mentioned above, and used as the classifier parameter in PLS-DA and SVM-classification methods. The number of the leaves used for building different models and their severity levels are shown in Table 1.

Table 1: Number of leaves with different severity levels. Zero severity means healthy leaves. Total number of leaves is 63 leaves.

	Leaves' severity levels						Total number of leaves
	0*	1	2	3	4	5	
Number of leaves	6	7	14	15	5	16	63

*: Healthy leaves

Trials were carried out using VIS, NIR and VIS/NIR spectra to get the ranges that give the best classification rates. It was found that the best classification rates' results were obtained using both VIS and NIR spectra.

Classification using PLS-DA

PLS-DA was used for classification of severity. It was found that PLS-DA could classify only leaves with the highest severity level (*i.e.* severity level 5) in a good classification rate (greater than 90%). This is might be due to the clear severe symptoms that appear on leaves' surface with severity degree 5, and the spectroscopy can sense this high severity.

Trials using PLS-DA to classify different groups of severity were carried out. Classification groups with severity 0, 1, 2 and group with severity 3, 4, 5, didn't give a good classification rate. However, it was found that classification of group consisted of all leaves with severity 0, 1, 2, 3 (*i.e.* 126 samples with triplicates) and group consisted of all leaves with severity 4 and 5 (*i.e.* 63 samples with triplicates) had a good classification rate, using three principals components (PCs). The former explained about 97 and 81% of the variance in X-data and Y-data, respectively. Random cross validation with 20 segments was used.

Table 2 and **Figure 2** show the classification rates (arranged as a confusion matrix), and scores plots of the validation set, respectively. The confusion matrix has information about the predicted and actual classifications of samples, with each row showing the instances in a predicted group (class), and each column representing the instances in an actual group (class). The sum of classification rates in each column should be 100%.

Table 2: Classification rates (%), explained in a confusion matrix, of different groups (group with severity 0, 1, 2, 3, and group with severity 4, 5) using PLS-DA, three principal components and random cross validation. Sum of each classification rate column is 100%.

	Actual groups	
Predicted groups	**Group with severity 0, 1, 2, 3**	**Group with severity 4, 5**
Group with severity 0, 1, 2, 3	***95***	**2**
Group with severity 4, 5	**5**	***98***

It can be seen from **Figure 2**, that a clear classification of different groups using three principal components (*i.e.* three factors). This classification result can be used for screening the leaves' severity, since it can predict the severity group of new testable leaves; groups with high severity (*i.e.* group with leaves' severity 4 and 5) or to with less severity (*i.e.* group with leaves' severity 0, 1, 2, 3).

Classification using SVM

SVM-classification method was able to classify disease severity using spectra data with a high accuracy and classification rates. The accuracy of training and validation sets **(Table 3)** were 87 and 71%, respectively.

A summary of the SVM-classification rates (%)are shown in a confusion matrix (**Table 4**).

The classification rates for 0, 1, 2, 3, 4, and 5 severity levels were 94, 90, 73, 79, 83 and 100%, respectively (**Figure 3**). The overall classification rate (*i.e.* average of all classification rates) is about 86%, which is relatively high and promising for predicting new severity classes.

