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PHYSIOLOGICAL ALTERATIONS IN *AVICENNIA MARINA* (FORSKI) VIERH ASSOCIATED WITH LEAF SPOT DISEASE CAUSED BY *ALTERNARIA ALTERNATA* (FR.) KEISSLER

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Abstract

The leaf spot disease caused by *Alternaria alternata* in *Avicennia marina* in Yanb'a city, Saudi Arabia showed significant alterations in chlorophyll a, chlorophyll b, and total pigments as compared to control plants. Glucose as well as fructose (monosaccharide hexose sugars) was increased significantly in the diseased leaves whereas sucrose (disaccharide), sorbitol (sugar alcohol) and inositol (carbocyclic polyol sugar) were decreased. The infection with *A. alternata* also caused a significant decrease in total lipids, triacylglycerol and sterol, however, diacylglycerol, sterol ester and non-esterified fatty acids increased significantly in diseased leaves. All phospholipid fractions decreased except phosphatidic acid, which increased as compared to healthy leaves. Gas chromatographic analysis of esterified fatty acids revealed the appearance of caprylic (C_8) and a significant increase in the amount of both palmitic (C_{10}) and palmitoleic ($C_{16:1}$). A significant increase in total saturated fatty acids was observed in the infected leaves of *A. marina* as compared to healthy leaves. Such biochemical alterations were directly related to the destructive effect of the causal organism and its mycotoxins on the chloroplast of *A. marina*.

Introduction

The mangrove [*Avicenna marina* (Forsk.) Vierh.] plants are found in the tropical intertidal forest communities like Saudi Arabia (SA). These plants are used as land builders and coastline stabilizers (Jithesh *et al*., 2006). Their main use is for the production of tannins, fire wood, and for deriving potential medicinal compounds (Mmom & Arokoyu, 2010). The leaves are used as fodder for animals (Amer, 2000).

The major disease of mangrove forests is leaf spot disease (LSD) caused by *Alternaria alternata* (Kjer *et al*., 2009). It causes major leaf defoliation and decrease in yield up to 40% (Shrestha *et al*., 2005). Severe LSD was found to occur on *A. marina* at the central industrial region of Yanb'a city, SA.

Such infections induce defense responses against the infection and cause alterations in the secondary metabolites of the host plant (Pati *et al*., 2008). The pathogen tries to manipulate the plant metabolism for its own need and causes an increased demand for assimilation in the plant (Swarbrick *et al*., 2006). The infection of leaf usually leads to the development of chlorotic and necrotic areas hence decrease in photosynthetic assimilate production (Ciuffetti *et al*., 2010). Such decrease in photosynthesis might be attributed to the destructive effect of the pathogen or its metabolites on chloroplasts (Chen *et al*., 2005), may be by blocking the electron transfer chain of the thylakoid membrane (Dai *et al*., 2004). The role of sugars in plant-pathogen interaction has been studied and it was seen that ketohexose variation during infection is directly related to the degree of pathogen infection and plant resistance. Srivastava & Pandey (2012) reported a pronounced decrease in the contents of total carbohydrates with significant increase in soluble and reducing sugars due to fungal infection.

Lipids are building blocks of biological membranes (Voet *et al*., 2006). They have important role in major physiological activities like respiration, energy transport, and photosynthesis (Radwan & Mangold, 1976). Thus, they play essential role in host-parasite relations (Manocha, 1980). The alterations in lipid metabolism have been used as an important criterion for studying plant-pathogen interaction and toxicity mechanisms of the metabolites produced by the pathogen (Kim *et al*., 2010, Kim, 2012).

The present study explores the physiological alterations in *Avicennia marina* associated with natural infection of *Alternaria alternata* (LSD) in Yanb'a city, SA.

Materials and Methods

Sample collection: Samples were collected from the central industrial region (Naturally infected plants) and 30 Km away, towards the north for healthy control plants in Yanb'a city, SA (Fig. 1).

Plant analysis: The quantitative estimation of photosynthetic pigments was estimated according to the method of Lichtenthaler & Wellburn (1983). The pigments were extracted from fresh leaves by acetone and the homogenate was centrifuged at 3000x*g* for 15 minutes. 1ml aliquot was mixed with 2ml acetone and the absorbance was read spectrophotometrically at 622, 664 and 440 nm wavelength.