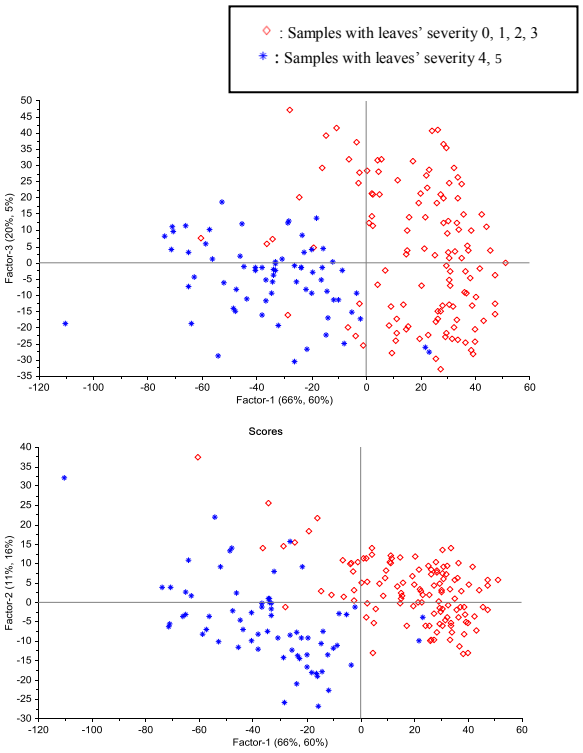

Figure 2: Scores plots of validation set of samples with different severity levels (group with leaves' severity 0, 1, 2, 3, and group with leaves' severity 4, 5) using PLS-DA, three principal components (*i.e.* three factors) and random cross validation. A: scores plot for first and second principal components. B: scores plot for first and third principal components.

It can be noticed that the classification had high rates when the severities were either low (*i.e.* severity levels 0, 1) or high (severity levels 4, 5). This can be explained by that the spectroscopy sensor may detect the extreme situations, *i.e.* low and high severities. Getting high classification rate by increasing disease severity is in agreement with Rumpf *et al.* (2010). The leaves with severity levels between high and low still had good classification rates, despite that they were less than the other classification rates.

Table 3: Accuracy (%) of training and validation sets using SVM-classification.

	Training set	Validation set
Accuracy (%)	**87**	**71**

Tables 3 and **4** show that VIS/NIR spectroscopy was able to identify and quantify of OLS severity with a high accuracy; 71% for both accuracy and 86% for averaged classification rate. SVM-classification was used to classify disease severity into two groups (group with severity 0, 1, 2, 3, and group with severity 4, 5). The classification rate was greater than 99%, which is higher than the classification rate using PLS-DA. This is due to the fact the SVM-classification can handle non-linearity issues.

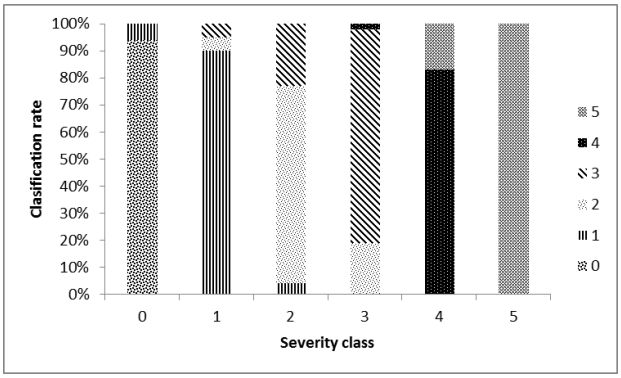

Figure 3: Classification rates (%) of different severity classes using SVM-classification.

Table 4: Classification rates (%) using SVM-classification of different severity class of olive leaf spot (OLS) severity explained in a confusion matrix. Sum of each classification rate column is 100%.

	Actual severity class					
Predicted severity class	**0**	**1**	**2**	**3**	**4**	**5**
0	***94***					
1	**6**	***90***	**4**			
2		**5**	***73***	**19**		
3		**5**	**23**	***79***		
4				**2**	***83***	
5					**17**	***100***

Further research is needed to investigate the possibility of identification and quantification of OLS in-situ using portable VIS/NIR spectroscopy.

Conclusion

VIS/NIR spectroscopy showed a high potential for sensing latent disease of olives. It could classify olives leave with different OLS' severities, with help of multivariate classification methods. Using PLS-DA (a linear classification method), with three principal components and random cross validation, a classification rate greater than 95% was achieved for different groups (group with severity 0, 1, 2, 3, and group with severity 4, 5). SVM-classification (a non-linear classification method) provided a high classification rates and high accuracy for OLS' severities. The classification rates for 0, 1, 2, 3, 4, and 5 severity levels were 94, 90, 73, 79, 83 and 100%, respectively. The overall classification rate was about 86%. This work revealed that VIS/NIR spectroscopy has a potential for evaluation of OLS under field conditions to detect infection of olive trees with OLS disease.

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