The oven dried (105^{o}C overnight) leaves were used for estimation of sugars. Soluble sugars were extracted in 80% (v/v) ethanol, the extract was transferred to 5 ml pear shaped flasks then completely dried in a stream of air and stored in desiccator until used for silylation as described by Holligan & Drew (1971). In this method, each sample was dissolved in 0.8ml anhydrous pyridine, then 0.2 ml of N-trimethylsilylimidazole – TSIM ($C_6H_{12}NSI$) - added as the

silylating reagent. The flasks were placed in a water bath in 60-70°C for 30 min. After cooling, the samples were transferred to small injections vials for gas liquid chromatography (GLC). The peak areas of both samples and standard were compared corresponding to retention time of standard sugars for qualitative as well as quantitative estimation of sugars. Standard sugars (arabinose, fructose, glucose, mannitol, sorbitol, inositol, sucrose, maltose, raffinose, and cellobiose) were used as references.

Total Lipids were extracted using chloroform:methanol (2:1, v/v), with 0.05% (w/v) of butylated hydroxytoluence (BHT; 2.6 di-tert-butyl-p-cresol). Total lipids were estimated using the charring method of Marsh & Weinstein (1966), with stearic acid (Sigma) as the standard. The neutral lipids in the extracts were separated on thin layer chromatography (TLC) plates (chloroform:methanol:water, 65:35:3; v/v/v, used as mobile phase). The qualitative estimation was carried by reaction of TLC plates with acid dichromate for clarification. The quantitative estimation was carried out spectrophotometricaIly according to Amenta (1964). Phospholipids were separated by two-dimensional chromatography with $CHCl_3$-MeOH - 28% (w/v) NH_4OH (13:5:1, v/v/v) for the first dimension and CHC_3- Me_2CO - MeOH - HOAc - H_20 (6:8:2:1, v/v/v/v/v) for the second dimension (Rouser *et al*., 1970). Fatty acid methyl esters were prepared by methanolysis in H_2SO_4-MeOH (Kates, 1972) and methyl esters were analyzed by gas liquid chromatography (GLC) (Perkin-Elmer Model 910, Perkin Elmer, Shelton, CT, USA) equipped with a flame ionization detector (Johnson & Stocks, 1971). Qualitative and quantitative analysis of peak fatty acid methyl esters was carried via comparing their retention times with those of an authentic methyl standard (Sigma Co., St. Louis, USA).

Statistical analysis: All experiments were repeated three times. The data were statistically analyzed using analysis of variance for a completely randomized design.

Results and Discussion

The results related to the effect of *A. alternata* on pigments are depicted in Table 1. A decrease of 42.3, 45.4 and 43.2% was observed in chl. 'a', 'b' and total pigment content respectively in diseased plants when compared to healthy plants. A minimum decrease of 2.9% was observed in carotenoid content in the diseased plants when compared to the control. Such decrease in photosynthetic pigments might be due to the negative effect of pathogen (*A. alternata*) or its mycotoxins on chloroplasts (Chen *et al*., 2005). It might be due to the reduction in the rate of electron transport in the non-cyclic photophosphorylation (Dai *et al*., 2004). Jia *et al*., (2013) and Zhao *et al*., (2013) also reported that *A. alternata* infection significantly decreases chlorophyll content in tomato and cotton respectively. Photosynthetic process seems to be quite important as it contributes to growth, development and yield of plants. Chlorophylls were proven to be a very useful tool for the evaluation of the effect of the biotic stresses on photosynthetic properties and plant metabolism (Zhu *et al*., 2012).

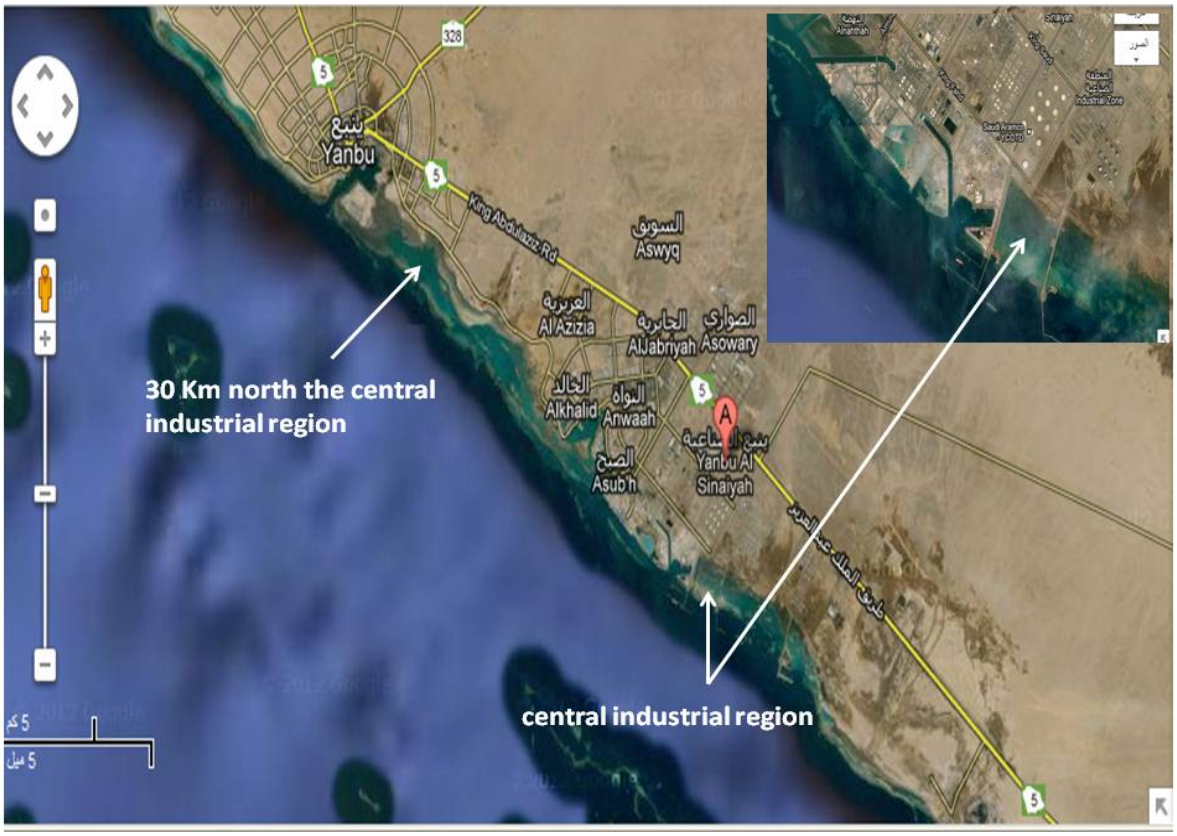

Fig. 1. Google map showing the locations of sampling areas.

Table 2 discusses the effect of *A. alternata* on sugar content of *A. marina*. Glucose and fructose showed an increase of 17.8% and 67.1% respectively in diseased plants as compared to the control. However, a decrease of 34.4% in sucrose, 57.8% in sorbitol and 50.6% in inositol was also observed in diseased plants as compared to the healthy plants. The change in sugar profile in our results indicated catabolic processes towards hydrolysis of sugars associated with the pathogen infection and appearance of LSD. Timmer *et al*., (2003) reported an increase in rate of respiration in the citrus fruits infected with *Alternaria,* as defense response of citrus. The increase in monosaccharide content as well as decrease in disaccharide content of diseased leaves indicated that phytopathogen produce carbohydrate hydrolytic enzymes during the penetration and degradation of the plant cells (Qin *et al*., 2007). The water stress developed during fungal infection of plant (Otani *et al*., 1995) play stimulatory effect on carbohydrate hydrolytic enzymes of host plant (Abdalla & El-Khoshiban, 2007) which have proved to increase monosaccharide contents in diseased plants compared to control plants. Furthermore, the production of carbohydrate hydrolytic enzymes by *A. alternata* during the infection increases the endogenous reducing and soluble sugar contents in diseased plants (Ijaz *et al.,* 2011; Chung, 2012).

The effect of *A. alternata* on total and neutral lipids of *A. marina* is presented in Table 3. The total lipids decreases by 33.5% in diseased plants as compared to the healthy ones. The neutral lipids, diacylglycerol (DG), sterol ester (SE) and non-esterified fatty acids (FAA) increases by 15.9, 31.4 and 48.8% respectively in diseased plants as compared to control plants. However, a decrease of 42.7% in triacylglycerol (TG) and 47.7% in sterol (S) was also observed in our study. Lipids are known to play an important role in the development of plant tolerance to biotic (Shah, 2005) and abiotic (Popov *et al*., 2012) stresses. A significant deterioration in the host lipid metabolism and induction of systemic physiological changes towards catabolism has been reported, suggesting a negative correlation between the fungal infection and the host lipid metabolism (Kim, 2012). Similar observations were also reported with the mycotoxins produced by *Alternaria alternata* in duckweed and susceptible varieties of tomato (Abbas *et al*., 1995). The alterations in total and neutral lipids by leaf pathogen might be due to decrease in photosynthesis (Chen *et al*., 2005; Kim *et al*., 2010).

Table 1. Effect of leaf spot disease caused by *A. alternata* on pigments system of *A. marina*.

Treatment	Pigments system (mg/g fresh weight)			
	Chlorophyll a	Chlorophyll b	Carotenoids	Total pigments
Healthy control	41.01	22.93	0.329	64.26
Diseased plant	23.64	12.50	0.339	36.47
LSD at 0.05	8.1549	7.5155	0.0871	10.34

Table 2. Effect of leaf spot disease caused by *A. alternata* on sugars content *A. marina*.

Treatment	Sugars content (µg/g fresh weight)				
	Glucose	Fructose	Sucrose	Sorbitol	Inositol
Healthy control	175	60.75	274.7	41.33	78.6
Diseased plant	213	184.8	180	17.43	38.75
LSD at 0.05	11.936	14.493	17.761	7.204	10.171

Table 3. Effect of leaf spot disease caused by *A. alternata* on total and neutral lipids of *A. marina*.

Treatment	Total lipids (mg/g fresh weight)	Neutral lipid fractions (µg/g fresh weight)				
		DG	TG	S	SE	FAA
Healthy control	35.33	670.64	868.98	766.41	804.7	225.21
Diseased plant	23.49	797.92	497.46	400.41	1173.2	440.14
LSD at 0.05	0.743	5.7777	34.723	51.807	42.07	143.88

DG, diacylglycerol; TG, triacylglycerol; S, sterol; SE, sterol ester; FAA, non-esterified fatty acids

The results pertaining to the effect of *A. alternata* on phospholipid fractions of *A. marina* is presented in Table 4. The phosphatidyl choline (PC); phosphatidyl ethanol amine (PE); phosphatidyl glycerol (PG); phosphatidyl inositol (PI); phosphatidyl serine (PS) are decreased by 49.4, 31.9, 44.3, 49.3 and 39.2% respectively in diseased plants as compared to control plants. In the present study, phosphatidic acid (PA) is the only phospholipid fraction, that showed an increase of 51.8% in diseased plants as compared to the control. As phospholipids are the most abundant membrane lipids which play significant role in membrane structure (Liu *et al*., 2008). An increase of PA in infected plants indicates alteration in phospholipid biosynthesis. The infection decreases the incorporation of phosphoric acid to lipid moiety, which leads to accumulation of free PA in the infected plant tissues (Miraazimova *et al*., 1997). Chung (2012) reported that *A. alternata* infection induced rapid lipid peroxidation and generation of ROS which could be ascribed to the enhancement of several phospholipid hydrolyzing enzymes that contribute to a decrease in the phospholipid fractions except PA (Canonne *et al*., 2011). Furthermore, the water deficit stress induced by phytotoxic metabolites from *Alternaria* species (Otani *et al*., 1995) plays a significant role in the alteration of phospholipid fractions of stressed plants (Navari-Izzo *et al*., 1993) and helps in defense mechanism against uncontrolled increase in membrane permeability (Belous & Bondarenko, 1982).

Table 4. Effect of leaf spot disease caused by *A. alternata* on phospholipids fractions of *A. marina*.

Treatment	Phospholipid fractions (µg/g fresh weight)					
	PA	PC	PE	PG	PI	PS
Healthy control	31.13	18.598	16.3	34.57	11.62	14.15
Diseased plant	64.64	9.407	11.090	19.243	5.895	8.607
LSD at 0.05	10.206	3.2229	2.6003	6.6304	2.0436	1.3503

PA, phosphatidic acid; PC, phosphatidyl choline; PE, phosphatidyl ethanol amine; PG, phosphatidyl glycerol; PI, phosphatidyl inositol; PS, phosphatidyl serine

Table 5. Effect of leaf spot disease caused by *A. alternata* on cellular fatty acids profile of *A. marina*.

Treatment	Fatty acid profile of *A. marina* (%)												
	Caprylic C8	Capric C10	Lauric C12	Myristic C14	Palmitic C16	Margaric C:17	Stearic C18	Oleic C18:1	Linoleic C18:2	α Linolenic C18:3	Arachidic C20	Arachidonic C20:4	Un-saturation %
Healthy control	0.00	0.00	0.00	0.00	2.65	0.55	14.68	10.02	9.04	17.09	17.78	28.19	64.34
Diseased plant	2.38	5.15	1.85	1.40	4.31	1.34	19.04	5.94	6.82	14.36	21.95	15.80	42.93
LSD at 0.05	0.2312	0.5827	0.3151	0.4779	0.4532	0.5623	1.6235	1.7169	1.0823	1.8328	1.9008	1.3163	

The results discussed in Table 5 revealed the significant increase of four saturated fatty acids namely palmitic ($C_{16:0}$), margaric (C_{17}), stearic ($C_{18:0}$) and arachidic ($C_{20:0}$) 38.5, 58.9, 22.8 and 18.99% respectively, as well as significant decrease in the unsaturated fatty acids namely oleic ($C_{18:1}$), linoleic ($C_{18:2}$), α linolenic ($C_{18:3}$) and arachidonic ($C_{20:4}$) fatty acids by 40.71, 24.55, 15.97 and 43.95% respectively. Such results of saturated fatty acids are in agreement with the observation of Joubert *et al*., (2011) in *Arabidopsis thaliana* infected with *Alternaria brassicicola*. The alteration in plant fatty acid profile directly relates to the resistance mechanism against pathogenic fungi (Reina-Pinto & Yephremov, 2009; Kim, 2012; Kim *et al*., 2013). The unsaturated fatty acids are considered essential membrane components and play important biophysical roles such as control of metabolism, mediator in signal transduction and fungal disease tolerance (Wu *et al*., 2009). Fatty acid hydroperoxides could be formed enzymatically by lipoxygenase activity of unsaturated fatty acids such as linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$), stimulated by the phytopathogens (Vick, 1993).

In conclusion, the present study uncovers the effects of LSD caused by *A. alternata* on pigments system, sugar fractions and lipid metabolism and is reported for the first time in mangrove leaves.

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References

Abbas, H.K., S.O. Duke and R.N. Paul. 1995. AAL-Toxin, a potent disrupts sphingolipid natural herbicide which metabolism of plants. *Pestic. Sci*., 43: 181-187.

Abdalla, M.M. and N.H. El-Khoshiban. 2007. The influence of water stress on growth, relative water content, photosynthetic pigments, some metabolic and hormonal contents of two *Triticium aestivum* cultivars. *J. Appl. Sci. Res*., 3: 2062-2074.

Amenta, J.S. 1964. A rapid method for quanti-cation of lipids separated by thin layer chromatography. *J. Lipid Res*., 5: 270-272.

Amer, A.F. 2000. Mangrove as a potential fodder crop in the Red Sea coastal areas of Egypt. *Egypt. J. Agri. Res*., 78: 521-528.

Belous, A.M. and V.A. Bondarenko. 1982. *Strukturnye izmeneniya biologicheskikh membran pri okhlazhdenii* (Structure changes in biological membranes under chilling), Kiev: Naukova Dumka, Kiev, pp. 255, in Germany.

Canonne, J., S.F. Nicolas and S. Rivas. 2011. Phospholipases in action during plant defense signaling. *Plant Signal. Behav*., 6: 13-18.

Chen, S., X. Dai, S. Qiang and Y. Tang. 2005. Effect of a nonhost-selective toxin from *Alternaria alternata* on chloroplast-electron transfer activity in *Eupatorium adenophorum*. *Plant Pathol*., 54: 671-677.

Chung K.R. 2012. Stress response and pathogenicity of the necrotrophic fungal pathogen *Alternaria alternata*. *Scientifica*, Article ID 635431,17pages http://dx.doi.org/10.6064/2012/635431

Ciuffetti, L.M., V.A. Manning, I. Pandelova, M.F. Betts and J.P. Martinez. 2010. Host- selective toxins, Ptr ToxA and Ptr ToxB, as necrotrophic effectors in the *Pyrenophora tritici-repentis*-wheat interaction. *New Phytol*., 187: 911-919.

Dai, X.B., S.G. Chen, S. Qiang, Q.F. An and R.X. Zhang. 2004. Effect of toxin from *Alternaria alternata* (Fr.) Keissler on leaf photosynthesis of *Eupatorium adenophorum* Spreng. *Acta Phytopathol. Sinica*, 34: 55-60.

Holligan, P.M. and E.A. Drew. 1971. Routine analysis by gas-liquid chromatography of soluble carbohydrates in extracts of plant tissues. II. Quantitative analysis of standard carbohydrates, and the separation and estimation of soluble sugars and polyois from a variety of plant tissues. *New Phytol*., 70: 271-297.

Ijaz, A., Z. Anwar, Y. Zafar, I. Hussain, A. Muhammad, M. Irshad and S. Mehmood. 2011. Optimization of cellulase enzyme production from corn cobs using *Alternaria alternata* by solid state fermentation. *J. Cell Molecular Biol*., 9: 51-56.

Jia, C., L. Zhang, L. Liu, J. Wang, C. Li and Q. Wang. 2013. Multiple phytohormone signalling pathways modulate susceptibility of tomato plants to *Alternaria alternata* f. sp. *lycopersici*. *Exp. Bot*., 64(2): 637-650.

Jithesh, M.N., S.R. Prashanth, K.R. Sivaprakash and A. Parida. 2006. Monitoring expression profiles of antioxidant genes

to salinity, iron, oxidative, light and hyperosmotic stresses in the highly salt tolerant grey mangrove, *Avicennia marina* (Forsk.) Vierh. by mRNA analysis. *Plant Cell Rep*., 25: 865-876.

Johnson, A. and R. Stocks. 1971. Gas-liquid chromatography of lipids. In: *Biochemistry and Methodology of Lipids*. (Eds.): A. Johnson & J. Davenport. Wiley Interscience, New York, NY.

Joubert, A., N. Bataille-Simoneau, C. Campion, T. Guillemette, P. Hudhomme, B. Iacomi-Vasilescu, T. Leroy, S. Pochon, P. Poupard and P. Simoneau. 2011. Cell wall integrity and high osmolarity glycerol pathways are required for adaptation of *Alternaria brassicicola* to cell wall stress caused by brassicaceous indolic phytoalexins. *Cell. Microbiol*., 13: 62-80.

Kates, M. 1972. Techniques of lipidology. In: *Laboratory Techniques in Biochemistry and Molecular Biology.* (Eds.): T.W. Work & E. Work. North-Holland Publishing Co., Amsterdam, the Netherlands.

Kim, D.W. 2012. *Lipid profiles in wheat cultivars resistant and susceptible to tan spot and the effect of disease on the profiles*. M.Sc. Thesis, Department of Plant Pathology College of Agriculture, Kansas State University, Manhatan, Kansas, USA.

Kim, D., R. Jeannotte, R. Welti and W.W. Bockus. 2013. Lipid profiles in wheat cultivars resistant and susceptible to tan spot and the effect of disease on the profiles. *Phytopathol*., 103(1): 74-80.

Kim, Y.M., N. Bouras, N.N. Kav and S.E. Strelkov. 2010. Inhibition of photosynthesis and modification of the wheat leaf proteome by Ptr ToxB: a host-specific toxin from the fungal pathogen *Pyrenophora tritici-repentis*. *Proteomics*, 10: 2911-2926.

Kjer, J., V. Wray, R.A. Edrada-Ebel, R. Ebel, A. Pretsc, W.H. Lin and P. Proksch. 2009. Xanalteric acid and related phenolic compounds from endophytic *Alternaria* sp. isolated from the mangrove plant *Sonneratia alba*. *J. Nat. Prod*., 72: 2053-2057.

Lichtenthaler, H.K. and A.R. Wellburn. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans*., 11: 591-592.

Liu, J., R.F. Epand, D. Durrant, D. Grossman, N.W. Chi, R.M. Epand and R.M. Lee. 2008. Role of phospholipid scramblase 3 in the regulation of tumor necrosis factor-alpha-induced apoptosis. *Biochem*., 47: 4518-4529.

Manocha, M.S. 1980. Role of lipids in host-parasite relations of a mycoparasite. *Physiol. Plant Pathol*., 17: 319-328.

Marsh, J.B. and D.B. Weinstein. 1966. Simple charring method for determination of lipids. *J. Lipid Res.*, 7: 574-576.

Navari-Izzo, F., M.F. Quartacci and R. Izzo. 1993. Lipid composition of plasma membranes isolated from sun ower seedlings grown under water stress. *Physiol. Plant*., 87: 508-514.

Miraazimova, K.H.T., S.D. Gusakova and A.I. Glushenkova. 1997. Post-infectional changes on the phospholipids of the leaves of cotton plant variety resistant to *Verticillium dahliae*. *Chem. Nat. Compd.*, (Engl. Transl. Khim. Prir. Soedin.), 33: 156-161.

Mmom, P.C. and S.B. Arokoyu. 2010. Mangrove forest depletion, biodiversity loss and traditional resources management practices in the Niger Delta, Nigeria. *Res. J. Appl. Sci., Eng. Technol*., 2: 28-34.

Otani, H., K. Kohomoto and M. Kodama. 1995. Alternaria toxins and their effects on host plants. *Can. J. Bot*., 73: 453-458.

Pati, P.K., M. Sharma, R.K. Salar, A. Sharma, A.P. Gupta and B. Singh. 2008. Studies on leaf spot disease of *Withania somnifera* and its impact on secondary metabolites. *Indian J. Microbiol.,* 48: 432-437.

Popov, V.N., O.V. Antipina, V.P. Pchelkin and V.D. Tsydendambaev. 2012. Changes in the content and composition of lipid fatty acids in tobacco leaves and roots at low temperature hardening. *Russ. J. Plant Physiol*., 59: 177-182.

Qin, G., S. Tian, Z. Chan and B. Li. 2007. Crucial role of antioxidant proteins and hydrolytic enzymes in pathogenicity of *Penicillium expansum*. *Mol. Cell. Proteomics*, 6(3): 425-438.

Radwan, S.S. and H.K. Mangold. 1976. The lipids of plant tissue cultures. *Adv. Lipid Res.*, 2: 171-211.

Reina-Pinto, J.J. and A. Yephremov. 2009. Surface lipids and plant defenses. *Plant Physiol. Biochem*., 47: 540-549.

Rouser, G., S. Fleischer and A. Yamamoto. 1970. Two dimensional thin layer chromatographic separation of polar lipids and determination of phospholipids and phosphorus analysis of spots. *Lipids*, 5: 494-496.

Shah, J. 2005. Lipids, lipases, and lipid-modifying enzymes in plant disease resistance. *Annu. Rev. Phytopathol*., 43:229-226.

Shrestha, S.K., L. Munk and S.B. Mathu. 2005. Role of weather on *Alternaria* Leaf Blight disease and its effect on yield and yield components of Mustard. *Nepal Agric. Res. J*., 6: 62-72.

Srivastava, A.K. and G.K. Pandey. 2012. Starch and glucose content and changes therein in the seeds of *Schleichera oleosa* (kusum) due to bioterioration by pathogenic fungi during storage. *Int. J. Recent Trends Sci. Tech*., 4: 5-8.

Swarbrick, P.J., P. Schulze-Lefert and J.D. Scholes. 2006. Metabolic consequences of susceptibility and resistance in barley leaves challenged with powdery mildew. *Plant, Cell Environ*., 29: 1061-1076.

Timmer, L.W., T.L. Peever, Z. Solel and K. Akimitsu. 2003. *Alternaria* diseases of citrus novel pathosystems. *Phytopathol. Mediterr*., 42: 99-112.

Vick, B.A. 1993. Oxygenated fatty acids of the lipoxygenase pathway. In: *Lipid Metabolism in Plants*. (Ed.): TSJr. Moore CRC Press, Boca Raton, FL, USA. pp. 167-191.

Voet, D., J.G. Voet and C.W. Pratt. 2006. Biological membranes and lipids. Pages 234- 281, In: *Fundamentals of Biochemistry*. Wiley, Hoboken.

Wu, Q., T. Liu, H. Liu and G. Zheng. 2009. Unsaturated fatty acid: Metabolism, synthesis and gene regulation. *Afr. J. Biotechnol*., 8: 1782-1785.

Zhao J., F. Zhao, G. Jian, Y. Ye, W. Zhang, J. Li and F. Qi. 2013. Intensified *Alternaria* spot disease under potassium deficiency conditions results in acceleration of cotton (*Gossypium hirsutum* L.) leaf senescence. *Aust. J. Crop Sci*., 7: 241-248.

Zhu, X.C., B. Song, S.Q. Liu, T.D. Liu and X. Zhou. 2012. Arbuscular mycorrhizae improves photosynthesis and water status of *Zea mays* L. under drought stress. *Plant Soil Environ*., 58: 186-191.

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